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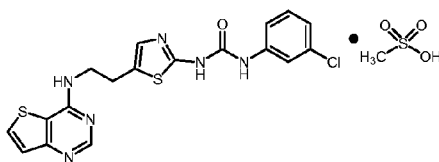
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(1)

(57) Abstract: The present invention provides Compound 1: (Formula should be inserted here) solid forms thereof, compositions thereof, and methods of using the same. The present invention also provides synthetic methods of preparing Compound 1 and intermediates thereto.

AURORA KINASE INHIBITORS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present invention claims priority to United States provisional application serial number 61/036,817, filed March 14, 2008, United States provisional application serial number 61/045,583, filed April 16, 2008, and United States provisional application serial number 61/053,658, filed May 15, 2008, the entirety of each of which is hereby incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0002] Aurora kinases constitute a family of serine-threonine kinases; members of the family are referred to herein collectively as Aurora kinase. Aurora kinase upregulation and/or amplification has been strongly associated with cancer. For example, Aurora kinase overexpression and/or amplification has been observed in cervical cancer, ovarian cancer, and neuroblastoma cell lines [Warner, S.L. et al., *Molecular Cancer Therapeutics* 2:589-95 (2003)]. Furthermore, Aurora kinase overexpression and/or amplification has been observed also in primary clinical isolates of cancers. Additionally, higher expression levels of Aurora kinase(s) have been associated with increased levels of aggressiveness in certain cancer types.

[0003] On a cellular level, Aurora kinases play crucial roles in mitotic cell division, both in ensuring accurate division of genomic material in the nucleus and also in division of cytoplasm (cytokinesis). Disruption of activity of the Aurora kinases leads to multiple mitotic defects including aberrant centrosome duplication, misalignment of chromosomes, inhibition of cytokinesis, and disruption of the spindle checkpoint. These defects in mitosis result in cells having abnormal counts of chromosomes (aneuploidy) and programmed cell death (apoptosis).

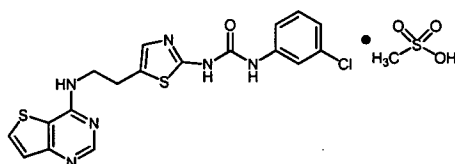
[0004] There are three mammalian Aurora gene products: Aurora A, Aurora B and Aurora C. Aurora A and B are essential in mitosis. The role of Aurora C is unclear; however, Aurora C can complement Aurora B kinase activity in mitosis.

[0005] Elevated expression of Aurora A transcripts and/or protein has been detected in a high percentage of colon, breast, ovarian, gastric, pancreatic, bladder and liver tumors, and the AURKA chromosome locus (20q13) is amplified in a subset of these tumors. Aurora A mRNA overexpression has also been reported to be associated with proliferative activity in mantle cell

lymphoma (MCL) and non-Hodgkin's lymphoma (NHL). Aurora B transcripts and/or protein have been found to be expressed at a high level in cancers of the thyroid, lung, prostate, endometrium, brain, and mouth, and in colorectal cancers. Aurora C is also expressed at high levels in primary tumors. Thus, there remains a need for developing a small-molecule antagonist of Aurora kinase activity as an oncology agent.

SUMMARY OF THE INVENTION

[0006] It has now been found that Compound 1:



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is particularly useful as an Aurora kinase ("Aurora") inhibitor and is therefore useful for treating disorders mediated by Aurora. Also provided herein are, among other things, solid forms of Compound 1, pharmaceutical compositions which comprise Compound 1, methods for making Compound 1 and intermediates thereof, and methods of using the same in the treatment of Aurora-mediated disorders. Such embodiments and others are described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] **Figure 1A** shows the inhibition of Aurora A; and **Figure 1B** shows the inhibition of Aurora B enzymatic activity *in vitro* by Compound 1, as measured by a homogeneous time-resolved fluorescence assay.

[0008] **Figure 2** shows a detail of co-crystal obtained of Aurora-A with Compound 2. The protein is depicted in ribbon form except for the DFG motif region (labeled on the lower right), which is shown as a Van der Waals surface. Compound 2 (center), is also depicted as a Van der Waals surface.

[0009] **Figure 3** shows HCT 116 cells exposed to DMSO vehicle (depicted in black line, black fill) or to 36 nM Compound 1 (depicted in gray line, white fill) for 16 hours. Cells were stained with propidium iodide and subjected to cell sorting. Cell count is plotted against total cell fluorescence.

[0010] **Figure 4** shows HCT 116 cells exposed to DMSO vehicle or to 16 nM Compound 1 for 72 hours, followed by staining for DNA (with propidium iodide) and tubulin (with anti-tubulin antibody).

[0011] **Figure 5** shows the dose-dependent effect in the amount of phosphohistone H3 in HCT 116 cells upon exposure to Compound 1, as measured by High Content Screening.

[0012] **Figures 6A and 6B** depicts the concentration of Compound 2 in tumor (black circles) and in plasma (gray diamonds) over time in HCT 116 tumor xenograft mice after IP administration of a 170 mg/kg dose of the compound. $T_{1/2}$ of Compound 2 in tumor and in plasma are also depicted in this Figure.

[0013] **Figure 7** shows Western blots of phosphorylation of histone H3 in HCT 116 tumor xenograft mice after IP administration of vehicle, 50 mg/kg of Compound 1, or 100 mg/kg Compound 1. Concentrations of Compound 1 in the tumor are shown below the blots. Blots are shown for 3 hours, 6 hours, and 10 hours after administration of the compound.

[0014] **Figure 8** depicts representative Caspase-3 (upper row) and hematoxylin and eosin (H&E) (lower row) sections prepared from tumors after completion of treatment with 170 mg/kg Compound 1 on a bi-weekly schedule for 3 consecutive weeks. Treatments were administered on Day 1, 4, 8, 11, 15, and 18, with tumors being excised Day 4, 11, 18, and 25 of study. All images were taken at 40X magnification.

[0015] **Figure 9** shows tumor volume (mm^3) at various times after implantation for HCT 116 colon cancer xenograft mice treated with vehicle (inverted triangles); treated with 125 mg/kg Compound 1 once a week for three weeks (squares); 150 mg/kg Compound 1 twice a week for three weeks (triangles); or 100 mg/kg per day, two times, with an interval of 9 days off between the two treatments.

[0016] **Figure 10A** shows pharmacokinetics of Compound 2 over time after intravenous administration in mouse (squares), rat (diamonds) and dog (circles). **Figure 10B** shows pharmacokinetics of Compound 2 in mice after intraperitoneal (IP), intravenous (IV), and oral (PO) administration. The routes of administration are depicted respectively as circles, squares, and triangles.

[0017] **Figure 11A** shows exposure of Compound 2 by female mice (squares), female dog (solid triangles), male dogs (open triangles), female rats (solid circles), and male rats (open

circles) as a function of the dose of Compound 1 administered. **Figure 11B** shows the AUClast, as defined herein, for female rats (solid squares), and male rats (open squares).

[0018] **Figure 12A** shows the mean percentage recovery of Compound 2 in rats over time in the following elimination pathways: bile (squares), feces (diamonds), and urine (triangles). **Figure 12B** shows amounts of radioactively-labeled Compound 2 and metabolites thereof as measured in rat bile. **Figure 12C** shows a map of the distribution of metabolites observed in samples of plasma, bile and urine from treated rats.

[0019] **Figure 13** shows a hypothetical example of measurement of drug cooperation. **Figure 13A** depicts effect of cooperation on EC₅₀ (effective concentration) curves; **Figure 13B** shows effect of cooperation on CI₅₀ (combination index) data. **Figure 13C** shows representative results for the hypothetical combinations.

[0020] **Figure 14A** shows High Content Screening (HCS) cell count data for Compound 1 as combined with various drugs in wild type (shown in black) and p53 -/- cells (shown in gray) HCT 116 colon cancer cells. Compound 1 was dosed first, and the combination drug was dosed second. Compound 1 dosed in combination with itself is depicted in open symbols; Compound 1 dosed with a different drug is shown in solid symbols. High/Low, High/High, and Low/High ratios of Compound 1 to combination drugs were used, as shown left to right. **Figure 14B** shows data from Compound 1 administered with other drugs: (i) as a co-dose; (ii) with Compound 1 administered prior to the combination drug; and (iii) with the combination drug administered prior to Compound 1. Results for High/Low, High/High, and Low/High ratios of Compound 1 are shown left to right. In addition, results are shown in the presence or in the absence of p53 RNAi.

[0021] **Figure 15A** shows results of a CellTiter Blue® cell proliferation assay using Compound 1 in combination with itself (open symbols) or a combination drug (solid symbols) in HCT 116 colon cancer cells. High/High and Low/High ratios of Compound 1 to combination drug are shown left to right. **Figure 15B** shows quantitative results for the experiment, including statistical significance.

[0022] **Figure 16** shows DNA morphologies of HCT 116 cells treated with (top row, left to right) DMSO vehicle, docetaxel, and vincristine, respectively; and with (bottom row, left to right) Compound 1, Compound 1 and docetaxel, and Compound 1 and vincristine, respectively.

Large arrows and small arrow indicate DNA morphologies of polyploidy and condensed chromatin, respectively.

[0023] **Figure 17** shows an HCT 116 mouse xenograft study. Mice were treated according to schedules presented schematically at the top of this Figure and described further herein, with vehicle (open squares); 10 mg/kg docetaxel administered as a single agent (solid circles); 42.5 mg/kg Compound 1 administered as a single agent (open circles); 10 mg/kg docetaxel administered prior to 42.5 mg/kg Compound 1 (inverted open triangles); and 42.5 mg/kg Compound 1 administered prior to 10 mg/kg docetaxel (open triangles).

[0024] **Figure 18** depicts an XRPD pattern obtained for Form A of Compound 1.

[0025] **Figure 19** depicts the DSC pattern obtained for Form A of Compound 1.

[0026] **Figure 20** depicts an XRPD pattern obtained for Form B of Compound 1.

[0027] **Figure 21** depicts the DSC pattern obtained for Form B of Compound 1.

[0028] **Figure 22** depicts an XRPD pattern obtained for Form C of Compound 1.

[0029] **Figure 23** depicts the DSC pattern obtained for Form C of Compound 1.

[0030] **Figure 24** depicts an XRPD pattern obtained for Form D of Compound 1.

[0031] **Figure 25** depicts the DSC pattern obtained for Form D of Compound 1.

[0032] **Figure 26** depicts an XRPD pattern obtained for Form E of Compound 1.

[0033] **Figure 27** depicts the DSC pattern obtained for Form E of Compound 1.

[0034] **Figure 28** depicts an XRPD pattern obtained for Form F of Compound 1.

[0035] **Figure 29** depicts the DSC pattern obtained for Form F of Compound 1.

[0036] **Figure 30** depicts an XRPD pattern obtained for Form G of Compound 1.

[0037] **Figure 31** depicts the DSC pattern obtained for Form G of Compound 1.

[0038] **Figure 32** depicts an XRPD pattern obtained for Form H of Compound 1.

[0039] **Figure 33** depicts the DSC pattern obtained for Form H of Compound 1.

[0040] **Figure 34** depicts an XRPD pattern obtained for Form I of Compound 1.

[0041] **Figure 35** depicts the DSC pattern obtained for Form I of Compound 1.

[0042] **Figure 36** depicts an XRPD pattern obtained for Form J of Compound 1.

[0043] **Figure 37** depicts the DSC pattern obtained for Form J of Compound 1.

[0044] **Figure 38** depicts an XRPD pattern obtained for Form K of Compound 1.

[0045] **Figure 39** depicts the DSC pattern obtained for Form K of Compound 1.

[0046] **Figure 40** depicts an XRPD pattern obtained for Form L of Compound 1.

[0047] **Figure 41** depicts the DSC pattern obtained for Form L of Compound 1.

[0048] **Figure 42** depicts photomicrographs of cells from HCT 116 xenograft mice treated with (top row) vehicle and (bottom row) Compound 1. **A)** shows epidermis (left) 4 days after treatment and (right) 18 days after treatment. **B)** shows bone marrow (left) eleven days after treatment and (right) eighteen days after treatment.

[0049] **Figure 43** shows correlation of plasma concentrations of Compound 1 with inhibition of phospho-histone H3 (pHH3) in tumor as measured in HCT 116 xenograft mice. **A)** A plot of (left y-axis and squares) plasma concentration of Compound 2 (μM) and (right y-axis and triangles) pHH3 levels one hour after administration against dose of Compound 1 administered. **B)** A plot of plasma concentration of Compound 2 plotted directly against pHH3 levels in U/mL one hour after administration of Compound 1. **C)** Western blots showing pHH3 and Histone H3 (HH3) levels after administration of Compound 1: (top blot, left to right) vehicle, 1 mg/kg, 2 mg/kg, 5 mg/kg to three HCT 116 mice for each dose; (bottom blot, left to right) vehicle, 10 mg/kg, and 20 mg/kg to three HCT 116 xenograft mice for each dose. **D)** Western blots showing pHH3 and HH3 levels, 6 hours, 9 hours, and 24 hours after administration of a single 170 mg/kg dose of Compound 1.

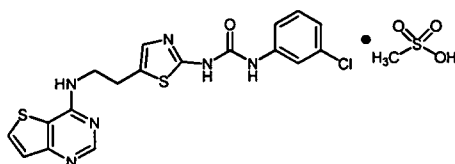
[0050] **Figure 44** shows induction of apoptosis in xenograft tumors after a single dose of Compound 1. **A)** Western blot showing cleaved PARP levels (as compared with β -actin control) for tumors from HCT 116 xenograft mice at 3 hours, 6 hours, and 12 hours after treatment with an IP dose of 170 mg/kg of Compound 1; three mice were treated at each dose. **B)** Western blot showing cleaved PARP and HH3 levels 2 hours, 6 hours and 24 hours after treatment of MV-4-11 xenograft mice with an IP dose of 50 mg/kg or 100 mg/kg of Compound 1; three mice were treated at each dose.

[0051] **Figure 45** shows observed form conversion from slurries and characterization of the various crystal forms observed.

DETAILED DESCRIPTION OF THE INVENTION

(1) Compound 1 and Compound 2

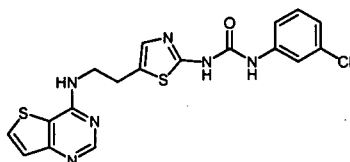
[0052] According to one embodiment, the present invention provides a mesylate salt of 1-(3-chlorophenyl)-3-{5-[2-(thieno[3,2-d]pyrimidin-4-ylamino)ethyl]thiazol-2-yl}-urea, referred to herein as "Compound 1":



1.

[0053] It has now been found that Compound 1, including compositions thereof, is particularly useful for treating disorders mediated by Aurora kinases. Compound 1 of the present invention is a novel small molecule that shows potent inhibition of Aurora kinases.

[0054] It will be appreciated by one of ordinary skill in the art that 1-(3-chlorophenyl)-3-{5-[2-(thieno[3,2-d]pyrimidin-4-ylamino)ethyl]thiazol-2-yl}-urea referred to herein as Compound 2:



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and methanesulfonic acid are ionically bonded to form Compound 1, i.e., the mesylate salt of Compound 2. Compound 2 is in the class of molecules described in US 2006/0035908 and WO 2006/036266, each of which is incorporated herein by reference for all that they disclose.

[0055] It is contemplated that Compound 1 can be provided in a variety of physical forms. For example, Compound 1 can be put into solution, suspension, or be provided in solid form. When Compound 1 is in solid form, said compound may be amorphous, crystalline, or a mixture thereof. Such solid forms are described in more detail below. Dosage amounts used in the compositions and methods provided herein are calculated based on Compound 2 (free base) rather than any particular salt form, even if it is the salt form itself that is used. For example, if a 750 mg/m² of Compound 1 is specified, the amount as used herein corresponds to the amount of Compound 1 that provides 750 mg/m² of the free base.

[0056] In general, Compound 1, and pharmaceutically acceptable compositions thereof, are useful as inhibitors (e.g., of Aurora kinases), and for the treatment of Aurora-mediated diseases or disorders including, but not limited to, cancers (e.g., bladder cancer, brain cancer, breast cancer, cervical cancer, colon cancer, esophageal cancer, head and neck cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, myeloma, neuroendocrine cancer (e.g.,

neuroblastoma), ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, sarcoma, skin cancer, stomach cancer, testicular cancer, thyroid cancer and uterine cancer); and hematological tumors (e.g., mantle cell lymphoma (MCL), Non-Hodgkin's lymphoma (NHL), Hodgkin's disease, acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL) or acute lymphoblastic lymphoma (ALL)).

Definitions

[0057] As used herein, the term "about", when used in reference to any degree 2-theta value recited herein, refers to the stated value ± 0.1 degree 2-theta.

[0058] As used herein, the term "anhydrous" refers to a form of a compound that is substantially free of water. It has been found that Compound 1 can exist as an anhydrous and nonsolvated crystalline form, referred to herein as Form A. As used herein, the term "substantially free of water" means that no significant amount of water is present. For example, in certain embodiments when the term "substantially free of water" is applied herein to a solid form, it means that water content in the crystalline structure is less than 0.5% of the weight of the solid. In some embodiments of the invention, the term "substantially free of water" means that the water content is less than 1% of the weight of the solid. One of ordinary skill in the art will appreciate that an anhydrous solid can contain various amounts of residual water wherein that water is not incorporated in the crystalline lattice. Such incorporation of residual water can depend upon the compound's hygroscopicity and storage conditions.

[0059] The term "carrier" refers to any chemical compound moiety consistent with the stability of Compound 1. In certain embodiments, the term "carrier" refers to a pharmaceutically acceptable carrier. An exemplary carrier herein is water.

[0060] The expression "dosage form" refers to means by which a formulation is stored and/or administered to a subject. For example, the formulation may be stored in a vial or syringe. The formulation may also be stored in a container which protects the formulation from light (e.g., UV light). Alternatively, a container or vial which itself is not necessarily protective from light may be stored in a secondary storage container (e.g., an outer box, bag, etc.) which protects the formulation from light.

[0061] The term "formulation" refers to a composition that includes at least one pharmaceutically active compound (e.g., at least Compound 1) in combination with one or more

excipients or other pharmaceutical additives for administration to a patient. In general, particular excipients and/or other pharmaceutical additives are typically selected with the aim of enabling a desired stability, release, distribution and/or activity of active compound(s) for applications.

[0062] The term “patient”, as used herein, means a mammal to which a formulation or composition comprising a formulation is administered, and includes humans.

[0063] As used herein, the term “polymorph” refers to different crystal structures achieved by a particular chemical entity. Specifically, polymorphs occur when a particular chemical compound can crystallize with more than one structural arrangement.

[0064] As used herein, the term “solvate” refers to a crystal form where a stoichiometric or non-stoichiometric amount of solvent, or mixture of solvents, is incorporated into the crystal structure. Similarly, the term “hydrate” refers to a crystal form where a stoichiometric or non-stoichiometric amount of water is incorporated into the crystal structure.

[0065] As used herein, the term “substantially all” when used to describe X-ray powder diffraction (“XRPD”) peaks of a compound means that the XRPD of that compound includes at least about 80% of the peaks when compared to a reference. For example, when an XRPD of a compound is said to include “substantially all” of the peaks in a reference list, or all of the peaks in a reference XRPD, it means that the XRPD of that compound includes at least 80% of the peaks in the specified reference. In other embodiments, the phrase “substantially all” means that the XRPD of that compound includes at least about 85, 90, 95, 97, 98, or 99% of the peaks when compared to a reference. Additionally, one skilled in the art will appreciate throughout, that XRPD peak intensities and relative intensities as listed herein may change with varying particle size and other relevant variables.

[0066] The term “substantially free of” when used herein in the context of a physical form of Compound 1 means that at least about 95% by weight of Compound 1 is in the specified solid form. In certain embodiments of the invention, the term “substantially free of” one or more other forms of Compound 1 means that at least about 97%, 98%, or 99% by weight of Compound 1 is in the specified solid form. For example, “substantially free of amorphous Compound 1” means that at least about 95% by weight of Compound 1 is crystalline. In certain embodiments of the invention, “substantially free of amorphous Compound 1” means that at least about 97%, 98%, or 99% by weight of Compound 1 is crystalline.

[0067] The term “substantially similar,” when used herein in the context of comparing X-ray powder diffraction or differential scanning calorimetry spectra obtained for a physical form of Compound 1, means that two spectra share defining characteristics sufficient to differentiate them from a spectrum obtained for a different form of Compound 1. In certain embodiments, the term “substantially similar” means that two spectra are the same.

[0068] As used herein, and unless otherwise specified, the terms “therapeutically effective amount” and “effective amount” of a compound refer to an amount sufficient to provide a therapeutic benefit in the treatment, prevention and/or management of a disease, to delay or minimize one or more symptoms associated with the disease or disorder to be treated. The terms “therapeutically effective amount” and “effective amount” can encompass an amount that improves overall therapy, reduces or avoids symptoms or causes of disease or disorder or enhances the therapeutic efficacy of another therapeutic agent.

[0069] The terms “treat” or “treating,” as used herein, refer to partially or completely alleviating, inhibiting, delaying onset of, reducing the incidence of, ameliorating and/or relieving a disorder or condition, or one or more symptoms of the disorder, disease or condition.

[0070] The expression “unit dose” as used herein refers to a physically discrete unit of a formulation appropriate for a subject to be treated. It will be understood, however, that the total daily usage of a formulation of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific effective dose level for any particular subject or organism will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of specific active compound employed; specific composition employed; age, body weight, general health, sex and diet of the subject; time of administration, and rate of excretion of the specific active compound employed; duration of the treatment; drugs and/or additional therapies used in combination or coincidental with specific compound(s) employed, and like factors well known in the medical arts.

(2) Solid Forms of Compound 1

[0071] It would be desirable to provide a solid form of Compound 1 that imparts characteristics such as improved aqueous solubility, stability and ease of formulation. In particular, such solid form may be thermodynamically stable in humid environments.

Additionally, such solid form may be stable at relative humidities below 90% and be readily isolated as a free-flowing solid.

[0072] It has been found that Compound 1 can exist in a variety of solid forms. Such forms include anhydrous, non-solvated, hydrated, and solvated forms. Such solid forms include crystalline and amorphous forms. In some embodiments, Compound 1 is an anhydrous and non-solvated crystalline form. All such solid forms of Compound 1 are contemplated under the present invention. In certain embodiments, the present invention provides Compound 1 as a mixture of one or more solid forms selected from crystalline and amorphous.

[0073] In certain embodiments of the present invention, Compound 1 is provided as a crystalline solid. In certain embodiments, Compound 1 is a crystalline solid substantially free of amorphous Compound 1.

[0074] In certain embodiments, the present invention provides Compound 1 as an anhydrous and non-solvated crystalline form. In some embodiments, such an anhydrous and non-solvated crystalline form is Form A. In certain embodiments, the present invention provides Form A of Compound 1 substantially free of other solid forms of Compound 1.

[0075] In some embodiments, the present invention provides Form A of Compound 1 characterized in that it has one or more, two or more, or three or more, peaks in its XRPD pattern selected from those at about 8.5, 13.2, 15.3, 15.6, 16.7, 20.2, 20.6, 25.2, 26.4 and 27.0 degrees 2-theta. In certain embodiments, the present invention provides Form A of Compound 1, substantially free of other forms of Compound 1.

[0076] In other embodiments, Form A of Compound 1 is characterized in that it has substantially all of the peaks in its XRPD pattern listed in Table 1, below.

Table 1. XRPD Peaks Form A

# Peak Data List							
peak no.	2Theta (deg)	d (Å)	I/I ₁	FWHM (deg)	Intensity (Counts)	Integrated Int (Counts)	
1	8.4678	10.43367	6	0.17720	269	3458	
2	10.3045	8.57770	1	0.20910	59	806	
3	12.6558	6.98886	3	0.21560	105	1358	
4	13.0200	6.79417	3	0.19200	110	1221	
5	13.2243	6.68966	10	0.21350	423	4400	
6	15.0400	5.88589	1	0.13000	49	453	
7	15.3000	5.78645	7	0.24880	292	3415	
8	15.5800	5.68308	7	0.20680	301	3143	
9	15.8580	5.58407	6	0.21600	233	2689	
10	16.4400	5.38768	3	0.22940	111	1533	
11	16.7269	5.29591	8	0.22900	333	3787	
12	17.7667	4.98824	4	0.22350	183	2366	
13	19.0584	4.65297	4	0.21900	163	2010	
14	19.5709	4.53226	5	0.21010	222	2521	
15	20.1577	4.40163	8	0.31790	349	5877	
16	20.6461	4.29860	21	0.29980	863	13866	
17	22.6000	3.93118	1	0.33340	56	1093	
18	22.8000	3.89715	2	0.20760	65	646	
19	23.6583	3.75767	2	0.19670	95	1139	
20	23.8908	3.72163	2	0.07270	80	298	
21	24.1800	3.67776	1	0.12440	55	862	
22	24.4200	3.64216	2	0.00000	68	0	
23	24.6200	3.61302	2	0.00000	65	0	
24	25.1659	3.53587	100	0.19490	4195	46845	
25	25.4800	3.49299	2	0.10600	95	1219	
26	25.9867	3.42602	2	0.18410	94	1030	
27	26.4191	3.37092	7	0.21350	280	3137	
28	27.0447	3.29435	7	0.22670	308	3871	
29	28.6015	3.11848	4	0.27500	188	2892	
30	30.1203	2.96460	6	0.29820	252	4411	
31	30.5800	2.92107	1	0.24000	49	714	
32	31.2458	2.86033	1	0.27830	44	658	
33	33.1618	2.69931	1	0.19640	59	839	
34	33.6641	2.66018	2	0.15970	66	653	
35	34.2166	2.61848	2	0.32670	73	1158	
36	34.7600	2.57878	2	0.13540	78	679	
37	35.0796	2.55601	4	0.21920	147	2167	
38	36.3556	2.46917	2	0.28370	97	1650	
39	37.1494	2.41821	2	0.20290	63	766	
40	37.8009	2.37802	2	0.16180	71	745	
41	39.9821	2.25317	1	0.29570	54	1198	
42	40.6153	2.21950	2	0.20600	102	1258	
43	41.4789	2.17526	2	0.28220	82	1373	
44	42.0600	2.14654	1	0.28000	60	1184	
45	43.4996	2.07878	2	0.24730	87	1250	

[0077] In some embodiments, the present invention provides Form A of Compound 1, having an X-ray diffraction pattern substantially similar to that depicted in Figure 18. In one aspect, the present invention provides Form A having a DSC pattern substantially similar to that depicted in Figure 19.

[0078] In certain embodiments, Compound 1 exists in at least one hydrate form. One such hydrate, i.e., as a monohydrate, is referred to herein as Form B. In certain embodiments, the present invention provides Form B of Compound 1. In some embodiments, the present invention provides Form B of Compound 1 characterized in that it has one or more, two or more, or three or more, peaks in its XRPD pattern selected from those at about 7.1, 10.5, 11.8, 17.0, 17.4, 18.0, 21.3, 23.7, 25.1, 25.8, 26.8, 27.4, and 27.7 degrees 2-theta. In certain embodiments, the present

invention provides Form B of Compound 1, substantially free of other forms of Compound 1. In certain embodiments, Form B is monohydrate solid form of Compound 1.

[0079] In other embodiments, Form B of Compound 1 is characterized in that it has substantially all of the peaks in its XRPD pattern listed in Table 2, below.

Table 2. XRPD Peaks Form B

# Peak no.	Data 2Theta (deg)	List d (Å)	I/I1	FWHM (deg)	Intensity (Counts)	Integrated Int (Counts)
1	6.6600	13.26120	4	0.16660	19	317
2	7.0543	12.52082	19	0.30860	102	1662
3	10.2400	8.63159	8	0.14860	42	463
4	10.5261	8.39761	34	0.27570	184	2654
5	11.4000	7.75576	4	0.14400	22	293
6	11.7888	7.50083	55	0.26760	294	4360
7	12.5150	7.06717	8	0.25000	43	681
8	13.3800	6.61217	5	0.19200	25	281
9	13.6200	6.49619	8	0.32000	41	584
10	14.1464	6.25562	9	0.24710	47	647
11	14.5789	6.07100	9	0.23210	48	634
12	16.4200	5.39419	5	0.53000	28	1214
13	16.9800	5.21753	47	0.27520	251	3455
14	17.3667	5.10222	100	0.27830	535	7775
15	18.0424	4.91263	16	0.27910	84	1425
16	18.8600	4.70147	4	0.18000	20	213
17	19.2450	4.60827	9	0.35000	49	926
18	20.4575	4.33780	4	0.18500	20	282
19	21.2582	4.17619	39	0.25150	206	3052
20	22.3268	3.97866	4	0.19640	21	231
21	23.6590	3.75756	32	0.27800	170	2696
22	24.4250	3.64143	4	0.23000	21	245
23	25.0681	3.54945	36	0.37870	195	3838
24	25.8430	3.44475	26	0.33400	138	2544
25	26.7610	3.32863	19	0.29400	100	1581
26	27.4400	3.24778	15	0.39200	78	1399
27	27.7200	3.21561	10	0.27340	51	801
28	29.3905	3.03653	6	0.40760	34	820
29	30.2236	2.95470	4	0.23930	19	314
30	31.6105	2.82815	7	0.25100	37	751
31	33.0166	2.71085	4	0.23330	19	341
32	34.5275	2.59561	4	0.20500	19	305
33	35.1650	2.55000	4	0.29000	19	390
34	37.8140	2.37723	5	0.30800	25	519
35	38.9500	2.31047	4	0.24000	22	309
36	39.9626	2.25423	3	0.34130	18	499
37	44.4620	2.03599	6	0.40400	32	711

[0080] In some embodiments, the present invention provides Form B of Compound 1, having an X-ray diffraction pattern substantially similar to that depicted in Figure 20. In one aspect, the present invention provides Form B having a DSC pattern substantially similar to that depicted in Figure 21.

[0081] In certain embodiments, the present invention provides Form C of Compound 1. In certain embodiments, the present invention provides Form C of Compound 1, substantially free of other forms of Compound 1. In certain embodiments, Form C is characterized in that it has one or more, two or more, or three or more, peaks in its XRPD pattern selected from those at about 8.8, 9.7, 14.6, 17.7, 18.2, 18.8, 19.2, 22.2, 23.5, 24.6, 25.1 and 25.5 degrees 2-theta.

[0082] In other embodiments, Form C of Compound 1 is characterized in that it has substantially all of the peaks in its XRPD pattern listed in Table 3, below.

Table 3. XRPD Peaks Form C

# Peak Data List							
peak no.	2Theta (deg)	d (Å)	I/I1	FWHM (deg)	Intensity (Counts)	Integrated I (Counts)	
1	4.8810	18.08985	7	0.25800	50	393	
2	8.1200	10.87978	3	1.44000	20	951	
3	8.8412	9.99384	25	0.30050	169	1169	
4	9.6784	9.13115	20	0.37840	133	1393	
5	10.4400	8.46668	4	0.28000	26	264	
6	11.0018	8.03556	16	0.31640	106	886	
7	11.5495	7.65570	14	0.31690	97	832	
8	12.2101	7.24295	17	0.32520	115	1219	
9	14.5762	6.07212	57	0.38720	384	3973	
10	15.5914	5.67895	3	0.30290	21	160	
11	16.3240	5.42570	11	0.26230	77	563	
12	17.6914	5.00930	25	0.27880	171	1267	
13	18.1677	4.87903	23	0.32450	158	1340	
14	18.7600	4.72630	37	0.30440	253	1650	
15	19.1600	4.62852	53	0.56180	356	4480	
16	20.2834	4.37464	19	0.26220	127	915	
17	20.8130	4.26450	13	0.30820	91	739	
18	22.1831	4.00411	44	0.35000	295	2822	
19	23.0400	3.85709	12	0.42000	83	888	
20	23.4800	3.78580	26	0.69540	177	2913	
21	24.0000	3.70494	15	0.00000	103	0	
22	24.6400	3.61014	67	0.40840	452	5085	
23	25.0800	3.54779	100	0.42820	676	6179	
24	25.4800	3.49299	41	0.30760	276	3218	
25	26.2800	3.38845	19	0.00000	130	0	
26	26.7200	3.33364	11	0.37340	71	1435	
27	27.3869	3.25396	12	0.29760	83	687	
28	27.9200	3.19303	4	0.24000	29	220	
29	28.6000	3.11864	9	0.32720	63	481	
30	28.9200	3.08485	11	0.38230	74	502	
31	29.3600	3.03961	9	0.72000	59	620	
32	29.7600	2.99966	5	0.26660	32	221	
33	30.4467	2.93355	3	0.24000	21	143	
34	30.9667	2.88547	5	0.28000	37	299	
35	32.0193	2.79297	8	0.26630	51	384	
36	33.1540	2.69993	3	0.26800	23	162	
37	33.6125	2.66414	5	0.26500	35	272	
38	34.9422	2.56574	11	0.37330	77	594	
39	35.2400	2.54474	3	0.24000	23	185	
40	36.4100	2.46561	8	0.42000	51	714	
41	37.2980	2.40892	5	0.47600	33	474	
42	38.6838	2.32575	9	0.37060	63	770	
43	40.6787	2.21618	6	0.27160	42	440	
44	43.5666	2.07574	5	0.33330	34	346	

[0083] In some embodiments, the present invention provides Form C of Compound 1, having an X-ray diffraction pattern substantially similar to that depicted in Figure 22. In one aspect, the present invention provides Form C having a DSC pattern substantially similar to that depicted in Figure 23. In some embodiments, Form C is characterized in that it has a melting point of 164 °C.

[0084] In certain embodiments, Compound 1 exists in at least one solvate form. In certain embodiments, the present invention provides Form D of Compound 1, as a dimethylacetamide (DMA) solvate. In certain embodiments, the present invention provides Form D of Compound 1.

[0085] In certain embodiments, the present invention provides Form **D** of Compound **1**. In certain embodiments, the present invention provides Form **D** of Compound **1**, substantially free of other forms of Compound **1**. In certain embodiments, Form **D** is characterized in that it has one or more, two or more, or three or more, peaks in its XRPD pattern selected from those at about 8.0, 9.8, 13.5, 13.9, 15.9, 16.2, 18.5, 20.7, 21.1, 24.4, 24.6, 25.0 and 26.3 degrees 2-theta.

[0086] In other embodiments, Form **D** of Compound **1** is characterized in that it has substantially all of the peaks in its XRPD pattern listed in Table 4, below.

Table 4. XRPD Peaks Form D

# Peak Data List	peak	2Theta	d	I/I1	FWHM	Intensity	Integrated I
	no.	(deg)	(Å)		(deg)	(Counts)	(Counts)
	1	3.4057	25.92209	3	0.07730	29	134
	2	3.8639	22.84909	5	0.10220	43	170
	3	4.1679	21.18317	4	0.11590	38	181
	4	4.7200	18.70653	7	0.14320	64	323
	5	4.8878	18.06470	16	0.23690	151	892
	6	5.8724	15.03789	3	0.12710	30	123
	7	6.2103	14.22042	3	0.08290	28	81
	8	7.4800	11.80917	3	0.17460	31	325
	9	7.9647	11.09157	27	0.18420	257	1530
	10	9.5600	9.24397	6	0.11780	56	248
	11	9.7824	9.03430	32	0.16300	303	1351
	12	12.1392	7.28509	7	0.15120	64	329
	13	13.4761	6.56523	24	0.20930	224	1345
	14	13.9025	6.36481	38	0.20770	359	2011
	15	14.2000	6.23213	10	0.14400	90	503
	16	14.6943	6.02358	11	0.16650	99	439
	17	14.9200	5.93296	6	0.20000	55	352
	18	15.5600	5.69034	6	0.11560	55	206
	19	15.8800	5.57639	22	0.23040	210	1233
	20	16.2354	5.45511	48	0.32780	448	3854
	21	17.6400	5.02378	3	0.09340	31	122
	22	17.8753	4.95818	11	0.21880	101	550
	23	18.2000	4.87044	13	0.20000	125	829
	24	18.5331	4.78365	69	0.22010	648	3878
	25	19.8984	4.45840	3	0.21030	31	185
	26	20.3547	4.35947	8	0.17600	75	344
	27	20.6800	4.29163	20	0.17460	188	1325
	28	21.1200	4.20320	28	0.15680	258	1431
	29	21.3600	4.15651	6	0.11420	54	504
	30	21.7516	4.08256	6	0.18330	54	266
	31	22.1622	4.00784	18	0.17170	166	770
	32	23.3487	3.80679	4	0.10250	36	117
	33	23.9794	3.70807	16	0.28120	150	1108
	34	24.3600	3.65099	25	0.15060	230	821
	35	24.6000	3.61592	40	0.20160	371	2330
	36	24.9636	3.56407	100	0.15520	936	4351
	37	25.6374	3.47190	4	0.09000	40	106
	38	26.2824	3.38814	51	0.18760	476	2724
	39	26.7791	3.32642	14	0.19240	131	810
	40	27.3415	3.25926	11	0.23960	107	637
	41	27.8267	3.20352	3	0.24000	31	242
	42	28.4657	3.13304	5	0.20700	47	302
	43	29.5208	3.02342	15	0.14720	140	678
	44	31.0692	2.87618	5	0.26150	46	515
	45	32.6307	2.74202	3	0.11000	29	202
	46	33.4725	2.67496	3	0.18500	30	259
	47	34.7906	2.57658	3	0.37070	31	494
	48	36.8708	2.43584	4	0.16960	39	240
	49	39.0209	2.30643	5	0.18360	44	210

[0087] In some embodiments, the present invention provides Form D of Compound 1, having an X-ray diffraction pattern substantially similar to that depicted in Figure 24. In one aspect, the present invention provides Form D having a DSC pattern substantially similar to that depicted in Figure 25.

[0088] In certain embodiments, Compound 1 exists in at least one solvate form. In certain embodiments, the present invention provides Form E of Compound 1, as a formamide solvate. In certain embodiments, the present invention provides Form E of Compound 1.

[0089] In certain embodiments, the present invention provides Form E of Compound 1. In certain embodiments, the present invention provides Form E of Compound 1, substantially free of other forms of Compound 1. In certain embodiments, Form E is characterized in that it has one or more, two or more, or three or more, peaks in its XRPD pattern selected from those at about 11.5, 12.7, 16.5, 17.2, 19.0, 19.3, 19.5, 22.2, 23.0, 25.4, 26.8 and 27.5 degrees 2-theta.

[0090] In other embodiments, Form E of Compound 1 is characterized in that it has substantially all of the peaks in its XRPD pattern listed in Table 5, below.

Table 5. XRPD Peaks Form E

# Peak	Data List						
peak no.	2Theta (deg)	d (Å)	I/I1	FWHM (deg)	Intensity (Counts)	Integrated Int (Counts)	Int
1	6.4010	13.79719	7	0.19350	209	1447	
2	8.6160	10.25454	6	0.18510	182	1117	
3	11.0000	8.03687	5	0.15680	165	1206	
4	11.5173	7.67703	61	0.17170	1833	9852	
5	12.6787	6.97629	15	0.18410	456	2594	
6	14.0166	6.31326	7	0.18440	206	1199	
7	16.1600	5.48039	6	0.12480	174	1036	
8	16.4704	5.37780	100	0.17070	3022	14441	
9	17.1901	5.15423	28	0.22660	838	5698	
10	17.9375	4.94112	8	0.19160	250	1377	
11	18.8000	4.71634	8	0.18580	255	3404	
12	19.0400	4.65742	17	0.00000	526	0	
13	19.2800	4.59999	24	0.23420	725	5354	
14	19.5200	4.54397	34	0.13880	1025	5077	
15	21.8000	4.07360	5	0.12680	143	834	
16	22.1590	4.00841	31	0.20220	951	6169	
17	22.6800	3.91750	11	0.15560	325	1876	
18	23.0310	3.85858	31	0.16990	931	4388	
19	23.3422	3.80784	6	0.18920	196	1258	
20	24.1450	3.68302	7	0.19960	221	1156	
21	24.4400	3.63922	4	0.12940	129	645	
22	25.0000	3.55896	7	0.18580	218	1790	
23	25.4193	3.50120	38	0.19730	1137	6420	
24	25.8000	3.45039	9	0.11460	284	1736	
25	26.8196	3.32149	31	0.19580	943	5335	
26	27.5266	3.23776	59	0.19200	1772	9986	
27	28.2140	3.16042	8	0.16870	232	1211	
28	28.9581	3.08088	6	0.16830	177	817	
29	29.8444	2.99137	4	0.19820	117	634	
30	30.2385	2.95328	7	0.20110	226	1411	
31	31.2905	2.85634	5	0.17900	149	1021	
32	33.1593	2.69951	14	0.17850	420	2265	
33	33.8499	2.64600	3	0.34390	102	865	
34	34.3901	2.60566	5	0.29280	163	1100	
35	34.7600	2.57878	3	0.20740	91	613	
36	35.7716	2.50813	3	0.22710	93	808	
37	37.3179	2.40768	3	0.34250	95	1029	
38	37.8887	2.37271	5	0.12960	156	567	
39	38.9600	2.30990	5	0.14000	144	749	
40	39.1600	2.29856	7	0.28120	216	1472	
41	39.5226	2.27830	7	0.19870	222	1253	
42	43.2000	2.09250	7	0.20180	214	1733	
43	43.3600	2.08515	4	0.09060	131	521	

[0091] In some embodiments, the present invention provides Form E of Compound 1, having an X-ray diffraction pattern substantially similar to that depicted in **Figure 26**. In one aspect, the present invention provides Form E having a DSC pattern substantially similar to that depicted in **Figure 27**.

[0092] In certain embodiments, the present invention provides Form F of Compound 1. In certain embodiments, the present invention provides Form F of Compound 1, substantially free of other forms of Compound 1. In certain embodiments, Form F is characterized in that it has one or more, two or more, or three or more, peaks in its XRPD pattern selected from those at about 9.8, 11.4, 13.0, 13.3, 17.1, 17.7, 18.0, 19.4 and 19.9 degrees 2-theta.

[0093] In other embodiments, Form F of Compound 1 is characterized in that it has substantially all of the peaks in its XRPD pattern listed in Table 6, below.

Table 6. XRPD Peaks Form F

# Peak no.	Data List 2Theta (deg)	d (Å)	I/I ₁	FWHM (deg)	Intensity (Counts)	Integrated I: (Counts)
1	3.4900	25.29614	4	0.08660	12	66
2	3.8273	23.06751	5	0.12130	15	107
3	4.0733	21.67492	7	0.13330	21	133
4	4.2150	20.94657	5	0.11000	16	82
5	4.4200	19.97551	4	0.12000	11	67
6	4.8024	18.38575	5	0.22710	16	162
7	5.0583	17.45617	7	0.14330	21	149
8	5.5600	15.88211	5	0.20000	14	153
9	5.7200	15.43821	5	0.14860	15	97
10	5.9010	14.96507	5	0.18200	14	122
11	6.2000	14.24403	4	0.06660	11	47
12	6.3600	13.88604	4	0.10800	12	110
13	6.5613	13.46046	4	0.09070	12	75
14	7.0533	12.52260	3	0.10670	10	101
15	7.5466	11.70510	4	0.09330	11	70
16	7.8033	11.32061	3	0.10000	10	67
17	9.3760	9.42496	7	0.15200	21	233
18	9.7532	9.06129	47	0.17360	136	1374
19	10.2750	8.60226	3	0.05000	9	52
20	10.8591	8.14083	3	0.06830	9	55
21	11.0400	8.00784	3	0.12000	10	165
22	11.3803	7.76914	53	0.16070	156	1467
23	11.8053	7.49038	5	0.12070	15	153
24	12.5966	7.02157	4	0.11330	12	84
25	12.8600	6.87834	6	0.13000	18	125
26	13.0400	6.78379	22	0.17340	63	723
27	13.3191	6.64226	100	0.19570	292	2854
28	13.6200	6.49619	12	0.16400	35	517
29	14.0133	6.31473	4	0.09330	11	94
30	14.3175	6.18124	3	0.08500	9	67
31	15.4545	5.72895	8	0.17900	24	239
32	16.0130	5.53037	6	0.11400	17	125
33	16.8600	5.25440	4	0.10660	13	91
34	17.1076	5.17890	32	0.20470	92	851
35	17.3200	5.11587	11	0.16000	33	333
36	17.7176	5.00195	34	0.24110	98	1196
37	18.0408	4.91306	61	0.18070	179	1706
38	18.6600	4.75140	11	0.17720	32	310
39	18.9400	4.68179	14	0.24660	40	536
40	19.4044	4.57077	24	0.37460	69	1228
41	19.8825	4.46193	18	0.24500	54	644
42	20.4558	4.33815	10	0.18170	29	285
43	20.8200	4.26308	11	0.20000	33	354
44	21.6225	4.10664	4	0.18500	11	168
45	22.4716	3.95335	14	0.17670	42	455
46	22.8600	3.88706	35	0.22660	101	1328
47	23.3512	3.80639	17	0.14250	51	463
48	23.6786	3.75449	17	0.17070	50	491
49	24.0066	3.70393	3	0.10670	9	71
50	24.5450	3.62389	4	0.15000	12	121
51	24.9606	3.56449	81	0.19450	236	2340
52	25.2783	3.52041	15	0.15670	43	380

Table 6 (cont).

peak no.	2Theta (deg)	d (Å)	I/I1	FWHM (deg)	Intensity (Counts)	Integrated Int (Counts)
53	25.6375	3.47189	24	0.24500	70	841
54	26.2159	3.39659	43	0.18050	127	1203
55	26.6073	3.34750	7	0.19870	20	271
56	27.3333	3.26022	7	0.13330	19	160
57	27.7223	3.21535	42	0.18660	122	1406
58	28.1550	3.16691	4	0.09000	11	95
59	28.7100	3.10694	10	0.22000	29	309
60	29.0600	3.07031	4	0.12000	11	76
61	31.4680	2.84064	4	0.09600	13	87
62	33.3325	2.68588	3	0.10500	10	91
63	34.9433	2.56567	4	0.11330	13	144
64	36.2200	2.47811	4	0.10000	11	77
65	36.5580	2.45597	4	0.11600	12	141
66	41.4066	2.17889	3	0.13330	10	111

[0094] In some embodiments, the present invention provides Form F of Compound 1, having an X-ray diffraction pattern substantially similar to that depicted in **Figure 28**. In one aspect, the present invention provides Form F having a DSC pattern substantially similar to that depicted in **Figure 29**.

[0095] As described above, Compound 1 exists in at least one hydrate form. One such hydrate, i.e., a monohydrate, is referred to herein as Form G. In certain embodiments, the present invention provides Form G of Compound 1. In certain embodiments, the present invention provides Form G of Compound 1, substantially free of other forms of Compound 1. In certain embodiments, Form G is characterized in that it has one or more, two or more, or three or more, peaks in its XRPD pattern selected from those at about 6.2, 11.9, 12.3, 16.7, 18.2, 18.5, 19.2, 22.3, 24.7, 26.0, 26.6 and 27.4 degrees 2-theta.

[0096] In other embodiments, Form G of Compound 1 is characterized in that it has substantially all of the peaks in its XRPD pattern listed in Table 7, below.

Table 7. XRPD Peaks Form G

# Peak Data List						
peak no.	2Theta (deg)	d (Å)	I/I1	FWHM (deg)	Intensity (Counts)	Integrated Int (Counts)
1	5.8400	15.12125	3	0.23000	41	382
2	6.1752	14.30117	18	0.20960	234	1396
3	8.8573	9.97571	4	0.17630	56	303
4	11.4000	7.75576	14	0.21940	185	1662
5	11.8537	7.45991	58	0.23210	760	5246
6	12.3194	7.17893	32	0.23430	422	2626
7	13.4638	6.57120	9	0.25760	116	911
8	14.3628	6.16185	6	0.37910	79	784
9	16.2800	5.44027	5	0.15200	68	390
10	16.7011	5.30403	100	0.21830	1317	8173
11	17.4036	5.09148	6	0.22940	80	527
12	17.7313	4.99812	8	0.16930	100	452
13	18.2000	4.87044	22	0.24700	291	1813
14	18.5425	4.78125	32	0.34900	417	3488
15	19.2077	4.61714	36	0.40820	471	5472
16	20.0247	4.43056	4	0.16610	54	239
17	20.6192	4.30414	6	0.18970	77	398
18	21.4453	4.14017	6	0.38930	78	703
19	21.8800	4.05889	7	0.24000	94	749
20	22.2800	3.98692	27	0.30700	356	2440
21	22.6000	3.93118	12	0.30720	152	1755
22	23.8400	3.72944	9	0.22060	119	1593
23	24.3200	3.65691	13	0.00000	172	0
24	24.7466	3.59483	31	0.35160	411	4319
25	25.1600	3.53669	7	0.14940	95	497
26	25.9972	3.42466	31	0.31320	402	3349
27	26.6361	3.34395	16	0.21580	214	1249
28	27.3617	3.25690	78	0.37020	1029	10166
29	28.1468	3.16781	9	0.25090	114	1108
30	32.3200	2.76767	4	0.17000	48	308
31	32.4800	2.75440	4	0.18280	55	273
32	33.7377	2.65454	9	0.25360	120	933
33	36.0813	2.48731	3	0.16270	41	276
34	39.5440	2.27712	3	0.59920	41	756
35	40.6493	2.21772	3	0.28530	40	342
36	41.8800	2.15535	6	0.29720	75	535
37	42.1200	2.14362	4	0.14580	49	224

[0097] In some embodiments, the present invention provides Form G of Compound 1, having an X-ray diffraction pattern substantially similar to that depicted in Figure 30. In one aspect, the present invention provides Form G having a DSC pattern substantially similar to that depicted in Figure 31.

[0098] As described above, Compound 1 exists in at least one solvate form. In certain embodiments, the present invention provides Form H of Compound 1, as an ethanol solvate. In certain embodiments, the present invention provides Form H of Compound 1. In certain embodiments, the present invention provides Form H of Compound 1, substantially free of other forms of Compound 1. In certain embodiments, Form H is characterized in that it has one or

more, two or more, or three or more, peaks in its XRPD pattern selected from those at about 9.8, 12.2, 13.6, 18.4, 18.7, 19.6, 20.0, 24.5, 24.8 and 28.7 degrees 2-theta.

[0099] In other embodiments, Form **H** of Compound **1** is characterized in that it has substantially all of the peaks in its XRPD pattern listed in Table 8, below.

Table 8. XRPD Peaks Form H

# Peak	Data List						
peak	2Theta	d	I/I1	FWHM	Intensity	Integrated Int	
no.	(deg)	(Å)		(deg)	(Counts)	(Counts)	
1	6.1433	14.37536	13	0.24670	217	1804	
2	9.7574	9.05740	22	0.23840	365	2877	
3	10.6481	8.30167	12	0.23900	205	1691	
4	12.2266	7.23321	26	0.23680	436	3293	
5	13.0800	6.76314	8	0.23320	128	969	
6	13.6072	6.50227	54	0.22920	898	6297	
7	15.4987	5.71271	4	0.18990	61	349	
8	16.2000	5.46695	6	0.17940	104	522	
9	16.4825	5.37388	14	0.25130	236	1524	
10	17.7241	5.00013	11	0.37570	182	1855	
11	18.3698	4.82581	41	0.27630	686	4682	
12	18.7231	4.73553	70	0.23420	1161	7439	
13	19.5600	4.53476	17	0.26900	276	2156	
14	19.9574	4.44535	21	0.30940	348	2701	
15	20.7471	4.27790	6	0.19170	104	534	
16	21.5200	4.12597	5	0.24500	81	719	
17	22.1072	4.01769	12	0.43360	205	2366	
18	22.9431	3.87316	6	0.18740	101	514	
19	23.6000	3.76682	11	0.23280	176	1882	
20	24.1200	3.68678	9	0.00000	153	0	
21	24.5200	3.62753	37	0.25260	607	4723	
22	24.8312	3.58277	100	0.24890	1658	11193	
23	26.2201	3.39605	37	0.27200	614	4390	
24	26.5200	3.35833	5	0.12500	76	591	
25	27.2224	3.27325	4	0.18210	68	356	
26	28.6625	3.11198	24	0.24470	403	2873	
27	29.2400	3.05182	4	0.21340	64	765	
28	30.2624	2.95100	8	0.24120	126	1073	
29	31.8122	2.81068	3	0.16080	54	429	
30	32.4000	2.76102	8	0.21600	125	558	
31	32.6000	2.74454	6	0.42660	92	1068	
32	35.1110	2.55380	6	0.23140	94	694	
33	38.7082	2.32434	9	0.27190	150	1257	
34	41.6466	2.16689	3	0.41330	50	1003	

[00100] In some embodiments, the present invention provides Form **H** of Compound **1**, having an X-ray diffraction pattern substantially similar to that depicted in **Figure 32**. In one aspect, the present invention provides Form **H** having a DSC pattern substantially similar to that depicted in **Figure 33**.

[00101] In certain embodiments, the present invention provides Form **I** of Compound **1**, as an acetic acid solvate. In certain embodiments, the present invention provides Form **I** of Compound **1**. In certain embodiments, the present invention provides Form **I** of Compound **1**, substantially free of other forms of Compound **1**. In certain embodiments, Form **I** is characterized in that it

has one or more, two or more, or three or more, peaks in its XRPD pattern selected from those at about 9.4, 13.3, 13.7, 17.0, 17.7, 18.8, 19.3, 20.7, 22.1, 22.5, 24.6, 24.8, 25.3, 26.7 and 29.8 degrees 2-theta.

[00102] In other embodiments, Form I of Compound 1 is characterized in that it has substantially all of the peaks in its XRPD pattern listed in Table 9, below.

Table 9. XRPD Peaks Form I

# Peak no.	Data List 2Theta (deg)	d (Å)	I/I1	FWHM (deg)	Intensity (Counts)	Integrated Int (Counts)
1	8.9200	9.90573	4	0.25720	60	800
2	9.4257	9.37538	24	0.20800	358	2067
3	10.3369	8.55089	11	0.22520	163	1084
4	10.7191	8.24684	6	0.23820	83	519
5	13.3200	6.64181	26	0.23920	387	2662
6	13.6515	6.48127	79	0.22170	1176	6875
7	14.5370	6.08840	3	0.21400	50	349
8	15.3723	5.75940	5	0.23040	67	484
9	17.0254	5.20372	29	0.20730	437	2597
10	17.7285	4.99890	26	0.20560	392	2365
11	18.5200	4.78700	4	0.12720	64	328
12	18.7887	4.71915	33	0.21530	486	2885
13	19.3060	4.59385	74	0.20590	1098	6023
14	19.9381	4.44961	18	0.22250	264	1635
15	20.6868	4.29023	38	0.21260	558	3138
16	21.3045	4.16722	4	0.19760	54	300
17	22.1356	4.01260	30	0.22890	444	2598
18	22.4844	3.95113	26	0.22510	393	2116
19	22.8862	3.88266	14	0.32180	215	1729
20	24.1228	3.68636	8	0.19340	122	751
21	24.6000	3.61592	25	0.33720	378	2535
22	24.8000	3.58721	26	0.21720	392	2224
23	25.3370	3.51238	100	0.20940	1485	9032
24	26.0463	3.41832	18	0.29910	260	2403
25	26.3600	3.37835	9	0.00000	140	0
26	26.6957	3.33662	44	0.21240	646	4156
27	27.8345	3.20264	11	0.17690	156	883
28	29.7752	2.99817	31	0.18640	456	2436
29	30.3575	2.94197	12	0.18550	172	1111
30	30.9729	2.88491	13	0.19280	197	1181
31	31.9623	2.79782	14	0.20380	215	1259
32	32.2780	2.77118	4	0.22800	52	365
33	33.0012	2.71208	10	0.19240	144	895
34	34.3481	2.60875	6	0.21630	90	570
35	34.9259	2.56690	4	0.27540	64	809
36	38.3297	2.34642	7	0.17950	108	603
37	38.9983	2.30772	4	0.25170	59	493
38	42.8875	2.10702	8	0.38220	117	1362
39	43.5852	2.07489	6	0.33280	96	1142

[00103] In some embodiments, the present invention provides Form I of Compound 1, having an X-ray diffraction pattern substantially similar to that depicted in Figure 34. In one aspect, the present invention provides Form I having a DSC pattern substantially similar to that depicted in Figure 35.

[00104] In certain embodiments, the present invention provides Form J of Compound 1, as a dimethylformamide (DMF) solvate. In certain embodiments, the present invention provides Form J of Compound 1. In certain embodiments, the present invention provides Form J of Compound 1, substantially free of other forms of Compound 1. In certain embodiments, Form J is characterized in that it has one or more, two or more, or three or more, peaks in its XRPD pattern selected from those at about 4.9, 8.0, 9.7, 13.0, 14.0, 16.0, 16.8, 17.8, 19.3, 20.6, 22.5, 23.0, 24.0, 25.6, 26.6 and 27.5 degrees 2-theta.

[00105] In other embodiments, Form J of Compound 1 is characterized in that it has substantially all of the peaks in its XRPD pattern listed in Table 10, below.

Table 10. XRPD Peaks Form J

# Peak Data List	peak no.	2Theta (deg)	d (Å)	I/I1	FWHM (deg)	Intensity (Counts)	Integrated Int (Counts)
	1	4.8657	18.14670	29	0.34630	185	1847
	2	7.9899	11.05664	42	0.36280	271	2796
	3	9.7224	9.08992	44	0.35060	284	2575
	4	10.3390	8.54916	4	0.37800	28	246
	5	12.2474	7.22097	9	0.29980	58	464
	6	12.9696	6.82046	19	0.33350	124	1096
	7	14.0017	6.31994	40	0.37050	259	2587
	8	14.5855	6.06826	8	0.30100	48	386
	9	15.2405	5.80891	8	0.33240	52	416
	10	16.0174	5.52886	21	0.34150	135	1157
	11	16.7606	5.28534	41	0.39870	260	2801
	12	17.7873	4.98251	17	0.41200	108	1555
	13	19.2987	4.59557	90	0.41810	578	6531
	14	20.5982	4.30848	36	0.35440	228	2039
	15	21.1782	4.19178	4	0.28360	26	189
	16	22.5082	3.94701	29	0.38530	188	1704
	17	23.0000	3.86371	16	0.38580	100	1161
	18	23.9938	3.70588	18	0.32100	114	1283
	19	25.5642	3.48168	100	0.85200	640	12268
	20	26.5885	3.34983	24	0.51240	154	2130
	21	27.4847	3.24260	17	0.38550	109	946
	22	28.0400	3.17963	6	0.65600	37	680
	23	29.2206	3.05380	12	0.45010	77	899
	24	30.6816	2.91163	7	0.63670	47	630
	25	31.5335	2.83488	5	0.28300	35	260
	26	33.6489	2.66134	8	0.38220	54	682
	27	34.8466	2.57256	4	0.30670	24	258
	28	35.7100	2.51232	7	0.50000	43	731
	29	37.0120	2.42688	5	0.29600	30	226
	30	37.8091	2.37752	5	0.42970	31	355
	31	38.6020	2.33049	5	0.36400	29	280
	32	41.0166	2.19870	8	0.35330	53	590

[00106] In some embodiments, the present invention provides Form J of Compound 1, having an X-ray diffraction pattern substantially similar to that depicted in Figure 36. In one aspect, the present invention provides Form J having a DSC pattern substantially similar to that depicted in Figure 37.

[00107] In certain embodiments, Compound 1 exists in at least one solvate form. In certain embodiments, the present invention provides Form K of Compound 1, as an

N-methylpyrrolidinone (NMP) solvate. In certain embodiments, the present invention provides Form **K** of Compound **1**. In certain embodiments, the present invention provides Form **K** of Compound **1**, substantially free of other forms of Compound **1**. In certain embodiments, Form **K** is characterized in that it has one or more, two or more, or three or more, peaks in its XRPD pattern selected from those at about 13.4, 13.9, 15.3, 16.8, 18.1, 21.3, 22.8, 24.5, 24.9, 25.2 and 28.6 degrees 2-theta.

[00108] In other embodiments, Form **K** of Compound **1** is characterized in that is has substantially all of the peaks in its XRPD pattern listed in Table 11, below.

Table 11. XRPD Peaks Form K

#	Peak Data List						
peak no.	2Theta (deg)	d (Å)	I/I1	FWHM (deg)	Intensity (Counts)	Integrated Int (Counts)	
1	7.2989	12.10177	2	0.27780	81	766	
2	9.1200	9.68894	4	0.27660	168	1221	
3	9.4273	9.37379	5	0.23870	185	1097	
4	11.3052	7.82058	9	0.21180	351	2319	
5	12.7054	6.96169	2	0.18910	87	549	
6	13.3906	6.60695	17	0.20920	696	4039	
7	13.8884	6.37124	12	0.25300	488	2959	
8	14.1600	6.24964	3	0.24620	132	1359	
9	14.7313	6.00853	7	0.26770	260	2172	
10	15.2912	5.78976	14	0.20500	564	3272	
11	16.0400	5.52112	9	0.21860	372	2103	
12	16.2400	5.45357	9	0.18220	356	2043	
13	16.8481	5.25808	10	0.20160	386	2142	
14	17.8000	4.97898	4	0.24820	156	1284	
15	18.1023	4.89651	10	0.21230	398	2165	
16	18.6400	4.75646	2	0.11200	65	217	
17	18.9234	4.68586	8	0.21730	321	1786	
18	19.2927	4.59699	3	0.19170	136	784	
19	20.0618	4.42246	7	0.26330	272	1832	
20	20.3600	4.35835	4	0.22840	143	998	
21	20.7196	4.28351	4	0.19650	160	899	
22	21.3167	4.16486	11	0.23700	443	2856	
23	21.8800	4.05889	5	0.32500	184	1440	
24	22.0400	4.02979	6	0.22500	228	993	
25	22.6000	3.93118	9	0.22580	358	1929	
26	22.8400	3.89041	10	0.17520	394	2202	
27	23.3200	3.81141	2	0.10160	72	322	
28	23.6740	3.75521	9	0.23040	355	2343	
29	24.4800	3.63337	12	0.39480	481	4516	
30	24.8800	3.57585	40	0.16060	1586	8250	
31	25.1653	3.53596	100	0.25070	3997	25825	
32	26.4744	3.36401	4	0.28110	152	1221	
33	26.8800	3.31416	3	0.15800	118	520	
34	27.1600	3.28062	2	0.14860	63	510	
35	27.8012	3.20640	3	0.19440	135	660	
36	28.2000	3.16196	3	0.13340	107	356	
37	28.5717	3.12166	10	0.27960	389	3164	
38	29.4758	3.02794	2	0.16300	79	498	
39	30.1628	2.96052	3	0.27810	128	1093	
40	30.7126	2.90876	3	0.18220	123	687	
41	32.2838	2.77069	2	0.35670	82	1143	
42	32.9136	2.71910	2	0.22190	67	381	
43	33.2846	2.68963	2	0.31920	65	488	
44	34.1600	2.62268	3	0.31060	103	865	
45	34.5268	2.59566	3	0.24370	116	877	

[00109] In some embodiments, the present invention provides Form K of Compound 1, having an X-ray diffraction pattern substantially similar to that depicted in Figure 38. In one aspect, the present invention provides Form K having a DSC pattern substantially similar to that depicted in Figure 39.

[00110] In certain embodiments, the present invention provides Form L of Compound 1, as a DMF solvate. In certain embodiments, the present invention provides Form L of Compound 1. In certain embodiments, the present invention provides Form L of Compound 1, substantially free of other forms of Compound 1. In certain embodiments, Form L is characterized in that it

has one or more, two or more, or three or more, peaks in its XRPD pattern selected from those at about 8.6, 13.1, 13.6, 14.3, 15.5, 17.1, 19.7, 21.0, 21.4, 22.0, 23.8, 25.7, 26.0, 26.3, 27.4 and 36.7 degrees 2-theta.

[00111] In other embodiments, Form L of Compound 1 is characterized in that it has substantially all of the peaks in its XRPD pattern listed in Table 12, below.

Table 12. XRPD Peaks Form L

# Peak no.	Data List 2Theta (deg)	d (Å)	I/I1	FWHM (deg)	Intensity (Counts)	Integrated Int (Counts)
1	8.6002	10.27334	17	0.21800	718	4810
2	11.2161	7.88251	3	0.20180	142	1221
3	13.0528	6.77717	7	0.21970	306	2104
4	13.5995	6.50593	7	0.30570	292	2295
5	14.3277	6.17687	8	0.22090	334	1928
6	14.6858	6.02704	5	0.23490	204	1295
7	15.4534	5.72935	8	0.35800	339	3103
8	17.1047	5.17978	21	0.21840	890	5746
9	18.3513	4.83063	3	0.25170	133	1308
10	19.0382	4.65786	6	0.20600	248	1347
11	19.7005	4.50274	15	0.24070	608	3802
12	20.1200	4.40979	4	0.25040	146	1548
13	21.0352	4.21995	20	0.28640	835	6052
14	21.3600	4.15651	13	0.39160	559	5224
15	22.0233	4.03280	35	0.21280	1450	8712
16	22.4000	3.96583	3	0.07640	131	496
17	23.7667	3.74078	28	0.23710	1157	8319
18	24.8000	3.58721	4	0.20260	180	1287
19	25.0800	3.54779	5	0.14180	191	943
20	25.6800	3.46624	7	0.25760	279	3153
21	25.9600	3.42949	7	0.00000	285	0
22	26.3309	3.38201	100	0.20210	4157	24328
23	27.3729	3.25559	11	0.19670	456	3726
24	28.8086	3.09653	4	0.24270	172	1606
25	31.9416	2.79959	4	0.27400	166	2105
26	34.4525	2.60108	3	0.20350	130	1099
27	35.3329	2.53826	3	0.22000	127	933
28	36.0702	2.48805	4	0.24830	169	1309
29	36.4400	2.46365	4	0.22900	148	814
30	36.7351	2.44453	7	0.24530	272	1775
31	38.4402	2.33993	4	0.21420	181	1064
32	39.1276	2.30039	3	0.23610	129	1947

[00112] In some embodiments, the present invention provides Form L of Compound 1, having an X-ray diffraction pattern substantially similar to that depicted in **Figure 40**. In one aspect, the present invention provides Form L having a DSC pattern substantially similar to that depicted in **Figure 41**.

[00113] In another embodiment, the present invention provides Compound 1 as an amorphous solid. Amorphous solids are well known to one of ordinary skill in the art and are typically prepared by such methods as lyophilization, melting and precipitation from supercritical fluid, among others.

[00114] In certain embodiments, the present invention provides a composition comprising Form A of Compound 1 and at least one or more other solid forms of Compound 1. In some embodiments, the present invention provides a composition comprising Form A and Form B. In other embodiments, the present invention provides a composition comprising Form A and amorphous Compound 1.

(3) Formulations

[00115] The present invention provides formulations and methods of administration of Compound 1. In certain embodiments, the present invention provides formulations that are suitable for parenteral administration of Compound 1. Formulations provided for parenteral administration include sterile solutions for injection, sterile suspensions for injection, sterile emulsions, and dispersions. In some embodiments, Compound 1 is formulated for intravenous administration. In some embodiments, Compound 1 is formulated for intravenous administration at a concentration of about 0.5 to about 5.0 mg/mL.

[00116] In certain embodiments, the solubility of Compound 1 in a formulation can be improved by the addition of solubilizing agents. Solubilizing agents are known to one skilled in the art and include cyclodextrins, nonionic surfactants, and the like. Cyclodextrins include, for example, sulfobutyl ether beta-cyclodextrin, sodium salt (e.g., Captisol®). Exemplary nonionic surfactants include Tween®-80 and PEG-400. Other illustrative formulations of Compound 1 of the present invention include 10%/30%/60%, 5%/30%/65%, and 2.5%/30%/67.5%, respectively, of Tween-80, PEG-400, and water.

[00117] In certain embodiments, the present invention provides a composition comprising Compound 2 or a pharmaceutically acceptable salt thereof, and a solubilizing agent.

[00118] In some embodiments, the present invention provides a composition comprising Compound 2 or a pharmaceutically acceptable salt thereof, and a cyclodextrin.

[00119] In some embodiments, the present invention provides a composition comprising Compound 2 or a pharmaceutically acceptable salt thereof, and a sulfobutyl ether beta-cyclodextrin, sodium salt.

[00120] In certain embodiments, the present invention provides a composition comprising Compound 1, and a solubilizing agent.

[00121] In some embodiments, the present invention provides a composition comprising Compound 1, and a cyclodextrin.

[00122] In some embodiments, the present invention provides a composition comprising Compound 1, and a sulfobutyl ether beta-cyclodextrin, sodium salt.

Additional components

[00123] In some embodiments, formulations may comprise one or more additional agents for modification and/or optimization of release and/or absorption characteristics. For example, incorporation of buffers, co-solvents, diluents, preservatives, and/or surfactants may facilitate dissolution, absorption, stability, and/or improved activity of active compound(s), and may be utilized in formulations of the invention. In some embodiments, where additional agents are included in a formulation, the amount of additional agents in the formulation may optionally include: buffers about 10% to about 90%, co-solvents about 1% to about 50%, diluents about 1% to about 10%, preservative agents about 0.1% to about 8%, and/or surfactants about 1% to about 30%, based upon total weight of the formulation, as applicable.

[00124] Suitable co-solvents (i.e., water-miscible solvents) are known in the art. For example, suitable co-solvents include, but are not limited to ethyl alcohol, propylene glycol.

[00125] Physiologically acceptable diluents may optionally be added to improve product characteristics. Physiologically acceptable diluents are known in the art and include, but are not limited to, sugars, inorganic salts and amino acids, and solutions of any of the foregoing. Representative examples of acceptable diluents include dextrose, mannitol, lactose, and sucrose, sodium chloride, sodium phosphate, and calcium chloride, arginine, tyrosine, and leucine, and the like, and aqueous solutions thereof.

[00126] Suitable preservatives are known in the art, and include, for example, benzyl alcohol, methyl paraben, propyl paraben, sodium salts of methyl paraben, thimerosal, chlorobutanol, and phenol. Suitable preservatives include but are not limited to: chlorobutanol (0.3-0.9% W/V), parabens (0.01-5.0% W/V), thimerosal (0.004-0.2% W/V), benzyl alcohol (0.5-5% W/V), phenol (0.1-1.0% W/V), and the like.

[00127] Suitable surfactants are also known in the art and include, e.g., poloxamer, polyoxyethylene ethers, polyoxyethylene sorbitan fatty acid esters polyoxyethylene fatty acid esters, polyethylene glycol fatty acid esters, polyoxyethylene hydrogenated castor oil,

polyoxyethylene alkyl ether, polysorbates, cetyl alcohol, glycerol fatty acid esters (e.g., triacetin, glycerol monostearate, and the like), polyoxymethylene stearate, sodium lauryl sulfate, sorbitan fatty acid esters, sucrose fatty acid esters, benzalkonium chloride, polyethoxylated castor oil, and docusate sodium, and the like, and combinations thereof. In some embodiments the formulation may further comprise a surfactant.

[00128] In certain embodiments, the present invention provides dosage forms including unit dose forms, dose-concentrates, etc. for parenteral administration wherein the dosage forms comprise Compound 1. Parenteral administration of provided formulations may include any of intravenous injection, intravenous infusion, intradermal, intralesional, intramuscular, subcutaneous injection, or depot administration of a unit dose. A unit dose may or may not constitute a single "dose" of active compound(s), as a prescribing doctor may choose to administer more than one, less than one, or precisely one unit dose in each dose (i.e., each instance of administration). For example, unit doses may be administered once, less than once, or more than once a day, for example, once per week, twice per week, once every other day (QOD), once per day, or 2, 3 or 4 times per day, or 1 or 2 times per day.

(4) Pharmaceutical Uses and Administration

[00129] As described above, Compound 1 is an inhibitor of Aurora kinases. As such, it is useful for treating diseases or conditions mediated by one or more Aurora kinases. Such diseases include, for example, cancers. In other embodiments of the methods provided herein, the cancer being treated is selected from the group consisting of bladder cancer, brain cancer, breast cancer, cervical cancer, colon cancer, esophageal cancer, head and neck cancer, leukemia, liver cancer, lung cancer (e.g., small cell and non-small cell lung cancers), lymphoma, melanoma, myeloma, neuroendocrine cancer (e.g., neuroblastoma), ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, sarcoma, skin cancer, stomach cancer, testicular cancer, thyroid cancer, and uterine cancer.

[00130] In certain embodiments, the patient has a solid tumor. For example, the method may be used to treat cancers of the brain, colon, lung, prostate, ovary, breast, cervix, and skin. In one embodiment, the lung cancer is a non-small cell lung cancer (NSCLC). In another embodiment, the skin cancer is a melanoma.

[00131] In other embodiments, the patient has a hematological tumor. In another embodiment, the patient has a lymphoma or leukemia. In certain embodiments the patient's hematological tumor is a mantle cell lymphoma (MCL), Non-Hodgkin's lymphoma (NHL), Hodgkin's disease, acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), or acute lymphoblastic lymphoma (ALL).

[00132] The invention is also directed to methods of treating cancer, comprising administering specific doses of Compound 1. These doses may be administered once or more than once. In one embodiment, the dose or doses are administered according to schedules described herein. Compositions of compounds formulated to contain the appropriate amount of compound so that the dose is readily administered are also envisaged.

[00133] In one aspect, the invention is directed to a method of treating cancer comprising administering to a patient Compound 1 or a composition thereof (e.g., a provided formulation herein) with a frequency of at least once every three weeks. In one embodiment, Compound 1 or a composition thereof is administered once every three weeks. In another embodiment, Compound 1 or a composition thereof is administered once every two weeks. In another embodiment, Compound 1 or a composition thereof is administered once per week. In another embodiment, Compound 1 or a composition thereof is administered twice per week. In another embodiment, the compound is administered daily.

[00134] In another embodiment, Compound 1 is administered to the patient in at least one cycle of once a day for five days. In another embodiment Compound 1 is administered in two cycles of once a day for five days, with at least one day between the two cycles wherein the compound is not administered. In another embodiment, Compound 1 is administered in at least two cycles, with two, three, four, five, six, seven, or eight days off between the two cycles. In another embodiment, Compound 1 is administered in at least two cycles, with nine days off between the two cycles.

[00135] The invention is also directed to methods of treating cancer comprising administering specific doses of Compound 1. Such doses may be administered once or more than once. In one embodiment, such dose or doses are administered according to schedules described herein. Compositions of compounds formulated to contain the appropriate amount of compound so that the dose is readily administered are also envisaged.

[00136] In another aspect, the invention is directed to a method for treating cancer in a patient, comprising administering to a patient having a cancer an effective amount of Compound 1.

[00137] In another aspect, the invention is directed to a method for treating cancer in a patient comprising administering to a patient having cancer a dose of about 10 mg/m²-3000 mg/m² of Compound 1. The dose may be administered as a composition comprising the dose of Compound 1 and one or more pharmaceutically acceptable carriers, diluents, or excipients.

[00138] In one embodiment, the dose is administered once a week. In another embodiment the dose administered once a week is 240 mg/m² - 2000 mg/m². In another embodiment, the dose administered once a week is about 480 mg/m² - 1800 mg/m². In another embodiment, the dose administered once a week is about 480 mg/m² - 1500 mg/m². In another embodiment, the dose administered once a week is about 480 mg/m² - 1200 mg/m². In another embodiment, the dose administered once a week is about 750 mg/m² - 1500 mg/m². In another embodiment, the dose administered once a week is about 960 mg/m² - 1200 mg/m².

[00139] In another embodiment, the dose is administered once a week for three weeks.

[00140] In another embodiment, the method of treating cancer comprises administering to a patient a dose of 30 mg/m² - 2000 mg/m² of Compound 1 administered in a cycle of once a week for three weeks, wherein there is at least one day off between cycles. In another embodiment, the method of treating cancer comprises administering to a patient a dose of 30 mg/m² - 750 mg/m² of Compound 1 administered in a cycle of once a week for three weeks, wherein there is at least one day off between cycles. In another aspect, the invention is directed to a method of treating cancer comprising administering to a patient a dose of 60 mg/m² - 750 mg/m² of Compound 1 administered in a cycle of once a week for three weeks, wherein there is at least one day off between cycles. In one embodiment, Compound 1 is administered on Day 1, Day 8, and Day 15 of three week cycle, with 7 days off between cycles. In other words, Compound 1 is administered on Day 1, Day 8, and Day 15 of a 21 day cycle, with 7 days off between cycles. In another embodiment, the dose administered on Day 1, Day 8, and Day 15 of the three week cycle with 7 days off between cycles is 200 mg/m² - 600 mg/m². In another embodiment, the dose administered on Day 1, Day 8, and Day 15 of the three week cycle with 7 days off between cycles is 300 mg/m² - 500 mg/m². In another embodiment, the dose administered on Day 1, Day 8, and Day 15 of the three week cycle with 7 days off between cycles is 350 mg/m² - 450 mg/m². In another embodiment the dose administered on Day 1, Day 8, and Day 15 of the three week

cycle with 7 days off between cycles is 300 mg/m² - 400 mg/m². In another embodiment, the dose administered on Day 1, Day 8, and Day 15 of the three week cycle with 7 days off between cycles is 400 mg/m² - 500 mg/m². In another embodiment, the dose administered on Day 1, Day 8, and Day 15 of the three week cycle with 7 days off between cycles is 500 mg/m² - 600 mg/m².

[00141] In another aspect, the invention is directed to a method comprising administering to a patient a dose of 30 mg/m² - 300 mg/m² of Compound 1. In one embodiment, the dose is administered once per day. In another embodiment, the dose administered once per day is 100 mg/m² - 300 mg/m². In another embodiment the dose administered once per day is 150 mg/m² - 250 mg/m². In another embodiment, the dose administered once per day is 100 mg/m² - 200 mg/m². In another embodiment, the dose administered once per day is 200 mg/m² - 300 mg/m². In other embodiments the doses are administered once per day for five days.

(5) Combination Therapy

[00142] It will also be appreciated that Compound 1 and pharmaceutically acceptable compositions comprising Compound 1 can be employed in complementary combination therapies with other active agents or medical procedures. Thus, Compound 1 and pharmaceutically acceptable compositions thereof can be administered concurrently with, prior to, or subsequent to, one or more other desired active agents or medical procedures. The particular combination of therapies (agents or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, Compound 1 may be administered concurrently with another active agent used to treat the same disorder), or they may achieve different effects (e.g., control of any adverse effects). Non-limiting examples of such agents and procedures include surgery, radiotherapy (e.g., gamma-radiation, neutron beam radiotherapy, electron beam radiotherapy, proton therapy, brachytherapy, and systemic radioisotopes), endocrine therapy, biologic response modifiers (interferons, interleukins, and tumor necrosis factor (TNF) to name a few examples), hyperthermia and cryotherapy, agents to attenuate any adverse effects (e.g., antiemetic agents), and other approved chemotherapeutic anticancer agents.

[00143] Examples of chemotherapeutic anticancer agents that may be used as second active agents in combination with Compound 1 include, but are not limited to, alkylating agents (e.g.,

mechlorethamine, chlorambucil, cyclophosphamide, melphalan, ifosfamide), antimetabolites (e.g., methotrexate), other aurora kinase inhibitors, purine antagonists and pyrimidine antagonists (e.g., 6-mercaptopurine, 5-fluorouracil, cytarabine, gemcitabine), spindle poisons (e.g., vinblastine, vincristine, vinorelbine, paclitaxel), podophyllotoxins (e.g., etoposide, irinotecan, topotecan), antibiotics (e.g., doxorubicin, daunorubicin, bleomycin, mitomycin), nitrosoureas (e.g., carmustine, lomustine), inorganic ions (e.g., platinum complexes such as cisplatin, carboplatin), enzymes (e.g., asparaginase), hormones (e.g., tamoxifen, leuprolide, flutamide, and megestrol), topoisomerase II inhibitors or poisons, EGFR (Her1, ErbB-1) inhibitors (e.g., gefitinib), antibodies (e.g., rituximab), IMiDs (e.g., thalidomide, lenalidomide), various targeted agents (e.g., HDAC inhibitors such as vorinostat), Bcl-2 inhibitors, VEGF inhibitors; proteasome inhibitors (e.g., bortezomib), cyclin dependent kinase (cdk) inhibitors (e.g., seliciclib), and dexamethasone.

[00144] Some specific anticancer agents that can be used in combination with Compound 1 include, but are not limited to: azacitidine (e.g., Vidaza[®]); bortezomib (e.g., Velcade[®]); capecitabine (e.g., Xeloda[®]); carboplatin (e.g., Paraplatin[®]); cisplatin (e.g., Platinol[®]); cyclophosphamide (e.g., Cytosan[®], Neosar[®]); cytarabine (e.g., Cytosar[®]), cytarabine liposomal (e.g., DepoCyt[®]), cytarabine ocfosfate or other formulations of the active moiety; doxorubicin, doxorubicin hydrochloride (e.g., Adriamycin[®]), liposomal doxorubicin hydrochloride (e.g., Doxil[®]); fludarabine, fludarabine phosphate (Fludara[®]); 5-fluorouracil (e.g., Adrucil[®]); gefitinib (e.g., Iressa[®]); gemcitabine hydrochloride (e.g., Gemzar[®]); irinotecan (CPT-11, camptothecin-11), irinotecan hydrochloride (e.g., Camptosar[®]); lenalidomide (e.g., Revlimid[®]); melphalan (e.g., Alkeran[®]); paclitaxel (e.g., Taxol[®]); paclitaxel protein-bound (e.g., Abraxane[®]); rituximab (e.g., Rituxan[®]); vorinostat (e.g., Zolinza[®]).

[00145] Other anticancer agents that can be used in combination with Compound 1 include, but are not limited to: acivicin; aclarubicin; acodazole hydrochloride; acronine; adalimumab (e.g., Humira[®]); adozelesin; alitretinoin (e.g., Panretin[®]); altretamine (hexamethylmelamine; e.g., Hexalen[®]); ambomycin; ametantrone acetate; aminoglutethimide (e.g., Cytadren[®]); amonafide malate (e.g., Xanafide[®]); amsacrine; anastrozole (e.g., Arimidex[®]); anthramycin; asparaginase (e.g., Kidrolase[®], Elspar[®]); asperlin; azetepa; azotomycin; batimastat; benzodepa; bevacizumab (e.g., Avastin[®]); bexarotene (e.g., Targetin[®]); bicalutamide (e.g., Casodex[®]); bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate (e.g., Blenoxane[®]); brequinar

sodium; bropirimine; busulfan (e.g., Busulfex[®], Myleran[®]); CD20 antibodies such as ofatumumab; CD23 antibodies such as lumiliximab; CD52 antibodies such as alemtuzumab (e.g., Campath[®]); CD80 antibodies such as galiximab; cactinomycin; calusterone; caracemide; carbetimer; carmustine (e.g., BiCNU[®]); carmustine implant (e.g., Gliadel[®] wafer); carubicin hydrochloride; carzelesin; cedefingol; celecoxib (COX-2 inhibitor, e.g., Celebrex[®]); chlorambucil (e.g., Leukeran[®]); cirolemycin; cladribine (e.g., Leustatin[®]); clofarabine; cloretazine; crisnatol; crisnatol mesylate; 4-hydroperoxycyclophosphamide; dacarbazine (e.g., DTIC-Dome[®]); dactinomycin (e.g., Cosmegen[®]); dasatanib (e.g., Sprycel[®]); daunorubicin hydrochloride (e.g., Cerubidine[®]), liposomal daunorubicin citrate (e.g., DaunoXome[®]); decitabine (e.g., Dacogen[®]); denileukin diftitox (e.g., Ontak[®]); dexormaplatin; dezaguanine, dezaguanine mesylate; diaziquone; droloxifene, droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; edrecolomab (Panorex[®]); eflornithine, eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride (e.g., Ellence[®]); erbulozole; erlotinib (e.g., Tarceva[®]); esorubicin hydrochloride; estramustine, estramustine phosphate sodium (e.g., Emcyt[®]), estramustine analogues; etanidazole; etoposide (VP-16; e.g., Toposar[®]), etoposide phosphate (e.g., Etopophos[®]); etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine (e.g., FUDR[®]); flurocitabine; flutamide (e.g., Eulexin[®]); fosquidone; fostriecin, fostriecin sodium; G250 monoclonal antibody; galiximab; gefitinib (e.g., Iressa[®]); gemtuzumab ozogamicin (Mylotarg[®]); goserelin acetate (Zoladex[®]); hydroxyurea (e.g., Droxia[®], Hydrea[®]); ibritumomab tiuxetan (e.g., Zevalin[®]) + ¹¹¹In or ⁹⁰Yt; idarubicin, idarubicin hydrochloride (e.g., Idamycin[®]); ifosfamide (e.g., Ifex[®]); ilmofosine; iproplatin; lanreotide, lanreotide acetate; lapatinib (e.g., Tykerb[®]); letrozole (e.g., Femara[®]); leuprolide acetate (e.g., Eligard[®], Viadur[®]); liarozole, liarozole hydrochloride; CD33 antibodies such as lintuzumab; lometrexol, lometrexol sodium; lomustine (e.g., CeeNu[®]); losoxantrone, losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine (nitrogen mustard, mustine), mechlorethamine hydrochloride (e.g., Mustargen[®]); megestrol acetate (e.g., Megace[®]); melengestrol acetate; menogaril; mercaptopurine (e.g., Purinethol[®]); methotrexate sodium (e.g., Rheumatrex[®]); metoprine; meturedopa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin (Mutamycin[®]), mitomycin analogues; mitosper; mitotane; mitoxantrone, mitoxantrone hydrochloride (e.g., Novantrone[®]); mycophenolic acid; nelarabine (Arranon[®]); nocodazole; nogalamycin; ormaplatin; oxisuran; panitumumab (e.g., Vectibix[®]); pegaspargase

(PEG-L-asparaginase; e.g., Oncaspar[®]); peliomycin; pemetrexed (e.g., Alimta[®]); pentamustine; peplomycin sulfate; perfosfamide; pertuzumab (e.g. Omnitarg[®]); pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride (e.g., Matulane[®]); puromycin; puromycin hydrochloride; pyrazofurin; R-roscovitine (seliciclib); riboprime;; safingol; safingol hydrochloride; semustine; simtrazene; sorafenib (e.g., Nexavar[®]); sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin (e.g., Zanosar[®]); sulofenur; sunitinib malate (e.g., Sutent[®]); talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; temozolomide (e.g., Temodar[®]); teniposide (e.g., Vumon[®]); teroxirone; testolactone; thalidomide (e.g., Thalomid[®]); thiamiprine; thioguanidine; 6-thioguanine; thiotepa (e.g., Thioplex[®]); tiazofurin; tipifarnib (e.g., Zarnestra[®]); tirapazamine; topotecan (e.g., Hycamtin[®]); toremifene, toremifene citrate (e.g., Fareston[®]); tositumomab+¹³¹I (e.g., Bexxar[®]); trastuzumab (e.g., Herceptin[®]); trestolone acetate; triciribine, triciribine phosphate; trimetrexate, trimetrexate glucuronate; triptorelin; troxacitabine (e.g., Troxatyl[®]); tubulazole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate (e.g., Velban[®]); vincristine (leurocristine) sulfate (e.g., Vincasar[®]); vindesine, vindesine sulfate; vinepidine sulfate; vinglycinatate sulfate; vinleurosine sulfate; vinorelbine tartrate (e.g., Navelbine[®]); vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin, zinostatin stimalamer; and zorubicin (rubidazone) hydrochloride.

[00146] Other anticancer agents that can be used in combination with Compound 1 include, but are not limited to: 20-epi-1,25-dihydroxyvitamin D3; 5-ethynyluracil; abiraterone acetate; acylfulvene, (hydroxymethyl)acylfulvene; adecypenol; ALL-TK antagonists; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; anagrelide (e.g., Agrylin[®]); andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; arsenic trioxide (e.g., Trisenox[®]); asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; BCR/ABL antagonists; benzochlorins; benzoylstauroporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bisantrene; bisaziridinylspermine; bisnafide; bistratene A;

brefeldin A or its prodrug breflate; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives (e.g., irinotecan); carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage-derived inhibitor; casein kinase inhibitors; castanospermine; cecropin B; cetorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; clarithromycin (e.g., Biaxin[®]); clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4, combretastatin analogues; conagenin; crambescidin 816; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytolytic factor; cytostatin; dacliximab (daclizumab; e.g., Zenapax[®]); dehydrodidemnin B; deslorelin; dexamethasone (e.g., Decadron[®]); dexifosfamide; dexrazoxane; dexverapamil; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; 9-dihydrotaxol; dioxamycin; diphenyl; docetaxel (e.g., Taxotere[®]); docosanol; doxifluridine; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; elemene; emitefur; epristeride; estrogen agonists; estrogen antagonists; exemestane (e.g., Aromasin[®]); fadrozole; filgrastim; finasteride; flavopiridol (alvocidib); flezelastine; fluasterone; fluorodaunorubicin hydrochloride; forfenimex; formestane; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganciclovir; ganirelix; gelatinase inhibitors; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idoxifene; idramantone; ilomastat; imatinib mesylate (e.g., Gleevec[®]); imiquimod (e.g., Aldara[®]), and other cytokine inducers; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons such as interferon alpha (e.g., Intron[®] A); pegylated interferon alfa-2b (e.g., PegIntron[®]); interleukins such as IL-2 (aldesleukin, e.g., Proleukin[®]); iobenguane; iododoxorubicin; 4-ipomeanol; iroplact; irsogladine; isobengazole; isohomohalicondrin B; jasplakinolide; kahalalide F; lamellarin-N triacetate; leinamycin; lenograstim; lentinan sulfate; leptolstatin; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lonidamine; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone (e.g., Mifeprex[®]); miltefosine; mirimostim; mitoguazone; mitolactol; mitonafide; mitotoxin fibroblast growth factor-saporin; mofarotene; cetuximab (e.g., Erbitux[®]); human chorionic

gonadotrophin; monophosphoryl lipid A+mycobacterium cell wall sk; mopidamol; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; nilutamide (e.g., Nilandron[®]); nisamycin; nitric oxide modulators; nitroxide antioxidants (e.g., tempol); nitrullyn; oblimersen (Genasense[®]); O6-benzylguanine; octreotide (e.g., Sandostatin[®]); octreotide acetate (e.g., Sandostatin LAR[®]); okicenone; oligonucleotides; onapristone; oracin; osaterone; oxaliplatin (e.g., Eloxatin[®]); oxaunomycin; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; panaxytriol; panomifene; parabactin; pazelliptine; peldesine; pentosan polysulfate sodium; pentostatin (e.g., Nipent[®]); pentozole; perflubron; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum-triamine complex; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitors, including microalgal PKC inhibitors; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed (e.g., Tomudex[®]); ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium (Re186); rhizoxin; ribozymes; RII retinamide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; saintopin; SarCNU; sarcophytol A; Sdi 1 mimetics; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; splenopentin; spongistatin 1; squalamine; steroids (e.g., prednisone, prednisolone); stipiamide; stromelysin inhibitors; sulfinosine; sulindac; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; tallimustine; tamoxifen, tamoxifen citrate (e.g., Nolvadex[®]), tamoxifen methiodide; tauromustine; tazarotene; tellurapyrylium; telomerase inhibitors; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrigan; thyroid stimulating hormone; tin ethyl etiopurpurin; titanocene bichloride; topsentin; translation inhibitors; tretinoin (all-trans retinoic acid, e.g., Vesanoide[®]); triacetyluridine; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor;

urokinase receptor antagonists; variolin B; velaresol; veramine; verdins; vinxaltine; vitaxin; zanoterone; and zilascorb.

[00147] For a more comprehensive discussion of updated cancer therapies see, *The Merck Manual*, Seventeenth Ed. 1999. See also the National Cancer Institute (NCI) website (<http://www.cancer.gov/drugdictionary/>) for a comprehensive list of oncology medicaments suitable as second active agents, and the U.S. Food and Drug Administration (FDA) website for a list of the FDA-approved oncology medicaments.

[00148] In other embodiments, the second active agent is a supportive care agent, such as an antiemetic agent or erythropoiesis stimulating agents. Specific antiemetic agents include, but are not limited to, phenothiazines, butyrophenones, benzodiazapines, corticosteroids, serotonin antagonists, cannabinoids, and NK1 receptor antagonists. Examples of phenothiazine antiemetic agents include, but are not limited to, prochlorperazine and trimethobenzamide. Examples of butyrophenone antiemetic agents include, but are not limited to, haloperidol. Examples of benzodiazapine antiemetic agents include, but are not limited to, lorazepam. Examples of corticosteroid antiemetic agents include, but are not limited to, dexamethasone. Examples of serotonin receptor (5-HT₃ receptor) antagonist antiemetic agents include, but are not limited to, dolasetron mesylate (e.g., Anzemet[®]), granisetron (e.g., Kytril[®]), itasetron, ondansetron (e.g., Zofran[®]), palonosetron (e.g., Aloxi[®]) ramosetron, tropisetron (e.g., Navoban[®]), batanopride, dazopride, renzapride. Examples of cannabinoid antiemetic agents include, but are not limited to, dronabinol. Examples of NK1 receptor antagonists include, but are not limited to, aprepitant (e.g., Emend[®]).

[00149] Other supportive care agents include agents that stimulate erythropoiesis or other hematopoietic processes, such as epoetin alfa (e.g., Epogen[®], Procrit[®]); G-CSF and recombinant forms such as filgrastim (e.g., Neupogen[®]), pegfilgrastim (e.g., Neulasta[®]), and lenofilgrastim; darbepoetin alfa (e.g., Aranesp[®]); and GM-CSF and recombinant forms such as sargramostim (e.g., Leukine[®]) or molgramostim. Other supportive care agents include chemoprotectant agents such as amifostine (e.g., Ethyl[®]), dexrazoxane (e.g., Zinecard[®]), leucovorin (folinic acid), and mesna (e.g., Mesnex[®]); thrombopoietic growth factors such as interleukin-11 (IL-11, oprelvekin, e.g., Neumega[®]); bisphosphonates such as pamidronate disodium (e.g., Aredia[®]), etidronate disodium (e.g., Didronel[®]) and zoledronic acid (e.g., Zometa[®]); and TNF antagonists, such as infliximab (e.g., Remicade[®]).

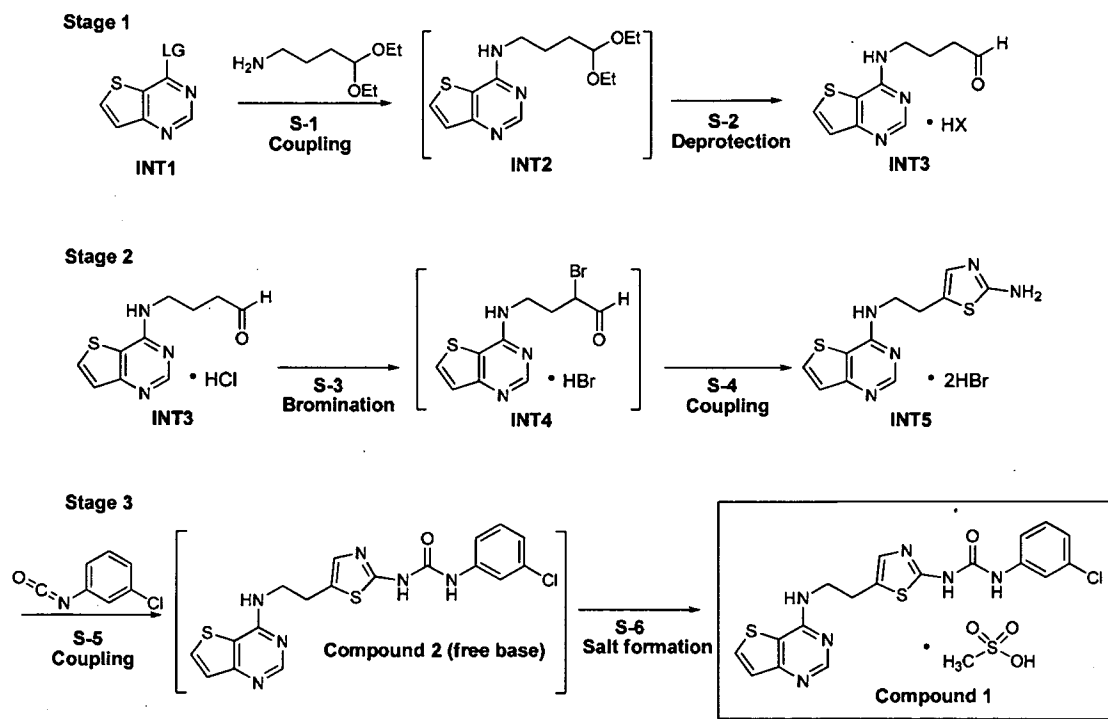
[00150] Tumor lysis syndrome (TLS) may be expected in the treatment of hematologic cancers, and supportive care treatment(s) to mitigate or prevent TLS or its component symptoms may be administered to patients treated with Compound 1 according to the invention. Treatments suitable for preventing or mitigating TLS (or any of the symptoms thereof, including hyperkalemia, hyperphosphatemia, hyperuricemia, hypocalcemia, and acute renal failure), include, for example, allopurinol (e.g., Zylprim[®]), rasburicase (e.g., Elitek[®]), and sodium polystyrene sulfonate (e.g., Kayexalate[®]).

[00151] Doses and dosing regimens of Compound 1 together with other active moieties and combinations thereof should depend on the specific indication being treated, age and condition of a patient, and severity of adverse effects, and may be adjusted accordingly by those of skill in the art. Examples of doses and dosing regimens for other active moieties can be found, for example, in *Physician's Desk Reference*, and will require adaptation for use in the methods of the invention.

[00152] While the active moieties mentioned herein as second active agents may be identified as free active moieties or as salt forms (including salts with hydrogen or coordination bonds) or other as non-covalent derivatives (e.g., chelates, complexes, and clathrates) of such active moieties, it is to be understood that the given representative commercial drug products are not limiting, and free active moieties, or salts or other derivative forms of the active moieties may alternatively be employed. Accordingly, reference to an active moiety should be understood to encompass not just the free active moiety but any pharmacologically acceptable salt or other derivative form that is consistent with the specified parameters of use.

(6) Methods for Preparing Compound 1

[00153] In one aspect, the present invention provides methods for preparing a Compound 1, according to the steps depicted in Scheme I.

Scheme I

[00154] In Scheme I above, LG and HX are as defined below and in classes and subclasses as described herein.

[00155] In one aspect, the present invention provides methods for preparing INT5, Compound 2 and Compound 1, according to the steps depicted in Scheme I above. In certain embodiments, the present invention provides a method for preparing Compound 2 comprising the steps of providing INT5 and coupling INT5 with 3-chlorophenyl-isocyanate to form Compound 2.

[00156] As depicted in step S-1, a compound of formula INT1 is coupled to aminobutyraldehyde diethyl actetal via a displacement of the LG moiety of formula INT1 to form INT2, where LG is a suitable leaving group. A "suitable leaving group" is a group that is subject to nucleophilic displacement, i.e., a chemical group that is readily displaced by an incoming chemical moiety, in this case, an amino moiety of aminobutyraldehyde diethyl actetal. Suitable leaving groups are well known in the art, e.g., see, *Advanced Organic Chemistry*, Jerry March, 5th Ed., pp. 351-357, John Wiley and Sons, N.Y. Such leaving groups include, but are not limited to, halogen and sulfonate esters. Examples of suitable leaving groups include chloro, iodo, bromo, fluoro, methanesulfonyloxy (mesyloxy), tosyloxy, triflyloxy, nitro-

phenylsulfonyloxy (nosyloxy), and bromo-phenylsulfonyloxy (brosyloxy). In another embodiment, a suitable leaving group is chlorine or tosyl.

[00157] According to an alternate embodiment, the suitable leaving group may be generated *in situ* within the reaction medium. For example, a leaving group may be generated *in situ* from a precursor of that compound wherein said precursor contains a group readily replaced by said leaving group *in situ*.

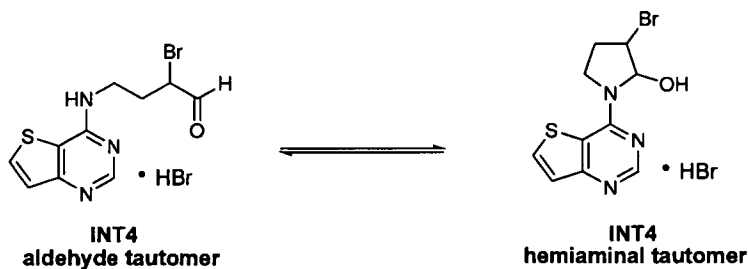
[00158] In step S-2, INT2 is deprotected using a suitable acid to form formula INT3. HX is a suitable acid, wherein X⁻ is the anion of said suitable acid. One skilled in the art would recognize that various mineral or organic acids are suitable for achieving the deprotection. In one embodiment, a suitable mineral or organic acid includes hydrobromic acid, sulfuric acid, methanesulfonic acid and the like. In one embodiment, the suitable acid is hydrochloric acid, wherein the anion X⁻ is chloride. One of ordinary skill in the art will appreciate that X⁻ can be derived from a variety of organic and inorganic acids. In certain embodiments, X⁻ is a suitable anion. Such anions include those derived from an inorganic acid such as trifluoroacetic acid, hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid or perchloric acid. It is also contemplated that such anions include those derived from an organic acid such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid, malonic acid, methanesulfonic acid, optionally substituted phenylsulfonic acids, sulfinic acid, optionally substituted phenylsulfinic acid, trifluoroacetic acid, trifluoromethanesulfonic (triflic) acid, optionally substituted benzoic acids, and the like. One of ordinary skill in the art will recognize that such salts are formed by other methods used in the art such as ion exchange.

[00159] For example, the general preparation of INT3 is as follows. INT1 combined with aminobutyraldehyde diethyl acetal in 2-propanol in the presence of triethylamine (TEA) at reflux temperature affords INT2. After an aqueous/organic workup (water/ethyl acetate and aqueous sodium chloride [NaCl]/ethyl acetate), treatment of crude acetal in tetrahydrofuran with aqueous HCl affords INT3 as an off-white crystalline solid. It has been surprisingly found that performing an aqueous/organic workup of INT2 at 45 °C to 50 °C prevents the precipitation of solids. It will be appreciated that INT3, although represented as the open aldehyde form in Scheme I, may be an equilibrium mixture of the aldehyde and hemiaminal tautomers shown below:

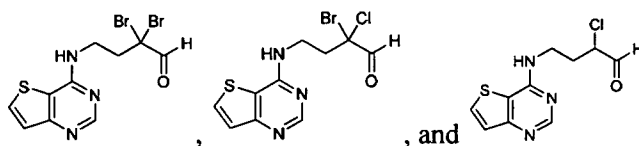
INT3 Aldehyde-Hemiaminal Tautomers.

[00160] In step S-3, INT3 is combined with a suitable brominating agent to form intermediate INT4. One skilled in the art would recognize that various organic acids are suitable for achieving the bromination. In one embodiment, a suitable organic acid includes propionic acid. In one embodiment, the suitable organic acid is acetic acid. One skilled in the art would recognize that various brominating agents are appropriate for such reaction. In certain embodiments, suitable brominating agents include dibromohydantoin and N-bromosuccinimide. In one embodiment, the brominating agent is bromine. One skilled in the art would recognize that the reaction may be performed at varied temperature ranges. In one embodiment, the reaction temperature range for heating is from about 80 °C to 90 °C. In one embodiment, the reaction temperature for heating is 85 °C. In one embodiment, the temperature range for cooling is from about 50 °C to about 55 °C.

[00161] For example, the general preparation of INT5 is as follows. INT3 is heated in acetic acid to afford a solution, cooled to 50 °C to 55 °C, and then a solution of bromine is added. Heat is removed, acetone and methyl *tert*-butyl ether (MTBE) are added to help induce crystallization, and the resulting solid INT4 is filtered. To INT4 and thiourea is added ethanol and water and the resulting slurry heated. The reaction mixture is then concentrated to azeotropically remove water, additional ethanol is added, and then MTBE is added to help induce crystallization. INT5 is isolated as a yellow solid. It will be appreciated that INT4, although represented as the open aldehyde form in Scheme I, may be an equilibrium mixture of the aldehyde and hemiaminal tautomers as shown below:

INT4 Aldehyde-Hemiaminal Tautomers.

[00162] In some embodiments, the present invention provides INT4 having less than about 30%, less than about 25%, less than about 10%, less than about 5%, or less than about 1%, by weight of any of the following compounds:



[00163] In step S-4 INT4 is coupled with thiourea to form a thiazole INT5 in a suitable solvent or solvent mixture. One skilled in the art would recognize that various solvents and/or solvent mixtures (with and without water) are appropriate for such reaction. In one embodiment, solvents and/or solvent mixtures include 100% ethanol; ethanol : water (70 : 30); 100% acetonitrile; acetonitrile : water (80 : 20). In one embodiment, the solvents and/or solvent mixture is ethanol : water (9 : 1). One skilled in the art would recognize that the reaction may be performed at varied temperature ranges. In one embodiment, the reaction temperature range for heating is from about 80 °C to reflux. In one embodiment, the reaction temperature is performed at reflux.

[00164] In step S-5, INT5 is coupled to 3-chlorophenyl-isocyanate to form Compound 2. One skilled in the art would recognize that various organic solvents are appropriate for such reaction. In one embodiment, such solvents include tetrahydrofuran (THF), dichloromethane (DCM), ethyl acetate, dimethylacetamide and 1,2-dichloroethane. In one embodiment, the solvent is acetonitrile. One skilled in the art would recognize that the reaction may be performed at varied temperature ranges. In one embodiment, the reaction temperature range is from about room temperature to about 80 °C. In one embodiment, the reaction temperature range is from about 50 °C to about 80 °C. In one embodiment, the reaction temperature range is from about 50 °C to about 55 °C. One skilled in the art would recognize that various solvents and/or solvent mixtures

are appropriate for reslurrying. In one embodiment, such solvents and/or solvent mixtures include 100% ethanol; acetone : methanol (50 : 50); ethanol : acetonitrile (50 : 50, or 20 : 80); methanol : DCM (50 : 50); and methanol : acetonitrile (10 : 90). In one embodiment, the solvent mixture is methanol : acetonitrile (1 : 1).

[00165] In step S-6 Compound 2 is combined with methanesulfonic acid in the presence of a suitable acid to form Compound 1 or other salt. One skilled in the art would recognize that various mineral or organic acids are suitable for achieving salt formation. In one embodiment, a suitable acid includes formic acid, propionic acid, and the like. In one embodiment, the suitable acid is acetic acid. One skilled in the art would recognize that the salt formation may be performed at varied temperature ranges. In one embodiment, the salt formation is performed at from about 60 °C to about 111 °C. In one embodiment, the reaction temperature range is from about 60 °C to about 65 °C. In one embodiment, the reaction temperature is about 65 °C. One skilled in the art would recognize that various organic solvents are appropriate for such salt formation. In one embodiment, such solvents include methylethylketone, EtOAc, MTBE and dimethylacetamide. In one embodiment, the solvent is acetone. One skilled in the art would recognize that the salt formation may be performed at varied temperature ranges. In one embodiment, the salt formation is performed at a temperature range of from about room temperature to about 56 °C. In one embodiment, the reaction performed at a temperature of about 56 °C.

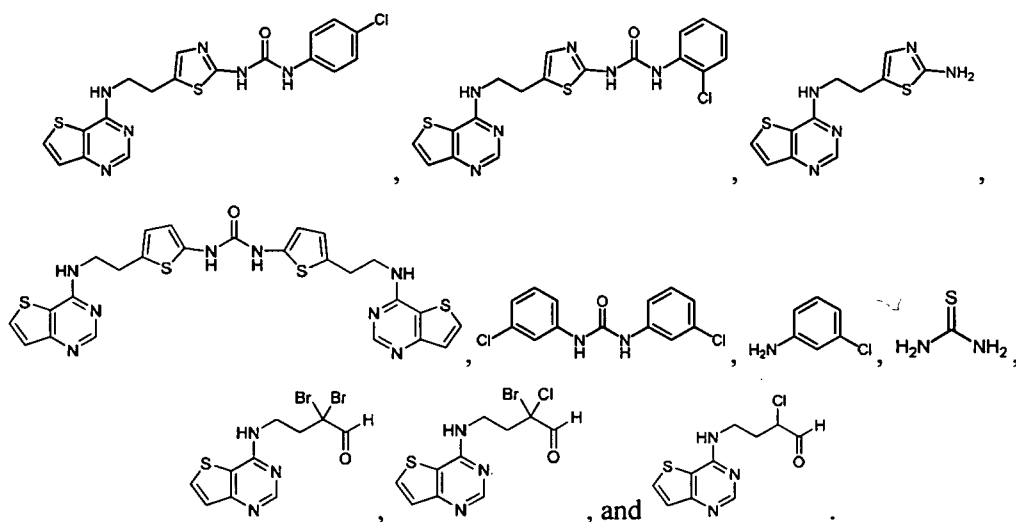
[00166] For example, the general preparation of Form A of Compound 1 is as follows. To a suspension of INT5 in acetonitrile is added (triethylamine) TEA and the mixture is warmed until a solution forms. 3-Chlorophenyl isocyanate is added at about 50 °C to 55 °C over 2 hours, and the mixture is then cooled and filtered. The collected solids are resuspended in hot 1:1 acetonitrile/methanol and the suspension is then cooled, filtered, and the collected solids washed with 1:1 acetonitrile/methanol to afford Compound 2. Compound 2 is dissolved in glacial acetic acid at about 60 °C to 65 °C and the solution is clarified by passing through an inline filter (10 µm).

[00167] To the resulting solution is added neat methanesulfonic acid, the mixture is cooled to about 50 °C to 55 °C, and acetone is added to induce crystallization. The suspension is cooled to ambient temperature and the resulting solids collected and washed with acetone. The solids are

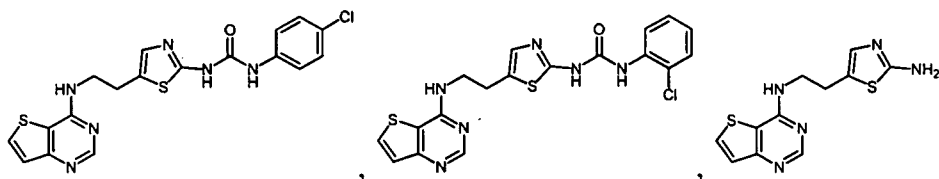
resuspended in acetone (ACS reagent grade) and the mixture distilled to azeotropically remove water. The solids are collected, washed with acetone (low water content), and dried in a vacuum oven at elevated temperature to obtain Compound 1. In certain embodiments, use of low water content acetone in the final wash step ensures the drug substance remains in its anhydrate form. Alternatively, the hydrate form of Compound 1 can be reconverted to the anhydrate by suspension in acetone followed by azeotropic distillation.

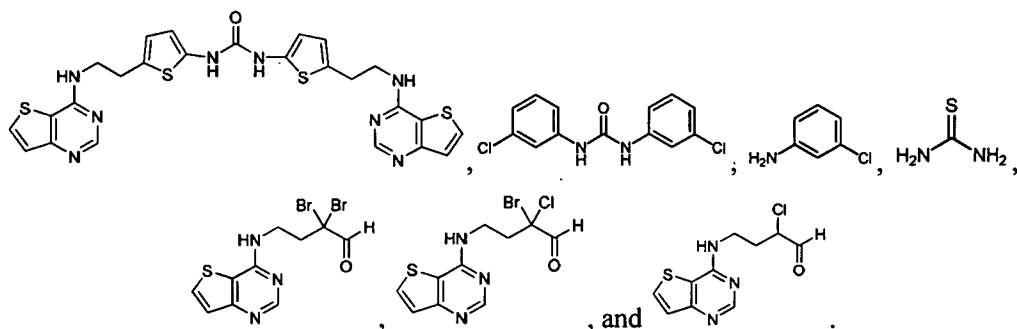
[00168] In certain embodiments, the present invention provides Compound 1 characterized in that it has ≤ 410 ppm acetonitrile, $\leq 3,000$ ppm methanol, $\leq 10,000$ ppm acetic acid, $\leq 5,000$ ppm acetone, or $\leq 5,000$ ppm triethylamine present as a residual solvent.

[00169] In other embodiments, the present invention provides Compound 1 having less than about 0.5%, less than about 0.15%, or less than about 0.10 %, by weight of any of the following compounds:

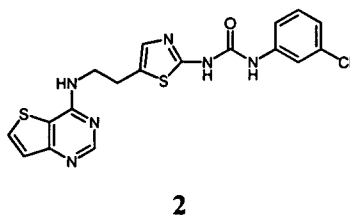


[00170] In certain embodiments the present invention provides a composition comprising Compound **1** and one or more of any of the following compounds:

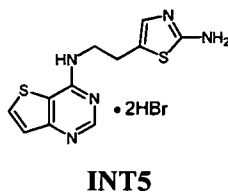




[00171] In certain embodiments, the present invention provides a method for preparing Compound 2:

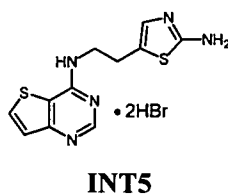


comprising the step of coupling INT5:



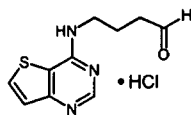
to 3-chlorophenyl-isocyanate to form Compound 2.

[00172] In certain embodiments, the present invention provides a method of preparing INT5:

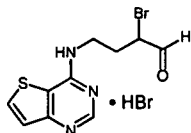


comprising the steps of:

(a) brominating INT3:

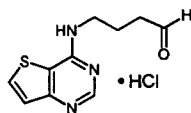
**INT3**

to form **INT4**:

**INT4,**

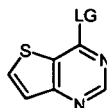
and (b) coupling **INT4** with thiourea to form **INT5**.

[00173] In some embodiments, the present invention provides a method of preparing **INT3**:

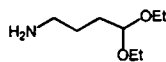
**INT3**

comprising the steps of:

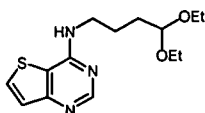
(a) coupling **INT1**:

**INT1**

wherein LG is a suitable leaving group, with

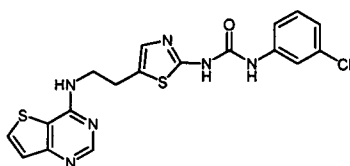


to form **INT2**:

**INT2**

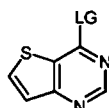
and (b) deprotecting **INT2** to form **INT3**.

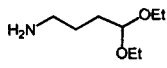
[00174] In certain embodiments, the present invention provides a method for preparing Compound 2:

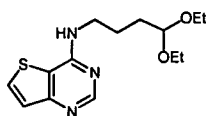
**2**

comprising the steps of:

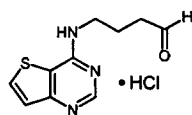
(a) coupling **INT1**:

**INT1**

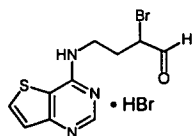
wherein LG is a suitable leaving group, with  to form **INT2**:

**INT2;**

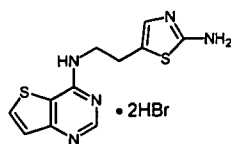
(b) deprotecting **INT2** to form **INT3**;

**INT3;**

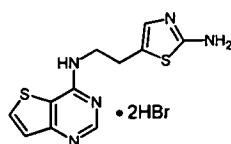
(c) brominating **INT3** to form **INT4**:

**INT4;**

(d) coupling **INT4** with thiourea to form **INT5**:

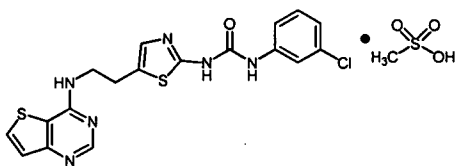
**INT5;**

and (e) coupling **INT5**:

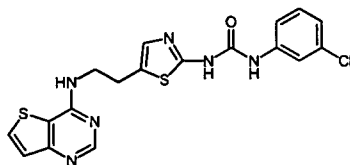
**INT5**

to 3-chlorophenyl-isocyanate to form Compound 2.

[00175] In some embodiments, the present invention provides a method of preparing Compound 1:

**1**

comprising the step of treating Compound 2:

**2**

with methanesulfonic acid.

[00176] The present disclosure now being generally described, it will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the disclosure herein.

EXEMPLIFICATION

[00177] The Aurora family of serine/threonine kinases (Aurora A, Aurora B, and Aurora C) plays a key role in cells orderly progression through mitosis. Elevated expression levels of Aurora kinases have been detected in a high percentage of melanoma, colon, breast, ovarian, gastric, and pancreatic tumors, and in a subset of these tumors the AURKA locus (20q13) is amplified. Compound 1, a novel aminothiazole-derived urea, is a selective inhibitor of Aurora kinases A, B, and C with IC₅₀ values in the low nanomolar range. Compound 1 potently inhibits cell proliferation and induces polyploidy (> 4N DNA) in a diverse panel of human cancer cell lines. The pharmacodynamic effects and in vivo activity of Compound 1 were investigated in human tumor xenograft models. Compound 1 displayed potent anti-tumor activity in HCT 116 (colon), PC-3 (prostate), CALU-6 (NSCLC) and MDA-MB-231 (breast) models. Tumor growth inhibition in these xenograft models ranged from 67.5 to 96.6% on a twice-weekly administration for 3 weeks. Following Compound 1 drug administration, endoreduplication and sustained pro-apoptotic effects measured by increased PARP cleavage and Caspase activation in tumor samples were observed. Compound 1-dependent effects in surrogate tissues were also evaluated as potential biomarkers and indicators of response; inhibition of histone H3 phosphorylation was observed in bone marrow and skin epidermis obtained from mice after exposure to Compound 1 at drug levels that are efficacious and well tolerated in xenograft models. Compound 1 displays favorable pharmacokinetics with measurable drug levels sustained for more than 96 hours post-dose in the HCT 116 tumor. These drug levels were associated with prolonged inhibition of histone H3 phosphorylation, an established substrate of Aurora Kinase B. Combined, these data suggest that Compound 1 may be an effective therapeutic agent for the treatment of diverse human malignancies.

Characterization Methods

[00178] Provided herein is an assortment of characterizing information to describe provided forms of Compound 1. It should be understood, however, that not all such information is required for one skilled in the art to determine that such particular form is present in a given composition, but that the determination of a particular form can be achieved using any portion of the characterizing information that one skilled in the art would recognize as sufficient for establishing the presence of a particular form, e.g., even a single distinguishing peak can be

sufficient for one skilled in the art to appreciate that such particular form is present. *United States Pharmacopeia* provides additional guidance with respect to characterization of crystalline forms (see X-Ray Diffraction, <941>. *United States Pharmacopeia*, 31st ed. Rockville, MD: United States Pharmacopeial Convention; 2008:372-374), which is incorporated herein by reference.

Instrumentation
[00179]

Instrument	Vendor/Model #
Differential Scanning Calorimeter	Mettler 822 [°] DSC
Thermal Gravimetric Analyzer	Mettler 851 [°] SDTA/TGA
X-Ray Powder Diffraction System	Shimadzu XRD-6000
Karl Fisher	Metrohm 756 KF Coulometer
Nuclear Magnetic Resonance Spectrometer	500 MHz Bruker AVANCE with 5mm BBO probe
Moisture Sorption Analysis	Hidden IGAsorp Moisture Sorption Instrument

Differential Scanning Calorimetry Analysis (DSC)

[00180] DSC analyses were carried out on the samples “as is”. Samples were weighed in an aluminum pan, covered with a pierced lid, and then crimped. Analysis conditions were 30 °C to 300 °C ramped at 10 °C/minute.

Thermal Gravimetric Analysis (TGA)

[00181] TGA analyses were carried out on the samples “as is”. Samples were weighed in an alumina crucible and analyzed from 30 °C to 230 °C and a ramp rate of 10 °C/minute.

X-Ray Powder Diffraction (XRPD)

[00182] Samples were analyzed “as is”. Samples were placed on Si zero-return ultra-micro sample holders and analyzed using the following conditions:

X-ray tube: Cu K α , 40 kV, 40 mA

Slits

Divergence Slit	1.00 deg
Scatter Slit	1.00 deg
Receiving Slit	0.30 mm

Scanning

Scan Range	3.0-45.0 deg
Scan Mode	Continuous
Step Size	0.04°
Scan Rate	2°/min

Dynamic Vapor Sorption (DVS)

[00183] DVS experiments were carried out on all available forms by first drying the sample at 0% RH and 25 °C until an equilibrium weight was reached or a maximum of four hours. The sample was then subjected to an isothermal (25 °C) adsorption scan from 10 to 90% RH in steps of 10% RH. The sample was allowed to equilibrate to an asymptotic weight at each point for a maximum of four hours. Following adsorption, a desorption scan from 85 to 0% RH (at 25 °C) was run in steps of -10%RH again allowing a maximum of four hours for equilibration to an asymptotic weight. The sample was then dried for two hours at 80 °C and the resulting solid analyzed by XRPD.

¹H Nuclear Magnetic Resonance (¹H NMR)

[00184] Samples (2–10 mg) were dissolved in DMSO-*d*₆ with 0.05% tetramethylsilane (TMS) for internal reference. ¹H NMR spectra were acquired at 500 MHz using 5 mm broadband observe (¹H-X) Z gradient probe. A 30 degree pulse with 20 ppm spectral width, 1.0 s repetition rate, and 16 to 64 transients were utilized in acquiring the spectra.

EXAMPLE 1**Preparation of Form A**

[00185] For all processes, a reactor, unless otherwise stated, refers to a 72-L, unjacketed, five-neck glass reactor equipped with a mechanical stirrer [19-mm glass stir shaft, poly-tetrafluoroethylene (PTFE) stir blade], drop-bottom valve, temperature probe, and nitrogen inlet.

All temperatures refer to internal temperatures unless otherwise stated. Where external cooling was applied, the reactor was placed in a steel cooling bath. For heating stages, the reactor was placed in a heating mantle and if applicable the reactor was equipped with a condenser. All table-top filter funnels were 24 inches in diameter and of polypropylene construction. All amber glass containers were fitted with a PTFE-lined closure.

Stage 1 Preparation of INT3

[00186] To a reactor was charged INT1 (2.00 kg, 11.72 mol), 2-propanol (20 L, 10 vol), triethylamine (1.96 L, 14.07 mol), and 4-aminobutyraldehyde diethyl acetal (2.36 kg, 14.65 mol), and a portion of 2-propanol was retained to rinse the weighing containers into the reactor. The batch was heated to 75 °C and maintained at 80 ± 5 °C for 3 hours 19 minutes prior to sampling. The analysis indicated that INT1 was 0.31% by conversion and met the specification of $\leq 2\%$ by conversion. The heating was turned off, and the batch allowed to cool overnight. The resultant suspension was concentrated via a rotary evaporator (water bath at 45 °C) to a slurry and the solvent chased with ethyl acetate (EtOAc) (50 L, 25 vol). A first portion of EtOAc (3 L) was used to rinse residue from the reactor, and was subsequently added to the bulb. The remaining EtOAc (47 L) portion was added to the reactor en route to the evaporator bulb.

[00187] The batch (net 5586 g) was diluted with EtOAc (35.35 L), to a total of volume of 40 L and transferred to the reactor and heating to 50 °C was initiated. EtOAc (34 L) was preheated (50 °C) in the reactor and the batch was readily soluble. Purified water (10 L, 5 vol.) was added to the reactor stirred for 16 minutes once the batch had reached 50 °C. The stirring was stopped and the phases settled and separated. Brine (10 L, 5 vol) was added to the reactor and once the batch had reheated to 50 °C (required 22 min), it was washed for 17 minutes. After the settled phases were separated, the batch was allowed to cool overnight.

[00188] The batch was concentrated via a rotary evaporator (water bath at 40 °C) to a slurry and the solvent chased with THF (50 L, 25 vol) using a similar method to that described above. The bulb was stored overnight under nitrogen at ambient temperature (net 3788 g). The batch was mobilized with THF and made up to a total of 40 L (required volume of THF was 36.5 L) and transferred to the reactor.

[00189] Hydrochloric acid (HCl) (2 N, 6.25 L, 3.13 vol, 1.06 equiv) was added to the yellow solution over 1 hour 2 minutes. An initial suspension formed after approximately 1 L had been

added and the addition rate was reduced, which resulted in the solids dissolving. The batch became turbid seven minutes after the addition was complete and four minutes later it was a thick yellow slurry. The stirring rate was increased to ensure that the solids mixed efficiently. HPLC analysis (TM-1486) after 4 hours 4 minutes indicated that the level of INT2 was $\leq 0.5\%$ (AUC). The cream-colored slurry was stirred for 5 hours 52 minutes at ambient temperature and then filtered through a 24-inch filter funnel (polypropylene) fitted with a PTFE filter cloth (wet with 3 L of THF).

[00190] Some solids passed through the filter cloth in the initial filtration and these were re-filtered. No further solids were observed in the filtrate. Once the batch was transferred to the filter, the reactor was rinsed with THF (10 L) and the rinse transferred to the filter cake. The cake was covered with a stainless-steel filter cover and a nitrogen sweep was passed over the batch. The batch (net wet weight 5645 g) was transferred to six glass drying trays and placed into a vacuum oven (50 ± 5 °C) and dried until the weight was constant (22 hr, 9 min). The batch was transferred to six amber glass bottles, blanketed with nitrogen, and stored at room temperature. Total yield of INT3=2.79 kg, 92% of theory.

Stage 2 Preparation of INT5

[00191] To a reactor was charged INT3 (2500 g, 9.70 mol) and glacial acetic acid (32.5 L, 13 vol). The batch was heated to 77.5 °C over 1 hour 36 minutes when a solution formed. The batch was then cooled to 50–55 °C over 6 hours 15 minutes and when the batch reached 53 °C, a solution of bromine in glacial acetic acid was added via a peristaltic pump over 45 minutes [bromine (1395 g, 8.73 mol) and glacial acetic acid (5.0 L, 2 vol)] using PTFE, polypropylene, and Pharmapure tubing. No significant exotherm or cooling was observed. The yellow solution was maintained at 50–55 °C during the HPLC analysis (TM-1493) for a total of 3 hours 56 minutes. After 1 hour 22 minutes, INT3 was above the specification of $\leq 4\%$ (AUC).

[00192] An additional charge of bromine (79 g) in acetic acid (280 mL) was performed. Thirty minutes later, INT3 was 2.03% (AUC) by HPLC analysis. The heating was stopped and acetone (12.5 L, 5 vol) was added to the batch via addition funnel. MTBE (12.5 L, 5 vol) was added to the batch. The resultant yellow suspension was allowed to cool to ≤ 30 °C and the batch filtered using a 24-inch, table-top filter (polypropylene) fitted with a PTFE cloth. The reactor was rinsed with acetone (6.25 L, 2.5 vol) and MTBE (6.25 L, 2.5 vol) and the rinse mixed in the

reactor. The rinse was applied to the cake. The yellow solid was transferred to six glass drying trays (net wet weight 3017 g) and dried in a vacuum oven at 50 °C to constant weight over 18 hours 58 minutes to give INT4 (2693 g, 73% of theory).

[00193] To a second reactor was charged INT4 (2694 g, 7.07 mol), ethanol (24.2 L, 200 Proof, 9 vol), thiourea (803 g, 10.54 mol), and purified water (2.7 L, 1 vol). The batch was heated to 78 ± 5 °C over 1 hour 19 minutes and maintained at that temperature range for 1 hour 53 minutes. The batch was sampled for HPLC analysis and INT4 was not detected [specification was $\leq 1\%$ (AUC)]. After a total of 3 hours 10 minutes, the heating was stopped and the batch cooled to <55 °C; a 10-L portion was cooled in a carboy and was concentrated ahead of the main batch. The batch was concentrated until all the batch was in the bulb (20-L) and then the ethanol rinse (26.9 L, 10 vol) was charged to the bulb. The batch was concentrated to a yellow slurry and the bulb was stored under nitrogen overnight. The batch was sampled for KF analysis which indicated a water content of 0.8% (specification $\leq 5\%$).

[00194] The batch was transferred to the second reactor in ethanol to give a total batch volume of 26.9 L (required 18 L ethanol, 200 Proof) and stirred at ambient temperature for 1 hour 22 minutes. MTBE (26.9 L, 10 vol) was added over 3 hours 12 minutes via an addition funnel (the funnel was fitted with a PTFE transfer tube to deliver the solvent between the outer side of the vortex and midway between the shaft and vessel wall). The yellow suspension was then cooled to 5–10 °C over 49 minutes and the batch was aged at this temperature range for 53 minutes ($T_{\min} = 6$ °C). The batch was filtered through a 24-inch, table-top filter (polypropylene) fitted with a PTFE cloth and the reactor and cake were rinsed with MTBE (26.9 L, 10 vol). The residue was transferred to six glass drying trays (net wet weight 3239 g) and dried at 50 °C to constant weight which required a total time of 18 hours 58 minutes. The yellow solid was transferred to three, amber, glass jars (80 oz.) and blanketed with nitrogen. Total Yield of INT5=2783 g, 90% of theory (65% over two steps).

Stage 3 Preparation of Compound 2

[00195] To a reactor was charged INT5 (2650 g, 6.03 mol) and acetonitrile (31.8 L, 21 vol, anhydrous) and heated to 50 ± 5 °C with stirring over 18 minutes. Triethylamine (1.770 L, 12.67 mol, 2.1 equiv, 99.5%) was added when the temperature was 54.5 °C over 2 hours 15 minutes.

An addition funnel fitted with a PTFE transfer tube was used to transfer the liquid close to the vortex. Eight minutes later, 3-chlorophenyl isocyanate (1854 g, 12.07 mol, 2.0 equiv, 99%) was added to the batch over 2 hours 3 minutes. HPLC analysis after 2 hours 12 minutes indicated **INT5** was 16.2% by conversion (specification $\leq 4\%$) and 3 hours 56 minutes from the time of addition of 3-chlorophenyl isocyanate, additional 3-chlorophenyl isocyanate (370 g, 2.41 mol, 0.4 equiv) was added over 26 minutes. The batch was sampled one hour later maintaining the temperature at 50 ± 5 °C and the level had reduced to 2.68% **INT4**. One hour 27 minutes from sampling, the heating was stopped and allowed to cool to <30 °C (required 5 hr 48 min).

[00196] The yellow suspension was filtered via a 24-inch, table-top filter fitted with a nylon cloth and the reactor and cake were rinsed with acetonitrile (26.5 L, 10 vol, ACS). The cake was covered with a stainless-steel filter cover under nitrogen (total filtration time 27 min). The residue was transferred back to the reactor and methanol (23.9 L, 9 vol, ACS) and acetonitrile (23.9 L, 9 vol, ACS) were added. The mixing solvents resulted in an endotherm to approximately 10 °C. The batch was heated to 50 ± 5 °C over 1 hour 2 minutes and maintained at that temperature for 6 hours 17 minutes with IPC sampling taking place after 3 hours 37 minutes. This indicated that **INT5** was 0.54% (AUC) and Compound **1** was 98.3% (AUC) and the heating was discontinued. The batch was allowed to cool to <30 °C overnight.

[00197] The light yellow suspension was filtered through a 24-inch, table-top filter fitted with a nylon cloth. Acetonitrile (6.7 L, 2.5 vol, ACS) and methanol (6.7 L, 2.5 vol, ACS) were charged to the reactor and mixed to rinse the reactor. The rinse was transferred to the filter cake, and was covered with a stainless-steel filter cover and a nitrogen sweep. The light yellow residue (wet-weight 2778 g) was transferred to six glass drying trays and dried under vacuum at 50 ± 5 °C for a total of 47 hours 9 minutes. The batch was sampled, transferred to three amber glass jars, blanketed with nitrogen and stored at room temperature. Total Yield of Compound **2** = 2194 g, 84% of theory.

Preparation of Compound 1 – final step

[00198] To the second reactor were charged Compound **2** (2136 g and 1564 g) and acetic acid (14.8 L, 4 vol, glacial) and were heated to 50–60 °C with stirring over 37 minutes. The resultant solution was clarified into a third reactor via a transfer pump equipped with a 10-micron filter

(Pall 12077) over four minutes. The batch was reheated to 60–65 °C over 19 minutes. Methanesulfonic acid (844 g, 0.228 wt) was added to the batch via an addition funnel over 1 hour 39 minutes maintaining the temperature at 60–65 °C. The batch was cooled to 50–55 °C over 1 hour 22 minutes and acetone (37 L, 10 vol, clarified) was then added over 2 hours 9 minutes maintaining the temperature at 50–55 °C. The batch became turbid after 14 L had been added and became a yellow suspension during 17–20 L. The heat was stopped and the batch cooled to <30 °C.

[00199] The batch was filtered via a 24-inch, table-top funnel fitted with a PTFE cloth and the reactor rinsed with acetone (18.5 L, clarified) and the rinse transferred to the cake. The dense yellow residue (net wet-weight 4975 g) was transferred to six glass drying trays and dried in a vacuum oven at 55 °C to constant weight (70 hr 51 min). The batch (3985 g) was stored in the oven with the heating discontinued under vacuum until required.

[00200] To a reactor were charged Compound 1 and acetone (63 L, 17 vol, clarified). The batch was heated to 57 ± 5 °C with stirring over 1 hour 50 minutes and distilled into a 12-L reactor whilst simultaneously adding additional remaining clarified acetone. After the addition of acetone, the batch was distilled with periodic draining of the 12-L reactor. Some of the distillate (~8 L) possibly escaped as vapor due to the nitrogen flow used to aid distillation. The final volume was gauged by distillation to a level on the reactor. The heating was stopped, the batch cooled to <30 °C and sampled for differential scanning calorimetry (DSC) analysis. The specification was met (consistent with reference).

[00201] The batch was filtered via a 24-inch, table-top funnel fitted with a PTFE cloth and the reactor rinsed with acetone (18.5 L, J.T. Baker, low water) and the rinse transferred to the cake. The cake was covered with a stainless-steel filter funnel and a nitrogen sweep applied. The dense yellow residue (net wet-weight 4594 g) was transferred to six glass drying trays and placed into a vacuum oven, dried at 55 °C to constant weight over 70 hours 21 minutes, and then sampled for IPC analysis. The batch was maintained in the oven at 55 ± 5 °C for 48 hours 54 minutes during the acquisition of the IPC data (total time at 55 ± 5 °C was 119 h 15 min). The batch of Compound 1 was packaged into two containers, each consisting of two 4 mil LDPE bags, cable ties, and a desiccant bag and blanketed under nitrogen. The amount per container was 2940 g and 1010 g (3950 g, 87% of theory from Compound 2).

[00202] The XRPD and DSC patterns obtained for Form A are depicted in Figures 18 and 19, respectively. Characteristics of Form A are summarized in Table 13.

Table 13. Summary of Characteristics for Crystalline Forms A-L

Form	Representative Solvents	Salt Ratio (¹ H NMR)	TGA Losses (wt %)	DSC Peaks (°C)	DVS Result	KF Wt % H ₂ O	Description
A	AcOH/Acetone	~1:1	0.0	229	No form change	<0.1	Anhydrate
B	Water slurries	~1:1	2.5, 0.2	170	No form change, low hygroscopicity	3.0	Mono-hydrate
C	AcOH/EtOAc	n/a	12.6	164	n/a	n/a	n/a
D	DMA	~1:1	14–19	~130, ~230	Converts to Form B	1.2	DMA solvate
E	Formamide	~0.9:1	No weight loss below 140 °C	~163, ~219	No form change, 8.0 wt % water uptake at 90% RH	0.44	Formamide solvate
F	AcOH	~1:1	4.0	164	n/a	n/a	n/a
G	MeOH	~1:1	4.0	131, 171(x), 212	No form change, low hygroscopicity	3.1	Mono-hydrate
H	EtOH	~1:1	3.6, 3.0	121, 163, 170(x), 179(x), 221, 227	Converts to Form G	0.50	Ethanol solvate
I	AcOH or AcOH slurry	~1:1	4.2, 4.3	81, 98, 164, 229	Converts to Form B	2.6	AcOH solvate or hydrate
J	DMF	~1:1	6.2	76, 129, 180	Converts to Form B	3.4	DMF solvate or hydrate
K	NMP slurry	~1:1	no weight loss below 90 °C	97, 116(x)	Converts to Form B	0.22	NMP solvate
L	DMF slurry	1:1	11.2	133, 232	Converts to Form B	<0.1	DMF solvate

n/a: data not available.

EXAMPLE 2

Preparation of Form B

[00203] Compound 1 (Form A, 291 mg) was dissolved in DMF (3 mL) at 55 °C followed by hot filtration and addition of THF (29 mL). This mixture was placed in the refrigerator for fast cooling and held at 4 °C for 16 hours. The resulting solids were isolated by filtration, dried in

vacuo (room temperature, 30 mm Hg) to afford Form **B** of Compound **1** (290.8 mg). The XRPD and DSC patterns obtained for Form **B** are depicted in **Figures 20** and **21**, respectively. Characteristics of Form **B** are summarized in Table 13.

EXAMPLE 3

Preparation of Form C

[00204] Compound **2** (500 mg) in acetic acid (5 mL) was heated to 55°C and then a solution of methanesulfonic acid (1.05 equivalents) in acetic acid (2 mL) was added. The solution was cooled to 42°C and then EtOAc (10 mL) was added, resulting in the formation of solids. The mixture was cooled to room temperature over 1 hour, filtered, and the solids washed with ethyl acetate (10 mL) then dried in a vacuum oven at 50°C to afford Form **C** of Compound **1** (650 mg). The XRPD and DSC patterns obtained for Form **C** are depicted in **Figures 22** and **23**, respectively. Characteristics of Form **C** are summarized in Table 13.

EXAMPLE 4

Preparation of Form D

[00205] Compound **1** (Form **A**, 204.3 mg) was weighed out into vial and dimethylacetamide (1.3 mL) was added until the material went into solution at 55 °C. The resulting solution was then clarified by hot filtration through a syringe filter (Millipore Millex-FH). After filtration, the vial was slowly cooled to room temperature at a rate of 20 °C per hour and further stirred at room temperature for 16 hours. The resulting solids were collected by vacuum filtration and dried (in vacuo, room temperature, 30 inches Hg) to afford Form **D** of Compound **1** (219.0 mg). The XRPD and DSC patterns obtained for Form **D** are depicted in **Figures 24** and **25**, respectively. Characteristics of Form **D** are summarized in Table 13.

EXAMPLE 5

Preparation of Form E

[00206] Compound **1** (Form **A**, 361 mg) was dissolved in formamide (4 mL) at 55 °C and held at this temperature with stirring for approximately one hour. After the initial dissolution, a precipitate was observed to form at 55 °C within five minutes. The resulting slurry was slowly cooled to room temperature at a rate of 20 °C per hour and further held at room temperature for

16 hours. The resulting solids were isolated by filtration, dried (in vacuo, room temperature, 30 mm Hg) to afford Form E of Compound 1 (305.2 mg). The XRPD and DSC patterns obtained for Form E are depicted in **Figures 26 and 27**, respectively. Characteristics of Form E are summarized in Table 13.

EXAMPLE 6

Preparation of Form F

[00207] Compound 1 (Form A, 30 mg) was weighed out into a vial and acetic acid (0.2 mL) was added until the material went into solution at 55 °C. The obtained solution was then slowly cooled to room temperature at a rate of 20 °C per hour and the resulting slurry further stirred at room temperature for 16 hours. The obtained solids were isolated by filtration, dried (in vacuo, room temperature, 30 mm Hg) to afford Form F of Compound 1 (16.7 mg). The XRPD and DSC patterns obtained for Form F are depicted in **Figures 28 and 29**, respectively. Characteristics of Form F are summarized in Table 13.

EXAMPLE 7

Preparation of Form G

[00208] Compound 1 (Form A, 153.5 mg) was weighed out into a vial and methanol (3.2 mL) was added to form a slurry. The slurry was stirred at 55 °C for one hour then slowly cooled to room temperature at a rate of 20 °C per hour and further held at this temperature for 16 hours. The resulting solids were collected by vacuum filtration and dried (in vacuo, room temperature, 30 inches Hg) to afford Form G of Compound 1 (145 mg). The XRPD and DSC patterns obtained for Form G are depicted in **Figures 30 and 31**, respectively. Characteristics of Form G are summarized in Table 13.

EXAMPLE 8

Preparation of Form H

[00209] Compound 1 (Form A, 193.4 mg) was weighed out into a vial and ethanol (3.2 mL) was added to form slurry. The slurry was stirred at 55 °C for one hour then slowly cooled to room temperature at a rate of 20 °C per hour and further held at this temperature for 16 hours.

The resulting solids were collected by vacuum filtration and dried (in vacuo, room temperature, 30 inches Hg) to afford Form H of Compound 1 (187.2 mg). The XRPD and DSC patterns obtained for Form H are depicted in Figures 32 and 33, respectively. Characteristics of Form H are summarized in Table 13.

EXAMPLE 9

Preparation of Form I

[00210] Compound 1 (Form A, 300 mg) was dissolved in acetic acid (2 mL) at 55 °C, stirred at this temperature for approximately one hour and slowly cooled to room temperature at a rate of 20°C per hour. The obtained slurry was then stirred at room temperature for 16 hours. The solids were isolated by filtration, dried (in vacuo, room temperature, 30 mm Hg) to afford Form I of Compound 1 (268 mg). The XRPD and DSC patterns obtained for Form I are depicted in Figures 34 and 35, respectively. Characteristics of Form I are summarized in Table 13.

EXAMPLE 10

Preparation of Form J

[00211] Compound 1 (Form A, 192.7 mg) was weighed out into a vial and DMF (1.4 mL) was added until the material went into solution at 55 °C. The resulting solution was filtered hot through a syringe filter (Millipore Millex-FH) then slowly cooled to room temperature at the rate of 20 °C per hour and further stirred at room temperature for 16 hours. The resulting solids were collected by vacuum filtration and dried (in vacuo, room temperature, 30 inches Hg) to afford Form J of Compound 1 (120 mg). The XRPD and DSC patterns obtained for Form J are depicted in Figures 36 and 37, respectively. Characteristics of Form J are summarized in Table 13.

EXAMPLE 11

Preparation of Form K

[00212] Compound 1 (Form A, 711 mg) was reslurried in N-methylpyrrolidine (1.5 mL) at room temperature for 19 hours. The resulting solids were isolated by filtration and dried (in vacuo, room temperature, 30 inches Hg) to afford Form K of Compound 1 (657 mg). The XRPD

and DSC patterns obtained for Form K are depicted in Figures 38 and 39, respectively. Characteristics of Form K are summarized in Table 13.

EXAMPLE 12

Preparation of Form L

[00213] Compound 1 (Form A, 500 mg) was reslurried in DMF (2.5 mL) at 40 °C for one week. The resulting solids were isolated by filtration and dried (in vacuo, room temperature, 30 inches Hg) to afford Form L of Compound 1 (405 mg). The XRPD and DSC patterns obtained for Form K are depicted in Figures 40 and 41, respectively. Characteristics of Form L are summarized in Table 13.

EXAMPLE 13

Solubility Screen

[00214] A solubility study of Compound 1 Form A in various solvents was executed to determine its solubility in various solvents. The results are summarized in Table 14. Compound 1 Form A was placed in vials and the chosen solvents were dispensed in 100 µL portions into the corresponding vials. The solvents were chosen based on differences in polarity and functionality and on their classification according to the International Conference on Harmonization (ICH), with preference given to class II and class III solvents. After each addition of solvent, the vials were visually inspected to assess dissolution and further heated to 55 °C to ensure dissolution.

[00215] Compound 1 Form A is soluble in DMF, NMP, DMA, formamide, AcOH and is sparingly soluble in methanol and ethanol. Compound 1 Form A showed poor solubility in THF, EtOAc, MeCN, acetone, MEK, IPA, water, dioxane, MTBE, IPAc, heptane, CH₂Cl₂ and toluene.

Table 14. Approximate Solubility of Compound 1 Form A

Solvent	Material Amount (mg)	Solvent Amount (mL)	Conc. (mg/mL)	Temp	Soluble	ICH Class
DMF	0.9	0.10	>9.00	RT	Yes	II
NMP	1.4	0.10	>14.00	RT	Yes	II
DMA	2.8	0.10	>28.00	RT	Yes	II
Formamide	1.6	0.10	>16.00	RT	Yes	
AcOH	1.7	0.10	>17.00	RT	Yes	III
MeOH	2.1	3.00	~0.70	55	Yes	II
THF	1.9	6.00	<0.32	55	No	II
EtOAc	1.5	6.00	<0.25	55	No	III
MeCN	2.5	6.00	<0.42	55	No	II
Acetone	2.2	6.00	<0.37	55	No	III
MEK	2.9	6.00	<0.48	55	No	III
IPA	1.8	6.00	<0.30	55	Partially	III
EtOH	1.9	3.00	~0.63	55	Yes	III
Water	1.7	6.00	<0.28	55	No	III
Dioxane	1.7	6.00	<0.28	55	No	II
MTBE	1.2	6.00	<0.20	55	No	III
IPAc	2.4	6.00	<0.40	55	No	III
Heptane	2.6	6.00	<0.43	55	No	III
DCM	2.6	6.00	<0.43	55	No	II
Toluene	2.0	6.00	<0.33	55	No	II

EXAMPLE 14**Single Solvent Recrystallization/Reslurry with Slow Cooling**

[00216] Based on the initial solubility study, seven solvents were selected for the slow cooling crystallization: DMF, NMP, DMA, formamide, AcOH, methanol, and ethanol. Compound 1 (approximately 30 mg) was weighed out into vials. and solvent was added until the material went into solution at elevated temperature (this applies to the primary solvents DMF, NMP, DMA, formamide, acetic acid); other solvents were added to form slurries and stirred at 55 °C for approximately two hours. The vials were then slowly cooled to room temperature at a rate of 20 °C/h and further stirred at room temperature for 16 hours. Table 15 shows all experimental details. Samples 15 and 16 using MeOH and EtOH respectively were filtered hot to remove some residual insoluble material and then were also slowly cooled to room temperature. After the cooling process, precipitates were isolated by filtration. Sample 2 did not produce any solid

and was therefore concentrated under a gentle nitrogen flow overnight. The recovered materials from all experiments were dried in vacuo at room temperature and 30 inches Hg.

[00217] Forms **D**, **E**, **F**, **G**, and **H** were obtained from single solvent recrystallizations from DMA, formamide, AcOH, MeOH, and EtOH, respectively. The unique XRPD patterns for these forms are shown in Figures 24, 26, 28, 30, and 32, respectively.

[00218] The single solvent recrystallization/reslurry from THF, EtOAc, MeCN, acetone, MEK, and IPA for Compound **1** produced samples showing XRPD patterns consistent with Form **A**. These samples were the same form as the starting material most likely due to the poor solubility of Compound **1** in these solvents.

[00219] Form **B** was produced from water. Form **B** was also produced from DMF and NMP, indicating that the residual water in these solvents is enough to trigger a form conversion to the hydrate.

Table 15. Single Solvent Recrystallization/Reslurry using a Slow Cooling Procedure

Solvent	Boiling Point	MSA Salt Amount (mg)	Solv. Amt (mL)	Conc. (mg/mL)	Temp (°C)	Precipit.	Form
DMF	153	26.2	0.20	131.00	55	Yes	B
NMP	82/10mm	39.1	0.20	195.50	55	No/evap	B
DMA	165	29.9	0.20	149.50	55	Yes (55 °C)	D
Formamide	210	36.1	0.40	90.25	55/100	Yes (55 °C)	E
AcOH	117	30.0	0.20	150.00	55	Yes	F
MeOH	64	24.3	0.50	48.60	55	Slurry	G
THF	65	28.7	0.50	57.40	55	Slurry	A
EtOAc	76	27.5	0.50	55.00	55	Slurry	A
MeCN	81	30.6	0.50	61.20	55	Slurry	A
Acetone	56	32.8	0.50	65.60	55	Slurry	A
MEK	80	22.3	0.50	44.60	55	Slurry	A
IPA	82	26.5	0.50	53.00	55	Slurry	A
EtOH	78	26.3	0.50	52.60	55	Slurry	H
Water	100	21.9	0.50	43.80	55	Slurry	B
MeOH*	64	32.5	7.0	4.64	62	Yes	G
EtOH*	78	30.8	7.0	4.40	75	Yes (42 °C)	H

*Samples were filtered hot

EXAMPLE 15

Binary Solvent Recrystallizations

[00220] Binary solvent recrystallizations of Compound 1 were performed using five primary solvents (DMF, NMP, DMA, formamide, and AcOH) and eight co-solvents (MeOH, EtOH, THF, EtOAc, MeCN, acetone, MEK, and IPA) with fast and slow cooling profiles. Tables 16–28 provide detailed information for these sets of experiments.

Fast Cooling Profile

[00221] Compound 1 (approximately 30 mg) was weighed out into vials, and primary solvent was added until the material went into solution at elevated temperature. After hot filtration, the anti-solvent was added portionwise until the solution became turbid or the vial was full. The vials were then placed in a refrigerator and held at 4 °C for 16 hours. After the cooling process, precipitates were isolated by filtration, and dried in vacuo at room temperature and 30 inches Hg. The vials without solids were evaporated to dryness using a gentle stream of nitrogen. The solids obtained were also dried in vacuo at ambient temperature and 30 inches Hg.

Slow Cooling Profile

[00222] Compound 1 (approximately 30 mg of Form A) was weighed into vials, and primary solvent was added until the material went into solution at elevated temperature. After a hot filtration, the anti-solvent was added portionwise until the solution became turbid or the vial was full, consistent with the fast cooling experiments. The vials were then slowly cooled to room temperature at a rate of 20 °C/h from 55 °C. After the cooling process, precipitates were isolated by filtration, and dried in vacuo at ambient temperature and 30 inches Hg. The vials without solids were evaporated to dryness or until a precipitate was formed using a gentle stream of nitrogen. The resultant solids were also dried in vacuo at room temperature and 30 inches Hg. All solids obtained were analyzed by XRPD to determine the physical form of the obtained material.

Formamide as Primary Solvent

[00223] As was observed during single solvent crystallizations, a minimum amount of formamide (0.3 mL) dissolved the starting material at 55 °C and very quickly produced a

precipitate, which was found to be Form E. In order to avoid premature crystallization of the material in this experiment, an additional amount of formamide was added at 100 °C before the addition of the anti-solvent, Table 16. After a hot filtration, the anti-solvent was added but the solution did not become turbid, even after reaching the maximum volume allowable by the size of the crystallization vials (8 mL). The vials were then placed in a refrigerator (4 °C) and held at this temperature for 16 hours, during which time no precipitation was observed. The solutions were transferred to larger vials (20 mL) and another 13 mL of the chosen anti-solvents were added to each vial. The resulting solutions were further held at 4 °C for 24 hours, during which time no precipitate was generated. All vials were evaporated to dryness using a gentle stream of nitrogen. The resulting solids were dried in vacuo at room temperature and 30 inches Hg and analyzed by XRPD. These forms were observed to be unique compared to single solvent crystallization but subsequent analysis by NMR indicated that the material was consistent with the free base and not the mesylate salt.

Table 16. Binary Solvent Recrystallizations of Compound 1 using Formamide as a Primary Solvent* and a Fast Cooling Procedure

Cmpd 1 Amount (mg)	Form-amide Amount (mL)	Anti-solvent	Antisolvent Amount (mL)	Appearance After Co-solvent Addition	Precipitate	Form	Recovery (mg)
28.4	3.50	MeOH	4+13	Clear	evap to ppt	FB	13.7
25.0	2.90	THF	4+13	Clear	evap to ppt	FB	11.8
29.6	3.50	EtOAc	4+13	Clear	evap to ppt	FB	14.8
25.0	2.90	MeCN	4+13	Clear	evap to ppt	FB	11.2
24.9	2.90	Acetone	4+13	Clear	evap to ppt	FB	11.2
30.0	3.50	MEK	4+13	Clear	evap to ppt	FB	12
23.9	2.90	IPA	4+13	Clear	evap to ppt	FB	10.3
29.7	3.50	EtOH	4+13	Clear	evap to ppt	FB	12.9

*Solids were dissolved at 100°C

FB - Free base, not a salt

[00224] Slow cooling procedure of binary solvent crystallizations with formamide as primary solvent afforded mostly Form E material with the exceptions of MeOH as anti-solvent which produced Form G, MeCN and IPA produced Form A and EtOH produced Form H. Table 17 provides a summary of the detailed information about this experiment.

Table 17. Binary Solvent Crystallizations of Compound 1 using Formamide as a Primary Solvent and a Slow Cooling Procedure

Cmpd 1 Amount (mg)	Formamide Amount (mL)	Anti-solvent	Antisolvent Amount (mL)	Appearance After Co-solvent Addition	Precipitate	Form	Recovery (mg)
26.6	0.3	MeOH	7.00	Clear	Yes	G	11.3
25.9	0.3	THF	1.00	Turbid	Yes	E	15.8
29.5	0.3	EtOAc	0.47	Turbid	Yes	E	18
25.4	0.3	MeCN	1.00	Turbid	Yes	A	14.1
28.8	0.3	Acetone	1.00	Turbid	Yes	E	18.7
29.0	0.3	MEK	1.00	Turbid	Yes	E	16
25.9	0.3	IPA	7.00	Clear	Yes	A	15.6
28.3	0.3	EtOH	7.00	Clear	Yes	H	14.6
27.7	0.3	n/a	n/a	n/a	Yes	E	12.2

DMF as Primary Solvent

[00225] Both fast cooling (Table 18) and slow cooling (Table 19) experiments using DMF as a primary solvent showed that MeOH (as anti-solvent) produces Form G, and EtOH produced Form H in slow cooling and Form B in fast cooling procedures. All other anti-solvents produced Form A in slow cooling procedure and Forms A or B in fast cooling procedures (see Table 16 and Table 21).

Table 18. Binary Solvent Recrystallizations of Compound 1 using DMF as a Primary Solvent and a Fast Cooling Procedure

Cmpd 1 Amount (mg)	DMF Amount (mL)	Anti-solvent	Antisolvent Amount (mL)	Appearance After Co-solvent Addition	Precipitate	Form	Recovery (mg)
26.5	0.3	MeOH	6.0	Clear	Small	G	12.5
29.1	0.3	THF	2.9	Turbid	Yes	B	18.8
24.4	0.3	EtOAc	0.6	Turbid	Yes	B+	14.0
30.7	0.3	MeCN	1.0	Turbid	Yes	A	21.2
26.7	0.3	Acetone	1.0	Turbid	Yes	A	15.1
25.0	0.3	MEK	1.5	Turbid	Yes	A	16.6
27.7	0.3	IPA	6.0	Clear	Small	B	15.5
28.2	0.3	EtOH	6.0	Clear	Small	B+	17.1

B+: Form B with extra diffraction peaks

Table 19. Binary Solvent Recrystallizations of Compound 1 using DMF as a Primary Solvent and a Slow Cooling Procedure

Cmpd 1 Amount (mg)	DMF Amount (mL)	Anti-solvent	Antisolvent Amount (mL)	Appearance After Co-solvent Addition	Precipitate	Form	Recovery (mg)
28.4	0.3	MeOH	7.0	Clear	Yes	G	15.2
28.3	0.3	THF	2.9	Turbid	Yes	A	20
28.5	0.3	EtOAc	0.6	Turbid	Yes	A	19.9
31.1	0.3	MeCN	1.0	Turbid	Yes	A	21.6
30.5	0.3	Acetone	1.0	Turbid	Yes	A	20.8
29.4	0.3	MEK	1.0	Turbid	Yes	A	20.3
25.2	0.3	IPA	7.0	Clear	Yes	A	17
31.4	0.3	EtOH	7.0	Clear	Yes	H	17.6

DMA as Primary Solvent

[00226] It was observed that most of the solvent mixtures in the binary solvent experiments with DMA as a primary solvent produced Form D with the exceptions of MeOH (Form G) and EtOH (Form H), and occasionally Forms A and B were also obtained (Table 20 and Table 21).

[00227] For the samples in Table 23, it was noted that after the dissolution of the starting material in DMA and stirring at 55 °C for 5–10 minutes, a very fine precipitate was formed in samples 1–3 and 5–8. In samples 1–3 and 5–6, this material went through the syringe filter (Millex-HV) during filtration. In the last two samples 7 and 8, this material was caught in the syringe filter.

Table 20. Binary Solvent Recrystallizations of Compound 1 using DMA as a Primary Solvent and a Fast Cooling Procedure

Cmpd 1 Amount (mg)	DMA Amount (mL)	Anti-solvent	Amount (mL)	Appearance After Co-solvent Addition	Precipitate	Form	Recovery (mg)
30.1	0.3	MeOH	3.00	Turbid	Yes	G	12.8
28.5	0.3	THF	1.00	Turbid	Yes	D	16.7
28.0	0.3	EtOAc	0.60	Turbid	Yes	D	16.2
26.5	0.3	MeCN	0.85	Turbid	Yes	B	14.8
30.2	0.3	Acetone	0.55	Turbid	Yes	D	18.2
27.5	0.3	MEK	0.65	Turbid	Yes	D	18.9
28.0	0.3	IPA	6.00	Turbid	Yes	B	16.8
24.9	0.3	EtOH	6.00	Clear	Yes	H	11.0

Table 21. Binary Solvent Crystallizations of Compound 1 using DMA as a Primary Solvent and a Slow Cooling Procedure

Cmpd 1 Amount (mg)	DMA Amount (mL)	Anti-solvent	Antisolvent Amount (mL)	Appearance After Co-solvent Addition	Precipitate	Form	Recovery (mg)
28.6	0.3	MeOH	7.00	Clear	Yes	G	11.6
29.6	0.3	THF	1.00	Turbid	Yes	D	20.5
27.8	0.3	EtOAc	0.60	Turbid	Yes	B+	19
25.3	0.3	MeCN	0.70	Turbid	Yes	D	14.6
31.2	0.3	Acetone	0.65	Turbid	Yes	D	25.2
31.5	0.3	MEK	0.65	Turbid	Yes	D	24.8
31.3	0.3	IPA	7.00	Clear	Yes	A	20.5
31.7	0.3	EtOH	7.00	Clear	Yes	H	15.8

B+: Form B with extra diffraction peaks

[00228] Fast cooling binary solvent crystallizations with DMA as the primary solvent were re-evaluated using a different crystallization technique. Each sample was dissolved in DMA without extra stirring at 55 °C for 5–10 minutes as was done before. Upon dissolution the solution was hot filtered through a syringe filter (Millex-FH) followed by fast addition of the anti-solvent. This was done to avoid any premature precipitation in the DMA. Table 22 summarizes the experimental details. The results were similar to the first experiment, with the exception that most of the solvents produced mixtures of Forms B and D instead of pure Form D.

Table 22. Binary Solvent Crystallizations of Compound 1 using DMA as a Primary Solvent and a Fast Cooling Procedure

Cmpd 1 Amount (mg)	DMA Amount (mL)	Anti-solvent	Antisolvent Amount (mL)	Appearance After Co-solvent Addition	Precipitate	Form	Recovery (mg)
28.3	0.3	MeOH	7.00	Clear	Yes	G	15.3
28.6	0.3	THF	1.00	Turbid	Yes	D	22.9
28.8	0.3	EtOAc	0.47	Turbid	Yes	B+D	20.6
28.4	0.3	MeCN	0.93	Turbid	Yes	B+D	20.3
26.1	0.3	Acetone	1.00	Turbid	Yes	B+D	18.6
26.2	0.3	MEK	1.00	Turbid	Yes	B+D	18.7
27.1	0.3	IPA	7.00	Clear	Yes	B	14.1
26.5	0.3	EtOH	7.00	Clear	Yes	H	12.2
26.5	0.3	n/a	n/a	n/a	Yes	B	18.3

NMP as Primary Solvent

[00229] Fast cooling binary solvent experiments (Table 23) using NMP as a primary solvent produced mainly Form B compared to slow cooling (Table 24) experiments which provided mostly Form A. Methanol and ethanol produced Forms G and H respectively in both fast and slow cooling experiments.

Table 23. Binary Solvent Recrystallizations of Compound 1 using NMP as a Primary Solvent and a Fast Cooling Procedure

Cmpd 1 Amount (mg)	NMP Amount (mL)	Anti-solvent	Antisolvent Amount (mL)	Appearance After Co-solvent Addition	Precipitate	Form	Recovery (mg)
26.1	0.3	MeOH	6.0	Clear	Small	G	10.0
30.2	0.3	THF	3.9	Turbid	Yes	B	17.0
28.3	0.3	EtOAc	0.6	Turbid	Yes	B+	16.4
30.1	0.3	MeCN	2.0	Turbid	Yes	A	16.0
28.1	0.3	Acetone	1.9	Turbid	Yes	B	13.0
26.3	0.3	MEK	2.5	Turbid	Yes	A	12.4
29.3	0.3	IPA	6.0	Clear	Small	B	15.7
27.2	0.3	EtOH	6.0	Clear	Small	B+	12.3

B+: Form B with extra diffraction peaks

Table 24. Binary Solvent Crystallizations of Compound 1 using NMP as a Primary Solvent and a Slow Cooling Procedure

Cmpd 1 Amount (mg)	NMP Amount (mL)	Anti-solvent	Antisolvent Amount (mL)	Appearance After Co-solvent Addition	Precipitate	Form	Recovery (mg)
26.3	0.3	MeOH	7.0	Clear	Yes	G	9.3
25.1	0.3	THF	5.0	Turbid	Yes	A	13.3
26.4	0.3	EtOAc	0.6	Turbid	Yes	B+	18.3
25.0	0.3	MeCN	2.0	Turbid	Yes	A	12.4
28.3	0.3	Acetone	1.95	Turbid	Yes	A	15.2
30.2	0.3	MEK	2.0	Turbid	Yes	A	16.2
30.0	0.3	IPA	7.0	Clear	Yes	A	14.3
26.9	0.3	EtOH	7.0	Clear	Yes	H	10.2

B+: Form B with extra diffraction peaks

AcOH as Primary Solvent

[00230] Most of the anti-solvents provided Form A in both fast cooling and slow cooling experiments using AcOH as primary solvent. Tables 25 and Table 26 summarize experimental details and results.

Table 25. Binary Solvent Recrystallizations of Compound 1 using AcOH as a Primary Solvent and a Fast Cooling Procedure

Cmpd 1 Amount (mg)	AcOH Amount (mL)	Anti-solvent	Antisolvent Amount (mL)	Appearance After Co-solvent Addition	Precipitate	Form	Recovery (mg)
30.8	0.3	MeOH	6.00	Clear	Yes	G	14.4
30.9	0.3	THF	0.65	Turbid	Yes	A	13.5
29.6	0.3	EtOAc	0.40	Turbid	Yes	B+	17.4
30.3	0.3	MeCN	3.00	Turbid	Yes	A	19.1
31.1	0.3	Acetone	2.00	Turbid	Yes	A	18.6
26.9	0.3	MEK	2.60	Turbid	Yes	A	15.8
27.1	0.3	IPA	6.00	Clear	Yes	B	19.1
30.2	0.3	EtOH	2.00	Turbid	Yes	H	19.6

B+: Form B with extra diffraction peaks

Table 26. Binary Solvent Crystallizations of Compound 1 using AcOH as a Primary Solvent and a Slow Cooling Procedure

Cmpd 1 Amount (mg)	AcOH Amount (mL)	Anti-solvent	Antisolvent Amount (mL)	Appearance After Co-solvent Addition	Precipitate	Form	Recovery (mg)
25.4	0.3	MeOH	7.00	Clear	Yes	G	12.9
27.0	0.3	THF	0.65	Turbid	Yes	A	14.4
26.3	0.3	EtOAc	0.40	Turbid	Yes	E	16.8
26.9	0.3	MeCN	4.00	Turbid	Yes	A	14.3
28.3	0.3	Acetone	2.00	Turbid	Yes	A	17.8
24.7	0.3	MEK	2.60	Turbid	Yes	A	11.8
27.4	0.3	IPA	7.00	Clear	Yes	A	16.8
26.6	0.3	EtOH	2.00	Turbid	Yes	G	16.5

EXAMPLE 16**Binary Solvent Crystallizations Using Water as a Co-Solvent**

[00231] In efforts to evaluate the propensity of Compound 1 for hydrate formation, six water miscible solvents (DMF, NMP, DMA, formamide, AcOH, and MeOH) were chosen for binary solvent crystallizations experiments using water as a co-solvent. Each solvent was pre-mixed with 2% and 10% water, for a total of 12 solvent combinations.

[00232] Compound 1 (25–30 mg) was weighed out into vials and the corresponding solvent mixture was added until the material went into solution at elevated temperature (55 °C). After a hot filtration through a syringe filter (Millex-FH), the vials were then placed in a refrigerator and

held at 4°C for 16 hours (fast cooling protocol) or slowly cooled to room temperature at a rate of 20 °C/h and further stirred at room temperature for 16 hours (slow cooling protocol). Tables 27 and 28 summarize the experimental details for both sets (fast and slow cooling). The isolated solids were collected by vacuum filtration. The vials without precipitates were evaporated to dryness using a gentle stream of nitrogen. All resultant solids were dried in vacuo at room temperature and 30 inches Hg.

[00233] The collected solids were analyzed by XRPD. Both fast and slow cooling experiments showed that aqueous DMF and acetic acid afforded Form B, aqueous formamide afforded Form E, aqueous methanol afforded Form G and aqueous DMA afforded Form D with only one exception of DMA/10% water, which afforded mostly Form B with some extra diffraction peaks. The residual material from aqueous NMP after evaporation under nitrogen flow was not analyzable as the material was an oil.

Table 27. Crystallizations with 2% and 10% Water using Fast Cooling Procedure

Material Amount (mg)	Solvent/% Water	Solvent Amount (mL)	Temp (°C)	Precipitate	Form	Recovery (mg)
28.6	DMF/2%	0.3	55	No	B	n/a
24.9	DMF/10%	0.3	75	Small	B	n/a
30.3	NMP/2%	0.3	55	No	n/a	n/a
29.1	NMP/10%	0.3	55	No	n/a	n/a
24.5	DMA/2%	0.3	55	Yes	D	10.9
29.8	DMA/10%	0.3	55	Yes	B+D	12.6
25.3	FA/2%	0.3	55	Yes	E	11.6
24.8	FA/10%	0.3	75	Yes	E	10.7
27.5	AcOH/2%	0.3	55	evap to dr.	B	n/a
24.5	AcOH/10%	0.3	55	evap to dr.	B+	n/a
24.6	MeOH/2%	7.3	64	Yes	G	14.0
27.6	MeOH/10%	7.3	55	Yes	G	13.6

FA: Formamide

n/a: not analyzable

B+: Form B with extra diffraction peaks

Table 28. Crystallizations with 2% and 10% Water Using Slow Cooling Procedure

Material Amount (mg)	Solvent/% Water	Solvent Amount (mL)	Temp (°C)	Precipitate	Form	Recovery (mg)
25.6	DMF/2%	0.3	55	evap to dr.	B+	n/a
28.6	DMF/10%	0.3	75	Yes	B	8.1
25.4	NMP/2%	0.3	55	evap to dr.	n/a	n/a
29.0	NMP/10%	0.3	55	evap to dr.	n/a	n/a
30.0	DMA/2%	0.3	55	Yes	D	11.4

28.0	DMA/10%	0.3	55	Yes	D	12
26.5	FA/2%	0.3	55	Yes	E	13.9
29.5	FA/10%	0.3	75	Yes	E	14.8
27.4	AcOH/2%	0.3	55	Yes	B	13.2
29.2	AcOH/10%	0.3	55	Yes	B	12.2
25.7	MeOH/2%	7.3	64	Yes	G	17.3
26.3	MeOH/10%	7.3	55	Yes	G	15.2

FA: Formamide

n/a: not analyzable

EXAMPLE 17**Reslurry of Form A in 20 Solvents**

[00234] The reslurry of Compound 1 Form A was conducted in 20 solvents: DMF, NMP, DMA, formamide, acetic acid, MeOH, EtOH, THF, EtOAc, MeCN, acetone, MEK, IPA, water, dioxane, MTBE, IPAc, heptane, CH₂Cl₂, and toluene. About 50–75 mg of Compound 1 was weighed into 2-dram amber vials. Various amounts of solvents were added to each vial to form slurries which were allowed to stir at room temperature for two weeks. The slurries were then filtered with the help of a gentle nitrogen flow. The samples were further dried in vacuo at room temperature for two hours, except the sample from formamide which was dried in vacuo for about 20 hours.

[00235] After two weeks, the samples were filtered and then analyzed by XRPD, DSC, and TGA. The results are summarized in Table 29. Three new forms were found from the slurry studies. These Forms I, K, and L were generated from AcOH, NMP, and DMF, respectively. The slurry samples from other solvents afforded results consistent with the single solvent recrystallizations. Form A remained unchanged after reslurry in THF, EtOAc, MeCN, acetone, MEK, IPA, dioxane, MTBE, IPAc, heptane, CH₂Cl₂, and toluene, most likely due to the poor solubility of SNS-314 mesylate in these solvents. Forms D, E, G, and H were generated from reslurry experiments in DMA, formamide, methanol, and ethanol, respectively. These results are consistent with those obtained in single solvent recrystallizations experiments using these solvents.

Table 29. Summary of XRPD, DSC and TGA Data for Slurries of Compound 1 Form A

Solvent	Form by XRPD	DSC Peaks (°C)	TGA Loss (Wt %)
DMF	L	140, 145(x), 226	5.7
NMP	K	90, 113	9.9, 6.0
DMA	D	91, 173	0.5, 0.7
Formamide	E	156, 210	3.9
AcOH	I	81, 105, 159, 174, 178(x), 219	3.1, 3.8, 4.4
MeOH	G	99, 169, 175(x), 216	1.5, 3.0
THF	A	226	0.0
EtOAc	A	228	0.0
MeCN	A	229	0.0
Acetone	A	228	0.0
MEK	A	229	0.0
IPA	A	229	0.0
EtOH	H	121, 161, 176, 219, 227	3.8, 2.4, 1.5
Water	B	87, 171	n/a
Dioxane	A	229	0.0
MTBE	A	229	0.0
IPAc	A	228	0.0
Heptane	A	228	0.0
DCM	A	229	0.0
Toluene	A	229	0.0

n/a: weight loss not available from the TGA thermogram most likely due to small sample amount.

EXAMPLE 18

Humidity-Controlled Form Conversion of Form A and Form B

[00236] In an attempt to determine the stability of Compound 1 Forms A and B at different humidity levels, chambers with five different humidities (0, 20, 52, 75, and 95% RH, Table 30) were set up for humidity-controlled form conversion for Form A and Form B samples of Compound 1. These chambers were allowed to equilibrate for at least 24 hours before the Form A and Form B samples of Compound 1 were placed in the chambers. The samples were monitored each week for a total duration of five weeks. Each sample was tested by XRPD, DSC, TGA, and Karl Fisher analysis.

Table 30. Set Up for Humidity Chambers

Humidity (% RH)	Reagent
0	Drierite
20	Saturated potassium acetate solution
52	Saturated NaHSO ₄ ·H ₂ O solution
75	Saturated NaCl solution
95	Saturated Na ₂ HPO ₄ ·12H ₂ O solution

[00237] The results obtained during the five weeks are summarized in Tables 31–35. For the first week samples, the XRPD patterns did not change for either Form A or Form B. However, the DSC, TGA, and KF results (Table 31) of Form A in 95% RH seemed to suggest the presence of the hydrate (Form B) in the sample. After the second week, the XRPD pattern of the Form A sample in 95% RH was consistent with Form B instead of Form A. This result, along with the DSC, TGA, and KF results (Table 32) for this sample suggested that Form A converted to Form B at 95% RH in two weeks. No form conversion was observed for Form A samples in the lower humidity (i.e., 0, 25, 52, and 75% RH) chambers or any Form B samples for the duration of five weeks.

Table 31. Summary of Analytical Results for Humidity Controlled Form Conversion for Form A and Form B of Compound 1 - Starting Point and Week 1

Starting Form	Humidity (% RH)	Week	XRPD Form	DSC Peaks (°C)	TGA Loss (Wt %)	KF (Wt % H ₂ O)
A	n/a	0	A	229	0.0	0.07
A	0	1	A	229	0.0	0.17
A	20	1	A	229	0.0	0.16
A	52	1	A	229	0.0	0.29
A	75	1	A	229	0.0	0.12
A	95	1	A	171, 228	0.1	1.5
B	n/a	0	B	170	2.5, 0.2	3.0
B	0	1	B	120, 170	2.4, 0.2	1.4
B	20	1	B	141, 171	2.7, 0.2	3.3
B	52	1	B	140, 171	2.7, 0.2	3.0
B	75	1	B	146, 172	2.3, 0.2	3.4
B	95	1	B	144, 171	2.4, 0.2	3.2

Table 32. Summary of Analytical Results for Humidity Controlled Form Conversion for Form A and Form B of Compound 1 - Week 2

Starting Form	Humidity (% RH)	Week	XRPD Form	DSC Peaks (°C)	TGA Loss (Wt %)	KF (Wt % H ₂ O)
A	0	2	A	229	0.0	<0.1
A	20	2	A	229	0.0	0.12
A	52	2	A	229	0.0	0.16
A	75	2	A	230	0.0	0.13
A	95	2	B	126, 171, 226	1.7, 0.2	3.2
B	0	2	B	142, 171	3.0, 0.2	3.4
B	20	2	B	119, 170	2.7, 0.3	3.7
B	52	2	B	130, 170	2.7, 0.2	3.6
B	75	2	B	133, 170	2.4, 0.3	3.4
B	95	2	B	136, 170	2.8, 0.3	3.5

Table 33. Summary of Analytical Results for Humidity Controlled Form Conversion for Form A and Form B of Compound 1 - Week 3

Starting Form	Humidity (% RH)	Week	XRPD Form	DSC Peaks (°C)	TGA Loss (Wt %)	KF (Wt % H ₂ O)
A	0	3	A	229	0.0	0.11
A	20	3	A	229	0.0	0.11
A	52	3	A	229	0.0	0.11
A	75	3	A	229	0.0	0.19
A	95	3	B	130, 171	2.1, 0.1	3.7
B	0	3	B	142, 171	2.5, 0.4	3.6
B	20	3	B	119, 170	2.9, 0.3	3.6
B	52	3	B	130, 170	2.9, 0.3	3.7
B	75	3	B	133, 170	2.8, 0.3	2.4
B	95	3	B	136, 170	3.2	4.6

Table 34. Summary of Analytical Results for Humidity Controlled Form Conversion for Form A and Form B of Compound 1 - Week 4

Starting Form	Humidity (% RH)	Week	XRPD Form	DSC Peaks (°C)	TGA Loss (Wt %)	KF (Wt % H ₂ O)
A	0	4	A	229	0.0	<0.1
A	20	4	A	228	0.0	<0.1
A	52	4	n/a	n/a	n/a	n/a
A	75	4	A	229	0.0	<0.1
A	95	4	B	122, 171	1.5	3.5
B	0	4	B	140, 171	2.8, 0.2	3.0
B	20	4	B	137, 170	2.7, 0.3	3.4
B	52	4	B	140, 171	2.5, 0.3	3.3

B	75	4	B	132, 171	2.6, 0.3	3.3
B	95	4	B	131, 170	2.3, 0.3	3.7

n/a: sample not available.

Table 35. Summary of Analytical Results for Humidity Controlled Form Conversion for Form A and Form B of Compound 1 - Week 5

Starting Form	Humidity (% RH)	Week	XRPD Form	DSC Peaks (°C)	TGA Loss (Wt %)	KF (Wt % H ₂ O)
A	0	5	A	229	0.0	0.1
A	20	5	A	229	0.0	0.2
A	52	5	n/a	n/a	n/a	n/a
A	75	5	A	229	0.0	0.3
A	95	5	B	126, 172	2.3, 0.2	3.9
B	0	5	B	143, 172	3.0, 0.3	3.4
B	20	5	B	128, 170	2.4, 0.3	3.5
B	52	5	B	120, 170	2.2, 0.3	3.6
B	75	5	n/a	n/a	n/a	n/a
B	95	5	B	148, 172	2.6, 0.3	4.7

n/a: sample not available.

EXAMPLE 19

Ripening Experiments and Relative Stability of Forms

[00238] In order to further investigate the relative stability of Forms A, B, E, and G, ripening experiments were performed in water and MEK as detailed in Table 36. In these experiments 10 to 40 mg of the samples were weighed into amber vials, and 0.8 mL water or 1 mL MEK was dispensed into each vial to form slurries. MEK was briefly dried using dried molecular sieves. The KF results showed 0.4 wt % of water in MEK after drying. The vials were capped with Teflon lined caps and sealed using a Parafilm[®] tape. After a week of stirring, the slurries were sampled, filtered, and analyzed by XRPD.

Table 36. Ripening Studies of Forms A, B, E, and G of Compound 1

Solvent	Sample Amount (mg)	Starting Form	Final Form
Water	20	B	B
Water	20	E	B
Water	20	G	B
Water	10+10	A+B	B
Water	10+10	A+E	B
Water	10+10	A+G	B
Water	10+10	B+E	B

Water	10+10	B+G	B
Water	10+10	E+G	B
MEK	20	B	B
MEK	20	E	B
MEK	20	G	A
MEK	10+10	A+B	B
MEK	10+10	A+E	B
MEK	10+10	A+G	A
MEK	10+10	B+E	B
MEK	10+10	B+G	B
MEK	10+10	E+G	A

[00239] The XRPD analysis showed that all slurries in water generated Form B. These results were consistent with the observations made during the polymorph study and during the process development studies.

[00240] The slurries in MEK starting with Form G, Forms A+G, or Forms E+G converted to Form A. The other six slurries in MEK (B, E, A+B, A+E, B+E, B+G) all converted to Form B. These results suggested that Form G is less stable than Form A and Form B. The slurry started with Forms A+B converted to Form B, indicating Form B is more stable than Form A.

[00241] Based on the characterization of various forms of Compound 1, the relative stability of the forms (A to L) can be ranked as shown in Figure 45. Detailed description and conversion conditions between the forms are summarized in Table 37.

Table 37. Description and Relative Stability of Crystalline Forms of Compound 1

Form	Description	Converts to	Conditions or Comments
A	Anhydrate	B	In water containing solvents or in anhydrous solvents with Form B seeding
B	Monohydrate	A	In anhydrous acetone (with distillation)
C	n/a	B	Under normal storage conditions
D	DMA solvate	B	At above 60% RH
E	Formamide solvate	G	After drying at 105 °C in vacuo
F	n/a	I	Crystallizations or slurries in AcOH all generated Form I, except one experiment which generated Form F, indicating Form F is less stable than Form I
G	Monohydrate	A (or B)	Converts to Form A in anhydrous solvents; converts to Form B in water containing solvents or anhydrous solvent with Form B

			seeding
H	Ethanol solvate	G	At above 90% RH
I	AcOH solvate or hydrate	B	At above 50% RH
J	DMF solvate or hydrate	B	At above 40% RH
K	NMP solvate	B	Gradually converts to Form B under humid environment.
L	DMF solvate	B	At above 80% RH

n/a: identification not available due to lack of materials.

EXAMPLE 20

Kinase assays

[00242] Compound 1 was tested for inhibitory activity against a panel of 219 kinases (Upstate Biotechnology, Dundee, UK). All screens were performed by incubating the kinase enzyme, Compound 1, and radiolabeled ATP together for typically 30-60 min. The final ATP concentration in the reaction was within 15 mM of the K_m for ATP, as calculated by Upstate.

[00243] It was determined that Compound 1 is a highly selective Aurora kinase inhibitor. Only 7 kinases out of the 219 show selectivity less than 100-fold. The respective IC_{50} values for these kinases are shown in Table 38.

Table 38

Kinase	IC_{50} (μM) in Radiometric Assay
Aurora A	0.001
TrkB	0.005
TrkA	0.012
Flt4	0.014
Fms	0.015
DDR2	0.082
Axl	0.084
c-Raf	0.100

[00244] Fourteen other kinases had an IC_{50} value between 0.100 μ M and 1 μ M. Compound 1 showed at least a 1000-fold selectivity over the remaining 197 kinases (i.e., $IC_{50} \geq 1 \mu$ M). These data suggest that Compound 1 has a low potential for off-target kinase related toxicities.

EXAMPLE 21**Aurora biochemical assays**

[00245] A Homogenous Time-Resolved Fluorescence (HTRF)-based biochemical IC₅₀ assay from Cisbio (Bedford, MA) was used to test for the kinase activity of the three isoforms of Aurora (Aurora A, B, and C) in the presence of Compound 1. A biotin-conjugated histone H3 peptide (Upstate Biotechnology) was used as a substrate.

[00246] Figure 1 shows representative Compound 1 IC₅₀ curves for (A) Aurora A and (B) Aurora B using the HTRF-based biochemical assay. As can be seen in this Figure, Compound 1 has an IC₅₀ of 0.0089 μ M for Aurora A, and has an IC₅₀ of 0.020 μ M for Aurora B.

[00247] Table 39 shows a summary of the results using the HTRF assay for Aurora A, Aurora B, and Aurora C. It can be seen from the data that Compound 1 is a potent Aurora kinase inhibitor.

Table 39

	Aurora-A	Aurora-B	Aurora-C
Average IC₅₀ (μM)	0.009	0.031	0.0034
Standard Deviation (SD)	0.002	0.007	NA
N	9	10	1

EXAMPLE 22**Crystallography**

[00248] Diffraction-quality crystals of Aurora A in complex Compound 2 were obtained by hanging-drop vapor diffusion at 20-25 °C. Diffraction data were collected under standard cryogenic conditions on RAXIS-IV, processed and scaled by using CrystalClear from Rigaku/Molecular Structure Corporation. The structures were determined from single-wavelength native diffraction experiments by molecular replacement with AMoRe using a search model from a previously determined structure.

[00249] A detail of a crystal structure of Aurora A with Compound 2 is provided in Figure 2. It can be seen from the structure that the compound is in an extended conformation. In particular, the inhibitor is located in the ATP (purine) binding pocket and extends into the substrate binding groove. Furthermore, the compound binds to the active conformation of Aurora A.

EXAMPLE 23**Flow Cytometry**

[00250] HCT 116 cells were seeded at 10,000 cells per well in 12-well plates and cells were incubated 24 hr at 37 °C. Compound 1 compound titration was achieved by making a 3-fold dilution series [in dimethyl sulfoxide (DMSO)], starting at 10 mM for a total of 11 concentrations (10 mM – 0.0002 mM) and one DMSO control. This series was diluted 1000X in RPMI-1640 containing 10% FBS (1X treatment concentration: 10 µM – 0.0002 µM).

[00251] Plates were removed from the incubator, growth media was aspirated, and 1 mL/well of 1X Compound 1 compound dilution series (in RPMI-1640/10% FBS) or no treatment control (RPMI-1640/10% FBS/0.1% DMSO) was added to cells. After 16 hrs, media was aspirated and placed in a labeled collection tube, cells were trypsinized with 100 µL trypsin for 5 min at room temperature, quenched with fresh media, and placed in the collection tube with their appropriate media aspirate. Cells were spun at 2000 RPM for 5 min, supernatant was aspirated, and cells were re-suspended in 50 µL 1X phosphate buffered saline (PBS) and 200 µL 100% methanol. Samples were then placed at -20 °C. Cells fixed in methanol were spun at 2000 RPM for 5 min, supernatant was removed and cells were washed with 500 µL 0.1% bovine serum albumin (BSA) in PBS. Cells were re-suspended in 100 µL propidium iodide (PI) staining solution [prepared from 10 µg/mL PI (Sigma #P4864), 100 µg/mL RNase (Sigma #R4642) in PBS] and incubated at 37° C for 1 hr. Cell populations were then analyzed by flow cytometry on Fluorescence-Activated Cell Sorter (FACS) instrumentation (FACSCalibur; Becton-Dickinson) according to common techniques.

[00252] The distribution of cells in the various phases of cell cycle was assessed by propidium iodide (PI) staining of DNA. The total intensity of PI was considered to reflect the DNA content of cells.

[00253] Data is shown in **Figure 3** from the 36 nM treatment sample. As can be seen from the Figure, exposure to Compound 1 caused cells to have a higher DNA content than exposure to DMSO vehicle. While the DMSO control-treated cells were predominantly distributed across 2N (G1) and 4N (G2/M) peaks, cells treated with 36 nM Compound 1 had predominantly 4N and 8N DNA content. This phenomenon is indicative of aberrant mitosis.

EXAMPLE 24

Fluorescent Imaging

[00254] HCT 116 cells were seeded at 6×10^4 cells/mL on coverslips in 12-well plates, and were treated with 16 nM Compound 1 or DMSO control for 72 hr. Cells were then fixed with 4% paraformaldehyde for 20 min at room temperature, washed with 1X PBS three times, permeabilized with 0.1% Triton[®] X-100 nonionic surfactant for 5 min at room temperature, washed with 1X PBS twice, blocked with 10% fetal bovine serum (FBS) in PBS for 2 hr at room temperature. The cells were incubated in a diluted alpha-tubulin primary antibody solution in 10% FBS for 2 days at 4 °C, and stained with DAPI (DNA/blue) and with a diluted FITC-labeled secondary antibody (tubulin/green) solutions in 10% FBS for 1 hr at room temperature away from light. Cells were then washed in 1X PBS and the coverslips were mounted on slides and analyzed with a Leica DMIRE2 fluorescence microscope with a 63X oil immersion objective. Images were captured on a Leica DFC300FX CCD camera and analyzed using Image-Pro software. For both images captured, the same objective was used.

[00255] As shown in **Figure 4**, treatment of the cells with the compound caused formation of large polyploid cells. A drastic increase in both nuclear and cellular area was observed when cells were treated with 16 nM Compound 1 for 72 hr as compared to vehicle. This increase in nuclear and cellular area indicates that the compound causes cell cycle defects that lead to abnormal cytokinesis and endoreduplication. These defects are consistent with Aurora kinase inhibition.

EXAMPLE 25

Phospho-Histone H3 Staining (High Content Screening)

[00256] Analysis of phospho-histone H3 (pHH3) levels was performed on adherent cells using high-content screening methodology. HCT 116 cells were plated at 1,000 cells per well in growth medium on 96-well poly-L-lysine plates and allowed overnight growth at 37 °C. Compound 1 titration was achieved by making a 3-fold dilution series (in DMSO) starting at 10 mM for a total of 11 concentrations (10 mM – 0.0002 mM) and one DMSO control. This series was diluted 1000X in RPMI-1640 containing 10% FBS (1X treatment concentration: 10 μM – 0.0002 μM). Plates were removed from the incubator, growth media was aspirated, and 100

μL/well of 1X compound dilution series (in RPMI-1640 with 10% FBS) or no treatment control (RPMI-1640 with 10% FBS/0.1% DMSO) was added to cells in duplicate wells.

[00257] Cells were treated with the various concentrations of Compound 1 for 1 hour. Then, medium was aspirated and cells were incubated in 100 μL/well 4% formaldehyde for 15 min at room temperature. After aspirating the fixation solution, cells were rinsed once in 100 μL/well 1X PBS and then incubated in 100 μL/well permeabilization buffer (0.5% Triton X-100 in 1X PBS) for 5 min at room temperature. This solution was aspirated and 100 μL/well of blocking buffer (10% FBS in 1X PBS) was added. Cells were incubated for 10-20 min at 37 °C. After aspirating the blocking buffer, the cells were incubated in 50 μL/well primary antibody solution (p-Histone H3 Cell Signaling # 9701 at 1:400 in 10% FBS) for 1-2 hr at 37 °C. Antibody solution was removed and cells were washed twice in 100 μL of 1X PBS. After removing the PBS, cells were incubated in 50 μL/well staining solution (1:100 secondary antibody/ 1:5000 Hoechst stain) for 35 min at room temperature away from light. Finally, cells were washed 3 times with 200 μL/well 1X PBS. Images were captured and pHH3 staining was analyzed using the Target Activation application and ArrayScan VTI™ instrument (Cellomics, Inc.). Data points taken from the parameter Mean_AveIntenCh2 were graphed in GraphPrism and fitted into an IC₅₀ equation.

[00258] As can be seen in **Figure 5**, phosphorylation of histone H3 on serine 10, a known Aurora B cellular target, was inhibited by treatment of cells with Compound 1. The EC₅₀ of the reduction of pHH3 is approximately 9 nM. The reduction in pHH3 levels likely reflects inhibition of Aurora B activity in HCT 116 cells by the compound.

EXAMPLE 26

Cellular Profile

[00259] Cellular proliferation was assessed using the Cell Proliferation ELISA, bromodeoxyuridine (BrdU) kit (Roche) including reagents, according to the kit protocol. Briefly, cells were treated with Compound 1 for 96 hr and labeled with BrdU for 2 hr before preparation for analysis.

[00260] For cell cycle analysis on adherent cells (HCT116, Calu-6, PC3, HeLa, A375, MiaPaca2, MDA-MB-231, and H1299), tumor cells were grown in 96-well tissue culture plates

overnight at 37 °C. The cells were then exposed to Compound 1 at 0.0002 to 10 μ M for 16 hours. Cells were fixed, stained, and analyzed. The percentage of cells with $\geq 4N$ DNA content as a function of concentration was fit to estimate EC₅₀. For cell cycle analysis on nonadherent cells or cells with irregular morphology (A2780, HL-60, CCRF-CEM, and HT-29), tumor cells were seeded in 12-well tissue culture plates overnight at 37 °C. The cells were then exposed to Compound 1 at 0.0002 to 10 μ M for 16 hours. Cells were trypsinized, collected, stained with propidium iodide, and analyzed by flow cytometry.

[00261] For analysis of pHH3 on adherent cells (HCT116, A375, and H1299), tumor cells were grown in 96-well tissue culture plates overnight at 37 °C. The cells were then exposed to Compound 1 at 0.0002 to 10 μ M for 1 hour. Cells were fixed, permeabilized and exposed to anti-pHH3 antibody and analyzed for pHH3 staining. Data were fit to an IC₅₀ equation. For analysis of pHH3 on nonadherent cells or cells with irregular morphology (A2780, Calu-6, and HT-29), tumor cells were seeded in 6-well tissue culture plates overnight at 37 °C. The cells were then exposed to Compound 1 at 0.0002 to 10 μ M for 1 hour. Cells were trypsinized, collected, lysed, and analyzed by immunoblotting.

[00262] For mitotic indexing, solid tumor cells were grown in 96-well tissue culture plates overnight at 37 °C. Cells were fixed, permeabilized, and exposed to fluorescently labeled antibody MPM2. The percentage of cells staining positive with this antibody was analyzed.

[00263] For analysis of Aurora A and Aurora B levels, tumor cells were grown in 12-well tissue culture plates overnight at 37 °C. Cells were harvested, separated by SDS-PAGE electrophoresis, and total Aurora kinase levels were analyzed by immunoblotting with antibodies to Aurora A and Aurora B.

[00264] The cellular effects of Compound 1 in a diverse panel of tumor cell lines are provided in Table 40.

Table 40

Tumor Type	Cell Line	BrdU IC ₅₀ (μ M)	Cell Cycle EC ₅₀ (μ M)	pHH3 IC ₅₀ (μ M)	MPM2 ⁺ Cells (%)	Aurora A vs. B Score ^a
Colon	HCT 116	0.0064	0.034	0.009	4.149	3
	HT29	0.0244	0.012	0.06	2.95	1
Lung	Calu-6	0.0133	0.013	0.06	1.928	2
	H1299	0.004	0.08	0.058	2.955	1

Prostate	PC3	0.0044	0.011	-	0.361	1
Ovarian	A2780	0.0018	0.0061	0.15	3.413	3
Breast	MDA-MB-231	0.0081	0.03	-	0.4275	1
Cervical	HeLa	0.0093	0.29	-	2.25	1
Pancreatic	MiaPaca2	0.0091	0.009	-	1.614	-
Melanoma	A375	0.0059	0.093	0.015	1.115	2
Leukemia	HL-60	- ^b	0.007	-	-	-
	CCRF-CEM	-	0.0042	-	-	2

^a Score of 1: Aurora A levels > Aurora B levels

2: Aurora A levels = Aurora B levels

3: Aurora A levels < Aurora B levels

^b A dash (-) indicates not tested.

[00265] It can be seen from Table 15 that Compound 1 shows low nanomolar anti-proliferative activity in a broad panel of cancer cell lines, with IC₅₀ values between 0.002 μ M and 0.01 μ M. Compound 1 also potently inhibits normal progression of cell cycle, and the phosphorylation of histone H3. The potency of Compound 1 in the assays of this example is independent of Aurora A and Aurora B levels, and the mitotic indices.

EXAMPLE 27

***In Vivo* Mouse Xenograft Assays**

[00266] The studies in Examples 20, 21, and 22 used female mice nu/nu athymic mice. Compound 1 was formulated fresh each week for dosing. The powder containing Compound 1 was added directly to a 30% aqueous cyclodextrin solution and sonicated at 50 °C for approximately 30 min until dissolved.

[00267] HCT 116 colorectal carcinoma cells were implanted in the animals' right hind flanks subcutaneously with 200 μ L of a 2.5×10^7 cells/mL suspension [1:1 Dulbecco's PBS (DPBS) with cells:Matrigel™]. For each of the studies of compound distribution, pHH3 levels, and tumor section microscopy, after the tumors reached an average volume of 500 mm³, the animals were weighed and sorted into randomized groups before initial dosing. Dosing schedules are provided separately for each of the studies in Examples 28, 29, and 30.

EXAMPLE 28

Distribution of Compound *In Vivo*

[00268] For the distribution studies depicted in **Figure 6A**, the mice were treated with a single dose of 170 mg/kg of Compound **1** intraperitoneally (IP). Terminal blood and tumor samples were harvested between 15 min and 96 hr.

[00269] Female nu/nu athymic mice received HCT 116 colorectal cancer cell suspension (1:1 DPBS with cells:Matrigel) as a subcutaneous injection in the right hind flank. When tumors reached an average volume of 500 mm³, mice were sorted into groups of 3 per time point. Compound **2** was extracted from tumor after homogenization with 10 x w/v PBS. Quantification of Compound **2** was done by HPLC-MS/MS after extraction from plasma and tumor homogenate with acetonitrile. For HPLC-MS/MS, the detector consisted of an API4000 (Sciex/ABI, Foster City, CA) triple quadrupole mass spectrometer using turbo electrospray ionization. Half-life estimates were made using the last 5 time points in tumor and last 3 time points in plasma.

[00270] It can be seen from **Figure 6A** that Compound **2** was preferentially retained in the tumor, i.e., the half-life of the compound is longer in the tumor as compared with its half-life in plasma (7.5 hr versus 4.7 hr, respectively).

[00271] For the distribution studies shown in **Figure 6B**, Female nu/nu athymic mice were administered 170 mg/kg Compound **1** IP with terminal plasma and skin collections between 15 min-16 hr post administration. It can be seen from **Figure 6B** that the plasma and skin PK profiles are similar. The similar profile allows PD readouts in the skin to be directly correlated with drug concentrations measured in the plasma.

EXAMPLE 29

Phospho-Histone H3 *In Vivo*

[00272] For the pHH3 studies depicted in **Figure 7**, the mice were treated IP with a single dose of either vehicle, 50 mg/kg of Compound **1**, or 100 mg/kg of Compound **1**, as labeled. It can be seen that at the 50 mg/kg and 100 mg/kg doses of Compound **1**, the level of pHH3 is decreased at 3 hr, 6 hr, and 10 hr post administration, as compared with the levels observed in vehicle-treated mice. The levels of compound in the tumor are provided below each lane; the levels of compound in the tumor are more than 20 times greater than the IC₅₀ for Aurora B *in vitro*.

EXAMPLE 30

Microscopy of Tumor Sections

[00273] For the microscopy assays depicted in **Figure 8**, the mice were treated either with vehicle or with a dose of Compound **1** of 170 mg/kg twice-weekly for three weeks. Following the treatment, tumors were harvested, placed in Streck fixative, paraffin embedded, sectioned, and transferred to slides. Tumor sections were stained with hematoxylin and eosin (H&E). Hematoxylin stains negatively charged nucleic acid structures, such as nuclei and ribosomes, blue, whereas eosin stains proteins pink. Treatments were administered on Day 1, 4, 8, 11, 15, and 18, with tumors being excised Day 4, 11, 18, and 25 of the study. All images in this Figure were taken at 40X magnification.

[00274] As the upper panel of **Figure 8** demonstrates, a significant increase (compared to vehicle) of caspase-3 positive cells were observed up to 18 days, indicating induction of apoptosis. As the lower panel of **Figure 8** demonstrates, large polyploid cells appeared by Day 4 and persisted for at least 25 days after treatment initiation, indicating successive rounds of endoreduplication.

EXAMPLE 31

In Vivo Efficacy

[00275] HCT 116 colon cancer cells [200 μ L of a 2.5×10^7 cells/mL suspension (1:1 DPBS with cells:Matrigel)] were subcutaneously implanted in the right hind flank of female nu/nu athymic mice. After 7 days, when tumors reached an average volume of approximately 200 mm³, animals were weighed, randomized by tumor volume ($l \times w \times h \times 0.52$), and assigned to the various study groups before initial dosing.

[00276] Compound **1** was tested for efficacy in HCT 116 xenograft mice on the following three schedules: a twice-weekly (biw) schedule for three weeks, a once-weekly (qw) schedule for three weeks, and a schedule of daily treatment for five days with a 9-day interval without drug administration (qd x5, 9 day off) with two cycles administered. The animals on the twice-weekly schedule received compound on Days 1, 4, 8, 11, 15 and 18. Doses were as shown in **Figure 9** and in Table 41. It can be seen from this Figure and the table that Compound **1** shows strong anti-tumor activity in HCT 116 xenograft mice on all dosing schedules tested.

Table 41

Dose (mg/kg)	Schedule	% TGI (Day 36)	TGD (days)
125	Qw x3	79.8	22.5
150	biw x3	95.6	32.5
100	qd x5, 9d off x2	91.6	45

TGI= Tumor Growth Inhibition

TGD= Tumor Growth Delay

[00277] Tumor Growth Inhibition (TGI) was determined by examining the tumor volume graph and calculating the percent of inhibition from the vehicle control group on the last day the control contained at least 75% of the animals. Percent TGI is then calculated with the following equation:

$$\%TGI = \frac{(\text{control } TV_i - \text{control } TV_f) - (\text{treatment } TV_i - \text{treatment } TV_f)}{(\text{control } TV_i - \text{control } TV_f)} \times 100$$

where TV_i is the average tumor volume on Day 10 and TV_f is the initial average tumor volume. ANOVA was performed to calculate statistical significance, defined as $p < 0.05$.

[00278] Time To Endpoint (TTE) was calculated for each individual animal to reach the predetermined study end point where the tumor volume becomes 1200 mm³ or 10% of body weight or a greater than 20% body weight loss for two sequential measurements. The TTE is calculated and the median value is recorded for the group. Tumor Growth Delay (TGD) is then calculated with the following equation:

$$TGD = \text{median } TTE_{\text{treatment}} - \text{median } TTE_{\text{control}}$$

[00279] Percent Tumor Growth Delay (%TGD) is calculated with the following equation:

$$\%TGD = \frac{\text{median } TTE_{\text{treatment}} - \text{median } TTE_{\text{control}}}{\text{median } TTE_{\text{control}}} \times 100$$

[00280] A Log Rank test was performed to calculate statistical significance, defined as $p < 0.05$.

[00281] The three dosing schedules for Compound 1 described above for the HCT 116 xenograft mice were also examined in mouse xenograft assays using other tumor types. Results for the twice-weekly for three weeks schedule and doses used are provided in Table 5 below.

[00282] A2780 ovarian cancer cells [200 μL of a 2.5×10^7 cells/mL suspension (1:1 DPBS with cells:Matrigel)] were implanted subcutaneously in the right hind flank of mice. After 7 days, when tumors reached an average volume of approximately 130 mm^3 , animals were weighed, randomized by tumor volume ($l \times w \times h \times 0.52$), and assigned to the various study groups before initial dosing.

[00283] A375 melanoma tumor fragments (1 mm^3) were implanted subcutaneously in the right hind flank of mice. After 9 days, when tumors reached an average volume of approximately 110 mm^3 , animals were weighed, randomized by tumor volume ($l \times w \times h \times 0.52$), and assigned to the various study groups before initial dosing.

[00284] MDA-MB-231 breast cancer cells [200 μL of a 2.5×10^7 cells/mL suspension (1:1 DPBS with cells:Matrigel)] were implanted subcutaneously in the right hind flank of mice. After 13 days, when tumors reached an average volume of approximately 95 mm^3 , animals were weighed, randomized by tumor volume ($l \times w \times h \times 0.52$), and assigned to the various study groups before initial dosing.

[00285] H1299 non-small cell lung cancer cells [200 μL of a 5×10^7 cells/mL suspension (1:1 DPBS with cells:Matrigel)] were implanted subcutaneously in the right hind flank. After 10 days, when tumors reached an average volume of approximately 100 mm^3 , animals were weighed, randomized by tumor volume ($l \times w \times h \times 0.52$), and assigned to the various study groups before initial dosing.

[00286] Calu 6 lung carcinoma cells [200 μL of a 5×10^7 cells/mL suspension (1:1 DPBS with cells:Matrigel)] were implanted subcutaneously in the right hind flank of mice. After 11 days, when tumors reached an average volume of approximately 150 mm^3 , animals were weighed, randomized by tumor volume ($l \times w \times h \times 0.52$), and assigned to the various study groups before initial dosing.

[00287] PC3 prostate tumor fragments (1 mm^3) were implanted subcutaneously in the right hind flank of mice. After 21 days, when tumors reached a volume of approximately 120 mm^3 , animals were weighed, randomized by tumor volume ($l \times w \times h \times 0.52$), and assigned to the various study groups before initial dosing.

[00288] As shown in Table 42, Compound 1 effected significant tumor growth inhibition in a dose-dependent manner ranging from 58-99% at well tolerated doses in a variety of mice xenograft models representing a range of tissue types.

Table 42

Cell line	Dose (mg/kg@ biw x 3)	% TGI	TGD (days)
HCT 116 (Colon)	150	95.6	32.5
	100	79.4	25.1
	75	67.5	22
A2780 (Ovarian)	170	53.8	9
	85	9.8	3.5
	42.5	0	0
A375 (Melanoma)	170	65.4	7.4
	85	20.6	0
	42.5	1.2	0
MDA-MB-231 (Breast)	170	73.8	14.1
	85	19.2	4.0
	42.5	20.6	3.5
H1299 (NSCLC)	170	69.1	5.7
	85	17.7	0
	42.5	0	0
CALU6 (NSCLC)	170	91.4	ND
	85	28.7	ND
	42.5	23.5	ND
PC3 (Prostate)	170	67.5	25
	85	63.6	12
	42.5	39.5	0

EXAMPLE 32

[00289] The human cell line MV-4-11 (human acute myeloid leukemia) was established as subcutaneous xenografts in nu/nu female mice. Animals were randomized by tumor volume and distributed into groups of ten animals each. Treatments were initiated when tumors averaged about 200 mm³ in volume. End points for each group were determined based on body weight nadir, adverse clinical observations, or tumor volumes exceeding maximum threshold of 2000 mm³.

[00290] Compound 1 was administered intraperitoneally (IP) biweekly (i.e. twice-weekly) for 3 weeks at a dose of 150 mg/kg. Responses were assessed by tumor growth inhibition (TGI) and tumor growth delay (TGD). TGI and TGD in the treatment group were evaluated against the vehicle control group. The treatment significantly delayed tumor growth compared to the vehicle. Percent tumor growth inhibition (% TGI) was 75.56 with a p-value of 0.0008, and the tumor growth delay was 10 days.

EXAMPLE 33**Nonclinical Pharmacokinetics, Distribution, and Excretion**

[00291] Pharmacokinetic studies were conducted in mice, rats and dogs after single and repeated administration of Compound 1. Pharmacokinetic parameters were estimated using noncompartmental analysis within WinNonlin v. 4.1. Quantification of Compound 2 was done by HPLC-MS/MS after extraction from plasma with acetonitrile. CD-1 mice, Sprague-Dawley rats, and beagle dogs were administered a single bolus intravenous injection of Compound 1 and blood sampled (terminal bleed, mouse, rat (exposure and gender data), n=3; serial bleed, rat and dog) between 5 min - 24 hours. Bioavailability profile in mice was determined after administration of 50 mg/kg IV, IP, and PO with blood sampling 15 min - 16 hr post administration. For rising dose experiments measuring exposure for each species, sets containing several animals were singly dosed at a given dose.

[00292] Results from single-dose experiments are shown in **Figure 10A**, Table 43, and in **Figure 10B** and Table 44, respectively. **Figure 10A** shows a decrease in plasma concentration of Compound 2 over time in mouse, rat, and dog after a single intravenous dose. Pharmacokinetic parameters for the study are provided in Table 43. C_0 is initial concentration extrapolated to time zero. AUC_{INF} is area under the plasma-concentration time curve from time zero extrapolated to the infinite time. CL is clearance; V_{ss} is steady state volume of distribution. $T_{1/2}$ is half-life.

Table 43

	MOUSE	RAT	DOG
Dose (mg/kg)	5	5	5
Dose (mg/m ²)	15	30	90
C_0 (μg/mL)	7.1	3.6	2.8
AUC_{INF} (μg*hr/mL)	1.8	2.1	12.3
CL (mL/min/kg)	47.6	41.2	6.8
V_{ss} (L/kg)	1.0	1.8	1.0
$T_{1/2}$ (hr)	1.4	0.8	1.1

[00293] In mice, a single dose of Compound 1 was administered intravenously, intraperitoneally, or orally, and decrease in plasma concentration of the compound over time is shown in **Figure 10B**. Pharmacokinetic parameters for the study are provided in Table 44.

Abbreviations are as in Table 18; F is fraction of dose absorbed. Compound 2 is rapidly and extensively distributed in both mice and rats when dosed IV, IP, or PO.

Table 44

	IV	IP	PO
Dose (mg/kg)	50	50	50
C ₀ (µg/mL)	42.0	-	-
AUC _{INF} (µg*hr/mL)	43.6	46.2	28.3
CL (mL/min/kg)	19.1	-	-
V _{ss} (L/kg)	1.2	-	-
T _{1/2} (hr)	0.8	0.7	3.3
F (%)	-	106	65

[00294] The results of rising dose pharmacokinetic studies are shown in **Figure 11** and in Table 45. In rising dose pharmacokinetic studies, Compound 2 displayed non-linear systemic exposure; the area under the concentration curve (AUC) increased more than dose linearly. As shown in **Figure 11A** this non-linear systemic exposure was most pronounced in rats and mice and occurs to a lesser extent in dogs. As can be seen in Table 45, the non-linear PK observed in rat correlated with changes in clearance.

[00295] Gender-related differences in pharmacokinetic parameters were observed in rodents and to a much lesser extent in dogs. As shown in **Figure 11B**, Female rats had 1.3- to 2-fold greater plasma AUC than male rats. "AUC last" on the plot corresponds to the area under the curve taken between the first and last measured time points.

Table 45

	Dose		
	60 mg/m ²	300 mg/m ²	600 mg/m ²
CL (mL/min/kg)	17.2	4.0	2.0
V _{ss} (L/kg)	1.3	1.1	1.1
T _{1/2} (hr)	1.4	1.7	5.9

EXAMPLE 34

Mass Balance and Elimination

[00296] ¹⁴C-Labeled Compound 1, with the label on the free base, was administered as a IV bolus dose of 50 mg/kg to male rats. Whole-body autoradiography indicated ¹⁴C-Compound 2-related radioactivity was widely distributed in tissues after an IV bolus dose with maximum concentrations observed 1 hour post dose.

[00297] Treated rats were further cannulated in femoral vein and the bile duct to allow for the evaluation of the rate and extent of elimination of total radioactivity from urine, bile, and feces. Total radioactivity was analyzed by liquid scintillation counting. Samples were also subject to HPLC-radiometric detection to elucidate the metabolic and elimination profile of Compound 2.

[00298] Results of elimination studies are shown in **Figure 12** and in Table 46. It can be seen from the **Figure 12A** and Table 46 that Compound 2 is predominately eliminated in bile (i.e., by biliary excretion).

Table 46

	Interval (hr)	Mean cumulative % recovery
Feces	0-24	11.8
	24-48	13.2
Bile	0-8	39.7
	8-24	68.5
	24-48	69.5
Urine	0-8	6.1
	8-24	9.4
	24-48	9.6
Cumulative Total	24	89.7
	48	92.3

[00299] **Figure 12B** shows various metabolites (M1-M11) of Compound 2 as observed in rat bile by HPLC. Preparation of samples for metabolite analysis was as follows. Plasma samples were extracted by protein precipitation with acetonitrile. The extraction was performed by adding ice cold acetonitrile (3 parts) to plasma (1 part v/v). After the samples were mixed using a benchtop vortex mixer the samples were centrifuged, the supernatants were transferred to silanized glass tubes, evaporated to dryness under nitrogen, and reconstituted in 50/50 acetonitrile/water solution. Urine samples were directly injected. Bile samples containing radioactivity were diluted in water prior to injection.

[00300] Metabolites in plasma, urine, and bile were separated on a reverse phase HPLC column with an Agilent 1000 system (Santa Clara, CA). Separation of Compound 2 and Compound 2-derived metabolites was achieved on a 250 x 4.6 mm 4 micron C18 Synergi Hydro column (Phenomenex, Torrance, CA) using mobile phase A of 0.1% formic acid in water and mobile phase B of acetonitrile. The flow rate was 0.75 mL/min with the following gradient: 0-2 min hold at 10% B followed by a linear gradient to 30% B at 45 min; 45-47 min ramping to 90% B

and held for 2 min; 49-50 min ramping from 90% to 10% B and held for 2 min; 52 to 55 min ramping to 90% B and back to 10% B at 57 min and held for the completion of the run. For ^{14}C detection the HPLC was coupled to a Radiomatic 610TR Flow Scintillation Analyzer equipped with a 500 μL liquid cell (PerkinElmer Life Sciences, Waltham, MA) using a scintillation fluid flow rate of 2.25 mL/min.

[00301] Taken together, **Figure 12A, 12B**, and Table 46 demonstrate that the majority of Compound **2** is eliminated as metabolized drug in rats. A graphical depiction of the location and distribution of the observed Compound **2** metabolites in rats, as mapped onto the circulating and elimination pathway, is provided in **Figure 12C**.

EXAMPLE 35

Protein Binding Studies

[00302] Studies were performed using the Rapid Equilibrium Dialysis (RED) device (Linden Bioscience). Inserts were soaked in water for 10 min x 2, then removed and drained immediately prior to use. Inserts were placed into a PTFE base plate prior to the addition of spiked matrix (Compound **1** in plasma at 15 μM) and buffer. All experiments were performed in duplicate and each chamber was sampled in duplicate. The samples were incubated for 4-6 h at 37 $^{\circ}\text{C}$ in a rotating incubator (100 rpm). Compound **2** was quantified using LC-MS/MS. Data from duplicate samples each sampled twice.

[00303] It was determined that in each of mouse plasma, dog plasma, and human plasma, Compound **2** is highly protein-bound. At the concentration used, the mean percentage of Compound **2** that is protein-bound is greater than or equal to 99.9% for each of mouse plasma, dog plasma, and human plasma.

EXAMPLE 36

Calculation of Combination Index

[00304] A combination index compares the concentration of compounds dosed in combination required for a given fractional effect to the concentration of single agent compound required to give the same fractional affect. In this application, the fractional effect is EC_{50} .

$$\text{CI}_{50} = \frac{\text{D}_1 \text{ Combo } \text{EC}_{50}}{\text{D}_1 \text{ EC}_{50}} + \frac{\text{D}_2 \text{ Combo } \text{EC}_{50}}{\text{D}_2 \text{ EC}_{50}}$$

[00305] The equation above represents the theoretical additive response for two mutually exclusive drugs, and takes into consideration the ratio at which the two compounds are dosed. When $CI_{50} = 1$, then drugs are additive, as if using twice as much of either drug alone. When $CI_{50} < 1$, less compound is required for a given fractional effect, and the combination is synergistic. When $CI_{50} > 1$, more compound is required, and the combination is antagonistic. The process by which CI_{50} values were determined in this application is described in the figures below which illustrate hypothetical outcomes for interactions of equipotent drugs (10 nM EC_{50}).

[00306] The following equations are examples of additive, antagonistic, and synergistic scenarios using the equation above, and where Drug 1 and Drug 2 are equipotent with an EC_{50} of 10 nM.

$$CI_{50} = \frac{5}{10} + \frac{5}{10} = 1 \quad \text{Additive}$$

$$CI_{50} = \frac{10}{10} + \frac{10}{10} = 2 \quad \text{Antagonistic}$$

$$CI_{50} = \frac{1.5}{10} + \frac{1.5}{10} = 0.3 \quad \text{Synergistic}$$

[00307] **Figure 13** shows an example of how interaction between two drugs can be determined by measuring corresponding dose-responses. **Figure 13A** generically depicts the interpretation of EC_{50} values for single agents and for combinations. **Figure 13B** generically depicts calculation of CI_{50} values for drug dosed with itself, or in combination with other drugs. Data from independent experiments may be plotted with 95% confidence intervals. **Figure 13C** generically depicts results from the Mann-Whitney test that was used to calculate a p-value and determine statistical significance from the additive internal control.

EXAMPLE 37

In Vitro Combination Studies

[00308] A colorectal carcinoma cell line, HCT 116 with either intact p53 (p53 +/+) or suppressed p53 (p53 -/-) protein levels, was treated *in vitro* with Compound 1 in combination with a panel of chemotherapeutic agents using either co-dosing or sequential dosing schedules,

as described in further detail below. High content cell imaging and a cell proliferation assay were used to measure the anti-proliferative effects of the compounds.

[00309] HCT 116 cells transfected with p53 RNAi or a control vector were cultured in DMEM, 10% FBS, and 1X antibiotic/antimycotic. Cells were plated in growth medium in black/clear Falcon® 384-well plates. Cells were treated to assess the effects of p53 status, drug dose ratios, and dose schedules. A dilution series of Compound 1 combined with a dilution series of various cytotoxics: gemcitabine (Gem), 5-fluorouracil (5-FU), docetaxel (DTX), vincristine (VIN), carboplatin (Carbo), SN38, daunomycin (Dauno), cisplatin (Cis), nocodazole (NOC), or Compound 1 (internal additive control) was applied to cells. The three dose ratios tested were (Compound 1/Panel), high/high, low/high, and high/low, where the “high” compound dose response is generated starting at 10X EC₅₀ and “low” compound is 1X EC₅₀. Dose schedules were tested by combining compounds as a co-dose (i.e. simultaneous administration), or sequential washout dose starting with either Compound 1 or a panel compound. All procedures were performed by a Tecan robotic platform.

Cell Count Assay

[00310] After overnight growth, cells were treated with compound for a total of 72 hours and incubated at 37 °C, 5% CO₂. Cells were fixed in 4% formaldehyde and stained with 1:4000 dilution of 10 mg/mL Hoechst 33342. HCS images were captured and data analyzed using the Target Activation application, object count per field parameter, on the ArrayScan VTI instrument (Cellomics, Inc.).

Proliferation Assay

[00311] Cells were plated and treated as described in the cell count assay with the exception of an extended incubation period of 6 days. A CellTiter Blue® cell viability assay (Promega) method was applied according to the manufacturer's instructions.

[00312] **Figure 14A** and **Figure 14B** shows results using the cell count assay for combination studies in HCT 116 cells conducted under three dosing ratios in the cell count assay. Studies were performed in p53 +/+ and p53 -/- (i.e. without and with p53 RNAi, respectively). It can be seen from the Figures 14A and 14B that conditional synergies were observed in vitro combined with gemcitabine (Gem), docetaxel (Dxtl), and vincristine (Vin). In other words, synergies with

the second agent were dependent in certain cases on the ratios of compounds used or p53 status of the cells.

[00313] **Figure 15** shows results obtained using the proliferation assay, demonstrating that microtubule targeted agents (i.e. spindle toxins) show synergy in combination with Compound 1 under certain conditions. These microtubule-targeted agents target the mitotic spindle in dividing cells. The sequence of administration was Compound 1, washout, and then docetaxel (DTX), vincristine (VIN), or nocodazole (NOC). High/High ratios of Compound 1/panel drug are on the left and Low/High ratios of Compound 1/panel drug are on the right.

[00314] **Figure 16** shows HCS images of HCT 116 cells treated with Compound 1, docetaxel (DTX), or vincristine (VIN), alone, and Compound 1 in combination with docetaxel or with vincristine. As can be seen from the figure, in cells that were treated with compound 1 alone or in combination, polyploidy was observed. Certain treatments and combinations of treatments also led to chromatin condensation or fragmentation.

[00315] In general, the most profound anti-proliferative effects were observed with Compound 1 and agents that disrupt microtubule polymerization such as vincristine and nocodazole. Statistically significant synergy was observed in p53 $-/-$ HCT 116 cells when Compound 1 was co-dosed with high doses of vincristine. Sequential dosing of Compound 1 followed by each chemotherapeutic compound showed significant synergy with vincristine and nocodazole, a trend toward synergy with docetaxel (i.e., under certain conditions), and additive anti-proliferative effects with carboplatin, gemcitabine, 5-fluorouracil, daunomycin, and the active metabolite of irinotecan, SN38.

EXAMPLE 38

In Vivo Combination Studies Using Compound 1 and Docetaxel

[00316] The *in vivo* anti-tumor activity of Compound 1 in combination with docetaxel (Taxotere[®]) was evaluated in female mice (nu/nu) subcutaneously implanted in the right hind flank region with 200 μ l of a 2.5×10^7 cells/mL suspension (1:1 DPBS with cells: Matrigel) of HCT 116 colorectal carcinoma cells. Treatments were initiated when tumors reached an average volume of 200 mm^3 ; mice were randomized into groups and treated with vehicle, Compound 1, docetaxel or with either sequential combination of Compound 1 and docetaxel administered with 24 hours separation. Results are shown in **Figure 17**.

[00317] End points for each group were determined based on body weight nadir, adverse clinical observations, or tumor volumes exceeding maximum threshold of 2000 mm³. Responses were assessed by tumor growth inhibition and tumor growth delay. TGI and TGD in the treatment group were evaluated against the vehicle control group.

[00318] Compound 1 was administered IP on day 0, 3, 7, 10, 14 and 17 at a dose of 42.5 mg/kg (shown as open circles, **Figure 17**); docetaxel was administered IP on day 0, 3, 7, 10 and 17 at a dose of 10 mg/kg (shown as solid circles, **Figure 17**). The sequence Compound 1 → docetaxel was accomplished by the administration IP of Compound 1 on day 0, 3, 10, 14 and 17 and of docetaxel on day 1, 4, 11, 15 and 18 (shown as open triangles, **Figure 17**). The sequence docetaxel → Compound 1 was accomplished by the IP administration of docetaxel on day 0, 3, 7 and 10 and of Compound 1 on day 1, 4, 8 and 11 (shown as open inverted triangles, **Figure 17**).

EXAMPLE 39

[00319] Compound 1 was formulated as a sterile, clear, colorless-to-yellow liquid for intravenous (IV) infusion. The formulation contained 10 mg/mL Compound 2 (the free base of Compound 1), 200 mg/mL of sulfobutyl ether beta-cyclodextrin, sodium salt (e.g., Captisol[®]) as a solublizing excipient, hydrochloric acid for pH adjustment, and Water for Injection (qs). The formulation had a pH of 3.0. In certain embodiments, the formulation for injection has a pH of about 2.5 to 3.5. The formulation for injection was manufactured without preservatives under current Good Manufacturing Practice (GMP). In certain embodiments, the formulation has a total impurity content of less than about 3% by weight.

[00320] Compound 1 formulation for injection was supplied in 25 mL Type 1 glass vials. Each vial contained sufficient Compound 2, at a concentration of 10 mg/mL, to permit administration of 200 mg of Compound 2 to a patient. A 6% fill overage was included for vial-needle-syringe withdrawal loss. Each single-use vial was labeled individually. The formulation is packaged in cartons that may contain multiple vials per carton. The cardboard carton also provides protection from light.

[00321] Before IV administration, Compound 1 formulation was diluted with 5% Dextrose in Water, USP, (D5W) to concentrations between 0.5 mg/mL and 5.0 mg/mL, measured as free base concentrations. Once prepared, these dilutions were stable for up to 32 hours, when stored at ambient conditions.

EXAMPLE 40

[00322] Compound 1 formulation for injection was administered weekly for 3 consecutive weeks of a 28-day cycle. In one embodiment, Compound 1 formulation for injection was given as a 3-hour infusion. In one embodiment, Compound 1 formulation for injection was given on Day 1, Day 8 and Day 15 of the 28-day cycle.

[00323] Pharmacokinetic (PK) evaluation was performed on Days 1 and 15. PK analysis showed that Compound 2 declines with a terminal half-life of 7 hours and has a moderate to low clearance. Pharmacokinetic parameters (including plasma exposure) were similar after the first and third-weekly dose administrations, indicating no change in Compound 2 disposition following repeated administration of Compound 1. At all dose levels time vs. concentration profiles showed spikes in plasma concentrations or a flat terminal phase, which is suggestive of entero-hepatic recirculation of Compound 2.

EXAMPLE 41

[00324] The activity of Compound 1 was studied in the human cell line HCT 116 established as subcutaneous xenografts in nu/nu female mice. For each study, animals were randomized by tumor volume and distributed into groups of ten animals each. Treatments were initiated when tumor volume averaged about 200 mm³. Compound 1 was administered intraperitoneally (IP) biweekly for 3 weeks (BIWx3) at a dose of 150 mg/kg.

Effects on Target Activity in tumors and normal tissues

[00325] Inhibition of histone H3 (HH3) phosphorylation was evaluated in HCT 116 xenograft tumors, mouse femur bone marrow, and mouse skin punch biopsy sections by immunohistochemistry (IHC). HCT 116 xenograft tumors, femurs, skin punches were excised from mice treated biweekly for three weeks BIW x 3 (on Days 1, 4, 8, 11, 15, and 18) with Compound 1 at a dose of 150 (skin) or 170 (bone marrow) mg/kg IP. The tumors were collected 6 hrs post-dose on day 4, 11, 18 and on day 25 (one week after completion of dosing phase of the experiment).

[00326] Phosphorylated histone H3 (pHH3) was detected by immunohistochemistry staining of tissue sections with the antibody # 9701 (Cell Signaling Technology, Inc.), which recognizes phosphorylation of Ser10 residue in histone H3 protein.

Effects in mouse skin punches

[00327] Photomicrographs of skin punches from *nu/nu* athymic mice after treatment with 150 mg/kg Compound 1 biweekly for 3 weeks. Three mice in each group were sacrificed on day 4 and day 18, 6 hours post-dose. Skin punches (8 mm) were fixed in formalin, trimmed, and sections stained to identify cells positive for histone H3 phosphorylation. The epidermis of mice exposed to Compound 1 displayed a decreased number of phospho-histone H3-positive cells; Compound 1 was able to reduce ~ 50 % the number of positively stained cells as compared with cells from vehicle-treated mice at day 4 and day 18 of the study (**Figure 42A**). On day 25 of the experiment, the number of positively stained cells in the epidermis of Compound 1-treated mice was still decreased compared to vehicle-treated mice.

Effects in mouse bone marrow

[00328] Photomicrographs of sections of mouse femurs after treatment with 170 mg/kg of Compound 1 on a BIW x 3 schedule show a significant drug-induced effects on histone H3 phosphorylation (**Figure 42B**). Bone marrow cells positive for this staining were 3 and 7 times less evident at day 11 and day 18, respectively, after treatment with 170 mg/kg Compound 1 IP as compared with vehicle treatment. Histone H3 phosphorylation had recovered to normal levels at day 25.

EXAMPLE 42

[00329] Clinical pharmacodynamic assessments were performed as follows. Skin punch biopsy samples were collected prior to (e.g., just prior to or up to 14 days prior to) treatment and during Cycle 1, Day 1 of treatment at between 3 and 7 hours after the start of the 3-hour infusion. Skin punch biopsy samples were analyzed for inhibition of histone H3 phosphorylation. Based on average *in vitro* cellular pHH3 EC₉₀ estimates, the target serum concentration is 1 μ M, which was achieved at all dose levels for a minimum duration of 4 hours. The duration for which the estimated target serum concentration threshold was achieved is provided in Table 47.

Table 47

Dose (mg/m²)	Duration of target serum concentration (hours)
30	4
60	8
120	10
240	20
480	20

[00330] Inhibition of pHH3 induced by administration of Compound 1 was observed in skin biopsies of patients treated at doses of 240 mg/m² and greater. At the 240 mg/m² dose level, serum Compound 2 levels exceeded the preclinical target inhibitory levels.

[00331] In addition to inhibition of phosphorylation of HH3, skin punch biopsies can also be tested for the appearance of polyploidy.

[00332] Patients with readily accessible tumors (such as skin, nodal, or liver metastases) undergo tumor biopsies. Tumor biopsy samples are obtained prior to treatment and on cycle 1, Day 22. Optionally, additional biopsies are also obtained. Tumor biopsy samples are analyzed for appearance of polyploidy and other markers of apoptosis or cell cycle changes.

[00333] In addition to skin punch and tumor biopsy samples, historic (e.g., paraffin-embedded pretreatment) samples, if available, are analyzed for baseline expression of proliferation and other markers of apoptosis or cell cycle changes. Samples may be assessed as shown in Table 48.

Table 48. Pharmacodynamic Assessments

Markers	Tissue Samples
pHH3	skin, tumor
Polyploidy	skin, tumor
Ki67	Tumor
Caspase	Tumor
Retinoblastoma (Rb)	Tumor
p53	Tumor

p21	Tumor
BCRA1	Tumor
Aurora A	Tumor

EXAMPLE 43

[00334] Mice received 200 μ L of a 5×10^6 HCT 116 colorectal cancer cell suspension (1:1 Dulbecco's phosphate-buffered saline with cells:Matrigel) as a subcutaneous injection in the right hind flank. When tumors reached an average volume of 400 mm³, mice were sorted into randomized groups of 3 per time point. For the dose escalation arm; mice were administered 1, 2, 5, 10, or 20 mg/kg Compound 1 IP. At 1 hr postdose tumor and plasma was collected and snap-frozen in liquid nitrogen and stored frozen at -80 °C until samples were processed for analysis. For the time-course arm, mice were administered an IP injection of 170 mg/kg Compound 1 followed by collection of plasma and tumor 6, 9, and 24 hr post-dose.

Western Blot Assay

[00335] Tumor samples were frozen on liquid nitrogen, and ground into a fine powder. Lysis buffer containing phosphatase inhibitors was added to the tumor powder before homogenization and a snap freeze cycle. The cellular debris was removed by centrifugation, and the protein concentration was measured using the BioRad DC Protein Assay. Twenty-five (25 μ g) of protein was loaded on NuPAGE 4-12% Bis-Tris Gel and separated by electrophoresis at a constant 200V. Protein was transferred to PVDF membrane at a constant 30 V for 1 hr using the Invitrogen XCell II Blot Module transfer system and, upon completion, the membranes were incubated with 5% milk in TBST (Tris-buffered saline with Tween) at room temperature for 1 hr. The membranes were incubated with antibody against pHH3 or total HH3 (#9701 and #9715, respectively, Cell Signaling Technology) in TBST, overnight at 4 °C. Membranes were washed in TBST, and then incubated with anti-rabbit IgG-HRP (#NA934V, GE HealthCare) in TBST for 1 hr at room temperature. Membranes were washed with TBST, and antibodies were detected with ECL Plus chemiluminescent detection system (Amersham), followed by exposure to Kodak BioMax film.

Western Blot Analysis

[00336] Films were visually assessed for total histone H3 (HH3) and histone H3 phosphorylation (pHH3). Total HH3 levels served as loading controls. Tumor pHH3 levels from mice treated with Compound 1 were compared to samples obtained from vehicle control mice.

ELISA Analysis

[00337] Phospho-histone H3 levels were determined using a commercial ELISA kit from (# KHO0671, Biosource/Invitrogen). The conditions were as described by the manufacturer with 100 µg total proteins from tumor lysates prepared as described in this Example.

[00338] **Preparation of Plasma Samples.** Plasma samples were extracted by protein precipitation with acetonitrile. The extraction was preformed by adding 3 parts ice cold acetonitrile containing internal standard (verapamil) to 1 part plasma (v/v). After the samples were mixed using a benchtop vortex mixer the samples were centrifuged, the supernatants were transferred and diluted with water prior to analysis of Compound 2 levels.

[00339] **HPLC-MS/MS.** Compound 2 in plasma was separated on a reverse phase HPLC column with an Agilent 1000 system (Santa Clara, CA). Chromatography achieved on a 30 x 2 mm 4 µm C18 Synergi Hydro-RP column (Phenomenex, Torrance, CA) using mobile phase A of 0.1 % formic acid in water, and mobile phase B, acetonitrile. The flow rate was 0.70 mL/min with the following gradient: linear gradient between 0-3.5 min starting at 95% A and ending at 60% A, followed by step to 5% A at 3.6 min and held until 4.49 min; the gradient was stepped to 95% A at 4.5 min and served as a wash cycle for the column. This wash cycle was repeated between 4.5 and 5.5 min, at which time the starting conditions were restored and the column allowed to equilibrate for 30 seconds prior to the next run. The detector consisted of an API4000 (Sciex/ABI, Foster City, CA) triple quadrupole mass spectrometer using positive mode turbo electrospray ionization.

[00340] As can be seen in **Figure 43**, increasing plasma concentrations of Compound 2 correlated with inhibition of phosphorylation of Histone H3 in tumor. **Figure 43A and C** show that low doses of Compound 1 administration modulated Histone H3 phosphorylation. **Figure 43B** demonstrates that 5 µM plasma concentration of Compound 2 produced maximal inhibition of phosphorylation of Histone H3. **Figure 43D** shows that at a single dose of 170 mg/kg

Compound 1, maximal inhibition of phospho-histone H3 in tumor was maintained for up to 24 hours.

EXAMPLE 44

[00341] PARP cleavage was measured in HCT 116 (colon carcinoma) and MV-4-11 tumor lysates by western blotting. Lysates were made from xenograft tumors excised from mice treated with a single dose of Compound 1 at a dose of 170 mg/kg IP for HCT 116 and 50 or 100 mg/kg IP for MV4-11. HCT 116 tumors were collected 3, 6 and 12 hrs post dosing; MV-4-11 tumors were collected at 2, 6 and 24 hrs post dosing. Time-dependent effects of Compound 1 on the expression levels of the indicated protein were measured.

[00342] Tumors were lysed in cell extraction buffer (Biosource # FNN0011) containing protease inhibitors (Sigma # P2714), and PMSF Phenylmethanesulfonyl fluoride (PMSF) [#P7626, Sigma]. Forty micrograms of protein for each sample was loaded and run on 4-12% Tris-Glycine NuPAGE gel (Invitrogen), in Novex Tris-Glycine running buffer (Invitrogen). After gel separation, proteins were electro-transferred to a PVDF membrane (Invitrogen). Proteins were detected by incubating membranes in primary and secondary antibodies as indicated in Tables 49 and 50 below.

Table 49. Primary antibodies

<i>Name and vendor (product #)</i>	<i>Dilution/Host</i>	<i>Buffer and incubation time</i>
Cl.PARP (Cell Signaling Technology #9541S)	1: 1,000 rabbit	5% milk/TBST o/n @ 4°
Actin (Abcam # Ab6276)	1: 10,000 mouse	5% milk/TBST for 1hr RT

Table 50. Secondary antibodies

<i>Name and vendor (product #)</i>	<i>Dilution</i>	<i>Buffer and incubation time</i>
Anti-rabbit (Amersham #NA934V)	1: 5,000	5 % milk/TBST for 1 hr RT
Anti-mouse (Amersham #NA931V)	1: 2,000	5 % milk/TBST for 1 hr RT
ECL detection reagent	Amersham	

[00343] **Figure 44A** shows that in HCT 116 tumor bearing mice treated with a single IP dose of 170 mg/kg Compound 1, that PARP cleavage became evident 3 hr after the dose, and is maintained for at least 12 hr after the dose. **Figure 44B** shows that in MV-4-11 tumor bearing

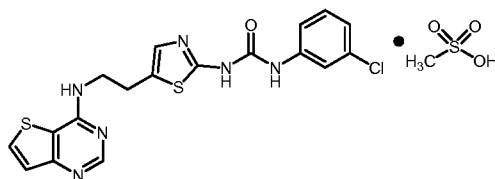
mice treated with a single IP dose (50 mg/kg or 100 mg/kg) of Compound 1, PARP cleavage was dose- and time-dependent.

[00344] While we have presented a number of embodiments of this invention, it is apparent that our basic teaching can be altered to provide other embodiments which utilize the compounds and methods of this invention. Therefore, it will be appreciated that the scope of this invention is to be defined by the appended claims rather than by the specific embodiments which have been represented by way of example.

CLAIMS

We claim:

1. Compound **1**:



2. A crystalline form of the compound of claim 1.
3. The form according to claim 2, wherein said form is anhydrous and nonsolvated.
4. The form according to claim 2, wherein said form is a hydrate.
5. The form according to claim 2, wherein said form is a solvate.
6. The form according to claim 2, wherein said form is anhydrous.
7. The form according to claim 3, wherein said form is Form **A** of Compound **1**.
8. The form according to claim 7, characterized in that it has one or more peaks in its XRPD pattern selected from those at about 8.5, 13.2, 15.3, 15.6, 16.7, 20.2, 20.6, 25.2, 26.4 and 27.0 degrees 2-theta and a differential scanning calorimetry pattern substantially similar to that depicted in **Figure 19**.
9. The form according to claim 7, characterized in that the form has an X-ray diffraction pattern substantially similar to that depicted **Figure 18**.
10. The form according to claim 4, wherein said form is Form **B** of Compound **1**.
11. The form according to claim 10, characterized in that it has one or more peaks in its XRPD pattern selected from those at about 7.1, 10.5, 11.8, 17.0, 17.4, 18.0, 21.3, 23.7, 25.1,

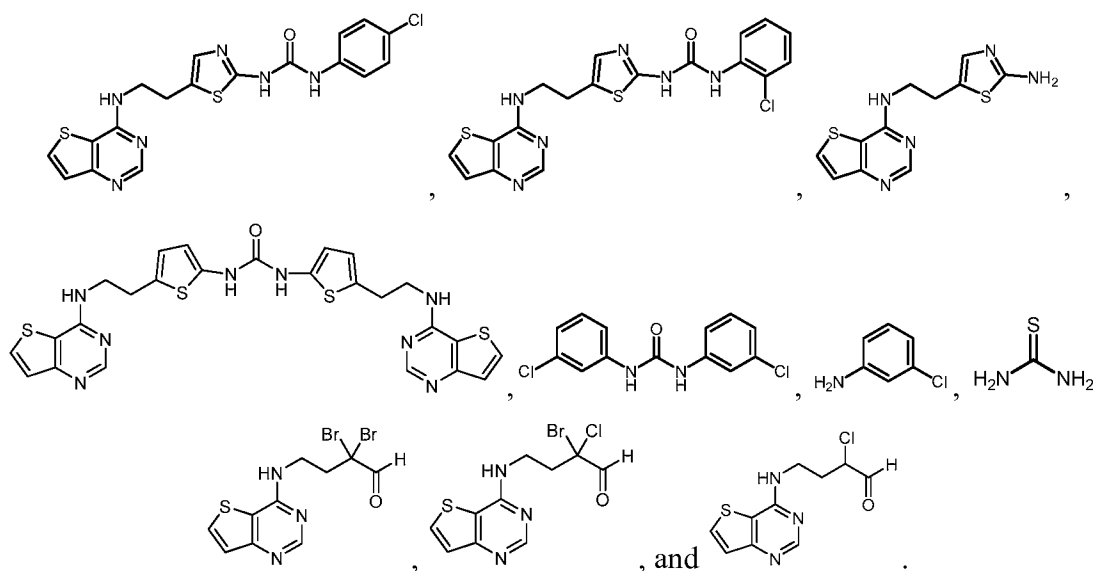
25.8, 26.8, 27.4, and 27.7 degrees 2-theta and a differential scanning calorimetry pattern substantially similar to that depicted in **Figure 21**.

12. The form according to claim 10, characterized in that the form has an X-ray diffraction pattern substantially similar to that depicted in **Figure 20**.

13. A composition comprising Form **A** of Compound **1** and at least one other solid form of Compound **1**.

14. The composition according to claim 13, wherein the other solid form is at least Form **B** of Compound **1**.

15. A composition comprising the compound according to claim 1, and one or more compounds selected from:



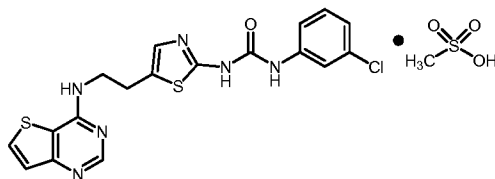
16. A composition comprising the compound according to claim 1 and a carrier.

17. A composition comprising the compound according to claim 1, and one or more pharmaceutically acceptable carriers, diluents, or excipients.

18. The composition according to claim 17, formulated for parenteral administration.

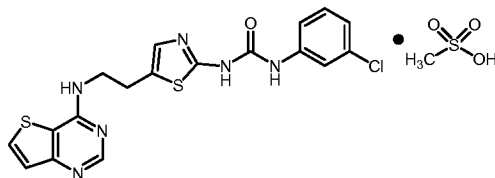
19. The composition according to claim 18, formulated for intravenous administration.
20. The composition according to claim 19, further comprising a solubility enhancer.
21. The composition according to claim 20, wherein the solubility enhancer comprises a cyclodextrin.
22. A method for treating cancer in a patient, comprising administering to the patient the composition according to claim 17.
23. The method according to claim 22, wherein the patient has a cancer characterized by a solid tumor or a hematological tumor.
24. The method according to claim 23, wherein the solid tumor cancer is selected from cancers of the colon, lung, prostate, ovary, breast, cervix, and skin.
25. The method according to claim 23 wherein the hematological tumor is a lymphoma or leukemia.
26. The method according to claim 25 wherein the lymphoma or leukemia is mantle cell lymphoma (MCL), Non-Hodgkin's lymphoma (NHL), Hodgkin's disease, acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL) or acute lymphoblastic lymphoma (ALL).
27. A method for treating cancer in a patient, comprising administering the composition according to claim 17 to a patient having a cancer selected from bladder cancer, brain cancer, breast cancer, cervical cancer, colon cancer, esophageal cancer, head and neck cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, myeloma, neuroendocrine cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, sarcoma, skin cancer, stomach cancer, testicular cancer, thyroid cancer, and uterine cancer.

28. A method for treating cancer in a patient, comprising administering to a patient having a cancer an effective amount of Compound 1:



1.

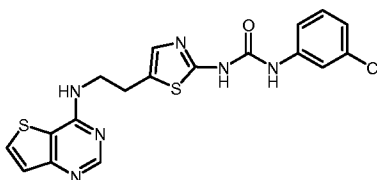
29. A method for treating cancer in a patient, comprising administering to a patient having cancer a dose of about 30 mg/m² -2000 mg/m² of Compound 1:



1.

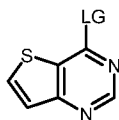
30. The method of claim 29 wherein the dose is administered once a week.
31. The method of claim 30 wherein the dose is administered once a week for three weeks.
32. The method of claim 30 wherein the dose administered is about 240 mg/m² - 2000 mg/m².
33. The method of claim 30 wherein the dose administered is about 480 mg/m² - 1800 mg/m².
34. The method of claim 33 wherein the dose administered is about 480 mg/m² - 1500 mg/m².

35. The method of claim 33 wherein the dose administered is about 480 mg/m^2 - 1200 mg/m^2 .
36. The method of claim 33 wherein the dose administered is about 750 mg/m^2 - 1500 mg/m^2 .
37. The method of claim 33 wherein the dose administered is about 960 mg/m^2 - 1200 mg/m^2 .
38. The method of claim 28, further comprising administering a dose of a second active agent.
39. The method of claim 28, wherein the composition is administered prior to the dose of the second active agent.
40. The method of claim 38, wherein the second active agent is a spindle poison.
41. The method of claim 38, wherein the second active agent is selected from docetaxel, gemcitabine, vincristine, nocodazole, carboplatin, 5-fluorouracil, daunomycin, cisplatin and SN38.
42. A method for preparing Compound **2**:

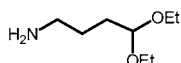
**2**

comprising the steps of:

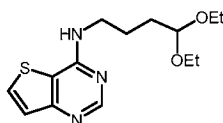
- (a) coupling **INT1**:

**INT1**

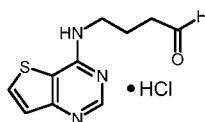
wherein LG is a suitable leaving group, with



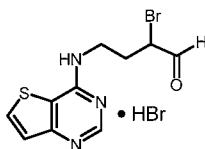
to form **INT2**:

**INT2;**

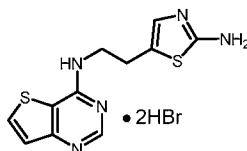
(b) deprotecting **INT2** to form **INT3**;

**INT3;**

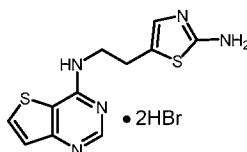
(c) brominating **INT3** to form **INT4**:

**INT4;**

(d) coupling **INT4** with thiourea to form **INT5**:

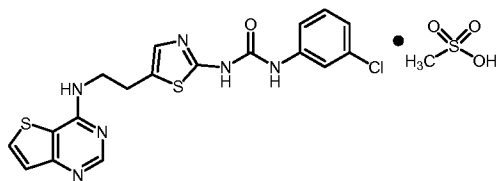
**INT5;**

and (e) coupling **INT5**:

**INT5**

to 3-chlorophenyl-isocyanate to form Compound **2**.

43. The method according to claim 42, further comprising the step of treating Compound **2** with methanesulfonic acid to form Compound **1**:

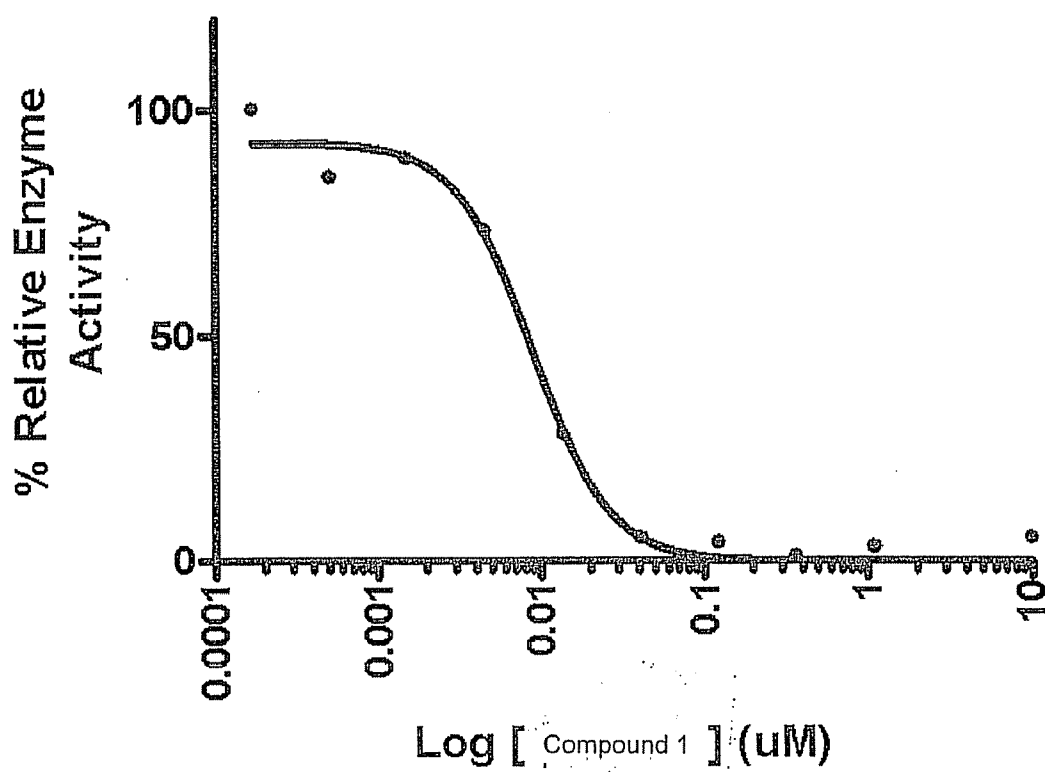


1.

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Figure 1A

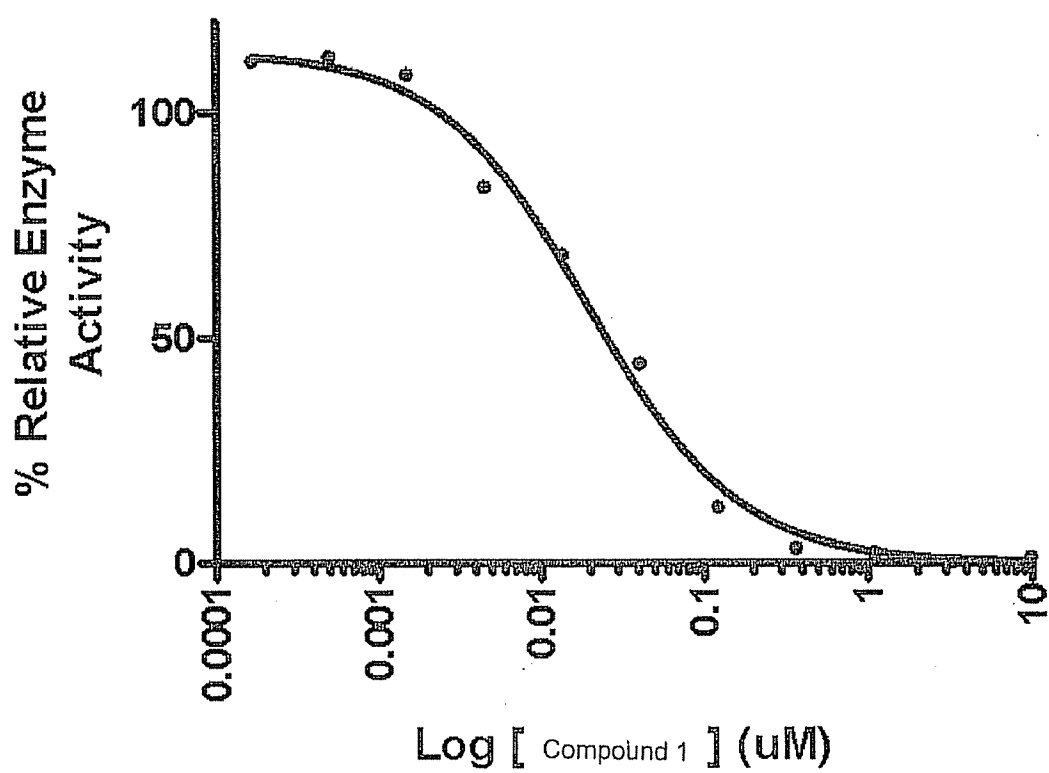
Aurora A

 $IC_{50}=0.0089 \mu M$ 

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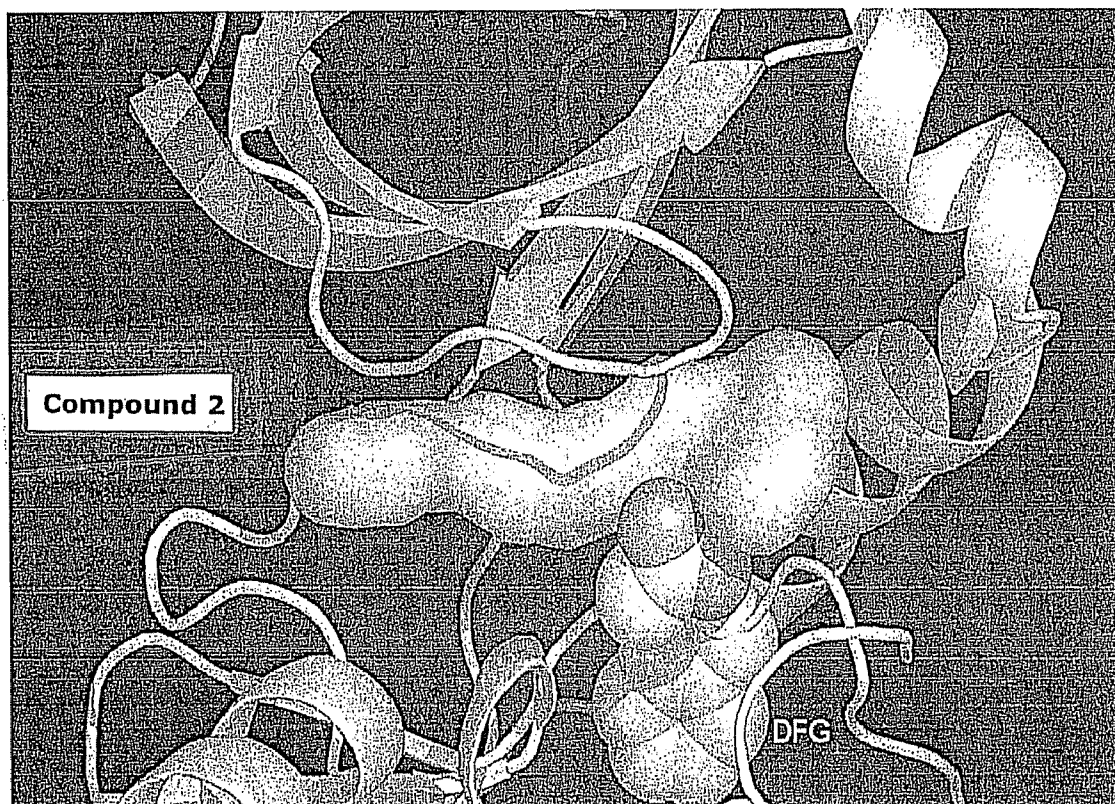
Figure 1B

Aurora B

 $IC_{50}=0.020\ \mu\text{M}$ 

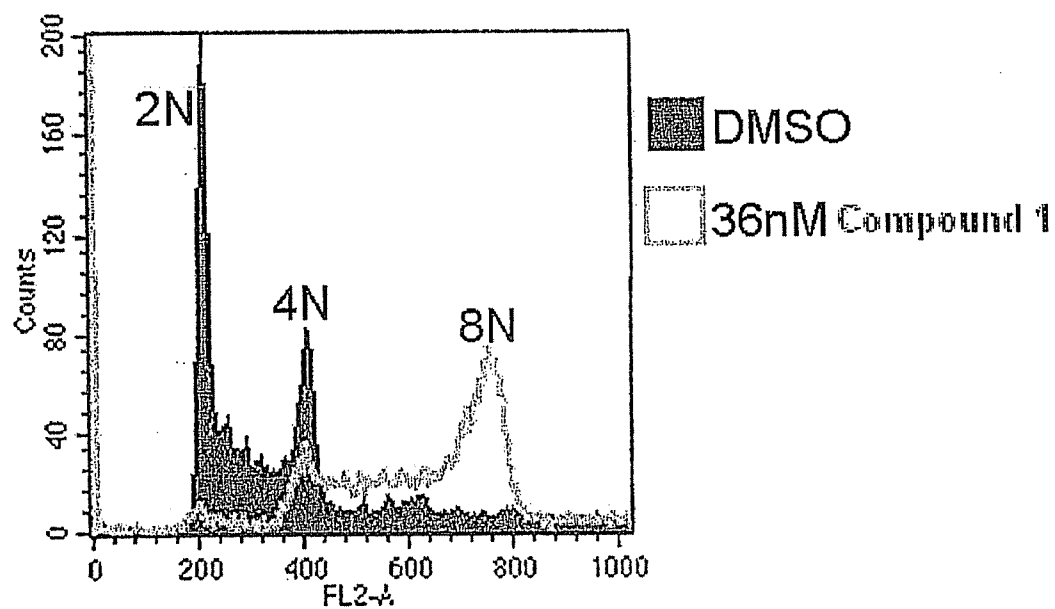
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Figure 2



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Figure 3



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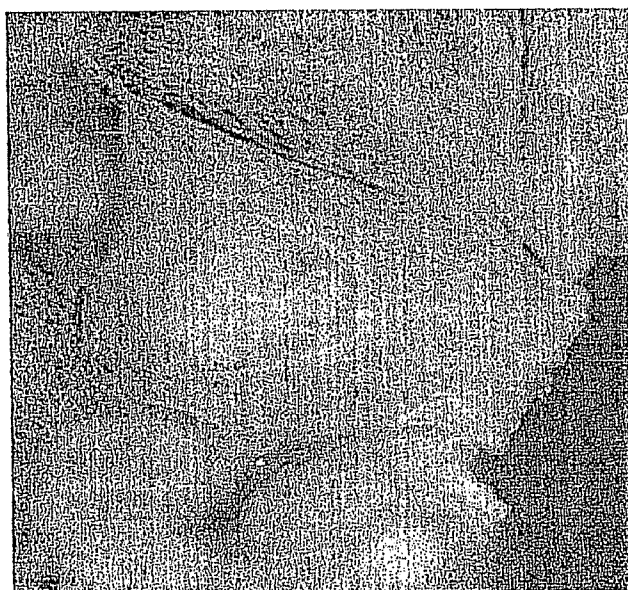
Figure 4

DMSO



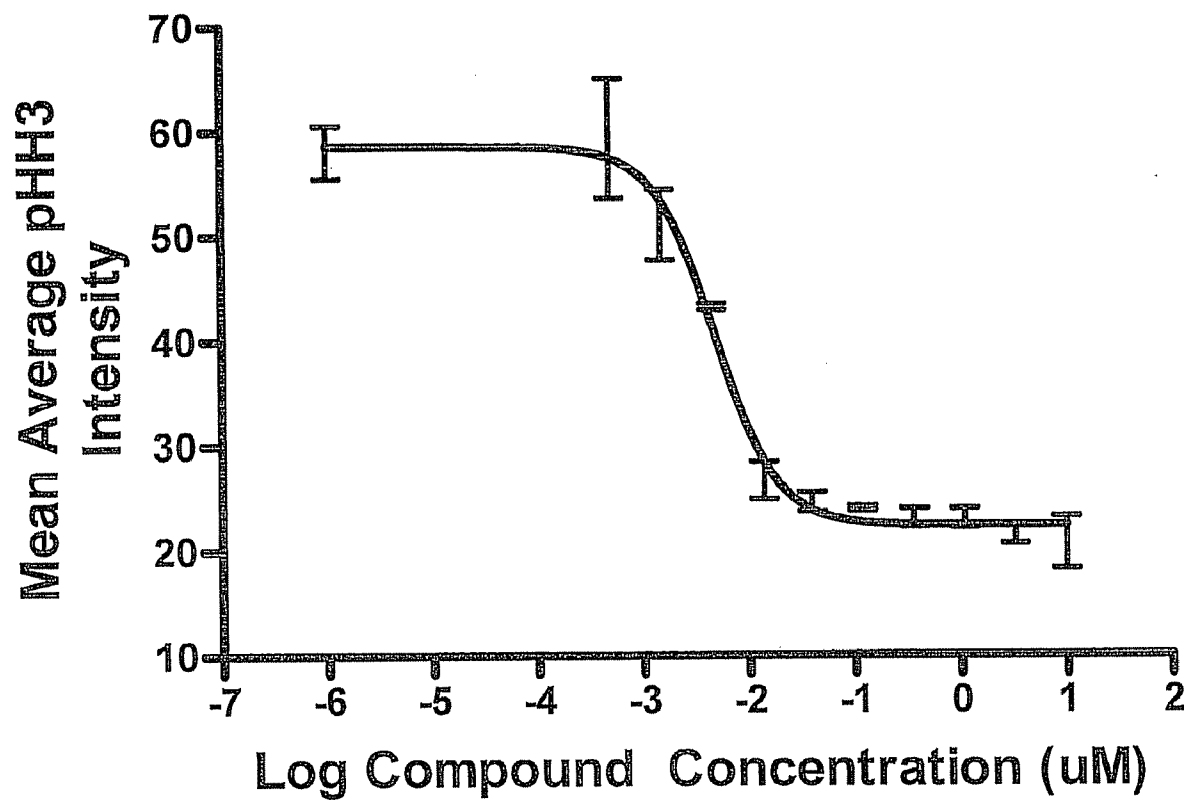
DNA
 α -tubulin

16 nM
Compound 1



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Figure 5



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Figure 6A

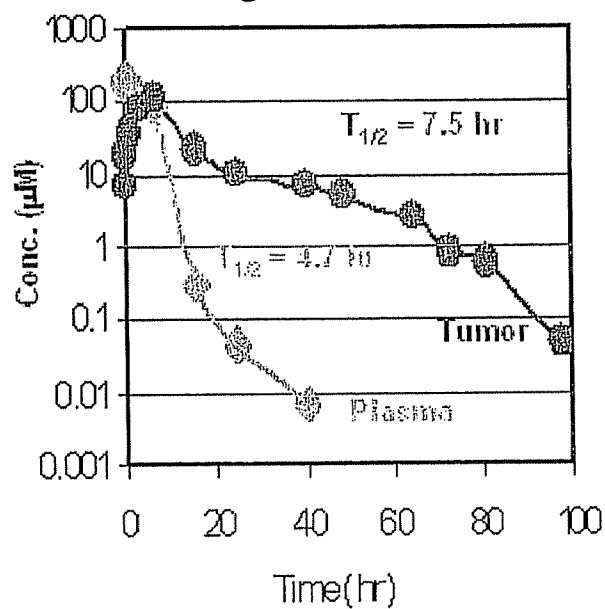
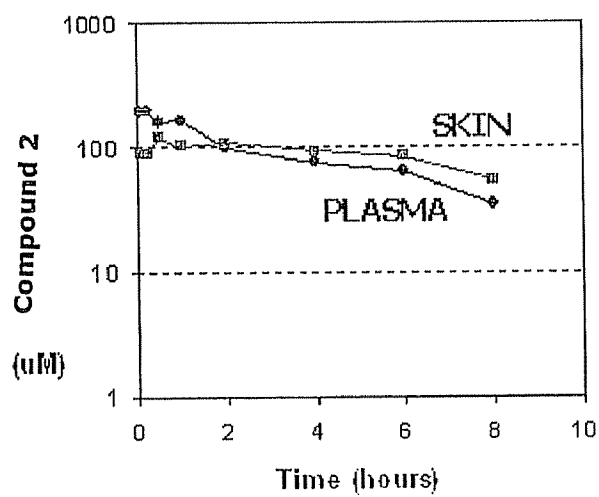
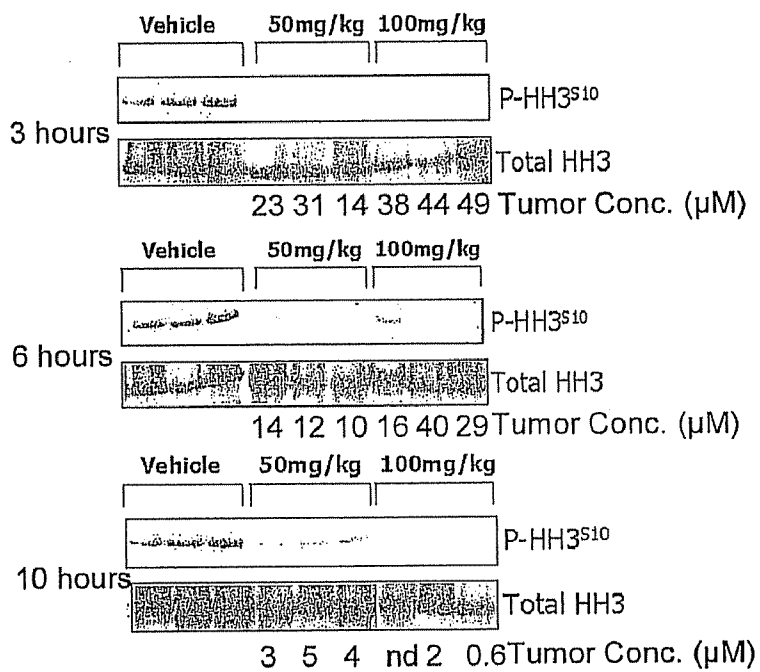


Figure 6B



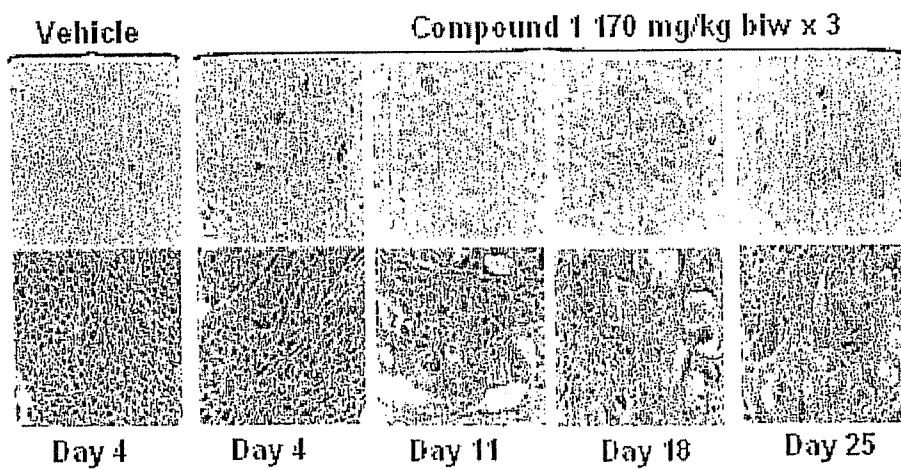
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Figure 7



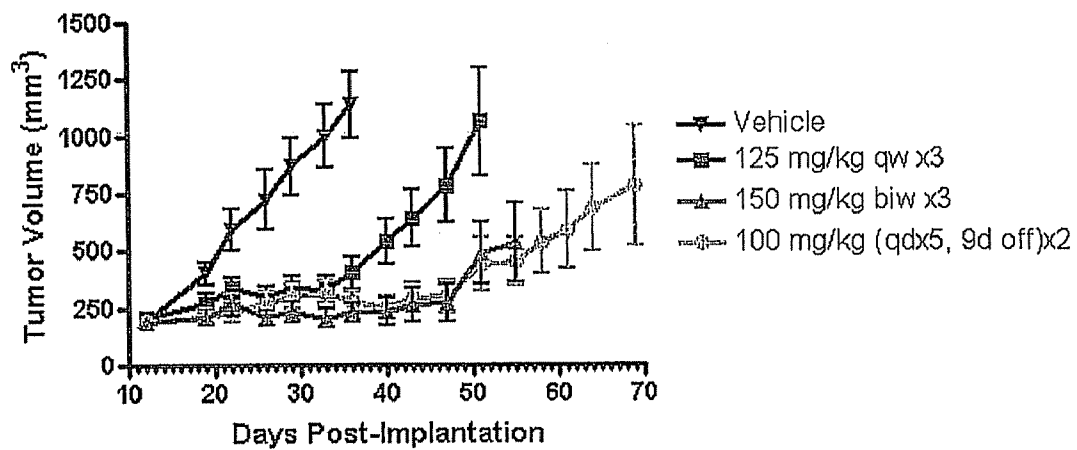
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Figure 8



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Figure 9



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Figure 10A

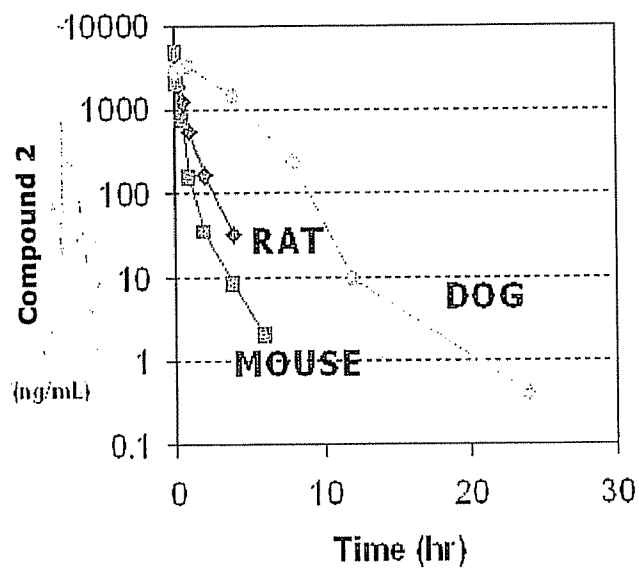
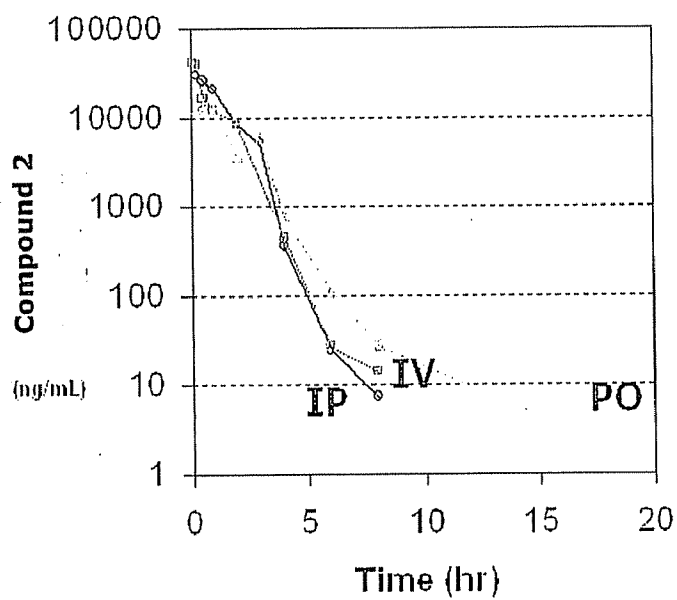


Figure 10B



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Figure 11A

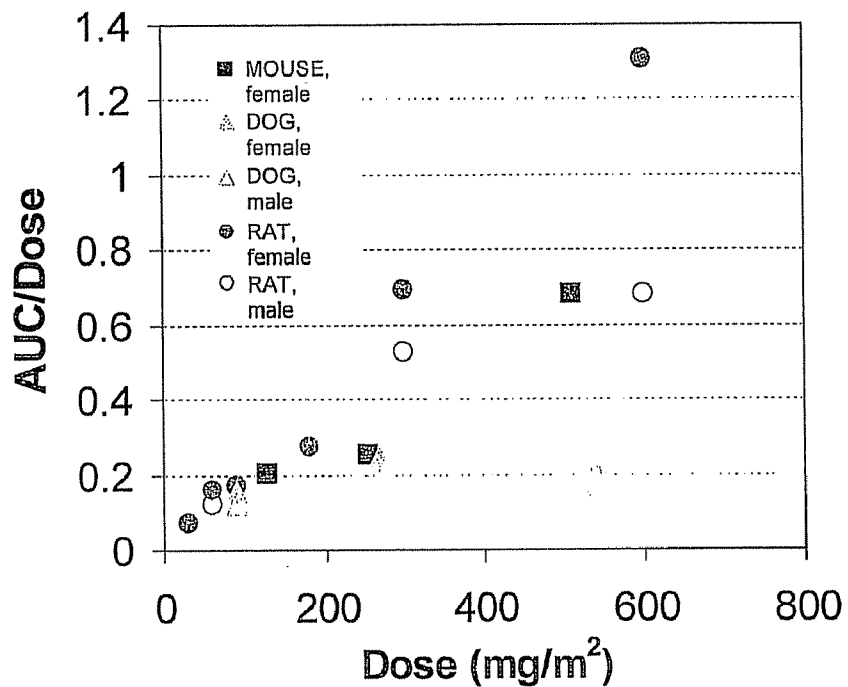
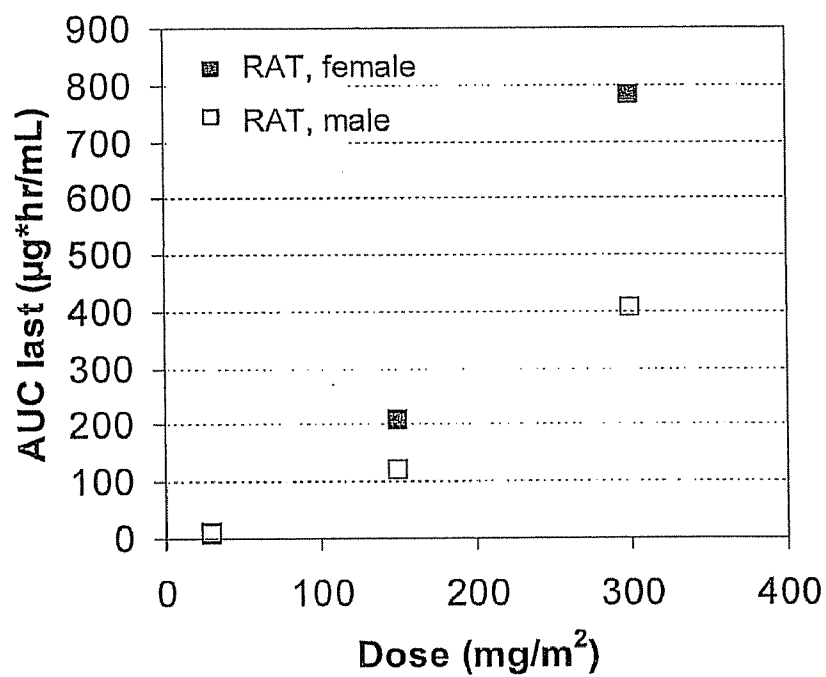


Figure 11B



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Figure 12A

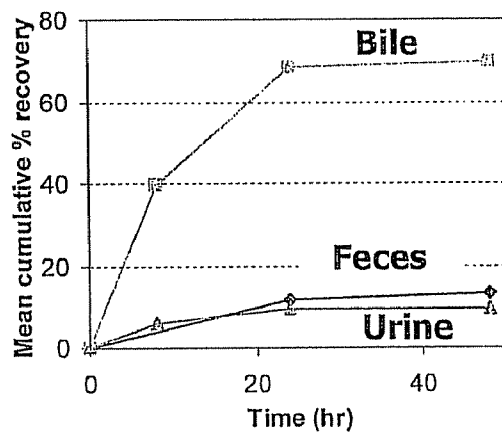


Figure 12B

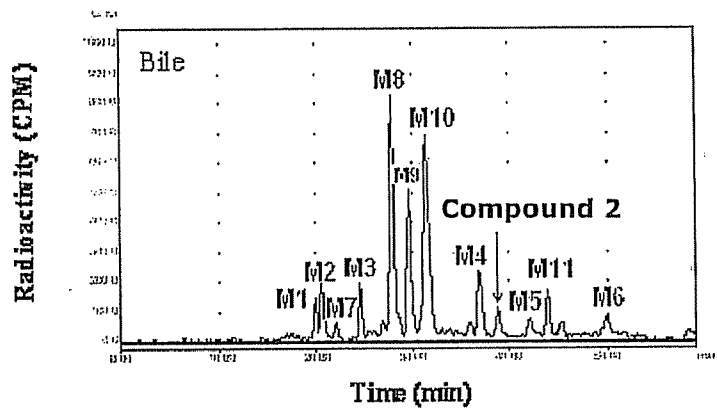
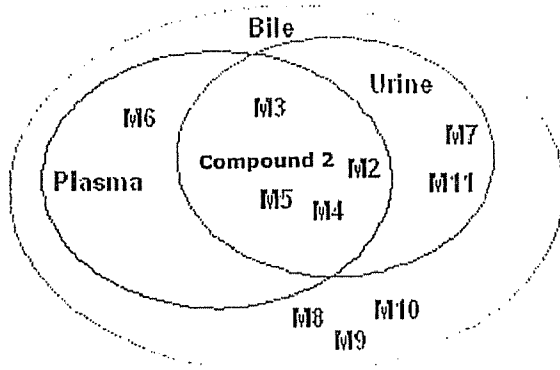


Figure 12C



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Figure 13A

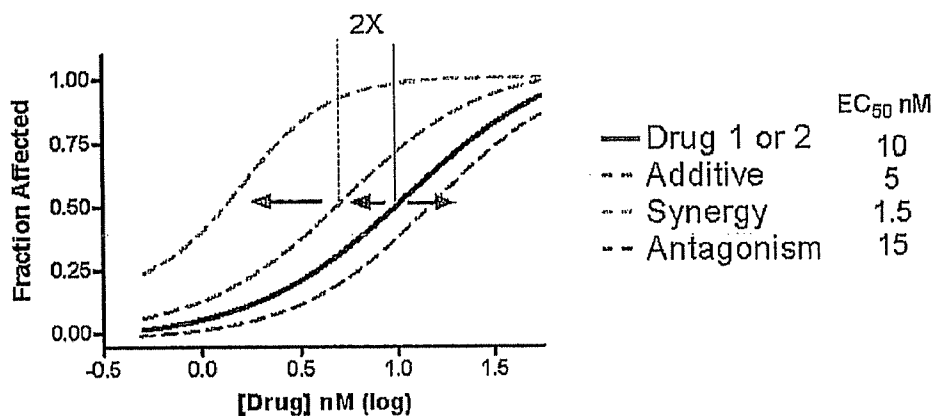


Figure 13B

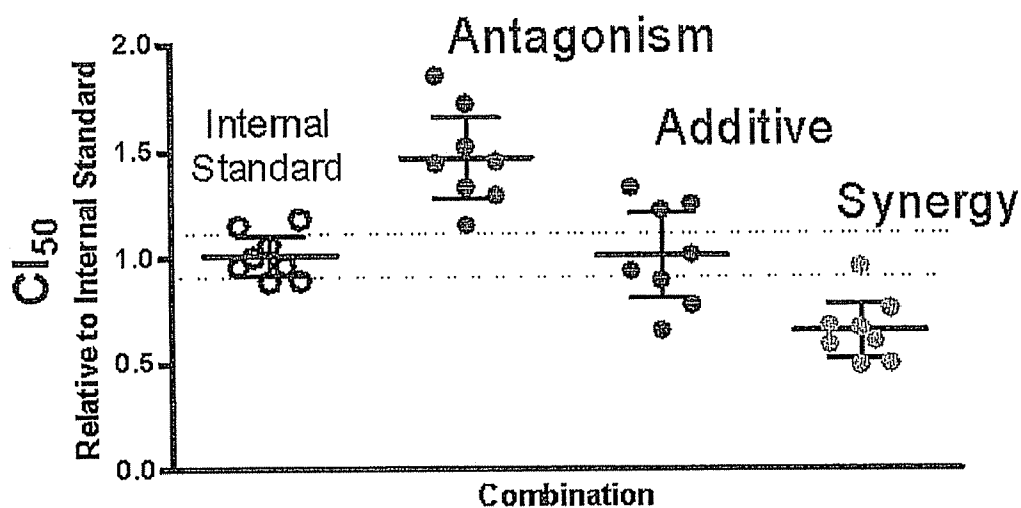


Figure 13C

Drug Combination	CI ₅₀	lower 95% CI	upper 95% CI	two tailed p-value	Result
Internal	1.00	0.71	1.29	1.0000	Additive
Drug 1	1.42	1.22	1.62	0.0003	Antagonistic
Drug 2	1.57	1.03	2.11	0.9591	Additive
Drug 3	0.57	0.03	1.11	0.0006	Synergistic

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Figure 14A

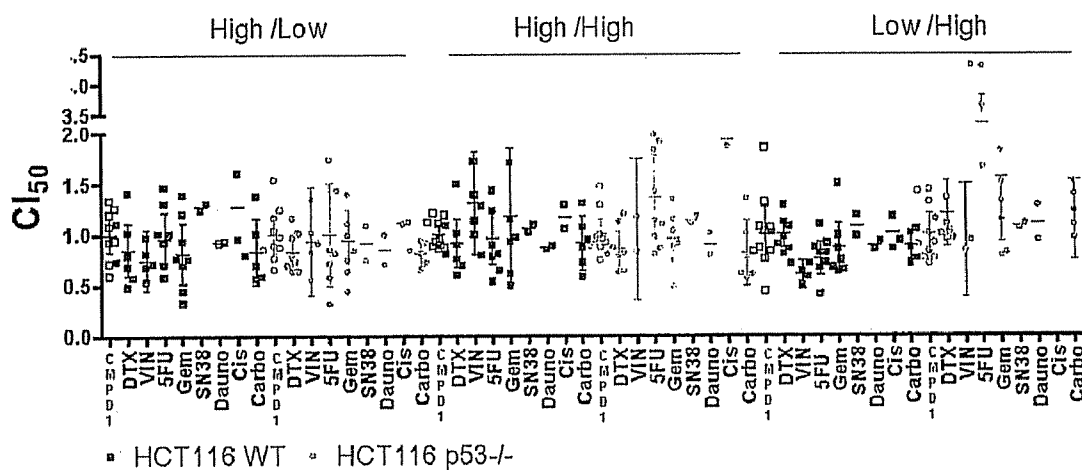


Figure 14B

		Compound 1	Panel	Compound 1	Panel	Compound 1	Panel
		Ratio	High	Low	High	Low	High
Schedule		p53 RNAi					
i. Codose Compound 1 Panel Drug	5FU	Antagonistic	Antagonistic	Antagonistic	Additive	Additive	Additive
	Carbo	Antagonistic	Antagonistic	Additive	Additive	Antagonistic	Antagonistic
	Gem	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Additive	Additive
	Dauno	Antagonistic	Antagonistic	Antagonistic	Additive	Additive	Additive
	SN38	Additive	Antagonistic	Additive	Antagonistic	Additive	Additive
	Dlx1	Antagonistic	Antagonistic	Additive	Antagonistic	Additive	Additive
	Vin	Antagonistic	Antagonistic	Additive	Synergistic	Additive	Synergistic
ii. Compound 1, washout, Panel Compound 1 Panel Drug	5FU	Additive	Additive	Additive	Additive	Additive	Antagonistic
	Carbo	Additive	Additive	Additive	Additive	Additive	Additive
	Gem	Additive	Additive	Additive	Additive	Additive	Additive
	Dlx1	Additive	Additive	Additive	Additive	Additive	Additive
	Vin	Additive	Additive	Additive	Additive	Synergistic	Additive
iii. Panel, washout, Compound 1 Panel Drug Compound 1	5FU	Additive	Additive	Additive	Additive	Additive	Additive
	Carbo	Additive	Additive	Additive	Additive	Antagonistic	Antagonistic
	Gem	Additive	Synergistic	Additive	Additive	Additive	Additive
	Dlx1	Additive	Additive	Additive	Additive	Additive	Additive
	Vin	Additive	Additive	Additive	Additive	Additive	Additive

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Figure 15A

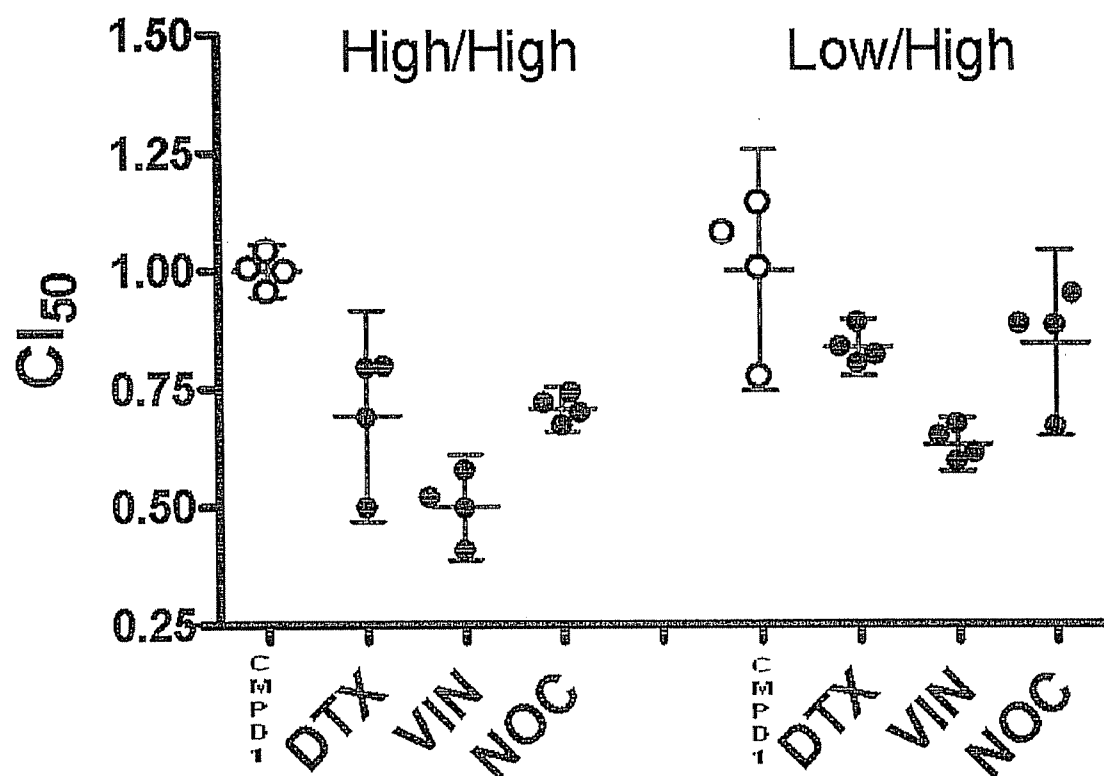
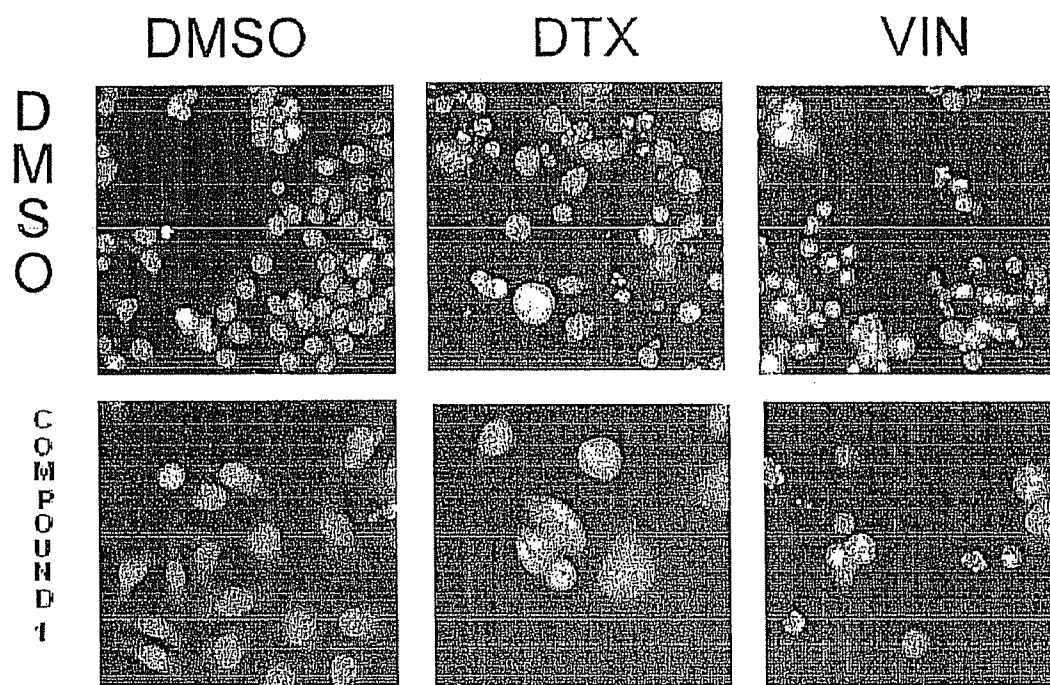


Figure 15B

Compound 1 Panel						Compound 1 Panel					
High High						Low High					
	Mean	lower	upper			Mean	lower	upper			
	CI50	95% CI	95% CI	p value	Result	CI50	95% CI	95% CI	p value	Result	
Compound 1	1.00	0.94	1.06			1.00	0.74	1.26			
DTX	0.69	0.47	0.92	0.0286	Synergistic	0.84	0.78	0.90	0.343	Additive	
VIN	0.50	0.39	0.61	0.0286	Synergistic	0.63	0.57	0.69	0.029	Synergistic	
NOC	0.71	0.66	0.75	0.0286	Synergistic	0.85	0.65	1.04	0.200	Additive	

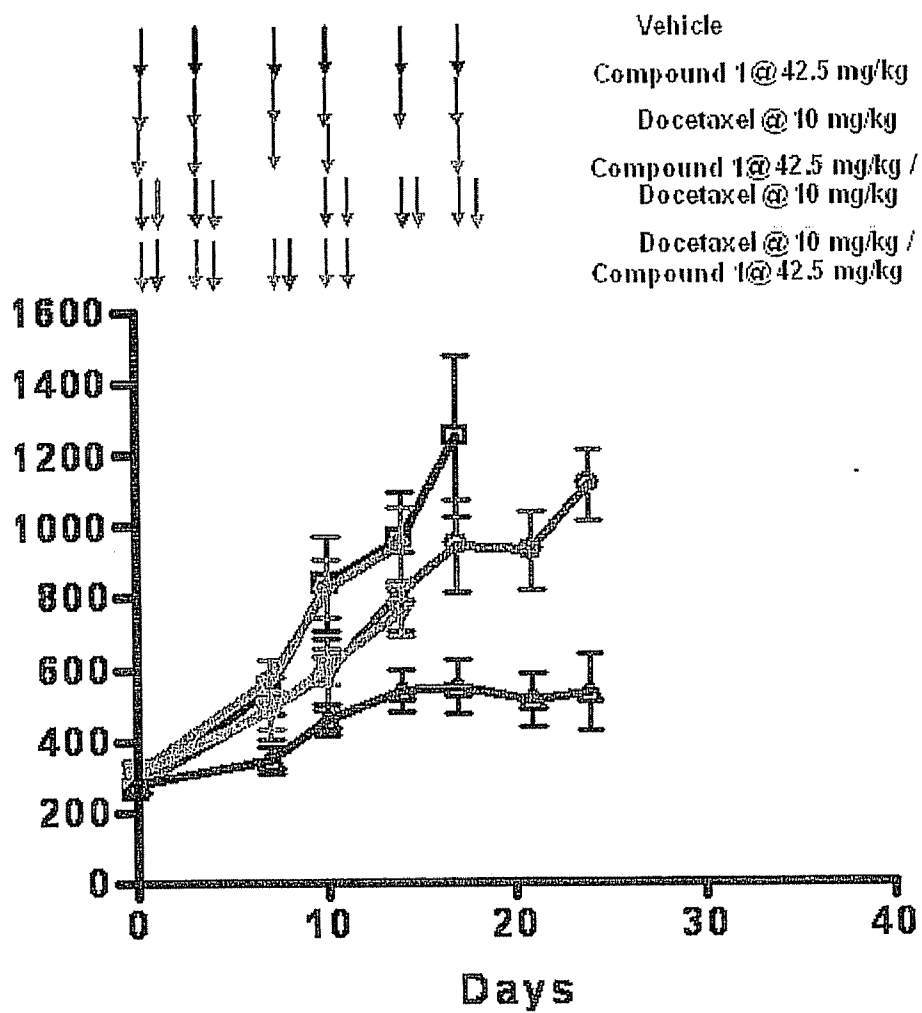
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Figure 16



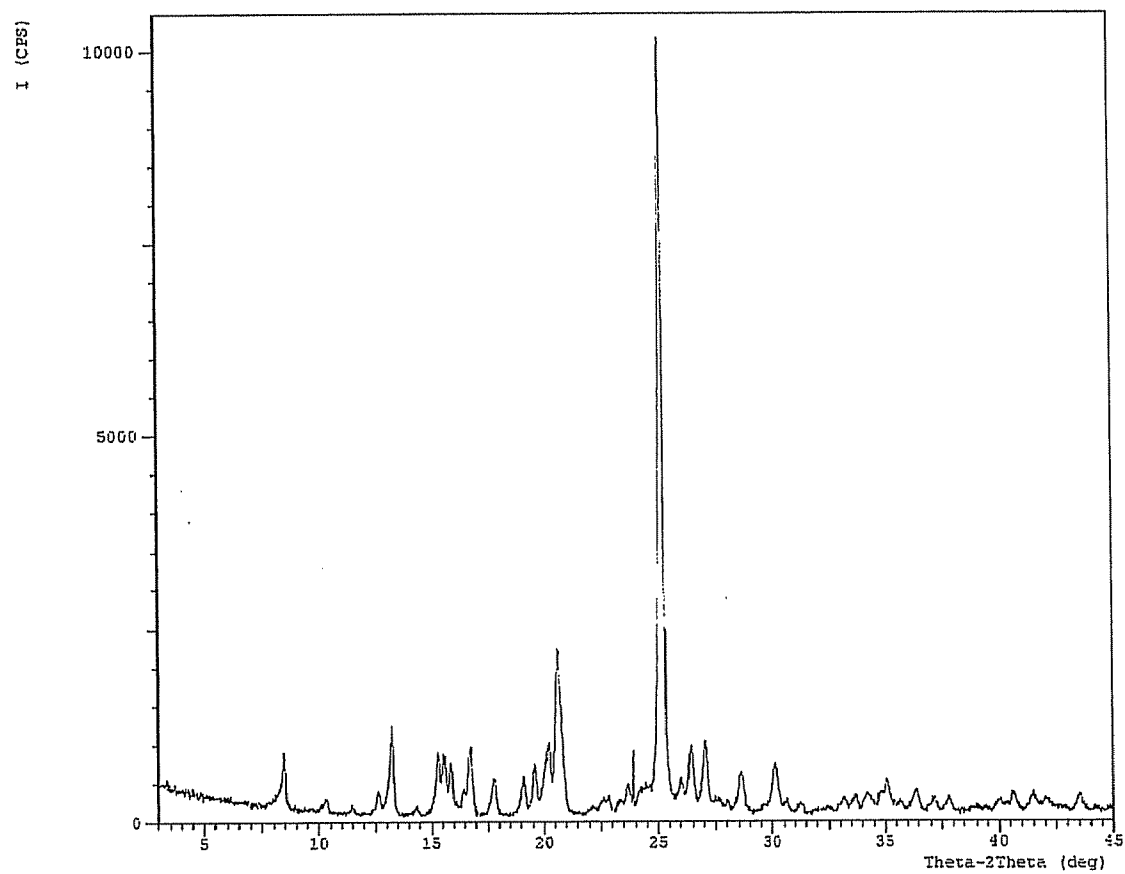
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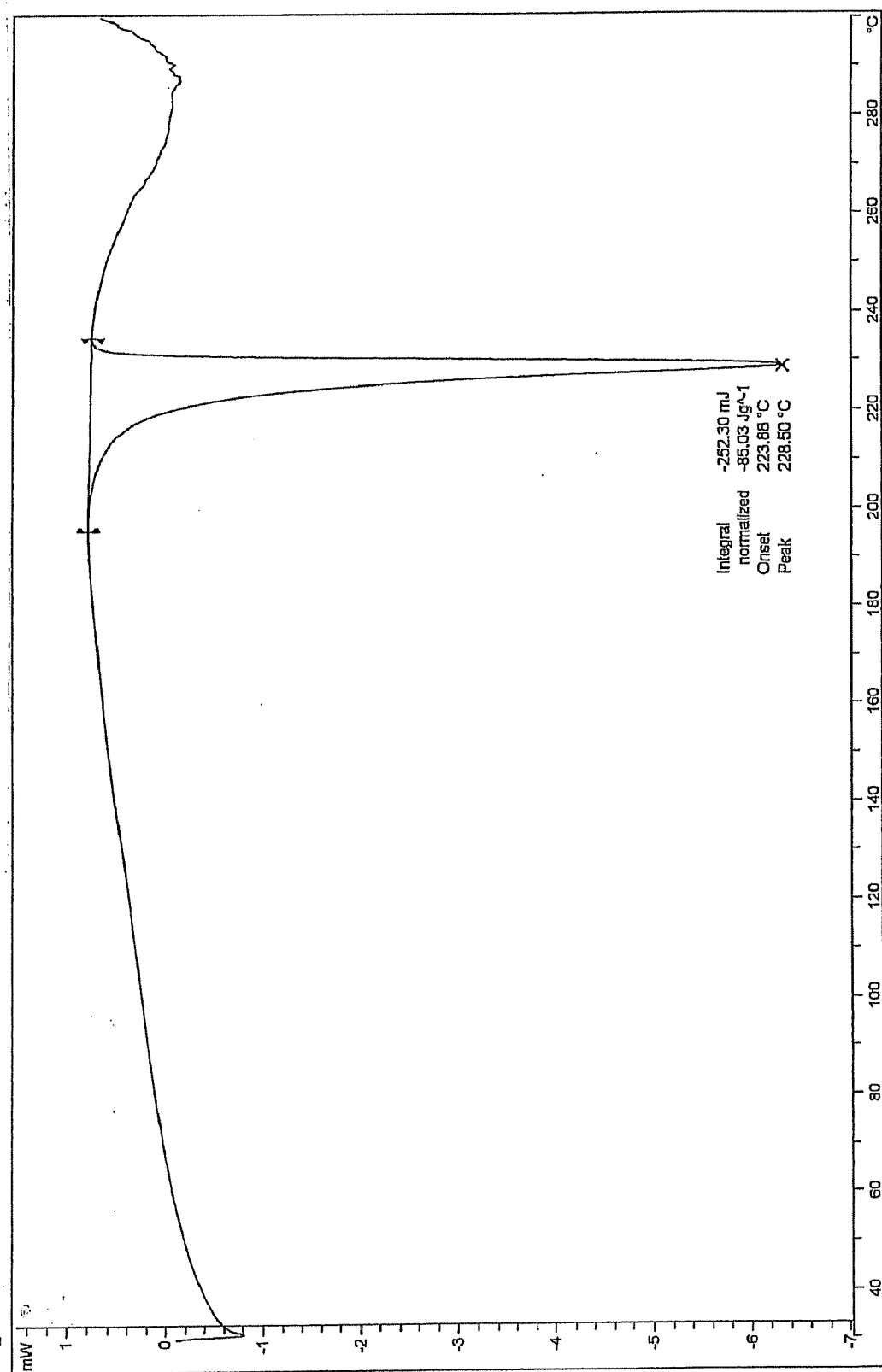
Figure 17



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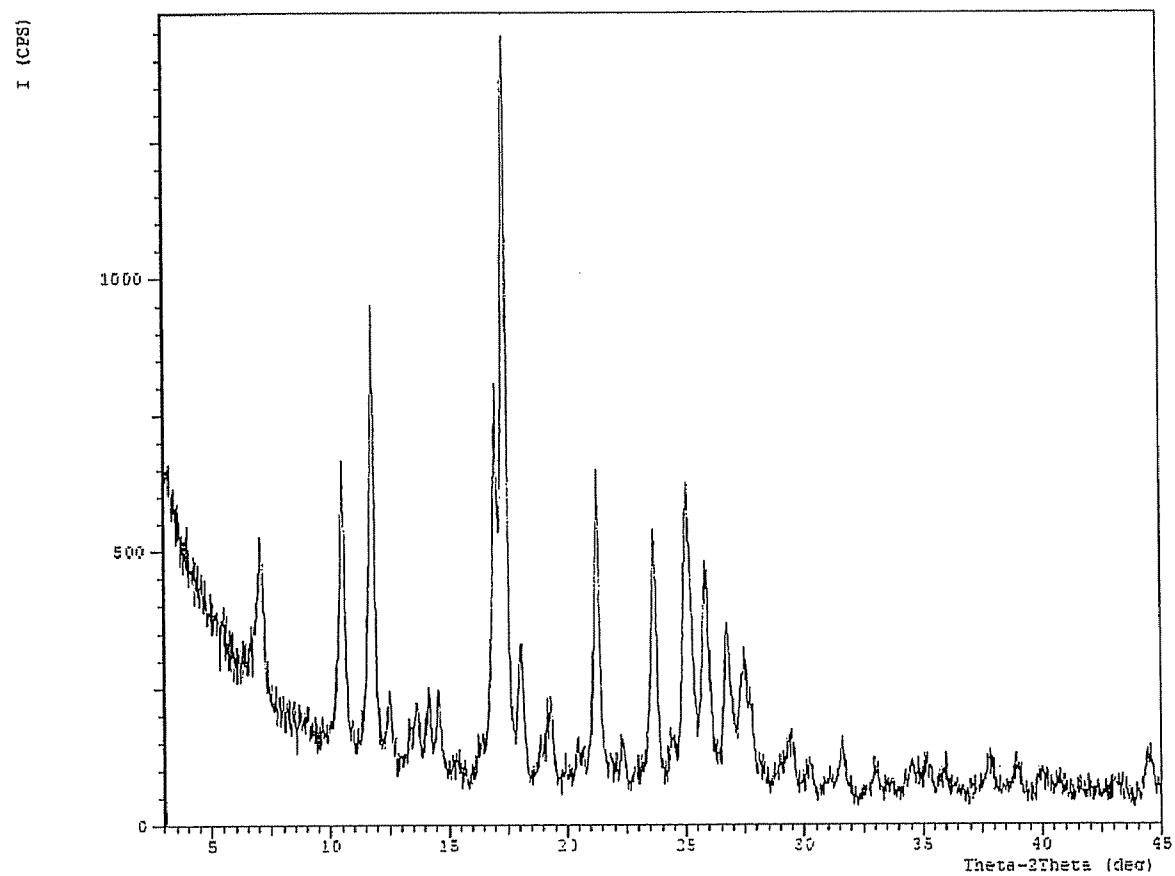
Figure 18



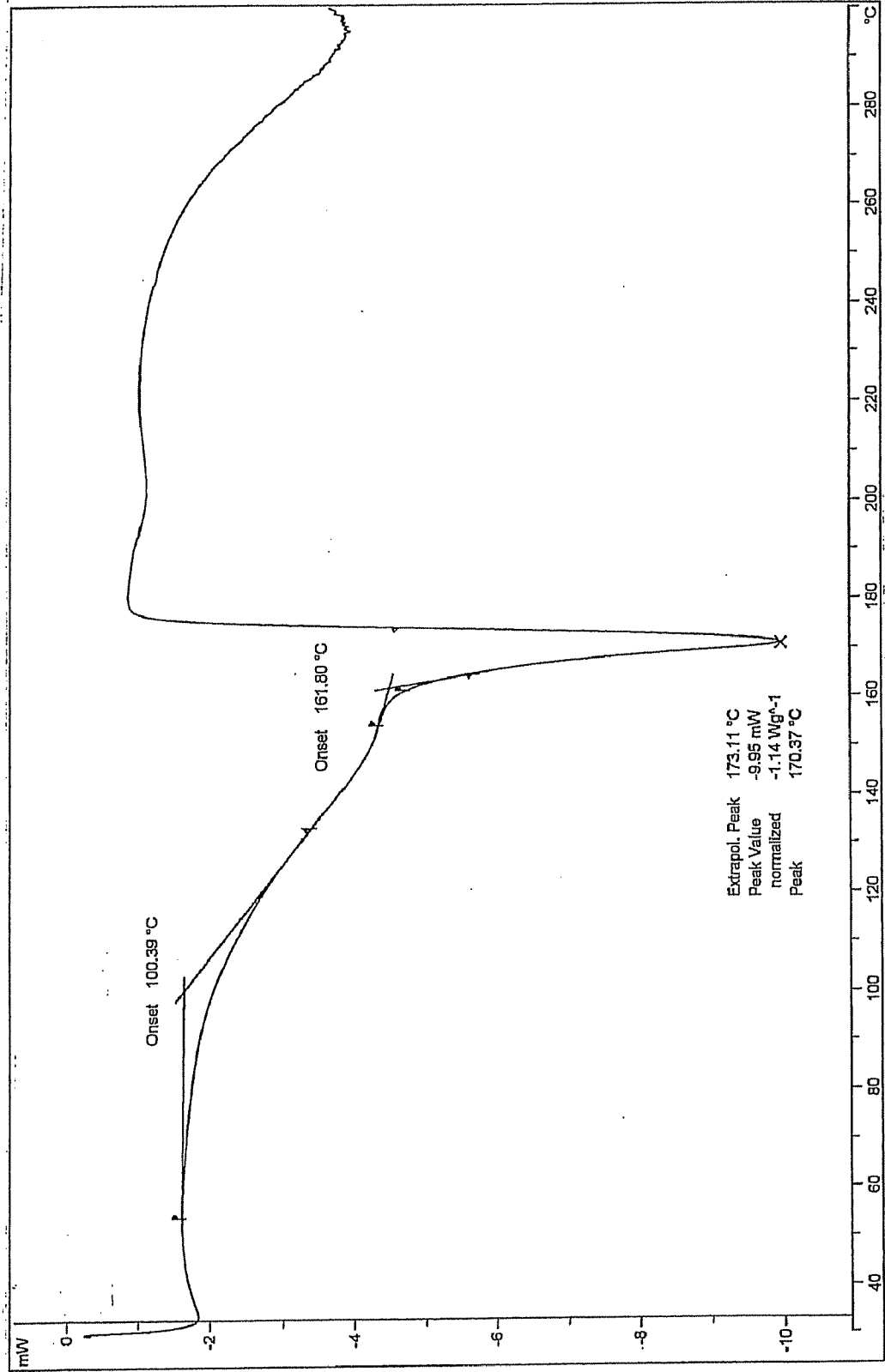
20/47
Figure 19

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Figure 20

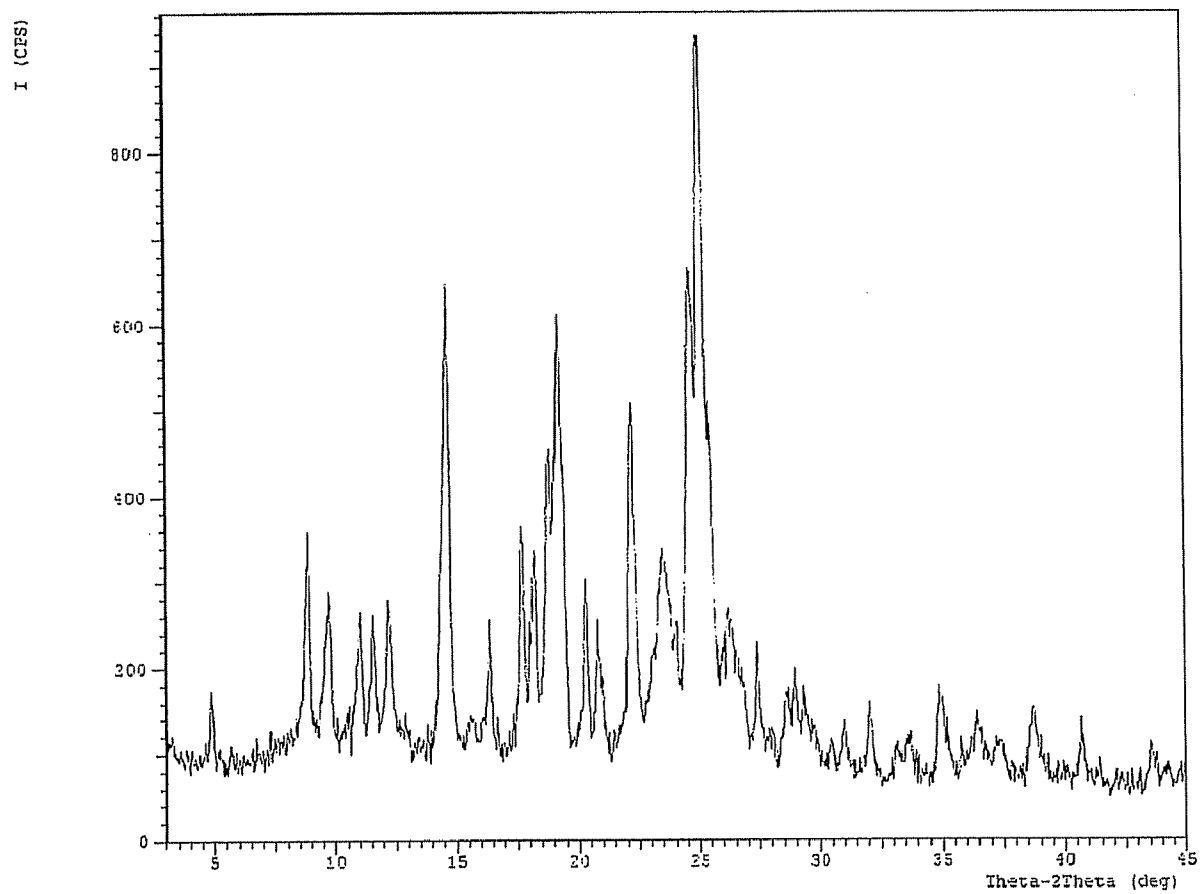


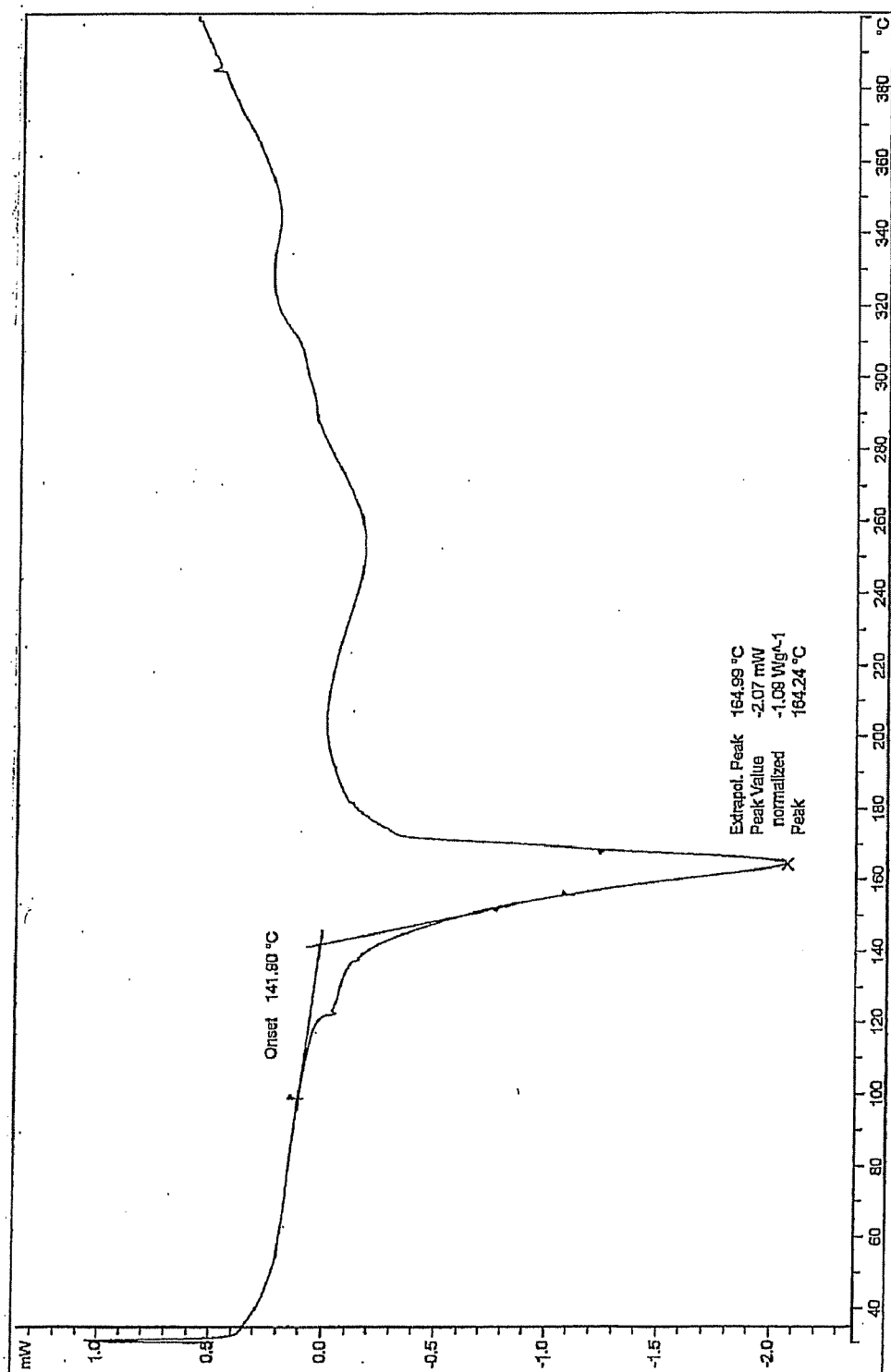
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Figure 21



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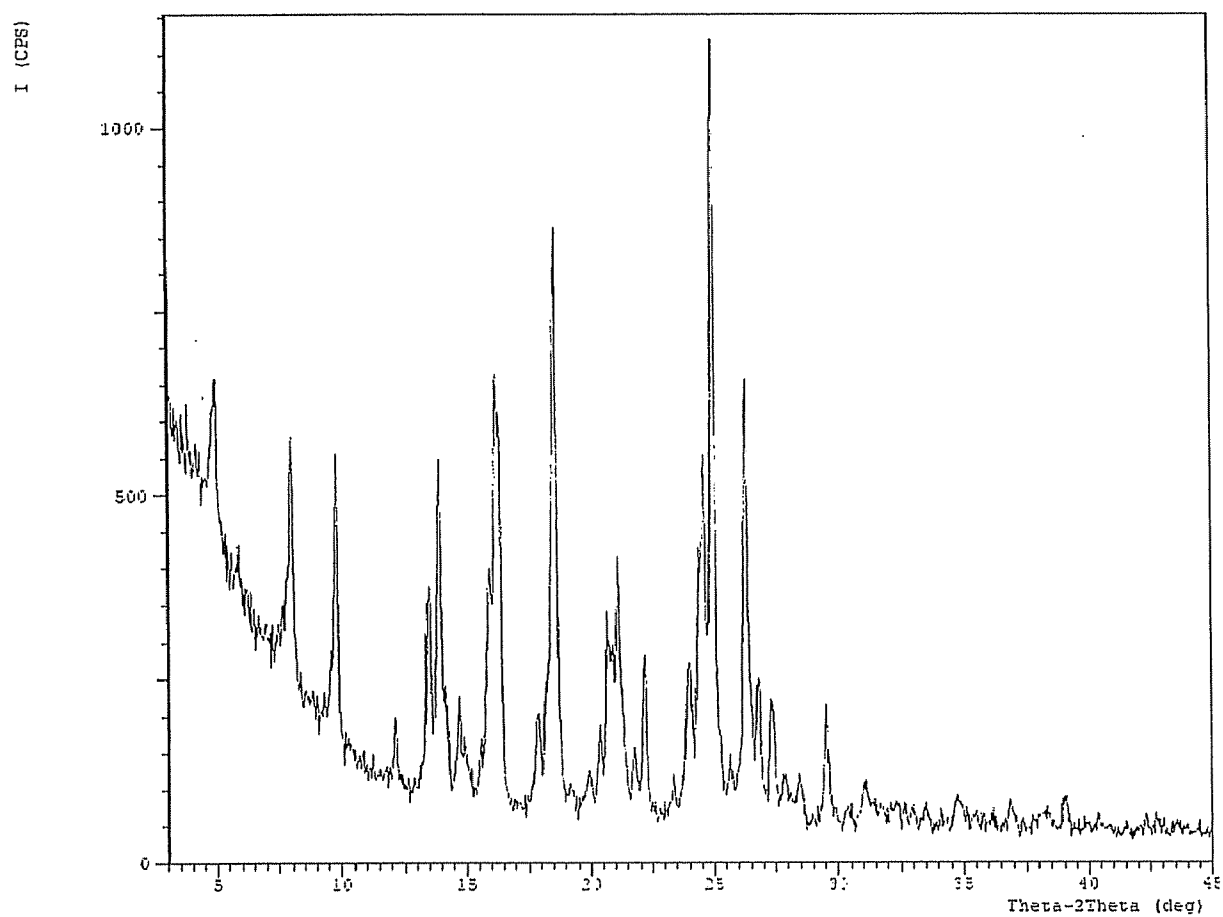
Figure 22



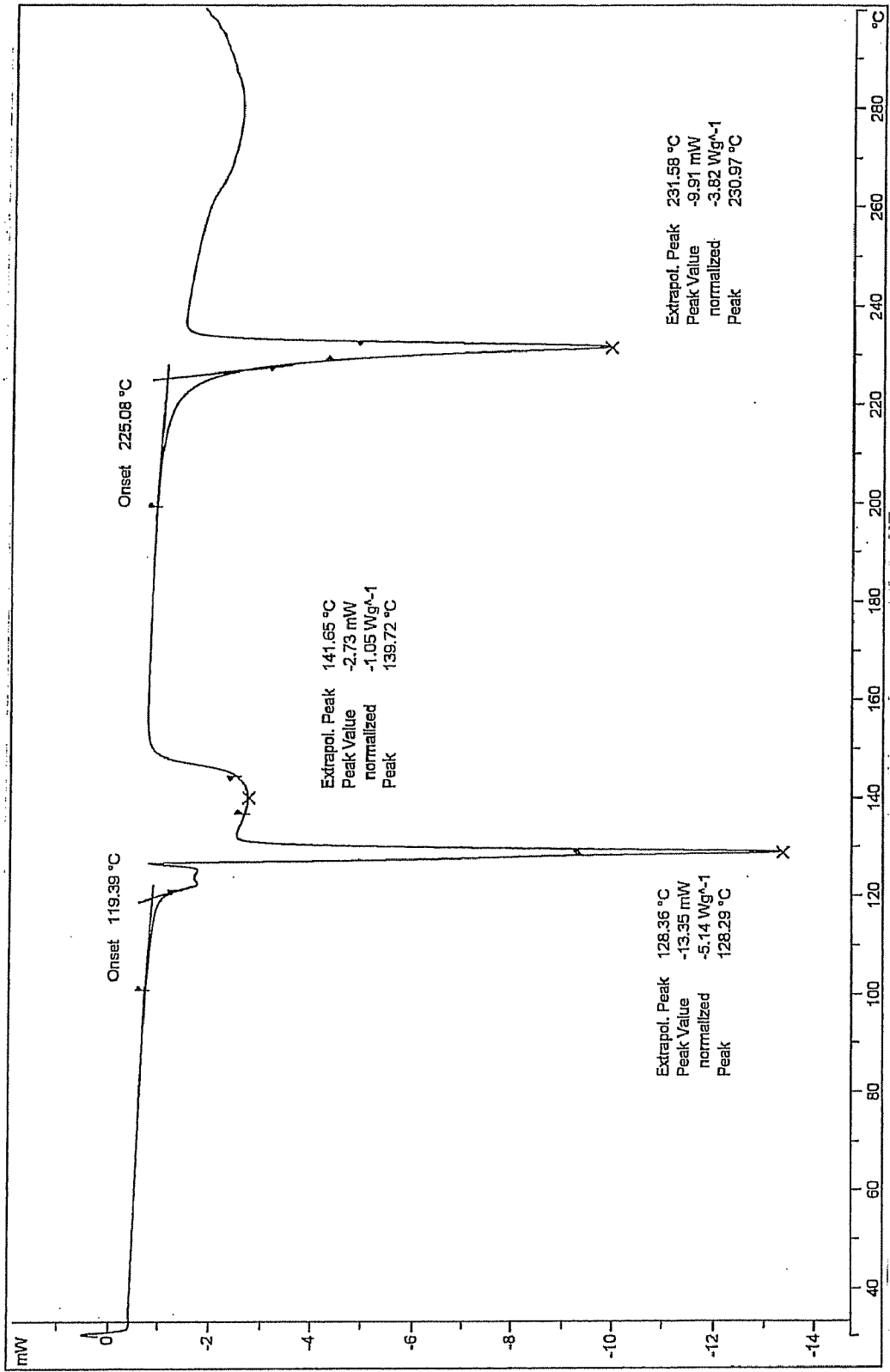
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Figure 23

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Figure 24

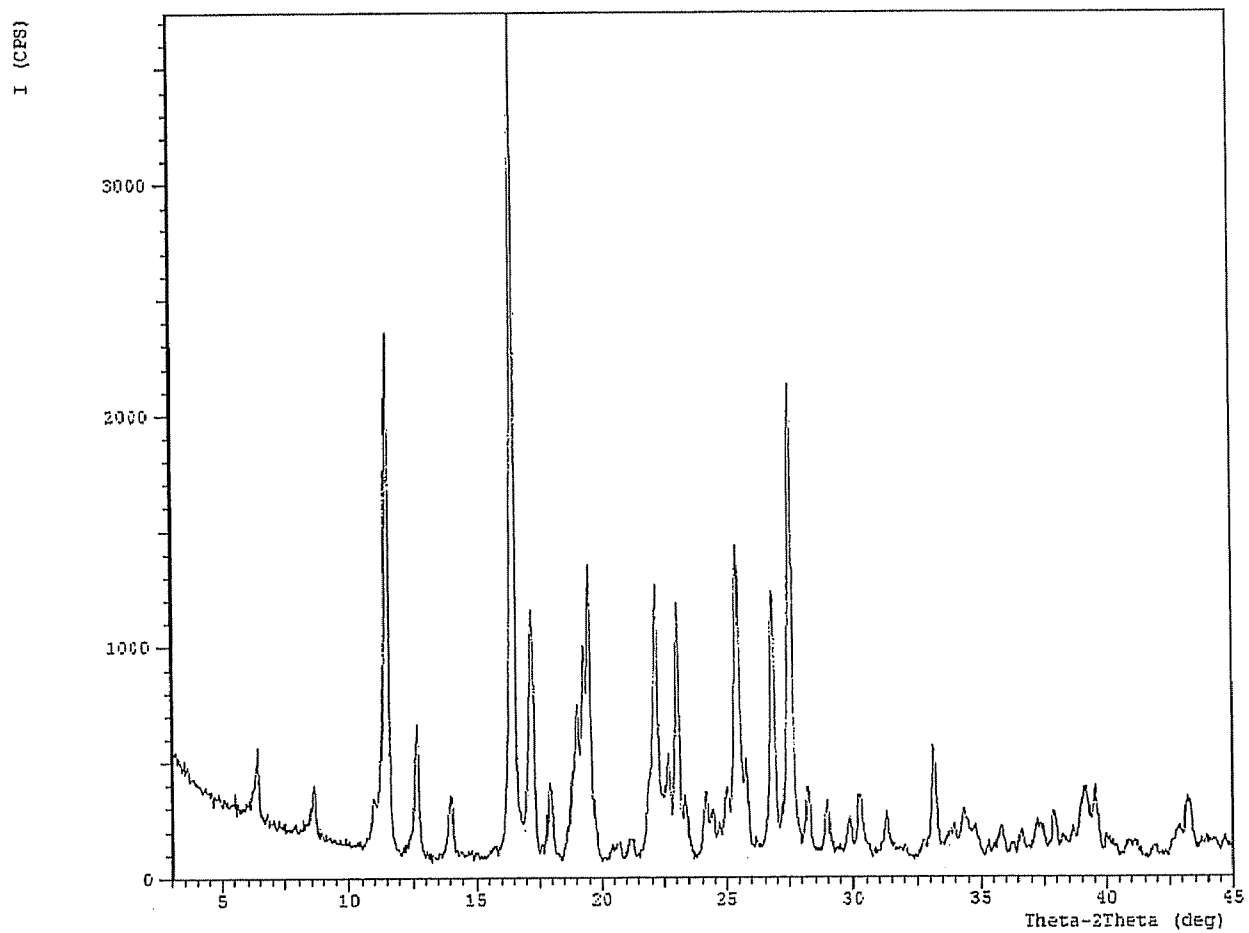


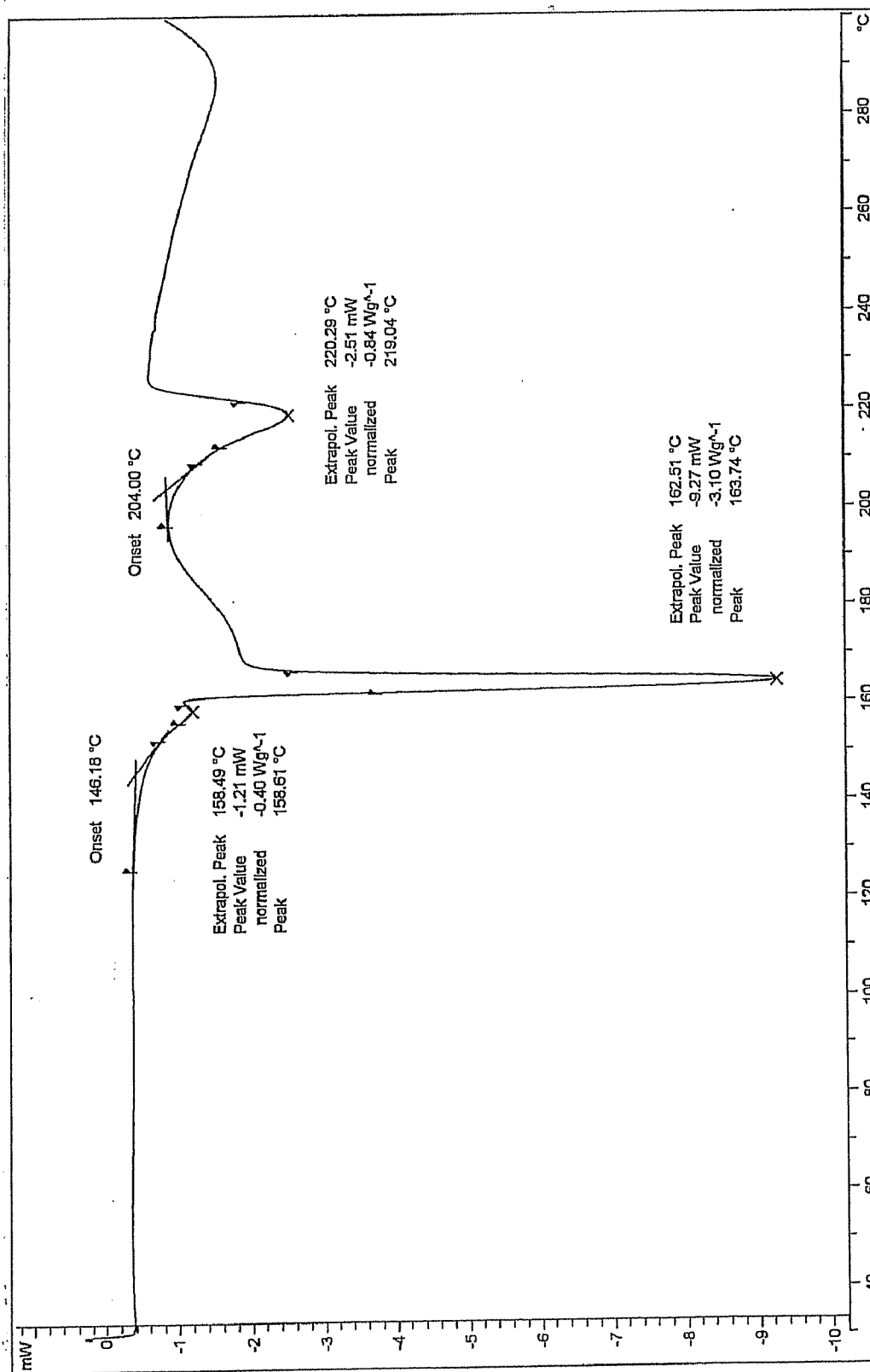
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Figure 25



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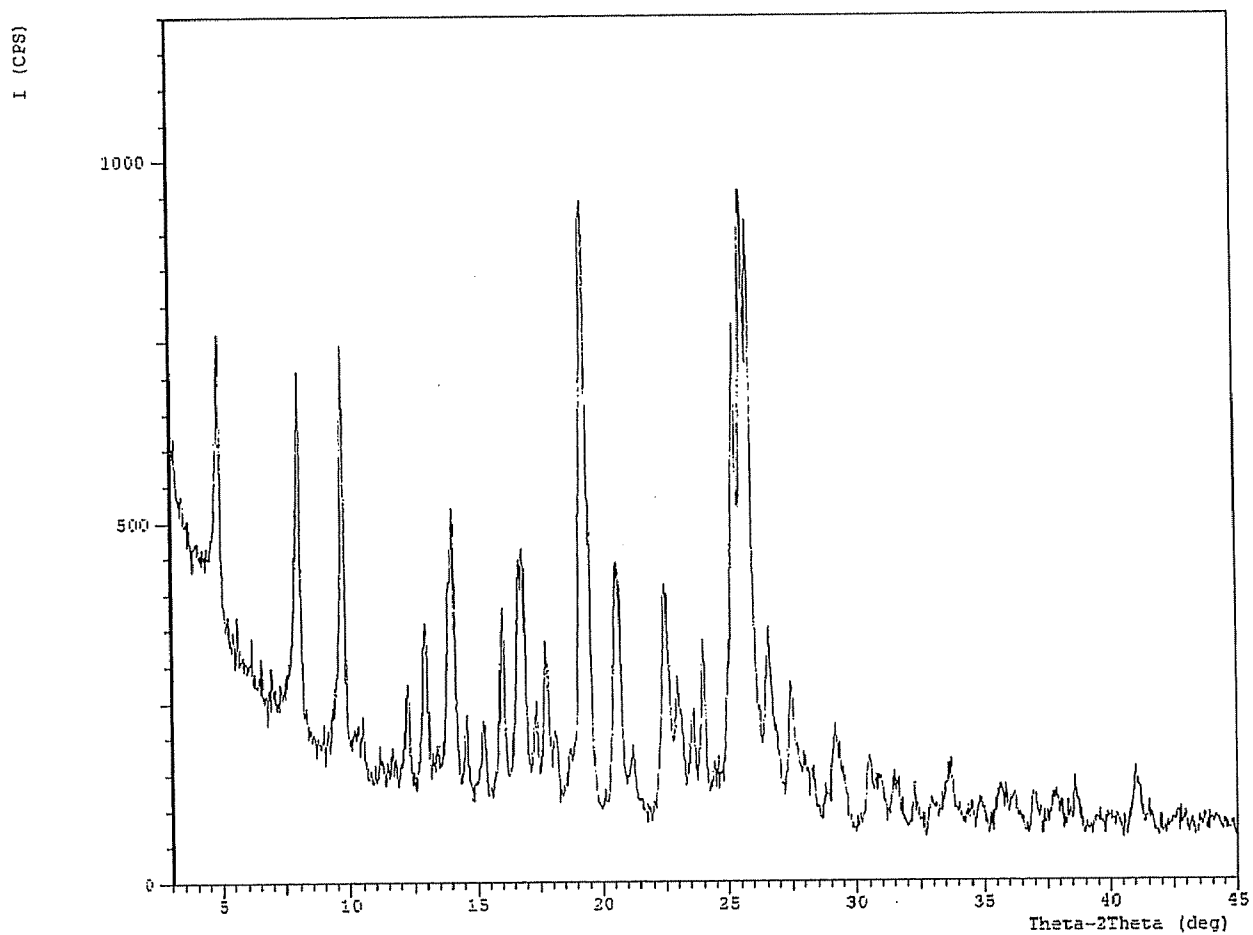
Figure 26

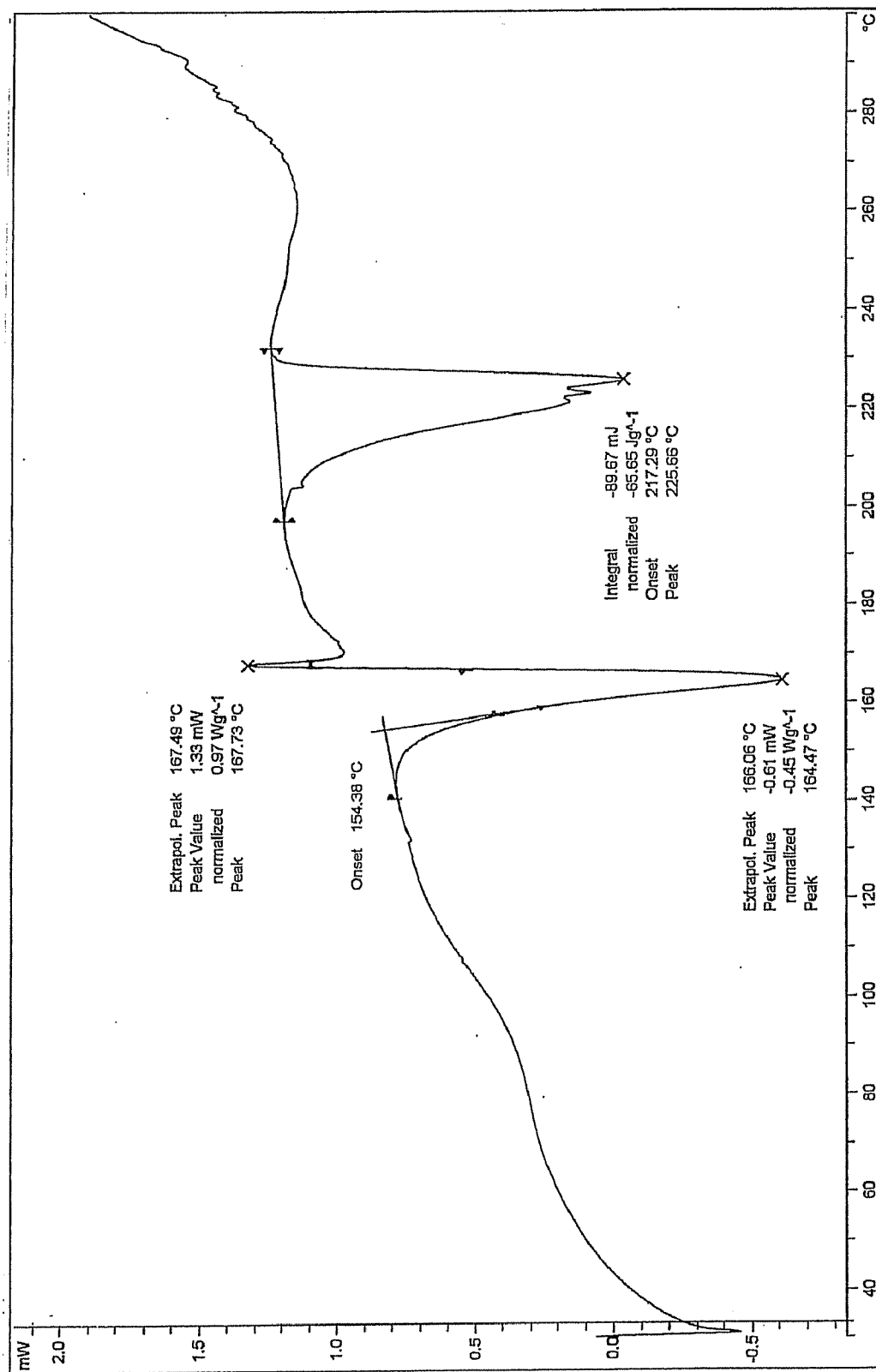


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Figure 27

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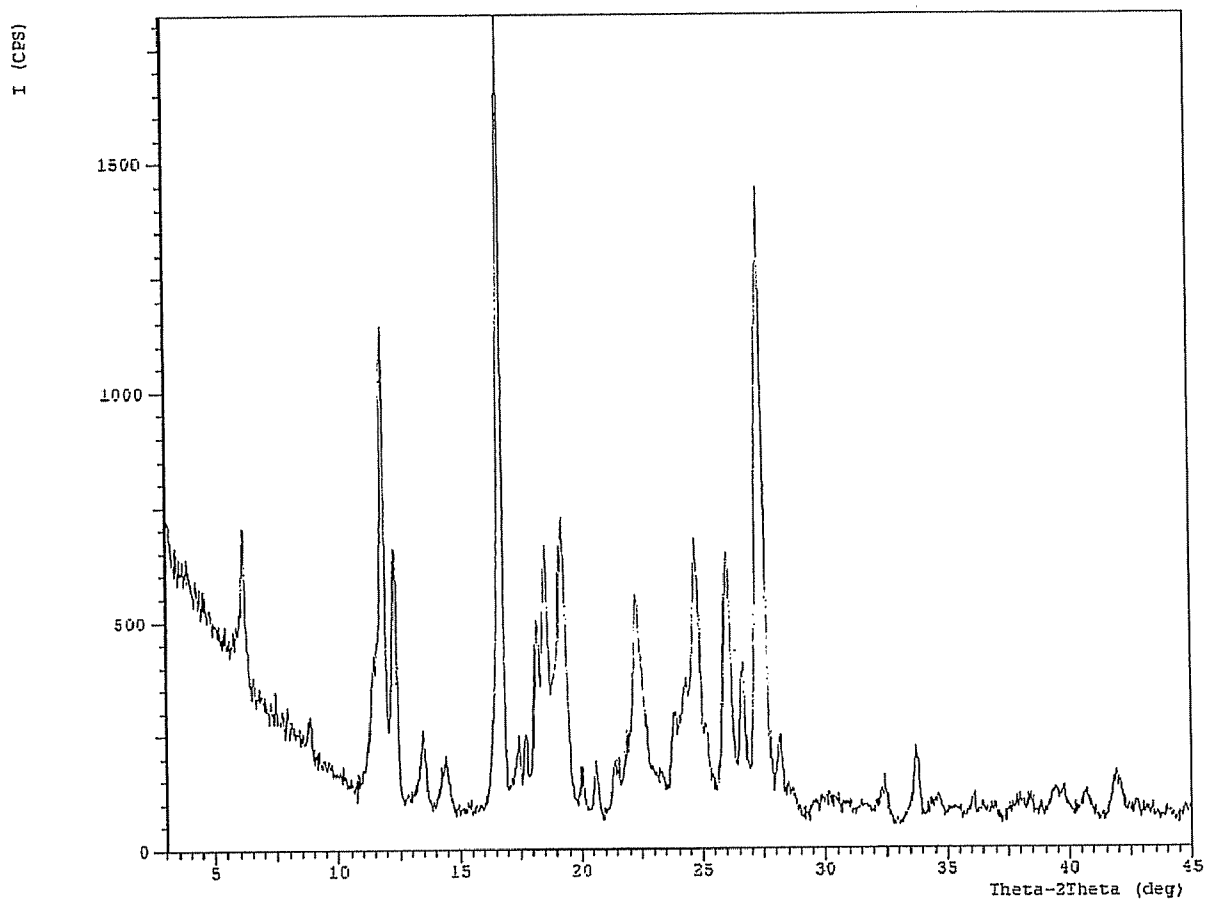
Figure 28

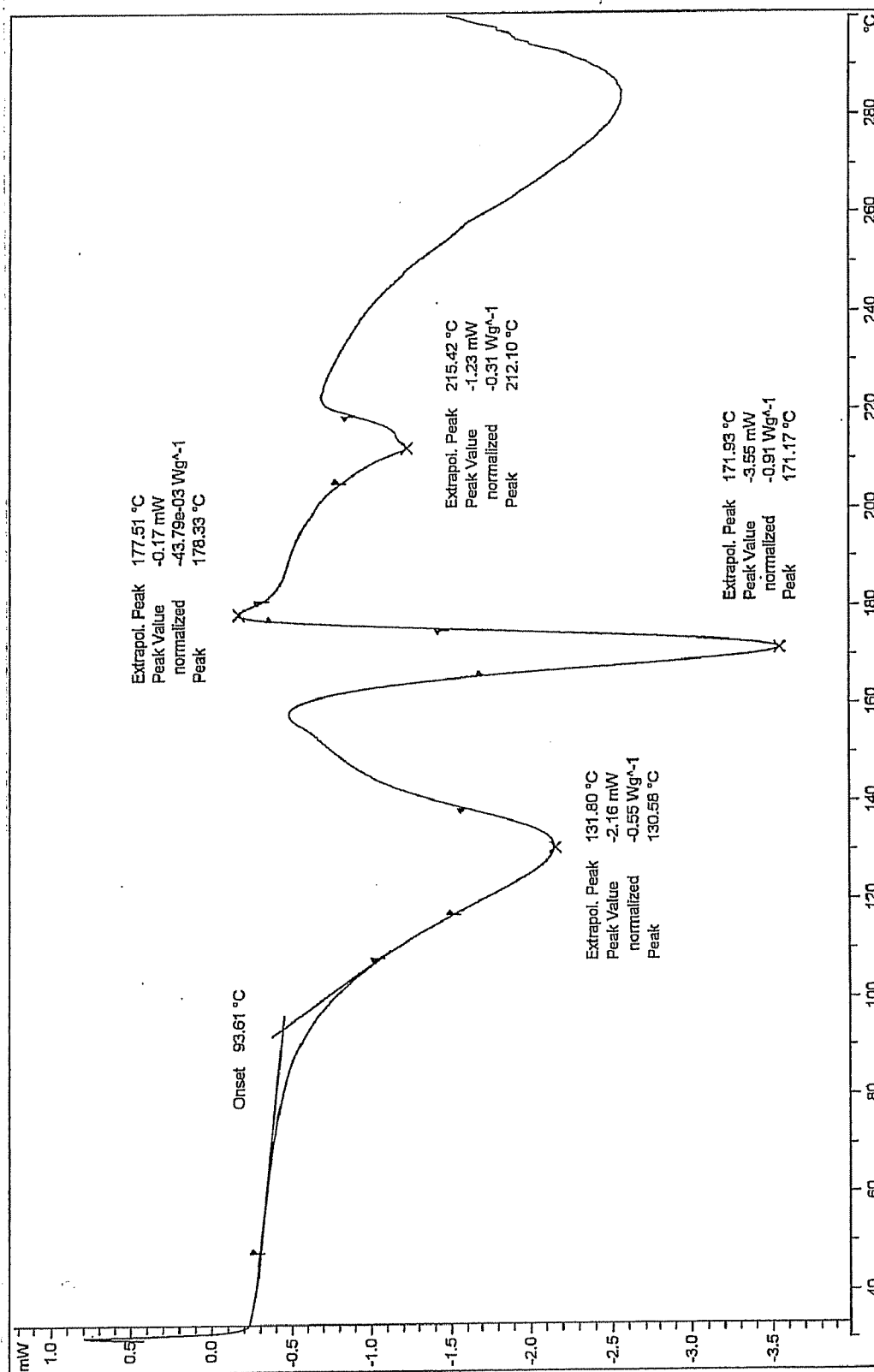


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Figure 29

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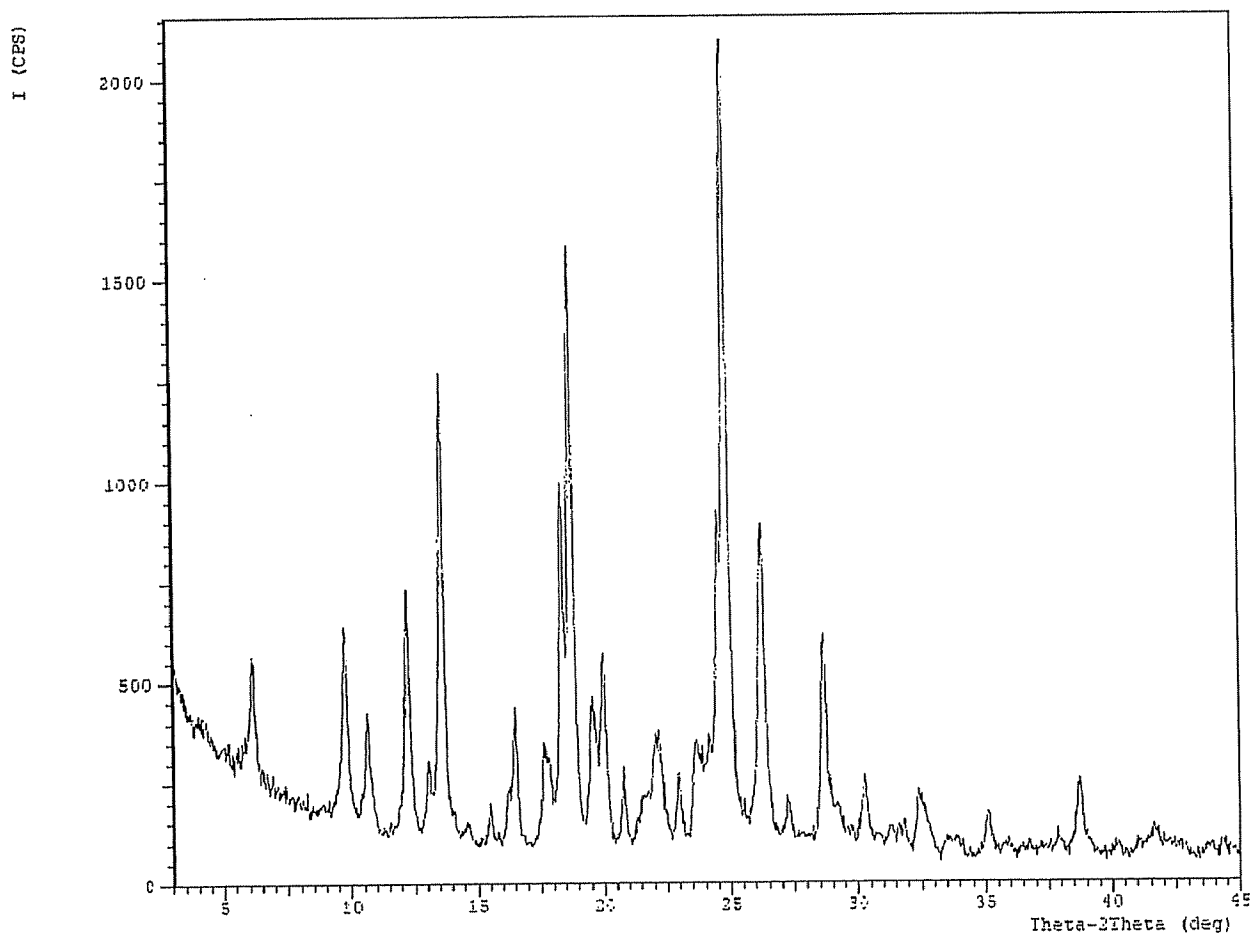
Figure 30

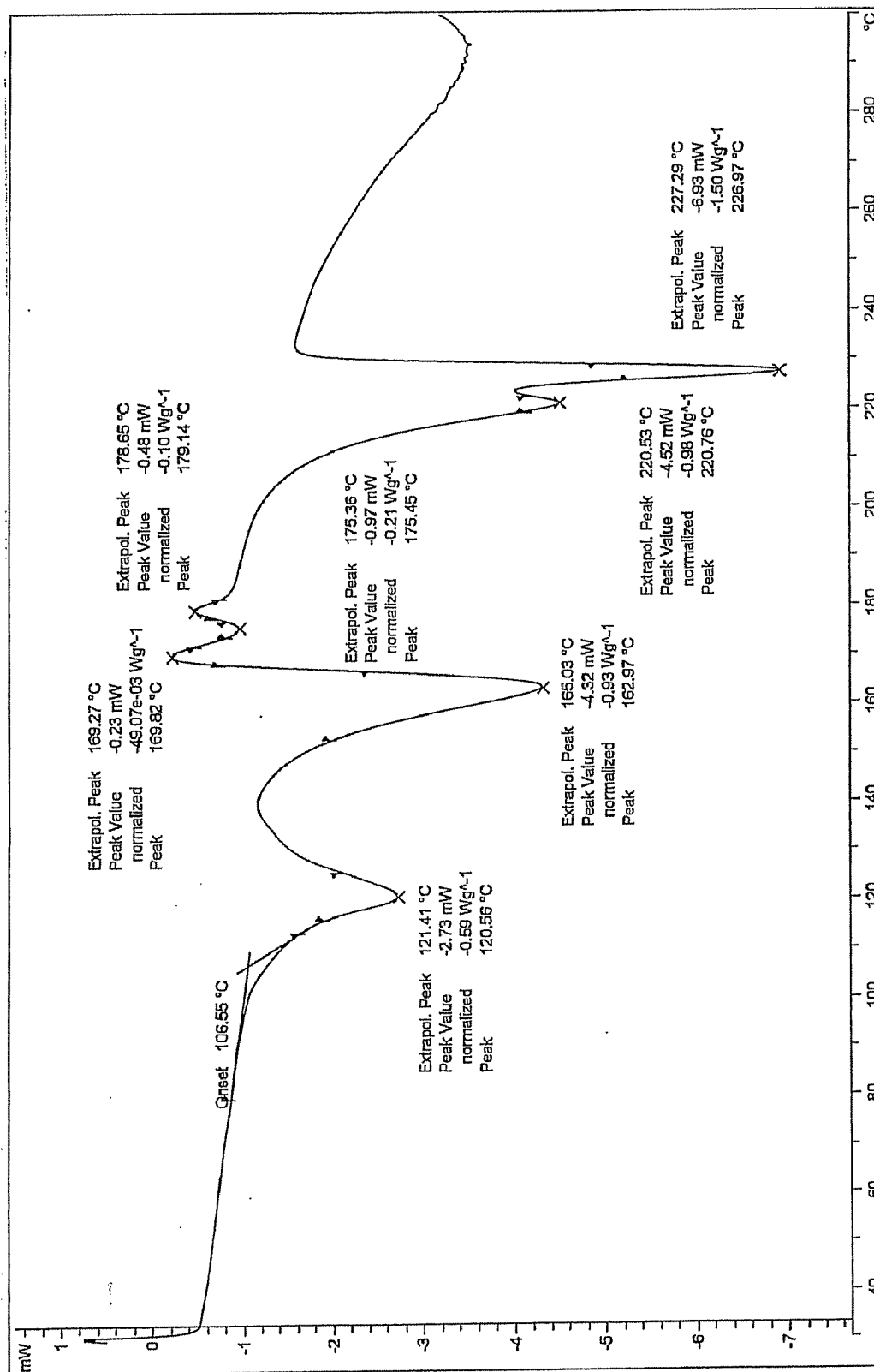


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Figure 31

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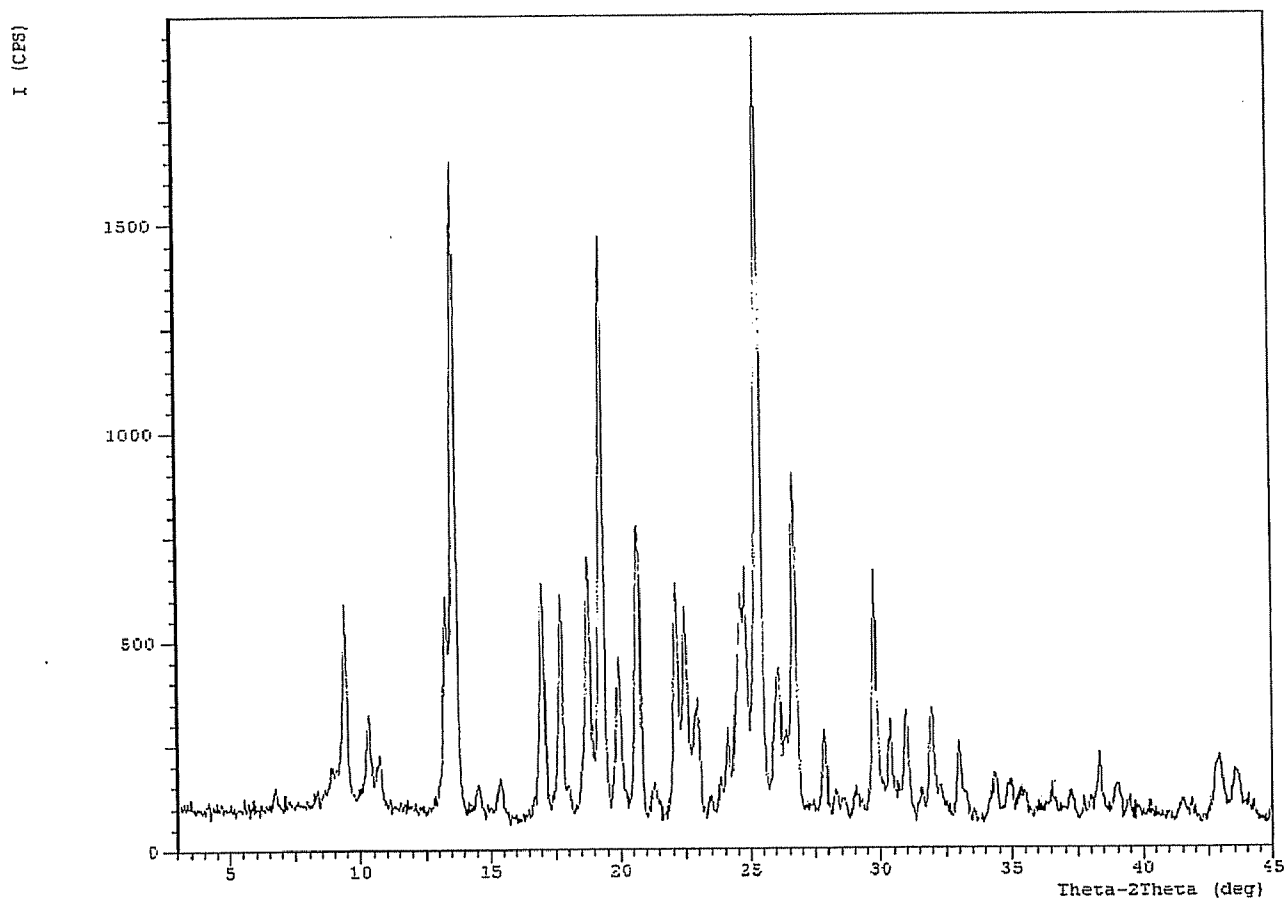
Figure 32

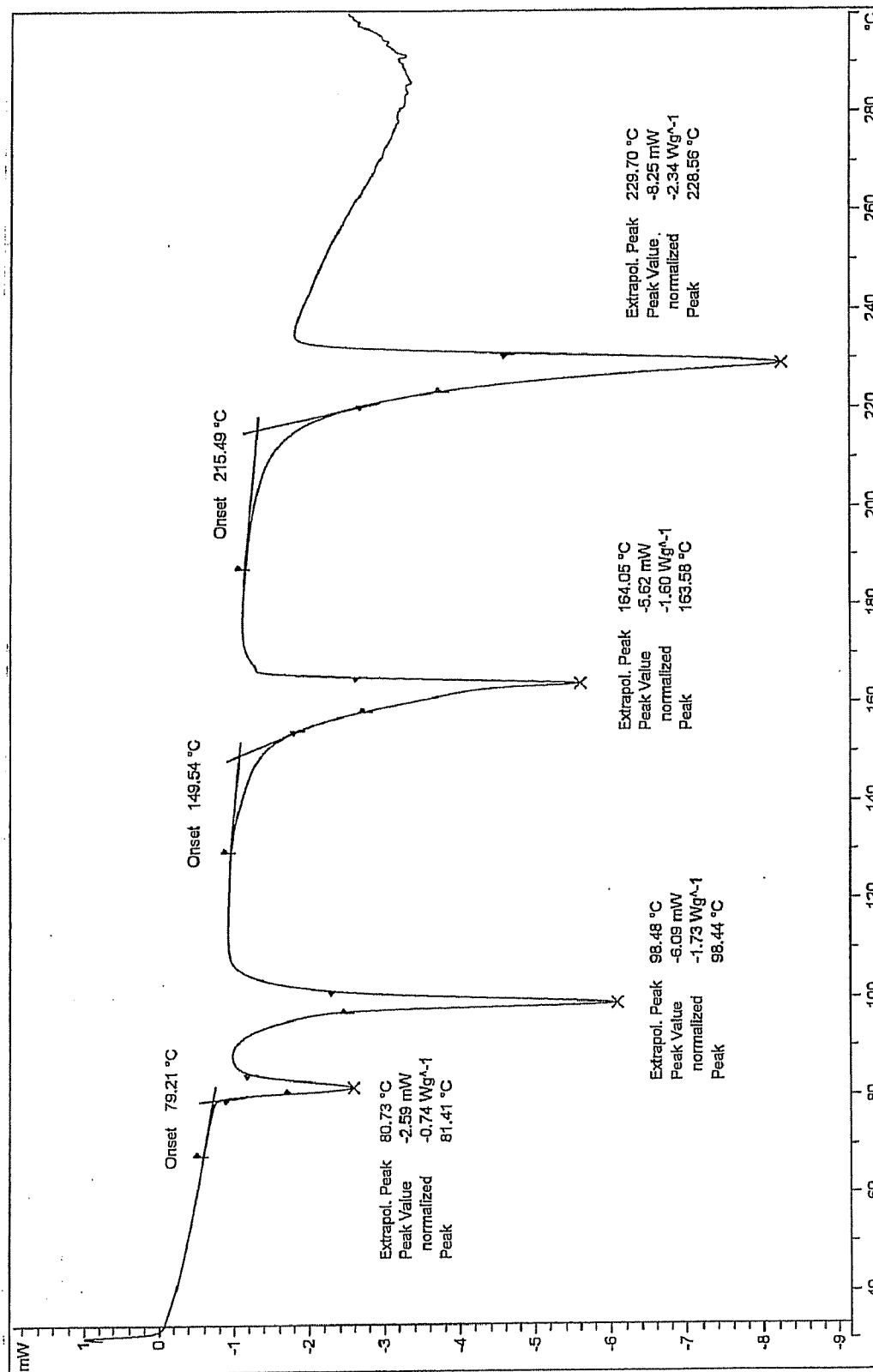


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Figure 33

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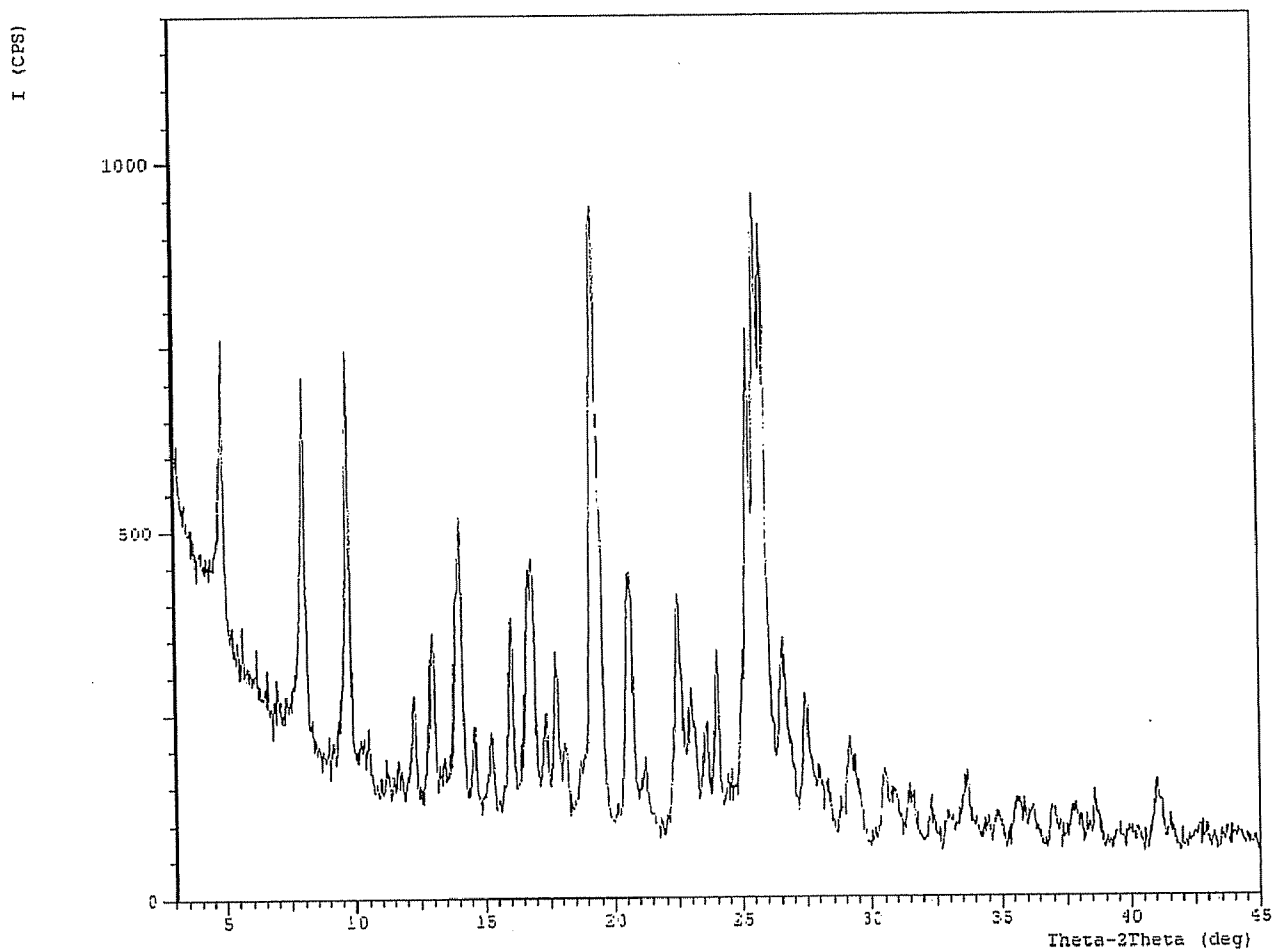
Figure 34



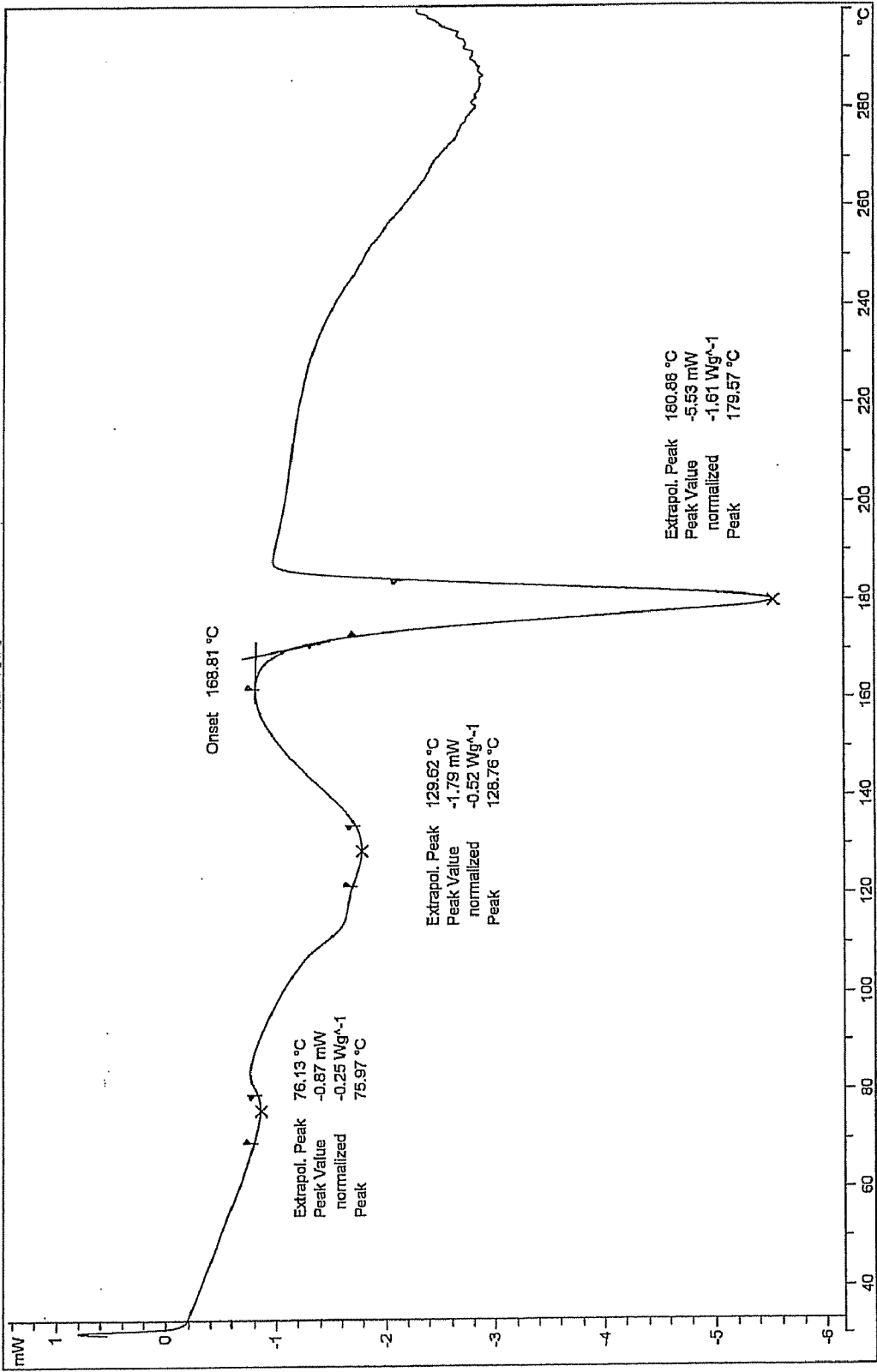
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Figure 35

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Figure 36

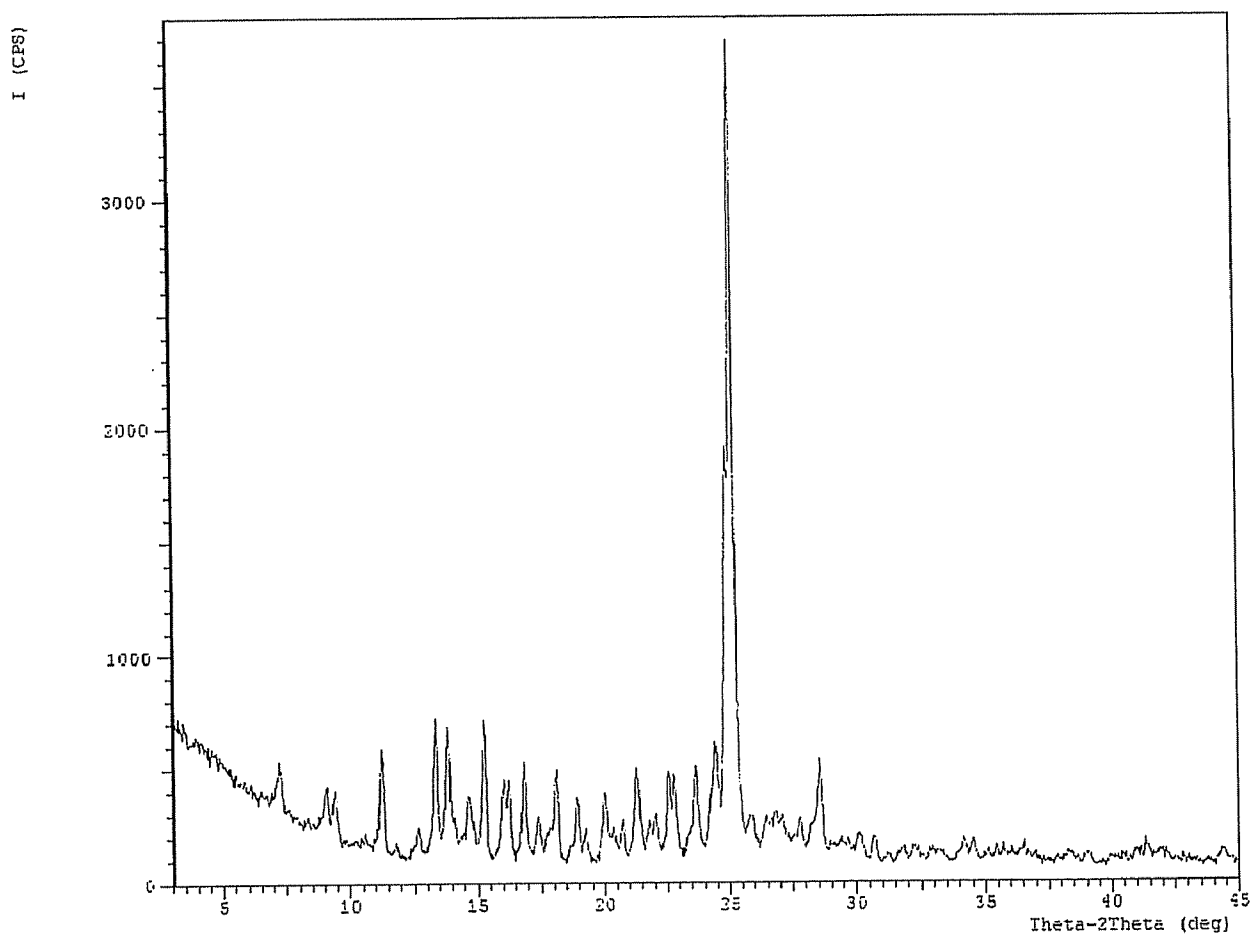


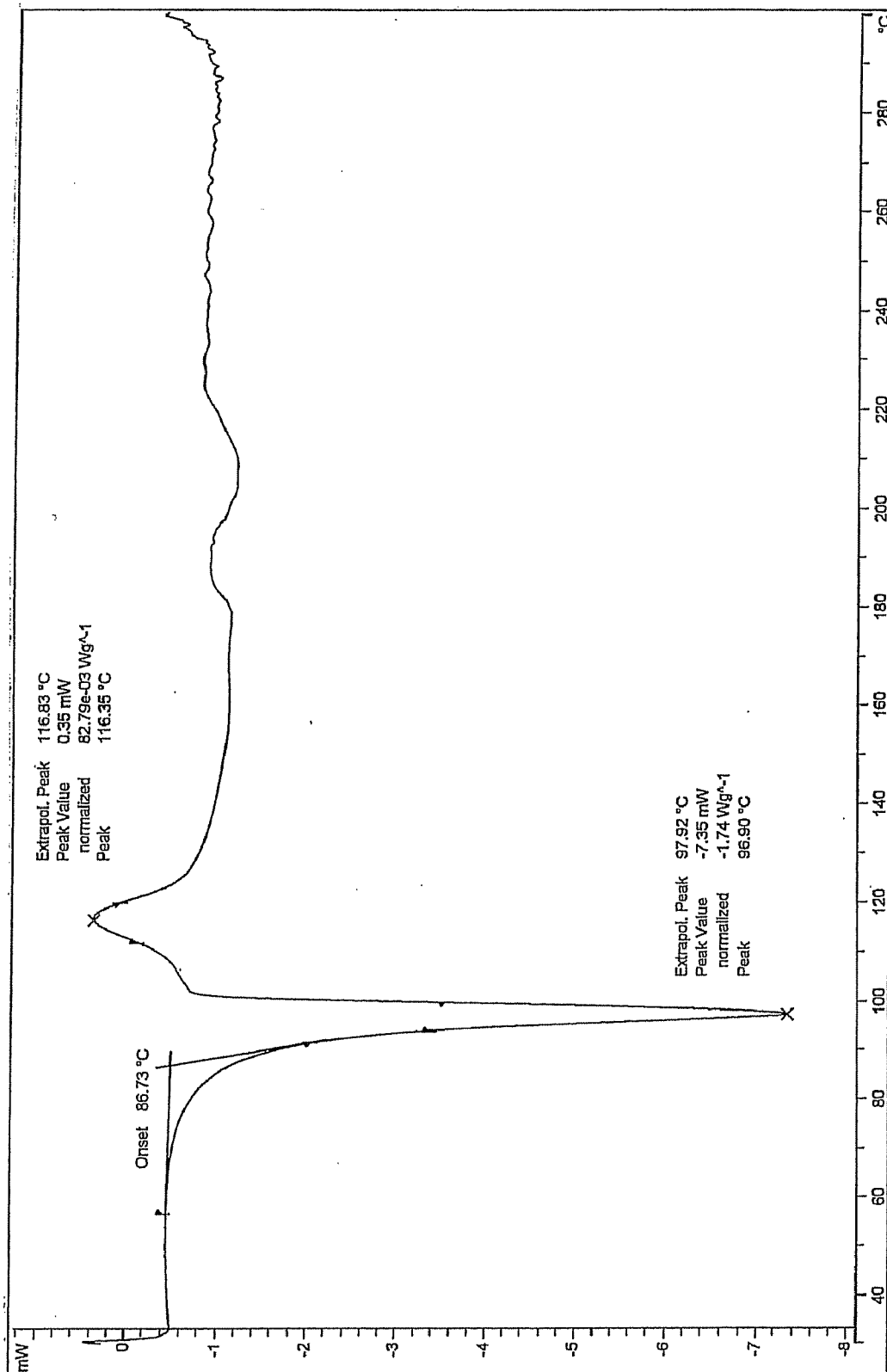
38/47
Figure 37



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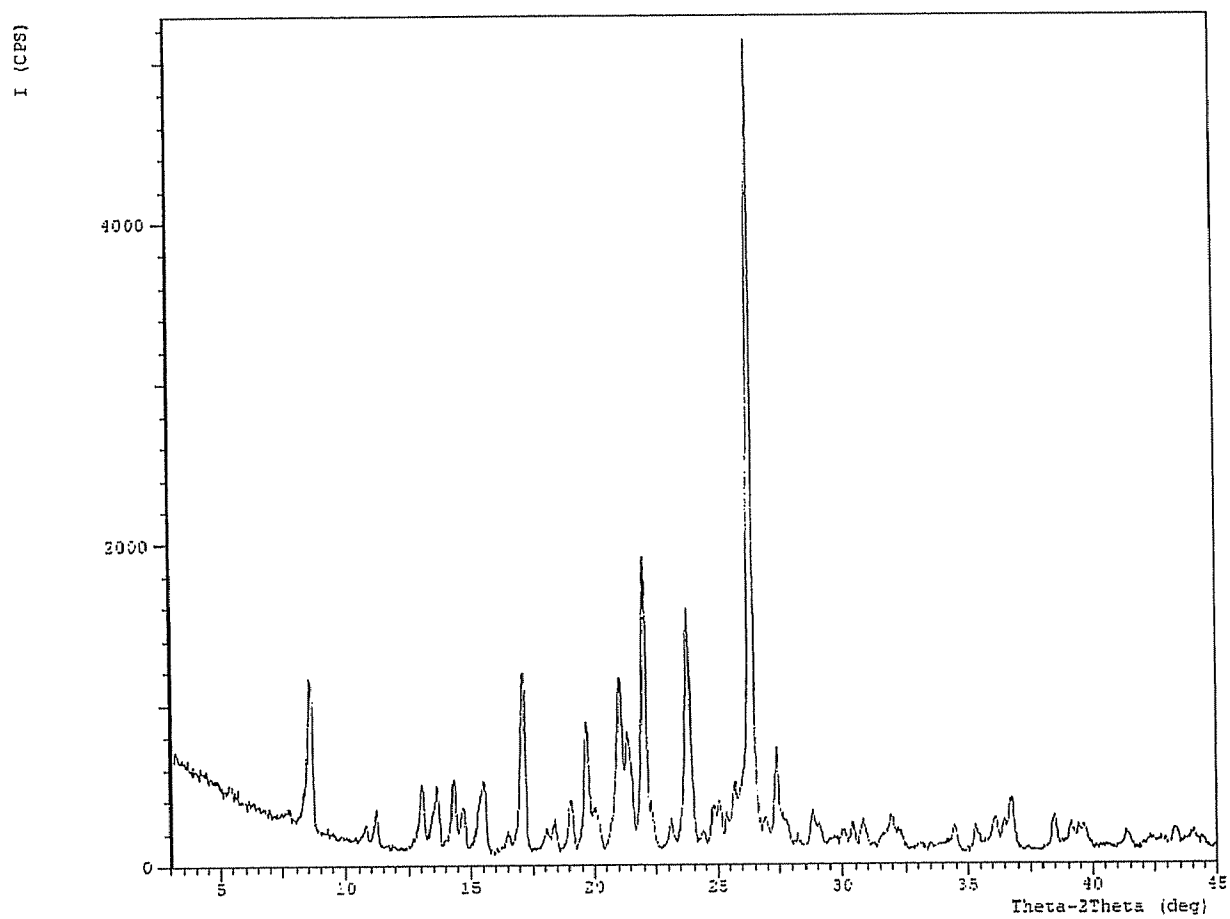
Figure 38

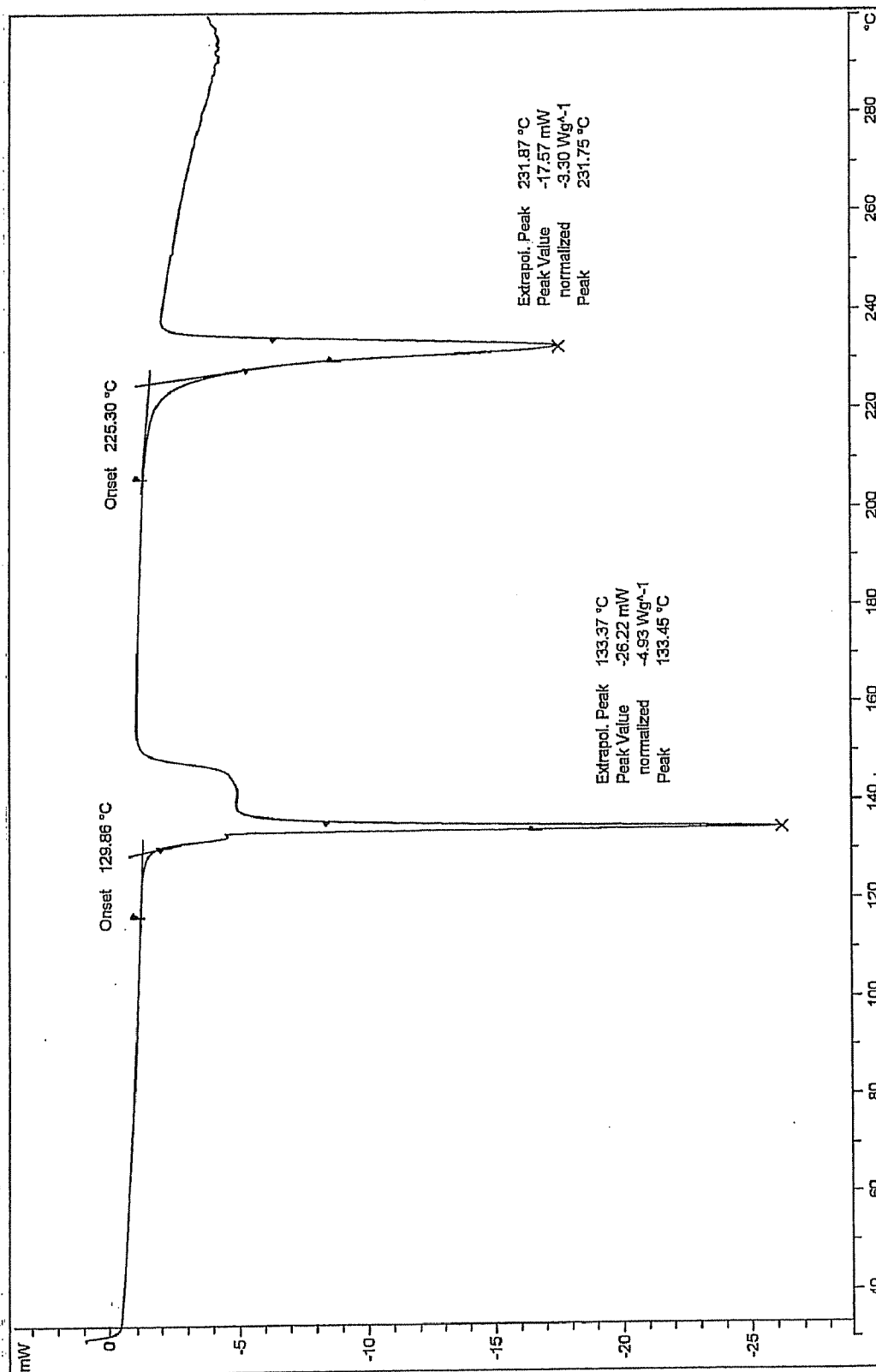


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Figure 39

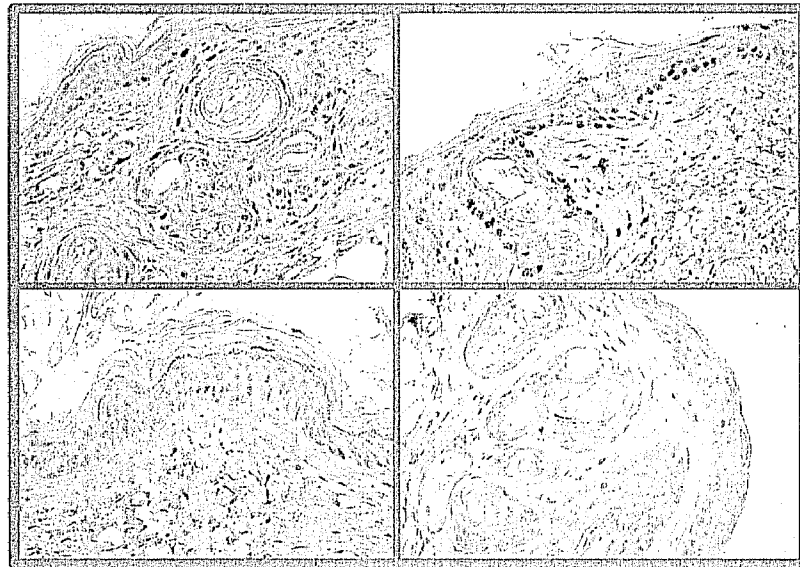
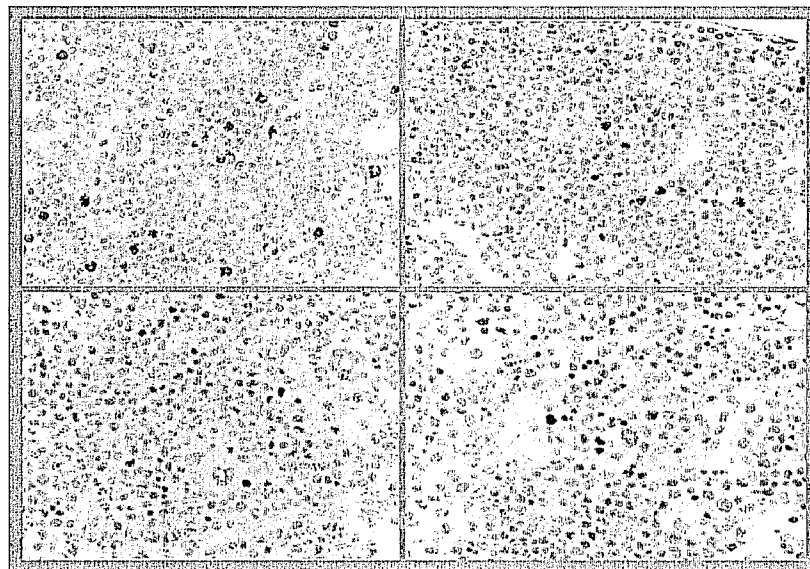
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Figure 40



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Figure 41

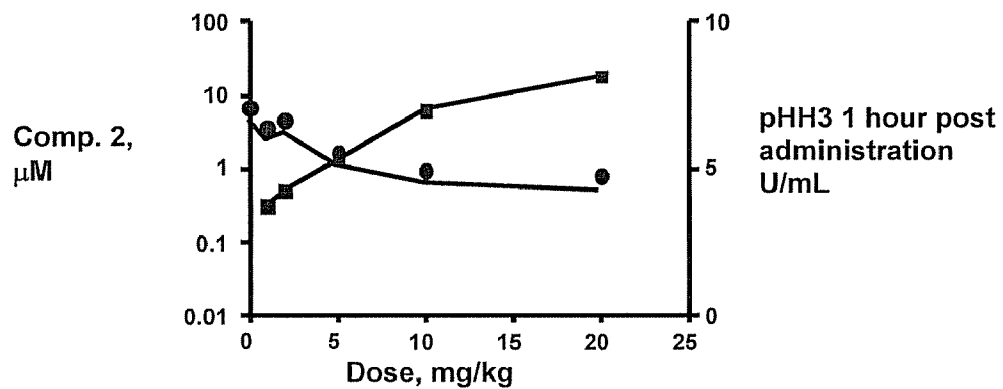
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Figure 42**A)****Day 4****Day 18****B)****Day 11****Day 18**

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Figure 43

A)



B)

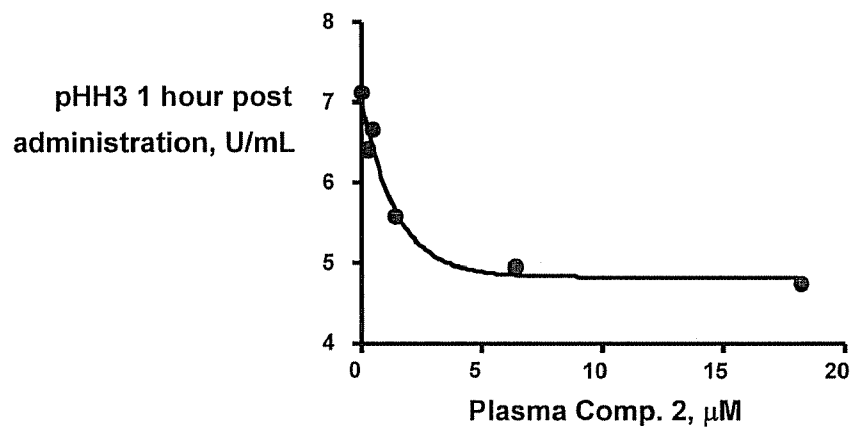
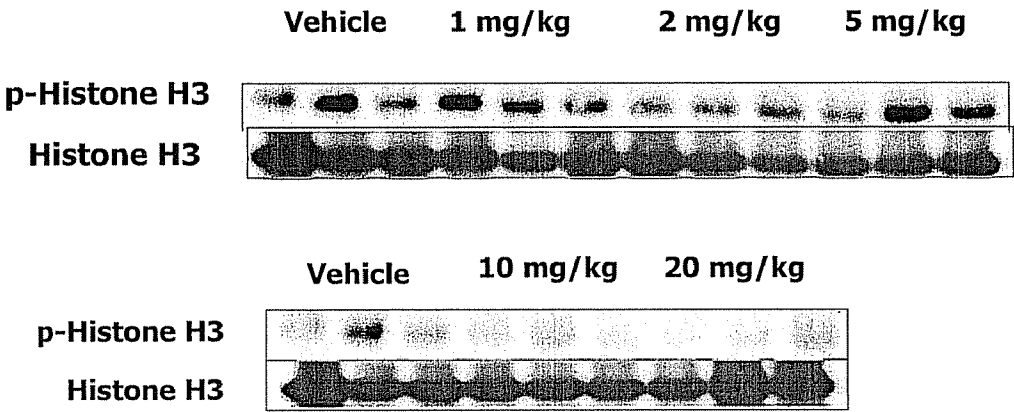


Figure 43, cont'd
C)



D)

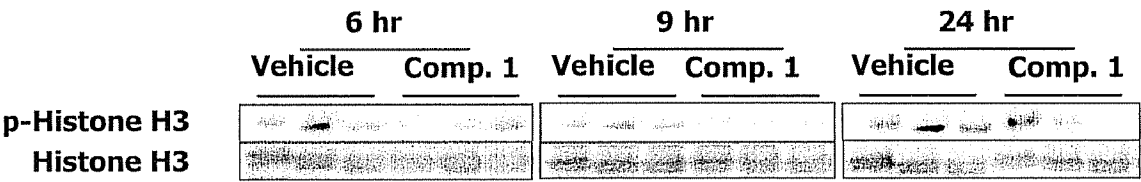
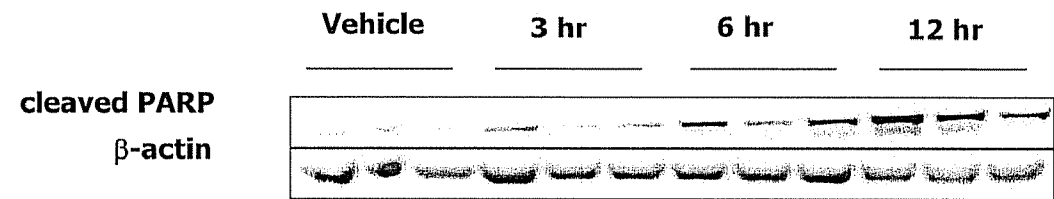
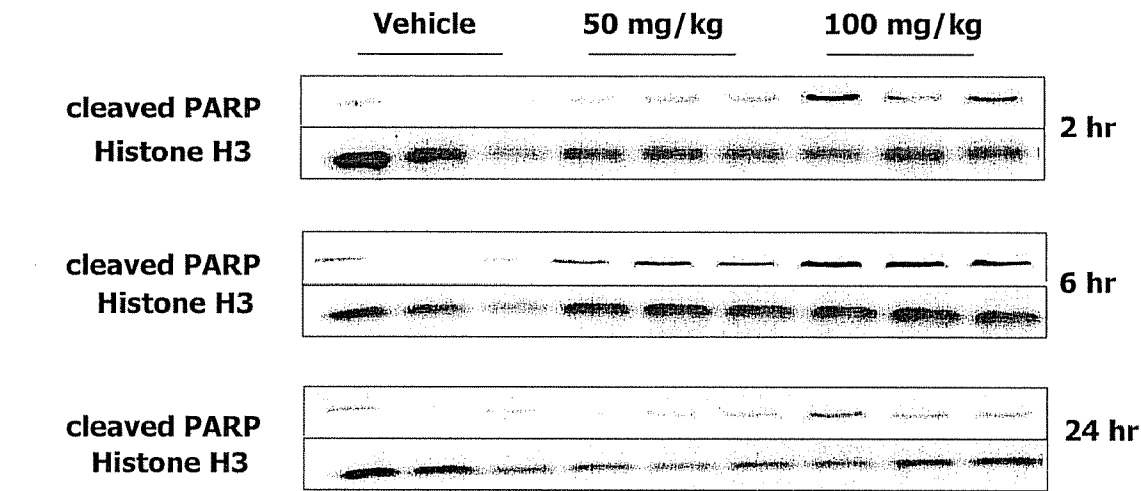


Figure 44

A)

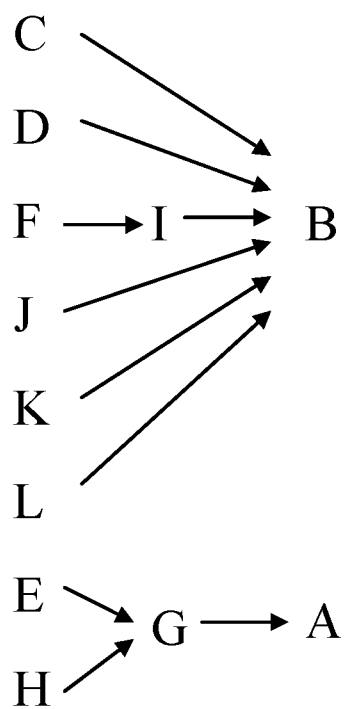


B)



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Figure 45



In slurries in aqueous containing solvents Form A converted to Form B.