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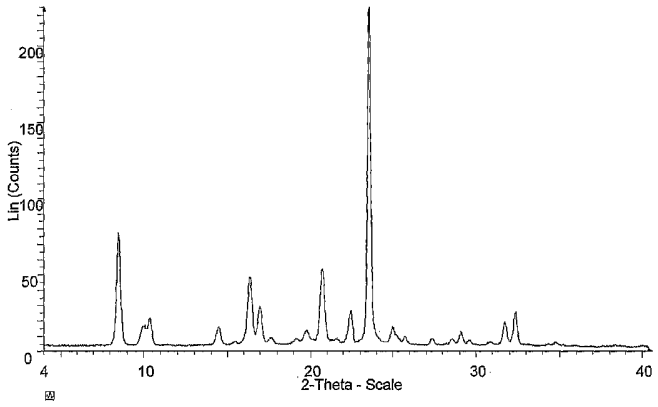

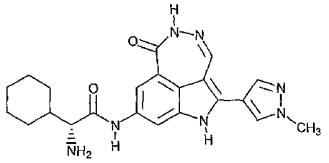

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(57) Abstract: The present invention relates to novel polymorphic forms and amorphous form of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide, and to processes for their preparation. Such polymorphic forms and amorphous form may be a component of a pharmaceutical composition and may be used to treat a cancer or a mammalian disease condition mediated by protein kinase activity.Formula (I).

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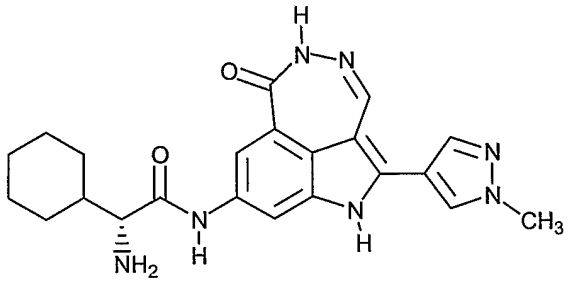
POLYMORPHIC FORMS OF (2R,Z)-2-AMINO-2-CYCLOHEXYL-N-(5-(1-METHYL-1H-PYRAZOL-4-YL)-1-OXO-2,6-DIHYDRO-1H-[1,2]DIAZEPINO[4,5,6-CD]INDOL-8-YL)ACETAMIDE

Field of the Invention

The present invention relates to novel polymorphic forms of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl) acetamide and to methods for their preparation. The invention is also directed to pharmaceutical compositions containing at least one polymorphic form and to the therapeutic use of such polymorphic forms and compositions.

Background of the Invention

The compound (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide (also referred to as "Compound 1"),



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as well as pharmaceutically acceptable salts thereof, is described in U.S. Patent No. 6,967,198, issued November 22, 2005, the disclosure of which is incorporated herein by reference.

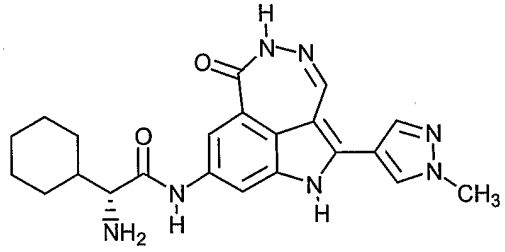
Many anticancer agents, as well as radiation therapy, cause DNA damage to cells, especially cancer cells. CHK1 inhibition enhances the anti-cancer effect of these anti-cancer agents or radiation therapy by abrogating the S and G_2 arrest of those DNA damaged cells and thus leading to mitotic catastrophe and cell death of these cells. (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide is a potent CHK1 protein kinase inhibitor. Use of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H[1,2]diazepino [4,5,6-cd]indol-8-yl)acetamide, a pharmaceutically acceptable salt or solvates thereof, or a mixture thereof, in combination with an anti-cancer agent or radiation therapy will greatly enhance the anti-cancer effect of the anti-cancer agent or radiation therapy.

A solid compound may exist in amorphous or crystalline forms. Each of the different crystalline forms of the same compound is considered a polymorphic form of the compound. Crystalline polymorphs are different crystalline forms of the same compound. Different polymorphic forms of the same active pharmaceutical ingredient (API) may have very different physical properties, such as thermodynamic stability, solubility, hygroscopicity as well as pharmacological properties such as oral bioavailability. These properties greatly influence the properties of a drug, in such areas as shelf life, cost of production, consistent dosage and even

effectiveness of the drug. Thus it is desirable to have polymorphic forms of a compound having good physical and pharmacological properties.

Summary of the Invention

In one embodiment, the present invention provides a crystalline form of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide, represented by Formula 1



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In another embodiment, the present invention provides a crystalline form of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide. Preferably, the crystalline form is a substantially pure polymorph of Form I. In one aspect of the embodiment, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 23.6 ± 0.1 and 8.5 ± 0.1. In another aspect of the embodiment, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 23.6 ± 0.1 , 8.5± 0.1 and 20.7 ± 0.1. In another aspect of the embodiment, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 23.6 ± 0.1, 8.5± 0.1, 20.7 ± 0.1 and 16.4± 0.1. In another aspect of the embodiment, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 23.6 ± 0.1, 8.5± 0.1, 20.7 ± 0.1, 16.4± 0.1 and 17.0± 0.1. In another aspect of the embodiment, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) essentially the same as shown in Figure 1. In another aspect of this embodiment, the crystalline form has a ^{13}C solid state NMR peak pattern comprising peaks at chemical shifts 175.0 ± 0.1, 137.6 ± 0.1, 134.9 ± 0.1, 110.3 ± 0.1, 106.3 ± 0.1, 41.1± 0.1 and 32.6 ± 0.1 ppm. In another aspect of this embodiment, the crystalline form has a ^{13}C solid state NMR peak pattern comprising at least three of the following seven peaks at chemical shifts 175.0 ± 0.1, 137.6 ± 0.1, 134.9 ± 0.1, 110.3 ± 0.1, 106.3 ± 0.1, 41.1± 0.1 and 32.6 ± 0.1 ppm. In another aspect of this embodiment, the crystalline form has a ^{13}C solid state NMR peak pattern comprising at least four of the following seven peaks at chemical shifts 175.0 ± 0.1, 137.6 ± 0.1, 134.9 ± 0.1, 110.3 ± 0.1, 106.3 ± 0.1, 41.1± 0.1 and 32.6 ± 0.1 ppm. In another aspect of this embodiment, the crystalline form has a ^{13}C solid state NMR peak pattern comprising at least five of the following seven peaks at chemical shifts 175.0 ± 0.1, 137.6 ± 0.1, 134.9 ± 0.1, 110.3 ± 0.1, 106.3 ± 0.1, 41.1± 0.1 and 32.6 ± 0.1 ppm. In another aspect of this embodiment, the crystalline form has a ^{13}C solid state NMR peak pattern comprising at least six of the following seven peaks at chemical shifts 175.0 ± 0.1, 137.6 ± 0.1, 134.9 ± 0.1, 110.3 ± 0.1, 106.3 ± 0.1, 41.1± 0.1 and 32.6 ± 0.1 ppm. In another aspect of this embodiment, the crystalline form has a ^{13}C solid state

NMR peak pattern comprising peaks at chemical shifts 175.0 ± 0.2, 137.6 ± 0.2, 134.9 ± 0.2, 110.3 ± 0.2, 106.3 ± 0.2, 41.1± 0.2 and 32.6 ± 0.2 ppm. In another aspect of this embodiment, the crystalline form has a ^{13}C solid state NMR peak pattern comprising at least three of the seven peaks at chemical shifts 175.0 ± 0.2, 137.6 ± 0.2, 134.9 ± 0.2, 110.3 ± 0.2, 106.3 ± 0.2, 41.1± 0.2 and 32.6 ± 0.2 ppm. In another aspect of this embodiment, the crystalline form has a ^{13}C solid state NMR peak pattern comprising at least four of the seven peaks at chemical shifts 175.0 ± 0.2, 137.6 ± 0.2, 134.9 ± 0.2, 110.3 ± 0.2, 106.3 ± 0.2, 41.1± 0.2 and 32.6 ± 0.2 ppm. In another aspect of this embodiment, the crystalline form has a ^{13}C solid state NMR peak pattern comprising at least five of the seven peaks at chemical shifts 175.0 ± 0.2, 137.6 ± 0.2, 134.9 ± 0.2, 110.3 ± 0.2, 106.3 ± 0.2, 41.1± 0.2 and 32.6 ± 0.2 ppm. In another aspect of this embodiment, the crystalline form has a ^{13}C solid state NMR peak pattern comprising at least six of the seven peaks at chemical shifts 175.0 ± 0.2, 137.6 ± 0.2, 134.9 ± 0.2, 110.3 ± 0.2, 106.3 ± 0.2, 41.1± 0.2 and 32.6 ± 0.2 ppm. In another aspect of this embodiment, the crystalline form has a ^{13}C solid state NMR peak pattern comprising peaks at chemical shifts position essentially the same as shown in Figure 4b.

In another embodiment, the present invention provides a crystalline form of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide. Preferably, the crystalline form is a substantially pure polymorph of Form II. In one aspect of this embodiment, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 25.3 ± 0.1 and 16.0 ± 0.1. In another aspect of the embodiment, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 25.3 ± 0.1, 16.0 ± 0.1, 13.9± 0.1 and 29.2± 0.1. In another aspect of the embodiment, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 25.3 ± 0.1, 16.0 ± 0.1, 13.9± 0.1, 29.2± 0.1 and 12.2± 0.1. In another aspect of the embodiment, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 25.3 ± 0.1, 16.0 ± 0.1, 13.9± 0.1, 29.2± 0.1 12.2± 0.1 and 16.8± 0.1. In another aspect of the embodiment, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 25.3 ± 0.1, 16.0 ± 0.1, 13.9± 0.1, 29.2± 0.1 12.2± 0.1, 16.8± 0.1, 6.9± 0.1 and 13.6± 0.1. In another aspect of the embodiment, the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) essentially the same as shown in Figure 2. In another aspect of the embodiment, the crystalline form has a ^{13}C solid state NMR peak pattern comprising peaks at chemical shifts 177.7 ± 0.1, 133.2 ± 0.1, 127.8 ± 0.1, 103.8 ± 0.1, and 22.7 ± 0.1ppm. In another aspect of the embodiment, the crystalline form has a ^{13}C solid state NMR peak pattern comprising at least three of the five peaks at chemical shifts 177.7 ± 0.1, 133.2 ± 0.1, 127.8 ± 0.1, 103.8 ± 0.1, and 22.7 ± 0.1ppm. In another aspect of the embodiment, the crystalline form has a ^{13}C solid state NMR peak pattern comprising at least four of the five peaks at chemical shifts 177.7 ± 0.1, 133.2 ± 0.1, 127.8 ± 0.1, 103.8 ± 0.1, and 22.7 ± 0.1ppm. In another aspect of the embodiment, the crystalline form has a ^{13}C solid state NMR peak pattern comprising peaks at chemical shifts 177.7 ± 0.2, 133.2 ± 0.2, 127.8 ± 0.2, 103.8 ± 0.2, and 22.7 ± 0.2ppm. In another aspect of the embodiment, the

crystalline form has a ^{13}C solid state NMR peak pattern comprising at least three of the five peaks at chemical shifts 177.7 ± 0.2, 133.2 ± 0.2, 127.8 ± 0.2, 103.8 ± 0.2, and 22.7 ± 0.2ppm. In another aspect of the embodiment, the crystalline form has a ^{13}C solid state NMR peak pattern comprising at least four of the five peaks at chemical shifts 177.7 ± 0.2, 133.2 ± 0.2, 127.8 ± 0.2, 103.8 ± 0.2, and 22.7 ± 0.2ppm. In another aspect of the embodiment, the crystalline form has a ^{13}C solid state NMR peak pattern comprising peaks at chemical shifts position essentially the same as shown in Figure 5b.

In another embodiment, the present invention provides an amorphous form of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide. Preferably, the amorphous form is substantially pure. In one aspect of the embodiment, the amorphous form has a ^{13}C solid state NMR peak pattern comprising peaks at chemical shifts 163.6 ± 0.2, 138.9 ± 0.2, 131.4 ± 0.2, 129.9 ± 0.2, and 30.8 ± 0.2 ppm. In another aspect of the embodiment, the amorphous form has a ^{13}C solid state NMR peak pattern comprising at least three of the five peaks at chemical shifts 163.6 ± 0.2, 138.9 ± 0.2, 131.4 ± 0.2, 129.9 ± 0.2, and 30.8 ± 0.2 ppm. In another aspect of the embodiment, the amorphous form has a ^{13}C solid state NMR peak pattern comprising at least four of the following peaks at chemical shifts 163.6 ± 0.2, 138.9 ± 0.2, 131.4 ± 0.2, 129.9 ± 0.2, and 30.8 ± 0.2 ppm. In another aspect of the embodiment, the amorphous form has a ^{13}C solid state NMR peak pattern comprising peaks at chemical shifts position essentially the same as shown in Figure 6.

In another embodiment, the present invention provides a solid form of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide (Compound 1), wherein the solid form comprises at least two forms selected from polymorphic Form I, polymorphic Form II and an amorphous form of Compound 1. In one aspect of this embodiment, the solid form comprises at least 10% of polymorph Form I. More preferably, the solid form comprises at least 20% of polymorph Form I. More preferably, the solid form comprises at least 30% of polymorph Form I. Even more preferably, the solid form comprises at least 30%, at least 40%, or at least 50% of polymorph Form I. Even more preferably, the solid form comprises at least 60%, at least 70% or at least 80% of polymorph Form I. Even more preferably, the solid form comprises at least 90% of polymorph Form I. Even more preferably, the solid form comprises at least 95% of polymorph Form I.

In another embodiment, the present invention provides a pharmaceutical composition comprising the polymorphic Form I, the polymorphic Form II, the amorphous form or the solid form of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide of the invention.

In another embodiment, the present invention provides a pharmaceutical composition comprising the solid form of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide of the invention.

In another embodiment, the present invention provides a method of treating cancer in a mammal comprising administering to the mammal in need thereof the pharmaceutical composition of the invention.

In another embodiment, the present invention provides a method of treating cancer in a mammal comprising administering to the mammal in need thereof a therapeutically effective amount of the pharmaceutical composition of the invention, in combination with a therapeutically effective amount of an anti-cancer treatment selected from an anti-cancer agent and radiation therapy. In one aspect of this embodiment, the anti-cancer treatment is an anti-cancer agent. Preferably, the anti-cancer agent is selected from the group consisting of Ara-c, VP-16, cis-platin, adriamycin, 2-chloro-2-deoxyadenosine, 9- (3-D-arabinosyl-2-fluoroadenine, carboplatin, gemcitabine, camptothecin, paclitaxel, BCNU, 5-fluorouracil, irinotecan, and doxorubicin. In another aspect of this embodiment, the anti-cancer treatment is radiation therapy.

In another embodiment, the present invention provides a method of treating a mammalian disease condition mediated by CHK1 protein kinase activity, comprising administering to a mammal in need thereof the polymorphic Form I, the polymorphic Form II, the amorphous form or the solid form of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide of the invention, in combination with a anti-cancer treatment selected from anti-cancer agent, radiation therapy and the combination thereof. In one aspect of this embodiment, the anti-cancer treatment is an anti-cancer agent. Preferably, the anti-cancer agent is selected from the group consisting of Ara-c, VP-16, cis-platin, adriamycin, 2-chloro-2-deoxyadenosine, 9- (3-D-arabinosyl-2-fluoroadenine, carboplatin, gemcitabine, camptothecin, paclitaxel, BCNU, 5-fluorouracil, irinotecan, and doxorubicin. In another aspect of this embodiment, the anti-cancer treatment is radiation therapy.

One of the ordinary skill in the art would appreciate that the chemical shifts of the ^{13}C solid state NMR of the polymorphic Form I, II or the amorphous form of Compound 1 may have some variance depending on the external reference used. In the claims of the present invention, the chemical shifts of the ^{13}C solid state NMR refer to those obtained when the upfield signal of adamantine at 29.5ppm is used as external reference.

The term "in combination with" refers to the relative timing of the administration of a first therapeutic treatment, such as (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide, a pharmaceutically acceptable salt or solvate thereof, or a mixture thereof, to the mammal in need, to that of a second therapeutic treatment, such as a anti-cancer agent or radiation therapy, the relative timing being those normally used in the field of medicine for combination therapy. In particular, relative timing can be sequential or simultaneous.

The term "hyperproliferative disorder" refers to abnormal cell growth that is independent of normal regulatory mechanisms (e.g., loss of contact inhibition), including the abnormal growth of normal cells and the growth of abnormal cells. This includes, but is not limited to, the abnormal growth of tumor cells (tumors), both benign and malignant. Examples of such benign proliferative diseases are psoriasis, benign prostatic hypertrophy, human papilloma virus (HPV), and restinosis.

The term "cancer" includes, but is not limited to, lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, uterine

cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, chronic or acute leukemia, lymphocytic lymphomas, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system (CNS), primary CNS lymphoma, spinal axis tumors, brain stem glioma, pituitary adenoma, or a combination of one or more of the foregoing cancers. In another embodiment of said method, said abnormal cell growth is a benign proliferative disease, including, but not limited to, psoriasis, benign prostatic hypertrophy or restinosis.

The term "mediated by CHK1 protein kinase activity" refers to biological or molecular processes that are regulated, modulated, or inhibited by CHK1 protein kinase activity.

The term "polymorph" refers to different crystalline forms of the same compound. "Polymorph" includes, but is not limited to, other solid state molecular forms including hydrates (e.g., bound water present in the crystalline structure) and solvates (e.g., bound solvents other than water) of the same compound.

The term "pharmaceutically acceptable, carrier, diluent, or vehicle" refers to a material (or materials) that may be included with a particular pharmaceutical agent to form a pharmaceutical composition, and may be solid or liquid. Exemplary of solid carriers are lactose, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are syrup, peanut oil, olive oil, water and the like. Similarly, the carrier or diluent may include time-delay or time-release material known in the art, such as glyceryl monostearate or glyceryl distearate alone or with a wax, ethylcellulose, hydroxypropylmethylcellulose, methylmethacrylate and the like.

The term "pharmaceutical composition" refers to a mixture of one or more of the compounds or polymorphs described herein, or physiologically/pharmaceutically acceptable salts or solvates thereof, with other chemical components, such as physiologically/pharmaceutically acceptable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

The term "radiation therapy" refers to medical use of radiation to control malignant cells.

The term "substantially pure" with reference to particular polymorphic forms or an amorphous form of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide means the polymorphic form or the amorphous form includes less than 10%, prefereably less than 5%, prefereably less than 3%, preferably less than 1% by weight of impurities, including other polymorphic forms of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide. Such purity may be determined, for example, by X-ray powder diffraction.

The term "therapeutically effective amount" generally refers to an amount of a compound, a pharmaceutically acceptable salt or solvate thereof, or a mixture thereof, being administered which will relieve to some extent one or more of the symptoms of the disorder being treated. In particular, when the term is used in describing a combination therapy, "therapeutically effective amount" refers to the amount of a particular therapeutic which will 1) enhance the therapeutic effect of another therapeutic such as an anti-cancer agent or radiation therapy, or 2) in combination with the other therapeutic, relieve to some extent one or more of the symptoms of the disorder being treated. In reference to the treatment of cancer, symptoms of the disease being treated includes a) reducing the size of the tumor; b) inhibiting (that is, slowing to some extent, preferably stopping) tumor metastasis; c) inhibiting to some extent (that is, slowing to some extent, preferably stopping) tumor growth, and d) relieving to some extent (or, preferably, eliminating) one or more symptoms associated with the cancer.

The term "2 theta value" or "2θ" refers to the peak position based on the experimental setup of the X-ray diffraction experiment described in the present invention, including but not limited to the radiation source used and the wavelength of the radiation source, and is a common abscissa unit in diffraction patterns. The experimental setup requires that if a reflection is diffracted when the incoming beam forms an angle theta (θ) with a certain lattice plane, the reflected beam is recorded at an angle 2 theta (2θ).

The terms "treat", "treating" and "treatment" refer to a method of alleviating or abrogating a cancer and/or its attendant symptoms. With regard particularly to cancer, these terms simply mean that the life expectancy of an individual affected with a cancer will be increased or that one or more of the symptoms of the disease will be reduced.

As used herein, the term "essentially the same" with reference to X-ray diffraction peak positions means that typical peak position and intensity variability are taken into account. For example, one skilled in the art will appreciate that the peak positions (2θ) will show some inter-apparatus variability, typically as much as 0.1°. Further, one skilled in the art will appreciate that relative peak intensities will show inter-apparatus variability as well as variability due to degree of crystallinity, preferred orientation, prepared sample surface, and other factors known to those skilled in the art, and should be taken as qualitative measures only. Similarly, as used herein, "essentially the same" with reference to solid state NMR spectra and Raman spectra is intended to also encompass the variabilities associated with these analytical techniques, which are known to those of skill in the art. For example, ^{13}C chemical shifts measured in solid state NMR will typically have a variability of 0.1 ppm, while Raman shifts will typically have a variability of 1 cm^{-1}.

Brief Description of the Drawings

Figure 1 is a X-Ray Powder Diffraction Pattern of polymorphic Form I of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide.

Figure 2 is a X-Ray Powder Diffraction Pattern of polymorphic Form II of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide.

Figure 3a is a X-Ray Powder Diffraction Pattern of an amorphous form (the first batch) of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide.

Figure 3b is a X-Ray Powder Diffraction Pattern of an amorphous form (the second batch) of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide.

Figure 4a is a Solid State ^{13}C Cross Polarization and Magic Angle Spinning (CP/MAS) NMR of polymorphic Form I (the first spectrum) of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide.

Figure 4b is a Solid State ^{13}C Cross Polarization and Magic Angle Spinning (CP/MAS) NMR of polymorphic Form I (the second spectrum) of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide.

Figure 5a is a Solid State ^{13}C CP/MAS NMR of polymorphic Form II (the first spectrum) of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide.

Figure 5b is a Solid State ^{13}C CP/MAS NMR of polymorphic Form II (the second spectrum) of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide.

Figure 6 is a solid State ^{13}C CP/MAS NMR of the amorphous form (the second batch) of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide.

Detailed Description of the Invention

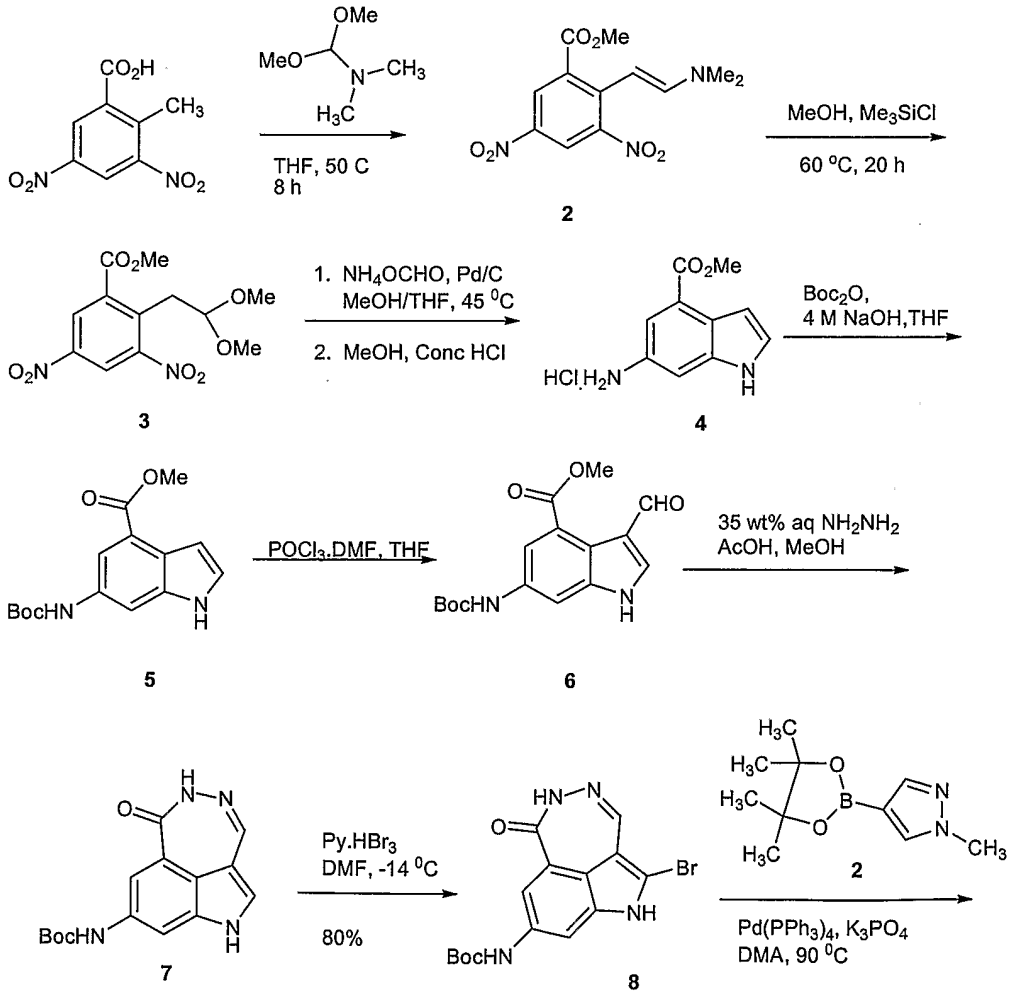
In this section, "BOC", "Boc" or "boc" refers to N-*tert*-butoxycarbonyl, "CBZ" refers to carbobenzyloxy, "DCE" refers to dichloroethane, "DCM" refers to dichloromethane, "DCC" refers to 1,3-dicyclohexylcarbodiimide, "DIC" refers to diisopropylcarbodiimide, "DIPEA" or "DIEA" refers to diisopropyl ethyl amine, DMA refers to N,N-dimethylacetamide, "DMAP" refers to 4-dimethyl amino pyridine, "DME" refers to 1,2-dimethoxyethane, "DMF" refers to dimethyl formamide, "DMSO" refers to dimethylsulfoxide, "DPPP" refers to 1,3-bis(diphenylphosphino)propane, "EDC" refers to 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, "HATU" refers to *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, "HBTU" refers to *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, "HOAc" refers to acetic acid, "HOBt" refers to 1-hydroxybenzotriazole hydrate, "IPA" refers to isopropyl alcohol, "LAH" refers to lithium aluminum hydride, "LiHMDS" refers to lithium bis(trimethylsilyl)amide, "MSA" means methanesulfonic acid; "MTBE" refers to methyl t-butyl ether, "NMP" refers to 1-methyl 2-pyrrolidinone, "TEA" refers to triethyl amine, "TFA" refers to trifluoro acetic acid, "TIPS" referst to triisopropylsilyl-. TMSCl refers to trimethyl silyl chloride, and "Trt" refers to triphenylmethyl-.

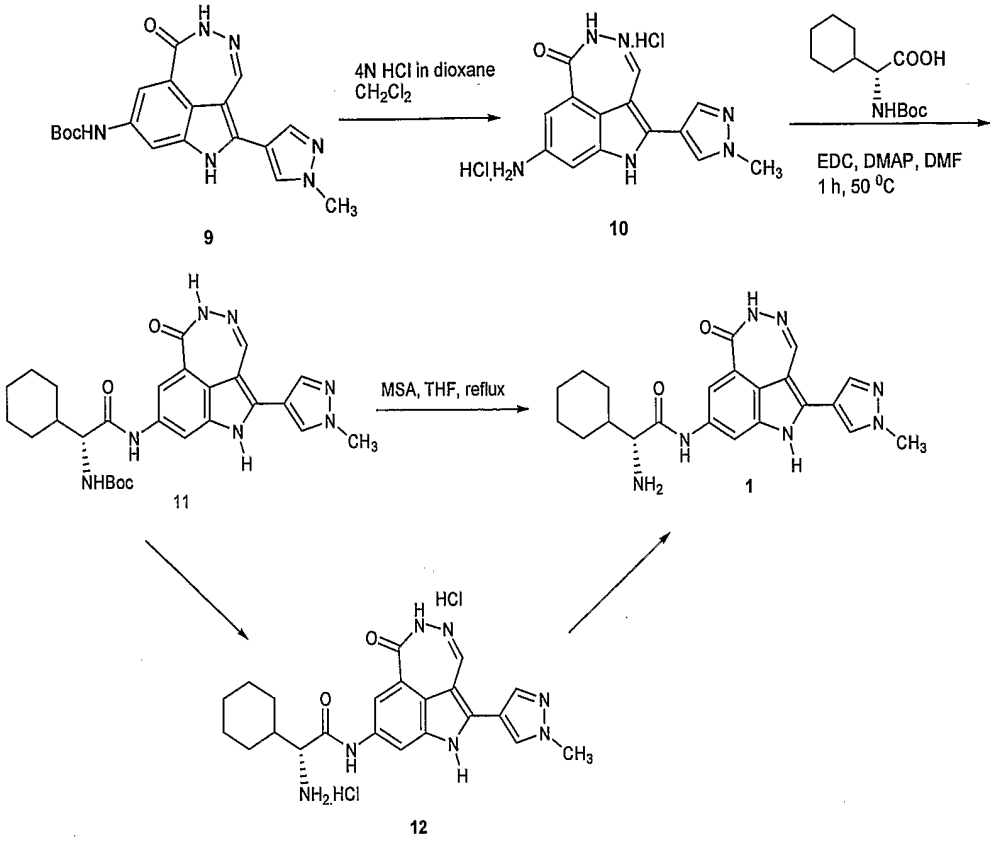
It has been found that the Compound 1 can exist in more than one polymorphic crystalline form as well as amorphous form. Processes for producing these polymorphic forms in high purity and characterization of these different polymorphic forms are described herein. Pharmaceutical formulations comprising Compound 1 are also provided.

I. Synthesis of Compound 1:

Synthetic route to make compound 12, the HCl salt of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl) acetamide, was described in United Patent No. 6,967,198, issued November 22, 2005. In the present invention, the said synthetic route was modified. In particular, the reaction conditions were improved, to fit the scale of the reactions. As shown in Scheme 1, Compound 1 can be made directly from compound 11, the N-Boc precursor of compound 12. Compound 1 can also be made from compound 12 by neutralizing the HCl salt. In either approach, depending on the work up and purification condition, different polymorphic forms of Compound 1 can be obtained.

Scheme 1





As illustrated in Scheme 1, 2-methyl-3,5-dinitrobenzoic acid reacts with N,N-dimethylformamide dimethyl acetal under heat to give compound 2. Solvents suitable for this reaction are DMF, DMA, MTBE, toluene and THF. Preferably, THF is used. Preferably, the reaction is carried out at around 50°C. The reaction mixture is concentrated under reduced pressure followed by addition of methanol. Compound 2 will then precipitate out as the product.

Compound 2 is then treated with an acid in methanol under heat to give compound 3. Typical acids that can be used here are TMSCl, HCl, MSA, H_2SO_4 and TFA. Preferably, TMSCl is used. During the course of the reaction, compound 3 crystallizes out from the reaction solution, thus significantly simplifies the isolation process.

In US 6,967,198, compound 3 was converted to compound 4 by a conventional hydrogenation reaction under Pd/C and H_2. However, this conventional hydrogenation reaction condition is difficult to control on large scale due to the fact that reduction of the two nitro groups is highly exothermic. In the present invention, compound 3 is converted to compound 4 by a transfer hydrogenation reaction followed by an acid-promoted cyclization reaction. Transfer hydrogenation reaction of compound 3 provides a dose-controlled procedure and therefore reduces thermal hazard. Different transfer hydrogenations reagents can be used, such as HCO_2NH_4 with Pd/C, $BH_3.NMe_3$ with $Pd(OH)_2$, hydrazine with $FeCl_3$/C and ammonium formate with Pd/C. Preferably, ammonium formate with Pd/C is used. Compound 3 can be reduced using ammonium formate with Pd/C in THF. The crude product is then cyclized to give compound 4 under a strong acid. A typical condition for this acid catalyzed cyclization is to use concentrated HCl in MeOH.

The primary amine group of compound 4 is then BOC-protected to give compound 5. In US 6, 967,198, this reaction was carried out using triethylamine as base and the reaction was carried out in acetonitrile. In the present invention, this reaction is carried out using more benign conditions of an inorganic base such as NaOH, and a solvent such as THF.

Compound 5 is converted to compound 6 by a Vilsmeier formylation reaction. In US 6,967,198, this reaction was carried out in methylene chloride, using 3 equivalents of the Vilsmeier reagent. Since neither compound 5 nor the reaction intermediate was very soluble in methylene chloride, this transformation in methylene chloride was a solid-to-solid reaction, which was quite often problematic in large scale. Furthermore, di-formylation occurred when compound 5 was treated with 3 equivalents of Vilsmeier reagent and additional hydrolysis step was required to convert di-formylated indole to compound 6. In the present invention, the reaction is carried out in a solvent in which the solubility of compound 5 is better. A typical solvent here is THF. Compound 5 is soluble in THF and therefore a solution-to-solid reaction is achieved. Mono-C-formylation takes place in preference to N-formylation to generate compound 6 directly when the amount of Vilsmeier reagent is reduced to 1.1 equivalents, thus simplified the reaction procedure significantly.

Compound 6 is converted to compound 7 by treating compound 6 with hydrazine under acidic condition. Hydrazine monohydrate as well 30% hydrazine aqueous solution can be used for this reaction. More preferably, the reaction is carried out in a dilute MeOH solution with excess hydrazine, preferably more than 5 equivalents.

Compound 7 is then brominated to give compound 8. In US 6,967,198, NBS was used as the bromination reagent. In the present invention, pyridinium tribromide is used instead as a cheaper alternative.

Compound 8 is then converted to compound 9 through Suzuki coupling. In US 6,967,198, $Pd(dppf)Cl_2$ was used. In the present invention, cheaper alternative $Pd(PPh_3)_4$ is used. Compound 8 is treated with 3 mol % $Pd(PPh_3)_4$ and K_3PO_4 in DMA. The residue palladium content in the crude product is typically very high (6000-8000 ppm). Due to the poor solubility of compound 9 in regular organic solvent, Florisol treatment for Pd removal proved to be not effective and required a large amount of solvent. A simple and effective water precipitation Pd removing procedure is developed in the present invention: 1) dissolve the crude product in a water immiscible solvent such as DMF, DMA, THF, DMSO DMA, preferably DMA, then add N-acetylcysteine to the resulting solution and stir for 1 hour; 2) add water to precipitate out product while the N-acetylcysteine-palladium complex remained in the aqueous solution. After the N-acetylcysteine treatment, the residual Pd level dropped below 400 ppm, which leads to the API Compound 1 with less than 20 ppm residual Pd.

Compound 9 is then treated with 4M HCl in dioxane and methylene chloride to give compound 10 as hydrogen chloride salt. It was found by Ion Chromatograph analysis that the hydrogen chloride content varied from batch to batch ranging from 2.1 to 2.8 ratio of hydrogen chloride to the corresponding free base. Other acids may also be used for this reaction, for example, sulfuric acid, sulfonic acids and TFA.

Compound 10 is then coupled with the boc protected hexyl amino acid to give compound 11. EDC is chosen as coupling agent. Many coupling conditions can be used such as EDC, HATU and DCC. In the present invention, EDC is used as the coupling agent, together with DMAP as catalyst. The required amount of DMAP should preferably be the same molar amount of hydrogen chloride presented in the starting amine salt. Large excess of DMAP in reaction mixture could cause significant racemization. Compound 11 is isolated by adding water into reaction mixture. Chiral HPLC analysis indicated the existence of ~1% racemized byproduct, which is removed by crystallization in the final step.

Compound 11 is then treated with a strong acid to remove the boc group. Conditions such as HCl/MeOH, HCl/EtOH, TFA/CH_2Cl_2 or MSA/THF can all be used. When compound 11 is treated with HCl/MeOH, or HCl/EtOH, the reaction mixture can be evaporated under reduced pressure to give compound 12, the HCl salt of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl) acetamide.

Compound 1 can be obtained directly from compound 11 by a basic aqueous work up of the de-boc reaction. For example, compound 11 is mixed with MSA and refluxed in THF to remove the Boc group. Upon completion of the reaction and cooling of the reaction mixture, aqueous solution of Na_2CO_3, K_2CO_3 or $NaHCO_3$ can be added to the reaction mixture. Subsequent standard work up will give Compound 1 in good purity and yield.

Compound 1 can also be obtained from compound 12 by mixing compound 12 with a basic aqueous solution, for example aqueous $NaHCO_3$, vigorously stirring the mixture followed by extracting the mixture with an organic solvent.

II. Preparation of Polymorphic forms I, II and the amorphous form of Compound 1.

A. Polymorphic Form I

Polymorphic Form I of Compound 1 can be prepared directly from compound 11 by removing the boc group under strong acidic condition followed by a basic aqueous work up. Typical strong acidic conditions to be used here are HCl/MeOH, HCl/EtOH, MSA/THF or TFA. Preferably, compound 11 is treated with methanesulfonic acid in THF to remove the boc group. After the reaction is complete, the mixture is basified with an aqueous inorganic base solution, such as aqueous solution of NaOH, Na_2CO_3, $NaHCO_3$, K_2CO_3 or $KHCO_3$. Preferably, 2 M aqueous sodium hydroxide or saturated $NaHCO_3$ solution is used. The organic phase is separated from aqueous phase and dried with magnesium sulfate. The organic solution is then reduced to a smaller volume and ethanol is added. The resulting solution is reduced to a smaller volume followed by further addition of ethanol. This volume reduction and addition of ethanol process is repeated until Compound 1 precipitates out as polymorphic Form I.

Polymorphic Form I of Compound 1 can also be prepared from compound 12. Compound 12 is dissolved in an aqueous inorganic base solution portion wise with vigorous stirring. Typical aqueous inorganic base solution used here are aqueous solution of NaOH, Na_2CO_3, $NaHCO_3$, K_2CO_3 or $KHCO_3$. Preferably, $NaHCO_3$ aqueous solution is used here. The mixture is extracted with large amount of an organic solvent such as EtOAc. The resulting organic solution is washed with brine and dried over Na_2SO_4 and filtered. The filtrate is

concentrated on vacuo to afford a yellow solid which is the amorphous form of Compound 1. This yellow solid is then dissolved in EtOH under heat, preferably around 75-80°C with vigorous stirring. The solution is cooled, preferably to room temperature of around 22°C and kept at 22°C for 12 hours. Polymorphic Form I will precipitate out.

B. Polymorphic Form II.

Compound 1 in polymorphic Form II may be obtained from compound 12 or compound 11 following similar procedure as described in previous paragraphs except that during the final working up procedure, polymorphic Form II forms in methanol at around 4°C.

C. The amorphous form.

Amorphous form of Compound 1 can be prepared directly from compound 12 following similar procedure of preparation of polymorphic Form I. After compound 12 is dissolved in an aqueous base solution portion wise with vigorous stirring, the mixture is extracted with large amount of EtOAc. The resulting EtOAc solution is washed with brine and dried and filtered. The filtrate is concentrated on vacuo to afford a yellow solid which is the amorphous form of Compound 1.

Amorphous form of Compound 1 can also be prepared from compound 11 following similar procedure of preparation of polymorphic Form I. Use EtOAc as the organic phase during the work up. After drying the organic phase, evaporating the solvent at around 40-70 °C affords Compound 1 in amorphous from.

The amorphous form of Compound 1 can also be made from polymorphic Form I of Compound 1 by dissolving the polymorphic Form I in THF until saturation followed by removing the solvent at 50°C.

III. Characterization and properties of different polymorphic forms of Compound 1:

Each solid form of Compound 1 can be characterized by one or more of the following: X-ray powder diffraction pattern (i.e., X-ray diffraction peaks at various diffraction angles (2θ)), melting point onset (and onset of dehydration for hydrated forms) as illustrated by endotherms of a Differential Scanning Calorimetry (DSC) thermogram, Raman spectral diagram pattern, aqueous solubility, light stability under International Conference on Harmonization (ICH) high intensity light conditions, and physical and chemical storage stability. For example, samples of polymorphic forms I, II and the amorphous form, of Compound 1 were each characterized by the positions and relative intensities of peaks in their X-ray powder diffraction patterns.

A. X-ray power diffraction pattern of the polymorphic forms.

The X-ray powder diffraction pattern for polymorphic Form I, From II and the first batch of the amorphous form of Compound 1 (Figure 3a) was measured on a Bruker AXS D8-Discover diffractometer with a Cu $K\alpha^{mean}$ radiation source of 1.5418Å wavelength operated at 40 kV and 40 mA. The samples were analyzed from angles 4-40 degrees (2θ) using a general area diffraction detector. The detector was set 30 cm from the sample. One of the ordinary skill in the art will appreciate that the peak positions (2θ) will show some inter-apparatus variability, typically as much as 0.1 degrees. Accordingly, where peak positions (2θ) are reported, such numbers are intended to encompass such inter-apparatus variability. Furthermore, where the crystalline forms

of the present invention are described as having a powder X-ray diffraction pattern essentially the same as that shown in a given figure, the term "essentially the same" is also intended to encompass such inter-apparatus variability in diffraction peak positions. Table 1 shows the 2θ values of polymorphic Form I and Form II. The amorphous form shows a continuous X-Ray power diffraction spectrum, where no 2θ value is collected.

The X-Ray powder diffraction patterns of the second batch of the amorphous form of Compound 1 is shown in Figure 3b. The preparation of the second batch of the amorphous form is described in Example 4b. Here, the powder X-ray diffraction pattern was generated with a Bruker D5000 diffractometer using Cu Kα radiation (wavelength = 1.5406 Å). The instrument was equipped with a line focus X-ray tube. The tube voltage and amperage were set to 38 kV and 38 mA, respectively. The divergence and scattering slits were set at 1 mm, and the receiving slit was set at 0.6 mm. Diffracted radiation was detected by a Sol-X energy dispersive X-ray detector. A theta two theta continuous scan at 2.4 °2θ /min (1 sec/0.04°2θ step) from 3.0 to 40 °2θ was used. Experiments were carried out at ambient temperature. An alumina standard (NIST standard reference material 1976) was analyzed to check the instrument alignment. Data were collected and analyzed using BRUKER AXS DIFFRAC PLUS software Version 2.0. The PXRD peak was selected using the peak maximum peak height.

Table 1: X-Ray Powder Diffraction of Polymorphic Forms I and II.

Polymorphic Form I		Polymorphic Form II	
2θ	Relative intensity	2θ	Relative intensity
8.5	33.5	6.9	21.2
10.4	10.1	10.8	9.8
14.4	7.2	12.2	26.3
16.4	21.5	13.6	21.2
17.0	12.6	13.9	28.7
20.7	23.1	16.0	40.0
22.4	11.5	16.8	23.8
23.6	100.0	18.2	11.8
25.0	6.8	20.1	12.0
31.8	8.3	20.8	11.9
32.4	10.9	21.9	10.2
		25.3	100.0
		27.9	13.1
		29.2	28.5

One skilled in the art will appreciate that relative peak intensities will show inter-apparatus variability as well as variability due to degree of crystallinity, preferred orientation, prepared sample surface, and other factors known to those skilled in the art, and should be taken as qualitative measures only.

Where a solid form comprises two or more polymorphs of the present invention, the X-ray diffraction pattern will have peaks characteristic of each of the individual polymorphs of the present invention. For example, a solid form that comprises two polymorphs will have a powder

X-ray diffraction pattern that is a convolution of the two X-ray diffraction patterns that correspond to the substantially pure polymorphic forms.

B. Solid State NMR (SSNMR) of the polymorphic forms.

Solid state NMR is a powerful tool to analyze solids. Different polymorphs often show significant chemical shifts differences in solid state ^{13}C cross polarization and magic angle spinning (CP/MAS) NMR. Solid state ^{13}C CP/MAS NMR was performed on polymorphic Form I and Form II, as well as the amorphous form.

For the polymorphic Form I, a first ^{13}C SSNMR spectrum was collected on a 600 MHz Bruker spectrometer and that chemical shifts were externally referenced to the methyl resonance of hexamethyl bezene at 17.36 ppm. A 500 MHz Bruker spectrometer was used for a second ^{13}C SSNMR spectrum of polymorphic Form I, a first and a second spectrum of polymorphic Form II and the spectrum of the second batch of the amorphous form of Compound 1 and the chemical shifts were externally referenced to the upfield signal of adamantine at 29.5ppm. Table 2a shows the chemical shifts of the first ^{13}C SSNMR spectrum of polymorphic Form I and the first ^{13}C SSNMR spectrum of polymorphic Form II. Table 2b shows the chemical shift of the second spectrum of polymorphic Form I, the second spectrum of polymorphic Form II and the spectrum of the second batch of the amorphous form of Compound 1. In Table 2b, peak intensity is defined as peak heights and can vary depending on the actual setup of the CPMAS experimental parameters.

Table 2a: Solid state ^{13}C CP/MAS NMR Chemical Shifts for polymorphic Form I (first spectrum) and polymorphic Form II (first spectrum) of Compound 1.

Peak number	Chemical Shift Form I (ppm)	Chemical Shift Form II (ppm)
1	177.1	177.7
2	168.0	165.7
3	142.8	140.7
4	139.6	136.6
5	137.0	135.3
6	130.7	133.2
7	128.3	127.8
8	127.3	125.2
9	116.0	114.4
10	112.4	112.5
11	108.5	108.9
12	64.6	103.8
13	43.2	58.7
14	40.9	39.7
15	34.8	37.9
16	28.9	30.3
17		22.7

Table 2b: Solid state ^{13}C CP/MAS NMR Chemical Shifts for polymorphic Form I (second spectrum), polymorphic Form II (second spectrum) and the amorphous form (second batch) of Compound 1.

Peak Number	^{13}C Chemical Shifts Form I [ppm]	Intensity	^{13}C Chemical Shifts Form II [ppm]	Intensity	^{13}C Chemical Shifts amorphous [ppm]	Intensity
1	175.0	4.65	177.7	6.59	175.8	2.53
2	166.0	4.17	165.7	7.78	166.2	1.3
3	140.8	3.46	140.7	10.95	163.6	5.6
4	137.6	3.59	136.6	9.45	138.9	4.45
5	134.9	6.91	135.3	8.67	135.5	7.42
6	128.7	3.83	133.2	8.21	131.4	6.89
7	126.3	4.89	127.8	12	129.9	7.19
8	125.2	7.25	125.2	8.34	125.9	5.58
9	113.7	6	114.4	10.58	112.2	6.9
10	110.3	4.64	112.5	7.72	108.6	6.9
11	106.3	3.53	108.9	10.43	61.3	2.37
12	62.4	3.62	103.8	6.17	40.1	3.42
13	41.1	4.76	58.7	5.68	37.7	5.75
14	38.8	5.4	39.7	5.57	30.8	4.42
15	32.6	4.35	37.9	8.77	27.1	12
16	26.8	12	30.3	5.62		
17			22.7	3.66		

C. Differential Scanning Calorimetry test of the polymorphic forms.

Different polymorphic forms of Compound 1 were also distinguished using differential scanning calorimetry (DSC). DSC is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference are measured as a function of temperature. DSC test is also frequently used to test the melting point of a solid.

In the present invention, DSC was performed using a TA Instruments Q-1000 DSC. The scanning rate for polymorphic Form I was 10°C/min from 25 °C to 300°C and under such condition, Polymorphic Form I has an onset melting point of 272°C. The scanning rate for polymorphic Form II was 40°C/min from 25 °C to 300°C, and under such condition, polymorphic Form II started melting at 242 °C, rapidly formed a new crystal form. We believe that the new crystal formed was polymorphic Form I. This was proved by the following test: Polymorphic Form II was heated to 260°C and the resulting solid was examined by PXRD. The PXRD result was identical to the PXRD result of polymorphic Form I. When the scanning rate for polymorphic Form II was set at 10°C/min from 25 °C to 300°C, polymorphic form II started melting at 243 °C, rapidly formed a new crystal form and then melted at 274°C and we believed the new crystal form was also polymorphic Form I.

D. Thermal Gravimetric Analysis of the polymorphic forms.

Thermal gravimetric analysis (TGA) is a testing procedure in which changes in weight of a specimen are recorded as the specimen is heated in air or in a controlled atmosphere such as nitrogen. Thermogravimetric curves (thermograms or TGA scan graphs) provide information regarding solvent and water content and the thermal stability of materials.

In the present invention, TGA was performed on Compound 1 polymorphic Form I and II using a TA Instruments TGA Q 500. The temperature was increased at 10°C/min from 25°C to 310°C and 350°C for polymorphic Form I and Form II, respectively. TGA analysis of polymorphic Form I showed that the crystalline Form I lost approximately 0.26% of total weight by the time the temperature reached 265°C. The degradation of polymorphic Form I occurred rapidly just after melt. TGA analysis of polymorphic Form II showed a loss of approximately 0.85% of the total weight by the time the temperature reached 275°C. The degradation of polymorphic Form II occurred rapidly after 275°C. These TGA results are consistent with the conclusion that both polymorphic Form I and polymorphic Form II are anhydrous/non-solvated forms.

E. Solubility of the different polymorphic forms.

Equilibrium solubility was tested on both polymorphic Form I and polymorphic Form II. Samples were prepared by dissolving each corresponding polymorph into or ethonal. Samples were stirred overnight at room temperature and centrifuged to get rid of any solid which was not dissolved. The solution was then analyzed by HPLC. The results are listed in Table 3.

Table 3: Solubility of Polymorphic Form I and Form II

Solvent	Polymorphic Form I solubility (mg/mL)	Polymorphic Form II solubility (mg/mL)
Water (pH= 8.35)	0.0015	0.0014
Ethanol	3.81	3.84

F. Hygroscopicity of the different polymorphic forms.

Different polymorphic forms of a compound may have different hygroscopic properties. Isothermal water sorption and desorption experiments of both polymorphic Form I and II of Compound 1, were performed on a Surface Measurements Systems Dynamic Vapor Sorption-1000 (DVS). Each polymorphic Form I and Form II was loaded on to the DVS instrument starting at 25°C of 0% RH. The humidity was increased step-by-step from 0% to 90%RH by stepping at 10% RH increments. After the sample weight stabilized at 90% RH, the humidity was decreased from 90%RH to 0% RH, stepwise, to complete a full cycle. The temperature remained constant of 25°C during the whole procedure. Weight gain during this experiment was used to determine hygroscopicity. A sample with weight gain of less than 2.0% from 0-90% RH is considered non-hygroscopic.

Polymorphic Form I and Form II showed an approximate 1.7% and 1.3% moisture gain from 0-90% RH, respectively.

F. Stability of the different polymorphic forms.

Solid state stability of polymorphic Form I of Compound 1 was investigated at 40°C, at 75% relative humidity (RH), in both open and closed vials for six weeks. The samples were analyzed by X-ray powder diffraction to check physical stability and HPLC for chemical stability. The results are presented in Table 4.

Table 4: Solid State Stability of Polymorphic Form I

	Close vial sample	Open vial sample
Physical stability determined by PXRD and DSC	No change	No change
Chemical stability determined by HPLC	99.3% unchanged	99.3% unchanged

Polymorphs are considered enantiotropic when one form is more thermodynamically stable at one temperature and the other form is more stable at another temperature. The temperature at which both polymorphs are equally stable is known as the transition temperature (T_t). Enantiotropy study was carried out for polymorphic Form I and Form II of Compound 1. A one-to-one mixture of Form I and Form II of 10-20 mg was added 1-2 mL of ethanol to form a slurry. Samples of such slurry were prepared and stirred at 70°C, 60°C, 50°C, 40°C, 30°C, room temperature (about 23°C) and 3.5°C respectively. The samples were then centrifuged. The supernatant was decanted and the remaining material was left to dry under vacuum at room temperature. The resulting materials were analyzed by PXRD. The results are summarized in the Table 5.

Table 5: Conversion between Form I and Form II

Temperature	Conversion direction	Time period	Completion of conversion
70°C	Form II → Form I	1 day	Complete
60°C	Form II → Form I	1 day	Complete
50°C	Form II → Form I	6 days	Complete
40°C	Form II → Form I	5 days	complete
30°C	Form II → Form I	11 days	Small amount of Form II remaining
23°C (RT)	Form I → Form II	12 days	Incomplete
3.5°C	Form I → Form II	7 days	Small amount of Form I remaining.

IV. Methods of Using the Polymorphs of the Invention

The inventive polymorphic forms of Compound 1 may be useful in all aspects that Compound 1 may be useful. The method of using Compound 1 was described in US 6,967,198 as method to use genus of compounds which contain Compound 1. It was described in US 6,967,198 that compounds of the invention therein may be used in combination with a therapeutically effective amount of an anti-neoplastic agent or radiation therapy to treat neoplasm in a mammal. It is within contemplation of the present invention, that the polymorphic forms and the pharmaceutical compositions of Compound 1 of the current invention may be used in combination with a therapeutically effective amount of an anti-neoplastic agent or radiation

therapy, as described in US 6,967,198 that could be used in combination with the compounds of the invention therein.

Examples

Example 1: Preparation of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide HCl salt (HCl salt of Compound 1)

Preparation of 2-(2-Dimethylamino-vinyl)-3,5-dinitro-benzoic acid methyl ester, compound 2: 2-Methyl-3,5-dinitro-benzoic acid (102.62 g, 0.45 mol) was dissolved in anhydrous THF (1200 mL). N,N-dimethylformamide dimethyl acetal (Aldrich, 94% purity, 3.0 eq, 172.4 g, 1.35 mol) was added under nitrogen atmosphere over 10 minutes at room temperature with stirring. Temperature rose to 29 °C from 22 °C. The solution was immediately heated to 50 °C and was stirred at this temperature for 8 hours behind a shield. The reaction solution was then cooled to room temperature and concentrated in a rotorvap to remove most of the THF and the unreacted N,N-dimethylformamide dimethyl acetal until ~250 g of crude material remained in the flask (water bath temperature was not allowed to exceed 30 °C). Methanol (400 ml) was added and the slurry was stirred at room temperature for 1 hour. The solid was collected by filtration, washed with cold methanol (60 ml) and dried in airflow to afford 109.08 g of purple solid (84% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.01(s, 6H), 3.93(s, 3H), 5.99(d, 1H, J= 13.5 Hz), 6.75(d, 1H, J= 13.2 Hz), 8.49(d, 1H, J= 2.4 Hz), 8.57(d, 1H, J= 2.4 Hz).

Preparation of 2-(2,2-Dimethoxy-ethyl)-3,5-dinitro-benzoic acid methyl ester compound 3: The purple solid 2 (92.25 g, 0.313 mol) was suspended in anhydrous MeOH (1000 mL). TMSCl (70.25 g, 0.65 mol, 2.1 eq) was added over 10 min. A clear solution was formed. The solution was heated at 60 °C (slow reflux) for 20 hours. HPLC analysis indicated the disappearance of starting material. Reaction mixture was cooled to room temperature and white solid precipitated out (often times, the product crystallized from the reaction mixture when reaction was near completion). The reaction mixture was stirred at room temperature for 1 h. The precipitated solid was collected by filtration, washed with cold MeOH (50 mL) and dried in airflow to afford 82.71 g of white solid. The mother liquor was concentrated to remove ~800 ml of solvent. Additional product precipitated out. The mixture was cooled to 0 °C and stirred for 1 h. The orange solid was then collected, washed with cold methanol (25 ml) and dried in airflow to giving another 5.58 g of the product. The combined yield for compound 3 was 88.29 g (90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.30(s, 6H), 3.73(d, 2H, J= 5.1 Hz), 4.00(s, 3H), 4.51(t, 1H, J= 5.1 Hz), 8.65(d, 1H, J= 2.4 Hz), 8.76(d, 1H, J= 2.4 Hz).

Preparation of 6-amino-1*H*-indole-4-carboxylic acid methyl ester hydrochloride compound 4: A 2-L Erlenmeyer flask was charged with 10% Pd/C wet catalyst (8.6 g), methanol (800 ml) and THF (160 ml). Ammonium formate (139.1 g, 2.21 mol) was then added with stirring and the mixture was heated to 35 °C. Water (100 ml) was added. Compound 3 was then added in small portions over 10 minutes. The reaction temperature was maintained between 40 °C and 45 °C by controlling the addition rate of 3 and proper cooling. The reaction mixture was stirred for 30 minutes after addition is complete. HPLC analysis indicated that the reaction was complete. The

reaction mixture was cooled to room temperature. The catalyst was filtered off through a Celite pad and the Celite pad was washed with MeOH (50 ml). The filtrate was concentrated to remove volatile components under reduced pressure. EtOAc (500 ml) and water (75 ml) were then added and the mixture was stirred for 5 minutes. Organic phase was separated and the aqueous phase was extracted with EtOAc (200 ml). The combined organic solution was concentrated to dryness to afford light-yellow oil (79.17 g). MeOH (100 ml) was added to the oil intermediate. The resulting solution was then added to the solution of concentrated aqueous HCl (37 wt%, 82.8 g) in MeOH (600 ml) while the temperature was maintained at 32 °C. The reaction mixture was stirred for 3 hours at 35 °C. Solid product precipitated out during this period. HPLC analysis indicated that the reaction was complete. The reaction mixture was cooled to room temperature. EtOAc (500 ml) was added and the resulting mixture was stirred for 30 minutes. The solid was collect by filtration to afford 45.07 g of product. The filtrate was concentrated to afford a paste (331 g). EtOAc (400 ml) was added to the paste and the mixture was stirred for 30 minutes. The solid was then collect by filtration to afford the 2nd crop of product 4 (16.28 g).). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.93(s, 3H), 6.97(d, 1H, J= 1.8 Hz), 7.65(m, 1H), 7.72(d, 1H, J= 1.8 Hz), 7.80(s, 1H), 11.81(s, br, 1H).

Preparation of 6-amino-1*H*-indole-4-carboxylic acid methyl ester hydrochloride 4 *via* direct hydrogenation of 2-(2-Dimethylamino-vinyl)-3,5-dinitro-benzoic acid methyl ester compound 2: Methanol (27 kg) and 2-(2,2-dimethoxy-vinyl)-3,5-dinitrobenzoic acid methyl ester 2 (10 kg, 33.87 mol) were charged into a reactor through the charge port. 10 % Palladium on Carbon Catalyst (0.4 kg) was then charged to the reactor as a slurry in water (2.5 ± 0.5 Kg). Ethyl Acetate (123 ± 3 kg) was added followed by addition of hydrogen portion wise. The internal temperature was maintained at approximately 0°C through cooling and controlling the rate of hydrogen addition. After pressure was constant for more than 30 min, hydrogen pressure was kept at 50-60 psi and the temperature was maintained at 0°C for 2 hours. The catalyst was filtered off through an in-line filter (Note: Catalyst is pyrrophoric. Do not allow the catalyst to pull dry.) The filtrate was concentrated to remove approximately 95 % of the solvent volume by vacuum distillation at an internal temperature of less than 35 °C. Ethyl acetate (130L) was added with stirring. 37% HCl (10kg) was then added slowly below 10°C. The resulting mixture was stirred at 10°C for 1 hour. The solids was filtered onto a plate filter and washed with methyl t-butyl ether (15L). The product (wet cake) was transferred to a vacuum dryer and dried at 23 ± 3 °C to afford 4.6 kg of product 4 (60% yield).

Preparation of 6-*tert*-Butoxycarbonylamino-1*H*-indole-4-carboxylic acid methyl ester 5: THF (600 ml) and 4 M aqueous NaOH solution (138 ml, 0.55 mol) were charged into a reaction flask. Indole HCl salt 4 (61.0 g, 0.269 mol) was then added and the mixture was stirred until solid was dissolved completely. $(BOC)_2O$ (70.43 g, 0.32 mol) was added slowly as solution in THF (50 ml) while temperature was maintained between 25 °C and 32 °C during addition. The resulting reaction mixture was stirred for 3 hours at room temperature. HPLC analysis indicated that the reaction was complete. Aqueous phase was separated. Organic phase was washed with saturated ammonium chloride aqueous solution (15 ml). The organic solution was then

concentrated to afford a paste (176 g). THF (40 ml) and heptane (800 ml) were added to the paste and the resulting mixture was stirred for 30 minutes. The solid was collected by filtration to afford 69.86 g of product 5 (90% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.50(s, 9H), 3.89(s, 3H), 6.83(s, 1H), 7.41(m, 1H), 7.89(s, 1H), 7.91(s, 1H), 9.38(s, 1H), 11.24(s, br, 1H).

Preparation of 6-tert-butoxycarbonylamino-3-formyl-1H-indole-4-carboxylic acid methyl ester compound 6: N, N-dimethylformamide (85 ml) was charged to a 250-ml flask and then cooled to -5 ^{0}C with an ice-acetone bath. Phosphorus oxychloride (35.15 g, 0.23 mol) was syringed into the flask over 10 min at 4 °C. After the addition was complete, the mixture was stirred at 0 °C for an additional 30 min. In another 2L 3-necked flask equipped with overhead stirrer and thermometer, indole 5 (60.57 g, 0.21 mol) and anhydrous THF (600 ml) were added and a clear solution was formed. The solution was cooled to –7 °C with an ice-acetone bath. To the stirred THF solution was cannulatede the cold pre-formed Vilsmeier reagent over 10 min while the temperature was maintained at –3 °C. After the addition was complete, a heavy precipitate formed within minutes. The reaction mixture was further stirred at 0 °C for 45 minutes. EtOAc (600 ml) was added to the reaction mixture followed by addition of cold aqueous NaOAc solution (3 M, 310 ml, 0.93 mol) with vigorously stirring. Temperature rose to 10 °C after quench. Cooling bath was removed. The reaction mixture was warmed to 21 °C over 20 minutes and stirred at this temperature for 2.5 h. Solids were completely dissolved. HPLC analysis indicated that all the intermediate was converted to product. Agitation was stopped and aqueous layer was separated. Organic layer was concentrated under reduced pressure to remove volatiles. A paste (226 g) was obtained. Water (30 ml) and EtOAc (70 ml) were added to the paste. The resulting mixture was stirred for 30 minutes. Solid was collected by filtration, washed with EtOAc (25 ml) and dried to afford 62.98 g of product (95% yield, 97% purity). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.50(s, 9H), 3.86(s, 3H), 7.68(d, 1H, J= 1.8 Hz), 7.97(s, 1H), 8.23(s, 1H), 9.56(s, 1H), 10.10(s, 1H), 12.25(s, br, 1H).

Preparation of *tert*-butyl 1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-ylcarbamate compound 7: MeOH (1200 ml), acetic acid (100 ml) and aldehyde 6 (62.98 g, 0.20 mol) were charged into a 2L flask. Aqueous hydrazine (35 wt%, 88.8 g, 0.97 mol) was then add with stirring. The mixture was heated to 60 °C and stirred for 3 hours. HPLC analysis indicated that the reaction was complete. The heating was stopped and the reaction mixture was cooled to room temperature. The mixture was stirred slowly at room temperature for 2 hours. The solid was collected by filtration to afford 46.0 g of product. The filtrate was concentrated to afford a paste (223 g). Water (250 ml) was added slowly with stirring. The mixture was stirred for additional 50 minutes to granulate solid. The solid was collected and dried to afford the 2nd crop of product (13.0 g). The combined yield for compound 7 was 99%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.49(s, 9H), 7.44(s, 1H), 7.51(d, 1H, J= 2.1 Hz), 7.62(s, 1H), 7.76(s, 1H), 9.45(s, 1H), 10.19(s, 1H), 11.63(s, br, 1H).

Preparation of tert-butyl 5-bromo-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-ylcarbamate 8: Compound 7 (45.0g, 0.15 mol) and N,N-dimethylformamide (0.45 L) were charged into a 5L-flask equipped with overhead stirrer, thermometer and nitrogen gas inlet. The mixture

was stirred to form a clear solution. The solution was then cooled to –14 °C under protection of nitrogen. Pyridinium tribromide (57.6g, 0.18 mol) was added portion-wise over 5 minutes under protection of nitrogen. Reaction temperature was controlled below –5 °C during addition. After addition was complete, the reaction mixture was stirred at 0 °C for 1.5 hours. HPLC analysis indicated that the reaction was complete. Water (0.225L) was added slowly to the reaction mixture while the temperature was maintained below 20 °C during addition. EtOAc (0.225L) was added to the reaction mixture at 15 °C followed by addition of aqueous potassium carbonate (Preparation: dissolve 20.73g K_2CO_3 in 60ml water, cool to 10 °C for use) in one portion. The mixture was stirred for 15 minutes to dissolve solid. Aqueous sodium sulfite solution (Preparation: dissolve 3.78g Na_2SO_3 in 18ml water) was then added and stirred for 10 minutes. Water (0.5 L) was added via addition funnel over 3 minutes while the temperature was maintained at 25-30 °C. Solid gradually precipitated out. The mixture was stirred for 20 minutes to granulate the solid. Additional water (0.625 L) was added to drive the precipitation to completion. The mixture was stirred for 60 minutes. The solid was collected by filtration, washed with water (0.3L) and dried in airflow for 90 minutes. The solid was dried in a vacuum oven at 60 °C with nitrogen breeze for 12 hours The crude product was suspended in acetone (0.19L) and stirred for 1 hour. Heptane (0.19L) was added slowly into the mixture. The resulting mixture was stirred for 1.5 hour. The solid was collected by filtration, washed with mixture of acetone (30ml) and heptane (30ml) and dried in airflow for 2 hours. The product was further dried in a vacuum oven at 50 °C for 12 hours. The solid weighed 45.5g (80% yield, 98.3% purity). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.53(s, 9H), 7.31(s, 1H), 7.70(d, 1H, J= 1.7 Hz), 7.77(s, 1H), 9.57(s, 1H), 10.47(s, 1H), 12.54(s, br, 1H).

Preparation of *tert*-butyl 5-(1-methyl-1*H*-pyrazol-4-yl)-1-oxo-2,6-dihydro-1*H*-[1,2]diazepino[4,5,6-cd]indol-8-ylcarbamate 9: Compound 8 (308.24g, 0.81 mol) and N,N-dimethylacetamide (2.5L) were added to a 10L flask equipped with overhead stirrer, thermometer and nitrogen gas inlet. A clear solution was formed. Water (0.625L) was added to the DMA solution with stirring while the temperature was controlled at 30 °C. 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (186g, 0.89 mol) and potassium phosphate (431.3g, 2.03 mol) were then added to the flask with stirring. The mixture was degassed by vacuuming the flask followed by nitrogen flush. The degassing process was repeated 3 times over 20 minutes. Tetrakis(triphenylphosphine) palladium (28.2g, 0.024 mol) was added under protection of nitrogen. The reaction mixture was degassed 3 times by vacuum/nitrogen cycle over 20 minutes. The reaction was heated at 90 °C under protection of nitrogen for 5 hours. HPLC analysis indicates the reaction is complete. The reaction mixture was cooled to room temperature. N-acetylcysteine (30g, 0.18 mol) was added into the flask and the mixture was stirred for 2 hours. Water (5L) was added over 30 minutes with stirring while the temperature was controlled at 30 °C. Solid precipitated out. The mixture was stirred for additional 2 hours to granulate solid. Solid was collected by filtration and washed with a mixture of DMA (0.1L) and water (0.3L), water (0.7L), and a mixture of acetone (0.3L) and water (0.3L). The filter cake was dried in airflow overnight and then dried in vacuum oven at 60 °C for 16 hours. The crude product was dissolved in DMA (1.5L). N-acetylcysteine (30g, 0.18 mol) was added into the DMA solution

and the solution was stirred for 2 hours. Water (0.8L) was added over 10 minutes with stirring while the temperature was controlled at 30 °C. Solid precipitated out. The mixture was stirred for 20 minutes to granulate solid. Additional water (2.2L) was added over 5 minutes to drive the precipitation to completion. The mixture was stirred for 1 hour. Solid was collected by filtration and washed sequentially with a mixture of DMA (0.2L) and water (0.4L), water (0.8L), and a mixture of acetone (0.4L) and water (0.4L). The compound was dried in vacuum oven at 60 °C until the water content is reduced to less than 1.5 wt%. The product weighed 286g (93% yield, 98% apparent purity). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.49(s, 9H), 3.92(s, 3H), 7.57(s, 1H), 7.65(d, 1H, J= 1.5 Hz), 7.68(d, 1H, J= 1.5 Hz), 7.90(s, 1H), 8.27(s, 1H), 9.40(s, 1H), 10.14(s, 1H), 11.75(s, br, 1H).

Preparation of 8-amino-5-(1-methyl-1*H*-pyrazol-4-yl)-2*H*-[1,2]diazepino[4,5,6-cd]indol-1(6*H*)-one hydrochloride 10: *tert*-butyl 5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-ylcarbamate 9 (236.06g, 0.62 mol) was charged into a 10L flask equipped with equipped with overhead stirrer, thermometer, and a bubbler. Dichloromethane (3.5L) was added and the mixture was stirred for 10 minutes. The suspension was then cooled to 10 °C followed by addition of 4M HCl in dioxane (2.4L) over 10 minutes. The temperature was not allowed to exceed 25 °C. The reaction mixture was stirred at room temperature for 16 hours. HPLC analysis indicates ~98% of starting material was consumed. The solid was collected by centrifuge under protection of nitrogen and washed with dichloromethane (2.2L). The product was then dried in vacuum oven at 40 °C to afford product (239.77g). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.94(s, 3H), 7.47(d, 1H, J= 1.8 Hz), 7.58(d, 1H, J= 1.8 Hz), 7.66(s, 1H), 8.00(s, 1H), 8.40(s, 1H), 10.42(s, 1H), 12.51(s, br, 1H).

Preparation of *tert*-butyl (R)-1-cyclohexyl-2-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-ylamino)-2-oxoethylcarbamate 11: 8-amino-5-(1-methyl-1*H*-pyrazol-4-yl)-2*H*-[1,2]diazepino[4,5,6-cd]indol-1(6*H*)-one hydrochloride 10 (224.35g, 0.64 mol, containing 25.7% of chloride determined by Ion Chromatography) was suspended in anhydrous DMF(2.2 L). 4-dimethylaminopyridine (197.08g, 1.65 mol) was added and the mixture was stirred for 30 minutes. Boc-D-cyclohexylglycine (179.80 g, 0.7 mol) was then added and the mixture was heated to 35 °C. 1-(3-dimethylaminopropyl)-3-ethylcarbondiimide (158.5 g, 0.83 mol) was added in one portion and the reaction mixture was heated at 50 °C for 1 hour. HPLC analysis indicates the reaction was complete. The reaction mixture was cooled to room temperature. Water (2.2 L) was added over 10 minutes while the temperature was maintained at 30 °C. The mixture was stirred for 20 minutes to granulate solid. Additional water (4.4 L) was added over 5 minutes to drive the precipitation to completion and the mixture was stirred for another 30 minutes. The solid was collect by filtration and washed with a mixture of DMF (0.5 L) and water (1.5 L), then water (1.0 L). The product was dried in vacuum oven at 60 °C for at least 48 hours to afford 293.91 g of product (94.2% HPLC purity). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.1(m, 5H), 1.36(s, 9H), 1.60(m, 6H), 3.93(s, 3H), 3.96(m, 1H), 7.59(s, 1H), 7.60(s, 1H), 7.92(s, 1H), 8.07(s, 1H), 8.30(s, 1H), 10.05(s, 1H), 10.20(s, 1H), 11.86(s, br, 1H).

Preparation of (2R)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide 12: Methansulfonic acid (44.4 g, 0.46 mol) was added to THF (690 mL) in a 2-L flask. *tert*-Butyl (R)-1-cyclohexyl-2-(5-(1-methyl-1*H*-pyrazol-4-yl)-1-oxo-2,6-dihydro-1*H*-[1,2]diazepino[4,5,6-cd]indol-8-ylamino)-2-oxoethylcarbamate 11 (30.0 g, 57.74 mmol) was added *via* powder funnel. THF (60 mL) was used to rinse the funnel and the sides of the flask. The flask was purged with nitrogen, and the reaction was heated to 65 °C and stirred for 18-24h. HPLC analysis revealed that the reaction was complete. The reaction was cooled to room temperature using a water bath. 2M NaOH (255 mL) was added over 30 minutes while maintaining the temperature at 20±5 °C. After stirring for 5 minutes, the mixture was transferred to a 2-L separatory funnel using THF for the rinse. The layers were separated. The aqueous phase was extracted with THF (60 mL). The organic fractions were combined and washed twice with saturated aqueous NaCl (2 x 60 mL). MeOH (80 mL) and $MgSO_4$ (42 g) were added. The mixture was stirred for 75 minutes, and then filtered through celite. The cake was washed with 9:1 THF/MeOH (3 x 50 mL), and the solution was transferred to a 2-L distillation flask. The solution was concentrated to a volume of 300 mL by distillation at 1 atmosphere. EtOH (450 mL) was added slowly, and the solution was cooled to 50 °C to crystallize the product. Once the product had crystallized, the mixture was re-heated and distilled down to a volume of 450 mL. The reaction solvent ratio was monitored by ^{1}H NMR. The distillation was stopped once THF content was reduced to 5 mol%. The resulting yellow suspension was cooled to 25 °C over 60 minutes and vacuum-filtered on paper. The filter cake was washed with EtOH (2 x 75 ml). The solids were transferred to a crystallizing dish and dried under vacuum at 55 °C for 16 hours. 13.55 g of compound 12 (54%) was obtained as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.14(m, 5H), 1.62(m, 6H), 3.10(d, 1H, J= 5.7 Hz), 3.93(s, 3H), 7.58(s, 1H), 7.59(s, 1H), 7.91(s, 1H), 8.11(s, 1H), 8.29(s, 1H), 10.19(s, 1H), 11.83(s, br, 1H).

Example 2: preparation of (2R)-2-amino-2-cyclohexyl-*N*-(5-(1-methyl-1*H*-pyrazol-4-yl)-1-oxo-2,6-dihydro-1*H*-[1,2] diazepino[4,5,6-*cd*]indol-8-yl)acetamide polymorphic Form I:

From HCl salt of Compound 1: To an aqueous solution of sodium bicarbonate (5%; 5 eq; ~18 mL) was added portion wise and with vigorous stirring, (2R)-2-amino-2-cyclohexyl-*N*-(5-(1-methyl-1*H*-pyrazol-4-yl)-1-oxo-2,6-dihydro-1*H*-[1,2] diazepino[4,5,6-*cd*]indol-8-yl)acetamide hydrochloride (1 g). To the mixture were added 500 mL of ethyl acetate and 80 mL of water. The suspension was vigorously stirred for 10-15 minutes and stirring was stopped. The organic phase was decanted carefully into another erlenmeyer and the process was repeated 3 more time, each time with 500 mL of ethyl acetate. The combined organic phase (~2000 mL) was washed with brine (100 mL), dried over sodium sulfate and filtered. The volatiles were removed in vacuo and the resulting yellow solid dried overnight to afford 670 mg of polymorphic Form I of Compound 1 (~84% yield).

From the amorphous form of Compound 1: amorphous form of (2R)-2-amino-2-cyclohexyl-*N*-(5-(1-methyl-1*H*-pyrazol-4-yl)-1-oxo-2,6-dihydro-1*H*-[1,2]diazepino[4,5,6-*cd*]indol-8-yl)acetamide (100 mg) was mixed with ethyl alcohol (1.5 mL) to form a suspension. The suspension was heated at 75-80 °C for five hours with vigorous stirring and let sit at 22 °C for

twelve hours. The solid was filtered and washed with 0.5 mL of ethyl alcohol. It was then dried with house vacuum for 24 hours and later, additionally dried at 25-30 °C on a vacuum pump for 12 hours to afford 66 mg of crystalline material.

Example 3: preparation of (2R)-2-amino-2-cyclohexyl-*N*-(5-(1-methyl-1*H*-pyrazol-4-yl)-1-oxo-2,6-dihydro-1*H*-[1,2] diazepino[4,5,6-*cd*]indol-8-yl)acetamide polymorphic Form II:

Polymorphic Form I of Compound 1, (2R)-2-amino-2-cyclohexyl-*N*-(5-(1-methyl-1*H*-pyrazol-4-yl)-1-oxo-2,6-dihydro-1*H*-[1,2] diazepino[4,5,6-*cd*]indol-8-yl)acetamide (50mg) was slurred in 1 mL of methanol at 4°C for 23 days. The slurry was centrifuged at 14,000 RPM to separate the solid. The solid was dried in a low temperature vacuum oven to give Form II.

Example 4a: Preparation of the first batch of amorphous form of (2R)-2-amino-2-cyclohexyl-*N*-(5-(1-methyl-1*H*-pyrazol-4-yl)-1-oxo-2,6-dihydro-1*H*-[1,2] diazepino[4,5,6-*cd*]indol-8-yl)acetamide amorphous form:

1.3 g of compound 11 (2 HCl) was dissolved in water (200 mL) at 22 °C followed by the addition of a saturated aqueous sodium bicarbonate solution (50 mL) and 500 mL of ethyl acetate. Two more spatulas of sodium bicarbonate were added and another 50 mL of water. After vigorous stirring, the mixture was filtered and the phases separated. The aqueous phase was re-extracted with ethyl acetate (two times 200 mL). The combined organic phase was washed with brine and dried over sodium sulfate. Filtration followed by evaporation of the volatiles up to 10 mL afforded a yellow solid, which was filtered and dried. Total amount of amorphous material obtained was 690 mg.

Example 4b: Preparation of the second batch of amorphous form of (2R)-2-amino-2-cyclohexyl-*N*-(5-(1-methyl-1*H*-pyrazol-4-yl)-1-oxo-2,6-dihydro-1*H*-[1,2] diazepino[4,5,6-*cd*]indol-8-yl)acetamide amorphous form:

749.2 mg of polymorphic Form I of Compound 1 was dissolved in 850 mL USP Grade ethanol. The solution was vacuum filtered using a Buchner funnel and Whatman Type 2 filter paper. The filtered solution was transferred to a 190 mm x 100 mm crystallization dish and allowed to evaporate under ambient conditions for 63 hours. 589.1 mg of an orange solid were recovered as the second batch of amorphous form of Compound 1.

Example 5. ^{13}C Solid State NMR of polymorphic Form I (the second spectrum), Form II (the second spectrum) and the amorphous form (the second batch) of Compound 1.

Samples were packed into a ZrO_2 rotors. Form I and amorphous samples were packed into 4mm rotors and the sample of form II was packed into 7mm rotor. The carbon spectra were collected at ambient conditions on a Bruker-Biospin 4 and 7mm CPMAS probes positioned into a wide-bore Bruker-Biospin Avance DSX 500 MHz NMR spectrometer. The rotors were placed at the magic angle and spun at 15.0 kHz (4mm rotors) and 7.0 kHz (7mm rotor). The fast spinning speed minimized the intensities of the spinning side bands. The number of scans was adjusted to obtain adequate S/N. The one dimensional ^{13}C spectra of form I and amorphous samples were collected using ^{1}H-^{13}C Cross-Polarization Magic Angle Spinning (CPMAS) which was followed by Total Suppression of Spinning side bands (TOSS) in the case of form II sample. The TOSS was applied to suppress the spinning side bands. To optimize the signal sensitivity, the cross-

polarization contact time was adjusted to 2.0 ms, and the decoupling field was set to approximately 75 kHz. 512 scans were acquired with recycle delay of 30s for form I, 256 scans were acquired with recycle delay of 30s for form II and 2048 scans were acquired with recycle delay of 3s for the amorphous sample. All spectra were referenced using an external sample of adamantane with its upfield signal set to 29.5 ppm.

Claims

We claim:

1. A crystalline form of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide.

2. The crystalline form of claim 1, wherein the crystalline form is a polymorph of Form I.

3. The crystalline form of claim 2, wherein the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 23.6 ± 0.1 and 8.5 ± 0.1.

4. The crystalline form of claim 2, wherein the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) essentially the same as shown in Figure 1.

5. The crystalline form of claim 2, wherein the crystalline form has a ^{13}C solid state NMR peak pattern comprising peaks at chemical shifts 175.0 ± 0.2, 137.6 ± 0.2, 134.9 ± 0.2, 110.3 ± 0.2, 106.3 ± 0.2, 41.1± 0.2 and 32.6 ± 0.2 ppm.

6. The crystalline form of claim 2, wherein the crystalline form has a ^{13}C solid state NMR peak pattern comprising at least three, at least four, at least five or at least six of the seven peaks at chemical shifts 175.0 ± 0.2, 137.6 ± 0.2, 134.9 ± 0.2, 110.3 ± 0.2, 106.3 ± 0.2, 41.1± 0.2 and 32.6 ± 0.2 ppm.

7. The crystalline form of claim 2, wherein the crystalline form has a ^{13}C solid state NMR peak pattern comprising peaks at chemical shifts position essentially the same as shown in Figure 4b.

8. The crystalline form of any of claims 2-7, wherein the crystalline form is a substantially pure polymorph of Form I.

9. The crystalline form of claim 1, wherein the crystalline form is a polymorph of Form II.

10. The crystalline form of claim 9, wherein the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) of 25.3 ± 0.1 and 16.0 ± 0.1.

11. The crystalline form of claim 9, wherein the crystalline form has a powder X-ray diffraction pattern comprising peaks at diffraction angles (2θ) essentially the same as shown in Figure 2.

12. The crystalline form of claim 9, wherein the crystalline form has a ^{13}C solid state NMR peak pattern comprising peaks at chemical shifts 177.7 ± 0.2, 133.2 ± 0.2, 127.8 ± 0.2, 103.8 ± 0.2, and 22.7 ± 0.2ppm.

13. The crystalline form of claim 9, wherein the crystalline form has a ^{13}C solid state NMR peak pattern comprising at least three or at least four of the five peaks at chemical shifts 177.7 ± 0.2, 133.2 ± 0.2, 127.8 ± 0.2, 103.8 ± 0.2, and 22.7 ± 0.2ppm.

14. The crystalline form of claim 9, wherein the crystalline form has a ^{13}C solid state NMR peak pattern comprising peaks at chemical shifts position essentially the same as shown in Figure 5b.

15. The crystalline form of any of claims 9-14, wherein the crystalline form is a substantially pure polymorph of Form II.

16. An amorphous form of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide.

17. The amorphous form of claim 16, wherein the amorphous form has a ^{13}C solid state NMR peak pattern comprising peaks at chemical shifts 163.6 ± 0.2, 138.9 ± 0.2, 131.4 ± 0.2, 129.9 ± 0.2, and 30.8 ± 0.2 ppm.

18. The amorphous form of claim 16, wherein the amorphous form has a ^{13}C solid state NMR peak pattern comprising at least three or at least four of the five peaks at chemical shifts 163.6 ± 0.2, 138.9 ± 0.2, 131.4 ± 0.2, 129.9 ± 0.2, and 30.8 ± 0.2 ppm.

19. The amorphous form of claim 16, wherein the amorphous form has a ^{13}C solid state NMR peak pattern comprising peaks at chemical shifts position essentially the same as shown in Figure 6.

20. The amorphous form of any of claims 16-19, wherein the amorphous form is substantially pure.

21. A solid form of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide comprising at least two forms selected from polymorph Form I, polymorph Form II and an amorphous form.

22. The solid form of claim 21, comprising at least 80% of polymorph Form I.

23. A pharmaceutical composition comprising a form of (2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide of any of claims 1-22.

24. A method of treating cancer in a mammal comprising administering to the mammal in need a therapeutically effective amount of a pharmaceutical composition of claim 23, in combination with a therapeutically effective amount of an anti-cancer treatment selected from an anti-cancer agent and radiation therapy.

25. A method of treating a mammalian disease condition mediated by CHK1 protein kinase activity, comprising administering to a mammal in need thereof a form of 2R,Z)-2-amino-2-cyclohexyl-N-(5-(1-methyl-1H-pyrazol-4-yl)-1-oxo-2,6-dihydro-1H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide of any of claims 1-23, in combination with a therapeutically effective amount of an anti-cancer treatment selected from an anti-cancer agent and radiation therapy.

Figure 1

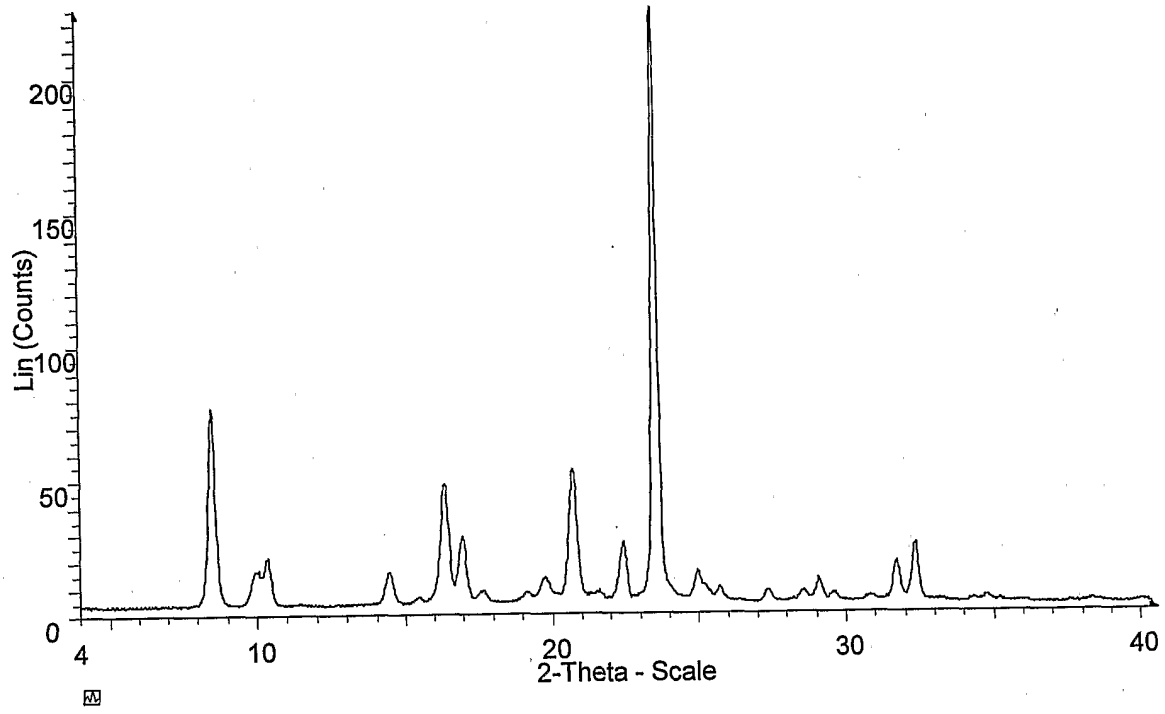


Figure 2

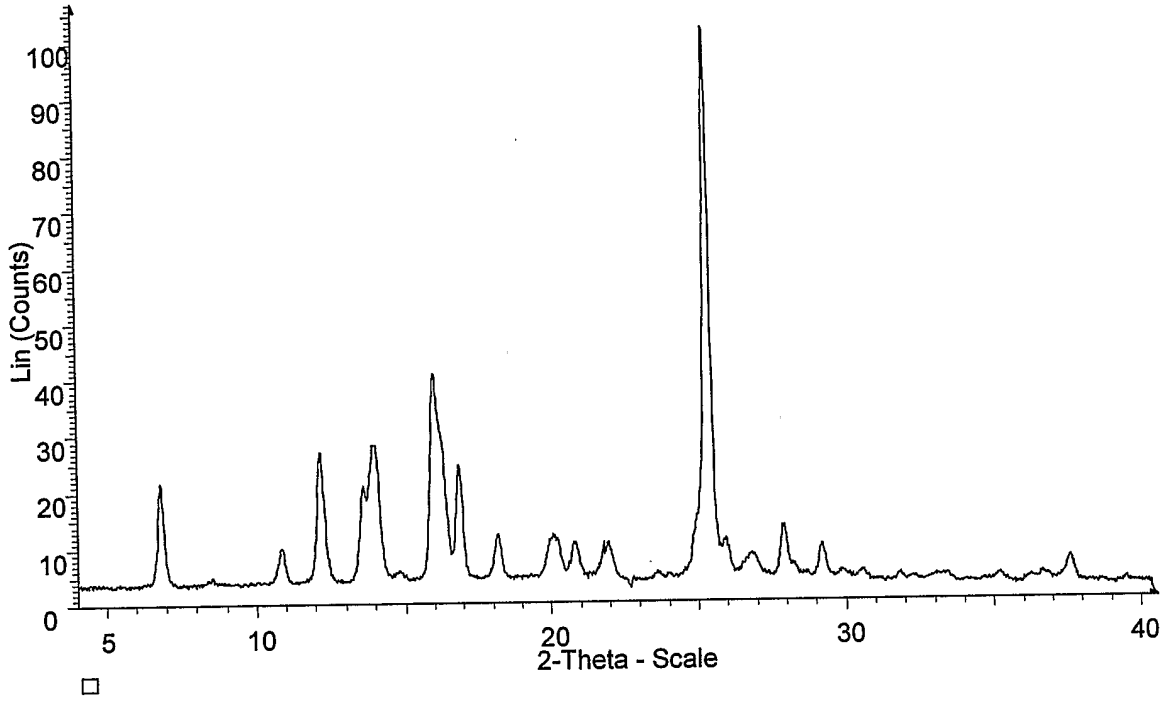

Lin (Counts)
100
90
80
70
60
50
40
30
20
10
0
5
10
20
30
40
2-Theta - Scale

Figure 3a

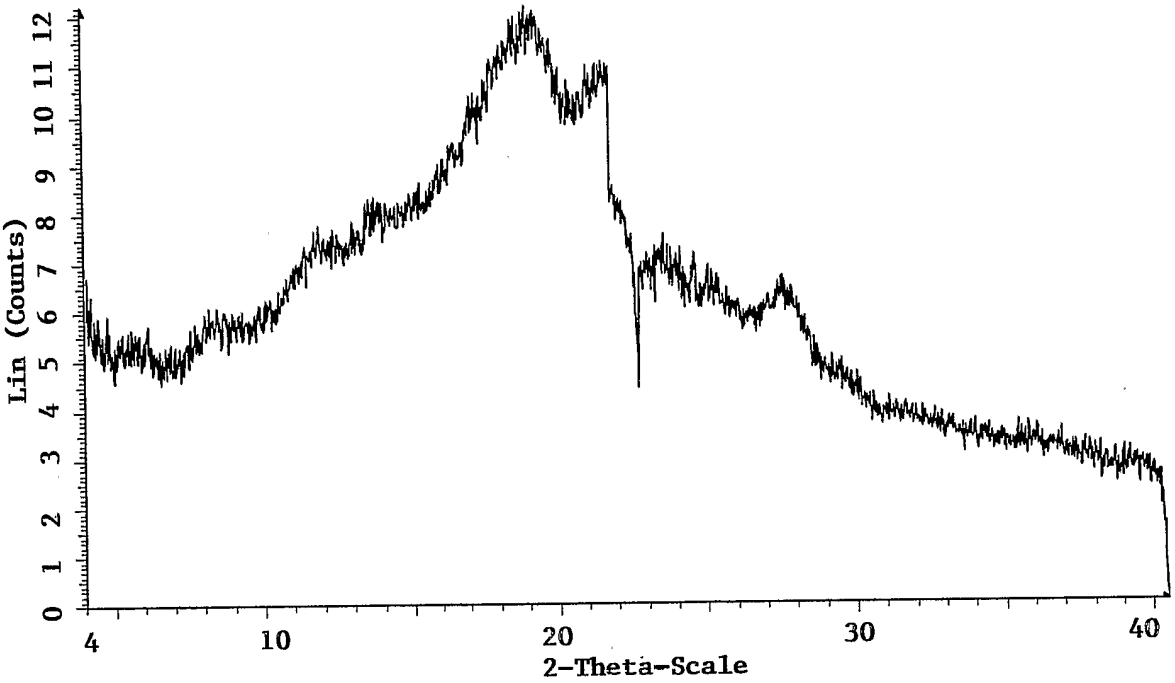


Figure 3b

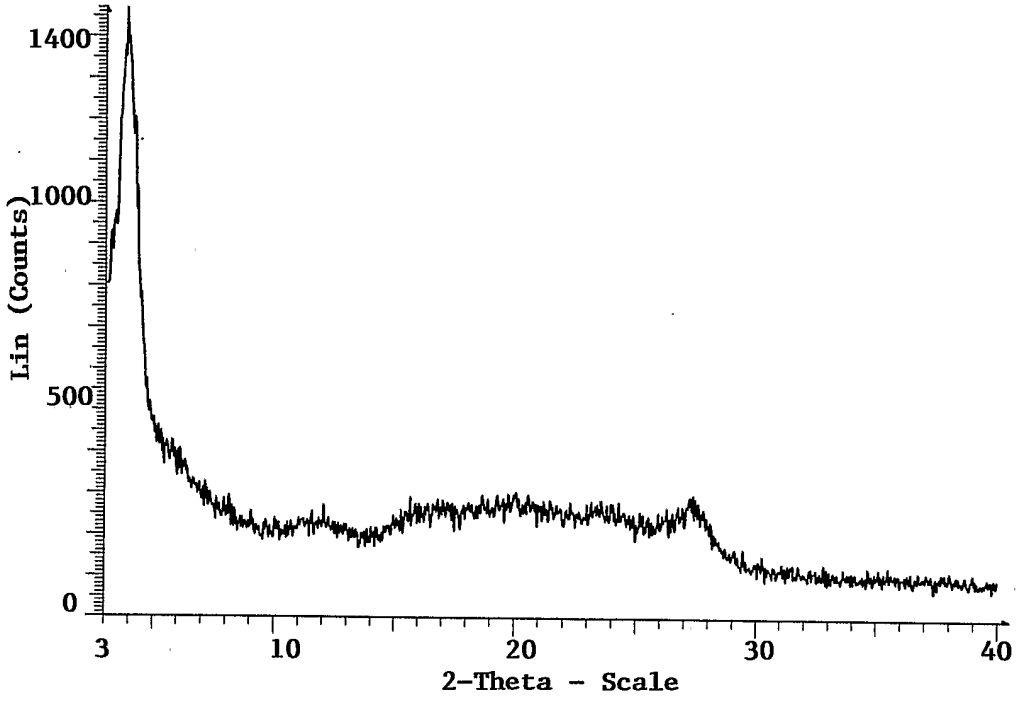


Figure 4a

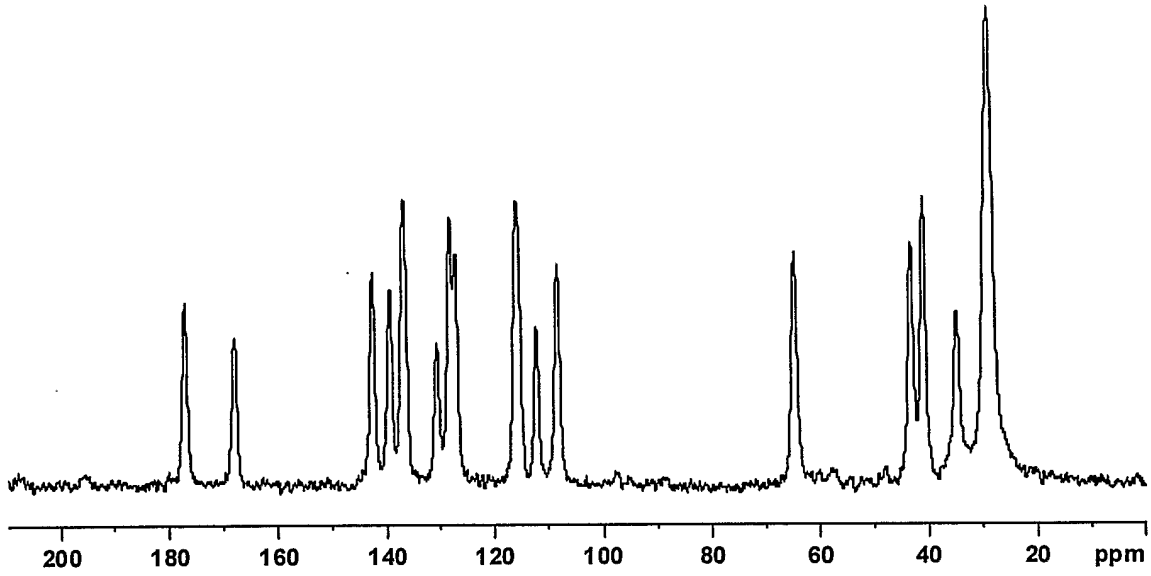


Figure 4b

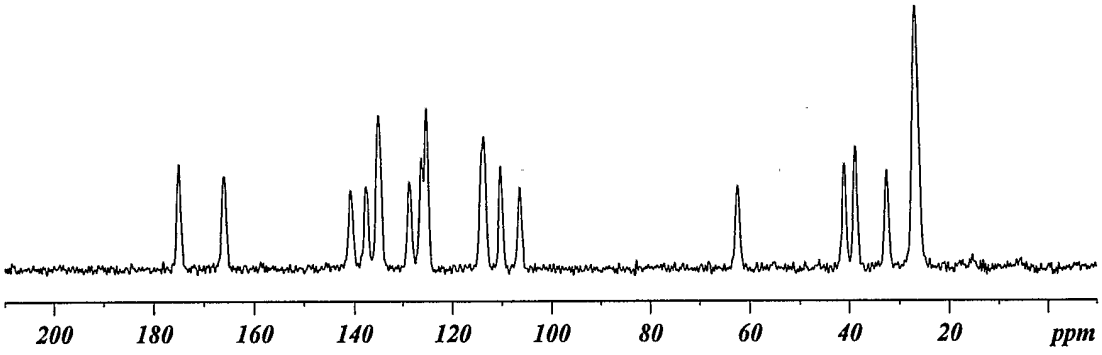


Figure 5a

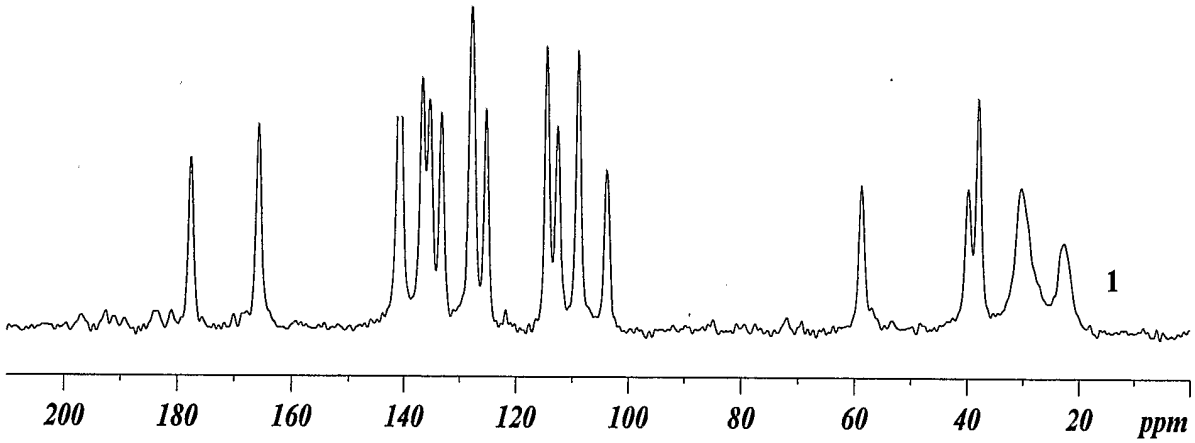


Figure 5b

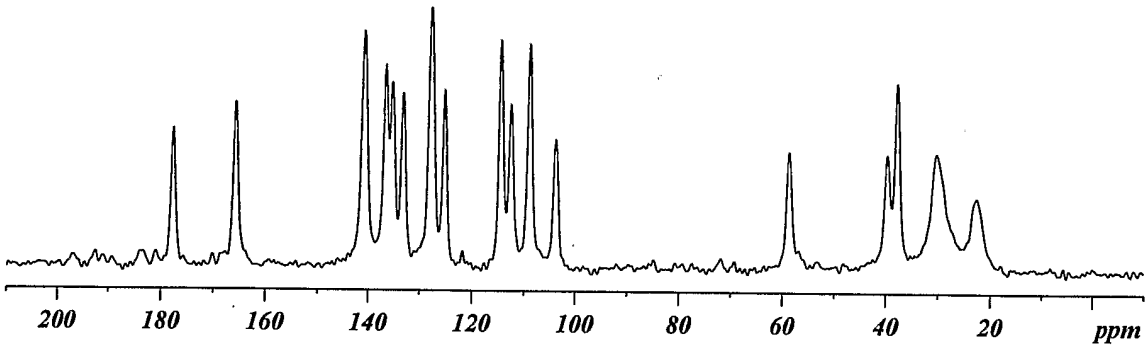
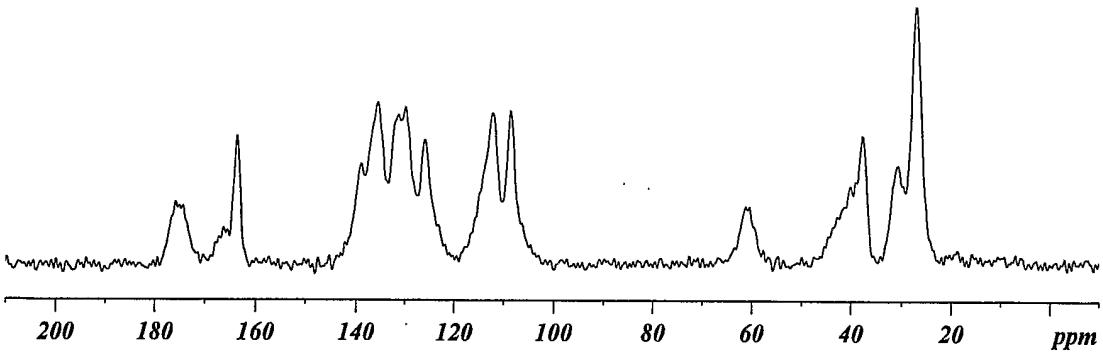


Figure 6



INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2007/000859

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D487/06 A61K31/5517 A61P35/00

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)
C07D 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, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 967 198 B2 (BENEDICT SUZANNE [US] ET AL BENEDICT SUZANNE [US] ET AL) 22 November 2005 (2005-11-22) cited in the application abstract; claim 1; example 258 column 49, line 17 - line 34	1-25

☐ Further documents are listed in the continuation of Box C.

☒ See patent family 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
10 August 2007	21/08/2007

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	Krische, Detlef

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2007/000859

Box II Observations where certain claims were found unsearchable (Continuation of item 2 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 24-25 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 III Observations where unity of invention is lacking (Continuation of item 3 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/IB2007/000859

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6967198 B2	22-11-2005	US 2006004052 A1	05-01-2006
		US 2005075499 A1	07-04-2005