S. MAJUMDAR & CO.
PATENT & TRADEMARK ATTORNEYS

Undertakings: Intellectual Property Laws,
Patents, Trademarks, Designs, Copyrights,
Licencing, Investigations, Litigations
**DOMESTIC AND INTERNATIONAL**

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The Controller of Patents
The Patent Office
Delhi

September 06, 2016

Dear Sir

Re: Representation u/s 25 (1) read with Rule 55 against
Patent Application **4412/DELNP/2007** dated November 8, 2006
(Nationalization date : June 8, 2007)
Applicant: NOVARTIS AG
Opponent: NATCO PHARMA LTD.
Our Ref: PII599

We wish to submit herewith a representation U/S 25(1) read with Rule 55 of The Patent Act. In reference to this we submit herewith following documents:

**(i)** Form 7A (in duplicate)
**(ii)** Representation under section 25(1) along with Annexure 1 to Annexure 3 and Exhibit I to Exhibit VI

The opposition may kindly be taken on record and proceeded to the next stage.

Yours faithfully,

Amrita Majumdar
Of S. Majumdar & Co.
Opponent's Agent

**Enclosures**: As above

**BEFORE THE CONTROLLER OF PATENTS**

**PATENT OFFICE**

**DELHI**

**PRE-GRANT OPPOSITION UNDER SECTION 25 (1) AGAINST PATENT**

**Application Number 4412/DELNP/2007 Nationalized On June 8, 2007**

NATCO PHARMA LTD., an Indian Company
at H. No: 8-2-112/A/32, Road No. 2, Banjara Hills,
Hyderabad – 500 034 ---------- **Opponent**

Vs

NOVARTIS AG., ---------- **Applicant**

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| | and annexed as **Exhibit III** | |
| 9. | Article titled: "High-throughput crystallization: polymorphs, salts, co-crystals and solvates of pharmaceutical solids", by Morissette et. al., first published in March 2004, in Advanced Drug Delivery Reviews, volume 56, pages 275-300, referred herein as D4 and annexed as **Exhibit IV** | 205-230 |
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Amrita Majumdar
Of S. Majumdar & Co.
Opponent's Agent

The Controller of Patents
The Patent Office,
Delhi

**FORM – 7A**

**THE PATENTS ACT, 1970**

**(39 OF 1970)**

**&**

**THE PATENTS RULES, 2003**

**REPRESENTATION FOR OPPOSITION TO GRANT OF PATENT**

**(See section 25 (1) and rule 55)**

We, NATCO PHARMA LTD., an Indian Company at H. No: 8-2-112/A/32, Road No. 2, Banjara Hills, Hyderabad – 500 034 hereby give representation by way of opposition to the grant of patent in respect of Patent Application No. **4412/DELNP/2007** dated November 8, 2006, (Nationalized on June 8, 2007) by **NOVARTIS AG.,** having office at Lichtstrasse 35, CH-4056, Basel, Switzerland.. It is published under section 11A in the Official Journal of Indian Patent Office dated August 24, 2007 and the publication date therein is mentioned as August 24, 2007

**The impugned Patent Application is opposed on the following grounds:-**

1. **Section 25(1) (e)-Obviousness/lack of inventive step**

   that the invention so far as claimed in any claim of the complete specification is obvious and clearly does not involve any inventive step, having regard to the matter published as mentioned in clause 25 (1) (b) or having regard to what was used in India before the priority date of the applicant's claim;

2. **25(1)(f) – Not an invention / Not patentable**

   that the subject of any claim of the complete specification is not an invention within the meaning of this Act, or is not patentable under this Act;

3. **25(1)(g) - Insufficiency**

   that the complete specification does not sufficiently and clearly describe the invention or the method by which it is to be performed;

4. **25(1)(h) – Failure to disclose information under Section 8**

   that the applicant has failed to disclose to the Controller the information required by section 8 or has furnished the information which in any material particular was false to his knowledge.

**Our address for service in India is**

S. Majumdar & Co. 5 Harish Mukherjee Road, Kolkata – 700 025, India, Phone: (033) 24557484; Fax: (033) 24557487; Email: cal@patentindia.com.

Dated this the 06th day of September 2016

Amrita Majumdar
Of S. Majumdar & Co.
Opponent's Agent

To
The Controller of Patents
The Patent Office,
At Delhi

BEFORE THE CONTROLLER OF PATENTS,
THE PATENT OFFICE, NEW DELHI

IN THE MATTER OF The Patents Act, 1970 *as amended by* The Patents (Amendment) Act 2005,

-And-

IN THE MATTER of The Patents Rules, 2006 (as amended by the Patents (Amendment) Rules 2006

-And-

IN THE MATTER of Indian Patent Application No. **4412/DELNP/2007** nationalized on June 8, 2007 from PCT Application No. PCT/US2006/043710 (Published as WO 2007056546 A1) filed on November 8, 2006, and claiming priority from the US Patent Application No. 60/735,093 dated November 9, 2005, 60/735,541 dated November 10, 2005, 60/789,332 dated April 4, 2006 and 60/822,086 dated August 11, 2006, assigned to **NOVARTIS AG.,** Lichtstrasse 35, CH-4056, Basel, Switzerland.

……**APPLICANT**

-And-

IN THE MATTER of Opposition to the grant of a patent thereto **NATCO PHARMA LTD**., an Indian Company at H. No: 8-2-112/A/32, Road No. 2, Banjara Hills, Hyderabad – 500 034, Andhra Pradesh, India.

……**OPPONENT**

**REPRESENTATION BY WAY OF OPPOSITION UNDER SECTION 25(1)**

We, Natco Pharma Ltd, H. No: 8-2-112/A/32, Road No. 2, Banjara Hills, Hyderabad – 500 034, Andhra Pradesh, India, (hereinafter called 'Opponent') make the following representation under Section 25(1) of The Patents Act in opposing the grant of patent on the application indicated in the cause title.

**1. THE OPPONENT'S BUSINESS AND ACTIVITIES**

The Opponent is a publicly traded company engaged in the research, development, manufacture and wholesale trade of pharmaceutical ingredients, intermediates and finished dosage forms. The Opponent is engaged in the research, development, manufacture and marketing of oncology drugs internationally and in India.

The Opponent has access to the latest technologies relating to manufacture of the drugs and medicines. The Opponent is also engaged in the research and development of medicines and pharmaceutical products and preparations.

**2. LOCUS STANDI**

*Locus standi* is not a condition precedent for an opposition under Section 25(1).

**3. GROUNDS OF OPPOSITION:**

The Impugned patent application is opposed by the Opponent on the following grounds enumerated in Section 25 (1) of The Patents Act, 1970 (hereinafter referred to as the "Act"):

1. **Section 25(1) (e)-Obviousness/lack of inventive step**

   that the invention so far as claimed in any claim of the complete specification is obvious and clearly does not involve any inventive step, having regard to the matter published as mentioned in clause 25 (1) (b) or having regard to what was used in India before the priority date of the applicant's claim;

2. **25(1)(f) – Not an invention / Not patentable**

that the subject of any claim of the complete specification is not an invention within the meaning of this Act, or is not patentable under this Act;

3. **25(1)(g) - Insufficiency**

that the complete specification does not sufficiently and clearly describe the invention or the method by which it is to be performed;

4. **25(1)(h) – Failure to disclose information under Section 8**

that the applicant has failed to disclose to the Controller the information required by section 8 or has furnished the information which in any material particular was false to his knowledge.

## 4. ANALYSIS OF INDIAN PATENT APPLICATION No. 4412/DELNP/2007:

**4.1.** The Opponent has learnt that the Applicant has filed an Indian National Phase Application No. **4412/DELNP/2007** (hereinafter also referred to as the 'impugned application'), which is currently pending before the Patent Office. The said patent application is entitled "PHARMACEUTICAL COMBINATIONS OF AN ANGIOTENSIN RECEPTOR ANTAGONIST AND AN NEP INHIBITOR" and is drawn to a pharmaceutical formulation comprising of a dual-acting compound having a formula: [((S)-N-valeryl-N-{[2'-(1-H-tetrazole-5-yl)-biphenyl-4-yl]-methyl}-valine). [(2R,4S)-5-biphenyl-4-yl-4-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester) $Na_{1-3}$.x $H_2O$ where x- 0-3, which is a combination of an angiotensin receptor antagonist valsartan and a neutral endopeptidase inhibitor (NEPi) (2R,4S)-5-biphenyl4-yl~5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester. The impugned application was filed on 8 June, 2007. It derives priority from 4 different applications being U.S. Provisional Application Nos. 60/735,093 dated November 9, 2005, 60/735,541 dated November 10, 2005, 60/789,332 dated April 4, 2006 and 60/822,086 dated August 11, 2006. The application was nationalized from PCT publication No. WO 2007056546 A1. The application originally contained a set of 29 claims. A copy of the specification of the impugned application is annexed hereto as **Annexure 1.**

**4.2.** It is stated that the alleged invention as disclosed in the impugned specification pertains to dual-acting compounds and combinations of angiotensin receptor blockers and neutral endopeptidase inhibitors (NEPi). In particular, these actives are linked via non-covalent bonding, or supramolecular complexes of the actives, also described as linked pro-drugs, such as mixed salts or co-crystals, as well as to pharmaceutical combinations containing such a dual-acting compound or combination, methods of preparing such dual-acting compounds and methods of treating a subject with such a dual-acting compound or combination.

**4.3.** In the background of invention, the applicant discloses the need for such combination therapy in view of available state of the art. The applicant discusses the mechanisms of action of the AT1 receptor inhibitors and neutral endopeptidase inhibitors and the advantages of such therapy over monotherapy since the nature of hypertensive vascular diseases is multifactorial. It further states that however combination of drugs having different modes of action does not necessarily have advantageous effects. Accordingly there was a need for efficacious combination therapy which does not have deleterious side effects.

**4.4.** To meet such need, the applicant provided a dual-acting compound, such as a supramolecular complex, comprising: (a) an angiotensin receptor antagonist; (b) a neutral endopeptidase inhibitor (NEPi); and optionally (c) a pharmaceutically acceptable cation. The impugned specification also discloses a process and compositions comprising such complexes. Interestingly the impugned specification also discloses physical combinations of angiotensin receptor antagonist and NEP inhibitors.

**4.5.** The allegedly inventive process for preparing the dual-acting compound, such as a supramolecular complex, comprises the below steps.

(i) dissolving an angiotensin receptor antagonist and a neutral endopeptidase inhibitor (NEPi) in a suitable solvent; (ii) dissolving a basic compound of Cat in a suitable solvent, wherein Cat is a cation; (iii) combining the solutions obtained in steps (i) and (ii); (iv) precipitation of the solid, and drying same to obtain the dual-acting compound; or alternatively obtaining the dual-acting compound by exchanging the solvent(s) employed in steps (i) and (ii) by (iva) evaporating the resulting solution to dryness; (va) re-dissolving the solid in a suitable solvent; (via) precipitation of the solid and drying same to obtain the dual-acting compound.

**4.6** In other embodiments the alleged invention provides linked pro-drugs comprising angiotensin receptor antagonist and NEP inhibitors, which are linked by a linking moiety.

**4.7** At page 4 of the specification the applicant discloses the preferred angiotensin receptor antagonists which are selected from the group consisting of valsartan, losartan, irbesartan, telmisartan, eprosartan, candesartan, olmesartan, saprisartan, tasosartan, elisartan and combinations thereof. At page 5 the applicant has disclosed the various NEPi which can be used in the alleged invention.

**4.8** The impugned specification describes the term "supramolecular complex" in the paragraph bridging pages 9 and 10 as “an interaction between the two pharmaceutically active agents, the cations and any other entity present such as a solvent, in particular water, by means of noncovalent, intermolecular bonding between them. This interaction leads to an association of the species present in the supramolecular complex distinguishing this complex over a physical mixture of the species.” Such noncovalent intermolecular bonding can be hydrogen bonding, van der Waals forces and π-π -stacking. Ionic bonds can also be present. It further states that the complex is crystalline and in this case is preferably a mixed crystal or co-crystal.

**4.9** The applicant at page 14 states that the preferred ARB is Valsartan and acknowledges its disclosure in U.S. Patent No 5,399,578 and EP 0 443 983. Further at pages 18 and 19, the impugned specification discloses the preferred NEPi and acknowledges a series of prior patents. The preferred NEPi is a compound of formula (II), which can exist in various isomeric forms and is described in U.S. Patent No. 5,217,996. The specification further discloses the X-ray powder diffraction pattern of the supramolecular complex of valsartan and the NEPi of formula II. At pages 34 and 35 the impugned specification puts forth the antihypertensive and neutral endopeptidase inhibitory activities of trisodium [3-((1S,3R)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(S)-3'-methyl-2'-(pentanoyl{2''-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate.

**4.10** Examples 1, 2 and 3 illustrate the preparation of trisodium [3-((1S,3R)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(S)-3'-

methyl-2'-(pentanoyl{2''-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate. It further discloses its physical characteristics. Example 4 illustrates the preparation of Linked Pro-Drug valsartan calcium salt and (2R,4S)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester.

**4.11** It is stated that the First Examination Report (FER) was issued on January 30, 2015. A copy of the FER is annexed hereto as **Annexure 2** (as downloaded from IPAIRS). The technical objections raised in the FER, the Ld. Controller are as under.

*"2. Claim 1 (and thus dependent claims) are not clear and succinct and sufficiently definitive to the scope of alleged invention in the absence of mention of any significant feature/components/characteristics of said product that reflects technological contribution to establish it as new product. [Requirements of Sec. 2(1) (j) and Sec. 10]*

*4. Claims 1-12,13,14-19 are mere new forms of the known compound and do not differ significantly in properties with regard to efficacy. Therefore, these Claims fall within the scope of such clause of section 3(d) of the Patent Act 1970 as amended in 2005.*

*5. Claims 12,13-19,20-21, 22-26,27-29 define a plurality of Distinct inventions.*

*6. Claims12,13,25,27 relates to an independent Invention.*

*7. Claim 1 and its dependent claims does not constitute an invention under section 2[1(j)] of Patents Act 1970 (as amended in 2005) as the claims are lacking in inventive step in the view of cited Patent documents: D1 : WO2006086456, D2: WO 03/059345 ,D3: EP-A1-0 443 983, D4: US-A-5 217 996, D5: J. Med. Chern. 1995, 38(1 0), 1689-1700*

*The present invention is directed to dual-acting compounds and combinations of angiotensin receptor blockiers and neutral endopeptidase inhibitors, in particular a dual acting molecule wherein the angiotensin receptor blocker and neutral endopeptidase inhibitor are linked via non-covalent ponding, or supramolecular complexes of angiotensin receptor blockers and neutral endopeptidase inhibitors, also described as linked pro-drugs, such as mixed salts or cocrystals, as well as to*

*pharmaceutical combinations containing such a dual-acting compound or combination, methods of preparing such dual-acting compounds and methods of treating a subject with such a dual-acting compound or combination.D1:discloses a combination comprising: (i) a renin inhibitor, or a pharmaceutically acceptable salt thereof; (ii) a neutral endopeptidase (NEP) inhibitor, or a pharmaceutically acceptable salt thereof; and optionally at least one therapeutic agent selected from the group consisting of (a) a diuretic, or a pharmaceutically acceptable salt thereof; and (b) an angiotensin II receptor blocker (ARB), or a pharmaceutically acceptable salt thereof; for the prevention of, delay the onset of and/or treatment of a disease or a condition mediated by angiotensin II and/or NEP activity, which method comprises administering to a warm-blooded animal, in need thereof, a therapeutically effective amount of a combination of this present invention.*

*D2: discloses a pharmaceutical composition comprising a combination of i the AT 1- antagonist valsartan or a pharmaceutically acceptable salt thereof and ii a NEP inhibitor or a pharmaceutically acceptable salt thereof and optionally a pharmaceutically acceptable carrier and to a method for the treatment or prevention of a condition or disease selected from the group consisting of hypertension, heart failure such as acute and chronic congestive heart failure, left ventricular dysfunction and hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation, atrial flutter, detrimental vascular remodeling, myocardial infarction and its sequelae, atherosclerosis, angina whether unstable or stable, renal insufficiency diabetic and non--diabetic, heart failure, angina pectoris, diabetes, secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions, such as diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, and also renal vascular hypertension, diabetic retinopathy, the management of other vascular disorders, such as migraine, peripheral vascular disease, Raynaud"s disease, luminal hyperplasia, cognitive dysfunction such as Alzheimer"s, glaucoma and stroke, comprising administering a therapeutically effective amount of the pharmaceutical composition to a mammal in need thereof.*

*D3: discloses Aromatic amide derivatives of formula (I) and their salts are new. (Where R1 =aliphatic hydrocarbon optionally substituted with halogen or OH, or a*

*cycloaliphatic or araliphatic hydrocarbon. X1 = CO, SO2, or OCO with the carbon linked to the N. X2 = aliphatic hydrocarbon (optionally substituted with OH, carboxy, NH2, guanidino, cycloaliphatic or aromatic hydrocarbon) or cycloaliphatic hydrocarbon, with a carbon of the aliphatic optionally bridged by a divalent aliphatic hydrocarbon. R2 = carboxy or its ester or amide derivative, NH2, substituted amino, formyl, acetal derivative of formyl, 1Htetrazol-5-yl, pyridyl, OH, ether, SR, SOR, SO2R, alkanoyl, sulphamoyl, N-substituted sulphamoyl, PO2H2 or PO3H2. R = H or aliphatic hydrocarbon. X3 = hydrocarbon. R3 = carboxy, 5-tetrazolyl, SO3H, PO2H2, PO3H2 or haloalkylsulphamoyl.; Rings A and B are each optionally substd. 121 compounds are specifically claimed, including (S)-N-(1-carboxy -2-methyl)-prop-1-yl) -N-pentanoyl-N- (2"-(1H-tetrazol-5-yl) biphenyl-4-ylmethyl) amine.*

*D4: discloses novel biaryl substituted 4-amino-butyric acid amide derivatives described below which arc useful as neutral endopeptidase (NEP) inhibitors, e.g. as inhibitors of the ANFdegrading enzyme in mammals, so as to prolong and potentate the diuretic, natriuretic and vasodilator properties of ANF in mammals, by inhibiting the degradation thereof to less active metabolites.*

*D5 discloses dicarboxylic acid dipeptide neutral endopeptidase inhibitors.*

*In view of cited documents D1-D5 claimed subject-matter therefore lacks novelty and inventive step in its entirety under section 2(1)(j) of the Patent Act 1970 as amended in 2005*

*8. Claim 1 and its dependent claims do anticipated by-prior claiming in the view of cited Patent documents: D1 : WO2006086456, D2: WO 03/059345,D3: EP-A1-0 443 983,D4: US-A-5 217 996, and D5: J. Med. Chern. 1995, 38(1 0), 1689-1700*

**4.12** As stated hereinbefore, the Applicant filed the response to the First Examination Report (FER) by a letter dated November 27, 2015 and amended the claims in view of the various objections. A copy of the Applicant's response to the FER along with the amended set of claims (as downloaded from IPAIRS) is annexed hereto as **Annexure 3**. The amended set of claims contains 17 claims out of which claims 1, 10, 12 and 15 are independent claims pertaining to a compound, compositions and a process.

**4.13** In response to the objection regarding the subject matter falling within Section 3(d) the applicant submitted that *claims do not fall under the purview of section 3(d) as compound of the present invention are completely novel, inventive and no compound having a dual mode of action was known to have been made using (S)-N-valeryl-N-{[2'-(lH-tetrazole5-yl)-biphenyl-4-yl]-methyl}-valine and (2R,4S)-5-biphenyl-4-yl-4-(3-carboxypropionylamino)-2-methylpentanoic acid ethyl ester. It further submitted that the compound claimed is a unique compound comprising two differently acting active molecules, e.g. hence having dual activities.*

**4.14** Further in reply to the objections on lack of novelty and inventive step (objections 7 and 8) in the First Examination Report, the Applicant submitted that with **"***the cited art failed to disclose a compound having both NEP inhibiting and angiotensin Il antagonistic activity within one (supra)molecular structure as described in the present invention"*

**4.15** In particular with regards to the inventive step, the Applicant stated that "*the cited prior art documents neither disclose the novel and unique compound having a dual mechanism of action of the present application nor a process for synthesis of such a compound. There is no teaching or enablement, in any of the cited prior art documents, in respect of such a compound much less a unique large compound having a supramolecular structure as claimed in the present invention. It is submitted that it would not have been possible for a person skilled in the art, as on the priority date of the present application, to arrive at the claimed compound and process of synthesis thereof in view of the cited prior art documents, either alone or in combination. The applicant further states that the chemical structure of the claimed compound is highly intricate and is stabilized by an involved network of ionic, hydrogen and coordination bonds, which has been described in various ways in the specification. The representative compound consists of six anions of AT-I antagonist, six anions of NEP inhibitor, 18 sodium cations, and 15 molecules of water. The claimed process provides a unique synthesizing route resulting in a unique supramolecular compound wherein the two anionic moieties are linked together with non-covalent bonds to form a single large and highly intricate molecular structure. It is submitted that synthesizing such a compound was unknown as on the priority date of the present application. In any*

*case, the process of D2 either alone or in view of the processes disclosed in other cited prior art documents cannot be said to motivate a person skilled to arrive at the claimed process, as on the priority date of the present application.*

**4.16** It is pertinent to note that during the prosecution of the impugned patent application, when faced with the cited prior art documents, the Applicant had to amend the claims by means of excluding the term "dual acting" and including that the compound of the present invention is nothing but a combination of two known drugs- the angiotensin receptor antagonist valsartan and the NEP inhibitor (2R, 4S)-5-biphenyl-4-yl-4-(3-carboxy-propionylamino)-2-methyl- pentanoic acid ethyl ester, co-existing together.

**4.17** The amended set of claims has 4 independent claims, namely, claim 1, claim 10, claim 12 and claim 15. Claim 1 recites a compound comprising valsartan and the NEP inhibitor (2R, 4S)-5-biphenyl-4-yl-4-(3-carboxy-propionylamino)-2-methyl- pentanoic acid ethyl ester. Claim 10 recites a composition comprising the compound of claim 1 along with a pharmaceutically acceptable additive. Claim 12 recites the process for preparation of the supramolecular complexes. Lastly, claim 15 recites a composition comprising the compound of claim 1 along with other active(s) selected from an anti-diabetic, anti-obesity, hypolimidemic agent and an anti-hypertensive agent; and at least one pharmaceutically acceptable additive.

## 5. PRIOR ART DOCUMENTS RELIED UPON:

The Opponent relies upon the following documents for supporting its case:

i. WO 2003/059345 published on July 24, 2003, assigned to Novartis Pharma GMBH, referred herein as D1 and annexed as Exhibit I;

ii. US 5217996 published on June 8th, 1993, assigned to Ciba-Geigy Corporation, referred herein as D2 and annexed as Exhibit II;

iii. WO 2002006253 published on January 24th, 2002, assigned to Novartis Ag & Novartis Pharma Gmbh, referred herein as D3 and annexed as Exhibit III;

iv. Article titled: "High-throughput crystallization: polymorphs, salts, co-crystals and solvates of pharmaceutical solids", by Morissette et. al., first published in March 2004, in Advanced Drug Delivery Reviews, volume 56, pages 275-300, referred herein as D4 and annexed as Exhibit IV;

v. Article titled: "Crystal engineering of the composition of pharmaceutical phases. Do pharmaceutical co-crystals represent a new path to improved medicines?", by Almarsson et. al., first published as an Advance Article on the web on 5th August 2004, in Chem. Commun., 2004, pages 1889 – 1896, referred herein as D5 and annexed as Exhibit V;

vi. Vishweshwar et. al., Crystal engineering of pharmaceutical co-crystals from polymorphic active pharmaceutical ingredients; Chem. Commun., 2005, 4601–4603 referred herein as D6 and annexed as Exhibit VI

**6. <u>GROUND I- Section 25(1)(e): Lack of inventive step: -</u>**

**6.1** It is stated that Exhibit I: WO 2003/059345 (D1), belonging to the applicant, teaches a pharmaceutical composition comprising a combination of an AT 1- antagonist valsartan or a pharmaceutically acceptable salt thereof and a NEP inhibitor or a pharmaceutically acceptable salt thereof and optionally a pharmaceutically acceptable carrier and to a method for the treatment or prevention of a condition or disease selected from the group consisting of hypertension, heart failure among other related diseases. As disclosed at page 2 of D1, it identifies the problems of prolonged and uncontrolled hypertension, which causes numerous pathological changes in target organs like the heart and kidneys. It further discloses that hypertension being a multifactorial disease, drugs with different modes of action have been combined. In view of such nature of hypertension, D1 provides a combination therapy of valsartan or a pharmaceutically acceptable salt thereof and a NEP inhibitor [(2R,4S)-5-biphenyl4-yl~5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester or a pharmaceutically acceptable salt thereof, which has less deleterious side effect.

**6.2** It is further stated that at page 7, D1 discloses the surprisingly improved therapeutic effect of the combination than the administration of valsartan, ACE inhibitors or NEP inhibitors alone. It also discloses the lessening of adverse effects and prolonged duration of action on administration of the combination.

**6.3** It is stated that the alleged invention also seeks to provide an efficacious combination therapy which does not have deleterious side effects. Accordingly, the applicants have disclosed and claimed a supramolecular complex of valsartan and

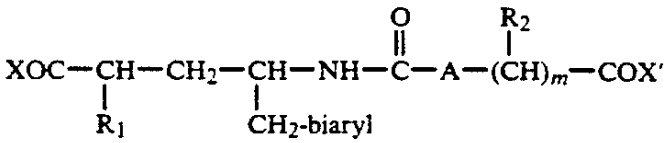
NEP inhibitor being (2R,4S)-5-biphenyl-4-yl-4-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester having the formula as under.

[((S)-N-valeryl-N-{[2'-(1-H-tetrazole-5-yl)-biphenyl-4-yl]-methyl}-valine).

[(2R,4S)-5-biphenyl-4-yl-4-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester) $Na_{1-3}.x\ H_2O$ where x- 0-3

Thus the mechanism of action of these two active drugs and their metabolism were already known from D1 at the time of the invention and the applicant merely combined the actives to form a supramolecular complex.

**6.4** It is stated that Exhibit II: US 5217996 (D2), an acknowledged prior art, teaches NEPi, which are useful antihypertensive or saluretic agents. These compounds are biaryl substituted 4-amino-butyric acid amide derivatives of formula I:




which prolong and potentate the diuretic, natriuretic and vasodilator properties of ANF in mammals, by inhibiting the degradation thereof to less active metabolites.

**6.5** In examples 7 and 8 and claim 6, it particularly teaches the sodium salt of the sacubitril [N-(3-Carboxy-1-oxopropyl)-(4S)-p-phenyl phenyl methyl)-4-amino-2R-methyl butanoic acid, ethyl ester]. Therefore, admittedly NEP inhibitors like (2R,4S)-5-biphenyl4-yl~5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester (Sacubitril) and its salts were known to be useful as anti-hypertensive agents.

**6.6** It is stated that Exhibit III: WO 2002006253 (D3) teaches valsartan, especially its various salt forms selected from the group consisting of the monosodium salt, the monopotassium salt, the disodium salt, the dipotassium salt, the magnesium salt, the calcium salt, the bis-diethylammonium salt, the bis-dipropylammonium salt, the bis- dibutylammonium salt, the mono-L-arginine salt, the bis-L-arginine salt, the mono-L-lysine salt and the bis-L-lysine salt, as well as salt mixtures thereof, which specifically forms hydrates such as di and tri hydrates.

Further at page 3, it teaches that the preferred valsartan salts are primarily in hemihydrate forms. The hydrate of valsartan di sodium salt with about 2.5 moles of water (Hemi Penta) has been well characterized in examples of D3. These valsartan salts in hydrate forms have been taught to have improved stability and handling properties, which in turn are desirable for pharmaceutical operations and applications. Further D3 teaches various combinations of valsartan disodium hydrate salts with other drugs like a dual angiotensin converting enzyme/neutral endopeptidase (ACE/NEP) inhibitor or a pharmaceutically acceptable salt thereof, for effective treatment of cardiovascular diseases and related conditions.

**6.7** It is stated that apart from teaching valsartan salts in hydrated form, specifically hemihydrate form, exhibiting desirable pharmaceutical and therapeutic efficacy against hypertension; D3 also teaches the effectiveness of combination therapy along with other ACE/NEP inhibitor for treating hypertension and cardio-vascular diseases.

**6.8** Therefore, the hydrated forms of valsartan, particularly the hemi-hydrates also with their benefits in combination therapy was known in the art at the date of the invention.

**6.9** It is stated that D1 and D3 provide a clear motivation to a person skilled in the art to formulate complexes of valsartan and an NEP inhibitor in order to achieve improved anti-hypertensive effect. Further, as taught in D2 and D3 the sodium salt of NEP inhibitor (2R,4S)-5-biphenyl4-yl~5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester or Sacubitril and valsartan disodium salts in hemihydrate forms were known at the time of the alleged invention. Furthermore, D1, D2 and D3 would motivate a person skilled in the art to combine the valsartan disodium salts in hemihydrate form with NEP inhibitors in an expectation to achieve improved efficacy.

**6.10** The opponent states that a combination drug comprising Valsartan-Sacubitril available on the market under the brand name Entresto©, which is incidentally manufactured and marketed by the applicant. Such compositions containing 24 mg of Sacubitril and 26 mg of valsartan as well as higher strengths are marketed in USA (49 mg/51 mg/97 mg/ 103 mg). It is imperative and surprising to note that in spite of having patents as well as marketing the

combination of Valsartan-Sacubitril, the applicant chose not to acknowledge such highly pertinent prior art.

**6.11** It is stated that Exhibit IV entitled "High-throughput crystallization: polymorphs, salts, co-crystals and solvates of pharmaceutical solids" (D4) teaches the use of high-throughput (HT) screening and combinatorial synthesis as emerging strategies in the pharmaceutical drug development arena. Diverse salt forms including co-crystals & hydrates have been reported in D4. Further, the teachings emphasize on the role of co-crystals of drugs and drug candidates in pharmaceutical development. The authors explain studies on hydrogen bonding between various synthetic moieties forming supramolecular synthons observed in co-crystals. These structures are formally molecular compounds (or co-crystals) but do not involve formation of covalent bonds or charge transfer from or to the active substance.

**6.12** D4 at pages 292 and 293 teaches the benefits and advantages of co-crystals over hydrates and solvates. It particularly teaches as follows, "in general, it is usually easier to initially prepare solvates than co-crystals, and indeed, solvates are often found as by-products of polymorph and salts screens. Co-crystals have been prepared by melt-crystallization, grinding and recrystallization from solvents. Solvent systems for co-crystals must dissolve all components, but must not interfere with the interactions necessary for co-crystal formation. The need to try many solvent combinations and the availability of multiple co-crystal formers creates a diversity that is ideally suited for exploration by HT systems. Co-crystals have the potential to be much more useful in pharmaceutical products than solvates or hydrates. The number of pharmaceutically acceptable solvents is very small, and because solvents tend to be more mobile and have higher vapour pressure, it is not unusual to observe dehydration/desolvation in solid dosage forms. Solvent loss frequently leads to amorphous compounds, which are less chemically stable and can crystallize into less soluble forms. In contrast, most co-crystal formers are unlikely to evaporate from solid dosage forms, making phase separation and other physical changes less likely."

**6.13** Thus, a person skilled in the art in view of D1, D3 and D4 will be have sufficient incentive to try a co-crystal of valsartan and (2R,4S)-5-biphenyl-4-yl-4-

(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester for better improved physicochemical properties.

**6.14** It is stated that Exhibit V entitled "Crystal engineering of the composition of pharmaceutical phases. Do pharmaceutical co-crystals represent a new path to improved medicines?" (D5) teaches that effective approaches in understanding and designing co-crystals is to apply the paradigm of supramolecular synthesis, in particular exploitation of supramolecular heterosynthons. At page 1889, column 2, it teaches that crystalline API's are strongly preferred due to their relative ease of isolation, better purity profile, which is inherent to the crystallization process and the physico-chemical stability.

**6.15** It makes particular reference to acids and amides in pharmaceutical actives, which are appropriate foci for design and synthesis. At page 1890, it teaches that the acid–amide supramolecular heterosynthon which have been exploited for the generation of co-crystals, the CSD (Cambridge Structural Database) reveals that there are 118 crystal structures in which both an acid and an amide moiety are present. 58 of these structures exhibit the acid–amide supramolecular heterosynthon, whereas only 11 structures exhibit the acid homodimer and only 28 exhibit the amide homodimer.

**6.16** It is stated that such statistics go to show the natural tendency of acid and an amide moiety to form supramolecular heterosynthon.

**6.17** It teaches that single component crystals that contain carboxylic acid or amide are prone to polymorphism even if one hydrogen bonding moiety is present. Furthermore, it is reveals that functional groups that are self-complementary are capable of forming supramolecular homosynthons at paragraph 2, left column, page 1890 of D5.

**6.18** It is stated the (2R,4S)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester contains both carboxylic acid and amide moieties and Valsartan contains carboxylic acid moiety, consequently being prone to polymorphism. Thus a person skilled in art in order to improve the stability and other properties will be motivated to combine the formulate a supramolecular complex in view of the teachings of D1, D2, D3, D4 and D5.

**6.19** It is thus stated that a person skilled in the art would be naturally inclined in the course of routine research to try various crystalline forms, including co-crystal of actives in expectation to improve its physicochemical properties among others. It would be particularly inclined to experiment with pharmaceutical actives having well-established efficacy. The impugned application has not emphasized on the physicochemical properties of the supramolecular complex, rather focusing on the improved efficacy of the complex over monotherapy with valsartan and NEP inhibitor (2R,4S)-5-biphenyl-4-yl-4-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester. It is stated that the impugned specification contains no comparative data whatsoever.

**6.20** It is further stated that in view of prior art, viz. D1 and the formulation marketed under Entresto©, it was incumbent on the applicant to provide comparative studies to support its assertions of enhanced therapeutic efficacy of the claimed compound. The applicant has merely stated that the present supramolecular complex as claimed has improved efficacy as opposed to valsartan and NEP inhibitor (2R,4S)-5-biphenyl-4-yl-4-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester individually. Such comparisons of combination therapy versus monotherapy were already reported in D1, which happens to be the applicant's patent. In view of such close prior art, the applicant ought to have provided comparative test data in order to establish the technical advancement brought about by the allegedly improved supramolecular complex.

**6.21** Thus, to summarize, a combination including a physical combination of valsartan and N-(3-Carboxy-1-oxopropyl)-(4s)-p-phenyl phenyl methyl)-4-amino-2R-methyl butanoic acid, ethyl ester is taught in D1 and D3. D4 and D5 teach the advantages and benefits of co-crystals or supramolecular complexes of pharmaceutical actives vis-à-vis physicochemical properties.

**6.22** Furthermore D2 teaches NEP inhibitor N-(3-Carboxy-1-oxopropyl)-(4s)-p-phenyl phenyl methyl)-4-amino-2R-methyl butanoic acid ethyl ester and its in mono sodium salt form to be effective in the treatment of hypertension. D3 also teaches valsartan in stable disodium hemihydrate salt form which is used as an anti-hypertensive agent.

**6.23** Therefore, D2 and D3 when read with D4 and D5, would motivate and incentivize a person skilled in the art to try co-crystals of these actives, particularly in view of the well-known improved efficacy of the combination of valsartan and NEPi Sacubitril as documented in D1.

**6.24** Further as stated before the impugned specification contains no comparative data in terms of the improved efficacy or decreased adverse effects of the compound [((S)-N-valeryl-N-{[2'-(1-H-tetrazole-5-yl)-biphenyl-4-yl]-methyl}-valine). [(2R,4S)-5-biphenyl-4-yl-4-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester) $Na_{1-3}.x\ H_2O$ where x- 0-3, over monotherapy, let alone with respect to the composition of valsartan and NEPi as disclosed in D1. In this context it is interesting to note that at page 8 of the impugned specification the applicant states that, *"One embodiment of the invention is directed to a physical combination comprising: (a) a pharmaceutically acceptable salt of an angiotensin receptor antagonist; and (b) a pharmaceutically acceptable salt of a neutral endopeptidase inhibitor (NEPi); wherein the pharmaceutically acceptable salt of the angiotensin receptor antagonist and the NEPi is the same and is selected from a salt of Na, K or* $NH_4$*."*

**6.25** Thus the impugned specification also covers physical combinations as one of its embodiments, thus effectively equating the activity of the supramolecular complex of the valsartan and N-(3-Carboxy-1-oxopropyl)-(4s)-p-phenyl phenyl methyl)-4-amino-2R-methyl butanoic acid ethyl ester with their physical combination, which happens to be disclosed in D1.

**6.26** Therefore, it is stated that the compound as claimed in claim 1 is devoid of an inventive step and is obvious to a person skilled in the art in view of the cited prior art and for the reasons stated hereinabove.

**6.27** It is stated that claims 2, 3, 4, 5 and 6 recite that the compound of claim 1 is a preferably formulated as a trisodium salt in amorphous or crystalline forms. The preferred crystalline form being hemipentahydrate. It is stated these features are merely preferred embodiments and do not have any inventive attributes. The preferred salts and crystalline forms can be arrived at by performing undue experiments and is wholly within the ambit of routine experimentation. Thus claims 2 to 6 are obvious to a person skilled in the art in view of he afore discussed

prior art and common general knowledge possessed by a person skilled in the art. It is stated that such forms are all the more obvious in absence of any data showing their significance with respect to other salt or crystalline forms. Therefore, it is stated that claims 2 to 6 of the impugned application are obvious to a person skilled in the art as they are devoid of an inventive step.

**6.28** It is stated that claims 7 to 9 recite the physical characteristics of the compound of claim 1. The claims merely recite the physical characterizations by way of the X-ray diffraction pattern, absorption band spectrum of the compound of claim 1 and do not have any inventiveness. Therefore, it is stated that claims 7 to 9 of the impugned application are obvious to a person skilled in the art as they are devoid of an inventive step.

**6.29** It is stated that claim 10 recites a composition comprising the compound of claim 1 along with at least one pharmaceutically acceptable additive. Claim 11 recites such additives which can be selected from diluents or fillers, disintegrants, glidants, lubricants, binders, colorants and mixtures thereof. It is stated that pharmaceutical compositions require such additives for their formation and it falls within the scope of routine experimentation to prepare such formulations. Reference is made to the compositions formed in D1, D2 and D3, wherein similar pharmaceutical additives or excipients. Further there is no comparative data over the compositions taught in D1 to exhibit the improved activity of the composition as claimed in claim 10. Therefore, it is stated that claims 10 and 11 of the impugned application are obvious to a person skilled in the art as they are devoid of an inventive step.

**6.30** It is stated that claim 12 recites the process comprising the steps of (i) dissolving an angiotensin receptor antagonist and a neutral endopeptidase inhibitor (NEPi) in a suitable solvent; (ii) dissolving a basic compound of Cat in a suitable solvent, wherein Cat is a cation; (iii) combining the solutions obtained in steps (i) and (ii); (iv) precipitation of the solid, and drying same to obtain the dual-acting compound; or alternatively obtaining the dual-acting compound by exchanging the solvent(s) employed in steps (i) and (ii) by (iva) evaporating the resulting solution to dryness; (va) re-dissolving the solid in a suitable solvent; (via) precipitation of the solid and drying same to obtain the dual-acting compound.

**6.31** The opponent states that Exhibit VI: Vishweshwar et. al.: Chem. Commun., 2005, 4601–4603 (D6) discloses use of *supramolecular heterosynthons, in particular the carboxylic acid–primary amide dimer*, to crystal engineer pharmaceutical co-crystals from pairs of APIs that are polymorphic in their pure forms. The study analyzes the co-crystal structure of Piracetam (amide) with p-hydroxybenzoic (carboxylic acid) 1:1, as provided in figure 2 page 4602, the presence of the acid–amide supramolecular heterosynthon is evident which in turn dimerizes to form a tetrameric motif sustained by N–H…O hydrogen bonding. The co-crytals are prepared by crystallization from acetonitrile via slow evaporation. These co-crystal can also be prepared via grinding or slurrying in water.

**6.32** It is thus stated that the preparation of co-crystals involves routine technique and experimentation. As taught in D6, it involves dissolution followed by evaporation of the solvent. Similar synthetic approach is followed in the present invention, where the Sacubitril monosodium and Valsartan disodium salts are dissolved in suitable solvent such as acetone and then co-crystallized via evaporation and drying. Addition of a few commonplace steps do not make a process novel and inventive especially in absence of any data as to its significance vis-à-vis the end product or efficiency of the process itself.

**6.33** Therefore, it is stated that claims 12 to 14 of the impugned application are obvious to a person skilled in the art as they are devoid of an inventive step.

**6.34** It is stated that claims 15 recites a pharmaceutical composition comprising the compound of claim 1 with a therapeutic agent selected from an anti-diabetic, a hypolipidemic agent, an anti-obesity agent and an anti-hypertensive agent and a pharmaceutically acceptable additive. Claims 16 and 17 recite that such therapeutic agent is amlodipine besylate and hydrochlorothiazide respectively. It is stated that combining pharmaceutical actives in order to effectively treat diseases or related diseases have been known in the art for a long time. It has also been acknowledged by the applicant. Reference is also made to D3 which teaches the effectiveness of combination therapy of valsartan along with other ACE/NEP inhibitor for treating hypertension and cardio-vascular diseases. Thus formulation of such compositions are within the skill of a person skilled in the art and do not require any undue

experimentation. Further in absence of any data demonstrating the activity of such compositions, the applicants have shown any improved disease management by administering these compositions. Therefore, it is stated that claims 15 to 17of the impugned application are obvious to a person skilled in the art as they are devoid of an inventive step.

**6.35** Therefore, it is stated that the claims 1 to 17 warrants rejection for being obvious to a person skilled in the art and for want of an inventive step.

**6.36** The impugned application ought to be rejected on this ground alone.

## 7. GROUND II - Section 25(1)(f): NOT PATENTABLE SUBJECT MATTER / NOT AN INVENTION:

The subject-matter of the claims 1 to 17 of the impugned application is not an invention within the meaning of this Act or is not patentable under this Act, based on the following grounds:

### 7.1 Section 2(1)(j)

The Opponent states that the claim 1 to 17 of the Impugned application are not an invention as they are devoid an inventive step for reasons stated in paragraphs under the preceding grounds of obviousness/lack of inventive step. The submissions are not being reiterated for the sake of brevity.

Therefore, it is stated that the claims of the impugned application warrant rejection for failing to meet the requirements of Section 2(1)(j).

### 7.2 Section 2(1)(ja)

The Opponent states that the claim 1 to 17 of the impugned application are not an invention as they are devoid of an inventive step for reasons stated in preceding paragraphs under the ground of obviousness/lack of inventive step, which are not reiterated for the sake of brevity.

Therefore, it is stated that the claims of the Impugned application warrant rejection for failing to meet the Section 2(1)(ja).

**7.3. Section 3(d)**

It is respectfully submitted that Claim 1 to 6 of the current set of claims of the impugned application are drawn towards a compound comprising of two known drugs Valsartan and N-(3-Carboxy-1-oxopropyl)-(4s)-p-phenyl phenyl methyl)-4-amino-2R-methyl butanoic acid, ethyl ester or its acid/salts (Sacubitril) in a supramolecular complex form.

In said complex form, these two known actives exist in co-crystal forms held together via noncovalent bonds, thus retaining their individual structural identities with known properties. Such a dual-acting compound comprising two known drugs complexed as a 'co-crystal' is merely a new form of a known substance, especially in view of that D1 discloses the physical combination of valsartan and (2R, 4S)-5-biphenyl-4-yl-4-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester and the properties of the combination.

The impugned specification contains no data whatsoever with regard to the efficacy of the claimed compound, let alone data on enhanced efficacy. The omission to acknowledge the closest prior art, i.e. D1, which incidentally belongs to the applicant is clearly a deliberate suppression with the ulterior motive of ever-greening. It is stated that the claim 1 is nothing but an attempt to evergreen the already existing and patented formulation comprising the same actives as claimed in claim of the impugned application. Thus the subject matter of claim 1 and its dependent claims clearly fall within the ambit of Section 3(d).

Therefore, the subject matter of claims 1 to 6 of the impugned application is not patentable under Section 3(d) and warrants rejection.

**7.4. Section 3(e)**

The subject matter of claims 10 and 11 and 15 to 17 of the impugned application recite compositions essentially comprising of the dual-acting compound of claim 1 with pharmaceutically acceptable additives and other actives. It is stated that such compositions are mere admixtures especially in absence of any data exhibiting synergistic activity. The composition comprising these two known actives in a complex form is a mere admixture of two known substances, which

result only in the aggregation of the known properties of those individual components and do not demonstrate any synergistic activity. A composition demonstrating mere aggregation of the known properties is not patentable under section 3(e).

Therefore, the subject matter of claims 10 and 11, and 15 to 17 fall squarely under Section 3(e) and warrants rejection.

The impugned application ought to be rejected on this ground alone.

**8. <u>GROUND V- Section 25(1)g: Insufficiency</u>**

**8.1** The complete specification of the impugned application does not sufficiently and clearly describe the invention or the method by which it is to be performed.

**8.2** It is stated that the method of preparing a composition comprising the dual-acting compound of the alleged invention as claimed in claims 10 and 15 is not described in the impugned specification, let alone being sufficiently and clearly described. Further the impugned specification does not demonstrate the preparation of these claimed compositions. Thus in absence of such data in the impugned specification, a person of average skill in the art has to conduct undue number of experiments to arrive at the best possible method for preparing the pharmaceutical composition. Furthermore, the impugned application also does not show any unexpected or superior therapeutic effect achieved by the claimed compositions over the combination drug compositions well-known in the art involving the same two actives.

**8.3** Even thought the applicant has claimed several hydrate forms in the claims, the specification only describes the preparation of only the hemipentahydrate form. It is stated that the applicant has claimed a range of molecular forms such as a hydrate, hemihydrate, monohydrate, sesquihydrate, dehydrate and trihydrate. Claim 1 on record encompasses several forms of the dual-acting compound, but the impugned specification does not sufficiently disclose the method of preparation of

all those compounds and the reaction conditions whereby all of the compounds of claim 1 may be prepared, except that for hemi-pentahydrate form. In absence of such preparation methods a person of average skill has to conduct undue experimentation in order to formulate such crystalline and salt forms.

**8.4** In summary, the impugned specification does not sufficiently disclose the preparation methods for all other hydrate forms of the compound claimed under the scope claim 1. Furthermore, there is no example illustrating pharmaceutical composition as allegedly claimed in claims 10 and 11 and 15 to 17. In absence of such examples, the claimed pharmaceutical compositions have not been properly enabled in the impugned specification. Therefore, it is stated that the impugned specification does not clearly and sufficiently disclose the invention.

**8.5** The impugned application ought to be rejected on this ground alone.

## 9. Section 25(1)(h): BREACH OF SECTION 8:

**9.1** The applicant is required to provide all the information regarding the prosecution of its equivalent applications till the grant of its Indian application to the Controller in writing within the prescribed time period which the applicant has apparently failed to do.

**9.2** Under section 8(1) of the Act, the applicant is under obligation to furnish to the Indian Patent Office details of corresponding foreign applications and also to furnish an undertaking under Section 8(1) (b) and subsequently furnish further details with respect to corresponding foreign applications including their status from time to time.

**9.3** It is stated that search or examination reports relating to the same or substantially the same invention is to be filed at the Patent Office (including Oppositions filed in other countries) under section 8 (2). It is stated that if such information has been willfully suppressed, the impugned application ought to be rejected on this ground alone.

**RELIEF SOUGHT**

In the circumstances aforesaid the applicant prays for the following reliefs:

1) Take on record the present representation;

2) Leave to file evidence;

3) Forward copy of reply of applicant and evidence if any and any amendments filed;

4) Leave to file a replication to the reply of the applicant and evidence

5) Grant of hearing.

6) Refusal of the application *in toto*;

7) Such other relief or reliefs as the Controller may deem appropriate.

Dated this 6th day of September 2016

Amrita Majumdar

Amrita Majumdar

Of S. Majumdar & Co.

(Opponent's Agent)

To

The Controller of Patents

The Patent Office Branch

Delhi

Enclosures:

- Annexure 1;
- Annexure 2;
- Annexure 3;
- Exhibit I;
- Exhibit II;
- Exhibit III;
- Exhibit IV;

- Exhibit V;
- Exhibit VI.

# ORGANIC COMPOUNDS

## Background of the Invention

### Field of the Invention

The present invention is directed to dual-acting compounds and combinations of angiotensin receptor blockers and neutral endopeptidase inhibitors, in particular a dual acting molecule wherein the angiotensin receptor blocker and neutral endopeptidase inhibitor are linked via non-covalent bonding, or supramolecular complexes of angiotensin receptor blockers and neutral endopeptidase inhibitors, also described as linked pro-drugs, such as mixed salts or co-crystals, as well as to pharmaceutical combinations containing such a dual-acting compound or combination, methods of preparing such dual-acting compounds and methods of treating a subject with such a dual-acting compound or combination. Specifically, the invention is directed to a dual acting compound or supramolecular complex of two active agents having the same or different modes of action in one molecule.

### Related Background Art

Angiotensin II Is a hormone that causes blood vessels to constrict. This, in turn, can result in high blood pressure and strain on the heart. It is known that angiotensin II interacts with specific receptors on the surface of target cells. Two receptor subtypes for angiotensin II, namely AT1 and AT2, have been identified thus far. In recent times, great efforts have been made to identify substances that bind to the AT1 receptor. Angiotensin receptor blockers (ARBs, angiotensin II antagonists) are now known to prevent angiotensin II from binding to its receptors in the walls of blood vessels, thereby resulting in lower blood pressure. Because of the inhibition of the AT1 receptor, such antagonists can be used, therefore, as anti-hypertensives or for the treatment of congestive heart failure, among other indications.

Neutral endopeptidase (EC 3.4.24.11; enkephalinase; atriopeptidase; NEP) is a zinc-containing metalloprotease that cleaves a variety of peptide substrates on the amino side of - hydrophobic residues [see *Pharmacol Rev,* Vol. 45, p. 87 (1993)]. Substrates for this enzyme include, but are not limited to, atrial natriuretic peptide (ANP, also known as ANF), brain natriuretic peptide (BNP), met- and leu-enkephalin, bradykinin, neurokinin A, endothelin-1 and substance P. ANP is a potent vasorelaxant and natriuretic agent [see

*J Hypertens*, Vol. 19, p. 1923 (2001)]. Infusion of ANP in normal subjects resulted in a reproducible, marked enhancement of natriuresis and diuresis, including increases in fractional excretion of sodium, urinary flow rate and glomerular filtration rate [see *J Clin Pharmacol,* Vol. 27, p. 927 (1987)]. However, ANP has a short half-life in circulation, and NEP in kidney cortex membranes has been shown to be the major enzyme responsible for degrading this peptide [see *Peptides,* Vol. 9, p. 173 (1988)]. Thus, inhibitors of NEP (neutral endopeptidase inhibitors, NEPi) should increase plasma levels of ANP and, hence, are expected to induce natriuretic and diuretic effects.

While substances, such as angiotensin receptor blockers and neutral endopeptidase inhibitors may be useful in the control of hypertension, essential hypertension is a polygenic disease and is not always controlled adequately by monotherapy. Approximately 333 million adults in economically developed countries and about 65 million Americans (1 in 3 adults) had high blood pressure in 2000 [see *Lancet,* Vol. 365, p. 217 (2005); and *Hypertension,* Vol. 44, p. 398 (2004)]. Prolonged and uncontrolled hypertensive vascular disease ultimately leads to a variety of pathological changes in target organs, such as the heart and kidney. Sustained hypertension can lead as well to an increased occurrence of stroke. Therefore, there is a strong need to evaluate the efficacy of anti-hypertensive therapy, an examination of additional cardiovascular endpoints, beyond those of blood pressure lowering, to get further insight into the benefits of combined treatment.

The nature of hypertensive vascular diseases is multifactorial. Under certain circumstances, drugs with different mechanisms of action have been combined. However, just considering any combination of drugs having different modes of action does not necessarily lead to combinations with advantageous effects. Accordingly, there is a need for efficacious combination therapy which does not have deleterious side effects.

## Summary of the Invention

In a first aspect, the present invention is directed to a dual-acting compound, such as a supramolecular complex, comprising:

(a) an angiotensin receptor antagonist;

(b) a neutral endopeptidase inhibitor (NEPi); and optionally

(c) a pharmaceutically acceptable cation.

The present invention is also directed to a dual-acting compound, such as a supramolecular complex, obtainable by:

(i) dissolving an angiotensin receptor antagonist and a neutral endopeptidase inhibitor (NEPi) in a suitable solvent;

(ii) dissolving a basic compound of Cat in a suitable solvent, wherein Cat is a cation;

(iii) combining the solutions obtained in steps (i) and (ii);

(iv) precipitation of the solid, and drying same to obtain the dual-acting compound; or alternatively

obtaining the dual-acting compound by exchanging the solvent(s) employed in steps (i) and (ii) by

(iva) evaporating the resulting solution to dryness;

(va) re-dissolving the solid in a suitable solvent;

(via) precipitation of the solid and drying same to obtain the dual-acting compound.

The present invention is also directed to linked pro-drugs comprising:

(a) an angiotensin receptor antagonist or a pharmaceutically acceptable salt thereof; and

(b) a NEPi or a pharmaceutically acceptable salt thereof, wherein the angiotensin receptor antagonist or a pharmaceutically acceptable salt thereof and the NEPi or a pharmaceutically acceptable salt thereof are linked by a linking moiety.

The present invention is also directed to a combination comprising:

(a) a pharmaceutically acceptable salt of an angiotensin receptor antagonist; and

(b) a pharmaceutically acceptable salt of a neutral endopeptidase inhibitor (NEPi);

wherein the pharmaceutically acceptable salt of the angiotensin receptor antagonist and the NEPi is the same and is selected from a salt of Na, K or $NH_4$.

In preferred embodiments, the angiotensin receptor antagonist and NEPi have acidic groups which facilitate formation of the dual acting compound, such as the supramolecular complex of the present invention.

Preferably, the angiotensin receptor antagonist is selected from the group consisting of valsartan, losartan, irbesartan, telmisartan, eprosartan, candesartan, olmesartan, saprisartan, tasosartan, elisartan and combinations thereof.

In preferred embodiments, the NEPi is selected from the group consisting of: SQ 28,603; N-[N-[1(*S*)-carboxyl-3-phenylpropyl]-(*S*)-phenylalanyl]-(*S*)-isoserine; *N*-[*N*-[(*S*)-carboxy-2-phenyl)ethyl]-(*S*)-phenylalanyl]-β-alanine; -[2(*S*)-mercaptomethyl-3-(2-methylphenyl)-propionyl]methionine; (*cis*-4-[[[1-[2-carboxy-3-(2-methoxyethoxy)propyl]-cyclopentyl]carbonyl]amino]-cyclohexanecarboxylic acid); thiorphan; retro-thiorphan; phosphoramidon; SQ 29072; -(3-carboxy-1-oxopropyl)-(4*S*)-*p*-phenylphenylmethyl)-4-amino-2*R*-methylbutanoic acid ethyl ester; (*S*)-*cis*-4-[1-[2-(5-indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido]-1-cyclohexanecarboxylic acid; 3-(1-[6-endo-hydroxymethylbicyclo[2,2,1]heptane-2-exo-carbamoyl]cyclopentyl)-2-(2-methoxyethyl)propanoic acid; *N*-(1-(3-(*N*-*t*-butoxycarbonyl-(*S*)-prolylamino)-2(*S*)-*t*-butoxy-carbonylpropyl)cyclopentanecarbonyl)-*O*-benzyl-(*S*)-serine methyl ester; 4-[[2-(mercaptomethyl)-1-*oxo*-3-phenylpropyl]amino]benzoic acid; 3-[1-(*cis*-4-carboxycarbonyl-*cis*-3-butylcyclohexyl-r-1-carbamoyl)cyclopentyl]-2*S*-(2-methoxyethoxymethyl)propanoic acid; ((2*S*)-2-(4-biphenylmethyl)-4-carboxy-5-phenoxyvaleryl)glycine; -(1-(*N*-hydroxycarbamoylmethyl)-1-cyclopentanecarbonyl)-phenylalanine; (*S*)-(2-biphenyl-4-yl)-1-(1*H*-tetrazol-5-yl)ethylamino) methylphosphonic acid; (*S*)-5-(*N*-(2-(phosphonomethylamino)-3-(4-biphenyl)propionyl)-2-aminoethyl)tetrazole; β-alanine; 3-[1,1'-biphenyl]-4-yl-*N*-[diphenoxyphosphinyl)methyl]-*L*-alanyl; N-(2-carboxy-4-thienyl)-3-mercapto-2-benzylpropanamide; 2-(2-mercaptomethyl-3-phenylpropionamido)thiazol-4-ylcarboxylic acid; (*L*)-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy)carbonyl)-2-phenylethyl)-*L*-phenylalanyl)-β-alanine; *N*-[*N*-[(*L*)-[1-[(2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy]carbonyl]-2-phenylethyl]-*L*-phenylalanyl]-(*R*)-alanine; -[*N*-[(*L*)-1-carboxy-2-phenylethyl]-*L*-phenylalanyl]-(*R*)-alanine; *N*-[2-acetylthiomethyl-3-(2-methyl-phenyl)propionyl]-methionine ethyl ester; *N*-[2-mercaptomethyl-3-(2-methylphenyl)propionyl]-methionine; [2(*S*)-mercaptomethyl-3-(2-methylphenyl)propanoyl]-(*S*)-isoserine; -(*S*)-[3-mercapto-2-(2-methylphenyl)propionyl]-(*S*)-2-methoxy-(R)-alanine; -[1-[[1(*S*)-benzyloxycarbonyl-3-phenylpropyl]amino]cyclopentylcarbonyl]-(*S*)-isoserine; N-[1-[[1(*S*)-carbonyl-3-phenylpropyl]amino]-cyclopentylcarbonyl]-(*S*)-isoserine; 1,1'-[dithiobis-[2(*S*)-(2-methylbenzyl)-1-*oxo*-3,1-propanediyl]]-*bis*-(*S*)-isoserine; 1,1'-[dithiobis-[2(*S*)-(2-methylbenzyl)-1-*oxo*-3,1-propanediyl]]-*bis*-(*S*)-methionine; *N*-(3-phenyl-2-(mercaptomethyl)-propionyl)-(*S*)-4-(methylmercapto)methionine; *N*-[2-acetylthiomethyl-3-phenyl-propionyl]-3-aminobenzoic acid; -[2-mercaptomethyl-3-phenyl-propionyl]-3-aminobenzoic acid; [1-(2-carboxy-4-phenylbutyl)-cyclopentane-carbonyl]-(*S*)-isoserine;

N[1-(acetylthiomethyl)cyclopentane-carbonyl]-(*S*)-methionine ethyl ester; 3(*S*)-[2-(acetylthiomethyl)-3-phenyl-propionyl]amimo-ε-caprolactam; -(2-acetylthiomethyl-3-(2-methylphenyl)propionyl)-methionine ethyl ester; and combinations thereof. Preferably, the dual-acting compound or combination , in particular the supramolecular complex, is a mixed salt or a co-crystal. It is also preferred that the linked pro-drug is a mixed salt or a co-crystal.

In a second aspect, the present invention is directed to pharmaceutical composition comprising

(a) the aforementioned dual-acting compound or combination, such as the aforementioned complex; and

(b) at least one pharmaceutically acceptable additive.

The present invention is also directed to pharmaceutical compositions comprising a linked pro-drug comprising:

(a) an angiotensin receptor antagonist or a pharmaceutically acceptable salt thereof;

(b) a NEPi or a pharmaceutically acceptable salt thereof,

wherein the angiotensin receptor antagonist or a pharmaceutically acceptable salt thereof and the NEPi or a pharmaceutically acceptable salt thereof are linked by a linking moiety; and

(c) at least one pharmaceutically acceptable additive.

In a third aspect, the present invention is directed to a method of preparing a dual-acting compound, in particular a supramolecular complex, comprising

(a) an angiotensin receptor antagonist;

(b) a neutral endopeptidase inhibitor (NEPi); and optionally

(c) a pharmaceutically acceptable cation selected from the group consisting of Na, K and $NH_4$;

said method comprising the steps of:

(i) dissolving an angiotensin receptor antagonist and a neutral endopeptidase inhibitor (NEPi) in a suitable solvent;

(ii) dissolving a basic compound of Cat in a suitable solvent, wherein Cat is a cation;

(iii) combining the solutions obtained in steps (i) and (ii);

(iv) precipitation of the solid, and drying same to obtain the dual-acting compound; or alternatively

obtaining the dual-acting compound by exchanging the solvent(s) employed in steps (i) and (ii) by

(iva) evaporating the resulting solution to dryness;

(va) re-dissolving the solid in a suitable solvent;

(via) precipitation of the solid and drying same to obtain the dual-acting compound.

The present invention is also directed to a method of making a linked pro-drug comprising:

(a) an angiotensin receptor antagonist or a pharmaceutically acceptable salt thereof;

(b) a NEPi or a pharmaceutically acceptable salt thereof, wherein the angiotensin receptor antagonist or a pharmaceutically acceptable salt thereof and the NEPi or a pharmaceutically acceptable salt thereof are linked by a linking moiety; and

comprising adding a linking moiety and a solvent to a mixture of an angiotensin receptor antagonist and a NEPi; and

(d) isolating the linked pro-drug.

In a fourth aspect, this invention is directed to a method of treating or preventing a disease or condition, such as hypertension, heart failure (acute and chronic), congestive heart failure, left ventricular dysfunction and hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation, atrial flutter, detrimental vascular remodeling, myocardial infarction and its sequelae, atherosclerosis, angina (unstable or stable), renal insufficiency (diabetic and non-diabetic), heart failure, angina pectoris, diabetes, secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions, such as diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, and also renal vascular hypertension, diabetic retinopathy, other vascular disorders, such as migraine, peripheral vascular disease, Raynaud's disease, luminal hyperplasia, cognitive dysfunction (such as Alzheimer's), glaucoma and stroke comprising administering the afore-mentioned dual-acting compound or combination , in particular the supramolecular complex, or the afore-mentioned linked pro-drug, preferably, the complex, to a subject in need of such treatment.

Figure 1 shows a pictorial representation of the unit cell of the supramolecular complex of trisodium [3-((1*S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(*S*)-3'-methyl-2'-(pentanoyl{2''-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate comprising two asymmetric units. The following color code is used: grey = carbon atom; blue = nitrogen atom; red -= oxygen atom; violet = sodium atom

## Detailed Description

The present invention relates to a dual-acting compound or combination, in particular a supramolecular complex, or linked pro-drug or in particular a supramolecular complex of two active agents with different mechanisms of action, namely an angiotensin receptor antagonist and a neutral endopeptidase inhibitor, which can form a unique molecular entity for the treatment of patients with various cardiovascular and/or renal diseases.

One embodiment of the invention is directed to a physical combination comprising:

(a) a pharmaceutically acceptable salt of an angiotensin receptor antagonist; and

(b) a pharmaceutically acceptable salt of a neutral endopeptidase inhibitor (NEPi);

wherein the pharmaceutically acceptable salt of the angiotensin receptor antagonist and the NEPi is the same and is selected from a salt of Na, K or $NH_4$.

Specifically, it is preferred that the two active agents are combined with each other so as to form a single dual-acting compound, in particular a supramolecular complex. By doing so, a new molecular or supramolecular entity is formed having distinct properties different to the above physical combination.

Thus, the present invention is directed to a dual-acting compound, in particular a supramolecular complex, comprising:

(a) an angiotensin receptor antagonist;

(b) a neutral endopeptidase inhibitor (NEPi); and

(c) a pharmaceutically acceptable cation preferably selected from the group consisting of Na, K and $NH_4$.

The present invention is also directed to a dual-acting compound, in particular a supramolecular complex, obtainable by:

(i) dissolving an angiotensin receptor antagonist and a neutral endopeptidase inhibitor (NEPi) in a suitable solvent;

(ii) dissolving a basic compound of Cat such as (Cat)OH, $(Cat)_2CO_3$, $(Cat)HCO_3$ in a suitable solvent, wherein Cat is a cation preferably selected from the group consisting of Na, K and $NH_4$;

(iii) combining the solutions obtained in steps (i) and (ii);

(iv) precipitation of the solid, and drying same to obtain the dual-acting compound; or alternatvely

obtaining the dual-acting compound by exchanging the solvent(s) employed in steps (i) and (ii) by

(iva) evaporating the resulting solution to dryness;

(va) re-dissolving the solid in a suitable solvent;

(via) precipitation of the solid and drying same to obtain the dual-acting compound.

The present invention is further directed to linked pro-drugs comprising:

(a) an angiotensin receptor antagonist or a pharmaceutically acceptable salt thereof; and

(b) a NEPi or a pharmaceutically acceptable salt thereof,

wherein the angiotensin receptor antagonist or a pharmaceutically acceptable salt thereof and the NEPi or a pharmaceutically acceptable salt thereof are linked by a linking moiety.

The two components are each linked to a linking moiety thereby creating a linked pro-drug. Preferably, the linked pro-drug is substantially pure; as used herein, "substantially pure" refers to at least 90%, more preferably at least 95% and most preferably at least 98% purity.

As one preferred embodiment of the present invention, the linked pro-drug has a structure such that by linking the two components with the linking moiety, a supramolecular complex is formed.

For the purpose of the present invention, the term "dual-acting compound" is intended to describe that these compounds have two different modes of action in one compound, one is

the angiotensin receptor blockade resulting from the ARB molecular moiety of the compound and the other is the neutral endopeptidase inhibition resulting from the NEPi molecular moiety of the compound.

For the purpose of the present invention, the term "compound" is intended to describe a chemical substance comprising covalent bonds within the two pharmaceutically active agents, the ARB and the NEPi molecular moieties, and non-covalent interactions between these two pharmaceutically active agents, the ARB and the NEPi molecular moieties. Typically, hydrogen bonding can be observed between the two pharmaceutically active agents, the ARB and the NEPi molecular moieties. Ionic bonds can be present between the cation and one or both of the two pharmaceutically active agents, the ARB and the NEPi molecular moieties. Other types of bonds may also be present within the compound such as van der Waals forces. For illustrative purposes, the dual-acting compound of the present invention could be represented as follows:

$$(ARB)\text{-}(L)_m\text{-}(NEPi)$$

wherein L is a linking rnoiety, such as a cation or is a noncovalent bond and m is an integer from 1 or more. In other words the ARB and NEPi moiety can be connected via non-covalent bonds such as hydrogen bonding. Alternatively or additionally they may be connected via a linking moiety such as a cation.

In one embodiment, the dual-acting compound may be considered to be a linked pro-drug, whereby the linking moiety, such as the cation, linking the two pharmaceutically active agents, the ARB and the NEPi, forms the pro-drug of these agents which are released once the linked pro-drug is ingested and absorbed.

In a preferred embodiment, the dual-acting compound is a complex, in particular a supramolecular complex.

For the purpose of the present invention, the term "supramolecular complex" is intended to describe an interaction between the two pharmaceutically active agents, the cations and any other entity present such as a solvent, in particular water, by means of noncovalent, intermolecular bonding between them. This interaction leads to an association of the species

present in the supramolecular complex distinguishing this complex over a physical mixture of the species.

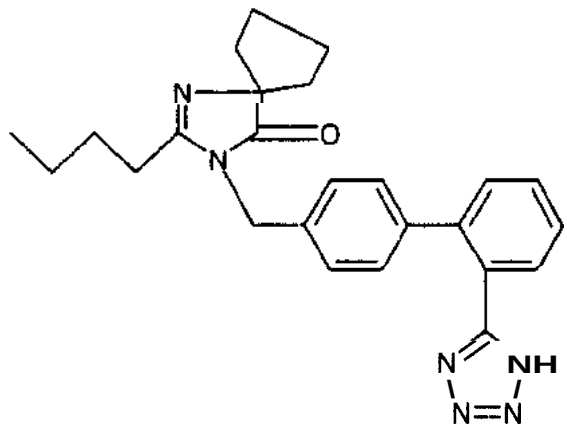
The noncovalent intermolecular bonding can be any interactions known in the art to form such supramolecular complexes, such as hydrogen bonding, van der Waals forces and $\pi$-$\pi$ -stacking. Ionic bonds can also be present. Preferably, there exists ionic bonding and additionally hydrogen bonding to form a network of interactions within the complex. The supramolecular complex exists preferably in the solid state but may also be present in liquid media. As a preferred embodiment of the invention, the complex is crystalline and in this case is preferably a mixed crystal or co-crystal.

Typically, the dual-acting compound, in particular the supramolecular complex shows properties such as melting point, IR spectrum etc. that are different from a physical mixture of the species.

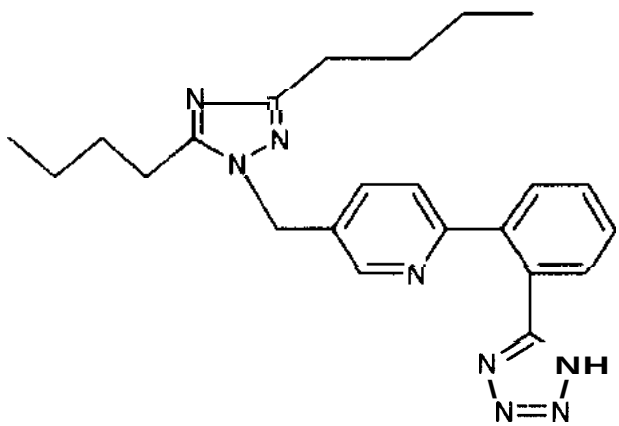
Preferably, the dual-acting compound, in particular the supramolecular complex, has a network of non-covalent bonds, in particular hydrogen bonds, between the two pharmaceutically active agents and any solvent, if present, preferably water. Moreover, it is preferred that the dual-acting compound, in particular the supramolecular complex, has a network of non-covalent bonds, in particular ionic and hydrogen bonds, between the two pharmaceutically active agents, the cation and any solvent, if present, preferably water. The cation is preferably coordinated to several oxygen ligands, thus, providing a linkage between these oxygen ligands. The oxygen ligands come from the carbonyl and carboxylate groups present in the two pharmaceutically active agents and preferably also from any solvent, if present, preferably water.

The dual acting compound comprises a molecular moiety of an angiotensin receptor antagonist. This means that a molecular moiety derived from an angiotensin receptor antagonist is participating in the build-up of the dual-acting compound. The angiotensin receptor antagonist is part of the compound and connected to the NEP inhibitor directly or indirectly via non-covalent bonds. For sake of convenience, throughout the application, the term "angiotensin receptor antagonist" will be used when describing this part of the compound. Angiotensin receptor antagonists (ARBs) suitable for use in the present invention

include, without limitation, valsartan, losartan, irbesartan, telmisartan, eprosartan, candesartan, olmesartan saprisartan, tasosartan, elisartan, the compound with the designation E-1477 of the following formula

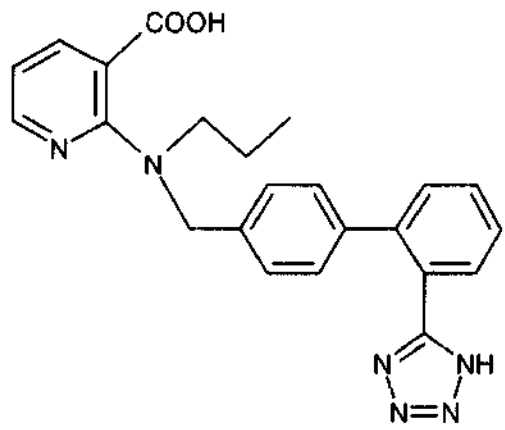


the compound with the designation SC-52458 of the following formula



and

the compound with the designation the compound ZD-8731 of the following formula




Suitable angiotensin II receptor antagonist also includes, but is not limited to, saralasin acetate, candesartan cilexetil, CGP-63170, EMD-66397, KT3-671, LR-B/081, valsartan, A-81282, BIBR-363, BIBS-222, BMS-184698, candesartan, CV-11194, EXP-3174, KW-3433, L-161177, L-162154, LR-B/057, LY-235656, PD-150304, U-96849, U-97018, UP-275-22, WAY-126227, WK-1492.2K, YM-31472, losartan potassium, E-4177, EMD-73495, eprosartan, HN-65021, irbesartan, L-159282, ME-3221, SL-91.0102, Tasosartan, Telmisartan, UP-269-6, YM-358, CGP-49870, GA-0056, L-159689, L-162234, L-162441, L-163007, PD-123177, A-81988, BMS-180560, CGP-38560A, CGP-48369, DA-2079, DE-3489, DuP-167, EXP-063, EXP-6155, EXP-6803, EXP-7711, EXP-9270, FK-739, HR-720, ICI-D6888, ICI-D7155, ICI-D8731, isoteoline, KRI-1177, L-158809, L-158978, L-159874, LR B087, LY-285434, LY-302289, LY-3I5995, RG-13647, RWJ-38970, RWJ-46458, S-8307, S-8308, saprisartan, saralasin, Sarmesin, WK-1360, X-6803, ZD-6888, ZD-7155, ZD-8731, BIBS39, Cl-996, DMP-811, DuP-532, EXP-929, L-163017, LY-301875, XH-148, XR-510, zolasartan and PD-123319.

Also included within the scope of this aspect of the invention are combinations of the above-identified ARBs.

ARBs to be used for preparing the combination or complex in accordance with the present invention can be purchased from commercial sources or can be prepared according to known methods. ARBs may be used for purposes of this invention in their free form, as well as in any suitable salt or ester form.

Preferred salts forms include acid addition salts. The compounds having at least one acid group (e.g., COOH or 5-tetrazolyl) can also form salts with bases. Suitable salts with bases

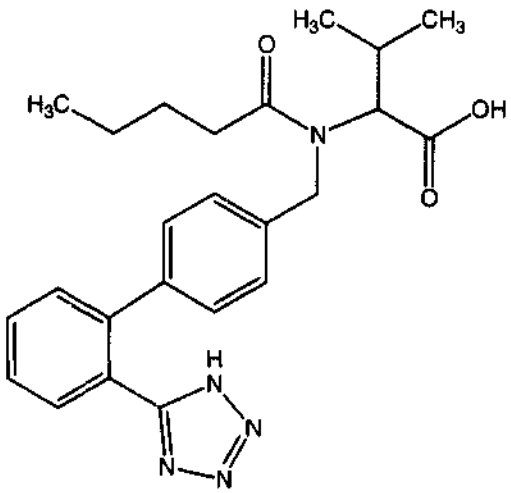
are, e.g., metal salts, such as alkali metal or alkaline earth metal salts, e.g., sodium, potassium, calcium or magnesium salts, or salts with ammonia or an organic amine, such as morpholine, thiomorpholine, piperidine, pyrrolidine, a mono-, di- or tri-lower alkylamine, e.g., ethyl-, tert-butyl-, diethyl-, diisopropyl-, triethyl-, tributyl- or dimethylpropylamine, or a mono-, di- or trihydroxy lower alkylamine, e.g., mono-, di- or tri-ethanolamine. Corresponding internal salts may furthermore be formed. Salts which are unsuitable for pharmaceutical uses but which can be employed, e.g., for the isolation or purification of free compounds I or their pharmaceutically acceptable salts, are also included. Even more preferred salts are, e.g., selected from the mono-sodium salt in amorphous form; di-sodium salt of valsartan in amorphous or crystalline form, especially in hydrate form, thereof.

Mono-potassium salt of valsartan in amorphous form; di-potassium salt of valsartan in amorphous or crystalline form, especially in hydrate form, thereof.

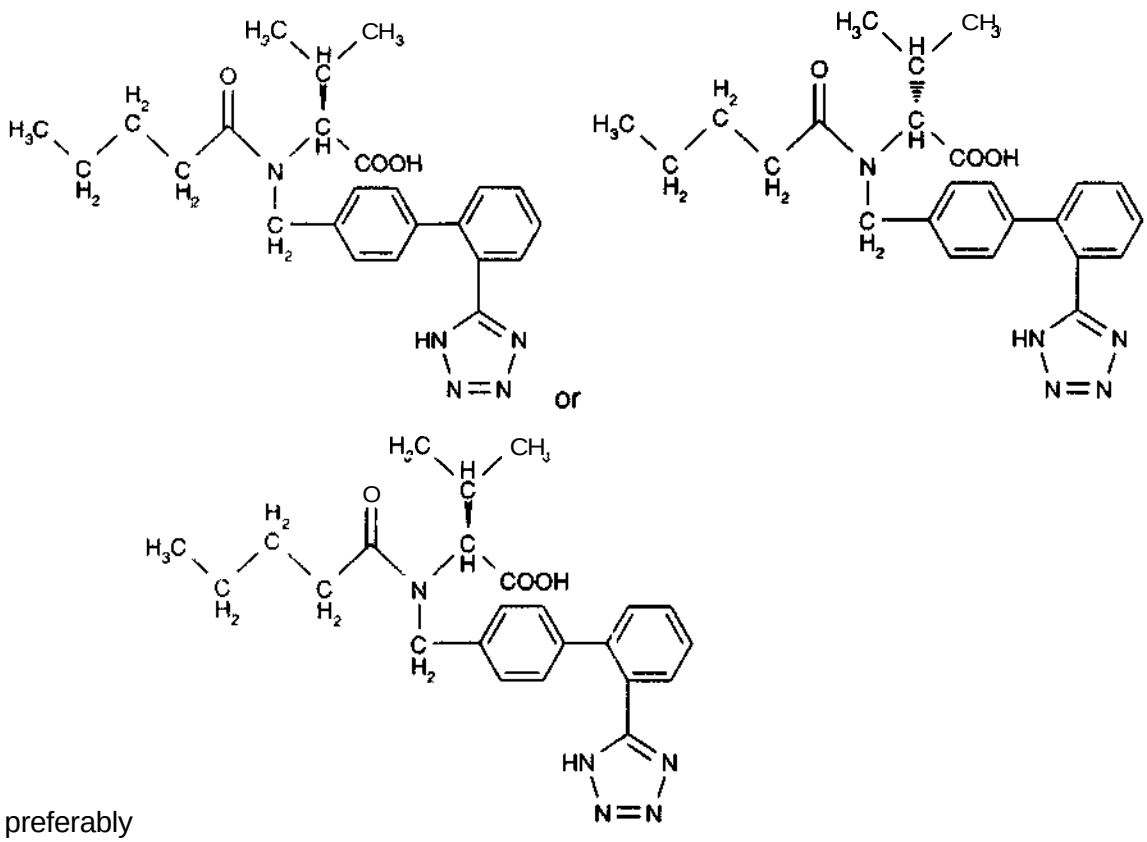
Calcium salt of valsartan in crystalline form, especially in hydrate form, primarily the tetrahydrate thereof; magnesium salt of valsartan in crystalline form, especially in hydrate form, primarily the hexahydrate thereof; calcium/magnesium mixed salt of valsartan in crystalline form, especially in hydrate form; *bis*-diethylammonium salt of valsartan in crystalline form, especially in hydrate form; *s*-dipropylammonium salt of valsartan in crystalline form, especially in hydrate form; *s*-dibutylammonium salt of valsartan in crystalline form, especially in hydrate form, primarily the hemihydrate thereof; mono-*L*-arginine salt of valsartan in amorphous form; *bis*-*L*-arginine salt of valsartan in amorphous form; mono-*L*-lysine salt of valsartan in amorphous form; *bis*-*L*-lysine salt of valsartan in amorphous form.

Preferably when preparing the dual-acting compound, in particular the complex according to the present invention, the free form of the ARB is used.

In a preferred embodiment of this invention, the angiotensin receptor blocker used in the combination or complex of the present invention is Valsartan the molecular structure of which is shown below



Valsartan may be in the racemic form or as one of the two isomers shown below



preferably

Valsartan ((*S*)-*N*-valeryl-*N*-{[2'-(1*H*-tetrazole-5-yl)-biphenyl-4-yl]-methyl}-valine) used according to the present invention can be purchased from commercial sources or can be prepared according to known methods. For example, the preparation of valsartan is described in U.S. Patent No 5,399,578 and EP 0 443 983, the entire disclosure of each of

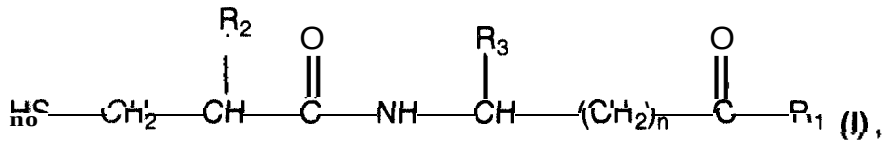
which is incorporated by reference herein. Valsartan may be used for purposes of this invention in its free acid form, as well as in any suitable salt form. Additionally, esters or other derivatives of the carboxylic grouping may be applied for the synthesis of linked pro-drugs, as well as salts and derivatives of the tetrazole grouping. Reference to ARBs includes reference to pharmaceutically acceptable salts thereof.

Preferably, the ARB is a diprotic acid. Thus, the angiotensin receptor blocker has a charge of 0, 1 or 2 depending on the pH of the solution.

In the combination of the present invention, the ARB is in the form of a pharmaceutically acceptable salt selected from Na, K or $NH_4$, preferably Na. This includes both the mono- and di-salt of these cations, preferably the di-salt. In particular in the case of valsartan this means that both the carboxylic acid moiety and the tetrazole moiety form the salt.

In the dual-acting compound, in particular the supramolecular complex of the present invention, typically the free form of the ARB is employed in the preparation and the cationic species present in the complex is introduced by using a base, e.g. (Cat)OH.

The dual acting compound comprises a molecular moiety of a neutral endopeptidase inhibitor. This means that a molecular moiety derived from a neutral endopeptidase inhibitor is participating in the build-up of the dual-acting compound. The neutral endopeptidase inhibitor is part of the compound and connected to the ARB directly or indirectly via non-covalent bonds. For sake of convenience, throughout the application, the term "neutral endopeptidase inhibitor" will be used when describing this part of the compound. Neutral endopeptidase inhibitors suitable for use in the present invention include those of formula (I)



(I),

wherein

$R_2$ is alkyl of 1-7 carbons, trifluoromethyl, phenyl, substituted phenyl, -$(CH_2)$1 to 4-phenyl, or -$(CH_2)$1 to 4-substituted phenyl;

$R_3$ is hydrogen, alkyl of 1-7 carbons, phenyl, substituted phenyl, -$(CH_2)$1 to 4-phenyl or -$(CH_2)$1 to 4-substituted phenyl;

$R_1$ is hydroxy, alkoxy of 1-7 carbons or $NH_2$;

n is an integer from 1-15;

and the term substituted phenyl refers to a substituent selected from lower alkyl of 1-4 carbons, lower alkoxy of 1-4 carbons, lower alkylthio of 1-4 carbons, hydroxy, Cl, Br or F.

Preferred neutral endopeptidase inhibitors of formula (I) include compounds, wherein

$R_2$ is benzyl;

$R_3$ is hydrogen;

n is an integer from 1-9; and

$R_1$ is hydroxy.

Another preferred neutral endopeptidase inhibitor is (3S,2'R)-3-{1-[2'-(ethoxycarbonyl)-4'-phenyl-butyl]-cyclopentan-1-carbonylamino}-2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepine-1-acetic acid or a pharmaceutically acceptable salt thereof.

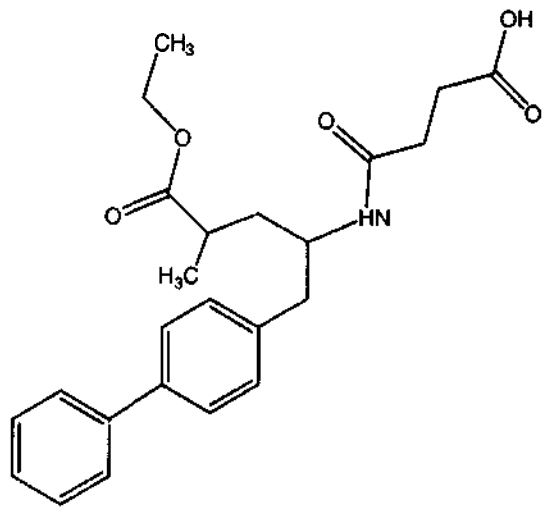
Preferred neutral endopeptidase inhibitors suitable for use in the present invention include, without limitation, SQ 28,603; N-[*N*-[1(*S*)-carboxyl-3-phenylpropyl]-(*S*)-phenylalanyl]-(*S*)-isoserine; N-[N-[((1*S*)-carboxy-2-phenyl)ethyl]-(*S*)-phenylalanyl]-β-alanine; N-[2(*S*)-mercaptomethyl-3-(2-methylphenyl)-propionyl]methionine; (*cis*-4-[[[1-[2-carboxy-3-(2-methoxyethoxy)propyl]-cyclopentyl]carbonyl]amino]-cyclohexanecarboxylic acid); thiorphan; retro-thiorphan; phosphoramidon; SQ 29072; (2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester; *N*-(3-carboxy-1-oxopropyl)-(4*S*)-*p*-phenylphenylmethyl)-4-amino-2*R*-methylbutanoic acid; (*S*)-*cis*-4-[1-[2-(5-indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido]-1-cyclohexanecarboxylic acid; 3-(1-[6-endo-hydroxymethylbicyclo[2,2,1]heptane-2-exo-carbamoyl]cyclopentyl)-2-(2-methoxyethyl)propanoic acid; *N*-(1-(3-(*N*-*t*-butoxycarbonyl-(*S*)-prolylamino)-2(*S*)-*t*-butoxy-carbonylpropyl)cyclopentanecarbonyl)-*O*-benzyl-(*S*)-serine methyl ester; 4-[[2-(mercaptomethyl)-1-*oxo*-3-phenylpropyl]amino]benzoic acid; 3-[1-(*cis*-4-carboxycarbonyl-*cis*-3-butylcyclohexyl-*r*-1-carbamoyl)cyclopentyl]-2*S*-(2-methoxyethoxymethyl)propanoic acid; ((2*S*)-2-(4-biphenylmethyl)-4-carboxy-5-phenoxyvaleryl)glycine; (1-(*N*-hydroxycarbamoylmethyl)-1-cyclopentanecarbonyl)-*L*-phenylalanine; (*S*)-(2-biphenyl-4-yl)-1-(1*H*-tetrazol-5-yl)ethylamino) methylphosphonic acid; (*S*)-5-(*N*-(2-(phosphonomethylamino)-3-(4-biphenyl)propionyl)-2-aminoethyl)tetrazole; β-alanine; 3-[1,1'-biphenyl]-4-yl-*N*-[diphenoxyphosphinyl)methyl]-*L*-alanyl; N(2-carboxy-4-

thienyl)-3-mercapto-2-benzylpropanamide; 2-(2-mercaptomethyl-3-phenylpropionamido)thiazol-4-ylcarboxylic acid; (*L*)-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy)carbonyl)-2-phenylethyl)-*L*-phenylalanyl)-β-alanine; *N*-[*N*-[(*L*)-[1-[(2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy]carbonyl]-2-phenylethyl]-*L*-phenylalanyl]-(*R*)-alanine; -[*N*-[(*L*)-1-carboxy-2-phenylethyl]-*L*-phenylalanyl]-(*R*)-alanine; N-[2-acetylthiomethyl-3-(2-methyl-phenyl)propionyl]-methionine ethyl ester; *N*-[2-mercaptomethyl-3-(2-methylphenyl)propionyl]-methionine; [2(*S*)-mercaptomethyl-3-(2-methylphenyl)propanoyl]-(*S*)-isoserine; *N*-(*S*)-[3-mercapto-2-(2-methylphenyl)propionyl]-(*S*)-2-methoxy-(*R*)-alanine; *N*-[1-[[1(*S*)-benzyloxycarbonyl-3-phenylpropyl]amino]cyclopentylcarbonyl]-(*S*)-isoserine; *N*-[1-[[1(*S*)-carbonyl-3-phenylpropyl]amino]-cyclopentylcarbonyl]-(*S*)-isoserine; 1,1'-[dithiobis [2(*S*)-(2-methylbenzyl)-1-*oxo*-3,1-propanediyl]]-*bis*-(*S*)-isoserine; 1,1'-[dithiobis-[2(*S*)-(2-methylbenzyl)-1-*oxo*-3,1-propanediyl]]-*bis*-(*S*)-methionine; *N*-(3-phenyl-2-(mercaptomethyl)-propionyl)-(*S*)-4-(methylmercapto)methionine; *N*-[2-acetylthiomethyl-3-phenyl-propionyl]-3-aminobenzoic acid; *N*-[2-mercaptomethyl-3-phenyl-propionyl]-3-aminobenzoic acid; *N*-[1-(2-carboxy 4-phenylbutyl)-cyclopentane-carbonyl]-(*S*)-isoserine; *N*-[1-(acetylthiomethyl)cyclopentane-carbonyl]-(*S*)-methionine ethyl ester; 3(*S*)-[2-(acetylthiomethyl)-3-phenyl-propionyl]amimo-ε-caprolactam; *N*-(2-acetylthiomethyl-3-(2-methylphenyl)propionyl)-methionine ethyl ester; and combinations thereof.

Neutral endopeptidase inhibitors can be purchased from commercial sources or can be prepared according to known methods, such as those set forth in any of U.S. Patent No. 4,722,810, U.S. Patent No. 5,223,516, U.S. Patent No. 4,610,816, U.S. Patent No. 4,929,641, South African Patent Application 84/0670, UK 69578, U.S. Patent No. 5,217,996, EP 00342850, GB 02218983, WO 92/14706, EP 00343911, JP 06234754, EP 00361365, WO 90/09374. JP 07157459, WO 94/15908, U.S. Patent No. 5,273,990, U.S. Patent No. 5,294,632, U.S. Patent No. 5,250,522, EP 00636621, WO 93/09101, EP 00590442, WO 93/10773, U.S. Patent No. 5,217,996, the disclosure of each of which is incorporated by reference. Neutral endopeptidase inhibitors may be used for purposes of this invention in their free form, as well as in any suitable salt form. Reference to neutral endopeptidase inhibitors includes reference to pharmaceutically acceptable salts thereof.

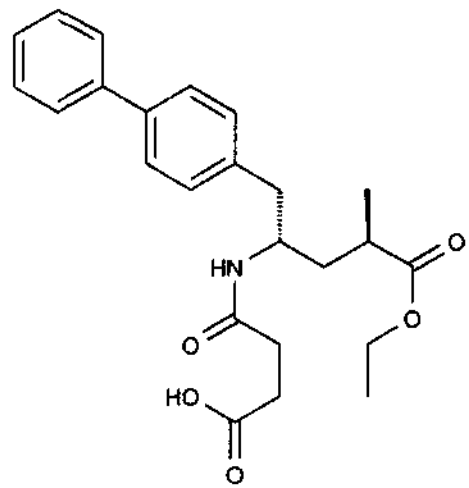
Additionally esters or other derivatives of any carboxylic grouping may be applied for the synthesis of linked pro-drugs, as well as salts and derivatives of any other acidic grouping. In a preferred embodiment of this invention, the NEPi is 5-biphenyl4-yl-5-(3-carboxy-

propionylamino)-2-methyl-pentanoic acid ethyl ester of formula (II) or the respective hydrolysed form 5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid.




**(II) .**

The compound of formula (II) can exist as the (2*R*,4*S*), (2*R*,4*S*), (2*R*,4*S*) or (2*R*,4*S*) isomer. Preferred is (2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester as shown below:




The compound of formula (II) is a specific inhibitor of NEP and is described in U.S. Patent No. 5,217,996. It can be purchased from commercial sources or can be prepared according to known methods. The compound of formula (II) may be used for purposes of this invention in its free form, as well as in any suitable salt or ester form.

Preferably the NEPi is a monoprotic acid. Thus, the NEPi has a charge of 0 or 1 depending on the pH of the solution.

In the combination of the present invention, the NEPi is in the form of a pharmaceutically acceptable salt selected from Na, K or $NH_4$, preferably Na.

In the dual-acting compound, in particular the supramolecular complex of the present invention, typically the free form of the NEPi is employed in the preparation and the cationic species present in the complex is introduced by using a base, (Cat)OH.

The dual acting compound preferably comprises non-covalent bonds between the ARB and the NEPi. Alternatively or in addition, it optionally comprises a linking moiety such as a pharmaceutically acceptable cation.

The linking moiety includes, but is not limited to, generally regarded as safe (GRAS) compounds or other pharmacologically acceptable compounds. The linking moiety may be an ion or a neutral molecule. In the case wherein the linking moiety is an ion the linked pro-drug is a salt and when the linking moiety is a neutral molecule the linked pro-drug is a co-crystal. Without being bound by any particular theory, the acidic portion of the ARB and NEPi donate a proton to the basic linking moiety such that all three components then become united to form one molecule. When the linked pro-drug is ingested by the subject intended to be treated the more acidic nature of the ingestion environment causes the linked pro-drug to separate into individual components concomitant with ingestion and absorption and therefore be converted into active agents to provide their beneficial biological action to treat the intended diseases.

In the case of a linked pro-drug salt or the dual-acting compound, the linking moiety or the cation, respectively, is preferably a positively charged mono-, di- or tri-valent cation, an organic base or an amino acid. Preferred cations (Cat) both for the linked pro-drug in general and the dual-acting compound, in particular the complex are basic cations, even more preferably metallic cations. Preferred metallic cations include, but are not limited to Na, K, Ca, Mg, Zn, Fe or $NH_4$. Amine bases and salt forming agents may also be employed, such as benzathine, hydrabamine, ethylenediamine, n-n-dibenzyl-ethylenediamine, L-arginine, choline hydroxide, N-methyl-glucamine, (Meglumine), L-Lysine, dimethylaminoethanol (Deanol), t-butylamine, diethylamine, 2-(diethylamino)-ethanol, 4-(2-

hydroxyethyl)-morpholine, Thromethanine (TRIS), 4-acetamidophenol, 2-amino-2-methyl-1,3-propanediol, 2-amino-2-methyl-propanol, benzylamine, cyclohexylamine, diethanolamine, ethanolamine, imidazole, piperazine and triethanolamine.

Most preferably, the cation is Na, K or $NH_4$, such as Na. In one embodiment Ca is preferred.

In the case of a linked pro-drug co-crystal, the linking moiety is may also be a neutral molecule which provides hydrogen-bonding functionality.

In one embodiment, the linked pro-drugs of this invention are represented as set forth below, wherein scheme (1) and (2) represent a salt and scheme (3) represents a co-crystal:

NEPi • Xa• ARB scheme (1)

NEPi • XaYb• ARB scheme (2)

NEPi • Zc• ARB scheme (3),

wherein

X is Ca, Mg, Zn or Fe;

Y is Na, K or NH4;

Z is a neutral molecule; and

a, b and c reflect the stoichiometry of the linked pro-drug, preferably, a, b and c are a valence of $1^+$, $2^+$ or $3^+$.

For the linked pro-drugs of schemes (1) and (2), above, preferably the NEPi is a monoprotic acid and ARB is a diprotic acid. The angiotensin receptor blocker has a charge of 0, 1 or 2 and the NEPi has a charge of 0 or 1 depending on the pH of the solution, while the overall molecule will be neutral. Ratios of ARB to NEPi will be 1:1, 1:2, 1:3, 3:1, 2:1, 1:1, preferably 1:1, 1:2 or 1:3, most preferably 1:1.

Multi-component salts, particularly with zinc and calcium have been reported in the literature, e.g., *Chem Pharm Bull,* Vol. 53, p. 654 (2005). These ions require a coordination geometry that facilitates the crystallization of multi-component systems. The metal ions have coordinating geometries governed by the atomic orbitals for each species

Valsartan comprises two acidic groupings: the carboxylic acid and the tetrazole. In one embodiment of this aspect of the present invention, the molecular structure of linked pro-drugs of valsartan and a NEPi comprise a linkage between the carboxylic acid and the linking moiety or a linkage between the tetrazole grouping and the linking moiety. In yet another embodiment, the linked pro-drug comprises a trivalent linking moiety linked to the valsartan carboxylic acid grouping, the tetrazole grouping and the NEPi grouping.

In an embodiment of this aspect of the invention, valsartan is linked to (2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester by a calcium salt ion.

In a preferred embodiment of the present application, the angiotensin receptor antagonist and the neutral endopeptidase inhibitor are present in a molar ratio of 1:1, 1:2, 1:3, 3:1, 2:1, more preferably 1:1 in the combination as well as in the supramolecular complex. This is also true for the linked pro-drug. Moreover, in the complex, angiotensin receptor antagonist, the . neutral endopeptidase inhibitor and the cation are present in a molar ratio of 1:1:1, 1:1:2, 1:1:3, more preferably 1:1:3. This applies equally to the linked pro-drug.

The combination or the dual-acting compound, in particular the complex of the present invention may contain a solvent. This is particularly preferred in the case of the dual-acting compound, in particular the complex, where the solvent may contribute to the intermolecular structure, e.g. the supramolecular interactions. Preferred solvents include water, methanol, ethanol, 2-propanol, acetone, ethyl acetate, methyl-*t*-butylether, acetonitrile, toluene, and methylene chloride, preferably water. If a solvent is present, one or more molecules per molecule of the active agent can be present. In this case, namely if a stoichiometric amount of the solvent is present, preferably 1, 2, 3, 4 or 5, more preferably 3, molecules of solvent, such as water, can be present per molecule of active agent. Alternatively, the solvent may be present in non-stoichiometric amounts. This means preferably any stoichiometric fraction of the solvent, such as 0.25, 0.5, 0.75, 1.25, 1.5, 1.75, 2.25, 2.5, 2.75, 3.25, 3.5, 3.75, 4.25, 4.5 and 4.75, preferably 2.5, molecules of solvent, such as water, can be present per molecule of active agent. If the dual-acting compound, in particular the complex is in the crystalline form, the solvent may be part of the molecular packing and be trapped in the crystal lattice.

Thus in a preferred embodiment of the present invention, the dual-acting compound, in particular the supramolecular complex is described by the sum formula:

[ARB(NEPi)]$Na_{1-3}$ • x$H_2O$, wherein x is 0, 1 , 2 or 3, such as 3, preferably

[ARB(NEPi)]$Na_3$ • x$H_2O$, wherein x is 0, 1 , 2 or 3, such as 3, more preferably

[valsartan ((2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester]Na$ • x $H_2O$, wherein x is 0, 1 , 2 or 3, such as 3.

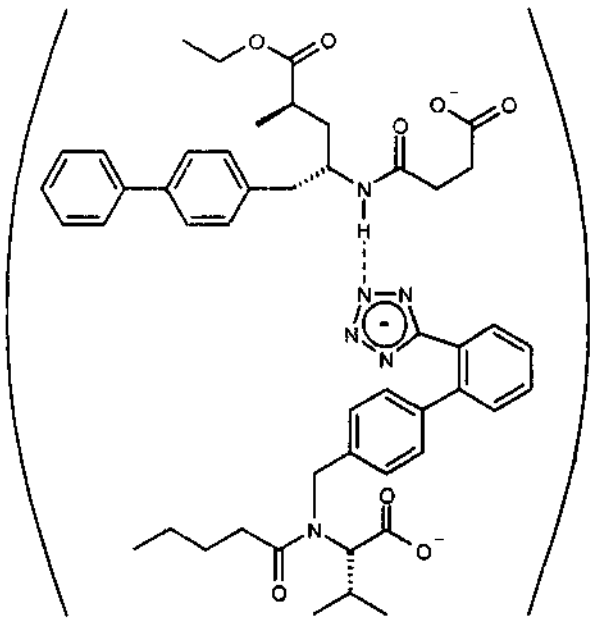
Thus in a preferred embodiment of the present invention, the dual-acting compound, in particular the supramolecular complex is described by the sum formula:

[ARB(NEPi)]$Na_{1-3}$ • x$H_2O$, wherein x is 0 to 3, such as 2.5, preferably

[ARB(NEPi)]$Na_3$ • x$H_2O$, wherein x is 0 to 3, such as 2.5, more preferably

[(*N*-valeryl-*N*-{[2'-(1*H*-tetrazole-5-yl)-biphenyl-4-yl]-methyl}-valine) (5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester]$Na_3$ • x $H_2O$, in particular [((*S*)-*N*-valeryl-*N*-{[2'-(1*H*-tetrazole-5-yl)-biphenyl-4-yl]-methyl}-valine) ((2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester]$Na_3$ • x $H_2O$, wherein x is 0 to 3, such as 2.5. In this most preferred example, the complex is termed trisodium [3-((1*S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(*S*)-3'-methyl-2'-(pentanoyl{2''-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate.

A simplified structure of trisodium [3-((1*S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(*S*)-3'-methyl-2'-(pentanoyl{2''-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate used to formally calculate the relative molecular mass, is shown below.



. 3 $Na^+$ . 2.5 $H_2O$

Valsartan comprises two acidic groupings: the carboxylic acid and the tetrazole. In one embodiment of this aspect of the present invention, the molecular structure of the dual-acting compound, in particular, the complex, of valsartan and a NEPi comprises an interaction between the carboxylic acid and the cation, such as Na, or the solvent, such as water, or a linkage between the tetrazole grouping and the cation, such as Na, or the solvent, such as water. In yet another embodiment, the dual-acting compound, in particular, the complex, comprises an interaction between the valsartan carboxylic acid grouping, the tetrazole grouping or the NEPi grouping and the cation, such as Na, or the solvent, such as water.

The combination or dual-acting compound, in particular, the complex, of the present invention is preferably in the solid form. In the solid state it can be in the crystalline, partially crystalline, amorphous, or polymorphous form, preferably in the crystalline form.

The dual-acting compound, in particular, the complex, of the present invention is distinct from a combination of an ARB and a NEPi obtained by simply physically mixing the two active agents. Thus, it can have different properties that make it particularly useful for manufacturing and therapeutic applications. The difference of the dual-acting compound, in particular, the complex, and the combination can be exemplified by the dual-acting compound of (*S*)-*N*-valeryl-*N*-{[2'-(1*H*-tetrazole-5-yl)-biphenyl-4-yl]-methyl}-valine and (2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester

which is characterized by very distinct spectral peaks and shifts that are not observed in the physical mixture.

Specifically, such a dual-acting compound is preferably characterized by an X-ray powder diffraction pattern taken with a Scintag XDS2000 powder diffractometer using Cu-Ka radiation (lamda=1.54056 A) with a Peltier-cooled Silicon detector at room temperature (25degree C). Scan range was from 1.5degree to 40degree in 2 theta with a scan rate of 3degree/minute. The most important reflections in the X-ray diffraction diagram comprise the following interlattice plane intervals:

The preferred characterization of trisodium [3-((1*S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(*S*)-3'-methyl-2'-(pentanoyl{2''-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate is obtained from the interlattice plane intervals d of the ascertained X-ray diffraction diagrams, whereby, in the following, average values 2Θ in [°] are indicated (error limit of ±0.2)

4.5, 5.5, 5.6, 9.9, 12.8, 15.7, 17.0, 17.1, 17.2, 18.3, 18.5, 19.8, 21.5, 21.7, 23.2, 23.3, 24.9, 25.3, 27.4, 27.9, 28.0, 30.2.

or with an error limit of ±0.1:

4.45, 5.52, 5.57, 9.94, 12.82, 15.66, 17.01, 17.12, 17.2, 18.32, 18.46, 19.76, 21.53, 21.72, 23.17, 23.27, 24.88, 25.3, 27.4, 27.88, 28.04, 30.2.

The most intensive reflections in the X-ray diffraction pattern show the following interlattice plane intervals:

2Θ in [º] : .4.5, 5.6, 12.8, 17.0, 17.2, 19.8, 21.5, 27.4, in particular 4.45, 5.57, 17.01, 17.2, 19.76, 21, 27.4.

A preferred method of checking the above-indicated average values of the interlattice plane intervals and intensities measured by experimentation from X-ray diffraction, for a given substance, consists in calculating these intervals and their intensities from the comprehensive single crystal structure determination. This structure determination yields ceU constants and atom positions, which enable the X-ray diffraction diagram corresponding to the solid to be calculated by means of computer-aided calculation methods. The program used is Powder Pattern within the application software Materials Studio (Accelrys). A

comparison of these data, namely the interlattice plane intervals and intensities of the most important lines of trisodium [3-((1*S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(*S*)-3'-methyl-2'-(pentanoyl{2''-(tetrazol-5-ylate)biphenyl-4'-ylrnethyl}amino)butyrate] hemipentahydrate, obtained from measurements and from calculating the single crystal data, is illustrated in the table below.

Table

| measured | | calculated | | measured | | calculated | |
|---|---|---|---|---|---|---|---|
| 2e in [°] | Intensity | 2Θ In [°] | Intensity | 2Θ in [°] | Intensity | 2Θ in [°3 | Intensity |
| 4.45 | very strong | 4.15 | very strong | 19.76 | strong | 19.6 | very weak |
| 5.52 | Strong | 5 | strong | 21.53 | weak | 19.8 | very weak |
| 5.57 | strong | 6.5 | strong | 21.72 | very weak | 21.4 | very weak |
| 9.94 | very weak | 9.75 | weak | 23.17 | weak | 23.1 | very weak |
| 12.82 | very strong | 12.6 | weak | 23.27 | weak | 23.15 | very weak |
| 15.66 | very weak | 15.05 | strong | 24.88 | very weak | | very weak |
| 17.01 | weak | 16.9 | very strong | 25.3 | weak | 25.3 | very weak |
| 17.12 | strong | 17.1 | strong | 27.4 | weak | 27.3 | very weak |
| 17.2 | weak | 17.15 | weak | 27.88 | very weak | 27.9 | very weak |
| 18.32 | weak | 18.25 | very | 28.04 | weak | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | weak | | | | |
| 18.46 | weak | 18.3 | weak | 30.2 | weak | | |

Relative intensity between 100% to 50% is referred to as very strong, 50% to 10% as strong, 10% to 5% as weak, and below 5% as very weak.

The invention relates to trisodium [3-((1*S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(*S*)-3'-methyl-2'-(pentanoyl{2''-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate, a crystalline solid which is characterized by the data and parameters obtained from single crystal X-ray analysis and X-ray powder patterns. An in-depth discussion of the theory of the methods of single crystal X-ray diffraction and the definition of the evaluated crystal data and the parameters may be found in Stout & Jensen, X-Ray Structure Determination; A Practical Guide, Mac Millian Co., New York, N.Y. (1968) chapter 3.

*Crystal data*

| | |
|---|---|
| sum formula | $C_{48}H_{55}N_6O_8Na_3 \cdot 2.5H_2O$ |
| molecular mass | 957.99 |
| crystal colour | colourless |
| crystal shape | tabular: hexagonal |
| crystal system | monoclinic |
| space group | $P2_1$ |
| Cell parameters | a=20.344 A |
| | b=42.018 Å |
| | c=20.374 A |
| | $\alpha = 90^0$ |
| | $\beta$=119.29° |

| | |
|---|---|
| | Y = 90° |
| volume of unit cell | 15190.03 Å$^3$ |
| Z (the number of asymmetric units in the unit cell) | 2 |
| calculated density | 1.26845 g/cm3 |

*Single crystal X-ray measurement data*

| | |
|---|---|
| diffractometer | Nonius KappaCCD |
| X-ray generator | Nonius FR571 X-ray generator with a copper rotating anode |
| temperature | 270 K and 150 K |

Notes:

Two data sets on two suitable single crystals were collected at two different temperatures to assure no phase change during cooling.

None of the hydrogen atoms on the water or amine nitrogen atoms were observed in the Fourier maps so they were not included in the refinement.

*Computer program used to solve the structure*

SHELXD ( Sheldrick, Göttingen)

In three dimensions, the unit cell is defined by three edge lengths a, b, and c, and three interaxial angles a, β, und γ. In this way, the volume of the unit cell $V_c$ is determined. A differentiated description of these crystal parameters is illustrated in chapter 3 of Stout & Jensen (see above). The details for trisodium [3-((1 *S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(*S*)-3'-methyl-2'-(pentanoyl{2''-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate from the single crystal measurements, especially the atom coordinates, the isotropic thermal parameters, the coordinates of the hydrogen atoms as well as the corresponding isotropic thermal parameters, show that a monoclinic unit cell exists, its cell content of twelve formula units of $C_{48}H_{55}N_6O_8Na_3 \bullet 2.5\ H_2O$ occurring as a result of two asymmetric units on two-fold positions.

The acentric space group $P2_1$ determined from the single crystal X-ray structure is a common space group for enantiomorphically pure molecules. In this space group there are two general positions which means that for twelve formula units in the unit cell there must be 18 sodium ions and 15 waters in the asymmetric unit.

A pictorial representation of the unit cell of the supramolecular complex of trisodium [3-((1*S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(*S*)-3'-methyl-2'-(pentanoyl{2''-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate comprising two asymmetric units is shown in Figure 1.

Based on the single crystal structure solution, the asymmetric unit of the trisodium [3-((1*S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(*S*)-3'-methyl-2'-(pentanoyl{2''-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate supramolecule comprises six each of ARB and NEPi moieties, 18 sodium atoms, and 15 water molecules. Trisodium [3-((1*S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-utylcarbamoyl)propionate-(*S*)-3'-methyl-2'-(pentanoyl{2''-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate may be considered a sodium supramolecular complex, coordinated by oxygen ligands. These oxygens come from twelve carboxylate groups and eighteen carbonyl groups of the above moieties, and from 13 of the 15 water molecules. The crystal is an infinite 3-dimensional network of these sodium complexes.

Such a compound may also be characterized by an infrared absorption spectrum obtained using Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectrometer (Nicolet Magna-IR 560) showing the following significant bands, expressed in reciprocal wave numbers ($cm^{-1}$):

2956 (w), 1711 (st), 1637 (st), 1597 (st), 1488 (w), 1459 (m), 1401 (st), 1357 (w), 1295 (m), 1266 (m), 1176 (w), 1085 (m), 1010 (w), 1942(w), 907 (w), 862 (w), 763 (st), 742 (m), 698 (m), 533 (st). Characteristic to the complex are in particular the following peaks 1711(st), 1637(st), 1597(st) and 1401(st). The error margin for all absorption bands of ATR-IR is $\pm$ 2 $cm^{-1}$. The intensities of the absorption bands are indicated as follows: (w) = weak; (m) = medium; and (st) = strong intensity.

Such a compound may also be characterized by a Raman spectrum measured by dispersive Raman spectrometer with 785 nm laser excitation source (Kaiser Optical Systems, Inc.) showing the following significant bands expressed in reciprocal wave numbers ($cm^{-1}$):

3061 (m), 2930 (m, broad), 1612 (st), 1523 (m), 1461 (w), 1427 (w), 1287 (st), 1195 (w), 1108 (w), 11053 (w), 1041 (w), 1011 (w), 997 (m), 866(w), 850 (w), 822 (w), 808 (w), 735 (w), 715 (w), 669 (w), 643 (w), 631 (w), 618 (w), 602 (w), 557 (w), 522 (w), 453 (w), 410 (w), 328 (w).

The error margin for all Raman bands is ± 2 $cm^{-1}$. The intensities of the absorption bands are indicated as follows: (w) = weak; (m) = medium; and (st) = strong intensity.

Such a compound may also be characterized by distinct melting properties measured by differential scanning calorimetry (DSC). Using Q1000 (TA Instruments) instrument, the melting onset temperature and the peak maximum temperature for such a complex are observed at 139°C and 145°C, respectively. The heating rate is 10 K/min.

The second embodiment of the present invention is directed to pharmaceutical compositions comprising a combination, a linked pro-drug or a dual-acting compound, in particular the complex as described herein and at least one pharmaceutically acceptable additive. The details regarding the combination and the complex, including the ARB and the NEPi, are as described above with regard to the first embodiment of the invention.

The pharmaceutical compositions according to the invention can be prepared in a manner known per se and are those suitable for enteral, such as oral or rectal, and parenteral administration to mammals (warm-blooded animals), including man, comprising a therapeutically effective amount of the combination or dual-acting compound, in particular the complex, alone or in combination with at least one pharmaceutically acceptable carrier, especially suitable for enteral or parenteral application. Typical oral formulations include tablets, capsules, syrups, elixirs and suspensions. Typical injectable formulations include solutions and suspensions.

Pharmaceutically acceptable additives suitable for use in the present invention include, without limitation and provided they are chemically inert so that they do not adversely affect the combination or the dual-acting compound, in particular the complex of the present invention, diluents or fillers, disintegrants, glidants, lubricants, binders, colorants and

combinations thereof. The amount of each additive in a solid dosage formulation may vary within ranges conventional in the art. Typical pharmaceutically acceptable carriers for use in the formulations described above are exemplified by: sugars, such as lactose, sucrose, mannitol and sorbitol; starches, such as cornstarch, tapioca starch and potato starch; cellulose and derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and methyl cellulose; calcium phosphates, such as dicalcium phosphate and tricalcium phosphate; sodium sulfate; calcium sulfate; polyvinylpyrrolidone; polyvinyl alcohol; stearic acid; alkaline earth metal stearates, such as magnesium stearate and calcium stearate; stearic acid; vegetable oils, such as peanut oil, cottonseed oil, sesame oil, olive oil and corn oil; non-ionic, cationic and anionic surfactants; ethylene glycol polymers; β-cyclodextrin; fatty alcohols; and hydrolyzed cereal solids, as well as other non-toxic compatible fillers, binders, disintegrants, buffers, preservatives, antioxidants, lubricants, flavoring agents and the like commonly used in pharmaceutical formulations.

Pharmaceutical preparations for enteral or parenteral administration are, e.g., in unit dose forms, such as coated tablets, tablets, capsules or suppositories and also ampoules. These are prepared in a manner which is known *per se,* e.g., using conventional mixing, granulation, coating, solubilizing or lyophilizing processes. Thus, pharmaceutical compositions for oral use can be obtained by combining the linked pro-drug, combination or dual-acting compound, in particular the complex with solid excipients, if desired, granulating a mixture which has been obtained, and, if required or necessary, processing the mixture or granulate into tablets or coated tablet cores after having added suitable auxiliary substances.

The dosage of the active compounds in the combination or dual-acting compound, in particular the complex can depend on a variety of factors, such as mode of administration, homeothermic species, age and/or individual condition. The projected efficacy in animal disease models ranges from about 0.1 mg/kg/day to about 1000 mg/kg/day given orally, and the projected dose for human treatment ranges from about 0.1 mg/day to about 2000 mg/day. Preferred ranges are from about 40 mg/day to about 960 mg/day of the linked pro-drug, preferably about 80 mg/day to about 640 mg/day. The ARB component is administered in a dosage of from about 40 mg/day to about 320 mg/day and the NEPi component is administered in a dosage of from about 40 mg/day to about 320 mg/day. More specifically, the dosages of ARB/NEPi, respectively, include 40 mg/40 mg, 80 mg/80 mg, 160 mg/160 mg, 320 mg/320 mg, 40 mg/80 mg, 80 mg/160 mg, 160 mg/320 mg, 320 mg/640 mg, 80 mg/40 mg, 160 mg/80 mg and 320 mg/160 mg, respectively. These dosages are "therapeutically effective amounts". Preferred dosages for the linked pro-drug, combination or dual-acting compound, in particular the complex of the pharmaceutical composition according to the present invention are therapeutically effective dosages.

The pharmaceutical compositions may contain in addition another therapeutic agent, e.g., each at an effective therapeutic dose as reported in the art. Such therapeutic agents include:

a) antidiabetic agents such as insulin, insulin derivatives and mimetics; insulin secretagogues such as the sulfonylureas, e.g., Glipizide, glyburide and Amaryl; insulinotropic sulfonylurea receptor ligands such as meglitinides, e.g., nateglinide and repaglinide; peroxisome proliferator-activated receptor (PPAR) ligands; protein tyrosine phosphatase-1B (PTP-1B) inhibitors such as PTP-112; GSK3 (glycogen synthase kinase-3) inhibitors such as SB-517955, SB-4195052, SB-216763, NN-57-05441 and NN-57-05445; RXR ligands such as GW-0791 and AGN-194204; sodium-dependent glucose cotransporter inhibitors such as T-1095; glycogen phosphorylase A inhibitors such as BAY R3401; biguanides such as metformin; alpha-glucosidase inhibitors such as acarbose; GLP-1 (glucagon like peptide-1), GLP-1 analogs such as Exendin-4 and GLP-1 mimetics; and DPPIV (dipeptidyl peptidase IV) inhibitors such as LAF237;

b) hypolipidemic agents such as 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitors, e.g., lovastatin, pitavastatin, simvastatin, pravastatin, cerivastatin, mevastatin, velostatin, fluvastatin, dalvastatin, atorvastatin, rosuvastatin and rivastatin; squalene

synthase inhibitors; FXR (farnesoid X receptor) and LXR (liver X receptor) ligands; cholestyramine; fibrates; nicotinic acid and aspirin;

c) anti-obesity agents such as orlistat; and

d) anti-hypertensive agents, e.g., loop diuretics such as ethacrynic acid, furosemide and torsemide; angiotensin converting enzyme (ACE) inhibitors such as benazepril, captopril, enalapril, fosinopril, lisinopril, moexipril, perinodopril, quinapril, ramipril and trandolapril; inhibitors of the Na-K-ATPase membrane pump such as digoxin; ACE/NEP inhibitors such as omapatrilat, sampatrilat and fasidotril; p-adrenergic receptor blockers such as acebutolol, atenolol, betaxolol, bisoprolol, metoprolol, nadolol, propranolol, sotalol and timolol; inotropic agents such as digoxin, dobutamine and milrinone; calcium channel blockers such as amlodipine, bepridil, diltiazem, felodipine, nicardipine, nimodipine, nifedipine, nisoldipine and verapamil; aldosterone receptor antagonists; and aldosterone synthase inhibitors. Most preferred combination partners are diuretics, such as hydrochlorothiazide, and/or calcium channel blockers, such as amlodipine or a salt thereof.

Other specific anti-diabetic compounds are described by Patel Mona in *Expert Opin Investig Drugs,* 2003, 12(4), 623-633, in the figures 1 to 7, which are herein incorporated by reference. A compound of the present invention may be administered either simultaneously, before or after the other active ingredient, either separately by the same or different route of administration or together in the same pharmaceutical formulation.

The structure of the therapeutic agents identified by code numbers, generic or trade names may be taken from the actual edition of the standard compendium "The Merck Index" or from databases, e.g., Patents International (e.g. IMS World Publications). The corresponding content thereof is hereby incorporated by reference.

Accordingly, the present invention provides pharmaceutical compositions in addition a therapeutically effective amount of another therapeutic agent, preferably selected from antidiabetics, hypoiipidemic agents, anti-obesity agents or anti-hypertensive agents, most preferably from antidiabetics, anti-hypertensive agents or hypoiipidemic agents as described above.

The person skilled in the pertinent art is fully enabled to select a relevant test model to prove the efficacy of a combination of the present invention in the hereinbefore and hereinafter indicated therapeutic indications.

Representative studies are carried out with trisodium [3-((1*S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(*S*)-3'-methyl-2'-(pentanoyl{2"-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate, e.g. applying the following methodology:

The antihypertensive and neutral endopeptidase 24.11 (NEP)-inhibitory activities of trisodium [3-((1*S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(*S*)-3'-methyl-2'-(pentanoyl{2"-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate is assessed in conscious rats. The blood pressure-lowering effect is evaluated in double-transgenic rats (dTGRs) that overexpress both human renin and its substrate, human angiotensinogen (Bohlender, et al, High human renin hypertension in transgenic rats. Hypertension; 29(1 Pt 2):428-34, 1997). Consequently, these animals exhibit an angiotensin II-dependent hypertension. The NEP-inhibitory effect of trisodium [3-((1*S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(*S*)-3'-methyl-2'-(pentanoyl{2"-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate is determined in conscious Sprague-Dawley rats infused with exogenous atrial natriuretic peptide (ANP). Potentiation of plasma ANP levels is used as an index of NEP inhibition in vivo. In both models, trisodium [3-((1*S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(*S*)-3'-methyl-2'-(pentanoyl{2"-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate is administered orally as a powder in gelatin mini capsules. The results are summarized below.

- Trisodium [3-((1*S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(*S*)-3'-methyl-2'-(pentanoyl{2"-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate exhibits a dose-dependent and long-lasting antihypertensive effect after oral administration in conscious dTGRs, a rat model of fulminant hypertension.
- Oral administration of trisodium [3-((1*S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(*S*)-3'-methyl-2'-(pentanoyl{2"-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate rapidly and dose-dependently inhibits NEP with a long duration of action, as reflected by its potentiation of plasma ANP

immunoreactivity (ANPir) in conscious Sprague-Dawley rats infused with exogenous ANP.

**Antihypertensive effect in vivo**

The dTGRs are instrumented with radiotelemetry transmitters for continuous measurement of arterial blood pressure and heart rate. Animals are randomly assigned to vehicle (empty capsule) or treatment (at 2, 6, 20 or 60 mg/kg, p.o.) groups. Baseline 24-hr mean arterial pressure (MAP) is approximately 170-180 mmHg in all groups. Trisodium [3-((1*S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(*S*)-3'-methyl-2'-(pentanoyl{2''-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate dose-dependently reduces MAP. The values obtained from the treatment groups are dose-dependent, and the results from the three highest doses are significantly different from the vehicle controls

**Inhibition of NEP in vivo**

The extent and duration of trisodium [3-((1*S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(*S*)-3'-methyl-2'-(pentanoyl{2''-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate for NEP inhibition in vivo is assessed with methodologies as described previously (Trapani, et al, CGS 35601 and its orally active prodrug CGS 37808 as triple inhibitors of endothelin-converting enzyme-1, neutral endopeptidase 24.11, and angiotensin-converting enzyme. J Cardiovasc Pharmacol; 44(Suppl 1):S211-5, 2004). Rat ANP(1-28) is infused intravenously at a rate of 450 ng/kg/min in conscious, chronically cannulated, male Sprague-Dawley rats. After one hour of infusion, rats are randomly assigned to one of six groups: untreated control, vehicle (empty capsule) control, or one of four doses of drug (2, 6, 20, or 60 mg/kg, p.o.). ANP infusion is continued for an additional eight hours. Blood samples are collected for measuring plasma ANPir by a commercial enzyme immunoassay kit at -60 min (i.e., before initiating ANP infusion), -30 min (after 30 min of ANP infusion), 0 min ("baseline"; after 60 min of ANP infusion but before dosing with drug or its vehicle), and at 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 hr post-dosing.

Before ANP infusion, ANPir is low (0.9-1.4 ng/ml) and similar in all six groups. ANP infusion rapidly (by 30 min) elevates ANPir to ~10 ng/ml. This ANPir level is sustained for the duration of the experiment in the untreated and vehicle control groups. In contrast, trisodium

[3-((1*S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(*S*)-3'-methyl-2'-(pentanoyl{2''-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate rapidly (within 15 min) and dose-dependently augments ANPir. In summary, orally administered LCZ696 rapidly and dose-dependently inhibited NEP with a long duration of action as reflected by the potentiation of plasma ANPir.

The available results indicate an unexpected therapeutic effect of a compound according to the invention.

In a third aspect, the present invention is directed to a method of making a linked pro-drug of an ARB or a pharmaceutically acceptable salt thereof and a NEPi or a pharmaceutically acceptable salt thereof comprising the steps of:

*(a)* adding an inorganic salt forming agent to a solvent to form a linked pro-drug salt forming solution;

(b) adding the salt forming solution to a mixture of an ARB and a NEPi such that the ARB and NEPi form a linked pro-drug; and

(c) isolating the linked pro-drug.

Preferably, the components are added in an equivalent amount.

The inorganic salt forming agent includes, but is not limited to, calcium hydroxide, zinc hydroxide, calcium methoxide, calcium acetate, calcium hydrogen carbonate, calcium formate, magnesium hydroxide, magnesium acetate, magnesium formate and magnesium hydrogen carbonate, sodium hydroxide, sodium methoxide, sodium acetate, sodium formate. The inorganic salt forming agent releases the linking moiety into the solvent such that when an ARB and a NEPi are present a linked pro-drug is formed.

Solvents included in the scope of the present invention include, but are not limited to, solvents in which the ARB, NEPi and inorganic salt forming agent preferably exhibit a lower solubility that allows the linked pro-drug to crystallize. Such solvents may comprise, but are not limited to, water, methanol, ethanol, 2-propanol, ethylacetate, methyl-*t*-butylether, acetonitrile, toluene, and methylene chloride and mixtures of such solvents.

The inorganic salt forming agent and the solvent when combined should have a pH which promotes linked pro-drug formation. The pH may be between about 2 and about 6, preferably between about 3 and about 5, most preferably between 3.9 and 4.7.

The linked pro-drug is isolated by crystallization and chromatography. Specific types of chromatography include, e.g., ligand specific resin chromatography, reverse phase resin chromatography and ion-exchange resin chromatography.

A specific example comprises contacting a divalent salt of one component with a mono-valent salt of the other component of the linked pro-drug. Specifically the mixed salt of valsartan and a mono-basic NEPi are synthesized by contacting the calcium salt of valsartan with the sodium salt of the NEPi component. Isolation of the desired mixed salt is carried out by selective crystallization or chromatography using ligand specific resins, reverse phase resins or ion-exchange resins. Similarly this process can be conducted with a monovalent salt of both components, such as the sodium salt of both components.

In another embodiment of this aspect of the invention, a co-crystal of the linked pro-drug is obtained. In a method of making a linked pro-drug co-crystal the inorganic salt forming agent is replaced with a neutral molecule which provides hydrogen binding properties. The solvent may be part of the molecular packing and be trapped in the crystal lattice.

In a preferred embodiment of the third aspect, the present invention is directed to a method of preparing a dual-acting compound comprising

(a) an angiotensin receptor antagonist;

(b) a neutral endopeptidase inhibitor (NEPi); and optionally

(c) a pharmaceutically acceptable cation;

said method comprising the steps of:

(i) dissolving an angiotensin receptor antagonist and a neutral endopeptidase inhibitor (NEPi) in a suitable solvent;

(ii) dissolving a basic compound of Cat in a suitable solvent, wherein Cat is a cation;

(iii) combining the solutions obtained in steps (i) and (ii);

(iv) precipitation of the solid, and drying same to obtain the dual-acting compound; or alternatively

obtaining the dual-acting compound by exchanging the solvent(s) employed in steps (i) and (ii) by

(iva) evaporating the resulting solution to dryness;

(va) re-dissolving the solid in a suitable solvent;

(via) precipitation of the solid and drying same to obtain the dual-acting compound.

The details regarding the complex, including the ARB, the NEPi and the cation, are as described above with regard to the first embodiment of the invention.

Preferably, in step (i) the ARB and the NEPi are added in an equivalent molar amount. Both the ARB and the NEPi are preferably used in the free form. The solvent used in step (i) may be any solvent that allows dissolution of both the ARB and the NEPi. Preferred solvents include those mentioned above, namely water, methanol, ethanol, 2-propanol, acetone, ethyl acetate, isoprapyl acetate, methyl-t-butylether, acetonitrile, toluene, DMF, NMF and methylene chloride and mixtures of such solvents, such as ethanol-water, methanol-water, 2-propanol-water, acetonitrile-water, acetone-water, 2-propanol-toluene, ethyl acetate-heptane, isopropyl acetate-acetone, methyl-t-butyl ether-heptane, methyl-t-butyl ether-ethanol, ethanol-heptane, acetone-ethyl acetate, actetone-cyclohexane, toluene-heptane, more preferably acetone.

Preferably, in step (ii) the basic compound of Cat is a compound capable of forming a salt with the acidic functionalities of the ARB and the NEPi. Examples include those mentioned above, such as calcium hydroxide, zinc hydroxide, calcium methoxide, calcium ethoxide, calcium acetate, calcium hydrogen carbonate, calcium formate, magnesium hydroxide, magnesium acetate, magnesium formate, magnesium hydrogen carbonate, sodium hydroxide, sodium carbonate, sodium hydrogen carbonate, sodium methoxide, sodium ethoxide, sodium acetate, sodium formate, potassium hydroxide, potassium carbonate, potassium hydrogen carbonate, potassium methoxide, potassium ethoxide, potassium acetate, potassium formate, ammonium hydroxide, ammonium methoxide, ammonium ethoxide, and ammonium carbonate. Perchlorates may also be used. Amine bases or salt forming agents such a those mentioned above may also be used, in particular benzathine, L-arginine, cholin, ethylene diamine, L-lysine or piperazine. Typically an inorganic base is

employed with Cat as specified herein. More preferably, the basic compound is (Cat)OH, $(Cat)_2CO_3$, $(Cat)HCO_3$, still more preferably Cat(OH), such as NaOH. The basic compound is employed in an amount of at least 3 equivalents relative to either the ARB or the NEPi, preferably it is employed in stoichiometric amount to obtain the dual-acting compound, in particular the complex with three cations. The solvent used in step (ii) may be any solvent or mixtures of solvents that allow dissolution of Cat(OH). Preferred solvents include water, methanol, ethanol, 2-propanol, acetone, ethylacetate, isopropyl acetate, methyl-*t*-butylether, acetonitrile, toluene, and methylene chloride and mixtures of such solvents, more preferably water.

In step (iii) the solutions obtained in steps (i) and (ii) are combined. This can take place by adding the solution obtained in step (i) to the solution obtained in step (ii) or vice versa, preferably, the solution obtained in step (ii) to the solution obtained in step (i).

According to the first alternative, once combined and preferably mixed, the dual-acting compound, in particular the complex precipitates in step (iv). This mixing and precipitation is typically effected by stirring the solutions for an appropriate amount of time such as 20 min to 6 h, preferably 30 min to 3 h, more preferably 2 h, at room temperature. It is advantageous to add seeds of the dual acting compound. This method facilitates precipitation.

In step (iv) according to this first alternative, a co-solvent is typically added. The co-solvent employed is a solvent in which the ARB and the NEPi in the complexed form exhibit a lower solubility that allows the compound to precipitate. Distillation, either continuous or stepwise, with replacement by this co-solvent results in a mixture predominantly of the co-solvent. Preferred solvents include ethanol, 2-propanol, acetone, ethylacetate, isopropyl acetate, methyl-*t*-butylether, acetonitrile, toluene, and methylene chloride and mixtures of such solvents, more preferably isopropyl acetate. Preferably, a minimum amount of solvent is employed to facilitate precipitation. The solid is collected, e.g. by filtration, and is dried to obtain the dual-acting compound, in particular the complex in accordance with the present invention. The drying step can be performed at room temperature or elevated temperature such as 30 to 60 °C, preferably 30 to 40 °C. Reduced pressure can be employed to facilitate removal of the solvent, preferably, drying is effected at ambient pressure or reduced pressure of e.g. 10 to 30 bar, such as 20 bar.

According to a second alternative, once combined and preferably mixed, the dual-acting compound, in particular the complex the mixture preferably forms a clear solution. This mixing is typically effected by stirring the solutions for an appropriate amount of time such as 20 min to 6 h, preferably 30 min to 3 h, more preferably 1 h, at room temperature. If necessary, the temperature may be raised so as to ensure a clear solution.

The obtained mixture is then further treated by solvent exchange to obtain the dual-acting compound, in particular the complex.

In step (iva) according to this second alternative, the solution is preferably evaporated to dryness at elevated temperatures such as > room temperature to 50 °C, more preferably 30 to 40 °C.

Preferably, in step (va) the solvent or solvent mixture employed is a solvent in which the ARB and the NEPi in the complexed form exhibit a lower solubility that allows the dual-acting compound, in particular the complex to precipitate. Preferred solvents include the ones mentioned above for step (i), such as water, ethanol, 2-propanol, acetone ethylacetate, isopropyl acetate, methyl-*t*-butylether, acetonitrile, toluene, and methylene chloride and mixtures of such solvents, more preferably isopropyl acetate. Preferably, a minimum amount of solvent or solvent mixture is employed to facilitate precipitation.

In step (via) precipitation can take place at room temperature. It can be effected by leaving the mixture standing or by agitating the mixture, preferably by agitating it. This is preferably effected by stinring and/or sonication. After precipitation, the solid is collected, e.g. by filtration, and is dried to obtain the compound in accordance with the present invention. The drying step can be performed at room temperature or elevated temperature such as 30 to 60 °C, preferably room temperature. Reduced pressure can be employed to facilitate removal of the solvent, preferably, drying is effected at ambient pressure.

In a fourth aspect, this invention is directed to a method of treating or preventing a disease or condition, such as hypertension, heart failure (acute and chronic) congestive heart failure, left ventricular dysfunction and hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation, atrial flutter, detrimental vascular remodeling, myocardial infarction and its sequelae, atherosclerosis, angina (unstable or stable), renal insufficiency (diabetic and non-diabetic), heart failure, angina

pectoris, diabetes, secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions, such as diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, and also renal vascular hypertension, diabetic retinopathy, other vascular disorders, such as migraine, peripheral vascular disease, Raynaud's disease, luminal hyperplasia, cognitive dysfunction (such as Alzheimer's), glaucoma and stroke comprising administering the afore-mentioned combination, linked pro-drug or he dual-acting compound, in particular the complex to a subject in need of such treatment.

The combination, linked pro-drug or he dual-acting compound, in particular the complex of the first embodiment may be administered alone or in the form of a pharmaceutical composition according to the second embodiment. Information regarding dosing, i.e., the therapeutically effective amount, *etc.*, is the same regardless of how the combination, linked pro-drug or he dual-acting compound, in particular the complex is administered.

The combination, linked pro-drug or he dual-acting compound, in particular the complex is beneficial over a combination of ARBs or neutral endopeptidase inhibitors alone or other ARB/NEPi combinations with regard to use as first line therapy, ease of formulation and ease of manufacture.

Specific embodiments of the invention will now be demonstrated by reference to the following examples. It should be understood that these examples are disclosed solely by way of illustrating the invention and should not be taken in any way to limit the scope of the present invention.

**Example 1**

**Preparation of [valsartan ((2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester]$Na_3$ • 2.5 $H_2O$**

The dual-acting compound of valsartan and (2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester is prepared by dissolving 0.42g of (2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester free acid (~95% purity) and 0.41 g of valsartan free acid in 40ml acetone. Separately, 0.111g of NaOH are dissolved in 7ml $H_2O$. The two solutions are combined and stirred at room temperature for 1 hour and a clear solution was obtained. The solution is evaporated at 35°C

to yield a glassy solid. The glassy solid residue is then charged with 40ml acetone and the resulting mixture is stirred and sonicated until precipitation occurred (~ 5 minutes). The precipitate was filtered and the solid is dried at room temperature in open air for 2 days until a constant mass of the crystalline solid is obtained.

Characterization by various methods could confirm the presence of both valsartan and (2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester and complex formation in contrast to a simple physical mixture. Significant spectral peaks for the complex are observed e.g. in the XRPD, IR, and Raman spectroscopy which are not present for the physical mixture. See below for details on the characterization.

## Example 2

**Alternative Preparation of [valsartan ((2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester]$Na_3$ • 2.5 $H_2O$**

The dual acting compound of valsartan and (2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester is prepared by dissolving 22.96 mmol of (2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester free acid (~95% purity) and valsartan (10.00 g; 22.96 mmol) in acetone (300 mL). The suspension is stirred at room temperature for 15 min to obtain a clear solution. A solution of NaOH (2.76 g; 68.90 mmol) in water (8 mL) water is then added to this solution over a period of 10 min. Solids start to precipitate in 10 min. Alternatively, precipitation can be induced by seeding. The suspension is stirred at 20-25 °C for 2 h. This suspension is concentrated at 15-30 °C under reduced pressure (180-250 mbar) to a batch volume of ~150 mL. Isopropyl acetate (150 mL) is then added to the batch and the suspension is concentrated again at 15-30 °C under reduced pressure (180-250 mbar) to a batch volume of ~150 mL. This operation (addition of 150 mL of isopropyl acetate to the batch and concentration) is repeated once again. The suspension is stirred at 20-25 °C for 1 h. The solids are collected by filtration under nitrogen over a Büchner funnel, washed with isopropyl acetate (20 mL), and dried at 35 °C under reduced pressure (20 mbar) to afford the compound.

Characterization revealed the same product as in Example 1.

**Example 3**

**Alternative Preparation of [valsartan ((2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester]$Na_3$ • 2.5 $H_2O$ using seeding**

A reactor is charged with 2.00 kg (2,323 mmol) of AHU377 calcium salt and 20 L of isopropyl acetate. The suspension is stirred at 23 ± 3 °C, and 4.56 L of 2 *N* HCl was added. The mixture is stirred at 23 ± 3 °C for 15 min to obtain a clear two-phase solution. The organic layer is separated and washed with 3 x 4.00 L of water. The organic layer is concentrated at 30-100 mbar and 22 ± 5 °C to ~3.5 L (3.47 kg) of AHU377 free acid isopropyl acetate solution as a colorless solution.

To the above reactor containing ~3.5 L (3.47 kg) of AHU377 free acid isopropyl acetate solution is added 1.984 kg (4,556 mmol) of Valsartan and 40 L of acetone. The reaction mixture is stirred at 23 ± 3 °C to obtain a clear solution which is filtered into a reactor. To the reaction mixture is added a solution of 547.6 g (13,690 mmol) of NaOH in 1.0 L of water at 23 ± 3 °C (which was pre-cooled to 20 ± 5 °C and in-line filtered) over a period of 15-30 min while maintaining the internal temperature at 20-28 °C (slightly exothermic). The flask is rinsed with 190 mL of water and added into the reaction mixture. The reaction mixture is stirred at 23 ± 3 °C for 15 min and a slurry of 4.0 g of [valsartan ((2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester]$Na_3$ • 2.5 $H_2O$ seeds in 50 mL of isopropyl acetate is added. The mixture is stirred at 23 ± 3 °C for 2 h to obtain a suspension. The suspension is heated to an internal temperature at 40 ± 3 °C over a period of 20 min and 20 L of isopropyl acetate is added over a period of 20 min while maintaining the internal temperature at 40 ± 3 °C . The suspension is stirred at this temperature for an additional 30 min. The mixture is concentrated at an internal temperature at 35 ± 5 °C (Tj 45 ± 5 °C) under reduced pressure (200-350 mbar) to ~35 L of a white slurry (solvent collected: ~25 L). Then 30 L of isopropyl acetate is added the mixture is concentrated at an internal temperature at 35 ± 5 °C ($T_j$ 45 ± 5 °C) under reduced pressure (100-250 mbar) to ~30 L of a white slurry (solvent collected: ~40 L). Again 40 L of isopropyl acetate is added and the mixture is concentrated at an internal temperature at 35 ± 5 °C (Tj 45 ± 5 °C) under reduced pressure (100*200 mbar) to ~30 L of a white slurry (solvent collected: ~30 L). The reaction

mixture is cooled to 23 ± 3 °C over ~20 min and stirred at this temperature for an additional 3 h. The solid is collected by filtration under nitrogen over a polypropylene pad on Büchner tunnel. The solid is washed with 2 X 5 L of isopropyl acetate and dried at 35 °C under reduced pressure (20 mbar) until isopropyl acetate content <0.5% to afford the above product as a white solid.

Characterization revealed the same product as in Example 1.

**X-ray powder diffraction**

Calculation of the interlattice plane intervals from the X-ray powder pattern taken with a Scintag XDS2000 powder diffractometer for the most important lines for the sample give the following results:

d in [ A ] : 21.2(s), 17.0(w), 7.1 (s), 5.2(w), 4.7(w), 4.6(w), 4.2(w), 3.5(w), 3.3(w)

The error margin for all interlattice plane intervals is ± 0.1 A. The intensities of the peaks are indicated as follows: (w) = weak; (m) = medium; and (st) = strong.

Average values 2Θ in [°] are indicated (error limit of ±0.2)

4.5, 5.5, 5.6, 9.9, 12.8, 15.7, 17.0, 17.1, 17.2, 18.3, 18.5, 19.8, 21.5, 21.7, 23.2, 23.3, 24.9, 25.3, 27.4, 27.9, 28.0, 30.2.

**Elemental analysis**

Elemental analysis gives the following measured values of the elements present in the sample. The findings of the elemental analysis, within the error limits, correspond to the overall formula of $H_{xx}N_xO_xNa_2)\cdot 2.5H_2O$

| | | | |
|---|---|---|---|
| Found | C: 60.05% | H: 6.24% | N: 8.80% |
| Calculated* | C: 60.18% | H: 6.31% | N: 8.77% |

**Infrared spectroscopy**

The infrared absorption spectrum for the sample obtained using Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectrometer (Nicolet Magna-IR 560) shows the following significant bands, expressed in reciprocal wave numbers ($cm^{-1}$ ):

2956 (w), 1711 (st), 1637 (st), 1597 (st), 1488 (w), 1459 (m), 1401 (st), 1357 (w), 1295 (m), 1266 (m), 1176 (w), 1085 (m), 1010 (w), 1942(w), 907 (w), 862 (w), 763 (st), 742 (m), 698 (m), 533 (st).

The error margin for all absorption bands of ATR-IR is ± 2 $cm^{-1}$.

The intensities of the absorption bands are indicated as follows: (w) = weak; (m) = medium; and (st) = strong intensity.

**Raman spectroscopy**

Raman spectrum of the sample measured by dispersive Raman spectrometer with 785 nm laser excitation source (Kaiser Optical Systems, Inc.) shows the following significant bands expressed in reciprocal wave numbers ($cm^{-1}$ ):

3061 (m), 2930 (m, broad), 1612 (st), 1523 (m), 1461 (w), 1427 (w), 1287 (st), 1195 (w), 1108 (w), 11053 (w), 1041 (w), 1011 (w), 997 (m), 866(w), 850 (w), 822 (w), 808 (w), 735 (w), 715 (w), 669 (w), 643 (w), 631 (w), 618 (w), 602 (w), 557 (w), 522 (w), 453 (w), 410 (w), 328 (w).

The error margin for all Raman bands is ± 2 $cm^{-1}$.

The intensities of the absorption bands are indicated as follows: (w) = weak; (m) = medium; and (st) = strong intensity.

**High Resolution CP-MAS $^{13}C$ NMR Spectroscopy**

The samples are investigated by high resolution CP-MAS (Cross Polarization Magic Angle Spinning) $^{13}C$ NMR spectroscopy using a Bruker-BioSpin AVANCE 500 NMR spectrometer equipped with a 300 Watt high power $^{1}H$, two 500 Watt high power X-amplifiers, necessary high power pre-amplifiers, a "MAS" controller and a 4 mm BioSolids high resolution Bruker probe.

Each sample is packed in a 4mm $ZrO_2$ rotor. Critical experimental parameters are 3 msec $^{13}C$ contact times, 12 KHz spinning speed at the magic angle,. a "ramped" contact time, using a "SPINAL64" $^{1}H$ decoupling scheme, a recycle delay of 10 secs and 1024 scans at 293 deg K. The chemical shifts are referenced with respect to an external Glycine carbonyl at 176.04 ppm.

High resolution CP-MAS $^{13}C$ NMR shows the following significant peaks (ppm):

179.0, 177.9 177.0, 176.7, 162.0, 141.0, 137.2, 129.6, 129.1, 126.7, 125.3, 64.0, 61.5, 60.4, 50.2, 46.4, 40.6, 38.6, 33.5, 32.4, 29.8, 28.7, 22.3, 20.2, 19.1, 17.8, 16.8, 13.1, 12.1, 11.1.

A physical mixture of individual Na salts of Valsartan and (2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester revealed a simple inert mixture of the two salts. However, the sample of the complex prepared in Example 1 exhibited distinctly different spectral features in comparison to a 1:1 mixture of the sodium salts.

**DSC and TGA**

As measured by differential scanning calorimetry (DSC) using Q1000 (TA Instruments) instrument, the melting onset temperature and the peak maximum temperature for the sample is observed at 139°C and 145°C, respectively.

As shown by DSC and thermogravimetric analysis (TGA), upon heating, the water of hydration is released in two steps: the first step occurs below 100°C and the second step above 120°C.

Both DSC and TGA instruments are operated at a heating rate of 10 K/min.

**Example 4**

**Preparation of Linked Pro-Drug of Scheme (1)**

Linked pro-drug of valsartan calcium salt and (2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester is prepared at room temperature by dissolving 114 mg of the calcium salt of valsartan and 86 mg of (2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester free acid in 2 mL methanol, followed by methanol evaporation. The glassy solid residue is then charged with 3 mL of acetonitrile and equilibrated by 10 min. sonication, followed by 20 hours of magnetic stirring.

Approximately 120 mg of white solids are collected by filtration. Liquid chromatography (LC) and elemental analysis indicate 1:1 ratio between (2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester and valsartan. The sample is amorphous by X-ray powder diffraction.

**Preparation of Linked Pro-Drug of Scheme (2)**

Linked pro-drug of valsartan calcium salt and (2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester and Tris is prepared at room temperature by dissolving 57 mg of the calcium salt of valsartan, 43 mg of (2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester free acid, and 12.6 mg of *tris*(hydroxymethyl)aminomethane (Tris) in 2 mL methanol, followed by methanol evaporation. The glassy solid residue is then charged with 3 mL of acetonitrile and equilibrated by 10 min. sonication, followed by 20 hours of magnetic stirring. Approximately 83 mg of white solids are collected by filtration. LC and elemental analysis indicate 1:1 ratio between (2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester and valsartan. The sample is amorphous by X-ray powder diffraction.

While the invention has been described above with reference to specific embodiments thereof, it is apparent that many changes, modifications, and variations can be made without departing from the inventive concept disclosed herein. Accordingly, it is intended to embrace all such changes, modifications and variations that fall within the spirit and broad scope of the appended claims. All patent applications, patents, and other publications cited herein are incorporated by reference in their entirety.

NEW CLAIMS 1 TO 29

1. A dual-acting compound having the sum formula [((*S*)-*N*-valeryl-*N*-{[2'-(1 *H*-tetrazole-5-yl)-biphenyl-4-yl]-methyl}-valine) ((2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester)]$Na_{1-3}$ • x $H_2O$, wherein x is 0 to 3.

2. The compound of claim 1 in the form of a complex.

3. The compound of claim 2 in the form of a supramolecular complex.

4. The compound of any of claims 1 to 3, wherein the (*S*)-*N*-valeryl-*N*-{[2'-(1 *H*-tetrazole-5-yl)-biphenyl-4-yl]-methyl}-valine moiety and the (2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester moiety are linked via non-covalent bonding.

5. The compound of any of claims 1 to 4, wherein the (*S*)-*N*-valeryl-*N*-{[2'-(1 *H*-tetrazole-5-yl)-biphenyl-4-yl]-methyl}-valine moiety and the (2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester moiety are linked via a sodium ion.

6. The compound of any of claims 1 to 5 in the crystalline, partially crystalline, amorphous, or polymorphous form, preferably in the crystalline form.

7. The compound of any of claims 1 to 6 in the form of a hydrate.

8. The compound of any of claims 1 to 7 having the sum formula [((*S*)-*N*-valeryl-*N*-{[2'-(1*H*-tetrazole-5-yl)-biphenyl-4-yl]-methyl}-valine) ((2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester]$Na_3$ • x $H_2O$, wherein x is 0 to 3.

9. The compound of claim 8, wherein x is 2.5.

10. The compound of claim 8 or 9 characterized by

an Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectrum having the following absorption bands expressed in reciprocal wave numbers ($cm^{-1}$)( ± 2 $cm^{-1}$): 2956 (w), 1711 (st), 1637 (st), 1597 (st), 1488 (w), 1459 (m), 1401 (st), 1357 (w), 1295 (m), 1266 (m), 1176 (w), 1085 (m), 1010 (w), 1942(w), 907 (w), 862 (w), 763 (st), 742 (m), 698 (m), 533 (st).

11. The compound of any of claims 8 to 10, characterized by

an X-ray powder diffraction pattern taken with a Scintag XDS2000 powder diffractometer comprising the following interlattice plane intervals:

d in [ A ] (± 0.1 A): 21.2(s), 17.0(w), 7.1(s), 5.2(w), 4.7(w), 4.6(w), 4.2(w), 3.5(w), 3.3(w).

12. A dual-acting compound having the sum formula (*N*-valeryl-*N*-{[2'-(1*H*-tetrazole-5-yl)-biphenyl-4-yl]-methyl}-valine) (5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester]$Na_3$ • x $H_2O$, wherein x is 0 to 3.

13. A dual-acting compound obtainable by:

(i) dissolving (*S*)-*N*-valeryl-*N*-{[2'-(1*H*-tetrazole-5-yl)-biphenyl-4-yl]-methyl}-valine or a salt thereof and (2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester or a salt thereof in a suitable solvent;

(ii) dissolving a basic Na compound in a suitable solvent;

(iii) combining the solutions obtained in steps (i) and (ii);

(iv) precipitation of the solid, and drying same to obtain the dual-acting compound; or alternatively

obtaining the dual-acting compound by exchanging the solvent(s) employed in steps (i) and (ii) by

(iva) evaporating the resulting solution to dryness;

(va) re-dissolving the solid in a suitable solvent;

(via) precipitation of the solid and drying same to obtain the dual-acting compound.

14. The compound of claim 13 in the form of a complex.

15. The compound of claim 14 in the form of a supramolecular complex.

16. The compound of any of claims 13 to 15 wherein the suitable solvent in steps (i) and (iv) is acetone.

17. The compound of any of claims 13 to 16 wherein the basic Na compound is NaOH, $Na_2CO_3$, $NaHCO_3$, NaOMe, NaOAc or NaOCHO.

18. The compound of any of claims 13 to 17 in the crystalline, partially crystalline, amorphous, or polymorphous form, preferably in the crystalline form.

19. The compound of any of claims 13 to 18 in the form of a hydrate.

20. A pharmaceutical composition comprising

(a) the compound according to claims 1 to 19; and

(b) at least one pharmaceutically acceptable additive.

21. The pharmaceutical composition of claim 20, wherein the pharmaceutically acceptable additive is selected from the group consisting of diluents or fillers, disintegrants, glidants, lubricants, binders, colorants arid combinations thereof.

22. A method of preparing the dual-acting compound according to any of claims 1 to 12, said method comprising the steps of:

(i) dissolving (*S*)-*N*-valeryl-*N*-{[2'-(1*H*-tetrazole-5-yl)-biphenyl-4-yl]-methyl}-valine or a salt thereof and (2*R*,4*S*)-5-biphenyl4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester or a salt thereof in a suitable solvent;

(ii) dissolving a basic Na compound in a suitable solvent;

(iii) combining the solutions obtained in steps (i) and (ii);

(iv) precipitation of the solid, and drying same to obtain the dual-acting compound; or alternatively

obtaining the dual-acting compound by exchanging the solvent(s) employed in steps (i) and (ii) by

(iva) evaporating the resulting solution to dryness;

(va) re-dissolving the solid in a suitable solvent;

(via) precipitation of the solid and drying same to obtain the dual-acting compound.

23. The method of claim 22 wherein the suitable solvent in steps (i) and/or (iva) is acetone.

24. The method of claim 66 or 67, wherein the basic Na compound is NaOH, $Na_2CO_3$, $NaHCO_3$, NaOMe, NaOAc or NaOCHO.

25. A method for the treatment or prevention of a condition or disease selected from the group consisting of hypertension, heart failure (acute and chronic), congestive heart failure, left ventricular dysfunction, hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation, atrial flutter, detrimental vascular remodeling, myocardial infarction and its sequelae, atherosclerosis, angina (unstable or stable), renal insufficiency (diabetic and non-diabetic), heart failure, angina pectoris, diabetes, secondary aldosteronism, primary and secondary pulmonary hypertension, diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, renal vascular hypertension, diabetic retinopathy, migraine, peripheral vascular disease, Raynaud's disease, luminal hyperplasia, cognitive dysfunction, glaucoma and stroke, comprising administering a therapeutically effective amount of the compound according to claims 1 to 19, to a mammal in need of such treatment.

26. The method of claim 25 for the treatment of hypertension.

27. A pharmaceutical composition comprising

(a) the compound according to claims 1 to 19;

(b) a therapeutic agent selected from an anti-diabetic, a hypolipidemic agent, an anti-obesity agent and an anti-hypertensive agent; and

(c) at least one pharmaceutically acceptable additive.

28. The pharmaceutical composition according to claim 27 wherein the therapeutic agent is amlodipine besylate.

29. The pharmaceutical composition according to claim 27, wherein the therapeutic agent is hydrochlorothiazide.

*Dated this 08th day of June 2007*

*Of Anand And Anand Advocates*
*Attorney for the Applicant*

**ANNEXURE - 2**

INTELLECTUAL PROPERTY INDIA

सत्यमेव जयते

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**Date : 30/01/2015**

To,
Anand & Anand,
Advocates,
B-41, Nizamuddin East,New Delhi-110013,India.

SUB : **Examination Report**

| | | |
|---|---|---|
| APPLICATION NUMBER | : | 4412/DELNP/2007 |
| DATE OF FILING | : | 08/06/2007 |
| DATE OF REQUEST FOR EXAMINATION | : | 06/11/2009 |
| DATE OF PUBLICATION | : | 24/08/2007 |

a. With reference to the RQ No. 9044/RQ-DEL/2009 Dated 06/11/2009in the above mentioned application for Grant of Patent , Examination has been conducted under Section 12 and 13 of the Patents Act 1970 , The following objections are hereby communicated

b. Observations:

1. Claims" part of the specification should be super scribed with "I /We claim":-.
2. Claim 1 (and thus dependent claims) are not clear and succinct and sufficiently definitive to the scope of alleged invention in the absence of mention of any significant feature/components/characteristics of said product that reflects technological contribution to establish it as new product. [Requirements of Sec. 2(1) (j) and Sec. 10]
3. Title inconsistent with description and claims. Title should be in accordance of claim.
4. Claims 1-12,13,14-19 are mere new forms of the known compound and do not differ significantly in properties with regard to efficacy. Therefore, these Claims fall within the scope of such clause of section 3(d) of the Patent Act 1970 as amended in 2005.
5. Claims 12,13-19,20-21, 22-26,27-29 define a plurality of Distinct inventions.
6. Claims12,13,25,27 relates to an independent Invention.
7. Claim 1 and its dependent claims does not constitute an invention under section 2[1(j)] of Patents Act 1970 (as amended in 2005) as the claims are lacking in inventive step in the view

of cited Patent documents :D1 : WO2006086456D2: WO 03/059345
D3: EP-A1-0 443 983
D4: US-A-5 217 996
D5: J. Med. Chern. 1995, 38(1 0), 1689-1700

The present invention is directed to dual-acting compounds and combinations of angiotensin receptor blockiers and neutral endopeptidase inhibitors, in particular a dual acting molecule wherein the angiotensin receptor blocker and neutral endopeptidase inhibitor are linked via non-covalent ponding, or supramolecular complexes of angiotensin receptor blockers and neutral endopeptidase inhibitors, also described as linked pro-drugs, such as mixed salts or co-crystals, as well as to pharmaceutical combinations containing such a dual-acting compound or combination, methods of preparing such dual-acting compounds and methods of treating a subject with such a dual-acting compound or combination.D1:discloses a combination comprising: (i) a renin inhibitor, or a pharmaceutically acceptable salt thereof; (ii) a neutral endopeptidase (NEP) inhibitor, or a pharmaceutically acceptable salt thereof; and optionally at least one therapeutic agent selected from the group consisting of (a) a diuretic, or a pharmaceutically acceptable salt thereof; and (b) an angiotensin II receptor blocker (ARB), or a pharmaceutically acceptable salt thereof; for the prevention of, delay the onset of and/or treatment of a disease or a condition mediated by angiotensin II and/or NEP activity, which method comprises administering to a warm-blooded animal, in need thereof, a therapeutically effective amount of a combination of this present invention.
D2:disclosesa pharmaceutical composition comprising a combination of i the AT 1- antagonist valsartan or a pharmaceutically acceptable salt thereof and ii a NEP inhibitor or a pharmaceutically acceptable salt thereof and optionally a pharmaceutically acceptable carrier and to a method for the treatment or prevention of a condition or disease selected from the group consisting of hypertension, heart failure such as acute and chronic congestive heart failure, left ventricular dysfunction and hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation, atrial flutter, detrimental vascular remodeling, myocardial infarction and its sequelae, atherosclerosis, angina whether unstable or stable, renal insufficiency diabetic and non--diabetic, heart failure, angina pectoris, diabetes, secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions, such as diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, and also renal vascular hypertension, diabetic retinopathy, the management of other vascular disorders, such as migraine, peripheral vascular disease, Raynaud"s disease, luminal hyperplasia, cognitive dysfunction such as Alzheimer"s, glaucoma and stroke, comprising administering a therapeutically effective amount of the pharmaceutical composition to a mammal in need thereof.
D3:discloses Aromatic amide derivatives of formula (I) and their salts are new. (Where R1 = aliphatic hydrocarbon optionally substituted with halogen or OH, or a cycloaliphatic or araliphatic hydrocarbon. X1 = CO, SO2, or OCO with the carbon linked to the N. X2 = aliphatic hydrocarbon (optionally substituted with OH, carboxy, NH2, guanidino, cycloaliphatic or aromatic hydrocarbon) or cycloaliphatic hydrocarbon, with a carbon of the aliphatic optionally bridged by a divalent aliphatic hydrocarbon. R2 = carboxy or its ester or amide derivative, NH2, substituted amino, formyl, acetal derivative of formyl, 1H-tetrazol-5-yl, pyridyl, OH, ether, SR, SOR, SO2R, alkanoyl, sulphamoyl, N-substituted sulphamoyl, PO2H2 or PO3H2. R = H or aliphatic hydrocarbon. X3 = hydrocarbon. R3 = carboxy, 5-tetrazolyl, SO3H, PO2H2, PO3H2 or haloalkylsulphamoyl.; Rings A and B are each optionally substd. 121 compounds are specifically claimed, including (S)-N-(1-carboxy -2-methyl)-prop-1-yl) -N-pentanoyl-N- (2"-(1H-tetrazol-5-yl) biphenyl-4-ylmethyl) amine.

D4: discloses novel biaryl substituted 4-amino-butyric acid amide derivatives described below which arc useful as neutral endopeptidase (NEP) inhibitors, e.g. as inhibitors of the ANF-degrading enzyme in mammals, so as to prolong and potentate the diuretic, natriuretic and vasodilator properties of ANF in mammals, by inhibiting the degradation thereof to less active metabolites.
D5 discloses dicarboxylic acid dipeptide neutral endopeptidase inhibitors.
In view of cited documents D1-D5 claimed subject-matter therefore lacks novelty and inventive step in its entirety under section 2(1)(j) of the Patent Act 1970 as amended in 2005

8. Claim 1 and its dependent claims do anticipated by-prior claiming in the view of cited Patent documents :D1 : WO2006086456D2: WO 03/059345
D3: EP-A1-0 443 983
D4: US-A-5 217 996
D5: J. Med. Chern. 1995, 38(1 0), 1689-1700
9. Form-3 filed on17/12/2013,23/05/2014, 06/06/2014 after prescribed time in Rule 12 of the Patents Rule,2006.So its not taken on record.

10. **Power of Authority with prescribedstamped dutyand the particulars of the case should be filed.**
11. Claim filed(1-29) in india are different from the claimfiled(1-85) in PCT application and remaining fees should be also paid for theall set of claims filed in PCT under section 138(2) and section 142(3) of the patentact 1970 as amended in 2005. Further consideration of the application issubject to filing of balance fees as applicable along with Form-13 and petitionfor condoning the irregularity of not entering National Phase as per Section *10(4A) of the Act.*

12. Complete specification should be filed afresh observing followings: Renumbering of pages, Scoring out the blank space with authorized signature, Deleting extraneous matter, typing of text of such specification as required by Rule 9 for margin (4 cm on top and left and 3 cm on right and bottom) in prescribed manner (with extra fees as applicable) and further amendments as desired by other objections in compliance to the requirements of the Act.

13. From-13 filed on 8/6/2007 and 16/1/2008 not filed in prescribed manner.So not allowed.
14. Details regarding the search and/or examination report including claims of the application allowed, as referred to in Rule 12(3) of the Patent Rule, 2003, in respect of same or substantially the same invention filed in all the major Patent offices along with appropriate translation where applicable, should be submitted within a period of Six months from the date of receipt of this communication as provided under section 8(2) of the Indian Patents Act.
15. Details regarding application for Patents which may be filed outside India from time to time for the same or substantially the same invention should be furnished within Six months from the date of filing of the said application under clause(b) of sub section(1) of secton 8 and rule 12(1) of Indian Patent Act.

c. You are requested to comply with the objections by filing your reply by way of explanation and/or amendments within 12 months from the date of issue of FER failing which you application will be treated as "Deemed to have been abandoned" under section 21(1) of the Act. The last Date is 01/02/2016.

d. You are advised to file your reply at the earliest so that the office can further proceed with application and complete the process within the prescribed period.

**( Dr. Rajesh Dixit )**

Deputy Controller of Patents & Designs

NOTE : All Communications to be sent to the Controller of Patents at INTELLECTUAL PROPERTY BUILDING Plot No. 32, Sector-14,Dwarka New Delhi - 110 078.

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230714

November 27, 2015

The Controller of Patents
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Plot No. 32, Sector 14, Dwarka
New Delhi-110078

**ASST. CONTROLLER: Dr. Rajesh Dixit**
**Final Period Expiring on: 30th January 2016**

Dear Sir,

Re: **Indian Patent Application No. 4412/DELNP/2007**
Filed on: 08.06.2007
Applicant: Novartis A.G
K&S Ref: IP32537/NC/sa

We write with reference to the official letter dated 30th January 2015. We now submit herewith the claim amendments along with the following reply:

To comply with objections raised in the Examination report and to expedite allowance, the Applicant has amended claims. The amended claims are attached herewith in a clear as well as marked up version. The Controller is kindly requested to consider the same and take on record.

The Applicant herewith deals with each of the objections in more details:

**Objections 1-2:**

According to the Examination report, claim 1 and its dependent claims are not clear and succinct, and do not define the characteristics of the product.

The Applicant respectfully disagrees, and submits that the amended claims are sufficiently definite and clear. The amended claims claim a definite product that is a "dual acting" compound "comprising the Angiotensin Receptor Antagonist valsartan and the NEP Inhibitor (2R,4S)-5-biphenyl-4-yl-4-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester" and having the sum formula [((S)-N-valeryl-N-{[2'-(1 H-tetrazole- 5-yl)-biphenyl-4-yl]-methyl}-valine) ((2R,4S)-5-biphenyl-4-yl-4-(3-carboxy-propionylamino)-2- methyl-pentanoic acid ethyl ester)]$Na_{1-3}$ • x $H_2O$, wherein x is 0 to 3.

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Further, dependent claims 2, 4 and 5 further define a specific "dual acting" compound wherein Na is 3 and X is 2.5. Claim 1 is directed towards a unique compound comprised of two active agents having different modes of action but in one large/super molecular structure. In other words, the present invention relates to a new entity, whose unique molecular structure combines two modes or mechanisms of action within one compound. The claimed compound is accordingly a "dual acting" compound also called ARNI (an angiotensin receptor-neprilysin inhibitor) since it is effective as an Angiotensin Receptor Blocker (ARB) as well as a Neutral Endopeptidase (NEP) inhibitors, and is used for the treatment of cardiovascular diseases.

The components of this product are clearly mentioned in claim 1 and its dependent claims. Accordingly, the claims fully meet the requirements of section 10. Further, claim 1 and its dependent claims are novel and inventive (as described in more details under Objections no. 7 & 8) and are capable of industrial application, and they thus meet the requirements of section 2(1)(j) as well.

**Objection 3**:
As per the Examination report, title is inconsistent with description and claims and title should be in accordance with claim.

To meet this objection, the Applicant has amended the title to read as *"A compound comprised of an Angiotensin Receptor Antagonist and a NEP Inhibitor"*. Further, the amended claims are in accordance with the title, which is also consistent with description.

Fresh Form-1, Form-2, and Abstract Page are also submitted herewith in view of the amendment in Title.

Respected Controller is requested to take all of the above on record and waive this objection.

**Objection 4:**

As per the Examination report, claims 1-12, 13, 14-19 are mere new forms of the known compound and do not differ significantly in properties with regard to efficacy and fall within the scope of section 3(d).

The Applicant disagrees and respectfully submits that the present claims do not fall under the purview of section 3(d) at all. As explained in more details under Objections 7-8, the compound of the present invention are completely novel, inventive and are not mere discovery of new form of a known compound as alleged in the Examination Report. It is submitted that as on the priority of the present application, no compound having a dual mode of action was known to have been made using (S)-N-valeryl-N-{[2'-(1H-tetrazole-5-yl)-biphenyl-4-yl]-methyl}-valine and (2R,4S)-5-biphenyl-4-yl-4-(3-carboxy-propionylamino)-2-methylpentanoic acid ethyl ester. The claimed compounds of the present invention are a result of extensive experimentation and research. It is, thus, submitted that the claimed compounds are not *"mere discovery"*.

As submitted above, the inventors of the present application have, for the first time, described a unique compound comprising two differently acting active molecules, e.g. hence having dual activities. Accordingly, the claimed compound is not a "*new form of any known compound*".

Without prejudice, to the best of the knowledge of the Applicant, there is no 'known compound' having both angiotensin receptor blocking and NEP inhibiting activities as on priority date, against which the efficacy of the claimed compounds can be shown.

In view of the above, it is submitted that provisions of Section 3(d) are not applicable to the present case. Accordingly, the present objection should be waived.

**Objections 5-6**:

As per the Examination report, claims 12, 13-19, 20-21, 22-26, 27-29 define plurality of distinct inventions and claims 12, 13, 25, 27 relate to an independent invention.

To meet the objection, claims have been amended and the Applicant respectfully submits that the amended claims do not define plurality of distinct inventions. Further, independent claims 12, 13, and 25 have been deleted and previous claim 27 (new claim 15) has been made dependent on claim 1.

The Applicant further submits that all the pending claims are linked by a common inventive concept. It is respectfully submitted that it is not barred by law for a patent invention to contain independent claims if they fulfill the criteria of novelty and inventiveness and share a common inventive step.

Thus, all these claims are linked by a common feature of the novel and inventive compound and thus do not define a plurality of distinct inventions.

In view of the above, these objections may be waived.

**Objections 7-8:**

According to the Examination report, claim 1 and its dependent claims lack inventive step and are anticipated by prior claiming in view of the following documents:

(i) D1: WO2006086456;
(ii) D2: WO 03/059345;
(iii) D3: EP-A1-0443983;
(iv) D4: US-A-5 217 996;
(v) D5: J. Med. Chem. 1995, 38(10), 1689-1700;

The Applicant respectfully submits that the presently amended claims are novel and do not lack inventive step over the cited documents D1 to D5. The submissions of the Applicant in respect of novelty and inventive step are as follows:

**The Present Invention**:
The present invention is directed towards a novel compound with a dual mode of action and suitable for treatment of cardiovascular diseases. The compound of the present invention has a unique structure combining two modes or mechanisms of action within. Further, the present invention also relates to the method by which this new compound is prepared, which has never been described before.

**Novelty**:
The Applicant distinguishes the present invention from each of the cited documents as below:

D1 (WO2006086456) is a document which is published on 17.8.2006 whereas the present invention claims a priority date of 09.11.2005. Thus, this document is not a valid prior art and relevant for assessment of novelty of the present invention.

Without prejudice and in any case, this document relates to a combination of individual compounds comprising a) a renin inhibitor, b) a NEP inhibitor and optionally at least one therapeutic agent selected from a diuretic or an angiotensin II receptor blocker. D1 teaches that a renin inhibitor and a NEP inhibitor may be combined together optionally with a diuretic or Angiotensin II receptor blocker. These may be combined together in a pharmaceutical composition or administered separately but as part of same therapeutic regimen. This further means that D1 discloses a combination of either a renin inhibitor and a neutral endopeptidase (NEP) inhibitor or a triple combination of a renin inhibitor, a neutral endopeptidase (NEP) inhibitor and an angiotensin II receptor blocker (ARB) or a triple combination of a renin inhibitor, a neutral endopeptidase (NEP) inhibitor and a diuretic. Thus, D1 nowhere discloses or teaches a compound having both NEP inhibiting and angiotensin II antagonistic activity within one (supra)molecular structure as described in the present invention.

D2 (WO 03/059345) relates to a pharmaceutical composition comprising a combination of (a) the AT-1 antagonist valsartan or a salt thereof, and (b) a NEP inhibitor, in particular (2R,4S)-5-biphenyl-4-yl-4-(3-carboxy-propionylamino)-2- methyl-pentanoic acid ethyl ester or a salt thereof. D2 only describes compositions containing valsartan and the respective NEP inhibitor as separate individual compounds. D2 also claims a kit comprising separate containers in a single package pharmaceutical composition, comprising in one container a pharmaceutical composition comprising a NEP inhibitor and in a second container a pharmaceutical composition comprising the AT 1 antagonist valsartan. On the contrary, the present invention is directed towards a unique novel compound combining the two modes of action of a NEP inhibitor and an AT1 antagonist. The compound of the present invention is a large compound having unique supramolecular structure, hitherto unknown. It is submitted that D2 nowhere discloses such a compound or any process of synthesis thereof as described in the present invention. Thus, the claimed compounds are novel over D2.

D3 (EP-A1-0443983) discloses preparation of Valsartan only. The said prior art is also present in the specification of the present invention at page 14 and is incorporated by

reference. D3 nowhere discloses a unique compound combining two modes of action as described in the present invention.

D4 (US-A-5 217 996) discloses Neutral endopeptidase inhibitors and has also been incorporated as reference in the specification (on page no. 17) of the present application. D4 nowhere discloses a unique compound having a dual mechanism of action as described in the present invention.

D5 (J. Med. Chem. 1995, 38(10), 1689-1700) again discloses synthesis of three series of dicarboxylic acid dipeptide neutral endopeptidase (NEP) inhibitors is described. D5 nowhere discloses a unique compound having a dual mechanism of action as described in the present invention.

In view of the above, the claimed compound having a dual mechanism of action of the present invention is novel over D1-D5.

Further, for the following reasons, the compound having a dual mechanism of action of the present invention is non-obvious and inventive over D1-D5.

**Inventive Step:**

As discussed above, the cited prior art documents neither disclose the novel and unique compound having a dual mechanism of action of the present application nor a process for synthesis of such a compound. There is no teaching or enablement, in any of the cited prior art documents, in respect of such a compound much less a unique large compound having a supramolecular structure as claimed in the present invention. It is submitted that it would not have been possible for a person skilled in the art, as on the priority date of the present application, to arrive at the claimed compound and process of synthesis thereof in view of the cited prior art documents, either alone or in combination.

As stated above, D1 was published after the prior date of the present application. Accordingly, D1 is not a valid prior art for assessment of inventive step.

As per the Examination report, D2 discloses a pharmaceutical composition comprising a combination of an AT 1 antagonist (valsartan) and a NEP inhibitor ((2R,4S)-5-biphenyl-4-yl-4-(3-carboxy-propionylamino)-2- methyl-pentanoic acid ethyl ester) and optionally pharmaceutically acceptable carrier and a method for treatment or prevention of a condition or diseases selected from a group of diseases using such the respective composition. As evident from the Examination report, D2 only disclosescomposition comprising a combination of the AT-1 antagonist and the NEP inhibitor as individual molecules, against the unique supramolecular compound as claimed in the present invention. There is no teaching or enablement in D2 in respect of a compound combining both actives into one compound, i.e. having a dual mechanism of action. The Examination report does not cite any teachings of D2 which would render the present invention obvious to a person skilled in the art, as on the priority date, when read with the teachings of other cited prior arts. The Applicant reserves its right to provide submission

in case any specific teaching of D2 or the cited prior arts is so indicated, at any time during the prosecution.

Without prejudice, in view of the following additional submissions in respect of inventive step, the instant objection is liable to be waived:

1) The person skilled in the art at the priority date of the application would understand the pharmaceutical composition as described in D2 as a combination of individual active ingredients in the form of a heterogeneous mixture of the respective compounds. In contrast, the present invention claims a unique compound combining the active components into a supramolecular structure.

2) The chemical structure of the claimed compound is highly intricate and is stabilized by an involved network of ionic, hydrogen and coordination bonds, which has been described in various ways in the specification. The representative compound consists of six anions of AT-1 antagonist, six anions of NEP inhibitor, 18 sodium cations, and 15 molecules of water, resulting in the molecular formula $C_{288}H_{330}N_{36}Na_{18}O_{48}\cdot 15H_2O$. Thus, it can be seen that the claimed compounds have a supramolecular structure distinct from the composition claimed in D2 and cannot be said to be obvious in view of teachings of D2.

3) The claimed process provides a unique synthesizing route resulting in a unique supramolecular compound wherein the two anionic moieties are linked together with non-covalent bonds to form a single large and highly intricate molecular structure. It is submitted that synthesizing such a compound was unknown as on the priority date of the present application. In any case, the process of D2 either alone or in view of the processes disclosed in other cited prior art documents cannot be said to motivate a person skilled to arrive at the claimed process, as on the priority date of the present application.

Thus, cited prior art documents in general, and D2 in particular do not teach or suggest the compound as claimed in the claim 1 of the present invention. Moreover, there is no teaching or suggestion in D2 how to synthesize the compound as claimed in the present invention. The process of preparation of the novel compound is not commonly used technical means.

Further, documents D3-D5, as explained above, do not teach or suggest a unique compound as claimed in the present invention. Moreover, D3-D5 do not teach or suggest a process of preparation of the compound as claimed in the present invention.

No part of any prior art cited by the Examiner (D1-D5) suggest or teach the compound having the formula [((S)-N-valeryl-N-{[2'-(1 H-tetrazole-5-yl)-biphenyl-4-yl]-methyl}-valine) ((2R,4S)-5-biphenyl-4-yl-4-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester)]$Na_{1-3}$ • x $H_2O$, wherein x is 0 to 3, or makes it evident that the said compound is obtainable as on the priority date of the present application.

It is further submitted that the inventors have developed the claimed unique compound of the present invention after extensive experimentation and research. The claimed compound having a dual mechanism of action was not a routine for the person skilled in the art as on the priority date of the present application and provides technological advancement as compared to the existing knowledge as on the priority date.

In summary and to reiterate, in view of the disclosures in the cited prior art documents, a skilled person would not have been motivated to develop a compound having dual modes or mechanism of action as claimed in the present invention. There is simply no pointer in the cited prior arts that would have prompted the skilled person to make such a compound as on its priority date. A skilled person would not have objectively inferred from the prior arts, without the benefit of hindsight, that a compound of the present invention could even be formed.

In addition, the Controller may note that the cited documents D1-D5 are the same documents that were also cited in ISR/IPRP and during EP prosecution. Further, documents D2 and D4 were also cited during the US prosecution. It is submitted that novelty and inventive step of the present invention over these cited documents has been already established before strong patent jurisdictions of US and EP, resulting into grant of corresponding US and EP applications. Further, the corresponding applications have already been granted in various countries worldwide (please refer to Form-3).

In view of the above comments, it is submitted that the claimed compound having a dual mechanism of action of the present invention is novel and inventive over cited documents D1-D5. Accordingly, respected Controller is requested to waive these objections.

**Objections 9-14:**

To meet the above objections, we submit herewith:

- CBR receipt for filing petition u/r 137 to condone the irregularity in filing Form-3 details;
- Copy of Power of attorney in favor of current agents K&S Partners was filed on November 18, 2015. A copy of cover letter is enclosed herewith;
- Balance fee payable with regards to 56 claims and CBR receipt for filing petition u/r 137 to condone the irregularity in paying claim fees pertaining to PCT claims;
- Marked up copy of claim amendments;
- Fresh pages of complete Specification in accordance with Patent Rules, 2003;
- Updated Form-3 was submitted on December 06, 2013; May 23, 2014; June 06, 2014; January 07, 2015, May 26, 2015, and November 18, 2015. Copies of cover letters are enclosed herewith.
- Section 8(2) details – The Applicant has already submitted the following details vide their letters dated May 26, 2015, July 27, 2015 and August 01, 2015:
    - US Letters Patent in respect of Patent NO. 8877938
    - European File Wrappers (in CD) in respect of application nos. 10176094.0 and 06827689.8
    - US file Wrappers (in CD) in respect of application nos. 60/735093, 11/722360, 60/735541 and 14/311788

- Japanese Office Action, Decision of Grant, Granted Claims and copy of Publication in respect of application no. 2008-516049
- Japanese Office Action, Decision of Rejection with rejected Claims and copy of Publication in respect of application no. 2011-177091;
- Japanese Pending Claims and copy of Publication in respect of application no. 2015-032678
- Australian Office Action, Notice of Acceptance, Grant Notice and copy of Patent in respect of application no. 2006311481
- Chinese Office Action and copy of Patent with Granted Claims in respect of application no. 200680001733.0
- Chinese Office Action, Amended Claims and copy of Publication in respect of application no. 201210191052.2
- Canadian Office Action, Notice of Allowance and copy of Patent in respect of application no. 2590511
- English translation of Russian Office Action, Decision of Grant, Granted Claims and copy of Patent in respect of application no. 2012107219
- English translation of Russian Office Action, Notice of Allowance, Granted Claims and copy of Patent in respect of application no. 2007123671
- International Search Report, Written Opinion and International Preliminary Report on Patentability
- Korean Office Actions with English translation and copy of Patent with Allowed Claims in respect of application no. 10-2007-7015021
- Korean Office Actions with English translation and copy of Publication and Amended Claims in respect of application no.10-2013-7025742
- Peru Prosecution History in respect of application no. 001388-2006/0in
- Egyptian Office Actions and Pending Claims in respect of application no. PCT 672/2007
- Israeli Prosecution History in respect of application no. 184027
- Israeli Office Actions and Pending Claims in respect of application no. 219782
- Colombian Prosecution History in respect of application no. 07-066.478
- Ecuador Prosecution History in respect of application no. SP-07-7556

Copies of cover letters for above are enclosed herewith.

In case the Controller requires any other documents or details pertaining to any corresponding applications, it is requested to contact the undersigned for further details.

Respected Controller is requested to waive these objections accordingly.

Without prejudice, the Applicant reserves the right to file any divisional application arising from the present invention.

In view of the foregoing, reconsideration of the application, withdrawal of the outstanding objections and rejections, allowance of claims and the prompt issuance of Letter Patent Document is respectfully requested.

Should the Controller believe that anything further is necessary in order to place the application in better condition for allowance or any issue is outstanding for resolution; the Controller is requested to kindly contact the undersigned for a discussion and/or offer a hearing u/s 14 of the Act before passing any adverse order against the Applicant.

Yours faithfully,

**Namrata Chadha**
**Of K&S Partners**
**Agent for the Applicant(s)**
**IN/PA/1904**

Enclosures

1. Amended Claims – clear version;
2. Amended Claims – Mark up version;
3. Fresh Form-1;
4. Fresh Form-2;
5. Fresh Specification pages in accordance with Patent Rules;
6. Retyped Abstract page, in accordance with Patent Rules;
7. Copy of cover letter dated November 18, 2015 submitting POA in favor of current agents K&S Partners;
8. Copies of cover letters dated December 06, 2013; May 23, 2014; June 06, 2014; January 07, 2015, May 26, 2015, and November 18, 2015 submitting Form-3 information;
9. Copy of CBR receipt for filing petition u/r 137 to condone the irregularity in paying claim fees pertaining to PCT claims;
10. Copy of CBR receipt for filing petition u/r 137 to condone the irregularity in filing Form-3 details;
11. Cheque of Rs. 98560/- for fee payable with respect to excess claims

**Total official fee: 56 claims* Rs. 1760/- = Rs. 98560/-**

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We claim:

1. A compound comprising the Angiotensin Receptor Antagonist valsartan and the NEP Inhibitor (2R,4S)-5-biphenyl-4-yl-4-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester having the formula [((S)-N-valeryl-N-{[2'-(1 H-tetrazole-5-yl)-biphenyl-4-yl]-methyl}-valine) ((2R,4S)-5-biphenyl-4-yl-4-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester)]$Na_{1-3}$ • x $H_2O$, wherein x is 0 to 3.

2. The compound as claimed in claim 1 having the formula [((S)-N-valeryl-N-{[2'-(1H-tetrazole-5-yl)-biphenyl-4-yl]-methyl}-valine) ((2R,4S)-5-biphenyl-4-yl-4-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester]$Na_3$ • x $H_2O$, wherein x is 0 to 3.

3. The compound as claimed in claim 2, wherein the compound is in crystalline or amorphous form.

4. The compound as claimed in claim 2 or 3, wherein x is 2.5.

5. The compound as claimed in claim 4, which is trisodium [3-((1*S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl)propionate-(*S*)-3'-methyl-2'-(pentanoyl{2''-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate.

6. The compound as claimed in claim 5, wherein the compound is in the crystalline form.

7. The compound as claimed in claim 6 characterized by an Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectrum having the following absorption bands expressed in reciprocal wave numbers ($cm^{-1}$)( ± 2 $cm^{-1}$): 1711 (st), 1637 (st), 1597 (st), 1401 (st).

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8. The compound as claimed in claim 7 wherein the Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectrum has the following absorption bands expressed in reciprocal wave numbers ($cm^{-1}$)( ± 2 $cm^{-1}$): 2956 (w), 1711 (st), 1637 (st), 1597 (st), 1488 (w), 1459 (m), 1401 (st), 1357 (w), 1295 (m), 1266 (m), 1176 (w), 1085 (m), 1010 (w), 942(w), 907 (w), 862 (w), 763 (st), 742 (m), 698 (m), 533 (st).

9. The compound as claimed in any of claims 6 to 8, characterized by an X-ray powder diffraction pattern taken with a Scintag XDS2000 powder diffractometer comprising the following interlattice plane intervals:
d in [ A ] (± 0.1 A): 21.2(s), 17.0(w), 7.1(s), 5.2(w), 4.7(w), 4.6(w), 4.2(w), 3.5(w), 3.3(w).

10. A pharmaceutical composition comprising the compound as claimed in any one of claims 1 to 9; and at least one pharmaceutically acceptable additive.

11. The pharmaceutical composition as claimed in claim 10, wherein the pharmaceutically acceptable additive is selected from the group consisting of diluents or fillers, disintegrants, glidants, lubricants, binders, colorants and combinations thereof.

12. A method of preparing the compound as claimed in any of claims 1 to 9, said method comprising the steps of:

(i) dissolving (S)-N-valeryl-N-{[2'-(1H-tetrazole-5-yl)-biphenyl-4-yl]-methyl}-valine or a salt thereof and (2R,4S)-5-biphenyl-4-yl-4-(3-carboxy-propionylamino)-2-methylpentanoic acid ethyl ester or a salt thereof in a suitable solvent;
(ii) dissolving a basic Na compound in a suitable solvent;
(iii) combining the solutions obtained in steps (i) and (ii);
(iv) precipitation of the solid, and drying same to obtain the dual-acting compound; or alternatively

obtaining the compound by exchanging the solvent(s) employed in steps (i) and (ii) by

(iva) evaporating the resulting solution to dryness;

(va) re-dissolving the solid in a suitable solvent;

(via) precipitation of the solid and drying same to obtain the compound.

13. The method as claimed in claim 12 wherein the suitable solvent in steps (i) and/or (iva) is acetone.

14. The method as claimed in claims 12 or 13, wherein the basic Na compound is NaOH, $Na_2CO_3$, $NaHCO_3$, NaOMe, NaOAc or NaOCHO.

15. A pharmaceutical composition comprising

(a) the compound as claimed in any one of claims 1 to 9;

(b) a therapeutic agent selected from an anti-diabetic, a hypolipidemic agent, an anti-obesity agent and an anti-hypertensive agent; and

(c) at least one pharmaceutically acceptable additive.

16. The pharmaceutical composition as claimed in claim 15 wherein the therapeutic agent is amlodipine besylate.

17. The pharmaceutical composition as claimed in claim 15, wherein the therapeutic agent is hydrochlorothiazide.

Dated this 26th day of November, 2015

NAMRATA CHADHA
OF K&S PARTNERS
AGENT FOR THE APPLICANT(S)

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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: PHARMACEUTICAL COMPOSITIONS COMPRISING VALSARTAN AND NEP INHIBITORS

(57) Abstract: The invention relates a pharmaceutical composition comprising a combination of (i) the AT 1- antagonist valsartan or a pharmaceutically acceptable salt thereof and (ii) a NEP inhibitor or a pharmaceutically acceptable salt thereof and optionally a pharmaceutically acceptable carrier and to a method for the treatment or prevention of a condition or disease selected from the group consisting of hypertension, heart failure such as (acute and chronic) congestive heart failure, left ventricular dysfunction and hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation, atrial flutter, detrimental vascular remodeling, myocardial infarction and its sequelae, atherosclerosis, angina (whether unstable or stable), renal insufficiency (diabetic and non-diabetic), heart failure, angina pectoris, diabetes, secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions, such as diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, and also renal vascular hypertension, diabetic retinopathy, the management of other vascular disorders, such as migraine, peripheral vascular disease, Raynaud's disease, luminal hyperplasia, cognitive dysfunction (such as Alzheimer's), glaucoma and stroke, comprising administering a therapeutically effective amount of the pharmaceutical composition to a mammal in need thereof.

WO 03/059345 A1

# PHARMACEUTICAL COMPOSITIONS COMPRISING VALSARTAN AND NEP INHIBITORS

Angiotensin II interacts with specific receptors on the surface of the target cell. It has been possible to identify receptor subtypes that are termed e.g. AT 1- and AT 2-receptors. In recent times great efforts have been made to identify substances that bind to the AT 1-receptor. Such active ingredients are often termed angiotensin II antagonists. Because of the inhibition of the AT 1-receptor such antagonists can be used e.g. as antihypertensives or for the treatment of congestive heart failure, among other indications. Angiotensin II antagonists are therefore understood to be those active ingredients which bind to the AT 1-receptor subtype.

Inhibitors of the renin angiotensin system are well known drugs that lower blood pressure and exert beneficial actions in hypertension and in congestive heart failure as described, for example, in N. Eng. J. Med. 316, 23 (1987) p. 1429-1435. A large number of peptide and non-peptide inhibitors of the renin angiotensin system are known, the most widely studied being the ACE inhibitors, which includes the drugs captopril, enalapril, lisinopril, benazepril and spirapril. Although a major mode of action of ACE inhibitors involves prevention of formation of the vasoconstrictor peptide Ang II, it has been reported in Hypertension, 16, 4 (1990) p. 363-370 that ACE cleaves a variety of peptide substrates, including the vasoactive peptides bradykinin and substance P. Prevention of the degradation of bradykinin by ACE inhibitors has been demonstrated, and the activity of the ACE inhibitors in some conditions has been reported in Circ. Res., 66, 1 (1990) p. 242-248 to be mediated by elevation of bradykinin levels rather than inhibition of Ang II formation. Consequently, it cannot be presumed that the effect of an ACE inhibitor is due solely to prevention of angiotensin formation and subsequent inhibition of the renin angiotensin system.

Neutral endopeptidase (EC 3.4.24.11; enkephalinase; atriopeptidase; NEP) is a zinc-containing metalloprotease that cleaves a variety of peptide substrates on the amino terminal side of aromatic amino acids. See Biochem. J., 241, (1987) p. 237-247. Substrates for this enzyme include, but are not limited to, atrial natriuretic factors (ANF, also known as ANP), brain natriuretic peptide (BNP), met and leu enkephalin, bradykinin, neurokinin A, and substance P.

ANPs are a family of vasodilator, diuretic and antihypertensive peptides which have been the subject of many recent reports in the literature, for example Annu. Rev. Pharm. Tox., 29, (1989) p. 23-54. One form, ANF 99-126, is a circulating peptide hormone which is released from the heart during conditions of cardiac distension. The function of ANF is to

maintain salt and water homeostasis as well as to regulate blood pressure. ANF is rapidly inactivated in the circulation by at least two processes: a receptor-mediated clearance reported in Am. J. Physiol., 256 (1989) p. R469-R475 and an enzymatic inactivation via NEP reported in Biochem. J., 243 (1987) p. 183-187. It has been previously demonstrated that inhibitors of NEP potentiate the hypotensive, diuretic, natriuretic and plasma ANF responses to pharmacological injection of ANF in experimental animals. The potentiation of ANF by two specific NEP inhibitors is reported by Sybertz et al. in J. Pharmacol. Exp. Ther. 250, 2 (1989) p. 624-631 and in Hypertension, 15, 2 (1990) p. 152-161, while the potentiation of ANF by NEP in general was disclosed in U.S. Patent No. 4,749,688. In U.S. Patent No. 4,740, 499 Olins disclosed the use of thiorphan and kelatorphan to potentiate atrial peptides. Moreover, NEP inhibitors lower blood pressure and exert ANF-like effects such as diuresis and increased cyclic guanosine 3',5'-monophosphate (cGMP) excretion in some forms of experimental hypertension. The antihypertensive action of NEP inhibitors is mediated through ANF because antibodies to ANF will neutralize the reduction in blood pressure.

Prolonged and uncontrolled hypertensive vascular disease ultimately leads to a variety of pathological changes in target organs such as the heart and kidney. Sustained hypertension can lead as well to an increased occurrence of stroke. Therefore, there is a strong need to evaluate the efficacy of antihypertensive therapy, an examination of additional cardiovascular endpoints, beyond those of blood pressure lowering, to get further insight into the benefits of combined treatment.

The nature of hypertensive vascular diseases is multifactorial. Under certain circumstances, drugs with different mechanisms of action have been combined. However, just considering any combination of drugs having different mode of action does not necessarily lead to combinations with advantageous effects. Accordingly, there is a need for more efficacious combination therapy which has less deleterious side effects.

In one aspect the present invention relates to pharmaceutical combinations comprising valsartan or pharmaceutically acceptable salts thereof and a neutral endopeptidase (NEP) inhibitor or a pharmaceutically effective salts thereof, optionally in the presence of a pharmaceutically acceptable carrier and pharmaceutical compositions comprising them.

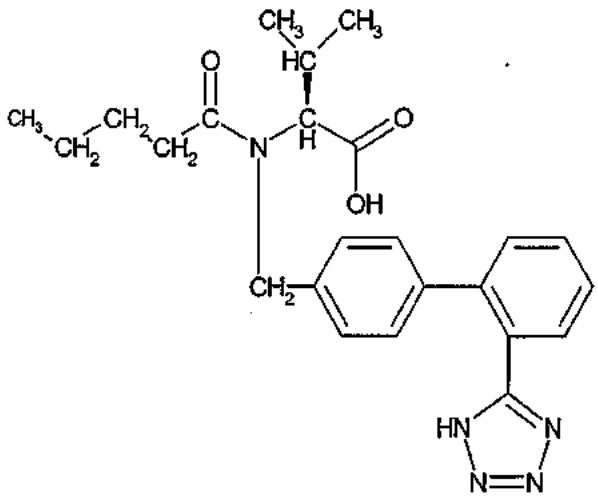
In another embodiment the present invention relates to methods of treating cardiac and renal related conditions by administration of the pharmaceutical composition comprising valsartan plus a NEP inhibitor or relates to the use of a pharmaceutical composition

comprising valsartan or pharmaceutically acceptable salts thereof and a neutral endopeptidase (NEP) inhibitor or a pharmaceutically effective salts thereof.

In another embodiment of the invention the present invention relates to a pharmaceutical composition comprising valsartan or pharmaceutically acceptable salts thereof and a neutral endopeptidase (NEP) inhibitor or a pharmaceutically effective salts thereof and a diuretic, especially hydrochlorothiazide.

Valsartan is the AT 1 receptor antagonist (S) –N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2;(1H-tetrazol-5-yl)biphenyl-4-yl-methyl]amine of formula (I)

and is disclosed in EP 0443983 A and United States Patent 5,399,578, the disclosures of

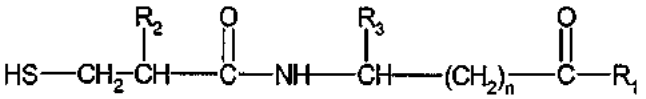


(I)

which are incorporated herein in their entirety as if set forth herein.

A NEP inhibitor useful in said combination is a compound of the formula (II)

(II)



and pharmaceutically acceptable salts thereof wherein:

$R_2$ is alkyl of 1 to 7 carbons, trifluoromethyl, phenyl, substituted phenyl, -$(CH_2)_{1\text{ to }4}$-phenyl, or -$(CH_2)_{1\text{ to }4}$-substituted phenyl;

$R_3$ is hydrogen, alkyl of 1 to 7 carbons, phenyl, substituted phenyl, -$(CH_2)_{1\text{ to }4}$-phenyl, or -$(CH_2)_{1\text{ to }4}$-substituted phenyl;

$R_1$ is hydroxy, alkoxy of 1 to 7 carbons, or $NH_2$;

n is an integer from 1 to 15; and

the term substituted phenyl refers to a substituent selected from lower alkyl of 1 to 4 carbons, lower alkoxy of 1 to 4 carbons, lower alkylthio of 1 to 4 carbons, hydroxy, Cl, Br, or F.

Preferred selective neutral endopeptidase inhibitors of formula II include compounds wherein:

$R_2$ is benzyl;

$R_3$ is hydrogen;

n is an integer from 1 to 9; and

$R_1$ is hydroxy.

Even more preferred selective neutral endopeptidase inhibitors of formula II are reported in the literature as SQ 28,603 which is the compound of formula II wherein:

$R_2$ is benzyl;

$R_3$ is hydrogen;

n is one; and

$R_1$ is hydroxy.

The preparation of the selective neutral endopeptidase inhibitors of formula II wherein $R_2$ is other than trifluoromethyl are disclosed by Delaney et al. in U.S. Patent No. 4,722,810. The preparation of the selective neutral endopeptidase inhibitors of formula II wherein $R_2$ is trifluoromethyl are disclosed by Delaney et al in U.S. Patent No. 5,223,516.

NEP inhibitors within the scope of the present invention include compounds disclosed in U.S. Patent No. 4,610,816, herein incorporated by reference, including in particular N-[N-[1(S)-carboxyl-3-phenylproplyl]-(S)-phenylalanyl]-(S)-isoserine and N-[N-[((1S)-carboxy-2-phenyl)ethyl]-(S)-phenylalanyl]-β-alanine; compounds disclosed in U.S. Patent No. 4,929,641, in particular N-[2(S)-mercaptomethyl-3-(2-methylphenyl)-propionyl]methionine; SQ 28603 (N-[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]-β-alanine), disclosed in South African Patent Application 84/0670; UK 69578 (cis-4-[[[1-[2-carboxy-3-(2-methoxyethoxy)propyl]-cyclopentyl]carbonyl]amino]-cyclohexanecarboxylic acid) and its active enantiomer(s); thiorphan and its enantiomers; retro-thiorphan; phosphoramidon; and SQ 29072 (7-[[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]amino]-heptanoic acid). Also suitable for use are any pro-drug forms of the above-listed NEP inhibitors, e.g., compounds in which one or more carboxylic acid groups are esterified.

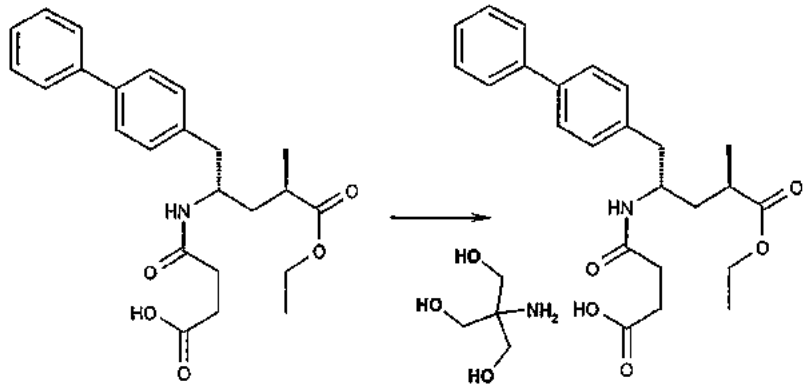
NEP inhibitors within the scope of the present invention also include the compounds disclosed in U.S. Patent No. 5,217,996, particularly, N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester and N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid or in each case a pharmaceutically acceptable salt thereof; the compounds disclosed in EP 00342850, particularly (S)-cis-4-[1-[2-(5-indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido]-1-cyclohexanecarboxylic acid; the compounds disclosed in GB 02218983, particularly 3-(1-[6-endo-hydroxymethylbicyclo[2,2,1]heptane-2-exo-carbamoyl]cyclopentyl)-2-(2-methoxyethyl)propanoic acid; the compounds disclosed in WO 92/14706, particularly N-(1-(3-(N-t-butoxycarbonyl-(S)-prolylamino)-2(S)-t-butoxy-carbonylpropyl)cyclopentanecarbonyl)-O-benzyl-(S)-serine methyl ester; the compounds disclosed in EP 00343911; the compounds disclosed in JP 06234754; the compounds disclosed in EP 00361365, particularly 4-[[2-(Mercaptomethyl)-1-oxo-3-phenylpropyl]amino]benzoic acid; the compounds disclosed in WO 90/09374, particularly 3-[1-(Cis-4-carboxycarbonyl-cis-3-butylcyclohexyl-r-1-carboamoyl)cyclopentyl]-2S-(2-methoxyethoxymethyl)propanoic acid; the compounds disclosed in JP 07157459, particularly N-((2S)-2-(4-biphenylmethyl)-4-carboxy-5-phenoxyvaleryl)glycine; the compounds disclosed in WO 94/15908 particularly N-(1-(N-hydroxycarbamoylmethyl)-1-cyclopentanecarbonyl)-L-phenylalanine; the compounds disclosed in U.S. Patent No. 5,273,990 particularly (S)-(2-biphenyl-4-yl)-1-(1H-tetrazol-5-yl)ethylamino) methylphosphonic acid; the compounds disclosed in U.S. Patent No. 5,294,632 particularly (S)-5-(N-(2-(phosphonomethylamino)-3-(4-biphenyl)propionyl)-2-aminoethyl)tetrazole; the compounds disclosed in U.S. Patent No. 5,250,522, particularly β-Alanine, 3-[1,1'-biphenyl]-4-yl-N-[diphenoxyphosphinyl)methyl]-L-alanyl; the compounds disclosed in EP 00636621, particularly N-(2-carboxy-4-thienyl)-3-mercapto-2-benzylpropanamide; the compounds disclosed in WO 93/09101, particularly 2-(2-mercaptomethyl-3-phenylpropionamido)thiazol-4-ylcarboxylic acid; the compounds disclosed in EP 00590442 particularly ((L)-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy)carbonyl)-2-phenylethyl)-L-phenylalanyl)-β-alanine, N-[N-[(L)-[1-[(2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy]carbonyl]-2-phenylethyl]-L-phenylalanyl]-(R)-alanine, N-[N-[(L)-1-carboxy-2-phenylethyl]-L-phenylalanyl]-(R)-alanine, N-[2-acetylthiomethyl-3-(2-methyl-phenyl)propionyl]-methionine ethyl ester, N-[2-mercaptomethyl-3-(2-methylphenyl)propioyl]-methionine, N-[2(S)-mercaptomethyl-3-(2-methylphenyl)propanoyl]-(S)-isoserine, N-(S)-[3-mercapto-2-(2-methylphenyl)propionyl]-(S)-2-methoxy-(R)-alanine, N-[1-[[1(S)-benzyloxycarbonyl-3-

phenylpropyl]amino]cyclopentylcarbonyl]-(S)-isoserine, N-[1-[[1(S)-carbonyl-3-phenylpropy]amino]-cyclopentylcarbonyl]-(S)-isoserine, 1,1'-[dithiobis-[2(S)-(2-methylbenzyl)-1-oxo-3,1-propanediyl]]-bis-(S)-isoserine, 1,1'-[dithiobis-[2(S)-(2-methylbenzyl)-1-oxo-3,1-propanediyl]]-bis-(S)-methionine, N-(3-phenyl-2-(mercaptomethyl)-propionyl)-(S)-4-(methylmercapto)methionine, N-[2-acetylthiomethyl-3-phenyl-propionyl]-3-aminobenzoic acid,
N-[2-mercaptomethyl-3-phenyl-propionyl]-3-aminobenzoic acid, N-[1-(2-carboxy-4-phenylbutyl)-cyclopentanecarbonyl]-(S)-isoserine, N-[1-(acetylthiomethyl)cyclopentane-carbonyl]-(S)-methionine ethyl ester, 3(S)-[2-(acetylthiomethyl)-3-phenyl-propionyl]amimo-e-caprolactam; and the compounds disclosed in WO 93/10773 particularly N-(2-acetylthiomethyl-3-(2-methylphenyl)propionyl)-methionine ethyl ester.

A diuretic is, for example, a thiazide derivative selected from the group consisting of chlorothiazide, hydrochlorothiazide, methylclothiazide, and chlorothalidon. The most preferred is hydrochlorothiazide.

The compounds to be combined can be present as pharmaceutically acceptable salts. If these compounds have, for example, at least one basic center, they can form acid addition salts. Corresponding acid addition salts can also be formed having, if desired, an additionally present basic center. The compounds having at least one acid group (for example COOH) can also form salts with bases. Corresponding internal salts may furthermore be formed, if a compound comprises e.g. both a carboxy and an amino group.

With respect to N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester, preferred salts include the sodium salt disclosed in U.S. Patent No. 5,217,996, the triethanolamine salt and the tris(hydroxymethyl)aminomethane salt. Preparation of the triethanolamine salt and the tris(hydroxymethyl)aminomethane salt may be carried out as follows:
Triethanolamine - To N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester (349 mg, 0.848 mmol) is added 5 ml of ethyl ether and 0.113 ml (0.848 mmol) of triethanolamine in 1 ml of ethyl acetate. The solid was collected and dried melting at 69-71 °C



Tris(hydroxymethyl) aminomethane - To N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester (3.2 g (7.78 mmol) is added 32 ml of ethyl acetate and 940 mg (7.78 mmol) tris(hydroxymethyl)aminomethane. The suspension is diluted with 45 ml of ethyl acetate and refluxed overnight (~20 hr). The reaction is cooled to 0°C, filtered, solid washed with ethyl acetate and dried melting at 114-115 °C.

The salts of N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester formed with triethanolamine and tris(hydroxymethyl) aminomethane are novel and can be used as NEP inhibitors. Another embodiment of the present invention are said new salts, their use as NEP inhibitors, especially for preventing and treating of conditions and disease associated with the inhibition on NEP, pharmaceutical composition comprising these salts and their combination with valsartan, especially for the treatment of conditions and diseases as disclosed for the combinations of the present invention hereinbefore or hereinafter.

It has surprisingly been found that, a combination of valsartan and a NEP inhibitor achieves greater therapeutic effect than the administration of valsartan, ACE inhibitors or NEP inhibitors alone and promotes less angioedema than is seen with the administration of a vasopeptidase inhibitor alone. Greater efficacy can also be documented as a prolonged duration of action. The duration of action can be monitored as either the time to return to baseline prior to the next dose or as the area under the curve (AUC) and is expressed as the product of the change in blood pressure in millimeters of mercury (change in mmHg) and the duration of the effect (minutes, hours or days).

Further benefits are that lower doses of the individual drugs to be combined according to the present invention can be used to reduce the dosage, for example, that the dosages need not only often be smaller but are also applied less frequently, or can be used

to diminish the incidence of side effects. The combined administration of valsartan or a pharmaceutically acceptable salt thereof and a NEP inhibitor or a pharmaceutically acceptable salt thereof results in a significant response in a greater percentage of treated patients, that is, a greater responder rate results, regardless of the underlying etiology of the condition. This is in accordance with the desires and requirements of the patients to be treated.

It can be shown that combination therapy with valsartan and a NEP inhibitor results in a more effective antihypertensive therapy (whether for malignant, essential, reno-vascular, diabetic, isolated systolic, or other secondary type of hypertension) through improved efficacy as well as a greater responder rate. The combination is also useful in the treatment or prevention of heart failure such as (acute and chronic) congestive heart failure, left ventricular dysfunction and hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation, atrial flutter or detrimental vascular remodeling. It can further be shown that a valsartan and NEP inhibitor therapy proves to be beneficial in the treatment and prevention of myocardial infarction and its sequelae. A valsartan plus NEP inhibitor combination is also useful in treating atherosclerosis, angina (whether stable or unstable), and renal insufficiency (diabetic and non-diabetic). Furthermore, combination therapy using valsartan and a NEP inhibitor can improve endothelial dysfunction, thereby providing benefit in diseases in which normal endothelial function is disrupted such as heart failure, angina pectoris and diabetes. Furthermore, the combination of the present invention may be used for the treatment or prevention of secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions, such as diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, and also renal vascular hypertension, diabetic retinopathy, the management of other vascular disorders, such as migraine, peripheral vascular disease, Raynaud's disease, luminal hyperplasia, cognitive dysfunction (such as Alzheimer's), glaucoma and stroke.

The structure of the active agents identified by generic or tradenames or code nos. may be taken from the actual edition of the standard compendium "The Merck Index" or from databases, e.g. Life Cycle Patents International (e.g. IMS World Publications). The corresponding content thereof is hereby incorporated by reference. Any person skilled in the art is fully enabled to identify the active agents and, based on these references, likewise enabled to manufacture and test the pharmaceutical indications and properties in standard test models, both in vitro and in vivo.

The subject matter of NEP inhibitors referred to in e.g. US patents, EP, , GB, JP or WO patent applications is herewith incorporated by reference, especially corresponding NEP inhibitors and pharmaceutically acceptable salts and pharmaceutical compositions thereof, that are claimed or disclosed in the working examples.

The person skilled in the pertinent art is fully enabled to select a relevant test model to prove the efficacy of a combination of the present invention in the hereinbefore and hereinafter indicated therapeutic indications.

Representative studies are carried out with a combination of valsartan and N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester, e.g. applying the following methodology:
Drug efficacy is assessed in various animal models including the deoxycorticosterone acetate - salt rat (DOCA-salt) and the spontaneously hypertensive rat (SHR), either maintained on a normal salt diet or with salt loading (4-8% salt in rat chow or 1% NaCl as drinking water).

The DOCA-salt test model utilizes either an acute or chronic study protocol. An acute study procedure involves assessment of the effects of various test substances over a six-hour experimental period using rats with indwelling femoral arterial and venous catheters. The Acute Study Procedure evaluates test substances for their ability to reduce blood pressure during the established phase of DOCA-salt hypertension. In contrast, the Chronic Study Procedure assesses the ability of test substances to prevent or delay the rise in blood pressure during the development phase of DOCA-salt hypertension. Therefore, blood pressure will be monitored in the chronic study procedure by means of a radiotransmitter. The radiotransmitter is surgically implanted into the abdominal aorta of rats, prior to the initiation of DOCA-salt treatment and thus, prior to the induction of hypertension. Blood pressure is chronically monitored for periods of up 6 weeks (approximately one week prior to DOCA-salt administration and for 5 weeks thereafter).

Rats are anesthetized with 2-3% isoflurane in oxygen inhalant followed by Amytal sodium (amobarbital) 100 mg/kg, ip. The level of anesthesia is assessed by a steady rhythmic breathing pattern.

Acute study procedure:

Rats undergo a unilateral nephrectomy at the time of DOCA implantation. Hair is clipped on the left flank and the back of the neck and scrubbed with sterile alcohol swabs and

povidone/iodine. During surgery rats are placed on a heating pad to maintain body temperature at 37 degrees C.

A 20mm incision is made through the skin and underlying muscle to expose the left kidney. The kidney is freed of surrounding tissue, exteriorized and two ligatures (3-0 silk) are tied securely around the renal artery and vein proximal to their juncture with the aorta. The renal artery and vein are then severed and the kidney removed. The muscle and skin wounds are closed with 4-0 silk suture and stainless steel wound clips, respectively. At the same time, a 15mm incision is made on the back of the neck and a 3-week-release pellet (Innovative Research of America, Sarasota, Florida) containing deoxycorticosterone acetate (100 mg/kg) is implanted subcutaneously. The wound is then closed with stainless-steel clips and both wounds are treated with povidone/iodine; the rats are given a post-surgical intramuscular injection of procaine penicillin G (100,000 U) and buprenorphine (0.05 – 0.1 mg/kg) s.c. The rats are immediately placed on 1% NaCl + 0.2% KCl drinking water; this treatment continues for at least 3 weeks at which time the animals have become hypertensive and available for experimentation.

Forty-eight hours prior to experimentation, animals are anesthetized with isoflurane and catheters are implanted in the femoral artery and vein for measuring arterial pressure, collection of blood, and administration of test compounds. Rats are allowed to recover for 48 hours while tethered in a Plexiglas home cage, which also serves as the experimental chamber.

Chronic study procedure:

This procedure is the same as above except that rats are implanted with a radiotransmitter, 7-10 days prior to the unilateral nephrectomy and initiation of DOCA and salt. In addition, rats do not undergo surgery for placement of femoral arterial and venous catheters. Radiotransmitters are implanted as described in M.K. Bazil, C. Krulan and R.L. Webb. Telemetric monitoring of cardiovascular parameters in conscious spontaneously hypertensive rats. J.Cardiovasc. Pharmacol. 22: 897-905, 1993.

Protocols are then set-up on the computer for measurement of blood pressure, heart rate, etc, at predetermined time points. Baseline data is collected at various time points and over various time intervals. For example, baseline or pre-dose values usually consist of data collection and averaging over 3 consecutive, 24-hour time periods prior to drug administration.

Blood pressure, heart rate and activity are determined at various pre-selected time points before, during, and after drug administration. All measurements are performed in unrestrained and undisturbed animals. The maximum study time, determined by battery life, could be as long as nine months. For studies of this duration, rats are dosed orally (1-3 ml/kg vehicle), no more than twice daily or drug is administered via the drinking water or mixed with food. For studies of a shorter duration, that is, up to 8 weeks, drugs are given via subcutaneously implanted osmotic minipumps. Osmotic minipumps are selected based on drug delivery rate and time. Valsartan dosages range from 1 to 10 mg/kg/day and N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester range from 10 to 50 mg/kg/day.

Additionally, SHR are utilized to study the effects of valsartan in combination with N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester. The hypertensive background of the SHR is modified either by chronic salt loading in an effort to suppress the renin angiotensin system (RAS) or chronic salt depletion to activate the RAS in the SHR. These manipulations will be carried out to more extensively evaluate the efficacy of the various test substances. Experiments performed in spontaneously hypertensive rats (SHR) are supplied by Taconic Farms, Germantown, New York (Tac:N(SHR)fBR). A radiotelemetric device (Data Sciences International, Inc., St. Paul, Minnesota) is implanted into the lower abdominal aorta of all test animals between the ages of 14 to 16 weeks of age. All SHR are allowed to recover from the surgical implantation procedure for at least 2 weeks prior to the initiation of the experiments. Cardiovascular parameters are continuously monitored via the radiotransmitter and transmitted to a receiver where the digitized signal is then collected and stored using a computerized data acquisition system. Blood pressure (mean arterial, systolic and diastolic pressure) and heart rate are monitored in conscious, freely moving and undisturbed SHR in their home cages. The arterial blood pressure and heart rate are measured every 10 minutes for 10 seconds and recorded. Data reported for each rat represent the mean values averaged over a 24 hour period and are made up of the 144-10 minute samples collected each day. The baseline values for blood pressure and heart rate consist of the average of three consecutive 24 hour readings taken prior to initiating the drug treatments. All rats are individually housed in a temperature and humidity controlled room and are maintained on a 12 hour light dark cycle.

In addition to the cardiovascular parameters, weekly determinations of body weight also are recorded in all rats. Treatments are administered in the drinking water, via daily oral gavage or in osmotic minipumps as stated above. If given in drinking water, water consumption is

measured five times per week. Valsartan and N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester doses for individual rats are then calculated based on water consumption for each rat, the concentration of drug substance in the drinking water, and individual body weights. All drug solutions in the drinking water are made up fresh every three to four days. Typical dosages for valsartan in drinking water range from 3 to 30 mg/kg/day whereas the dosage of N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester is highly dependent upon the specific agent used. In most situations, a daily dose will not exceed 50 mg/kg/day when administered as the monotherapy. In combination, lower dosages of each agent are used and correspondingly, valsartan is given in the range of 1 to 30 mg/kg/day and N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester in dosages below 50 mg/kg/day. However, in cases wherein the responder rate is increased with combination treatment, the dosages are identical to those used as monotherapy.

When drugs are administered by oral gavage, the dose of valsartan ranges from 1 to 50 mg/kg/day and N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester does not exceed 100 mg/kg/day.

Upon completion of the chronic studies, SHR or DOCA-salt rats are anesthetized and the heart rapidly removed. After separation and removal of the atrial appendages, left ventricle and left plus right ventricle (total) are weighed and recorded. Left ventricular and total ventricular mass are then normalized to body weight and reported. All values reported for blood pressure and cardiac mass represent the group mean $\pm$ sem.

Vascular function and structure are evaluated after treatment to assess the beneficial effects of the combination. SHR are studied according to the methods described by Intengan HD, Thibault G, Li JS, Schiffrin EL, Circulation 1999, 100 (22): 2267-2275. Similarly, the methodology for assessing vascular function in DOCA-salt rats is described in Intengan HD, Park JB, Schiffrin, EL, Hypertension, 1999, 34(4 Part 2): 907-913.

The available results indicate an unexpected therapeutic effect of a combination according to the invention.

In one aspect is the object of this invention to provide a pharmaceutical combination composition, e.g. for the treatment or prevention of a condition or disease selected from the group consisting of hypertension, heart failure such as (acute and chronic) congestive heart failure, left ventricular dysfunction and hypertrophic cardiomyopathy, diabetic cardiac

myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation, atrial flutter, detrimental vascular remodeling, myocardial infarction and its sequelae, atherosclerosis, angina (whether unstable or stable), renal insufficiency (diabetic and non- diabetic), heart failure, angina pectoris, diabetes, secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions, such as diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, and also renal vascular hypertension, diabetic retinopathy, the management of other vascular disorders, such as migraine, peripheral vascular disease, Raynaud's disease, luminal hyperplasia, cognitive dysfunction (such as Alzheimer's), glaucoma and stroke which composition comprises (i) the AT 1-antagonists valsartan or a pharmaceutically acceptable salt thereof and (ii) a NEP inhibitor or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier. A further active ingredient may be a diuretic, especially hydrochlorothiazide.

In this composition, components (i) and (ii) can be obtained and administered together, one after the other or separately in one combined unit dose form or in two separate unit dose forms. The unit dose form may also be a fixed combination.

A further aspect of the present invention is a method for the treatment or prevention of a condition or disease selected from the group consisting of hypertension, heart failure such as (acute and chronic) congestive heart failure, left ventricular dysfunction and hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation, atrial flutter, detrimental vascular remodeling, myocardial infarction and its sequelae, atherosclerosis, angina (whether unstable or stable), renal insufficiency (diabetic and non- diabetic), heart failure, angina pectoris, diabetes, secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions, such as diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, and also renal vascular hypertension, diabetic retinopathy, the management of other vascular disorders, such as migraine, peripheral vascular disease, Raynaud's disease, luminal hyperplasia, cognitive dysfunction (such as Alzheimer's), glaucoma and stroke, comprising administering a therapeutically effective amount of combination of (i) the AT 1-antagonists valsartan or a pharmaceutically acceptable salt thereof and (ii) a NEP inhibitor or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier to a mammal in need of such treatment.

A therapeutically effective amount of each of the component of the combination of the present invention may be administered simultaneously or sequentially and in any order.

The corresponding active ingredient or a pharmaceutically acceptable salt thereof may also be used in form of a hydrate or include other solvents used for crystallization.

The pharmaceutical compositions according to the invention can be prepared in a manner known per se and are those suitable for enteral, such as oral or rectal, and parenteral administration to mammals (warm-blooded animals), including man, comprising a therapeutically effective amount of the pharmacologically active compound, alone or in combination with one or more pharmaceutically acceptable carriers, especially suitable for enteral or parenteral application. Typical oral formulations include tablets, capsules, syrups, elixirs and suspensions. Typical injectable formulations include solutions and suspensions.

The typical pharmaceutically acceptable carriers for use in the formulations described above are exemplified by: sugars such as lactose, sucrose, mannitol and sorbitol; starches such as cornstarch, tapioca starch and potato starch; cellulose and derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and methyl cellulose; calcium phosphates such as dicalcium phosphate and tricalcium phosphate; sodium sulfate; calcium sulfate; polyvinylpyrrolidone; polyvinyl alcohol; stearic acid; alkaline earth metal stearates such as magnesium stearate and calcium stearate; stearic acid; vegetable oils such as peanut oil, cottonseed oil, sesame oil, olive oil and corn oil; non-ionic, cationic and anionic surfactants; ethylene glycol polymers; betacyclodextrin; fatty alcohols; and hydrolyzed cereal solids, as well as other non-toxic compatible fillers, binders, disintegrants, buffers, preservatives, antioxidants, lubricants, flavoring agents, and the like commonly used in pharmaceutical formulations.

The invention also relates to combining separate pharmaceutical compositions in kit form. That is a kit combining two separate units: a valsartan pharmaceutical composition and a NEP inhibitor pharmaceutical composition. The kit form is particularly advantageous when the separate components must be administered in different dosage forms (e.g. parenteral valsartan formulation and oral NEP formulation) or are administered at different dosage intervals.

These pharmaceutical preparations are for enteral, such as oral, and also rectal or parenteral, administration to homeotherms, with the preparations comprising the pharmacological active compound either alone or together with customary pharmaceutical auxiliary substances. For example, the pharmaceutical preparations consist of from about 0.1 % to 90 %, preferably of from about 1 % to about 80 %, of the active compounds.

Pharmaceutical preparations for enteral or parenteral administration are, for example, in unit dose forms, such as coated tablets, tablets, capsules or suppositories and also ampoules. These are prepared in a manner which is known per se, for example using conventional mixing, granulation, coating, solubulizing or lyophilizing processes. Thus, pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipients, if desired granulating a mixture which has been obtained, and, if required or necessary, processing the mixture or granulate into tablets or coated tablet cores after having added suitable auxiliary substances.

The dosage of the active compound can depend on a variety of factors, such as mode of administration, homeothermic species, age and/or individual condition.

Preferred dosages for the active ingredients of the pharmaceutical combination according to the present invention are therapeutically effective dosages, especially those which are commercially available.

Normally, in the case of oral administration, an approximate daily dose of from about 1 mg to about 360 mg is to be estimated e.g. for a patient of approximately 75 kg in weight.

Valsartan is supplied in the form of suitable dosage unit form, for example, a capsule or tablet, and comprising a therapeutically effective amount, e.g. from about 20 to about 320 mg, of valsartan which may be applied to patients. The application of the active ingredient may occur up to three times a day, starting e.g. with a daily dose of 20 mg or 40 mg of valsartan, increasing via 80 mg daily and further to 160 mg daily up to 320 mg daily. Preferably, valsartan is applied once a day or twice a day in heart failure patients with a dose of 80 mg or 160 mg, respectively, each. Corresponding doses may be taken, for example, in the morning, at mid-day or in the evening. Preferred is q.d. or b.i.d. administration in heart failure.

In case of NEP inhibitors, preferred dosage unit forms are, for example, tablets or capsules comprising e.g. from about 20 mg to about 800 mg, preferably from about 50 mg to about 700 mg, even more preferably from about 100 mg to about 600 mg and even more preferably from about 100 mg to about 300 mg, administered once a day.

In case of diuretics, preferred dosage unit forms are, for example, tablets or capsules comprising e.g. from about 5 mg to about 50 mg, preferably from about 6.25 mg to about 25 mg. A daily dose of 6.25 mg, 12.5 mg or 25 mg of hydrochlorothiazide is preferably administered once a day.

The above doses encompass a therapeutically effective amount of the active ingredients of the present invention.

The following examples illustrate the above-described invention; however, it is not intended to restrict the scope of this invention in any manner.

Formulation Example 1:

Film-Coated Tablets:

| Components | Composition Per Unit (mg) | Standards |
|---|---|---|
| Granulation | | |
| Valsartan [= active ingredient] | 80.00 | |
| Microcrystalline cellulose/ Avicel PH 102 | 54.00 | NF, Ph. Eur |
| Crospovidone | 20.00 | NF, Ph. Eur |
| Colloidal anhydrous silica / colloidal silicon dioxide / Aerosil 200 | 0.75 | Ph. Eur/ NF |
| Magnesium stearate | 2.5 | NF, Ph. Eur |
| Blending | | |
| Colloidal anhydrous silica / colloidal silicon dioxide / Aerosil 200 | 0.75 | Ph. Eur/ NF |
| Magnesium stearate | 2.00 | NF, Ph. Eur |
| Coating | | |
| Purified water *) | - | |
| DIOLACK pale red 00F34899 | 7.00 | |
| Total tablet mass | 167.00 | |

*) Removed during processing.

The film-coated tablet is manufactured e.g. as follows:

A mixture of valsartan, microcrystalline cellulose, crospovidone, part of the colloidal anhydrous silica/colloidal silicon dioxide/Aerosile 200, silicon dioxide and magnesium stearate is premixed in a diffusion mixer and then sieve through a screening mill. The resulting mixture is again pre-mixed in a diffusion mixer, compacted in a roller compactor and then sieve through a screening mill. To the resulting mixture, the rest of the colloidal anhydrous silica/colloidal silicon dioxide/Aerosile 200 are added and the final blend is made

in a diffusion mixer. The whole mixture is compressed in a rotary tabletting machine and the tablets are coated with a film by using Diolack pale red in a perforated pan.

Formulation Example 2:

Film-coated tablets:

| Components | Composition Per Unit (mg) | Standards |
|---|---|---|
| Granulation | | |
| Valsartan [= active ingredient] | 160.00 | |
| Microcrystalline cellulose/ Avicel PH 102 | 108.00 | NF, Ph. Eur |
| Crospovidone | 40.00 | NF, Ph. Eur |
| Colloidal anhydrous silica / colloidal silicon dioxide / Aerosil 200 | 1.50 | Ph. Eur/ NF |
| Magnesium stearate | 5.00 | NF, Ph. Eur |
| Blending | | |
| Colloidal anhydrous silica / colloidal silicon dioxide / Aerosil 200 | 1.50 | Ph. Eur/ NF |
| Magnesium stearate | 4.00 | NF, Ph. Eur |
| Coating | | |
| Opadry Light Brown 00F33172 | 10.00 | |
| Total tablet mass | 330.00 | |

The film-coated tablet is manufactured e.g. as described in Formulation Example 1.

Formulation Example 3:

Film-Coated Tablets:

| Components | Composition Per Unit (mg) | Standards |
|---|---|---|
| Core: Internal phase | | |
| Valsartan [= active ingredient] | 40.00 | |
| Silica, colloidal anhydrous (Colloidal silicon dioxide) [= Glidant] | 1.00 | Ph. Eur, USP/NF |
| Magnesium stearate [= Lubricant] | 2.00 | USP/NF |
| Crospovidone [Disintegrant] | 20.00 | Ph. Eur |
| Microcrystalline cellulose [= Binding agent] | 124.00 | USP/NF |
| External phase | | |
| Silica, colloidal anhydrous, (Colloidal silicon dioxide) [= Glidant] | 1.00 | Ph. Eur, USP/NF |
| Magnesium stearate [Lubricant] | 2.00 | USP/NF |
| Film coating | | |
| Opadry® brown OOF 16711*) | 9.40 | |
| Purified Water**) | - | |
| Total tablet mass | 199.44 | |

*) The composition of the Opadry® brown OOF16711 coloring agent is tabulated below.

**) Removed during processing

Opadry® Composition:

| Ingredient | Approximate % Composition |
|---|---|
| Iron oxide, black (C.I. No. 77499, E 172) | 0.50 |
| Iron oxide, brown (C.I. No. 77499, E 172 | 0.50 |
| Iron oxide, red (C.I. No. 77491, E 172) | 0.50 |
| Iron oxide, yellow (C.I. No. 77492, E 172) | 0.50 |
| Macrogolum (Ph. Eur) | 4.00 |
| Titanium dioxide (C.I. No. 77891, E 171) | 14.00 |
| Hypromellose (Ph. Eur) | 80.00 |

The film-coated tablet is manufactured e.g. as described in Formulation Example 1.

Formulation Example 4:

Capsules:

| Components | Composition Per Unit (mg) |
|---|---|
| Valsartan [= active ingredient] | 80.00 |
| Microcrystalline cellulose | 25.10 |
| Crospovidone | 13.00 |
| Povidone | 12.50 |
| Magnesium stearate | 1.30 |
| Sodium lauryl sulphate | 0.60 |
| Shell: | |
| Iron oxide, red (C.I. No. 77491, EC No. E 172) | 0.123 |
| Iron oxide, yellow (C.I. No. 77492, EC No. E 172) | 0.123 |
| Iron oxide, black (C.I. No. 77499, EC No. E 172) | 0.245 |
| Titanium dioxide | 1.540 |
| Gelatin | 74.969 |
| Total tablet mass | 209.50 |

The tablet is manufactured e.g. as follows:

Granulation/Drying

Valsartan and microcrystallin cellulose are spray-granulated in a fluidized bed granulator with a granulating solution consisting of povidone and sodium lauryl sulphate dissolved in purified water. The granulate obtained is dried in a fluidized bed dryer.

Milling/Blending

The dried granulate is milled together with crospovidone and magnesium stearate. The mass is then blended in a conical srew type mixer for approximately 10 minutes.

Encapsulation

The empty hard gelatin capsules are filled with the blended bulk granules under controlled temperature and humidity conditions. The filed capsules are dedusted, visually inspected, weightchecked and quarantined until by Quality assurance department.

Formulation Example 5:

Capsules:

| Components | Composition Per Unit (mg) |
|---|---|
| Valsartan [= active ingredient] | 160.00 |
| Microcrystalline cellulose | 50.20 |
| Crospovidone | 26.00 |
| Povidone | 25.00 |
| Magnesium stearate | 2.60 |
| Sodium lauryl sulphate | 1.20 |
| Shell | |
| Iron oxide, red (C.I. No. 77491, EC No. E 172) | 0.123 |
| Iron oxide, yellow (C.I. No. 77492, EC No. E 172) | 0.123 |
| Iron oxide, black (C.I. No. 77499, EC No. E 172) | 0.245 |
| Titanium dioxide | 1.540 |

| | |
|---|---|
| Gelatin | 74.969 |
| Total tablet mass | 342.00 |

The formulation is manufactured e.g. as described in Formulation Example 4.

Formulation Example 6:

Hard Gelatine Capsule:

| Components | Composition Per Unit (mg) |
|---|---|
| Valsartan [= active ingredient] | 80.00 |
| Sodium laurylsulphate | 0.60 |
| Magnesium stearate | 1.30 |
| Povidone | 12.50 |
| Crospovidone | 13.00 |
| Microcrystalline cellulose | 21.10 |
| Total tablet mass | 130.00 |

Formulation Example 7:

A hard gelatin capsule, comprising as active ingredient e.g. (S)-N-(1-carboxy-2-methylprop-1-yl)-N-pentanoyl-N-[2'(1H-tetrazol-5-yl)biphenyl-4-yl-methyl]amine, can be formulated, for example, as follows:

Composition:

| | |
|---|---|
| (1) valsartan | 80.0 mg |
| (2) microcrystalline cellulose | 110.0 mg |
| (3) polyvidone K30 | 45.2 mg |
| (4) sodium lauryl sulfate | 1.2 mg |
| (5) crospovidone | 26.0 mg |
| (6) magnesium stearate | 2.6 mg |

Components (1) and (2) are granulated with a solution of components (3) and (4) in water. The components (5) and (6) are added to the dry granulate and the mixture is filled into size 1 hard gelatin capsules.

All publications and patents mentioned herein are incorporate by reference in their entirety as if set forth in full herein.

What is claimed is:

1. A pharmaceutical composition comprising (*i*) the AT 1-antagonist valsartan or a pharmaceutically acceptable salt thereof and (ii) a NEP inhibitor or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

2. The pharmaceutical composition of claim 1, wherein the NEP inhibitor is selected from the group consisting of SQ 28,603, N-[N-[1(S)-carboxyl-3-phenylproplyl]-(S)-phenylalanyl]-(S)-isoserine, N-[N-[((1S)-carboxy-2-phenyl)ethyl]-(S)-phenylalanyl]-β-alanine, N-[2(S)-mercaptomethyl-3-(2-methylphenyl)-propionyl]methionine, (cis-4-[[[1-[2-carboxy-3-(2-methoxyethoxy)propyl]-cyclopentyl]carbonyl]amino]-cyclohexanecarboxylic acid), thiorphan, retro-thiorphan, phosphoramidon, SQ 29072, N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester, (S)-cis-4-[1-[2-(5-indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido]-1-cyclohexanecarboxylic acid, 3-(1-[6-endo-hydroxymethylbicyclo[2,2,1]heptane-2-exo-carbamoyl]cyclopentyl)-2-(2-methoxyethyl)propanoic acid, N-(1-(3-(N-t-butoxycarbonyl-(S)-prolylamino)-2(S)-t-butoxy-carbonylpropyl)cyclopentanecarbonyl)-O-benzyl-(S)-serine methyl ester, 4-[[2-(Mercaptomethyl)-1-oxo-3-phenylpropyl]amino]benzoic acid, 3-[1-(Cis-4-carboxycarbonyl-cis-3-butylcyclohexyl-r-1-carboamoyl)cyclopentyl]-2S-(2-methoxyethoxymethyl)propanoic acid, N-((2S)-2-(4-biphenylmethyl)-4-carboxy-5-phenoxyvaleryl)glycine, N-(1-(N-hydroxycarbamoylmethyl)-1-cyclopentanecarbonyl)-L-phenylalanine, (S)-(2-biphenyl-4-yl)-1-(1H-tetrazol-5-yl)ethylamino) methylphosphonic acid, (S)-5-(N-(2-(phosphonomethylamino)-3-(4-biphenyl)propionyl)-2-aminoethyl)tetrazole, β-Alanine, 3-[1,1'-biphenyl]-4-yl-N-[diphenoxyphosphinyl)methyl]-L-alanyl, N-(2-carboxy-4-thienyl)-3-mercapto-2-benzylpropanamide, 2-(2-mercaptomethyl-3-phenylpropionamido)thiazol-4-ylcarboxylic acid, (L)-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy)carbonyl)-2-phenylethyl)-L-phenylalanyl)-β-alanine, N-[N-[(L)-[1-[(2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy]carbonyl]-2-phenylethyl]-L-phenylalanyl]-(R)-alanine, N-[N-[(L)-1-carboxy-2-phenylethyl]-L-phenylalanyl]-(R)-alanine, N-[2-acetylthiomethyl-3-(2-methyl-phenyl)propionyl]-methionine ethyl ester, N-[2-mercaptomethyl-3-(2-methylphenyl)propioyl]-methionine, N-[2(S)-mercaptomethyl-3-(2-methylphenyl)propanoyl]-(S)-isoserine, N-(S)-[3-mercapto-2-(2-methylphenyl)propionyl]-(S)-2-methoxy-(R)-alanine, N-[1-[[1(S)-benzyloxycarbonyl-3-phenylpropyl]amino]cyclopentylcarbonyl]-(S)-isoserine, N-[1-[[1(S)-carbonyl-3-

phenylpropy]amino]-cyclopentylcarbonyl]-(S)-isoserine, 1,1'-[dithiobis-[2(S)-(2-methylbenzyl)-1-oxo-3,1-propanediyl]]-bis-(S)-isoserine, 1,1'-[dithiobis-[2(S)-(2-methylbenzyl)-1-oxo-3,1-propanediyl]]-bis-(S)-methionine, N-(3-phenyl-2-(mercaptomethyl)-propionyl)-(S)-4-(methylmercapto)methionine, N-[2-acetylthiomethyl-3-phenyl-propionyl]-3-aminobenzoic acid, N-[2-mercaptomethyl-3-phenyl-propionyl]-3-aminobenzoic acid, N-[1-(2-carboxy-4-phenylbutyl)-cyclopentanecarbonyl]-(S)-isoserine, N-[1-(acetylthiomethyl)cyclopentane-carbonyl]-(S)-methionine ethyl ester, 3(S)-[2-(acetylthiomethyl)-3-phenyl-propionyl]amimo-ε-caprolactam and N-(2-acetylthiomethyl-3-(2-methylphenyl)propionyl)-methionine ethyl ester, or in each case, a pharmaceutically acceptable salt thereof.

3. The pharmaceutical composition of claim 2, wherein the NEP inhibitor is N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester is a triethanolamine or tris(hydroxymethyl)aminomethane salt thereof or N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid or a pharmaceutically acceptable salt thereof.

4. The pharmaceutical composition of claim 1 further comprising a diuretic.

5. A kit comprising in separate containers in a single package pharmaceutical compositions comprising in one container a pharmaceutical composition comprising a NEP inhibitor and in a second container a pharmaceutical composition comprising valsartan.

6. A method for the treatment or prevention of a condition or disease selected from the group consisting of hypertension, heart failure such as (acute and chronic) congestive heart failure, left ventricular dysfunction and hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation, atrial flutter, detrimental vascular remodeling, myocardial infarction and its sequelae, atherosclerosis, angina (whether unstable or stable), renal insufficiency (diabetic and non- diabetic), heart failure, angina pectoris, diabetes, secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions, such as diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, and also renal vascular hypertension, diabetic retinopathy, the management of other vascular disorders, such as migraine, peripheral vascular disease, Raynaud's disease, luminal hyperplasia, cognitive dysfunction (such as Alzheimer's), glaucoma and stroke,

comprising administering a therapeutically effective amount of combination of (i) the AT 1-antagonists valsartan or a pharmaceutically acceptable salt thereof and (ii) a NEP inhibitor or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier to a mammal in need of such treatment.

7. A method as claimed in claim 6, wherein the NEP inhibitor is selected from the group consisting of SQ 28,603, N-[N-[1(S)-carboxyl-3-phenylproplyl]-(S)-phenylalanyl]-(S)-isoserine, N-[N-[((1S)-carboxy-2-phenyl)ethyl]-(S)-phenylalanyl]-β-alanine, N-[2(S)-mercaptomethyl-3-(2-methylphenyl)-propionyl]methionine, (cis-4-[[[1-[2-carboxy-3-(2-methoxyethoxy)propyl]-cyclopentyl]carbonyl]amino]-cyclohexanecarboxylic acid), thiorphan, retro-thiorphan, phosphoramidon, SQ 29072, N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester, (S)-cis-4-[1-[2-(5-indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido]-1-cyclohexanecarboxylic acid, 3-(1-[6-endo-hydroxymethylbicyclo[2,2,1]heptane-2-exo-carbamoyl]cyclopentyl)-2-(2-methoxyethyl)propanoic acid, N-(1-(3-(N-t-butoxycarbonyl-(S)-prolylamino)-2(S)-t-butoxy-carbonylpropyl)cyclopentanecarbonyl)-O-benzyl-(S)-serine methyl ester, 4-[[2-(Mercaptomethyl)-1-oxo-3-phenylpropyl]amino]benzoic acid, 3-[1-(Cis-4-carboxycarbonyl-cis-3-butylcyclohexyl-r-1-carboamoyl)cyclopentyl]-2S-(2-methoxyethoxymethyl)propanoic acid, N-((2S)-2-(4-biphenylmethyl)-4-carboxy-5-phenoxyvaleryl)glycine, N-(1-(N-hydroxycarbamoylmethyl)-1-cyclopentanecarbonyl)-L-phenylalanine, (S)-(2-biphenyl-4-yl)-1-(1H-tetrazol-5-yl)ethylamino) methylphosphonic acid, (S)-5-(N-(2-(phosphonomethylamino)-3-(4-biphenyl)propionyl)-2-aminoethyl)tetrazole, β-Alanine, 3-[1,1'-biphenyl]-4-yl-N-[diphenoxyphosphinyl)methyl]-L-alanyl, N-(2-carboxy-4-thienyl)-3-mercapto-2-benzylpropanamide, 2-(2-mercaptomethyl-3-phenylpropionamido)thiazol-4-ylcarboxylic acid, (L)-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy)carbonyl)-2-phenylethyl)-L-phenylalanyl)-β-alanine, N-[N-[(L)-[1-[(2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy]carbonyl]-2-phenylethyl]-L-phenylalanyl]-(R)-alanine, N-[N-[(L)-1-carboxy-2-phenylethyl]-L-phenylalanyl]-(R)-alanine, N-[2-acetylthiomethyl-3-(2-methyl-phenyl)propionyl]-methionine ethyl ester, N-[2-mercaptomethyl-3-(2-methylphenyl)propioyl]-methionine, N-[2(S)-mercaptomethyl-3-(2-methylphenyl)propanoyl]-(S)-isoserine, N-(S)-[3-mercapto-2-(2-methylphenyl)propionyl]-(S)-2-methoxy-(R)-alanine, N-[1-[[1(S)-benzyloxycarbonyl-3-phenylpropyl]amino]cyclopentylcarbonyl]-(S)-isoserine, N-[1-[[1(S)-carbonyl-3-phenylpropy]amino]-cyclopentylcarbonyl]-(S)-isoserine, 1,1'-[dithiobis-[2(S)-(2-methylbenzyl)-

1-oxo-3,1-propanediyl]]-bis-(S)-isoserine, 1,1'-[dithiobis-[2(S)-(2-methylbenzyl)-1-oxo-3,1-propanediyl]]-bis-(S)-methionine, N-(3-phenyl-2-(mercaptomethyl)-propionyl)-(S)-4-(methylmercapto)methionine, N-[2-acetylthiomethyl-3-phenyl-propionyl]-3-aminobenzoic acid, N-[2-mercaptomethyl-3-phenyl-propionyl]-3-aminobenzoic acid, N-[1-(2-carboxy-4-phenylbutyl)-cyclopentanecarbonyl]-(S)-isoserine, N-[1-(acetylthiomethyl)cyclopentane-carbonyl]-(S)-methionine ethyl ester, 3(S)-[2-(acetylthiomethyl)-3-phenyl-propionyl]amimo-ε-caprolactam and N-(2-acetylthiomethyl-3-(2-methylphenyl)propionyl)-methionine ethyl ester, and in each case, a pharmaceutically acceptable salt thereof.

8. The method of claim 6, wherein the NEP inhibitor is N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester is a triethanolamine or tris(hydroxymethyl)aminomethane salt thereof or N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid or a pharmaceutically acceptable salt thereof.

9. A triethanolamine salt of N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester

10. A tris(hydroxymethyl)aminomethane salt of N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester.

11. A pharmaceutical composition comprising the salt of claim 9.

12. A pharmaceutical composition comprising the salt of claim 10.

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/00415

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/41 A61K31/192 A61K31/216 A61P9/12 A61P9/10
A61P13/12 A61P25/28 A61P3/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 01 74348 A (BRISTOL MYERS SQUIBB CO ;WOLF ROBERT A (US); CHANG PAUL I (US); RE) 11 October 2001 (2001-10-11) page 1, line 9,10 page 14, line 8; claims 1,3 page 8, line 6 page 12, line 1,2 --- | 1,4-6 |
| Y | US 5 217 996 A (KSANDER GARY) 8 June 1993 (1993-06-08) cited in the application claims 5,6 --- | 1-12 |
| X | EP 0 498 361 A (SCHERING CORP) 12 August 1992 (1992-08-12) | 1-8 |
| Y | claims 1,2,4,5,9 column 14, line 41-54 --- -/-- | 1-12 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 13 May 2003 | 20/05/2003 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL – 2280 HV Rijswijk<br>Tel. (+31–70) 340–2040, Tx. 31 651 epo nl,<br>Fax: (+31–70) 340–3016 | Veronese, A |

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/00415

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | EP 0 726 072 A (SQUIBB BRISTOL MYERS CO)<br>14 August 1996 (1996-08-14)<br>claim 1; examples 1,2 | 1-8 |
| P,X | WO 02 092622 A (NOVARTIS ERFIND VERWALT GMBH ;NOVARTIS AG (CH); FINK CYNTHIA ANNE)<br>21 November 2002 (2002-11-21)<br>page 23, line 14<br>page 1, paragraphs 1-3<br>claims 1,12 | 1,5,6 |
| P,X | WO 02 06253 A (NOVARTIS ERFIND VERWALT GMBH ;MARTI ERWIN (CH); NOVARTIS AG (CH);)<br>24 January 2002 (2002-01-24)<br>claims 1,12<br>See page 36, ACE/NEP inhibitor | 1,5,6 |

International Application No. PCT/EP 03 /00415

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1,2, 5, 7, relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the NEP inhibitors having the formula II as disclosed in the description at page 3, and the ones disclosed in claim 3, 8-12.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 03/00415

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 6-8 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 03/00415

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US005217996A

# United States Patent [19]

**Ksander**

[11] **Patent Number: 5,217,996**

[45] **Date of Patent: Jun. 8, 1993**

[54] **BIARYL SUBSTITUTED 4-AMINO-BUTYRIC ACID AMIDES**

[75] Inventor: **Gary Ksander**, Milford, N.J.

[73] Assignee: **Ciba-Geigy Corporation**, Ardsley, N.Y.

[21] Appl. No.: **824,132**

[22] Filed: **Jan. 22, 1992**

[51] **Int. Cl.**[5] ..... **C07C 229/34**; A61K 31/235

[52] **U.S. Cl.** ..... **514/533**; 514/563; 546/335; 549/452; 558/267; 558/275; 560/41; 562/450

[58] **Field of Search** ..... 560/41, 39; 562/450; 549/77, 452, 496; 546/335; 558/267, 275; 514/533, 563

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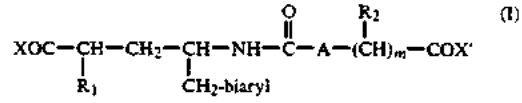
*Assistant Examiner*—B. Frazier
*Attorney, Agent, or Firm*—Norbert Gruenfeld

[57] **ABSTRACT**

The invention relates to biaryl substituted 4-amino-butyric acid derivatives of formula I



(I)

wherein COX and COX' independently represent carboxyl or carboxyl derivatized in form of a pharmaceutically acceptable ester or amide; $R_1$ represents hydrogen, lower alkyl, $C_3$-$C_7$-cycloalkyl-lower alkyl, aryl-lower alkyl, biaryl-lower alkyl, lower alkoxy, aryl-lower alkoxy, aryloxy, N-lower alkylamino, N,N-di-lower alkylamino, N-aryl-lower alkylamino, N,N-di-aryl-lower alkylamino, N-arylamino, N,N-diarylamino, lower alkanoylamino, aryl-lower alkanoylamino or aroylamino; $R_2$ represents hydrogen, hydroxy, lower alkoxy, lower alkyl, aryl-lower alkyl, $C_3$-$C_7$-cycloalkyl-lower alkyl, amino-lower alkyl, hydroxy-lower alkyl, lower alkylthio-lower alkyl, lower alkoxy-lower alkyl, aryl-lower alkylthio-lower alkyl or aryl-lower alkoxy-lower alkyl; biaryl represents phenyl substituted by carbocyclic or heterocyclic aryl; A represents a direct bond, lower alkylene, phenylene or cyclohexylene; m represents 1 or zero, provided that m represents 1 when A is a direct bond; or pharmaceutically acceptable salts thereof; pharmaceutical compositions comprising said compounds; methods for the preparation of said compounds and for the preparation of intermediates; and methods of treating disorders in mammals which are responsive to the inhibition of neutral endopeptidases by administration of said compounds to mammals in need of such treatment.

**11 Claims, No Drawings**

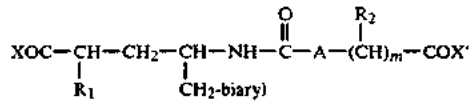
# BIARYL SUBSTITUTED 4-AMINO-BUTYRIC ACID AMIDES

## SUMMARY OF THE INVENTION

Endogenous atrial natriuretic peptides (ANP), also called atrial natriuretic factors (ANF) have diuretic, natriuretic and vasorelaxant functions in mammals. The natural ANF peptides are metabolically inactivated, in particular by a degrading enzyme which has been recognized to correspond to the enzyme neutral endopeptidase (NEP) EC 3.4. 24.11, also responsible for e.g. the metabolic inactivation of enkephalins.

The aim of the present invention is to provide novel biaryl substituted 4-amino-butyric acid amide derivatives described below which are useful as neutral endopeptidase (NEP) inhibitors, e.g. as inhibitors of the ANF-degrading enzyme in mammals, so as to prolong and potentiate the diuretic, natriuretic and vasodilator properties of ANF in mammals, by inhibiting the degradation thereof to less active metabolites. The compounds of the invention are thus particularly useful for the treatment of conditions and disorders responsive to the inhibition of neutral endopeptidase EC 3.4. 24.11, particularly cardiovascular disorders, such as hypertension, renal insufficiency including edema and salt retention, pulmonary edema and congestive heart failure. By virtue of their inhibition of neutral endopeptidase, the compounds of the invention may also be useful for the treatment of pain, depression and certain psychotic conditions. Other potential indications include the treatment of angina, premenstrual syndrome, Meniere's disease, hyperaldosteronism, hypercalciuria, ascites, glaucoma, asthma, inflammations and gastrointestinal disorders such as diarrhea, irritable bowel syndrome and gastric hyperacidity.

The present invention relates to biaryl substituted 4-amino-butyric acid derivatives of formula I



(I)

wherein COX and COX' independently represent carboxyl or carboxyl derivatized in form of a pharmaceutically acceptable ester or amide; $R_1$ represents hydrogen, lower alkyl, $C_3$-$C_7$-cycloalkyl-lower alkyl, aryl-lower alkyl, biaryl-lower alkyl, lower alkoxy, aryl-lower alkoxy, aryloxy, N-lower alkylamino, N,N-di-lower alkylamino, N-aryl-lower alkylamino, N,N-di-aryl-lower alkylamino, N-arylamino, N,N-diarylamino, lower alkanoylamino, aryl-lower alkanoylamino or aroylamino; $R_2$ represents hydrogen, hydroxy, lower alkoxy, lower alkyl, aryl-lower alkyl, $C_3$-$C_7$-cycloalkyl-lower alkyl, amino-lower alkyl, hydroxy-lower alkyl, lower alkylthio-lower alkyl, lower alkoxy-lower alkyl, aryl-lower alkylthio-lower alkyl or aryl-lower alkoxy-lower alkyl; biaryl represents phenyl substituted by carbocyclic or heterocyclic aryl; A represents a direct bond, lower alkylene, phenylene or cyclohexylene; m represents 1 or zero, provided that m represents 1 when A is a direct bond; or a pharmaceutically acceptable salt thereof.

Pharmaceutically acceptable ester and amide derivatives are preferably prodrug derivatives, such being convertible by solvolysis or under physiological conditions to the free carboxylic acids of formula I wherein COX and/or COX' represent carboxyl.

Compounds of formula I and derivatives thereof, depending on the nature of substituents, possess one or more asymmetric carbon atoms. The resulting diastereoisomers and optical antipodes are encompassed by the instant invention.



## DETAILED DESCRIPTION OF THE INVENTION

The definitions used herein, unless denoted otherwise, have the following meanings within the scope of the present invention.

The term biaryl represents phenyl substituted by carbocyclic aryl or heterocyclic aryl as defined herein, ortho, meta or para to the point of attachment of the phenyl ring, advantageously para; biaryl is also represented as the $-C_6H_4-R_3$ substituent in formulae herein.

Carbocyclic aryl preferably represents preferably monocyclic carbocyclic aryl or optionally substituted naphthyl.

Monocyclic carbocyclic aryl represents optionally substituted phenyl, being preferably phenyl or phenyl substituted by one to three substituents, such being advantageously lower alkyl, hydroxy, lower alkoxy, lower alkanoyloxy, halogen, cyano, trifluoromethyl, lower alkanoylamino or lower alkoxycarbonyl. Monocyclic carbocyclic aryl particularly preferably represents phenyl or phenyl substituted by lower alkyl, lower alkoxy, hydroxy, halogen, cyano or trifluoromethyl.

Optionally substituted naphthyl represents 1- or 2-naphthyl or 1- or 2-naphthyl preferably substituted by lower alkyl, lower alkoxy or halogen.

Heterocyclic aryl represents preferably monocyclic heterocyclic aryl such as optionally substituted thienyl, indolyl, imidazolyl, furanyl, pyridyl, pyrrolyl or N-lower alkylpyrrolyl.

Optionally substituted furanyl represents 2- or 3-furanyl or 2- or 3-furanyl preferably substituted by lower alkyl.

Optionally substituted pyridyl represents 2-, 3- or 4-pyridyl or 2-, 3- or 4-pyridyl preferably substituted by lower alkyl, halogen or cyano.

Optionally substituted thienyl represents 2- or 3-thienyl or 2- or 3-thienyl preferably substituted by lower alkyl.

Optionally substituted indolyl represents preferably 2- or 3-indolyl or 2- or 3-indolyl preferably substituted by lower alkyl, lower alkoxy or halogen.

Optionally substituted imidazolyl is preferably 1- or 2-imidazolyl or 1- or 2-imidazolyl preferably substituted by lower alkyl.

Aryl as in aryl-lower alkyl, aryl-lower alkoxy, aryloxy, N-arylamino, N,N-diarylamino, aryl-lower alkoxycarbonyl or aryl-lower alkanoylamino is preferably phenyl or phenyl substituted by one or two of lower alkyl, lower alkoxy, hydroxy, lower alkanoyloxy, halogen, trifluoromethyl, cyano, lower alkanoylamino or lower alkoxycarbonyl.

The term "lower" referred to herein in connection with organic radicals or compounds respectively defines such with up to and including 7, preferably up and including 4 and advantageously one or two carbon atoms. Such may be straight chain or branched.

A lower alkyl group preferably contains 1-4 carbon atoms and represents e.g. ethyl, n- or iso-propyl, n-, iso-, sec.- or tert.-butyl or advantageously methyl.

A lower alkoxy group preferably contains 1–4 carbon atoms and represents for example methoxy, n-propoxy, isopropoxy, n-, iso-, sec.- or tert.-butoxy or advantageously ethoxy.

Aryl-lower alkyl is advantageously benzyl or phenethyl optionally substituted by one or two of lower alkyl, lower alkoxy, hydroxy, lower alkanoyloxy, halogen or trifluoromethyl.

Aryl-lower alkoxy represents advantageously e.g. benzyloxy, benzyloxy substituted by lower alkyl, lower alkoxy, lower alkanoyloxy, halogen or trifluoromethyl, or pyridylmethoxy.

Aryloxy preferably represents phenoxy or phenoxy substituted by lower alkyl, lower alkoxy, lower alkanoyloxy, halogen or trifluoromethyl.

N-arylamino and N,N-diarylamino represent advantageously N-phenylamino or N,N-diphenylamino optionally substituted in the phenyl moiety or phenyl moieties by lower alkyl, lower alkoxy, hydroxy, lower alkanoyloxy, halogen or trifluoromethyl.

The term $C_3$–$C_7$-cycloalkyl represents a saturated cyclic hydrocarbon radical which contains 3 to 7 and preferably 5 to 7 ring carbon and is, most preferably, cyclopentyl or cyclohexyl.

The term cycloalkyl-lower alkyl represents preferably 1- or 2-(cyclopentyl or cyclohexyl)ethyl, 1-, 2- or 3-(cyclopentyl or cyclohexyl)propyl, or 1-, 2-, 3- or 4-(cyclopentyl or cyclohexyl)-butyl.

Amino-lower alkyl represents preferably amino-(ethyl, propyl or butyl), particularly omega-amino-(ethyl, propyl or butyl).

A N-lower alkylamino group preferably contains 1–4 carbon atoms in the lower alkyl portion and represents, for example, N-n-propyl-amino, N-iso-propylamino, N-n-butylamino, N-tert.-butylamino and advantageously N-methylamino or N-ethylamino.

A N,N-di-lower alkylamino group preferably contains 1–4 carbon atoms in each lower alkyl portion and represents, for example, N,N-dimethylamino, N-methyl-N-ethylamino and advantageously N,N-diethylamino.

Hydroxy-lower alkyl is for example 2-hydroxyethyl and preferably hydroxymethyl.

Lower alkylthio as in lower alkylthio-lower alkyl represents advantageously $C_1$–$C_4$-alkylthio and preferably methylthio or ethylthio.

Lower alkylene represents branched or straight chain alkylene of 1 to 7 carbon atoms, advantageously straight chain (or linear) alkylene, such as methylene, ethylene, propylene, butylene, pentylene or hexylene and most preferably straight chain $C_1$–$C_4$-alkylene.

Phenylene represents preferably 1,3 or 1,4-phenylene, advantageously 1,4-phenylene.

Cyclohexylene represents preferably 1,4-cyclohexylene.

Halogen (halo) preferably represents fluoro or chloro, but may also be bromo or iodo.

Lower alkanoyloxy advantageously contains 2 to 5 carbon atoms and is preferably acetoxy, pivaloyloxy or propionyloxy.

Lower alkanoylamino advantageously contains 2 to 5 carbon atoms and is preferably acetylamino or propionylamino.

A lower alkoxycarbonyl group preferably contains 1 to 4 carbon atoms in the alkoxy portion and represents, for example, methoxycarbonyl, n-propoxycarbonyl, iso-propoxycarbonyl or advantageously ethoxycarbonyl.

Aroylamino is preferably benzoylamino or benzoylamino substituted on the benzene ring by lower alkyl, lower alkoxy, halogen or trifluoromethyl.

Carboxyl esterified in form of a pharmaceutically acceptable ester, represents advantageously a prodrug ester that may be convertible by solvolysis or under physiological conditions to the free carboxylic acid, such being preferably $C_1$–$C_{20}$-alkoxycarbonyl, advantageously lower alkoxycarbonyl; (amino, acylamino, mono-or di-lower alkylamino)-lower alkoxycarbonyl; carboxy-lower alkoxycarbonyl, e.g. alpha-carboxy-lower alkoxycarbonyl; lower alkoxycarbonyl-lower alkoxycarbonyl, e.g. alpha-lower alkoxycarbonyl-lower alkoxycarbonyl; α-(di-lower alkylamino, amino, mono-lower alkylamino, morpholino, piperidino, pyrrolidino, 1-lower alkylpiperazino)-carbonyl-lower alkoxycarbonyl; aryl-lower alkoxycarbonyl, preferably optionally (halo, lower alkyl or lower alkoxy)-substituted benzyloxycarbonyl, or pyridylmethoxycarbonyl; 1-(hydroxy, lower alkanoyloxy or lower alkoxy)-lower alkoxycarbonyl, e.g. pivaloyloxymethoxycarbonyl; (hydroxy, lower alkanoyloxy or lower alkoxy)-lower alkoxymethoxycarbonyl; bicycloalkoxycarbonyl-lower alkoxycarbonyl, e.g. bicyclo[2,2,1]-heptyloxycarbonyl-lower alkoxycarbonyl, especially bicyclo-[2,2,1]-heptyloxycarbonylmethoxycarbonyl such as bornyloxycarbonylmethoxycarbonyl; 1-(lower alkoxycarbonyloxy)-lower alkoxycarbonyl; 5-indanyloxycarbonyl; 3-phthalidoxycarbonyl and (lower alkyl, lower alkoxy or halo)-substituted 3-phthalidoxycarbonyl; polyhydroxy-lower alkoxycarbonyl or protected polyhydroxy-lower alkoxycarbonyl in which polyhydroxy-lower alkoxy and protected polyhydroxy-lower alkoxy represent preferably dihydroxypropyloxy or trihydroxybutyloxy wherein hydroxy groups are free or one or more, as appropriate, are protected in form of esters, e.g. a lower alkanoyl or a benzoyl ester, in form of ethers, e.g. a lower alkyl or benzyl ether, or, in case two vicinal hydroxy groups are involved, in the form of acetals or ketals, e.g. a lower alkylidene, a benzylidene or a 5- or 6-membered cycloalkylidene derivative.

Protected polyhydroxy-lower alkoxycarbonyl advantageously represents (2,2-dimethyl-1,3-dioxolan-4-yl)-methoxycarbonyl.

Acyl as in acyloxy or acylamino represents preferably lower alkanoyl, carbocyclic aryl-lower alkanoyl, aroyl, lower alkoxycarbonyl or aryl-lower alkoxycarbonyl, advantageously lower alkanoyl. Lower alkoxycarbonyl for acyl is preferably t-butoxycarbonyl (abbreviated t-BOC). Aryl-lower alkoxycarbonyl for acyl is preferably benzyloxycarbonyl (abbreviated CBZ).

Carboxy-lower alkoxycarbonyl represents advantageously e.g. 1-carboxyethoxycarbonyl.

Lower alkoxycarbonyl-lower alkoxycarbonyl represents advantageously e.g. 1-(ethoxycarbonyl)ethoxycarbonyl.

Amino-lower alkoxycarbonyl, mono-lower alkylamino-lower alkoxycarbonyl, di-(lower)alkylamino-lower alkoxycarbonyl advantageously represent e.g. aminoethoxycarbonyl, ethylaminoethoxycarbonyl, diethylaminoethoxycarbonyl.

Lower alkylidene is preferably isopropylidene.

Cycloalkylidene is preferably cyclohexylidene.

Carboxyl esterified in form of a pharmaceutically acceptable prodrug ester represents most advantageously $C_1$–$C_4$-alkoxycarbonyl, phenyloxycarbonyl, benzyloxycarbonyl optionally substituted on phenyl by lower alkyl, lower alkoxy, halo or trifluoromethyl,

pivaloyloxymethoxycarbonyl, 1-($C_2$–$C_4$-alkanoyloxy)-ethoxycarbonyl, (2,2-dimethyl-1,3-dioxolan-4-yl)-methoxycarbonyl, 5-indanyloxycarbonyl, 3-phthalidoxycarbonyl, bornyloxycarbonylmethoxycarbonyl, 1-($C_1$–$C_4$-alkoxycarbonyloxy)-ethoxycarbonyl or 3-pyridylmethoxycarbonyl.

Carboxyl derivatized in the form of a pharmaceutically acceptable amide represents preferably carbamoyl or N-substituted carbamoyl, advantageously [lower alkylamino, arylamino, di-lower alkylamino, morpholino, N-lower alkylpiperazino, pyrrolidino, piperidino, perhydroazepino, (amino or acylamino)-lower alkylamino or aryl-lower alkylamino]-carbonyl.

Pharmaceutically acceptable salts are either pharmaceutically acceptable acid addition salts for any basic compounds of the invention or salts derived from pharmaceutically acceptable bases for any acidic compounds of the invention.

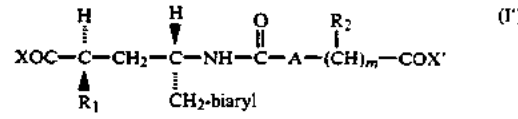
Pharmaceutically acceptable salts of basic compounds of the invention are acid addition salts, which are preferably such of therapeutically acceptable inorganic or organic acids, such as strong mineral acids, for example hydrohalic, e.g. hydrochloric or hydro-bromic acid, sulfuric, phosphoric or nitric acid; aliphatic or aromatic carboxylic or sulfonic acids, e.g. formic, acetic, propionic, succinic, glycollic, lactic, malic, tartaric, gluconic, citric, maleic, fumaric, pyruvic, phenylacetic, benzoic, 4-aminobenzoic, anthranilic, 4-hydroxybenzoic, salicylic, 4-aminosalicylic, pamoic, nicotinic, methanesulfonic, ethanesulfonic, hydroxyethanesulfonic, 1,2-ethanedisulfonic acid, benzenesulfonic, p-toluenesulfonic, naphthalenesulfonic, sulfanilic, cyclohexylsulfamic acid, or ascorbic acid.

Pharmaceutically acceptable salts of the acidic compounds of the invention, e.g. those having a free carboxyl group are salts formed with pharmaceutically acceptable bases, e.g. alkali metal salts (e.g. sodium, potassium salts), alkaline earth metal salts (e.g. magnesium, calcium salts), ammonium salts, mono-, di- or tri-lower (alkyl or hydroxyalkyl)-ammonium salts (e.g. ethanolammonium, diethanolammonium, triethanolammonium, tromethamine salts).

The compounds of the invention, of formula I and derivatives thereof may contain several asymmetric carbon atoms, depending on the nature of the substituents. Thus the compounds of the invention exist in the form of geometric isomers, racemates, diastereoisomers, pure enantiomers or mixtures thereof, all of which are within the scope of the invention.

For example, the compounds of formula I exist in isomeric forms, e.g. wherein the asymmetric carbon atom on the butyryl chain bearing the $R_1$ and/or biarylmethyl groups may either exist in the S or R configuration. The compounds of the invention, e.g. those of formula I having said two asymmetric centers exist as two different racemic diastereoisomeric forms which may be called erythro and threo depending on the relative orientation of the $R_1$ and biarylmethyl substituents of the chain. Each of the two racemates consists of the optically active enantiomers (or antipodes) having (S,S), (R,R), (R,S) or (S,R) configurations, respectively.

Preferred is the threo racemic form and particularly the enantiomeric form depicted in formula I'

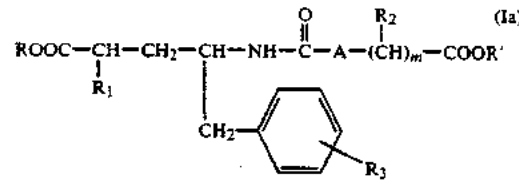


(I')

wherein COX, COX', $R_1$, $R_2$, A, biaryl and m have the meanings as defined herein above for compounds of formula I. The compounds of formulae Ia, Ib, Ic, Id, Ie and If given below are present as well, preferably in the enantiomeric form depicted in formula I'.

Illustrative thereof, in the above compounds of formula I wherein $R_1$ is lower alkyl, the carbon atom carrying said substituent is assigned the (R)-configuration; and the carbon atom carrying the biarylmethyl substituent is assigned the (S)-configuration.

More particularly, the present invention is concerned with and has for its object the compounds of formula Ia



(Ia)

wherein COOR and COOR' independently represent carboxyl or carboxyl derivatized in form of a pharmaceutically acceptable ester; $R_1$ represents hydrogen, lower alkyl, lower alkoxy, N-lower alkylamino, lower alkanoylamino, aryl-lower alkyl, aryl-lower alkoxy, aryloxy, N-arylamino or aroylamino wherein aryl in each case represents phenyl optionally substituted by lower alkyl, lower alkoxy, halogen, hydroxy, cyano, acyloxy or trifluoromethyl, or aryl represents thienyl or furanyl optionally substituted by lower alkyl; $R_2$ represents hydrogen, hydroxy, lower alkyl or aryl-lower alkyl wherein aryl independently has the meaning given above under $R_1$; $R_3$ represents phenyl, or phenyl substituted by lower alkyl, lower alkoxy, halogen, cyano, acyloxy or trifluoromethyl; or $R_3$ represents thienyl or furanyl optionally substituted by lower alkyl; A represents a direct bond, lower alkylene, 1,4-phenylene or 1,4-cyclohexylene; m represents 1 or zero provided that m represents 1 when A is a direct bond; or a pharmaceutically acceptable salt thereof.

Advantageously, $R_3$ is located in the para position.

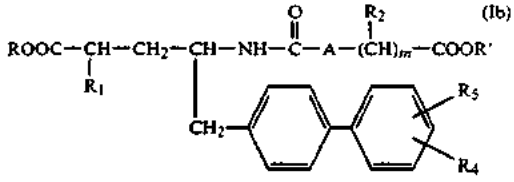
Particularly preferred embodiments of the invention as described above relate to:

a) compounds wherein $R_3$ is phenyl or phenyl substituted by lower alkyl, lower alkoxy, halogen, cyano, acyloxy or trifluoromethyl;

b) compounds wherein A is lower alkylene, m represents 1 or zero, and $R_2$ represents hydrogen, lower alkyl, hydroxy or lower alkoxy.

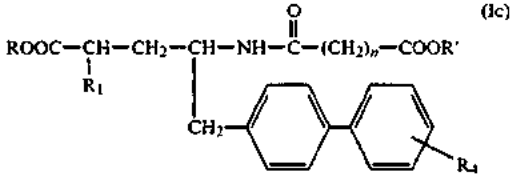
c) compounds wherein $R_1$ represents hydrogen, lower alkyl, lower alkoxy or aryl-lower alkyl wherein aryl represents phenyl optionally substituted by one or two of lower alkyl, lower alkoxy, halogen, hydroxy, cyano, acyloxy or trifluoromethyl; most preferably compounds wherein $R_1$ represents lower alkoxy or lower alkyl.

A particular embodiment of the invention relates to compounds of formula Ib



wherein COOR and COOR' independently represent carboxyl or carboxyl derivatized in form of a pharmaceutically acceptable ester; $R_1$ is hydrogen, lower alkyl, lower alkoxy or aryl-lower alkyl wherein aryl represents phenyl optionally substituted by lower alkyl, lower alkoxy, halogen, hydroxy, cyano, acyloxy or trifluoromethyl; $R_2$ represents hydrogen, hydroxy or lower alkoxy; $R_4$ and $R_5$ independently represent hydrogen, lower alkyl, hydroxy, lower alkoxy, halogen, cyano or trifluoromethyl; A represents lower alkylene; m represents 1 or zero; or a pharmaceutical acceptable salt thereof.

Particularly preferred are compounds of formula Ic

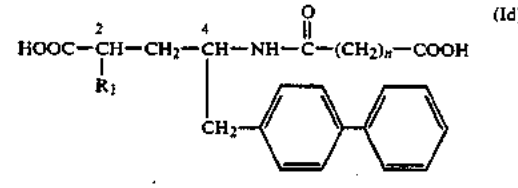


wherein COOR and COOR' independently represent carboxyl or carboxyl derivatized in form of a pharmaceutically acceptable ester; $R_1$ is lower alkyl or lower alkoxy; $R_4$ represents hydrogen, lower alkyl, lower alkoxy, halogen, or trifluoromethyl; n represents an integer 1 through 6; or a pharmaceutical acceptable salt thereof.

Preferred are compounds of formula Ic wherein COOR and COOR' independently represent carboxyl, $C_1$–$C_{20}$-alkoxycarbonyl, (carbocyclic or heterocyclic aryl)-lower alkoxycarbonyl, (di-lower alkylamino, N-lower alkylpiperazino, morpholino, pyrrolidino, piperidino or perhydrazepino)-$C_2$ to $C_4$-alkoxycarbonyl, dihydroxypropyloxycarbonyl protected in form of a ketal, 5-indanyloxycarbonyl, 3-phthalidoxycarbonyl, bicycloalkoxycarbonyl-lower alkoxycarbonyl, α-(lower alkoxycarbonyl or di-lower alkylaminocarbonyl)-lower alkoxycarbonyl, 1-(lower alkoxycarbonyloxy)-lower alkoxycarbonyl or 1-(lower alkanoyloxy)-lower alkoxycarbonyl; or a pharmaceutically acceptable salt thereof.

Particularly preferred are said compounds of formula Ic wherein COOR and COOR' independently represent carboxyl, $C_1$–$C_4$-alkoxycarbonyl, 3-pyridylmethoxycarbonyl, benzyloxycarbonyl optionally substituted on phenyl by lower alkyl, lower alkoxy, halo or trifluoromethyl, 5-indanyloxycarbonyl, 1-($C_2$–$C_5$-alkanoyloxy)-ethoxycarbonyl, 3-phthalidoxycarbonyl, (2,2'-dimethyl-1,3-dioxolan-4-yl)-methoxycarbonyl, bornyloxycarbonylmethoxycarbonyl, 1-($C_1$–$C_4$-alkoxycarbonyloxy)-ethoxycarbonyl; or a pharmaceutically acceptable salt thereof.

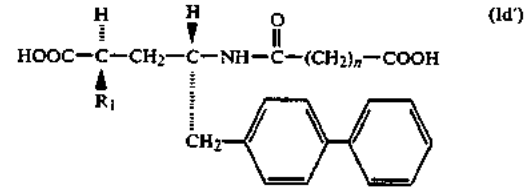
A preferred embodiment of the invention relates to compounds of formula Id



wherein $R_1$ is lower alkyl; n is an integer 1 through 4; or a pharmaceutically acceptable mono- or di-ester derivative thereof in which one or two of the acidic hydroxy groups of the carboxyl functional groups are esterified in form of a mono- or di-pharmaceutically acceptable ester; or a pharmaceutically acceptable salt thereof; or an optical antipode thereof.

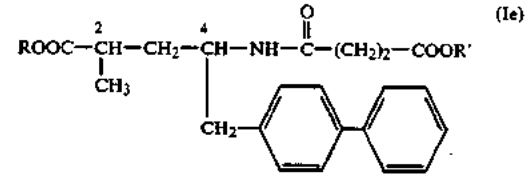
Preferred are said compounds of formula Id wherein $R_1$ is methyl and n is 2; and mono- or di-esters thereof.

As discussed before, the butyric acid compounds of e.g. formula Id exist in two distinct diastereomeric forms which may be called erythro and threo. Preferred are e.g. the compounds of formula Id as the threo diastereomer (racemate), more particularly as the enantiomeric form having the R-configuration at C-atom 2 and the S-configuration at C-atom 4 and wherein the butyryl portion is as depicted in formula Id'



wherein $R_1$ and n are as defined under formula Id; or a pharmaceutical acceptable mono-or diester derivative thereof; or a pharmaceutical acceptable salt thereof.

Particularly preferred are compounds of formula Ie



wherein COOR and COOR' independently represent carboxyl or carboxyl esterified in form of a pharmaceutical acceptable prodrug ester; or a pharmaceutically acceptable salt thereof.

Particularly preferred embodiments of the invention as described above relate to:

(a) compounds of the above formula Ie wherein R and R' independently represent hydrogen, $C_1$–$C_4$-alkyl, benzyl optionally substituted on phenyl by lower alkyl, lower alkoxy, halo or trifluoromethyl, pivaloyloxymethyl, 1-($C_2$–$C_4$-alkanoyloxy)-ethyl, (2,2-dimethyl-1,3-dioxolan-4-yl)-methyl, 5-indanyl, 3-phthalidyl, bornyloxycarbonylmethyl, 1-($C_1$–$C_4$-alkoxycarbonyloxy)-ethyl or 3-pyridylmethyl; or a pharmaceutically acceptable salt thereof;

(b) compounds of the above formula Ie wherein COOR' is carboxyl; and COOR represents carboxyl or carboxyl derivatized in form of a pharma-

ceutically acceptable ester; or a pharmaceutically acceptable salt thereof;

(c) compounds of the above formula Ie having the R-configuration at C-atom 2 and the S-configuration at C-atom 4;

(d) the compound according to the above formula Ie wherein COOR is ethoxycarbonyl and COOR' is carboxyl, namely being 4-[N-(3-carboxy-1-oxopropyl)amino]-4-(p-phenylphenylmethyl)-2-methylbutanoic acid ethyl ester, the (2R,4S)antipode thereof or a pharmaceutical acceptable salt thereof.

The novel compounds of the invention are pharmacologically potent neutral endopeptidase enzyme inhibitors which inhibit e.g. the degradation of atrial natriuretic factors (ANF) in mammals. They thus potentiate the diuretic and natriuretic effect of exogenous or endogenous ANF in mammals.

The compounds of the invention are thus particularly useful in mammals as diuretic, natriuretic (saluretic) and antihypertensive agents for the treatment of e.g. hypertension, congestive heart failure and edema.

As neutral endopeptidase inhibitors, the compounds are also e.g. enkephalinase inhibitors so as to inhibit the degradation of endogenous enkephalins and may thus also be useful for the treatment of pain in mammals.

The above-cited properties are demonstrable in vitro and in vivo tests, using advantageously mammals, e.g. mice, rats, dogs, monkeys or isolated organs, tissues and preparations thereof. Said compounds can be applied in vitro in the form of solutions, e.g. preferably aqueous solutions, and in vivo either enterally, parenterally, advantageously intravenously, e.g. as a suspension or in aqueous solution. The dosage in vitro may range between about $10^{-4}$ molar and $10^{-9}$ molar concentrations. The dosage in vivo may range depending on the route of administration, between about 0.01 and 50 mg/kg, advantageously between about 1.0 and 25 mg/kg.

The analgesic activity can be determined by measuring the potentiation of the analgesic effects of enkephalin and derivatives thereof, and by classical analgesic tests, such as the phenyl-p-benzoquinone induced writing test [J. Pharmacol. Exp. Therap. 125, 237 (1959)] and the hot plate test in the mouse [J. Pharmacol. Exp. Therap. 107, 385 (1953).

The antihypertensive activity can be determined in the spontaneously hypertensive rat, Goldblatt rat or Goldblatt dog by direct measurement of blood pressure. Advantageously, the effect is measured in the DOCA-salt hypertensive rat and/or renal hypertensive rat or dog model.

The diuretic (saluretic) activity can be determined in standard diuretic screens, e.g. as described in "New Antihypertensive Drugs", Spectrum Publications, 1976, pages 307–321, or by measuring the potentiation of atrial natriuretic factor-induced natriuresis and diuresis in the rat.

The potentiation of ANF can also be determined by measuring the increase in ANF plasma level achieved.

The in vitro inhibition of neutral endopeptidase (NEP) 3.4.24.11 can be determined as follows:

Neutral endopeptidase 3.4.24.11 activity is determined by the hydrolysis of the substrate glutaryl-Ala-Ala-Phe-2-naphthylamide (GAAP) using a modified procedure of Orlowski and Wilk (1981). The incubation mixture (total volume 125 µl) contains 4.2 µg of protein (rat kidney cortex membranes prepared by method of Maeda et al, 1983), 50 mM tris buffer, pH 7.4 at 25° C., 500 µM substrate (final concentration), and leucine aminopeptidase M (2.5 µg). The mixture is incubated for 10 minutes at 25° C. and 100 µl of fast garnet (250 µg fast garnet/ml of 10% Tween 20 in 1M sodium acetate, pH 4.2) is added. Enzyme activity is measured spectrophotometrically at 540 nm. One unit of NEP 24.11 activity is defined as 1 nmol of 2-naphthylamine released per minute at 25° C. at pH 7.4. $IC_{50}$ values are determined, i.e. the concentration of test compound required for 50% inhibition of the release of 2-naphthylamine.

Neutral endopeptidase activity is also determined using ANF as a substrate. A trial natriuretic factor degrading activity is determined by measuring the disappearance of rat-ANF (r-ANF) using a 3 minute reverse phase-HPLC separation. An aliquot of the enzyme in 50 mM Tris HCl buffer, pH 7.4, is preincubated at 37° C. for 2 minutes and the reaction is initiated by the addition of 4 nmol of r-ANF in a total volume of 50 µl. The reaction is terminated after 4 minutes with the addition of 30 µl of 0.27% trifluoroacetic acid (TFA). Forty microliters of the mixture is injected into a reverse phase-HPLC and analyzed using a C4 cartridge in a 3 minute, isocratic separation. Twenty-three percent of buffer B (0.1% TFA in 80% acetonitrile) is used. Buffer A is 0.1% TFA in water. One unit of activity is defined as the hydrolysis of 1 nmol of r-ANF per minute at 37° C. at pH 7.4. $IC_{50}$ values are determined, i.e. the concentration of test compound required for 50% inhibition of the hydrolysis of ANF.

The test compound is dissolved in dimethyl sulfoxide or 0.25M sodium bicarbonate solution, and the solution is diluted with pH 7.4 buffer to the desired concentration.

In vitro testing is most appropriate for the free carboxylic acids of the invention.

The effect of the compounds of the invention on rat plasma ANF concentration can be determined as follows:

Male Sprague-Dawley rats (275–390 g) are anesthetized with ketamine (150 mg/kg)/acepromazine (10%) and instrumented with catheters in the femoral artery and vein to obtain blood samples and infuse ANF, respectively. The rats are tethered with a swivel system and are allowed to recover for 24 hours before being studied in the conscious, unrestrained state.

In this assay, plasma ANF levels are determined in the presence and absence of NEP inhibition. On the day of study, all rats are infused continuously with ANF at 450 ng/kg/min. i.v. for the entire 5 hours of the experiment. Sixty minutes after beginning the infusion, blood samples for baseline ANF measurements are obtained (time 0) and the rats are then randomly divided into groups treated with the test compound or vehicle. Additional blood samples are taken 30, 60, 120, 180 and 240 minutes after administration of the test compound.

Plasma concentrations are determined by a specific radioimmunoassay. The plasma is diluted (×12.5, ×25 and ×50) in buffer containing: 50 mM Tris (pH 6.8), 154 mM NaCl, 0.3% bovine serum albumin, 0.01% EDTA. One hundred microliters of standards [rANF (99–126)] or samples are added to 100 µl of rabbit anti-rANF serum and incubated at 4° C. for 16 hours. Ten thousand cpm of [$^{125}$I]rANF are then added to the reaction mixture which is incubated at 4° C. for an additional 24 hours. Goat anti-rabbit IgG serum coupled to paramagnetic particles is added to the reaction mixture and bound [$^{125}$I]rANF is pelleted by exposing the mixture to

an attracting magnetic rack. The supernatant is decanted and the pellets counted in a gamma counter. All determinations are performed in duplicate. Plasma ANF levels are expressed as a percent of those measured in vehicle-treated animals which received ANF alone (450 ng/kg/min i.v.).

Illustrative of the invention, N-(3-carboxy-1-oxopropyl)-(4S)-(p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester at doses of about 1–30 mg/kg p.o., administered in 10% ethanol/polyethylene glycol (PEG) 400, produces significant increases in plasma ANF levels.

The antihypertensive effect can be determined in desoxycorticosterone acetate (DOCA)-salt hypertensive rats.

DOCA-salt hypertensive rats (280–380 g) are prepared by the standard method. Rats underwent a unilateral nephrectomy and one week later are implanted with silastic pellets containing 100 mg/kg of DOCA. The rats are maintained on 1% NaCl/0.2% KCl drinking water for three to five weeks until sustained hypertension is established. The antihypertensive activity is evaluated at this time.

Two days before an experiment, the rats are anesthetized with methoxyflurane and instrumented with catheters in the femoral artery to measure arterial blood pressure. Forty-eight hours later, baseline arterial pressure and heart rate are recorded during a 1 hour period. The test compound (30 mg/kg p.o.) or vehicle is then administered and the same cardiovascular parameters are monitored for an additional 5 hours.

Illustrative of the invention, N-(3-carboxy-1-oxopropyl)-(4S)-(p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester at a dose of 30 mg/kg p.o., administered in PEG 400, produces a significant reduction in blood pressure in the DOCA-salt hypertensive rat model.

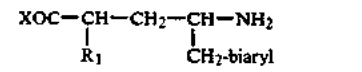
The potentiation of the natriuretic effect of ANF can be determined as follows:

Male Sprague-Dawley rats (280–360 g) are anesthetized with Inactin (100 mg/kg i.p.) and instrumented with catheters in the femoral artery, femoral vein and urinary bladder to measure arterial pressure, administer ANF and collect urine, respectively. A continuous infusion of normal saline (33 μl/min) is maintained throughout the experiment to promote diuresis and sodium excretion. The experimental protocol consists of an initial 15 minute collection period (designated as pre-control) followed by three additional collection periods. Immediately after completion of the pre-control period, test compound or vehicle is administered; nothing is done for the next 45 minutes. Then, blood pressure and renal measurements are obtained during a second collection period (designated control; 15 min). At the conclusion of this period, ANF is administered (1 μg/kg i.v. bolus) to all animals and arterial pressure and renal parameters are determined during two consecutive 15 minutes collection periods.

Mean arterial pressure, urine flow and urinary sodium excretion are determined for all collection periods. Blood pressure is measured with a Gould p50 pressure transducer, urine flow is determined gravimetrically, sodium concentration is measured by flame photometry, and urinary sodium excretion is calculated as the product of urine flow and urine sodium concentration.

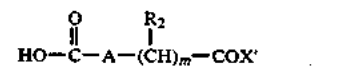
The compounds of the invention are thus particularly useful as inhibitors of neutral endopeptidase, enhancing the potency and duration of action of artrial natriuretic peptide(s). The compounds are therefore particularly useful for the treatment of cardiovascular disorders such as hypertension, edema and salt retention, and cardiac conditions such as congestive heart failure.

The compounds of the invention of formula I may be prepared using the following process which comprises: condensing a compound of formula II



(II)

wherein COX, $R_1$ and biaryl have the meaning as defined above, in temporarily protected form if required; with a compound of formula III



(III)

or a reactive functional derivative thereof, wherein A, $R_2$, m and COX' have the meaning as defined above, in temporarily protected form if required; and, if temporarily protecting any interfering reactive group(s), removing said protecting group(s), and then isolating the resulting inventive compound; and, if desired, converting any resulting compound into another compound of the invention, and/or, if desired, converting a resulting free compound into a salt or a resulting salt into the free compound or into another salt, and/or, if desired, separating a mixture of isomers or racemates obtained into the single isomers or racemates, and/or, id desired, resolving a racemate obtained into the optical antipodes.

In starting compounds and intermediates which are converted to the compounds of the invention in a manner described herein, functional groups present, such as carboxyl, amino and hydroxy groups, are optionally protected by conventional protecting groups that are common in preparative organic chemistry. Protected carboxyl, amino and hydroxy groups are those that can be converted under mild conditions into free carboxyl, amino and hydroxy groups without other undesired side reactions taking place.

The purpose of introducing protecting groups is to protect the functional groups from undesired reactions with reaction components and under the conditions used for carrying out a desired chemical transformation. The need and choice of protecting groups for a particular reaction is known to those skilled in the art and depends on the nature of the functional group to be protected (carboxyl group, amino group etc.), the structure and stability of the molecule of which the substituent is a part, and the reaction conditions.

Well-known protecting groups that meet these conditions and their introduction and removal are described, for example, in J. F. W. McOmie, "Protective Groups in Organic Chemistry", Plenum Press, London, New York 1973, T. W. Greene, "Protective Groups in Organic Synthesis", Wiley, New York 1984, and also in "The Peptides", Vol. I, Schroeder and Luebke, Academic Press, London, New York, 1965.

The preparation of compounds of the invention according to the above process, i.e. the condensation of an amine of formula II with the acid of formula III, or a functional reactive derivative thereof, is carried out by methodology well-known for peptide synthesis.

Reactive functional derivatives of compounds of formula III are preferably halides, anhydrides such as succinic anhydride, glutaric anhydride, or mixed anhydrides such as the pivaloyl, alkoxycarbonyl or cyanoacetyl anhydride.

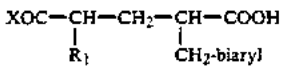
The condensation of an amine of formula II with a free carboxylic acid of formula III is carried out advantageously in the presence of a condensing agent such as dicyclohexylcarbodiimide or N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide and hydroxybenzotriazole in an inert polar solvent such as dimethylformamide or methylene chloride, preferably at room temperature.

The condensation of an amine of formula II with a reactive functional derivative of an acid of formula III in the form of an acid halide, advantageously an acid chloride, anhydride or mixed anhydride, is carried out in an inert solvent such as toluene or methylene chloride, advantageously in the presence of a base, e.g. an inorganic base such as potassium carbonate or an organic base such as triethylamine or pyridine, preferably at room temperature.

The starting materials of formula III are acids or functional derivatives thereof known in the art or which may be prepared by conventional methods known in the art.

The starting materials of formula II are known or, if new, may be prepared according to conventional methods, e.g., those illustrated by the examples herein.

For example, the compounds of formula II may be prepared by converting a compound of formula IV

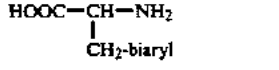


(IV)

wherein COX, $R_1$ and biaryl have the meaning mentioned above, in temporarily protected form if required, into a suitable carboxylic acid amide or carboxylic acid azide and then subjecting this compound to a Hofmann reaction or to a Curtius rearrangement in a manner well known in the art. The compounds of formula IV are known, for example, from U.S. Pat. No. 5,021,430 or may be prepared analogous to the methods described therein.

In a preferred alternative route, the starting materials of formula II may be prepared by

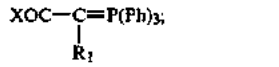
(a) reducing the carboxylic group of a biarylalanine of formula V



(V)

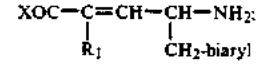
in temporarily protected form if required, to yield the respective aldehyde;

(b) subsequently reacting said aldehyde with a triphenylphosphonium compound of formula VI



(VI)

(c) hydrogenating the resulting compound of formula VII



(VII)

and, if temporarily protecting any interfering reactive group(s), removing said protective group(s) and then isolating the resulting product. In the above formulae V, VI and VII, the variables COX, $R_1$ and biaryl have the meaning as defined under formula I. The above reaction steps (a), (b) and (c) are carried out by methodology well-known in the art.

For example, in step (a) the compound of formula V, advantageously an amino protected compound of formula V, is reacted first of all with a hydroxylamine or a salt thereof, e.g. with N,O-dimethylhydroxylamine hydrochloride; the resulting hydroxylamine amide is then reduced to the aldehyde in a conventionel manner, e.g. with lithium aluminum hydride.

Reaction step (b) represents a conventional Wittig reaction which may be performed in a manner known in the art.

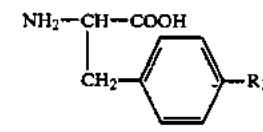
Reaction step (c) as well represents a commonly known hydrogenation reaction which may be performed e.g. with molecular hydrogen in the presence of a suitable catalyst such as palladium/charcoal.

Biarylalanines of formula V are either known in the art or can be prepared according to methods reported in the art.

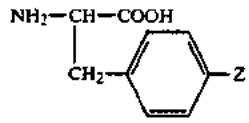
As to the preparation of the biarylalanines of formula V as starting materials in optically active form, such can be prepared e.g. by resolution or by one of the following methods:

(a) Adapting a method described in Tetrahedron Letters 1988, 6075, a biarylmethanol, e.g. 4-biphenylylmethanol, is converted to a reactive derivative, e.g. the bromide, which is then condensed with an N-acyl derivative of 2,3-diphenyl-6-oxomorpholine, e.g. the N-carbobenzyloxy-(2R,3S)-isomer, in the presence of a strong base such as sodium bis-trimethylsilylamide, to yield e.g. N-carbobenzyloxy-2(R),3(S),5(S)-6-oxo-2,3-diphenyl-5-(4-biphenylylmethyl)-morpholine. Catalytic hydrogenolysis, e.g. using hydrogen and palladium on charcoal as catalyst, yields the optically active (S)-(+)-4-biphenylalanine.

(b) Alternatively, using the Pd (0)-catalyzed cross-coupling reaction described in Tetrahedron Letters 31, 1665 (1990), J. Organic Chemistry 55, 906 (1990) and Tetrahedron 45, 6670 (1989) as developed by W. Shieh et al, the substantially optically pure chiral biarylalanines, of the formula



or the N-acyl and/or carboxy ester derivatives thereof wherein $R_3$ has meaning as defined hereinabove, can be prepared by: condensing a reactive esterified optically active tyrosine derivative of the formula



wherein the amino and carboxy groups are in protected form (as N-acyl and esterified carboxy ester derivatives), and Z represents reactive esterified hydroxy (advantageously trifluoromethylsulfonyloxy) with an aryl boronic acid in which aryl corresponds to $R_3$ as defined above, in the presence of a palladium (0) catalyst, in particular tetrakis(triphenylphosphine)palladium (0), and in the presence of an anhydrous base (such as an alkali metal carbonate), in an inert solvent (such as xylene or toluene) at an elevated temperature ranging from about 50° to 150° C., and removing any protecting groups as required.

For example, N-t-butoxycarbonyl-tyrosine methyl ester is first converted to N-t-butoxycarbonyl-4-trifluoromethylsulfonyloxy-phenylalanine methyl ester (N-t-butoxycarbonyltyrosine triflate methyl ester). This compound is then condensed with an arylboronic acid (e.g. phenylboronic acid) in the presence of anhydrous potassium carbonate, and tetrakis (triphenylphosphine) palladium (0) complex as catalyst, in toluene preferably at an elevated temperature, advantageously at about 100° to obtain N-t-butoxycarbonyl-4-biphenylalanine methyl ester. After N-deacylation, substantially optically pure 4-biphenylalanine methyl ester is obtained with a configuration corresponding to that of the tyrosine derivative used as starting material.

The arylboronic acids are either commercial or can be prepared as described in the literature, e.g. J. Org. Chem. 49,5237 (1984).

The triphenylphosphonium compounds of formula VI are either known in the art or can be prepared according to methods reported in the art.

Compounds of the invention wherein COX or COX' represent carboxyl derivatized in form of a pharmaceutically acceptable amide can also be prepared according to the above methods using corresponding starting materials wherein COX or COX' represent carbamoyl or N-substituted carbamoyl.

The compounds of the invention so obtained, can be converted into each other according to conventional methods. Thus, for example, resulting amides or esters may be hydrolyzed with aqueous alkalies, such as alkali metal carbonates or hydroxides. Resulting free acids may be esterified with e.g. said unsubstituted or substituted alkanols or reactive esterified derivatives thereof such as alkyl halides, or diazoalkanes. Free acids are also converted into said metal, ammonium or acid addition salts in conventional manner.

Thus, any resulting free acid or base can be converted into a corresponding metal, ammonium or acid addition salt respectively, by reacting it with an equivalent amount of the corresponding base, basic salt, acid or ion exchange preparation, e.g. said free acids with alkali or ammonium hydroxides or carbonates, or e.g. free amines with said inorganic or organic acids respectively. Any resulting salt may also be converted into the free compound, by liberating the latter with stronger acids or bases, respectively. In view of the close relationship between the free compounds and the salts thereof, whenever a compound of the invention, or intermediate, is referred to in this context, a corresponding salt is also intended, provided such is possible or appropriate under the circumstances.

The compounds, including their salts, may also be obtained in the form of their hydrates, or include other solvent used for the crystallization. Furthermore, the functional derivatives of the free acids of formula I, wherein the carboxy groups are esterified by identical or different radicals may be prepared by condensing a free acid of formula I or a mono- or di-ester derivative thereof with an esterifying agent of the formula VIII

$$R_6—Z \qquad \text{(VIII)}$$

wherein Z represents hydroxy or a reactive esterified hydroxyl group; and $R_6$ represents an esterifying radical as defined herein for the carboxylic esters (encompassed e.g. by COX or COX' representing esterified carboxy), in particular said non-aromatic radicals.

A reactive esterified hydroxyl group, such as Z in a compound of the formula VIII, is a hydroxyl group esterified by a strong inorganic or organic acid. Corresponding Z groups are in particular halo, for example chloro, bromo or preferably iodo, also sulfonyloxy groups, such as lower alkyl- or arylsulfonyloxy groups, for example (methane-, ethane-, benzene- or toluene-) sulfonyloxy groups, also the trifluoromethylsulfonyloxy group.

The esterification of the carboxyl groups, optionally in salt form, with a compound of formula VIII wherein Z represents a reactive esterified hydroxyl group, is performed in a manner known per se, in the presence of for example an organic base, such as an organic amine, for example a tertiary amine, such as tri-lower alkylamine, for example trimethylamine, triethylamine or ethyl-di-isopropylamine, an N,N-di-lower-alkyl-aniline, for example N,N-di-methylaniline, a cyclic tertiary amine, such as an N-lower-alkylated morpholine, for example N-methyl-morpholine, a base of the pyridine type, for example pyridine, an inorganic base, for example hydroxides, carbonates, or hydrogen carbonates of alkali metals or alkaline-earth metals, for example sodium, potassium or calcium hydroxide, carbonate or hydrogen carbonate, or a quaternary ammonium base, such as a tetraalkylammonium hydroxide, carbonate or hydrogen carbonate, for example in which alkyl is e.g. methyl, ethyl, propyl, isopropyl, butyl, or the like, or an alkali metal salt of bis-trialkylsilylamide (e.g. trimethyl) optionally in the presence of a crown ether such as 18-crown-6 in a suitable inert solvent or solvent mixture, e.g. acetonitrile, toluene, and the like.

A trifunctional free acid, e.g. of the formula I, or a monoester or diester thereof, is preferably first converted into a salt of one of the stated organic or inorganic bases, especially into the sodium or potassium salt, and is then reacted with a compound of the formula VIII. The compounds of formula VIII are known or can be prepared by methods well-known to the art.

A compound of the formula or VIII wherein Z is a reactive esterified hydroxyl group can be prepared in situ. For example, a compound of the formula VIII wherein Z is chloro can be converted by treatment with sodium iodide in a solvent, for example in acetone or acetonitrile, into a compound of the formula VIII wherein Z is iodo; or esterification can be carried out with a chloro compound of the formula VIII in the presence of sodium iodide.

Esterification of a compound with a free carboxyl group using in excess an alcohol of formula VIII

(wherein Z represents hydroxy) is carried out in a manner known per se, e.g. in the presence of an acid catalyst e.g. sulfuric acid or boron trifluoride etherate, preferably at an elevated temperature, advantageously ranging from about 40° C. to 100° C. Alternately, the esterification of a compound with a free carboxyl group can be carried out with at least an equimolar amount of the alcohol in the presence of a condensing agent such as dicyclohexylcarbodiimide or N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide in a polar solvent such as methylene chloride, in the presence of a base if required, e.g. such as 4-(dimethylamino)pyridine.

Conversely, carboxylic acid esters can be converted to compounds of the invention with a free carboxy group using methods and conditions generally known in the art and illustrated herein. Depending on type of ester involved, useful reagents include aqueous acids or bases; also anhydrous reagents such as trialkylsilyl halides, hydrobromic acid in glacial acetic acid; also hydrogen and a hydrogenolysis catalyst. For instance, trialkyl esters can be converted to the free trifunctional acids by treatment with hydrobromic acid in glacial acetic acid, e.g. at room temperature or elevated temperature. Also trialkyl esters can be converted to the mono esters wherein carboxy only remains esterified, by treatment with e.g. trimethylsilyl bromide at room temperature.

Any benzyl esters can be selectively hydrogenolyzed with e.g. hydrogen in the presence of a catalyst such as palladium on charcoal.

In the case mixtures of stereoisomers or optical isomers of the above compounds are obtained, these can be separated into the single isomers by methods in themselves known, e.g., by fractional distillation, crystallization and/or chromatography. Racemic products can be resolved into the optical antipodes, for example, by separation of diastereomeric salts thereof, e.g., for basic compounds by the fractional crystallization of d- or l-(tartrate, mandelate or camphorsulfonate) salts, or for acidic compounds by fractional crystallization of d- or l-(alpha-methylbenzylamine, cinchonidine, cinchonine, quinine, quinidine, ephedrine, dehydroabietylamine, brucine or strychnine)-salts.

The above-mentioned reactions are carried out according to standard methods, in the presence or absence of diluents, preferably such as are inert to the reagents and are solvents thereof, of catalysts, alkaline or acidic condensing or said other agents respectively and/or inert atmospheres, at low temperatures, room temperature or elevated temperatures, preferably near the boiling point of the solvents used, at atmospheric or superatmospheric pressure.

The invention further includes any variant of said processes, in which an intermediate product obtainable at any stage of the process is used as a starting material and any remaining steps are carried out, or the process is discontinued at any stage thereof, or in which the starting materials are formed under the reaction conditions, or in which the reaction components are used in the form of their salts or optically pure antipodes. Mainly those starting materials should be used in said reactions, that lead to the formation of those compounds indicated above as being preferred.

The present invention additionally relates to the use in mammals of the compounds of the invention and their pharmaceutically acceptable, non-toxic acid addition salts, or pharmaceutical compositions thereof, as medicaments, e.g. as neutral endopeptidase inhibitors, e.g. for the treatment of cardiovascular disorders such as hypertension, edema, salt retention and congestive heart failure.

The present invention also relates to the use of the compounds of the invention for the preparation of pharmaceutical compositions especially pharmaceutical compositions having neutral endopeptidase inhibiting activity, and e.g. antihypertensive or saluretic activity.

The pharmaceutical compositions according to the invention are those suitable for enteral, such as oral or rectal, transdermal and parenteral administration to mammals, including man, for the treatment of cardiovascular disorders, such as hypertension, comprising an effective amount of a pharmacologically active compound of the invention or a pharmaceutically acceptable salt thereof, alone or in combination with one or more pharmaceutically acceptable carriers.

The pharmacologically active compounds of the invention are useful in the manufacture of pharmaceutical compositions comprising an effective amount thereof in conjunction or admixture with excipients or carriers suitable for either enteral or parenteral application. Preferred are tablets and gelatin capsules comprising the active ingredient together with a) diluents, e.g. lactose, dextrose, sucrose, mannitol, sorbitol, cellulose and/or glycine; b) lubricants, e.g. silica, talcum, stearic acid, its magnesium or calcium salts and/or polyethyleneglycol; for tablets also c) binders, e.g. magnesium aluminum silicate, starch paste, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone; if desired, d) disintegrants, e.g. starches, agar, alginic acid or its sodium salt, or effervescent mixtures; and/or e) absorbents, colorants, flavors and sweeteners. Injectable compositions are preferably aqueous isotonic solutions or suspensions, and suppositories are advantageously prepared from fatty emulsions or suspensions. Said compositions may be sterilized and/or contain adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for regulating the osmotic pressure and/or buffers. In addition, the compositions may also contain other therapeutically valuable substances. Said compositions are prepared according to conventional mixing, granulating or coating methods, respectively, and contain about 0.1 to 75%, preferably about 1 to 50%, of the active ingredient.

Suitable formulations for transdermal application include an effective amount of a compound of the invention with carrier. Advantageous carriers include absorbable pharmacologically acceptable solvents to assist passage through the skin of the host. Characteristically, transdermal devices are in the form of a bandage comprising a backing member, a reservoir containing the compound, optionally with carriers, optionally a rate controlling barrier to deliver the compound to the skin of the host at a controlled and predetermined rate over a prolonged period of time, and means to secure the device to the skin.

A unit dosage for a mammal of about 50 to 70 kg may contain between about 10 and 100 mg of the active ingredient. The dosage of active compound is dependent on the species of warm-blooded animal (mammal), the body weight, age and individual condition, and on the form of administration.

The following examples are intended to illustrate the invention and are not to be construed as being limitations thereon. Temperatures are given in degrees Centigrade. If not mentioned otherwise, all evaporations are

performed under reduced pressure, preferably between about 15 and 100 mm Hg. Optical rotations are measured at room temperature at 589 nm (D line of sodium), 365 nm or other wavelengths as specified in the examples.

The prefixes R and S are used to indicate the absolute configuration at each asymmetric center.

## EXAMPLE 1

To a solution of N-(3-carbo(t)butoxy-1-oxopropyl)-(4S)-(p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester (0.80 g) in 15 ml of $CH_2Cl_2$ at room temperature are added 3 ml of trifluoroacetic acid. The mixture is stirred overnight and concentrated. The residue is dissolved in tetrahydrofuran (THF), and 6.5 ml of 1N NaOH is added. The mixture is concentrated and triturated with ether. The solid can be recrystallized from methylene chloride-hexane to give sodium N-(3-carboxyl-1-oxopropyl)-(4S)-(p-phenylphenylmethyl)-4-amino-2R-methyl butanoic acid ethyl ester melting at 159°–160° C.; $[\alpha]_D^{20} = -11.4°$ (methanol).

The starting material is prepared as follows:

A solution of α-t-BOC-(R)-tyrosine methyl ester (5.9 g, 20 mmol) and pyridine (8 mL, 100 mmol) in methylene chloride (30 mL) is cooled to 0°–5° C. Trifluoromethanesulfonic anhydride (4 mL, 23 mmol) is added at 0°–5° C., and the resulting mixture is held for another 30 minutes. The reaction mixture is diluted with water (60 mL) and methylene chloride (100 mL), and washed sequentially with 0.5N sodium hydroxide solution (1×50 mL), water (1×60 mL), 10% citric acid solution (2×75 mL) and water (1×60 mL). The organic phase is dried over $MgSO_4$ and concentrated to an oil. The oil is purified by column chromatography (silica gel, hexane/ethyl acetate, 2:1 to give methyl(R)-2-(t-butoxycarbonylamino)-3-[4-(trifluoromethylsulfonyloxy)phenyl]-propionate which crystallizes on standing; m.p. 46°–48° C.; $[\alpha]^{20}_D - 36.01°$ (c=1, $CHCl_3$).

Nitrogen is passed through a suspension of (R)-2-(t-butoxycarbonylamino)-3-[4-(trifluoromethylsulfonyloxy)-phenyl]-propionate (1.75 mmol), phenylboronic acid (3.5 mmol), anhydrous potassium carbonate (2.63 mmol) and toluene (17 mL) for 15 minutes. Tetrakis(triphenylphosphine)palladium(0) is added, and the mixture is heated at 85°–90° for 3 hours. The reaction mixture is cooled to 25° C., diluted with ethyl acetate (17 mL) and washed sequentially with saturated sodium bicarbonate (1×20 mL), water (1×20 mL), 10% citric acid (1×20 mL), water (1×20 mL) and saturated sodium chloride solution (1×20 mL). The organic phase is concentrated, and the residue is purified by column chromatography (silica gel, hexane/ethyl acetate 2:1) to yield methyl (R)-2-(t-butoxycarbonylamino)-3-(p-phenylphenyl)-propionate which can also be called N-(R)-t-butoxycarbonyl-(p-phenylphenyl)-alanine methyl ester.

To a solution of N-(R)-t-butoxycarbonyl-(p-phenylphenyl)-alanine methyl ester (6.8 g) in 60 ml of THF and 20 ml of methanol are added 20 ml of aqueous 1N sodium hydroxide solution. The mixture is stirred for 1 h at room temperature and then acidified with 21 ml of 1N hydrochloric acid. The aqueous solution is extracted 3× with ethyl acetate. The combined organic extracts are dried ($MgSO_4$), filtered and concentrated to give N-(R)-t-butoxycarbonyl-(p-phenylphenyl)-alanine, m.p. 98°–99° C.; $[\alpha]^{20}_D - 18.59°$ (c=1, methanol).

To a solution of N-(R)-t-butoxycarbonyl-(p-phenylphenyl)-alanine (4.8 g) in 70 ml of methylene chloride ($CH_2Cl_2$) at 0° C. with 1.65 g of N,O-dimethylhydroxylamine HCl, 1.7 g of triethylamine and 2.85 g of hydroxybenzotriazole are added 5.37 g of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride. The mixture is stirred 17 h at room temperature. The mixture is concentrated taken up in ethyl acetate (EtOAc) and washed with saturated sodium bicarbonate, 1N HCl and brine, then dried ($MgSO_4$), filtered and concentrated to give N-(R)-t-butoxycarbonyl-(p-phenylphenyl)-alanine N,O-dimethyl hydroxylamine amide.

To a 0° C. solution of N-(R)-t-butoxycarbonyl-(p-phenylphenyl)-alanine N,O-dimethyl hydroxylamine amide (5.2 g) in 250 ml of diethyl ether are added 0.64 g of lithium aluminum hydride. The reaction is stirred for 30 min. and quenched with aqueous potassium hydrogen sulfate. The mixture is stirred for additional 5 min., poured onto 1N HCl, extracted (3×) with EtOAc, dried ($MgSO_4$), filtered, and concentrated to give N-(R)-4-t-butoxycarbonyl-(p-phenylphenyl)-alanine carboxaldehyde as a colorless oil.

To a 0° C. solution of N-(R)-t-butoxycarbonyl-(p-phenylphenyl)-alanine carboxaldehyde (4.4 g) in 200 ml of $CH_2Cl_2$ are added 10 g of carboethoxyethylidene phenyl phosphorane. The mixture is warmed to room temperature, stirred for 1 h, washed with brine, dried ($MgSO_4$), filtered and concentrated. The residue is chromatographed on silica gel eluting with (1:2) ether:-hexane to give N-t-butoxycarbonyl-(4R)-(p-phenylphenylmethyl)-4-amino-2-methyl-2-butenoic acid ethyl ester.

A solution of N-t-butoxycarbonyl-(4R)-(p-phenylphenylmethyl)-4-amino-2-methyl-2-butenoic acid ethyl ester (4.2 g) in 400 ml of ethanol is suspended with 2.0 g of 5% palladium on charcoal and then is hydrogenated at 50 psi for 6 h. The catalyst is removed by filtration and the filtrate is concentrated to give N-t-butoxycarbonyl(4S)-(p-phenylphenylmethyl)-4-amino-2-methylbutanoic acid ethyl ester as a 80:20 mixture of diastereomers.

To the N-t-butoxycarbonyl(4S)-(p-phenylphenylmethyl)-4-amino-2-methylbutanoic acid ethyl ester (4.2 g) in 40 ml of $CH_2Cl_2$ at 0° C. is bubbled dry hydrogen chloride gas for 15 min. The mixture is stirred 2 h and concentrated to give (4S)-(p-phenylphenylmethyl)-4-amino-2-methylbutanoic acid ethyl ester hydrochloride as a 80:20 mixture of diastereomers.

To a room temperature solution of the above amine salt (3.12 g) in 15 ml of $CH_2Cl_2$ and 15 ml of pyridine are added 13.5 g of succinic anhydride. The mixture is stirred for 17 h, concentrated, dissolved in ethyl acetate, washed with 1N HCl and brine, and dried ($MgSO_4$) to give N-(3-carboxy-1-oxopropyl)-(4S)-(p-phenylphenylmethyl)-4-amino-2-methylbutanoic acid ethyl ester as a 80:20 mixture of diastereomers.

The above N-(3-carboxy-1-oxopropyl)-(4S)-(p-phenylphenylmethyl)-4-amino-2-methylbutanoic acid ethyl ester diastereomeric mixture (3.9 g) and N,N-dimethylformamide-di-t-butyl acetal (8.8 ml) are heated at 80° C. in 40 ml of toluene for 2 h. The mixture is poured onto ice-1N HCl, extracted with ether, chromatographed on silica gel eluting with (2:1) toluene:ethyl acetate to give N-(3-carbo(t)butoxy-1-oxopropyl)-(4S)-(p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester as the more polar mate-

rial and the corresponding (S,S) diastereomer as the less polar material.

## EXAMPLE 2

To a solution of N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid ethyl ester (0.33 g) in 20 ml of (1:1) ethanol:tetrahydrofuran (THF) at room temperature are added 5 ml of 1N sodium hydroxide solution (NaOH) and stirred for 17 h. The mixture is concentrated, dissolved in water and washed with ether. The aqueous layer is acidified with 1N hydrochloric acid (HCl), extracted 3× with ethyl acetate (EtOAc), dried over magnesium sulfate ($MgSO_4$), filtered and concentrated. The residue is triturated with ether to yield N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid melting at 158°–164° C., $[\alpha]_D^{20} = -23.5°$ (methanol).

## EXAMPLE 3

Following the procedures described in Examples 1 or 2, the following compounds are prepared:

N-(3-carboxy-1-oxopropyl)-(4S)-(p-phenylphenylmethyl)-4-amino-2S-methylbutanoic acid melting at 165°–167° C.;

N-(3-carboxy-1-oxopropyl)-(4S)-[p-(4-methylphenyl)-phenylmethyl]-4-amino-2R-methyl butanoic acid melting at 165°–170° C., $[\alpha]_D^{20} = -18.4°$ (c=1, methanol);

N-(3-carboxy-1-oxopropyl)-(4R)-p-phenylphenylmethyl-4-amino-2S-methylbutanoic acid, melting at 145°–149° C.;

N-(3-carboxyl-1-oxopropyl)-(4R)-p-phenylphenylmethyl-4-amino-(2R)-methylbutanoic acid, melting at 162°–165° C.;

N-(3-carboxy-1-oxopropyl)-4(S,R)-p-phenylphenylmethyl-4-amino-2(S,R)-methyl butanoic acid, melting at 165°–167° C.;

Sodium N-(3-carboxy-1-oxopropyl)-4(S,R)-p-phenylphenylmethyl-4-amino-2(S,R)-methylbutanoic acid ethyl ester, melting at 165°–167° C.;

Sodium N-(3-carboxy-1-oxopropyl)-(4R)-p-phenylphenylmethyl-4-amino-2S-methylbutanoic acid ethyl ester, melting at 117°–120° C.;

N-(3-ethoxycarbonyl-1-oxopropyl)-(4S)-(p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid, melting at 178°–190° C.;

N-(2-carboxy-1-oxoethyl)-(4S)-p-phenylphenylmethyl-4-amino-2(S,R)-methylbutanoic acid, melting at 160°–161° C.;

N-(5-carboxy-1-oxopentyl)-(4S)-p-phenylphenylmethyl-4-amino-2R-methylbutanoic acid, melting at 124°–127° C.;

Sodium N-(3-carboxy-1-oxopropyl)-4(S,R)-p-phenylphenylmethyl-4-amino-2(S,R)-methoxybutanoic acid, melting at 180°–185° C.;

Sodium N-(3-carboxy-1-oxopropyl)-4(S,R)-p-phenylphenylmethyl-4-amino-2(S,R)-methoxybutanoic acid indanyl ester, melting at 134°–136° C.;

N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-butanoic acid, melting at 163°–166° C.;

N-(3-carboxy-3-hydroxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-2R-methylbutanoic acid, melting at 156°–170° C.

## EXAMPLE 4

Following the procedures described in example 1 except substituting glutaric anhydride for succinic anhydride, the following compounds are prepared:

N-(4-carboxy-1-oxobutyl)-(4S)-p-phenylphenylmethyl-4-amino-2R-methylbutanoic acid, melting at 152°–155° C.

Sodium N-(4-carboxy-1-oxobutyl)-(4S)-p-phenylphenylmethyl-4-amino-2R-methylbutanoic acid ethyl ester, melting at 68°–72° C.

## EXAMPLE 5

Following the procedures described in example 1 except substituting carbobutoxyethylidene phenyl phosphorane for carboethoxyethylidene phenyl phosphorane, the following compound is prepared:

Sodium N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-2R-methylbutanoic acid n-butyl ester, melting at 155°–165° C.

## EXAMPLE 6

To a room temperature solution of N-t-butoxycarbonyl-(4R)-p-phenylphenylmethyl-4-amino-2-methyl-2-butenoic acid ethyl ester (0.50 g) in 2 ml ethanol and 4 ml THF are added 2.0 ml of 1N NaOH. The reaction is stirred until the disappearance of starting material monitored by thin layer chromatography. The mixture is concentrated, dissolved in sodium bicarbonate and washed with ether. The aqueous layer is acidified with 3N HCl and extracted (3×) with ethyl acetate. The organic extracts are washed with brine, dried ($MgSO_4$), filtered and concentrated to give N-t-butoxycarbonyl-(4R)-p-phenylphenylmethyl-4-amino-2-methyl-2-butenoic acid.

To a room temperature solution of N-t-butoxycarbonyl-(4R)-p-phenylphenylmethyl-4-amino-2-methyl-2-butenoic acid (0.30 g) in 10 ml of $CH_2Cl_2$ are added 0.123 g of dimethyl aminopyridine, 0.203 g of 5-indanol and 0.387 g of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride. The mixture is stirred overnight, and then is concentrated and taken up in ethyl acetate. The organics are washed with saturated sodium bicarbonate (2×), 1N HCl (2×) and brine (2×), dried ($MgSO_4$), filtered, concemtrated and chromatographed on silica gel eluting with (1:4) ethyl acetate:hexane to give N-t-butoxycarbonyl-(4R)-p-phenylphenylmethyl-4-amino-2-methyl-2-butenoic acid indanyl ester. This material is converted to sodium N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-2R-methylbutanoic acid indanyl ester melting at 60°–65° C. according to the procedures described in example 1.

## EXAMPLE 7

To a solution of (4S)-p-phenylphenylmethyl-4-amino-2-methylbutanoic acid ethyl ester hydrochloride (0.84 g) in 10 ml of methylene chloride are added 0.58 g of adipic acid mono methyl ester, 0.293 g of triethylamine, 0.49 g of hydroxybenzotriazole and 0.928 g of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride. The reaction is stirred at room temperature overnight. The mixture is concentrated and the residue is taken up in ethyl acetate. The organics are washed with sodium bicarbonate, 1N HCl, brine, dried ($MgSO_4$), filtered and evaporated. The residue is chromatographed on silica gel eluting with (1:2) ethyl acetate:hexane to give the more polar diastereomer N-(5-carbomethoxy-1-oxopentyl)-(4S)-p-phenylphenylmethyl-

4-amino-2R-methylbutanoic acid ethyl ester. The less polar (S,S) diastereomer is also isolated.

To a solution of N-(5-carbomethoxy-1-oxopentyl)-(4S)-p-phenylphenylmethyl-4-amino-2R-methylbutanoic acid ethyl ester (0.58 g) in 10 ml of THF and 10 ml of ethanol are added 4.0 ml of 1N NaOH. The reaction is stirred overnight. The mixture is concentrated taken up in water and washed with ether (2×). The aqueous layer is acidified with 2N HCl and extracted with ethyl acetate (2×). The organics are dried ($MgSO_4$), filtered, concentrated and recrystallized from methylene chloride-ether to give N-(5-carboxy-1-oxopentyl)-(4S)-p-phenylphenylmethyl-4-amino-2R-methylbutanoic acid, melting at 124°-127° C.

## EXAMPLE 8

Preparation of 1,000 capsules each containing 50 mg of the active ingredient, as follows:

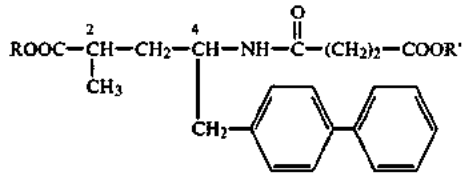
| | |
|---|---|
| N-(3-carboxy-1-oxopropyl)-(4S)-(p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester sodium salt | 50.00 g |
| Lactose | 187.00 g |
| Modified starch | 80.00 g |
| Magnesium stearate | 3.00 g |

Procedure: All the powders are passed through a screen with openings of 0.6 mm. The drug substance is placed in a suitable mixer and mixed first with the magnesium stearate, then with the lactose and starch until homogenous. No. 2 hard gelatin capsules are filled with 300 mg of said mixture each, using a capsule filling machine.

Analogously capsules are prepared, containing about 10-100 mg of the other compounds disclosed and exemplified herein, e.g. the compounds of examples 1-5.

What is claimed is:

1. A compound of formula Ie



(Ie)

wherein COOR and COOR' independently represent carboxyl or carboxyl esterified in form of a pharmaceutical acceptable prodrug ester, or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 wherein R and R' independently represent hydrogen, $C_1$-$C_4$-alkyl, benzyl optionally substituted on phenyl by lower alkyl, lower alkoxy, halo or trifluoromethyl, pivaloyloxymethyl, 1-($C_2$-$C_4$-alkanoyloxy)-ethyl, (2,2-dimethyl-1,3-dioxolan-4-yl)-methyl, 5-indanyl, 3-phthalidyl, bornyloxycarbonylmethyl, 1-($C_1$-$C_4$-alkoxycarbonyloxy)-ethyl or 3-pyridylmethyl; or a pharmaceutically acceptable salt thereof.

3. A compound according to claim 1 wherein COOR' is carboxyl; and COOR represents carboxyl or carboxyl derivatized in form of a pharmaceutically acceptable ester; or a pharmaceutically acceptable salt thereof.

4. A compound according to claim 1 having the R-configuration at C-atom 2 and the S-configuration at C-atom 4.

5. A compound according to claim 1, being N-(3-carboxy-1-oxopropyl)-4-(p-phenylphenylmethyl)-4-amino-2-methylbutanoic acid ethyl ester, the (2R,4S)-antipode thereof, or a pharmaceutical acceptable salt thereof.

6. A compound according to claim 4 being N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl-4-amino-(2R) -methylbutanoic acid or a pharmaceutically acceptable salt thereof.

7. A compound according to claim 4 being N-(3-carboxy-1-oxopropyl)-(4S)-(p-phenylphenylmethyl)-4-amino-(2R) -methylbutanoic acid ethyl ester or a pharmaceutically acceptable salt thereof.

8. A neutral endopeptidase inhibiting pharmaceutical composition comprising an effective neutral endopeptidase inhibiting amount of a compound of claim 1, in combination with one or more pharmaceutically acceptable carriers.

9. A neutral endopeptidase inhibiting pharmaceutical composition according to claim 8 comprising an effective neutral endopeptidase inhibiting amount of N-(3-carboxy-1-oxopropyl)-4-(p-phenylphenylmethyl)-4-amino-2-methylbutanoic acid ethyl ester, the (2R,4S)-antipode thereof, or a pharmaceutical acceptable salt thereof.

10. A method of treating cardiovascular disorders which comprises administering to a mammal in need of such treatment an effective neutral endopeptidase inhibiting amount of a compound of claim 1 in combination with one or more pharmaceutically acceptable carriers.

11. A method of treating cardiovascular disorders according to claim 10 which comprises administering to a mammal in need of such treatment an effective neutral endopeptidase inhibiting amount of 4-[N-(3-carboxy-1-oxopropyl)-amino]4-(p-phenylphenylmethyl)-2-methylbutanoic acid ethyl ester, the (2R,4S)-antipode thereof or a pharmaceutical acceptable salt thereof.

* * * * *

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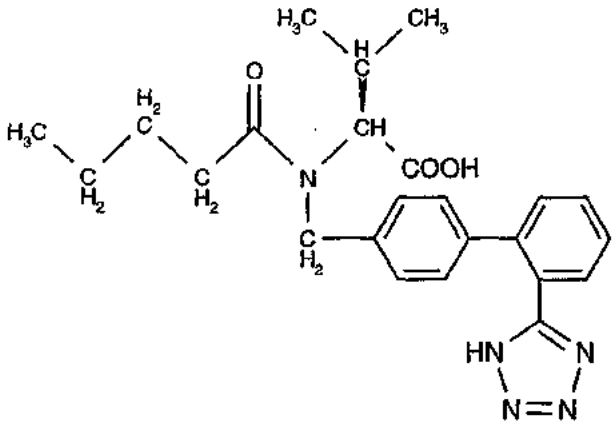
(54) Title: VALSARTAN SALTS

(57) Abstract: The invention relates to new salts of valsartan or crystalline, also partly crystalline and amorphous salts of valsartan, the respective production and usage, and pharmaceutical preparations containing such a salt.

# VALSARTAN SALTS

The invention relates to new salts of the $AT_1$ receptor antagonist (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-yl-methyl]-amine (valsartan) of formula



(I).

The active ingredient valsartan is the free acid which is described specifically in EP 0443983, especially in example 16; it has two acidic hydrogen atoms: (i) the hydrogen atom (H atom) of the carboxyl group, and (ii) that of the tetrazole ring. Accordingly, one acidic H atom (primarily the carboxyl H atom) or both acidic H atoms may be replaced by a monovalent or higher valent, e.g. divalent, cation. Mixed salts may also be formed.

EP 443983 does not disclose any specific salts of valsartan. Also, it does not mention any special properties of salts. Meanwhile, the active ingredient valsartan has been introduced as an anti-hypertensive agent in a series of countries under the trade name DIOVAN.

The free acid valsartan has a melting point in a closed crucible of 80 to 95°C and in an open crucible of 105 to 110°C and a melting enthalpy of 12 kJ/mol. The optical rotation is $[\alpha]^{20}_D = (-70 \pm 2)^0$ for a concentration of c = 1% in methanol.

The density of the valsartan crystals and of the salt hydrates was determined by a helium pycnometer (Accupyc 1330 of Micromeritics, Norcross, GA, USA). The density for the crystals of the free acid valsartan is 1.20 ± 0.02.

The X-ray diffraction diagram consists essentially of a very broad, diffuse Xray reflection; the free acid is therefore characterised as almost amorphous under X-ray. The melting point linked with the measured melting enthalpy of 12 kJ/mol unequivocally confirm the existence of a considerable residual arrangement in the particles or structural domains for the free acid valsartan.

There is a need for more stable, e.g. crystalline forms of valsartan, which are even easier to manage in the drying or grinding processes following the final stage of the chemical preparation process and also in the steps for preparing the pharmaceutical formulations. Many futile attempts have been made to find improved forms through salt formation, the forms ideally being as crystalline as possible, as well as physically and chemically stable. Only the salts according to the invention, their solvates and polymorphous forms thereof exhibit the desired improved properties.

The formation of salts of valsartan with the desired advantageous properties has proved to be difficult. In the majority of cases, for example, amorphous salts with little stability are obtained (such as hard foams, waxes or oils). Extensive research has shown that the salts of valsartan according to the invention have proved to be particularly advantageous compared with the free acid valsartan.

The objects of the present invention are salts of valsartan which are selected from the group consisting of the monosodium salt, the monopotassium salt, the dipotassium salt, the magnesium salt, the calcium salt, the bis-diethylammonium salt, the bis-dipropylammonium salt, the bis-dibutylammonium salt, the mono-L-arginine salt, the bis-L-arginine salt, the mono-L-lysine salt and the bis-L-lysine salt, as well as salt mixtures, or respectively, an amorphous form, a solvate, especially hydrate, as well as a polymorphous form thereof, the respective production and usage, and pharmaceutical preparations containing such salts.

The objects of the present invention are salts of valsartan which are selected from the group consisting of the monosodium salt, the monopotassium salt, the dipotassium salt, the magnesium salt, the calcium salt, the bis-diethylammonium salt, the bis-dipropylammonium salt, the bis-dibutylammoniumsalt, the mono-L-arginine salt, the bis-L-arginine salt, the

mono-L-lysine salt and the bis-L-lysine salt, or respectively, an amorphous form, a solvate, especially hydrate, as well as a polymorphous form thereof.

Salt mixtures are (i) single salt forms from different cations selected from the above group or (ii) mixtures of those single salt forms which exist for example in the form of conglomerates.

Preferred salts are for example selected from the
*mono-sodium salt in amorphous form;*
di-sodium salt of valsartan in amorphous or crystalline form, especially in hydrate form, thereof.
Mono-potassium salt of valsartan in amorphous form;
di-potassium salt of valsartan in amorphous or crystalline form, especially in hydrate form, thereof.
calcium salt of valsartan in crystalline form, especially in hydrate form, primarily the tetrahydrate thereof;
magnesium salt of valsartan in crystalline form, especially in hydrate form, primarily the hexahydrate thereof;
calcium/magnesium mixed salt of valsartan in crystalline form, especially in hydrate form;
bis-diethylammonium salt of valsartan in crystalline form, especially in hydrate form;
bis-dipropylammonium salt of valsartan in crystalline form, especially in hydrate form;
bis-dibutylammonium salt of valsartan in crystalline form, especially in hydrate form, primarily the hemihydrate thereof;
mono-L-arginine salt of valsartan in amorphous form;
bis-L-arginine salt of valsartan in amorphous form;
mono-L-lysine salt of valsartan in amorphous form;
bis-L-lysine salt of valsartan in amorphous form.

The salts according to the invention preferably exist in isolated and essentially pure form, for example in a degree of purity of >95%, preferably >98%, primarily >99%. The enantiomer purity of the salts according to the invention is >98%, preferably >99%.

Compared with the free acid, the salts according to the invention, or the amorphous forms, solvates such as salt hydrates, and also the corresponding polymorphous forms thereof,

have unexpectedly advantageous properties. Under given conditions, the crystalline salts and crystalline salt hydrates have a clear melting point which is linked with a marked, endothermic melting enthalpy. The crystalline salts according to the invention are stable and are of better quality than valsartan also during storage and distribution. The amorphous or partially amorphous salts have limited stability, i.e. as the solid, they have a restricted stability range. To be stabilised, they require certain measures which can be achieved for example by galenic formulations.

In addition, both the crystalline and the amorphous salts according to the invention have a high degree of dissociation in water and thus substantially improved water solubility. These properties are of advantage, since on the one hand the dissolving process is quicker and on the other hand a smaller amount of water is required for such solutions. Furthermore, the higher water solubility can, under certain conditions, also lead to increased biological availability of the salts or salt hydrates in the case of solid dosage forms. Improved properties are beneficial especially to the patients. Furthermore, some of the salts according to the invention have proved to be exceptionally physically stable, particularly the alkaline earth salts. For different relative humidities at room temperature and also at a slightly higher temperatures, the salt hydrates according to the invention show practically no water absorption or water loss over a wide range of humidities and for periods of a few hours, e.g. four hours. Also, for example, the melting point of the salts according to the invention will not be changed by storing under different relative humidities.

Improved physicochemical properties of certain salts or certain salt hydrates are of great importance both when they are produced as a pharmaceutically active substance and when producing, storing and applying the galenic preparation. In this way, starting with improved constancy of the physical parameters, an even higher quality of the formulations can be guaranteed. The high stability of the salts or salt hydrates also give the possibility of attaining economic advantages by enabling simpler process steps to be carried out during working up. The high crystallinity of certain salt hydrates allows the use of a choice of analytical methods, especially the various X-ray methods, the usage of which permits a clear and simple analysis of their release to be made. This factor is also of great importance to the quality of the active substance and its galenic forms during production, storage and administration to the patients. In addition, complex provisions for stabilising the active ingredient in the galenic formulations can be avoided.

The invention accordingly relates to crystalline, also partly crystalline and amorphous salts of valsartan.

As well as the solvates, such as hydrates, the invention also relates to polymorphous forms of the salts according to the invention.

Solvates and also hydrates of the salts according to the invention may be present, for example, as hemi-, mono-, di-, tri-, tetra-, penta-, hexa-solvates or hydrates, respectively. Solvents used for crystallisation, such as alcohols, especially methanol, ethanol, aldehydes, ketones, especially acetone, esters, e.g. ethyl acetate, may be embedded in the crystal grating. The extent to which a selected solvent or water leads to a solvate or hydrate in crystallisation and in the subsequent process steps or leads directly to the free acid is generally unpredictable and depends on the combinations of process conditions and the various interactions between valsartan and the selected solvent, especially water. The respective stability of the resulting crystalline or amorphous solids in the form of salts, solvates and hydrates, as well as the corresponding salt solvates or salt hydrates, must be determined by experimentation. It is thus not possible to focus solely on the chemical composition and the stoichiometric ratio of the molecules in the resulting solid, since under these circumstances both differing crystalline solids and differing amorphous substances may be produced.

The description salt hydrates for corresponding hydrates may be preferred, as water molecules in the crystal structure are bound by strong intermolecular forces and thereby represent an essential element of structure formation of these crystals which, in part, are extraordinarily stable. However, water molecules are also existing in certain crystal lattices which are bound by rather weak intermolecular forces. Such molecules are more or less integrated in the crystal structure forming, but to a lower energetic effect. The water content in amorphous solids can, in general, be clearly determined, as in crystalline hydrates, but is heavily dependent on the drying and ambient conditions. In contrast, in the case of stable hydrates, there are clear stoichiometric ratios between the pharmaceutical active substance and the water. In many cases these ratios do not fulfil completely the stoichiometric value, normally it is approached by lower values compared to theory because of certain crystal defects. The ratio of organic molecules to water molecules for the weaker bound water may

vary to a considerable extend, for example, extending over di-, tri- or tetra-hydrates. On the other hand, in amorphous solids, the molecular structure classification of water is not stoichiometric; the classification may however also be stoichiometric only by chance.

In some cases, it is not possible to classify the exact stoichiometry of the water molecules, since layer structures form, e.g. in the alkali metal salts, especially in the potassium salt, so that the embedded water molecules cannot be determined in defined form.

For the crystalline solids having identical chemical composition, the different resulting crystal gratings are summarised by the term polymorphism.

Any reference hereinbefore and hereinafter, to the salts according to the invention is to be understood as referring also to the corresponding solvates, such as hydrates, and polymorphous modifications, and also amorphous forms, as appropriate and expedient.

Especially preferred are the tetrahydrate of the calcium salt of valsartan and the hexahydrate of the magnesium salt of valsartan.

The X-ray diffraction diagram of powders of these two salt hydrates has a number of discrete X-ray reflections, and practically no signs of non-crystalline or amorphous portions. The degree of crystallisation of these defined salt hydrates is therefore surprisingly high. Equally, relatively large crystals may be cultured from certain salt hydrates, and in the crystallographic sense these are single crystals. Such single crystals allow the structure of the solid to be determined. It is effected by computer-aided evaluation of the reflection intensities measured by an X-ray diffractometer.

This process for determining the structure of a crystal enables, under normal conditions such as high physical, chemical and enantiomeric purity of the gauged crystals, a clear determination of the structure to be carried out on a molecular or atomic level, namely symmetry and size of the elementary cells, atom positions and temperature factors, and from the ascertained cell volume, the X-ray-photographic density is shown on the basis of a molecular weight. At the same time, the X-ray-photographic structure determination supplies details of its quality.

The outstanding properties of these two salt hydrates are based on the crystals, which form these salts by incorporating four or six water molecules per valsartan molecule. Thus, practically perfect three-dimensional crystal gratings are produced. These two salts have water solubility that is several times better than the free acid of valsartan, and this is especially surprisingly at high melting points and melting enthalpies, which are eight or five times greater than the free acid. The extraordinary crystal gratings of these two salt hydrates are the basis for the chemical and physical stability of these two compounds.

The particularly notable salt hydrate is the tetrahydrate of the calcium salt of valsartan. In a closed specimen container, for a heating rate of $T_r = 10\ K \cdot min^{-1}$ it has a melting point of 205 ± 1.5 °C and a melting enthalpy of 98 ± 4 $kJ \cdot Mol^{-1}$. The tetrahydrate of the calcium salt of valsartan is not stable at elevated temperatures both in respect of the hydrate water and in respect of the structure of the molecule. The indicated melting point is a hydrate melting point which can only be measured in a closed specimen container. Gold containers with a wall thickness of 0.2 mm were used; after weighing in samples of between 2 and 4 mg salt hydrate, they were sealed by cold welding. These gold containers have an internal free volume of ca. 22 microlitres. The amounts of the sample and the volume of the pressurised containers must be suitably adapted, so that strong dehydration of the salt hydrates cannot take place during measurement of the melting point. The partial pressure of the water at 205° Celsius is ca. 18 bar, so that with an open container in DSC (Differential Scanning Calorimeter) during measurement of the melting point, conversion to the anhydrate takes place. If the data from several heating rates ($T_r$ = 10, 20, 40 $K \cdot min^{-1}$) are extrapolated to a continuously rapid heating rate, a melting point of 213 ± 2 °C and a melting enthalpy of 124 ± 5 $kJ \cdot Mol^{-1}$ result. Both the high hydrate melting point and the amount of the melting enthalpy are an expression of the exceptional stability of the crystal grating of the tetrahydrate of the calcium salt of valsartan. These two thermodynamic characteristics illustrate the advantageous physical properties, compared to the free acid, with the two corresponding data, namely a melting point in the closed system of 90°C and a melting enthalpy of 12 $kJ \cdot Mol^{-1}$. These thermodynamic data, together with the X-ray data, prove the high stability of this crystal grating. They are the foundation for the special physical and chemical resistance of the tetrahydrate of the calcium salt of valsartan.

A measurement of the infrared absorption spectrum of the tetrahydrate of the calcium salt of valsartan in a potassium bromide compressed tablet shows the following significant

bands expressed in reciprocal wave numbers ($cm^{-1}$ ): 3750 – 3000 (st); 3400 – 2500 (st); 1800 – 1520 (st); 1500 – 1380 (st); 1380 – 1310 (m); 1290 – 1220 (w); 1220 – 1190 (w); 1190 – 1160 (w); 1160 – 1120 (w); 1120 – 1050 (w); 1030 – 990 (m); 989 – 960 (w), 950 – 920 (w); 780 – 715 (m); 710 – 470 (m). The intensities of the absorption bands are indicated as follows: (w) = weak; (m) = medium; and (st) = strong intensity. Measurement of the infrared spectrum likewise took place by means of ATR-IR (Attenuated Total Reflection-Infrared Spectroscopy) using the instrument Spektrum BX from Perkin-Elmer Corp., Beaconsfield, Bucks, England.

The tetrahydrate of the calcium salt of valsartan has the following absorption bands expressed in reciprocal wave numbers ($cm^{-1}$):

3594 (w); 3306 (w); 3054 (w); 2953 (w); 2870 (w); 1621 (st); 1578 (m); 1458 (m); 1441 (m); 1417 (m); 1364 (m); 1336 (w); 1319 (w); 1274 (w); 1241 (w); 1211 (w); 1180 (w); 1149 (w); 1137 (w); 1106 (w); 1099 (w); 1012 (m); 1002 (w); 974 (w); 966 (w); 955 (w); 941 (w); 863 (w); 855 (w); 844 (w); 824 (w); 791 (w); 784 (w); 758 (m); 738 (m); 696 (m); 666 (m).

The intensities of the absorption bands are indicated as follows: (w) = weak; (m) = medium and (st) = strong intensity.

The most intensive absorption bands of the ATR-IR spectroscopy are shown by the following values expressed in reciprocal wave numbers ($cm^{-1}$): 3306 (w); 1621 (st); 1578 (m); 1458 (m); 1441 (m); 1417 (m); 1364 (m); 1319 (w); 1274 (w); 1211 (w); 1180 (w); 1137 (w); 1012 (m); 1002 (w); 758 (m); 738 (m); 696 (m); 666 (m).

The error margin for all absorption bands of ATR-IR is ± 2 $cm^{-1}$.

The water content is in theory 13.2% for the tetrahydrate of the calcium salt of valsartan. Using the thermo-scale TGS-2 ( Perkin-Elmer Corp. , Norwalk, CT USA ) the water content was determined as 12.9 %. A total formula was calculated from this $(C_{24}H_{27}N_5O_3)^{2-}$ $Ca^{2+}$• (3.9 ± 0.1) $H_2O$.

Using thermogravimetry, in a water-free $N_2$ atmosphere, the weight loss, i.e. the water loss for the tetrahydrate as a function of temperature, was measured at a heating rate of 10 K•$min^{-1}$. The results are illustrated in table 1.

Table 1

| temperature [° C] | weight loss or water loss in % |
|---|---|

| | |
|---|---|
| 25 | 0 |
| 50 | 0 |
| 75 | 0.5 |
| 100 | 3.5 |
| 125 | 10.2 |
| 150 | 12.4 |
| 175 | 12.8 |
| 200 | 12.9 |
| 225 | 12.9 |
| 250 | 13.0 |
| 275 | 13.2 |

The solubility of the tetrahydrate of the calcium salt of valsartan in water-ethanol mixtures is illustrated in Table 2 for a temperature of 22°C.

Table 2

| vol-% ethanol in water | solubility of the tetrahydrate of the calcium salt of valsartan in g/l solution at 22°C |
|---|---|
| 0 | 9 ( pH = 7.4 ) |
| 10 | 9 |
| 30 | 14 |
| 50 | 46 |

A comparison of the solubilities of the two most important salts according to the invention and the free acid in distilled water is illustrated in Table 3.

Table 3

| Compound | solubility in g/l solution at 22°C |
|---|---|
| valsartan | 0.17 |
| tetrahydrate of the calcium salt of valsartan | 9 |
| hexahydrate of the magnesium salt of valsartan | 59 |

Further characterisation of the tetrahydrate of the calcium salt of valsartan is effected using the interlattice plane intervals determined by a X-ray powder pattern. Measurement of the

X-ray powder patterns was made with a Guinier camera (FR 552 from Enraf Nonius, Delft, NL) on an X-ray film in transmission geometry, using Cu-$Ka_1$ radiation at room temperature. Evaluation of the films for calculation of the interlattice plane intervals is made both visually and by a Line-Scanner (Johansson Täby, S), and the reflection intensities are determined simultaneously.

The preferred characterisation of the tetrahydrate of the calcium salt of valsartan is obtained from the interlattice plane intervals d of the ascertained X-ray diffraction diagrams, whereby, in the following, average values are indicated with the appropriate error limits.

d in [Å] : 16.1±0.3, 9.9±0.2, 9.4±0.2, 8.03±0.1, 7.71±0.1, 7.03±0.1, 6.50±0.1, 6.33±0.1, 6.20±0.05, 5.87±0.05, 5.74±0.05, 5.67±0.05, 5.20±0.05, 5.05±0.05, 4.95±0.05, 4.73±0.05, 4.55±0.05, 4.33±0.05, 4.15±0.05, 4.12±0.05, 3.95±0.05, 3.91±0.05, 3.87±0.05, 3.35±0.05.

The most intensive reflections in the X-ray diffraction diagram show the following interlattice plane intervals:

d in [Å] : 16.1±0.3, 9.9±0.2, 9.4±0.2, 7.03±0.1, 6.50±0.1, 5.87±0.05, 5.74±0.05, 4.95±0.05, 4.73±0.05, 4.33±0.05, 4.15±0.05, 4.12±0.05, 3.95±0.05.

A preferred method of checking the above-indicated average values of the interlattice plane intervals and intensities measured by experimentation from X-ray diffraction diagrams with a Guinier camera, for a given substance, consists in calculating these intervals and their intensities from the comprehensive single crystal structure determination. This structure determination yields cell constants and atom positions, which enable the X-ray diffraction diagram corresponding to the solid to be calculated by means of computer-aided calculation methods (programme CaRine Crystallography, Université de Compiègne, France). A comparison of these data, namely the interlattice plane intervals and intensities of the most important lines of the tetrahydrate of the calcium salt of valsartan, obtained from measurements with the Guinier camera and from calculating the single crystal data, is illustrated in Table 4.

Table 4

| measured | | calculated | | measured | | calculated | |
|---|---|---|---|---|---|---|---|
| d in [Å] | Intensity | d in [Å] | Intensity | d in [Å] | Intensity | d in [Å] | Intensity |
| 16.10 | very strong | 16.02 | very strong | 5.67 | very weak | 5.658 | very weak |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 9.89 | strong | 9.88 | very strong | 5.20 | very weak | 5.199 | very weak |
| 9.38 | average | 9.37 | average | 5.05 | very weak | 5.040 | very weak |
| 8.03 | weak | 8.02 | average | 4.95 | average | 4.943 | weak |
| 7.71 | weak | 7.70 | weak | 4.73 | weak | 4.724 | weak |
| 7.03 | average | 7.01 | average | 4.55 | weak | 4.539 | weak |
| 6.50 | average | 6.49 | average | 4.33 | weak | 4.338 | weak |
| 6.33 | weak | 6.33 | weak | 4.15 | strong | 4.150 | strong |
| 6.20 | very weak | 6.19 | very weak | 4.12 | weak | 4.114 | weak |
| 5.87 | average | 5.862 | average | 3.95 | average | 3.941 | average |
| 5.74 | average | 5.738 | average | 3.35 | weak | 3.349 | weak |

The invention relates to the crystalline tetrahydrate of the calcium salt of (S)-N-(1-carboxy-2-methylprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amine, a crystalline solid which is clearly characterised by the data and parameters obtained from single crystal X-ray analysis and X-ray powder patterns. An in-depth discussion of the theory of the methods of single crystal X-ray diffraction and the definition of the evaluated crystal data and the parameters may be found in Stout & Jensen, X-Ray Structure Determination; A Practical Guide, Mac Millian Co., New York, N.Y. (1968) chapter 3.

The data and parameters of the single crystal X-ray structure determination for the tetrahydrate of the calcium salt of valsartan are contained in Table 5.

Table 5

Crystal data and parameters of the tetrahydrate of the calcium salt of valsartan

| | |
|---|---|
| *Crystal data* | |
| sum formula | $(C_{24}H_{27}N_5O_3)^{2-} Ca^{2+} \cdot 4 H_2O$ |
| molecular mass | 545.65 |
| crystal colour | colourless |
| crystal shape | flat prisms |

| | |
|---|---|
| crystal system | monoclinic |
| space group | $P2_1$ |
| size of the single crystal | 0.42 • 0.39 • 0.17 $mm^3$ |
| dimensions and angle of elementary cell | a = 10.127(2) Å |
| | b = 8.596(2) Å |
| | c = 32.214(6) Å |
| | $\alpha = 90^0$ |
| | $\beta = 95.34(3)^\circ$ |
| | $\gamma = 90^\circ$ |
| volume of elementary cell | $V_c = 2792.1(10)$ $Å^3$ |
| number of molecules in the elementary cell | 4 |
| F (000) | 1160 |
| measurement range of cell parameters (Θ) | $7.47\text{-}16.50^\circ$ |
| calculated density | 1.298 ($g \cdot cm^{-3}$) |
| linear absorption coefficient | 0.274 $mm^{-1}$ |

*X-ray measurement data*

| | |
|---|---|
| diffractometer | Enraf Nonius CAD4 |
| X-radiation ( graphite monochromator ) | MoKα |
| wavelength | 0.71073 |
| temperature | 295 K |
| scan range (θ) | $1.27 - 31.99^0$ |
| scan mode | ω / 2 Θ |
| reflections collected/unique | 19384 / 18562 |
| number of significant reflections ( $I > 2\sigma(I)$ ) | 10268 |
| variation in intensity | 1.7 % |
| absorption correction | numeric |

*Structure refinement*

| | |
|---|---|
| method | full matrix, least squares, $F^2$ |
| number of parameters | 893 |
| agreement index (R) | 6.2 % |
| weighted agreement index ($R_w$) | 14.4 % |

| | |
|---|---|
| S factor (Goodness of fit) | 1.085 |
| number of reflections used | 18562 |
| treatment of all hydrogen atoms in the molecule, including in the water molecules | all found by difference-Fourier calculation, almost all isotropically refined, a few theoretically fixed (riding) |
| extinction correction | none |
| maximum/minimum residual electron density in conclusive difference-Fourier calculation | 0.662 / - 0.495 ( e•Å$^{-3}$ ) |
| absolute structure parameters | 0.00 (4) |

*Computer programmes used*
SHELXS 86 ( Sheldrick, Göttingen, 1990 )
SHELXL 96 ( Sheldrick, Göttingen, 1996 )
SCHAKAL 86 ( Keller, Freiburg 1986 )
PLATON ( Spek, Acta Cryst., 1990 )

The elementary cell is defined by six parameters, namely by the grating constants a, b and c, and by the axial angle, namely by a, β, und γ. In this way, the volume of the elementary cell $V_c$ is determined. A differentiated description of these crystal parameters is illustrated in chapter 3 of Stout & Jensen (see above). The details for the tetrahydrate of the calcium salt of valsartan from the single crystal measurements, especially the atom coordinates, the isotropic thermal parameters, the coordinates of the hydrogen atoms as well as the corresponding isotropic thermal parameters, show that a monoclinic elementary cell exists, its cell content of four formula units $Ca^{2+}$ valsartan$^{2-}$ • 4 $H_20$ occurring as a result of two crystallographic independent units on two-fold positions.

Given the acentric space group $P2_1$ determined from the single crystal X-ray structure determination, a racemate is ruled out. Thus the enantiomeric purity of the S-configuration for the crystalline tetrahydrate of the calcium salt of (S)-N-(1-carboxy-2-methylprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amine is verified.

An essential feature for the quality of a pure active substance both for the physical-chemical procedures such as drying, sieving, grinding, and in the galenic processes which are carried

out with pharmaceutical excipients, namely in mixing processes, in granulation, in spray-drying, in tabletting, is the water absorption or water loss of this active substance depending on temperature and the relative humidity of the environment in question. With certain formulations, free and bound water is without doubt introduced with excipients and/or water is added to the process mass for reasons associated with the respective formulation process. In this way, the pharmaceutical active substance is exposed to free water over rather long periods of time, depending on the temperature of the different activity (partial vapour pressure).

A clear characterisation of this property is achieved by means of isothermal measurements over predetermined time intervals and predetermined relative humidity using dynamic vapour sorption (DVS-1 from the company Surface Measurement Systems LTD, Marlow, Buckinghamshire, UK). Table 6 illustrates the mass change, i.e. the water absorption or loss as a function of relative humidity at 25°C for a sample of 9.5 mg of the tetrahydrate of the calcium salt of valsartan and for a period of 4 hours. The following cycles of changes in relative humidity are shown: 40-90; 90-0; 0-90; 90-0 % relative humidity:

Table 6

| relative humidity in % | water absorption or loss in % | relative humidity in % | water absorption or Abgabe in % |
|---|---|---|---|
| 40 | 0.04 | 10 | 0.00 |
| 50 | 0.04 | 0 | -0.01 |
| 60 | 0.03 | 10 | 0.00 |
| 70 | 0.02 | 20 | 0.00 |
| 80 | 0.02 | 30 | 0.00 |
| 90 | 0.00 | 40 | 0.00 |
| 80 | 0.02 | 50 | 0.00 |
| 70 | 0.02 | 60 | 0.01 |
| 60 | 0.02 | 70 | 0.00 |
| 50 | 0.02 | 80 | -0.01 |
| 40 | 0.02 | 90 | -0.02 |
| 30 | 0.01 | 0 | -0.02 |
| 20 | 0.01 | (starting value) | 0.00 |

The measurement error of this sorption method based on thermogravimetry is about 0.1%. Therefore, the tetrahydrate of the calcium salt of valsartan under the conditions employed, which are realistic from a pharmaceutical-galenic point of view, shows no measurable water absorption or loss. This is surprising to a large extent, since the tetrahydrate, which has incorporated about 13% of bound water in the crystal structure, is totally indifferent to water even at extreme values of relative humidity. This property is crucial in the final stages of chemical manufacture and also in practice in all galenic process stages of the different dosage forms. This exceptional stability similarly benefits the patients through the constant availability of the active ingredient.

The intrinsic dissolving rates of the calcium salt of valsartan at pH 1, pH 4.5 and pH 6.8 show improved values over those of valsartan.

The exceptional stability of the calcium salt of valsartan, especially the tetrahydrate thereof, towards water may also be shown in stability tests. In these, the water content of the tetrahydrate of the calcium salt of valsartan remains constant both in an open container and in a sealed ampoule after four weeks at 40°C and 75% relative humidity.

Owing to the advantageous crystallinity of the calcium salt, especially the tetrahydrate thereof, this salt is suitable for pressing directly to form corresponding tablet formulations.

In addition, an improved dissolving profile in a tablet can be assured. In studies of the dissolving profile, it was established that the calcium salt, especially the tetrahydrate thereof, is released by 100% from a film-coated tablet within 15 minutes.

Of the group of new-type crystalline solids, a magnesium salt hydrate of valsartan is preferred, in particular the hexahydrate. The thermal behaviour of this salt hydrate in the region of the melting point shows a certain chemical and physical instability. The thermal data are thus dependent on the measurement conditions. In the sealed gold specimen container with an internal free volume of ca. 22 microlitres, with a sample of 2 to 4 mg and with a heating rate of $T_r = 10\ K \cdot min^{-1}$, the melting point of the hexahydrate of the magnesium salt of valsarten is 132 ± 1.5° Celsius and the melting enthalpy is 56 ± 3 kJ·Mol$^{-}$

[1]. The melting enthalpy which is about 5 times higher than the free acid of valsartan, together with the significantly higher melting point of the hexahydrate of the magnesium salt of valsartan is a measure of the stability of the new-type crystal grating at around room temperature.

The optical rotation of the hexahydrate of the magnesium salt of valsartan in methanol as a 1% solution at 20°C is $[\alpha]^{20}_D = -14°$.

A measurement of the infrared absorption spectrum of the hexahydrate of the magnesium salt of valsartan in a potassium bromide compressed tablet shows the following significant bands expressed in reciprocal wave numbers ($cm^{-1}$): 3800 – 3000 (st); 3000 – 2500 (st); 1800 – 1500 (st); 1500 – 1440 (m); 1440 – 1300 (m); 1280 – 1240 (w); 1240 – 1190 (w); 1190 – 1150 (w); 1120 – 1070 (w); 1050 – 990 (w); 990 – 960 (w); 960 – 920 (w); 920 – 700 (m); 700 – 590 (w); 590 – 550 (w).
The intensities of the absorption bands are indicated as follows: (w) = weak; (m) = medium; and (st) = strong intensity.

Measurement of the infrared spectrum likewise took place by means of ATR-IR (Attenuated Total Reflection-Infrared Spectroscopy) using the instrument Spektrum BX from Perkin-Elmer Corp., Beaconsfield, Bucks, England.

The hexahydrate of the magnesium salt of valsartan has the following absorption bands expressed in reciprocal wave numbers ($cm^{-1}$):
3378 (m); 3274 (m); 2956 (m); 2871 (w); 2357 (w); 1684 (w); 1619 (st); 1557 (m); 1464 (m); 1419 (m); 1394 (st); 1374 (m); 1339 (w); 1319 (w); 1300 (w); 1288 (w); 1271 (w) 1255 (w); 1223 (w); 1210 (w); 1175 (m); 1140 (w); 1106 (w); 1047 (w); 1024 (w); 1015 (w); 1005 (w); 989 (w); 975 (w); 955 (w); 941 (w); 888 (w); 856 (w); 836 (m); 820 (w); 766 (st); 751 (m); 741 (st); 732 (st).
The intensities of the absorption bands are indicated as follows: (w) = weak; (m) = medium and (st) = strong intensity.

The most intensive absorption bands of the ATR-IR spectroscopy are shown by the following values expressed in reciprocal wave numbers ($cm^{-1}$): 3378 (m); 3274 (m);

2956 (m); 1619 (st); 1557 (m); 1464 (m); 1419 (m); 1394 (st); 1271 (w); 1175 (m); 1015 (w); 975 (w); 836 (m); 766 (st); 751 (m); 741 (st); 732 (st).
The error margin for all absorption bands of ATR-IR is ± 2 $cm^{-1}$.

The theoretical water content of the hexahydrate of the magnesium salt of valsartan is 19.1%. Using a coupled instrument based on thermogravimetry-Fourier transformation-infrared-spectroscopy (TG-FTIR, IFS 28 from the companies Netzsch Gerätebau GmbH, Selb, Bayern and Bruker Optik GmbH, Karlsruhe ), whilst simultaneously measuring the weight loss and identifying the material component given up, using infrared spectroscopy (release of water), the water content was determined at 18.5 %, conforming well with the theoretical value. For the hexahydrate, this corresponds to a molar ratio of 5.8 ± 0.2 mols $H_2O$ per mol magnesium salt.

Table 7 illustrates the water loss of the hexahydrate of the magnesium salt of valsartan depending on temperature, using the weight loss measured in an $N_2$ atmosphere on a thermogravimetric thermal analysis instrument for a heating rate of 10 $K°min^{-1}$. From the TG-FTIR measurement, the correlation of the weight loss is assured solely by the release of water.

Table 7

| temperature [° C] | weight loss or water release in % |
|---|---|
| 25 | 0 |
| 50 | 1.2 |
| 75 | 4.2 |
| 100 | 11.0 |
| 125 | 16.7 |
| 150 | 17.7 |
| 175 | 18.3 |
| 200 | 18.5 |
| 225 | 18.7 |
| 250 | 18.9 |
| 275 | 19.3 |

The hexahydrate of the magnesium salt of valsartan has a solubility in distilled water at 22°C of 59 g per litre of solution for a pH value of 9.3.

The crystalline form of the hexahydrate of the magnesium salt of valsartan is clearly characterised by the interlattice plane intervals calculated from the lines in an X-ray powder pattern. The measurement and analysis methods used are the same as those used for the tetrahydrate of the calcium salt of valsartan.

This preferred characterisation of the hexahydrate of the magnesium salt of valsartan is obtained from the interlattice plane intervals d, whereby, in the following, average values are indicated with the appropriate error limits:
d in [Å]: 19.7±0.3, 10.1±0.2, 9.8±0.2, 7.28±0.1, 6.48±0.1, 6.00±0.1, 5.81±0.1, 5.68±0.1, 5.40±0.05, 5.22 ±0.05, 5.12±0.05, 5.03±0.05, 4.88±0.05, 4.33±0.05, 4.22±0.05, 4.18±0.05, 4.08±0.05, 3.95±0.05, 3.46±0.05, 3.42±0.05.

The most intensive reflections in the X-ray diffraction diagram show the following interlattice plane intervals:
d in [Å] : 19.7±0.3, 10.11±0.2, 9.8±0.2, 7.28±0.1, 5.81±0.05, 5.68±0.05, 5.03±0.05, 4.88±0.05, 4.18±0.05, 4.08±0.05, 3.46 ±0.05.

A preferred method of checking the above-indicated average values of the interlattice plane intervals and intensities measured by experimentation from X-ray diffraction diagrams with a Guinier camera, for a given substance, consists in calculating these intervals and their intensities from the comprehensive single crystal structure determination. This structure determination yields cell constants and atom positions, which enable the X-ray diffraction diagram corresponding to the solid to be calculated by means of computer-aided calculation methods (programme CaRine Crystallography, Université de Compiègne, France). A comparison of these data, namely the interlattice plane intervals and intensities of the most important lines of the hexahydrate of the magnesium salt of valsartan, obtained from measurements with the Guinier camera and from calculating the single crystal data, is illustrated in Table 8.

Table 8

| measured | | calculated | | measured | | calculated | |
|---|---|---|---|---|---|---|---|
| d in [Å] | Intensity | d in [Å] | Intensity | d in [Å] | Intensity | d in [Å] | Intensity |
| 19.7 | very strong | 19.66 | very strong | 5.12 | weak | 5.124 | weak |
| 10.11 | average | 10.09 | average | 5.03 | strong | 5.032 | very strong |
| 9.83 | average | 9.84 | very strong | 4.88 | strong | 4.878 | very strong |
| 7.28 | average | 7.27 | average | 4.33 | very weak | 4.341 | weak |
| 6.48 | weak | 6.46 | weak | 4.22 | weak | 4.215 | weak |
| 6.00 | weak | 6.00 | weak | 4.18 | average | 4.181 | average |
| 5.81 | average | 5.805 | average | 4.08 | average | 4.079 | average |
| 5.68 | average | 5.676 | strong | 3.95 | weak | 3.946 | weak |
| 5.40 | very weak | 5.391 | very weak | 3.46 | average | 3.463 | average |
| 5.22 | weak | 5.217 | weak | 3.42 | weak | 3.428 | weak |

The invention relates in particular to the crystalline hexahydrate of the magnesium salt of (S)-N-(1-carboxy-2-methylprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amine, a crystalline solid which is clearly characterised by the data and parameters obtained from single crystal X-ray analysis. An in-depth discussion of the theory of the methods of single crystal X-ray diffraction and the definition of the evaluated crystal data and the parameters may be found in Stout & Jensen, X-Ray Structure Determination; A Practical Guide, Mac Millian Co., New York, N.Y. (1968) chapter 3.

The data and parameters of the single crystal X-ray analysis for the magnesium-valsartan-hexahydrate are given in Table 9.

Table 9

Crystal data and parameters of the hexahydrate of the magnesium salt of valsartan

*Crystal data*

| | |
|---|---|
| sum formula | $(C_{24}H_{27}N_5O_3)^{2-}Mg^{2+} \cdot 6H_2O$ |
| molecular mass | 565.91 |

| | |
|---|---|
| crystal colour | colourless |
| crystal shape | flat prisms |
| crystal system | monoclinic |
| space group | C2 |
| size of the single crystal | 0.013 • 0.50 • 0.108 $mm^3$ |
| dimensions and angle of elementary cell | a = 40.075(8) Å |
| | b = 7.400(1) Å |
| | c = 10.275(2) Å |
| | $\alpha$ = 90 $^0$ |
| | $\beta$ = 100.85(3) $^0$ |
| | $\gamma$ = 90 $^0$ |
| volume of elementary cell | $V_c$ = 2992.6(9) $Å^3$ |
| number of molecules in the elementary cell | 4 |
| F (000) | 1208 |
| measurement range of cell parameters ($\Theta$) | 2.82 –11.15 $^0$ |
| calculated density | 1.256 ($g \cdot cm^{-3}$) |
| linear absorption coefficient | 0.114 $mm^{-1}$ |

*X-ray measurement data*

| | |
|---|---|
| *diffractometer* | *Enraf Nonius CAD4* |
| X-radiation ( graphite monochromator ) | MoK$\alpha$ |
| wavelength | 0.71073 |
| temperature | 295 K |
| scan range ($\theta$) | 1.03 – 26.00 $^0$ |
| scan mode | $\omega$ / 2 $\Theta$ |
| reflections collected/unique | 5954 / 5868 |
| number of significant reflections ( I > 2$\sigma$(I) ) | 1341 |
| variation in intensity | <1 % |
| absorption correction | numeric |

*Structure refinement*

| | |
|---|---|
| method | full matrix, least squares, $F^2$ |

| | |
|---|---|
| number of parameters | 243 |
| agreement index (R) | 10.7 % |
| weighted agreement index ($R_W$) | 13.8 % |
| S factor (Goodness of fit) | 1.001 |
| number of reflections used | 5868 |
| determination of hydrogen atoms | majority according to the "riding" model, nine H-atoms from water molecules isotropically refined from difference-Fourier calculation |
| extinction correction | 0.00098 (10) |
| maximum/minimum residual electron density in final difference-Fourier calculation | 0.473 / - 0.614 ( $e \cdot Å^{-3}$ ) |
| absolute structure parameters | 0.0(10) |

*Computer programmes used*

SHELXS 86 (Sheldrick, Göttingen, 1990)

SHELXL 96 (Sheldrick, Göttingen, 1996)

SCHAKAL 86 (Keller, Freiburg 1986)

PLATON (Spek, Acta Cryst., 1990)

The elementary cell is defined by six parameters, namely by the grating constants a, b and c, and by the axial angle, namely by a, β, und γ. In this way, the volume of the elementary cell $V_c$ is determined. A differentiated description of these crystal parameters is illustrated in chapter 3 of Stout & Jensen (see above).

The details for the hexahydrate of the magnesium salt of valsartan from the single crystal measurements, especially the atom coordinates, the isotropic thermal parameters, the coordinates of the hydrogen atoms as well as the corresponding isotropic thermal parameters, show that a monoclinic elementary cell exists, its cell content occurring from four formula units $Mg^{2+}$ Valsartan • 6 $H_2O$ .

Given the acentric space group C2 determined from the single crystal X-ray structure determination, a racemate is ruled out. Thus the enantiomeric purity of the S-configuration for the crystalline hexahydrate of the magnesium salt of valsartan is proved.

Table 10 illustrates the mass change, i.e. the water absorption or loss as a function of relative humidity at 25°C for a sample of 9.5 mg of magnesium-valsartan-hexahydrate and for a period of 4 hours (h). The following cycles of changes in relative humidity are shown: 40-90; 90-0; 0-90; 90-0 % relative humidity:

Table 10

| relative humidity in % | water absorption or loss in % | relative humidity in % | water absorption or loss in % |
|---|---|---|---|
| 40 | 0.06 | 10 | -0.12 |
| 50 | 0.14 | 0 | -4.3 |
| 60 | 0.19 | 10 | -0.79 |
| 70 | 0.25 | 20 | -0.14 |
| 80 | 0.41 | 30 | -0.05 |
| 90 | 0.58 | 40 | 0.02 |
| 80 | 0.32 | 50 | 0.09 |
| 70 | 0.22 | 60 | 0.14 |
| 60 | 0.14 | 70 | 0.20 |
| 50 | 0.08 | 80 | 0.28 |
| 40 | 0.16 | 90 | 0.51 |
| 30 | -0.03 | 0 | -3.68 |
| 20 | -0.07 | (starting value) | -0.01 |

The measurement error of this sorption method based on thermogravimetry is about 0.1%. Therefore, the hexahydrate of the magnesium salt of valsartan under the conditions employed, which are realistic from a pharmaceutical-galenic point of view, shows weak, reproducible water absorption or water loss in a range of 20 to 80% relative humidity. This is surprising to a large extent, since the hexahydrate, which has incorporated about 19% bound water in the crystal structure, reversibly absorbs or releases water even at extreme values of relative humidity and is relatively insensitive at an average range of relative humidity. This characteristic enables an uncomplicated physical-chemical process to be developed and allows a choice of the best dosage forms for the patients.

The exceptional stability of the magnesium salt of valsartan, especially the hexahydrate thereof, towards water may also be shown in stability tests In these, the water content of the hexahydrate of the magnesium salt of valsartan remains constant both in an open container and in a sealed ampoule after four weeks at 40°C and 75% relative humidity.

Owing to the advantageous crystallinity of the magnesium salt, especially the hexahydrate thereof, this salt is suitable for pressing directly to form corresponding tablet formulations.

In addition, an improved dissolving profile in a tablet can be assured. In studies of the dissolving profile, it was established that the magnesium salt, especially the hexahydrate thereof, is released by 100% from a film-coated tablet within 15 minutes.

In addition, the magnesium salt of valsartan, especially the hexahydrate thereof, shows an advantageous compression hardness profile.

Calcium/magnesium mixed salts of valsartan also have advantageous properties, for example uniform crystal conglomerates may be produced. These may be advantageously used in the galenic formulation.

The intrinsic dissolving rates of the di-potassium salt of valsartan at pH 1, pH 4.5 and pH 6.8 show improved values over those of valsartan.

A further object of the invention is the preparation of the salts according to the invention.

The salts according to the invention, including amorphous or crystalline forms thereof, may be prepared as follows:

To form the salt, the process is carried out in a solvent system, in which the two reactants, namely the acid valsartan and the respective base, are sufficiently soluble. It is expedient to use a solvent or solvent mixture, in which the resulting salt is only slightly soluble or not soluble at all, in order to achieve crystallisation or precipitation. One variant for the salt according to the invention would be to use a solvent in which this salt is very soluble, and to subsequently add an anti-solvent to this solution, that is a solvent in which the resulting salt has only poor solubility. A further variant for salt crystallisation consists in concentrating the

salt solution, for example by heating, if necessary under reduced pressure, or by slowly evaporating the solvent, e.g. at room temperature, or by seeding with the addition of seeding crystals, or by setting up water activity required for hydrate formation.

The solvents that may be used are for example $C_1$-$C_5$-alkanols, preferably ethanol and isopropanol, as well as $C_1$-$C_5$-dialkylketones, preferably acetone and mixtures thereof with water.

The antisolvents for salt crystallisation may be for example $C_3$-$C_7$-alkylnitriles, especially acetonitrile, esters, especially $C_2$-$C_7$-alkanecarboxylic acid-$C_1$-$C_5$-alkylester, such as ethyl or isopropyl acetate, di-($C_1$-$C_5$-alkyl)-ethers, such as tert.-butylmethylether, furthermore tetrahydrofuran, and $C_5$-$C_8$-alkanes, especially pentane, hexane or heptane.

To produce hydrates, a dissolving and crystallising process is used in particular, or a water-equilibrating crystallisation process.

The dissolving and crystallising process is characterised in that
(i) valsartan and the appropriate base are brought to a reaction in a preferably water-containing, organic solvent,
(ii) the solvent system is concentrated, for example by heating, if necessary under reduced pressure and by seeding with seeding crystals or by slowly evaporating, e.g. at room temperature, then crystallisation or precipitation is initiated and
(iii) the salt obtained is isolated.

In the dissolving and crystallising process, the water-containing, organic solvent system employed is advantageously a mixtures of alcohols, such as ethanol, and water, or or alkyl-nitrile, especially acetonitrile, and water.

The equilibrating crystallisation process for producing hydrates is characterised in that
(i) valsartan and the appropriate base are added to a water-containing organic solvent,
(ii) the solvent is concentrated, for example by heating, if necessary under reduced pressure or by slowly evaporating, e.g. at room temperature,
(iii) the residue of evaporation is equilibrated with the required amount of water by

(a) suspending the residue of evaporation, which is advantageously still warm, and which still contains some water, in an appropriate solvent or
(b) by equilibrating the water excess in the solvent;
whereby in a) and b) the existing or added water is present in a quantity in which the water dissolves in the organic solvent and does not form an additional phase; and
(iv) the salt obtained is isolated.

The solvent system used as the water-containing organic solvent advantageously comprises mixtures of suitable alcohols, such as $C_1$-$C_7$-alkanols, especially ethanol, and water.

An appropriate solvent for equilibration is, for example, an ester such as $C_1$-$C_7$-alkanecarboxylic acid-$C_1$-$C_7$-alkylester, especially ethyl acetate, or a ketone such as di-$C_1$-$C_5$-alkylketone, especially acetone.

The equilibration process is notable for example for its high yields and outstanding reproducibility.

When producing the mono-alkali metal salts according to the present invention, predominantly amorphous forms are obtained. On the other hand, the di-alkali metal salts and alkaline earth metal salts of the present invention may also be obtained in crystalline form and are in the form of hydrates throughout, from appropriate solvents that are conventionally used in production processes, such as esters, e.g. $C_1$-$C_7$-alkanecarboxylic acid-$C_1$-$C_7$-alkylesters, especially ethyl acetate, ketones, e.g. di-$C_1$-$C_5$-alkylketones, especially acetone, $C_3$-$C_7$-alkylnitriles, especially acetonitrile, or ethers, e.g. di-($C_1$-$C_5$-alkyl)-ethers, such as tert.-butylmethylether, also tetrahydrofuran, or mixtures of solvents. By using the dissolving and crystallising process, or the water-equilibrating crystallisation process, the defined hydrates, which are present in crystalline and in polymorphous forms, may be obtained reproducibly.

The preparation of the hydrate-free bis-dialkylammonium salts of the present invention is advantageously effected in one step by using an appropriate solvent which is optionally mixed with an antisolvent. In this way, crystalline salts are obtained.

As a rule, the amino acid salts of the present invention are obtained in amorphous form.

The processes for forming salts are likewise objects of the present invention.

These salts or salt hydrates according to the invention are obtained for example by neutralising the acid valsartan with a base corresponding to the respective cation. This neutralisation is suitably effected in an aqueous medium, e.g. in water or a mixture of water and a solvent in which valsartan is more soluble than in water. Salts with weaker bases may be converted into other salts either by treating with stronger bases or by treating with acids and then neutralising with other bases.

Crystallisation, especially of the alkaline earth salt hydrates, is effected in water or an aqueous medium, which consists of water and at least one solvent that is miscible or partially miscible with water, i.e. not too non-polar, e.g. an alkanol such as methanol, ethanol, propanol, isopropanol, butanol, acetone, methyl ethyl ketone, acetonitrile, DMF, DMSO. The alkanol portion amounts to about 10 to 90, or 20 to 70, advantageously 30 to 50% by volume. For higher alkanols, the less polar solvent may also be present in lower concentrations. Owing to the restricted water-solubility of valsartan, the process frequently takes place in suspensions, or if valsartan is soluble in the other solvent component, in a solution.

In one embodiment, for example to produce the calcium salt of valsartan, an aqueous solution of valsartan is neutralised with a calcium hydroxide solution at room temperature and the solution is left to crystallise. In a preferred procedure, crystallisation is effected from a solvent mixture of water/ethanol, the ethanol proportion amounting to ca. 30 to 50% by volume. In an especially preferred form, crystallisation is effected in a closed system by transporting through a low temperature gradient (especially 1-2°C at 40°C) in 30% by volume of ethanol.

In a preferred variant, crystallisation may be optimised, e.g. accelerated, by adding at least one seed crystal.

The salts according to the invention may be used e.g. in the form of pharmaceutical preparations, which contain the active substance e.g. in a therapeutically effective amount

of the active substance, optionally together with a pharmaceutically acceptable carrier, for example with an inorganic or organic, solid or optionally also liquid pharmaceutically acceptable carrier, which is suitable for enteral, e.g. oral, or parenteral administration.

The invention relates in particular to a pharmaceutical composition, especially in a solid dosage unit, preferably for oral administration, optionally together with a pharmaceutically acceptable carrier.

Pharmaceutical preparations of this kind may be used for example for the prophylaxis and treatment of diseases or conditions which may be inhibited by blocking the $AT_1$ receptor for example

a disease or condition selected from the group consisting of

(a) hypertension, congestive heart failure, renal failure, especially chronic renal failure, restenosis after percutaneous transluminal angioplasty, and restenosis after coronary artery bypass surgery;

(b) atherosclerosis, insulin resistance and syndrome X, diabetes mellitus type 2, obesity, nephropathy, renal failure, e.g. chronic renal failure, hypothyroidism, survival post myocardial infarction (MI), coronary heart diseases, hypertension in the elderly, familial dyslipidemic hypertension, increase of formation of collagen, fibrosis, and remodeling following hypertension (antiproliferative effect of the combination), all these diseases or conditions associated with or without hypertension;

(c) endothelial dysfunction with or without hypertension,

(d) hyperlipidemia, hyperlipoproteinemia, atherosclerosis and hypercholesterolemia, and

(e) glaucoma.

Primary usages are for the treatment of high blood pressure and congestive heart failure, as well as post-myocardial infarction.

The person skilled in the pertinent art is fully enabled to select a relevant and standard animal test model to prove the hereinbefore and hereinafter indicated therapeutic indications and beneficial effects.

The pharmaceutical activities as effected by administration of representatives of the salts of the present invention or of the combination of active agents used according to the present

invention can be demonstrated e.g. by using corresponding pharmacological models known in the pertinent art. The person skilled in the pertinent art is fully enabled to select a relevant animal test model to prove the hereinbefore and hereinafter indicated therapeutic indications and beneficial effects.

These beneficial effects can, for example, be demonstrated in the test model as disclosed by G. Jeremic et al. in J. Cardovasc. Pharmacol. 27:347-354, 1996.

For example, the valuable potential of the salts or combinations of the present invention for the prevention and treatment of myocardial infarction can be found using the following test model.

Study design

In the study to be performed, permanent coronary artery occlusion (CAO) in rats is used as a model of acute myocardial infarction. The experiments are carried out with 5 treatment groups characterized by following features:

- sham-operated animals
- CAO + vehicle
- CAO + a salt according to the present invention,

optionally

- CAO + a salt according to the present invention + a combination partner.

During the study following variables are measured:

- infarct size
- LV chamber volume
- interstitial and perivascular collagen density in spared LV myocardium
- COL-I and COL-III protein content in spared LV myocardium by Western blot
- cardiomyocytes cross-sectional area and length in sections of LV myocardium
- plasma concentrations of renin and aldosterone
- urine concentration of sodium, potassium and aldosterone
- blood pressure in conscious animals
- LV and carotid blood pressure in anesthetized animals.

Methodology

**Infarct size:** Six µm-thick transverse histological sections of the left ventricle are stained with nitroblue tetrazolium and acquired by a B/W XC-77CE CCD video camera (Sony). The resulting image is processed on a KS 300 image analysis system (Carl Zeiss Vision) using a software specifically developed (Porzio *et al.*, 1995). A single operator blinded to treatment interactively defines the boundaries of the interventricular septum, and the infarcted area on each section is semiautomatically identified as the area of unstained ventricular tissue. The software automatically calculates for each component of the ventricular section defined as the chamber, septum, infarcted area, infarcted LV wall and viable LV wall, a set of geometric parameters (Porzio *et al.*, 1995).

**Histology:** Hearts are fixed in situ, by retrograde perfusion with buffered 4% formaldehyde after arrest in diastole by i.v. injection of 0.5 M KCl. After fixation, the left ventricle (LV) and the free wall of the right ventricle are separately weighed; LV longer diameter is measured with a caliper. LV histological sections are stained with hematoxylin & eosin for qualitative examination and to quantify cardiomyocytes cross-sectional area with a semi-automated image analysis routine. Interstitial collagen deposition in LV is evaluated on Sirius red stained sections with a semi-automated image analysis routine (Masson *et al.*, 1998).

**Collagen content in LV spared myocardium:** LV tissue in the spared myocardium is homogenized, subjected to PAGE-SDS electrophoresis and electroblotted onto nitrocellulose membrane. The blots are exposed to primary antibodies, i.e. rabbit anti-rat collagen type I or type III antiserum (Chemicon). The primary antibodies are recognized by secondary antibodies conjugated to alkaline phosphatase (for colagen type I) or peroxidase (collagen type III).

**Left ventricular chamber volume:** LV chamber volume is determined in hearts arrested in diastole (KCl) and fixed in formalin under a hydrostatic pressure equivalent to the measured LV end-diastolic pressure. A metric rod is inserted into the LV to measure LV inner length. The transverse diameters of the LV chamber are measured in two 1-mm thick transverse sections near to the base and the apex of the ventricle (Jeremic *et al.*, 1996). The chamber volume is computed from an equation integrating transverse diameters and inner length.

**Systemic and Left ventricular hemodynamics:** A microtip pressure transducer (Millar SPC-320) connected to a recorder (Windograf, Gould Electronics) is inserted into the right carotid artery to record systolic and diastolic blood pressures. The pressure transducer is advanced into the LV to measure LV systolic (LVSP) and end-diastolic (LVEDP) pressures, the first derivative of LV pressure over time (+dP/dt) and heart rate.

**Non-invasive blood pressure:** Systolic blood pressure and heart rate are measured by the tail-cuff method (Letica LE 5002) in conscious rats.

**Urine electrolytes, hormones:** Rats are individually housed in metabolic cages and 24-h urine collected on 1 ml HCl 6N. Water intake is measured. Urine catecholamines are extracted on Bondelut $C_{18}$ columns (Varian), separated by HPLC (Apex-II C18, 3 µm, 50x4.5 mm analytical column, Jones Chromatography) and quantified with an electrochemical detector (Coulochem II, ESA) (Goldstein *et al.*, 1981). Plasma and urine aldosterone, and plasma angiotensin II is determined with specific radioimmunoassays (Aldoctk-2, DiaSorin and Angiotensin II, Nichols Diagnostics). Urine sodium and potassium are measured by flamme photometry.

**Sample size**

10 animals analyzable in each treatment groups are sufficient to detect biologically significant differences. Only rats with an infarct size of at least 10% of the LV section area are included in the final analysis.

Endothelial dysfunction is being acknowledged as a critical factor in vascular diseases. The endothelium plays a bimodal role as the source of various hormones or by-products with opposing effects: vasodilation and vasoconstriction, inhibition or promotion of growth, fibrinolysis or thrombogenesis, production of anti-oxidants or oxidising agents. Genetically predisposed hypertensive animals with endothelial dysfunction constitute a valid model for assessing the efficacy of a cardiovascular therapy.

Endothelial disfunction is characterized by, for example, increased oxidative stress, causing decreased nitric oxide, increased factors involved in coagulation or fibrinolysis such as plasminogen activating inhibitor-1 (PAI-1), tissue factor (TF), tissue plasminogen activator (tPA), increased adhesion molecules such as ICAM and VCAM, increased growth factors

such as bFGF, TGFb, PDGF, VEGF, all factors causing cell growth inflammation and fibrosis.

The treatment e.g. of endothelian dysfunction can be demonstrated in the following pharmacological test:

Material and methods
Male 20-24 week-old SHR, purchased from RCC Ldt (Füllingsdorf, Switzerland), are maintained in a temperature- and light-controlled room with free access to rat chow (Nafag 9331, Gossau, Switzerland) and tap water. The experiment is performed in accordance with the NIH guidelines and approved by the Canton Veterinary office (Bew 161, Kantonales Veterinäramt, Liestal, Switzerland). All rats are treated with the NO synthesis inhibitor L-NAME (Sigma Chemicals) administered in drinking water (50 mg/l) for 12 weeks. The average daily dose of L-NAME calculated from the water consumed was 2.5 mg/kg/d (range 2.1-2.7 ).

The rats can be divided into 2 or 3 groups: group 1, control (n = e.g. 40); Group 2, a salt according to the present invention; n = e.g. 40); for testing combinations Group 3, combination partner;(n = e.g. 30). The drugs are administered in drinking fluid. The pressure effect of Ang II at 1 mg/kg obtained in controls normotensive rats can be reduced after treatment with a salt according to the present invention (Gervais et al. 1999).

Body weight is measured every week. Systolic blood pressure and heart rate are recorded by tail cuff plethysmography 3 and 2 weeks before starting the study and at 2 weeks after drug administration. Urine is collected over a 24 hour period from rats kept in individual (metabolic) cages the week before starting treatment and at weeks 4 and 12 for volume measurement and protein, creatinine, sodium and potassium determination using standard laboratory methods. At the same time points, blood samples are withdrawn from the retro-orbital plexus (maximum 1 ml) for creatinine, $Na^+$ and $K^+$ assays.

Ten rats from each group are sacrificed at 4 weeks for collection of kidney and heart for morphological analysis. The remaining rats are sacrificed at 12 weeks. Cardiac and kidney weight is recorded. Terminal blood sampling is performed in 5 % EDTA at 4 (morphometry

study) and 12 (end of the study) weeks for aldosterone, determination by radioimmunoassay using a DPC coat-a-count aldosterone-RIA kit (Bühlmann, Switzerland).

**Statistical analysis:**

All data are expressed as mean ± SEM. Statistical analysis is performed using a one-way ANOVA, followed by a Duncan's multiple range test and a Newman-Keuls test, 7for comparison between the different groups. Results with a probability value of less than 0.05 are deemed statistically significant.

An improvement of regression of artherosclerosis without effecting the serum lipid levels can, for example, be demonstrated by using the animal model as disclosed by H. Kano et al. in Biochemical and Biophysical Research Communications 259, 414-419 (1999).

That the salts or combinations according to the present invention can be used for the regression of a cholesterol diet-induced atherosclerosis, can be demonstrated using the test model described, e.g., by C. Jiang et al. in Br. J. Pharmacol. (1991), 104, 1033-1037.

That the salts or combinations according to the present invention can be used for the treatment of renal failure, especially chronic renal failure, can be demonstrated using the test model described, e.g., by D. Cohen et al. in Journal of Cardiovascular Pharmacology, 32: 87-95 (1998).

The present pharmaceutical preparations which, if so desired, may contain further pharmacologically active substances, are prepared in a manner known *per se*, for example by means of conventional mixing, granulating, coating, dissolving or lyophilising processes, and contain from about 0.1% to 100%, especially from about 1% to about 50%, of lyophilisates up to 100% of the active substance.

The invention similarly relates to compositions containing the salts according to the invention.

The invention similarly relates to the use of the salts according to the invention preferably for the production of pharmaceutical preparations, especially for the prophylaxis and also for the treatment of diseases or conditions which may be inhibited by blocking the $AT_1$

receptor. Primary usages are for the treatment of high blood pressure and congestive heart failure, as well as post-myocardial infarction.

The invention similarly relates to the use for the prophylaxis and treatment of diseases or conditions which may be inhibited by blocking the $AT_1$ receptor, characterised in that a patient, including a human patient, requiring such treatment is administered with a therapeutically effective amount of a salt according to the invention, optionally in combination with at least one composition for the treatment of cardiovascular diseases and related conditions and diseases listed hereinbefore or hereinafter.

The invention similarly relates to combinations, e.g. pharmaceutical combinations, containing a salt of the present invention or in each case a pharmaceutically acceptable salt thereof in combination with at least one composition for the treatment of cardiovascular diseases and related conditions and diseases as listed hereinbefore or hereinafter, or in each case a pharmaceutically acceptable salt thereof. Combinations with other compositions for the treatment of cardiovascular diseases and related conditions and diseases as listed hereinbefore or hereinafter, or in each case a pharmaceutically acceptable salt thereof, are likewise objects of the present invention.

The combination may be made for example with the following compositions, selected from the group consisting of a:

(i) HMG-Co-A reductase inhibitor or a pharmaceutically acceptable salt thereof,

(ii) angiotensin converting enzyme (ACE) inhibitor or a pharmaceutically acceptable salt thereof,

(iii) calcium channel blocker or a pharmaceutically acceptable salt thereof,

(iv) aldosterone synthase inhibitor or a pharmaceutically acceptable salt thereof,

(v) aldosterone antagonist or a pharmaceutically acceptable salt thereof,

(vi) dual angiotensin converting enzyme/neutral endopeptidase (ACE/NEP) inhibitor or a pharmaceutically acceptable salt thereof,

(vii) endothelin antagonist or a pharmaceutically acceptable salt thereof,

(viii) renin inhibitor or a pharmaceutically acceptable salt thereof, and

(ix) diuretic or a pharmaceutically acceptable salt thereof.

HMG-Co-A reductase inhibitors (also called β-hydroxy-β-methylglutaryl-co-enzyme-A reductase inhibitors) are understood to be those active agents that may be used to lower the lipid levels including cholesterol in blood.

The class of HMG-Co-A reductase inhibitors comprises compounds having differing structural features. For example, mention may be made of the compounds that are selected from the group consisting of atorvastatin, cerivastatin, compactin, dalvastatin, dihydrocompactin, fluindostatin, fluvastatin, lovastatin, pitavastatin, mevastatin, pravastatin, rivastatin, simvastatin, and velostatin, or, in each case, a pharmaceutically acceptable salt thereof.

Preferred HMG-Co-A reductase inhibitors are those agents which have been marketed, most preferred is fluvastatin and pitavastatin or, in each case, a pharmaceutically acceptable salt thereof.

The interruption of the enzymatic degradation of angiotensin I to angiotensin II with so-called ACE-inhibitors (also called angiotensin converting enzyme inhibitors) is a successful variant for the regulation of blood pressure and thus also makes available a therapeutic method for the treatment of congestive heart failure.

The class of ACE inhibitors comprises compounds having differing structural features. For example, mention may be made of the compounds which are selected from the group consisting alacepril, benazepril, benazeprilat, captopril, ceronapril, cilazapril, delapril, enalapril, enaprilat, fosinopril, imidapril, lisinopril, moveltopril, perindopril, quinapril, ramipril, spirapril, temocapril, and trandolapril, or, in each case, a pharmaceutically acceptable salt thereof.

Preferred ACE inhibitors are those agents that have been marketed, most preferred are benazepril and enalapril.

The class of CCBs essentially comprises dihydropyridines (DHPs) and non-DHPs such as diltiazem-type and verapamil-type CCBs.

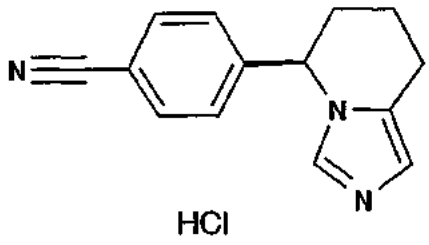
A CCB useful in said combination is preferably a DHP representative selected from the group consisting of amlodipine, felodipine, ryosidine, isradipine, lacidipine, nicardipine, nifedipine, niguldipine, niludipine, nimodipine, nisoldipine, nitrendipine, and nivaldipine, and is preferably a non-DHP representative selected from the group consisting of flunarizine, prenylamine, diltiazem, fendiline, gallopamil, mibefradil, anipamil, tiapamil and verapamil, and in each case, a pharmaceutically acceptable salt thereof. All these CCBs are therapeutically used, e.g. as anti-hypertensive, anti-angina pectoris or anti-arrhythmic drugs. Preferred CCBs comprise amlodipine, diltiazem, isradipine, nicardipine, nifedipine, nimodipine, nisoldipine, nitrendipine, and verapamil, or, e.g. dependent on the specific CCB, a pharmaceutically acceptable salt thereof. Especially preferred as DHP is amlodipine or a pharmaceutically acceptable salt, especially the besylate, thereof. An especially preferred representative of non-DHPs is verapamil or a pharmaceutically acceptable salt, especially the hydrochloride, thereof.

Aldosterone synthase inhibitor is an enzyme that converts corticosterone to aldosterone to by hydroxylating cortocosterone to form 18-OH-corticosterone and 18-OH-corticosterone to aldosterone. The class of aldosterone synthase inhibitors is known to be applied for the treatment of hypertension and primary aldosteronism comprises both steroidal and non-steroidal aldosterone synthase inhibitors, the later being most preferred.

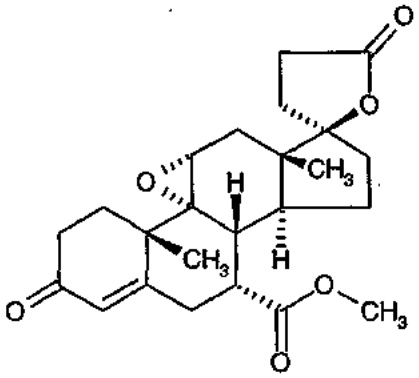
Preference is given to commercially available aldosterone synthase inhibitors or those aldosterone synthase inhibitors that have been approved by the health authorities.

The class of aldosterone synthase inhibitors comprises compounds having differing structural features. For example, mention may be made of the compounds which are selected from the group consisting of the non-steroidal aromatase inhibitors anastrozole, fadrozole (including the (+)-enantiomer thereof), as well as the steroidal aromatase inhibitor exemestane, or, in each case where applicable, a pharmaceutically acceptable salt thereof.

The most preferred non-steroidal aldosterone synthase inhibitor is the (+)-enantiomer of the hydrochloride of fadrozole (US patents 4617307 and 4889861) of formula




A preferred steroidal aldosterone antagonist is eplerenone of the formula

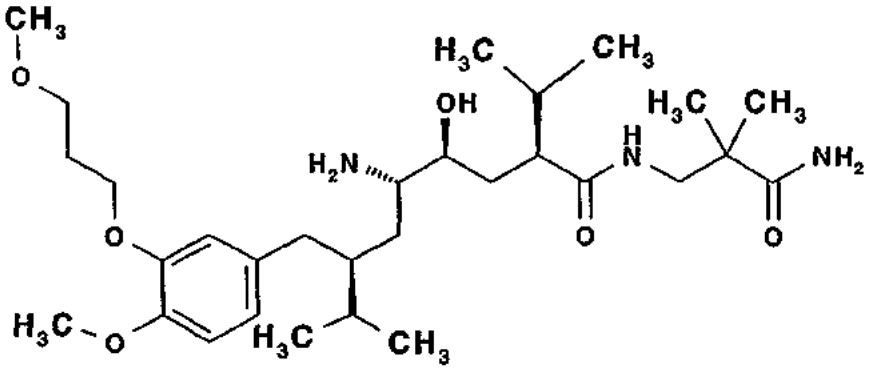



or spironolactone.

A preferred dual angiotensin converting enzyme/neutral endopetidase (ACE/NEP) inhibitor is, for example, omapatrilate (cf. EP 629627), fasidotril or fasidotrilate, or, if appropriable, a pharmaceutically acceptable salt thereof.

A preferred endothelin antagonist is, for example, bosentan (cf. EP 526708 A), furthermore, tezosentan (cf. WO 96/19459), or in each case, a pharmaceutically acceptable salt thereof.

A renin inhibitor is, for example, a non-peptidic renin inhibitor such as the compound of formula



chemically defined as 2(S),4(S),5(S),7(S)-N-(3-amino-2,2-dimethyl-3-oxopropyl)-2,7-di(1-methylethyl)-4-hydroxy-5-amino-8-[4-methoxy-3-(3-methoxy-propoxy)phenyl]-octanamide. This representative is specifically disclosed in EP 678503 A. Especially preferred is the hemi-fumarate salt thereof.

A diuretic is, for example, a thiazide derivative selected from the group consisting of chlorothiazide, hydrochlorothiazide, methylclothiazide, and chlorothalidon. The most preferred is hydrochlorothiazide.

Preferably, the jointly therapeutically effective amounts of the active agents according to the combination of the present invention can be administered simultaneously or sequentially in any order, separately or in a fixed combination.

The structure of the active agents identified by generic or tradenames may be taken from the actual edition of the standard compendium "The Merck Index" or from databases, e.g. Patents International (e.g. IMS World Publications). The corresponding content thereof is hereby incorporated by reference. Any person skilled in the art is fully enabled to identify the active agents and, based on these references, likewise enabled to manufacture and test the pharmaceutical indications and properties in standard test models, both in vitro and in vivo.

The corresponding active ingredients or a pharmaceutically acceptable salts thereof may also be used in form of a solvate, such as a hydrate or including other solvents, used for crystallization.

The compounds to be combined can be present as pharmaceutically acceptable salts. If these compounds have, for example, at least one basic center, they can form acid addition salts. Corresponding acid addition salts can also be formed having, if desired, an additionally present basic center. The compounds having an acid group (for example COOH) can also form salts with bases.

In a variation thereof, the present invention likewise relates to a "kit-of-parts", for example, in the sense that the components to be combined according to the present invention can be dosed independently or by use of different fixed combinations with distinguished amounts of the components, i.e. simultaneously or at different time points. The parts of the kit of parts can then e.g. be administered simultaneously or chronologically staggered, that is at different time points and with equal or different time intervals for any part of the kit of parts. Preferably, the time intervals are chosen such that the effect on the treated disease or condition in the combined use of the parts is larger than the effect that would be obtained by use of only any one of the components.

The invention furthermore relates to a commercial package comprising the combination according to the present invention together with instructions for simultaneous, separate or sequential use.

Dosaging may depend on various factors, such as mode of application, species, age and/or individual condition. For oral application, the doses to be administered daily are between ca. 0.25 and 10 mg/kg, and for warm-blooded animals with a body weight of ca. 70 kg, preferably between ca. 20 mg and 500 mg, especially 40mg, 80mg, 160mg and 320mg based on the free acid.

The invention is illustrated in particular by the examples and also relates to the new compounds named in the examples and to their usage and to methods for the preparation thereof.

The following examples serve to illustrate the invention without limiting the invention in any way.

For example, the di-potassium salt of valsartan is formed, especially a hydrate thereof. The di-potassium salt is noted in particular for its marked water solubility. The crystalline tetrahydrate of the di-potassium salt of valsartan, with a melting point of 135.0°C, may be mentioned in particular. According to elementary analysis, a certain sample of this hydrate has a water content of 3.72 mols of water per mol of di-potassium salt. For high relative humidity at room temperature, the tetrahydrate is formed and for low values of relative humidity, the anhydrate of the di-potassium salt is formed.

A magnesium salt of valsartan is similarly produced, in this instance as an amorphous solid with 3.4% $H_2O$. The temperature of glass transition, as a mean value of the stage of the specific heat of 0.85 J • [g • $^0$ C]$^{-1}$ is 167 °C. No melting point is observed. Both facts, namely the glass transition and the absence of a melting point, together with the measured value of the change in specific heat, confirm that this magnesium salt of valsartan is practically 100% amorphous. According to a stereo-specific chromatography method, the enantiomer purity of this amorphous magnesium salt has been determined as 83%.

Example 1:

Production of the calcium salt as the tetrahydrate *in situ* of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

21.775 g of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine are dissolved at room temperature in 300 ml of ethanol. By careful addition of 300 ml of water, the ethanol concentration is reduced to 50% by volume. Using a magnetic stirrer, 3.89 g of $Ca(OH)_2$ are added slowly in small portions to this clear, slightly acidic (pH 4) solution, so that the pH value temporarily does not exceed a value of ca. 8. Because it absorbs $CO_2$ from the air, the $Ca(OH)_2$ used contains traces of $CaCO_3$; therefore the added amount includes an excess of 5%. After adding the stoichiometric amount of $Ca(OH)_2$, the pH is ca. 6, and after adding the excess it rises to 7. The solution becomes turbid through the small amount of finely divided $CaCO_3$, which is removed through a folded filter. The product contained in the solution crystallises continuously upon removal of the alcohol content by allowing to stand at room temperature. The procedure can be accelerated by using a flat dish in a recirculating air drier at 40°C. After concentrating to ca. one half, the alcohol content of the solution drops to ca. 10% by

volume and most of the product crystallises. It is filtered, rinsed for a short time with 10% by volume ethanol and dried at 40°C until reaching a constant weight. (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine calcium salt tetrahydrate is obtained.

The melting point for the tetrahydrate of the calcium salt of valsartan, produced according to example 1, for a heating rate of 10 $K \cdot min^{-1}$ and in a closed specimen container with a small internal volume is determined as 205°C and the melting enthalpy as 92 $kJ \cdot Mol^{-1}$.
The density of the crystals of the calcium-valsartan-tetrahydrate produced according to example 1, determined by a helium pycnometer, is 1.297 $g \cdot cm^{-3}$. This value conforms to the theoretically calculated value of 1.298 $g \cdot cm^{-3}$ calculated from the single crystal structure.
The optical rotation of the tetrahydrate of the calcium salt of valsartan according to example 1 is measured in methanol as a 1% solution $[a]^{20}_{D} = +1^{\circ}$.
The enantiomer purity of the salt hydrate produced according to example 1 is determined by a stereo-specific HPLC method. The stereo-specific separation is achieved by a chiral column (Chiral AGP). The enantiomer purity is determined as ee = 100%.

Calculation of the interlattice plane intervals from the X-ray powder pattern taken with a Guinier camera is as follows for the most important lines for this batch of the tetrahydrate of the calcium salt of valsartan:
d in [ Å ] : 16.27, 9.90, 9.39, 8.04, 7.71, 7.05, 6.49, 6.34, 6.2, 5.87, 5.75, 5.66, 5.20, 5.05, 4.95, 4.73, 4.55, 4.33, 4.15, 4.12, 3.95, 3.91, 3.87, 3.35.

Elementary analysis gives the following measured values of the elements present in calcium-valsartan-tetrahydrate and of water. The water evaluation was carried out at 130°C after expulsion. The findings of the elementary analysis, within the error limits, correspond to the sum formula $(C_{24}H_{27}N_5O_3)^{2-} Ca^{2+} \cdot 4 H_2O$.

| | % found | % calculated |
|---|---|---|
| C | 52.82 | 52.83 |
| H | 6.42 | 6.47 |
| N | 12.91 | 12.83 |
| O | 20.20 | 20.53 |
| water | 13.25 | 13.21 |

| | | |
|---|---|---|
| Ca | 7.03 | 7.35 |

Example 2:

Production of the magnesium salt as the hexahydrate *in situ* of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

43.55 g of valsartan [(S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine] are dissolved at room temperature in 600 ml of 50% by volume ethanol (from absolute ethanol - see Merck and quarz-bidistilled water). The slightly turbid solution becomes clear after adding a further 50 ml of 50% ethanol. Using a magnetic stirrer, 4.03 g or 0.1 M MgO (Merck p.a.) are slowly added in small portions to this slightly acidic solution with a pH value of 4. The pH value hereby rises to ca. 6. The process is effected with an excess of 10%, i.e. a further 0.40 g of MgO are added. This excess is not fully dissolved, and the pH value rises to ca. 7.5. The small residue is filtered from the solution through a folded filter and washed with 50 ml of 50% ethanol.

The combined clear solution is carefully concentrated at 40°C whilst stirring with a magnetic stirrer in a large crystallisation dish. Towards the end of this procedure, the solution has a tendency to harden into a glassy gel. Scratching with a glass rod induces the *in situ* crystallisation in this phase, which may be recognised by the white colour of the crystalline solid thus formed. The product is dried at 50°C in a recirculating air drier until reaching a constant weight. The yield of magnesium-valsartan-hexahydrate is 53.7 g or 95% based on the valsartan employed as the free acid.

The melting point for the salt hydrate produced according to example2, namely the magnesium-valsartan-hexahydrate, for a heating rate of 10 $K \cdot min^{-1}$ in a sealed sample container with a small internal volume, in an amount of 2.24 mg, was measured at 132°C and the melting enthalpy at 64 $kJ \cdot Mol^{-1}$.

The density of the crystals of the hexahydrate of the magnesium salt of valsartan produced according to example 2, determined by a helium pycnometer, is 1.273 $g \cdot cm^{-3}$. This value conforms to the theoretically calculated value of 1.256 $g \cdot cm^{-3}$ calculated from the single crystal structure.

The optical rotation of the magnesium-valsartan-hexahydrate produced according to example 2 is measured in methanol as a 1% solution $[a]^{20}_D = -14°$.
The enantiomer purity of the salt hydrate produced according to example 2 is determined by a stereo-specific HPLC method. The stereo-specific separation is achieved by a chiral column (Chiral AGP). The enantiomer purity is determined as ee = 99.6 %.

Calculation of the interlattice plane intervals from the X-ray powder pattern taken with a Guinier camera is as follows for the most important lines for this batch of the magnesium valsartan hexahydrate:
d in [ Å ] : 19.78, 10.13, 9.84, 7.28, 6.00, 5.81, 5.67, 5.21, 5.04, 4.88, 4.21, 4.18, 4.08, 3.95, 3.46, 3.42.

Elementary analysis gives the following measured values of the elements present in the hexahydrate of the magnesium salt of valsartan and of water. The water evaluation is carried out at 130°C after expulsion. The findings of the elementary analysis, within the error limits, correspond to the sum formula $(C_{24}H_{27}N_5O_3)^{2-} Mg^{2+} \cdot 6 H_2O$.

| | % found | % calculated |
|---|---|---|
| C | 51.03 | 50.94 |
| H | 7.00 | 6.95 |
| N | 12.45 | 12.38 |
| O | 25.02 | 25.44 |
| water | 19.08 | 19.10 |
| Mg | 4.35 | 4.29 |

Example 3:
Production of the hydrate of di-potassium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine (3.5 ± 1.0 mole $H_2O$)

5 g of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine are dissolved whilst heating gently in 11.5 ml of 2 normal potassium hydroxide solution and mixed with 320 ml of acetonitrile. The mixture is heated for 5 minutes to reflux (turbid solution), left without stirring for 3 days at room temperature (seeding) and then left for 24 hours at 0°C. The mother liquor is decanted. The

crystallisate is washed twice with acetonitrile and then dried in the air for 36 hours until reaching a constant weight. (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine dipotassium salt hydrate is obtained (3.7 mols water per mol dipotassium salt). The melting point in a closed specimen container is 135°C.

Elementary analysis: $C_{24}$ $H_{27}$ $N_5$ $O_3$ $K_2$, 3.72 $H_2O$, molar mass 578.72

| | % found | % calculated |
|---|---|---|
| C | 49.90 | 49.81 |
| H | 5.92 | 6.00 |
| N | 12.14 | 12.10 |
| O | 18.55 | 18.58 |
| water | 11.58 | 11.58 |
| K | 13.50 | 13.51 |

X-ray diffraction diagram measured with the diffractometer Scintag Inc., Cupertino, CA 95014, US, using CuKα radiation.
Reflection lines and intensities of the most important lines of the hydrate of the di-potassium salt of valsartan, values given in 2θ in °:

| 2θ in ° | Intensity |
|---|---|
| 4.6 | strong |
| 8.8 | medium |
| 9.2 | strong |
| 11.1 | weak |
| 12.5 | weak |
| 14.8 | strong |
| 15.3 | weak |
| 16.4 | medium |
| 17.8 | strong |
| 18.2 | medium |
| 18.4 | medium |
| 18.9 | medium |

| | |
|---|---|
| 20.4 | medium |
| 21.1 | weak |
| 21.3 | medium |
| 22.3 | weak |
| 22.5 | strong |
| 23.1 | medium |
| 23.9 | strong |
| 25.6 | weak |
| 26.6 | strong |
| 26.9 | medium |
| 28.1 | medium |

Preferred are hydrates comprising the medium and strong intensity peaks.

Table 11:

Crystal data and parameters of the hydrate of the di-potassium salt of valsartan

| Crystal data | |
|---|---|
| sum formula | $(C_{24}H_{27}N_5O_3)^{2-}2K^+ . x\ H_2O$ (x=3.5±1.0) |
| molecular mass | 574.78 |
| crystal system | orthorhombic |
| space group | $P2_12_12$ |
| a (Å) | 38.555(2) |
| b (Å) | 7.577(1) |
| c (Å) | 10.064(1) |
| V ($Å^3$) | 2940.0(5) |
| Z | 4 |
| F(000) | 1212 |
| $D_{calc.}$ ($g.cm^{-3}$) | 1.286 |
| number of reflections for cell parameters | 25 |
| θ range for cell parameters (°) | 30-38 |
| μ ($mm^{-1}$) | 3.24 |
| Temperature (°C) | 23 |
| crystal shape | prisms |

| | |
|---|---|
| crystal size (mm) | 0.63x0.20x0.14 |
| crystal colour | colourless |
| | |
| *Data collection* | |
| diffractometer | Enraf Nonius CAD4 |
| radiation ( graphite monochromator ) | CuKα |
| wave length (Å) | 1.54178 |
| scan mode | ω/2θ |
| scan range (θ) | 3-74 |
| absorption correction | none |
| number of measured reflections | 3450 |
| number of observed reflections (I>2σ(I)) | 2867 |
| h range | -48→0 |
| k range | -9→0 |
| l range | -12→0 |
| number of standard reflections | 3 every 120 mins |
| variation in intensity | ±5% |
| | |
| *Structure refinement* | |
| refinement method | refinement on $F^2$, complete matrix |
| number of parameters | 341 |
| R | 0.069 |
| $R_W$ | 0.182 |
| S | 1.57 |
| number of reflections used | 2867 |
| treatment of H-atoms | "riding", apart from those of the water molecules ,which were ignored |
| $\Delta/\sigma_{max}$ | 0.24 |
| extinction correction | 0.0010(5) |
| maximum/minimum residual electron density in final difference-Fourier calculation | 0.815/-0.676(eÅ$^{-3}$) |
| absolute structure parameters | -0.02(4) |

*Programmes used*

SHELXS86 (Sheldrick, Göttingen),
XHELXL93 (Sheldrick, Göttingen),
SCHAKAL92 (Keller, Freiburg)

Example 4:

Production of the di-potassium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

25 g of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine are dissolved in 200 ml of ethanol. 50 ml of water are added, the solution cooled to 0°C and then mixed with 57.4 ml of 2 normal potassium hydroxide solution. The mixture is concentrated by evaporation on a rotary evaporator, evaporated again with each of toluene and acetonitrile, and dried in a high vacuum for 15 minutes at 50°C. The product is dissolved in 290 ml of a hot mixture of acetonitrile/water (95:5), mixed with an additional 110 ml of acetonitrile, allowed to cool and seeded at ca. 30°C. The mixture is left to stand for 4 days at room temperature and filtered by suction. The residue is washed with acetonitrile/water (95:5) and dried in a high vacuum at 80°C. (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine dipotassium salt is obtained as a white powder. Melting point >300°C.

Elementary analysis: The material obtained is hygroscopic and can be equilibrated in the air ($C_{24}$ $H_{27}$ $N_5$ $O_3$ $K_2$, 3.96 mols $H_2O$).

| | % found | % calculated |
|---|---|---|
| C | 49.15 | 49.44 |
| H | 6.02 | 6.04 |
| N | 11.91 | 12.01 |
| O | 19.18 | 19.1 |
| water | 12.23 | 12.24 |
| K | 13.4 | 13.41 |

Example 5:

Production of the di-sodium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

1 g of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine is dissolved in 50 ml of ethanol, mixed with 2.3 ml of 2 normal sodium hydroxide solution and concentrated by evaporation, and the residue is evaporated with each of ethanol and ethyl acetate. The white residue is stirred in hot acetonitrile and filtered by suction at room temperature. Drying in a high vacuum at 80°C over night yields (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine disodium salt as a white powder. Melting point from 260°C, brownish discolouration at 295°C.

Elementary analysis: The material obtained (hygroscopic) can be equilibrated in the air ($C_{24}$ $H_{27}$ $N_5$ $O_3$ $Na_2$, 5.36 mols $H_2O$, molar mass 576.05)

| | % found | % calculated |
|---|---|---|
| C | 49.79 | 50.04 |
| H | 6.51 | 6.60 |
| N | 12.00 | 12.16 |
| O | 23.44 | 23.22 |
| water | 16.75 | 16.76 |
| Na | 8.09 | 7.98 |

Example 6:

Production of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

5 g of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine are added to a suspension of 0.666 g of magnesium hydroxide in 20 ml of water. 40 ml of methanol are added, then the mixture is stirred for 2 hours at room temperature and concentrated. The residue is dissolved in methanol, filtered through a hard filter, concentrated and evaporated with acetonitrile. The product is stirred with hot

acetonitrile, filtered by suction at room temperature and dried in a high vacuum at 90°C over night. (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine magnesium salt is obtained as a white powder. Melting point: The sample becomes brownish upon heating and vitrifies towards 300°C.

Elementary analysis: $C_{24}$ $H_{27}$ $N_5$ $O_3$ Mg, 0.89 mols $H_2O$, molar mass: 473.85

| | % found | % calculated |
|---|---|---|
| C | 61.26 | 60.83 |
| H | 6.13 | 6.12 |
| N | 14.88 | 14.78 |
| O | | 13.13 |
| water | 3.39 | 3.38 |
| Mg | 4.74 | 5.13 |

Example 7:

Production of the calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

5 g of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine are added to a suspension of 0.851 g of calcium hydroxide in 20 ml of water and then mixed with 200 ml of ethanol. The mixture is stirred for one hour at room temperature, concentrated by evaporation to dryness (re-evaporation with acetonitrile), stirred in hot acetonitrile (with a trace each of ethanol and water) and filtered by suction at room temperature.

0.95 g of the salt are heated to reflux in 20 ml of acetonitrile/water (1:1), whereby the mixture almost dissolves. The mixture is allowed to cool to room temperature, mixed with 20 ml of acetonitrile, filtered by suction and washed twice with acetonitrile/water (1:1) and dried over night in a high vacuum at 80°C. Melting point: from 300°C (decomposition).

Elementary analysis: $C_{24}$ $H_{27}$ $N_5$ $O_3$ Ca, 1.71 mols $H_2O$, molar mass 504.39 (water evaluation carried out after expulsion at 150°C).

| | % found | % calculated |
|---|---|---|
| C | 56.88 | 57.15 |
| H | 6.13 | 6.08 |
| N | 13.89 | 13.88 |
| O | | 14.94 |
| water | 6.12 | 6.11 |
| Ca | 7.94 | 7.95 |

Example 8:

Production of the mono-potassium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

2 g of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine are suspended in 20 ml of water and mixed with 2.296 ml of a 2 normal potassium hydroxide solution. The mixture is stirred for 30 minutes and mixed with 50 ml of ethanol, whereupon a colourless solution is obtained. The mixture is concentrated by evaporation, evaporated once more with acetonitrile and lyophilised from tert.-butanol (with a trace of water).

Elementary analysis (after equilibration in the air). $C_{24}$ $H_{27}$ $N_5$ $O_3$ Ca, 1.69 mols $H_2O$, molar mass 504.06 (water evaluation carried out after expulsion at 150°C).

| | % found | % calculated |
|---|---|---|
| C | 57.30 | 57.19 |
| H | 6.35 | 6.27 |
| N | 13.61 | 13.89 |
| O | 14.58 | 14.89 |
| water | 6.04 | 6.04 |
| K | 7.72 | 7.76 |

Example 9:

Production of the magnesium salt as the hexahydrate of valsartan by a water-equilibrating process.

1600 g of valsartan and 6820 g of isopropanol are stirred to form a suspension in a mixing container at room temperature, and added to an 80 litre glass receptacle with a stirrer. The mixing container is rinsed with 3919 g of isopropanol in portions and the rinsing solution added to the main mixture. After adding 3800 g of deionised water, the mixture is transformed into a homogeneous solution by stirring. Then, 156.3 g of magnesium oxide, suspended in 1520 g of deionised water, are added and the suspension supplemented with 1000 g of deionised water. By slowly stirring at room temperature, the magnesium oxide goes into solution. The pH value of the resulting solution is ca. 7.2. By adding a further 2.5 g of magnesium oxide in small portions, the pH value is raised to ca. 8.3. The resulting mixture is turbid owing to undissolved particles of unknown type in the magnesium oxide.

This mixture is transferred through a candle filter to a 35 litre enamel boiler and the glass receptacle and the transfer tube are rinsed with 885 g of isopropanol and 1122 g of deionised water. For mild concentration, a vacuum is created in the boiler to an initial theoretical value of 89-100 mbar. With a temperature of the heating medium of 45-50°C and a boiling temperature of the mixture of 37-40°C, a total of 13.66 kg of aqueous isopropanol is distilled. By lowering the distillation pressure to a final value of 10 mbar and simultaneously raising the heating medium temperature to 65°C, the amount of distillate is increased to a total of 17.12 kg. 9300 g of ethyl acetate, followed by 14.9 g of valsartan Mg salt hexahydrate as seeding crystals, are added to the boiler content whilst stirring. Finally, a further 6680 g of ethyl acetate are dispensed in and cooling is effected to room temperature whilst stirring. The stirring procedure is maintained for at least 24 hours. The suspension is then filtered through Büchner filters. A moist filter cake is thus obtained. The boiler is rinsed with 1171 g of ethyl acetate and the rinsing mixture is used to wash the filter cake. Drying of a partial amount on metal sheets in a vacuum drying chamber at 50 mbar pressure and 40°C oven temperature for 6.5 hours until reaching a constant weight yields a dry substance.

The physical data, especially the X-ray powder pattern, correspond to the magnesium hexahydrate salt of example 2.

Example 10:

Production of the calcium salt of valsartan as the tetrahydrate.

1600 g of valsartan and 7000 g of ethanol are stirred to form a suspension in a mixing container at room temperature, and added to a 35 litre enamel boiler with a stirrer. The mixing container is rinsed with 2000 g of ethanol in portions and the rinsing solution added to the main mixture. After adding 9000 g of deionised water, the mixture is transformed into a homogeneous solution by stirring. Then, 272 g of calcium hydroxide, suspended in 1500 g of deionised water, are added and the suspension supplemented with 1300 g of deionised water. By slowly stirring at room temperature, the calcium hydroxide goes into solution. The pH value of the resulting solution is ca. 6.9. By adding a further 9.6 g of calcium hydroxide, the pH value is raised to ca. 10.6. The resulting mixture is turbid owing to undissolved particles (calcium carbonate) in the calcium hydroxide. This mixture is transferred through a candle filter to a 35 litre enamel boiler and the glass receptacle and the transfer tube are rinsed with a solution of 1048 g of ethanol and 1000 g of deionised water. For mild concentration, a vacuum is created in the boiler to a theoretical value of 100-120 mbar. With a temperature of the heating medium of ca. 50°C and a boiling temperature of the mixture of max. 44°C, a total of 11.32 kg of aqueous ethanol is distilled. The dissolved salt crystallises spontaneously during the course of distillation. The suspension present at the end of distillation is cooled to ca. 5°C whilst stirring, and is stirred for ca. 16 hours at 5°C. The suspension is then filtered through Büchner filters. The boiler is rinsed with a mixture of 3600 ml of deionised water and 400 ml of ethanol, the mixture being cooled to 5°C, and the rinsing mixture is used to wash the filter cake. A moist filter cake is thus obtained. Drying of a partial amount on metal sheets in a vacuum drying chamber at 50 mbar pressure and 40°C oven temperature for 24 hours until reaching a constant weight yields a dry substance.

The physical data, especially the X-ray powder pattern, correspond to the calcium tetrahydrate salt of example 1.

Example 11:

Hydrate of valsartan disodium salt (2.4 ± 1.0 mole $H_2O$):

50 ml of 2N sodium hydroxide solution are added dropwise at ca. 25°C to a solution of 21.5 g of valsartan in 200 ml of isopropanol. The clear solution (pH ca. 7.2) is concentrated under vacuum at ca. 40°C. The amorphous residue of the disodium salt is suspended in 100 ml of isopropanol, and water is removed by concentrating under vacuum once more at ca. 40°C and degassing. The amorphous residue is suspended in 75 ml of acetone and 2 ml of water at ca. 40°C. At ca. 25-30°C, 200 ml of tert.-butylmethylether are added, whereby constituents that are initially smeary are gradually transformed into a crystalline suspension. After stirring over night at ca. 25°C, the suspension is cooled to 10°C and after ca. 1 hour is filtered by suction whilst excluding atmospheric moisture. Washing then takes place with 20 ml of tert.-butylmethylether. The moist filter cake is dried over night at ca. 30 mbar and at 30°C. A colourless, slightly hygroscopic crystal powder is obtained.

Elementary analysis: $C_{24}$ $H_{27}$ $N_5$ $O_3$ $Na_2$, 2.44 mols $H_2O$

| | % found | % calculated |
|---|---|---|
| C | 55.03 | 55.07 |
| H | 6.16 | 6.14 |
| N | 13.38 | 13.38 |
| O | | 16.63 |
| water | 8.40 | 8.41 |
| Na | 8.67 | 8.78 |

X-ray diffraction diagram (reflection lines and intensities of the most important lines) of the crystalline hydrate of the disodium salt of valsartan measured with the diffractometer Scintag Inc. Cupertino, CA 95014, US, using CuKα radiation:

| 2θ | Intensity |
|---|---|
| 4.7 | strong |
| 9.1 | strong |
| 13.3 | weak |
| 13.7 | weak |
| 15.6 | medium |
| 16.4 | medium |

| | |
|---|---|
| 17.2 | medium |
| 17.9 | medium |
| 18.7 | medium |
| 19.6 | medium |
| 21.3 | medium |
| 21.9 | medium |
| 22.8 | strong |
| 24.0 | weak |
| 24.8 | weak |
| 25.5 | weak |
| 26.5 | medium |
| 26.8 | weak |
| 27.3 | weak |
| 27.8 | weak |
| 28.6 | weak |
| 29.4 | weak |
| 29.9 | medium |

Example 12:

Hydrate of the valsartan dipotassium salt (3.4 ± 1.0 mole $H_2O$):

6.9 g of potassium carbonate are added at ca. 25°C to the solution of 21.7 g of valsartan in 150 ml of acetone and 20 ml of water. After stirring for 2 hours at ca. 25°C, an almost clear solution is obtained, which is concentrated in a vacuum at ca. 50°C bath temperature. 55 ml of acetone are added to the residue (29.3 g) which contains residual water, and at ca. 35°C, over the course of ca. two hours, a total of 250 ml of tert.-butylmethylether is dispensed in. After stirring at ca. 25°C, the easily stirrable crystal suspension is cooled to 10°C, stirred for at least one hour, filtered by suction and washed with 20 ml of tert.butylmethylether. The moist filter cake is dried over night at ca. 30 mbar and at 30°C. A colourless, slightly hygroscopic crystal powder is obtained.

Elementary analysis: $C_{24}$ $H_{27}$ $N_5$ $O_3$ $K_2$, 3.42 mols $H_2O$

| | % found | % calculated |
|---|---|---|
| C | 50.37 | 50.28 |
| H | 5.87 | 5.95 |
| N | 12.24 | 12.22 |
| O | | 17.92 |
| water | 10.76 | 10.75 |
| K | 13.4 | 13.64 |

X-ray diffraction diagram measured with the diffractometer Scintag Inc., Cupertino, CA 95014, US using a CuKα radiation.

Reflection lines and intensities of the most important lines of the hydrate of the di-potassium salt of valsartan, values given in 2θ in °:

| 2θ in ° | Intensity |
|---|---|
| 4.9 | strong |
| 9.4 | strong |
| 11.4 | weak |
| 12.8 | weak |
| 14.0 | weak |
| 15.0 | weak |
| 15.6 | weak |
| 16.6 | medium |
| 18.0 | weak |
| 18.5 | weak |
| 18.9 | weak |
| 20.7 | weak |
| 21.5 | weak |
| 22.0 | weak |
| 22.7 | medium |
| 23.3 | weak |
| 24.1 | medium |
| 25.6 | weak |
| 25.8 | weak |

| | |
|---|---|
| 27.1 | medium |
| 29.4 | weak |

Preferred are hydrates comprising medium and strong intensity peaks.

Example 13:

Valsartan calcium/magnesium mixed salt:

21.5 g of valsartan in 200 ml of isopropanol and 100 ml of water are stirred for ca. 3 hours at ca. 25°C with 1.5 g of magnesium hydroxide and 1.9 g of calcium hydroxide. The practically clear solution is concentrated in a vacuum at ca. 50°C. A total of 240 ml of ethyl acetate is added with stirring to the still warm, semi-solid residue which contains residual water. Upon stirring over night at ca. 25°C, initially sticky constituents are transformed into a homogeneous suspension. The suspension is filtered by suction and washed with 20 ml of ethyl acetate. The moist filter cake is dried in a vacuum at 30-40°C. A colourless crystal powder is obtained.

The X-ray diffraction diagram corresponds to a conglomerate of calcium tetrahydrate and magnesium hexahydrate from example 1 and 2.

Example 14:

Valsartan bis-diethylammonium salt:

1.5 g of diethylamine are added dropwise at ca. 25°C to the solution of 4.35 g of valsartan in 60 ml of acetone. After a short time, crystallisation slowly sets in. After stirring over night, the crystallisate is filtered by suction at ca. 20°C, washed with cold acetone and dried in a vacuum at ca. 50°C. A colourless crystal powder is obtained.

Elementary analysis: $C_{32}$ $H_{51}$ $N_7$ $O_3$, 0.1 mols $H_2O$

| | % found | % calculated |
|---|---|---|
| C | 65.82 | 65.84 |
| H | 8.90 | 8.84 |
| N | 16.84 | 16.80 |

| | | |
|---|---|---|
| O | | 8.52 |
| water | 0.34 | 0.34 |

X-ray diffraction diagram (reflection lines and intensities of the most important lines) of the crystalline bis-diethylammonium salt

| 2θ | Intensity |
|---|---|
| 4.7 | weak |
| 8.5 | strong |
| 9.3 | strong |
| 10.8 | strong |
| 11.3 | weak |
| 13.4 | strong |
| 14.0 | medium |
| 14.3 | weak |
| 14.9 | medium |
| 17.1 | medium |
| 17.4 | medium |
| 17.6 | medium |
| 18.3 | weak |
| 19.0 | medium |
| 20.0 | weak |
| 21.2 | medium |
| 21.6 | weak |
| 22.4 | medium |
| 22.7 | weak |
| 24.9 | medium |
| 25.2 | weak |
| 27.0 | weak |

Example 15:

Valsartan bis-dipropylammonium salt:

2.1 g of dipropylamine are added dropwise at 25°C to the solution of 4.35 g of valsartan in 60 ml of acetone. When crystallisation has set in, the temperature is raised for a brief

period to 40°C and is allowed to drop to room temperature over ca. 2 hours. After stirring over night, the crystallisate is filtered by suction, washed twice with 15 ml of acetone and dried in a vacuum at ca. 40°C. Granular crystals are obtained.

Elementary analysis: $C_{36}$ $H_{69}$ $N_7$ $O_3$, 0.05 mols $H_2O$

| | % found | % calculated |
|---|---|---|
| C | 67.74 | 67.69 |
| H | 9.32 | 9.33 |
| N | 15.36 | 15.35 |
| O | | 7.64 |
| water | 0.13 | 0.14 |

X-ray diffraction diagram (reflection lines and intensities of the most important lines) of the crystalline bis-dipropylammonium salt

| 2θ | Intensity |
|---|---|
| 8.5 | strong |
| 8.9 | weak |
| 9.4 | strong |
| 10.0 | medium |
| 11.2 | weak |
| 11.6 | weak |
| 12.5 | weak |
| 13.2 | strong |
| 13.9 | strong |
| 14.3 | weak |
| 14.7 | weak |
| 15.1 | weak |
| 15.6 | weak |
| 16.0 | weak |
| 17.0 | medium |
| 17.9 | medium |
| 18.7 | strong |
| 19.9 | weak |

| | |
|---|---|
| 20.4 | weak |
| 20.6 | weak |
| 21.0 | strong |
| 21.7 | weak |
| 22.3 | medium |
| 23.1 | strong |
| 24.5 | weak |
| 25.5 | medium |
| 25.8 | weak |
| 26.7 | weak |
| 28.6 | weak |

Example 16:

Bis-dibutylammonium salt of valsartan:

A solution of 2.15 g of valsartan in 30 ml of acetone is mixed with 1.4 g of dibutylamine at ca. 25°C. Crystallisation sets in after a short time, and the thick suspension is gradually diluted with 20 ml of isopropyl acetate over ca. 1 hour. After stirring for 4 hours at ca. 25°C, the crystals are removed by suction, washed twice with 10 ml of isopropyl acetate and dried in a vacuum at 50°C. A colourless, slightly hygroscopic crystal powder is obtained.

Elementary analysis: $C_{40}$ $H_{67}$ $N_7$ $O_3$, 0.5 mols $H_2O$

| | % found | % calculated |
|---|---|---|
| C | 68.25 | 68.30 |
| H | 9.79 | 9.75 |
| N | 13.89 | 13.94 |
| O | | 8.01 |
| water | 1.33 | 1.33 |

X-ray diffraction diagram (reflection lines and intensities of the most important lines) of the crystalline bis-dibutylammonium salt

| 2θ | Intensity |
|---|---|
| 7.5 | very strong |
| 8.5 | medium |
| 9.7 | strong |
| 12.7 | strong |
| 13.3 | weak |
| 14.1 | strong |
| 15.1 | medium |
| 16.4 | strong |
| 17.7 | weak |
| 18.2 | weak |
| 19.5 | strong |
| 19.9 | medium |
| 20.5 | medium |
| 21.4 | medium |
| 21.9 | medium |
| 22.2 | medium |
| 22.6 | medium |
| 23.0 | strong |
| 23.7 | weak |
| 24.2 | weak |
| 24.7 | medium |
| 25.7 | medium |
| 26.0 | weak |
| 26.5 | weak |
| 28.8 | weak |

Formulation example 1:

Directly compressed tablet:

| No. | Ingredient | proportion per batch [g] | proportion per tablet core [mg] |
|---|---|---|---|
| 1 | valsartan calcium salt tetrahydrate | 134.24 | 80 |

| | | | |
|---|---|---|---|
| 2 | Avicel PH 102 (microcrystalline cellulose) | 60.408 | 36 |
| 3 | lactose (crystalline) | 96.1494 | 57.3 |
| 4 | crospovidone | 7.551 | 4.5 |
| 5 | aerosil 200 (silica, colloidal anhydrous) | 0.839 | 0.5 |
| 6 | magnesium stearate (vegetable) | 6.2086 | 3.7 |

Ingredient no. 1 is sieved through a 0.5 mm sieve and mixed for 15 minutes in a Turbula with ingredients 1-6. Tablets are compress using a single punch tablet press with punches of a diameter of 8mm.

Formulation example 2:

Tablet produced by roller compaction:

| No. | Ingredient | proportion per batch [g] | proportion per tablet core [mg] |
|---|---|---|---|
| 1 | valsartan magnesium salt hexahydrate | 400 | 80 |
| 2 | Avicel PH 102 (microcrystalline cellulose) | 270 | 54 |
| 3 | crospovidone | 75 | 15 |
| 4 | aerosil 200 (silica, colloidal anhydrous) | 7.5 | 1.5 |
| 5 | magnesium stearate | 15 | 3 |
| 6 | magnesium stearate | 7.5 | 1.5 |

Ingredients no. 1-5 are mixed for 50 minutes and compacted on a Freund roller compactor. The band is milled and after admixing ingredient no 6, compressed into tablets using a single punch tablet press with punches of a diameter of 8mm.

What we claim is:

1. A salt of valsartan, selected from the group consisting of the monosodium salt, the monopotassium salt, the disodium salt, the dipotassium salt, the magnesium salt, the calcium salt, the bis-diethylammonium salt, the bis-dipropylammonium salt, the bis-dibutylammonium salt, the mono-L-arginine salt, the bis-L-arginine salt, the mono-L-lysine salt and the bis-L-lysine salt, as well as salt mixtures thereof.

2. A salt according to claim 1 in crystalline, partially crystalline or amorphous form.

3. The calcium salt or the magnesium salt of valsartan according to claim 1.

4. The tetrahydrate of the calcium salt of valsartan according to claim 3.

5. The tetrahydrate according to claim 4, characterised by
(i) an X-ray powder pattern taken with a Guinier camera comprising the following interlattice plane intervals:
d in [Å]: 16.1±0.3, 9.9±0.2, 9.4±0.2, 7.03±0.1, 6.50±0.1, 5.87±0.05, 5.74±0.05, 4.95±0.05, 4.73±0.05, 4.33±0.05, 4.15±0.05, 4.12±0.05, 3.95±0.05; or
(ii) an ATR-IR spectrum having the following absorption bands expressed in reciprocal wave numbers ($cm^{-1}$):
1621 (st); 1578 (m); 1458 (m); 1441 (m); 1417 (m); 1364 (m); 1012 (m); 758 (m); 738 (m); 696 (m); 666 (m).

6. The hexahydrate of the magnesium salt of valsartan according to claim 1.

7. The hexahydrate according to claim 6, characterised by
(i) an X-ray powder pattern taken with a Guinier camera comprising the following interlattice plane intervals:
d in [Å] : 19.7±0.3, 10.11±0.2, 9.8±0.2, 7.28±0.1, 5.81±0.05, 5.68±0.05, 5.03±0.05, 4.88±0.05, 4.18±0.05, 4.08±0.05, 3.46 ±0.05; or

(ii) an ATR-IR spectrum having the following absorption bands expressed in reciprocal wave numbers ($cm^{-1}$):
3378 (m); 3274 (m); 2956 (m); 1619 (st); 1557 (m); 1464 (m); 1419 (m); 1394 (st); 1374 (m); 1175 (m); 836 (m); 820 (s); 766 (st); 751 (m); 741 (st); 732 (st).

8. A salt according to one of claims 1-7 in the form of a solvate.

9. A salt according to one of claims 1-8 in the form of a hydrate.

10. A salt according to one of claims 1-9 in a form selected from the group consisting of
(i) a crystalline form;
(ii) a partly crystalline form;
(iii) an amorphous form; and
(iv) a polymorphous form.

11. Pharmaceutical preparation containing a compound according to one of claims 1 to 10 and a pharmaceutically acceptable excipient or additive.

12. Pharmaceutical preparation according to claim 11, containing a salt according to one of claims 1-9 in combination with at least one composition selected from the group consisting of a:
(i) HMG-Co-A reductase inhibitor or a pharmaceutically acceptable salt thereof,
(ii) angiotensin converting enzyme (ACE) inhibitor or a pharmaceutically acceptable salt thereof,
(iii) calcium channel blocker or a pharmaceutically acceptable salt thereof,
(iv) aldosterone synthase inhibitor or a pharmaceutically acceptable salt thereof,
(v) aldosterone antagonist or a pharmaceutically acceptable salt thereof,
(vi) dual angiotensin converting enzyme/neutral endopeptidase (ACE/NEP) inhibitor or a pharmaceutically acceptable salt thereof,
(vii) endothelin antagonist or a pharmaceutically acceptable salt thereof,
(viii) renin inhibitor or a pharmaceutically acceptable salt thereof, and
(ix) diuretic or a pharmaceutically acceptable salt thereof.

13. Use of a compound according to one of claims 1 to 10 in the preparation of a medicament for the prophylaxis or treatment of diseases and conditions which can be inhibited by blocking the $AT_1$ receptor.

14. Process for the manufacture of a salt according to claim 1, characterised in that

(i) valsartan and the appropriate base are added to a water-containing organic solvent,

(ii) the solvent is concentrated, for example by heating, if necessary under reduced pressure or by slowly evaporating, e.g. at room temperature,

(iii) the residue of evaporation is equilibrated with the required amount of water by

(a) suspending the residue of evaporation, which is advantageously still warm, and which still contains some water, in an appropriate solvent or

(b) by equilibrating the water excess in the solvent;

whereby in a) and b) the existing or added water is present in a quantity in which the water dissolves in the organic solvent and does not form an additional phase; and

(iv) the salt obtained is isolated.

# INTERNATIONAL SEARCH REPORT

onal Application No
PCT/EP 01/08253

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D257/04 A61K31/41 A61P9/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 26 November 2001 | 04/12/2001 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Lauro, P |

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Ional Application No
PCT/EP 01/08253

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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# High-throughput crystallization: polymorphs, salts, co-crystals and solvates of pharmaceutical solids

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**Abstract**

The concepts of high-throughput (HT) screening and combinatorial synthesis have been integrated into the pharmaceutical discovery process, but are not yet commonplace in the pharmaceutical development arena. Emerging strategies to speed pharmaceutical development and capture solid form diversity of pharmaceutical substances have resulted in the emergence of HT crystallization technologies. The primary type of diversity often refers to polymorphs, which are different crystal forms of the same chemical composition. However, diverse salt forms, co-crystals, hydrates and solvates are also amenable to study in HT crystallization systems. The impact of form diversity encompasses issues of stability and bioavailability, as well as development considerations such as process definition, formulation design, patent protection and regulatory control. This review highlights the opportunities and challenges of HT crystallization technologies as they apply to pharmaceutical research and development.


*Keywords:* High-throughput; Crystallization; Polymorph; Solvate; Salt; Co-crystal

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## 1. Introduction

Active pharmaceutical ingredients (APIs) are frequently delivered to the patient in the solid-state as part of an approved dosage form (e.g., tablets, capsules, etc.). Solids provide a convenient, compact and generally stable format to store an API or a drug product. Understanding and controlling the solid-state chemistry of APIs, both as pure drug substances and in formulated products, is therefore an important aspect of the drug development process. APIs can exist in a variety of distinct solid forms, including polymorphs, solvates, hydrates, salts, co-crystals and amorphous solids. Each form displays unique physicochemical properties that can profoundly influence the bioavailability, manufacturability purification, stability and other performance characteristics of the drug [1]. Hence, it is critical to understand the relationship between the particular solid form of a compound and its functional properties. Discovery and characterization of the diversity of solid forms of a drug substance provide options from which to select a form that exhibits the appropriate balance of critical properties for development into the drug product. Importantly, the desired properties may vary with each mode of delivery (i.e., oral, pulmonary, parenteral, transdermal, etc.), such that the solid form may differ for each optimized dosage form. Given these options, the choice and design of pharmaceutical solid forms can be critically important to successful drug development.

Solid form discovery and design depends on the nature of the molecule of interest and type of physical property challenges faced in its development. The preferred solid form is generally the thermodynamically most stable crystalline form of the compound [1,2]. However, the stable crystal form of the parent compound may exhibit inadequate solubility or dissolution rate resulting in poor oral absorption, particularly for water-insoluble compounds. In this case, alternative solid forms may be investigated. For ionizable compounds, preparation of salt forms using pharmaceutically acceptable acids and bases is a common strategy to improve bioavailability [1,3,4]. Like the parent compound, pharmaceutical salts may exist in several polymorphic, solvated and/or hydrated forms.

Most APIs and their salts are purified and isolated by crystallization from an appropriate solvent during the final step in the synthetic process. A large number of factors can influence crystal nucleation and growth during this process, including the composition of the crystallization medium and the process(es) used to generate supersaturation and promote crystallization [1,5–13]. The most notable variables of composition and processing are summarized in Table 1. Solid form screening is used to understand the effects that these variables have on the polymorphic outcome of a crystallization experiment, so that a robust process can be identified to produce the desired crystal form. Traditionally, the study of solid form diversity of active compounds has relied on the use of a variety of common process methods for generation of new forms, coupled with modern characterization methods for analysis of the solids produced [2,14]. Most often, however, a combination of solvent recrystallization (cooling or evaporative, as well as slurry conversion) and thermal analysis (e.g., hot stage microscopy, differential scanning calorimetry) are employed for initial form screening. Such methods are inherently slow and only allow exploration of a small fraction of the composition and process space that can contribute to form diversity. Before suggesting a form for development, scientists may have carried out only a few dozen crystallization experiments and possibly prepared a handful of different salts of a compound. The main reasons for the limited number of experiments are the constraints on availability of compound and scientists' analytical capacity in a given time frame, and they are therefore often forced to make form selection decisions on incomplete data. Accordingly, it is not surprising that unexpected and undesired outcomes can, and do, occur later on in development.

Despite more than a century of research [15], the fundamental mechanisms and molecular properties that drive crystal form diversity, specifically the nucleation of polymorphic forms, are not well under-

Table 1
Crystallization composition and processing variables [1,2,8]

| Composition type | | Process variables[a] | | | | |
|---|---|---|---|---|---|---|
| Polymorph/ solvates | Salts/ co-crystals | Thermal | Anti-solvent | Evaporation | Slurry conversion | Other variables |
| ▪ Solvent/ solvent combinations | ▪ Counter-ion type | ▪ Heating rate | ▪ Anti-solvent type | ▪ Rate of evaporation | ▪ Solvent type | ▪ Mixing rate |
| ▪ Degree of supersaturation | ▪ Acid/base ratio | ▪ Cooling rate | ▪ Rate of anti-solvent addition | ▪ Evaporation time | ▪ Incubation temperature | ▪ Impeller design |
| ▪ Additive type | ▪ Solvent/ solvent combinations | ▪ Maximum temperature | ▪ Temperature of anti-solvent addition | ▪ Carrier gas | ▪ Incubation time | ▪ Crystallization vessel design (including capillaries, etc.) |
| ▪ Additive concentration | ▪ Degree of super-saturation<br>▪ Additive type and concentration<br>▪ pH<br>▪ Ionic strength | ▪ Incubation temperature(s)<br>▪ Incubation time | ▪ Time of anti-solvent addition | ▪ Surface-volume ratio | ▪ Thermal cycling and gradients | |

[a] Applicable to all types of screens.

stood [13,16]. As a result, predictive methods of assessing polymorphic behavior of pharmaceutical compounds by ab initio calculations remain a formidable challenge. Even in cases where the existence of a crystalline form is predicted, the stability relative to other crystalline packing arrangements has been difficult to estimate with accuracy [17]. Moreover, the prediction of packing structures for multicomponent (e.g., solvates, hydrates, co-crystals) or ionic systems is not yet possible [17]. Due to these limitations, solid form discovery remains an experimental exercise, where manual screening methods are employed to explore form diversity of a compound.

Control over solid form throughout the drug development process is of paramount importance. Reliable preparation and preservation of the desired form of the drug substance must be demonstrated, and has become increasingly scrutinized by regulatory agencies as more sensitive and quantitative solid-state analytical methods have become available [18]. Many strategies to influence and control the crystallization process to produce the solid form of interest have been reported. Some examples include stereochemical control using tailor-made auxiliaries [19–21], targeted solvent recrystallization [22–24], and templating using a variety of surfaces (e.g., organic single crystal substrates [25], surfaces of metastable crystal faces [25,26], inorganic crystal surfaces [27] and polymeric materials [28]). Recent studies have also begun to uncover the role of reaction byproducts and other impurities in determining polymorphic outcome and crystal properties [29–32], and in fact, it has been shown that in some cases such species can stabilize metastable crystal forms [33,34]. In addition, new processing methods continue to be developed to improve discovery and characterization of new forms, including precipitation by supercritical fluid [35,36], laser induced nucleation [37–39] and capillary crystallization [40–42]. However, there remains a lack of fundamental understanding of the nucleation process and the specific factors that contribute to crystallization of diverse forms of a compound [13,21,23]. In order to fully control the crystallization process, the link between the physical or chemical processes that influence nucleation and crystal growth needs to be better established. It is in this area that new experimental methodologies have the potential to enable development of this knowledge base.

There is reason to believe that the already complicated landscape of pharmaceutical solid forms will become even more complex in the future. It is now increasingly appreciated that hydrogen bonded co-crystal structures between active agents and molecules other than water or solvent can be prepared. For example, co-crystals of aspirin, *rac*-ibuprofen and

*rac*-flurbiprofen have been prepared by disrupting the carboxylic acid dimers using 4,4′ -bipyridine [43]. These structures are formally molecular compounds (or co-crystals) but do not involve formation of covalent bonds or charge transfer from or to the active substance. Recent demonstrations of these principles with drug compounds have been published [43–45].

Exploration of a given compound's polymorphs, hydrates, solvates, salts, co-crystals and combinations of all of these appears intractable by conventional experimental methods, and as the number of potential methods for exploring and controlling crystal form diversity continue to expand, existing strategies will become increasingly inadequate. In an effort to understand form diversity in a more comprehensive manner, high-throughput (HT) crystallization systems have recently been developed. This methodology uses a combinatorial approach to solid form generation, where large arrays of conditions and compositions are processed in parallel. Experiments are performed at small scale to reduce the material demand and to afford the largest number of conditions possible. The large number of crystallization trials performed in these experiments reflects the reality that nucleation rate has an extremely non-linear dependence on the experimental conditions, and as such, the probability of a chance occurrence of a particular form is increased by a HT approach. Supersaturation (solubility) and induction time of the various possible solid forms are independently controlled by these conditions, resulting in highly non-linear time dependence of crystallization. In addition, the combinatorial approach permits exploration of a chemical continuum, where use of many solvent mixtures may allow one to assess what underlying physical or chemical processes are required to produce a particular solid form. Once a variety of conditions that can be used to produce a given crystal form on the microscale are identified in the HT screen, scale-up studies are typically conducted to optimize the process for laboratory scale production.

In this review, the development and application of novel HT crystallization technologies for exploration of solid form diversity are discussed. The operational features of a fully integrated, automated HT crystallization system are presented, highlighting the design requirements for hardware and software components, as well as general specifications for consumables. Case studies are used to illustrate the benefits and capabilities of the approach, including salt selection in early lead optimization (ELO) and pre-clinical development, polymorph and solvate screening in highly polymorphic systems, comprehensive discovery of crystal forms to reduce the risk of late displays of polymorphism, comparison of experimental and predictive methods of solid form discovery, and engineering of co-crystals. The need for post-screening characterization of crystal forms to enable ranking and selection of the most suitable form for development is briefly reviewed. Finally, the implications of HT crystallization technologies on the future of solid form screening processes, intellectual property protection and regulatory compliance are discussed.

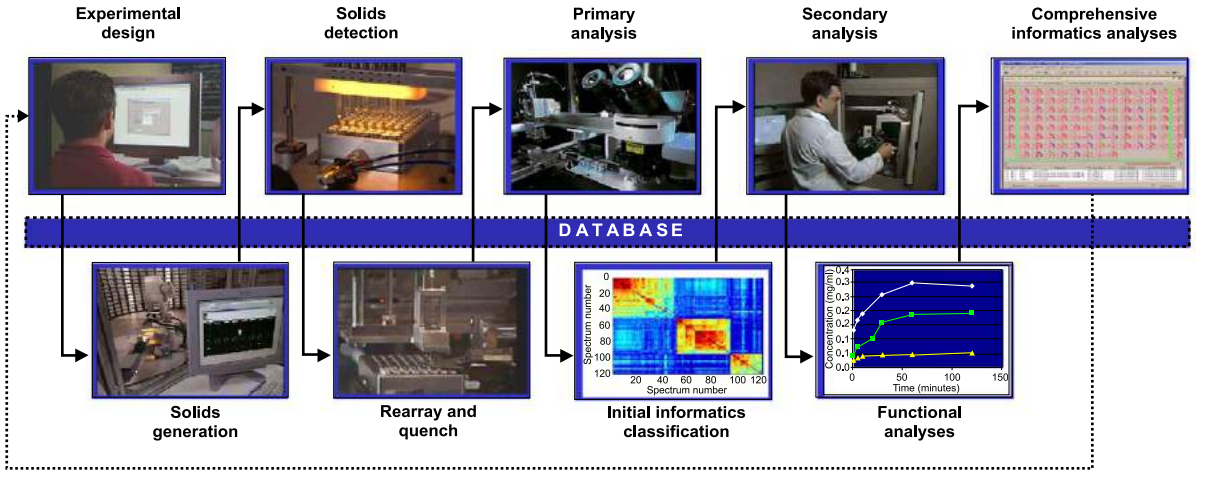
## 2. Development of high-throughput crystallization technologies

HT crystallization systems have been developed to more rapidly and comprehensively explore the multiparameter space that contributes to solid form diversity [40,46–51]. In its simplest description, HT crystallization can be broken down into three key experimental steps: *design* of experiment (DOE), *execution* of experimental protocols and *analysis* of data. Systems designed to carry out these experiments generally consist of both hardware and software components that drive and track experimentation, and permit data storage, retrieval and analysis. Such systems should be designed to be flexible and scalable to ensure that a variety of experimental procedures can be carried out either serially or concurrently. Thus, the system can be employed at various stages of drug development, where differences exist in the quality and quantity of compound available. While it is highly desirable to have the ability to mine and model experimental data, and to use the subsequent knowledge to guide further experiments, not all HT crystallization systems are equipped with these features. In Section 3, the hardware and software considerations for design and development of a fully integrated, informatics-driven HT crystallization system are described.

While the concepts of HT screening are widely applied in the pharmaceutical industry, most notably in the drug discovery arena [52], the application of

HT approaches to drug development, in particular solid form screening, are just beginning to be realized. These latter approaches, however, are more akin to HT experimentation than HT screening. Hence, several important distinctions, which reflect on the design of HT experimental systems, need to be made. First, the goal of HT screening is to get a small number of successful outcomes, which are then passed on to the next stage of development. Little effort is typically made to learn why certain outcomes were positive and why others were negative. In contrast, HT experimentation, such as HT crystallization, is carried out with the goal of having each point in the experiment produce multiple types of data that can be interpreted, and the interpretation used to guide the experimental process to a successful conclusion. Second, unlike traditional HT screening assays where experiments are generally conducted under constant experimental conditions, HT crystallization experiments for solid form discovery are best conducted using a variety of process methods, each having varying experimental conditions (e.g., temperature variations as a function of time) over the course of the experiment. These additional process variables permit maximal diversity in the experimental space, increasing the likelihood that comprehensive coverage will be achieved. Finally, there is a distinction to be made in terms of relative "hit rates". In both HT screening and HT crystallization, a "hit" can be thought of as a set of conditions that gives rise to a desired result. In HT screening, the desired result is typically an activity, or potency, that exceeds a predefined threshold. In HT crystallization, a hit is defined as the formation of a solid. The typical observed hit rate of HT screening is on the order of 0.1% of the total number of samples analyzed. In contrast, HT crystallization experiments can yield hit rates ranging from tens of percents to nearly 100%, depending on the type of experiment and the process mode(s) used. For example, while only a handful of compounds from a selection of thousands may exhibit the required potency, 10–50% of crystallization trials may yield solids. In fact, the range of wells that yield solids is very wide, depending on process mode and experimental time scale, as will be discussed in subsequent sections. The impact of these differences is manifested in the design and operational requirements of HT experimentation systems.

A fully integrated HT crystallization system consists of a number of components, including experimental design and execution software, robotic dispensing and handling hardware, automated high-speed micro-analytical tools, end-to-end sample tracking and integrated cheminformatics analysis software for data visualization, modeling and mining. A schematic overview detailing the workflow of such a system is depicted in Scheme 1 [53]. These features are supported by a comprehensive informatics foun-




Scheme 1. A schematic illustration of the workflow of a fully integrated HT crystallization system [53].

dation that is used to handle the large quantities of data generated. Specifically, informatics tools are used to design statistically relevant and diverse experiments, drive the automation hardware to perform the specified operations, and provide an analytical function to analyze, compare and sort the results of experiments. An important feature of these systems is the ability to mine and model experimental data and use the knowledge generated to guide further experiments. These functions are supported by use of a relational database that provides a mechanism of communication between system components.

When designing a HT crystallization experiment, or set of experiments, a large variety of parameters of composition and process are involved. Experimental designs must be aimed at covering a large multifactorial parameter space, with the goal of determining which experimental factors affect the desired outcome. In practice, it is desirable to place constraints on the experimental space, making common statistical design methods such as full or partial factorial designs inappropriate or impractical. For example, hardware limitations, including minimum and maximum dispense volumes or masses and accessible temperature ranges, as well as constraints related to chemical compatibility (i.e., reactivity of components, miscibility, etc.) or toxicity limits of components (if appropriate), need to be considered. Thus, alternative DOE methods that can accommodate such constraints are required. D-optimal design [54,55] is an example of a DOE algorithm that can take a set of constraints, such as the ones described above, in combination with a target analytical model and determine the optimal set of experimental points to test. Another commonly used DOE algorithm is diversity generation, with which the experimentalist selects a set of pertinent chemical properties and uses the algorithm to evenly spread experimental points over the chosen property space. In addition, some systems utilize a solubility calculator tool to estimate the solubility of the API in the given solvent/additive mixture. The calculated information is then used to select the appropriate concentration of API in each mixture so that it is supersaturated with respect to the reference phase at the harvest temperature. Here, the driving force for crystallization can also be varied by tailoring the composition of each sample based on the API solubility in that mixture. With such DOE tools, experiments may be designed to effectively and simultaneously explore the diverse composition and process space described in Table 1.

Ideally, DOE algorithms should also incorporate prior knowledge or experimental results, which have been stored in a database as a set of rules or models, to limit an experimental space to have certain predicted characteristics. For example, over the course of time, a regression model may be developed between a set of known or calculated chemical properties and a parameter of experimental interest. The model could be used during the design of a new experiment in order to test only those chemicals that are predicted to give a desirable result. Since a large number of factors need to be considered during experimental design, the DOE interface available to the scientist must not only be flexible and easy to use, but must also offer tools that aid design efficiency and effectiveness and permit input of scientific knowledge generated over time.

At the end of the experimental design process, the resulting set of experimental conditions is translated into a series of commands for the HT systems, and stored in a relational database for later retrieval by the software that controls the automation. When an experiment is activated, the overall operation of the automation systems is managed by the HT informatics system, which is responsible for physical operation of the HT platforms as well as data tracking and storage.

Execution of experimental commands is carried out by automated laboratory equipment that comprises the HT crystallization system. Specialized automated systems perform several of the functions in a sequence of events that make up the experiment. Each station is controlled through an interface to the informatics system that ensures the samples are processed at the correct stations, in the correct order, with the selected experimental parameters being followed. Parameters of operation are recorded, including the time at which an action is taken. After execution of the experimental steps, the software interface retrieves any pertinent information generated by the automated platform, such as assay results or operational parameters, stores these data in the relational database, and updates the status of the experiment to reflect the completion of operations.

In general, the hardware required for a HT crystallization system is comprised of four major functional elements: sample preparation, solids generation, solids detection and sample analysis. Sample preparation

involves adding the compound of interest (API) to the diverse set of conditions used to conduct crystallization studies. Typically, the API is dispensed as a solution in a suitable solvent, followed by solvent removal to yield the solid API. Solvent removal can be achieved by passive evaporation or by controlled active evaporation (e.g., use of a vortex dryer). Alternatively, the API can be delivered in the solid state with suitable powder handling systems. Depending on the amount of saturation desired, the crystallization vessel used, and the API's solubility in solvents or solvent mixtures of interest, API masses ranging from a few hundreds of micrograms to several milligrams will be present in each vessel. Once the API has been delivered to the crystallization vessels (tubes, vials or microwell plates), combinations of solvents and/or additives are added to each vessel. By taking advantage of the power of combinatorial approaches, large numbers of unique combinations can be dispensed from manageable sets of starting materials.

Compatibility of equipment components (syringes, dispense tips, tubing, etc.) and consumables (plates, tubes, etc.) with solvents and other compounds is a key hurdle faced in the development of combinatorial crystallization for small molecules. Unlike protein crystallization systems [56,57], which are commonly based on the sitting-drop method in aqueous media, small molecule crystallization employs a range of crystallization additives and processes. The additives include organic solvents with varying properties (e.g., alcohols, acetone, hexane, ethyl acetate, etc.), water, acids, bases and co-crystal formers, as well as other compounds (e.g., small molecule templating agents, surfactants, pharmaceutical excipients, etc.). This wide range of materials needs to be handled by appropriate liquid handling techniques to enable the combinatorial assembly previously mentioned. Ideally, liquid transfers are achieved using multichannel pipettors with individually controllable channels. Depending on the crystallization vessel design, the volumes of reagents dispensed will be as low as a few microliters to as high as several hundred microliters.

Potential for cross-contamination and tendency toward unwanted solvent evaporation from crystallization wells are challenges that need to be addressed in a HT crystallization system. A large number of the solvents used to crystallize small molecules have high vapor pressure under ordinary laboratory conditions. Sealing of the crystallization vessels is key to being able to control composition during crystallization from these solvents. Due to solvent fugacity, vessels need to be protected from ingress of the components of neighboring wells. These problems have been solved by different means, such as sealing of individual tubes with a Teflon-backed crimp seal [40] or O-rings/gasket seals and clamped covers [47,51].

HT crystallization must enable several process modes that are compatible with the compound (e.g., chemical stability, thermal stability, etc.). In some cases, multiple modes of operation may be combined. The most common modes of solids generation will be discussed below, including thermal cooling crystallization, anti-solvent and evaporative crystallization. Less common process modes include melt crystallization, flash or quench cooling and template-directed crystallization. It is important to note that generation of maximal diversity in solid form requires multiple modes of operation [6,18,58].

In thermally induced cooling crystallization, samples created in the sample preparation process described above are subjected to temperature ramps. Prior to beginning the temperature ramp, samples are exposed to an elevated temperature for a short period of time in order to dissolve the API in the crystallization medium. Although dissolution can be achieved most simply by diffusion and convection from the heating process, addition of external energy can speed up the process (e.g., sonication). Samples may be optically inspected (see Fig. 1) and vessels that contain undissolved solids can be flagged in the database for further analysis. For instance, undissolved samples may be treated as slurry conversion experiments and monitored over time for crystal form changes. The thermal cycle is then initiated, using controlled cooling to induce supersaturation. In this mode of crystallization, samples continually experience an under cooling and, based on the level of supersaturation in the vessel, may recrystallize at a given temperature after a period of time. Thermal crystallization tends to generate a cumulative number of samples that are produced over time in a fashion approximating a square root function, as illustrated in Fig. 2. This means that initially there is a small bolus of ''hits'', after which the rate of crystallization tails off over a period of time, typically in days to weeks. This results in a manageable hit rate

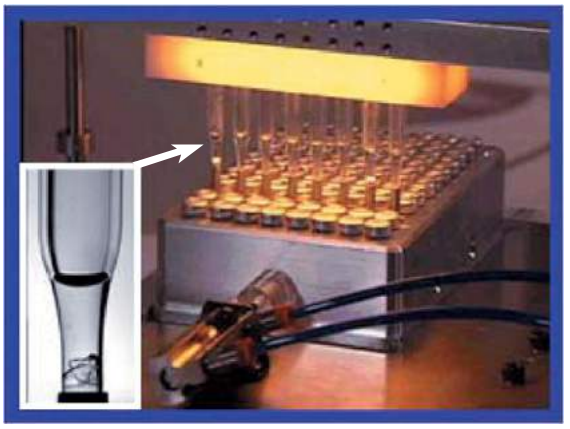


Fig. 1. Photo of optical inspection station. (Inset shows close up of crystallization vessel that contains crystals.) (Courtesy of Trans-Form Pharmaceuticals, 2002.)

for analysis, on the order of approximately 10% in aggregate. This mode of solids generation has the lowest throughput rate, typically, because experiments span days to weeks, with system residence times of months being possible.

In contrast, anti-solvent addition, also known as "crash-out" (or "drown out") crystallization, relies on the fact that an API is soluble to varying degrees in the crystallization medium, but is largely insoluble in a particular solvent or solvents (e.g., the anti-solvent). As a result, this mode of crystallization can operate at high-throughput rates, with samples being turned around hourly. When crystallization vessels containing API in reagent mixtures are exposed to aliquots of anti-solvent, nearly all vessels will contain API that has precipitated out of solution. This creates a challenge to the analytical process, as the near 100% hit rate leads to a large bolus of samples. There are, however, advantages to this mode of solids generation, such as the ability to produce microfine crystallites and amorphous solids, should they be desired.

Lastly, evaporative crystallization can be carried out on the combinatorial array of samples. This mode of operation relies on gradually increasing the concentration of API in the vessel to achieve supersaturation and to increase the degree of supersaturation (by preferential evaporation) in order to induce crystallization. Concentration of samples can be achieved either passively or actively by controlled flow of inert gas while maintaining temperature. With evaporative methods, differential rates of solvent loss from mixtures result in unknown composition of the crystallization medium at the time of crystal nucleation. In addition, the degree of supersaturation changes over the course of the experiment, often resulting in the appearance of multiple crystal forms. The evaporative mode of solids generation typically produces throughput and hit rates intermediate between the thermal and anti-solvent processes.

As suggested above, in appropriately configured HT crystallization systems, several process modes may be used in series or in parallel [40]. Frequently, the preparation of replicate plates (in some systems "daughter" plates [47,51]) is necessary for parallel processing by different process modes. Systems may be additionally equipped with the ability to serially process sample arrays using different process modes [59]. This feature is particularly attractive for cases where only small quantities of sample are available, increasing the drive to generate useful information from every sample. Here, samples may be processed by optimal modes first (e.g., thermal crystallization), then a secondary process step can be applied to maximize the hit rate. Another example where this feature is useful is in the case of salt selection, especially in early drug discovery. Upon the addition of salt forming acids or bases, the solubility of the compound is modulated by in-situ salt formation, often resulting in reduced or non-existent driving forces for crystallization (e.g., subsaturation) of the salt species, particularly in polar

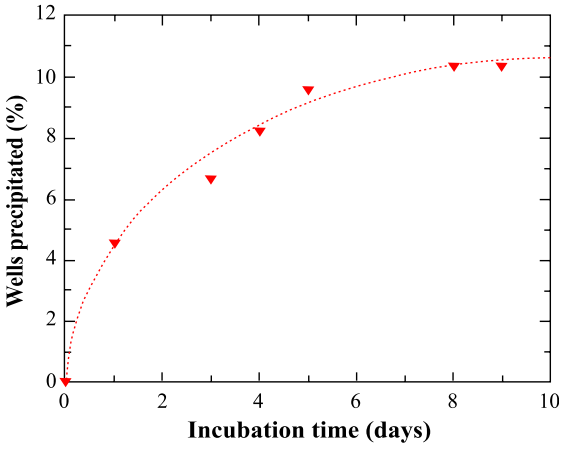



Fig. 2. Typical rate of appearance of solids during a thermally driven HT crystallization experiment [65].

solvents. It should be noted that rapid onset of supersaturation can be experienced in any of the process modes discussed and can result in oiling out or precipitation of amorphous solids, rather than generation of crystalline solids. Thus, it is important to monitor and control the crystallization conditions throughout the experiment.

In general, the percentage of wells that yield solids varies, depending on process mode and experimental time scale. For example, evaporative modes usually result in a solid in virtually every vessel, while slow undercooling results in far fewer (on the order of low percents). The differences in hit rates between these process methods arise in part from the differences in the supersaturation attained. For evaporative crystallization, supersaturation is achieved in all cases as the concentration of the active compound is continuously increased as solvent is evaporated. In contrast, the composition of wells processed by thermal crystallization is fixed. In some cases, because there is limited data on the precise state of supersaturation for each of the large variety of experimental compositions and potential crystal forms, some wells may remain subsaturated during the process. For these wells, additional process steps, such as partial evaporation or anti-solvent addition, may be employed to generate supersaturation to yield a solid. In contrast, as mentioned previously, a fraction of the wells may not go fully into solution at elevated temperatures. In this case, the temperature of the system may be raised to achieve full dissolution, additional solvent may be added to solubilize residual solids or the samples may simply be monitored for slurry conversion over time. To overcome these challenges, we have developed a solubility calculator tool using group contribution theory to estimate the solubility of the reference solid phase at specified temperatures in each solvent composition. These data are then used at the DOE step to define the viable concentrations of the active compound for crystallization (i.e., minimum concentration required to achieve saturation and maximum solubility limit or concentration) in each solvent mixture. Additionally, the timescale of the experiment has a significant impact on the observed hit rate. Hit rates will approach 100% for viable crystallization conditions in the limit of infinite time, but in practice most experiments are conducted over days to weeks, so observed hit rates reflect this temporal influence. In fact, similar behavior is observed in manual experimentation. Note that only some HT crystallization systems are configured to permit selective sampling of "hits", providing the ability to further incubate un-crystallized samples to monitor for slow growing crystal forms.

Solids detection can be achieved by examining each sample using machine vision systems. Samples may be monitored over time to detect precipitation in vessels that were previously devoid of solids. This simple, yet robust process can rapidly and non-destructively determine state changes in the crystallization vessels and signal when a particular vessel or set of vessels is ready for solid-state analysis. Depending on the sample array configuration, the signaling of "hits" results in harvesting of samples by one of two approaches. In the "cherry-picking" approach, only those samples that have been flagged as containing solids are selected for further processing [40]. In contrast, using a sacrificial approach the entire plate must be moved forward after a predetermined fraction of the samples in that array have produced precipitates [47,51]. The latter, of course, can be carried out without an online detection system. Here, samples can be processed in batches, without regard to whether there are actually solids present in a vessel. This simple process approach is effective, but has significant limitations, the primary of which being that samples are destroyed after a fixed amount of time regardless of their state. Hence, it is advantageous to employ an online detection and harvest system so that samples can be differentially and asynchronously processed, with only those vessels containing solids undergoing analysis [40,60].

Sample analysis is the final action in execution of the HT crystallization process. Depending on the mode of operation and the choice of analytical measurements employed, this process may involve several steps. Most HT crystallization systems use Raman spectroscopy and/or powder X-ray diffraction (PXRD) for primary analysis of harvested solid-state samples. Both techniques have advantages and disadvantages in terms of their ability to discriminate between forms of a solid (i.e., polymorphs, salt forms, solvates, hydrates) [1,14,61]. The rate of generation of samples for analysis likely dictates which technique is used for the primary approach. Generally speaking, Raman spectroscopy can be employed in a more rapid fashion than PXRD, since acquisition times for Raman are considerably less dependent on sample size, as is depicted in

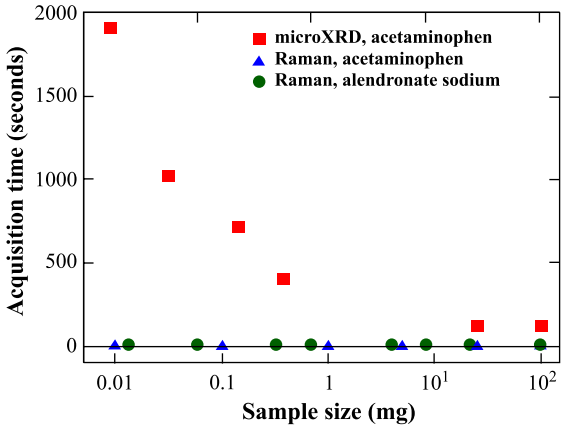



Fig. 3. Comparison of acquisition times of Raman and X-ray powder diffraction data as a function of mass of API [65]. (Data collected on D/Max Rapid, Contact Rigaku/MSC, 9009 New Trails Drive, The Woodlands, TX, USA 77381-5209).

Fig. 3. In addition, plate-based PXRD methods are susceptible to problems with preferred orientation effects, which may prevent accurate classification of samples. As a result, Raman spectroscopy methods are often used as a primary means of characterization in HT crystallization systems. Although one disadvantage of the Raman technique is interference due to fluorescent samples, the wavelength of the excitation laser can be changed to the near-IR to reduce fluorescence of problematic samples. Recent advances in PXRD instrumentation, brought on by the increasing demands of HT crystallization, make it possible to achieve similar analysis timescales with PXRD and Raman, on the order of less than one minute per sample depending on the capabilities of particular instruments used. Clearly, the best option is to employ both methods for initial sample evaluation, which can be realized with the appropriate informatics structure, as described in Section 3.

Once the primary solid-state characterization data are collected and stored, samples are generally classified into groups (or bins) that display similar characteristics (e.g., Raman spectra or powder X-ray diffraction patterns) using informatics tools. A variety of methods can be used to accomplish the binning. For instance, Raman spectra may be compared (based on relevant features or over the entire spectral range) and clustered using calculated similarity measures, such as Tanimoto coefficients. In one method [40,60,61], each Raman spectrum, which represents the contents of an individual well at a given time, is filtered to remove background and to accentuate Raman peaks and shoulders. Peaks are then located and assigned a wavenumber using standard derivative methods and the amplitude of each peak is calculated. These data are used to calculate a similarity (or distance) measure related to the Tanimoto coefficient, from which the Raman spectra are binned into groups of similar samples using a classification algorithm such as hierarchical clustering. This method often uses peak positions, rather than amplitudes to discriminate between different patterns in order to reduce the significance of potential preferred orientation effects, which can result in modulation of relative peak intensity for certain crystallographic planes. The window over which two peaks are considered to be at the same position (e.g., 1 $cm^{-1}$ wavenumber), as well as a minimum height for a filtered peak to be considered for clustering, can be selected by the user, allowing regions of interest (e.g., spectral ranges) to be explored in greater detail. With appropriate settings, a Raman spectrum that has only one peak or feature in a slightly different location than observed in other patterns can be differentiated and binned as unique, indicating a different or new crystal form. During clustering, each spectrum is assigned an arbitrary number, i.e., a sorted spectrum number, for ease of tracking, and the resultant clusters are graphed as shown in Fig. 4, where the red-colored regions repre-

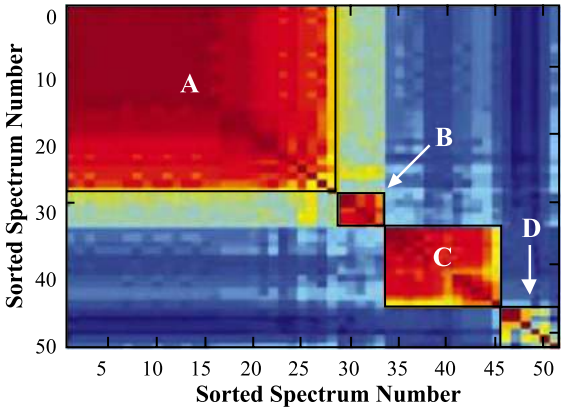



Fig. 4. Raman cluster diagram showing *n*-by-*n* matrix of sorted spectrum numbers for all samples resulting from the HT polymorph screen of Ritonavir. Clusters are indicated by warm-colored (red) regions, which have been outlined to guide the eye, and indicate different solid forms [65].

sent bins of similar samples. Alternatively, the results from several analytical methods such as Raman and PXRD can be used to simultaneously classify samples.

Regardless of the choice of primary analytical method, and in keeping with traditional methodologies for solid form screening, it is necessary to further characterize the solids generated in HT crystallization systems to accurately determine their solid form and properties. Most HT systems integrate multiple analytical methods as part of the screening process. These so-called secondary analytical methods often include thermal property measurement (e.g., melting point) and optical microscopy (for crystallinity, habit, etc.). Depending on how the samples are processed and the degree of computerized support, these techniques may be applied to all samples, or a subset of selected samples. For systems that analyze all samples by secondary techniques, several HT plate-based methods for optical microscopy and melting point determination have been developed [47,51]. It is important to note that, in this case, all samples are destroyed during characterization of the melting point. When replicates are retained, the functional properties such as dissolution rate and hygroscopicity can be analyzed using either manual or HT methods. (For more information on functional analysis, see Section 4 on post-screening analyses and form selection.)

With the aid of informatics tools, the data sets obtained can be used to generate information about the experimental space. Software interfaces that allow access to the data permit classification and regression analysis to be performed. The results are displayed in high-dimensional visualization tools that can be used to guide further experiments toward optimizing processes to make each form. For instance, sample composition and processing information can be linked to the resulting crystal form and morphology. Correlation of trends between experimental factors and the products can lead to hypotheses that can be used to direct the design of follow-up experiments. An example of this was reported by Peterson et al. [40], where the knowledge gained from iterative experiments was used to drive new experimental designs, which ultimately yielded the desired outcome, i.e., the isolation and characterization of the highly unstable form III of acetaminophen (paracetamol).

While these new methodologies provide unprecedented capabilities for solids form discovery, it is clear that there remains a need for some level of manual processing, particularly in the case of detailed form characterization such as single crystal structure determination, scale-up of the desired form and understanding the effects of downstream processing on potential form conversion. HT methods provide the landscape of possible forms and their properties and should be used in conjunction with traditional methods to enable rapid, efficient selection of the optimal form for development.

## 3. Applications of high-throughput crystallization screening in pharmaceutical research and development: case studies

HT technologies offer unprecedented capabilities for form discovery and characterization. Potential applications range across the entire pharmaceutical value chain, including screening of active molecules in discovery during ELO, form selection for preclinical candidates, final form optimization for early clinical candidates, process chemistry development of crystallization processes for bulk drug and intermediates, as well as identification of new or enabling solid forms for product life cycle management. While numerous impact points have been identified, only limited information on the use and performance of HT form screening systems is available in the literature, indicating that the benefits of these new methodologies have just begun to be realized. In the following sections, case studies on the application of HT crystallization systems are reviewed. Special attention is given to the implications of new form discoveries.

### *3.1. High-throughput salt selection*

Preparation of salt forms of an active compound is commonly used to modulate physicochemical properties. In most cases, the goal is to increase solubility (or dissolution rate) to improve bioavailability or to enhance the manufacturability of poorly soluble ionizable compounds [1,3,4]. Salts may also be employed to increase chemical stability [3] or to reduce the solubility of a given compound for certain applications (e.g., sustained release dosage forms) [62]. Thus, it is important to consider the route of administration and

dosage form requirements when selecting a salt form for development. Since the choice of counter-ion affects the properties of salt forms [3,4], salt selection studies involve the preparation of a number of different salts using a variety of pharmaceutically acceptable acids or bases with differing properties (e.g., acidity/basicity, molecular size, shape, flexibility, etc.). The relevant physicochemical properties of each salt are characterized, including degree of crystallinity, hygroscopicity, aqueous solubility, crystal habit, and physical and chemical stability. Based on these properties of the salt forms, their suitability for development can be evaluated. Several strategies for streamlining and optimizing salt selection procedures have been reported, including in-situ techniques for ranking the solubility of salts [63], tiered approaches in which the least time-consuming studies are carried out first and used to remove from consideration salts that are not viable [64]. One issue not readily considered by existing strategies is the polymorphism and solvate forming behavior of the different salt forms of a compound, which could be used as an additional criterion when more than one salt may be viable, but the degree of polymorphism and solvate formation of each may become a criterion for form selection.

HT crystallization technologies have been used to more rapidly and comprehensively identify the range of salt forms that may be prepared for a given compound or series of compounds, and characterize their crystal form diversity (polymorphs, solvates, hydrates). However, only a few studies have been published or presented. Several HT salt selection studies on well-characterized pharmaceutical compounds have been carried out to demonstrate the power of these technologies in solid form discovery. For example, in a small HT study (i.e., 96 wells) on the antibacterial sulfathiazole, salt formation was explored using varying stoichiometric ratios of pharmaceutically acceptable organic and mineral bases in an array of solvent conditions [65]. The screen resulted in the rapid identification and characterization of 10 salt forms and showed that the salts exhibited a range of melting points depending on the counter-ion type and stoichiometric ratio. Similar HT salt selection experiments on caffeine and naproxen resulted in the identification of numerous salts of each compound [47,50,51].

In the discovery phase, HT crystallization has been used to identify soluble salt forms of compounds during ELO to facilitate early animal dosing, thereby providing the ability to uncover underlying chemical and/or biological responses elicited by candidate molecules, including toxicity or efflux [46,59]. Such information permits rapid identification of problematic compounds or scaffolds, allowing resources to be directed to projects with greater opportunity for success. HT crystallization can facilitate selection of leads that are more likely to survive preclinical development. HT crystallization has been used successfully to identify multiple new salt forms and the polymorphs and solvates of each compound belonging to two discovery programs using less than 200 mg of compound per screen [59]. Approximately 150–200 experiments were performed on each compound using a library of pharmaceutically acceptable acids or bases with an array of solvent compositions and process conditions. Each screen resulted in discovery of multiple new salt forms, and in some cases polymorphs and solvates. Interestingly, similar salt types were identified for each compound in a given series, as illustrated in Fig. 5, where the frequency of occurrence is plotted as a function of counter-ion for each discovery series. Clear trends in the degree of solid form diversity of salt forms, including polymorphism and solvation behavior, were also evident within each compound series. These data indicate the potential for identifying salts suitable for most compounds tested in a particular scaffold or series, based on analysis of only a portion of the series, i.e., a platform-based approach to salt selection, provided the chemistry surrounding the ionizable functionality is not significantly altered during further structure–activity relationship (SAR) development. Furthermore, solubility measurements of each salt form in physiologically relevant fluids allowed ranking of salt forms in a given series, and comparison of salts between series was also possible. The average turnaround time per screen was approximately 2 weeks, such that feedback on the physicochemical properties of each compound was provided to the medicinal chemists on a similar time scale as potency, selectivity and metabolism screens.

Salt selection is normally part of the standard preformulation studies carried out during preclinical development, where rapid identification of the possible salts of a compound and their properties can facilitate product development. To further facilitate

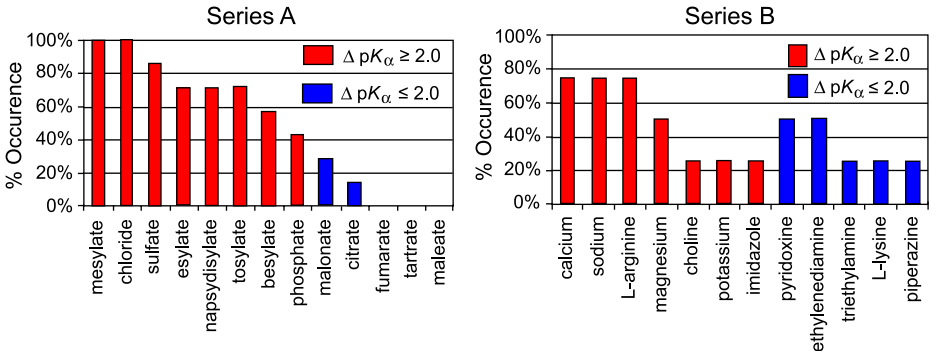



Fig. 5. Frequency of occurrence (%) plotted as a function of the counter-ion of the salt for compounds from discovery series A and B [59].

such studies, a microplate technique capable of investigating an array of conditions has been developed to determine which counter-ion and solvent conditions can be used to prepare crystalline salts of the compound [66]. Each plate is prepared by first depositing approximately 0.5 mg of compound into each well using an appropriate amount of stock solution. The counter-ion type is systematically varied along the rows of the plate and different crystallization solvents are deposited down the columns of the plate. Crystallization is monitored by optical microscopy over the course of the evaporative crystallization, which can be accelerated by flowing a stream of dry nitrogen over the plate. Once salt forms are identified, they are scaled up for more detailed characterization.

The microplate approach was demonstrated by Bastin et al. [66] through several examples, however little detail of the specific screening protocol and results was provided. All three of the reported examples are on compounds that are weak bases with p$K_a$ between 4.1 and 5.3. Only a small number of stable, crystalline salts could be prepared for the two very weak bases (i.e., p$K_a$ < 4.25), as opposed to the larger variety found for the stronger base. In each case, the salt forms were scaled-up for more detailed analysis and comparison to the respective free base compound to determine the optimal form for development. This approach provides a useful mechanism for preliminary, small-scale salt formation studies. Both the crystallization media and process modes accessible by the technique are somewhat limited, resulting in a narrow exploration of experimental conditions for salt formation. For example, only solvents compatible with plate materials can be used, thereby reducing the probability that a crystalline phase can be identified. In addition, current protocols only provide for evaporative crystallization, likely due to difficulties with sealing of the plates. In this case, the composition of the crystallization medium is not well controlled. The utility of HT crystallization in ELO, although demonstrated by initial reports of feasibility, is less well documented than the use of HT on later stage compounds.

### *3.2. Solid form discovery in highly polymorphic systems*

The statement by the late Walter McCrone in 1965 that "the number of forms of a given molecule is proportional to the time, money and experiments spent on that compound" [67] has gained credence in recent years, as illustrated by the significant increase in reported crystal form diversity of pharmaceutical solids. Depending on when alternative solid forms of a compound are identified, the appearance of a novel form may or may not be a welcomed discovery. Occurrence of a new form in research or early development is potentially enabling. At later stages, the appearance of new forms, particularly stable ones that are not bioequivalent or deemed unprocessable, can have catastrophic consequences for product performance as well as regulatory compliance (e.g., control of crystal form). Additionally, recent rulings on the use of alternative, commercially viable solid forms not protected by patents from

innovator companies have opened the market to generic competition [68–79]. In order to mitigate these risks, and to save time and reduce costs, many pharmaceutical companies have begun to re-evaluate their strategies for solid form screening and are looking to HT crystallization technologies to address the needs for more rapid and comprehensive exploration. In this section, the application of HT crystallization to highly polymorphic systems is reviewed, including specific cases of compounds exhibiting latent polymorphism.

Polymorphic systems are quite common among many types of organic crystals [7]. For the purposes of this review, compounds exhibiting more than three polymorphic forms will be classified as being "highly polymorphic". While only a handful of well-known organic compounds are considered for practical purposes to be non-polymorphic, e.g., aspirin [80,81], sucrose and naphthalene [7], it should be stressed that one will never be able to exclude the possibility of polymorphs appearing, even a century after the initial discovery of the compound. So far, no polymorphs of aspirin have been found, despite the proposal by Payne et al. [80] that polymorphic forms may exist. In contrast, acetaminophen form III was observed by Burger in 1982 using thermal microscopy [82], but it took another 20 years for a crystal structure to be proposed [40]. Many reports exist on the polymorphic nature of specific drug compounds with one or two alternative packing modes for the same chemical composition. However, literature examples of compounds with more than three packing modes are considerably rarer, as will be summarized shortly. It should be noted that the increased number of reports on highly polymorphic compounds in recent years is likely the result of enhanced screening practices and more sensitive characterization techniques.

Highly polymorphic compounds present several challenges in drug development. First, the generation of different forms is often not a simultaneous event, but rather a gradual evolution of form diversity leading to the branding of a compound as being highly polymorphic. Consequently, once more than one form is identified, concern is raised that additional forms may eventually be discovered. For instance, the 13 polymorphs of phenobarbitone evolved over ca. 13 years [7], and a fourth polymorph of carbamazepine was reported in 2002, a full two decades after the publication of the structures of the initial three forms [83]. Second, selection of the preferred form of a highly polymorphic compound for development demands a complex set of thermodynamic and kinetic investigations, due to the geometric increase in the number of stability relationships that need to be established. More complexity arises when some polymorphic pairs are enantiotropic, exhibiting a switch in the identity of the stable form as a function of temperature. Third, concerns over bio-performance and the impact of a large number of polymorphs on processing lead to regulatory issues that need to be addressed. Decision trees [58] have been established to aid scientists in assessing the impact of polymorphic change and have been incorporated into the ICH guidelines [84]. Lastly, the analytical challenge of monitoring polymorph content in the dosage form increases as the number of possible forms grows, particularly with low dose compounds where the concentration of drug in the formulation is small.

The literature on highly polymorphic pharmaceuticals is relatively sparse, but several examples of compounds known to have four or more polymorphic forms are available in the literature and are summarized in Table 2. In addition to these drug examples, the pharmaceutical ingredients mannitol and aspartame have been shown to exhibit 4 and 5 polymorphs, respectively [7]. The phenomenon in inactive exci-

Table 2
Examples of highly polymorphic drug compounds in the literature

| Compound | Number of reported polymorphs | Other forms | Reference(s) |
|---|---|---|---|
| Phenobarbitone | 13 | | [7,p.255] |
| Cimetidine | 7 | Hydrates | [7,p.73] |
| 'ROY' | 7 | 7th form found after the initial publication | [111,112] |
| Sulfathiazole | 5 | Numerous solvates | [113] |
| Carbamazepine | 4 | Dihydrate and numerous solvates | [28,45,83,85] |
| MK-996 | 9 | Hydrate | [87] |
| MK-A | 4 | 2 hydrates and numerous solvates | [86] |

pients may well be under-appreciated due to lack of study.

In general, pharmaceutical polymorphism is likely to be underreported in the literature, since much of the polymorphism research is carried out in companies. As a result of growing interest in the subject and advances in techniques to study polymorphism, it is expected that reports of extreme form diversity will grow. Conferences on the subject, such as the ACS ProSpectives symposium, reflect the appreciation for the complexities introduced by the appearance of polymorphism in important materials such as pharmaceuticals. Work has recently commenced to understand the opportunities and challenges of using HT technologies in pursuit of rapid identification and characterization of the large number of forms presented by highly polymorphic compounds. Three published case studies and two examples that are in press at the time of this review will be highlighted.

Form IV of carbamazepine was reportedly discovered as the result of crystallization trials in the presence of hydroxypropyl cellulose HPC [83]. Subsequent to this publication, Lang et al. [28] published the use of polymers to influence polymorphic form using a 96-well plate system for the screening of polymorphs of carbamazepine and acetaminophen. In all, 84 different polymers were employed to direct nucleation. Form IV of carbamazepine was found to crystallize from methanol in the presence of hydroxypropyl cellulose, poly(4-methylpentene), poly(*R*-methylstyrene) or poly(*p*-phenylene ether-sulfone). Using the same approach, the monoclinic and orthorhombic forms I and II, respectively, of acetaminophen were also isolated. While observation of metastable form III was not reported in this study, the strategy of employing polymeric additives is of interest, as it can direct the course of crystallization and because polymeric impurities may be in contact with a drug substance and/or formulation at various points in development.

Another approach, reported by Anquetil et al. [85], identified selective conditions for the crystallization of carbamazepine polymorphs forms I and III, as well as the dihydrate, from methanol and/or methanol/water solutions by thermal processing in a microliter cell format (i.e., 35–100 μl). Optical laser trapping was used in situ to target the microcrystals for real-time form analysis using Raman spectroscopy. The crystallization process was monitored optically and with Raman spectroscopy as a function of temperature and time. The study revealed the conversion of form I to form III, as evidenced by a change in characteristic crystal habit from needles to prisms. Raman spectroscopy on the solution phase measured the saturation solubility of each crystal form produced. Although only several experiments were carried out in this study, the authors advance the microfluidic cell format as a potentially viable system for HT polymorph screening.

A third report details the use of in situ Raman spectroscopy to optimize process conditions. The compound MK-A has four anhydrous polymorphs and several other forms, including two hydrates and numerous solvates [86]. The study gives an example of the complex thermodynamic relationships (monotropic and enantiotropic pairs) that can exist in highly polymorphic systems and demonstrates the power of in-situ methods for monitoring the crystallization process.

The angiotensin-II antagonist MK-996 is an example of a highly polymorphic compound (Table 2) [87]. The structure of MK-996, depicted in Fig. 6, contains seven rotatable bonds, the conformations of which could lead to many configurations for crystal packing. HT crystallization experiments with MK-996 in 96-well arrays comprising over 1500 discrete recrystallization trials from a set of 21 solvents or solvent mixtures yielded 186 solids, which were harvested over a period of 7 days [87]. PXRD analysis of these solids suggested the presence of at least 18 distinct

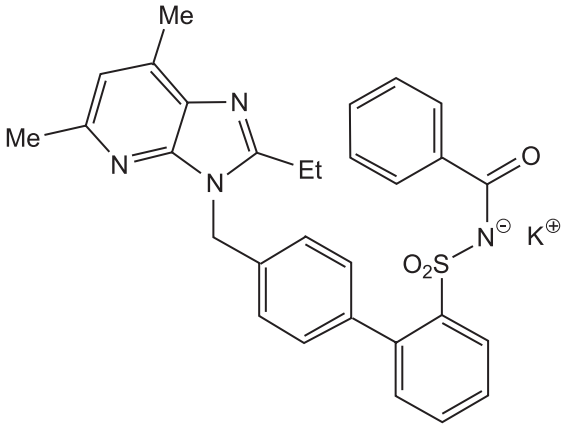



Fig. 6. The molecular structure of the angiotensin-II antagonist MK-996 [87].

forms, some resulting from solvent-mediated recrystallization. A hydrate (originally named form I), obtained by slurry conversion in the presence of aqueous solvent mixtures in the HT experiments, was the form previously selected for pharmaceutical development. Importantly, a form (form D) reported by the innovator [87] to be a "disappearing polymorph" [88] once form I appeared, was also found in the HT screen. Clearly, sufficient experimentation with rationally selected diverse conditions affords the possibility to regenerate elusive forms.

Sertraline HCl, the active ingredient in the antidepressant Zoloft®, is found in various crystal forms. The molecular structure for Sertraline HCl is illustrated in Fig. 7. Information on various solid phases can be found in patent disclosures filed by several companies [89–92]. Survey of these documents, which published between 1992 and 2001, reveals data for 27 purported crystal forms of Sertraline HCl, including 17 polymorphs, 4 solvates, 6 hydrates and the amorphous solid. Further analysis and comparison of characterization data for the various forms presented in the patents revealed that mixtures have been mistaken for real polymorphs on at least two occasions, and at least two polymorphs were disclosed more than once (by different workers each time). In addition, the hydrate forms reported were not readily identified as polymorphic and many of the forms are likely transient, e.g., only identified by variable-temperature and humidity-controlled XRD. With the help of HT crystallization, the extent of true polymorphism of the HCl salt was estimated at eight forms so far [92]. Two new solvates were also found in the HT studies. Care should be taken in isolation of such forms, particularly at small to intermediate scale, as desolvation of solvates due to aggressive drying during processing may cause one to overlook solvated forms [93]. Comparing the results of the HT study to the congruence of historical data, one can conclude that HT screening gives rise to relevant forms of the drug in a time frame of weeks rather than years. One metastable form, polymorph IV, remained elusive in the hands of the authors [92]. The lack of observation of form IV may be due to a subtle purity difference between early batches at Pfizer and the materials available for testing in the HT screen. Clearly, impurity effects should be explored further [32].

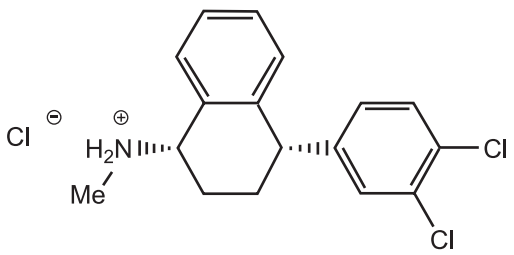



Fig. 7. The molecular structure of the selective serotonin reuptake inhibitor (SSRI) sertraline HCl.

To date, HT studies on highly polymorphic materials highlight the importance of varying processing conditions (including solvent conditions, degree of supersaturation, method of crystallization, desolvation of solvates, inclusion of additives, thermal microscopy, etc.) to find as many forms as possible. It has been shown that multiple process modes, including HT processing, coupled with detailed follow-up characterization studies of form stability, facilitate insight into crystal form diversity [40]. Such a multimode strategy becomes valuable in the quest for the most comprehensive dataset possible for a given pharmaceutical material.

Undoubtedly, the definition of highly polymorphic materials and their frequency will evolve in the age of HT crystallization [40,60] and with the aid of ever improved solid-state analytical capabilities [18,94,95]. The value of employing multiple processing techniques to elucidate as many crystal forms as possible will be demonstrated, as it is expected that no single technique will generate all forms of a given compound. Without doubt, HT crystallization strategies will be used, as a complement to other techniques, to identify issues of polymorphism early, thus allowing drug development scientists to react appropriately to information on form diversity of their compounds.

### 3.3. Avoiding latent polymorphism

Very few cases of latent polymorphism have been reported in the literature. It is likely that many more instances of the phenomenon have occurred, but unless product development was slowed, product performance was impacted, or generic competition was threatened, a spotlight is not usually cast on the issue. As an example of a public polymorph issue, form 2 of ranitidine hydrochloride was discovered 2–

3 years into development but it was (and is) the form still marketed by GlaxoSmithKline [75,76,96]. Paroxetine hydrochloride hemihydrate, the active ingredient in Paxil®, was discovered during development after only an anhydrate had been known for a number of years [97]. The hemihydrate is the form marketed by the innovator, but recent litigations have occurred between the innovator company and generic competition around the anhydrate form.

One of the most recognized cases of latent polymorphism occurred with Abbott Laboratories' Norvir®. Two years after entry into the market, a previously unknown, but thermodynamically more stable, polymorph of the active ingredient (Ritonavir) appeared. This new form (form II) was approximately 50% less soluble in the hydroalcoholic formulation vehicle, resulting in poor dissolution behavior and eventual withdrawal of the original Norvir® capsule from the market [98]. At some considerable cost, a new formulation of Norvir® using form II was eventually developed and launched [99]. In a recent HT crystallization study on Ritonavir, a total of five forms were found: both known polymorphs and three previously unknown forms [99]. The HT polymorph screen, which consisted of 2000 experiments was carried out with less than 2 g of the API and used multiple, and sometimes combined, process methods. The three new forms were described as a metastable polymorph, a crystalline solvate and a non-stoichiometric hydrate. Interestingly, the solvate was easily converted to form I via the hydrate phase using a simple washing procedure, and provided an unusual route to prepare the form I "disappearing polymorph" [88]. Since the crystals of form I prepared using this method retained the small needle morphology of the solvate, the authors suggest that the process may offer a potential strategy for particle size and morphology control. The results of this study emphasize the need for more comprehensive studies of form diversity in the early stages of drug development to avoid risks of form conversion downstream, and highlight the advantage of combining parallel HT crystallization experimentation with detailed physicochemical analyses to identify the diversity of solid forms in which a given molecule can exist. Clearly, late stage discovery of new forms or form conversion can have serious competitive and regulatory implications (e.g., process control), especially in cases where the new forms are not bioequivalent.

### *3.4. Prediction of crystallization and polymorphism: applications to pharmaceutical form studies*

Crystal structure prediction is a challenging area of research. Due to the overwhelming influence of packing forces in determining crystal structure, it remains extremely difficult to predict the structural impact of subtle conformational effects and weak interactions between adjacent molecules in a crystalline arrangement. Although significant progress has been made in the last decade, crystal structures are by and large not reliably predictable from first principles [88]. While this important area of theoretical research is too large a topic to be considered in detail here, a brief overview of the successes and challenges will be presented, and the potential for using HT crystallization as a validation to aid model development will be highlighted. For a more detailed discussion on polymorph and crystal structure prediction, refer to the article by Price [100] in this issue.

Polymorph prediction of pharmaceuticals is thwarted by the complexity of active pharmaceutical molecules. The number of degrees of freedom in torsion angles and the molecule count in the unit cell (which can be deduced by such techniques as solid-state NMR [94]) are frequently too great to allow computations on a reasonable time scale. Additionally, predictions are typically carried out one space group at a time. This limitation is mitigated by the fact that over 90% of the organic compounds in the Cambridge Structural Database (CSD) [101] crystallize in only a few space groups [100]. We know of only one example where predictions have been extended to multicomponent systems [102]. The prevalence of multicomponents systems, some of which have charge transfer (salts) and many of which exist as hydrates, solvates or mixed hydrate/solvates, essentially limits the usefulness of the prediction methods to neutral compounds. Various other technical issues remain as the science of crystal structure prediction matures [100]. Some of these issues were highlighted in two blind tests that were conducted in recent years to determine the accuracy and robustness of crystal structure prediction [103]. In the latest round, 17 methods were used to predict structure, yielding only three correct predictions [104]. For one of the compounds used in the study, experimental characterization of a second, more stable, polymorph provided the key to the correct prediction by three participating

research groups. The structure could have easily been overlooked, leading to the misinterpretation of the results as an apparent failure of the computational methods. Thus, compounds that are amenable to structure prediction are not always studied experimentally to the extent necessary to ensure that the relevant forms have in fact been discovered and characterized ahead of computational studies.

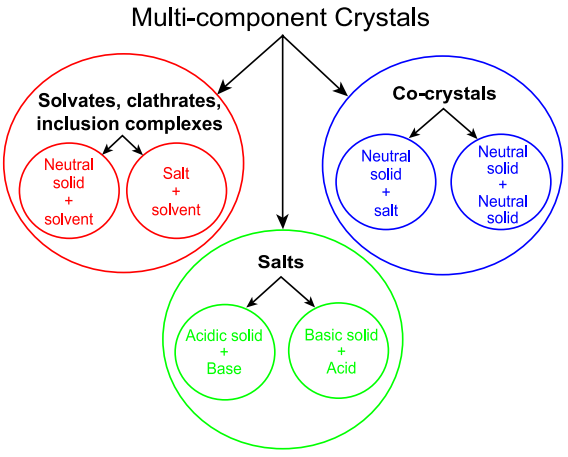
Despite the challenges, a few methods have been developed that allow structure prediction of small, relatively rigid organic compounds with only a few functional groups in several important space groups [17,105,106]. Polymorph Predictor™ has been implemented within the commercial software Cerius2 (C2 Polymorph by Accelrys). In general, current prediction methods generate large ensembles of different packing arrangements along with calculations of relative energetics. In reality, many of the calculated structures are not observed, giving the appearance of over-prediction of polymorphism. This was apparently the case with acetaminophen (paracetamol) [107]. In their study of the drug, Beyer et al. [107] calculated 14 structures, 2 of which were the known monoclinic (stable) and orthorhombic forms. The remaining 12 structures were considered as candidates for the metastable form III, which had been observed by thermal microscopy methods [82] but for which diffraction data were unavailable. Using calculations of mechanical properties and morphology, Beyer et al. separated the 12 energetically feasible structures into two groups, based on the likelihood of each structure to exist as a stable form. Shortly after the publication of the prediction study, the experimental powder pattern of form III became available [40]. Rietveld refinement and comparison of the experimental diffraction results with the theoretical powder patterns published by Beyer et al. yielded a monoclinic structure solution for form III. This structure is in fact part of the prediction set, but was considered an unlikely contender based on its extreme plate-like morphology. The potential for complementarity of HT crystallization and polymorph prediction is evident from these studies. In one sense, polymorph prediction can serve as a yardstick for ‘‘risk assessment’’ when it comes to form diversity, but inevitably one will require experimental data to assess the scope of polymorphism that can be elicited and the precise relative stabilities of different crystalline arrangements.

Opportunities do exist for current use of predictions in solid form discovery. For instance, certain hydrogen-bonding motifs or molecular layer types may be observed in predicted structures. Such information can be used to aid the design of crystallization experiments. It might be desirable to employ a particular type of interaction with salt selection or co-crystal formation by the strategic selection of crystallization conditions, solvents, additives and processing methods [22,23]. In addition, since transient or metastable crystalline species may be difficult to characterize accurately, one may use predicted structures to estimate various physical data. For example, powder diffraction patterns may be used to assist the accurate description of these metastable forms [40]. Continued development of theoretical methods coupled with validation of the predictions by extensive crystallization screening will lead to better models and computational methods. At present, experimental methods must still be relied upon to assess the potential form diversity of a given compound. It will be important to concurrently push the limits on theoretical prediction and HT crystallization, in order to advance our understanding of the nature and extent of polymorphism in pharmaceutical compounds.

### 3.5. Engineering of co-crystals

Co-crystals of drugs and drug candidates represent a new type of material for pharmaceutical development. They are part of a broader family of multicomponent crystals that also includes salts, solvates, clathrates, inclusion crystals and hydrates as shown in Scheme 2. The primary difference between solvates and co-crystals is the physical state of the isolated pure components: if one component is a liquid at room temperature, the crystals are designated as solvates; if both components are solids at room temperature, the crystals are designated as co-crystals. While at first glance these differences may seem trivial, they have profound impact on preparation, stability and ultimately on the ability to develop products.

In general, it is usually easier to initially prepare solvates than co-crystals, and indeed, solvates are often found as by-products of polymorph and salts screens. Co-crystals have been prepared by melt-crystallization, grinding and recrystallization from solvents [1]. Sol-



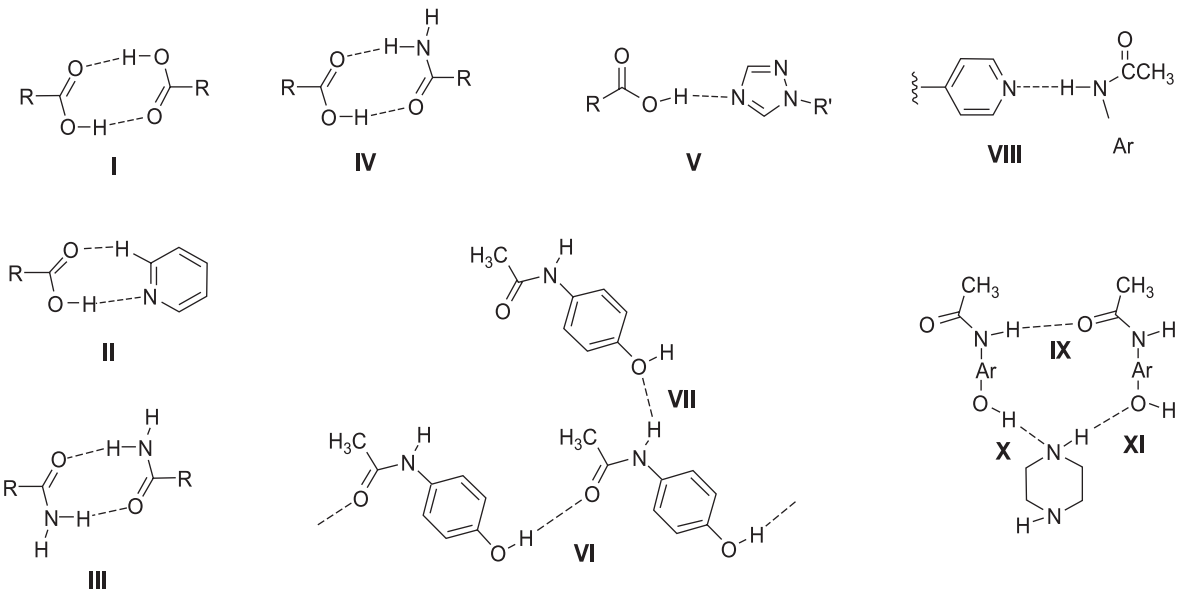

Scheme 2. Types of multicomponent crystals.

vent systems for co-crystals must dissolve all components, but must not interfere with the interactions necessary for co-crystal formation. The need to try many solvent combinations and the availability of multiple co-crystal formers creates a diversity that is ideally suited for exploration by HT systems.

Co-crystals have the potential to be much more useful in pharmaceutical products than solvates or hydrates. The number of pharmaceutically acceptable solvents is very small, and because solvents tend to be more mobile and have higher vapor pressure, it is not unusual to observe dehydration/desolvation in solid dosage forms. Solvent loss frequently leads to amorphous compounds, which are less chemically stable and can crystallize into less soluble forms. In contrast, most co-crystal formers are unlikely to evaporate from solid dosage forms, making phase separation and other physical changes less likely.

Examples of co-crystals have existed in conductive organic crystals, non-linear optical crystals, dyes, photographic materials pigments and agrochemicals for some time [7]. Two recent papers by Fleischman et al. [43, 45] emphasize the importance of understanding "supramolecular synthons" in synthesizing co-crystals containing pharmaceutical agents. For example, the ability to insert 4,4′ -bipyridine between the carboxylic acid dimers of aspirin, *rac*-ibuprofen and *rac*-flurbiprofen was recently reported [43]. The three examples clearly demonstrate the generality of the use of a pyridine-carboxylic acid heterosynthon II (Scheme 3) to replace a dicarboxylic acid dimer homosynthon I. A second study focused on finding multiple solvates and co-crystals of carbamazepine [45]. Carbamazepine polymorphs crystallize as amide dimers, each of which ties up the polar amide functional groups through homosynthon III. Crystal structures shows that each dimer contains a peripheral H-bond donor and acceptor pair that remain unused due to geometric constraints imposed by the drug molecule. Simple H-bond acceptor solvents like acetone and DMSO insert themselves to fill voids between the adjacent pairs of dimers [45]. Multiple co-crystals formers having hydrogen bond acceptors likewise insert themselves into the void. The homosynthon can also be broken to form heterosynthon IV, an amide-carboxylic acid dimer [45]. This was achieved to form solvates with acetic, formic and butyric acids, and co-crystals with trimesic and nitro-isophthalic acid.

A recent study of adducts of acetaminophen (paracetamol) with ethers and amines provides additional examples of supramolecular synthons for co-crystal formation [108]. While amide-amide homosynthon could have formed, both known forms of the pure material consist of linear head-to-tail chains held together through motif VI; the chains are cross-linked through synthon VII. The linear chain structure is preserved in co-crystals with 4,4′ bipyridine, but the cross-linking interaction VII is replaced by VIII, in which the 4,4′ bipyridine is hydrogen bonded to the amide hydrogen. The chains remain cross-




Scheme 3. Supramolecular synthons observed in co-crystals.

linked but only through pi-stacking interactions between 4,4′ bipyridine pairs on neighboring chains. In co-crystals with piperazine, the acetaminophen forms head-to-head chains through IX. Each chain is joined to the next through a layer of piperazine molecules that interact through heterosynthons X and XI. The paper also includes many solvates that will not be reviewed here, but their synthons should be applicable to co-crystal formation.

The above studies focused on demonstrating the use of supramolecular synthons to create novel crystalline phases. The variety of structures observed provides hope that some forms will have superior performance in pharmaceutical dosage forms. However, the studies stop short of providing data on the physical properties, such as solubility, necessary to evaluate their utility. Furthermore, only the saccharin and nicotinamide co-crystals of carbamazepine represent pharmaceutically acceptable co-crystals. Crystals containing two drugs may appear to be a good technique for making combination products of two drugs, but unless the two drugs are dosed only in stoichiometric ratios consistent with the co-crystal composition, such crystals would still need to be co-formulated with at least one of the bulk drugs in order to satisfy the clinical requirements.

We recently reported on the discovery and dissolution properties of pharmaceutically acceptable co-crystals consisting of hydrogen-bonded trimers of two molecules of *cis*-itraconazole and one molecule of a 1,4-dicarboxylic acid resulting from a HT crystallization screen [44]. The crystal structure of the succinic acid co-crystal (Fig. 8) revealed an unanticipated interaction between the triazole of itraconazole and the carboxylic acid (heterosynthon V in Scheme 3). The extended succinic acid molecule fills a pocket, bridging the triazole groups. The interaction between the 1,4-diacid and the strongest base on itraconazole (piperazine) is absent in the co-crystal structure. Other 1,4-diacids including fumaric acid, L-malic acid and L-, D- and DL-tartaric acids also yielded co-crystals with itraconazole, but co-crystals could not be made from maleic acid with *Z*-regiochemistry, or from 1,3- or 1,5-dicarboxylic acids. Hence, geometric fit appears to be more important than acid-base chemistry in directing crystallization of the compounds of itraconazole with 1,4-dicarboxylic acids.

Identification of multiple crystal forms of the same drug with acceptable solubility, dissolution rate and stability enables selection of the optimal form for dosage form development. To demonstrate this feature, the dissolution of itraconazole co-crystals in

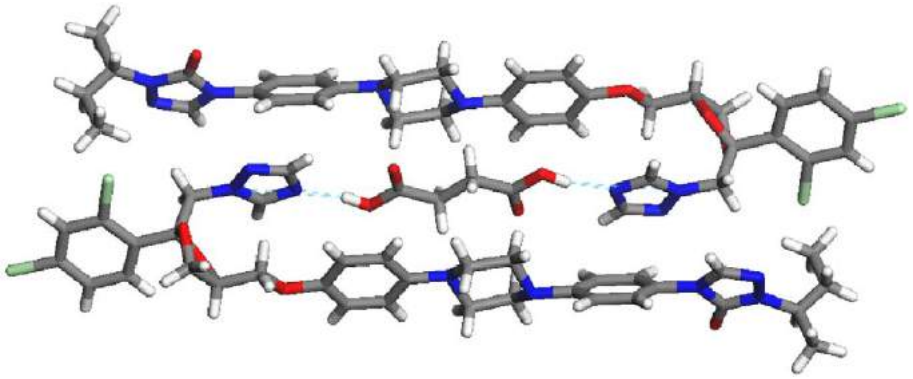


Fig. 8. Trimer unit of the itraconazole succinic acid co-crystal from single crystal X-ray structure (from [44], with permission).

aqueous medium was studied to assess their potential impact on bioavailability of the drug from a solid dosage form. Fig. 9 compares the dissolution profiles of the co-crystals into 0.1 N HCl to those of crystalline itraconazole-free base (95 % of all crystalline particles $< 10$ μm) and commercial Sporanox® beads (amorphous itraconazole). The malic acid co-crystal rivals the dissolution of the commercial product. In general, the co-crystals behave more similarly to Sporanox® than the crystalline-free base. The co-crystal forms achieve and sustain 4- to 20-fold higher concentrations than that achieved from the crystalline-free base. The practical implication is significant, since the ability to form a supersaturated solution, even transiently, can have dramatic impact on absorption and bioavailability.

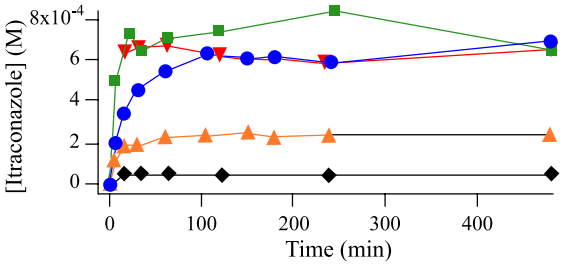



Fig. 9. Dissolution profiles into 0.1 N HCl at 25 °C plotted as itraconazole concentration ([itraconazole]) as a function of time for Sporanox® beads (■), crystalline itraconazole-free base (♦) and co-crystals of itraconazole with L-malic acid (▼), L-tartaric acid (●) and succinic acid (▲) (from [44], with permission).

Co-crystals represent a class of pharmaceutical materials of interest, both in terms of projected diversity and applicability. The study of co-crystals, along with polymorphs, solvates, salts and hydrates, is perfectly suited to HT crystallization experimentation and should be considered part of the form selection processes.

## 4. Post-screening analyses and form selection

Several functional characteristics must be considered in the selection of a suitable crystal form for a pharmaceutical dosage form. HT crystallization has the potential to create a larger pool of crystal forms for which functional parameters, such as dissolution rate, chemical stability, flow and compressibility, must be determined and compared. Strategies to accomplish ranking of the numerous forms must be devised. An example is the adaptation of HT for solubility measurement. The plot in Fig. 9 illustrates results of a plate-based kinetic dissolution assay in which various forms of a compound were placed in simulated gastric fluid and monitored for dissolution as a function of time. The schematic in Fig. 10 shows how such an analysis can be accomplished in a 96-well filter plate. The concentration at a given time point is determined after filtration of the suspension by quantification using either UV or HPLC with UV detection.

While the entire plate is filtered at one time, different time points can be achieved by timing the addition of dissolution medium such that the aliquot

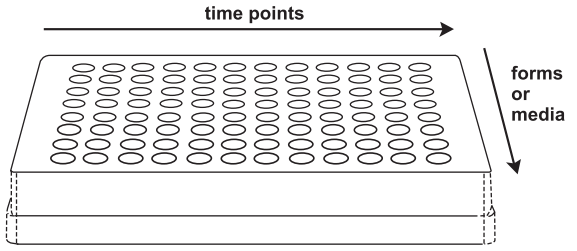



Fig. 10. Schematic of a 96-well dissolution filter plate.

for the longest time point desired is dispensed first and the shortest one comes last. Instead of varying the form along one axis of the plate, one can choose to study the dissolution of a single form into several different media (see Fig. 10). Equilibrium solubility can be determined in a variety of solvents and at different temperatures using a similar principle to the dissolution plate. A demonstration has been provided using automated React-IR analysis [109]. Other functional parameters, such as solid-state stability and thermal properties, can be adapted to HT. Such systems for ranking the stability of forms generated from HT crystallization await publication and review at a future date.

## 5. Summary and outlook

HT crystallization methodologies are capable of screening hundreds or thousands of crystallization conditions in parallel using small amounts of compound for the identification and characterization of diverse forms of active pharmaceutical ingredients. As demonstrated by numerous case studies from several stages of pharmaceutical development, such technologies have begun to show promise in enabling more comprehensive exploration of solid form diversity. The technologies are likely to provide a landscape of potential operating conditions from which scientists and engineers can design robust and scalable processes for transfer to manufacturing.

The ability to conduct extensive crystallizations with small amounts of material using a variety of solvents, additives and conditions necessarily generates large sets of data. However, the information by itself is of limited value, unless it can be properly analyzed. In order to extract maximum knowledge from the studies, it is essential to have the ability to design experiments, track samples in the process, collect the data in a relational database, and mine the information using statistical techniques and models in property space that assist the scientist to maximize the value of the data. Such models attempt to fit an output variable to physical properties or descriptors using techniques similar to those used in traditional quantitative structure activity relationships (QSAR). These models can be *carefully* extended to mixtures containing compounds that were not included in the original experiments if validation suggests that the models are sufficiently stable. Significant models that are found in the analysis of the data can be stored in the database for later retrieval and use to direct iterative experiments. The power of this approach becomes increasingly more visible when several properties are being co-optimized, as can be very important in the pharmaceutical development process where such properties as oral bioavailability, stability and processability need to be reconciled. The availability of a map of conditions that lead to the formation of different forms (salts, hydrates, solvates, polymorphs, co-crystals) of the drug can be valuable to the process chemists or engineers as they develop scalable processes to produce materials suitable for development and registration.

For many years, the value of composition of matter (CoM) patents on new chemical entities, including where appropriate, pharmaceutically acceptable salts, has been well appreciated. However, it is only within the last decade or so that the application of CoM patents has been significantly extended to cover all forms of the compound, including hydrates, solvates, co-crystals and polymorphs. Unlike salts, which for the most part can be prophetically claimed based on an understanding of the chemical structure of the compound and its ionization constants, the existence and identity of hydrates, solvates, co-crystals and polymorphs have defied prediction. Therefore, in order to obtain patent protection on these forms, some of which may have significantly different properties and relevance as development candidates, it is essential to prepare them, identify conditions for making them and evaluate their properties as valuable new pharmaceutical materials.

In general, discrete crystal forms are considered non-obvious and patentable. Given the diversity and greater complexity of chemical structures of today's

drug candidates [110], coupled with the advanced technology to identify novel forms, it is common to find multiple forms of drugs [61], some similar, some dramatically different in terms of their in vivo performance. These forms are all candidates for separate intellectual property protection. Therefore, it is incumbent on the innovator of a new drug candidate to identify and patent these forms in order to optimally protect their investment in the compound. Recent case studies suggest that identifying and patenting all forms of new chemical entities should be a primary strategy of all innovators of novel drugs. In this regard, the use of HT crystallization technologies for rapid, comprehensive discovery and characterization of solids form diversity offers significant advantages for the development of a strong intellectual property position.

With the advent of HT crystallization methods, appreciation for the landscape of physical form for drug development has begun to change. Use of these systems has the potential to facilitate drug development by saving valuable time in selecting the optimal physical or chemical form of a given compound. HT systems that generate rich datasets offer the ability to develop a more fundamental understanding of the crystallization process, based on knowledge generated from large numbers of experiments on diverse compounds. Having such information at an early stage minimizes the risk of process modifications resulting in form changes and provides the opportunity to gain more comprehensive intellectual property coverage. In addition, comprehensive form data help address important regulatory questions related to the number of solid forms of an API and the relationships between them.

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FEATURE ARTICLE

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# Crystal engineering of the composition of pharmaceutical phases. Do pharmaceutical co-crystals represent a new path to improved medicines?

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**The evolution of crystal engineering into a form of supramolecular synthesis is discussed in the context of problems and opportunities in the pharmaceutical industry. Specifically, it has become clear that a wide array of multiple component pharmaceutical phases, so called pharmaceutical co-crystals, can be rationally designed using crystal engineering, and the strategy affords new intellectual property and enhanced properties for pharmaceutical substances.**

## 1 Introduction

"Benzoic acid and other carboxylic acids have been shown to be associated to double molecules in solution in certain solvents, such as benzene, chloroform, carbon tetrachloride and carbon disulfide...Benzoic acid exists in the monomeric form in solution in acetone, acetic acid, ethyl ether, ethyl alcohol, ethyl acetate and phenol; in these solutions the single molecules are stabilized by hydrogen bond formation with the solvent." (Linus Pauling in *The Nature of the Chemical Bond*, 2nd edition, Cornell University Press, 1948.)

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In terms of intrinsic value, active pharmaceutical ingredients (API's) are among the most valuable materials on the planet. It is therefore surprising that the growing field of crystal engineering[1–3] and its ability to produce new and potentially valuable materials has only addressed API's within the last two years.[4–9] Pharmaceuticals are generally comprised of an API, a formulation containing inactive ingredients as a carrier system, and a package for market performance and appeal. The vast majority of API's occur as solids. Crystalline API's are strongly preferred due to their relative ease of isolation, the rejection of impurities inherent to the crystallization process and the physico-chemical stability that the crystalline solid state affords. The problems that arise with the use of crystalline material are usually related to poor solubility properties and the existence of more than one crystalline form of an API. In terms of regulatory approval crystalline forms of an API have traditionally been limited to polymorphs, salts and stoichiometric solvates (pseudopolymorphs).[10] However, crystal engineering affords a paradigm for rapid development of a fourth class of API's, that of pharmaceutical co-crystals.

Crystal engineering can be defined as application of the concepts of supramolecular chemistry to the solid state with particular emphasis upon the idea that crystalline solids are *de facto* manifestations of self-assembly. Crystal structures can therefore be regarded as the result of a series of weak but directional molecular recognition events. With understanding comes the possibility of design and it is the advent of supramolecular synthesis[1–3] that facilitates the rational design of new structures and compositions. The roots of crystal engineering can be traced at least as far back as the 1930's, when Pauling defined the chemical bond in both covalent and noncovalent terms.[11] The term "crystal engineering" was coined by Pepinsky in 1955[12] but was not implemented until Schmidt studied a series of solid state reactions in crystalline solids.[13] Indeed, solvent free synthesis continues to represent an active area of research in the context of crystal engineering.[14,15] Based upon literature citations,† it is apparent that crystal engineering enjoyed rapid growth during the 1990's, especially in terms of organic solids and metal-organic solids but also in terms of organometallic[16] and inorganic structures.[17]

What are pharmaceutical co-crystals? Herein we define pharmaceutical co-crystals as being a subset of a broader group of multi-component crystals that also includes salts, solvates (pseudopolymorphs), clathrates, inclusion crystals and hydrates. In a supramolecular context, solvates and pharmaceutical co-crystals are related to one another in that at least two components of the crystal interact by hydrogen bonding and, possibly, other non-covalent interactions rather than by ion-pairing. Neutral compounds and salt forms alike have the potential to be solvated (*i.e.* interact with solvent molecules) or co-crystallized (*i.e.* interact with a co-crystal former). Solvate molecules and co-crystal formers can include organic acids or bases that remain in their neutral form within the multi-component crystal. The primary difference is the physical state of the isolated pure components: if one component is a liquid at room temperature, the crystals are referred to as solvates; if both components are solids at room temperature, the products are

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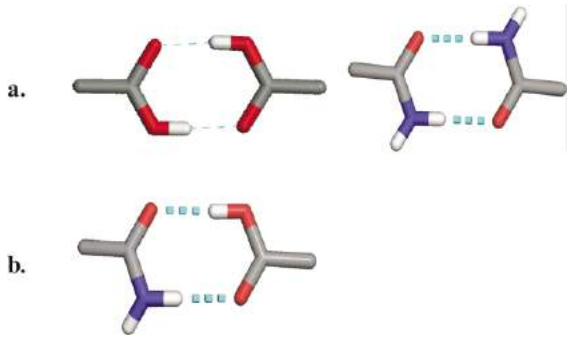
referred to as co-crystals. While at first glance these differences may seem inconsequential, they have profound impact on the preparation, stability, and ultimately on developability of products. Furthermore, whereas solvates are commonplace because they often occur as a serendipitous result of crystallization from solution, co-crystals, especially pharmaceutical co-crystals, represent a relatively unexplored class of compounds. On the other hand, as will become clear herein, pharmaceutical co-crystals can be rationally designed and there are many more potential co-crystal formers than there are solvents or counterions.

The complex nature of API structures means that they inherently contain exterior functional groups that engage in molecular recognition events. Indeed, it is the very presence of these functional groups that affords biological activity but also provides an ability to engage in more than one supramolecular event with itself, a solvent molecule or co-crystal former, thereby forming polymorphs, solvates or co-crystals, respectively. It is important to note that there are two basic types of molecular recognition that facilitate the formation of polymorphs, solvates and co-crystals. Functional groups that are self-complementary are capable of forming *supramolecular homosynthons*. For example, as revealed by Scheme 1a, carboxylic acid moieties and amide moieties can




**Scheme 1** The formation of supramolecular synthons between acids and amides: (a) supramolecular homosynthons as exhibited by acid–acid and amide–amide dimers; (b) supramolecular heterosynthons as exhibited by acid–amide dimers.

form homodimers *via* a two-point donor-acceptor molecular recognition path. However, it is also possible for functional groups to engage with a different but complementary functional group, as noted by Pauling. Indeed, carboxylic acids and amides are complementary with each other and can interact through formation of a *supramolecular heterosynthon* (Scheme 1b). This particular motif has been studied for some time in the context of co-crystals.[18]

In this contribution we detail the current and potential impact of crystal engineering on our understanding of polymorphs, solvates and co-crystals with particular emphasis upon API's. Carboxylic acid and amide moieties are widely encountered in API's and studied in model compounds. They will therefore be used extensively in this contribution even though it should be remembered that they represent just a microcosm of the functional group diversity that exists in API's.

## 2 Crystal engineering in the context of polymorphs

"A solid crystalline phase of a given compound resulting from the possibility of at least two different arrangements of the molecules of that compound in the solid state" (W.C. McCrone in *Physics and Chemistry of the Organic Solid State*, Vol II, Wiley Interscience, New York, 725–726, 1965.)

McCrone's definition of a polymorph as presented above is particularly appropriate in the context of drugs, since the existence of highly functional API's invites multiple modes of self-organization and amounts to promiscuity in self-assembly. It is this feature and conformational flexibility that are the primary driving forces for the existence of crystal polymorphism. It is therefore not surprising that it is well and long documented that API's can exist in several polymorphic, solvated and/or hydrated forms.[10,18] This tendency for polymorphism represents both a problem and an opportunity in pharmaceutical research. Lack of reliability of manufacturing and physical (and sometimes chemical) instability of a given polymorph can be an issue for a drug developer, while a novel polymorph in the hands of a competitor can provide options for generic pharmaceutical competition.

We shall focus upon polymorphism from a supramolecular perspective with emphasis upon two functional groups that are commonly encountered in API's: carboxylic acids and amides.

### 2.1 Structures in which carboxylic acids are involved in self-organization.

Carboxylic acid moieties represent perhaps the longest and most widely studied functional group in terms of our understanding of hydrogen bonding in both solution and the solid state.[11] In the context of crystal structures, carboxylic acids exhibit a remarkable range of diversity in their supramolecular chemistry and this in turn leads to observation of polymorphs in even the most simple of chemical structures. There are two primary modes for carboxylic acids to self-organize in the form of supramolecular homosynthons: the dimer and the catemer. Such "supramolecular isomerism" is the origin of polymorphism exhibited by the two polymorphs of chloroacetic acid (Fig. 1). Fig. 1a illustrates the dimer motif which

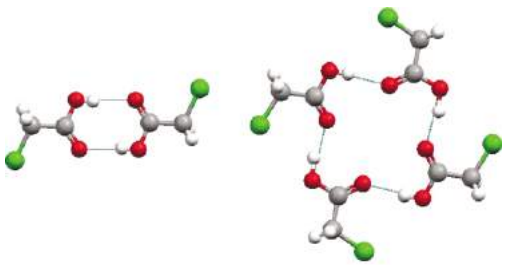



**Fig. 1** The self-organization modes seen in the two reported polymorphs of chloroacetic acid: (a) centrosymmetric dimer; (b) catemer motif, which leads to a tetrameric assembly.

occurs in one polymorph[19] whereas Fig. 1b presents the second form, in which a catemer supramolecular synthon results in the formation of a tetrameric supramolecular assembly.[20] It should be noted that carboxylic acid polymorphs are not always a consequence of isomerism in supramolecular homosynthons. For example, they can result from factors such as different crystal packing arrangements of dimer motifs or, if appropriate, torsional flexibility, which can afford conformational polymorphism.[21] Nevertheless, there are other simple carboxylic acids that exhibit polymorphism because of dimer/catemer supramolecular isomerism (*e.g.* hydroxybenzoic acid,[22] oxalic acid[23] and tetrolic acid[24]).

The story does not end there: whereas there are over 4000 entries in the Cambridge Structural Database[25] (CSD) of crystal structures in which at least one carboxylic acid moiety is present, 1179 exhibit the dimer motif (29.4%) and only 86 exhibit the catemer motif (2.1%). In other words, the formation of supramolecular homosynthons is not the dominant supramolecular event in the solid state even if it might be in solution. An analysis of the remaining carboxylic acid containing crystal structures reveals that they typically form supramolecular structures that involve a carboxylic acid and a different functional group, *i.e.* they form *supramolecular heterosynthons*. The ability of a molecule to engage in either supramolecular homosynthons or supramolecular heterosynthons represents another avenue for the existence of polymorphism.

Polymorphism in molecules which contain multiple functional groups is exemplified by Fig. 2, which presents the monoclinic and

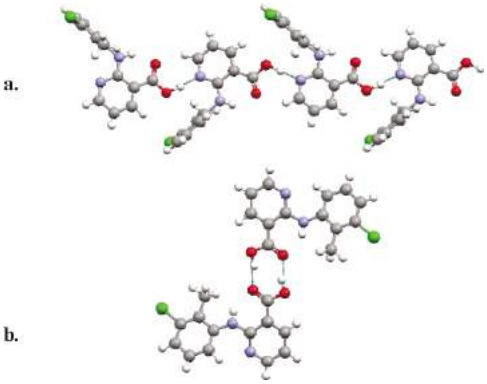
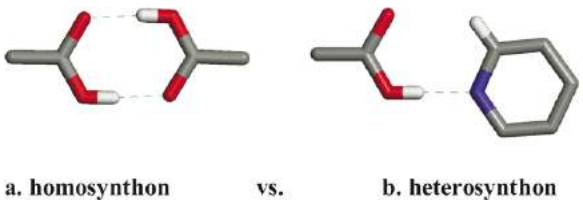



**Fig. 2** The monoclinic (a) and triclinic (b) forms of 2-(2-methyl-3-chloroanilino)-nicotinic acid, an analgesic/anti-inflammatory molecule.

triclinic forms of 2-(2-methyl-3-chloroanilino)-nicotinic acid,[26] a molecule that exhibits analgesic and anti-inflammatory properties. Fig. 2 reveals that 2-(2-methyl-3-chloroanilino)-nicotinic acid can self-organize *via* either supramolecular homosynthons or supramolecular heterosynthons: (a) generation of head-to-tail chains sustained by a carboxylic acid–pyridine supramolecular heterosynthon; (b) formation of centrosymmetric dimers sustained by the carboxylic acid supramolecular homosynthon.

It is important to emphasize the distinction between supramolecular homosynthons and supramolecular heterosynthons since the latter represent a possible entry into the realm of multiple-component crystals and a diverse range of compositions of matter and physical properties. That carboxylic acids represent such a large subset of the CSD makes it possible to ask an important question: are supramolecular heterosynthons not just rational but also predictable? In the context of the pyridine–carboxylic supramolecular heterosynthon the CSD reveals that there are 424 compounds that contain both a carboxylic acid and an aromatic nitrogen base. 198 of these compounds (46.7%) exhibit the supramolecular heterosynthon rather than one of the carboxylic acid supramolecular homosynthons (Scheme 2). When one considers that many of the compounds in this dataset contain multiple functional groups this is a remarkably high rate of occurence.




**Scheme 2** The homosynthon *vs.* heterosynthon motifs observed in crystal structures of compounds in which both carboxylic acids and pyridine moieties are present. The heterosynthon dominates, occurring in 119/245 crystal structures whereas the homosynthon occurs in only 10 crystal structures.

## 2.2 Structures in which primary amides are involved in self-organization

Primary amides are also well represented in the CSD, with 1152 entries. The dominant supramolecular homosynthon is the centrosymmetric dimer as presented in Scheme 1. This homosynthon contains complementary hydrogen bond donors and acceptors and is capable of further self-assembly, thereby generating supramolecular tapes or sheets. Fig. 3a illustrates how chloroacetamide forms a tape network based upon self-organization of homodimers.[27–30] Interestingly, chloroacetamide also exhibits polymorphism and for the same fundamental reason as chloroacetic acid: it exhibits a catemer structure as well as a homodimer structure.[31] The

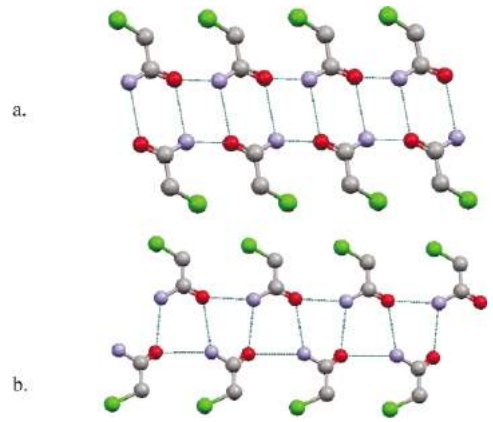



**Fig. 3** The self-organization modes seen in two polymorphs of chloroacetamide: (a) centrosymmetric dimer that self-assembles as 1-D tapes; (b) catemer motif, which also forms 1-D tapes.

polymorphic form of chloroacetamide that is the result of catemer motifs is illustrated in Fig. 3b. It reveals that the superstructure is also that of a tape. The two forms of chloroacetamide crystallize in the same space group with almost identical cell parameters. This is an extremely unusual situation and is presumably related to the fact that the two tapes are similar in terms of dimensions and exterior features.

Chloroacetic acid and chloroacetamide serve as illustrations of how even small molecules with only one hydrogen bonding group can generate polymorphs based upon supramolecular isomerism. A similar analogy can be found in API's that contain acid and amide moieties. Piracetam, a learning process drug, is an amide-containing API that exemplifies the type of polymorphism that occurs when supramolecular isomerism occurs in supramolecular homosynthons. There are three forms of Piracetam reported in the CSD.[32,33] Two of these forms exist as tapes that are sustained by the amide homodimer and NH···O=C(carboxamide) hydrogen bonds (Fig. 4a).[32] The third form is sustained by catemer chains that are crosslinked by N–H···O=C(carboxamide) hydrogen bonds (Fig. 4b).[33] The superstructure can therefore be described as hydrogen bonded sheets.

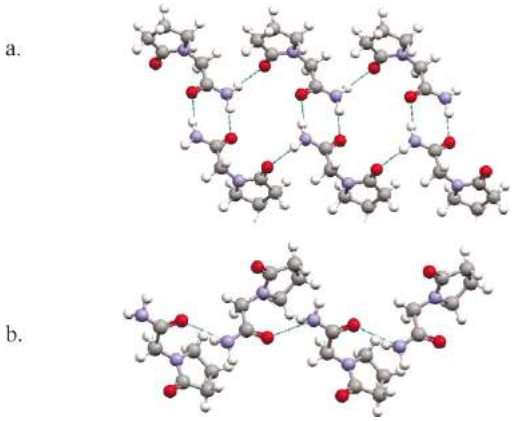



**Fig. 4** The network structures formed by Piracetam: (a) homodimers form supramolecular tapes two forms; (b) 1-D chains sustained by the catemer motif are found in the third form.

To summarize the points made thus far:

• Single component crystals that contain carboxylic acid or amide moieties are prone to polymorphism even if only one hydrogen bonding moiety is present and supramolecular homosynthons are the primary molecular recognition events.

• In the case of API's, the situation is further complicated by the presence of additional hydrogen bonding moieties, which can lead to the formation of supramolecular heterosynthons.

• Carboxylic acid and amide groups were chosen as examples, because they are prevalent in the CSD and in API's. However, the points made thus far can be regarded as being generally relevant. For example, we recently reported[34] how alcohol–ether heterosynthons can afford polymorphic forms of butylated hydroxy anisole, an antioxidant that is commonly used in solid dosage forms of API's.[35,36] The difference between the two forms is striking: form I exists as the result of 4-fold helical chains: form II contains discrete hexamers.

How one might exploit supramolecular heterosynthons for the crystal engineering of new compositions of matter will form the basis of the remainder of this contribution.

## 3 Crystal engineering in the context of co-crystals

"Supramolecular synthons are structural units within supermolecules that can be formed and/or assembled by known or conceivable synthetic operations involving intermolecular interactions". (Gautam R. Desiraju *Angew. Chem. Int. Ed. Engl.*, 34, 2311, 1995.)

How does one develop a strategy for the preparation of co-crystals? Solvates are frequently encounted but are typically the result of serendipity rather than design and are often found as by-products of polymorph and salt screens. Co-crystals, on the other hand, are less ubiquitous but are more prone to rational design. Co-crystals have been prepared by melt-crystallization, by grinding[37] and by recrystallization from solvents.[14,15] Pharmaceutical co-crystals have the potential to be much more useful in pharmaceutical products than solvates or hydrates. First, the number of pharmaceutically acceptable solvents is very small. Secondly, solvents tend to be more mobile and have higher vapour pressures than small molecule co-crystal formers. It is not unusual to observe dehydration/desolvation of hydrates/solvates in solid dosage forms, depending on storage conditions. Solvent loss frequently leads to amorphous compounds, which are generally less chemically stable and can crystallize into less soluble forms. In contrast to solvents, most co-crystal formers are unlikely to evaporate from solid dosage forms, making phase separation less likely.

### 3.1 Co-crystals based upon acids or amides

As suggested earlier, an effective approach to understanding and designing co-crystals is to apply the paradigm of supramolecular synthesis, in particular exploitation of supramolecular heterosynthons. The ubiquity of acids and amides in the CSD makes them appropriate foci for design and synthesis. Indeed, the acid–amide supramolecular heterosynthon illustrated in Scheme 1a has been exploited by several groups for the generation of co-crystals[18,38–41] and the CSD reveals that there are 118 crystal structures in which both an acid and an amide moiety are present. Remarkably, 58 of these structures exhibit the acid–amide supramolecular heterosynthon whereas only 11 structures exhibit the acid homodimer and only 28 exhibit the amide homodimer. Fig. 5 presents two

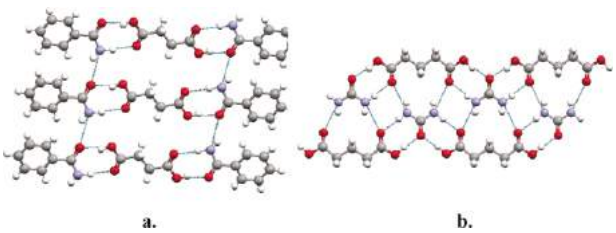



**Fig. 5** Two examples of co-crystals that are sustained by the acid–amide supramolecular heterosynthon: (a) succinic acid : benzamide (1:2); (b) urea : glutaric acid (1:1).

prototypal examples of co-crystals that are sustained by the acid–amide supramolecular heterosynthon: succinic acid : benzamide[18] and urea : glutaric acid.[38] Acid–amide supramolecular heterosynthons are not the only examples of robust heterosynthons that are favored over the parent homosynthons. Acid–pyridine supramolecular heterosynthons, a subset of the acid–aromatic amine set described earlier, occur in 119 of the 245 crystal structures that contain both functional groups. Remarkably, only 10 of these 245 structures contain acid–acid homosynthons (Scheme 2).

Representative examples of co-crystals that are sustained by the pyridine–carboxylic acid supramolecular synthon are presented in Fig. 6. Maleic acid : 4,4′-bipyridine forms a discrete 2:1 adduct[42]

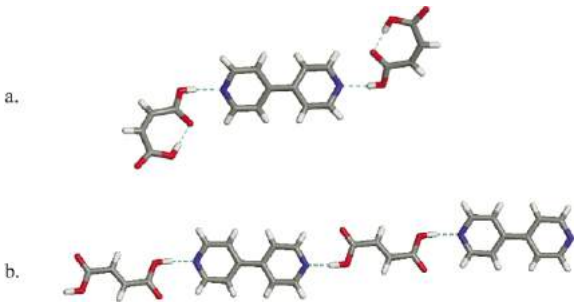



**Fig. 6** Two examples of co-crystal structures formed by the acid–pyridine supramolecular heterosynthon: (a) maleic acid: 4,4′-bipyridine; (b) fumaric acid : 4,4′-bipyridine.

whereas fumaric acid : 4,4′-bipyridine forms in 1:1 stoichiometry and thereby generates a 1-D chain.[42]

### 3.2 Functional co-crystals

Examples of co-crystals have existed in conductive organic crystals, non-linear optical crystals, dyes, pigments and agrochemicals for some time[43] but have only recently been applied to API's. Several recent papers emphasize the importance of understanding supramolecular heterosynthons in the synthesis of pharmaceutical co-crystals. For example, the ability to insert 4,4′-bipyridine and related molecules between the carboxylic acid dimers of aspirin, *rac*-ibuprofen, and *rac*-flurbiprofen was recently reported.[6] Fig. 7 illustrates two of these structures, which further demonstrate the ability of the pyridine–carboxylic acid heterosynthon to compete with a carboxylic acid dimer homosynthon (Scheme 2).

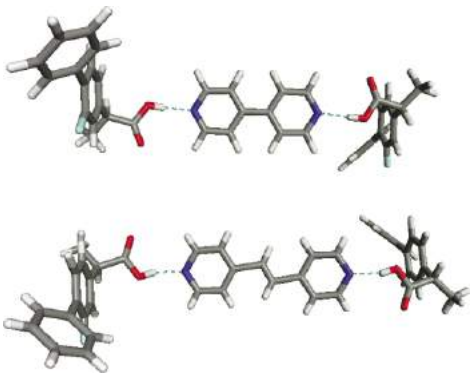


**Fig. 7** The 2:1 supramolecular adducts formed by flurbiprofen and 4,4′-bipyridine (top) and 4,4′-dipyridylethane (bottom). Similar structures occur for ibuprofen and aspirin.

A second study focused on finding multiple solvates and co-crystals of carbamazepine.[5] Carbamazepine represents an excellent test case since four polymorphs and two solvates of carbamazepine have been reported in the literature. In all of the compounds for which structural data is available, carbamazepine molecules crystallize as amide dimers (Fig. 8). The crystal structures illustrate that each dimer contains a peripheral H-bond donor and acceptor pair that is unsatisfied due to geometric constraints imposed by the drug molecule. Simple H-bond acceptor solvents like acetone and DMSO insert themselves to fill voids between the adjacent pairs of dimers. Multiple co-crystal formers having hydrogen bonding

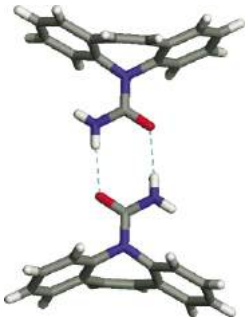


**Fig. 8** The carbamazepine dimers that exist in all previously reported solvates and polymorphs of carbamazepine.

groups likewise insert themselves into the void, including saccharin and nicotinamide. The amide homosynthon can also be broken to form heterosynthon **Ib**. This was achieved to form solvates with acetic, formic, and butyric acids and co-crystals with trimesic and nitroisophthalic acid. The crystal structures of the carbamazepine : saccharin co-crystal and the formic acid solvate are illustrated in Fig. 9.

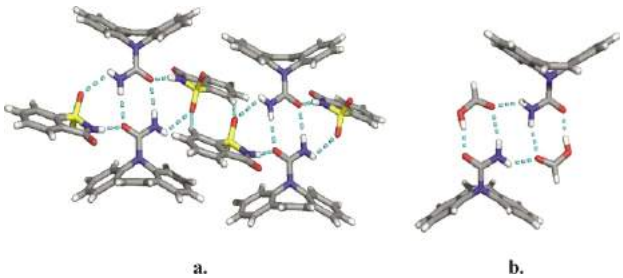
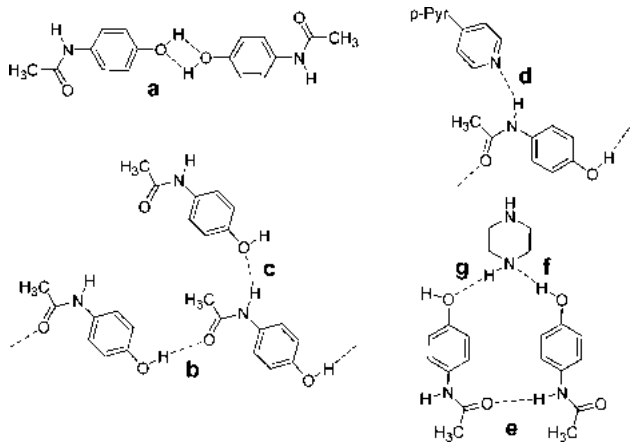



**Fig. 9** Examples of the supramolecular adducts formed in the crystal structures of co-crystals and solvates of carbamazepine: (a) saccharin co-crystal; (b) carbamazepine:formic acid solvate.

A study of adducts of acetaminophen (paracetamol) with ethers and amines provides additional examples of supramolecular synthons for co-crystal formation (Scheme 3).[9] While supramolecular homosynthon **IIIa** could have formed, both known forms of the pure material consist of linear head-to-tail chains held together through motif **IIIb**; the chains are cross-linked through synthon **IIIc**. The linear chain structure is preserved in co-crystals with 4,4′-bipyridine, but the cross-linking interaction **IIIc** is replaced by **IIId**, in which the 4,4′-bipyridine is hydrogen bonded to the amide hydrogen. The chains remain cross-linked but only through pi-stacking interactions between 4,4′-bipyridine pairs on neighboring chains. In co-crystals with piperazine, the acetaminophen forms head-to-head chains through **IIIe**. Each chain is joined to the next through a layer of piperazine molecules that interact through heterosynthons **IIIf** and **IIIg**. The paper also includes many solvates that will not be reviewed here, but their supramolecular synthons should also be applicable in the context of co-crystal design and formation.




**Scheme 3** The supramolecular synthons observed in co-crystals of acetaminophen (paracetamol): **IIIa–c** occur in polymorphs whereas **IIId** and **IIIe** occur in co-crystals.

The analysis of molecules for complementarity of supramolecular synthons represents a valuable approach to screening that a knowledgeable scientist can exploit to narrow the search for co-crystals. However, an early study of 1:1 molecular complexes between the antibacterial agents trimethoprim (TMP) and sulfamethoxypyridazine (SMP) highlights the need to explore the space beyond those leading to expected interactions.[44] Each complex contains an 8-membered, hydrogen-bonded ring joining the two molecules as shown in Fig. 10. The specific ring structures formed

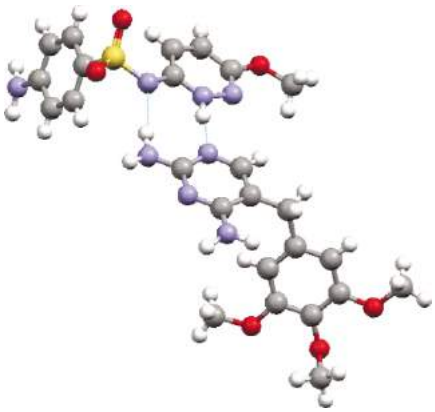


**Fig. 10** The 8-membered hydrogen-bonded ring that links antibacterial agents trimethoprim (TMP) and sulfamethoxypyridazine (SMP).

are not those that might have been predicted by inspection of the structures of the neutral molecules. Instead, the synthons are derived from the 2-aminopyridine of TMP and the zwitterionic form of SMP involving the sulfonamide ($pK_a$ ~ 7) and pyridazine ($pK_a$ ~ 2). The zwitterion is a thermodynamically unfavorable form of SMP in aqueous solution. This example of assembly through an unstable intermediate underscores the limitation of the approach of analyzing co-crystal formation solely on the basis of $pK_a$ arguments. A more comprehensive approach is needed. HT crystallization offers the possibility to uncover unexpected interactions by screening against a full library of pharmaceutically acceptable molecules instead of limiting the studies to co-crystal formers with perceived complementarity.

The more comprehensive approach to study expected and unexpected co-crystal formation events is high-throughput (HT) crystallization. The discovery of pharmaceutically acceptable co-crystals consisting of hydrogen-bonded trimers of two molecules of *cis*-itraconazole, a triazole anti-fungal agent, and a molecule of a 1,4-dicarboxylic acid resulting from a HT crystallization screen was recently reported.[8] The crystal structure of the succinic acid co-crystal (Fig. 11) reveals a supramolecular heterosynthon between the triazole of each pair of drug molecule and carboxylic acid moieties on a single diacid molecule. The extended succinic acid molecule fills a pocket, while bridging the triazole groups. The interaction between the 1,4-diacid and the strongest base on itraconazole (piperazine) is not observed in the co-crystal structure. Other 1,4-diacids capable of extended (anti-) conformations also yielded co-crystals with itraconazole, while co-crystals could not be made from maleic acid with *Z*-regiochemistry, or from 1,3- or 1,5-dicarboxylic acids. Hence, structural fit appears to be far more important than acid–base strength complementarity for co-crystallization of itraconazole with 1,4-dicarboxylic acids.

The structures presented herein demonstrate that pharmaceutical co-crystals represent an interesting and emerging class of pharma-

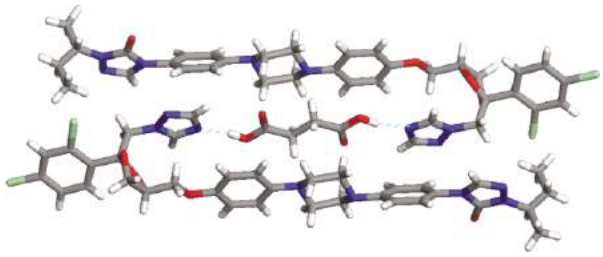


**Fig. 11** The 2:1 supramolecular adduct formed by itraconazole and succinic acid.

ceutical materials in terms of rational design, projected diversity and applicability. Furthermore, the study of pharmaceutical co-crystals, along with polymorphs, solvates, salts and hydrates, is perfectly suited to HT crystallization experimentation and could in the future be considered an integral part of form selection processes in pharmaceutical research and development.

## 4 Conclusions and future directions

"What would the properties of materials be if we could really arrange the atoms the way we want them?…we will get an enormously greater range of possible properties that substances can have, and of different things that we can do." (Richard P. Feynman, December 29, 1959).

In the world of pharmaceuticals, the opportunity presented by co-crystals appears to be significant. Published examples of pharmaceutical co-crystals are few as yet, but we now believe the approach can be applied broadly to API's. The design and selection of optimal pharmaceutical materials based on supramolecular synthesis is a relatively low-risk strategy, because the approach employs principles of molecular recognition and self-assembly rather than creating covalent bonds. Therefore, there are no covalent modifications of the API in question. Nevertheless, some big questions remain:

### 4.1 How large is the space of pharmaceutical co-crystals?

Compared with the space of salt forms, solvates and polymorphs, how large is the space of pharmaceutical co-crystals? Polymorphism tendency of pharmaceutical substances varies greatly, but the general observation is that most compounds are at some time or another going to display polymorphic behavior. Typically, the extent of polymorphism of pharmaceuticals is limited to a handful of different crystal forms. A recent review classifies highly polymorphic materials as having 4 or more forms.[45] Solvates (including hydrates) can be more numerous, and in certain cases very large numbers of solvates can be observed. Indeed, one study suggests that sulfathiazole is inordinately promiscuous in terms of solvate formation, with over one hundred solvates found.[46] Salt forms can be numerous as well, with over 90 acids and 30 bases being considered suitable for pharmaceutical salt selection.[47] Examples of compounds possessing a dozen or more crystalline salt forms have been published.[48,49] It is important to remember that salt formation is generally directed at one acidic or basic functional group. In contrast, co-crystals can simultaneously address multiple functional groups (synthons) in a single drug molecule. In addition, the space is not limited to binary combinations (such as acid–base pairs) since tertiary and quaternary co-crystals are realistic possibilities. Co-crystal formers for pharmaceutical use remain to be enumerated fully, but we argue that well over a hundred solid materials with GRAS status (including food additives and other well-accepted substances) can be employed. Even more provocatively, one might consider using sub-therapeutic amounts of eminently safe drug substances, such as aspirin and acetaminophen, as legitimate co-crystal formers, thus expanding the arsenal even further. Taken together with the high dimensionality and resulting combinatorial nature of supramolecular assembly, the space of pharmaceutical co-crystals would appear to be extremely large: one can easily envision thousands of possibilities for any given drug with at least two synthons present in the molecule. Such diversity will probably be best addressed with combinatorial methodologies, such as high-throughput crystallization.

### 4.2 Can there be rational, directed design of pharmaceutical co-crystal phases?

This is another question which relates to the prospect for design. Crystal structures are inherently unpredictable, but the interactions that occur prior to a crystal forming or growing are predictable. An analogy can be drawn to salt selection,[47,50] in which p$K_a$ arguments are used to select acid–base pairs that can be converted to salt compounds. The prediction of the proton transfer event is based on solution data, but the occurrence of a crystalline salt form cannot be predicted *a priori*. Based on the examples of rational synthon selection presented here, it follows that strategies of rational design of co-crystal experimentation are viable.

### 4.3 Are pharmaceutical co-crystals more or less prone to polymorphism than other pharmaceutical phases?

This question will not have a direct answer, because to prove the absence of polymorphism is tantamount to "proving the negative". But if one considers the argument that compounds have a lower degree of self-complementarity than complementarity to a rationally selected co-crystal former, one might suspect that a compound polymorphic in the pure state could display a decreased tendency to polymorphism as a co-crystal relative to the pure phase. Support for or defeat of this argument will involve significant research. Initial indications are that polymorphic substances may provide good candidates for co-crystal formation.[39a] As an example, carbamazepine can exist as four well characterized polymorphs[51] and a dihydrate.[52] This drug was recently converted to many co-crystals.[5] In terms of assessing polymorphism, one co-crystal of carbamazepine and saccharin has only displayed one packing arrangement, despite testing *via* HT crystallization in over 2000 experiments.[53] In contrast, two co-crystal structures of a *N*,*N*′-bis(para-bromophenyl)melamine-diethylbarbital demonstrate how a specific heterosynthon between the two molecules is robust, but packing of the tapes into a crystalline arrangement can lead to two discrete polymorphs.[54] Hence, there may be opportunity to reduce the practical extent of polymorphism of drug compounds specifically by co-crystal formation although there may be exceptions.

### 4.4 What opportunities exist for tuning physico-chemical properties by pharmaceutical co-crystal formation?

This is perhaps the most important question, because it is after all the complex interplay of form, function and performance attributes that determine success (or failure) of a particular pharmaceutical formulation.[55] Issues ranging from *poor solubility* and inadequate dissolution properties to lack of crystallinity and attendant instability plague the industry.[56] Poor aqueous solubility is a growing problem in the industry and it is having an impact on the productivity of drug research. The solubility issue hampers preclinical study of a new drug candidate, and can limit dosing and bioavailability. New strategies to deal with these problems are badly needed. Why are API's increasingly found to be of low solublility? There are varying ways to speculate around this question, but some would point to the methodologies that are now employed to discover pharmacologically active compounds. *In*

*vitro* assays have largely replaced *in vivo* animal screens as means to discover active compounds. The challenge of drug delivery is not addressed until there is a real desire to advance a compound into the development process. In addition, combinatorial chemistry and application of genomics have generated molecular targets for which the most potent lead compounds are inherently poorly soluble. Attempts to engineer compounds *via* medicinal chemistry avenues often lead to frustration, as the *in vitro* activity is frequently lost with increased water solubility. In the end, a discovery project may end up advancing a compound for which the stable crystal form exhibits inadequate aqueous solubility or dissolution rate that leads to poor oral absorption or inability to deliver by other routes (*e.g.* injection or inhalation). The most common strategy currently employed for improving bioavailability and optimizing drug delivery is to prepare salt forms of ionizable compounds, using pharmaceutically acceptable acids and bases. However, in the case of compounds that cannot form stable salts in aqueous medium, pharmaceutical scientists are left with few good options for material design, and must resort to particle size reduction to the nanometer range, deliberate amorphization, or solubilization in non-aqueous vehicles. These processes lead to formulations that have more physico-chemical problems than crystalline preparation.[55]

In terms of addressing the *stability issue*, one of the most important challenges presently is crystallization of compounds that are amorphous. In general, amorphs are undesirable forms due to physical instability (at the very least, there is the theoretical possibility of the material crystallizing at some point). Chemical reactivity can be significantly increased in amorphous states relative to crystalline forms.[57] In addition, amorphous forms tend to be hygroscopic and have low powder densities, giving rise to significant processing challenges. One reason for the resistance to crystallization is undoubtedly the mismatch that can occur in the number of hydrogen bond donors and acceptors in a molecule. In such cases, a solvate (or series of solvates) that leads to more satisfied hydrogen bond arrangements may be produced, while the pure, desolvated substance remains amorphous. An example of this situation is the HCl salt of the ACE inhibitor quinapril HCl.[57b] The opportunity exists to use co-crystallization to replace the solvate, while taking advantage of the supramolecular synthons that are suggested by the solvate structures. Given a co-crystal form thus obtained, one can expect the crystallinity of the material to result in greater stability and other desirable properties as compared with the amorphous form. In terms of solubility, amorphous compounds can have significant advantage over crystalline forms.[58,59] Though this advantage could find use in isolated cases, the lack of a crystalline form and concern over phase changes make the use of amorphous drugs in market formulations undesirable. When a crystalline form of a pure phase exhibits poor solubility or slow dissolution rate in aqueous media that translates to inadequate bioperformance, the strategies of salt selection and co-crystal formation should be considered. While salts can be made of acidic and basic drugs, the large space of non-ionizable compounds are generally candidates for co-crystal exploration. The *in vitro* dissolution profile of carbamazepine-saccharin co-crystal[60] illustrates the superior dissolution of the drug molecule in that context as compared with one of the pure anhydrous polymorphs. While the polymorph transiently supersaturates in the aqueous medium and subsequently precipitates to eventually form the known dihydrate, the co-crystal supersaturates to a sustained two-fold equilibrium solubility of the dihydrate. Such supersaturation behavior has been found to influence the bioavailability of carbamazepine.[61] While equilibrium solubility of drug compounds in co-crystals may be less affected than in the context of salt forms, the kinetic aspects of solubilization provide the key to many successful formulation strategies, such as oral immediate or controlled release. Co-crystals clearly open up a vast space of possibilities for exploring the range of dissolution characteristics, and facilitate co-optimization with other parameters, such as stability and processability.

To summarize, despite the need for a greater understanding and control of the crystalline phases for pharmaceutical development, the concepts of supramolecular synthesis and crystal engineering have remained underexploited in the world of drugs. As presented herein, applying the concept of supramolecular synthesis to the development of pharmaceutical co-crystals would seem to represent a new paradigm that would address both intellectual and property issues related to drug development and delivery, especially when supramolecular synthesis is coupled with HT screening technologies.

## Notes and references

† There has been steady growth in occurrence of the term "crystal engineering" in titles, keywords or abstracts as follows for 1989–2003, respectively: 4, 6, 6, 14, 26, 22, 19, 45, 59, 67, 96, 93, 129, 129, 133. The actual number of papers addressing the general subject of crystal engineering is much larger.

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# Crystal engineering of pharmaceutical co-crystals from polymorphic active pharmaceutical ingredients†

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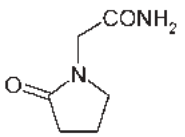
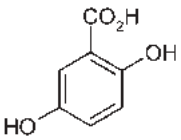
**The carboxylic acid–primary amide supramolecular heterosynthon is exploited for the generation of pharmaceutical co-crystals that contain two active pharmaceutical ingredients that are polymorphic in their pure forms.**

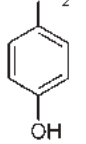
That crystal engineering has matured into a form of supramolecular synthesis is the consequence of several decades of research focused upon gaining a better understanding of the forces that sustain and direct crystal structures.[1] The fundamental precept of crystal engineering is that crystals are in effect "supermolecules",[2] the result of a series of directional, and therefore predictable, molecular recognition events or supramolecular synthons.[3] A salient feature of crystal engineered structures is that they are designed from first principles and can therefore consist of a diverse range of chemical components as exemplified by coordination polymers (*i.e.*, metals and organic ligands),[4] polymers sustained by organometallic linkages[5] and hydrogen bonded organic networks.[1d,e,6] Active pharmaceutical ingredients, APIs, are extremely valuable materials so it is perhaps surprising that crystal engineering has only recently addressed APIs *via* development of a fourth class of API, pharmaceutical co-crystals.[7] Whereas co-crystals have long been known as *addition compounds*[8] or *organic molecular compounds*[9] the Cambridge Structural Database, CSD,[10] indicates that they remain relatively unexplored with very few entries prior to 1960 and even now there are only *ca.* 1450 hydrogen bonded co-crystals *vs.* almost 35,000 hydrates. The potential benefits of co-crystals include the generation of novel NLO materials,[11] solvent-free organic synthesis,[12] modification of photographic films[13] and formulation of APIs,[7,14] which is the focus of this contribution.



Piracetam, **1**



Gentisic acid, **2**



*p*-Hydroxybenzoic acid, **3**

Pharmaceutical co-crystals, *i.e.*, co-crystals that are formed between an API and a co-crystal former that is a solid under ambient conditions, represent a new paradigm in API formulation that might address important intellectual and physical property issues in the context of drug development and delivery. In this contribution we demonstrate how the carboxylic acid–primary amide supramolecular heterosynthon[7c] can be exploited to generate pharmaceutical co-crystals of a polymorphic API, piracetam, **1**,[15] in which the co-crystal formers are also polymorphic and APIs in their own right: gentisic acid, **2**,[16] and *p*-hydroxybenzoic acid, **3**.[17]

Piracetam, (2-oxo-1-pyrrolidinyl)acetamide, **1**, is a nootropic drug that works to boost intelligence by stimulating the central nervous system.[18] Four polymorphic forms of **1** have been reported[15] although only three, refcode BISMEV, have been deposited in the CSD. No co-crystals, solvates or hydrates have been reported although one study suggests that **1** may exhibit as many as 6 polymorphs.[19] Gentisic acid, 2,5-dihydroxybenzoic acid, **2**, is an aspirin metabolite that exhibits NSAID activity.[20] Gentisic acid exhibits two polymorphic forms[21] and forms co-crystals with piperazine-2,5-dione and L-proline.[22] Single crystals of the 1:1 co-crystal of piracetam and gentisic acid, **4**, were obtained *via* slow evaporation from acetonitrile. Co-crystal **4** can also be prepared *via* grinding or slurrying in water. Co-crystal **4** was characterized by IR, melting point, DSC, PXRD and single crystal X-ray diffraction.[23] The carboxylic acid–amide supramolecular heterosynthon has been long documented[24] and 71 of the 153 structures in the CSD that contain both a carboxylic acid and a primary amide are sustained by this interaction. It would therefore be unsurprising if the acid–amide supramolecular heterosynthon were to occur in **4** and, as revealed by Fig. 1a, this is indeed the case. The remaining H-bond donors are satisfied as follows: the 2-hydroxy group of gentisic acid forms an intramolecular hydrogen bond and acts an acceptor to the *anti*-oriented NH of the primary amide; the 5-hydroxy group of gentisic acid serves as a hydrogen bond donor to the ring carbonyl of piracetam (Fig. 1b). The resulting network exhibits 4,4-topology and it is 2-fold interpenetrated (Fig. 1c).

Piracetam also forms a 1:1 co-crystal with *p*-hydroxybenzoic acid, **5**. Co-crystal **5** can be crystallized from acetonitrile *via* slow evaporation. Co-crystal **5** can also be prepared *via* grinding or slurrying in water. The crystal structure of **5**[25] reveals the presence of the acid–amide supramolecular heterosynthon which in turn dimerizes to form a tetrameric motif sustained by N–H···O hydrogen bonding (Fig. 2). This tetrameric motif is found in 10 (14%) of the 71 structures in the CSD that contain acid–amide supramolecular heterosynthons.[26] The ring carbonyl of piracetam molecules and the hydroxy group of **3** H-bond each tetramer to four others, thereby affording a 3-fold interpenetrated network.

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† Electronic supplementary information (ESI) available: Experimental details of solvent drop grinding, PXRD spectra and interpenetration in **5**. See http://dx.doi.org/10.1039/b501304f

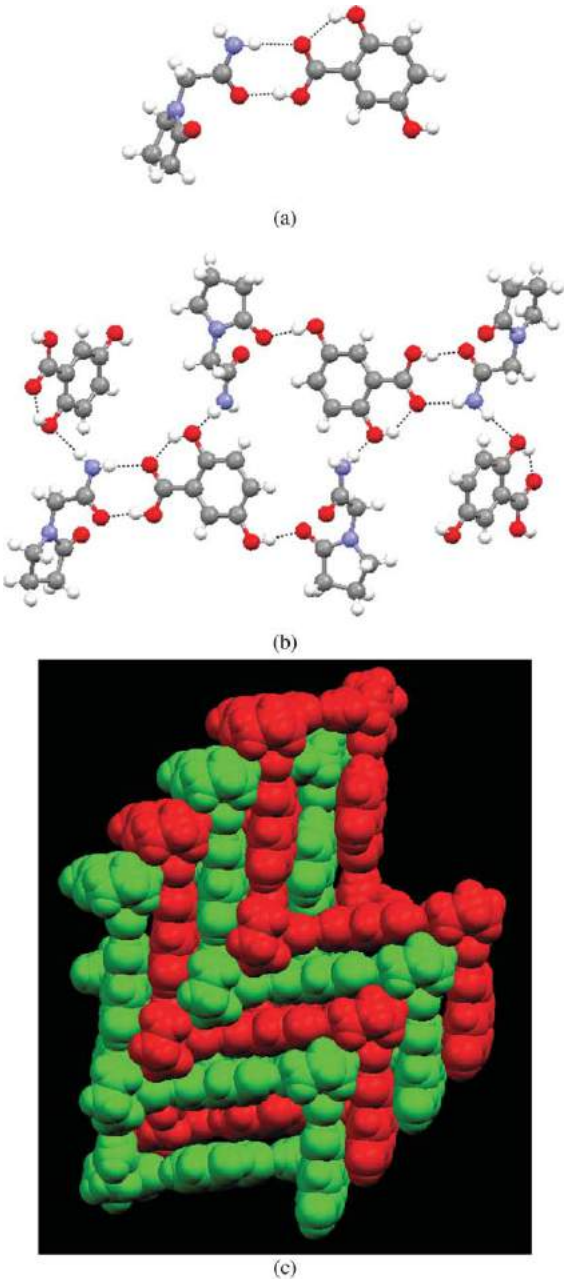


**Fig. 1** (a) The carboxylic acid–amide supramolecular heterosynthon in the 1:1 co-crystal of piracetam and gentisic acid, **4**. Structural parameters: O–$H_{acid}$···O = 2.590(15) Å, N–$H_{syn}$···O = 2.907(18) Å, N–$H_{anti}$···O = 2.944(19) Å. (b) A portion of the hydrogen bond network in **4**. (c) A space-filling model of the 2-fold interpenetration that occurs in **4**.

That APIs are promiscuous in the context of polymorphism is a critical issue for the pharmaceutical industry:[27] from a regulatory perspective it has been established that bioactivity can change between forms; from an intellectual property perspective, polymorphic forms are established in law as discrete materials and new forms can be patented. We have therefore investigated the general occurrence of polymorphism in existing co-crystals. A CSD search revealed only eleven examples of polymorphism in hydrogen bonded co-crystals for which coordinates are available for two or more forms.[28] Interestingly, the polymorphism in these eleven co-crystals can be attributed to conformational flexibility or different

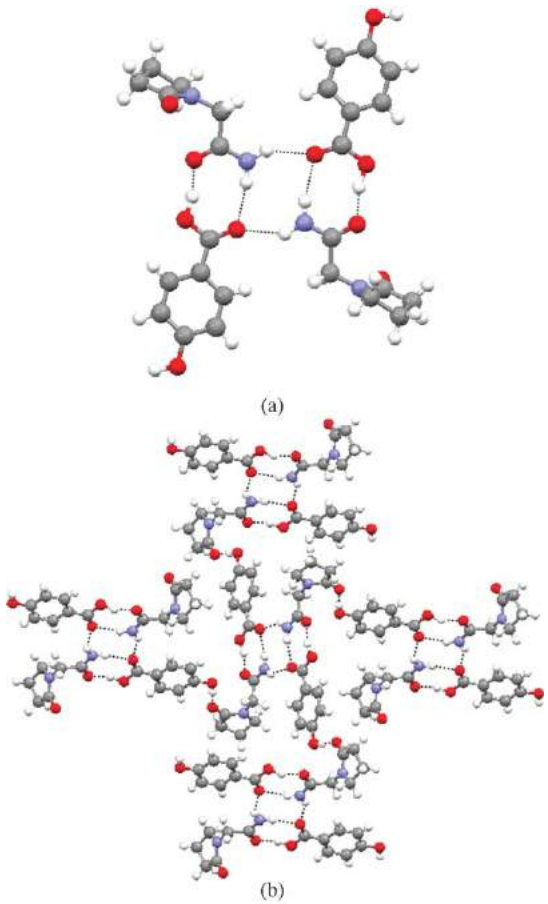


**Fig. 2** (a) The carboxylic acid–amide supramolecular heterosynthon in the tetrameric motif that sustains **5**. Structural parameters: O–$H_{acid}$···O = 2.598(3)Å, N–$H_{syn}$···O = 2.955(3) Å, N–$H_{anti}$···O = 2.908(3) Å. (b) A portion of the hydrogen bond network in **5**.

packing between layers, *i.e.*, the hydrogen bonded supramolecular synthons are consistent. Co-crystals **4** and **5** were therefore screened for the existence of polymorphs using solvent-drop grinding, a technique that has been shown to be able to generate and control polymorphism.[29] Mechanical grinding experiments were conducted in reaction vessels by adding gentisic acid or *p*-hydroxybenzoic acid to solid piracetam form A. A group of 23 solvents (water, acetone, methanol, ethanol, ethyl acetate, *n*-hexane, toluene, acetonitrile, tetrahydrofuran, isopropyl acetate, benzyl alcohol, nitromethane, dimethyl amine, 2-butanol, ethyl formate, acetic acid, methyl ethyl ketone, methyl *tert*-butyl ether, chlorobenzene, *N*-methyl pyrrolidone, 1,2-dichloroethane, dimethyl sulfoxide and dimethoxyethane) was evaluated by adding a different solvent to each well. The samples were ground for 20 minutes and characterized using powder X-ray diffraction. Co-crystals **4** and **5** were also obtained by slurrying 0.62 mmol of piracetam and 0.62 mmol of gentisic acid or *p*-hydroxybenzoic acid in water (100μL) for 60 or 16 hours, respectively. Co-crystals **4** or **5** were obtained from all grinding and slurrying reactions as a mixture with one or both of the starting materials, *i.e.*, co-crystals **4** and **5** do not exhibit polymorphism based on a series of solvent-mediated grinding experiments (See ESI for experimental details and PXRD spectra†).

In summary, we address herein the use of supramolecular heterosynthons, in particular the carboxylic acid–primary amide

 

dimer, to crystal engineer pharmaceutical co-crystals from pairs of APIs that are polymorphic in their pure forms. An analysis of the CSD and evaluation of new pharmaceutical co-crystals suggests that these co-crystals are robust enough to be prepared *via* solution, slurry or solid-state methods and that they appear to be less prone to polymorphism than the corresponding single component APIs. However, it should be stressed that the amount of data available concerning the extent of polymorphism in co-crystals remains minimal and that one will not be able to make definitive conclusions even if exhaustive high throughput screenings are conducted.

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‡ See http://dx.doi.org/10.1039/b501304f for crystallographic data in CIF or other electronic format.


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23 Crystal data for **4**: $C_{13}H_{16}N_2O_6$, $M$ = 296.28, monoclinic, $a$ = 27.896(3), $b$ = 5.1762(5), $c$ = 19.7879(18) Å, $\beta$ = 101.090(2)°, $V$ = 2803.9(4) Å$^3$, $T$ = 100 K, space group $C2/c$, $Z$ = 8, $\rho_{calc}$ = 1.404 g cm$^{-3}$, $\mu$(Mo-K$_\alpha$) = 0.112 mm$^{-1}$, 7622 reflections measured, 2873 unique reflections, 2417 observed reflections [$I > 2\sigma(I)$], $R_1$ obs = 0.043, w$R_2$ obs = 0.108. CCDC 262605.‡ Microcrystals of **4** can also be obtained by grinding **1** and **2** in a 1:1 ratio for four minutes in a ball mill or by melting the two components.

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25 Crystal data for **5**: $C_{13}H_{16}N_2O_5$, $M$ = 280.28, monoclinic, $a$ = 14.780(3), $b$ = 5.5029(12), $c$ = 17.068(4) Å, $\beta$ = 109.557(4)°, $V$ = 1308.0(5) Å$^3$, $T$ = 100 K, space group $P2_1/n$, $Z$ = 4, $\rho_{calc}$ = 1.423 g cm$^{-3}$, $\mu$(Mo-K$_\alpha$) = 0.11 mm$^{-1}$, 4995 reflections measured, 2220 unique reflections, 1753 observed reflections [$I > 2\sigma(I)$], $R_1$ obs = 0.048, w$R_2$ obs = 0.109. CCDC 262606.‡ Microcrystals of **5** can also be obtained by grinding **1** and **3** in a 1:1 ratio for four minutes in a ball mill or by melting the two components.

26 CSD refcodes: AJALAY, FURAOX, NEMTEE, RAHGEM, UNEZIW, UNEZOC, UNEZUI, UROXAM, XEWTIC and ZODWIY.

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