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Inventor(s)	OSTOVIC; Drazen et al.

CRYSTALLINE FORM OF TEGAVIVINT, METHOD OF PREPARATION, AND USE THEREOF

Abstract

The present invention relates to crystalline forms of (9E,10E)-2,7-bis((3,5-dimethylpiperidin-1-yl)sulfonyl)anthracene-9,10-dione dioxime, pharmaceutical compositions comprising the crystalline form, processes for preparing the crystalline form and methods of use therefore.

Inventors: **OSTOVIC; Drazen (Redwood City, CA), Sukumar; Gowri (Spring, TX)**

Applicant: **Iterion Therapeutics, Inc.** (Houston, TX)

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Background/Summary

FIELD OF THE INVENTION

[0001] The present invention relates to crystalline forms of tegavivint, aka, (9E,10E)-2,7-bis((3,5-dimethylpiperidin-1-yl)sulfonyl)anthracene-9,10-dione dioxime, pharmaceutical compositions comprising the crystalline form, processes for preparing the crystalline form, and methods of use thereof.

BACKGROUND OF THE INVENTION

[0002] Cancer is the second leading cause of death in the United States. It presents complex challenges for the development of new therapies. Cancer is characterized by the abnormal growth of malignant cells that have undergone a series of genetic changes that lead to growth of tumor mass and metastatic properties.

[0003] Beta-catenin (β-catenin) is part of a complex of proteins that constitute adherens junctions (AJs). AJs are necessary for the creation and maintenance of epithelial cell layers by regulating cell growth and adhesion between cells. β-catenin also anchors the actin cytoskeleton and may be responsible for transmitting the contact inhibition signal that causes cells to stop dividing once the epithelial sheet is complete.

[0004] Wnt/β-catenin pathway has been shown to play a role in cancer. Aberrant β-catenin signaling plays an important role in tumorigenesis. In particular, colorectal cancer is estimated to have greater than 80% mutations in the β-catenin pathway, leading to unregulated oncogenic signaling. Aberrant β-catenin signaling has been shown to be involved in various cancer types, including but not limited to, melanoma, breast, lung, colon, liver, gastric, myeloma, multiple myeloma, chronic myelogenous leukemia, chronic lymphocytic leukemia, T-cell non-Hodgkin lymphomas, colorectal and acute myeloid leukemia (AML) cancers. Further, aberrant Wnt/β-catenin signaling has been found in a large number of other disorders, including osteoporosis, osteoarthritis, polycystic kidney disease, diabetes, schizophrenia, vascular disease, cardiac disease, hyperproliferative disorders, neurodegenerative diseases, and fibrotic diseases including but not limited to idiopathic pulmonary fibrosis (IPF), Dupuytren's contracture, Nonalcoholic steatohepatitis (NASH), and others. Myeloproliferative neoplasms (MPNs) are a closely related group of hematological malignancies in which the bone marrow cells that produce the body's blood cells develop and function abnormally. The three main myeloproliferative neoplasms are Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF). A gene mutation in JAK2 is present in most PV patients and 50% of ET and PMF patients. The beta catenin pathway is activated in MPN in many cases and required for survival of these cells.

[0005] Tegavivint and related compounds are described, for example, in U.S. Pat. No. 8,129,519. Tegavivint has the following structural formula:

##STR00001##

The chemical name is (9E,10E)-2,7-bis((3,5-dimethylpiperidin-1-yl)sulfonyl)anthracene-9,10-dione dioxime

[0006] The molecular formula of tegavivint is C.sub.28H.sub.36N.sub.4O.sub.6S.sub.2.

[0007] The molecular mass of tegavivint is 588.20763 amu.

[0008] The small scale chemical synthesis of tegavivint had been disclosed in U.S. Pat. No. 8,129,519. The drug substance/Active Pharmaceutical Ingredient (API) has good chemical and physical stability. However, there is a major concern about physical stability of nanoparticle formulations of tegavivint over time that can manifest as crystal growth (Oswald ripening), or a polymorphic change, which can result in the increase in large particle count, or in generation of unfavorable particle morphology during long term storage of the formulated drug. Thus, there remains a need to perform crystal investigation to explore suitable/relevant polymorph(s) of

tegavivint that would be feasible for milling and formulation development to yield a formulation with good long term physical stability. The present invention advantageously addresses this need.

SUMMARY OF THE INVENTION

[0009] The present application discloses an invention to address the foregoing challenges and need by providing a crystalline single polymorphic form of tegavivint, referred to throughout this application as Form IV. The current formulation of tegavivint is a nanosuspension created utilizing a milling process. While Form I (BC-2059 obtained from chemical synthesis as is) has been currently utilized as the starting material for the milling process and the end product obtained from milling is nanosuspension of Form I. However, the inventors of present invention have unexpectedly found that there are specific advantages of utilizing Form IV (in comparison to Form I) as the starting material for the milling process to prepare a nanosuspension of tegavivint.

[0010] The main advantage is that Form IV is sufficiently unstable so that Form IV gets converted to Form I when milled at an elevated temperature (60° C.). Thus, the system will undergo a full solvent-mediated recrystallization from Form IV to Form I. The crystals for Form I will grow "bottom-up" as they are milled, so the chance of getting any unmilled larger crystals would be significantly diminished. In other words, it is beneficial to utilize Form IV as the starting material because it will eventually be converted to Form I and the only Form I crystals would come from re-crystallization from Form IV. Thus, facilitating suspension with a single polymorph form generated through milling at elevated temperatures would in turn enhance the stability of the suspension.

[0011] Thus, in one embodiment, the invention provides a crystalline form of tegavivint, referred to as Form IV which has an X-ray powder diffraction pattern (XRPD) comprising diffraction peaks having °2θ angles selected from the group consisting of 5.0+−0.2°; 7.5+−0.2°; 7.7+−0.2°; 10.2+−0.2°; 14.8+−0.2°; 15.2+−0.2°; 15.4+−0.2°; 18.0+−0.2°; 20.0+−0.2°; 20.5+−0.2°; and 22.2+−0.2°.

[0012] In one embodiment, Form IV can be a single crystal.

[0013] In one embodiment, Form IV is a trihydrate.

[0014] In another embodiment, Form IV has an endothermic peak at about 115.9° C.

[0015] In another embodiment, Form IV has an onset of exothermic peak at about 147.1° C.

[0016] In yet another embodiment, exothermic decomposition of Form IV starts at about 280° C.

[0017] In yet another embodiment, the invention provides a nanosuspension of tegavivint wherein the nanosuspension was prepared by a process comprising using Form IV as the starting material and milling Form IV at a temperature of between about 40° C. and about 60° C., most preferably at about 60° C.

[0018] In one embodiment, if the milling process is done at temperature of less than about 60° C., the nanosuspension has to further undergo the annealing process at or above 60° C.

[0019] In another embodiment of the invention, pharmaceutical compositions are provided for use in the methods comprising stable nanosuspensions of Form I prepared using Form IV as the starting material, and pharmaceutically acceptable excipient.

[0020] In another embodiment of the invention, provided herein are methods for preventing, treating or ameliorating cancer or tumor metastasis in a mammal in need thereof comprising administering to said mammal an effective amount of the compositions of the invention.

Description

BRIEF DESCRIPTION OF THE FIGURES

[0021] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0022] FIG. **1** illustrates an X-ray powder diffraction (XRPD) pattern of Form I of tegavivint.

[0023] FIG. **2** illustrates a differential scanning calorimetry (DSC) curve and Thermogravimetric

Analysis (TGA) curve of Form I.
[0024] FIG. **3** shows a Polarized Light Microscopy (PLM) image of Form I.
[0025] FIG. **4** shows XRPD patterns overlay of Form II preparation.
[0026] FIG. **5** shows XRPD patterns overlay of Form III preparation.
[0027] FIG. **6** illustrates a DSC curve and TGA curve of Form III.
[0028] FIG. **7** shows a PLM image of Form III.
[0029] FIG. **8** shows XRPD patterns overlay of Form III samples after drying.
[0030] FIG. **9**A shows XRPD patterns of Form IV samples.
[0031] FIG. **9**B shows a DSC curve and TGA curve of Form IV.
[0032] FIG. **9**C shows a Dynamic Vapor Sorption (DVS) profile of Form IV.
[0033] FIG. **9**D shows a PLM image of Form IV.
[0034] FIG. **9**E shows Variable Temperature X-ray Powder Diffraction (VT-XRPD) profile of Form IV.
[0035] FIG. **9**F shows XRPD patterns overlay of Form IV sample after VT-XRPD and exposure to ambient conditions.
[0036] FIG. **10** shows XRPD pattern of Form V.
[0037] FIG. **11** shows XRPD pattern of Form VI.
[0038] FIG. **12**A illustrates XRPD pattern of amorphous sample.
[0039] FIG. **12**B shows a modulate DSC (mDSC) curve of amorphous sample.
[0040] FIG. **13**A shows XRPD pattern of solid obtained from slurry competition in water.
[0041] FIG. **13**B shows XRPD pattern of solid obtained from slurry competition in ACN/water (1:1, v/v).
[0042] FIG. **13**C shows XRPD pattern of solid obtained from slurry competition in ACN/water (1:3, v/v).
[0043] FIG. **14**A is a Particle Size Distribution (PSD) plot of Form I sample milled at 5° C.
[0044] FIG. **14**B is a PSD plot of Form I sample milled at RT.
[0045] FIG. **14**C is a PSD plot of Form I sample milled at 60° C.
[0046] FIG. **14**D is a PSD plot of Form IV sample milled at 5° C.
[0047] FIG. **14**E is a PSD plot of Form IV sample milled at RT.
[0048] FIG. **14**F is a PSD plot of Form IV sample milled at 60° C.
[0049] FIG. **14**G is a PLM image of Form I and Form IV.
[0050] FIG. **14**H is a PLM image of Form I sample milled at 5° C.
[0051] FIG. **14**I is a PLM image of Form I sample milled at RT.
[0052] FIG. **14**J is a PLM image of Form I sample milled at 60° C.
[0053] FIG. **14**K is a PLM image of Form IV sample milled at 5° C.
[0054] FIG. **14**L is a PLM image of Form IV sample milled at RT.
[0055] FIG. **14**M is a PLM image of Form IV sample milled at 60° C.
[0056] FIG. **14**N shows XRPD patterns overlay of Form I sample milled at 5° C.
[0057] FIG. **14**O shows XRPD patterns overlay of Form I sample milled at RT.
[0058] FIG. **14**P shows XRPD patterns overlay of Form I sample milled at 60° C.
[0059] FIG. **14**R shows XRPD patterns overlay of Form IV sample milled at 5° C.
[0060] FIG. **14**S shows XRPD patterns overlay of Form IV sample milled at RT.
[0061] FIG. **14**T shows XRPD patterns overlay of Form IV sample milled at 60° C.

DETAILED DESCRIPTION OF THE INVENTION

[0062] The present invention relates to crystalline forms of tegavivint. In particular, the present invention relates to a crystalline form designated Form IV of tegavivint, pharmaceutical compositions comprising the crystalline form, processes for preparing the crystalline form and methods of use thereof.
[0063] In one embodiment, the crystalline form of tegavivint is designated as Form IV, which has an X-ray powder diffraction pattern (XRPD) comprising diffraction peaks having °2θ angle values

independently selected from the group consisting of 5.0+−0.2°; 7.5+−0.2°; 14.8+−0.2°; 15.2+−0.2°; 15.4+−0.2°; 20.0+−0.2°; and 22.2+−0.2°.

[0064] In one embodiment, Form IV has an XRPD comprising diffraction peaks having °2θ angle values independently selected from the group consisting of 5.0+−0.2°; 7.5+−0.2°; 7.7+−0.2°; 14.8+−0.2°; 15.2+−0.2°; 15.4+−0.2°; 20.0+−0.2°; and 22.2+−0.2°.

[0065] In one embodiment, Form IV has an XRPD comprising diffraction peaks having °2θ angle values independently selected from the group consisting of 5.0+−0.2°; 7.5+−0.2°; 7.7+−0.2°; 10.2+−0.2°; 14.8+−0.2°; 15.2+−0.2°; 15.4+−0.2°; 20.0+−0.2°; and 22.2+−0.2°.

[0066] In another embodiment, Form IV has an XRPD comprising diffraction peaks having °2θ angle values independently selected from the group consisting of 5.0+−0.2°; 7.5+−0.2°; 7.7+−0.2°; 10.2+−0.2°; 14.8+−0.2°; 15.2+−0.2°; 15.4+−0.2°; 18.0+−0.2°; 20.0+−0.2°; and 22.2+−0.2°.

[0067] In another embodiment, Form IV has an XRPD comprising diffraction peaks having °2θ angle values independently selected from the group consisting of 5.0+−0.2°; 7.5+−0.2°; 7.7+−0.2°; 10.2+−0.2°; 14.8+−0.2°; 15.2+−0.2°; 15.4+−0.2°; 18.0+−0.2°; 20.0+−0.2°; 20.5+−0.2°; and 22.2+−0.2°.

[0068] In another embodiment, Form IV has an XRPD pattern substantially as shown in FIG. **9**A.

[0069] In another embodiment, Form IV is characterized by having an endotherm with a peak maximum at approximately 115.9° C. by differential scanning calorimetry (DSC).

[0070] In another embodiment, Form IV is characterized by having an onset of exothermic peak at approximately 147.1° C. by DSC.

[0071] In one embodiment, Form IV is characterized by having a DSC thermogram substantially as shown in FIG. **9**B.

[0072] In one embodiment, Form IV is a trihydrate which is isolated from solvents with high water activity. The crystal morphology is needles. The trihydrate dehydrates thermally with the half-point of dehydration at about 60° C.

[0073] In yet another embodiment, the invention provides a nanosuspension of tegavivint wherein the nanosuspension was prepared by a process comprising using Form IV as the starting material and milling Form IV at a temperature of between about 40° C. and about 60° C., most preferably at about 60° C.

[0074] In one embodiment, if the milling process is done a temperature of less than about 60° C., the nanosuspension has to further undergo the annealing process at or above 60° C.

[0075] In another embodiment of the invention, pharmaceutical compositions are provided for use in the methods comprising stable nanosuspensions of Form I prepared using Form IV as the starting material, and pharmaceutically acceptable excipient.

[0076] In another embodiment of the invention, provided herein are methods for preventing, treating or ameliorating cancer or tumor metastasis in a mammal in need thereof comprising administering to said mammal an effective amount of the compositions of the invention.

[0077] The crystalline forms of tegavivint may be formulated by any method well known in the art and may be prepared for administration by any route, including, without limitation, parenteral, oral, sublingual, transdermal, topical, intranasal, intratracheal, or intrarectal. In certain embodiments, the crystalline form of tegavivint is administered intravenously in a hospital setting. In one embodiment, administration may be by the oral route.

[0078] The characteristics of the carrier will depend on the route of administration. As used herein, the term “pharmaceutically acceptable” means a non-toxic material that is compatible with a biological system such as a cell, cell culture, tissue, or organism, and that does not interfere with the effectiveness of the biological activity of the active ingredient(s). Thus, compositions may contain, in addition to the inhibitor, diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The preparation of pharmaceutically acceptable formulations is described in, e.g., Remington's Pharmaceutical Sciences, 18th Edition, ed. A. Gennaro, Mack Publishing Co., Easton, Pa., 1990.

[0079] The pharmaceutical compositions comprising a crystalline form of tegavivint may be used in the methods of use described herein.
[0080] The active compound is included in the pharmaceutically acceptable carrier or diluent in an amount sufficient to deliver to a patient a therapeutically effective amount without causing serious toxic effects in the patient treated. The effective dosage range of the pharmaceutically acceptable derivatives can be calculated based on the weight of the parent compound to be delivered. If the derivative exhibits activity in itself, the effective dosage can be estimated as above using the weight of the derivative, or by other means known to those skilled in the art.
[0081] In some embodiments of any of the methods described herein, before treatment with the compositions or methods of the invention, the patient may be treated with one or more of a chemotherapy, a targeted anticancer agent, radiation therapy, and surgery, and optionally, the prior treatment was unsuccessful; and/or the patient has been administered surgery and optionally, the surgery was unsuccessful; and/or the patient has been treated with a platinum-based chemotherapeutic agent, and optionally, the patient has been previously determined to be non-responsive to treatment with the platinum-based chemotherapeutic agent; and/or the patient has been treated with a kinase inhibitor, and optionally, the prior treatment with the kinase inhibitor was unsuccessful; and/or the patient was treated with one or more other therapeutic agent(s).
Definitions
[0082] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. All patents, patent applications, and publications referred to herein are incorporated by reference.
[0083] As used herein, “tegavivint” refers to (9E,10E)-2,7-bis((3,5-dimethylpiperidin-1-yl)sulfonyl)anthracene-9,10-dione dioxime.
[0084] As used herein, the term “Form IV” or “Crystalline Form IV” when used alone refers to Crystalline Form IV of (9E,10E)-2,7-bis((3,5-dimethylpiperidin-1-yl)sulfonyl)anthracene-9,10-dione dioxime.
[0085] As used herein, the term “subject,” “individual,” or “patient,” used interchangeably, refers to any animal, including mammals such as mice, rats, other rodents, rabbits, dogs, cats, swine, cattle, sheep, horses, primates, and humans. In some embodiments, the patient is a human. In some embodiments, the subject has experienced and/or exhibited at least one symptom of the disease or disorder to be treated and/or prevented. In some embodiments, the subject is suspected of having a multi-tyrosine kinase-associated cancer.
[0086] As used herein, a “therapeutically effective amount” of a crystalline form of tegavivint is an amount that is sufficient to ameliorate, or in some manner reduce a symptom or stop or reverse progression of a condition, or negatively modulate or inhibit the activity of a multi-tyrosine kinase. Such amount may be administered as a single dosage or may be administered according to a regimen, whereby it is effective.
[0087] As used herein, “treatment” means any manner in which the symptoms or pathology of a condition, disorder or disease are ameliorated or otherwise beneficially altered. Treatment also encompasses any pharmaceutical use of the compositions herein.
[0088] As used herein, amelioration of the symptoms of a particular disorder by administration of a particular pharmaceutical composition refers to any lessening, whether permanent or temporary, lasting or transient that can be attributed to or associated with administration of the composition.
[0089] As used herein, the term “about” when used to modify a numerically defined parameter (e.g., the dose of a crystalline form of tegavivint detailed herein or a pharmaceutically acceptable salt thereof, or the length of treatment time described herein) means that the parameter may vary by as much as 10% below or above the stated numerical value for that parameter. For example, a dose of about 5 mg/kg may vary between 4.5 mg/kg and 5.5 mg/kg. “About” when used at the beginning of a listing of parameters is meant to modify each parameter. For example, about 0.5 mg, 0.75 mg or 1.0 mg means about 0.5 mg, about 0.75 mg or about 1.0 mg. Likewise, about 5% or more, 10%

or more, 15% or more, 20% or more, and 25% or more means about 5% or more, about 10% or more, about 15% or more, about 20% or more, and about 25% or more.

[0090] As used herein, the term “about” when used in reference to XRPD peak positions refers to the inherent variability of peaks depending on the calibration of the instrument, processes used to prepare the crystalline forms of the present invention, age of the crystalline forms and the type of instrument used in the analysis. The variability of the instrumentation used for XRPD analysis was about +0.2° 20.

[0091] As used herein, the term “about” when used in reference to DSC endothermic peak onset refers to the inherent variability of peaks depending on the calibration of the instrument, method used to prepare the samples of the present invention, and the type of instrument used in the analysis. The variability of the instrumentation used for DSC analysis was about +2° C.

GENERAL METHODS

[0092] The general methods outlined below were used in the exemplified Examples, unless otherwise noted.

[0093] Crystalline forms of the present invention may be prepared using a variety of methods well known to those skilled in the art including crystallization or recrystallization from a suitable solvent or by sublimation. A wide variety of techniques may be employed, including those in the exemplified Examples, for crystallization or recrystallization including evaporation of a water-miscible or a water-immiscible solvent, crystal seeding in a supersaturated solvent mixture, decreasing the temperature of the solvent mixture, or freeze drying the solvent mixture.

[0094] In the present invention, crystallization may be done with or without crystal seed. The crystal seed may come from any previous batch of the desired crystalline form. The addition of crystal seed may not affect the preparation of the crystalline forms in the present invention. The sample was recovered after completion of the isotherm and re-analyzed by XRPD.

ABBREVIATIONS AND ACRONYMS

TABLE-US-00001 Category Abbreviations Full Name/Description Analytical DSC Differential Scanning Calorimetry Techniques DVS Dynamic Vapor Sorption NMR Nuclear Magnetic Resonance PLM Polarized light microscopy PSD Particle Size Distribution TGA Thermogravimetric Analysis XRPD X-ray Powder Diffraction VT-XRPD Variable Temperature X-ray Powder Diffraction ACN Acetonitrile CHCl.sub.3 Chloroform DMF Dimethylforamide DMSO Dimethylsulfoxide EtOAc Ethyl acetate EtOH Ethanol IPA Isopropyl alcohol Solvent MEK Methyl ethyl ketone MeOH Methanol MTBE Methyl-tert-butyl ether THF Tetrahydrofuran Other RT Room temperature v/v percent volume ratio

[0095] The following Examples are intended to illustrate further certain embodiments of the invention and are not intended to limit the scope of the invention.

Example 1

Investigation of Form I of Tegavivint

[0096] This Example illustrates the investigation of Form I of tegavivint.

[0097] Starting material (Form I of tegavivint) was characterized by XRPD, TGA, DSC and PLM. XRPD pattern displayed in FIG. **1** showed the starting material was crystalline and confirmed to be Form I. TGA and DSC curves are displayed in FIG. **2**. A weight loss of 0.4% up to 150° C. was observed on TGA curve, and DSC result showed no melting endotherm before decomposition. Based on the characterization results, Form I was speculated to be an anhydrate. PLM image displayed in FIG. **3** showed irregular fine particles with partial aggregation for Form I sample.

Example 2

Preparation of Form II of Tegavivint

[0098] Attempts to prepare Form II of tegavivint were performed in four conditions. The detailed results are shown in FIG. **4** and Table 1. The results suggested that Form II was quite challenging to be re-prepared or metastable.

TABLE-US-00002 TABLE 1 Summary of Form II Preparation Attempts Anti-solvent, ID Method

Solvent, mL mL Results 803759-05-A1 Anti-solvent EtOH, 2.5 H.sub.2O, 2 Form III addition 803759-05-A2 Anti-solvent EtOH, 2.5 H.sub.2O, 2 Form I addition 803759-05-A3 Solution vapor EtOH, 2 H.sub.2O Form I diffusion 803759-05-A4 Slow EtOH, 2 n-heptane, 2 Form I evaporation

Example 3

Preparation of Form III of Tegavivint

[0099] Form III samples (803759-03-A and 803759-05-A1) were prepared via anti-solvent addition in MeOH/H.sub.2O and EtOH/H.sub.2O systems, and the XRPD results are displayed in FIG. **5**. The TGA/DSC results of Form III (803759-05-A1) are displayed in FIG. **6**. A weight loss of 8.2% up to 100° C. was observed on TGA. One endotherm at 64.5° C. and one exotherm at 158.6° C. were observed before decomposition on DSC. The PLM image displayed in FIG. **7** showed needle-like and fine particles with aggregation for Form III sample (803759-05-A1).

[0100] Since Form III samples could be obtained from different solvent systems and converted to Form IV after vacuum drying at RT or Form V after exposure to ambient condition at RT (FIG. **8**), Form III might be isomorphic.

Example 4

Preparation of Form IV of Tegavivint

[0101] Form IV (803759-13-B) sample was re-prepared at 2-g scale. The detailed procedures were as follows:

[0102] 1. Weigh 2.0 g 803759-01-A sample into a 1-L reactor.

[0103] 2. Charge 200 mL EtOH, and stir at RT with 300 rpm to obtain a clear solution

[0104] 3. Charge 100 mL water.

[0105] 4. Add 90.2 mg Form IV seed, a suspension was observed.

[0106] 5. Charge 100 mL water over 1 h.

[0107] 6. Keep slurry for 2 h.

[0108] 7. Filter and test XRPD of the wet cake.

[0109] 8. Transfer the wet cake into a 1-L reactor, charge 200 mL water and slurry overnight.

[0110] 9. Filter and vacuum dry for 4 hrs. 1.9 g solids were obtained (803759-13-B, Form IV).

[0111] The XRPD results of Form IV are displayed in FIG. **9**A. The TGA/DSC results were displayed in FIG. **9**B. A weight loss of 8.4% up to 150° C. could be observed on TGA. One endotherm at 115.9° C. (peak) and one exotherm at 147.1° C. (onset) before decomposition were observed on DSC. DVS result displayed in FIG. **9**C showed that: 1) two platforms were observed indicating two potential hydrate forms existing. 2) A water uptake of 9.1% was observed at 25° C./80% RH, which was consistent with TGA weight loss of Form IV. The PLM image displayed in FIG. **9**D showed needle-like particles was observed for Form IV sample (803759-13-B).

[0112] VT-XRPD test was employed for further investigation of Form IV, the results displayed in FIG. **9**E and FIG. **9**F showed that 1) Form IV sample partially converted to a new form at 30° C. under N.sub.2, and the new form was named as Form VI. 2) After heating to 75 and 120° C., pure Form VI was observed. 3) After cooling to 30° C., Form VI was still observed. 4) After exposure to ambient condition, Form VI converted to Form IV quickly. Thus, Form IV is believed to be a hydrate.

Example 5

Preparation of Form V of Tegavivint

[0113] Form V sample (803759-03-A 22Apr) was obtained via drying Form III sample (803759-03-A) at ambient condition, the XRPD pattern was displayed in FIG. **10**. The TGA and DSC data were not collected due to limited solid.

Example 6

Preparation of Form VI of Tegavivint

[0114] Form VI sample (803759-02-B_N2 back_30.0° C.) was obtained during VT-XRPD test for Form IV sample (803759-02-B). The XRPD pattern is displayed in FIG. **11**. After exposure to ambient condition, Form VI converted to Form IV quickly (FIG. **9**F). Since Form VI was unstable

under ambient condition, no further characterization data was collected for Form VI, and Form VI was speculated to be anhydrate.

Example 7

Preparation of Amorphous Form of Tegavivint

[0115] Amorphous sample (803759-04-B3 dry) was prepared by reverse anti-solvent addition in DMSO/H.sub.2O system and vacuum drying at RT. The XRPD pattern is displayed in FIG. **12**A. The mDSC result displayed in FIG. **12**B showed the Tg of the amorphous sample was 65.0° C. (middle temperature).

Example 8

Slurry Competition Experiments

[0116] To determine the most stable form under high water activity at RT, slurry competition of Form I, III, IV and V was performed in three solvent systems (water, ACN/H.sub.2O (1:1, v/v) and ACN/H.sub.2O (1:3, v/v)) with high water activity at RT.

[0117] The detailed procedure was as follows: [0118] 1) Prepare saturated solution with Form I sample (803759-01-A) in three solvent systems. [0119] 2) Add about 10 mg of each form into 1 mL corresponding saturated solution. [0120] 3) Slurry and check the XRPD of the wet cake after one day and six days.

[0121] The results are displayed in Table 2, FIG. **13**A, FIG. **13**B and FIG. **13**C. The results showed: [0122] 1) Form IV was obtained from water system. [0123] 2) A new form was obtained from ACN/H.sub.2O (1:1, v/v) system, which was speculated to be ACN solvate. [0124] 3) Form I was obtained from ACN/H.sub.2O (1:3, v/v) system.

TABLE-US-00003 TABLE 2 Summary of slurry competition ACN/H.sub.2O ACN/H.sub.2O Starting Material H.sub.2O (1:1, v/v) (1:3, v/v) Form I (803759-01-A), Form IV New form Form I Form III (803759-05-A1), (speculated to be Form IV (803759-02-B) and ACN solvate) Form V (803759-03-A22Apr)

Example 9

Ball Milling Experiments

[0125] Previously, Form I was milled to a very small particle size and started converting to Form IV in aqueous suspension with unacceptable particle size growth during storage. Therefore, ball milling of Form I and Form IV was performed to evaluate the form stability and particle size growth. The detailed procedure of ball milling was as follows. [0126] 1. Suspend Form I and Form IV sample in 1% Poloxamer 188 water solution (50 mg/mL), separately. [0127] 2. Add ˜12 mL suspension into a 50-mL tube which contained milling beads (the volume of milling beads is around 30 mL). After 12 mL suspension was added, the liquid surface just covered the beads. [0128] 3. Roll the 50-mL tube (containing beads and suspension) at 5° C., RT and 60° C. with 30 rpm. [0129] 4. Sample ˜0.8 mL suspension at 1, 2, 4, 24 h using 1-mL syringe. [0130] 5. Test XRPD, PSD and PLM for the suspension.

[0131] The results displayed in Table 3 and in FIGS. **14**A to **14**T showed that: [0132] 1) form conversion was observed for Form IV at RT after 24 h and 60° C. after 2 h. [0133] 2) the particle size was decreased during milling. [0134] 3) Aggregation was observed for Form I at 5° C. and Form IV at 5° C. and RT after 24 h.

[0135] Therefore, ball milling of Form I sample at elevated temperature (60° C.) was recommended to reduce the particle size.

[0136] Additionally, the anticipated outcome is that milling of Form I at 60° C. or higher should prevent formation crystal seeds for the undesirable Form IV and result in a highly crystalline milled material that is annealed and free of high energy particles and free of amorphous material.

[0137] Ball milling of Form IV at elevated temperature (60° C.) confirmed conversion to Form I.

TABLE-US-00004 TABLE 3 Summary of ball milling for Form I and Form IV at different temperatures Experiment ID 803759-15-A 803759-14-A1 803759-14-A2 Initial Form Form 1 Temperature, ° C. 5 RT 60 1 h Form Form I Form I Form I D 90 (μm) 9.4 22.4 22.6 2 h Form

Form I Form I Form I D 90 (μm) 9.0 21.4 6.6 4 h Form Form I Form I Form I D 90 (μm) 5.2 14.2 5.3 24 h Form Form I Form I Form I D 90 (μm) 5.6 2.8 2.0 Experiment ID 803759-15-B 803759-14-B1 803759-14-B2 Initial Form Form IV Temperature, ° C. 5 RT 60 1 h Form Form IV Form IV Form IV D 90 (μm) 33.7 6.6 3.9 2 h Form Form IV Form IV Form I + IV D 90 (μm) 17.4 4.0 4.0 4 h Form Form IV Form IV Form I D 90 (μm) 5.8 3.4 2.0 24 h Form Form IV Amorphous Form I D 90 (μm) 9.8 5.8 0.6

[0138] To summarize, five crystal forms and amorphous sample of tegavivint were obtained. The summary is displayed in Table 4.

TABLE-US-00005 TABLE 4 Summary of tegavivint polymorphs Endotherm Polymorph (ID) Weight Loss (%) (° C., onset) Form Form I (803759-01-A) 0.4 (150) ND Anhydrate Form III (803759-05-A1) 8.2 (100) .sup. 64.5, 158.6* Unidentified Form IV (803759-13-B) 8.4 (100) 115.9.sup.#, 147.1* Hydrate Form V (803759-03-A 22 Apr) NA NA Unidentified Form VI (803759-02-B_N2back_30.0° C.) NA NA Anhydrate Amorphous (803759-04-B3 dry) NA 65.0** Amorphous ND: no thermal event was observed before decomposition. *exothermic peak. .sup.#peak temperature. **glass transition temperature (middle temperature). NA: the data was not collected

APPENDIX

Instruments and Methods

XRPD

[0139] For XRPD analysis, PANalytical X'Pert.sup.3 X-ray powder diffractometer was used. The XRPD parameters used are listed in Table 5.

TABLE-US-00006 TABLE 5 Parameters for XRPD test X′ Pert# Parameters (reflection mode) X-Ray Cu, Kα, Kα1 (Å): 1.540598: Kα2 (Å): 1.544426 Kα2/Kα1 intensity ration: 0.50 X-Ray tube setting 45 KV, 40 mA Divergence slit 1/8° Scan mode Continuous Scan range (°2Theta) 3°~40° Scan step time (s) 46.667 Step size (°2Theta) 0.0263° Test time (min) ~5 min

TGA, DSC and mDSC

[0140] TGA data were collected using a TA Discovery 5500/Q5000 TGA from TA Instruments. DSC and mDSC were performed using a TA Discovery 2500 DSC from TA Instruments. Detailed parameters used are listed in Table 6 and Table 7.

TABLE-US-00007 TABLE 6 Parameters for TGA and DSC test Parameters TGA DSC Method Ramp Ramp Sample pan Aluminum, open Aluminum crimped Temperature RT~350° C. 25~300° C. Heating rate 10° C./min 10° C./min Purge gas N.sub.2 N.sub.2

TABLE-US-00008 TABLE 7 Parameters for mDSC test Parameters mDSC Method Conventional Sample pan Aluminum crimped Temperature 25~200° C. Period 60 s Heating rate 3° C./min Purge gas N.sub.2

DVS

[0141] DVS was measured via a SMS (Surface Measurement Systems) DVS Intrinsic. The relative humidity at 25° C. were calibrated against deliquescence point of LiCl, Mg(NO.sub.3).sub.2 and KCl. Parameters for DVS test were listed in Table 8.

TABLE-US-00009 TABLE 8 Parameters for DVS test Parameters DVS Temperature 25° C. Sample size 10~20 mg Gas and flow rate N.sub.2, 200 mL/min dm/dr 0.002%/min Min. dm/dt stability duration 10 min Max. equilibrium time 180 min RH range 0% RH to 95% RH RH step size 10% RH from 0% RH to 95% RH 5% RH from 95% RH to 0% RH

PLM

[0142] PLM images were captured using Axio Scope A1 microscope from Carl Zeiss German.

PSD

[0143] Microtrac S3500 with SDC (Sample Delivery Controller) was used for PSD test and the method is shown in Table 9.

TABLE-US-00010 TABLE 9 PSD Method Parameters/Values Parameters/Values Distriction: Volume Runtime: 10 sections/run Dispersive solvent: water Particle size coordinate: Standard Run

number: 3 runs, average Solvent refractive index: 1.33 Transparency: Trans Residuals: Enabled Particle refractive index: 1.59 Flow rate: 60%* Particle Shape: Irregular Filter: On Sonication power: NA Sonication time: NA *60% of the maximum flow rate (65 mL/s)

[0144] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

Claims

1. A crystalline form of a compound having the following formula: ##STR00002## wherein the [001] crystalline form is designated as Form IV and has an X-ray powder diffraction pattern (XRPD) comprising diffraction peaks having °2θ angle values independently selected from the group consisting of 5.0+−0.2°; 7.5+−0.2°; 14.8+−0.2°; 15.2+−0.2°; 15.4+−0.2°; 20.0+−0.2°; and 22.2+−0.2°.

2. The crystalline form according to claim 1, wherein Form IV has an X-ray powder diffraction pattern (XRPD) comprising diffraction peaks having °2θ angle values independently selected from the group consisting of 5.0+−0.2°; 7.5+−0.2°; 7.7+−0.2°; 14.8+−0.2°; 15.2+−0.2°; 15.4+−0.2°; 20.0+−0.2°; and 22.2+−0.2°.

3. The crystalline form according to claim 2, wherein Form IV has an X-ray powder diffraction pattern (XRPD) comprising diffraction peaks having °2θ angle values independently selected from the group consisting of 5.0+−0.2°; 7.5+−0.2°; 7.7+−0.2°; 10.2+−0.2°; 14.8+−0.2°; 15.2+−0.2°; 15.4+−0.2°; 20.0+−0.2°; and 22.2+−0.2°.

4. The crystalline form according to claim 2, wherein Form IV has an X-ray powder diffraction pattern (XRPD) comprising diffraction peaks having °2θ angle values independently selected from the group consisting of 5.0+−0.2°; 7.5+−0.2°; 7.7+−0.2°; 10.2+−0.2°; 14.8+−0.2°; 15.2+−0.2°; 15.4+−0.2°; 18.0+−0.2°; 20.0+−0.2°; and 22.2+−0.2°.

5. The crystalline form according to claim 2, wherein Form IV has an X-ray powder diffraction pattern (XRPD) comprising diffraction peaks having °2θ angle values independently selected from the group consisting of 5.0+−0.2°; 7.5+−0.2°; 7.7+−0.2°; 10.2+−0.2°; 14.8+−0.2°; 15.2+−0.2°; 15.4+−0.2°; 18.0+−0.2°; 20.0+−0.2°; 20.5+−0.2°; and 22.2+−0.2°.

6. The crystalline form according to claim 1, wherein Form IV has an XRPD pattern substantially as shown in FIG. **9**A.

7. The crystalline form according to claim 1, wherein Form IV is characterized by having an endotherm with a peak maximum at approximately 115.9° C. by differential scanning calorimetry (DSC).

8. The crystalline form according to claim 1, wherein Form IV has a DSC thermogram substantially as shown in FIG. **9**B.

9. The crystalline form according to claim 1, wherein Form IV is characterized by having an onset of exothermic peak at approximately 147.1° C. by differential scanning calorimetry (DSC).

10. A nanosuspension of tegavivint prepared by a process comprising using Form IV as the starting material and milling Form IV at a temperature of between about 40° C. and about 60° C., most preferably at about 60° C.

11. A pharmaceutical composition, comprising a therapeutically effective amount of a stable crystalline form of (9E,10E)-2,7-bis((3,5-dimethylpiperidin-1-yl)sulfonyl)anthracene-9,10-dione dioxime prepared by a process utilizing Form IV as the starting material, and a pharmaceutically acceptable excipient and/or diluent.

12. A method for preventing, treating or ameliorating cancer or tumor metastasis in a mammal in

need thereof comprising administering to said mammal an effective amount of the crystalline form according to claim 1 or the pharmaceutical composition according to claim **12**.

13. The method of claim 12, wherein the cancer is acute myeloid leukemia (AML).