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(54) Title: JNK INHIBITORS AS ANTICANCER AGENTS

(57) Abstract: Compounds that inhibit JNK, e.g., JNK2 and/or JNK3, such as fused thiophenes, and methods of making and using the compounds are provided.



JNK INHIBITORS AS ANTICANCER AGENTS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing date of U.S. application No. 62/865,659, filed on June 24, 2019, the disclosure of which is incorporated by reference herein.

10 BACKGROUND

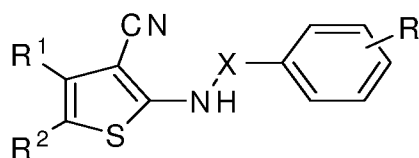
Protein kinases, catalyzing the transfer of the terminal phosphate group of ATP to specific amino acid residues in target proteins, are the largest family of enzymes encoded by the human genome. The phosphorylation of the target protein results in modified activity, degradation, localization or association with
15 other molecules. Among the mitogen-activated protein kinases (MAPKs) is the c-Jun N-terminal kinase (JNK) family that includes at least three proteins (JNK1, JNK2 and JNK3) that are encoded by three separate genes *jnk1* (*Mapk8*), *jnk2* (*Mapk9*) and *jnk3* (*Mapk10*), and are alternatively spliced to create at least many variants. JNK1 and JNK2 are expressed in most tissues, while the expression of
20 JNK3 is largely restricted to brain, heart and testes.

JNKs regulate many physiological processes, including inflammatory responses, morphogenesis, cell proliferation, differentiation, survival and death, and persistent activation of JNKs is involved in cancer development and progression. However, JNK1 and JNK2 may have distinct or even opposing
25 functions in different types of cancer. Some JNK inhibitors have a lack of specificity and cellular toxicity, which is less than desirable for therapeutics.

SUMMARY

The disclosure provides a compound of formula (I):

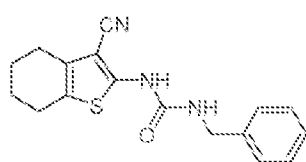
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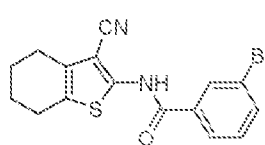
wherein R is hydrogen, halo, alkyl, alkenyl, alkynyl, alkoxy, haloalkyl, or substituted alkyl, alkenyl or alkynyl, or a carbocycle or heterocycle; wherein X comprises an alkyl, carbonyl, or amide; and wherein R¹ and R² independently are alkyl, alkenyl, alkynyl, alkoxy, or substituted alkyl or R¹ and R² together form a C6 or C7 ring; or a pharmaceutically acceptable salt thereof. In one embodiment, R is hydrogen, halo, (C1-C6)alkyl, or substituted (C1-C6)alkyl. In one embodiment, halo is Br, F or I. In one embodiment, R is a carbocycle, e.g., an aryl such as a C5, C6, C7, or C8 aryl. In one embodiment, R is a substituted carbocycle. In one embodiment, R is not benzyl. In one embodiment, R is not hydrogen. In one embodiment, X is (C1-C6)alkyl, CONH or CONH(C1-C6)alkyl. In one embodiment, X is substituted (C1-C6)alkyl, or substituted CONH(C1-C6)alkyl. In one embodiment, X has a chain length of no more than 6 atoms. In one embodiment, R1 and R2 together form a C7 ring. In one embodiment, R1 and R2 together form a C6 ring. In one embodiment, R1 and R2 together form an aryl ring. In one embodiment, the compound is a benzamide. In one embodiment, the compound is a benzylamine. In one embodiment, the compound comprises urea. In one embodiment, the compound is a fused thiophene. In one embodiment, the compound inhibits JNK2 and/or JNK3. In one embodiment, the compound is not N-(3-cyano-4,5,6,7-tetrahydro-1-benzothien-2-yl)1-naphthamide. In one embodiment, the X is not COCONHNCC=C(C₆H₅). Also provided is a composition having the compound. In one embodiment, the composition comprises nanoparticles having the compound. In one embodiment, the nanoparticles have a diameter from about 100 nm to about 250 nm. In one embodiment, the nanoparticles are formed of a synthetic polymer, e.g., lactic acid, glycolic acid, caproic acid, a polyanhydride, or a combination thereof.

N-(3-cyano-4,5,6,7-tetrahydro-1-benzothien-2-yl)1-naphthamide was found to be a potent JNK inhibitor with pIC₅₀ values of 6.5 and 6.7 for JNK2 and JNK3, respectively. As disclosed herein, certain fused thiophenes, e.g., having formula (I) or (II), including those that inhibit one or more distinct JNKs, have anti-cancer activity. In one embodiment, a series of 3-cyano-4,5,6,7-tetrahydro-1-benzothiophene with 2-benzamides, 2-benzylamines or 2-urea moieties were designed as potential anticancer agents through inhibition of JNKs. *In vitro* anticancer screening using a MTS assay against A549 cell line showed that an

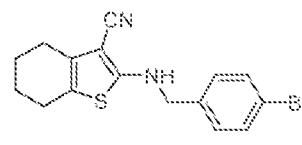
exemplary benzyl urea (1), 3-bromobenzamide (2) and 4-bromobenzylamine (3) were the most active members among the ureas, benzamides and benzylamines, with IC₅₀ values of 1.6, 7.6 and 2.7 μM, respectively. The inhibitory activity of the synthesized compounds against JNK2 and JNK3 was determined. The selective JNK inhibitors disclosed herein are useful as anticancer agents.

IC₅₀ = 1.6 μM

(1)

IC₅₀ = 7.6 μM

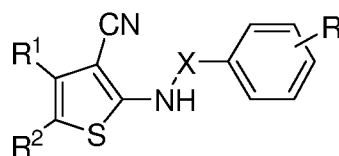
(2)

IC₅₀ = 2.7 μM

(3)

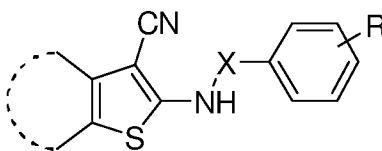
In one embodiment, the disclosure provides for a compound of formula

(I):



- 10 wherein X= CO; CH₂; CONH; or CONHCH₂;
 wherein R= 2-I; 2-Br; 3-Br; 4-Br; 2-F; C₁₀H₇; C₆H₅; 2-BrC₆H₄; 4-CH₃C₆H₄; 4-OCH₃C₆H₄; 4-n-C₄H₉C₆H₄; or C₆H₅CH₂. In one embodiment, X comprises alkyl, carbonyl or an amide. In one embodiment, X is CO, CH₂CONH or CONHCH₂. In one embodiment, R is halo, hydrogen, alkyl, e.g., C₁-C₁₀ alkyl, alkenyl,
 15 substituted alkyl or substituted alkenyl. In one embodiment, R¹ and R² together form a C₆ or C₇ ring. In one embodiment, R¹ and R² together form an aryl ring. In one embodiment, R¹ and R² together form a heterocyclic ring.

In one embodiment, the disclosure provides for a compound of formula (II) which is a fused thiophene:



20

In one embodiment, X comprises a carbonyl or an amide. In one embodiment, X comprises one carbonyl group. In one embodiment, X is CO, CH₂CONH or CONHCH₂. In one embodiment, R is halo, hydrogen, alkyl, e.g., C₁-C₁₀ alkyl, alkenyl, substituted alkyl, substituted alkenyl, or a C₆ carbocycle that is
 25 optionally substituted.

Also provided is a method to prevent, inhibit or treat cancer in a mammal, comprising administering to the mammal a composition having an effective amount of the compound, e.g., a fused thiophene. In one embodiment, the amount of effective to inhibit cancer. In one embodiment, the mammal is a human. In one embodiment, the compound comprises a benzamide. In one embodiment, the compound comprises a benzylamine. In one embodiment, the compound comprises urea. In one embodiment, the composition is intravenously administered. In one embodiment, the composition is locally administered. In one embodiment, the composition is injected. In one embodiment, the composition is orally administered. In one embodiment, the composition comprises nanoparticles or microparticles. In one embodiment, the particles are formed of a synthetic polymer. In one embodiment, the particles comprise lactic acid, glycolic acid, caproic acid, a polyanhydride, or a combination thereof. In one embodiment, the particles comprise lactic acid and glycolic acid, polycaprolactone or poly lactic acid. In one embodiment, the particles comprise PEI. In one embodiment, the particles are formed of a natural polymer. In one embodiment, the particles are formed of collagen, proteoglycan, alginate, chitosan or extracellular matrix. In one embodiment, the particles are formed of polystyrene. In one embodiment, the particles comprise N-(3-cyano-4,5,6,7-tetrahydro-1-benzothien-2-yl)1-naphthamide. In one embodiment, the cancer is melanoma, ovarian cancer or renal cancer. In one embodiment, the cancer is leukemia, lung cancer or colon cancer. In one embodiment, the compound inhibits JNK2 and/or JNK3.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Synthesis of benzamides 2a,b-5a,b, benzylamines 6a,b-11a,b and ureas 12a,b-17a,b.

Figure 2. Exemplary structures and exemplary substituents in Formula (I).

Figure 3. Screening results for compound 4a.

Figure 4. Screening results for compound 6a.

Figure 5. Screening results for compound 6b.

Figure 6. Screening results for compound 9a.

Figure 7. Screening results for compound 13b.

Figure 8. Screening results for compound 17a.

Figure 9. GI₅₀ values.

Figure 10. Dose response curves.

Figure 11. *In vitro* testing results.

Figure 12. Screening results for compound 17a.

5 Figure 13. GI₅₀ values.

Figure 14. Dose response curves.

Figure 15. *In vitro* testing results.

Figure 16. MTS cell viability testing of exemplary benzamides using A549 cell line.

10 Figure 17. MTS cell viability testing of exemplary benzylamines using A549 cell line.

Figure 18. MTS cell viability testing of exemplar ureas using A549 cell line.

15 Figure 19. Cell cycle analysis of cells treated with compound 17a (SA10).

Figure 20. Detection of apoptosis in A549 cells treated with compound 17a at 10 μ M.

Figure 21. *In vivo* antitumor efficacy of compound 17a using Ct-26 murine colon carcinoma.

20 Figure 22. *In vivo* antitumor efficacy of compound 17a using Ct-26 murine colon carcinoma.

Figure 23. 17a nanoparticle (NP) formulation using PLGA RG 502 H⁺.

Figure 24. Cell viability testing using compound 17a.

25 Figure 25. Cell viability testing for compound 11 SA10 (benzyl urea derivative) and its nanoparticle formulation using MTS assay B16F10 murine melanoma cell line (48 hours treatment).

DETAILED DESCRIPTION

Definitions

30 A composition is comprised of "substantially all" of a particular compound, or a particular form a compound (e.g., an isomer) when a composition comprises at least about 90%, 95%, 99%, or 99.9%, of the particular composition on a weight basis. A composition comprises a "mixture" of compounds, or forms of the same compound, when each compound (e.g.,

isomer) represents at least about 10% of the composition on a weight basis. A compound of the invention, can be prepared as an acid salt or as a base salt, as well as in free acid or free base forms. In solution, certain of the compounds of the invention may exist as zwitterions, wherein counter ions are provided by the solvent molecules themselves, or from other ions dissolved or suspended in the solvent.

As used herein, "pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and the like.

The pharmaceutically acceptable salts of the compounds useful in the present invention can be synthesized from the parent compound, which contains a basic or acidic moiety, by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media for example ether, ethyl acetate, ethanol, isopropanol, or acetonitrile. Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, PA, p. 1418 (1985), the disclosure of which is hereby incorporated by reference.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the

tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication commensurate with a reasonable benefit/risk ratio.

The following definitions are used, unless otherwise described: halo or
5 halogen is fluoro, chloro, bromo, or iodo. Alkyl, alkoxy, alkenyl, alkynyl, etc. denote both straight and branched groups; but reference to an individual radical such as "propyl" embraces only the straight chain radical, a branched chain isomer such as "isopropyl" being specifically referred to. Aryl denotes a phenyl
10 radical or an ortho-fused bicyclic carbocyclic radical having about nine to ten ring atoms in which at least one ring is aromatic. Het can be heteroaryl, which encompasses a radical attached via a ring carbon of a monocyclic aromatic ring containing five or six ring atoms consisting of carbon and one to four heteroatoms each selected from the group consisting of non-peroxide oxygen,
15 sulfur, and N(X) wherein X is absent or is H, O, (C₁-C₄)alkyl, phenyl or benzyl, as well as a radical of an ortho-fused bicyclic heterocycle of about eight to ten ring atoms derived therefrom, particularly a benz-derivative or one derived by fusing a propylene, trimethylene, or tetramethylene diradical thereto.

It will be appreciated by those skilled in the art that compounds of the invention having a chiral center may exist in and be isolated in optically active
20 and racemic forms. Some compounds may exhibit polymorphism. It is to be understood that the present invention encompasses any racemic, optically-active, polymorphic, or stereoisomeric form, or mixtures thereof, of a compound of the invention, which possess the useful properties described herein, it being well known in the art how to prepare optically active forms (for example, by
25 resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase) and how to determine agonist activity using the standard tests described herein, or using other similar tests which are well known in the art. It is also understood by those of skill in the art that the
30 compounds described herein include their various tautomers, which can exist in various states of equilibrium with each other.

"Therapeutically effective amount" is intended to include an amount of a composition useful in the present invention or an amount of the combination of compounds, e.g., to treat or prevent the disease or disorder, or to treat the

symptoms of the disease or disorder, in a host. As used herein, "treating" or "treat" includes (i) preventing a pathologic condition from occurring (e.g. prophylaxis); (ii) inhibiting the pathologic condition or arresting its development; (iii) relieving the pathologic condition; and/or diminishing symptoms associated with the pathologic condition.

As used herein, the term "patient" refers to organisms to be treated by the methods of the present invention. Such organisms include, but are not limited to, mammals such as humans. In the context of the invention, the term "subject" generally refers to an individual who will receive or who has received treatment (e.g., administration of a composition of the invention).

"Stable compound" and "stable structure" are meant to indicate a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into an efficacious therapeutic agent. Only stable compounds are contemplated by the present invention.

In cases where compounds are sufficiently basic or acidic to form acid or base salts, use of the compounds as salts may be appropriate. Examples of acceptable salts are organic acid addition salts formed with acids which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, α -ketoglutarate, and α -glycerophosphate. Suitable inorganic salts may also be formed, including hydrochloride, sulfate, nitrate, bicarbonate, and carbonate salts.

Acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can also be made.

Alkyl includes straight or branched C₁₋₁₀ alkyl groups, e.g., methyl, ethyl, propyl, butyl, pentyl, isopropyl, isobutyl, 1-methylpropyl, 3-methylbutyl, hexyl, and the like.

Lower alkyl includes straight or branched C₁₋₆ alkyl groups, e.g., methyl, ethyl, propyl, 1-methylethyl, butyl, 1-methylpropyl, 2-methylpropyl, 1,1-dimethylethyl, pentyl, 1-methylbutyl, 2-methylbutyl, 3-methylbutyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl, 2,2-dimethylpropyl, and the like.

The term "alkylene" refers to a divalent straight or branched hydrocarbon chain (e.g., ethylene: $-\text{CH}_2-\text{CH}_2-$).

C₃₋₇ Cycloalkyl includes groups such as, cyclopropyl, cyclopentyl, cyclohexyl, cycloheptyl, and the like, and alkyl-substituted C₃₋₇ cycloalkyl group, e.g., straight or branched C₁₋₆ alkyl group such as methyl, ethyl, propyl, butyl or pentyl, and C₅₋₇ cycloalkyl group such as, cyclopentyl or cyclohexyl, and the like.

Lower alkoxy includes C₁₋₆ alkoxy groups, such as methoxy, ethoxy or propoxy, and the like.

Lower alkanoyl includes C₁₋₆ alkanoyl groups, such as formyl, acetyl, propanoyl, butanoyl, pentanoyl or hexanoyl, and the like.

C₇₋₁₁ aroyl, includes groups such as benzoyl or naphthoyl;

Lower alkoxycarbonyl includes C₂₋₇ alkoxycarbonyl groups, such as methoxycarbonyl, ethoxycarbonyl or propoxycarbonyl, and the like.

Lower alkylamino group means amino group substituted by C₁₋₆ alkyl group, such as, methylamino, ethylamino, propylamino, butylamino, and the like.

Di(lower alkyl)amino group means amino group substituted by the same or different and C₁₋₆ alkyl group (e.g., dimethylamino, diethylamino, ethylmethylamino).

Lower alkylcarbamoyl group means carbamoyl group substituted by C₁₋₆ alkyl group (e.g., methylcarbamoyl, ethylcarbamoyl, propylcarbamoyl, butylcarbamoyl).

Di(lower alkyl)carbamoyl group means carbamoyl group substituted by the same or different and C₁₋₆ alkyl group (e.g., dimethylcarbamoyl, diethylcarbamoyl, ethylmethylcarbamoyl).

Halogen atom means halogen atom such as fluorine atom, chlorine atom, bromine atom or iodine atom.

Aryl refers to a C₆₋₁₀ monocyclic or fused cyclic aryl group, such as phenyl, indenyl, or naphthyl, and the like.

Heterocyclic or heterocycle refers to monocyclic saturated heterocyclic groups, or unsaturated monocyclic or fused heterocyclic group containing at least one heteroatom, e.g., 0-3 nitrogen atoms ($-\text{NR}^{\text{d}}$ - where R^d is H, alkyl, or Y² as defined herein), 0-1 oxygen atom ($-\text{O}-$), and 0-1 sulfur atom ($-\text{S}-$). Non-

limiting examples of saturated monocyclic heterocyclic group includes 5 or 6 membered saturated heterocyclic group, such as tetrahydrofuranyl, pyrrolidinyl, morpholinyl, piperidyl, piperazinyl or pyrazolidinyl. Non-limiting examples of unsaturated monocyclic heterocyclic group includes 5 or 6 membered

5 unsaturated heterocyclic group, such as furyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, thienyl, pyridyl or pyrimidinyl. Non-limiting examples of unsaturated fused heterocyclic groups includes unsaturated bicyclic heterocyclic group, such as indolyl, isoindolyl, quinolyl, benzothiazolyl, chromanyl, benzofuranyl, and the like. A Het group can be a saturated heterocyclic group or an unsaturated

10 heterocyclic group, such as a heteroaryl group.

R^2 and R^1 in formula (I) taken together can form a cyclic or heterocyclic ring, or an aryl or heteroaryl ring. Non-limiting examples of heterocyclic rings include 5 or 6 membered saturated heterocyclic rings, such as 1-pyrrolidinyl, 4-morpholinyl, 1-piperidyl, 1-piperazinyl or 1-pyrazolidinyl, 5 or 6 membered

15 unsaturated heterocyclic rings such as 1-imidazolyl, and the like.

The alkyl, alkenyl, or cyclic, e.g., aryl, or heterocyclic, groups of R, or R^1 and/or R^2 , can be optionally substituted with one or more substituents, wherein the substituents are the same or different, and include lower alkyl; cycloalkyl, hydroxyl; hydroxy C_{1-6} alkylene, such as hydroxymethyl, 2-hydroxyethyl or 3-hydroxypropyl; lower alkoxy; C_{1-6} alkoxy C_{1-6} alkyl, such as 2-methoxyethyl, 2-ethoxyethyl or 3-methoxypropyl; amino; alkylamino; dialkyl amino; cyano;

20 nitro; acyl; carboxyl; lower alkoxycarbonyl; halogen; mercapto; C_{1-6} alkylthio, such as, methylthio, ethylthio, propylthio or butylthio; substituted C_{1-6} alkylthio, such as methoxyethylthio, methylthioethylthio, hydroxyethylthio or

25 chloroethylthio; aryl; substituted C_{6-10} monocyclic or fused-cyclic aryl, such as 4-hydroxyphenyl, 4-methoxyphenyl, 4-fluorophenyl, 4-chlorophenyl or 3,4-dichlorophenyl; 5-6 membered unsaturated heterocyclic, such as furyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, thienyl, pyridyl or pyrimidinyl; and bicyclic unsaturated heterocyclic, such as indolyl, isoindolyl, quinolyl, benzothiazolyl,

30 chromanyl, benzofuranyl or phthalimino. In certain embodiments, one or more of the above groups can be expressly excluded as a substituent of various other groups of the formulas.

The alkyl, alkenyl, aryl, non-aryl cyclic, or heterocyclic groups of R can be optionally substituted with one or more substituents, wherein the substituents

are the same or different, and include hydroxyl; C₁₋₆ alkoxy, such as methoxy, ethoxy or propoxy; carboxyl; C₂₋₇ alkoxy carbonyl, such as methoxycarbonyl, ethoxycarbonyl or propoxycarbonyl) or halogen.

The alkyl, alkenyl, aryl, non-aryl cyclic, or heterocyclic groups of R can
 5 be optionally substituted with one or more substituents, wherein the substituents are the same or different, and include C₃₋₆ cycloalkyl; hydroxyl; C₁₋₆ alkoxy; amino; cyano; aryl; substituted aryl, such as 4-hydroxyphenyl, 4-methoxyphenyl, 4-chlorophenyl or 3,4-dichlorophenyl; nitro or halogen.

The ring formed together with R² and R¹ can be optionally substituted
 10 with one or more substituents, wherein the substituents are the same or different, and include C₁₋₆ alkyl; hydroxy C₁₋₆ alkylene; C₁₋₆ alkoxy C₁₋₆ alkylene; hydroxyl; C₁₋₆ alkoxy; or cyano.

A specific value for each of X, R, R¹ and/or R² independently is 2-hydroxyethyl, 3-hydroxypropyl, 4-hydroxybutyl, 2-aminoethyl, 3-aminopropyl,
 15 4-aminobutyl, methoxymethyl, 2-methoxyethyl, 3-methoxypropyl, ethoxymethyl, 2-ethoxyethyl, methylthiomethyl, 2-methylthioethyl, 3-methylthiopropyl, 2-fluoroethyl, 3-fluoropropyl, 2,2,2-trifluoroethyl, cyanomethyl, 2-cyanoethyl, 3-cyanopropyl, methoxycarbonylmethyl, 2-methoxycarbonylethyl, 3-methoxycarbonylpropyl, benzyl, phenethyl, 4-
 20 pyridylmethyl, cyclohexylmethyl, 2-thienylmethyl, 4-methoxyphenylmethyl, 4-hydroxyphenylmethyl, 4-fluorophenylmethyl, or 4-chlorophenylmethyl.

A specific value for each of X, R, R¹ and/or R² is independently hydrogen, CH₃-, CH₃-CH₂-, CH₃CH₂CH₂-, hydroxyC₁₋₄alkylene, or C₁₋₄alkoxyC₁₋₄alkylene.

25 Another specific value for value for each of X, R, R¹ and/or R² independently is hydrogen, CH₃-, CH₃-CH₂-, CH₃-O-CH₂CH₂- or CH₃-CH₂-O-CH₂CH₂-.

A specific value for value for R, R¹ or R² is hydrogen, halogen, or C₁₋₄alkyl.

30 Another specific value for R is hydrogen, chloro, bromo, fluoro, iodo, C-(CO)-NH, (CO)-NH-CH₃, CH₃-, or CH₃-CH₂-.

A specific value for R is halo.

A specific value for R is hydrogen.

A specific value for R is C₁₋₆alkyl, or substituted C₁₋₆alkyl.

A specific value for R is C₁₋₆alkoxy or substituted C₁₋₆alkoxy.

A specific value for R is Br, F, or I.

A specific value for R is a carbocycle, e.g., C₅₋₇ carbocycle.

5 A specific value for R is a substituted carbocycle, e.g., C₅₋₇ carbocycle.

A specific value for R is a heterocycle, e.g., having a ring with 5-7 atoms.

A specific value for R is a substituted heterocycle.

A specific value for R is an aryl, e.g., C₅₋₇ aryl.

10 A specific value for R is a substituted aryl.

A specific value for value for R¹ and R² is a C₆ cyclic group, e.g., a cyclohexane.

A specific value for value for R¹ and R² is a C₇ cyclic group, e.g., a cycloheptane.

15 Specific substituents for substitution on the alkyl, aryl or other cyclic groups are hydroxy, C₁₋₆alkyl, hydroxyC₁₋₆alkylene, C₁₋₆alkoxy, C₁₋₆alkoxyC₁₋₆alkylene, C₃₋₆cycloalkyl, amino, cyano, halogen, or aryl.

A specific value for X is an amide, amine or imide group.

20 Another specific value for X is hydrogen, C₁₋₆alkyl, C-(CO)-NH, (CO)-NH-CH₃, CH₃-, or CH₃-CH₂-.

A specific value for X is a chain having up to about 12 atoms; wherein the atoms are selected from the group consisting of carbon, sulfur, nitrogen, and non-peroxide oxygen.

A specific value for X is a carboxyl or carbonyl group.

25 A specific value for X is a bond or a chain; wherein the atoms are selected from the group consisting of carbon, nitrogen, sulfur, non-peroxide oxygen, and phosphorous.

Another specific value for X is a bond or a chain having from about 3 to about 6 atoms.

30 Another specific value for X is a bond or a chain having from about 1 to about 5 atoms.

Exemplary Delivery Vehicles

Delivery vehicles for fused thiophenes or JNK inhibitor, e.g., compounds of formula (I) or (II), include, for example, naturally occurring or synthetic

polymers that form microparticles, nanoparticles, or other macromolecular complexes capable of mediating delivery of the compounds. Vehicles can also comprise other components or functionalities that further modulate, or that otherwise provide beneficial properties.

- 5 In one embodiment, the delivery vehicle is a naturally occurring polymer, e.g., formed of materials including but not limited to albumin, collagen, fibrin, alginate, extracellular matrix (ECM), e.g., xenogeneic ECM, hyaluronan (hyaluronic acid), chitosan, gelatin, keratin, potato starch hydrolyzed for use in electrophoresis, or agar-agar (agarose). In one embodiment, the delivery vehicle
- 10 comprises a hydrogel. In one embodiment, the composition comprises a naturally occurring polymer. For example, the compounds may be in nanoparticles or microparticles. Table 1 provides exemplary materials for delivery vehicles that are formed of naturally occurring polymers and materials for particles.

15 Table 1

Particle class	Materials
Natural materials or derivatives	Chitosan
	Dextran
	Gelatine
	Albumin
	Alginates
	Liposomes
	Starch
Polymer carriers	Poly(lactic acid)
	Poly(cyano)acrylates
	Polyethyleneimine
	Block copolymers
	Polycaprolactone

An exemplary polycaprolactone is methoxy poly(ethylene glycol)/poly(epsilon caprolactone). An exemplary poly lactic acid is poly(D,L-lactic-co-glycolic)acid (PLGA).

- 20 Some examples of materials for particle formation include but are not limited to agar acrylic polymers, polyacrylic acid, poly acryl methacrylate,

gelatin, poly(lactic acid), pectin(poly glycolic acid), cellulose derivatives, cellulose acetate phthalate, nitrate, ethyl cellulose, hydroxyl ethyl cellulose, hydroxypropylcellulose, hydroxyl propyl methyl cellulose, hydroxypropylmethylcellulose phthalate, methyl cellulose, sodium

5 carboxymethylcellulose, poly(ortho esters), polyurethanes, poly(ethylene glycol), poly(ethylene vinyl acetate), polydimethylsiloxane, poly(vinyl acetate phthalate), polyvinyl alcohol, polyvinyl pyrrolidone, and shellac. Soluble starch and its derivatives for particle preparation include amylopectin, amylopectin and carboxy methyl starch.

10 In one embodiment, the polymers in the nanoparticles or microparticles are biodegradable. Examples of biodegradable polymers useful in particles preparation include synthetic polymers, e.g., polyesters, poly(ortho esters), polyanhydrides, or polyphosphazenes; natural polymers including proteins (e.g., collagen, gelatin, and albumin), or polysaccharides (e.g., starch, dextran,

15 hyaluronic acid, and chitosan). For instance, a biocompatible polymer includes poly (lactic) acid (PLA), poly (glycolic acid) (PLGA). Natural polymers that may be employed in particles (or as the delivery vehicle) include but are not limited to albumin, chitin, starch, collagen, chitosan, dextran, gelatin, hyaluronic acid, dextran, fibrinogen, alginic acid, casein, fibrin, and polyanhydrides.

20 In one embodiment, the delivery vehicle is a hydrogel. Hydrogels can be classified as those with chemically crosslinked networks having permanent junctions or those with physical networks having transient junctions arising from polymer chain entanglements or physical interactions, e.g., ionic interactions, hydrogen bonds or hydrophobic interactions. Natural materials useful in

25 hydrogels include natural polymers, which are biocompatible, biodegradable, support cellular activities, and include proteins like fibrin, collagen and gelatin, and polysaccharides like starch, alginate and agarose.

In one embodiment, the delivery vehicle comprises inorganic nanoparticles, e.g., calcium phosphate or silica particles; polymers including but

30 not limited to poly(lactic-co-glycolic acid) (PLGA), polylactic acid (PLA), linear and/or branched PEI with differing molecular weights (e.g., 2, 22 and 25 kDa), dendrimers such as polyamidoamine (PAMAM) and polymethoacrylates; lipids including but not limited to cationic liposomes, cationic emulsions, DOTAP, DOTMA, DMRIE, DOSPA, distearoylphosphatidylcholine (DSPC), DOPE, or

DC-cholesterol; peptide based vectors including but not limited to Poly-L-lysine or protamine; or poly(β -amino ester), chitosan, PEI-polyethylene glycol, PEI-mannose-dextrose, DOTAP-cholesterol or RNAiMAX.

In one embodiment, the delivery vehicle is a glycopolymer-based
 5 delivery vehicle, poly(glycoamidoamine)s (PGAAs), that have the ability to complex with various polynucleotide types and form nanoparticles. These materials are created by polymerizing the methylester or lactone derivatives of various carbohydrates (D-glucarate (D), *meso*-galactarate (G), D-mannarate (M), and L-tartarate (T)) with a series of oligoethyleneamine monomers (containing
 10 between 1-4 ethylenamines. A subset composed of these carbohydrates and four ethyleneamines in the polymer repeat units yielded exceptional delivery efficiency.

In one embodiment, the delivery vehicle comprises polyethyleneimine (PEI), Polyamidoamine (PAMAM), PEI-PEG, PEI-PEG-mannose, dextran-PEI,
 15 OVA conjugate, PLGA microparticles, or PLGA microparticles coated with PAMAM.

In one embodiment, the delivery vehicle comprises a cationic lipid, e.g., *N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium (DOTMA), 2,3-dioleoyloxy-*N*-[2-spermine carboxamide] ethyl-*N,N*-dimethyl-1-
 20 propanammonium trifluoroacetate (DOSPA, Lipofectamine); 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP); *N*-[1-(2,3-dimyristloxy) propyl]; *N,N*-dimethyl-*N*-(2-hydroxyethyl) ammonium bromide (DMRIE), 3- β -[*N*-(*N,N'*-dimethylaminoethane) carbamoyl] cholesterol (DC-Chol); dioctadecyl amidoglyceryl spermine (DOGS, Transfectam); or
 25 imethyldioctadecylammonium bromide (DDAB). The positively charged hydrophilic head group of cationic lipids usually consists of monoamine such as tertiary and quaternary amines, polyamine, amidinium, or guanidinium group. A series of pyridinium lipids have been developed. In addition to pyridinium cationic lipids, other types of heterocyclic head group include imidazole,
 30 piperazine and amino acid. The main function of cationic head groups is to condense negatively charged nucleic acids by means of electrostatic interaction to slightly positively charged nanoparticles, leading to enhanced cellular uptake and endosomal escape.

Lipids having two linear fatty acid chains, such as DOTMA, DOTAP and SAINT-2, or DODAC, may be employed as a delivery vehicle, as well as tetraalkyl lipid chain surfactant, the dimer of *N,N*-dioleoyl-*N,N*-dimethylammonium chloride (DODAC). All the *trans*-orientated lipids
5 regardless of their hydrophobic chain lengths ($C_{16:1}$, $C_{18:1}$ and $C_{20:1}$) appear to enhance the transfection efficiency compared with their *cis*-orientated counterparts.

The structures of cationic polymers useful as a delivery vehicle include but are not limited to linear polymers such as chitosan and linear
10 poly(ethyleneimine), branched polymers such as branch poly(ethyleneimine) (PEI), circle-like polymers such as cyclodextrin, network (crosslinked) type polymers such as crosslinked poly(amino acid) (PAA), and dendrimers. Dendrimers consist of a central core molecule, from which several highly branched arms 'grow' to form a tree-like structure with a manner of symmetry or
15 asymmetry. Examples of dendrimers include polyamidoamine (PAMAM) and polypropylenimine (PPI) dendrimers.

DOPE and cholesterol are commonly used neutral co-lipids for preparing cationic liposomes. Branched PEI-cholesterol water-soluble lipopolymer conjugates self-assemble into cationic micelles. Pluronic (poloxamer), a non-
20 ionic polymer and SP1017, which is the combination of Pluronics L61 and F127, may also be used.

In one embodiment, PLGA particles are employed to increase the encapsulation frequency although complex formation with PLL may also increase the encapsulation efficiency. Other cationic materials, for example,
25 PEI, DOTMA, DC-Chol, or CTAB, may be used to make nanospheres.

In one embodiment, the particles comprise at least one polymeric material. In one embodiment, the polymeric material is biodegradable. In one embodiment, polymeric materials include: silk, elastin, chitin, chitosan, poly(α -hydroxy acids), poly(anhydrides), and poly(orthoesters). In one embodiment,
30 the biodegradable microparticle may comprise polyethylene glycol, poly(lactic acid), poly(glycolic acid), copolymers of lactic and glycolic acid, copolymers of lactic and glycolic acid with polyethylene glycol, poly(ϵ -caprolactone), poly(3-hydroxybutyrate), poly(*p*-dioxanone), polypropylene fumarate, poly(orthoesters), polyol/diketene acetals addition polymers, poly(sebacic

anhydride) (PSA), poly(carboxybiscarboxyphenoxyphenoxy hexone (PCPP) poly[bis (p-carboxyphenoxy) methane] (PCPM), copolymers of SA, CPP and CPM, poly(amino acids), poly(pseudo amino acids), polyphosphazenes, derivatives of poly[(dichloro)phosphazenes] and poly[(organo) phosphazenes],
 5 poly-hydroxybutyric acid, or S-caproic acid, polylactide-co-glycolide, polylactic acid, and polyethylene glycol. Polyesters may be employed. In one embodiment, PLGA is employed, e.g., PLGA 75:25, PLGA 50:50 and PLGA 85:15.

Exemplary Particle Sizes (Diameters)

10 In one embodiment, the particle is a nanoparticle. In one embodiment, the particle may be about 50 nm to less than about 1000 nm, about 100 nm to about 900 nm, about 400 nm to about 800 nm, or about 500 nm to about 700 nm, in diameter. In various aspects, the nanoparticles which range in size from about
 15 diameter, about 1 nm to about 250 nm in mean diameter, about 1 nm to about 240 nm in mean diameter, about 1 nm to about 230 nm in mean diameter, about 1 nm to about 220 nm in mean diameter, about 1 nm to about 210 nm in mean diameter, about 1 nm to about 200 nm in mean diameter, about 1 nm to about 190 nm in mean diameter, about 1 nm to about 180 nm in mean diameter, about 1 nm to about 170 nm in mean diameter, about 1 nm to about 160 nm in mean diameter, about
 20 1 nm to about 150 nm in mean diameter, about 1 nm to about 140 nm in mean diameter, about 1 nm to about 130 nm in mean diameter, about 1 nm to about 120 nm in mean diameter, about 1 nm to about 110 nm in mean diameter, about 1 nm to about 100 nm in mean diameter, about 1 nm to about 90 nm in mean diameter, about 1 nm to about 80 nm in mean diameter, about 1 nm to about 70
 25 nm in mean diameter, about 1 nm to about 60 nm in mean diameter, about 1 nm to about 50 nm in mean diameter, about 1 nm to about 40 nm in mean diameter, about 1 nm to about 30 nm in mean diameter, or about 1 nm to about 20 nm in mean diameter, about 1 nm to about 10 nm in mean diameter. In other aspects, the size of the nanoparticles is from about 5 nm to about 150 nm (mean
 30 diameter), from about 5 to about 50 nm, from about 10 to about 30 nm. The size of the nanoparticles may be from about 5 nm to about 150 nm (mean diameter), from about 30 to about 100 nm, from about 40 to about 80 nm. The size of the nanoparticles may be from about 25 nm to about 200 nm (mean diameter), from about 30 to about 150 nm, from about 50 to about 100 nm, or from about 75 nm

to about 125 nm. The size of the nanoparticles may be from about 125 nm to about 250 nm (mean diameter), from about 150 to about 220 nm, from about 175 to about 250 nm, or from about 175 nm to about 225 nm.

5 Microparticles, in contrast to nanoparticles, for use in the composition of the invention are 1.0 μm up to about 100 μm , and in one embodiment up to about 3.0 μm .

Compositions, Formulations and Routes of Administration

 The compositions of this invention are administered in a therapeutically effective amount to a subject in need of treatment. Administration of the
10 compositions of the invention can be via any of suitable route of administration, particularly parenterally, for example, intravenously, intra-arterially, intraperitoneally, intrathecally, intraventricularly, intraurethrally, intrasternally, intracranially, intratumorally, intramuscularly, or subcutaneously. Such administration may be as a single bolus injection, multiple injections, or as a
15 short- or long-duration infusion. Implantable devices (e.g., implantable infusion pumps) may also be employed for the periodic parenteral delivery over time of equivalent or varying dosages of the particular formulation. For such parenteral administration, the compositions may be formulated as a sterile solution in water or another suitable solvent or mixture of solvents. The solution may contain
20 other substances such as salts, sugars (particularly glucose or mannitol), to make the solution isotonic with blood, buffering agents such as acetic, citric, and/or phosphoric acids and their sodium salts, and preservatives.

 The compositions of the invention can be formulated as pharmaceutical compositions and administered to a mammalian host, such as a human patient in
25 a variety of forms adapted to the chosen route of administration, i.e., orally or parenterally, by intravenous, intramuscular, topical or subcutaneous routes.

 Thus, the present compositions may be systemically administered, e.g., orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or
30 soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the active compound may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions

and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of active compound in such therapeutically useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and devices.

The active compound may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the active compound or its salts can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form should be sterile, fluid and

stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it may include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

For topical administration, the present compositions may be applied in pure form, i.e., when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Useful liquid carriers include water, alcohols or glycols or water-alcohol/glycol blends, in which the present compositions can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

5 In addition, in one embodiment, the invention provides various dosage formulations for inhalation delivery. For example, formulations may be designed for aerosol use in devices such as metered-dose inhalers, dry powder inhalers and nebulizers.

Examples of useful dermatological compositions which can be used to
10 deliver the compositions of the invention to the skin are known to the art; for example, see Jacquet et al. (U.S. Pat. No. 4,608,392), Geria (U.S. Pat. No. 4,992,478), Smith et al. (U.S. Pat. No. 4,559,157) and Wortzman (U.S. Pat. No. 4,820,508).

Useful dosages of the compositions of the invention can be determined
15 by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949.

Generally, the concentration of the composition(s) of the invention in a liquid composition, such as a lotion, will be from about 0.1-25 wt-%, or from
20 about 0.5-10 wt-%. The concentration in a semi-solid or solid composition such as a gel or a powder will be about 0.1-5 wt-%, or about 0.5-2.5 wt-%.

The active ingredient may be administered to achieve peak plasma concentrations of the active compound of from about 0.5 to about 75 μ M, about 1 to 50 μ M, or about 2 to about 30 μ M. This may be achieved, for example, by
25 the intravenous injection of a 0.05 to 5% solution of the active ingredient, optionally in saline, or orally administered as a bolus containing about 1-100 mg of the active ingredient. Desirable blood levels may be maintained by continuous infusion to provide about 0.01-5.0 mg/kg/hr or by intermittent infusions containing about 0.4-15 mg/kg of the active ingredient(s).

30 The amount of the compound, or an active salt or derivative thereof, required for use in treatment will vary not only with the particular salt selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician. In general, however, a suitable

dose will be in the range of from about 0.5 to about 100 mg/kg, e.g., from about 10 to about 75 mg/kg of body weight per day, such as 3 to about 50 mg per kilogram body weight of the recipient per day, in the range of 6 to 90 mg/kg/day, or in the range of 15 to 60 mg/kg/day.

- 5 The composition is conveniently administered in unit dosage form; for example, containing 5 to 1000 mg, conveniently 10 to 750 mg, most conveniently, 50 to 500 mg of active ingredient per unit dosage form.

 The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three,
10 four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations; such as multiple inhalations from an insufflator or by application of a plurality of drops into the eye. The dose, and perhaps the dose frequency, will also vary according to the age, body weight, condition, and response of the individual patient. In general,
15 the total daily dose range for a compound or compounds of formula (I) or (II), for the conditions described herein, may be from about 50 mg to about 5000 mg, in single or divided doses. A daily dose range may be about 100 mg to about 4000 mg, or about 1000-3000 mg, in single or divided doses, e.g., 750 mg every 6 hr of orally administered composition. This can achieve plasma levels of
20 about 500-750 uM, which can be effective to kill cancer cells. In managing the patient, the therapy should be initiated at a lower dose and increased depending on the patient's global response.

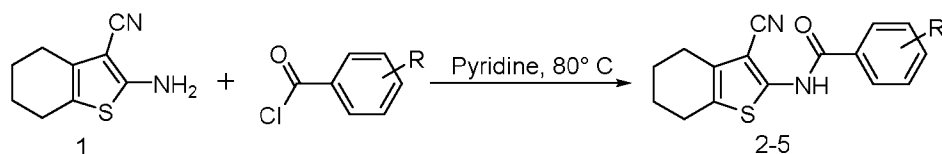
 As described above, compositions are useful in the treatment or prevention of a disease or disorder in, for example, humans or other mammals (e.g., bovine,
25 canine, equine, feline, ovine, and porcine animals), and perhaps other animals as well. Depending on the particular compound, the composition will, for example, be useful for treating cancer.

 The invention will be described by the following non-limiting example.

30

Example

Synthesis:



5

	2	3	4	5
R	2-I	2-Br	3-Br	4-Br

Scheme 1.

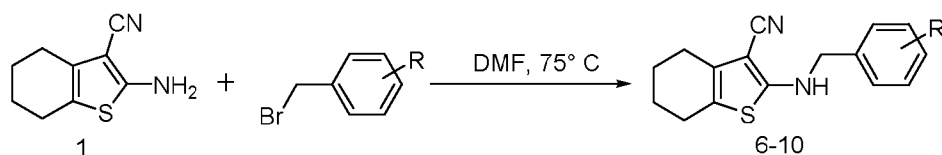
Synthesis of

benzamide derivatives

10 General procedure for synthesis of **2-5**

Compound **1** was synthesized as previously reported in Gewald et al. (1966). Compound **1** (0.003 mol) and the corresponding acid chloride (0.003 mol) were heated at 80°C in pyridine (10 mL) for 16-18 h. The reaction mixture was cooled and poured into 200 mL of 1M HCl, then the aqueous mixture was

15 extracted with chloroform (75 mL x 3). The combined organic layers were concentrated under reduced pressure and the crude product was purified by flash column chromatography using ethylacetate/hexanes as eluent solvents to give **2-5** as yellow to white solids.



20

	6	7	8	9	10
R	2-F	2-I	2-Br	3-Br	4-Br

Scheme 2.

Synthesis of

benzylamine derivatives

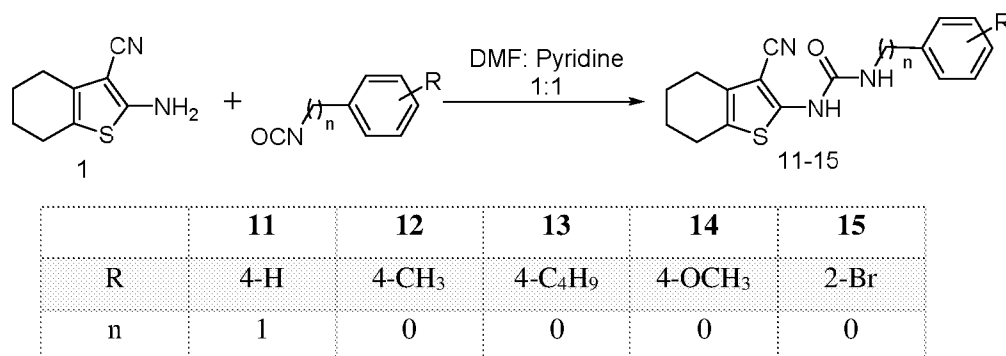
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General procedure for synthesis of **6-10**

Compound **1** (0.003 mol) and the corresponding benzyl bromide (0.003 mol) were heated at 75°C in DMF (10 mL) for 24 h. The reaction mixture was cooled and poured into 200 mL of brine solution, then the aqueous mixture was

extracted with chloroform (75 mL x 3). The combined organic layers were concentrated under reduced pressure and the crude product was purified by flash column chromatography using ethylacetate/hexanes as eluent solvents to give **6-10** as yellow solids.

5



Scheme 3. Synthesis of urea derivatives

10

General procedure for synthesis of **11-15**

Compound **1** (0.003 mol) and the corresponding isocyanate (0.003 mol) were stirred at room temperature in a mixture of DMF:pyridine (10 mL 1:1) for 24-36 h. The reaction mixture was cooled and poured into 200 mL of 1M HCl, then the aqueous mixture was extracted with chloroform (75 mL x 3). The combined organic layers were concentrated under reduced pressure and the crude product was purified by flash column chromatography using ethylacetate/hexanes as eluent solvents to give **11-15** as white solids.

15

Biological Screening:

In vitro antitumor screening:

The synthesized derivatives were tested for their antitumor activity adopting MTS assay against A549 adenocarcinoma cell line following the reported assay method

- 5 In Riss et al. Results are shown in Table 2. See Figure 25.

Compound	IC ₅₀	Compound	IC ₅₀
2	>75 uM	9	>75 uM
3	61.77 uM	10	2.7 uM
4	7.6 uM	11	1.6 uM
5	53.22 uM	12	ND
6	>75 uM	13	ND
7	30.7 uM	14	>75 uM
8	84.08 uM	15	>75 uM

Table 2. IC₅₀ values of the tested compounds **2-15**

ND Not determined

References

- 10 K. Gewald, E. Schinke, H. Bottcher, 2-Aminothiopheneaus methlenaktiven nitrilen carbonyl verbindungen and schwefet, Chem. Ber. 99 (1966) 94-100.
- T.L. Riss, and R.A. Moravec, Comparison of MTT, XTT, and a novel tetrazolium compound for MTS for *in vitro* proliferation and chemosensitivity assays. Mol.
- 15 Biol. Cell (Suppl.) (1992) 3, 184a.

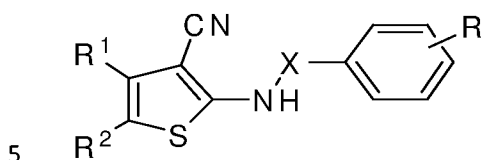
All publications, patents and patent applications are incorporated herein by reference. While in the foregoing specification, this invention has been

20 described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details herein may be varied considerably without departing from the basic principles of the invention.

25

WHAT IS CLAIMED IS:

1. A compound of formula (I):



or a pharmaceutically acceptable salt thereof,

wherein R is halo, alkyl, alkenyl, alkynyl, alkoxy, haloalkyl, substituted alkyl, carbocycle or heterocycle;

10 wherein X comprises an alkyl, carbonyl, or amide; and

wherein R¹ and R² independently are alkyl, alkenyl, alkynyl, alkoxy, or substituted alkyl or R¹ and R² together form a C6 or C7 ring.

2. The compound of claim 1 wherein R is halo, (C1-C6)alkyl, substituted (C1-C6)alkyl, or C5-C7 carbocycle.

15

3. The compound of claim 2 wherein halo is Br, F or I.

4. The compound of claim 1, 2 or 3 wherein X is (C1-C6)alkyl, CONH or CONH(C1-C6) alkyl.

20

5. The compound of any one of claims 1 to 4 wherein R¹ and R² together form a C7 ring

25 6. The compound of any one of claims 1 to 4 wherein R¹ and R² together form a C6 ring

7. The compound of any one of claims 1 to 4 wherein R¹ and R² together form an aryl ring.

30

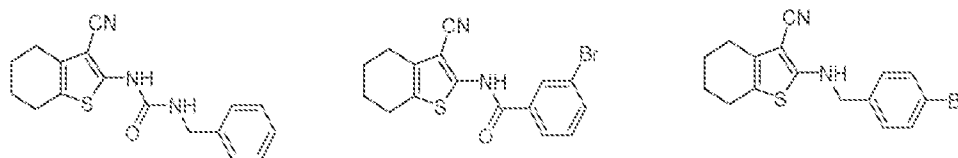
8. The compound of any one of claims 1 to 4 which is a benzylamide.

9. The compound of any one of claims 1 to 4 which is a benzylamine.

10. The compound of any one of claims 1 to 4 which comprises urea.

5

11. The compound of any one of claims 1 to 4 which is one of:



10 12. A composition having the compound of any one of claims 1 to 11.

13. The composition of claim 12 which comprises nanoparticles having the compound.

15 14. The composition of claim 13 wherein the nanoparticles have a diameter from about 100 nm to about 250 nm.

15. The composition of claim 13 or 14 wherein the nanoparticles are formed of a synthetic polymer.

20

16. The composition of claim 15 wherein the polymer comprises lactic acid, glycolic acid, caproic acid, a polyanhydride, or a combination thereof.

17. A pharmaceutical composition comprising the compound of any one of
25 claims 1 to 11.

18. The composition of claim 17 which comprises nanoparticles having the compound.

19. The composition of claim 18 wherein the nanoparticles have a diameter
30 from about 100 nm to about 250 nm.

20. The composition of claim 18 or 19 wherein the nanoparticles are formed of a synthetic polymer.
21. The composition of claim 20 wherein the polymer comprises lactic acid,
5 glycolic acid, caproic acid, a polyanhydride, or a combination thereof.
22. The composition of any one of claims 17 to 21 further comprising a pharmaceutically acceptable carrier.
- 10 23. A method to prevent, inhibit or treat cancer in a mammal, comprising administering to the mammal a composition having an effective amount of the compound of any one of claims 1 to 11 or the composition of any one of claims 12 to 21.
- 15 24. The method of claim 23 wherein the amount of effective to inhibit cancer.
25. The method of claim 23 or 24 wherein the mammal is a human.
- 20 26. The method of any one of claims 23 to 25 wherein the compound comprises a benzamide.
27. The method of any one of claims 23 to 25 wherein the compound comprises a benzylamine.
- 25 28. The method of any one of claims 23 to 25 wherein the compound comprises urea.
29. The method of any one of claims 23 to 28 wherein the composition is
30 intravenously administered.
30. The method of any one of claims 23 to 28 wherein the composition is locally administered.

31. The method of any one of claims 23 to 28 wherein the composition is injected.
32. The method of any one of claims 23 to 28 wherein the composition is orally administered.
33. The method of any one of claims 23 to 32 wherein the composition comprises nanoparticles or microparticles.
34. The method of claim 33 wherein the particles are formed of a synthetic polymer.
35. The method of claim 33 wherein the particles comprise lactic acid, glycolic acid, caproic acid, a polyanhydride, or a combination thereof.
36. The method of claim 34 wherein the particles comprise lactic acid and glycolic acid, polycaprolactone or poly lactic acid.
37. The method of claim 34 wherein the particles comprise PEI.
38. The method of claim 33 wherein the particles are formed of a natural polymer.
39. The method of claim 33 wherein the particles are formed of collagen, proteoglycan, alginate, chitosan or extracellular matrix.
40. The method of claim 33 wherein the particles are formed of polystyrene.
41. The method of any one of claims 23 to 40 wherein the cancer is melanoma, ovarian cancer or renal cancer.
42. The method of any one of claims 23 to 40 wherein the cancer is leukemia, lung cancer or colon cancer.

43. The method of any one of claims 23 to 40 wherein the compound inhibits JNK2 and/or JNK3.

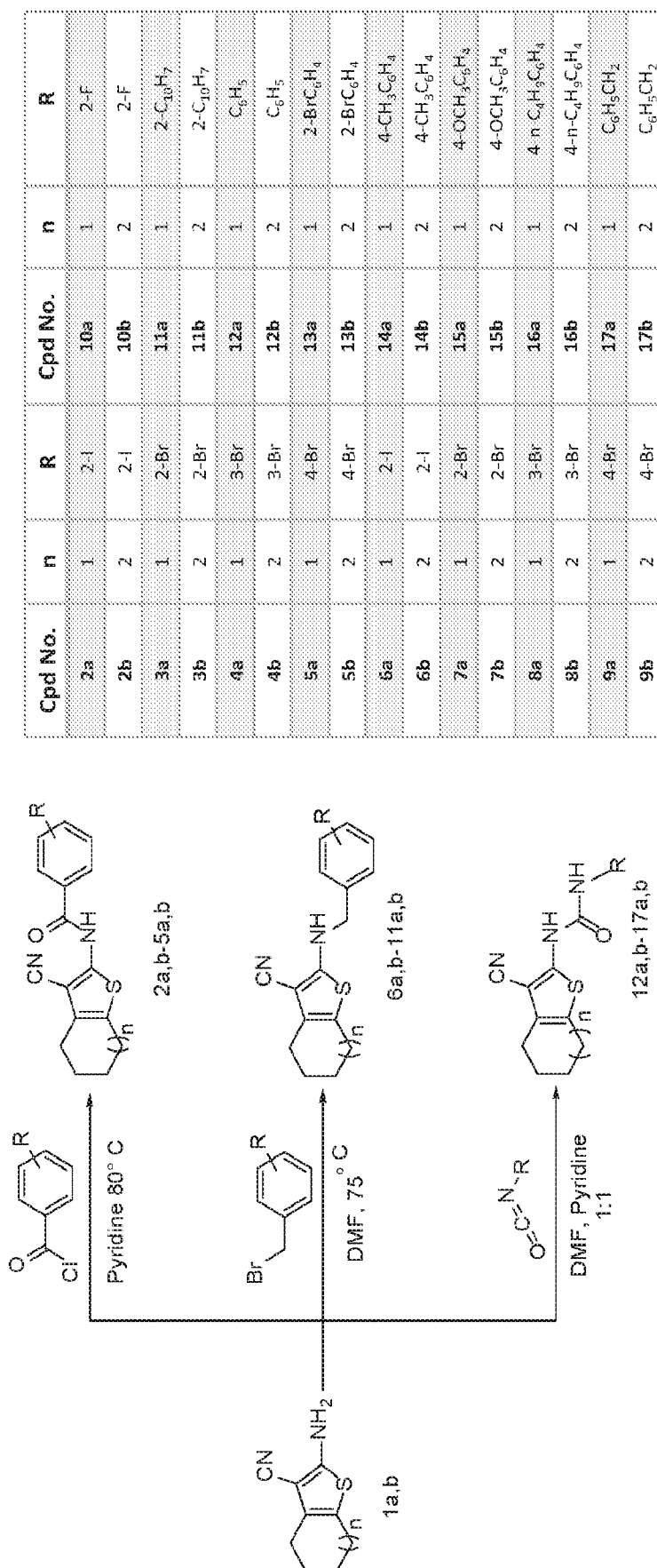
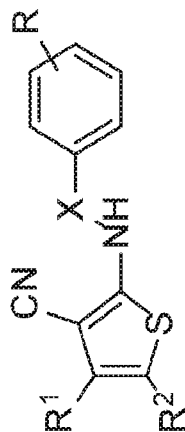


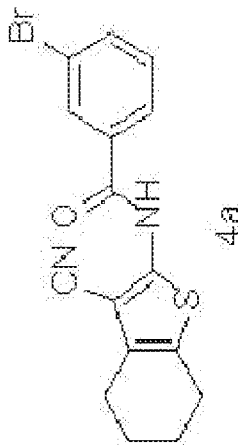
Figure 1. Synthesis of benzamides 2a,b-5a,b, benzylamines 6a,b-11a,b and ureas 12a,b-17a,b

Figure 2. Formula
(I)



X= CO; CH₂CONH; CONHCH₂

R= 2-I; 2-Br; 3-Br; 4-Br; 2-F; C₁₀H₇; C₆H₅; 2-BrC₆H₄; 4-CH₃C₆H₄; 4-OCH₃C₆H₄; 4-n-C₄H₉C₆H₄; C₆H₅CH₂



4 (NSC: 813478)

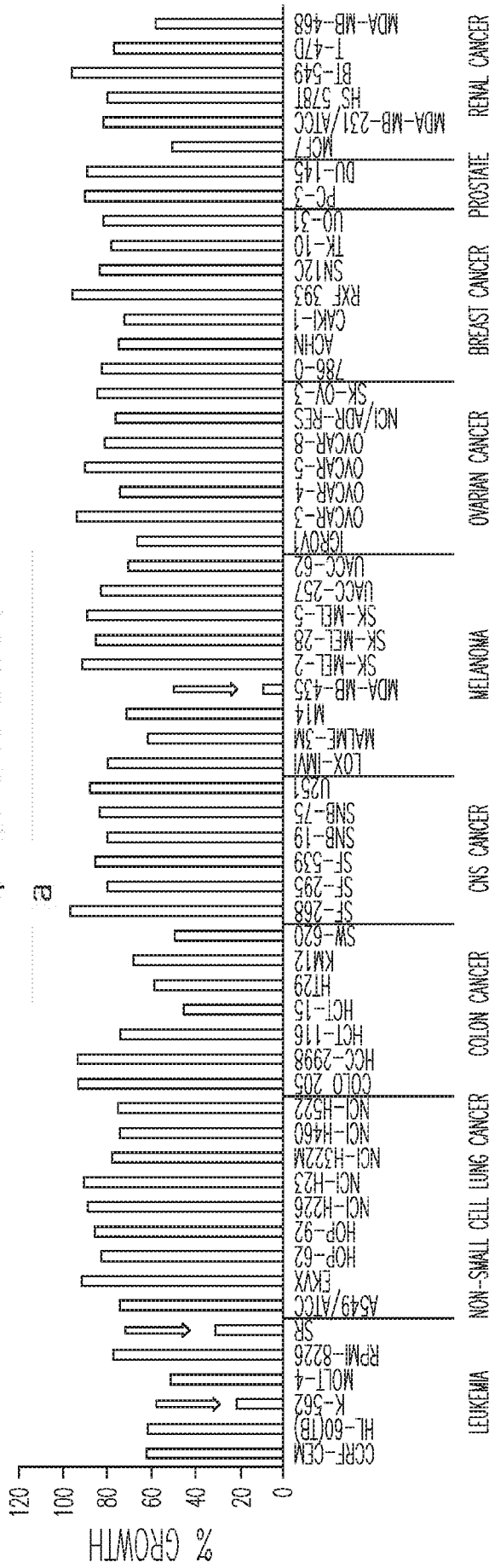
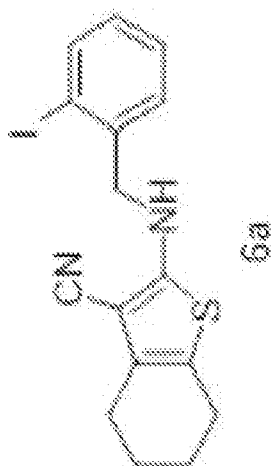


Fig. 3



6a (NSC: 813479)

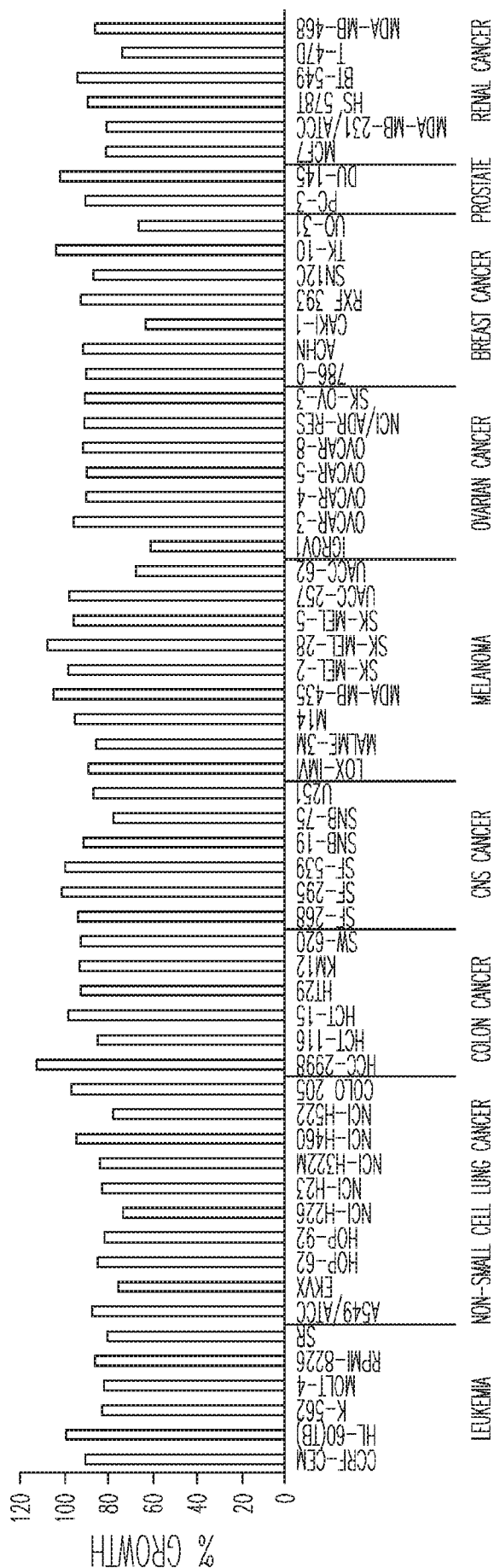


Fig. 4

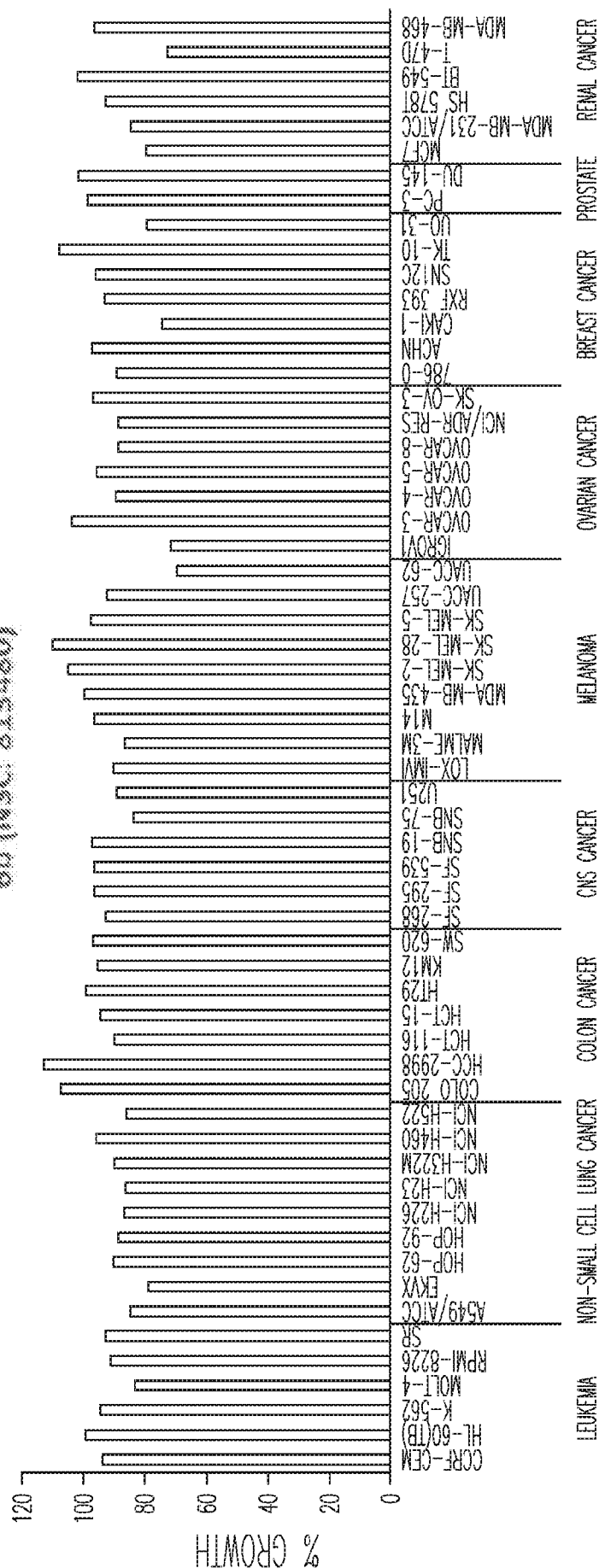
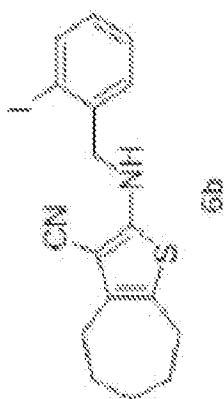
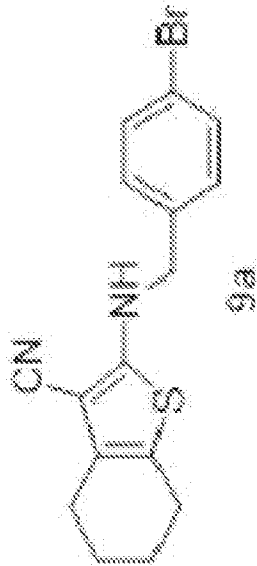


Fig. 5



9a (NSC: 814543)

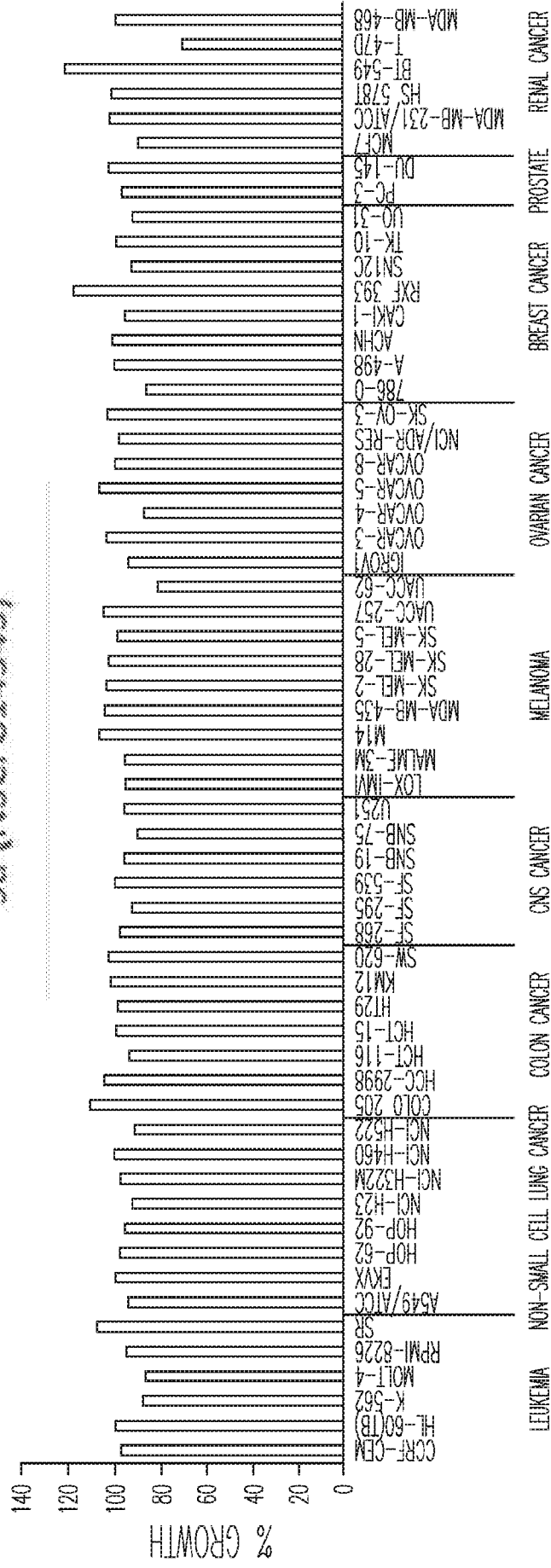
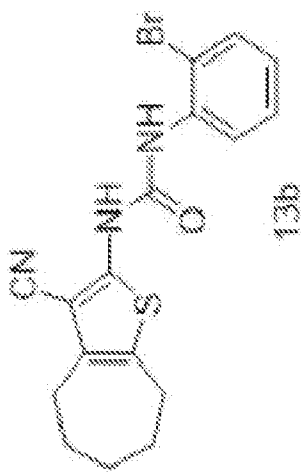


Fig. 6



13b (NSC: 813477)

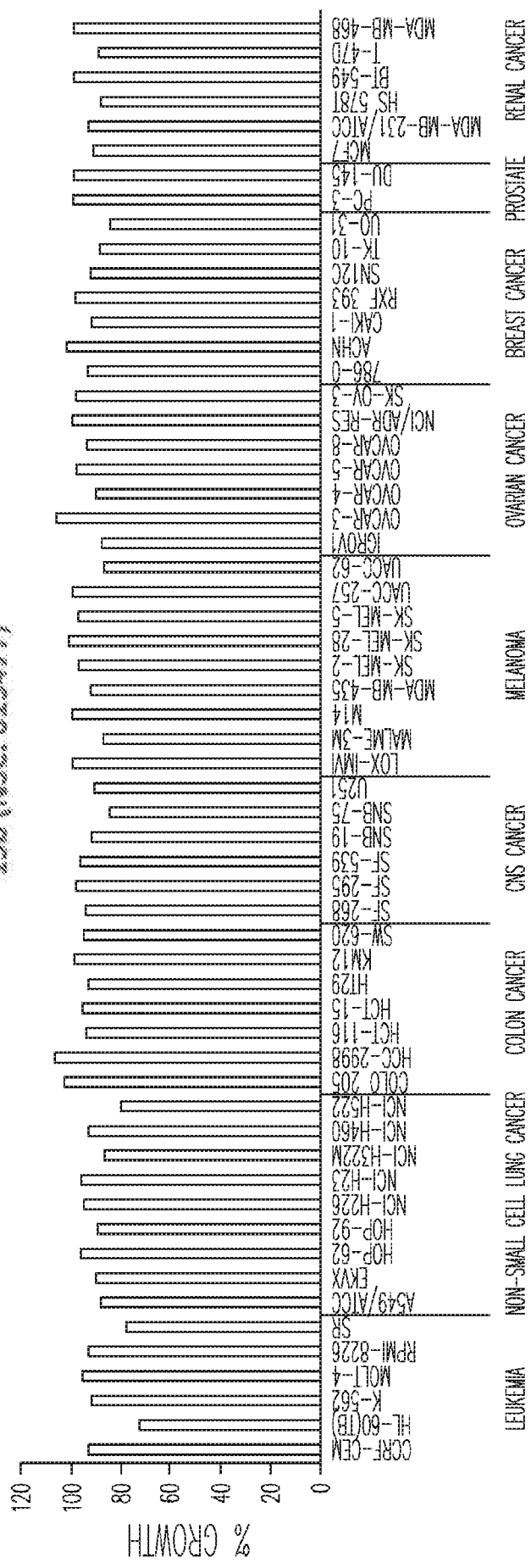
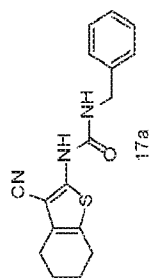
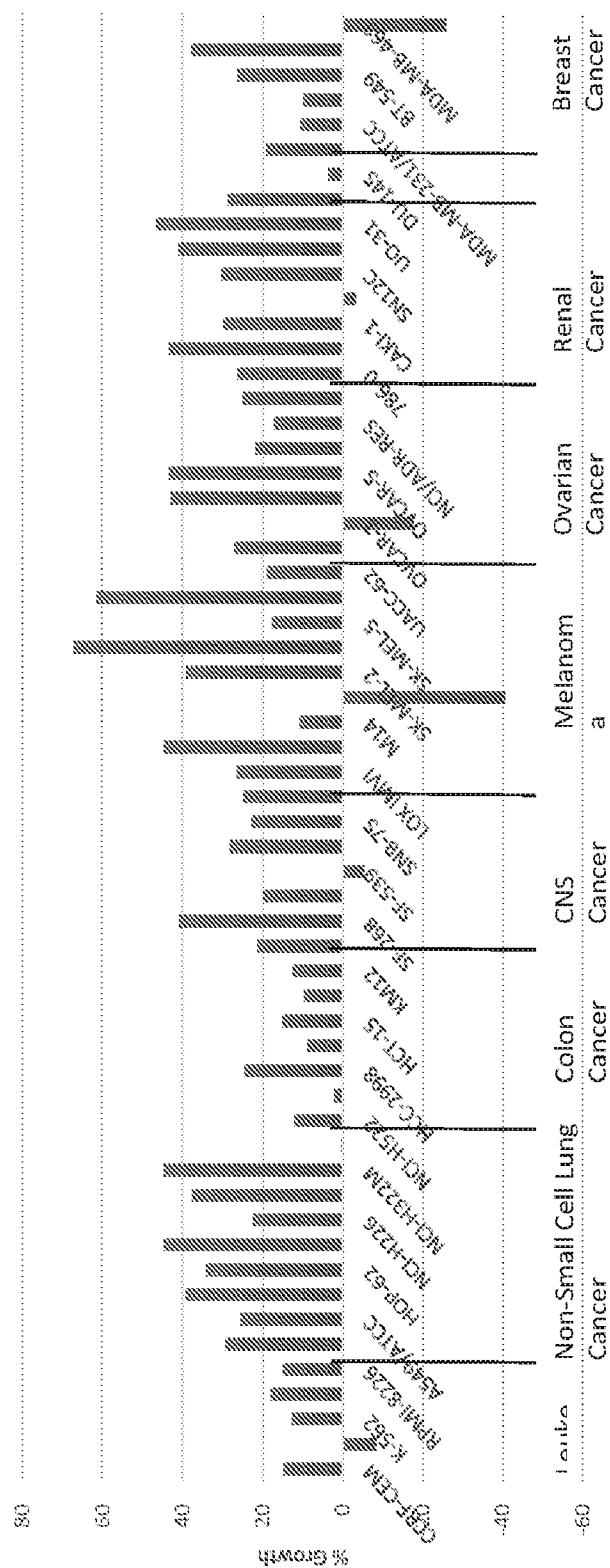


Fig. 7



17a (NSC: 813475)

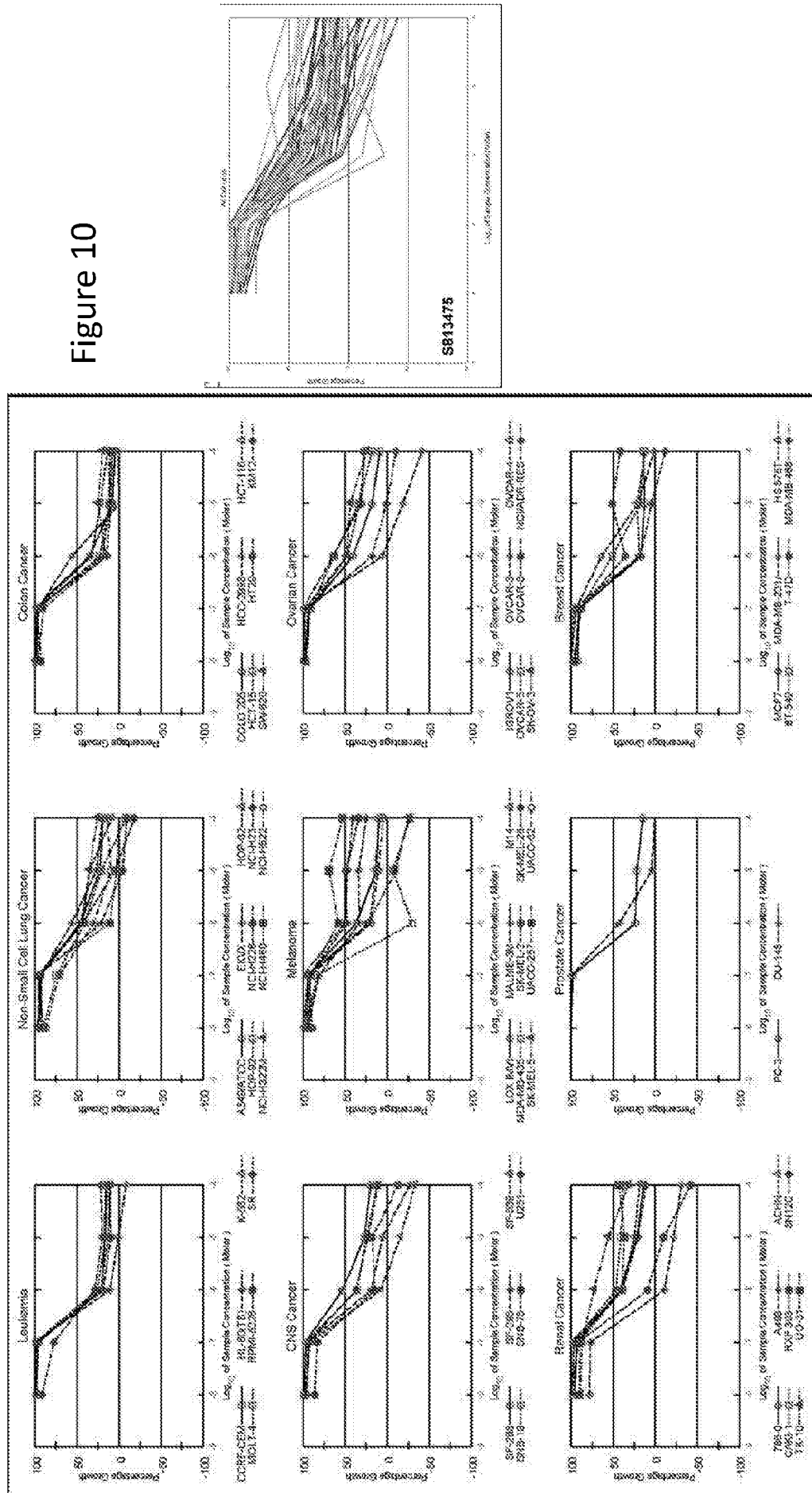
Figure 8

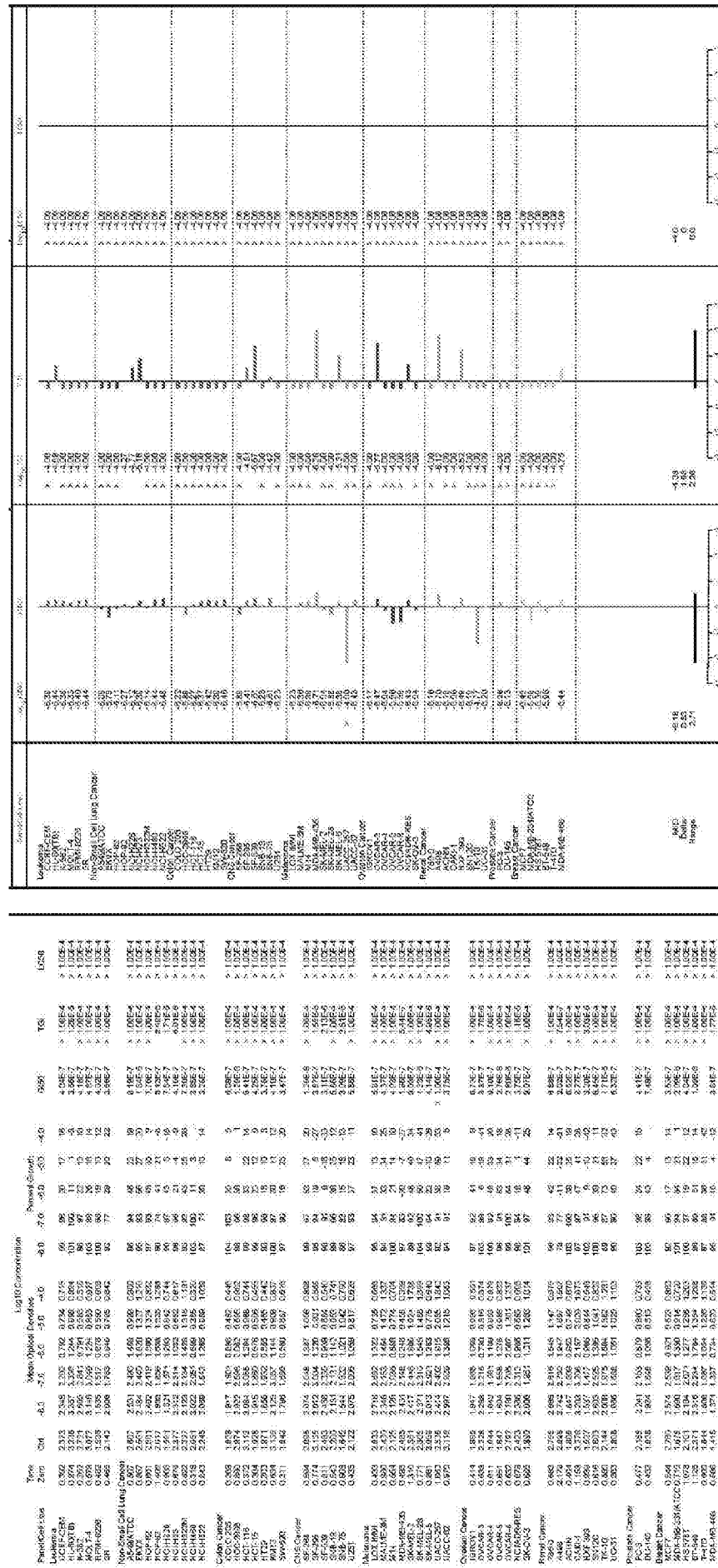


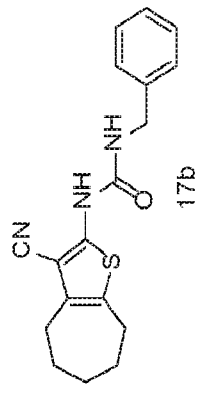
Cell Line	GI ₅₀ values (µM)	Cell Line	GI ₅₀ values (µM)
Leukemia		Melanoma	
CCRF-CEM	0.40738	M14	0.4073
HL-60(TB)	0.36307	MDA-MB-435	0.1949
K-562	0.41687	SK-MEL-2	0.9120
MOLT-4	0.46773	SK-MEL-28	1.318255
RPMI-8226	0.3981	SK-MEL-5	0.4168
SR	0.3630	UACC-257	>100
Non-Small Cell Lung Cancer		UACC-62	0.3715
A549/ATCC	0.8128	Ovarian Cancer	
EKVX	1.6218	IGROV1	0.6760
HOP-62	0.7762	OVCAR-3	0.3388
HOP-92	0.5370	OVCAR-4	0.9120
NCI-H226	0.7585	OVCAR-5	2.754
NCI-H23	0.4073	OVCAR-8	2.5703
NCI-H322M	0.7244	NCI/ADR-RES	0.3715
NCI-H460	0.3630	SK-OV-3	0.9120
NCI-H522	0.3311	Renal Cancer	
Colon Cancer		786-0	0.6918
COLO 205	0.6025	A498	0.1995
HCC-2998	1.2882	ACHN	0.6606
HCT-116	0.5370	CAR1-1	0.8709
HCT-15	0.4265	RAF 393	0.3235
HT29	0.3801	SN 12C	0.6456
KM12	0.4073	TK-10	0.1698
SW-620	0.3467	UO-31	0.6309
CNS Cancer		Prostate Cancer	
SF-268	1.2882	PC-3	0.4365
SF-295	0.3890	DU-145	0.7413
SF-539	0.3090	Breast Cancer	
SNB-19	0.5888	MCF-7	0.3548
SNB-75	0.3090	MDA-MB-231/ATCC	2.0892
U251	0.5888	HS 578T	0.4073
Melanoma		BT-549	1.0964
LOX IMVI	0.5888	T-47D	ND
MALME-3M	0.4365	MDA-MB-468	0.3630

Figure
9

Figure 10

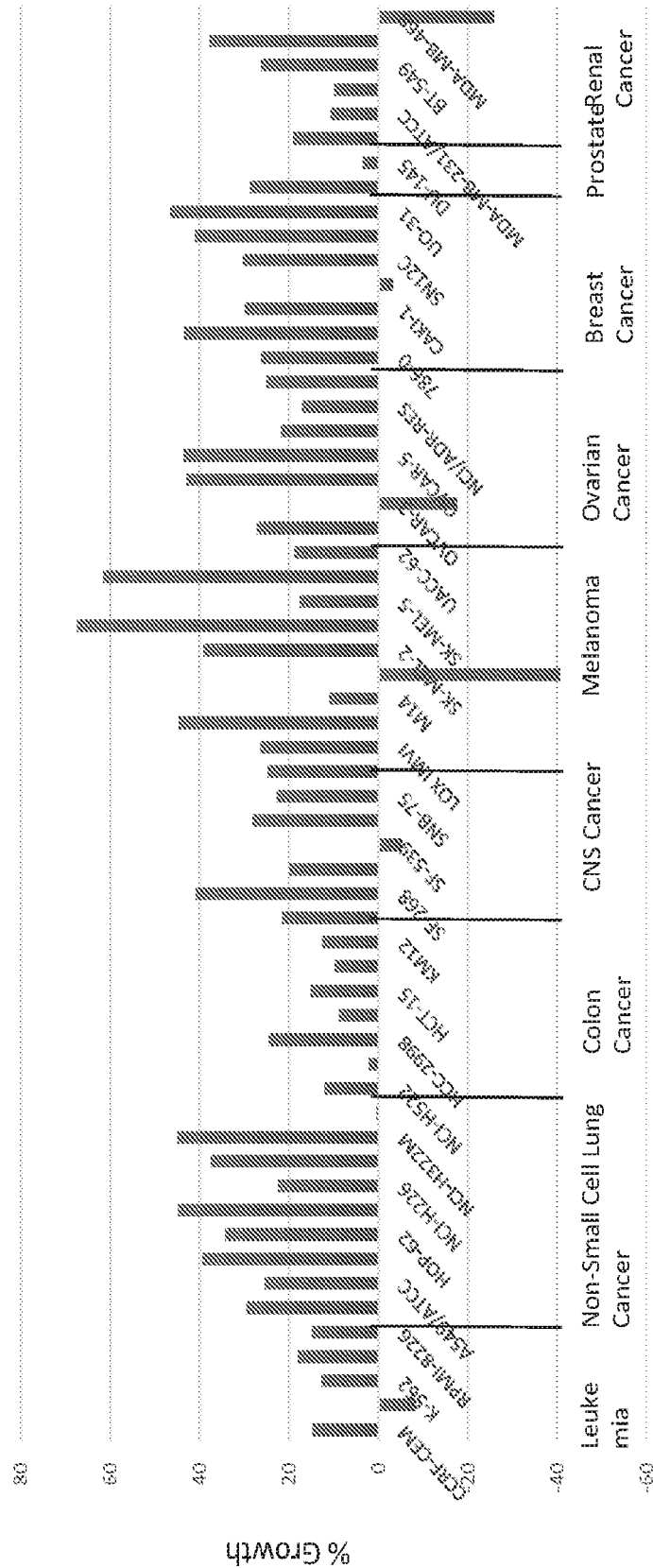






17b (NSC: 813476)

Figure 12



Cell Lines	GI ₅₀ values (μM)	Cell Lines	GI ₅₀ values (μM)
Leukemia		Melanoma	
CCRF-CEM	0.35	M14	0.392
HL-60(TB)	0.29	MDA-MB-435	1.72
K-562	0.356	SK-MEL-2	0.789
MOLT-4	0.393	SK-MEL-28	1.86
RPMI-8226	0.362	SK-MEL-5	0.368
SR	0.329	UACC-257	1.91
Non-Small Cell Lung Cancer		UACC-62	0.493
A549/ATCC	0.609	Ovarian Cancer	
EKVX	0.406	IGROV1	0.652
HOP-62	0.611	OVCAR-3	0.345
HOP-92	0.337	OVCAR-4	0.865
NCI-H226	0.712	OVCAR-5	1.85
NCI-H23	0.453	OVCAR-8	1.27
NCI-H322M	0.436	NCI/ADR-RES	0.394
NCI-H460	0.298	SK-OV-3	0.653
NCI-H522	0.500	Renal Cancer	
Colon Cancer		786-0	0.563
COLO 205	0.487	A498	0.176
HCC-2998	1.56	ACHN	0.631
HCT-116	0.538	CAKI-1	0.629
HCT-15	0.400	RXF 393	0.262
HT29	0.400	SN 12C	0.627
KM12	0.427	TK-10	0.592
SW-620	0.383	UO-31	0.581
CNS Cancer		Prostate Cancer	
SF-268	1.22	PC-3	0.388
SF-295	0.338	DU 145	0.514
SF-539	0.290	Breast Cancer	
SNB-19	0.560	MCF-7	0.332
SNB-75	0.252	MDA-MB-231/ATCC	0.770
U251	0.457	HS 578T	0.314
Melanoma		BT-549	0.717
LOX IMVI	0.579	T-47D	0.325
MALME-3M	0.414	MDA-MB-468	0.296

Figure
13

Figure 15

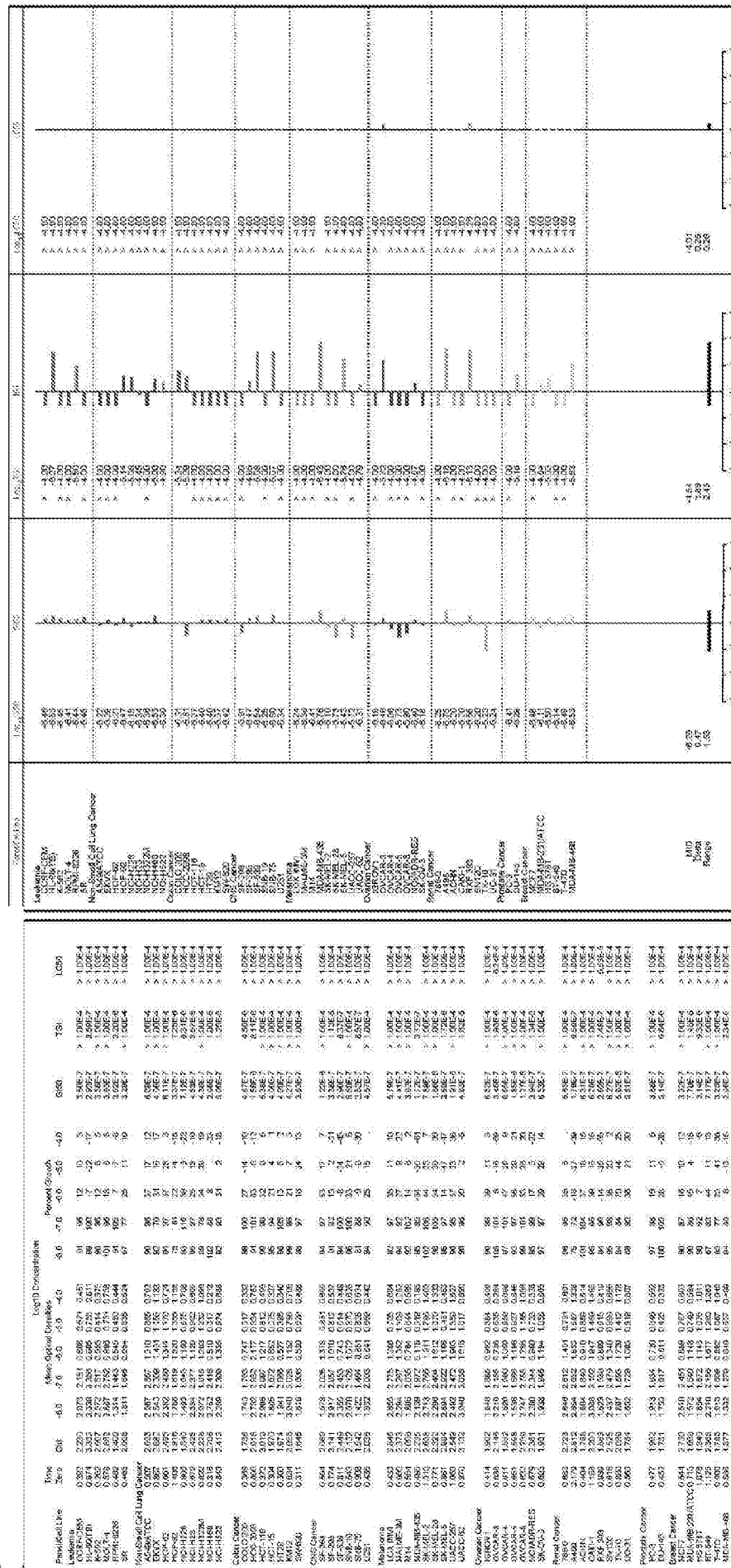
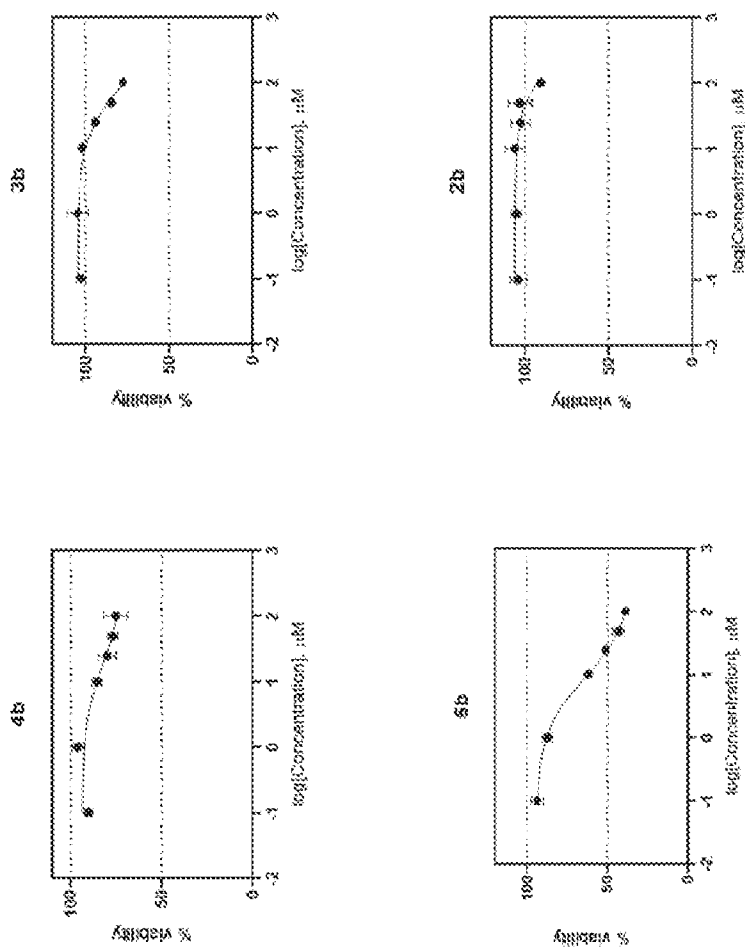


Figure 16



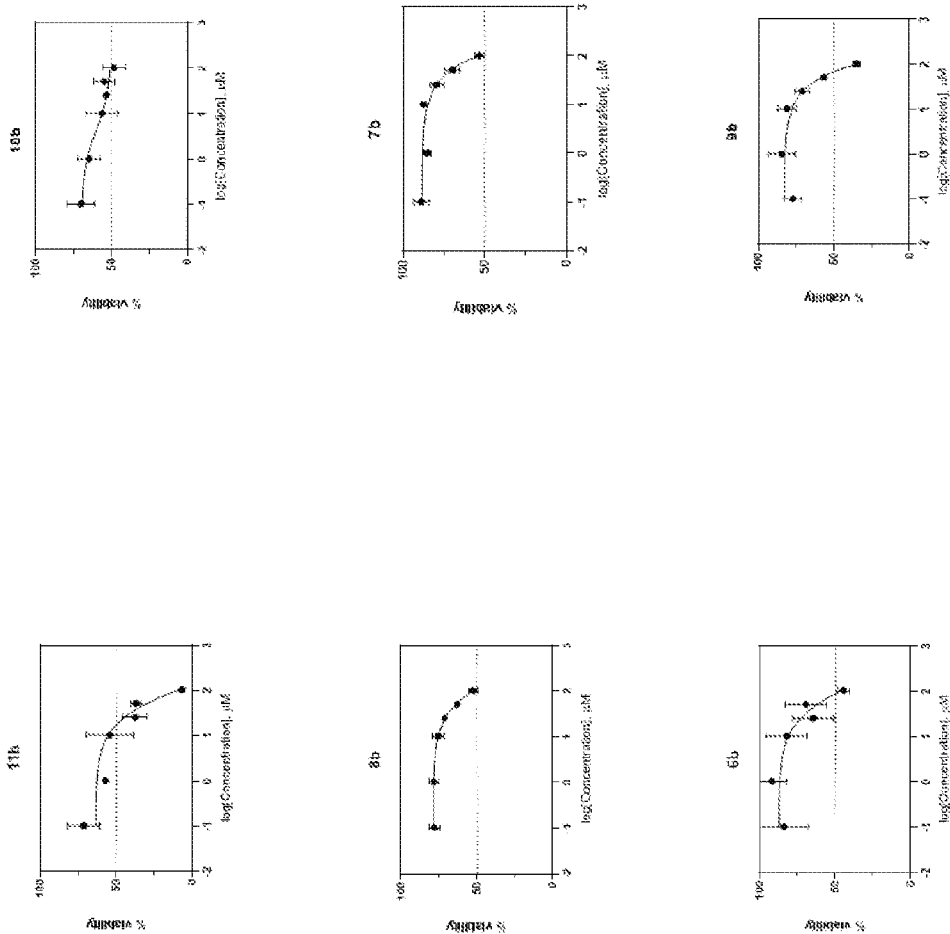


Figure 17

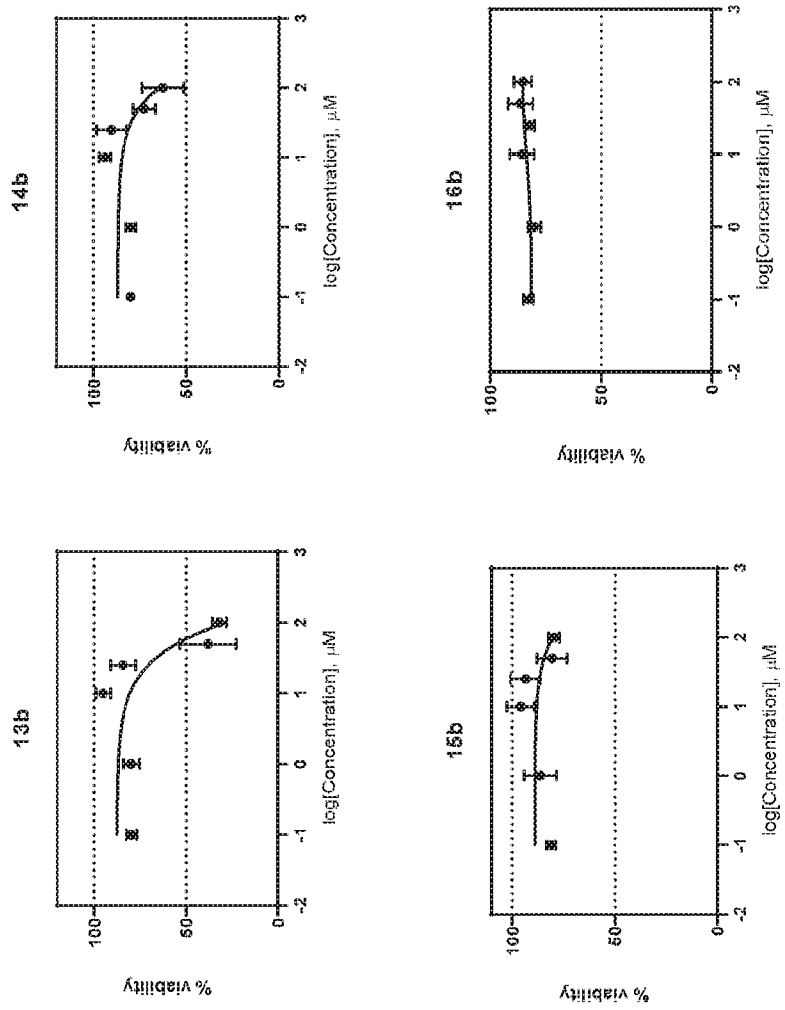


Figure 18

Figure 19. Cell Cycle analysis of cells treated with 17a (SA10)

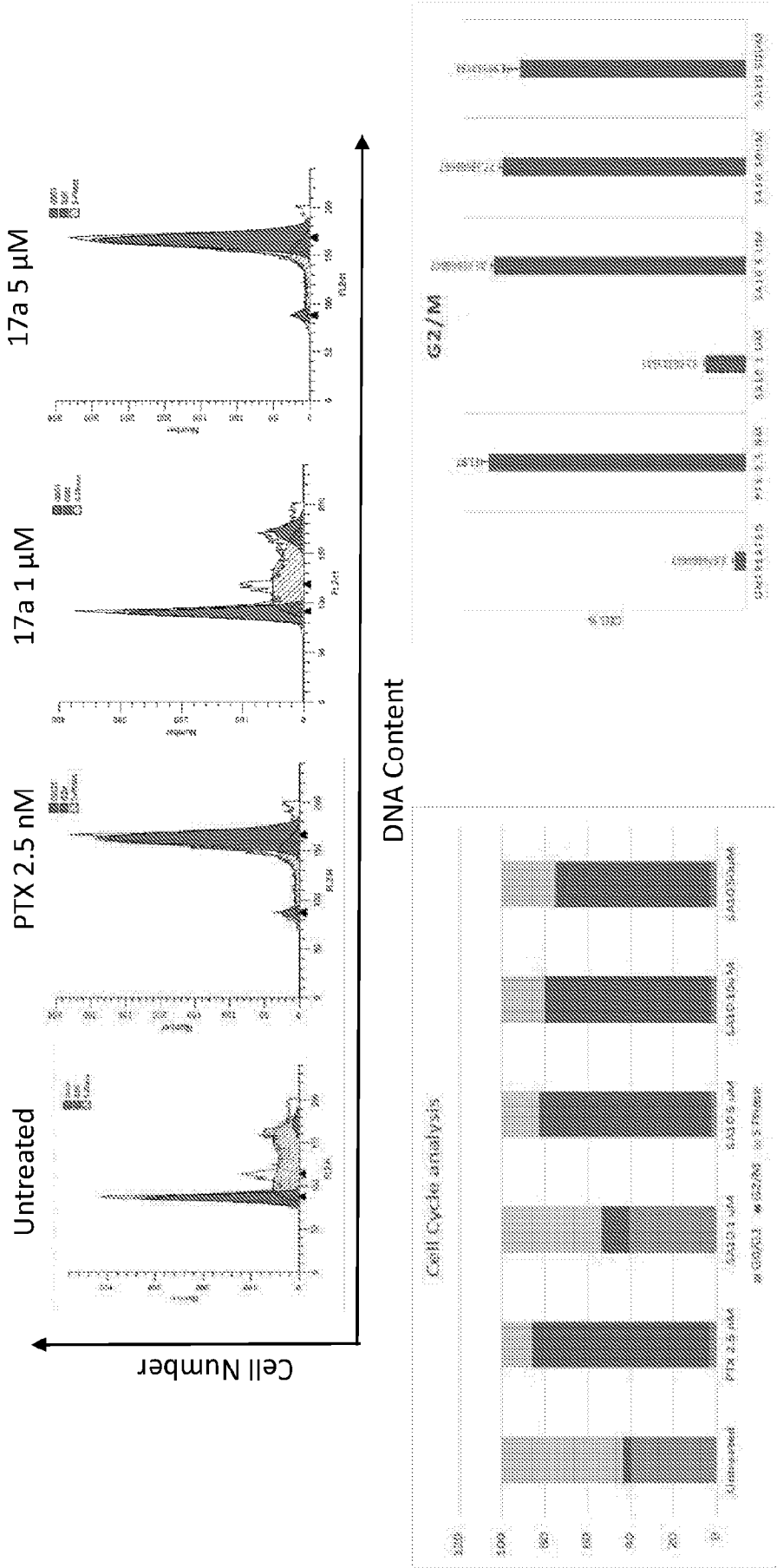
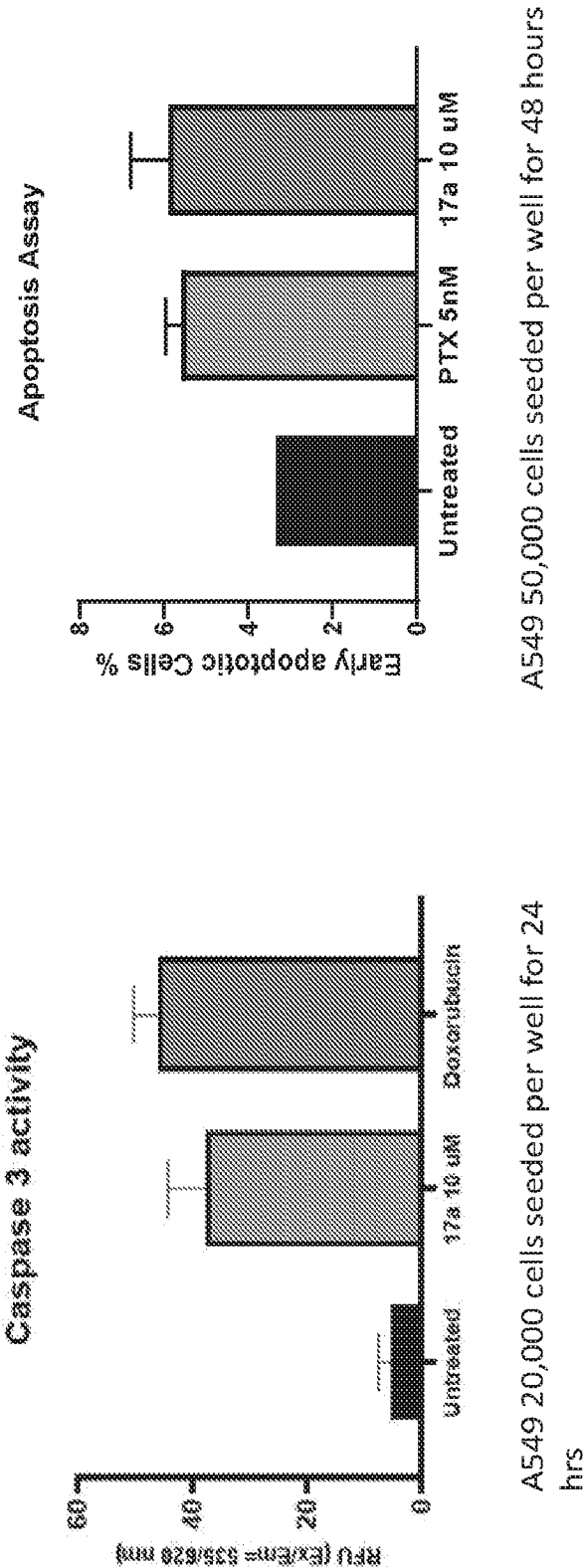


Figure 20. Detection of apoptosis in A549 cells treated with 17a at 10 μ M



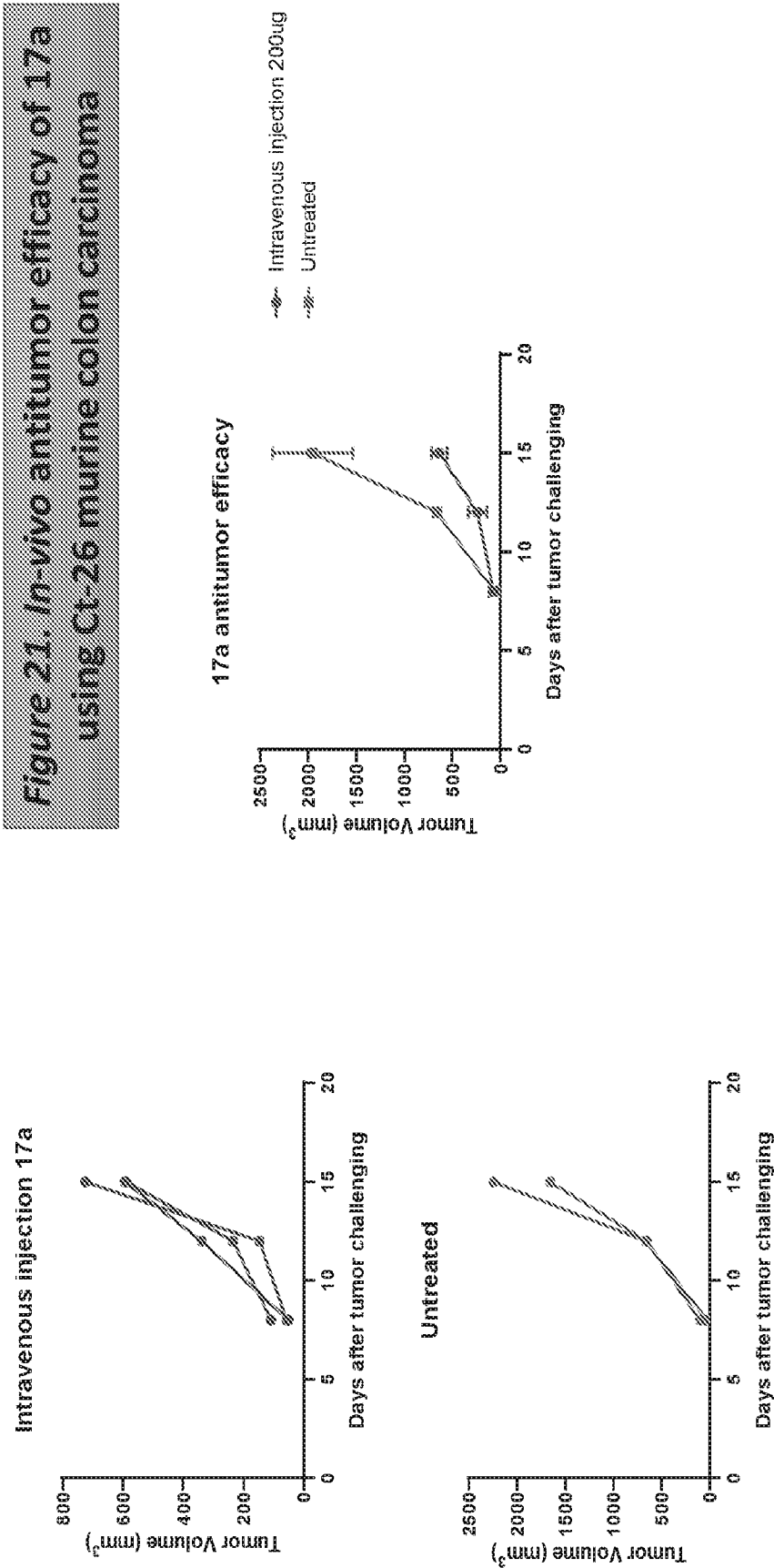
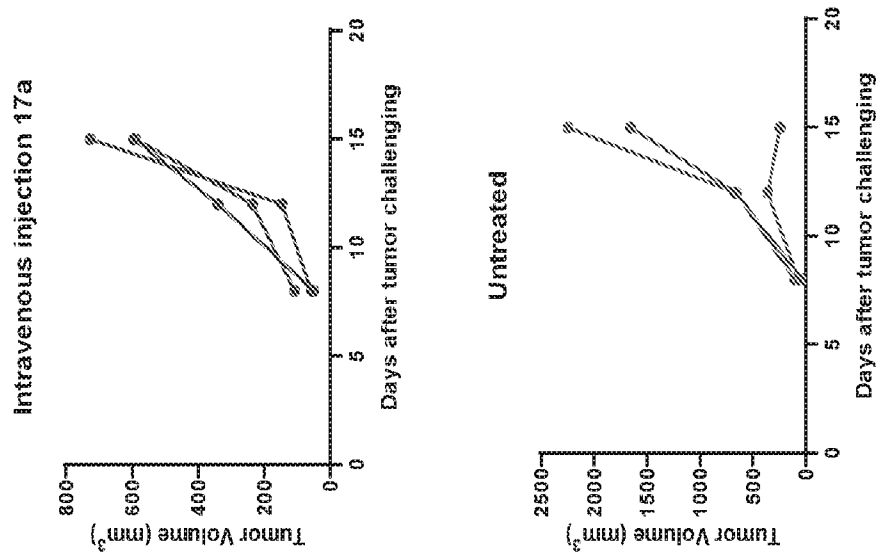


Figure 22. *In-vivo* antitumor efficacy of 17a using Ct-26 murine colon carcinoma



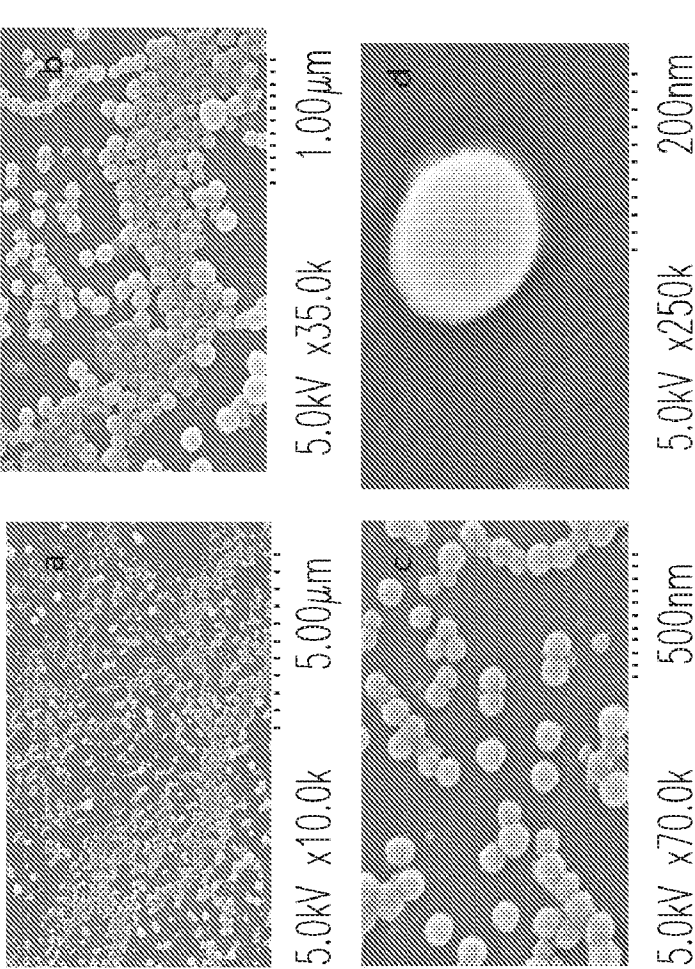
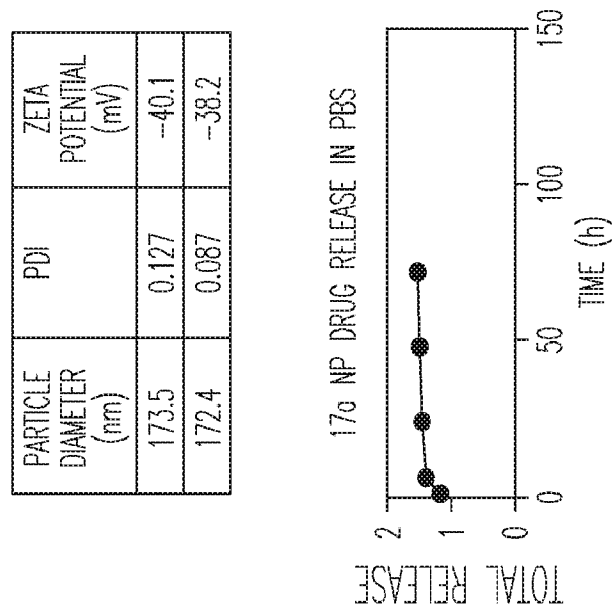


Fig. 23

Figure 24

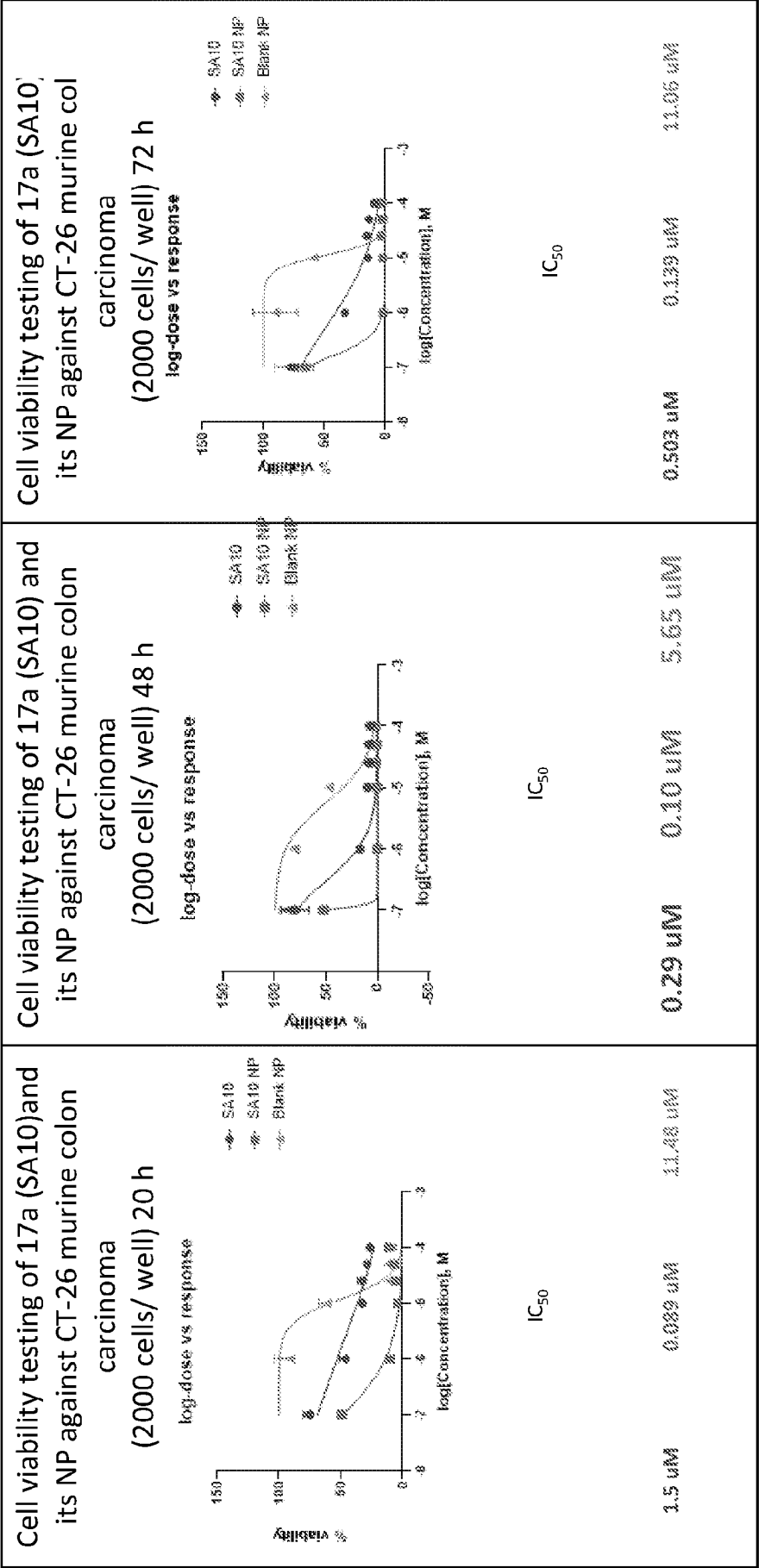


Figure 25

Cell viability testing for compound 11 SA10 (benzyl urea derivative) and its nanoparticle formulation using MTS assay B16F10 murine melanoma cell line (48 hours treatment)		
Formulation or compound	Graph	IC ₅₀
SA10 NP F#2 (TPGS)	log-dose vs response % viability log[Concentration], M Blank NP SA10 NP	1.46 μ M
Blank NP F#2 (TPGS)		30.46 μ M
SA10 alone	log-dose vs response % viability log[Concentration], M	6.35 μ M

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/039373

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D333/66 C07D333/80 A61P35/00 A61K31/381
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ANGELL ET AL: "N-(3-Cyano-4,5,6,7-tetrahydro-1-benzothie n-2-yl)amides as potent, selective, inhibitors of JNK2 and JNK3", BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, PERGAMON, AMSTERDAM, NL, vol. 17, no. 5, 14 February 2007 (2007-02-14), pages 1296-1301, XP005888468, ISSN: 0960-894X, DOI: 10.1016/J.BMCL.2006.12.003 page 1296, paragraph 3; table 1; compounds 5d-h ----- -/--	1-43



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

17 August 2020

Date of mailing of the international search report

16/10/2020

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Stroeter, Thomas

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANAND ACHARYA ET AL: "One-Pot Synthesis of Functionalized Benzo[b]thiophenes and Their Hetero-Fused Analogues via Intramolecular Copper-Catalyzed S-Arylation of In Situ Generated Enethiolates", THE JOURNAL OF ORGANIC CHEMISTRY, vol. 80, no. 5, 17 February 2015 (2015-02-17), pages 2884-2892, XP055722613, US ISSN: 0022-3263, DOI: 10.1021/acs.joc.5b00032 table 2 (entry 23) -----	1,4,6,7, 12
X	BONAGIRI SARAIAH ET AL: "One-Pot Synthesis of 2-(Aryl/Alkyl)amino-3-cyanobenzo[b]thiophenes and Their Hetero-Fused Analogues by Pd-Catalyzed Intramolecular Oxidative C-H Functionalization/Arylthiolation : One-Pot Synthesis of 2-(Aryl/Alkyl)amino-3-cyanobenzo[b]thiophenes and Their Hetero-Fused Analogues by Pd-Catalyzed In", EUROPEAN JOURNAL OF ORGANIC CHEMISTRY, vol. 2017, no. 37, 9 October 2017 (2017-10-09), pages 5679-5688, XP055722619, DE ISSN: 1434-193X, DOI: 10.1002/ejoc.201700963 table 2; compounds 4l, 4n, 4n' -----	1,4,6,7, 12
X	DEBASHRUTI BANDYOPADHYAY ET AL: "Nickel catalyzed site selective C-H functionalization of [alpha]-aryl-thioamides", ORGANIC & BIOMOLECULAR CHEMISTRY, vol. 16, no. 35, 1 January 2018 (2018-01-01), pages 6405-6409, XP055722623, ISSN: 1477-0520, DOI: 10.1039/C8OB01712C table 4; compounds 6e-6g -----	1,4,6,7, 12
Y	WO 02/083667 A2 (VERTEX PHARMA [US]; CAO JINGRONG [US] ET AL.) 24 October 2002 (2002-10-24) tables 1, 3, 5; compounds 7-9, IIIa7-9, IVa7-9 -----	1-43

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/039373

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-43(partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-43(partially)

claimed subject-matter directed to a compound of formula (I)
wherein X = (C1-C6)alkyl

2. claims: 1-4, 6-43(all partially)

claimed subject-matter directed to a compound of formula (I)
wherein X = CONH or CONH(C1-C6)alkyl

3. claims: 1-3, 8-10, 12-43(all partially)

claimed subject-matter directed to a compound of formula (I)
wherein X = C0 and R1 and R2 do not together form a ring

4. claims: 1-3, 5, 8-10, 12-43(all partially)

claimed subject-matter directed to a compound of formula (I)
wherein X = C0 and R1 and R2 together form a C7 ring

5. claims: 1-3, 6-10, 12-43(all partially)

claimed subject-matter directed to a compound of formula (I)
wherein X = C0, R1 and R2 together form a C6 ring and R =
alkyl, alkenyl, alkynyl, haloalkyl, subst. alkyl, alkoxy,
carbocycle or heterocycle

6. claims: 1-3, 6-43(all partially)

claimed subject-matter directed to a compound of formula (I)
wherein X = C0, R1 and R2 together form a C6 ring and R =
halo

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2020/039373

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 02083667	A2	24-10-2002	
		AR 035824 A1	14-07-2004
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		US 2004023963 A1	05-02-2004
		WO 02083667 A2	24-10-2002
