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(57) Abstract

The present invention relates to methods of increasing the bioavailability of the most stable crystalline form of a compound. The invention also relates to particles of the most stable crystalline form of a compound having an average particle size of less than 400 nm. The invention further relates to pharmaceutical compositions comprising these particles and the use of such pharmaceutical compositions for treating diseases, such as HIV.

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METHODS OF INCREASING THE BIOAVAILABILITY OF STABLE CRYSTAL POLYMORPHS OF A COMPOUND

TECHNICAL FIELD OF THE INVENTION

The present invention relates to methods of increasing the bioavailability of the most stable crystalline form of a compound. The invention also relates to particles of the most stable crystalline form of a compound having an average particle size of less than 400 nm. The invention further relates to pharmaceutical compositions comprising these particles and the use of such pharmaceutical compositions for treating diseases, such as HIV.

BACKGROUND OF THE INVENTION

The morphology of a solid form of a drug is extremely important in the determination of its bioavailability and stability when that drug is formulated into a dosage form. A solid form of an organic drug may exist in either a crystalline or amorphous form. In a crystalline form, a drug may exist one of seven different crystal states. The particular crystal state that a solid drug assumes depends upon the choice of solvent that is used for recrystallization and/or the choice of solvent pair

used for precipitation. This phenomenon is referred to as polymorphism.

The various crystal polymorphic forms that a drug may assumes have different stabilities, different oral bioavailability and different solubilities. For example, the formulation of the antibiotic chloramphenicol marketed by Parke-Davis uses a metastable polymorph of the drug which has higher dissolution rates and higher solubility than other polymorphic forms. Generic manufacturers of chloramphenicol used a more stable, less soluble form of the drug which resulted in poorer oral bioavailability than the Parke-Davis formulation. This particular incident led the FDA to mandate dissolution testing for all generic products.

The metastable crystal form of a drug is often believed to possess the highest bioavailability based upon its faster dissolution rate. This allows one to achieve supersaturated concentrations. The ability to successfully formulate the metastable form of a drug depends upon the stability of that from e.g., the rate of transition between the metastable and a more stable, less bioavailable polymorphic form. If the transition rate is high, the drug will tend to convert to a more stable, less soluble form and crystallize out of the supersaturated system.

Various methods are known for the production of metastable polymorphs. In enantiomorphs, the metastable form can be frequently obtained by holding the crystal at an elevated temperature until the transition occurs. Seeding or the addition of a solvent often accelerates this transition. In monotropic systems, sublimation or supercooling offers

the most successful route for the preparation of the metastable form.

The best means of stabilizing the metastable form of a drug is to create small crystals at the time of production. Attempting to reduce the size of the crystals after they are formed by mechanical processes, such as milling, is likely to bring about a transformation to the more stable polymorph which will result in a loss of dissolution, thus oral bioavailability.

Although the metastable form is preferred form from a biopharmaceutical point of view, the chosen polymorph must also be stable in a kinetic sense. The latter can only be achieved in a solid dosage form. The most stable form, which is also the least soluble, is likely to produce "liquid" preparations which would be most stable over time, but less orally bioavailable. The use of the metastable forms in solution dosage forms will cause physical instability because a supersaturated solution will be obtained, which will later crystallize out over time and/or temperature range; making their use inadvisable in solution dosage forms. The use of metastable polymorph in suspension dosage forms is not recommended because crystal growth is likely, resulting in caking. For obvious reasons, the use of the wrong polymorph in other pharmaceutical dosage forms such as creams and suppositories, will cause crystal growth and/or changes in melting characteristics resulting in a physical instability. Thus, from a pharmaceutical development point of view, the most stable polymorph in a solid dosage form (yielding the lower bioavailability) is the preferred crystal form.

United States patent 5,145,684 describes solid particle forms of drugs that are smaller than 400 nm as a potential solution to the low bioavailability of insoluble drugs. Although this "nanoparticle" technology is theoretically useful in increasing the bioavailability of any insoluble or poorly soluble drug, in practice many insoluble drugs do not exhibit increased bioavailability when milled into particles smaller than 400 nm. Moreover, that document does not address the problem of differing bioavailabilities of different crystal forms of the same drug.

Thus, there is a great need for methodologies which will increase the bioavailability of the most stable crystal form of a compound to a level equivalent to that of the metastable form.

SUMMARY OF THE INVENTION

Applicant has solved the problems set forth above by discovering that reducing the particle size of the most stable crystalline form of a compound to less than about 400 nm increases its bioavailability a level similar to that of a solution of the metastable crystalline form of that compound. This is surprising and unexpected in that reducing the particle size of the metastable form of the compound causes little if any increase in its bioavailability.

In addition to providing methods for increasing the bioavailability of the most stable crystalline form of a compound, the present invention also provides solid and liquid suspension dosage forms of that crystal form which have been prepared by those methods.

Because of the higher bioavailability, an effective dose of the drugs prepared by the methods of this invention can be contained in smaller volume and/or fewer capsules or tablets. This, in turn, increases patient compliance.

In addition, suspension formulations of the drugs prepared by the methods of this invention, can be better taste-masked because the intense bitterness of a solubilized form of a drug is avoided.

DETAILED DESCRIPTION OF THE INVENTION

According to one embodiment, the invention provides a method of increasing the bioavailability of the most stable crystalline form of a compound to be administered to a patient. This method comprises the steps of: (a) dispersing said most stable crystalline form of said compound in a liquid dispersion medium; and (b) wet grinding said compound in the presence of a rigid grinding media having an average particle size of less than 3 mm; and a surface modifier, so as to reduce the particle size of said compound to an effective average particle size less than about 400 nm.

The term "patient" as used herein, refers to a mammal. Preferably a "patient" is a human being.

Preferably, the compound utilized in this method is the most stable crystalline form of an aspartyl protease inhibitor. More preferably, the compound is an inhibitor of HIV protease. Examples of HIV protease inhibitors include, but are not limited to VX-478 (Vertex, also known as 141W94 (Glaxo-Wellcome) and KVX-478 (Kissei)), saquinavir (Ro 31-8959, Roche), indinavir (L-735,524, Merck)), ritonavir (ABT 538, Abbott), nelfinavir (AG 1343, Agouron), palinavir (Bila

2011 BS), U-103017 (Upjohn), XM 412 (DuPont Merck), XM 450 (DuPont Merck), BMS 186318 (Bristol-Meyers Squibb), CPG 53,437 (Ciba Geigy), CPG 61,755 (Ciba Geigy), CPG 70,726 (Ciba Geigy), ABT 378 (Abbott), GS 3333 (Gilead Sciences), GS 3403 (Gilead Sciences), GS 4023 (Gilead Sciences), GS 4035 (Gilead Sciences), GS 4145 (Gilead Sciences), GS 4234 (Gilead Sciences), and GS 4263 (Gilead Sciences). Most preferably, the inhibitor used in this method is VX-478.

In the first step of this method, the most stable crystalline form of a compound is dispersed in a liquid media. It is preferred, but not essential, that the particle size of the compound be less than about 100 µm as determined by sieve analysis when first dispersed in the liquid media. If the particle size is greater than about 100 µm, then it is preferred that the particles of the compound be reduced in size to less than 100 µm using a conventional milling method such as airjet or fragmentation milling.

The most stable crystalline form of the compound is then be added to a liquid medium in which it is essentially insoluble to form a premix. The concentration of the compound in the liquid medium can vary from about 0.1-60%, and preferably is from 5-30% (w/w). A preferred liquid dispersion medium is water. However, the invention can be practiced with other liquid media in which the most stable crystalline form of a compound is poorly soluble and dispersible including, for example, aqueous salt solutions, safflower oil and solvents such as ethanol, t-butanol, hexane and glycol. The pH of the aqueous dispersion media can be adjusted by techniques known in the art.

A surface modifier is also utilized in this method. The surface modifier should adhere to, but not

form a chemical bond with, the compound. The surface modifier may be added prior to dispersion of the compound in liquid media; following dispersion, but prior to wet milling; or after wet milling. Thus, according to an alternate embodiment, the invention provides a method of increasing the bioavailability of the most stable crystalline form of a compound to be administered to a patient comprising the steps of: (a) dispersing said most stable crystalline form of said compound in a liquid dispersion medium; (b) wet grinding said compound in the presence of a rigid grinding media having an average particle size of less than 3 mm; and (c) mixing said wet grinded dispersion of said compound with a surface modifier, to form particles having an effective particle size of less than about 400 nm.

It is preferred, however, that the surface modifier be added to the premix prior to wet milling, either before or after dispersion of the compound in the liquid media. The concentration of the surface modifier can vary from about 0.1 to about 90%, and preferably is 1-75%, more preferably 20-60%, by weight based on the total combined weight of the compound and surface modifier. The apparent viscosity of the premix suspension is preferably less than about 1000 centipoise.

The surface modifier is adsorbed on the surface of the most stable crystalline form of a compound in an amount sufficient to maintain an effective average particle size of less than about 400 nm. As used herein, particle size refers to a number average particle size as measured by conventional particle size measuring techniques well known to those skilled in the art, such as sedimentation field flow

fractionation, photon correlation spectroscopy, or disk centrifugation. By "an effective average particle size of less than about 400 nm" it is meant that at least 90% of the particles have a weight average particle size of less than about 400 nm when measured by the above-noted techniques. In preferred embodiments of the invention, the effective average particle size is less than about 250 nm. With reference to the effective average particle size, it is preferred that at least 95% and, more preferably, at least 99% of the particles have a particle size less than the effective average, e.g., 400 nm. In particularly preferred embodiments, essentially all of the particles have a size less than 400 nm.

Suitable surface modifiers are set forth in United States patent 5,145,684, the disclosure of which is herein incorporated by reference. Such surface modifiers are preferably be selected from known organic and inorganic pharmaceutical excipients. Such excipients include various polymers, low molecular weight oligomers, natural products and surfactants. Preferred surface modifiers include nonionic and anionic surfactants. Representative examples of excipients include gelatin, casein, lecithin (phosphatides), gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glyceryl monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, e.g., macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, e.g., the commercially available Tweens, polyethylene glycols, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium

dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethycellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, and polyvinylpyrrolidone (PVP). Most of these excipients are described in detail in the Handbook of Pharmaceutical Excipients, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain, the Pharmaceutical Press, 1986, the disclosure of which is hereby incorporated by reference in its entirety. The surface modifiers are commercially available and/or can be prepared by techniques known in the art.

Particularly preferred surface modifiers include polyvinyl pyrrolidone, Pluronic F68 and F108, which are block copolymers of ethylene oxide and propylene oxide, Tetronic 908, which is a tetrafunctional block copolymer derived from sequential addition of ethylene oxide and propylene oxide to ethylenediamine, dextran, lecithin, Aerosol OT, which is a dioctyl ester of sodium sulfosuccinic acid, available from American Cyanamid, Duponol P, which is a sodium lauryl sulfate, available from DuPont, Triton X-200, which is an alkyl aryl polyether sulfonate, available from Rohm and Haas, Tween 80, which is a polyoxyethylene sorbitan fatty acid ester, available from ICI Specialty Chemicals, and Carbowax 3350 and 934, which are polyethylene glycols available from Union Carbide.

Surface modifiers which have found to be particularly useful include polyvinylpyrrolidone, Pluronic F-68, and lecithin.

The premix is then subjected to wet grinding in the presence of a rigid grinding media. The rigid grinding media is preferably spherical or particulate in form preferably having an average size less than about 3 mm and, more preferably, less than about 1 mm. Such media desirably can provide the particles of the invention with shorter processing times and impart less wear to the milling equipment. The selection of material for the grinding media is not believed to be critical.

Suitable grinding media includes, but is not limited to, zirconium oxide, such as 95% ZrO stabilized with magnesia, zirconium silicate, glass grinding media, stainless steel, titania, alumina, and 95% ZrO stabilized with yttrium. Preferred media have a density greater than about 3 g/cm^3.

It is preferred that the premix be used directly when a ball mill is used for attrition. Alternatively, the compound and the surface modifier may be dispersed in the liquid medium using suitable agitation, e.g., a roller mill or a Cowles type mixer, until a homogeneous dispersion is observed in which there are no large agglomerates visible to the naked eye. It is preferred that the premix be subjected to such a premilling dispersion step when a recirculating media mill is used for attrition.

The mechanical means applied to reduce the particle size of the drug substance conveniently can take the form of a dispersion mill. Suitable dispersion mills include a ball mill, an attritor mill, a vibratory mill, and media mills such as a sand mill and a bead mill. A media mill is preferred due to the relatively shorter milling time required to provide the intended result, i.e., the desired reduction in

particle size. For media milling, the apparent viscosity of the premix preferably is from about 100 to about 1000 centipoise. For ball milling, the apparent viscosity of the premix preferably is from about 1 up to about 100 centipoise. Such ranges tend to afford an optimal balance between efficient particle fragmentation and media erosion.

The attrition time can vary widely and depends primarily upon the particular mechanical means and processing conditions selected. For ball mills, processing times of up to five days or longer may be required. On the other hand, processing times of less than 1 day (residence times of one minute up to several hours) have provided the desired results using a high shear media mill.

The particles must be reduced in size at a temperature which does not significantly degrade the protease inhibitor. Processing temperatures of less than about 30-40° C are ordinarily preferred. If desired, the processing equipment can be cooled with conventional cooling equipment. The method is conveniently carried out under conditions of ambient temperature and at processing pressures which are safe and effective for the milling process. For example, ambient processing pressures are typical of ball mills, attritor mills and vibratory mills. Processing pressures up to about 20 psi (1.4 kg/cm^2) are typical of media milling.

According to another embodiment, the invention provides a pharmaceutical composition in a liquid suspension dosage form comprising: particles consisting essentially of 99.9-10% by weight of the most stable crystalline form of a compound, having a solubility in water of less than 10 mg/ml, said

compound having a non-crosslinked surface modifier adsorbed on the surface thereof in an amount of 0.1-90% by weight and sufficient to maintain an effective average particle size of less than about 400 nm; and a pharmaceutically acceptable adjuvant useful for preparing a liquid suspension dosage form.

Preferably, the compound utilized in this composition is an aspartyl protease inhibitor. Even more preferably, the compound is an HIV protease inhibitor, particularly one of those set forth above. The most preferred compound in these compositions is VX-478

The preparation of the particles utilized in this composition is carried out by the method described above. The amount of the most stable crystalline form of a compound in these compositions is that which will produce a desired effect when the composition is administered to a patient. For example, an amount effective to inhibit an aspartyl protease in a patient is between about 5 - 500 mg/kg/day. Preferably, an effective amount is between about 10 - 100 mg/kg/day. Most preferably, an effective amount is between about 20 - 60 mg/kg/day.

Preferably, the particles in a unit dosage form of the composition will contain a sufficient amount of the compound so that less than 20 unit dosage forms need be administered in a day to achieve whatever is determined to be an effective amount. The term "unit dosage form", as used herein with respect to a solid dosage form, refers to a single tablet, capsule or pill. With respect to a suspension formulation, that term refers to less than 2.5 ml of the suspension.

For example, it is preferred that greater than 150 mg of the most stable crystalline form of an aspartyl protease inhibitor be present in a unit dosage form. More preferably, a single dosage form will contain greater than 400 mg of an aspartyl protease inhibitor.

Suitable pharmaceutically acceptable carriers for use in the pharmaceutical compositions of this invention are well known to those skilled in the art. These include non-toxic physiologically acceptable carriers, adjuvants or vehicles for parenteral injection, for oral administration in solid or liquid form, for rectal administration, and the like.

In one preferred embodiment, the pharmaceutical compositions of this invention are in a tablet or capsule form. In this embodiment, the pharmaceutically acceptable carrier is a standard excipient. Standard excipients useful to manufacture particles of the most stable crystalline form of a compound into a tablet or capsule form include fillers, such as sugars (e.g., lactose or sucrose) or celluloses (e.g., microcrystalline or starch); disintegrants (e.g., Cab-O-Sil); lubricants (e.g, talc or magnesium stearate); and other necessary surfactants, such as sucrose esters, crodesta or sorbitanisters (e.g., Span/Tween).

According to another preferred embodiment, the pharmaceutical compositions of this invention are in a suspension formulation. Particles of the stable crystalline form of a compound may be more easily taste masked than solutions of the corresponding salts (or solutions of less stable crystalline forms to the extent they can be solubilized). Thus, suspension

formulations of these particles are ideally suited for pediatric formulations.

In these preferred formulations the pharmaceutically acceptable carriers include suspending agents, such as sodium methyl cellulose, methyl cellulose, gum acacia or tragacanth; sweeteners, such as sorbitol, sucrose, aspartame or saccharin; flavors, such as bitter orange, strawberry citrus, or mocha; preservatives, such as methyl and propyl parabenz, sodium benzoate, or parabenzoic acid; antioxidants, such as sodium sulfite, tocopherol, butyl hydroxy toluene, or butyl hydroxy anisole); necessary surfactants, such as Tween, Span, or crodestas; anticaking agents, such as sodium citrate or citric acid; cosolvents/vehicle systems, such as glycerin, ethanol, propylene glycol, polyethylene glycols, water, medium chain triglycerides, etc.

According to another embodiment, the invention provides particles consisting essentially of 99.9-10% by weight of the most stable crystalline form of a compound having a solubility in water of less than 10 mg/ml, said compound having a non-crosslinked surface modifier adsorbed on the surface thereof in an amount of 0.1-90% by weight and sufficient to maintain an effective average particle size of less than about 400 nm. Preferred compounds used to make these particles are set forth above.

These particles are made by the methods described above.

The particles of this invention and the compositions which comprise them may be employed in a conventional manner for the treatment or prophylaxis of a disease which is normally treated or prevented with more soluble forms of the compound.

When the compound utilized in the particles is an aspartyl protease inhibitor, the particles and the compositions which employ them may be used in the treatment or prevention of viral diseases, such as HIV and HTLV, which depend on aspartyl proteases for obligatory events in their life cycle. Such methods of treatment, their dosage levels and requirements may be selected by those of ordinary skill in the art from available methods and techniques. For example, the aspartyl protease inhibitor-containing particles of this invention may be combined with a pharmaceutically acceptable adjuvant for administration to a virally-infected patient in a pharmaceutically acceptable manner and in an amount effective to lessen the severity of the viral infection or to alleviate pathological effects associated with HIV infection.

Alternatively, the aspartyl protease inhibitor-containing particles of this invention may be used in prophylactics and methods for protecting individuals against viral infection during a specific event, such as childbirth, or over an extended period of time. The particles may be employed in such prophylactics either alone or together with other antiretroviral agents to enhance the efficacy of each agent. As such, these preferred particles of the invention can be administered as agents for treating or preventing HIV infection in a mammal.

The preferred aspartyl protease inhibitor-containing particles of this invention may be administered to a healthy or HIV-infected patient either alone or in combination with other anti-viral agents which interfere with the replication cycle of HIV. The additional agent may be part of the same composition which comprises the particles of this

invention or it may be part of a separate composition which is administered to the patient sequentially or concurrently with the particle-containing composition. Thus, the terms "in combination with" and "coadministered with", as used herein, refer to both single and multiple dosage forms.

By administering the preferred aspartyl protease inhibitor-containing particles of this invention with other anti-viral agents which target different events in the viral life cycle, the therapeutic effect of these particles is potentiated. For instance, the co-administered anti-viral agent can be one which targets early events in the life cycle of the virus, such as cell entry, reverse transcription and viral DNA integration into cellular DNA. Anti-HIV agents targeting such early life cycle events include, didanosine (ddI), dideoxycytidine (ddC), d4T, zidovudine (AZT), 3TC, 935U83, 1592U89, 524W91, polysulfated polysaccharides, sT4 (soluble CD4), ganiclovir, trisodium phosphonoformate, eflornithine, ribavirin, acyclovir, alpha interferon and trimenotrexate. Additionally, non-nucleoside inhibitors of reverse transcriptase, such as TIBO, delavirdine (U90) or nevirapine, may be used to potentiate the effect of the particles of this invention, as may viral uncoating inhibitors, inhibitors of trans-activating proteins such as tat or rev, or inhibitors of the viral integrase.

Combination therapies according to this invention exert an additive or synergistic effect in inhibiting HIV replication because each component agent of the combination acts on a different site of HIV replication. The use of such combination therapies also advantageously reduces the dosage of a given

conventional anti-retroviral agent which would be required for a desired therapeutic or prophylactic effect, as compared to when that agent is administered as a monotherapy. Such combinations may reduce or eliminate the side effects of conventional single anti-retroviral agent therapies, while not interfering with the anti-retroviral activity of those agents. These combinations reduce potential of resistance to single agent therapies, while minimizing any associated toxicity. These combinations may also increase the efficacy of the conventional agent without increasing the associated toxicity. Preferred combination therapies include the administration of the particles of this invention with AZT, ddI, ddC, d4T, 3TC, 935U83, 1592U89, 524W91 or a combination thereof.

Alternatively, the aspartyl protease inhibitor-containing particles of this invention may also be co-administered with other HIV protease inhibitors such as saquinavir (Ro 31-8959, Roche), L-735,524 (Merck), ABT 538 (A-80538, Abbott), AG 1341 (Agouron), XM 412 (DuPont Merck), XM 450 (DuPont Merck), BMS 186318 (Bristol-Meyers Squibb) and CPG 53,437 (Ciba Geigy) or prodrugs of these or related particles to increase the effect of therapy or prophylaxis against various viral mutants or members of HIV quasi species.

Preferably, the preferred aspartyl protease inhibitor-containing particles of this invention are administered as a single agent or in combination with retroviral reverse transcriptase inhibitors, such as derivatives of AZT, or other HIV aspartyl protease inhibitors, including multiple combinations comprising from 3-5 agents. We believe that the co-administration of these preferred particles of this invention with

retroviral reverse transcriptase inhibitors or HIV aspartyl protease inhibitors may exert a substantial additive or synergistic effect, thereby preventing, substantially reducing, or completely eliminating viral replication or infection or both, and symptoms associated therewith.

The aspartyl protease inhibitor-containing particles of this invention can also be administered in combination with immunomodulators and immunostimulators (e.g., bropirimine, anti-human alpha interferon antibody, IL-2, GM-CSF, interferon alpha, diethyldithiocarbamate, tumor necrosis factor, naltrexone, tuscarasol, and rEPO); and antibiotics (e.g., pentamidine isethiorate) to prevent or combat infection and disease associated with HIV infections, such as AIDS and ARC.

According to yet another embodiment, the invention provides methods for inhibiting aspartyl proteases, in particular inhibiting aspartyl proteases in a human. These include viral aspartyl proteases that essential for the life cycle of certain viruses, such as HIV and other AIDS-like diseases caused by retroviruses, such as simian immunodeficiency viruses, HTLV-I and HTLV-II; renin; and aspartyl proteases that process endothelin precursors. Preferably, the methods of this invention are used to treat or prevent HIV infections in humans.

In order that this invention be more fully understood, the following examples are set forth. These examples are for the purpose of illustration only and are not to be construed as limiting the scope of the invention in any way.

EXAMPLE 1

Synthesis of VX-478

The synthesis of the aspartyl protease inhibitor VX-478 is disclosed in United States patent 5,585,397. It is referred to as compound #168 in that document. The essentials of that synthesis are set forth below.

A solution of 102 mg of N-((2 syn, 3S)-2-hydroxy-4-phenyl-3-((S)-tetrahydrofuran 3-yl-oxycarbonylaminobutyl-amine in 4:1 CH_2Cl_2/saturated aqueous $NaHCO_3$ was treated sequentially, at ambient temperature under an atmosphere of nitrogen, with 65 mg of p-nitrobenzenesulfonyl chloride and 51 mg of sodium bicarbonate. The mixture was stirred for 14 h, diluted with CH_2Cl_2, washed with saturated NaCl, then dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by low pressure silica gel chromatography using 20% diethyl ether/CH_2Cl_2 as eluent to provide 124 mg of product as a white solid. TLC: Rf = 0.36, 20% diethyl ether/CH_2Cl_2. HPLC: Rt = 15.15 min. (1H)-NMR (CDCl3) consistent with structure.

A solution of 124 mg of the resultant compound in ethyl acetate was treated, at ambient temperature, with 13 mg of 10% palladium on carbon. The mixture was stirred for 14 h under an atmosphere of hydrogen, filtered through a pad of Celite filter agent, and concentrated in vacuo. The residue was subjected to preparative HPLC to yield 82 mg of 4-Amino-N-((2 syn,3S)-2-Hydroxy-4-phenyl-3-((S)-tetrahydrofuran-3-yl-oxycarbonylamino)-butyl)-N-isobutyl-benzenesulfonzmide (VX-478) as a white solid.

TLC: Rf = 0.10, 20% ether/CH_2Cl_2. HPLC: Rt = 13.16 min. (1H)-NMR ($CDCl_3$) consistent with structure.

EXAMPLE 2

Pharmaceutical Formulations of VX-478

The manufacturing procedures of VX-478, yielded a metastable polymorph (Form I). Under the conditions of manufacturing VX-478 into a solution form in a soft gelatin capsule, Form I has an apparent solubility of >200 mg/ml in the vehicle used to solubilize the drug. Upon long term storage of these capsules, and further modifications to the chemical manufacturing process, a new more stable polymorph (Form V) was produced.

Under similar processing conditions, Form V has approximately one-half the solubility of Form I in the vehicle used to manufacture the soft gelatin capsules. The use of Form V led to a reduction in the drug load per capsule due to reduced solubility, as well as some changes in the matrix composition used to dissolve VX-478. These changes reduced the excellent oral bioavailability of the drug, as well as an increased capsule burden on the patients. This, in turn, is likely to lead to poor patient compliance, thus reducing the therapeutic benefit. Furthermore, the use of supersaturated solution of the Form V polymorph, has the potential to crystallize out of solution upon storage and/or shipping, thus further reducing bioavailability.

When the metastable polymorph of VX-478 (Form I) was prepared in a traditional solid dosage forms using micronized powders of Form I, it had negligible oral bioavailability. This lack of any oral

bioavailability in a solid dosage form with the metastable polymorph suggested that a less soluble, more stable polymorph of VX-478, such as Form V, would also possess negligible oral bioavailability.

EXAMPLE 3

Nanoparticle Pharmaceutical Formulations of the Form V Polymorph of VX-478

The solid form of the most stable polymorph (Form V) of VX-478 (2.0% w/v) was subjected to wet milling in the presence of the surface modifiers hydroxypropyl cellulose (HPC-L) (1.0% w/v) and SDS (0.01% w/v). The resulting particles had a mean particle size of 157 nm.

The particles were then formulated into a suspension formulation and administered to Sprague-Dawley rats (364 mg/kg). The pharmacokinetic parameters of that suspension were then compared to a solution formulation of the metastable Form I of VX-478 administered to rats at concentrations of 100 and 500 mg/kg. The data is presented in Table 1 below.

Table 1: Statistical summary of selected pharmacokinetic parameters following oral administration of a solution of VX-478 metastable polymorph (Form I) and a novel suspension formulation of the more stable polymorph (Form V) in Sprague-Dawley rats.

Pharmacokinetic Parameter	VX-478 metastable (Form I) "Solution" Formulation Oral Bioavailability		VX-478 (Form V) Suspension Oral Bioavailability
Dose (mg/kg)	100	500*	364
AUC (μg.hr/mL)	52.6 ± 23.8	78.4 ± 18.5	92.2 ± 46.3
C_{max} (μg/mL)	8.5 ± 2.4	N/A	10.2 ± 1.9
Half-life (hrs)	3.0 ± 1.0	N/A	3.9**

* Dose in mg/kg; $AUC_{(0-12)}$ calculated from a 1 month rat toxicology study with VX-478

** Harmonic mean

As shown in Table 1, the bioavailability (AUC) following oral administration of a suspension of nanoparticles of the more stable polymorph (Form V) is surprisingly and unexpectedly similar to that obtained for a solution formulation of the metastable Form I of VX-478. This surprising result in relative oral bioavailability, means that the more stable and, therefore, pharmaceutically preferred Form V, may be formulated into a suspension and/or solid dosage form such as capsules, tablets, etc., without the expected loss in oral bioavailability from not using the metastable (biopharmaceutically preferred) Form I of VX-478.

Qualitatively similar results will be obtained for the most stable crystalline polymorph of

any compound. Thus, the methods, pharmaceutical compositions and nanosized particles disclosed herein allow the most stable crystalline form of any compound to be formulated into traditional solid or liquid dosage forms, without having the conventionally expected reduction in oral bioavailability.

While we have hereinbefore described a number of embodiments of this invention, it is apparent that our basic constructions can be altered to provide other embodiments of this invention. Therefore, it will be appreciated that the scope of this invention is to be defined by the claims appended hereto rather than by the specific embodiments which have been presented hereinbefore by way of example.

CLAIMS

I/We claim:

1. A method of increasing the bioavailability of the most stable crystalline form of a compound to be administered to a patient, comprising the steps of:

(a) dispersing said most stable crystalline form of said compound in a liquid dispersion medium; and

(b) wet grinding said compound in the presence of:

(i) a rigid grinding media having an average particle size of less than 3 mm; and

(ii) a surface modifier,

so as to reduce the particle size of said compound to an effective average particle size less than about 400 nm.

2. A method of increasing the bioavailability of the most stable crystalline form of a compound to be administered to a patient comprising the steps of:

(a) dispersing said most stable crystalline form of said compound in a liquid dispersion medium;

(b) wet grinding said compound in the presence of a rigid grinding media having an average particle size of less than 3 mm; and

(c) mixing said wet grinded dispersion of said compound with a surface modifier,

to form particles having an effective particle size of less than about 400 nm.

3. The method according to claim 1 or 2, wherein said compound is an aspartyl protease inhibitor.

4. The method according to claim 3, wherein said aspartyl protease inhibitor is selected from VX-478, saquinavir, indinavir, ritonavir, nelfinavir, palinavir, U-103017, XM 412, XM 450, BMS 186318, CPG 53,437, CPG 61,755, CPG 70,726, ABT 378, GS 3333, GS 3403, GS 4023, GS 4035, GS 4145, GS 4234, or GS 4263.

5. The method according to claim 3, wherein said aspartyl protease inhibitor is VX-478.

6. A pharmaceutical composition in a solid dosage form comprising:

(a) particles consisting essentially of 99.9-10% by weight of the most stable crystalline form of a compound, having a solubility in water of less than 10 mg/ml, said compound having a non-crosslinked surface modifier adsorbed on the surface thereof in an amount of 0.1-90% by weight and sufficient to maintain an effective average particle size of less than about 400 nm; and

(b) a pharmaceutically acceptable adjuvant useful for preparing a solid dosage form.

7. A pharmaceutical composition in a liquid suspension dosage form comprising:

(a) particles consisting essentially of 99.9-10% by weight of the most stable crystalline form of a compound, having a solubility in water of less than 10 mg/ml, said compound having a non-crosslinked surface modifier adsorbed on the surface thereof in an

amount of 0.1-90% by weight and sufficient to maintain an effective average particle size of less than about 400 nm; and

(b) a pharmaceutically acceptable adjuvant useful for preparing a liquid suspension dosage form.

8. The pharmaceutical composition according to claim 5 or 6, wherein said compound is an aspartyl protease inhibitor.

9. The pharmaceutical composition according to claim 8, wherein said aspartyl protease inhibitor is selected from VX-478, saquinavir, indinavir, ritonavir, nelfinavir, palinavir, U-103017, XM 412, XM 450, BMS 186318, CPG 53,437, CPG 61,755, CPG 70,726, ABT 378, GS 3333, GS 3403, GS 4023, GS 4035, GS 4145, GS 4234, or GS 4263.

10. The pharmaceutical composition according to claim 8, wherein said aspartyl protease inhibitor is VX-478.

11. A method of treating a human suffering from an HIV infection comprising the step of administering to said human a pharmaceutical composition according to claim 8.

12. A method of treating a human suffering from an HIV infection comprising the step of administering to said human a pharmaceutical composition according to claim 9 or 10.

13. Particles consisting essentially of 99.9-10% by weight of the most stable crystalline form of a compound having a solubility in water of less than 10 mg/ml, said compound having a non-crosslinked surface modifier adsorbed on the surface thereof in an amount of 0.1-90% by weight and sufficient to maintain an effective average particle size of less than about 400 nm.

14. The particles according to claim 13, wherein said compound is an aspartyl protease inhibitor.

15. The particles according to claim 14, wherein said aspartyl protease inhibitor is selected from VX-478, saquinavir, indinavir, ritonavir, nelfinavir, palinavir, U-103017, XM 412, XM 450, BMS 186318, CPG 53,437, CPG 61,755, CPG 70,726, ABT 378, GS 3333, GS 3403, GS 4023, GS 4035, GS 4145, GS 4234, or GS 4263.

16. The particles according to claim 14, wherein said aspartyl protease inhibitor is VX-478.

INTERNATIONAL SEARCH REPORT

Interna ıl Application No
PCT/US 98/12474

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/635 A61K9/14 A61K9/51

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 6 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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 145 684 A (LIVERSIDGE GARY G ET AL) 8 September 1992 cited in the application see column 2, line 32-37 see column 2, line 54-56 see column 3, line 38-45 see column 3, line 53 - column 4, line 7 see column 6, line 18-36 see column 7, line 1-3 see column 8, line 3-9 see column 13, line 54 - column 14, line 4 see claims --- -/--	1,2,6,7, 13

[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
29 September 1998	12/10/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	La Gaetana, R

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

INTERNATIONAL SEARCH REPORT

Interna' I Application No
PCT/US 98/12474

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 534 270 A (DE CASTRO LAN) 9 July 1996 see column 3, line 4-20 see column 3, line 34-55 see column 4, line 13-16 see column 4, line 29-44 see column 6, line 16-19 see claims	1,2,6,7, 13
A	US 5 610 193 A (AL-RAZZAK LAMAN A ET AL) 11 March 1997 see column 1, line 13-23 see column 2, line 15-30 see column 3, line 60 - column 4, line 16 see column 9, line 41 - column 10, line 2 see claim 1	3-5, 8-12, 14-16
A	WO 97 20554 A (VERTEX PHARMA) 12 June 1997 see page 4, line 11-16 see page 13, line 15 - page 14, line 4 see page 19, line 4-33 see claims	3-5, 8-12, 14-16
P,A	WO 97 35587 A (GLAXO GROUP LTD ;ROY ARUP K (US); TILLMAN LLOYD GARY (US)) 2 October 1997 see page 1, line 4-9 see page 3, line 19 - page 4, line 6 see example 1 see claims	3-5, 8-12, 14-16
T	MUELLER BU ET AL: "A phase I/II study of the protease inhibitor indinavir in children with HIV infection" PEDIATRICS, vol. 102, no. 1pt1, 1 July 1998, pages 101-109, XP002078932 see abstract see page 103, column 1, paragraph "Pharmacokinetic studies" see page 104, column 2, paragraph "pharmacokinetics" see page 8, column 1, lines 11-14	3,4, 8-11,14, 15

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INTERNATIONAL SEARCH REPORT

Inte. ional application No.
PCT/US 98/ 12474

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:

Remark: Although claim(s) 11 &12
is(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. [ ] 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:

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

Internat' l Application No
PCT/US 98/12474

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