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(54) Title: CHROMOBOX PROTEIN INHIBITORS AND USES THEREOF

(57) Abstract: Provided herein are compounds useful as inhibitors of CBX. Also described are pharmaceutical compositions and medical uses of these compounds.

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CHROMOBOX PROTEIN INHIBITORS AND USES THEREOF

Related Application

This application claims the benefit of priority to U.S. Provisional Patent Application serial number 62/399,857, filed September 26, 2016, which is hereby incorporated herein by reference in its entirety.

Background

The polycomb complexes have been shown to be important for the maintenance of cellular identity and play important roles in stem cell differentiation. Two canonical polycomb complexes have been described in mammalian cells, polycomb repressive complex 1 and 2 (PRC1 and PRC2). The catalytic component of PRC2 is the Enhancer of Zeste Homologue 2 (EZH2), which mediates trimethylation of H3K27me3. This mark is considered a repressive histone modification and is usually found at the promoters of developmental genes in adult organisms. H3K27me3 at promoters serves as a docking site for chromobox (CBX) proteins, which all contain a conserved chromodomain responsible for binding to the methylated lysine. CBX proteins are one component of PRC1 and recruit the complex to its target genes. Two other PRC1 subunits, RING1b and BMI1, coordinate to catalyze ubiquitination of lysine 119 of histone 2A, which contributes to gene silencing. Levels of H3K27me3 are found to be deregulated in many cancers, with the repressive mark occupying tumor suppressor genes, allowing the cancerous cells to escape cell cycle checkpoints and continue uncontrolled growth. Numerous examples exist in cancer biology where hyperactivity of the polycomb complex has been linked to the pathogenesis of cancer.

Overexpression of EZH2, resulting in high global levels of H3K27me3, is observed in metastatic breast and prostate cancer and has been implicated in the development of oral squamous carcinoma and colorectal cancer. BMI1 is elevated in squamous cell carcinomas, neuroblastoma, and bladder tumors; furthermore, several studies describe its deregulation in leukemia. Mutations of EZH2 that increase catalytic activity on H3K27me2 toward elaboration of high levels of H3K27me3 have been observed in non-Hodgkin's lymphoma, and render these cancers sensitive to EZH2 inhibition. Additionally, inactivating mutations of UTX, one of two validated H3K27me3 demethylases, have been observed in numerous

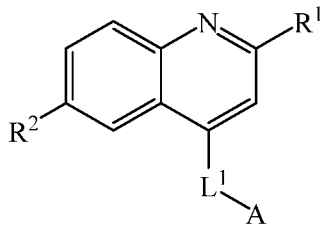
solid and hematologic malignancies, lending further evidence for the oncogenic activity of PRC2.

Interestingly, recent research has identified a putative tumor suppressor function for PRC2 in T-cell acute lymphoblastic leukemia. As such, chemical probes of H3K27me3 biology are urgently needed for mechanistic and translational research. Recent studies demonstrate that EZH2 knockdown in murine models of a genetically-defined subtype of acute leukemia (MLL-AF9) results in a less aggressive phenotype and cellular differentiation. PRC2 target genes are reactivated by RNA interference, including the tumor suppressors p16 and p19. Additionally, the well-studied MYC gene set that is associated with aggressive cancers was observed to be suppressed following EZH2 inactivation. The effect was generally modest as EZH1 could compensate and residual H3K27me3 was present in the EZH2 knockdown mice. An alternative knockdown of EED, another PRC2 subunit led to complete depletion of H3K27me3, and a dramatic response in the mouse model of leukemia. These data would suggest that, rather than blocking the methyltransferase activity of EZH2 in cancer, a more effective approach may be inhibition of CBX chromodomain binding to the H3K27me3 mark placed by EZH1 and EZH2. In support of this hypothesis, a discrete chromobox homolog (CBX8) has been shown to be required for MLL-AF9 induced leukemogenesis, using genetic constructs. Importantly, these studies using Cbx8-deficient mice failed to identify a lethal or debilitating phenotype, even with respect to hematopoiesis, supporting a putative therapeutic window for CBX inhibitors. As CBX chromodomains are the only reported chromatin readers for H3K27me3, molecules that inhibit the ability of CBX to bind H3K27me3 would serve as valuable chemical probes of polycomb biology in cancer, as well as in stem cell and developmental biology. CBX inhibitors could be used to address the hypothesis that polycomb readers are promising nodes for pharmacologic intervention in H3K27me3 enriched cancers.

Thus, there is a continuing need for pharmacologic agents that antagonize CBX chromodomains and that can be used to manipulate CBX in therapeutic or experimental applications.

Summary of Invention

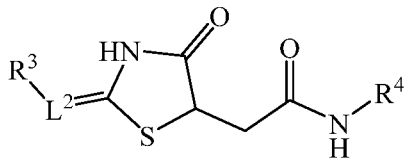
In one aspect, the invention relates to compounds having the structure of Formula I or a pharmaceutically acceptable salt thereof:



I

wherein R^1, R^2, L^1 and A are defined herein.

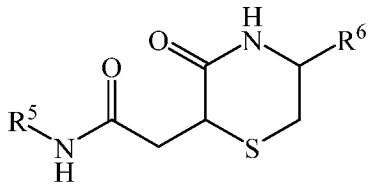
In another aspect, the invention relates to compounds having the structure of Formula II or a pharmaceutically acceptable salt thereof:



II

wherein R^3, R^4, and L^2 are defined herein.

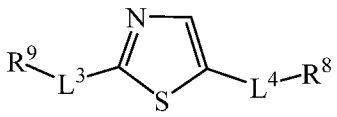
In another aspect, the invention relates to compounds having the structure of Formula III or a pharmaceutically acceptable salt thereof:



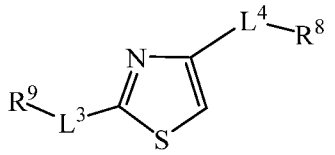
III

wherein R^5 and R^6 are defined herein.

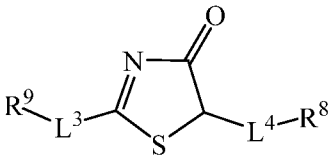
In another aspect, the invention relates to compounds having the structure of Formula IV, V, or IX or a pharmaceutically acceptable salt thereof:



IV



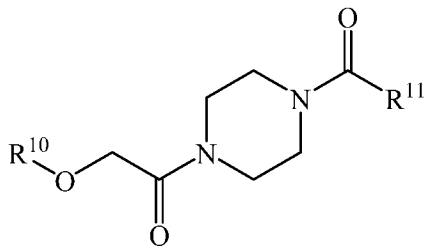
V



IX

wherein R^8, R^9, L^3 and L^4 are defined herein.

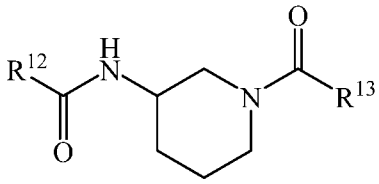
In another aspect, the invention relates to compounds having a structure of Formula VI or a pharmaceutically acceptable salt thereof:



VI

wherein R^{10} and R^{11} are defined herein.

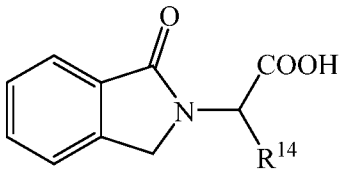
In another aspect, the invention relates to compounds having a structure of Formula VII or a pharmaceutically acceptable salt thereof:



VII

wherein R^{12} and R^{13} are defined herein.

In another aspect, the invention relates to compounds having a structure of Formula VIII or a pharmaceutically acceptable salt thereof:



VIII

wherein R^{14} is defined herein.

In another aspect, the invention relates to pharmaceutical compositions of a compound of one of Formulas I-IX and a pharmaceutically acceptable carrier.

The invention also relates to methods of treating or preventing a disease or condition comprising administering a compound or composition of the invention. In certain embodiments, the disease is cancer. The invention further relates to methods of inhibiting proliferation of a cancer cell, comprising contacting a cancer cell with a compound or composition of the invention.

The invention also relates to methods of inhibiting CBX, comprising contacting a cell with a compound or composition of the invention.

Brief Description of the Figures

Figure 1 is a schematic representation of the CBX AlphaScreen Assay.

Figure 2 includes three compounds identifying scaffolds selected for optimization.

Figure 3 is a plot of the results for the AlphaScreen assay for compounds ACV-106 (circle), ACV-105 (triangle), ACV-108 (square), ACV-110 (triangle).

Figures 4A-4D are plots of the results for the AlphaScreen assay for ACV-037 (Fig. 4A), ACV-036 (Fig. 4 B), ACV-038 (Fig. 4C), and ACV-044 (Fig. 4D).

Figures 5A-5D are plots of the results for the AlphaScreen assay for ACV-47-C (Fig. 5A), ACV-047-E1 (Fig. 5B), ACV-047-D (Fig. 5C), and ACV-047-E2 (Fig. 5D).

Figure 6 is a plot the results for the AlphaScreen assay for certain compounds of Formula II.

Figure 7 is a plot the results for the AlphaScreen assay for certain compounds of Formula I.

Figure 8 is a plot if the initial Cap Scan Results for certain compounds of Formula II.

Figure 9 is a plot the results for the AlphaScreen assay for certain compounds of Formula II.

Figure 10 is a bar graph depicting data representing decreased viability of HCT292 human lung cancer cells after 96 hours in the presence of certain compounds of the invention at various doses.

Figure 11 is a plot depicting data representing decreased viability of HCT292 human lung cancer cells in the presence of certain compounds of the invention.

Figure 12 is a bar graph depicting data representing decreased viability of HCT292 human lung cancer cells after 96 hours in the presence of certain compounds of the invention at various doses.

Figure 13 is a plot of the results for the AlphaScreen assay for certain compounds of Formulas I, II, IV and V.

Figure 14 is a bar graph depicting data representing decreased viability of HCT292 human lung cancer cells after 96 hours in the presence of certain compounds of the invention at various doses.

Figure 15 is a plot of the ^{15}N NMR spectrum vs. the ^{1}H NMR spectrum, which shows binding between ACV-2-112 with CBX7.

Figure 16 is a plot of the results for the AlphaScreen assay for certain compounds disclosed herein in comparison to a known compound.

Figure 17 is a plot of the results for the AlphaScreen assay for certain compounds disclosed herein.

Figure 18 is a bar graph depicting data representing decreased viability of HCT292 human lung cancer cells after 96 hours in the presence of certain compounds of the invention at various doses.

Figure 19 is a bar graph depicting data representing decreased viability of HCT292 human lung cancer cells after 96 hours in the presence of certain compounds of the invention at various doses.

Figure 20 shows the ITC data for CBX7 with certain small molecule inhibitors disclosed herein.

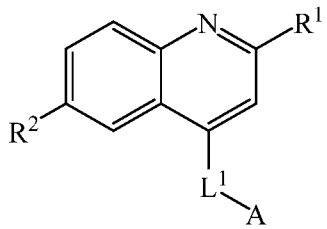
Figures 21A-21D show the biological activity of certain compounds of the invention in cancer cell lines sensitive to CBX inhibition and insensitive to CBX inhibition.

Detailed Description of the Invention

In certain aspects, the invention provides various novel compounds, and pharmaceutical compositions thereof. In particular, such compounds are useful as CBX inhibitors, and thus can be used to treat or prevent a disease or condition (e.g., cancer).

I. COMPOUNDS

In certain embodiments, the invention relates to compounds having the structure of Formula (I), or a pharmaceutically acceptable salt thereof:



I

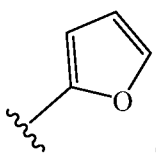
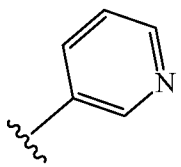
wherein

R^1 is optionally substituted aryl or heteroaryl;

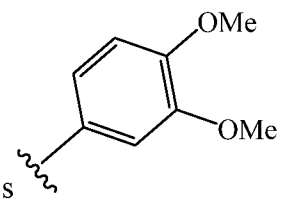
R^2 is H, halo, optionally substituted aryl, or optionally substituted heteroaryl;

L^1 is -C(O)-, -C(O)NH-, optionally substituted -C(O)NH-alkylene-, or -C(O)NHNCH-; and

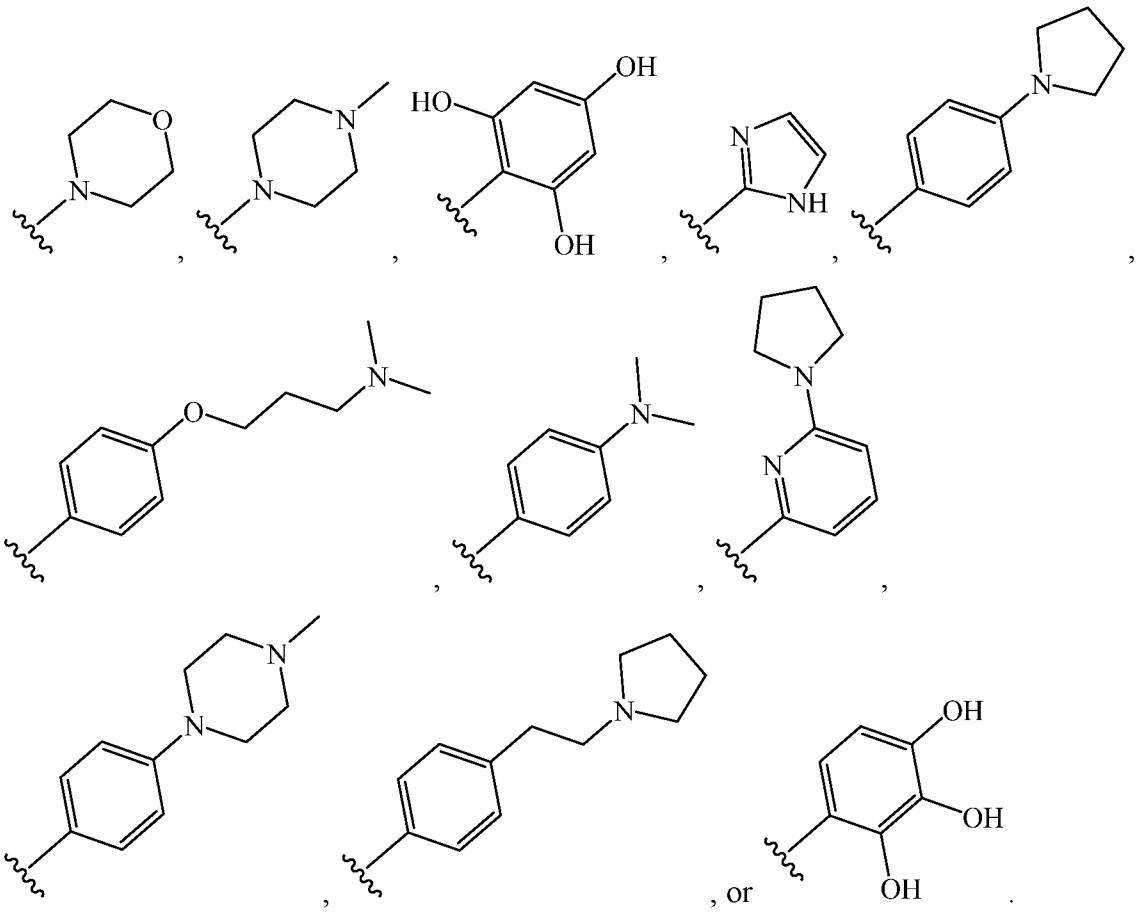
A is OH or optionally substituted heterocyclyl, aryl, or heteroaryl.

In certain embodiments, R^1 is  or  .

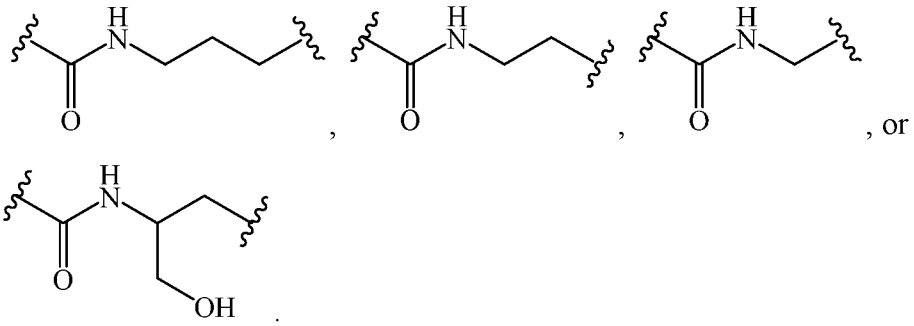
In certain embodiments, R^2 is Br.

In certain embodiments, R^2 is  .

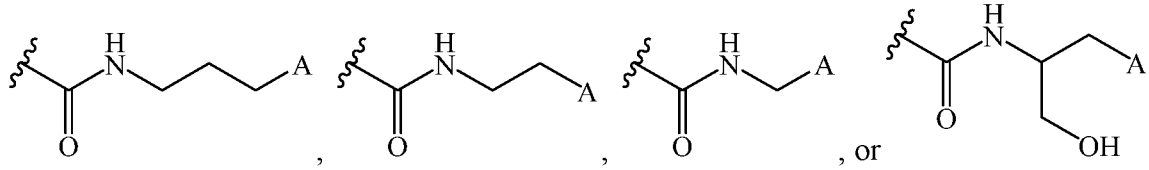
In certain embodiments, A is

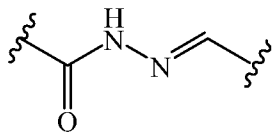


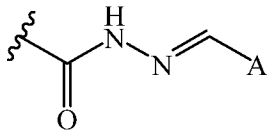
In certain embodiments, L^1 is



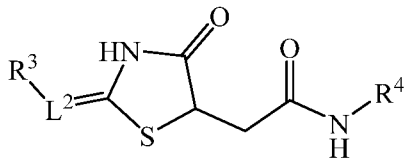
In certain embodiments, L^1-A is



In certain embodiments, L^1 is  .

In certain embodiments, L^1-A is  .

In certain embodiments, the invention relates to compounds having the structure compound having a structure of Formula II or a pharmaceutically acceptable salt thereof:



II

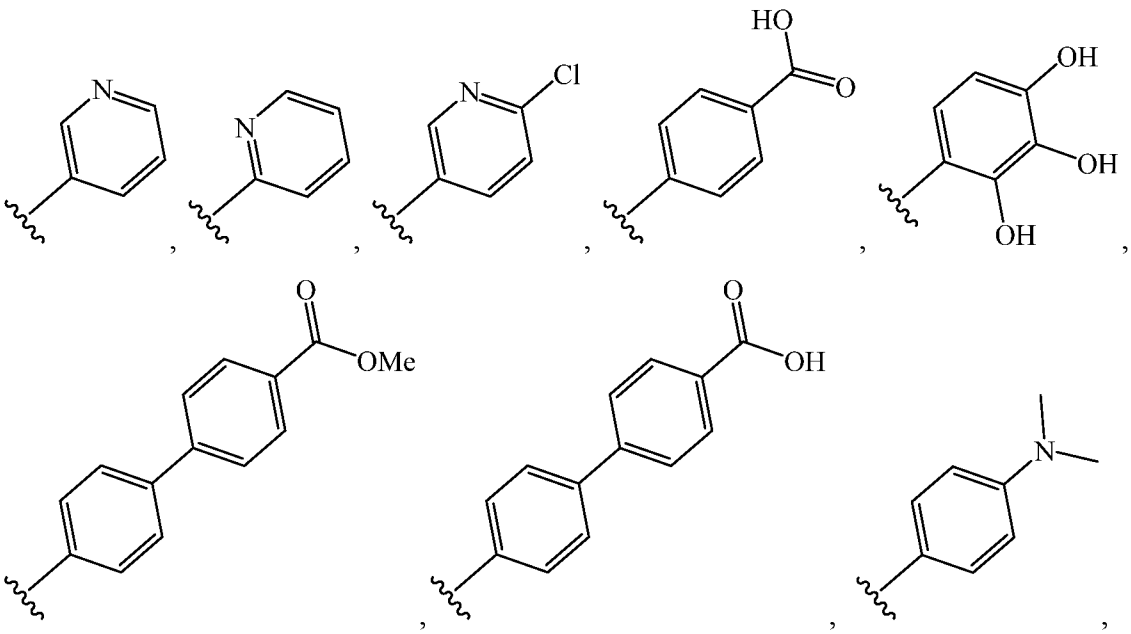
wherein

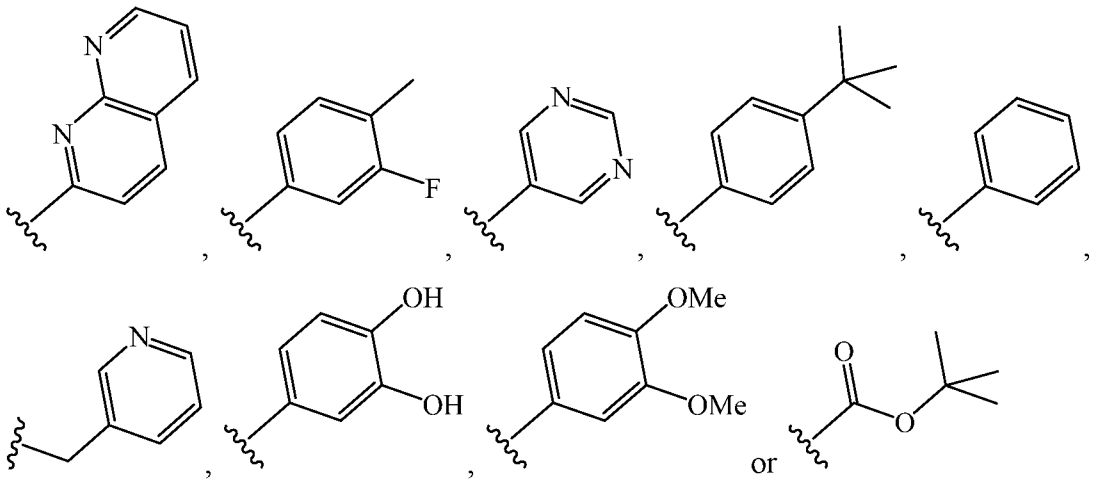
R^3 is optionally substituted aryl, heteroaryl, -C(O)-aryl, alkyl, or alkoxycarbonyl;

R^4 is optionally substituted alkyl, aryl or heteroaryl; and

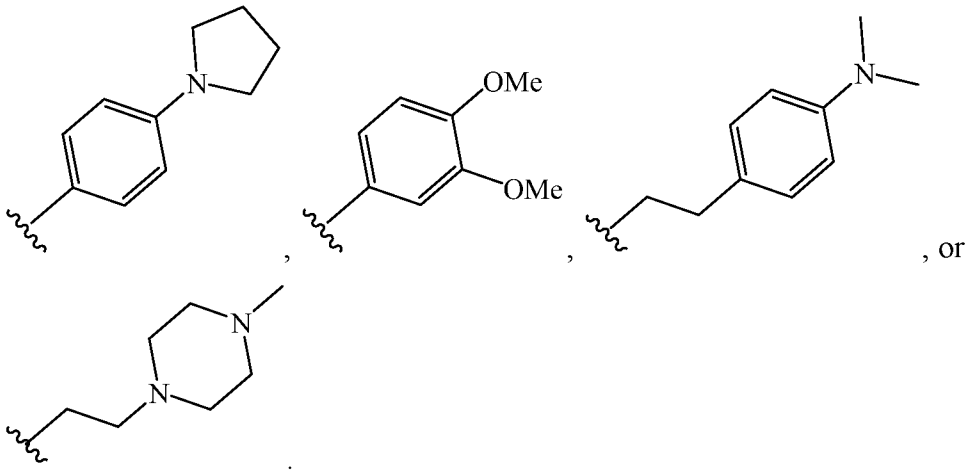
L^2 is =N-C(O)-, =N-NCH-, =N-, or =N-NH-.

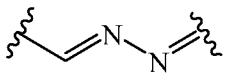
In certain embodiments, R^3 is



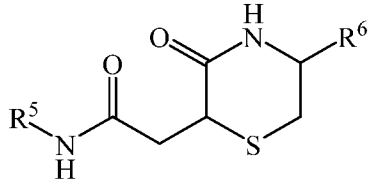


In certain embodiments, R^4 is



In certain embodiments, L^2 is  .

In certain embodiments, the invention relates to compounds having a structure of Formula III or a pharmaceutically acceptable salt thereof:



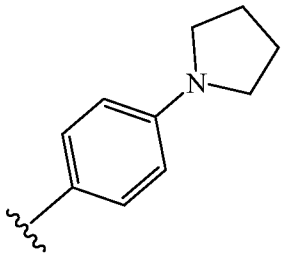
III

wherein

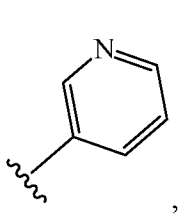
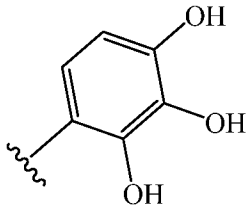
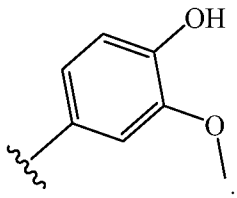
R^5 is optionally substituted aryl or heteroaryl;

R^6 is -C(O)NH-NCH-R^7 or -C(O)O-alkyl; and

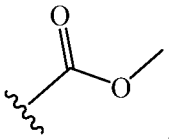
R^7 is optionally substituted aryl or heteroaryl.

In certain embodiments, R^5 is  .

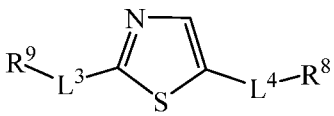
In certain embodiments, R^7 is

 ,  , or  .

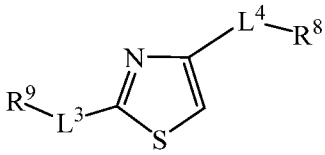
In certain embodiments, R^6 is

 .

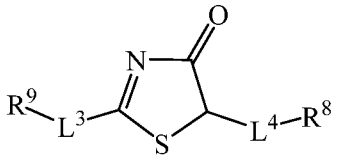
In certain embodiments, the invention relates to compounds having a structure of Formula IV, V, IX or a pharmaceutically acceptable salt thereof:



IV



V



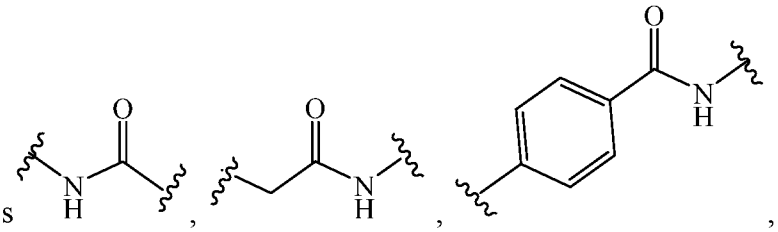
IX

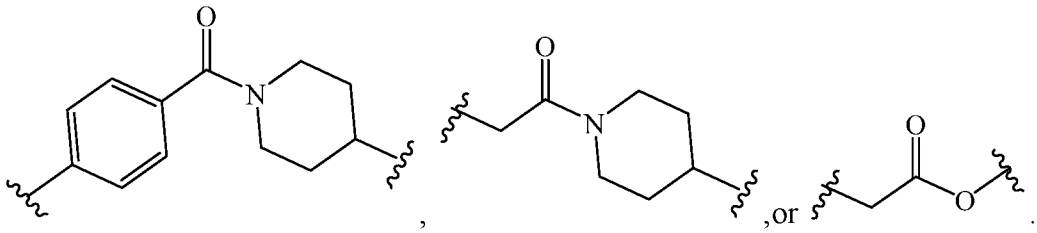
wherein

R^8 is optionally substituted alkyl, aryl, or heteroaryl ;

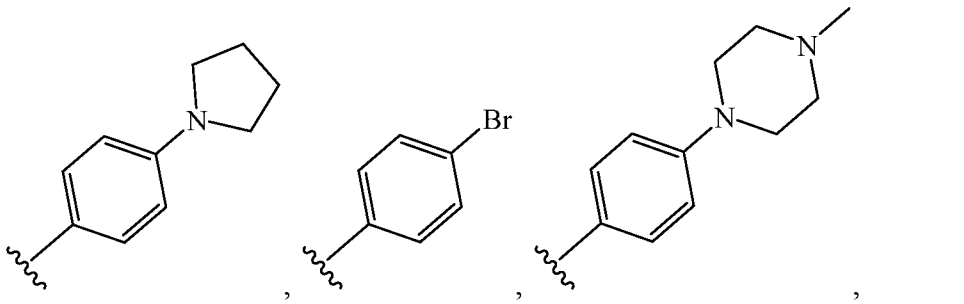
R^9 is optionally substituted aryl or heteroaryl;

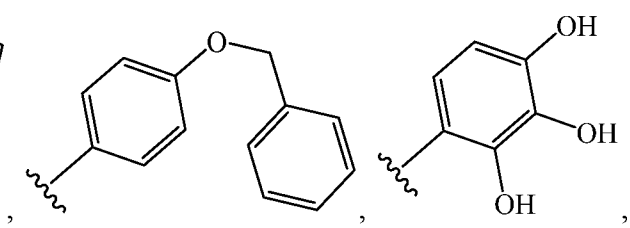
L^3 is -NH-CO-NH-, -NH-CO-, -NH-NCH_2-, -NH-, -CH_2-, or -CH_2-NH-CO-; and

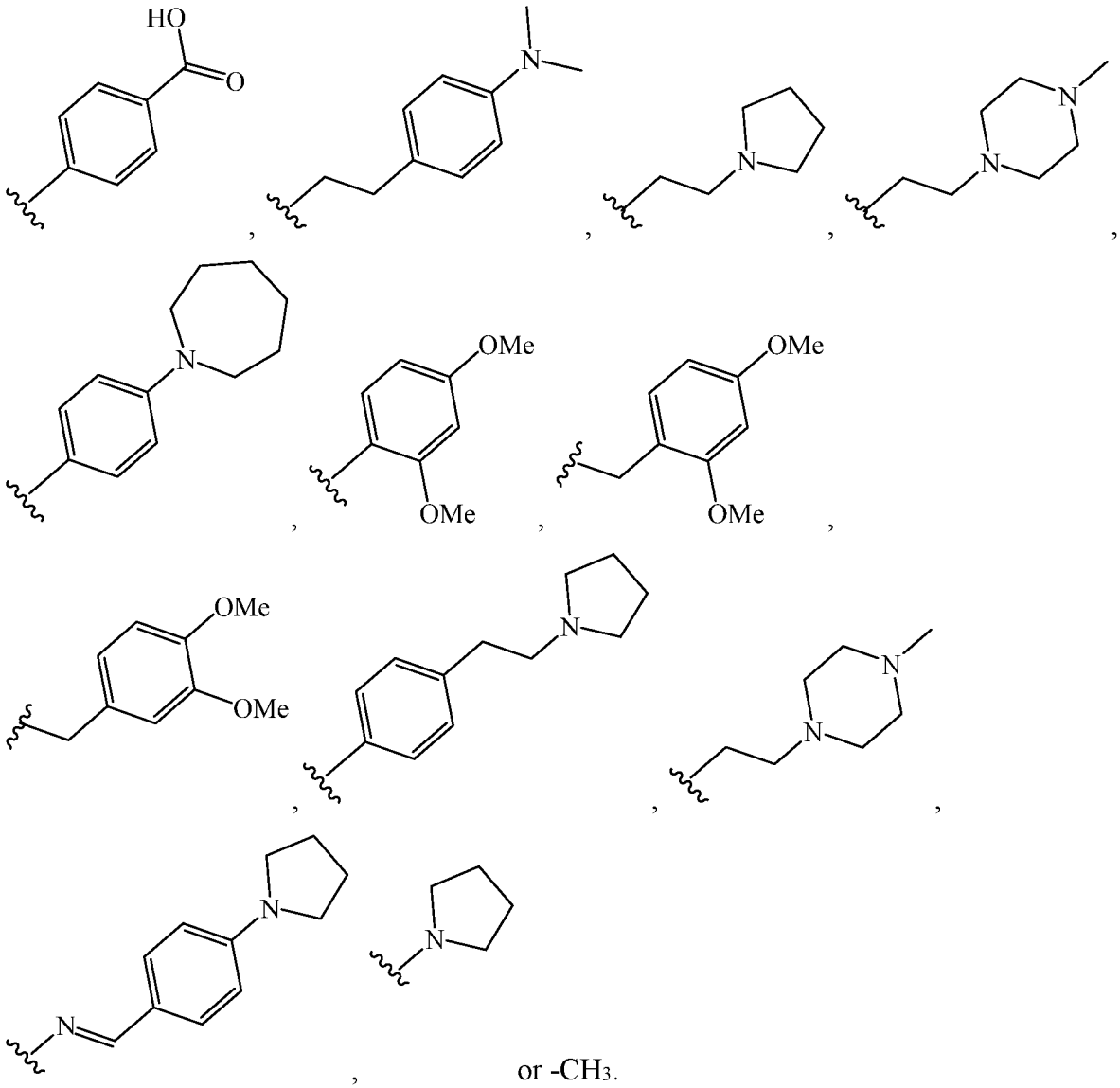
L^4 is absent or is 



In certain embodiments, R^8 is

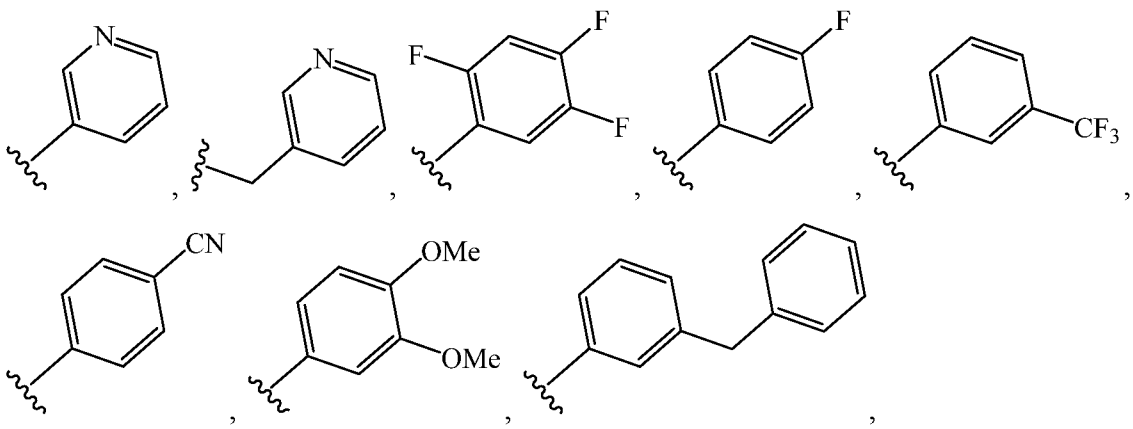


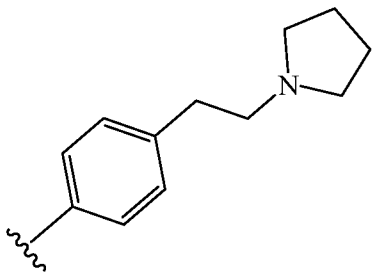
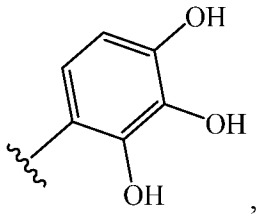
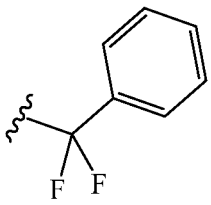
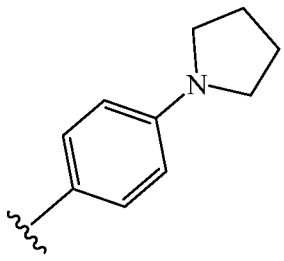
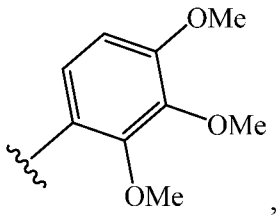
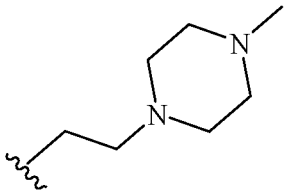
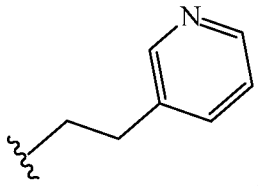
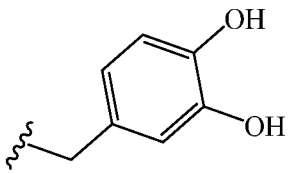
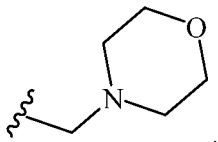




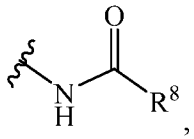
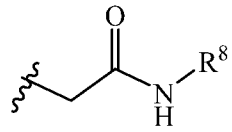
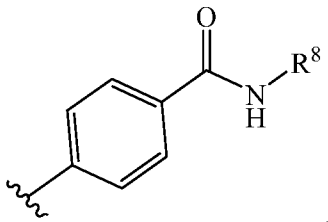
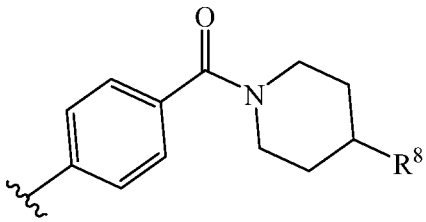
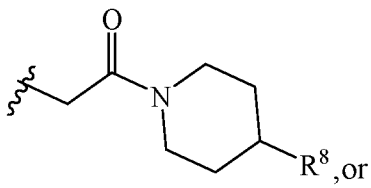
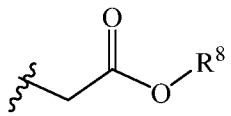
, or -CH_3.

In certain embodiments, R^9 is

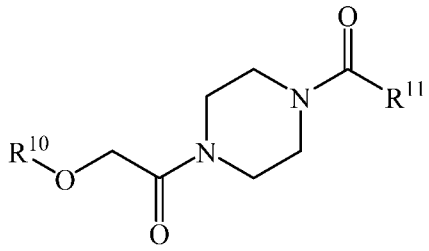


In certain embodiments, L^3-R^9 is -NH-CO-NH-R^9, -NH-CO-R^9, -NH-NCH_2-R^9, or -CH_2-NH-CO-R^9.

In certain embodiments, L^4 is 





.

In certain embodiments, the invention relates to compounds having a structure of Formula VI or a pharmaceutically acceptable salt thereof:



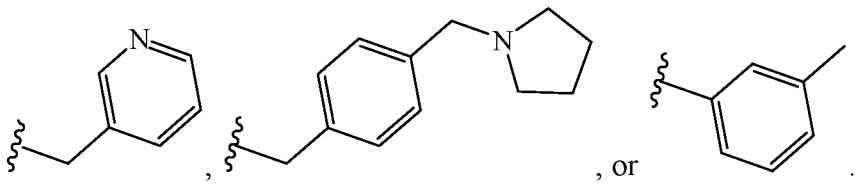
VI

wherein

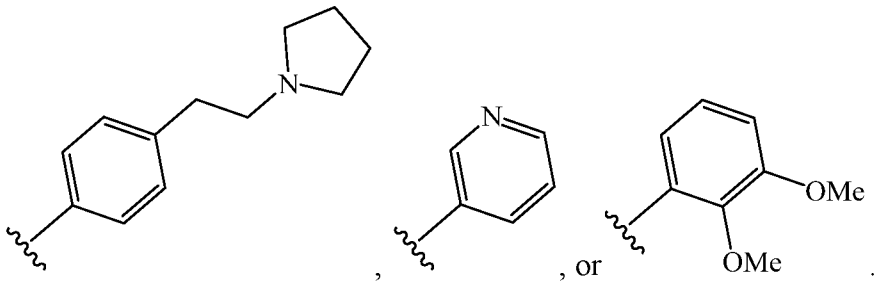
R^{10} is optionally substituted alkyl, aryl or heteroaryl; and

R^{11} is optionally substituted aryl or heteroaryl.

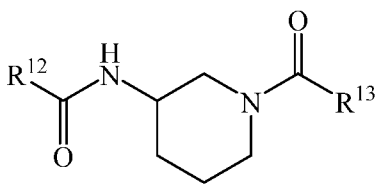
In certain embodiments, R^{10} is



In certain embodiments, R^{11} is



In certain embodiments, the invention relates to compounds having a structure of Formula VII or a pharmaceutically acceptable salt thereof:



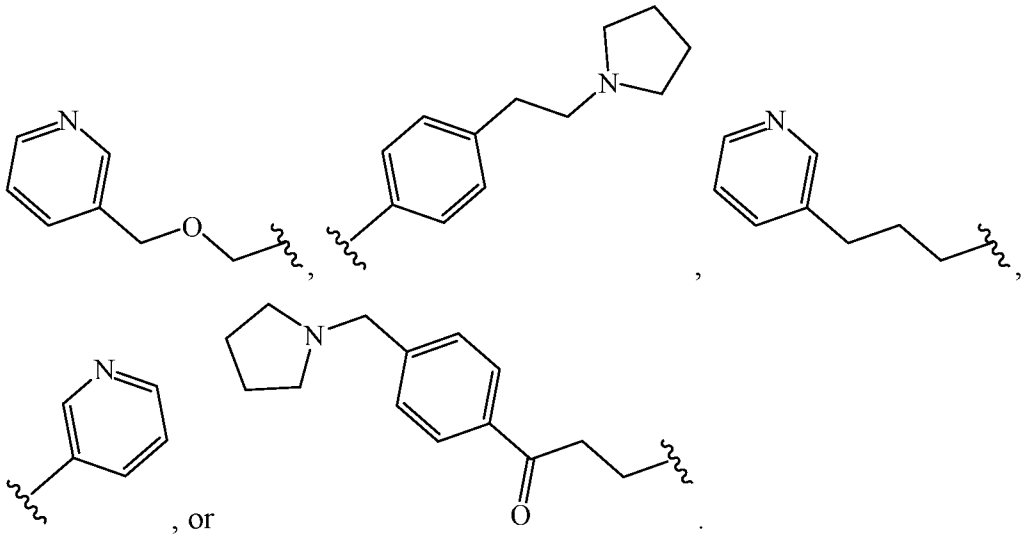
VII

wherein

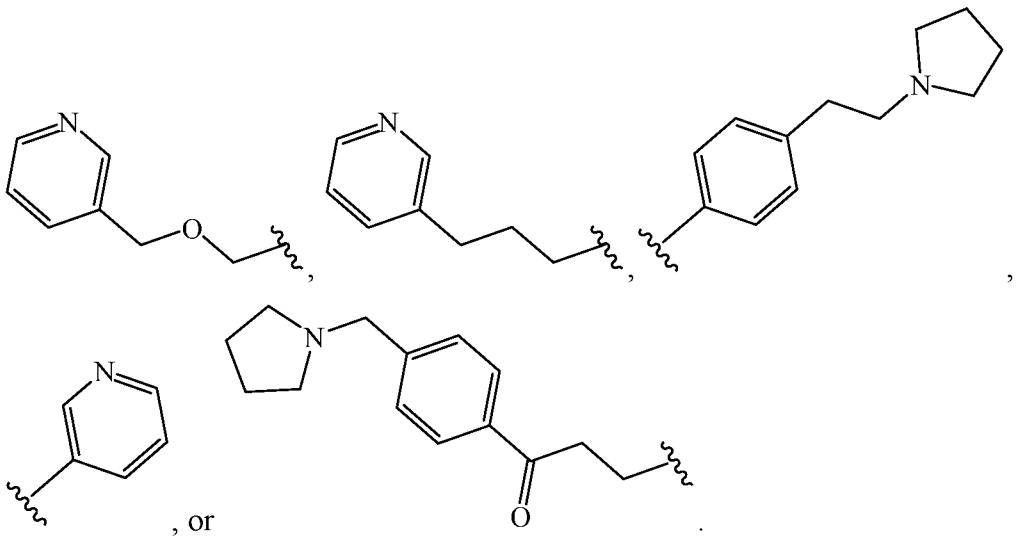
R^{12} is optionally substituted alkyl, aryl or heteroaryl; and

R^{13} is optionally substituted aryl or heteroaryl.

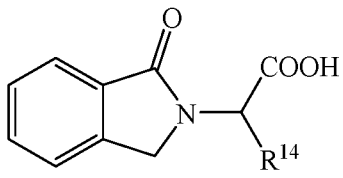
In certain embodiments, R^{13} is



In certain embodiments, R^{12} is

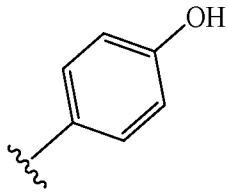
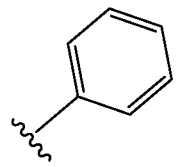
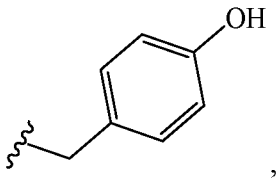
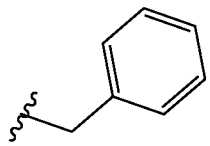
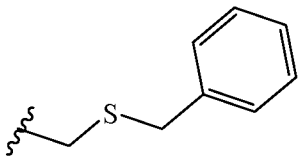


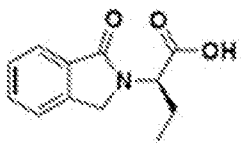
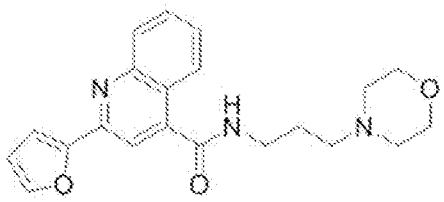
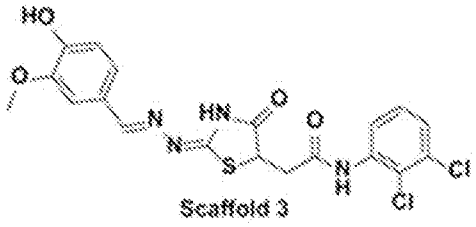
In certain embodiments, the invention relates to compounds having a structure of Formula VIII or a pharmaceutically acceptable salt thereof:



VIII

wherein R^{14} is optionally substituted alkyl or aryl.

In certain embodiments, R^{14} is -ethyl,  ,  ,  ,  , or  .

In certain embodiments, the compound is any of those described herein provided that the compound is not  ,  , or 

.

In certain embodiments, compounds of the invention may be racemic. In certain embodiments, compounds of the invention may be enriched in one enantiomer. For example, a compound of the invention may have greater than 30% ee, 40% ee, 50% ee, 60% ee, 70% ee, 80% ee, 90% ee, or even 95% or greater ee. The compounds of the invention have more than one stereocenter. Consequently, compounds of the invention may be enriched in one or more diastereomer. For example, a compound of the invention may

have greater than 30% de, 40% de, 50% de, 60% de, 70% de, 80% de, 90% de, or even 95% or greater de.

In certain embodiments, as will be described in detail below, the present invention relates to methods of treating or preventing a disease or condition with a compound of Formula I, or a pharmaceutically acceptable salt thereof. In certain embodiments, the therapeutic preparation may be enriched to provide predominantly one enantiomer of a compound of one of Formulas I-IX. An enantiomerically enriched mixture may comprise, for example, at least 60 mol percent of one enantiomer, or more preferably at least 75, 90, 95, or even 99 mol percent. In certain embodiments, the compound enriched in one enantiomer is substantially free of the other enantiomer, wherein substantially free means that the substance in question makes up less than 10%, or less than 5%, or less than 4%, or less than 3%, or less than 2%, or less than 1% as compared to the amount of the other enantiomer, *e.g.*, in the composition or compound mixture. For example, if a composition or compound mixture contains 98 grams of a first enantiomer and 2 grams of a second enantiomer, it would be said to contain 98 mol percent of the first enantiomer and only 2% of the second enantiomer.

In certain embodiments, the therapeutic preparation may be enriched to provide predominantly one diastereomer of a compound of one of Formulas I-IX. A diastereomerically enriched mixture may comprise, for example, at least 60 mol percent of one diastereomer, or more preferably at least 75, 90, 95, or even 99 mol percent.

In certain embodiments, the present invention provides a pharmaceutical preparation suitable for use in a human patient in the treatment of a disease or condition, comprising an effective amount of any compound of one of Formulas I-IX, and one or more pharmaceutically acceptable excipients. In certain embodiments, the pharmaceutical preparations may be for use in treating or preventing a condition or disease as described herein. In certain embodiments, the pharmaceutical preparations have a low enough pyrogen activity to be suitable for use in a human patient.

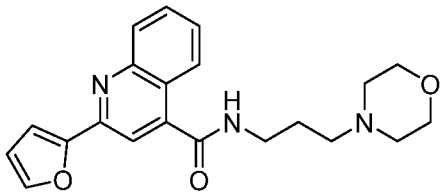
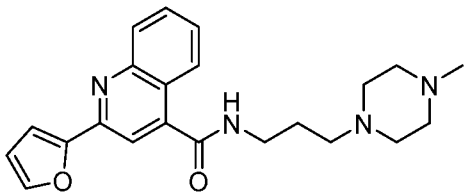
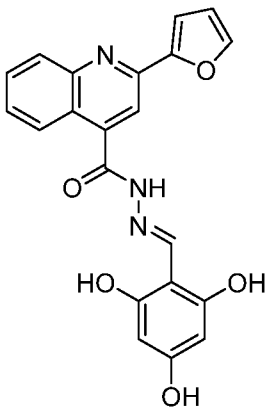
Compounds of any of the above structures may be used in the manufacture of medicaments for the treatment of any diseases or conditions disclosed herein.

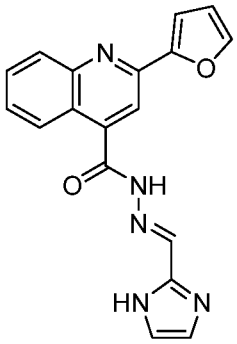
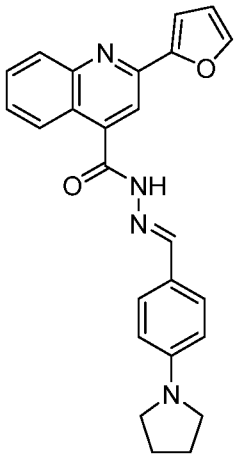
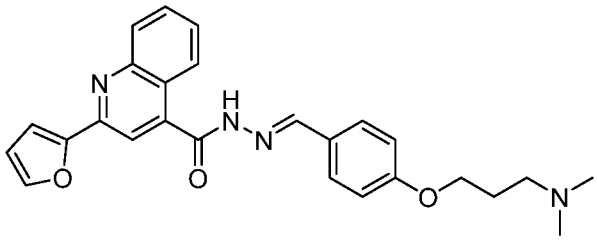
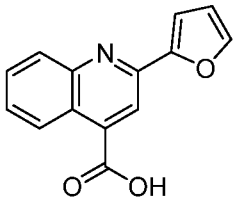
Exemplary compounds of the invention are depicted in Table 1. The compounds of Table 1 are understood to encompass both the free base and the conjugate acid. For example, the compounds in Table 1 may be depicted as complexes or salts with trifluoroacetic acid or hydrochloric acid, but the compounds in their corresponding free

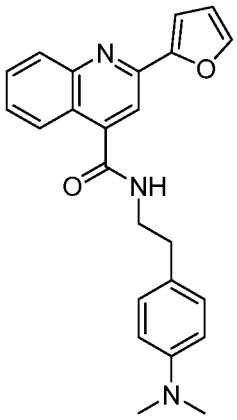
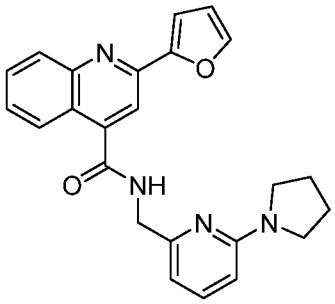
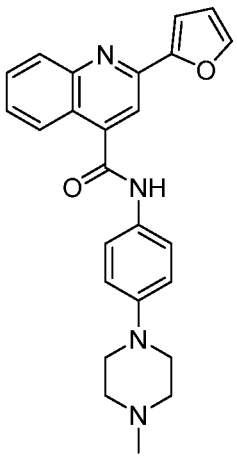
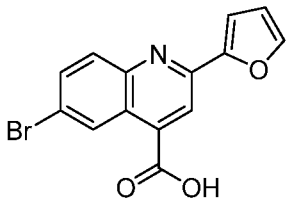
base forms or as salts with other acids are equally within the scope of the invention. Compounds may be isolated in either the free base form, as a salt (e.g., a hydrochloride salt) or in both forms. In the chemical structures shown below, standard chemical abbreviations are sometimes used.

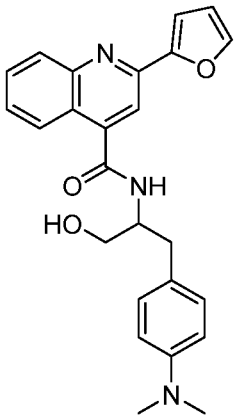
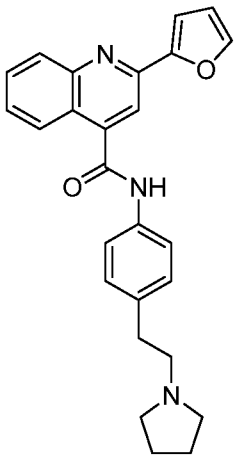
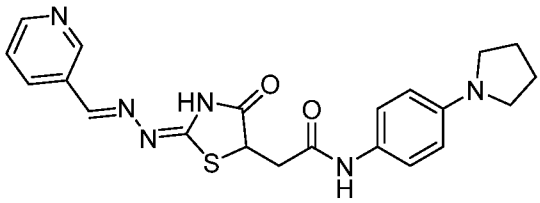
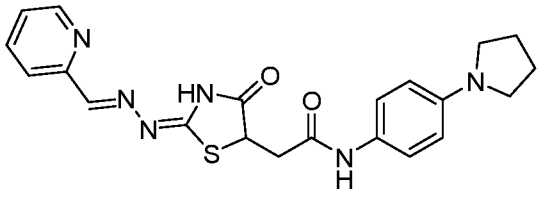
5

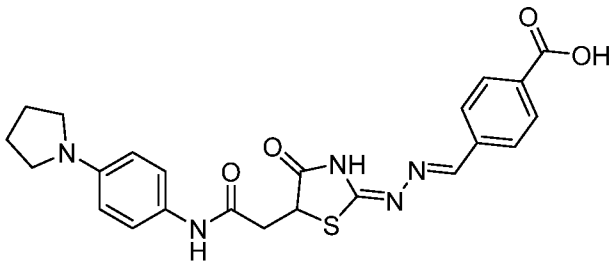
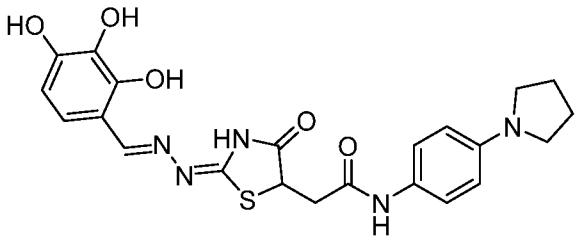
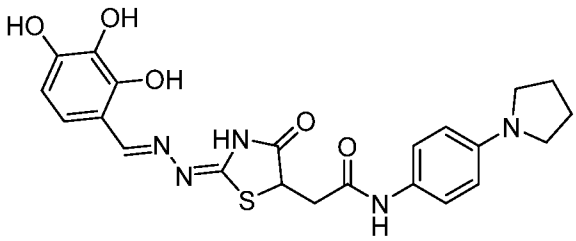
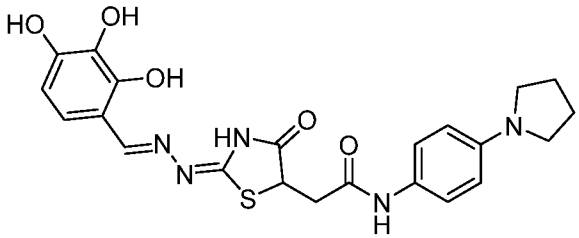
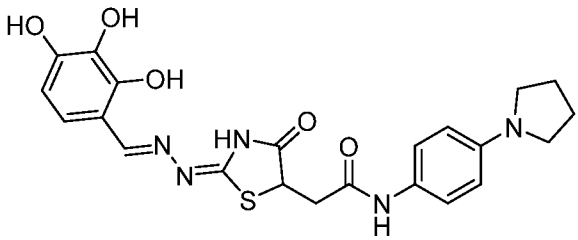
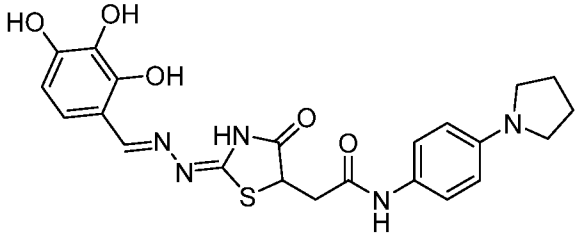
Table 1. *Exemplary Compounds*

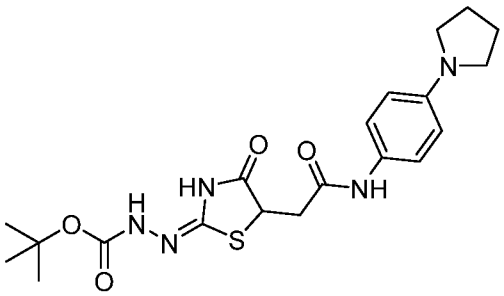
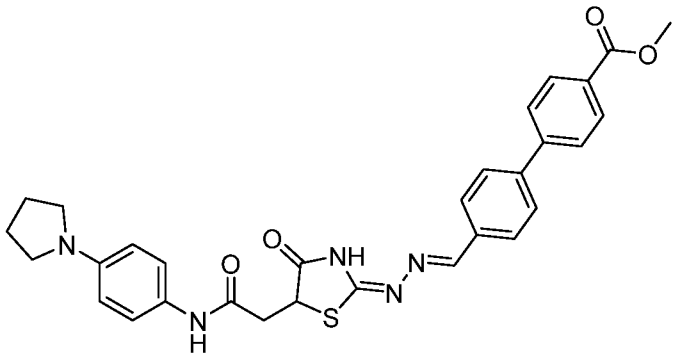
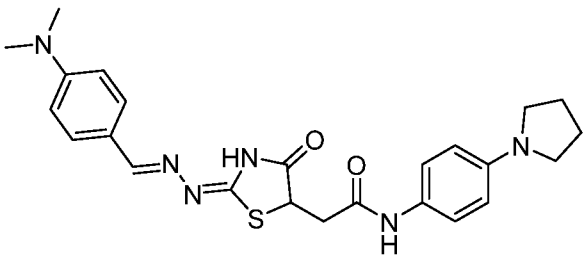
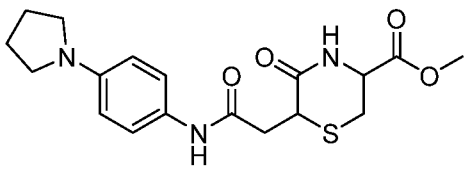
Structure	Compound Name	Alternate name
	ACV-01	ACV-1-180
	ACV-02	ACV-1-182
	ACV-03	ACV-1-183-A

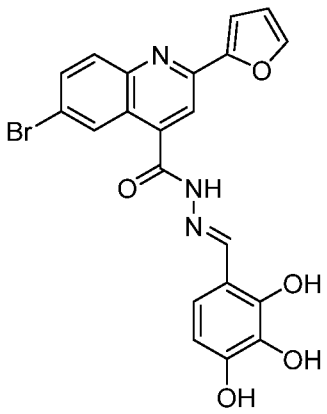
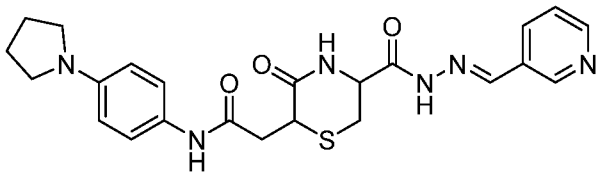
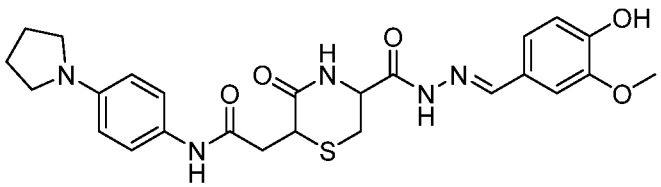
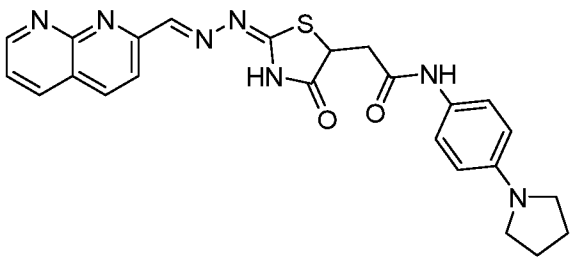
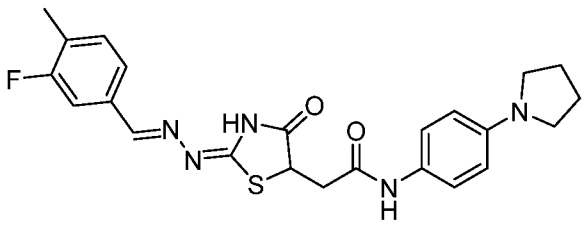
	ACV-04	ACV-1-183-B
	ACV-05	ACV-1-183-D
	ACV-06	ACV-1-183-E
	ACV-07 (1)	ACV-1-190, AJF-109
	ACV-07 (2)	ACV-2-192

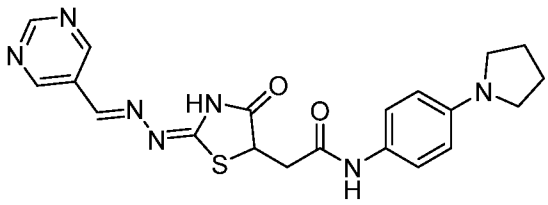
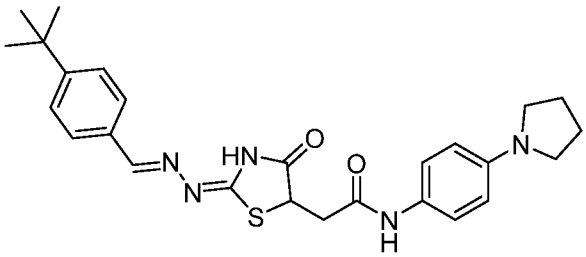
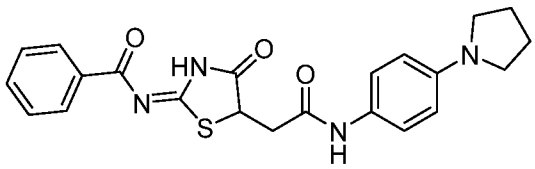
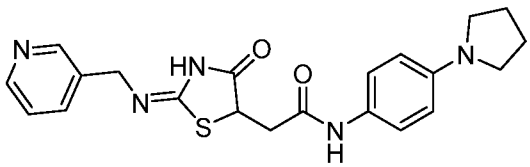
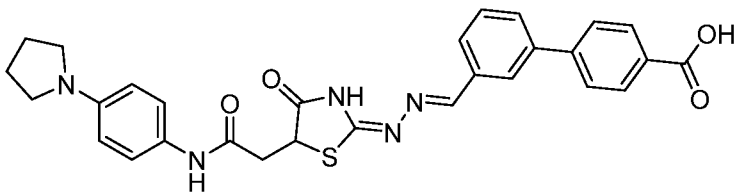
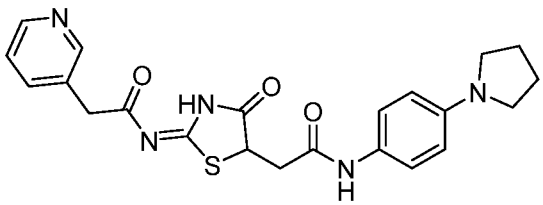
	ACV-08	ACV-1-191
	ACV-09	ACV-1-195-A
	ACV-10	ACV-1-195-D
	ACV-11	ACV-1-202

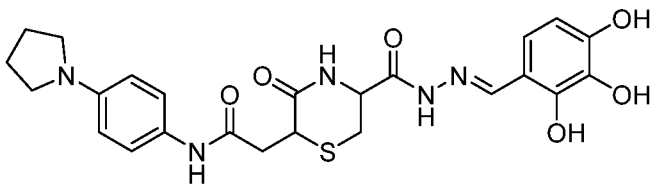
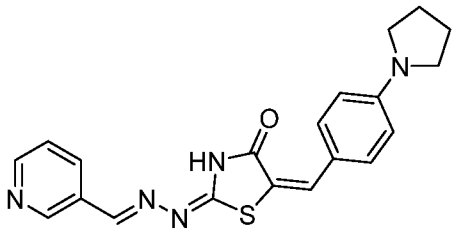
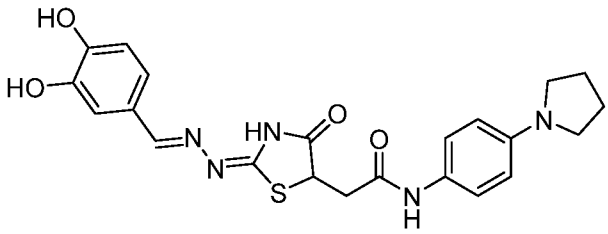
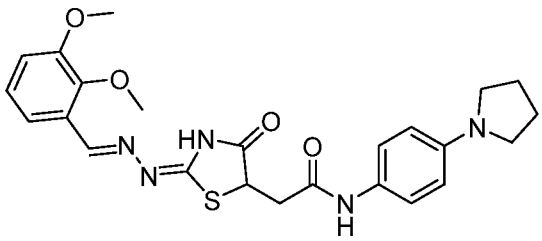
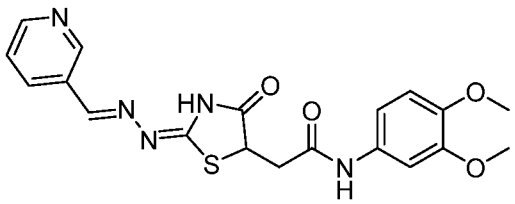
	ACV-12	ACV-1-204
	ACV-13	ACV-1-205
	ACV-14 (1)	ACV-1-242, F1
	ACV-14 (2)	ACV-2-129
	ACV-15	ACV-1-258-A, E12

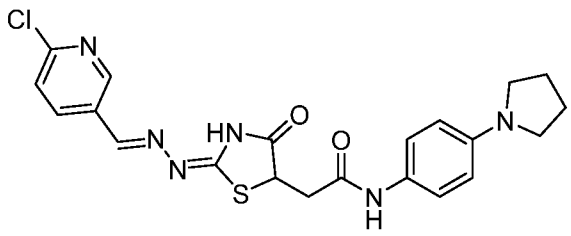
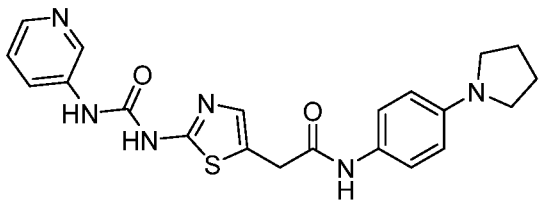
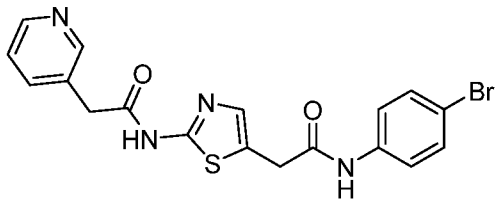
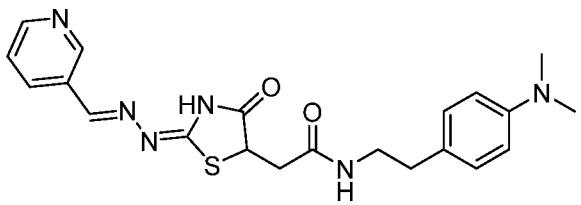
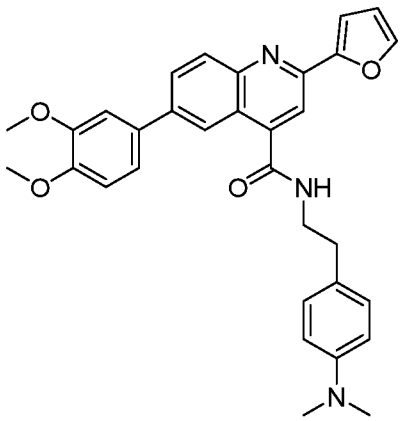
	ACV-16	ACV-1-258-B
	ACV-17 (1)	ACV-1-258-C, A5
	ACV-17 (2)	ACV-2-165
	ACV-17(3)	ACV-2-189
	ACV-17(4)	ACV-2-206-A
	ACV-17(CP)	ACV-258C (CP)

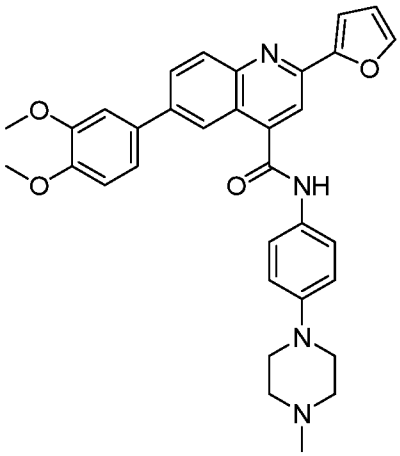
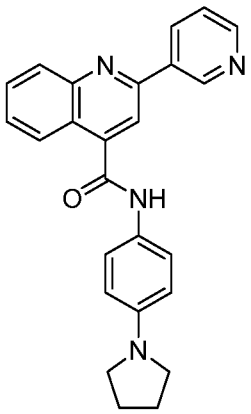
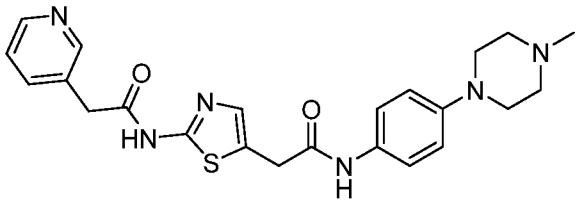
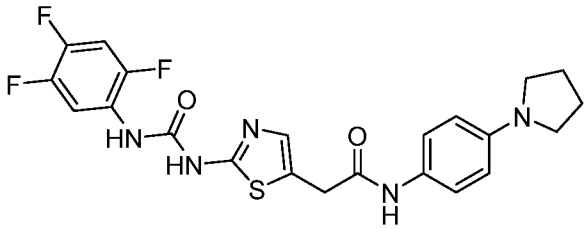
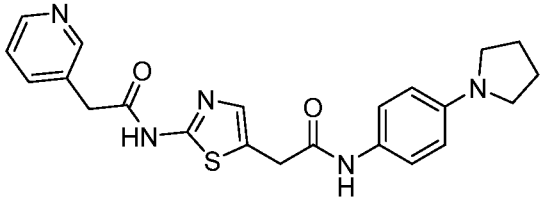
stereoisomer of ACV-17	ACV-17* (P1)	ACV-258C (P1)
The other stereoisomer of ACV-17	ACV-17* (P2)	ACV-258C (P2)
	ACV-18	ACV-1-259
FALK(me3)SK peptide	ACV-19	ACV-1-261
	ACV-20	ACV-1-273
	ACV-21	ACV-1-283-B
	ACV-22	**ACV-1-285-D1**/ACV-1-285-MIX

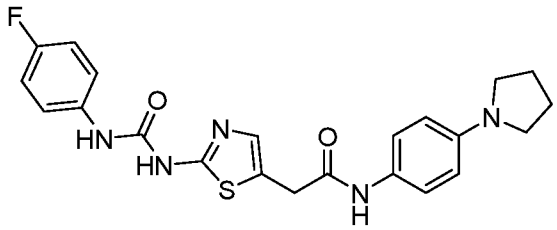
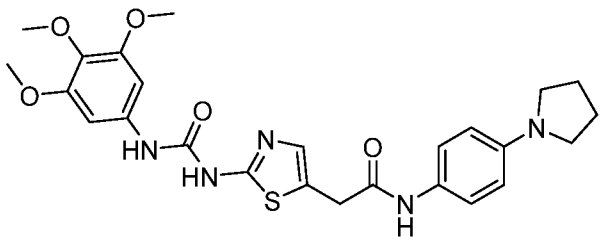
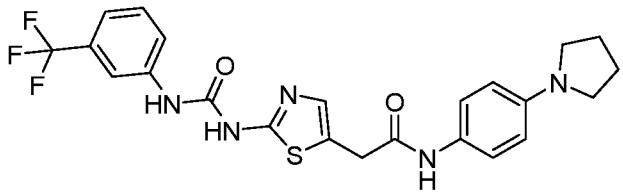
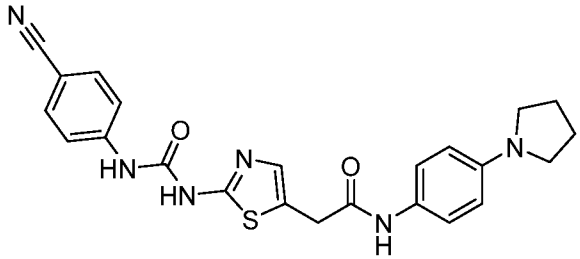
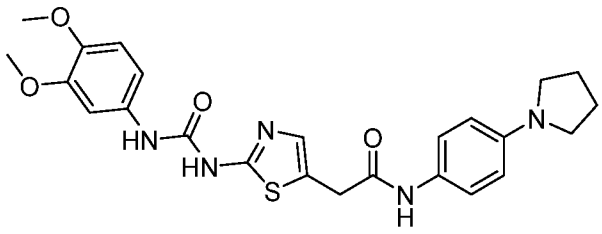
	ACV-22	ACV-1-285-D1/**ACV-1-285-MIX**
	ACV-23	ACV-1-288
	ACV-24	ACV-2-007-A
	ACV-25	ACV-2-007-B
	ACV-26	ACV-2-011-A
	ACV-27	ACV-2-011-B

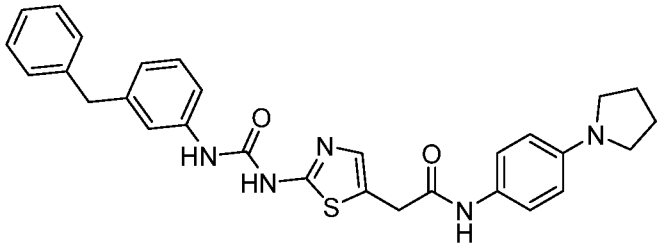
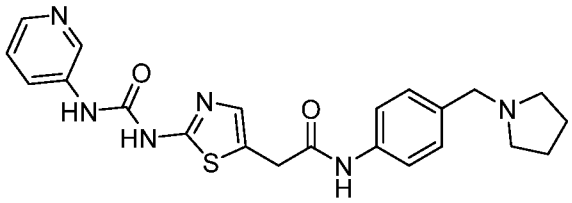
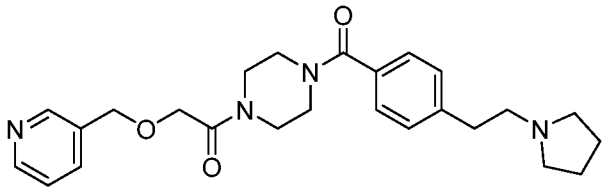
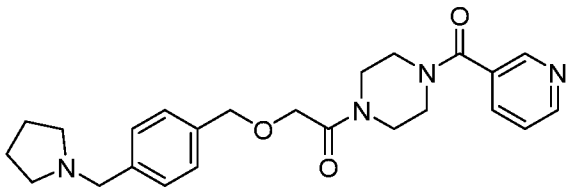
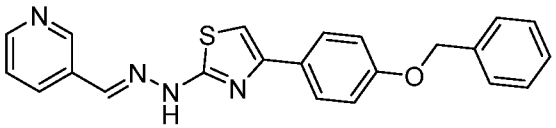
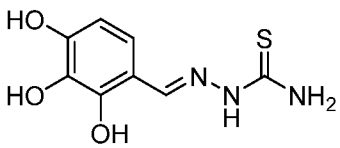
	ACV-28	ACV-2-015
	ACV-29	ACV-2-016-**A**/B
	ACV-29	ACV-2-016-A/**B**
	ACV-30	ACV-2-019
	ACV-31	ACV-2-023
	ACV-32	ACV-2-024
	ACV-33	ACV-2-026

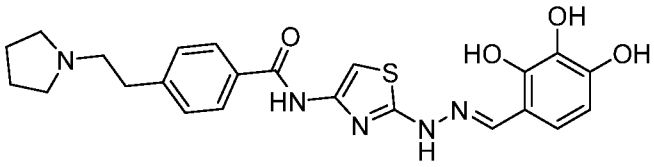
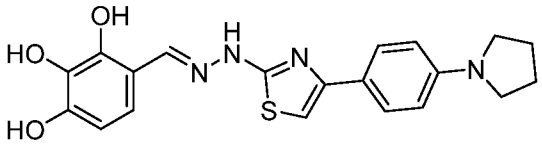
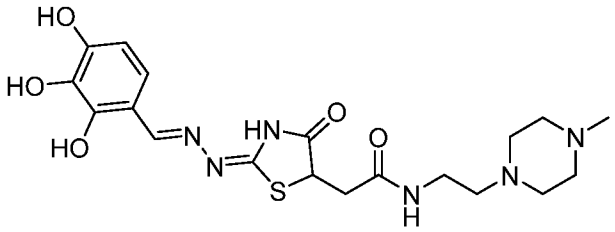
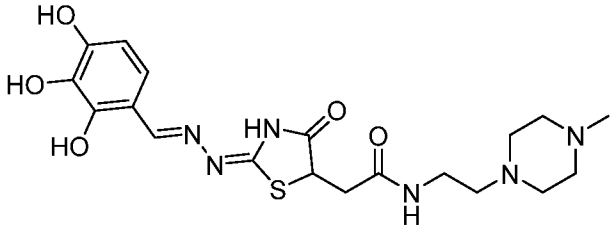
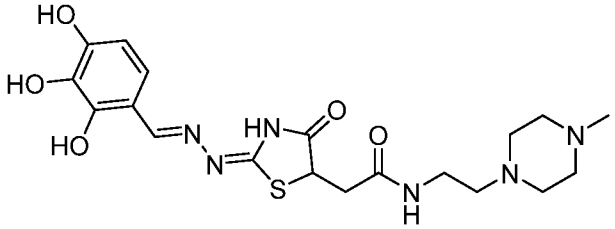
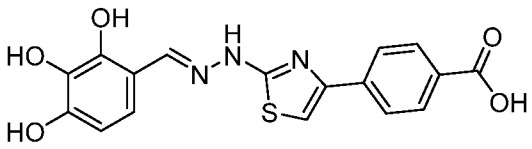
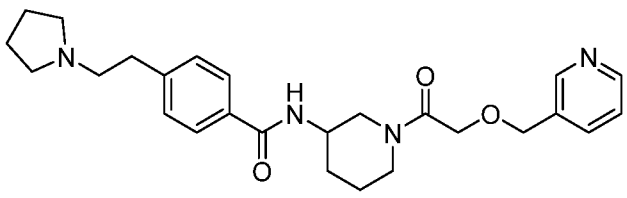
	ACV-34	ACV-2-029
	ACV-35	ACV-2-049
	ACV-36 (1)	ACV-2-082
	ACV-36 (2)	ACV-2-163
	ACV-37	ACV-2-083
	ACV-38	ACV-2-084

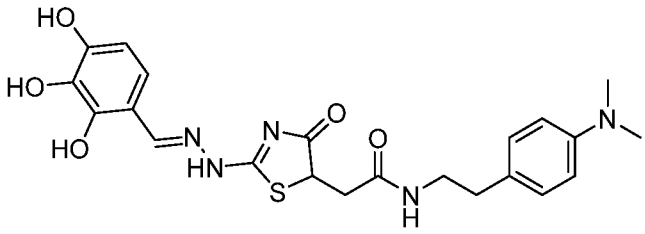
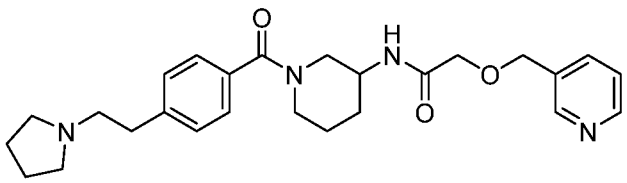
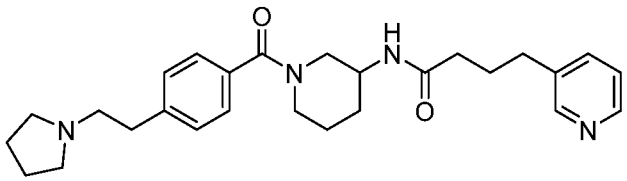
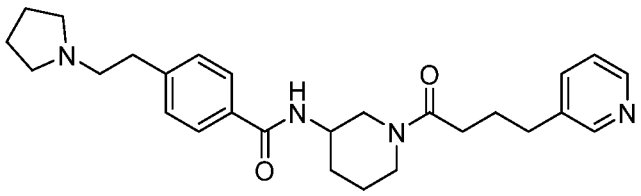
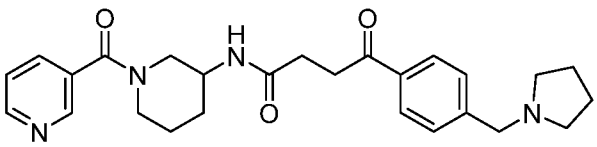
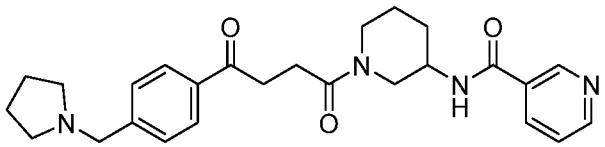
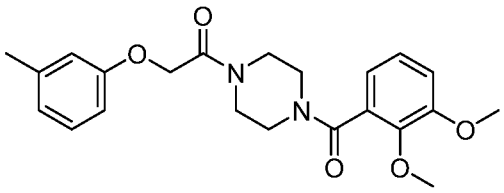
	ACV-39	ACV-2-108
	ACV-40	ACV-2-112
	ACV-41	ACV-2-115
	ACV-42	ACV-2-121
	ACV-43	ACV-2-123

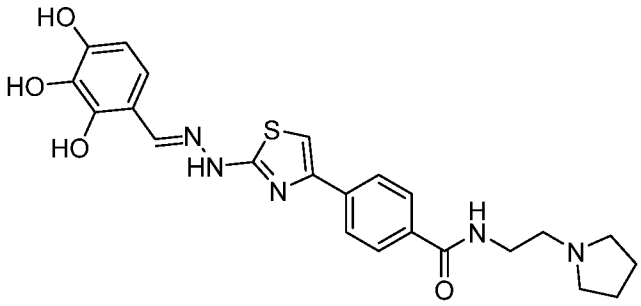
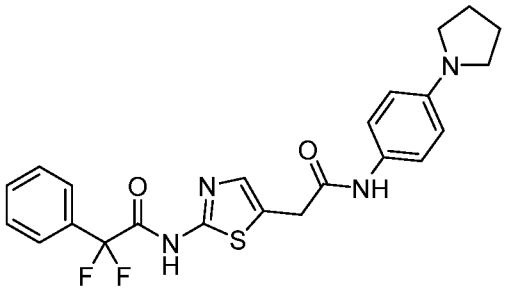
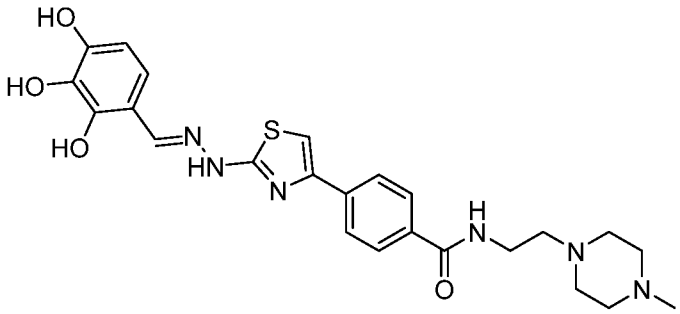
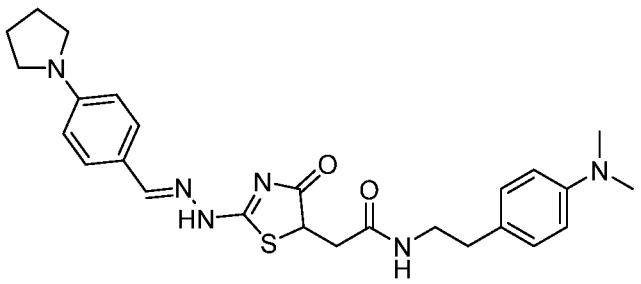
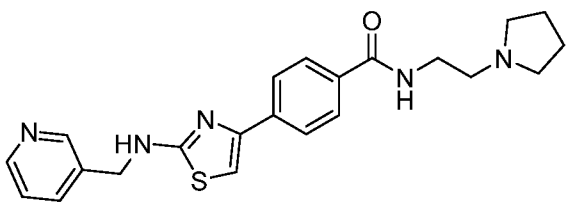
	ACV-44	ACV-2-127
	ACV-45	ACV-2-132
	ACV-46	ACV-2-138
	ACV-47	ACV-2-142
	ACV-48 (1)	ACV-2-147

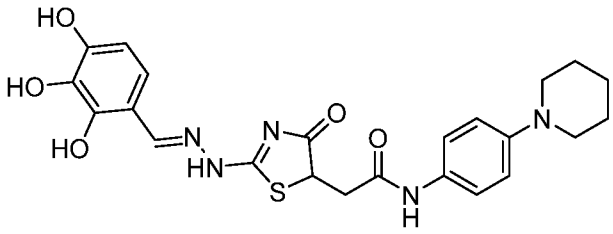
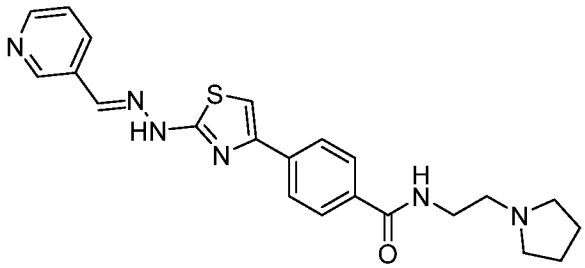
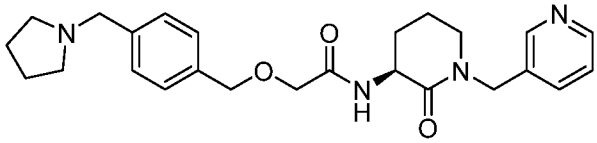
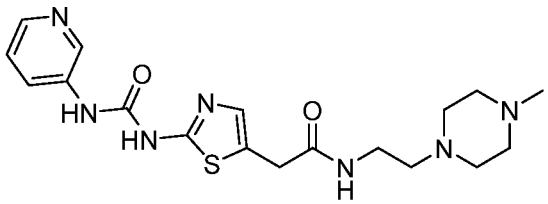
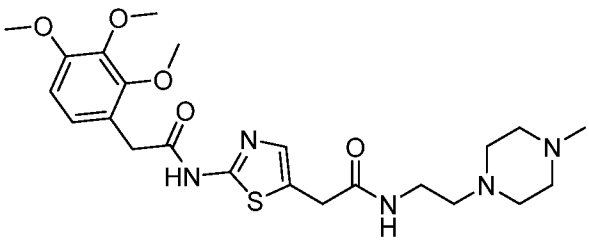
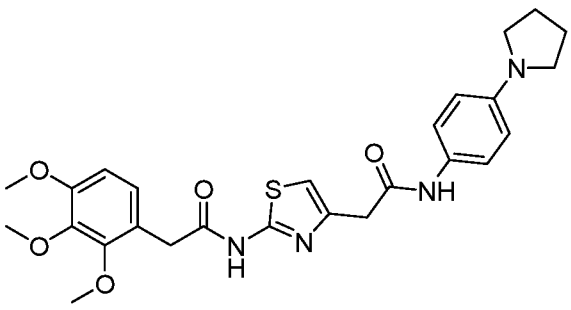
	ACV-48 (2)	ACV-2-147
	ACV-49	ACV-2-150
	ACV-50	ACV-2-151
	ACV-51	ACV-2-152
	ACV-52	ACV-2-154
	ACV-53	ACV-2-155

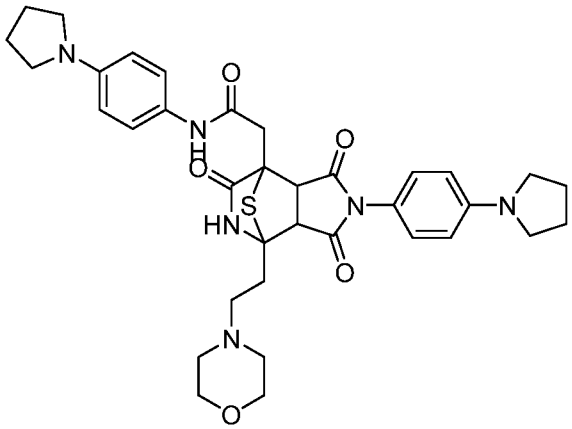
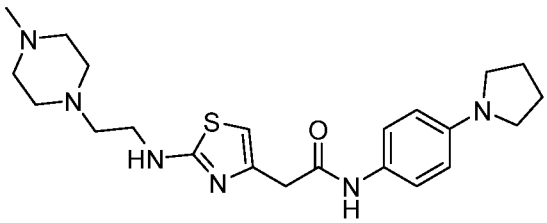
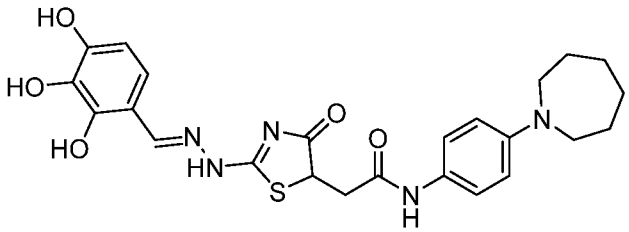
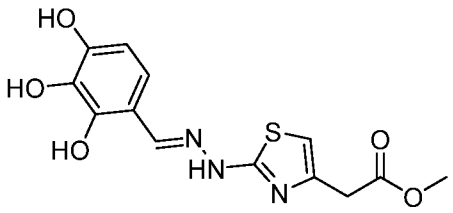
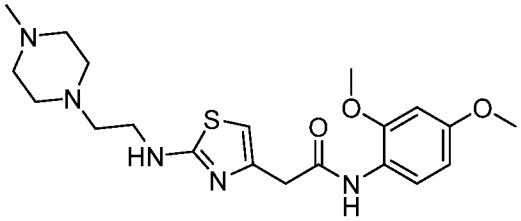
	ACV-54	ACV-2-156
	ACV-55	ACV-2-160
	ACV-56	CBX-01
	ACV-57	CBX-02
a synthetic peptide with the sequence FALK(me3)S capped with a phenyl methyl ester on the N terminus	ACV-58	ACV-2-130
	ACV-59	ACV-2-179
	ACV-60	ACV-2-185

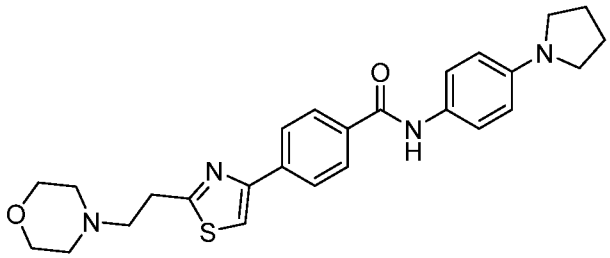
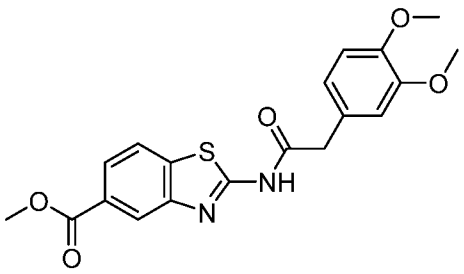
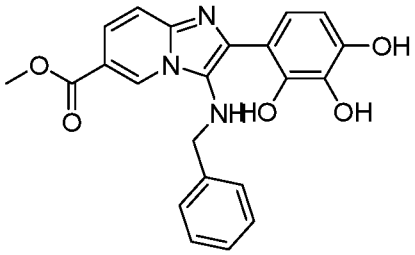
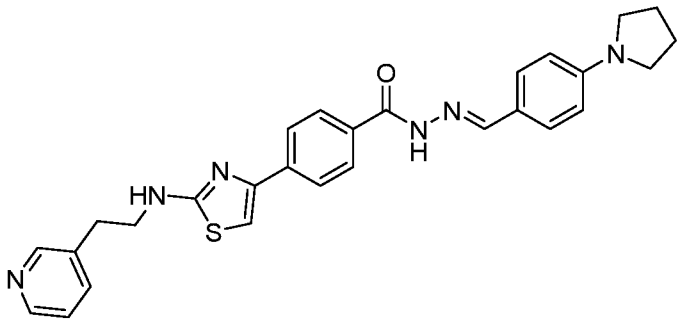
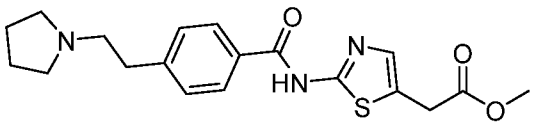
	ACV-61	ACV-2-188
	ACV-62	ACV-2-191
	ACV-63	ACV-2-195
	ACV-63 (2)	ACV-3-018
	ACV-63 (CP)	ACV-2-195 (CP)
	ACV-64	ACV-2-203
	ACV-65	CBX-03a

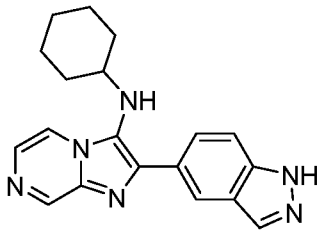
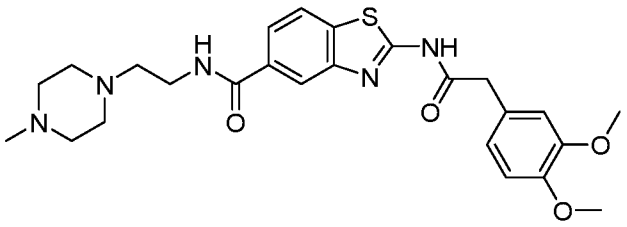
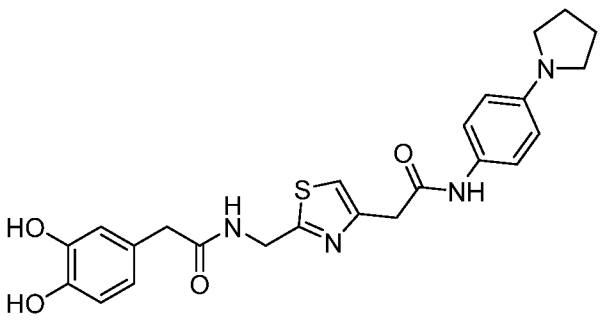
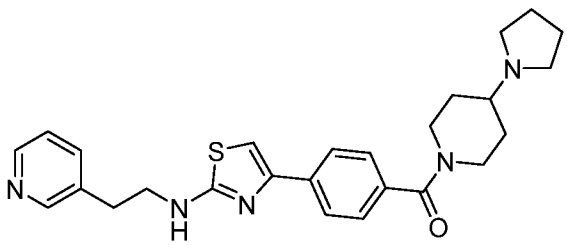
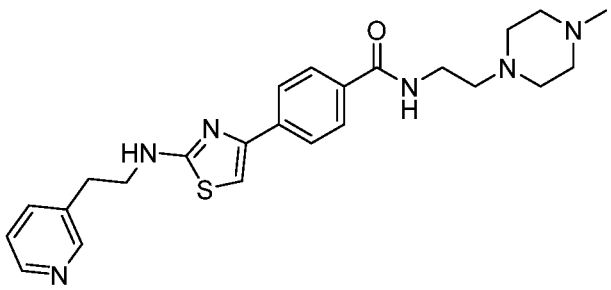
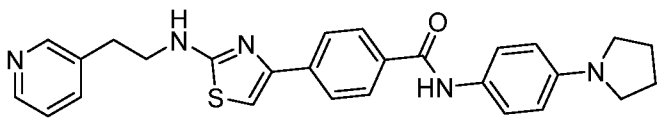
	ACV-66	ACV-2-204
	ACV-67	CBX-03
	ACV-68	CBX-04
	ACV-69	CBX-04a
	ACV-70	CBX-06
	ACV-71	CBX-06a
	ACV-72	CBX-07

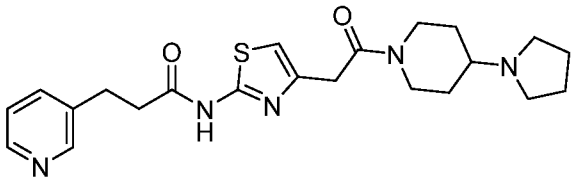
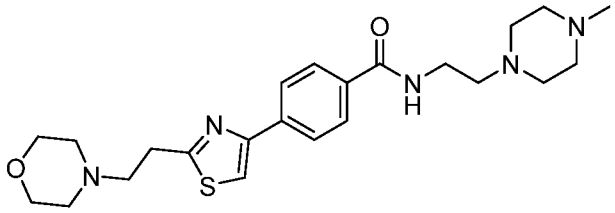
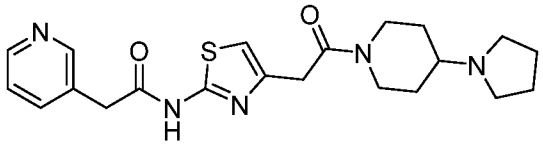
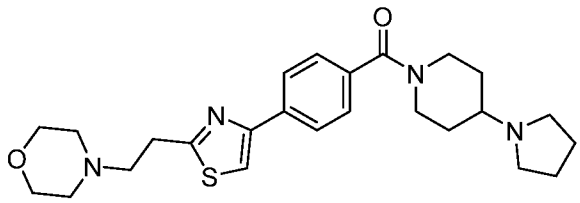
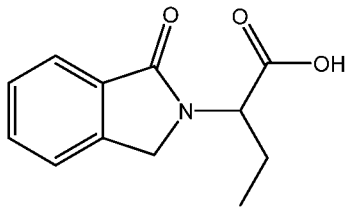
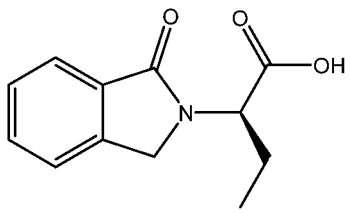
	ACV-73	ACV-2-224
	ACV-74	ACV-2-231
	ACV-75	ACV-2-233
	ACV-76	ACV-2-247
	ACV-77	ACV-2-251

	ACV-78	ACV-2-254
	ACV-79	ACV-2-270
	ACV-80	CBX-05
	ACV-81	ACV-2-287
	ACV-82	ACV-2-288
	ACV-83	ACV-2-293

	ACV-84	ACV-2-294
	ACV-85	ACV-3-019
	ACV-86	ACV-3-024
	ACV-87	ACV-3-027
	ACV-88	ACV-3-042

	ACV-89	ACV-3-048
	ACV-90	ACV-3-060
	ACV-91	ACV-3-061
	ACV-92	ACV-3-074
	ACV-93	ACV-2-144

	ACV-94	ACV-3-073
	ACV-95	ACV-3-090
	ACV-96	ACV-3-096
	ACV-97	ACV-3-104
	ACV-98	ACV-3-106
	ACV-99	ACV-3-107

	ACV-100	ACV-3-122
	ACV-101	ACV-3-123
	ACV-102	ACV-3-124
	ACV-103	ACV-3-126
	ACV-104	076
	ACV-105	ACV-1-037

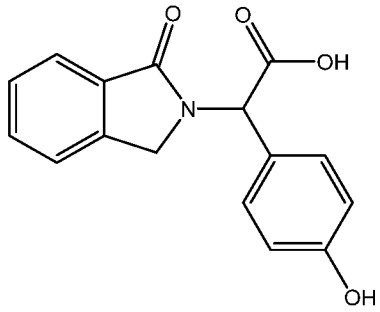
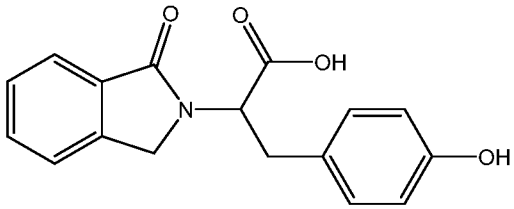
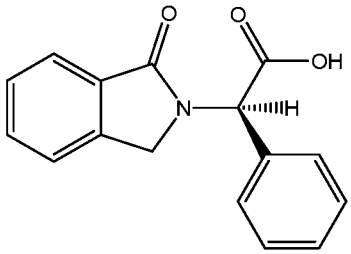
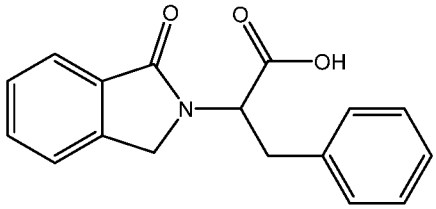
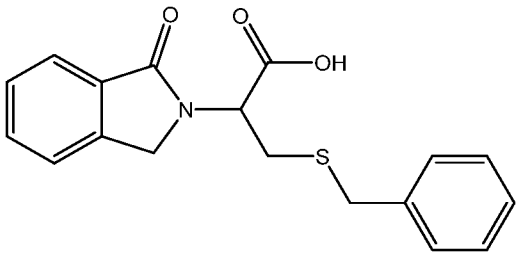
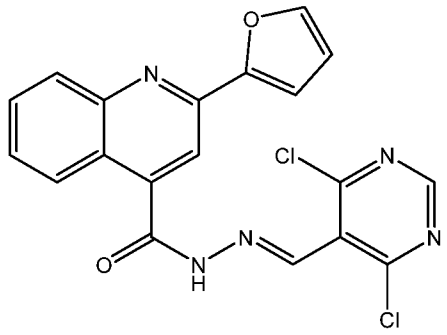
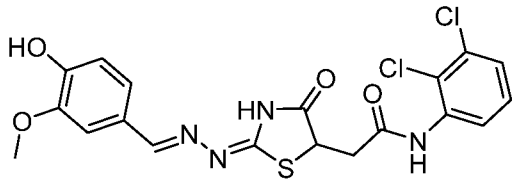
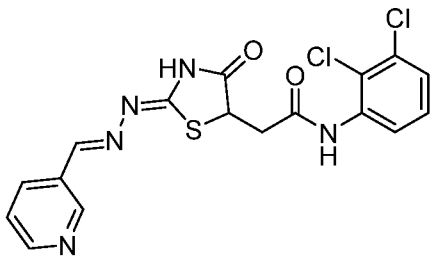
	ACV-106	
	ACV-107	295
	ACV-108	ACV-1-056
	ACV-109	017
	ACV-110	821

Table 2 - Other Structures

I			V56
			JQ-1
			JQIII-263-A

II. PHARMACEUTICAL COMPOSITIONS

In certain embodiments, the present invention provides pharmaceutical compositions comprising a compound of one of Formulas I-IX and a pharmaceutically acceptable carrier.

The compositions and methods of the present invention may be utilized to treat an individual in need thereof. In certain embodiments, the individual is a mammal such as a human, or a non-human mammal. When administered to an animal, such as a human, the composition or the compound is preferably administered as a pharmaceutical composition comprising, for example, a compound of the invention and a pharmaceutically acceptable

carrier. Pharmaceutically acceptable carriers are well known in the art and include, for example, aqueous solutions such as water or physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, oils such as olive oil, or injectable organic esters. In a preferred embodiment, when such pharmaceutical compositions are for human administration, particularly for invasive routes of administration (i.e., routes, such as injection or implantation, that circumvent transport or diffusion through an epithelial barrier), the aqueous solution is pyrogen-free, or substantially pyrogen-free. The excipients can be chosen, for example, to effect delayed release of an agent or to selectively target one or more cells, tissues or organs. The pharmaceutical composition can be in dosage unit form such as tablet, capsule (including sprinkle capsule and gelatin capsule), granule, lyophile for reconstitution, powder, solution, syrup, suppository, injection or the like. The composition can also be present in a transdermal delivery system, e.g., a skin patch. The composition can also be present in a solution suitable for topical administration, such as an eye drop.

A pharmaceutically acceptable carrier can contain physiologically acceptable agents that act, for example, to stabilize, increase solubility or to increase the absorption of a compound such as a compound of the invention. Such physiologically acceptable agents include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins or other stabilizers or excipients. The choice of a pharmaceutically acceptable carrier, including a physiologically acceptable agent, depends, for example, on the route of administration of the composition. The preparation or pharmaceutical composition can be a self-emulsifying drug delivery system or a self-microemulsifying drug delivery system. The pharmaceutical composition (preparation) also can be a liposome or other polymer matrix, which can have incorporated therein, for example, a compound of the invention. Liposomes, for example, which comprise phospholipids or other lipids, are nontoxic, physiologically acceptable and metabolizable carriers that are relatively simple to make and administer.

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 phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

A pharmaceutical composition (preparation) can be administered to a subject by any of a number of routes of administration including, for example, orally (for example, drenches as in aqueous or non-aqueous solutions or suspensions, tablets, capsules (including sprinkle capsules and gelatin capsules), boluses, powders, granules, pastes for application to the tongue); absorption through the oral mucosa (e.g., sublingually); anally, rectally or vaginally (for example, as a pessary, cream or foam); parenterally (including intramuscularly, intravenously, subcutaneously or intrathecally as, for example, a sterile solution or suspension); nasally; intraperitoneally; subcutaneously; transdermally (for example as a patch applied to the skin); and topically (for example, as a cream, ointment or spray applied to the skin, or as an eye drop). The compound may also be formulated for inhalation. In certain embodiments, a compound may be simply dissolved or suspended in sterile water. Details of appropriate routes of administration and compositions suitable for same can be found in, for example, U.S. Pat. Nos. 6,110,973, 5,731,000, 5,541,231, 5,427,798, 5,358,970 and 4,172,896, as well as in patents cited therein.

The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form

will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient that can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 1 percent to about ninety-nine percent of active ingredient, preferably from about 5 percent to about 70 percent, most preferably from about 10 percent to about 30 percent.

Methods of preparing these formulations or compositions include the step of bringing into association an active compound, such as a compound of the invention, with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules (including sprinkle capsules and gelatin capsules), cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), lyophile, powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. Compositions or compounds may also be administered as a bolus, electuary or paste.

To prepare solid dosage forms for oral administration (capsules (including sprinkle capsules and gelatin capsules), tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols,

sodium lauryl sulfate, and mixtures thereof; (10) complexing agents, such as, modified and unmodified cyclodextrins; and (11) coloring agents. In the case of capsules (including sprinkle capsules and gelatin capsules), tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions, such as dragees, capsules (including sprinkle capsules and gelatin capsules), pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions that can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms useful for oral administration include pharmaceutically acceptable emulsions, lyophiles for reconstitution, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents,

cyclodextrins and derivatives thereof, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations of the pharmaceutical compositions for rectal, vaginal, or urethral administration may be presented as a suppository, which may be prepared by mixing one or more active compounds with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound.

Formulations of the pharmaceutical compositions for administration to the mouth may be presented as a mouthwash, or an oral spray, or an oral ointment.

Alternatively or additionally, compositions can be formulated for delivery via a catheter, stent, wire, or other intraluminal device. Delivery via such devices may be especially useful for delivery to the bladder, urethra, ureter, rectum, or intestine.

Formulations which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants that may be required.

The ointments, pastes, creams and gels may contain, in addition to an active compound, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch,

tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to an active compound, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the active compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the compound in a polymer matrix or gel.

Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention. Exemplary ophthalmic formulations are described in U.S. Publication Nos. 2005/0080056, 2005/0059744, 2005/0031697 and 2005/004074 and U.S. Patent No. 6,583,124, the contents of which are incorporated herein by reference. If desired, liquid ophthalmic formulations have properties similar to that of lacrimal fluids, aqueous humor or vitreous humor or are compatible with such fluids. A preferred route of administration is local administration (*e.g.*, topical administration, such as eye drops, or administration via an implant).

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

Pharmaceutical compositions suitable for parenteral administration comprise one or more active compounds in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which

render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents that delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution, which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsulated matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions that are compatible with body tissue.

For use in the methods of this invention, active compounds can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

Methods of introduction may also be provided by rechargeable or biodegradable devices. Various slow release polymeric devices have been developed and tested *in vivo* in recent years for the controlled delivery of drugs, including proteinacious biopharmaceuticals. A variety of biocompatible polymers (including hydrogels), including both biodegradable and non-degradable polymers, can be used to form an implant for the sustained release of a compound at a particular target site.

Actual dosage levels of the active ingredients in the pharmaceutical compositions may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular compound or combination of compounds employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound(s) being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound(s) employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the therapeutically effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the pharmaceutical composition or compound at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. By "therapeutically effective amount" is meant the concentration of a compound that is sufficient to elicit the desired therapeutic effect. It is generally understood that the effective amount of the compound will vary according to the weight, sex, age, and medical history of the subject. Other factors which influence the effective amount may include, but are not limited to, the severity of the patient's condition, the disorder being treated, the stability of the compound, and, if desired, another type of therapeutic agent being administered with the compound of the invention. A larger total dose can be delivered by multiple administrations of the agent. Methods to determine efficacy and dosage are known to those

skilled in the art (Isselbacher *et al.* (1996) Harrison's Principles of Internal Medicine 13 ed., 1814-1882, herein incorporated by reference).

In general, a suitable daily dose of an active compound used in the compositions and methods of the invention will be that amount of the compound that is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above.

If desired, the effective daily dose of the active compound may be administered as one, two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. In certain embodiments of the present invention, the active compound may be administered two or three times daily. In preferred embodiments, the active compound will be administered once daily.

The patient receiving this treatment is any animal in need, including primates, in particular humans, and other mammals such as equines, cattle, swine and sheep; and poultry and pets in general.

This invention includes the use of pharmaceutically acceptable salts of compounds of the invention in the compositions and methods of the present invention. The term "pharmaceutically acceptable salt" as used herein includes salts derived from inorganic or organic acids including, for example, hydrochloric, hydrobromic, sulfuric, nitric, perchloric, phosphoric, formic, acetic, lactic, maleic, fumaric, succinic, tartaric, glycolic, salicylic, citric, methanesulfonic, benzenesulfonic, benzoic, malonic, trifluoroacetic, trichloroacetic, naphthalene-2-sulfonic, and other acids. Pharmaceutically acceptable salt forms can include forms wherein the ratio of molecules comprising the salt is not 1:1. For example, the salt may comprise more than one inorganic or organic acid molecule per molecule of base, such as two hydrochloric acid molecules per molecule of compound of Formula I or Formula II. As another example, the salt may comprise less than one inorganic or organic acid molecule per molecule of base, such as two molecules of compound of Formula I or Formula II per molecule of tartaric acid.

In further embodiments, contemplated salts of the invention include, but are not limited to, alkyl, dialkyl, trialkyl or tetra-alkyl ammonium salts. In certain embodiments, contemplated salts of the invention include, but are not limited to, L-arginine, benenthamine, benzathine, betaine, calcium hydroxide, choline, deanol, diethanolamine, diethylamine, 2-(diethylamino)ethanol, ethanolamine, ethylenediamine, N-methylglucamine, hydrabamine, 1H-imidazole, lithium, L-lysine, magnesium, 4-(2-

hydroxyethyl)morpholine, piperazine, potassium, 1-(2-hydroxyethyl)pyrrolidine, sodium, triethanolamine, tromethamine, and zinc salts. In certain embodiments, contemplated salts of the invention include, but are not limited to, Na, Ca, K, Mg, Zn or other metal salts.

The pharmaceutically acceptable acid addition salts can also exist as various solvates, such as with water, methanol, ethanol, dimethylformamide, and the like. Mixtures of such solvates can also be prepared. The source of such solvate can be from the solvent of crystallization, inherent in the solvent of preparation or crystallization, or adventitious to such solvent.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically acceptable antioxidants include: (1) water-soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal-chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

III. USES OF COMPOUNDS AND COMPOSITIONS

In certain aspects, the invention provides methods of treating or preventing a disease or condition, comprising administering to a subject a compound of one of Formulas I-IX, e.g., in a therapeutically effective amount or a composition comprising a compound of one of Formulas I-IX.

In some embodiments, the disease is cancer. In some embodiments, the cancer is breast cancer, prostate cancer, oral squamous carcinoma, colorectal cancer, squamous cell carcinomas, neuroblastoma, bladder tumors, leukemia, non-Hodgkin's lymphoma, and solid and hematologic malignancies.

In certain embodiments, the cancer is a solid tumor. The subject is generally one who has been diagnosed as having a cancerous tumor or one who has been previously treated for a cancerous tumor (e.g., where the tumor has been previously removed by

surgery). The cancerous tumor may be a primary tumor and/or a secondary (e.g., metastatic) tumor.

In certain embodiments, the subject is a mammal, e.g., a human.

In certain embodiments, the invention provides methods of inhibiting proliferation of a cancerous cell comprising contacting a cancerous cell with an effective amount of a compound of one of Formulas I-IX.

The invention also provides methods of inhibiting proliferation of a cancer cell, comprising contacting a cancer cell with a compound of one of Formulas I-IX or a composition comprising a compound of one of Formulas I-IX.

The invention also provides methods of inhibiting CBX, comprising contacting a cell with a compound of one of Formulas I-IX. Such methods may be performed *in vivo* or *in vitro*.

IV. DEFINITIONS

The term "acyl" is art-recognized and refers to a group represented by the general formula hydrocarbylC(O)-, preferably alkylC(O)-.

The term "acylamino" is art-recognized and refers to an amino group substituted with an acyl group and may be represented, for example, by the formula hydrocarbylC(O)NH-.

The term "acyloxy" is art-recognized and refers to a group represented by the general formula hydrocarbylC(O)O-, preferably alkylC(O)O-.

The term "alkoxy" refers to an alkyl group, preferably a lower alkyl group, having an oxygen attached thereto. Representative alkoxy groups include methoxy, $-OCF_3$, ethoxy, propoxy, tert-butoxy and the like.

The term "cycloalkyloxy" refers to a cycloakyl group having an oxygen attached thereto.

The term "alkoxyalkyl" refers to an alkyl group substituted with an alkoxy group and may be represented by the general formula alkyl-O-alkyl.

The term "alkylaminoalkyl" refers to an alkyl group substituted with an alkylamino group.

The term "alkenyl", as used herein, refers to an aliphatic group containing at least one double bond and is intended to include both "unsubstituted alkenyls" and "substituted alkenyls", the latter of which refers to alkenyl moieties having substituents replacing a

hydrogen on one or more carbons of the alkenyl group. Such substituents may occur on one or more carbons that are included or not included in one or more double bonds. Moreover, such substituents include all those contemplated for alkyl groups, as discussed below, except where stability is prohibitive. For example, substitution of alkenyl groups by one or more alkyl, carbocyclyl, aryl, heterocyclyl, or heteroaryl groups is contemplated.

An “alkyl” group or “alkane” is a straight chained or branched non-aromatic hydrocarbon which is completely saturated. Typically, a straight chained or branched alkyl group has from 1 to about 20 carbon atoms, preferably from 1 to about 10 unless otherwise defined. Examples of straight chained and branched alkyl groups include methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, tert-butyl, pentyl, hexyl, pentyl and octyl. A C_1-C_6 straight chained or branched alkyl group is also referred to as a "lower alkyl" group.

Moreover, the term "alkyl" (or "lower alkyl") as used throughout the specification, examples, and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents, if not otherwise specified, can include, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxycarbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxyl, a phosphoryl, a phosphate, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. For instance, the substituents of a substituted alkyl may include substituted and unsubstituted forms of amino, azido, imino, amido, phosphoryl (including phosphonate and phosphinate), sulfonyl (including sulfate, sulfonamido, sulfamoyl and sulfonate), and silyl groups, as well as ethers, alkylthios, carbonyls (including ketones, aldehydes, carboxylates, and esters), -CF_3, -CN and the like. Exemplary substituted alkyls are described below. Cycloalkyls can be further substituted with alkyls, alkenyls, alkoxys, alkylthios, aminoalkyls, carbonyl-substituted alkyls, -CF_3, -CN, and the like.

The term “C_{x-y}” when used in conjunction with a chemical moiety, such as acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups that contain from x to y carbons in the chain. For example, the term “C_{x-y}alkyl” refers to substituted or unsubstituted saturated hydrocarbon groups, including straight-chain alkyl and branched-

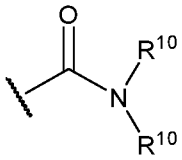
chain alkyl groups that contain from x to y carbons in the chain, including haloalkyl groups such as trifluoromethyl and 2,2,2-trifluoroethyl, etc. C_0 alkyl indicates a hydrogen where the group is in a terminal position, a bond if internal. The terms "C_{2-y}alkenyl" and "C_{2-y}alkynyl" refer to substituted or unsubstituted unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

The term "alkylamino", as used herein, refers to an amino group substituted with at least one alkyl group.

The term "alkylthio", as used herein, refers to a thiol group substituted with an alkyl group and may be represented by the general formula alkylS-.

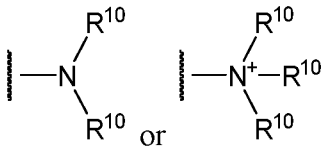
The term "alkynyl", as used herein, refers to an aliphatic group containing at least one triple bond and is intended to include both "unsubstituted alkynyls" and "substituted alkynyls", the latter of which refers to alkynyl moieties having substituents replacing a hydrogen on one or more carbons of the alkynyl group. Such substituents may occur on one or more carbons that are included or not included in one or more triple bonds. Moreover, such substituents include all those contemplated for alkyl groups, as discussed above, except where stability is prohibitive. For example, substitution of alkynyl groups by one or more alkyl, carbocyclyl, aryl, heterocyclyl, or heteroaryl groups is contemplated.

The term "amide", as used herein, refers to a group



wherein each R^{10} independently represent a hydrogen or hydrocarbyl group, or two R^{10} are taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

The terms "amine" and "amino" are art-recognized and refer to both unsubstituted and substituted amines and salts thereof, e.g., a moiety that can be represented by



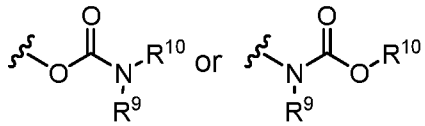
wherein each R^{10} independently represents a hydrogen or a hydrocarbyl group, or two R^{10} are taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

The term "aminoalkyl", as used herein, refers to an alkyl group substituted with an amino group.

The term "aralkyl", as used herein, refers to an alkyl group substituted with an aryl group.

The term "aryl" as used herein include substituted or unsubstituted single-ring aromatic groups in which each atom of the ring is carbon. Preferably the ring is a 5- to 7-membered ring, more preferably a 6-membered ring. The term "aryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is aromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Aryl groups include benzene, naphthalene, phenanthrene, phenol, aniline, and the like.

The term "carbamate" is art-recognized and refers to a group



wherein R^9 and R^{10} independently represent hydrogen or a hydrocarbyl group, such as an alkyl group, or R^9 and R^{10} taken together with the intervening atom(s) complete a heterocycle having from 4 to 8 atoms in the ring structure.

The terms "carbocycle", and "carbocyclic", as used herein, refers to a saturated or unsaturated ring in which each atom of the ring is carbon. The term carbocycle includes both aromatic carbocycles and non-aromatic carbocycles. Non-aromatic carbocycles include both cycloalkane rings, in which all carbon atoms are saturated, and cycloalkene rings, which contain at least one double bond. "Carbocycle" includes 5-7 membered monocyclic and 8-12 membered bicyclic rings. Each ring of a bicyclic carbocycle may be selected from saturated, unsaturated and aromatic rings. Carbocycle includes bicyclic molecules in which one, two or three or more atoms are shared between the two rings. The term "fused carbocycle" refers to a bicyclic carbocycle in which each of the rings shares two adjacent atoms with the other ring. Each ring of a fused carbocycle may be selected from saturated, unsaturated and aromatic rings. In an exemplary embodiment, an aromatic

ring, e.g., phenyl, may be fused to a saturated or unsaturated ring, e.g., cyclohexane, cyclopentane, or cyclohexene. Any combination of saturated, unsaturated and aromatic bicyclic rings, as valence permits, is included in the definition of carbocyclic. Exemplary "carbocycles" include cyclopentane, cyclohexane, bicyclo[2.2.1]heptane, 1,5-cyclooctadiene, 1,2,3,4-tetrahydronaphthalene, bicyclo[4.2.0]oct-3-ene, naphthalene and adamantane. Exemplary fused carbocycles include decalin, naphthalene, 1,2,3,4-tetrahydronaphthalene, bicyclo[4.2.0]octane, 4,5,6,7-tetrahydro-1H-indene and bicyclo[4.1.0]hept-3-ene. "Carbocycles" may be substituted at any one or more positions capable of bearing a hydrogen atom.

A "cycloalkyl" group is a cyclic hydrocarbon which is completely saturated. "Cycloalkyl" includes monocyclic and bicyclic rings. Typically, a monocyclic cycloalkyl group has from 3 to about 10 carbon atoms, more typically 3 to 8 carbon atoms unless otherwise defined. The second ring of a bicyclic cycloalkyl may be selected from saturated, unsaturated and aromatic rings. Cycloalkyl includes bicyclic molecules in which one, two or three or more atoms are shared between the two rings. The term "fused cycloalkyl" refers to a bicyclic cycloalkyl in which each of the rings shares two adjacent atoms with the other ring. The second ring of a fused bicyclic cycloalkyl may be selected from saturated, unsaturated and aromatic rings. A "cycloalkenyl" group is a cyclic hydrocarbon containing one or more double bonds.

The term "carbocyclylalkyl", as used herein, refers to an alkyl group substituted with a carbocycle group.

The term "carbonate" is art-recognized and refers to a group $-OCO_2-R^{10}$, wherein R^{10} represents a hydrocarbyl group.

The term "carboxy", as used herein, refers to a group represented by the formula $-CO_2H$.

The term "ester", as used herein, refers to a group $-C(O)OR^{10}$ wherein R^{10} represents a hydrocarbyl group.

The term "ether", as used herein, refers to a hydrocarbyl group linked through an oxygen to another hydrocarbyl group. Accordingly, an ether substituent of a hydrocarbyl group may be hydrocarbyl-O-. Ethers may be either symmetrical or unsymmetrical. Examples of ethers include, but are not limited to, heterocycle-O-heterocycle and aryl-O-heterocycle. Ethers include "alkoxyalkyl" groups, which may be represented by the general formula alkyl-O-alkyl.

The terms “halo” and “halogen” as used herein means halogen and includes chloro, fluoro, bromo, and iodo.

The terms “hetaralkyl” and “heteroaralkyl”, as used herein, refers to an alkyl group substituted with a hetaryl group.

The term "heteroalkyl", as used herein, refers to a saturated or unsaturated chain of carbon atoms and at least one heteroatom, wherein no two heteroatoms are adjacent.

The term “heteroalkylamino”, as used herein, refers to an amino group substituted with a heteralkyl group.

The terms “heteroaryl” and “hetaryl” include substituted or unsubstituted aromatic single ring structures, preferably 5- to 7-membered rings, more preferably 5- to 6-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. The terms “heteroaryl” and “hetaryl” also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heteroaromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heteroaryl groups include, for example, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, benzimidazole, quinoline, isoquinoline, quinoxaline, quinazoline, indole, isoindole, indazole, benzoxazole, pyrazine, pyridazine, purine, and pyrimidine, and the like.

The term “heteroatom” as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, and sulfur.

The terms “heterocyclyl”, “heterocycle”, and “heterocyclic” refer to substituted or unsubstituted non-aromatic ring structures, preferably 3- to 10-membered rings, more preferably 3- to 7-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. The terms “heterocyclyl” and “heterocyclic” also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heterocyclic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heterocyclyl groups include, for example, piperidine, piperazine, pyrrolidine, morpholine, lactones, lactams, and the like. Heterocyclyl groups can also be substituted by oxo groups. For example, “heterocyclyl” encompasses both pyrrolidine and pyrrolidinone.

The term "heterocycloalkyl", as used herein, refers to an alkyl group substituted with a heterocycle group.

The term "heterocycloalkylamino", as used herein refers to an amino group substituted with a heterocycloalkyl group.

The term "hydrocarbyl", as used herein, refers to a group that is bonded through a carbon atom that does not have a =O or =S substituent, and typically has at least one carbon-hydrogen bond and a primarily carbon backbone, but may optionally include heteroatoms. Thus, groups like methyl, ethoxyethyl, 2-pyridyl, and trifluoromethyl are considered to be hydrocarbyl for the purposes of this application, but substituents such as acetyl (which has a =O substituent on the linking carbon) and ethoxy (which is linked through oxygen, not carbon) are not. Hydrocarbyl groups include, but are not limited to aryl, heteroaryl, carbocycle, heterocyclyl, alkyl, alkenyl, alkynyl, and combinations thereof.

The term "hydroxyalkyl", as used herein, refers to an alkyl group substituted with a hydroxy group.

The term "lower" when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups where there are ten or fewer non-hydrogen atoms in the substituent, preferably six or fewer. A "lower alkyl", for example, refers to an alkyl group that contains ten or fewer carbon atoms, preferably six or fewer. In certain embodiments, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy substituents defined herein are respectively lower acyl, lower acyloxy, lower alkyl, lower alkenyl, lower alkynyl, or lower alkoxy, whether they appear alone or in combination with other substituents, such as in the recitations hydroxyalkyl and aralkyl (in which case, for example, the atoms within the aryl group are not counted when counting the carbon atoms in the alkyl substituent).

As used herein, the term "oxo" refers to a carbonyl group. When an oxo substituent occurs on an otherwise saturated group, such as with an oxo-substituted cycloalkyl group (e.g., 3-oxo-cyclobutyl), the substituted group is still intended to be a saturated group. When a group is referred to as being substituted by an "oxo" group, this can mean that a carbonyl moiety (i.e., -C(=O)-) replaces a methylene unit (i.e., $-CH_2-$).

The terms "polycyclyl", "polycycle", and "polycyclic" refer to two or more rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls) in which two or more atoms are common to two adjoining rings, e.g., the rings are "fused rings". Each of the rings of the polycycle can be substituted or unsubstituted. In certain

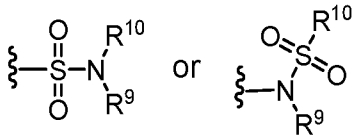
embodiments, each ring of the polycycle contains from 3 to 10 atoms in the ring, preferably from 5 to 7.

The term "silyl" refers to a silicon moiety with three hydrocarbyl moieties attached thereto.

The term "substituted" refers to moieties having substituents replacing a hydrogen on one or more carbons of the backbone. It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and non-aromatic substituents of organic compounds. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. Substituents can include any substituents described herein, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxycarbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxyl, a phosphoryl, a phosphate, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that substituents can themselves be substituted, if appropriate. Unless specifically stated as "unsubstituted," references to chemical moieties herein are understood to include substituted variants. For example, reference to an "aryl" group or moiety implicitly includes both substituted and unsubstituted variants.

The term "sulfate" is art-recognized and refers to the group $-OSO_3H$, or a pharmaceutically acceptable salt thereof.

The term "sulfonamide" is art-recognized and refers to the group represented by the general formulae



wherein R^9 and R^{10} independently represents hydrogen or hydrocarbyl, such as alkyl, or R^9 and R^{10} taken together with the intervening atom(s) complete a heterocycle having from 4 to 8 atoms in the ring structure.

The term "sulfoxide" is art-recognized and refers to the group -S(O)-R^{10}, wherein R^{10} represents a hydrocarbyl.

The term "sulfonate" is art-recognized and refers to the group SO_3H, or a pharmaceutically acceptable salt thereof.

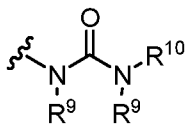
The term "sulfone" is art-recognized and refers to the group -$S(O)_2$-R^{10}, wherein R^{10} represents a hydrocarbyl.

The term "thioalkyl", as used herein, refers to an alkyl group substituted with a thiol group.

The term "thioester", as used herein, refers to a group -C(O)SR^{10} or -SC(O)R^{10} wherein R^{10} represents a hydrocarbyl.

The term "thioether", as used herein, is equivalent to an ether, wherein the oxygen is replaced with a sulfur.

The term "urea" is art-recognized and may be represented by the general formula



wherein R^9 and R^{10} independently represent hydrogen or a hydrocarbyl, such as alkyl, or either occurrence of R^9 taken together with R^{10} and the intervening atom(s) complete a heterocycle having from 4 to 8 atoms in the ring structure.

"Protecting group" refers to a group of atoms that, when attached to a reactive functional group in a molecule, mask, reduce or prevent the reactivity of the functional group. Typically, a protecting group may be selectively removed as desired during the course of a synthesis. Examples of protecting groups can be found in Greene and Wuts, *Protective Groups in Organic Chemistry,* 3rd Ed., 1999, John Wiley & Sons, NY and Harrison et al., *Compendium of Synthetic Organic Methods*, Vols. 1-8, 1971-1996, John

Wiley & Sons, NY. Representative nitrogen protecting groups include, but are not limited to, formyl, acetyl, trifluoroacetyl, benzyl, benzyloxycarbonyl ("CBZ"), tert-butoxycarbonyl ("Boc"), trimethylsilyl ("TMS"), 2-trimethylsilyl-ethanesulfonyl ("TES"), trityl and substituted trityl groups, allyloxycarbonyl, 9-fluorenylmethyloxycarbonyl ("FMOC"), nitro-veratryloxycarbonyl ("NVOC") and the like. Representative hydroxyl protecting groups include, but are not limited to, those where the hydroxyl group is either acylated (esterified) or alkylated such as benzyl and trityl ethers, as well as alkyl ethers, tetrahydropyranyl ethers, trialkylsilyl ethers (e.g., TMS or TIPS groups), glycol ethers, such as ethylene glycol and propylene glycol derivatives and allyl ethers.

As used herein, a therapeutic that "prevents" a disorder or condition refers to a compound that, in a statistical sample, reduces the occurrence of the disorder or condition in the treated sample relative to an untreated control sample, or delays the onset or reduces the severity of one or more symptoms of the disorder or condition relative to the untreated control sample.

The term "treating" includes prophylactic and/or therapeutic treatments. The term "prophylactic or therapeutic" treatment is art-recognized and includes administration to the host of one or more of the subject compositions. If it is administered prior to clinical manifestation of the unwanted condition (e.g., disease or other unwanted state of the host animal) then the treatment is prophylactic (i.e., it protects the host against developing the unwanted condition), whereas if it is administered after manifestation of the unwanted condition, the treatment is therapeutic, (i.e., it is intended to diminish, ameliorate, or stabilize the existing unwanted condition or side effects thereof).

The term "prodrug" is intended to encompass compounds which, under physiologic conditions, are converted into the therapeutically active agents of the present invention (e.g., a compound of one of Formulas I-IX). A common method for making a prodrug is to include one or more selected moieties which are hydrolyzed under physiologic conditions to reveal the desired molecule. In other embodiments, the prodrug is converted by an enzymatic activity of the host animal. For example, esters or carbonates (e.g., esters or carbonates of alcohols or carboxylic acids) are preferred prodrugs of the present invention. In certain embodiments, some or all of the compounds of one of Formulas I-IX in a formulation represented above can be replaced with the corresponding suitable prodrug, e.g., wherein a hydroxyl in the parent compound is presented as an ester or a carbonate or carboxylic acid present in the parent compound is presented as an ester.

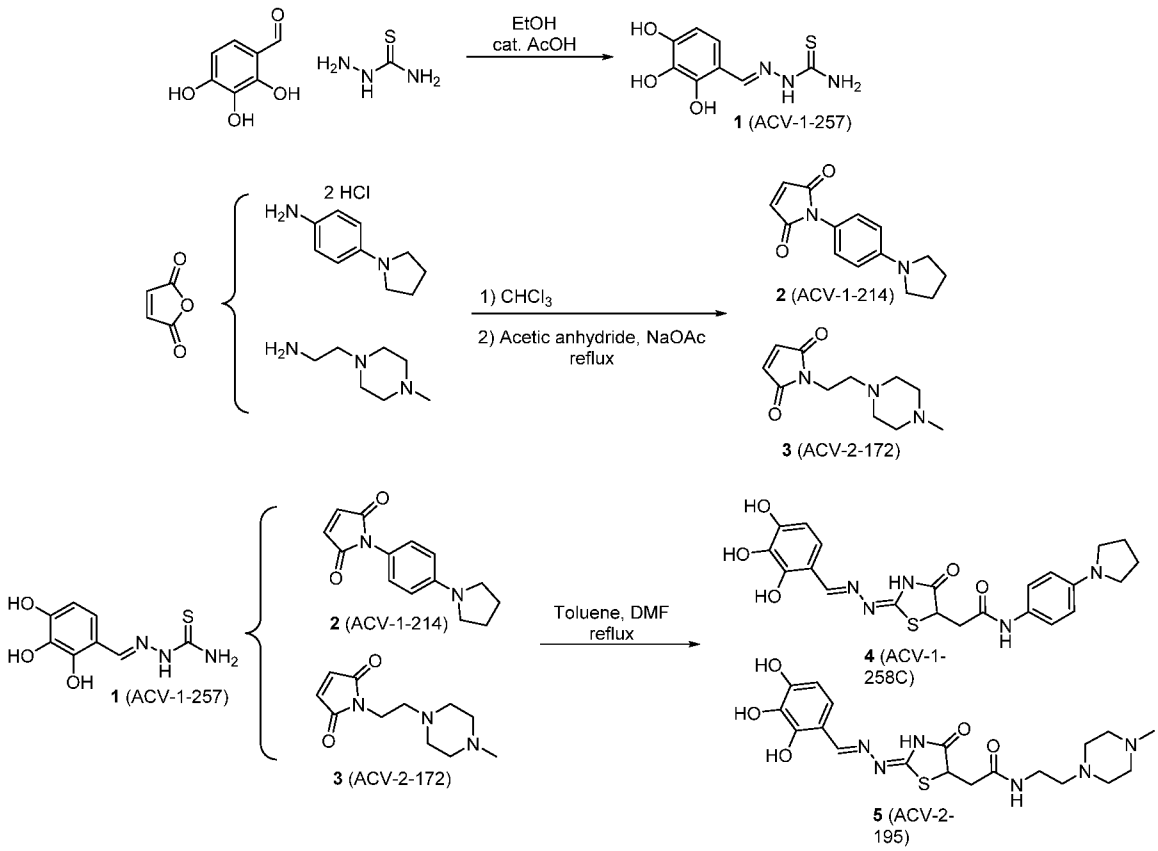
Examples

Examples of compounds of Formulas I-IX or pharmaceutically acceptable salts thereof having useful biological activity are listed above in Table 1. The preparation of these compounds can be realized by one of skilled in the art of organic synthesis using known techniques and methodology.

Example 1: Chemical Syntheses

A general procedure used in the methods to prepare various compounds of the invention are described below.

Scheme 1



Synthesis of ACV-1-258C/ACV-2-195

(1) 2-(2,3,4-trihydroxybenzylidene)hydrazine-1-carbothioamide:

2,3,4-trihydroxybenzaldehyde (338.3 mg, 2.20 mmol) and thiosemicarbazide (200.3 mg, 2.20 mmol) were combined and suspended in EtOH (4.4 mL) and a few drops of acetic acid were then added. The reaction was stirred at room temperature for 45 minutes. The reaction

was then filtered and the precipitate was washed with hexanes to afford **1** as a white powder. MS: m/z $(M+1)^+$: 228.24. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 6.42 (d, J = 8.61 Hz, 1H) 6.88 (d, J = 8.61 Hz, 1H) 8.13 (s, 1H).

(2) 1-(4-(pyrrolidin-1-yl)phenyl)-1H-pyrrole-2,5-dione:

Maleic anhydride (121.0 mg, 1.23 mmol) was dissolved in CHCl3 (6.5 mL) and 4-(pyrrolidin-1-yl)aniline dihydrochloride (199.3 mg, 1.23 mmol) was then added. The reaction was stirred at room temperature for 3 hours. The reaction was filtered and the solid was resuspended in acetic anhydride (6 mL). To the reaction was then added sodium acetate (103 mg). The reaction was heated to reflux under N2 for 2 hours. After reflux, H2O (20 mL) was added to the reaction. A black precipitate was formed and the reaction was filtered to afford **2** as a dark brown/black solid. MS: m/z $(M+1)^+$: 243.59. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 2.04 (dt, J = 6.65, 3.33 Hz, 4H) 6.59–6.64 (m, 2H) 6.90 (s, 2H) 7.02-7.07 (m, 2H).

(3) 1-(2-(4-methylpiperazin-1-yl)ethyl)-1H-pyrrole-2,5-dione:

Maleic anhydride (501.9 mg, 5.11 mmol) was dissolved in CHCl3 (25 mL) and 2-(4-methylpiperazin-1-yl)ethan-1-amine (767 µL, 5.11 mmol) was then added. The reaction was stirred at room temperature for 2 hours. The solvent was removed in vacuo and the crude solid was resuspended in acetic anhydride (25 mL). To the reaction was then added sodium acetate (250 mg). The reaction was heated to reflux under N2 for 3 hours. After reflux, the reaction was cooled to room temperature and washed with hexanes to obtain **3** (crude) as a dark brown/black oil. MS: m/z $(M+1)^+$: 224.28

(4)2-(4-oxo-2-((2,3,4-trihydroxybenzylidene)hydrazono)thiazolidin-5-yl)-N-(4-(pyrrolidin-1-yl)phenyl) acetamide:

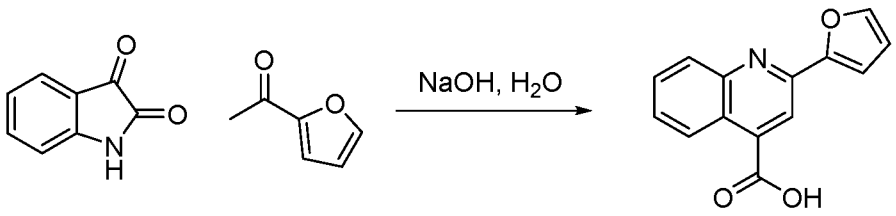
2 (50.0 mg, 0.21 mmol) and **1** (47.0 mg, 0.21 mmol) were combined and dissolved in DMSO (1 mL). The reaction was heated at 80°C overnight. The reaction was then purified via HPLC to afford the pure product **4** (8.8 mg). MS: m/z $(M+1)^+$: 470.37. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.28 (s, 3 H) 2.37 (br t, J=6.71 Hz, 2 H) 2.62 - 2.72 (m, 1 H) 2.98 (dd, J=16.17, 3.66 Hz, 1 H) 3.18 (q, J=6.51 Hz, 2 H) 4.38 (dd, J=10.07, 3.66 Hz, 1 H) 6.40 (d, J=8.24 Hz, 1 H) 6.83 (d, J=8.54 Hz, 1 H) 8.03 (br t, J=5.49 Hz, 1 H) 8.15 (s, 1 H) 8.41 - 8.47 (m, 1 H) 11.18 (br s, 1 H).

(5)N-(2-(4-methylpiperazin-1-yl)ethyl)-2-(4-oxo-2-((2,3,4-trihydroxybenzylidene)hydrazono)thiazolidin -5-yl)acetamide:

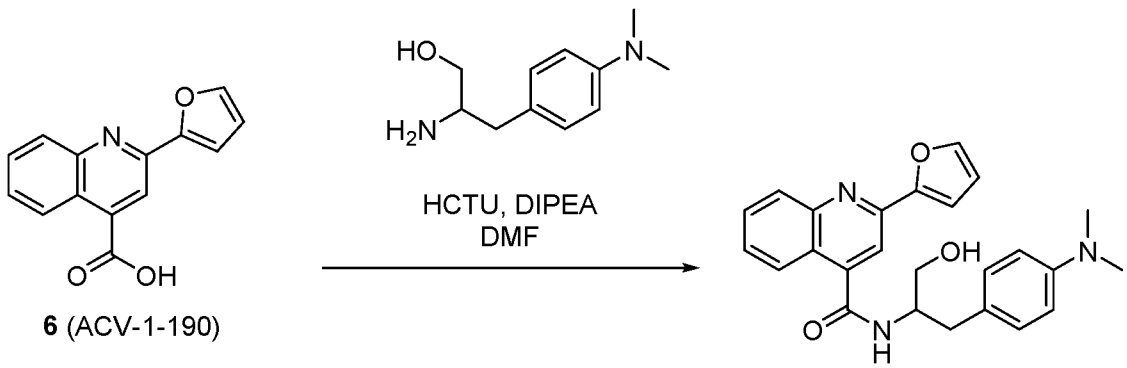
3 (22.5 mg, 0.1 mmol) and **1** (22.8 mg, 0.1 mmol) were combined and dissolved in Toluene (0.5 mL) and DMF (0.5 mL). The reaction was heated to reflux for 10 minutes in the microwave. The crude was purified via HPLC to afford the pure product **5** (6.0 mg). MS: m/z $(M+1)^+$: 451.35. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.15 (s, 1 H) 1.23 (s, 1 H) 1.93 (dt, *J*=6.48, 3.32 Hz, 4 H) 2.73 (d, *J*=0.61 Hz, 1 H) 2.84 - 2.91 (m, 1 H) 3.14 - 3.23 (m, 5 H) 3.29 (s, 3 H) 4.48 (br dd, *J*=10.22, 3.20 Hz, 1 H) 6.39 (d, *J*=8.54 Hz, 1 H) 6.48 (d, *J*=8.85 Hz, 2 H) 6.83 (d, *J*=8.54 Hz, 1 H) 7.32 - 7.39 (m, 2 H) 8.46 (s, 1 H) 8.58 (s, 1 H) 9.53 (s, 1 H) 9.81 (s, 1 H) 11.23 (s, 1 H) 12.00 (s, 1 H).

Synthesis of ACV-1-204

Scheme 2



6 (ACV-1-190)



6 (ACV-1-190)

7 (ACV-1-204)

(6) 2-(furan-2-yl)quinoline-4-carboxylic acid:

Isatin (249.8 mg, 1.70 mmol) and NaOH (341.6 mg, 8.50 mmol) were combined and suspended in H2O (3.5 mL) and heated to 50°C. When the solution became a clear, 2-

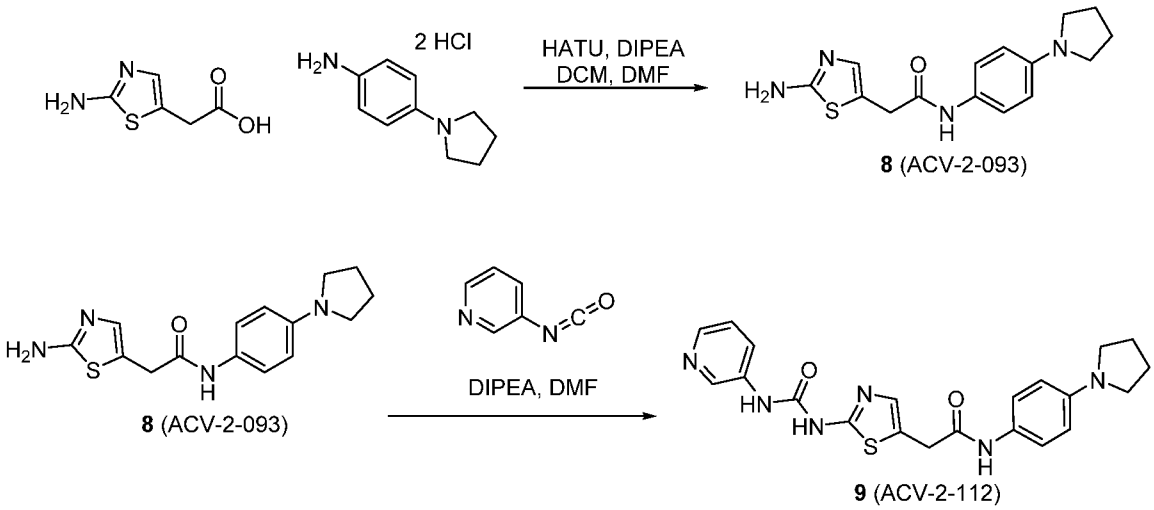
acetylfuran (170.4 µL, 1.70 mmol) was added and the reaction was stirred at 50°C overnight. The reaction was then filtered to afford **6**. MS: m/z (M+1) $^{+}$: 240.55. ^{1}H NMR (600 MHz, METHANOL-d_4) δ ppm 6.67 (dd, J = 3.23, 1.47 Hz, 1H) 7.33 (d, J = 3.52 Hz, 1H) 7.55 (t, J = 7.63 Hz, 1H) 7.72 (t, J = 7.63 Hz, 1H) 7.76 (d, J = 1.76 Hz, 1H) 7.97 (s, 1H) 8.05 (d, J = 8.22 Hz, 1H) 8.36 (d, J = 8.22 Hz, 1H).

(7) N-(1-(4-(dimethylamino)phenyl)-3-hydroxypropan-2-yl)-2-(furan-2-yl)quinoline-4-carboxamide:

6 (61.7 mg, 0.26 mmol) and HCTU (213.3 mg, 0.52 mmol) were combined and dissolved in DMF (1 mL). DIPEA (224.3 µL, 1.29 mmol) was then added and the reaction was stirred at room temperature for a few minutes at which point a solution of 2-amino-3-(4-(dimethylamino)phenyl)propan-1-ol (50.0 mg, 0.26 mmol) in DMF (0.3 mL) was added. The reaction was stirred at room temperature for 2 hours. The reaction was diluted with EtOAc (40 mL) and then washed with H2O (5 mL) and brine (5 mL). The organic layer was collected, dried over Na2SO4, filtered and concentrated to dryness. The crude material was then purified via silica gel column chromatography (0-15% MeOH:DCM) to afford the pure product **7** (2.3 mg). MS: m/z (M+1) $^{+}$: 416.69. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 2.66 (dd, J = 13.69, 10.17 Hz, 1H) 2.81 (s, 1H) 2.89 (s, 1H) 2.94 (s, 6H) 2.98-3.04 (m, 1H) 3.74 (dd, J = 14.28, 5.67 Hz, 2H) 4.47-4.57 (m, 1H) 6.69 (dd, J = 3.52, 1.96 Hz, 1H) 6.78 – 6.81 (m, 2H) 7.15-7.20 (m, 2H) 7.31-7.35 (m, 1H) 7.40-7.45 (m, 1H) 7.56 (d, J = 7.43 Hz, 1H) 7.73 (ddd, J = 8.51, 6.95, 1.37 Hz, 1H) 7.79 (dd, J = 1.96, 0.78 Hz, 1H) 7.80 (s, 1H) 7.90 (s, 1H) 8.04 (d, J = 8.22 Hz, 1H).

Synthesis of ACV-2-112

Scheme 3



(8) 2-(2-aminothiazol-5-yl)-N-(4-(pyrrolidin-1-yl)phenyl)acetamide:

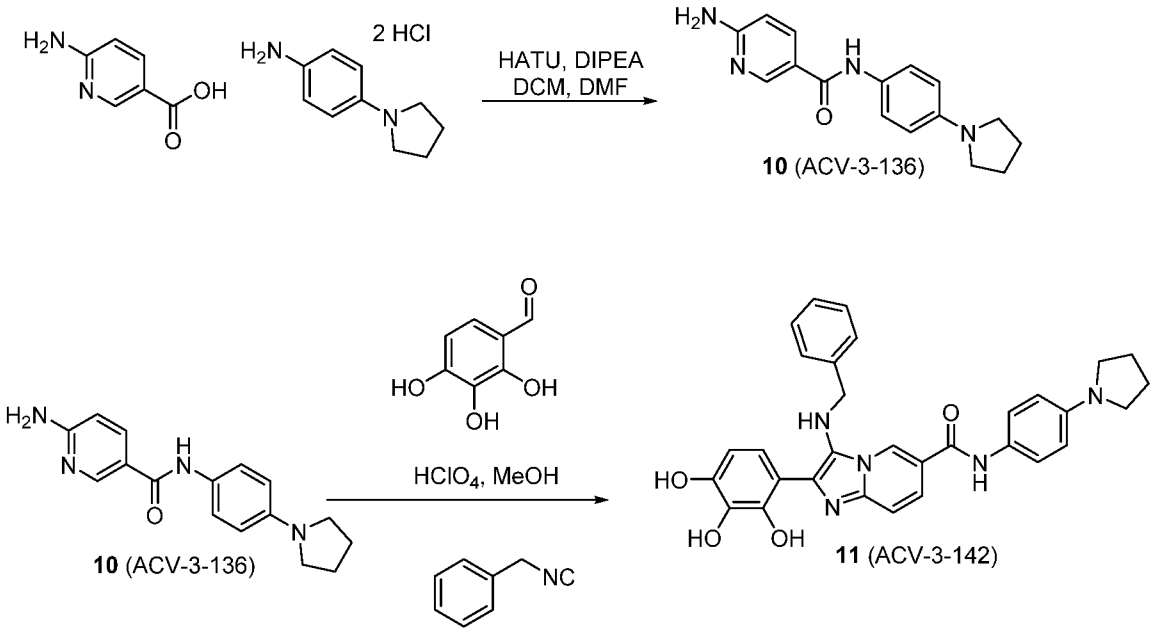
2-aminothiazole-5-acetic acid (50.5 mg, 0.32 mmol) was added to a solution of the 4-(pyrrolidin-1-yl)aniline dihydrochloride (151.0 mg, 0.64 mmol)and DIPEA (334.0 µL, 1.92 mmol) in DCM (1 mL). DMF (1 mL) was added to the reaction followed by HATU (146.7 mg, 0.38 mmol) and the reaction was stirred at room temperature for 2 hours. The reaction was diluted with DCM (10 mL) and washed with H2O and brine (10 mL each). The organic layer was dried over Na2SO4, filtered and concentrated to dryness. The crude material was purified via silica gel column chromatography (0-15% MeOH:DCM) to afford **8**. MS: m/z $(M+1)^+$: 303.24

(9) 2-(2-(3-(pyridin-3-yl)ureido)thiazol-5-yl)-N-(4-(pyrrolidin-1-yl)phenyl)acetamide:

8 (10.0 mg, 0.033 mmol) was dissolved in DMF (0.66 mL). To the reaction was then added DIPEA (28.8 µL, 0.165 mmol) followed by the pyridine-3-isocyanate (12.5 mg, 0.104 mmol). The reaction was stirred at room temperature for 3 hours. The crude reaction was purified via HPLC to afford pure **9** (8.5 mg). MS: m/z $(M+1)^+$: 423.25

Synthesis of ACV-3-142

Scheme 4



(10) 6-amino-N-(4-(pyrrolidin-1-yl)phenyl)nicotinamide:

4-(pyrrolidin-1-yl)aniline dihydrochloride (263.0 mg, 1.12 mmol) was suspended in DCM (1.5 mL). DIPEA (650.7 µL, 3.74 mmol) was added to neutralize the HCl salts giving a clear brown solution. The solution was then transferred to a vessel containing 6-aminonicotinic acid (51.6 mg, 0.37 mmol). DMF (0.5 mL) was added followed by HATU (172.4 mg, 0.45 mmol) and the reaction was stirred at room temperature for 4 hours. The reaction was transferred to a separatory funnel and diluted with DCM then washed with H2O, saturated NaHCO3, and brine. The organic layer was collected, dried over Na2SO4, filtered, and concentrated to dryness. The crude material was purified via silica gel column chromatography (0-10% MeOH:DCM) to afford **10**. MS: m/z $(M+1)^+$: 283.31. ^{1}H NMR (500 MHz, METHANOL-*d*$_4$) δ ppm 1.31-1.38 (m, 3H) 2.00-2.07 (m, 4H) 3.24-3.30 (m, 4H) 6.48–6.72 (m, 3H) 7.39 (d, *J* = 8.85 Hz, 2H) 7.97 (dd, *J* = 8.85, 2.44 Hz, 1H) 8.52 (d, *J* = 2.14 Hz, 1H).

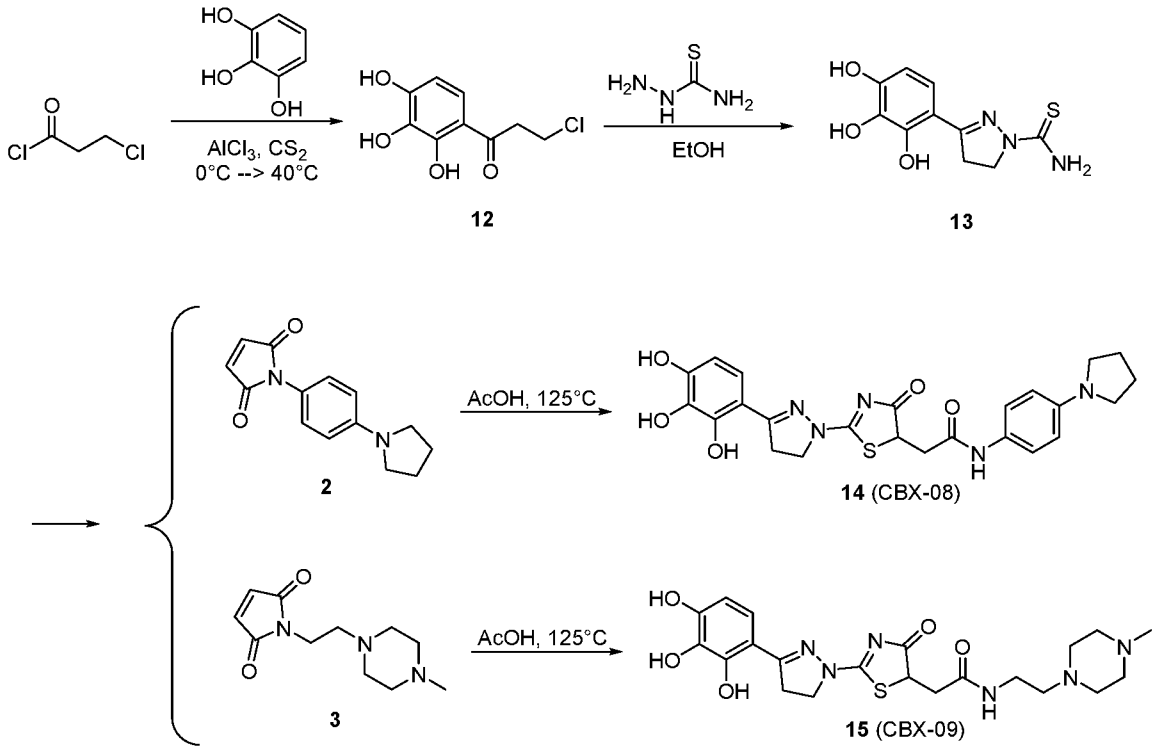
(11) 3-(benzylamino)-N-(4-(pyrrolidin-1-yl)phenyl)-2-(2,3,4-trihydroxyphenyl)imidazo[1,2-a]pyridine-6-carboxamide:

10 (20.9 mg, 0.07 mmol) and the 2,3,4-trihydroxybenzaldehyde (21.2 mg, 0.14 mmol) were combined and suspended in MeOH (1 mL). To the reaction was then added the benzyl

isocyanide (12.7 µL, 0.10 mmol) followed by HClO4 (9.1 µL of a 1M solution in MeOH , 0.01 mmol). The reaction was stirred at room temperature overnight. The reaction was transferred to a separatory funnel and diluted with DCM then washed with H2O, saturated NaHCO3, and brine. The organic layer was collected, dried over Na2SO4, filtered, and concentrated to dryness. The crude material was purified via silica gel column chromatography (0-100% EtOAc:Hexanes) to afford **11** (2.2 mg). MS: m/z $(M+1)^+$: 536.36. ^{1}H NMR (500 MHz, METHANOL-d_4) δ ppm 2.00-2.06 (m, 4H) 3.28 (t, J = 6.41 Hz, 4H) 4.11-4.17 (m,2H) 6.47 (d, J = 8.54 Hz, 1H) 6.59 (d, J = 8.54 Hz, 2H) 7.13-7.20 (m, 3H) 7.21-7.26 (m,2H) 7.42 (dd, J = 8.54, 5.80 Hz, 3H) 7.48 (d, J = 9.16 Hz, 1H) 7.69 (dd, J = 9.31, 1.68 Hz, 1H) 8.65 (s, 1H).

Synthesis of CBX-08/CBX-09

Scheme 5



(12) 3-chloro-1-(2,3,4-trihydroxyphenyl)propan-1-one:

Benzene-1,2,3-triol (1.94 g, 15.4 mmol) and 3-chloropropanoyl chloride (2 g, 15.4 mmol) were combined in CS_2. The reaction was placed in an ice bath and $AlCl_3$ (6.16 g, 46.2 mmol) was added. The reaction was stirred at 0°C for 10 minutes and then heated to 40°C overnight. The crude material was purified via Prep-TLC to afford **12** as a yellow solid.

(13) 3-(2,3,4-trihydroxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide:

12 (200 mg, 0.92 mmol) was suspended in EtOH and thiosemicarbazide (85.2 mg, 0.92 mmol) was added. The reaction was heated to 60°C overnight. The crude reaction was purified via prep-HPLC to afford **13**.

(14) 2-(4-oxo-2-(3-(2,3,4-trihydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4,5-dihydrothiazol-5-yl)-N-(4-(pyrrolidin-1-yl)phenyl)acetamide (CBX-08):

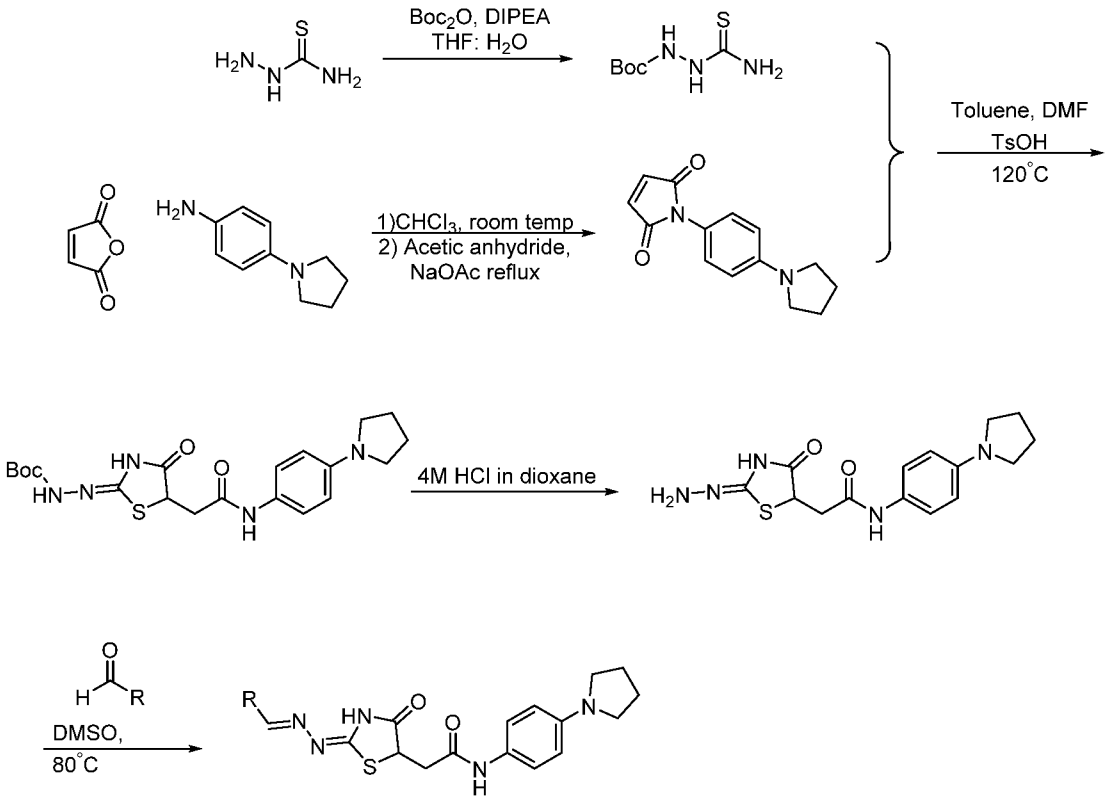
13 (50 mg, 0.20 mmol) and **2** (47.8 mg, 0.20 mmol) were combined and suspended in acetic acid. The reaction was heated to 125°C for 4 hours. The crude material was purified via prep-HPLC to afford **14** as a brown solid (1 mg).

(15)N-(2-(4-methylpiperazin-1-yl)ethyl)-2-(4-oxo-2-(3-(2,3,4-trihydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4,5-dihydrothiazol-5-yl)acetamide (CBX-09):

13 (50 mg, 0.20 mmol) and **3** (44.0 mg, 0.20 mmol) were combined and suspended in acetic acid. The reaction was heated to 125°C for 4 hours. The crude material was purified via prep-HPLC to afford **15** as an off-white solid (4.8 mg).

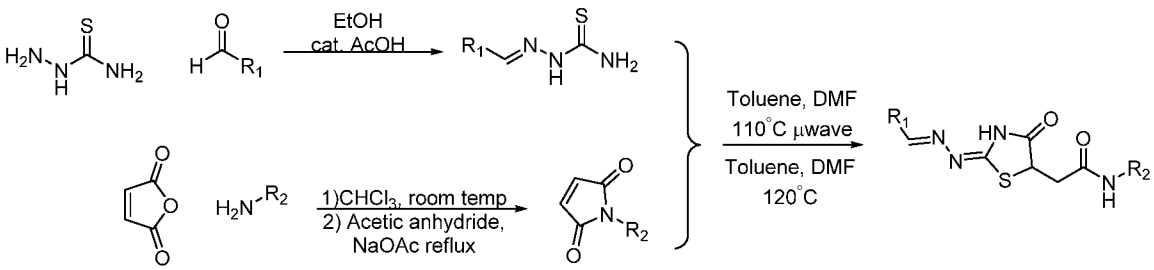
Synthesis of exemplary compounds of Formula II

Scheme 6



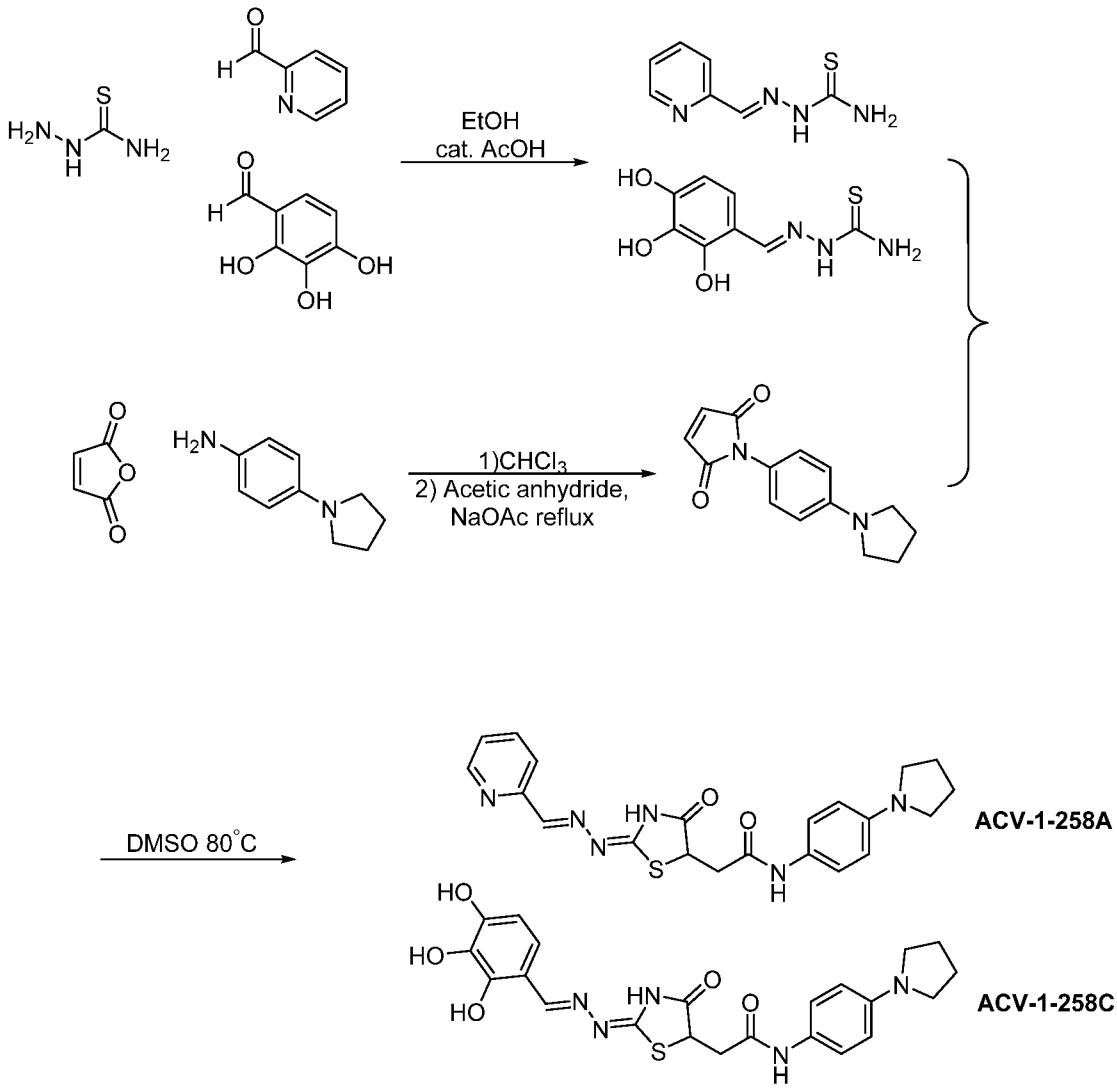
Alternate Synthesis of exemplary compounds of Formula II

Scheme 7



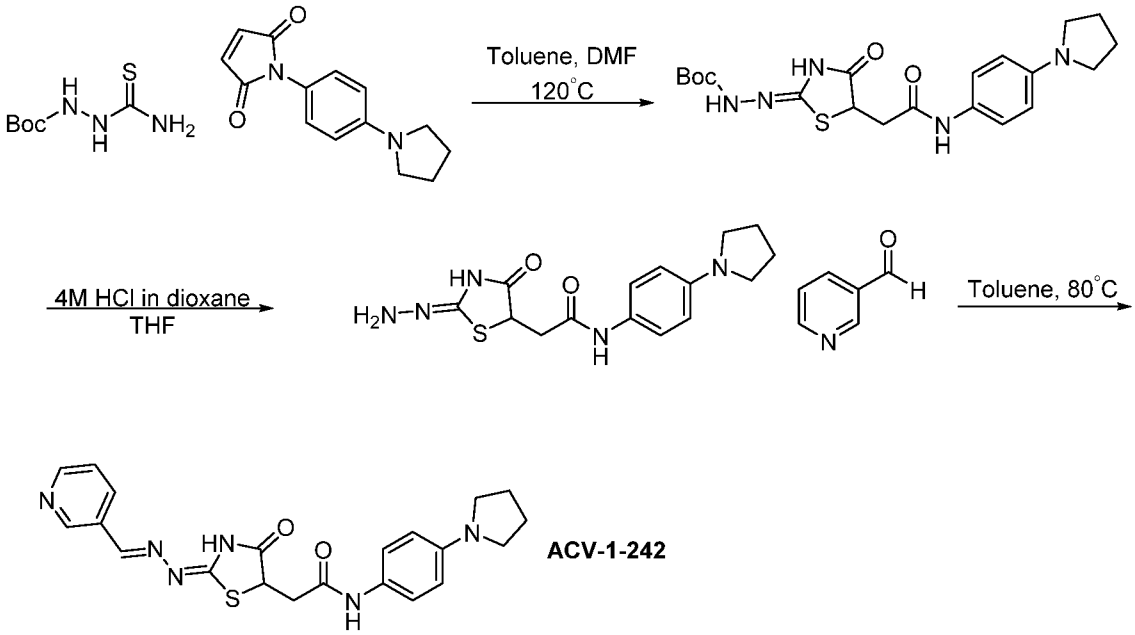
Synthesis of ACV-1-258A and ACV-1-258C

Scheme 8



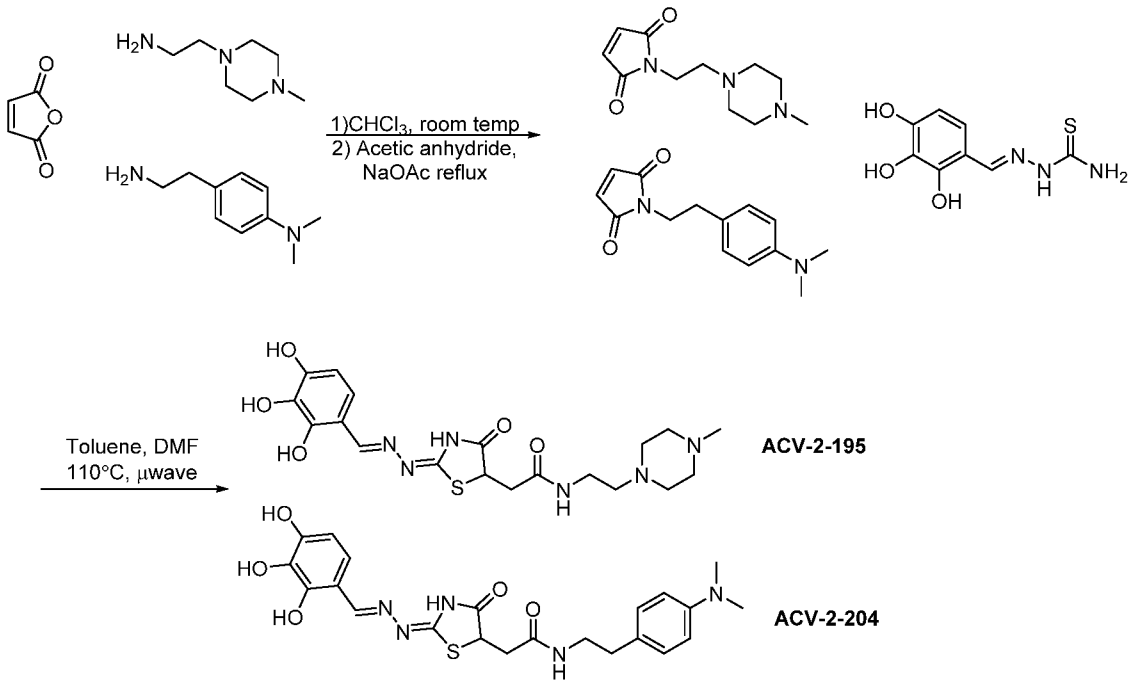
Synthesis of ACV-1-258A and ACV-1-242

Scheme 9



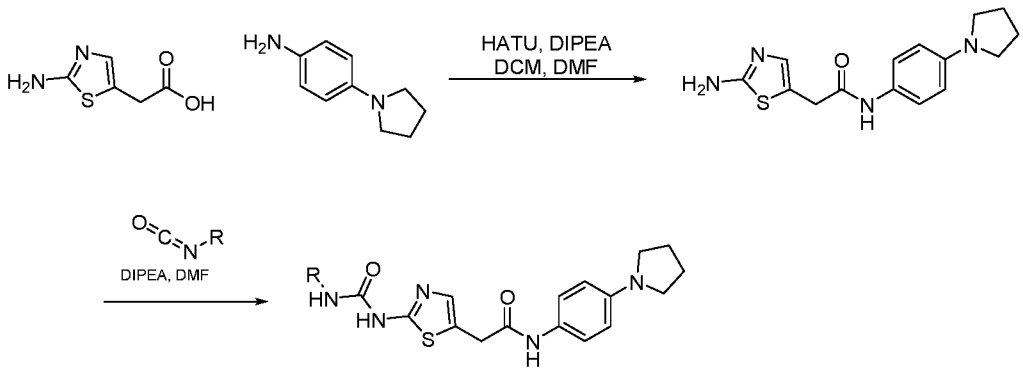
Synthesis of ACV-2-195 and ACV-2-204

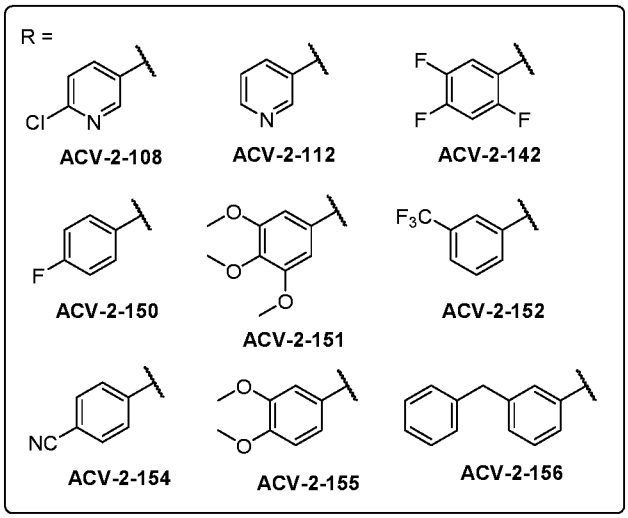
Scheme 10



Synthesis of ACV-2-108, ACV-2-112, ACV-2-142, ACV-2-150, ACV-2-151, ACV-2-152, ACV-2-154, ACV-2-155, and ACV-2-156

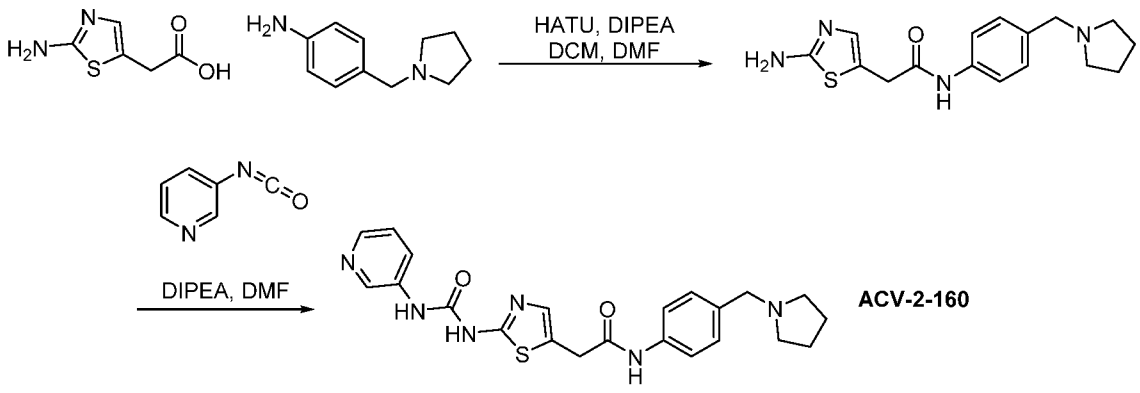
Scheme 11





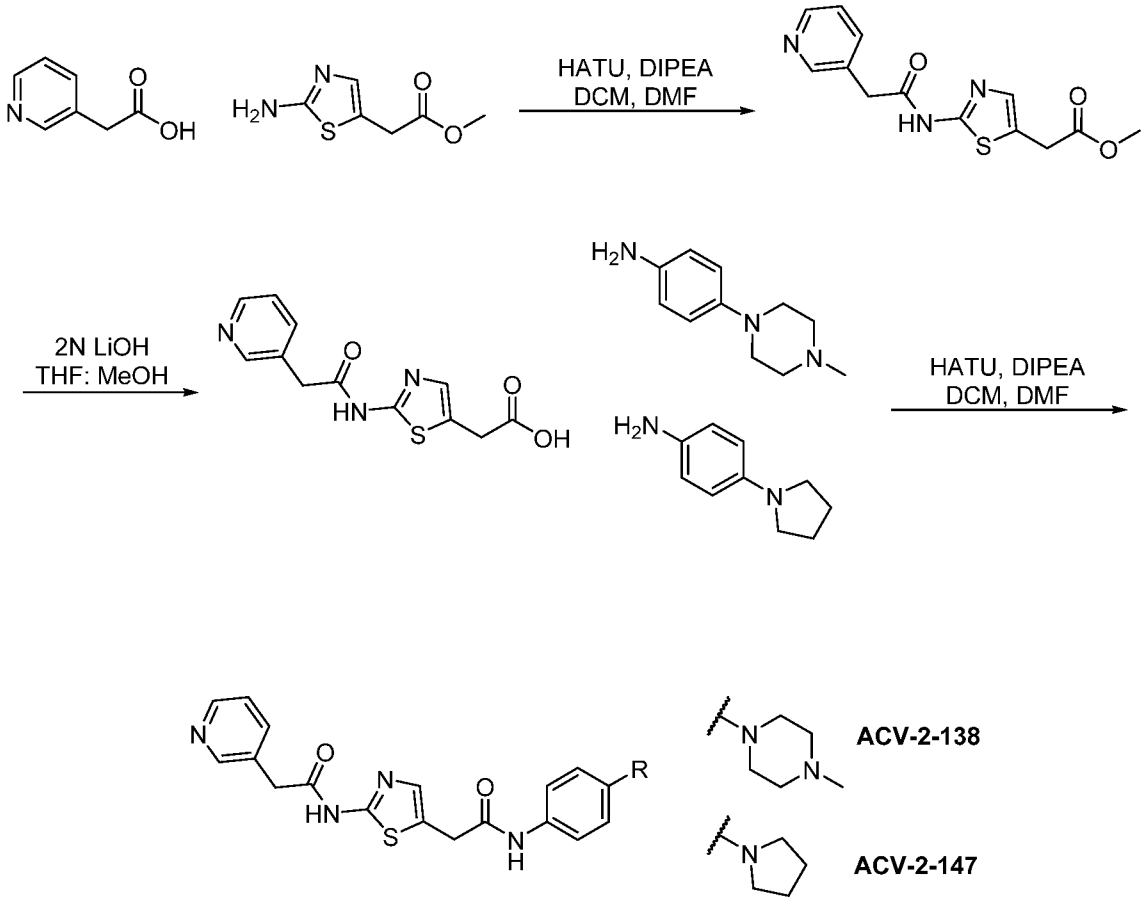
Synthesis of ACV-2-160

Scheme 12



Synthesis of ACV-2-138 and ACV-2-147

Scheme 13



Example 2

A biochemical assay was developed based on nanoparticle AlphaScreen assay to monitor the binding of recombinant CBX chromodomain (CBX7) to a methylated peptide (H3K27me3).

The primary assay uses recombinant CBX chromodomain and synthetic, trimethylated H3 histone tail peptide. When CBX/histone tail-binding occurs, AlphaScreen beads are brought in close proximity, resulting in singlet molecular oxygen transfer and production of a luminescent signal. Assay positives inhibiting the binding interaction are therefore expected to prevent complex formation, diminishing signal intensity. The AlphaScreen technology features an anti-Stokes shift in detection: due to energy transfer between beads by singlet oxygen and internal chemical reactions within the acceptor bead, the emission wavelength is blue-shifted relative to the excitation wavelength, which is impossible in

traditional fluorescent assays. The relatively long path length of 200 nm that singlet molecular oxygen can travel enables reporting on full protein complexes and not just single target binding. The assay proves highly amenable to miniaturization. Neither the small molecules being tested nor the target protein, CBX7, need to be chemically, conformationally, or behaviorally altered by tethering them to a surface or reporter that may decrease physiological relevance and beget screening artifacts. The primary assay monitors binding of the chromodomain of CBX7 to a histone tail containing a trimethylated lysine at K27, the biologically relevant interaction through which PRC binds to chromatin to repress transcription. This is a robust proximity-based assay that utilizes AlphaScreen technology (PerkinElmer). The assay consists of His_6-CBX7 bound to a Ni^{++} acceptor bead and the biotinylated histone tail bound to a streptavadin donor bead. Upon CBX7/H3K27me3 binding, the steptavidin bead comes into proximity with the Ni^{++} bead to luminesce upon light excitation (680 nM; *see below*).

This assay was tested for Z' and reproducibility over time in smaller libraries before moving on to larger libraries. In total, the number of distinct compounds screened is greater than 250,000 across the libraries.

The protocol for the CBX7 AlphaScreen was as follows: a standard alpha buffer (50mM HEPES, 150mM NaCl, 0.1% w/v BSA, 0.01% w/v Tween20) at pH 8.0 and make up two stock solutions at 2x final concentration in the alpha buffer. Solution A contains Human His6-CBX7 is used with biotinylated-H3K27me3 (residues 20-34), at 100nM and 50nM respectively. Solution B contains 20nM streptavidin donor beads and 20nM nickel acceptor beads. For 384 well assay formats, 10uL of solution A is added to each well of the assay plate and the plate is spun at 1000 rpm for 30 seconds. 100 nL of experimental compounds from stock plates are delivered by robotic pin transfer using a Janus Workstation (PerkinElmer), allowing the compounds to interact with CBX7 binding prior to assay measurement, followed by another spin and an incubation room temperature. Finally, 10uL of solution B is added to each well, the plate is spun again and then incubated at room temperature. AlphaScreen measurements are performed on an Envision 2104 (PerkinElmer) utilizing the manufacturer's protocol that has the correct excitation and emission wavelengths, cutoff filters, delay time, etc. A crosstalk calculation is also done through the Envision software to correct for luminescence for adjacent wells while reading the plate. The signal is then normalized to DMSO control wells on the compound plate prior to creation of the IC50 curves.

The assay reports on inhibition of binding with high fidelity (Z' of 0.88) in a dose-responsive manner. Assay positives from the primary screen were retested at 10-point dose response in the primary assay, to determine relative potencies and to screen for artifacts resulting from certain forms of assay interference. Additionally, all assay positives were studied for assay-specific activity (so-called "Alpha inhibition"), using a familiar, commercially available assay involving a biotinylated poly-histidine peptide which cross-links donor and acceptor beads. All compounds which scored in this assay were eliminated for non-selective activity (e.g. metal chelators, oxygen quenchers, biotin mimetics). From the libraries screened, we found a few different scaffolds that looked promising and brought them forward for further medicinal chemistry and structure activity relationship studies.

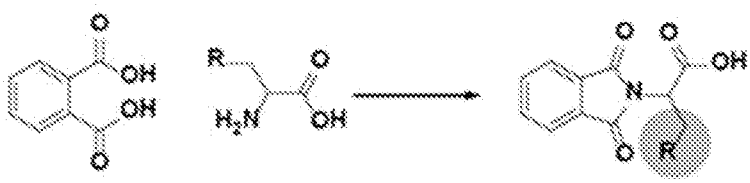
The assay performed well in the under high throughput conditions. Assay signal is stable for over 24 hours, although all measurements were made within 6 hours of the final addition of assay reagents. The assay proved robust over time. The Z' value, which is a measurement of the assay signal-to-noise taking into account variability in the positive and negative control values, was consistently above 0.8 for the duration of the screening. A value of 0.5 is generally considered acceptable to identify assay positives. Compound data between the two assay replicates were highly reproducible and correlative.

The overall hit rate in was 0.9%, higher than most biochemical screens, but was expected due to the rate of compounds interfering with AlphaScreen detection. All the assays that were run have also been screened against other proteins using AlphaScreen technology (bromodomains and methyltransfersases). Compounds that are active in all three screens were removed from consideration and annotated as 'AlphaScreen inhibitors'. This filter decreased the hit rate to 0.4%, and a set of 3 scaffolds (Figure 2) was assessed for potency and selectivity in biochemical and cellular assays.

Example 3

In order to further test and confirm the binding potency of the three 'hit' scaffolds to CBX, a collection of compounds was designed and synthesized for each scaffold to explore the structure-activity relationship (SAR). For Scaffold 1, a synthetic route was established to produce a variety of amides (Scheme 14). However, modification at the R position (highlighted) did not improve activity over the original compound. Further modification of the acid motif of Scaffold 1 also completely destroyed the compound's activity as assayed by the CBX AlphaScreen (Figure 1). The narrow SAR around Scaffold 1 prompted the exploration of other scaffolds.

Scheme 14



Example 4

Next, the SAR around Scaffold 2 was explored by utilizing a hydrazone library synthesis strategy or 'cap scanning' method to produce more than 400 analogues of Scaffold 2 in a high-throughput manner. Among these compounds, several molecules were identified with improved binding affinity for CBX. Among these compounds, ACV-1-183A and ACV-1-183D provided more than 5-fold improvement in binding affinity compared to the original scaffold (Figure 7). The same 'cap scanning' synthesis strategy was utilized to explore the optimization of Scaffold 3. The resulting library of compounds was screened via the CBX AlphaScreen assay and a series of promising molecules were identified, such as JQI-III-263-A, with improved activity (Figure 8).

Encouraged by the SAR established through library optimization of the three hit scaffolds, novel compounds were generated by combining the motifs that improved activity into the backbone of Scaffold 3. A new series of compounds were designed and synthesized based on computational modeling of inhibitor compounds with the literature reported crystal structure of CBX. This novel set of molecules has largely improved binding affinity for CBX (Figure 9). Notably, ACV-1-285 possesses low micromolar binding affinity with CBX. The binding affinity of the small molecule was further confirmed by isothermal titration calorimetry (Figure 20).

Example 5

To predict the interaction between compounds and CBX protein, a 15N labeled CBX was generated for use in NMR studies. The labeled protein was used to map the interaction between the small molecule inhibitor and CBX protein . Compound ACV-2-112, a potentially more stable produced significant peak relocation on CBX protein NMR, which further confirmed the binding of small molecule toward the CBX.

Briefly, an overnight culture of His6CBX7 was grown in 5 ml of LB with antibiotic. The inoculated 1 liter of the 15N base media (2mM MgSO4, 100uM CaCl2, 1g 15NH4Cl,

25 ml 20% w/v Glucose in PBS) plus antibiotic with the overnight culture and incubated at 37C. The temperature of the media was reduced to 23°C at OD = 4.0 and induced at OD = 7 with 1mM isopropyl-thio-β-D-galactopyranoside and incubated for an additional 12 hours. The culture was then pelleted and protein purification was started. Each pellet was resuspended in 40 ml lysis buffer (250 mM NaCl in PBS plus Halt protease inhibitor) and homogenized for 1 hour at 4C. Cell lysis was accomplished by sonication on ice, the sonication protocol was a 5 second pulse at amplitude = 40, 30 second rest, for 10 minutes total sonication time. Unclarified lysate was mixed with 3-4 ml Ni-NTA superflow Resin (Qiagen). The mixture was incubated overnight on ice. The next day it was loaded onto an empty column and washed with 20 ml wash buffer A (250mM NaCl, 10mM imidizole in PBS) and 5 ml wash buffer B (250mM NaCl, 50mM imidazole in PBS). Samples were eluted from the resin by exposure to 2-3 column volumes of elution buffer (250mM NaCl, 500mM imidazole in PBS) and collected in fractions. Elution fractions containing protein were confirmed by a protein gel and the protein was dialyzed overnight in dialysis buffer (300mM NaCl, 500mM tris(2-carboxyethyl)phosphine in PBS). The N15 labeled protein was titrated with the ligand ACV-2-112 and HSQC spectra were recorded for each point using the 500 Hz NMR located in the HMS East Quad NMR facility. Figure 15 shows preliminary data from the experiment that seems to indicate the compound ACV-2-112 is binding into the aromatic cage of the CBX protein which is the same area that coordinates the trimethylated lysine residue of the peptide.

The amino acids interacted with small molecule were assigned based literature reported NMR structure of CBX, and identified the binding site of small molecule on CBX.

Example 6

Lastly, the biological activity of lead compounds was assessed in cancer cells lines determined to be sensitive to CBX inhibition. First, a cell line screening for CBX2 and CBX3 dependence/sensitivity was conducted using shRNAs. It was found that the HCT292 human lung cancer cell line was sensitive to CBX2 and CBX3 knockdown, while the NCI-H1792 human lung cancer cell line was not. These cell lines were utilized to evaluate the potential cellular activity of a panel of CBX inhibitors (Tables 3-9).

Briefly, HCT292 cells were treated 24 hours after passage; the compounds in DMSO were added directly to their media. Initially, which cells caused cell death was checked by fixing and staining the cells with crystal violet 96-hours post treatment and then reading the

absorbance using the Envision and comparing the signal to a DMSO control. This was done as a preliminary viability screen prior to the ATPlite assay to determine which compounds HCT292 cells were and H661 cells were not sensitive to. Then, cell viability assays were performed using the ATPlite kit to determine which compounds decreased cell viability. The small molecule CBX inhibitors that are disclosed herein effectively inhibit the growth of HCT292 cells in the similar manner as the shRNA. On the other hand, these molecules did not show any activity against the insensitive cell line, NCI-H17912 (Figure 21). Thus, a cellular system to evaluate the activity of small molecule CBX inhibitors was established.

Example 7

The protocol for the CBX7 Fluorescence Polarization assay is as follows: A standard FP buffer (20mM Tris, 250mM NaCl, 0.01% w/v Tween20) at pH 8.0 is created and make up one stock solution at 1x final concentration in the FP buffer. The solution contains Human His_6-CBX7 used with FITC-VARKme3SA, at 250nM and 10nM respectively. For 384 well assay formats, 10uL of solution is added to each well of the assay plate and the plate is spun at 1000 rpm for 30 seconds. 100 nL of experimental ccompounds from stock plates are delivered by robotic pin transfer using a Janus Workstation (PerkinElmer), allowing the compounds to interact with CBX7 binding prior to assay measurement, followed by another spin and an incubation room temperature. Fluorescence Polarization measurements are performed on an Envision 2104 (PerkinElmer) utilizing the manufacturer's protocol that has the correct excitation and emission wavelengths, cutoff filters, delay time, etc. A crosstalk calculation is also done through the Envision software to correct for luminescence for adjacent wells while reading the plate. The signal is then normalized to DMSO control wells on the compound plate prior to creation of the IC_{50} curves.

Table 3

Compound, dose	% Viability
DMSO	100
2-121, 50uM	95.8

1-258C, 10uM	72.2
1-258C, 50uM	33.0
2-165, 50uM	58.8
258C (CP), 50uM	20.3

Table 4

Compound	**IC50 (uM)**
ACV-2-165 (258C)	0.3337
ACV-2-129 (242)	115.3
ACV-2-112	83.93
ACV-2-121	1001

Table 5

Compound, dose	**% Viability**
DMSO	100
2-121, 50uM	95.8
2-195, 10uM	94.2
2-195, 50uM	43.5
2-195 (CP), 10uM	34.9
2-195 (CP), 20uM	41.0
2-195 (CP), 50uM	60.6

5

Table 6

Compound, dose	**% Viability**
DMSO	100
2-121, 50uM	95.8

2-112, 10uM	99.0
2-112, 50uM	82.9

Table 7

Compound, dose	% Viability
DMSO	100
2-121, 50uM	95.8
3-048, 10uM	31.3
3-048, 20uM	29.0
3-048, 50uM	28.0

Table 8

Compound, dose	% Viability
DMSO	100
2-121, 50uM	95.8
3-061, 10uM	33.5
3-061, 20uM	30.5
3-061, 50uM	18.0

5

Table 9

Compound Name	Alternate Name	AVG CBX7 Alpha IC50 (M)	AVG CBX2 Alpha IC50	CBX7 FP
ACV-01	ACV-1-180	2.83E+01	1.03E-04	
ACV-02	ACV-1-182	1.44E-01	5.54E-05	
ACV-03	ACV-1-183-A	4.45E-07	2.15E-05	
ACV-04	ACV-1-183-B	3.52E-07	2.55E-06	
ACV-05	ACV-1-183-D	1.88E-06	0.00E+00	

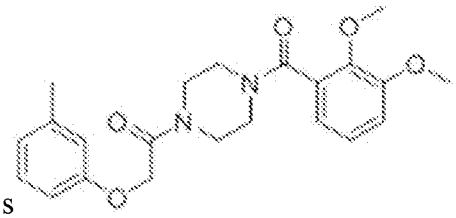
ACV-06	ACV-1-183-E	1.36E-05	1.44E-03	
ACV-07 (1)	ACV-1-190			
ACV-07 (2)	ACV-2-192	1.94E-04	8.55E-02	
ACV-08	ACV-1-191	1.03E-05	4.77E-05	
ACV-09	ACV-1-195-A	7.39E-06	2.82E-05	
ACV-10	ACV-1-195-D	2.02E-05	9.88E-05	
ACV-11	ACV-1-202	8.51E-06		
ACV-12	ACV-1-204	6.10E-06	0.00E+00	
ACV-13	ACV-1-205	6.95E-05	3.18E-01	
ACV-14 (1)	ACV-1-242	4.41E-06	1.52E-05	3.04E-04
ACV-14 (2)	ACV-2-129	1.02E-05	1.97E-05	
ACV-15	ACV-1-258-A	3.73E-05	5.52E-06	
ACV-16	ACV-1-258-B	6.79E-06	2.04E-05	
ACV-17 (1)	ACV-1-258-C	6.97E-05	7.87E-07	1.20E-04
ACV-17 (2)	ACV-2-165	9.30E-07	5.72E-07	
ACV-17(3)	ACV-2-189	1.01E-06	3.79E-07	
ACV-17(4)	ACV-2-206-A	1.56E-06	2.60E-07	
ACV-17(CP)	ACV-258C (CP)	1.74E-06		
ACV-17* (P1)	ACV-258C (P1)	2.36E-06		
ACV-17* (P2)	ACV-258C (P2)	3.37E-06		
ACV-18	ACV-1-259	6.91E+01	3.67E-05	
ACV-19	ACV-1-261	3.35E-03	9.42E-07	1.52E-04
ACV-20	ACV-1-273	2.86E-05	5.56E-06	
ACV-21	ACV-1-283-B	3.69E-06	2.40E-04	
ACV-22	ACV-1-285-D1/ACV-1-285-MIX	1.29E-02	1.17E-04	
ACV-22	ACV-1-285-D1/ACV-1-285-MIX	5.85E-06	2.89E-04	
ACV-23	ACV-1-288	0.00E+00	2.00E-06	

ACV-24	ACV-2-007-A	6.53E-05	4.37E-05	0.00E+00
ACV-25	ACV-2-007-B	6.09E-05	2.51E-05	0.00E+00
ACV-26	ACV-2-011-A	1.66E-05	2.28E-05	2.26E+02
ACV-27	ACV-2-011-B	3.39E-05	3.51E-05	2.89E+02
ACV-28	ACV-2-015	4.46E-05	5.60E-05	6.67E-03
ACV-29	ACV-2-016-A/B	3.90E-05	3.84E-05	1.39E+03
ACV-29	ACV-2-016-A/B	1.55E-05	1.65E-05	8.00E+00
ACV-30	ACV-2-019	3.90E-05	2.09E-05	
ACV-31	ACV-2-023	4.04E-04	2.05E-04	
ACV-32	ACV-2-024	2.35E-05	2.29E-05	
ACV-33	ACV-2-026	5.62E-05	6.25E-05	
ACV-34	ACV-2-029	7.90E-06	1.03E-07	
ACV-35	ACV-2-049	3.05E-06	2.21E-02	
ACV-36 (1)	ACV-2-082	1.00E-05	1.70E-05	
ACV-36 (2)	ACV-2-163	2.45E-05	2.88E-05	
ACV-37	ACV-2-083	1.14E-04	6.66E-05	
ACV-38	ACV-2-084	1.65E-04		
ACV-39	ACV-2-108	2.63E-05	1.25E-05	
ACV-40	ACV-2-112	1.28E-05	1.85E-05	
ACV-41	ACV-2-115	1.45E-03	2.56E-04	
ACV-42	ACV-2-121	1.55E-04	7.22E-05	
ACV-43	ACV-2-123	9.10E-03	6.73E-03	
ACV-44	ACV-2-127	1.01E-04	7.82E-05	
ACV-45	ACV-2-132	1.15E-01	3.13E-03	
ACV-46	ACV-2-138	8.84E-05	1.39E+13	
ACV-47	ACV-2-142	1.44E-02	1.04E-02	
ACV-48 (1)	ACV-2-147	4.03E-04	1.89E-04	
ACV-48 (2)	ACV-2-147	6.07E-04	2.59E-04	
ACV-49	ACV-2-150	1.72E-05	1.28E-05	
ACV-50	ACV-2-151	7.20E-06	1.31E-05	

ACV-51	ACV-2-152	2.41E-05	3.57E-05	
ACV-52	ACV-2-154	6.58E-06	9.34E-06	
ACV-53	ACV-2-155	1.25E-05	4.79E-05	
ACV-54	ACV-2-156	9.31E-06	7.99E-06	
ACV-55	ACV-2-160	2.30E-05	4.81E-05	
ACV-56	CBX-01	6.41E-05	0.00E+00	
ACV-57	CBX-02	3.36E-04	0.00E+00	
ACV-58	ACV-2-130	1.75E-08	1.93E-08	
ACV-59	ACV-2-179	7.59E-04	5.51E-05	
ACV-60	ACV-2-185	3.64E-07	1.67E-07	
ACV-61	ACV-2-188	3.94E-06	1.93E-06	
ACV-62	ACV-2-191	8.61E-07	6.09E-07	
ACV-63	ACV-2-195	1.83E-07	6.86E-08	
ACV-63 (2)	ACV-3-018	2.25E-05		
ACV-63 (CP)	ACV-2-195 (CP)	5.31E-06		
ACV-64	ACV-2-203	5.22E-06	2.93E-05	
ACV-65	CBX-03a		2.99E-10	
ACV-66	ACV-2-204	1.19E-05	3.44E-07	
ACV-67	CBX-03	0.00E+00	1.89E-02	
ACV-68	CBX-04	3.96E+12		
ACV-69	CBX-04a	1.37E+00	0.00E+00	
ACV-70	CBX-06	4.67E-05	1.46E-05	
ACV-71	CBX-06a	4.92E-03	0.00E+00	
ACV-72	CBX-07	1.24E-04		
ACV-73	ACV-2-224	1.65E-06	1.96E-07	
ACV-74	ACV-2-231	1.67E-04	7.21E-05	
ACV-75	ACV-2-233	6.86E-08		
ACV-76	ACV-2-247	3.76E-06		
ACV-77	ACV-2-251			
ACV-78	ACV-2-254	2.25E-07		
ACV-79	ACV-2-270	3.01E-06		

ACV-80	CBX-05	2.86E-11		
ACV-81	ACV-2-287	1.04E-04		
ACV-82	ACV-2-288	5.08E-05		
ACV-83	ACV-2-293	2.39E-05		
ACV-84	ACV-2-294	7.26E-06		
ACV-85	ACV-3-019	5.48E-05		
ACV-86	ACV-3-024	1.18E-05		
ACV-87	ACV-3-027	7.26E-06		
ACV-88	ACV-3-042	2.46E-04		
ACV-89	ACV-3-048	1.29E-05		
ACV-90	ACV-3-060	6.71E-01		
ACV-91	ACV-3-061	1.20E-05		
ACV-92	ACV-3-074	1.70E-04		
ACV-93	ACV-2-144	4.40E-04		
ACV-94	ACV-3-073	1.23E-04		
ACV-95	ACV-3-090	8.79E-04		
ACV-96	ACV-3-096	7.93E-05		
ACV-97	ACV-3-104	8.17E-05		
ACV-98	ACV-3-106	1.21E-04		
ACV-99	ACV-3-107	1.64E-05		
ACV-100	ACV-3-122			
ACV-101	ACV-3-123			
ACV-102	ACV-3-124			
ACV-103	ACV-3-126			
	ACV-3-140			
	ACV-3-142	6.60E-07		
	CBX-08	8.35E-07		
	CBX-09	5.34E-05		
	ACV-3-141	4.44E-05		
	ACV-3-145	2.73E-04		
	ACV-3-147	6.65E-06		
	ACV-3-191	1.06E-05		

	ACV-3-194	1.69E+01		
	ACV-3-195	3.56E+14		
	ACV-3-200	9.55E+03		
	ACV-3-205	6.83E-05		
	ACV-3-215	9.92E-05		
	ACV-3-217	5.23E-05		

CBX-07 is  .

Incorporation by Reference

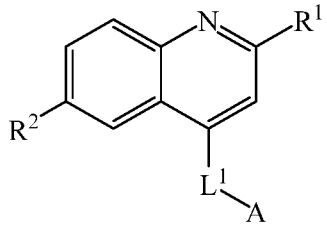
All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

Equivalents

While specific embodiments of the subject invention have been discussed, the above specification is illustrative and not restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of this specification and the claims below. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

Claims:

1. A compound having a structure of Formula I or a pharmaceutically acceptable salt thereof:



I

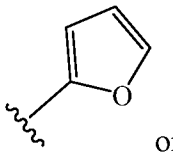
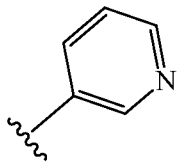
wherein

R^1 is optionally substituted aryl or heteroaryl;

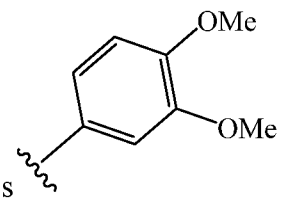
R^2 is H, halo, optionally substituted aryl, or optionally substituted heteroaryl;

L^1 is -C(O)-, -C(O)NH-, optionally substituted -C(O)NH-alkylene-, or -C(O)NHNCH-; and

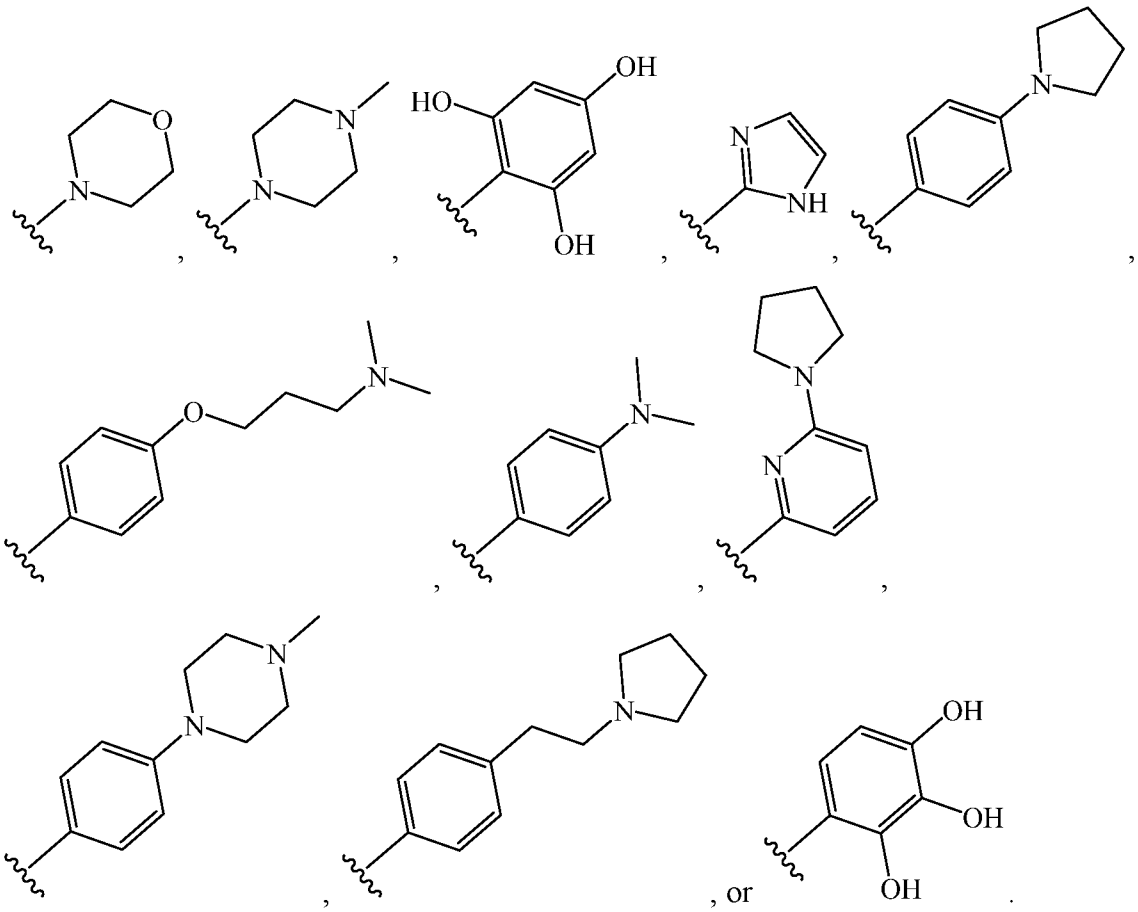
A is OH or optionally substituted heterocyclyl, aryl, or heteroaryl.

2. The compound of claim 1, wherein R^1 is  or  .

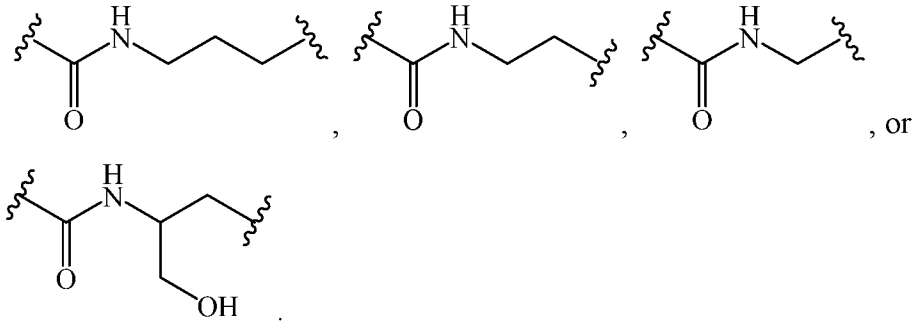
3. The compound of any preceding claim wherein R^2 is Br.

4. The compound of any one of claims 1 or 2, wherein R^2 is  .

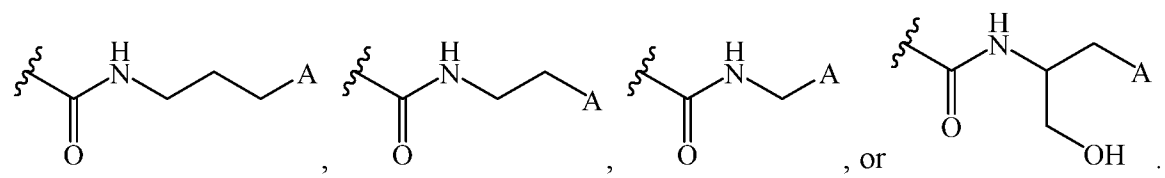
5. The compound of any preceding claim wherein A is

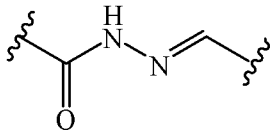


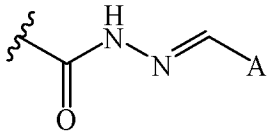
6. The compound of any preceding claim wherein L^1 is



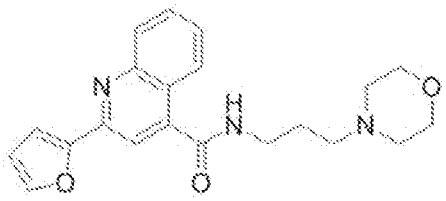
7. The compound of claim 6 wherein L^1-A is



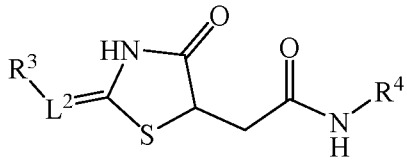
8. The compound of any one of claims 1-5, wherein L^1 is  .

9. The compound of claim 8, wherein L^1-A is  .

10. The compound of any preceding claim, wherein the compound is not

 .

11. A compound having a structure of Formula II or a pharmaceutically acceptable salt thereof:



II

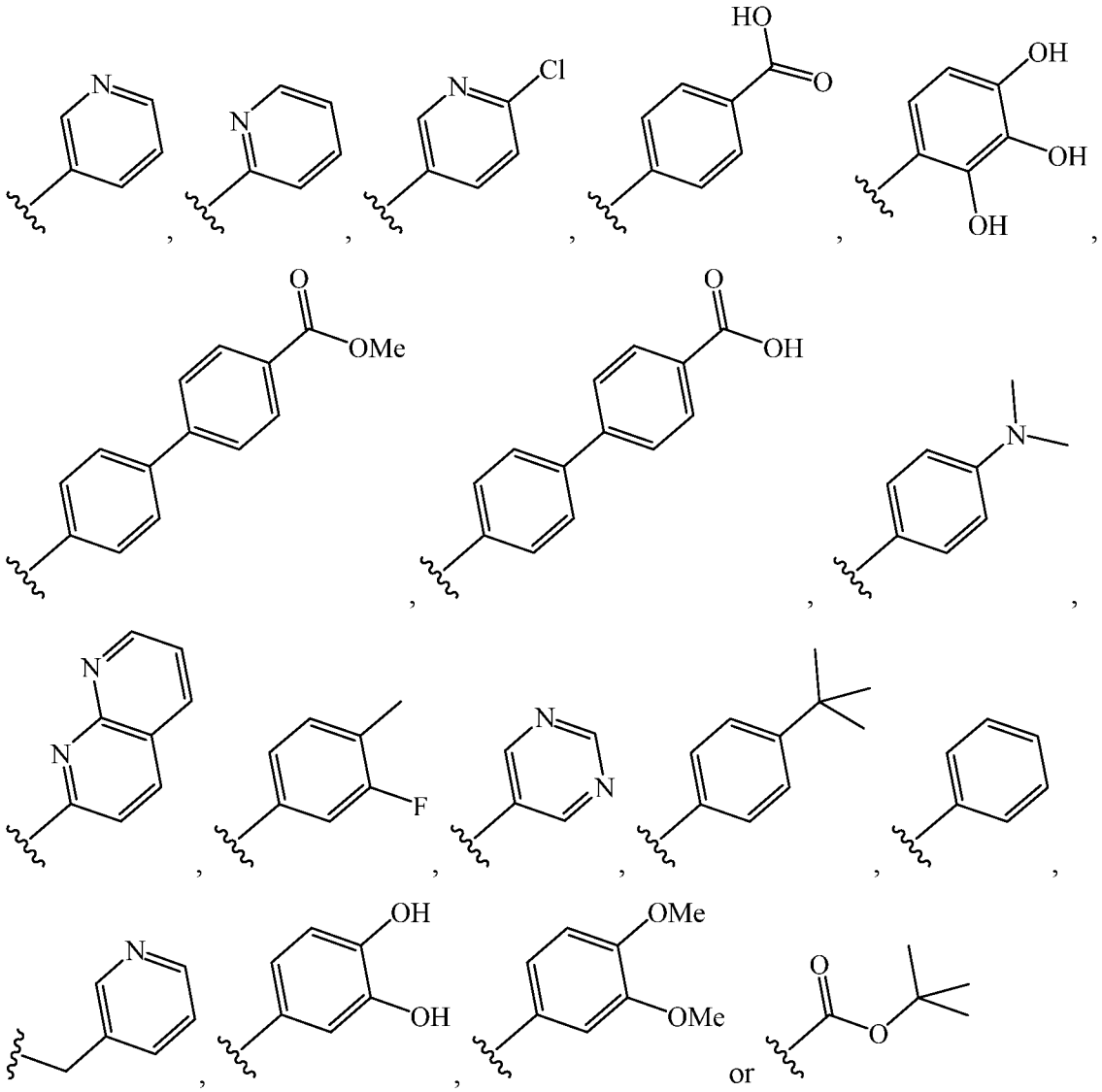
wherein

R^3 is optionally substituted aryl, heteroaryl, -C(O)-aryl, alkyl, or alkoxycarbonyl;

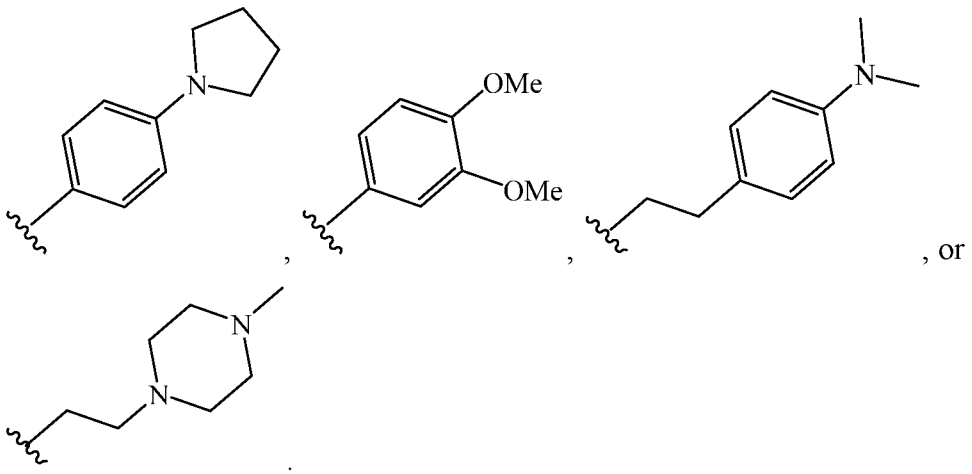
R^4 is optionally substituted alkyl, aryl or heteroaryl; and

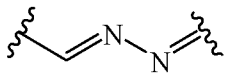
L^2 is =N-C(O)-, =N-NCH-, =N-, or =N-NH-.

12. The compound of claim 11, wherein R^3 is

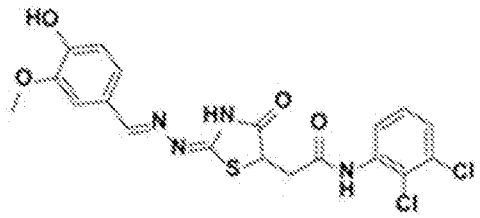


13. The compound of any one of claims 11 or 12, wherein R^4 is

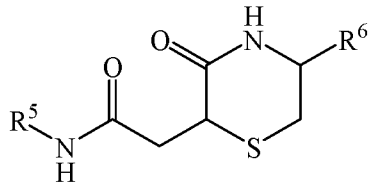


14. The compound of any one of claims 12-13, wherein L^2 is  .

15. The compound of any one of claims 12-14, wherein the compound is not



16. A compound have a structure of Formula III or a pharmaceutically acceptable salt thereof:



III

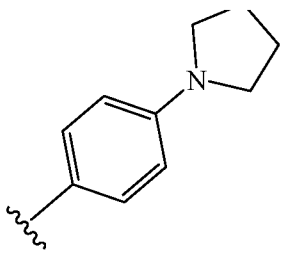
wherein

R^5 is optionally substituted aryl or heteroaryl;

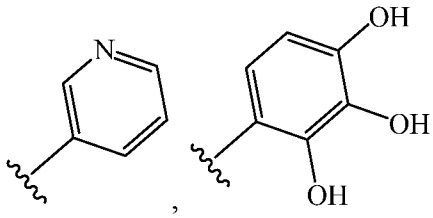
R^6 is -C(O)NH-NCH-R^7 or -C(O)O-alkyl; and

R^7 is optionally substituted aryl or heteroaryl.

17. The compound of claim 16, wherein R^5 is

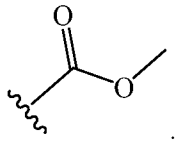


18. The compound of any one of claims 16 or 17, wherein R^7 is



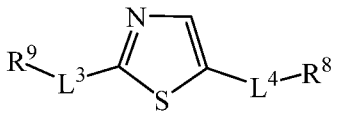
, , or .

19. The compound of any one of claims 16 or 17, wherein R^6 is

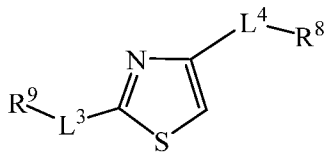


.

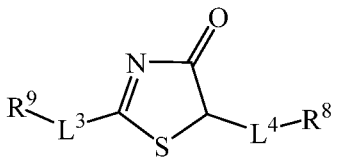
20. A compound having a structure of Formula IV or V or a pharmaceutically acceptable salt thereof:



IV



V



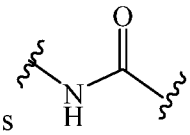
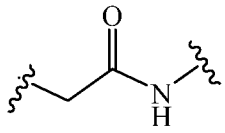
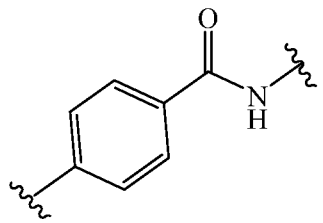
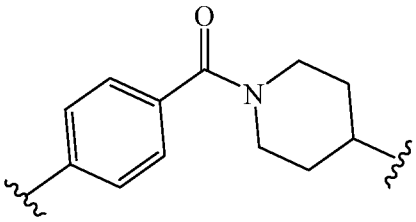
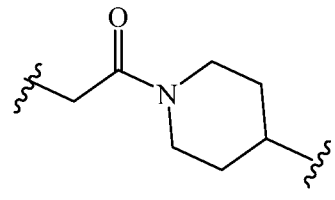
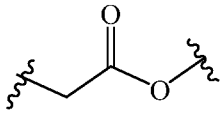
IX

wherein

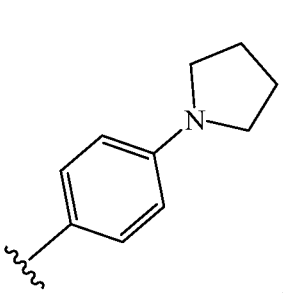
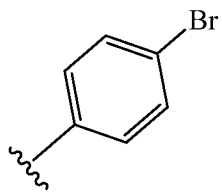
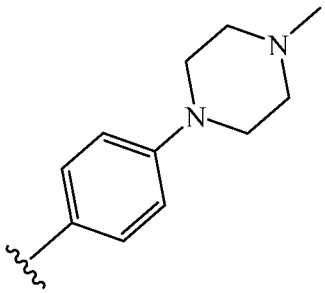
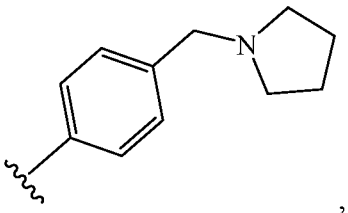
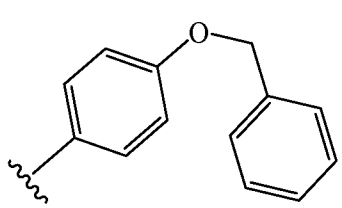
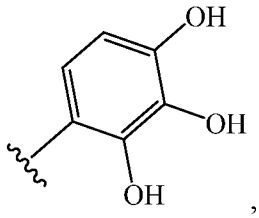
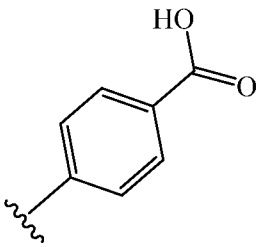
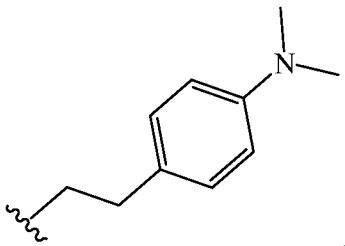
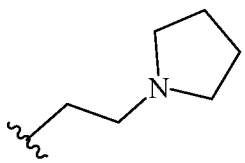
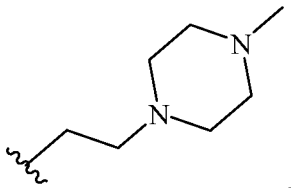
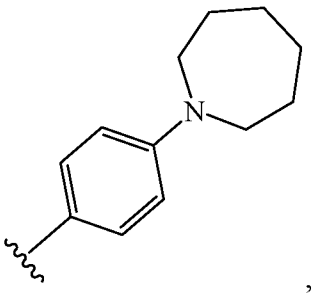
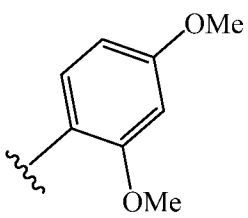
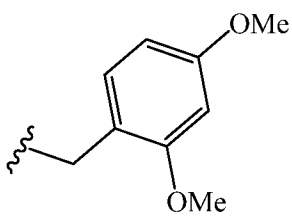
R^8 is optionally substituted alkyl, aryl, or heteroaryl ;

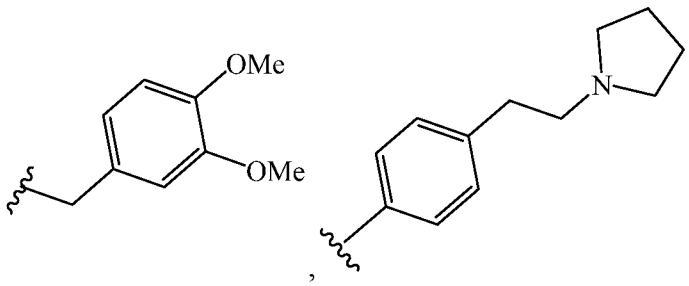
R^9 is optionally substituted aryl or heteroaryl;

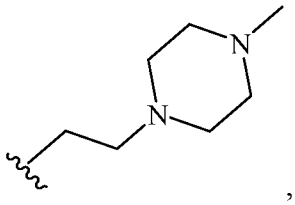
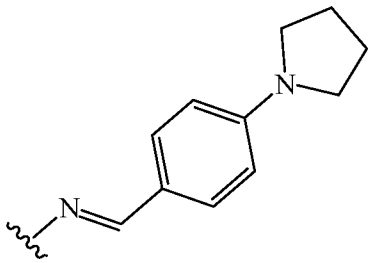
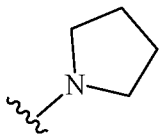
L^3 is -NH-CO-NH-, -NH-CO-, -NH-NCH_2-, -NH-, -CH_2-, or -CH_2-NH-CO-; and

L^4 is absent or is  ,  ,  ,  ,  , or  .

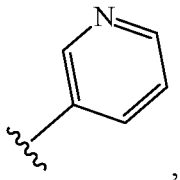
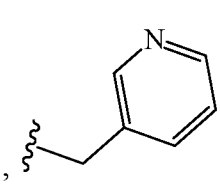
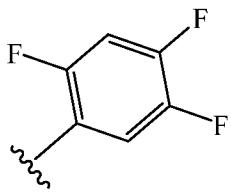
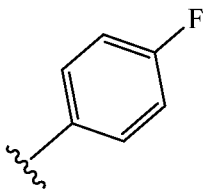
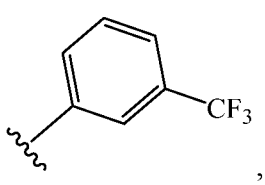
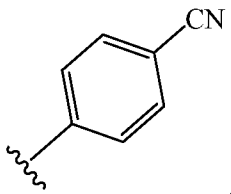
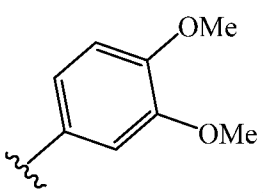
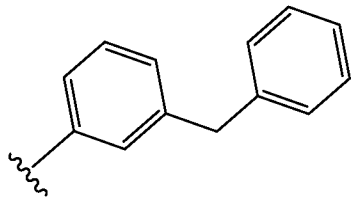
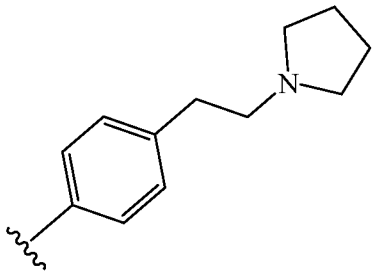
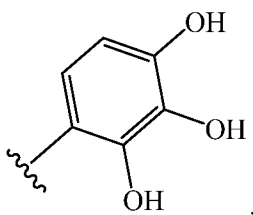
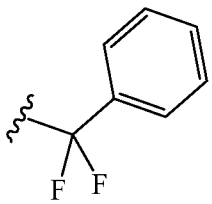
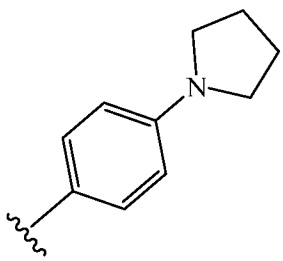
21. The compound of claim 20, wherein R^8 is

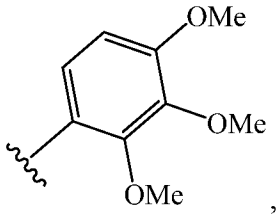
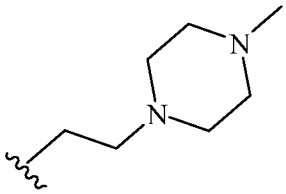
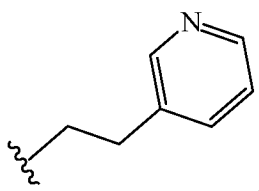
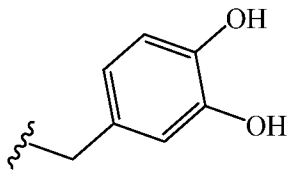
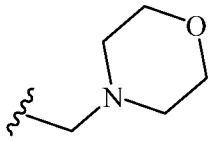
 ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  .



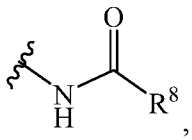
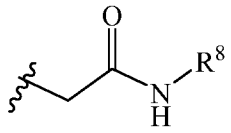
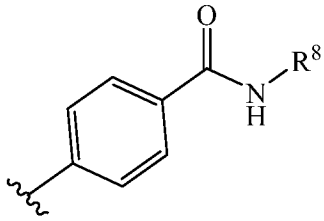
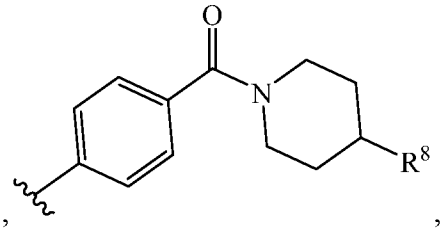



or -CH_3.

22. The compound of any one of claims 20 or 21, wherein R^9 is





, , , , or

.

23. The compound of any one of claims 20-22, wherein L^3-R^9 is -NH-CO-NH-R^9, -NH-CO-R^9, -NH-NCH_2-R^9, or -CH_2-NH-CO-R^9.

24. The compound of any one of claims 20-23, wherein L^4 is  ,



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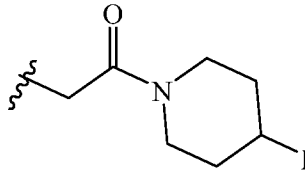
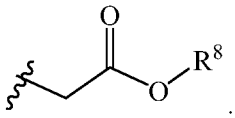
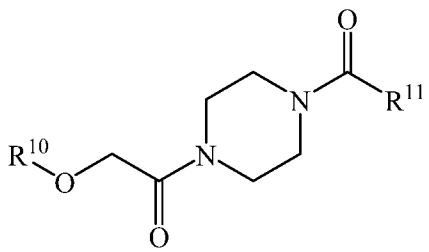


.

25. A compound having a structure of Formula VI or a pharmaceutically acceptable salt thereof:



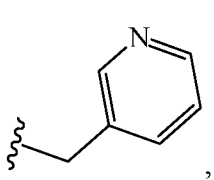
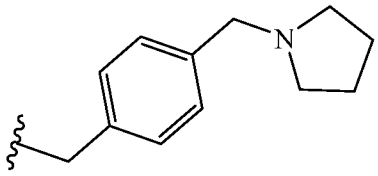
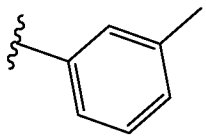
VI

wherein

R^{10} is optionally substituted alkyl, aryl or heteroaryl; and

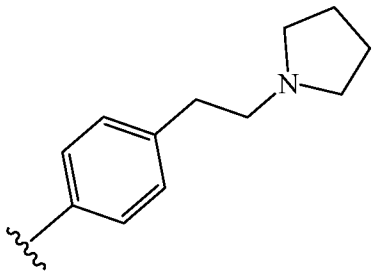
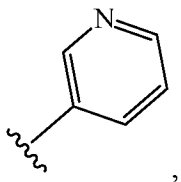
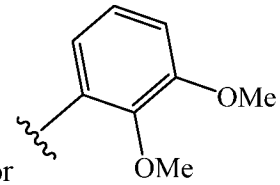
R^{11} is optionally substituted aryl or heteroaryl.

26. The compound of claim 25, wherein R^{10} is

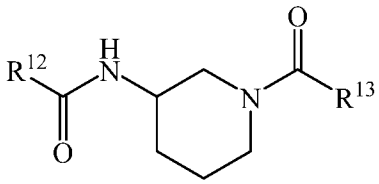
, , or .

27. The compound of any one of claims 25 or 26, wherein R^{11} is

, , or .

28. A compound having a structure of Formula VII or a pharmaceutically acceptable salt thereof:



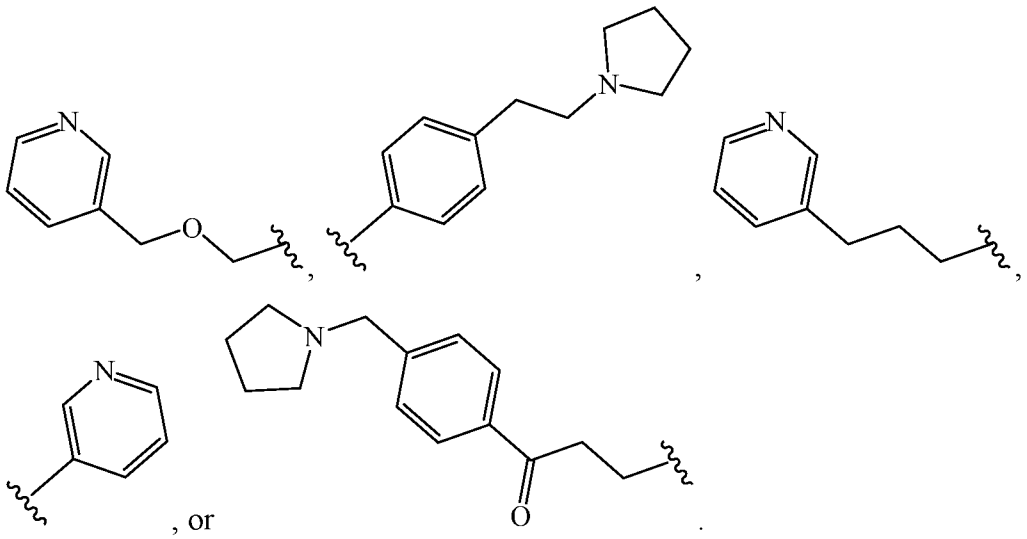
VII

wherein

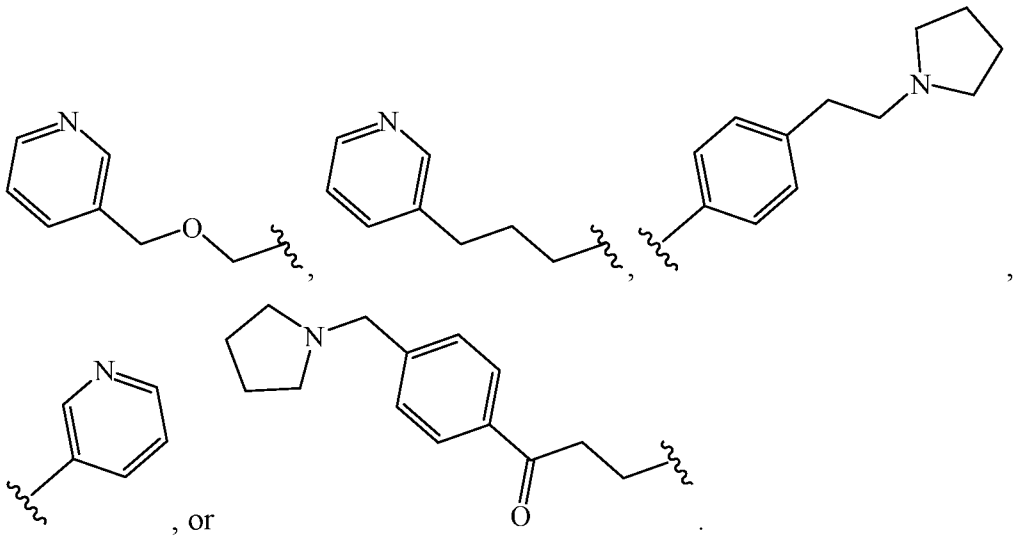
R^{12} is optionally substituted alkyl, aryl or heteroaryl; and

R^{13} is optionally substituted aryl or heteroaryl.

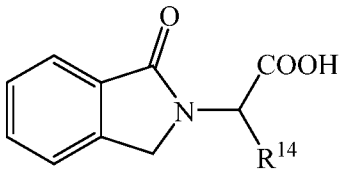
29. The compound of claim 28, wherein R^{13} is



30. The compound of any one of claims 28 or 29, wherein R^{12} is

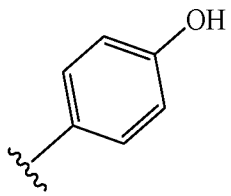
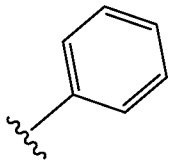
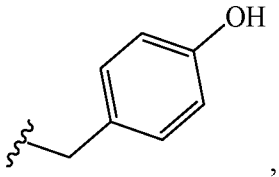
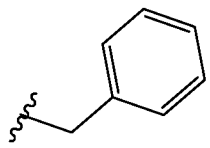
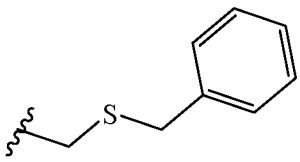


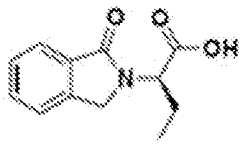
31. A compound having a structure of Formula VIII or a pharmaceutically acceptable salt thereof:



VIII

wherein R^{14} is optionally substituted alkyl or aryl.

32. The compound of claim 31, wherein R^{14} is -ethyl,  ,  ,  ,  , or  .

33. The compound of any one of claims 31 or 32, wherein the compound is not  .

34. A compound depicted in Table 1 or a pharmaceutically acceptable salt thereof.

35. A pharmaceutical composition comprising a compound of any preceding claim and a pharmaceutically acceptable carrier.

36. A method of treating or preventing a disease or condition comprising administering to a subject a compound of any one of claims 1-34 or a composition of claim 35.

37. The method of claim 36, wherein the disease is cancer.

38. The method of claim 37, wherein the cancer is selected from breast cancer, prostate cancer, oral squamous carcinoma, colorectal cancer, squamous cell carcinomas, neuroblastoma, bladder tumors, leukemia, non-Hodgkin's lymphoma, and solid and hematologic malignancies.

39. A method of inhibiting proliferation of a cancer cell, comprising contacting a cancer cell with a compound of any one of claims 1-34 or a composition of claim 35.

40. A method of inhibiting CBX, comprising contacting a cell with a compound of any one of claims 1-34 or a composition of claim 35.

Figure 1

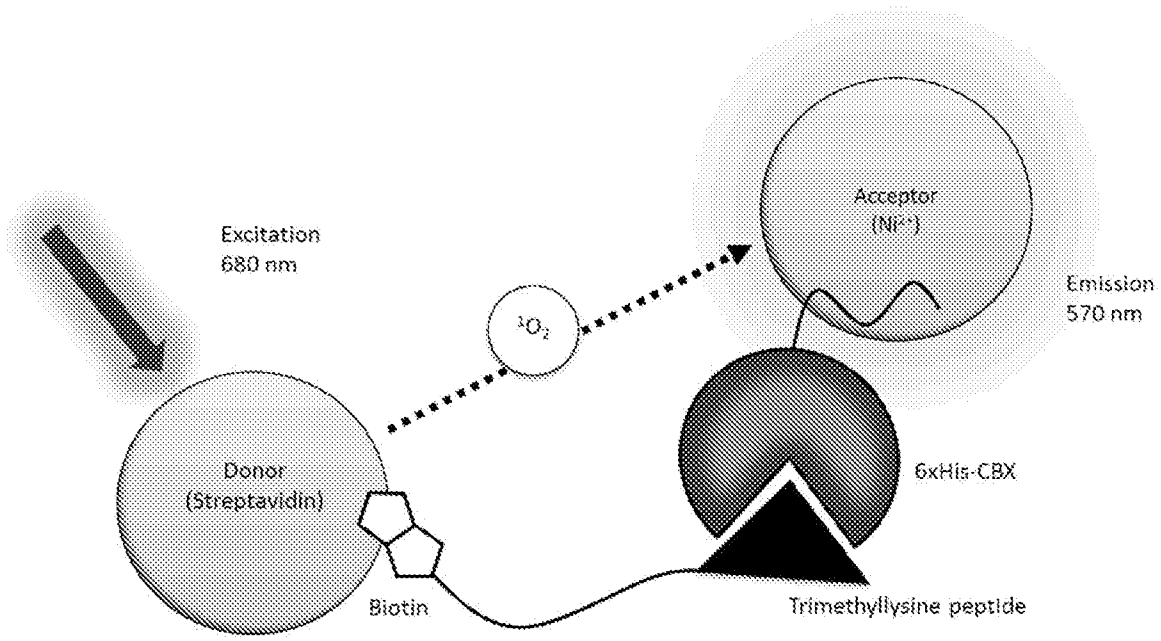

Excitation
680 nm
$^1O_2$
Acceptor
(Ni^{++})
Emission
570 nm
Donor
(Streptavidin)
6xHis-CBX
Biotin
Trimethyllysine peptide

Figure 2

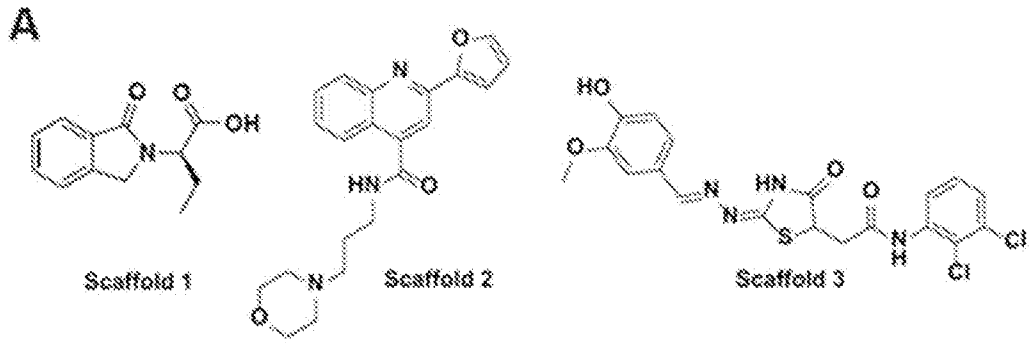


Figure 3

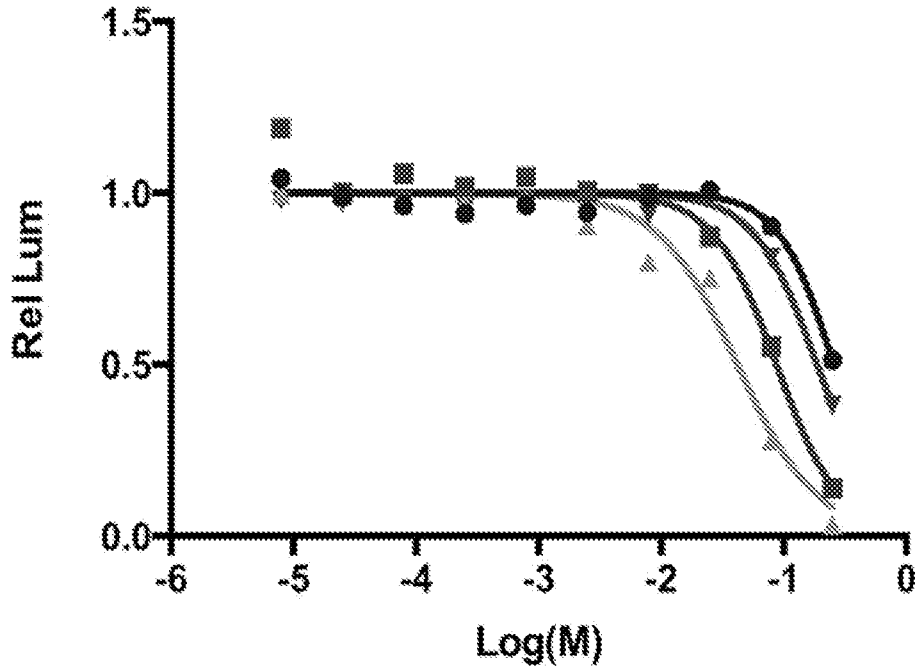


Fig. 4A

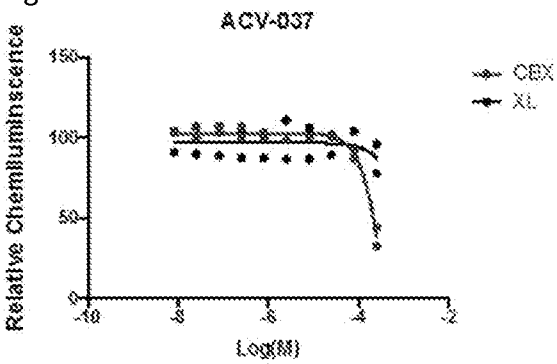


Fig. 4C

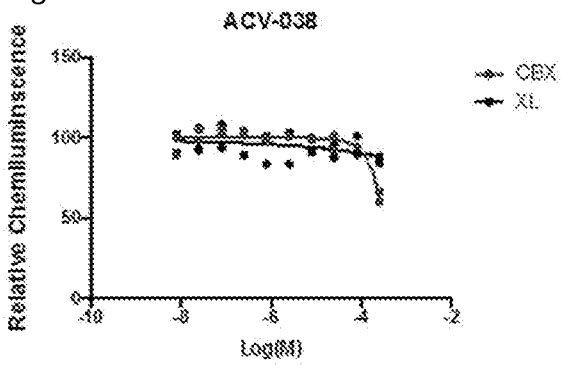


Fig. 4B

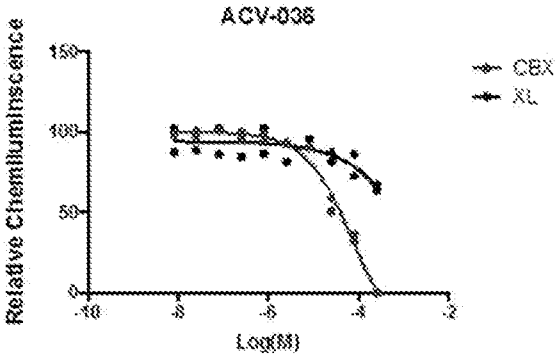


Fig. 4D

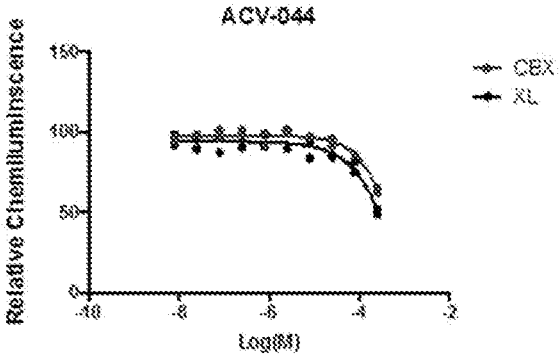


Fig. 5A

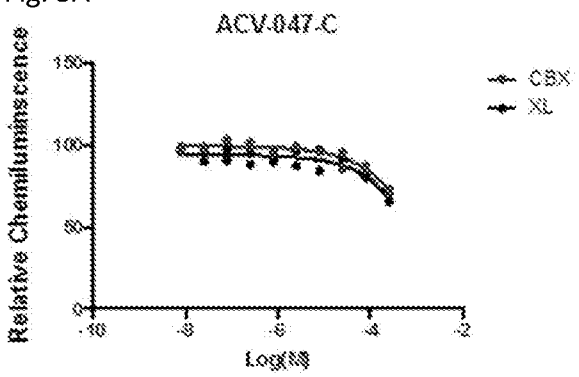


Fig. 5C

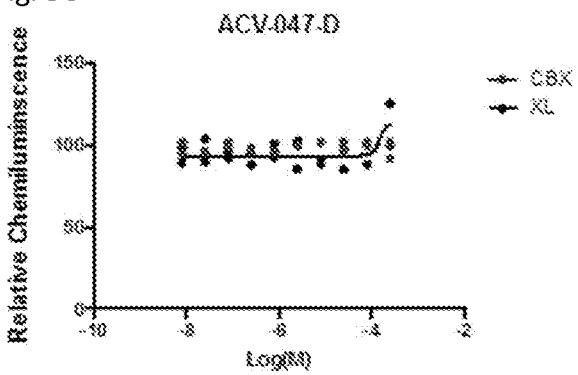


Fig. 5B

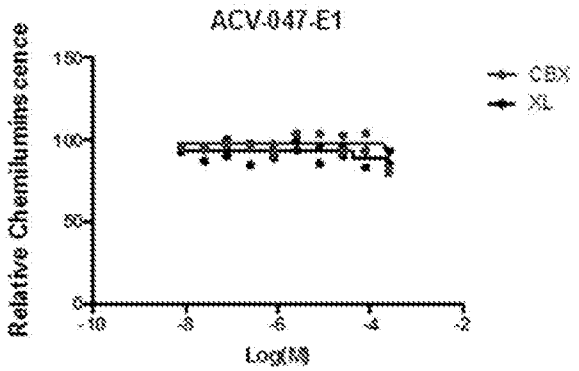


Fig. 5D

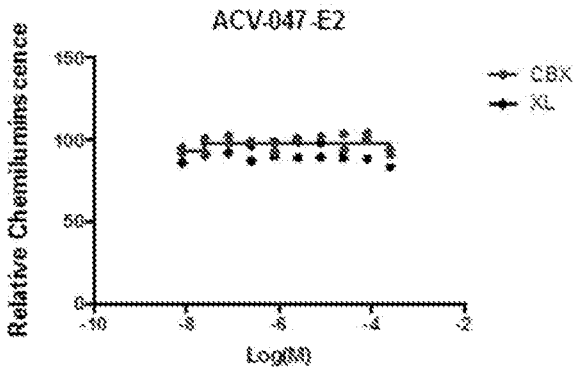


Figure 6

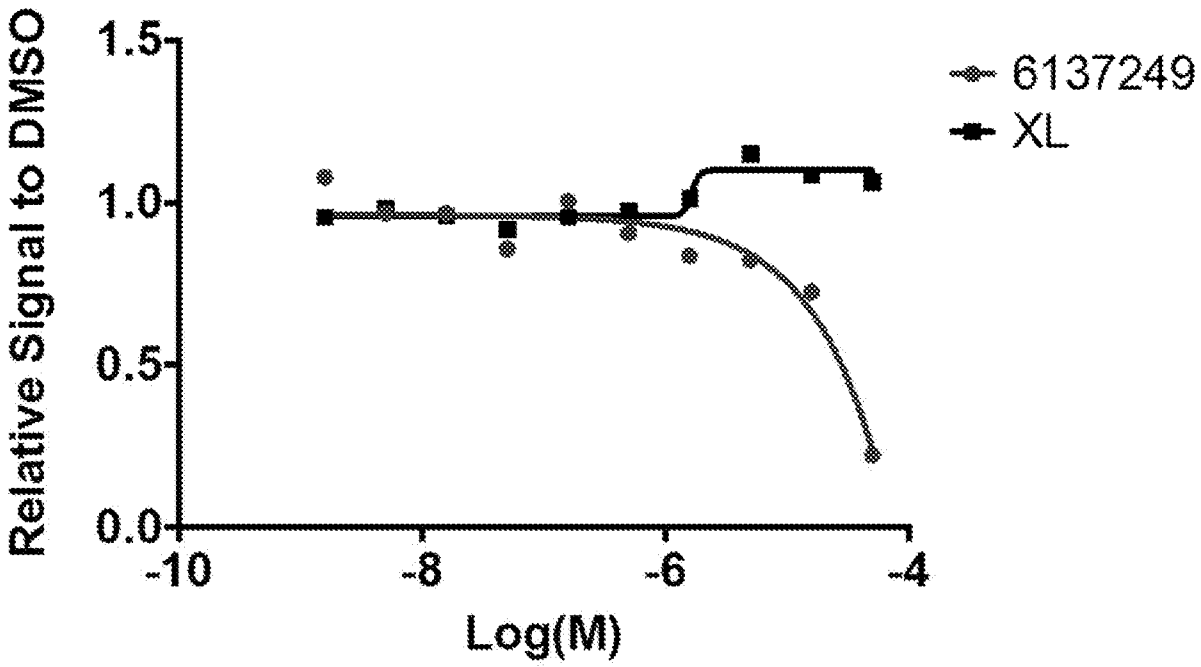
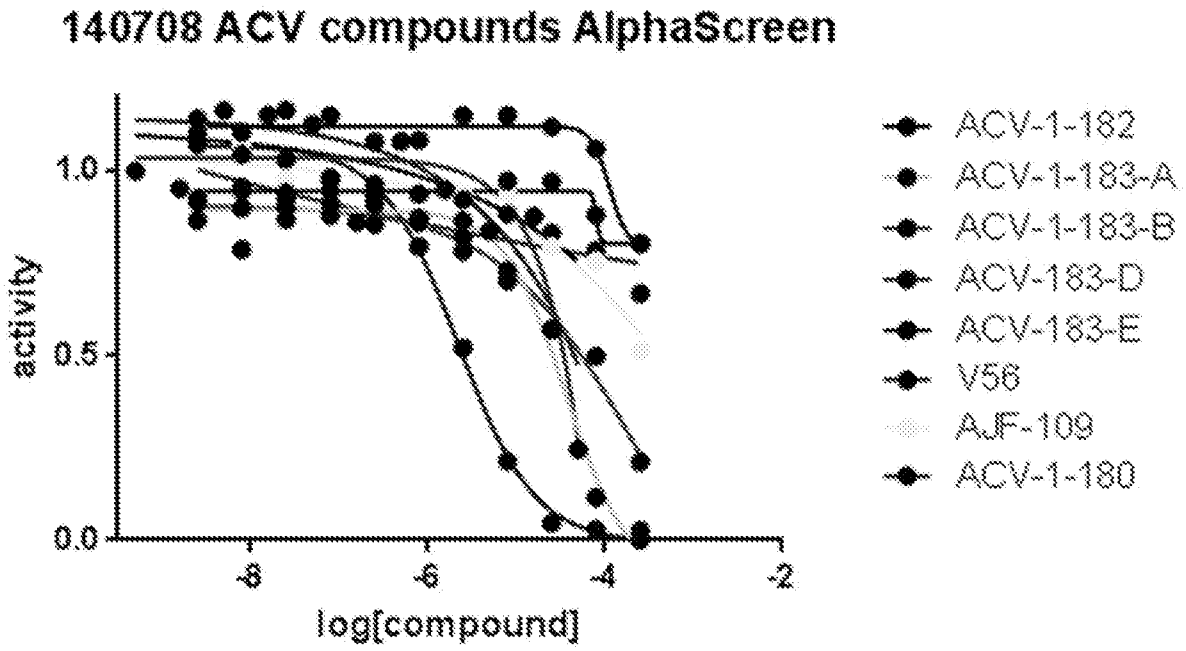


Figure 7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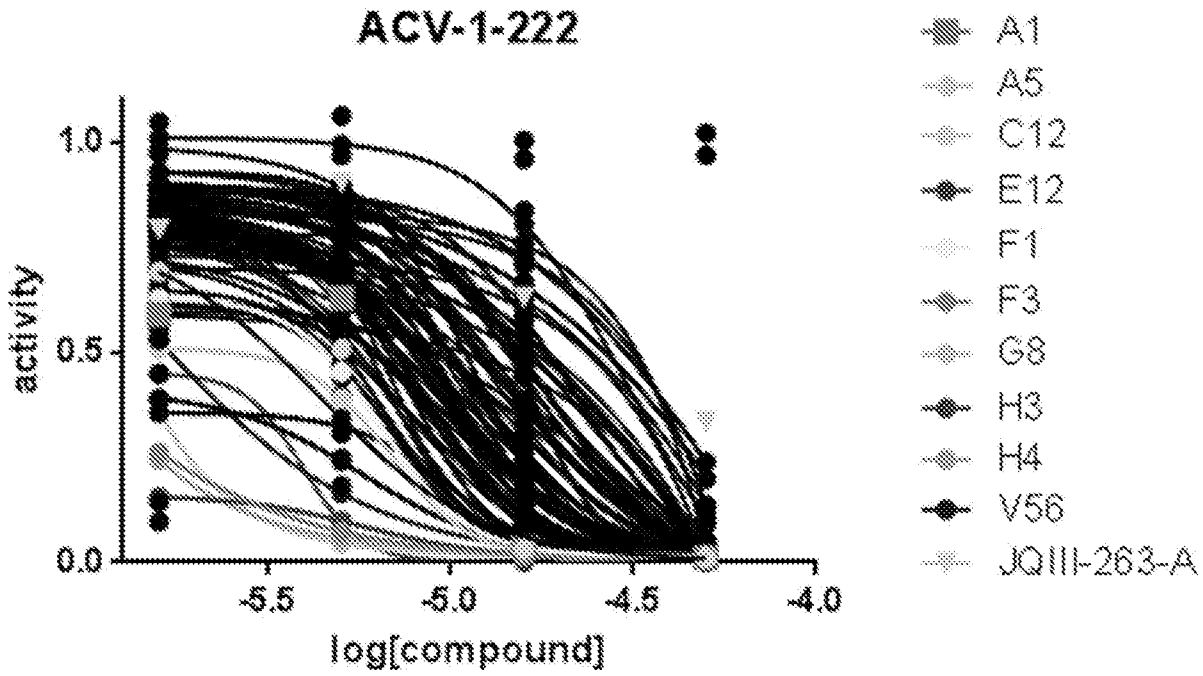
0.5
0.0
-8
-6
-4
-2
log[compound]
ACV-1-182
ACV-1-183-A
ACV-1-183-B
ACV-183-D
ACV-183-E
V56
AJF-109
ACV-1-180

Figure 8


ACV-1-222
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-4.0
log[compound]
A1
A5
C12
E12
F1
F3
G8
H3
H4
V56
JQ1I-263-A

Figure 9

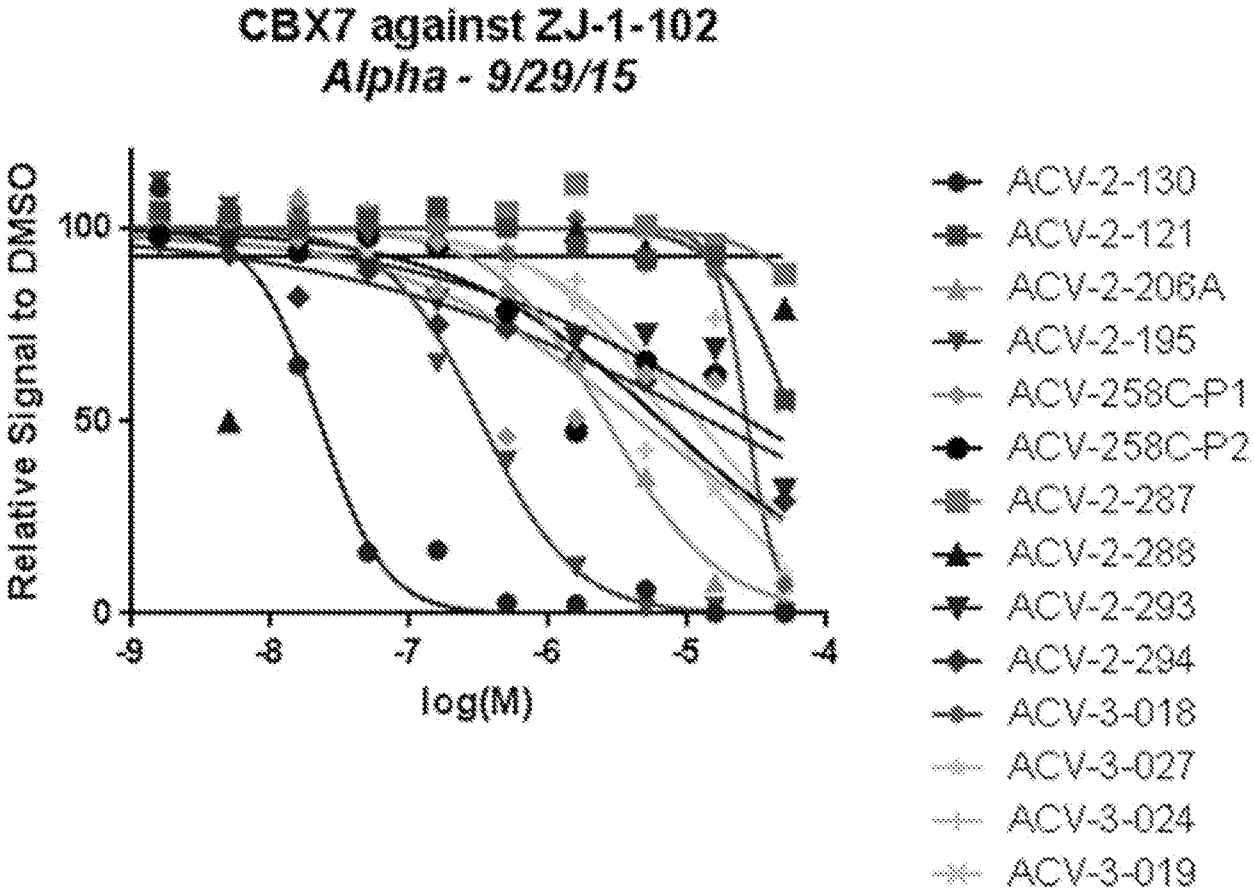

CBX7 against ZJ-1-102
Alpha - 9/29/15
Relative Signal to DMSO
100
50
0
-9
-8
-7
-6
-5
-4
log(M)
ACV-2-130
ACV-2-121
ACV-2-206A
ACV-2-195
ACV-258C-P1
ACV-258C-P2
ACV-2-287
ACV-2-288
ACV-2-293
ACV-2-294
ACV-3-018
ACV-3-027
ACV-3-024
ACV-3-019

Figure 10

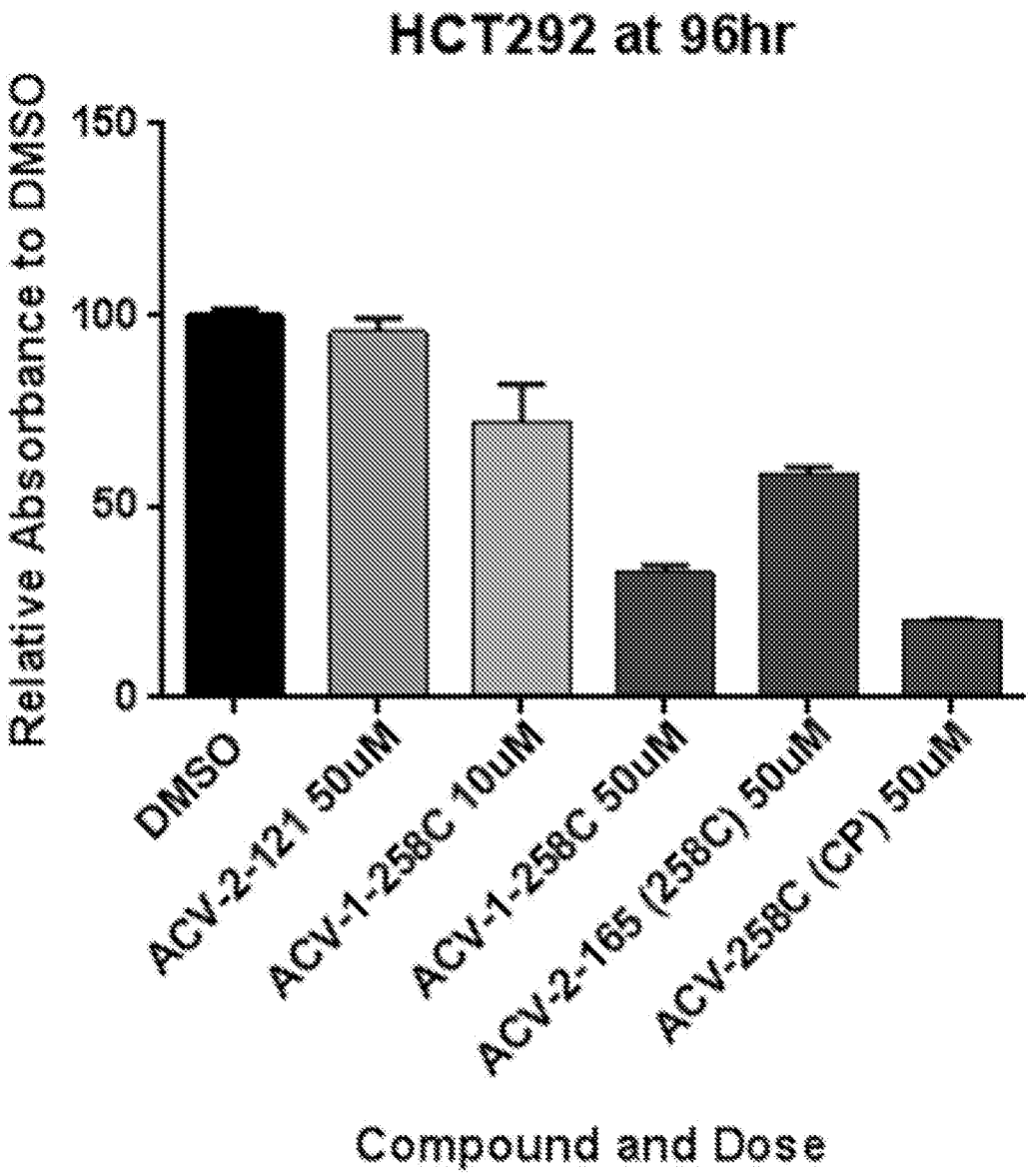

HCT292 at 96hr
Relative Absorbance to DMSO
150
100
50
0
DMSO
ACV-2-121 50uM
ACV-1-258C 10uM
ACV-1-258C 50uM
ACV-2-165 (258C) 50uM
ACV-258C (CP) 50uM
Compound and Dose

Figure 11

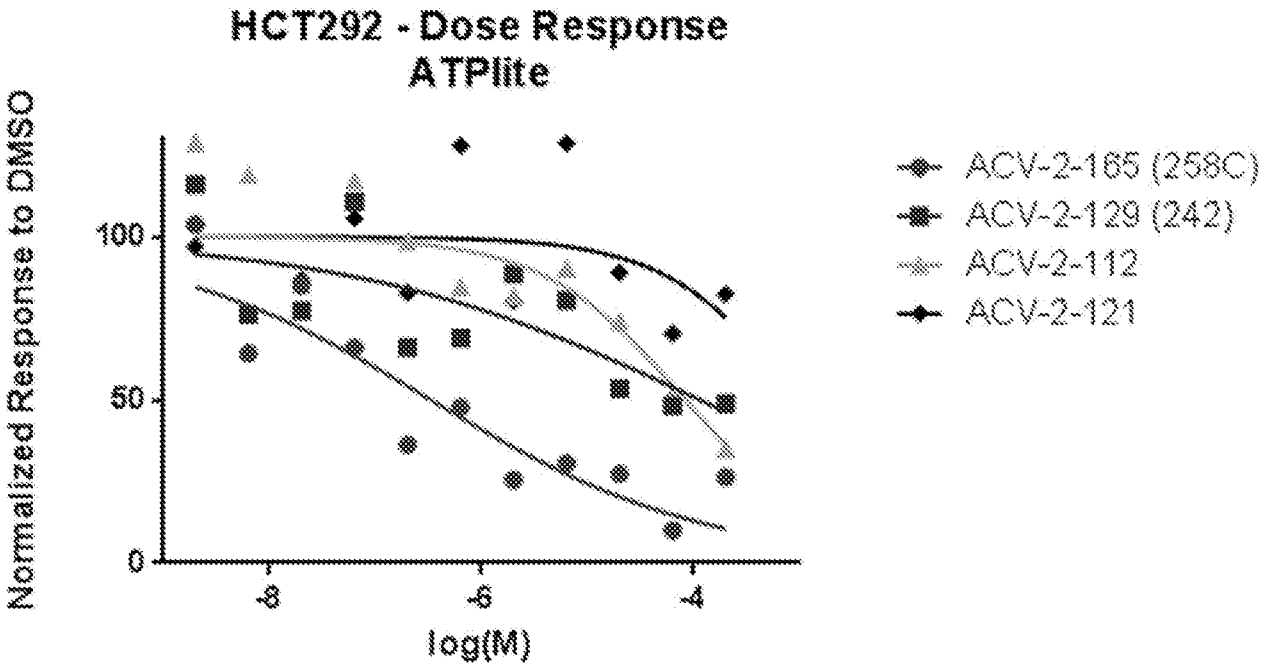

HCT292 - Dose Response
ATPlite
Normalized Response to DMSO
100
50
0
-8
-6
-4
log(M)
ACV-2-165 (258C)
ACV-2-129 (242)
ACV-2-112
ACV-2-121

Figure 12

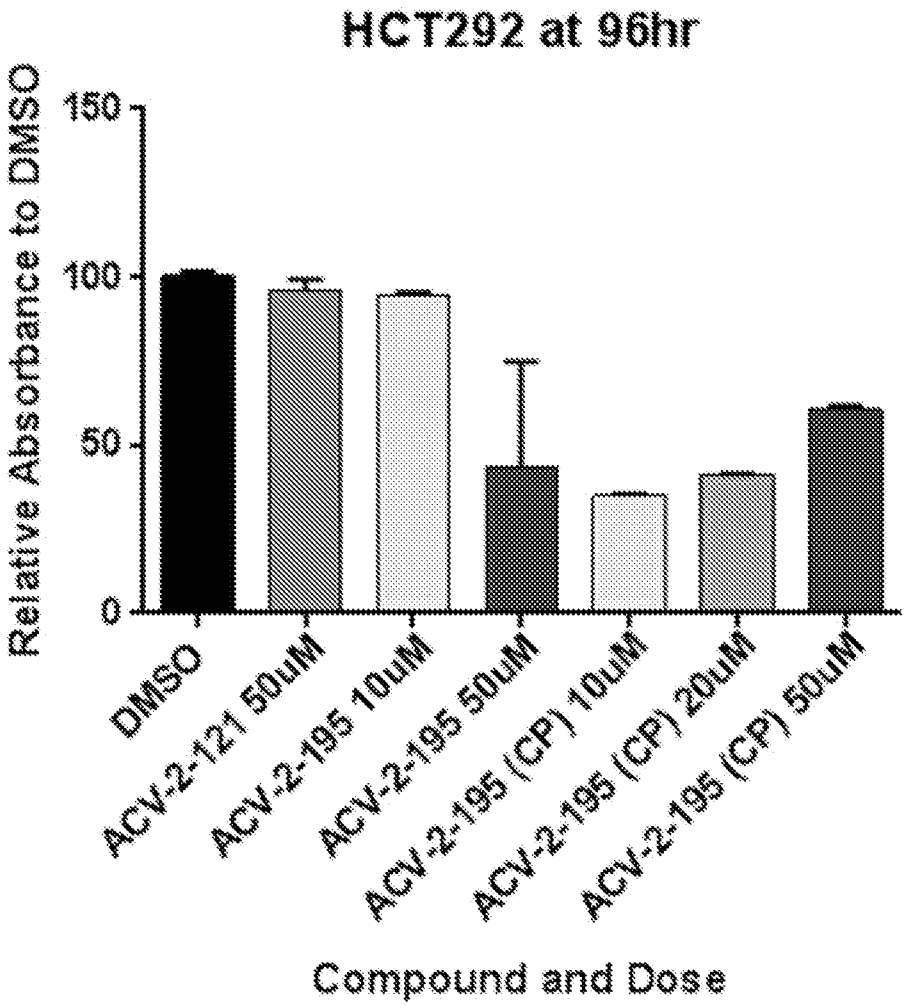

HCT292 at 96hr
Relative Absorbance to DMSO
150
100
50
0
DMSO
ACV-2-121 50uM
ACV-2-195 10uM
ACV-2-195 50uM
ACV-2-195 (CP) 10uM
ACV-2-195 (CP) 20uM
ACV-2-195 (CP) 50uM
Compound and Dose

Figure 13

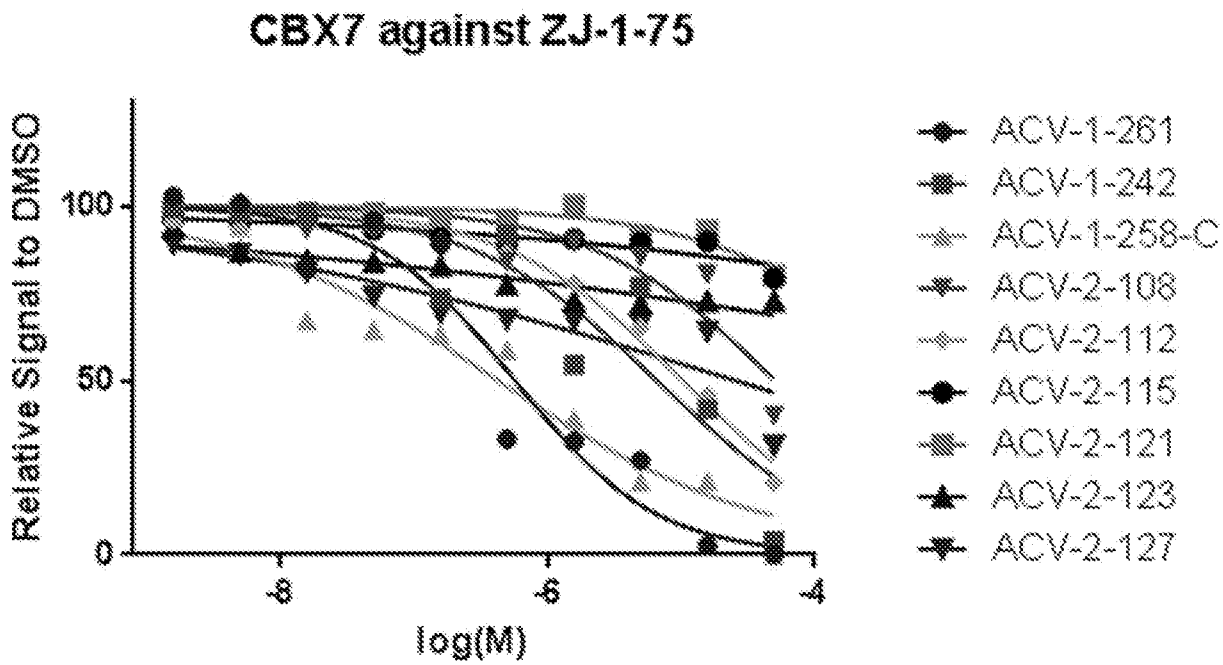

CBX7 against ZJ-1-75
Relative Signal to DMSO
100
50
0
-8
-6
-4
log(M)
ACV-1-261
ACV-1-242
ACV-1-258-C
ACV-2-108
ACV-2-112
ACV-2-115
ACV-2-121
ACV-2-123
ACV-2-127

Figure 14

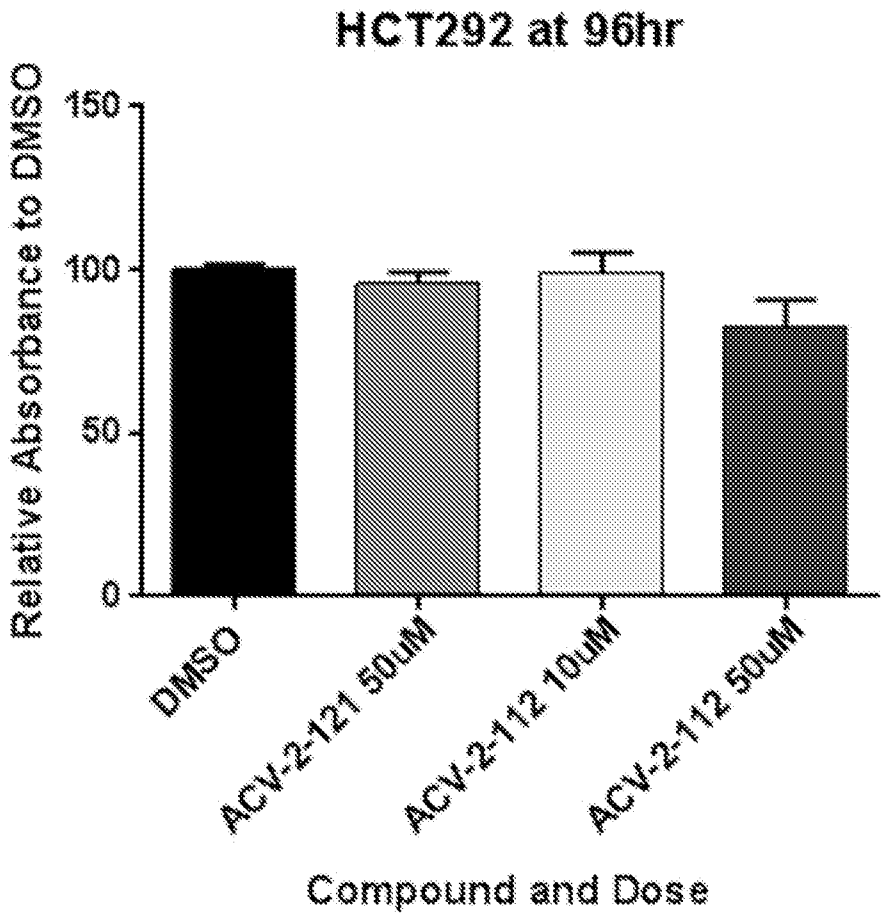

HCT292 at 96hr
Relative Absorbance to DMSO
150
100
50
0
DMSO
ACV-2-121 50uM
ACV-2-112 10uM
ACV-2-112 50uM
Compound and Dose

Figure 15

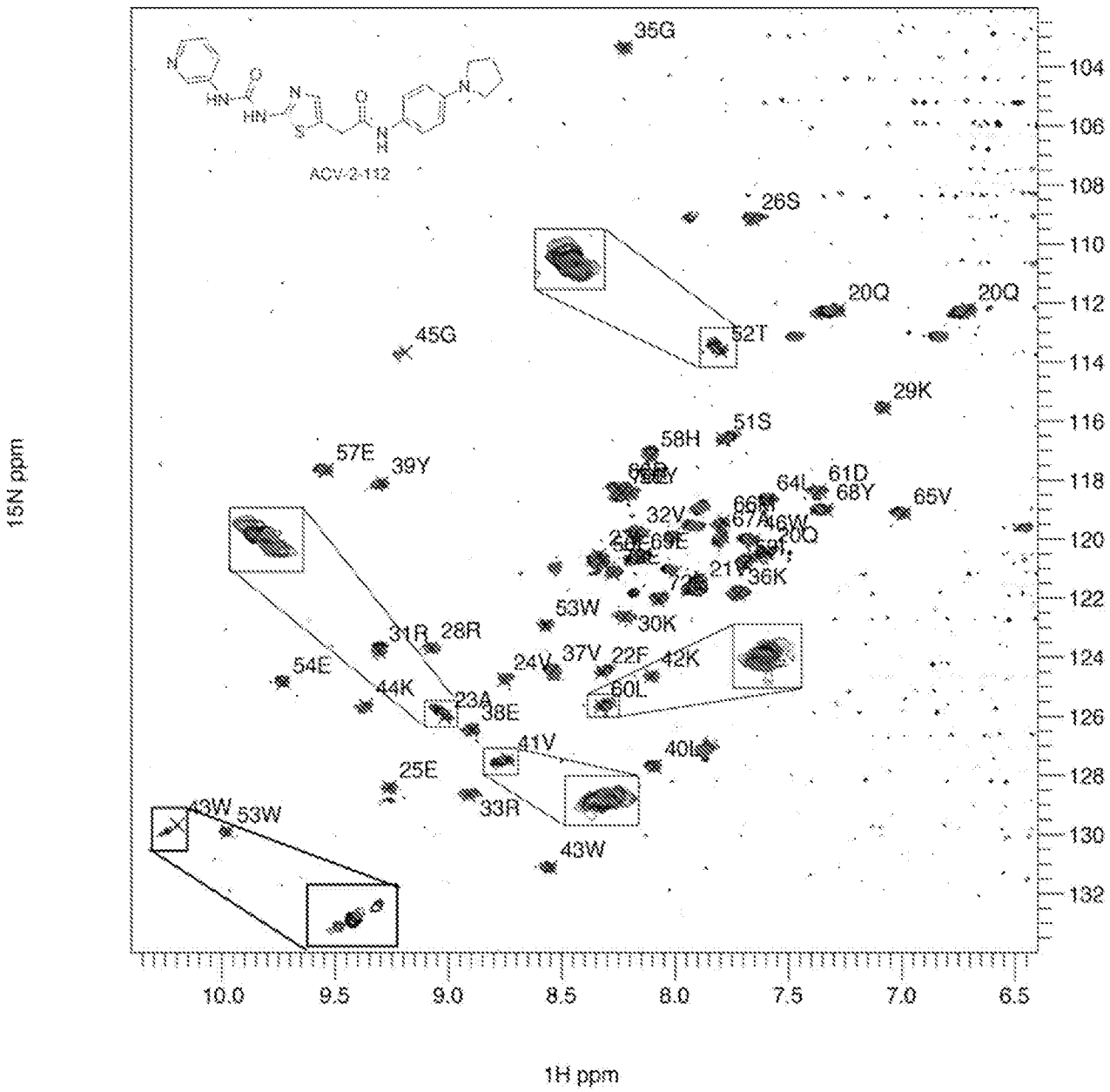

ACV-2-112
35G
26S
20Q
20Q
52T
45G
29K
51S
58H
57E
39Y
64L
61D
68Y
65V
32V
67A
46W
20Q
36K
53W
30K
31R
28R
54E
24V
37V
22F
42K
44K
23A
38E
60L
41V
40I
25E
33R
43W
43W
53W
104
106
108
110
112
114
116
118
120
122
124
126
128
130
132
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
15N ppm
1H ppm

Figure 16

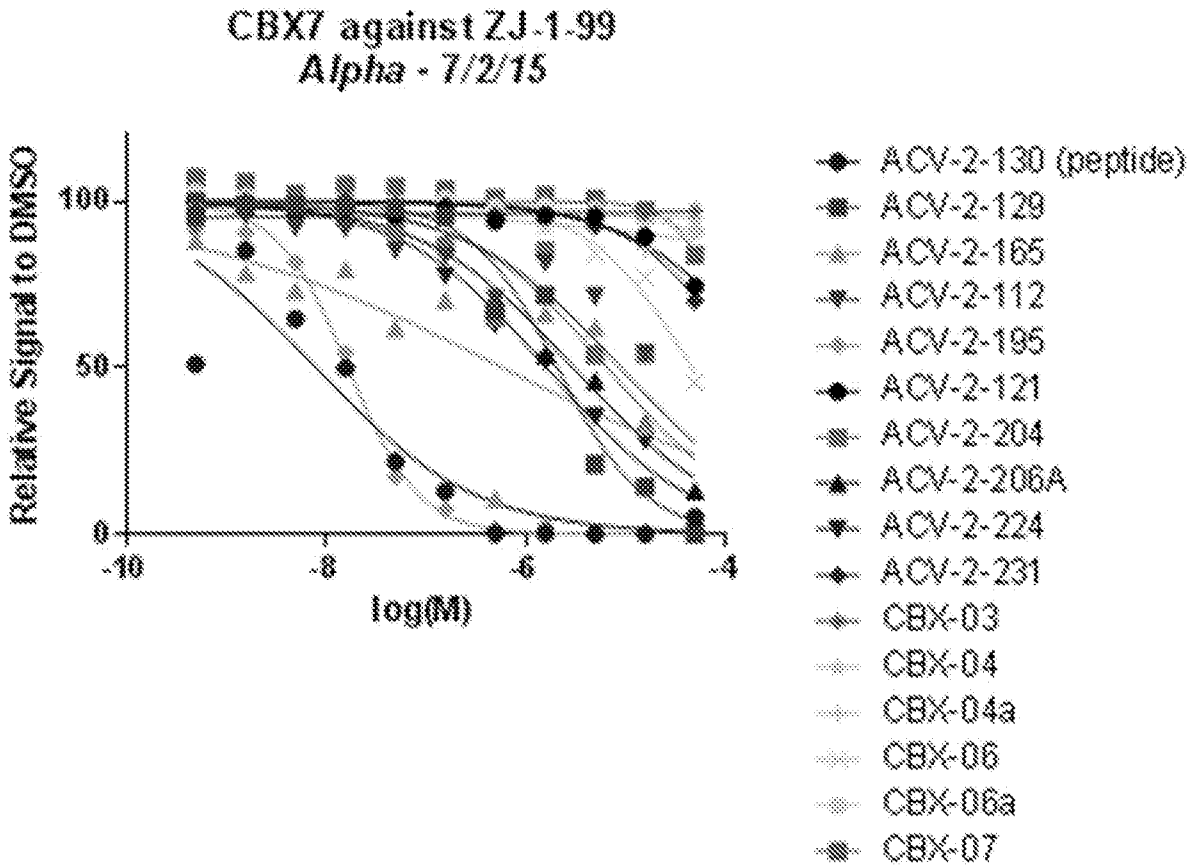

CBX7 against ZJ-1-99
Alpha - 7/2/15
Relative Signal to DMSO
100
50
0
-10
-8
-6
-4
log(M)
ACV-2-130 (peptide)
ACV-2-129
ACV-2-165
ACV-2-112
ACV-2-195
ACV-2-121
ACV-2-204
ACV-2-206A
ACV-2-224
ACV-2-231
CBX-03
CBX-04
CBX-04a
CBX-06
CBX-06a
CBX-07

Figure 17

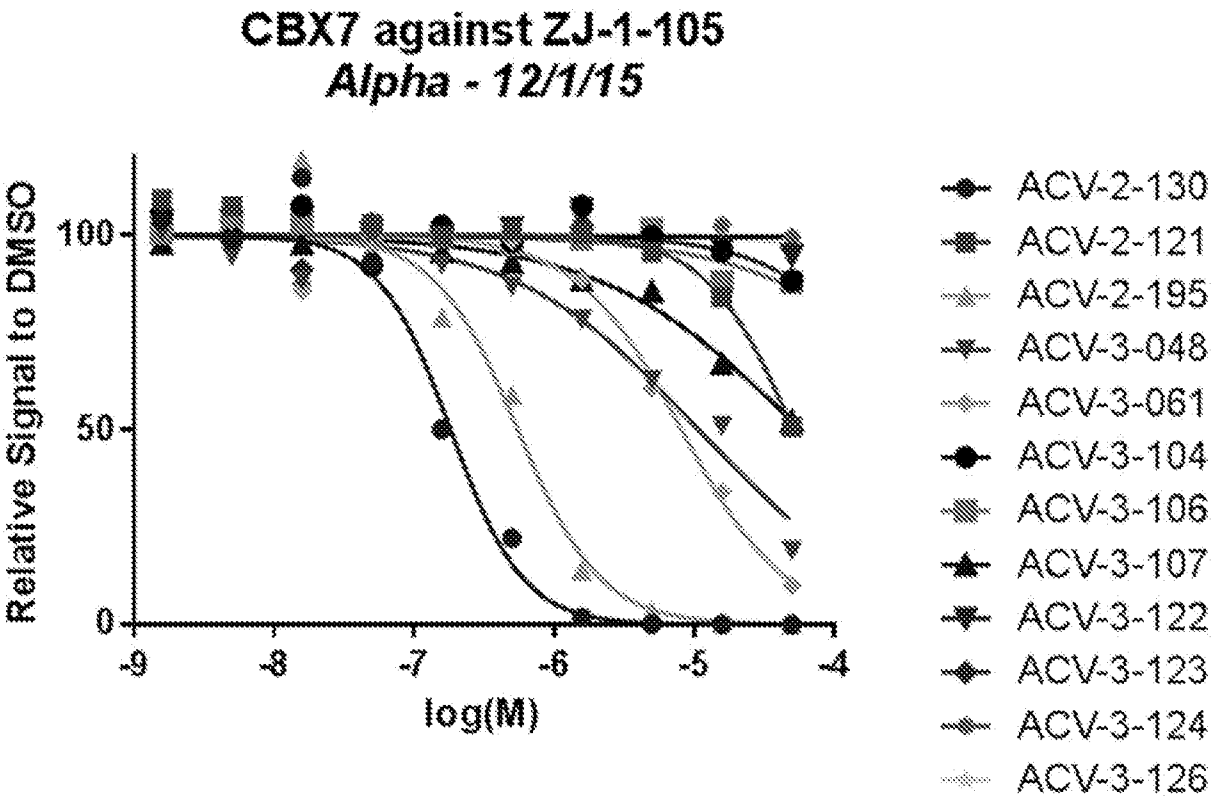

CBX7 against ZJ-1-105
Alpha - 12/1/15
Relative Signal to DMSO
100
50
0
-9
-8
-7
-6
-5
-4
log(M)
ACV-2-130
ACV-2-121
ACV-2-195
ACV-3-048
ACV-3-061
ACV-3-104
ACV-3-106
ACV-3-107
ACV-3-122
ACV-3-123
ACV-3-124
ACV-3-126

Figure 18

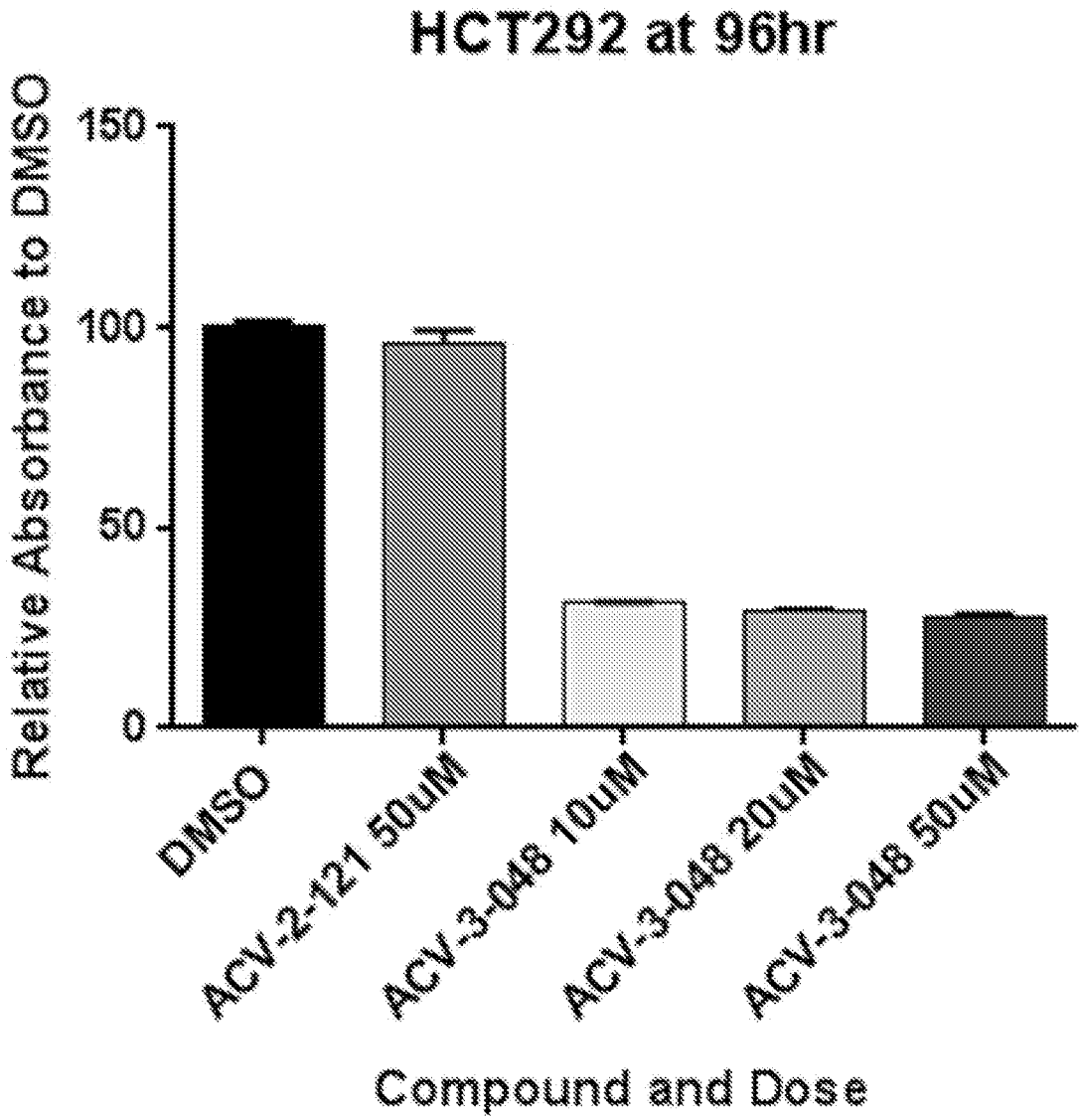

HCT292 at 96hr
Relative Absorbance to DMSO
150
100
50
0
DMSO
ACV-2-121 50uM
ACV-3-048 10uM
ACV-3-048 20uM
ACV-3-048 50uM
Compound and Dose

Figure 19

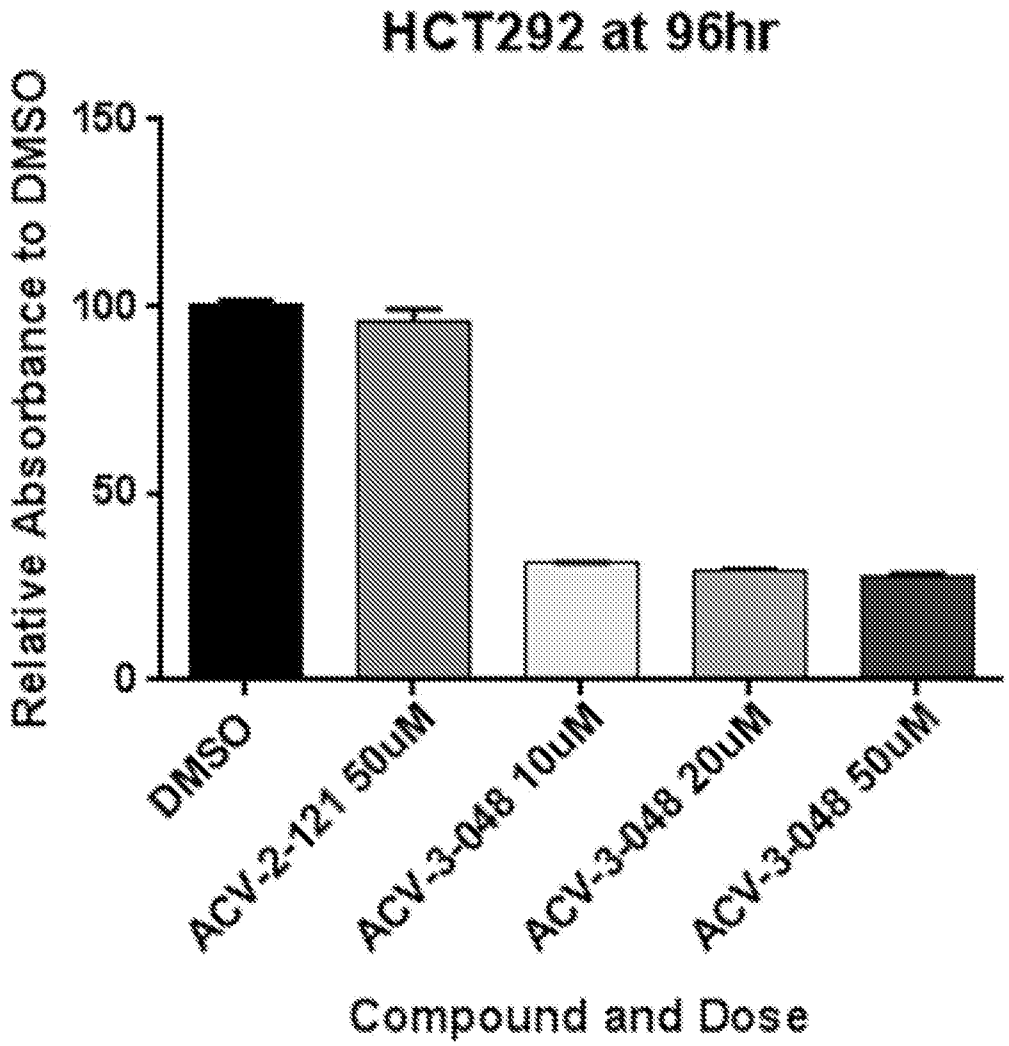

HCT292 at 96hr
Relative Absorbance to DMSO
150
100
50
0
DMSO
ACV-2-121 50uM
ACV-3-048 10uM
ACV-3-048 20uM
ACV-3-048 50uM
Compound and Dose

Figure 20

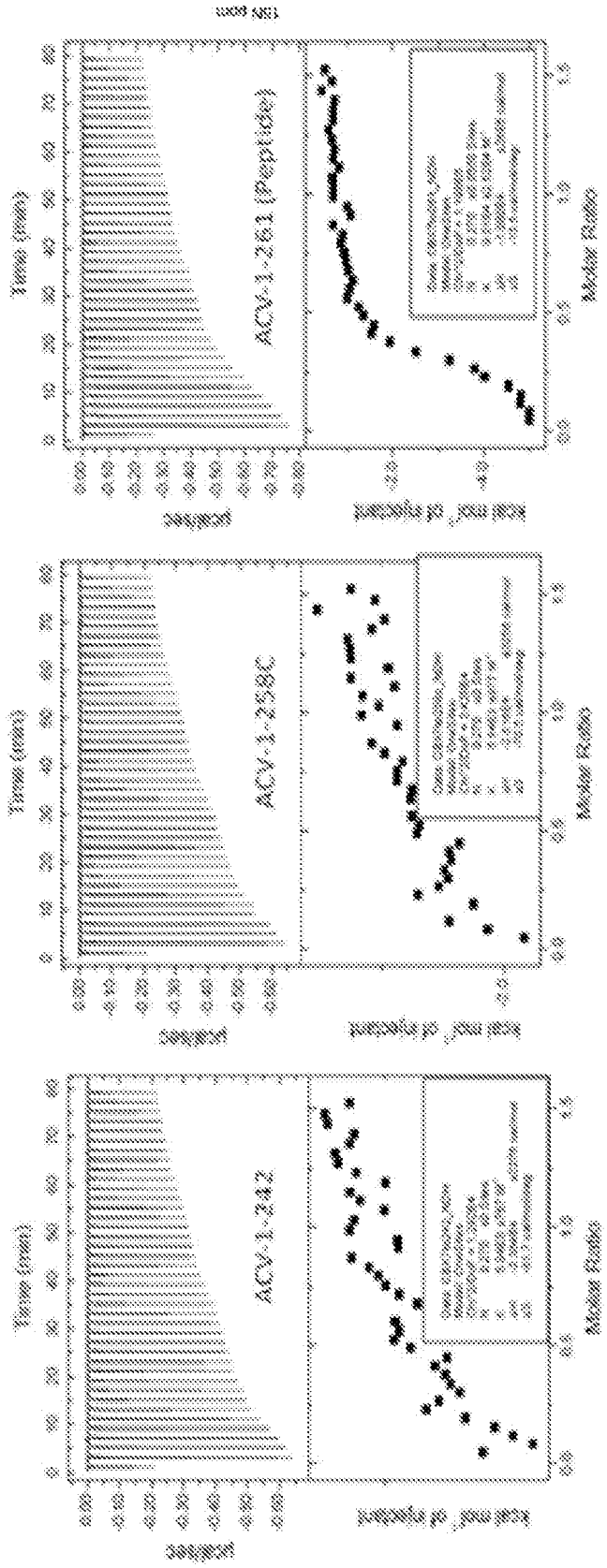


Fig. 21A

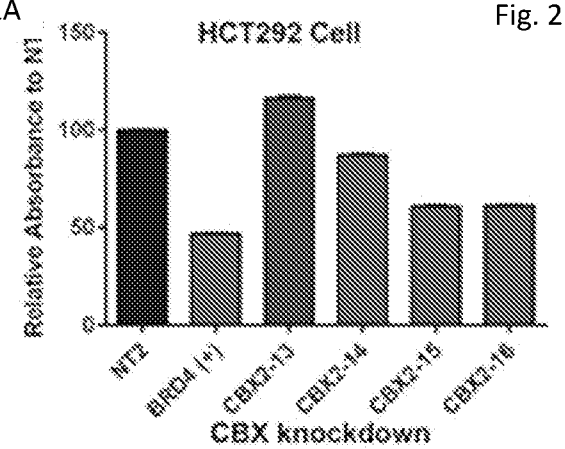


Fig. 21B

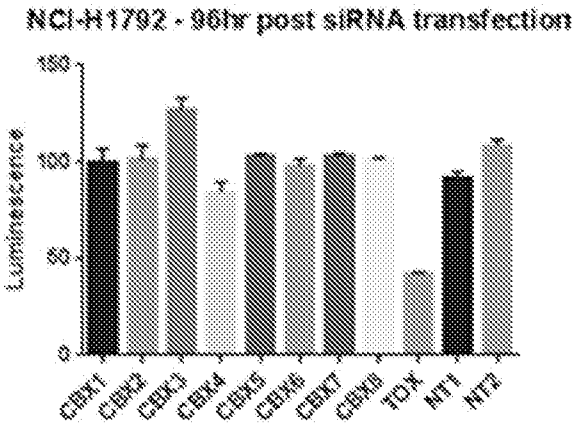


Fig. 21C

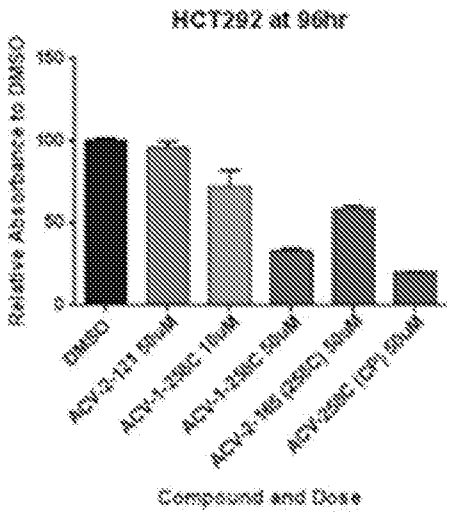
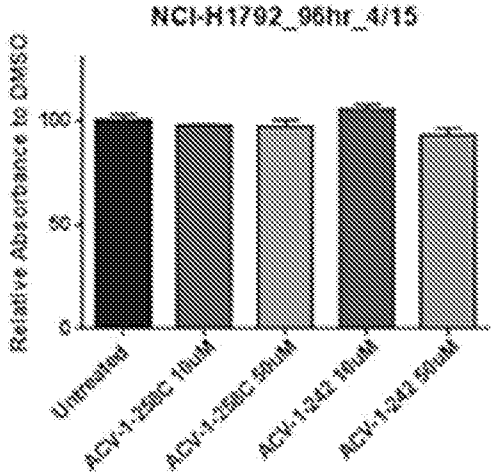


Fig. 21D



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/53229

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/535, A61P 37/00 (2017.01)
CPC - G01N 33/5047, G01N 33/5023, C12Q 1/6897

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)
See Search History Document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 8,846,320 B2 (Kosmeder et al.) 30 September 2014 (30.09.2014); col 10, ln 5-10	1
A	US 2014/356322 A1 (YALE UNIVERSITY) 04 December 2014 (04.12.2014); entire document	1
A	WO 2013/059944 A1 (BRITISH COLUMBIA CANCER AGENCY BRANCH) 02 May 2013 (02.05.2013); entire document	1
A	US 2016/0130261 A1 (GLAXOSMITHKLINE INTELLECTUAL PROPERTY (NO.2) LIMITED) 12 May 2016 (12.05.2016); entire document	1

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
03 January 2018	29 JAN 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/53229

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.: 5-10, 14-15, 23-24, 35-40
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 supplemental page)

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 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

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.

--continued from Box No. III--

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I+: Claims 1-4 and 34 (in part), directed to a compound of claim 1, having the structure of formula I. The compound of claim 1 will be searched to the extent that it encompasses the first species of claim 1, represented by a compound of formula I wherein R1 is optionally substituted aryl; R2 is H; L1 is C(O) and A is OH. It is believed that claim 1 reads on this first named invention, and thus these claims will be searched without fee. Applicant is invited to elect additional compounds of claim 1, wherein each additional compound elected will require one additional invention fee. Applicants must specify the claims that encompass any additionally elected compound. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first claimed invention to be searched. Additionally, an exemplary election wherein different actual variables are selected is suggested. An exemplary election would be a compound of claim 1, represented by a compound of formula I wherein R1 is heteroaryl; R2 is H; L1 is C(O) and A is OH (i.e., claims 1-2 and 34).

Group II: Claims 11-13 and 34 (in part), directed to a compound of claim 11, having the structure of formula II.

Group III: Claims 16-19 and 34 (in part), directed to a compound of claim 16, having the structure of formula III.

Group IV: Claims 20-22 and 34 (in part), directed to a compound of claim 20, having the structure of formula IV or V.

Group V: Claims 25-27 and 34 (in part), directed to a compound of claim 25, having the structure of formula VI.

Group VI: Claims 28-30 and 34 (in part), directed to a compound of claim 28, having the structure of formula VII.

Group VII: Claims 31-33 and 34 (in part), directed to a compound of claim 31, having the structure of formula VIII.

The group of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I+ includes the technical feature of a unique composition of claim I, formula I, which is not required by any other invention of Group I+ or II-VII.

Group II includes the technical feature of a compound of claim 11, formula II, which is not required by any other invention of Group I+ or III-VII.

Group III includes the technical feature of a compound of claim 16, formula III, which is not required by any other invention of Group I+, II, or IV-VII.

Group IV includes the technical feature of a compound of claim 20, formula IV or V, which is not required by any other invention of Group I+, II-III, or V-VII.

Group V includes the technical feature of a compound of claim 25, formula VI, which is not required by any other invention of Group I+, II -IV, or VI-VII.

Group VI includes the technical feature of a compound of claim 28, formula VII, which is not required by any other invention of Group I+, II-V, or VII.

Group VII includes the technical feature of a compound of claim 31, formula VIII, which is not required by any other invention of Group I+, or II-VI.

Common technical features:

The inventions of Group I+ share the technical feature of a compound of claim 1.

These shared technical features, however, do not provide a contribution over the prior art, as being anticipated by US 8,846,320 B2 to Kosmeder et al. (hereinafter Kosmeder). Kosmeder discloses a compound of formula I wherein R1 is optionally substituted aryl; R2 is H; L1 is C(O); and A is OH (col 10, ln 5-10).

The inventions of Groups I+ and II-VII share the technical feature of a heterocycle/heteroaryl comprising a carbonyl substituent which is a compound of Table 1.

--continued on following page--

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These shared technical features, however, do not provide a contribution over the prior art, as being anticipated by US 2014/356322 A1 to YALE UNIVERSITY (hereinafter Yale). Yale discloses a heterocycle/heteroaryl comprising a carbonyl substituent which is a compound of Table 1, compound ACV-104 (para [0455]: Table, 2nd compound listed: (S)-2-(1-oxoisoindolin-2-yl)butanoic acid).

As said compound and compositions were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the inventions of Groups I+ or II-VII. The inventions of Group I+ and II-VII thus lack unity under PCT Rule 13.

Note Re: Item 4: Claims 5-10, 14-15, 23-24 and 35-40 have been found to be unsearchable because they are not drafted in accordance with the second and third sentences of Rule 6.4(a).