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(54) Title: SMALL MOLECULE AGONISTS AND ANTAGONISTS OF NR2F6 ACTIVITY IN NON-HUMANS

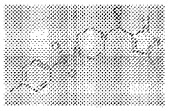

FIG. 1A

(57) Abstract: The present technology is directed to modulators of nuclear receptor activity, specifically to the modulation of NR2F6 activity and NR2F6 utilizing compounds, and the immune modulation and modulation of cancer stem cell activity through administration of compounds described herein. In certain embodiments the present disclosure is directed to methods of using small molecule compounds as immune modulators.

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TITLE

Small Molecule Agonists and Antagonists of NR2F6 Activity in Non-Humans

BACKGROUND

[0001] The present technology relates to agonists and antagonists of nuclear receptor activity, specifically to the modulation of NR2F6 activity and NR2F6 utilizing compounds, and the immune modulation and modulation of cancer stem cell activity through administration of compounds described herein.

[0002] Many drugs administered to treat diseases or conditions are targeted against differences between a diseased cell and a normal cell. T cells of the immune system are known to recognize and interact with specific molecules through receptors (*e.g.*, a T cell receptor in complex with a CD3 dimer) which, upon recognition or interaction with these molecules, result in the activation of the T cell to perform various immune activities. Innate immune cells are cells of the immune system that are known to be activated by one or more agents (*e.g.*, allergens, chemicals produced upon injury (*e.g.*, opioids and alcohols), polymyxins, crosslinked IgE, crosslinked complement proteins, cytokines produced by T cells or other immune cells (*e.g.*, interferon-γ), DAMPs, or PAMPs) that activate downstream signaling pathway(s) in the innate immune cell and result in the activation of one or more immune activities of the innate immune cell.

[0003] Both T cells and innate immune cells play a role in a mammal's immune defense. For example, the immune activities of an innate immune cell can protect a mammal against infectious diseases. The immune activities of a T cell can protect a mammal against, for example, infectious diseases and cancer.

[0004] Adoptive cell therapy is a method of treatment that includes harvesting one or more different types of immune cells from a mammal, culturing and/or manipulating the harvested immune cells ex vivo, and administering the cultured and/or manipulated immune cells back to the mammal. The manipulating of a harvested immune cell ex vivo can include introducing a recombinant nucleic acid into the immune cell.

[0005] Molecularly targeted therapeutics represent a new approach to discovering anti-cancer drugs. Using this approach, small molecules are designed to inhibit directly the very oncogenetic proteins that are mutated or overexpressed in specific tumor cell types. By targeting specific molecular defects or conditions found within tumor cells, this approach can yield therapies tailored to each tumor's genetic makeup. A complementary strategy involves searching for genotype-selective anti-tumor agents that become lethal to tumor cells only in the presence of specific oncoproteins or only in the absence of specific tumor oppressors. Such genotype-selective compounds might target oncoproteins directly, or target other critical proteins involved in oncoprotein-linked signaling networks.

[0006] The immune system is comprised of activatory and inhibitory mechanisms that can allow for control of immune responses and subsequent inhibition of responses after clearance of the immune target. The central event stimulating immune responses is the antigen-specific activation of naive $CD4^+$ T cells subsequent to binding antigen presenting cell MHC containing antigenic peptide. The $CD4^+$ T cell, also known as the "helper T cell," helps to coordinate the activation of the adaptive immune response, playing a role in the stimulation of cytotoxic CD8+ T cells, whose role includes destroying host cells affected by cancer, viruses, and intracellular bacteria, as well as stimulating B cell maturation to eventual plasma cell differentiation. Antibodies can be critical molecules in clearance of extracellular pathogens such as various bacteria and parasites.

[0007] Under many circumstances, naive $CD4^+$ T cells require two distinct signals to proliferate and differentiate into the armed effector cells that mediate adaptive immunity. Signal 1 of this two-signal model is antigen-specific and is generated by interaction of the TCR with antigenic peptide presented in context with MHC II antigens. This results in transduction of TCR intracellular signals leading to production of IL-2 and T cell activation. Signal 2 is referred to as a "costimulatory" signal because, while essential, it does not necessarily induce any functional response in T cells.

[0008] The best characterized costimulatory signal 2 is generated through the T cell surface molecule CD28. CD28 delivers a costimulatory signal upon interaction with CD80 or CD86 present

on B cells, macrophages, or dendritic cells. Activation of the TCR in the presence of costimulatory signals leads to T cell clonal expansion and initiation of effector functions such as IL-2 production.

[0009] For cancer, immune inhibitory mechanisms, termed "immune checkpoints," are prematurely activated in order for the tumor to escape immune attack. Two immune checkpoints exist: a) CTLA-4, which sends an inhibitory signal to T cells upon binding CD80 or CD86 on antigen presenting cells; and b) PD-1, which binds to PD-1 ligand on tumor cells, stromal cells, or antigen presenting cells.

[0010] CTLA-4 is related to CD28, however instead of activating T cells in a co-stimulatory manner, it leads to inhibition or co-inhibition of T cells.

[0011] Nuclear receptor subfamily 2, group F, member 6 (NR2F6), also known as nuclear orphan receptor Ear2, is an orphan member of the nuclear receptor (NR) superfamily of ligand-activated receptors, which exhibit a common modular structure and are involved in various homeostatic functions, but also play a role in oncogenesis and cancer propagation. Specifically, studies have shown that members of the NR family regulate development, reproduction, and metabolism of lipids, drugs and energy. The importance of this family of proteins in metabolic disease is exemplified by NR ligands used in the clinic or under exploratory development for the treatment of diabetes mellitus, dyslipidemia, hypercholesterolemia, or other metabolic abnormalities.

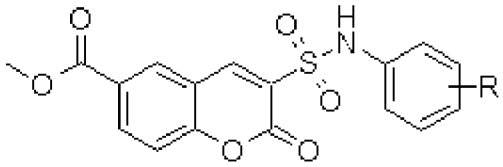
[0012] Genetic studies in humans and rodents support the notion that NRs control a wide variety of metabolic processes by regulating the expression of genes encoding key enzymes, transporters and other proteins involved in metabolic homeostasis. Genomic sequence availability has led to the identification of 48 NRs encoded by the human genome and 49 NRs encoded by the mouse genome.

[0013] The present disclosure is directed to, in certain embodiments, methods of using small molecule compounds as immune modulators; as well as to compounds, solid forms and compositions thereof that are immune modulators and that exhibit desirable characteristics thereof; as well as to methods of making the compounds, solid forms and composition thereof.

SUMMARY OF THE DISCLOSED TECHNOLOGY

[0014] In certain embodiments, the present technology is directed compounds discussed and described herein, which compounds have been found to modulate the immune system. These compounds can include any of the following:

[0015]



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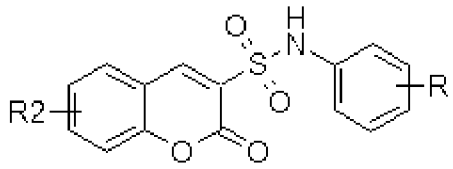

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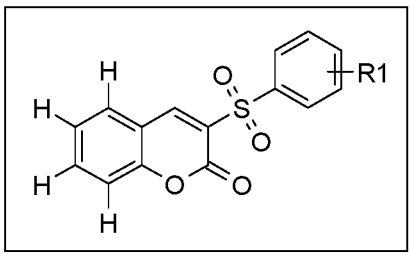

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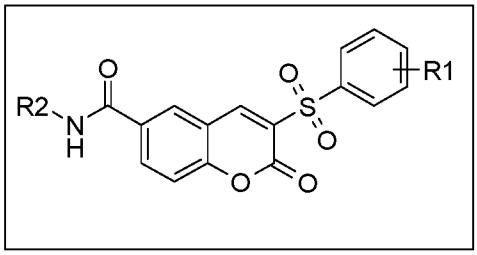

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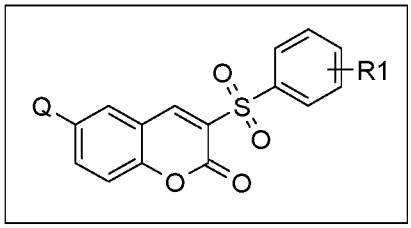

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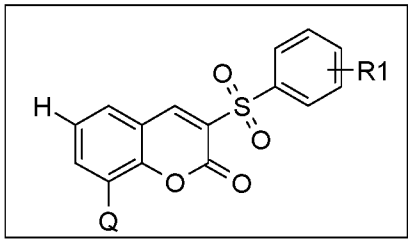

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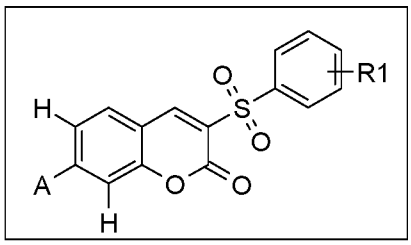

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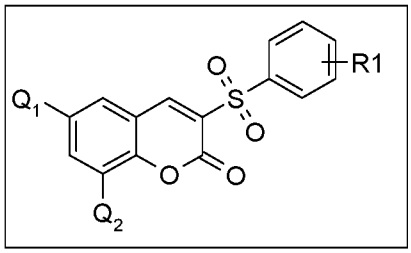

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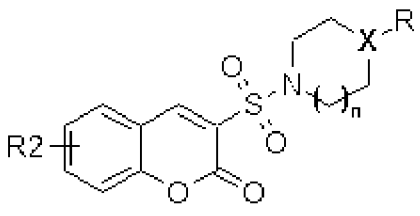

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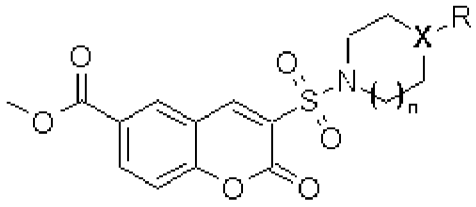

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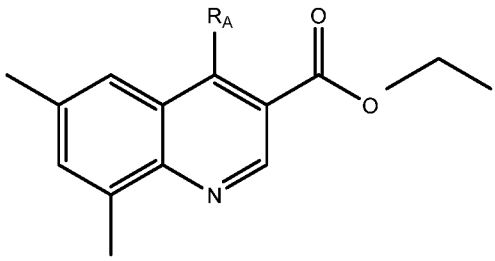

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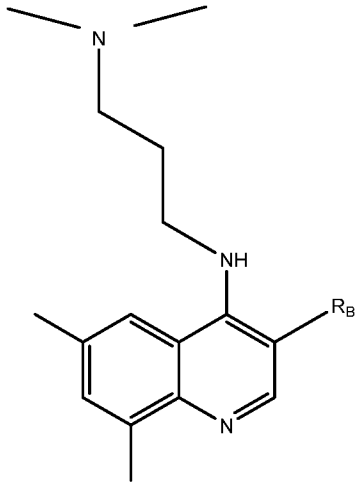

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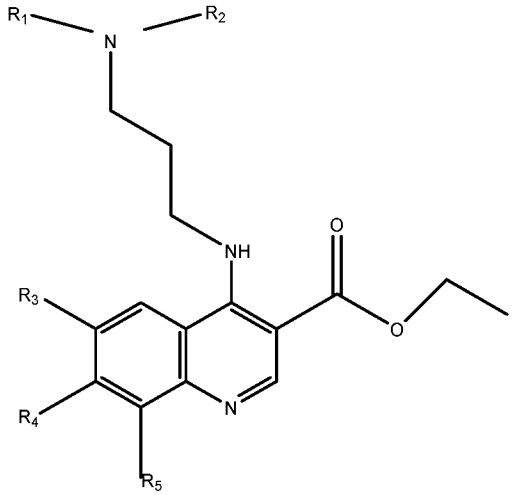

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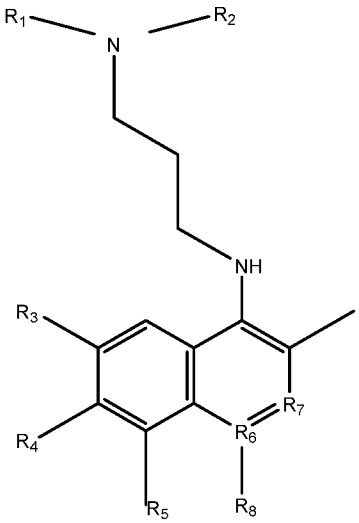

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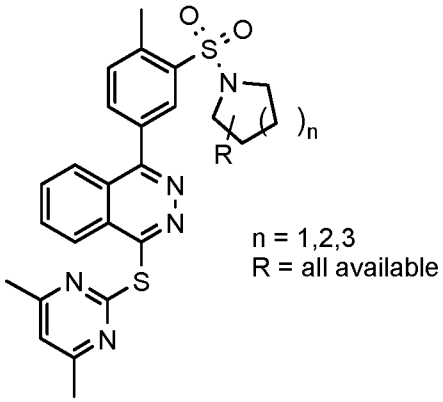

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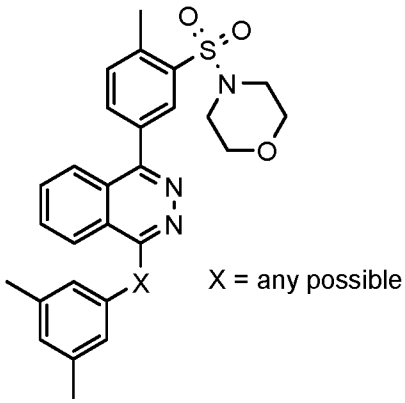

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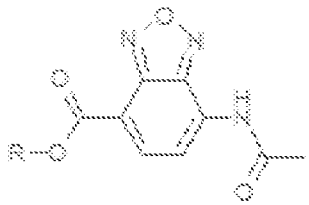

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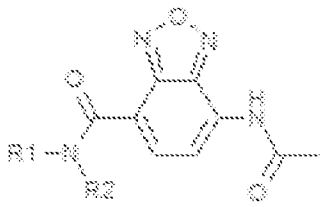

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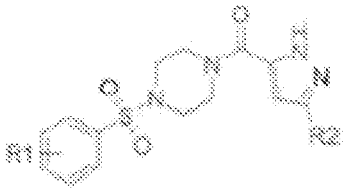

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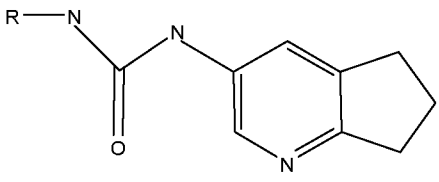
[0016]



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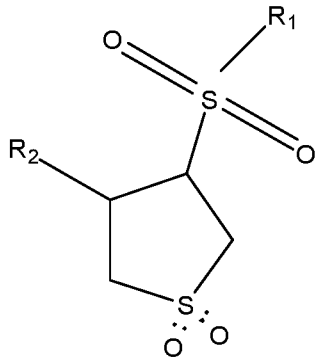
[0017]



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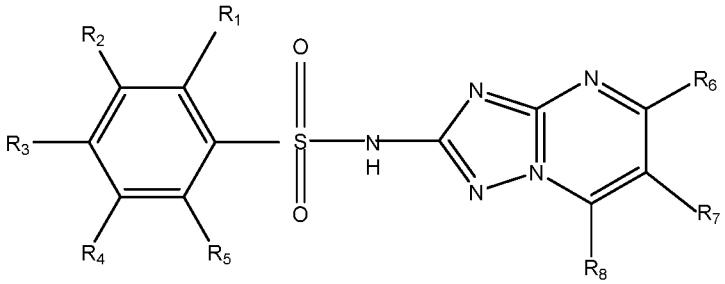

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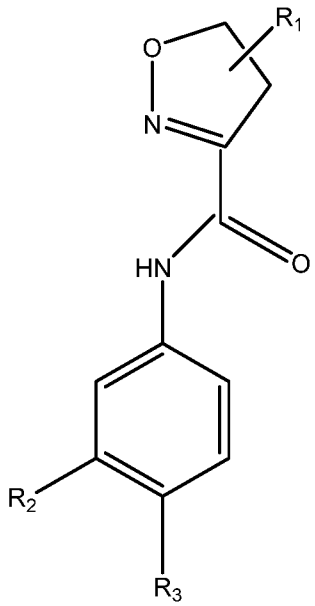

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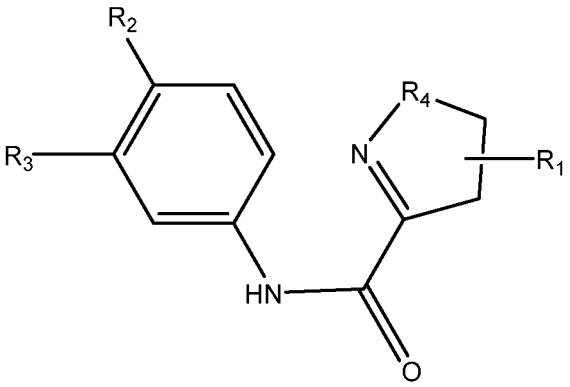

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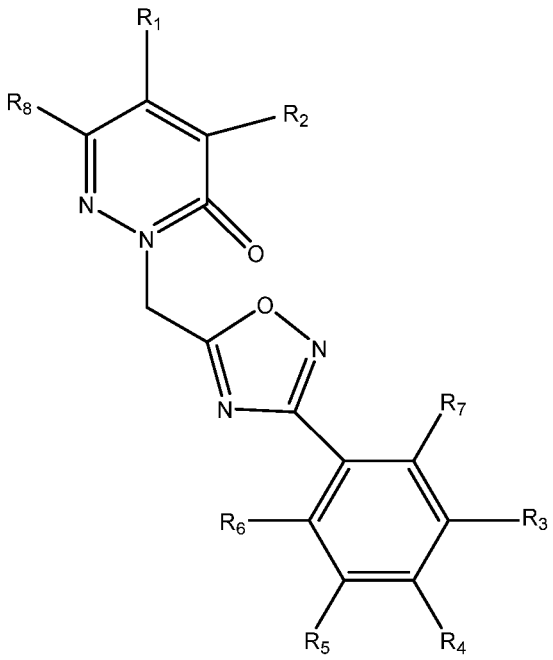

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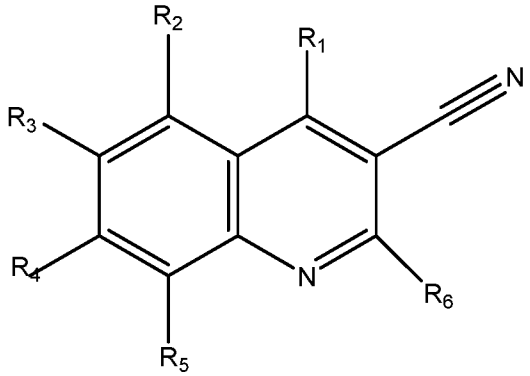

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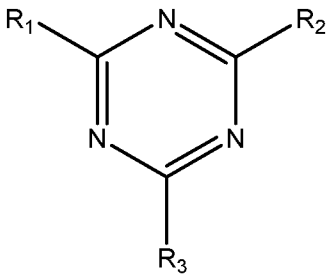


(XXV)

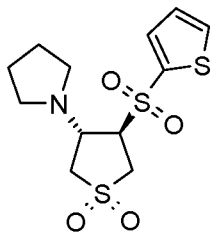
[0018] In various embodiments, in any of the above, moieties R, RA, RB, R1-R8, X, Q, Q1, Q2, A can be any of the following: C, H, N, O, S, a halogen, an alkyl group, a substituted alkyl group, a cyclic alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, an ester, an aldehyde, a ketone, a carboxylic acid, an amide, an amine, an ether, a thiol or a nitrile. In various embodiments, in any of the above, n can be an integer 1, 2, 3, 4, 5 or 6.

[0019] In various embodiments, any of R, RA, RB, R1-R8, X, Q, Q1, Q2, or A can be any of the following: Me, OMe, Br, N, H, Cl, F or NO_2. In certain embodiments, any of R, RA, RB, R1-R8, X, Q, Q1, Q2, or A can be any of the following: 4-Me, 4-OMe, 4-Br, 4-t-Bu, 3,4-di-Me, 4-Cl, 3,4-di-Cl, 3-Cl-4-F, 2-F, 3-Cl, 3-CH_3-4-F, a thiazole, an isothiazole or a dithiolane.

[0020] In various embodiments, any of R1 and R2 can have the values shown in any of the Figures, for example, **FIGS. 1A-1F, FIGS. 3A-3F, FIGS. 4A-4L, FIGS. 5A** and **5B**, **FIGS. 6A-6F, FIGS. 7A-7O, FIGS. 8A-8M, FIGS. 9A-9J, FIGS. 10A-10J** or **FIGS. 11A-11G**.

[0021] In certain embodiments, the present technology is directed a compound having a structure of any of the following, or a pharmaceutically acceptable salt thereof:

[0022]



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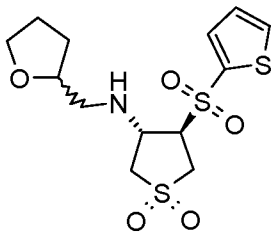

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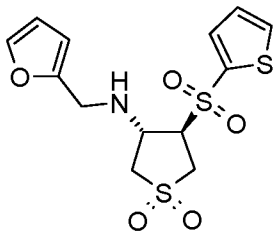

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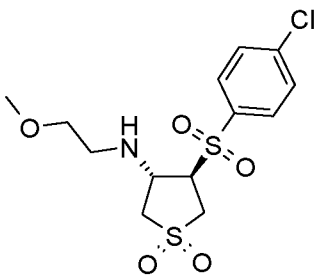

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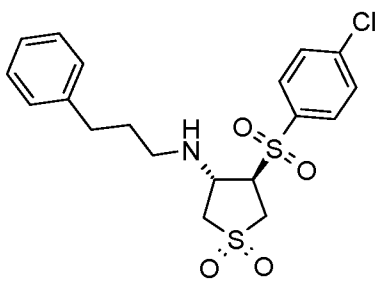

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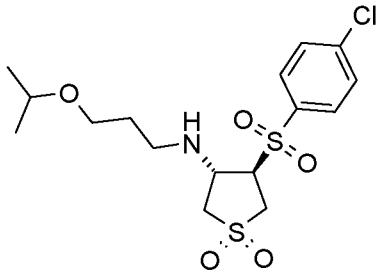

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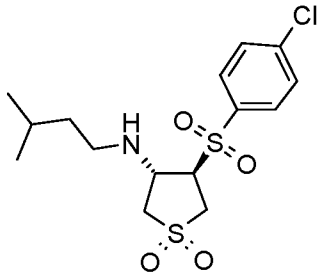

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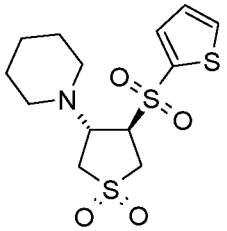

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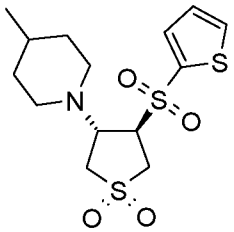

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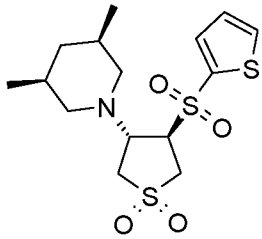

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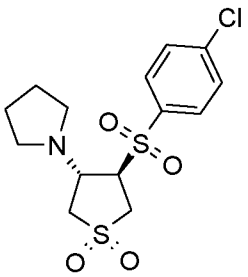
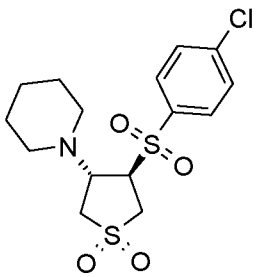
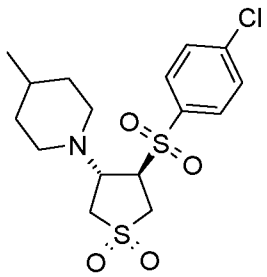


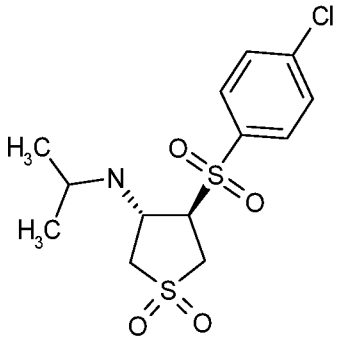
Compound D122



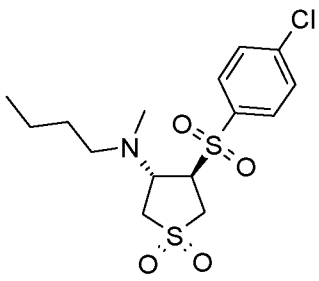
Compound D123



Compound D125



Compound D118



Compound D132

[0023] In certain embodiments, the present technology is directed to a novel compound, any solid form thereof, and any formulation or composition thereof, that is useful as agonists or antagonists of nuclear receptor activity, specifically to the modulation of NR2F6 activity and NR2F6 utilizing compounds.

[0024] In certain embodiments, the present technology is directed to methods of modulating the immune system or modulating cancer cell activity using compounds that alter activity of NR2F6.

[0025] In certain embodiments, the present technology is directed to methods of "reprogramming" the immune cells in a patient to attack tumors or other invasive cells. Such "reprogramming" can include: (a) extraction of an amount of a patient's cellular material (including, but not limited to: blood, which itself includes blood serum, plasma red blood cells, white blood cells and platelets), (b) isolating specific immune cells from the cellular material; (c) inhibiting or activating the NR2F6 target in the extracted immune cells; and (d) re-administering the immune cells (for example, by injection) to the patient's body.

[0026] In certain embodiments, the present technology is directed to methods treating or reducing the effect of an autoimmune response, reaction, disease or disorder, the method comprising any of the steps discussed herein, or activating the NR2F6 target in isolated immune cells by binding them with a compound according to the present technology.

[0027] In certain embodiments, the present technology is directed to methods of shrinking (reducing the size of) a tumor, increasing or decreasing the activity of a cell, initiating or inducing an immune response, destroying a cancer cell, reducing the effect of a disease, alleviating a symptom of a disease, treating a disorder, as well as methods of inducing a cell in a patient's body to do any of these, the method comprising administering a compound herein to a tumor, contacting a compound herein with a cell, or any other steps discussed herein. In various embodiments, these methods can comprise: comprising the steps of:

(a) extracting an amount of a patient's cellular material;

(b) isolating immune cells from the cellular material;

(c) activating the NR2F6 target in the isolated immune cells by binding them with a compound of claim 1; and

(d) re-administering the isolated immune cells to the patient's body.

[0028] In certain embodiments, the present technology is directed to methods of treating or reducing the effect of a reaction, disease or disorder, the method comprising activating the NR2F6 target in immune cells by contacting them with a compound herein.

[0029] In certain embodiments, the present technology is directed to a pharmaceutical composition comprising a compound described herein, with a pharmaceutically acceptable carrier or excipient.

[0030] The methods herein can, in various embodiments, involve humans or non-humans as subjects. As used herein, "non-human" means any living thing other than a human, for example, a mammal.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] **FIGS. 1A-1F** show certain compounds that have been found to be effective for the purposes of the present technology.

[0032] **FIG. 2A** shows a certain compound that has been found to be effective for the purposes of the present technology. **FIG. 2B** shows different domains, or portions of a base compound, that were substituted with different moieties to ascertain whether these made a difference in the activity of such compound.

[0033] **FIG. 3A** shows a certain compound that has been found to be effective for the purposes of the present technology. **FIGS. 3B-3F** show various additional compounds formed from substitution of different moieties.

[0034] **FIGS. 4A-4L** show additional compounds that were found to be useful in accordance with the embodiments herein.

[0035] **FIGS. 5A** and **5B** show additional embodiments of compounds, along with (in the case of **FIG. 5A**) different values of moieties R1 and R2.

[0036] **FIGS. 6A-6F** show additional compounds that were found to be useful in accordance with the embodiments herein.

[0037] **FIGS. 7A-7O** show additional compounds that were found to be useful in accordance with the embodiments herein.

[0038] **FIGS. 8A-8M** show additional compounds that were found to be useful in accordance with the embodiments herein.

[0039] **FIGS. 9A-9J** show additional compounds that were found to be useful in accordance with the embodiments herein.

[0040] **FIGS. 10A-10J** show additional compounds that were found to be useful in accordance with the embodiments herein.

[0041] **FIGS. 11A-11G** show additional compounds that were found to be useful in accordance with the embodiments herein.

[0042] **FIG. 12** shows a certain compound that has been found to be effective for the purposes of the present technology; and different domains, or portions of a base compound, that were substituted with different moieties to ascertain whether these made a difference in the activity of such compound.

[0043] **FIG. 13A** shows a certain compound that has been found to be effective for the purposes of the present technology. **FIGS. 13B-13H** show various additional compounds formed from substitution of different moieties.

[0044] **FIG. 14A** shows a certain compound that has been found to be effective for the purposes of the present technology. **FIGS. 14B-14D** show various additional compounds formed from substitution of different moieties.

[0045] **FIGS. 15A-15G** show additional compounds that were found to be useful in accordance with the embodiments herein

[0046] **FIGS. 16A** and **16B** show charts of cytokines release by hPBMC and cytotox for Compound C11, which was identified as a useful compound in accordance with the embodiments

herein. Results were repeated in follow set screen from fresh powder. 5 direct analogs were available (top structure in **FIG. 16A**).

[0047] **FIGS. 17A** and **17B** show analogues and other related compounds to Compound C11.

[0048] **FIGS. 18A and 18B** show charts of cytokines release by hPBMC and cytotox for Compound 18. For cytokines release and cytotox on hPBMCs compound was tested at 1.25, 2.5, 5, 10, 25 and 50 uM in duplicates. For cytotox on HEK293, HEK293 pGL4 and HEK293 NR2F6 (full length) compound was tested from 50 uM with dilution step 3.16 in duplicates. Human PBMC were activated by 10 ng/mL PMA + 500 ng/mL ionomycin. Data were normalized to controls with (100%) w/o compounds.

[0049] **FIGS. 19A** and **19B** show the results of Dog's PBMC ELISA and cytotoxicity experiments. All compounds were tested at 5, 10, 25 and 50 uM in duplicates on activated by 10 ng/mL PMA + 500 ng/mL ionomycin dogs PBMC (1 x 106 cells/mL). Cell culture supernates were removed and frozen for further ELISA analysis and remained cells were analyzed. Compounds without cytotox were chosen for cytokine release inhibition analysis. Compound Z92 was also analyzed at 5uM and 10uM.

[0050] **FIGS. 20A** and **20B** show results of cytokines release by hPBMC and cytotox for Compound Z95. For cytokines release and cytotox on hPBMCs compound was tested at 1.25, 2.5, 5, 10, 25 and 50 uM in duplicates. For cytotox on HEK293, HEK293 pGL4 and HEK293 NR2F6 (full length) compound was tested from 50 uM with dilution step 3.16 in duplicates. Human PBMC were activated by 10 ng/mL PMA + 500 ng/mL ionomycin. Data were normalized to controls with (100%) without compounds.

[0051] **FIGS. 21A-21D** show human and dog results of a cytokine release experiment – parent compound for dogs and human PBMC, for Compound D28. All compounds were tested at 5, 10, 25 and 50 uM in duplicates. Dog PBMC (1 x 106 cells/mL) were activated by 10 ng/mL PMA + 500 ng/mL ionomycin. Data were normalized to controls with (100%) without (0%) PMA + ionomycin activation.

[0052] **FIGS. 22A** and **22B** show results of cytokines release by hPBMC and cytotox for Compound Z17. For cytokines release and cytotox on hPBMCs compound was tested at 1.25, 2.5, 5, 10, 25 and 50 uM in duplicates. For cytotox on HEK293, HEK293 pGL4 and HEK293 NR2F6 (full length) compound was tested from 50 uM with dilution step 3.16 in duplicates. Human PBMC were activated by 10 ng/mL PMA + 500 ng/mL ionomycin. Data were normalized to controls with (100%) without compounds.

[0053] **FIGS. 23A** and **23B** show results of cytokines release by hPBMC and cytotox for Compound Z33. For cytokines release and cytotox on hPBMCs compound was tested at 1.25, 2.5, 5, 10, 25 and 50 uM in duplicates. For cytotox on HEK293, HEK293 pGL4 and HEK293 NR2F6 (full length) compound was tested from 50 uM with dilution step 3.16 in duplicates. Human PBMC were activated by 10 ng/mL PMA + 500 ng/mL ionomycin. Data were normalized to controls with (100%) without compounds.

[0054] **FIG. 24** shows results of a cytokines release by hPBMC for Compound E56.

[0055] **FIGS. 25A** and **25B** show results of cytokines release by hPBMC and cytotox for Compound Z96. For cytokines release and cytotox on hPBMCs compound was tested at 1.25, 2.5, 5, 10, 25 and 50 uM in duplicates. For cytotox on HEK293, HEK293 pGL4 and HEK293 NR2F6 (full length) compound was tested from 50 uM with dilution step 3.16 in duplicates. Human PBMC were activated by 10 ng/mL PMA + 500 ng/mL ionomycin. Data were normalized to controls without (100%) compounds.

[0056] **FIGS. 26A** and **26B** show results of cytokine release by hPBMC and cytotox for Compound Z97.

[0057] **FIGS. 27A-27D** show NR2F6 and LBD transient transfection, respectively, for Compound D28. **FIGS. 27E and 27F** show toxicity of Compound D28.

[0058] **FIGS. 28A-28D** show the results of a cytokine release experiment for dog and human PBMC. All compounds were tested at 5, 10, 25 and 50 uM in duplicates. Dog PBMC (1 x 106 cells/mL) were activated by 10 ng/mL PMA + 500 ng/mL ionomycin. Data were normalized to controls with (100%) /without (0%) PMA + ionomycin activation.

[0059] **FIGS. 29, 30 31A, 31B and 32** show exemplary methods of formulating the compounds that have been discussed herein.

[0060] **FIGS. 33A** and **33B** and **34A-34D** show NR2F6 and LBD transient transfection, respectively, for Compound E21.

[0061] **FIGS. 35A** to **35D** show the results of a cytokine release experiment for dog and human PBMC, for Compound E21.

[0062] **FIG. 36** shows additional compounds related to compound E21 that were tested herein.

[0063] **FIG. 37** shows additional compounds that were synthesized and tested according to the present embodiments.

[0064] **FIG. 38A** and **38B**, **39A-39D** and **40A-40D** show results of testing on Compound F1.

[0065] **FIG. 41** shows the general SAR strategy for testing Compound F1 and compounds related to it in structure; by dividing the active molecule into four domains (Domains A through D), and evaluating each domain independently to establish SAR trends.

[0066] **FIG. 42** shows an exemplary synthesis of a boronate compound, and the results of other exemplary syntheses of compounds comprising boronate, and the relative proportions of resultant compounds.

[0067] **FIG. 43** shows methods of synthesis of certain compounds found to be useful for the embodiments herein.

[0068] **FIGS. 44A** and **44B** and **45A-D** show NR2F6 and LBD transient transfection, respectively, for Compound P1.

[0069] **FIGS. 46A** and **46B** show the results of a cytokine release experiment for dog and human PBMC, for Compound P1.

[0070] **FIGS. 47A** and **47B** show NR2F6 agonist activity of various compounds discussed herein.

[0071] **FIGS. 48-51** show synthesis of various additional compounds discussed herein.

[0072] **FIGS. 52A** and **52B** show synthesis of Compounds Z119, Z120, Z121 and Z123.

[0073] **FIG. 53-55** shows synthesis of additional compounds herein.

[0074] **FIGS. 56A** and **56B** show Nr2F6 agonist activity for various compounds discussed herein.

[0075] **FIGS. 57** and **58** show synthesis of various compounds discussed herein.

[0076] **FIGS. 59A-D** show further results for firefly, renila and pGL4 for Compounds D104, D118, D122 and D137.

[0077] **FIGS. 60A-60C** show further results for cytokines release inhibition for Compounds D136, D140, D141, D142, Z155 and Z166.

[0078] **FIGS. 61A-61E** show further results of cytokine release by hPBMC for Compound D136.

[0079] **FIGS. 62A** and **62B** show stability in plasma (human plasma and rat plasma) and various pH solubility data for Compound D136.

[0080] **FIGS. 63A** and **63B** show stability in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF), respectively, for Compound D136.

[0081] **FIGS. 64A** and **64B** show microsomal stability in human liver microsomes and rat liver microsomes, respectively, for Compound D136.

[0082] **FIGS. 65A** and **65B** show data for PK in rat plasma for Compound D136.

[0083] **FIGS. 66A** and **66B** show data for PK in mice plasma for Compound D136.

[0084] **FIGS. 67-70** show synthesis of additional compounds herein.

DETAILED DESCRIPTION

[0085] As used herein, "disease" or "disorder" are used interchangeably and mean a disorder of structure or function in any living thing (including but not limited to a human, animal, or plant), especially one that produces specific signs or symptoms or that affects a specific location and is not simply a direct result of physical injury.

[0086] As used herein, "mammal" means a warm-blooded vertebrate animal of a class that is distinguished by the possession of hair or fur, the secretion of milk by females for the nourishment of the young, and (typically) the birth of live young. As used herein, "human" means a person. As used

herein, "animal" or "non-human mammal" means any non-human animal, including but not limited to: a canine (*e.g.*, a dog), a feline (*e.g.*, a cat), a rodent, an ungulate (*e.g.*, a cow or ox), an equine (*e.g.*, a horse), or a primate.

[0087] As used herein, "modulator" means a molecule that alters the basal activity of NR2F6either positively (activates it or increases it) or negatively (represses, suppresses or decreases it). "Modulating" means the act of the modulator, either positive or negative. A compound of the technology herein can be, in various embodiments, a modulator of NR2F6, for example, at an effective concentration or in an effective amount, but not be a modulator of any other receptor, or not a modulator at any other amount of NR2F6 or any other receptor. This can provide selectivity of effect of a compound of the technology herein when administered to a patient for treatment of any disease.

[0088] In certain embodiments, one diastereomer or one enantiomer of a compound of the present technology can display superior biological activity compared with the other. When required, separation of the diastereomeric mixture or the racemic material can be achieved by HPLC, optionally using a chiral column or by using a resolving agent such as camphonic chloride for the resolution of enantiomers. A chiral compound described herein can also be directly synthesized using a chiral catalyst or a chiral ligand.

[0089] In certain embodiments, one deuterated or tritiated compound of the present technology can display superior biological activity compared with one or more others. When required, separation of the material can be achieved by one of ordinary skill in the art.

[0090] In certain embodiments, the present technology is directed to methods of modulating the immune system using compounds that alter activity of NR2F6.

[0091] In certain embodiments, compounds herein can be utilized for stimulation of NR2F6 activity, alone, or in combination with, for example, PKC activation. In certain embodiments, the compounds herein can be utilized for inhibition of NR2F6 activity, alone or in combination with, for example, anti-PD1, anti-PDL1 or anti-CTLA4 antibodies.

[0092] In other embodiments, the methods are directed to the stimulation of NR2F6 for, *e.g.*, induction of immune inhibition, or stimulation of cellular proliferation without significant induction of differentiation. Inhibition of NR2F6 can be desirable in situations where a clinician seeks to augment immune response, or induce cellular differentiation.

[0093] In other embodiments, inhibition of NR2F6 expression can be desirable in situations where inhibition of cancer or cancer stem cells is desired.

[0094] In certain embodiments, activation of NR2F6 expression can be desirable in situations where inhibition of the immune system is desired, for example, in connection with autoimmune disorders.

[0095] The interplay between the activation or deactivation of NRs by different structural classes of endogenous ligands, such as the steroid and thyroid hormones, lipids, vitamins and other biochemicals, is an important part of their function. The 48 NR family members are classified into subgroups based on the identification of endogenous ligands for each receptor. The endocrine receptors include the steroid hormone receptors that bind steroid hormones and the heterodimeric receptors that partner with the retinoid X receptor and bind thyroid hormones, retinoids, and vitamin D.

[0096] The identification of specific endogenous ligands for the endocrine receptors has facilitated the design and development of selective receptor modulators (SRMs) that exhibit tissue-specific agonist or antagonist activities and are used for treatment of hormone-/hormone receptor-dependent diseases. Tamoxifen is one of many selective estrogen receptor (ER) modulators used in endocrine therapies for treating ER-positive breast cancer patients.

[0097] Adopted orphan receptors are a subtype of NRs that are subdivided into groups based on their ligands. The lipid sensor receptor subtypes and their ligands include retinoid X receptor (9-*cis*-retinoic acid), peroxisome proliferator-activated receptors (PPARs) (fatty acids), liver X receptor (oxysterols), farnesoid X receptor (bile acids), and pregnane X receptor, which binds cholesterol derivatives.

[0098] Retinoid X receptors have been found in various cancer stem cells and methodologies for their utilization, as well as ligands/synthetic ligands targeting them, have been developed. Any of these can be utilized by one of skill in the art to practice the methods of the present technology, which provides compounds useful for modulating the NR2F6 nuclear receptor. Methods of modulating PPARs are also amenable to utilization in the context of the current technology, whose methodologies can, in various embodiments, be adapted for use with the compounds discussed herein for treatment of cancer or immune modulation.

[0099] With regard to PPARs, three subtypes of the PPAR family are PPARα, PPARγ, and PPARδ. PPARγ is abundantly expressed in many cell types, where it regulates lipid metabolism, glucose homeostasis, tumor progression, and inflammation. Polyunsaturated fatty acids, eicosanoids, prostaglandins, and linoleic acid have been identified as endogenous ligands for PPARγ. The thiazolidinedione class of compounds function as high-affinity synthetic agonists for PPARγ subsequent to exposure to specific ligands. PPARγ forms a heterodimer complex with retinoid X receptor, which then mediates the target gene expression. In terms of immune modulation, in certain embodiments, NR2F6 specific compounds can be substituted for those described for PPAR.

[00100] The enigmatic orphan receptor subtype can include the constitutive androstane receptor (androstane and many drugs or xenobiotics), hepatocyte nuclear factor-4, and steroidogenic factor-1/liver receptor homolog 1(LRH-1) (phospholipids), retinoid acid-related orphan receptor (cholesterol and retinoic acids), and estrogen-related receptor (estrogens). These can be useful in methods of performing immunotherapy that include NR2F6 modulators.

[00101] The orphan receptors are the third class of NRs. The crystal structure of the ligand-binding domain of the orphan receptor Nurr1 (NR4A2) shows that several hydrophobic residues protrude into the ligand-binding pocket, and a typical coactivator-binding site is lacking, suggesting that some orphan receptors may not bind ligands.

[00102] Like other NR classes, the orphan receptors play important roles in cellular homeostasis and diseases including cancer, and several recent reports document the expression and potential functions of orphan receptors in different tumors and cancer cell lines. Breast tumors are

routinely classified as ER^+ or ER^-, and expression of ER has prognostic significance that influences selection of therapeutic regimens. However, analysis of ER^+ and ER^- tumors for expression (mRNA) of all 48 NRs also demonstrated the important prognostic significance of several orphan receptors. The NR4A (Nur77/TR3, Nurr1, and Nor1) and NR2F6 [v-erbA-related protein (EAR2)] receptors are uniquely overexpressed in (ER^+ and ER^- combined) tumors. Moreover, Nur77, EAR2, and chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) are among a limited group of NRs that are prognostic for breast cancer classification and histologic grade, and COUP-TFII expression was a positive prognostic factor for tamoxifen-treated ER^+ breast cancer patients.

[00103] Examination of lung tumor and nontumor tissue indicated highly variable NR expression; however, gene combinations and individual receptors, such as the orphan receptor small heterodimer partner (SHP, NR0B2), predicted enhanced survival for early-stage lung cancer patients. Moreover, expression of Nur77 in normal lung epithelium from patients has been shown to be an indicator for good prognosis.

[00104] NR profiling of the NCI60 cancer cell panel demonstrated that relative expression levels of some orphan receptors also correlated with drug sensitivity. For example, cancer cell sensitivity to microtubule-disrupting drugs has been found to be enhanced in cells expressing low levels of NR2F6 and COUP-TFII, whereas high levels of the orphan receptor tailless (TLX, NR2E1) correlated with sensitivity to 9-fluoroprednisolone.

[00105] As used herein, the term "NR2F6" means "nuclear receptor subfamily 2, group F, member 6" or "Ear2." Nuclear receptors are transcription factors that regulate the expression of specific target genes, thereby orchestrating a wide array of cellular processes including cellular activation, development and disease progression. The nuclear receptor super-family includes receptors that bind to hormones and orphan receptors with yet undefined endogenous ligands. As discussed in the present disclosure, NR2F6 can be a target in cancer immunotherapy or autoimmune suppression.

[00106] The COUP-TF orphan receptors are preferentially expressed in the central nervous system and organs that depend on the interaction between mesenchyme and epithelial layers. The three mammalian COUP-TF family members are NR2F1/Ear3, NR2F2/Arp1 and NR2F6. The

established target genes of said COUP-TF family members are apolipoproteins and retinoic acid-, peroxisome-, oxytocin-, estrogen- and vitamin D receptors. By yeast 1-hybrid screen and in vitro assays with recombinant NR2F6, it was found that the TGACCT direct-repeat motif is the DNA binding sequence of NR2F6, and that overexpression of NR2F6 induces repression of the renin gene transcription in a DNA-binding-specific manner. Wild type human/animal NR2F6 is known to possess the following nucleotide sequence: 1 gtgcagcccg tgcccccgc gcgccggggc cgaatgcgcg ccgcgtaggg tcccccgggc 61 cgagaggggt gcccggaggg aagagcgcgg tgggggcgcc ccggccccgc tgccctgggg 121 ctatggccat ggtgaccggc ggctggggcg gccccggcgg cgacacgaac ggcgtggaca 181 aggcgggcgg ctacccgcgc gcggccgagg acgactcggc ctcgcccccc ggtgccgcca 241 gcgacgccga gccgggcgac gaggagcggc cggggctgca ggtggactgc gtggtgtgcg 301 gggacaagtc gagcggcaag cattacggtg tcttcacctg cgagggctgc aagagctttt 361 tcaagcgaag catccgccgc aacctcagct acacctgccg gtccaaccgt gactgccaga 421 tcgaccagca ccaccggaac cagtgccagt actgccgtct caagaagtgc ttccgggtgg 481 gcatgaggaa ggaggcggtg cagcgcggcc gcatcccgca ctcgctgcct ggtgccgtgg 541 ccgcctcctc gggcagcccc ccgggctcgg cgctggcggc agtggcgagc ggcggagacc 601 tcttcccggg gcagccggtg tccgaactga tcgcgcagct gctgcgcgct gagccctacc 661 ctgcggcggc cggacgcttc ggcgcagggg gcggcgcggc gggcgcggtg ctgggcatcg 721 acaacgtgtg cgagctggcg gcgcggctgc tcttcagcac cgtggagtgg gcgcgccacg 781 cgcccttctt ccccgagctg ccggtggccg accaggtggc gctgctgcgc ctgagctgga 841 gcgagctctt cgtgctgaac gcggcgcagg cggcgctgcc cctgcacacg gcgccgctac 901 tggccgccgc cggcctccac gccgcgccta tggccgccga gcgcgccgtg gctttcatgg 961 accaggtgcg cgccttccag gaggaggtgg acaagctggg ccgcctgcag gtcgactcgg 1021 ccgagtatgg ctgcctcaag gccatcgcgc tttcacgcc cgacgcctgt ggcctctcag 1081 acccggccca cgttgagagc ctgcaggaga aggcgcaggt ggccctcacc gagtatgtgc 1141 gggcgcagta cccgtcccag ccccagcgct tcgggcgcct gctgctgcgg ctccccgccc 1201 tgcgcgcggt ccctgcctcc ctcatctccc agctgttctt catgcgcctg gtggggaaga 1261 cgcccattga gacactgatc agagacatgc tgctgtcggg gagtaccttc aactggccct 1321 acggctcggg ccagtgacca tgacggggcc acgtgtgctg tggccaggcc tgcagacaga 1381 cctcaaggga cagggaatgc tgaggcctcg aggggcctcc cggggcccag gactctggct 1441 tctctcctca gacttctatt ttttaaagac tgtgaaatgt ttgtcttttc tgttttttaa 1501 atgatcatga aaccaaaaag agactgatca tccaggcctc agcctcatcc tccccaggac 1561 ccctgtccag gatggagggt ccaatcctag gacagccttg ttcctcagca cccctagcat 1621 gaacttgtgg gatggtgggg ttggcttccc tggcatgatg gacaaaggcc tggcgtcggc 1681 cagaggggct gctccagtgg gcaggggtag

ctagcgtgtg ccaggcagat cctctggaca 1741 cgtaacctat gtcagacact acatgatgac tcaaggccaa taataaagac atttcctacc 1801 tgca, which corresponds to the following amino acid sequence:

MAMVTGGWGGPGGDTNGVDKAGGYPRAAEDDSASPPGAASDAEPGD
EERPGLQVDCVVCGDKSSGKHYGVFTCEGCKSFFKRSIRRNLSYTC
RSNRDCQIDQHHRNQCQYCRLKKCFRVGMRKEAVQRGRIPHSLPGA
VAASSGSPPGSALAAVASGGDLFPGQPVSELIAQLLRAEPYPAAAG
RFGAGGGAAGAVLGIDNVCELAARLLFSTVEWARHAPFFPELPVAD
QVALLRLSWSELFVLNAAQAALPLHTAPLLAAAGLHAAPMAAERAV
AFMDQVRAFQEQVDKLGRLQVDSAEYGCLKAIALFTPDACGLSDPA
HVESLQEKAQVALTEYVRAQYPSQPQRFGRLLLRLPALRAVPASLI
SQLFFMRLVGKTPIETLIRDMLLSGSTFNWPYGSGQ (SEQ ID NO: 1).

[00107] Accordingly, in certain embodiments the present technology is directed to compounds that bind to a portion or all of an NR2F6 molecule; or any molecule that is, in various embodiments, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the amino acid sequence of NR2F6.

[00108] As used herein, the terms "agonist" or "activator" are used interchangeably and mean a compound or substance capable of fully or partially stimulating the physiologic activity of one or more specific receptors. In the context of the present disclosure, an agonist can therefore stimulate the physiological activity of a receptor such as NR2F6 upon binding of said compound substance to said receptor. As further discussed herein, an "agonist" or "activator" can be used to "activate," "stimulate" or "increase activity" of a cell.

[00109] In certain embodiments, binding of an "agonist" or "activator" to a given receptor, *e.g.*, NR2F6, can mimic the action of an endogenous ligand binding to said receptor. As used herein, accordingly, the term "agonist" also encompasses partial agonists or co-agonists or co-activators. In addition, however, an "agonist" or "activator" of NR2F6 as used herein can also be capable of stimulating the function of a given receptor, such as NR2F6, by inducing or enhancing the expression of the nucleic acid molecule encoding for said receptor. Thus, an agonist or activator of NR2F6 can,

in certain embodiments, lead to an increased expression level of NR2F6 (*e.g.*, increased level of NR2F6 mRNA, NR2F6 protein) which is reflected in an increased activity of NR2F6. This increased activity can be measured or detected by the methods herein.

[00110] Accordingly, an activator of NR2F6 in accordance with the present technology can, in certain embodiments, also encompass transcriptional activators of NR2F6 expression that are capable of enhancing NR2F6 function. As mentioned above, "agonist" includes a partial agonist. "Partial agonists" mean candidate molecules that behave like agonists, but that, even at high concentrations, cannot activate NR2F6 to the same extent as a full agonist. As described below, the increased expression or activity of NR2F6 by an agonist or activator of NR2F6 can lead to decreased activity (or expression) of components of the NR2F6-dependent signaling pathway; in particular the activity of NF-AT and AP-1 can be decreased. NF-AT/AP-1 regulate transcription/expression of further "downstream" components of the NR2F6-dependent signaling pathway, such as IL-2, IL-17 and/or IFN-gamma. A decrease in NF-AT/AP-1 activity can result in a decreased transcription of these "downstream" components (*e.g.*, IL-2, IL-17 and/or IFN-gamma) which in turn leads to a suppression of an immune response.

[00111] In certain embodiments, an agonist or activator of NR2F6 can lead to suppression of an immune response. Hence, the use of potent agonists/activators of NR2F6 can lead to a higher expression or activity of NR2F6.

[00112] In certain embodiments NR2F6 can be considered its own "agonist" or "activator." For example, in certain embodiments, overexpression of NR2F6 can lead to enhanced NR2F6 activity, thus agonizing NR2F6 function. Accordingly, NR2F6 as defined herein can, in certain embodiments, be used for the treatment of a disease related to an augmented immune response.

[00113] For example, NR2F6 can be used in accordance with certain embodiments of the present technology, wherein NR2F6 is any of the following: (a) a polypeptide comprising an amino acid encoded by a nucleic acid molecule having the nucleic acid sequence of NR2F6; (b) a polypeptide having an amino acid sequence of NR2F6; (c) a polypeptide encoded by a nucleic acid molecule encoding a peptide having an NR2F6 amino acid sequence; (d) a polypeptide comprising an

amino acid encoded by a nucleic acid molecule hybridizing to the complementary strand of nucleic acid molecules as defined in (a) or (c) and encoding a NR2F6 or a functional fragment thereof; (e) a polypeptide having at least 60% homology to the polypeptide of any one of (a) to (d), whereby said polypeptide is a NR2F6 or a functional fragment thereof; or (f) a polypeptide comprising an amino acid encoded by a nucleic acid molecule being degenerate as a result of the genetic code to the nucleotide sequence of a nucleic acid molecule as defined in (a), (c) and (d). As described herein, the increase of NR2F6 activity can lead to a decreased activity of NF-AT/AP-1 (and other components of the NR2F6-dependent signaling pathway) which in turn can result in a suppressed immune response.

[00114] An exemplary transfection of $CD4^+$ T cells with a construct for the overexpression of NR2F6 is also shown in the appended examples. As demonstrated therein, overexpression (about 5-fold increase over normal expression level) can lead to a diminished IL-2 activity/expression and consequently to a reduced IL-2 amount, resulting in a reduced immune response.

[00115] Therefore, agonists/activators of NR2F6 are useful in the treatment of diseases where suppression of the immune response is desired (*e.g.*, diseases with an overstimulated immune response, such as allergies and multiple sclerosis). As used herein. the term "overexpression" means that the NR2F6 activity/expression is, in various embodiments, at least 1-fold, at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, or at least 25-fold increased in comparison to a (control) standard value as defined herein, wherein a value of 25 fold expression level or greater over normal can be considered as a maximum overexpression level.

[00116] As used herein, "antagonist" or "inhibitor" are used interchangeably and mean a compound or substance capable of fully or partially suppressing or inhibiting the physiologic activity of one or more specific receptors. In the context of the present disclosure, an antagonist can therefore suppress the physiological activity of a receptor upon binding of said compound substance to said receptor but does not activate the receptor and therefore blocks the activity of other agonists. As further discussed herein, an "antagonist" or "inhibitor" can be used to "deactivate," "inhibit," "suppress" or "decrease activity" of a cell.

[00117] As used herein, the terms "immune response" or "immune reaction" are used interchangeably and mean the response or reaction of the immune system to an antigen. In the case of an immune response, immune cells are activated in such way that one or more specific functions of said immune cells can be induced. The "immune cells" can include, but are not limited to, B cells, T cells, neutrophils, eosinophils, basophils, mast cells, macrophages and dendritic cells. In certain embodiments, said "specific function(s) of activated immune cells" can include, but are not limited to, secretion of antibodies, presentation of antigen, proliferation of said immune cells, secretion of cytokines such as interleukin-2 (IL-2), interleukin-17 (IL-17), interleukin-18 (IL-18), or interferon gamma (IFNgamma), expression of regulatory-, activation- or adhesion molecules, and the ability to induce apoptosis or cytolysis.

[00118] As used herein, the term "antigen" means any substance capable of inducing an immune response. An antigen typically is associated with a foreign substance (*i.e.* a "non-self antigen"). However, an own body-derived substance (*i.e.*, a "self antigen") can also induce an immune response.

[00119] As used herein, accordingly, the term "immune response" also encompasses autoimmune responses or autoimmune reactions. For example, in certain embodiments, the technology herein is directed to a method of treating or reducing the effect of an autoimmune response, reaction, disease or disorder, the method comprising activating the NR2F6 target in isolated immune cells by binding them with a compound according to the present technology.

[00120] As used herein, "treating a cancer," "inhibiting cancer" or "reducing cancer growth" are used interchangeably and mean inhibiting or preventing oncogenic activity of cancer cells. Oncogenic activity can comprise stimulating migration, invasion, drug resistance, cell survival, anchorage-independent growth, non-responsiveness to cell death signals, angiogenesis, or combinations thereof of the cancer cells. In various embodiments, agents suitable for use in treating a cancer or reducing the growth rate of a tumor include, but are not limited to, small organic molecules, peptides, proteins, peptidomimetics, nucleic acids, antibodies and combinations thereof. In various embodiments, such agents can be formulated with a pharmaceutically acceptable carrier, and can be

administered: intravenously, orally, buccally, sublingually, parenterally, by inhalation, by nasal administration, by insufflation, by topical application, transdermally, by cutaneous injection, or by local administration. An agent can additionally be administered in conjunction with one or more anti-cancer chemotherapeutic agent in an additive or synergistic manner.

[00121] As used herein, "cancer," "cancer cell," "tumor" and "tumor cell" are used interchangeably and mean a group of diseases characterized by uncontrolled, abnormal growth of cells (*e.g.*, a neoplasia). These can include solid tumor cancer, liquid tumor cancer and metastatic disease. In some forms of cancer, the cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body ("metastatic cancer"). As used herein, "ex vivo activated lymphocytes," "lymphocytes with enhanced antitumor activity" and "dendritic cell cytokine induced killers" are used interchangeably and mean composition of cells that have been activated ex vivo and subsequently reintroduced within the context of the current disclosure. Although the word "lymphocyte" is used, this also includes heterogeneous cells that have been expanded during the ex vivo culturing process including dendritic cells, NKT cells, gamma delta T cells, and various other innate and adaptive immune cells.

[00122] As used herein, "cancer" means any disease caused by uncontrolled division or growth of abnormal cells, and any malignant growth or tumor resultant from such uncontrolled division or growth. As used herein, "cancer" includes all types of cancer or neoplasm or malignant tumors found in animals, including leukemias, carcinomas and sarcomas. Examples of cancers include, but are not limited to: cancer of the brain, skin (including melanoma), breast, cervix, head and neck, kidney, lung, non-small cell lung, mesothelioma, sarcoma, any internal organ (including bladder, stomach, liver, pancreas, uterus, ovary, prostate, colon) and Medulloblastoma.

[00123] As used herein, "leukemia" means a broadly progressive, malignant disease of the hematopoietic organs or systems and is generally characterized by a distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow. Leukemia diseases include, but are not limited to: acute nonlymphocytic leukemia, chronic lymphocytic leukemia, acute granulocytic leukemia, chronic granulocytic leukemia, acute promyelocytic leukemia, adult T-cell

leukemia, B cell lymphoma, aleukemic leukemia, a leukocythemic leukemia, basophilic leukemia, blast cell leukemia, bovine leukemia, chronic myelocytic leukemia, leukemia cutis, embryonal leukemia, eosinophilic leukemia, Gross' leukemia, Rieder cell leukemia, Schilling's leukemia, stem cell leukemia, subleukemic leukemia, undifferentiated cell leukemia, hairy-cell leukemia, hemoblastic leukemia, hemocytoblastic leukemia, histiocytic leukemia, stem cell leukemia, acute monocytic leukemia, leukopenic leukemia, lymphatic leukemia, lymphoblastic leukemia, lymphocytic leukemia, lymphogenous leukemia, lymphoid leukemia, lymphosarcoma cell leukemia, mast cell leukemia, megakaryocytic leukemia, micromyeloblastic leukemia, monocytic leukemia, myeloblastic leukemia, myelocytic leukemia, chronic myeloid leukemia, myeloid granulocytic leukemia, myelomonocytic leukemia, Naegeli leukemia, plasma cell leukemia, plasmacytic leukemia, and promyelocytic leukemia.

[00124] As used herein, the term "carcinoma" means a malignant new growth made up of epithelial cells tending to infiltrate the surrounding tissues, or resist physiological and non-physiological cell death signals and give rise to metastases. Exemplary carcinomas include, but are not limited to: acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiennoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniform carcinoma, gelatinous carcinoma, giant cell carcinoma, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrmcous carcinoma,

carcinoma villosum, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypemephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, nasopharyngeal carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhous carcinoma, and carcinoma scroti.

[00125] As used herein, the term, "sarcoma" means a tumor which is made up of a substance like the embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar, heterogeneous, or homogeneous substance. Sarcomas include, but are not limited to: chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, endometrial sarcoma, stromal sarcoma, Ewing's sarcoma, fascial sarcoma, fibroblastic sarcoma, giant cell sarcoma, Abernethy's sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms' tumor sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented hemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, and telangiectaltic sarcoma. Additional exemplary neoplasias include, for example, Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, breast cancer, ovarian cancer, lung cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, small-cell lung tumors, primary brain tumors, stomach

cancer, colon cancer, malignant pancreatic insulanoma, malignant carcinoid, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, cervical cancer, endometrial cancer, and adrenal cortical cancer.

[00126] In some particular embodiments of the present technology, the cancer treated is a melanoma. As used herein, the term "melanoma" means a tumor arising from the melanocytic system of the skin or other organs. Melanomas include, for example, Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, nodular melanoma subungual melanoma, and superficial spreading melanoma. As used herein, the term "polypeptide" is used interchangeably with "peptide," "altered peptide ligand" and "fluorocarbonated peptides."

[00127] As used herein, the term "pharmaceutically acceptable carrier" means any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the therapeutic compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions herein.

[00128] As used herein, the terms "T cell" or "T lymphocyte" are used interchangeably, and mean a cell derived from thymus among lymphocytes involved in an immune response. In various embodiments a T cell includes any of: a CD8-positive T cell (cytotoxic T cell: CTL), a $CD4^+$ T cell (helper T cell), a suppressor T cell, a regulatory T cell such as a controlling T cell, an effector cell, a naive T cell, a memory T cell, an alpha (α) beta (β) T cell expressing TCR α and β chains, and a gamma (γ) delta (δ) T cell expressing TCR γ and δ chains.

[00129] In certain embodiments, the T cell includes a precursor cell of a T cell in which differentiation into a T cell is directed. Examples of "cell populations containing T cells" include, in addition to body fluids such as blood (peripheral blood, umbilical blood etc.) and bone marrow fluids, cell populations containing peripheral blood mononuclear cells (PBMC), hematopoietic cells,

hematopoietic stem cells, umbilical blood mononuclear cells etc., which have been collected, isolated, purified or induced from the body fluids.

[00130] Further, a variety of cell populations containing T cells and derived from hematopoietic cells can be used in connection with the embodiments of the present technology. These cells may have been activated by cytokine such as IL-2 in vivo or ex vivo, and can be collected in any known way, for example, collected from a living body; obtained via ex vivo culture, for example, a T cell population obtained by a method herein; or obtained by freeze preservation.

[00131] As used herein, the term “antibody” means both intact molecules as well as fragments thereof that include the antigen-binding site. Whole antibody structure is often given as H_2L_2 and refers to the fact that antibodies commonly comprise 2 light (L) amino acid chains and 2 heavy (H) amino acid chains. Both chains have regions capable of interacting with a structurally complementary antigenic target. The regions interacting with the target are referred to as "variable" or "V" regions and are characterized by differences in amino acid sequence from antibodies of different antigenic specificity. The variable regions of either H or L chains contain the amino acid sequences capable of specifically binding to antigenic targets. Within these sequences are smaller sequences dubbed "hypervariable" because of their extreme variability between antibodies of differing specificity. Such hypervariable regions are also referred to as "complementarity determining regions" or "CDR" regions. These CDR regions account for the basic specificity of the antibody for a particular antigenic determinant structure. The CDRs represent non-contiguous stretches of amino acids within the variable regions but, regardless of species, the positional locations of these critical amino acid sequences within the variable heavy and light chain regions have been found to have similar locations within the amino acid sequences of the variable chains. The variable heavy and light chains of all antibodies each have 3 CDR regions, each non-contiguous with the others (termed L1, L2, L3, H1, H2, H3) for the respective light (L) and heavy (H) chains.

[00132] In various embodiments, the antibodies discussed herein can also be wholly synthetic, wherein the polypeptide chains of the antibodies are synthesized and, possibly, optimized for binding to the polypeptides disclosed herein as being receptors. Such antibodies can be, in various

embodiments, chimeric or humanized antibodies, and can be fully tetrameric in structure, or can be dimeric and comprise only a single heavy and a single light chain.

[00133] As used herein, the terms "effective amount" or "therapeutically effective amount" are used interchangeably and mean a dosage sufficient to treat, inhibit, or alleviate one or more symptoms of a disease state being treated or to otherwise provide a desired pharmacologic or physiologic effect, especially enhancing T cell response to a selected antigen. The precise dosage in any given embodiment can vary according to a variety of factors such as subject-dependent variables (*e.g.*, age, immune system health, etc.), the disease, and the treatment being administered.

[00134] As used herein, the terms "individual," "host," "subject" and "patient" are used interchangeably and mean a mammal, including, but not limited to, primates, for example, human beings, as well as rodents, such as mice and rats, and other laboratory animals or any other animals mentioned herein. In various embodiments, the compounds or compositions discussed herein can be general compounds or compositions useful for any purpose, pharmaceutical compounds or compositions, or animal (*e.g.*, veterinary) compounds or compositions.

[00135] As used herein, "treat," "treating" or "treatment" means an alleviation of symptoms associated with a disorder or disease, or inhibition of further progression or worsening of those symptoms, or prevention or prophylaxis of the disease or disorder; and includes: (i) preventing a pathologic condition from occurring (*e.g.*, prophylaxis); (ii) inhibiting the pathologic condition or arresting its development (*e.g.*, slowing or stopping proliferation of cancer cells or tumor growth); (iii) relieving the pathologic condition; or (iv) diminishing symptoms associated with the pathologic condition.

[00136] As used herein, the term "treatment regimen" means a treatment of a disease or a method for achieving a desired physiological change, such as increased or decreased response of the immune system to an antigen or immunogen, such as an increase or decrease in the number or activity of one or more cells, or cell types, that are involved in such response. In various embodiments discussed herein, the treatment or method comprises administering to an animal, such as a mammal, a sufficient amount of one or more (in certain embodiments two or more) chemical agents or

components of said regimen to effectively treat a disease or to produce said physiological change. In certain embodiments, the two or more agents or components are administered together, such as part of the same composition, or administered separately and independently at the same time or at different times (*i.e.*, administration of each agent or component is separated by a finite period of time from one or more of the agents or components). In certain embodiments, administration of said one or more agents or components achieves a result greater than that of any of said agents or components when administered alone or in isolation.

[00137] As used herein, the terms "anergy" or "unresponsiveness" are used interchangeably and include unresponsiveness to an immune cell to stimulation, for example, stimulation by an activation receptor or cytokine. Anergy can occur due to, for example, exposure to an immune suppressor or exposure to an antigen in a high dose. Such anergy is generally antigen-specific, and can continue even after completion of exposure to a tolerized antigen. For example, the anergy in a T cell and/or NK cell can be characterized by failure of production of cytokine, *e.g.*, interleukin (IL)-2. The T cell anergy and/or NK cell anergy can occur in part when a first signal (signal via TCR or CD-3) is received in the absence of a second signal (costimulatory signal) upon exposure of a T cell and/or NK cell to an antigen.

[00138] As used herein, the terms "enhanced function of a T cell," "enhanced cytotoxicity" and "augmented activity" are used interchangeably and mean that the effector function of the T cell or NK cell is improved. In certain embodiments, the enhanced function of the T cell or NK cell can include any of the following: an improvement in the proliferation rate of the T cell or NK cell, an increase in the production amount of cytokine, or an improvement in cytotoxity. Further, in certain embodiments the enhanced function of the T cell or NK cell includes cancellation or suppression of tolerance of the T cell or NK cell in the suppressed state such as the anergy (unresponsive) state, or the rest state, that is, transfer of the T cell or NK cell from the suppressed state into the state where the T cell or NK cell responds to stimulation from the outside.

[00139] As used herein, "expression" means generation of mRNA by transcription from nucleic acids such as genes, polynucleotides, and oligonucleotides, or generation of a protein or a

polypeptide by transcription from mRNA. Expression can be detected by any method including RT-PCR, Northern Blot, or in situ hybridization. As used herein, "suppression of expression" means a decrease of a transcription product or a translation product in a significant amount as compared with the case of no suppression. The suppression of expression herein shows, in various embodiments, a decrease of a transcription product or a translation product in amounts of 30% or more, 50% or more, 70% or more, or 90% or more.

[00140] As used herein, "augmented immune response" means characterized by a particularly strong response or reaction of the immune system to the presence of an antigen. Under normal, non-pathological conditions, immune responses are regulated in a tightly controlled fashion. Moreover, immune responses are self-limiting and decline in time after exposure to the antigen. In case of an "augmented immune response" however, the immune response can be hypersensitive, *i.e.*, the immune response can cause damage to the organism's own cells or tissue in presence of an antigen. Furthermore, in some cases of an "augmented immune response," for example in auto-immune diseases or disorders or in transplant rejects (and the like), the immune system can fail to distinguish between self and non-self substances. As used herein, "disease related to an augmented immune response" accordingly relates to any disease or disorder in which an augmented immune response is etiological for, associated with, secondary to or the resultant of said disorder.

[00141] In certain embodiments, an augmented immune response can be determined by directly or indirectly measuring parameters that are indicative for the magnitude of the immune response or reaction to an antigen, and comparing the outcome of said measurement with the outcome of the same test in a physiologically normal subject. Parameters indicative for the magnitude of the immune response/reaction can include, but are not limited to: the presence or quantity of (specific) antibodies; the presence or quantity of (specific) immune cells; the presence or quantity of (specific) cytokines; or the presence or quantity of (specific) regulatory-, activation- or adhesion molecules.

[00142] For a disease to be related to an augmented immune response, accordingly, said augmented immune response can be detectable preceding, during or following said disease. In certain embodiments, the disease related to an augmented immune response is any of the following:

[00143] -- an acute or chronic transplant rejection, including septic shock, infections caused by bacteria including MRSA and viruses;

[00144] -- a dermatological disease, for example, psoriasis, atopic dermatitis or contact allergy;

[00145] -- T- and B-cell-mediated inflammatory disease, for example, asthma or chronic obstructive pulmonary disease (COPD);

[00146] -- graft-versus-host disease, for example, acute (or fulminant) graft-versus-host disease or chronic graft-versus-host disease; or

[00147] -- auto-immune disease, for example, multiple sclerosis, inflammatory bowel disease, like ulcerative colitis or Behcet's disease; vasculitis, lupus erythematosus, pemphigus vulgaris, pemphigus foliaceus, myasthenia gravis, polymyositis, mixed collective tissue disease (MCTD) rheumatoid arthritis, diabetes mellitus (whether Type 1 or Type 2), celiac disease, celiac sprue disease, atherosclerosis, Goodpasture's syndrome, Grave's disease, autoimmune hepatitis/hepatic autoimmune diseases, autoimmune thrombocytopenic purpura, granulomatosis (*e.g.*, morbus Wegener), Sjogren's Syndrome, scleroderma, alopecia areata or autoimmune hemolytic anemia.

[00148] Immune responses can be exquisitely controlled, requiring multiple finely tuned levels of activation as well as inactivation signals. In T lymphocytes among these signaling networks, T cell receptor (TCR) stimulation activates NF-AT/AP-1, a family of transcription factors that is of particular importance during immune cell activation. NF-AT mediates the transcriptional induction of "cell fate-determining genes," which govern as diverse outcomes as activation, anergy or apoptosis. Mechanistically, the rise of intracellular Ca^{2+} triggered by antigen binding to the TCR can lead to the activation of calcineurin's phosphatase activity. This leads to dephosphorylation of phospho-sites within the N-terminal regulatory domain on NF-AT and, subsequently, nuclear import of NF-AT. Upon transient stimuli, however, feedback inhibition, mediated via GSK3 (glycogen synthase kinase 3), CK1 (casein kinase 1) and DYRK (dual-specificity tyrosine phosphorylation-regulated kinase) protein kinases can counter-regulate NF-AT nuclear occupancy by rephosphorylation, which induces

the nuclear export of NF-AT and the abort of immune activation-associated gene transcription. NF-AT family members are also subject to regulation in the nucleus through their ability to directly interact with other transcriptional regulatory factors. NF-AT requires a protein partner for high-affinity binding at most DNA sites. NF-AT complexes mostly contain cell type- or cell lineage--specific protein binding partners. In cardiac, skeletal, and smooth muscle cells, NF-AT forms complexes with GATA proteins.

[00149] Accordingly, in certain embodiments the present technology is directed to agonists or activators of NR2F6 for the treatment of a disease related to an augmented immune response. In other embodiments, the present technology is directed to the use of an agonist or activator of NR2F6 for the preparation of a medicament for the treatment of a disease related to an augmented immune response. The utilization of NR2F6 modulating compounds for alteration of immune response can be utilized by administering in patients suffering from cancer in which increased efficacy of a cancer vaccine is desired. In these situations, inhibition of NR2F6 is desirable, optionally in addition to immune stimulation. Thus, in various embodiments, the compositions herein can comprise any of the following:

[00150] (a) agonists/activators of NR2F6;

[00151] (b) antagonists/inhibitors of NR2F6;

[00152] (c) agonists/activators of NR2F6 in combination with: (i) one or more additional immune enhancers (ii) CAR-T cell therapy (which can reduce side effects); or (iii) autologous cell therapies, *e.g.*, dendritic cells, anti-PD1 and antiCTLA4 antibodies, PMBC, or umbilical vein cord blood-derived cells.

[00153] (d) antagonists/inhibitors of NR2F6 in combination with: (i) one or more additional immune suppressants; (ii) CAR-T cell therapy (which can reduce side effects); or (iii) autologous cell therapies, *e.g.*, dendritic cells, anti-PD1 and antiCTLA4 antibodies, PMBC, or umbilical vein cord blood-derived cells.

[00154] Accordingly, in certain embodiments, inhibitor compounds of NR2F6 are administered with a cancer antigen, said cancer antigens include ROBO-4. In certain embodiments,

the antigens can be used to replace ROBO-4. These can include any of the following: a) Fos-related antigen 1; b) LCK; c) FAP; d) VEGFR2; e) NA17; f) PDGFR-beta; g) PAP; h) MAD-CT-2; i) Tie-2; j) PSA; k) protamine 2; l)legumain; m) endosialin; n) prostate stem cell antigen; o)carbonic anhydrase IX; p) STn; q) Page4; r) proteinase 3; s) GM3 ganglioside; t) tyrosinase; u) MART1; v) gp100; w) SART3; x) RGS5; y)SSX2; z) Globol1; aa) Tn; ab) CEA; ac) hCG; ad) PRAME; ae) XAGE-1; af) AKAP-4; ag) TRP-2; ah) B7H3; ai) sperm fibrous sheath protein; aj) CYP1B1; ak)HMWMAA; al) sLe(a); am) MAGE A1; an) GD2; ao) PSMA; ap) mesothelin; aq) fucosyl GM1; ar) GD3; as) sperm protein 17; at) NY-ESO-1; au) PAX5; av) AFP; aw) polysialic acid; ax) EpCAM; ay) MAGE-A3; az) mutant p53; ba) ras; bb) mutant ras; bc) NY-BR1; bd) PAX3; be) HER2/neu; bf) OY-TES1; bg) HPV E6 E7; bh) PLAC1; bi) hTERT; bj) BORIS; bk) ML-IAP; bl) idiotype of b cell lymphoma or multiple myeloma; bm) EphA2; bn) EGFRvIII; bo) cyclin B1; bp) RhoC; bq) androgen receptor; br) surviving; bs) MYCN; bt) wildtype p53; bu) LMP2; bv) ETV6-AML; bw) MUC1; bx) BCR-ABL; by) ALK; bz) WT1; ca) ERG (TMPRSS2 ETS fusion gene); cb) sarcoma translocation breakpoint; cc) STEAP; cd) OFA/iLRP; and ce) Chondroitin sulfate proteoglycan 4 (CSPG4).

[00155] In certain embodiments, the assessment of compounds for NR2F6 modulating activity is performed utilizing means known in the art, such as described in U.S, Patent No. 9,091,696. Compounds useful for the screening and modification for enhanced NR2F6 modulatory activity include: CAR Agonists such as 5β-Dihydroprogesterone, 6,7-Dimethylesculetin, Amiodarone, Artemisinin, Benfuracarb, Carbamazepine, Carvedilol, Chlorpromazine, Chrysin, CITCO, Clotrimazole, Cyclophosphamide, Cypermethrin, DHEA, Efavirenz, Ellagic acid, Griseofulvin, Methoxychlor, Mifepristone, Nefazodone, Nevirapine, Nicardipine, Octicizer, Permethrin, Phenobarbital, Phenytoin, Reserpine, TCPOBOP, Telmisartan, Tolnaftate, Troglitazone, Valproic acid. CAR Antagonists such as 3,17β-Estradiol, 3α-Androstanol, 3α-Androstenol, 3β-Androstanol, 17-Androstanol, AITC, Ethinyl estradiol, Meclizine, Nigramide J, Okadaic acid, PK-11195, S-07662, T-0901317. FXR Agonists such as Bile acids, Cafestol, Chenodeoxycholic acid, Fexaramine, GW-4064, Obeticholic acid. FXR Antagonists such as Guggulsterone. LXR Agonists such as 22R-Hydroxycholesterol, 24S-Hydroxycholesterol, 27-Hydroxycholesterol, Cholestenoic acid, DMHCA,

GW-3965, Hypocholamide, T-0901317. PPAR-alpha Agonists such as 15-HETE, 15-HpETE, Aleglitazar, Aluminum clofibrate, Arachidonic acid, Bezafibrate, Clofibrate, CP-775146, DHEA, Elafibranor, Fenofibrate, Gemfibrozil, GW-7647, Leukotriene B4, LG-101506, LG-100754, Lobeglitazone, Muraglitazar, Oleylethanolamide, Palmitoylethanolamide, Pemafibrate, Perfluorononanoic acid, Perfluorooctanoic acid, Pioglitazone, Saroglitazar, Sodelglitazar, Tesaglitazar, Tetradecylthioacetic acid, Troglitazone, WY-14643. PPAR-alpha Antagonists such as GW-6471, MK-886. PPAR-delta Agonists such as 15-HETE, 15-HpETE, Arachidonic acid, Bezafibrate, Elafibranor, GW-0742, GW-501516, L-165,041, LG-101506, MBX-8025, Sodelglitazar, Tetradecylthioacetic acid. PPAR-delta Antagonists such as FH-535, GSK-0660, GSK-3787. PPAR gamma agonists such as 5-Oxo-ETE, 5-Oxo-15-hydroxy-ETE, 15-Deoxy-Δ12,14-prostaglandin J2, 15-HETE, 15-HpETE, Aleglitazar, Arachidonic acid, Berberine, Bezafibrate, Ciglitazone, Darglitazone, Edaglitazone, Etalocib, GW-1929, Ibuprofen, LG-100268, LG-100754, LG-101506, Lobeglitazone, Muraglitazar, nTZDpa, Perfluorononanoic, acid, Pioglitazone, Prostaglandin J2, Rosiglitazone, RS5444, Saroglitazar, Sodelglitazar, Telmisartan, Tesaglitazar, Troglitazone. SSPARMS such as BADGE, EPI-001, INT-131, MK-0533, S26948. PPAR gamma antagonists such as FH-535, GW-9662, SR-202, T-0070907. PPAR nonselective agonists such as Ciprofibrate, Clinofibrate, Clofibride, Englitazone, Etofibrate, Farglitazar, Netoglitazone, Ronifibrate, Rivoglitazone, Simfibrate. PXR Agonists such as 5α-Dihydroprogesterone, 5β-Dihydroprogesterone, 17α-Hydroxypregnenolone ,17α-Hydroxyprogesterone, Δ4-Androstenedione, Δ5-Androstenediol, Δ5-Androstenedione, AA-861, Allopregnanolone, Alpha-Lipoic acid, Ambrisentan, AMI-193, Amlodipine besylate, Antimycotics, Artemisinin, Aurothioglucose, Bile acids, Bithionol, Bosentan, Bumecaine, Cafestol, Cephaloridine, Cephradine, Chlorpromazine, Ciglitazone, Clindamycin, Clofenvinfos, Chloroxine, Clotrimazole, Colforsin, Corticosterone, Cyclophosphamide, Cyproterone acetate, Demecolcine, Dexamethasone, DHEA, DHEA-S, Dibunate sodium, Diclazuril, Dicloxacillin, Dimercaprol, Dinaline, Docetaxel, Docusate calcium, Dodecylbenzenesulfonic acid, Dronabinol, Droxidopa, Eburnamonine, Ecopipam, Enzacamene, Epothilone B, Erythromycin, Famprofazone, Febantel, Felodipine, Fenbendazole, Fentanyl, Flucloxacillin, Fluorometholone, Griseofulvin,

Haloprogin, Hetacillin potassium, Hyperforin (Hypericum perforatum), Indinavir sulfate, Lasalocid sodium, Levothyroxine, Linolenic acid, LOE-908, Loratadine, Lovastatin, Meclizine, Methacycline, Methylprednisolone, Metyrapone, Mevastatin, Mifepristone, Nafcillin, Nicardipine, Nicotine, Nifedipine, Nilvadipine, Nisoldipine, Norelgestromin, Omeprazole, Orlistat, Oxatomide, Paclitaxel, Phenobarbital, Plicamycin, Prednisolone, Pregnanolone, Pregnenolone, Pregnenolone 16α-carbonitrile, Proadifen, Progesterone, Reserpine, Reverse triiodothyronine Rifampicin, Rifaximin, Rimexolone, Riodipine, Ritonavir, Simvastatin, Sirolimus, Spironolactone, Spiroxatrine, SR-12813, Suberoylanilide, Sulfisoxazole, Suramin, Tacrolimus, Tenylidone, Terconazole, Testosterone isocaproate, Tetracycline, Thiamylal sodium, Thiothixene, Thonzonium bromide, Tianeptine, Troglitazone, Troleandomycin, Tropanyl 3,5-dimethulbenzoate, Zafirlukast, Zearalanol. PXR Antagonist such as Ketoconazole. RAR Agonists such as 9CDHRA, 9-cis-Retinoic acid (alitretinoin), AC-261066, AC-55649, Acitretin, Adapalene, all-trans-Retinoic acid (tretinoin), AM-580, BMS-493, BMS-753, BMS-961, CD-1530, CD-2314, CD-437, Ch-55, EC 23, Etretinate, Fenretinide, Isotretinoin, Palovarotene, Retinoic acid, Retinol (vitamin A), Tamibarotene, Tazarotene, Tazarotenic acid, TTNPB. RAR Antagonists such as BMS-195614, BMS-493, CD-2665, ER-50891, LE-135, MM-11253. RXR Agonists such as 9CDHRA, 9-cis-Retinoic acid (alitretinoin), all-trans-Retinoic acid (tretinoin), Bexarotene, CD 3254, Docosahexaenoic acid, Fluorobexarotene, Isotretinoin, LG-100268, LG-101506, LG-100754, Retinoic acid, Retinol (vitamin A), SR-11237. RXR Antagonists such as HX-531, HX-630, LG-100754, PA-452, UVI-3003. TR Agonists such as Dextrothyroxine, GC-1, Levothyroxine, Liothyronine, Thyroxine, Tiratricol, Triiodothyronine.

[00156] Other compounds useful for modulation of NR2F6 activity include: 5-tert-butyl-N-[(6-fluoro-4H-1,3-benzodioxin-8-yl)methyl]-2-methylpyrazole-3-carboxamide, ST50775950, ethyl 4-(cyclohexylamino)-2-(3,5-dimethylpyrazol-1-yl)pyrimidine-5-carboxylate, ethyl 4-(cyclopentylamino)-2-(3,5-dimethylpyrazol-1-yl)pyrimidine-5-carboxylate, AGN-PC-09SAX3, SMR000064686, AGN-PC-0NLTEQ, T6090485, MLS002548992, 5,6-dimethyl-4-[4-[2-(4-methylphenoxy)ethyl]piperazin-1-yl]thieno[2,3-d]pyrimidine, MLS002473459, MLS001030349, 4-(3,4-dihydro-1H-isoquinolin-2-yl)-5H-pyrimido[5,4-b]indole, 4-(3,4-Dihydro-1H-isoquinolin-2-yl)-8-

fluoro-5H-pyrimido[5,4-b]indole, 4-[4-(4-methoxyphenyl)piperazino]-5H-pyrimido[5,4-b]indole, 4-[4-(1,3-benzodioxol-5-ylmethyl)piperazin-1-yl]-7-methoxy-5H-pyrimido[5,4-b]indole, SMR000044829,8-fluoro-N-(3-propan-2-yloxypropyl)-5H-pyrimido[5,4-b]indol-4-amine, GNF-Pf-1678, MLS003116118, 2-[4-(5-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-yl)piperazin-1-yl]-1,3-benzothiazole, 5-methyl-3,6-diphenylpyrazolo[1,5-a]pyrimidin-7-amine, 4-[4-[(4-chlorophenyl)methyl]piperazin-1-yl]-1-[(4-methylphenyl)methyl]pyrazolo[3,4-d]pyrimidine, MLS002632722, MLS002477203,MLS003120814, AGN-PC-07AHX3, MLS003120821, MLS003120807, MLS003120811, MLS003120820, ethyl 4-[[1-(2,4-dimethylphenyl)pyrazolo[3,4-d]pyrimidin-4-yl]amino]piperidine-1-carboxylate, N-[2-(3,4-dimethoxyphenyl)ethyl]thieno[2,3-d]pyrimidin-4-amine, N-[2-(3,4-dimethoxyphenyl)ethyl]-6-methylthieno[2,3-d]pyrimidin-4-amine hydrochloride, N-(1-phenylethyl)quinazolin-4-amine, AG-F-87638, ZINC03428816, CHEMBL493153, ST50323391, N-Benzylquinazolin-4-amine, ST50483228, N-[4-(2-methyl-1-methylsulfonyl-2,3-dihydroindol-5-yl)-1,3-thiazol-2-yl]-2-thiophen-2-ylacetamide, F0558-0175, AC1MLRO7, 4-(2-methylimidazo[1,2-a]pyridin-3-yl)-N-(3-methylphenyl)-1,3-thiazol-2-amine, AGN-PC-09PPXW, Compound 15Jf, AC1MEEXM, ST50941838, [2-[(3-carbamoylthiophen-2-yl)amino]-2-oxoethyl] 2-naphthalen-1-ylacetate, F0239-0029, AC1OBZ0O, ST4126227, 1-[(4-bromophenyl)methyl]-2-methylbenzimidazole, SMR000718391, MLS002694437, Chlormidazole, 2-methyl-1-(2-methylbenzyl)-1H-benzimidazole, MLS003119103, Ambcb90456311, AGN-PC-04RX4B, MLS001122505, Ambcb81049924, AGN-PC-04RX7E, Ambcb42757923, MLS001124721, 7-benzyl-4-chloro-5,6-dimethyl-7H-pyrrolo[2,3-d]pyrimidine, AGN-PC-04V4GP, MLS000562030, AGN-PC-00YPMB, T5400648, MLS003107990, AC1NUNJE, MLS002701851, SMR000185185, STK850401,[(3-bromobenzyl)sulfanyl][(4-fluorophenyl)amino]methylidene, propanedinitrile, AC1NXBLH, CAS-66-81-9, Cycloheximide, ACTIPHENOL, MLS001032885, MLS000553012, SMR000285129, MLS000688479, MLS002702480, GNF-Pf-4659, MLS002702449, T0501-4035, MLS000712179, AGN-PC-00MQWB, AGN-PC-0NKU3S, T0503-0850, T0501-5798, SMR000212173, 3,3'-Diethylthiazolinocarbocyanine iodide, 2-methyl-3,5-bis(4-methylphenyl)isoxazol-2-ium, MLS000705900, SMR000211540, AGN-PC-00PL3I, AGN-PC-

0NJNZK, SMR000354849, T0503-1204, MLS000688685, GNF-Pf-4078, T0503-3525, T0503-4982, T0501-7391, GNF-Pf-3268, TCMDC-125620, 1-[1,1'-Biphenyl]-4-yl-2-(4-imino-1(4H)-pyridinyl)ethanone, SMR000036350, MLS000080109, MLS000080126, Ambcb40308772, MLS000733369, Ambcb20390854, MLS000732313, AGN-PC-04RYS6, Ambcb33735952, AGN-PC-04RYKA, MLS000733096, Ambcb63657849, MLS001090213, T6132867, MLS003678910, AC1OXF5M, SMR000218920, MLS000037490, Boc-KS, MLS000734694, AGN-PC-087SDW, ISUPSL100073, 4-{[5,7-bis(trifluoromethyl) benzenol, BAS 07204618, MLS001144057, MLS001250118, SMR000041809, SMR000635220, MLS003120011, T5546966, 4-chloro-N-(4-chlorobenzyl)-1,3-dimethyl-1H-pyrazole-5-carboxamide, 3-(Toluene-4-sulfonylmethyl)-2,3-dihydro-benzo[4,5]imidazo[2,1-b]thiazole, T0508-0735, Carboxyamidotriazole, MLS003116132, F0850-5968, Verrucarin A 9,10-epoxide, MLS002702133, Ossamycin, MLS002702060, Dihydrorotenone, SMR000623161, Pyridaben, ASN 09858385, T6069554, T6302989, SMR000629820, SMR000629835, MLS001028777, MLS001028747, MLS001028806, SMR000625125, T5403634, T5459762, T5626573, T5337170, SMR000093473, T6120097, N-[2-[2-[2,5-dimethyl-1-(thiophen-2-ylmethyl)pyrrol-3-yl]-2-oxoethoxy]phenyl]acetamide, MLS000575323, N-[4-[2-[2,5-dimethyl-1-(thiophen-2-ylmethyl)pyrrol-3-yl]-2-oxoethyl]sulfanylphenyl]acetamide, SMR000274842, T5565081, 6-chloro-N-[3-[(4-methoxyphenyl)sulfamoyl]phenyl]pyridine-3-carboxamide, N-methyl-N-[(1,3,5-trimethylpyrazol-4-yl)methyl]naphthalene-2-sulfonamide, T6099016, T6094971, ASN 04448329, SMR000241542, AGN-PC-03RL0E, AGN-PC-080KFN, T6151837, AGN-PC-0KIUAY, N-[4-(4-methylphenyl)-1,3-thiazol-2-yl]-1-thiophen-2-ylsulfonylpiperidine-4-carboxamide, 5-(3,5-dimethylpiperidin-1-yl)sulfonyl-N,N-diethyl-3-methyl-1-benzofuran-2-carboxamide, SMR000124769, N-(1-benzylpiperidin-4-yl)-1-(5-chloro-2-methylphenyl)sulfonylpiperidine-4-carboxamide, MLS001095722, 4-ethoxy-N-(pyridin-4-ylmethyl)benzenesulfonamide, 4-chloro-3-ethoxy-N-(pyridin-4-ylmethyl)benzenesulfonamide, 2,4,6-trimethyl-N-(pyridin-4-ylmethyl)benzenesulfonamide, BAS 05598377, 4-bromo-2,5-dimethyl-N-(pyridin-4-ylmethyl)benzenesulfonamide, MLS000735463, MLS000687652, AGN-PC-093SBW, AG-401/42008258, 5L-526S, 2-[[5-(3-chloro-1-benzothiophen-2-yl)-1,3,4-oxadiazol-2-

yl]sulfanyl]acetonitrile, 2-(5-Pyridin-3-yl-[1,3,4]thiadiazol-2-ylsulfanyl)-N-quinolin-4-yl-acetamide, 2-[[5-(benzotriazol-1-ylmethyl)-1,3,4-oxadiazol-2-yl]sulfanyl]-N-[(4-chlorophenyl)methyl]-N-phenylacetamide, 2-[[5-(benzotriazol-1-ylmethyl)-1,3,4-oxadiazol-2-yl]sulfanyl]-N-[(4-fluorophenyl)methyl]-N-phenylacetamide, SR-01000288264, 2-(1-cyclopropyltetrazol-5-yl)sulfanyl-1-[4-[(4-propan-2-ylphenyl)methyl]piperazin-1-yl]ethanone, N-(2,4-difluorophenyl)-4-[5-(trifluoromethyl)pyridin-2-yl]-1,4-diazepane-1-carbothioamide, T0512-9975, [[2,7-bis(2-morpholin-4-ylethoxy)fluoren-9-ylidene]amino]thiourea, MLS001018548, T0507-0244, 4-(4-acetylphenyl)-N-(4-phenoxyphenyl)piperazine-1-carbothioamide, N-(3-ethoxypropyl)-4-[4-(4-fluorophenyl)-1,3-thiazol-2-yl]piperazine-1-carbothioamide, (+)-Emetine dihydrochloride hydrate, MLS002302684, 4-(6-chloro-1,3-benzothiazol-2-yl)-N-(2-chloro-6-methylphenyl)-1,4-diazepane-1-carboxamide, N-(3-chloro-2-methylphenyl)-4-(3-phenyl-1,2,4-thiadiazol-5-yl)-1,4-diazepane-1-carboxamide, MLS000692856, bjm-csc-19, MLS002701991, and MLS000586514. Additional compounds include 6-formylindolo (3,2-B) carbazole, 4-hydroxyphenylretinamide, 3,5-Dilodo-L-tyrosine, Rifampicin, and Z30972355.

[00157] Another aspect of the present disclosure is a pharmaceutical composition comprising a NR2F6 modulator, such as a NR2F6 inhibitor or NR2F6 activator, for use in the methods described herein. Accordingly, in certain embodiments, the present technology provides a pharmaceutical composition comprising an effective amount of a NR2F6 inhibitor or NR2F6 activator in admixture with a pharmaceutically acceptable carrier, excipient or diluent. Such pharmaceutical composition can be, in various embodiments, a composition suitable for administering to a human, or to an animal (i.e., a veterinary pharmaceutical composition).

[00158] In various embodiments, the pharmaceutical compositions herein can be used to inhibit NR2F6; or to activate NR2F6.

[00159] In certain embodiments, the pharmaceutical composition is used to treat a disease or a hematopoietic condition as described herein. The NR2F6 inhibitors or NR2F6 activators can be formulated into pharmaceutical compositions for administration to subjects in a biologically compatible form suitable for administration in vivo.

[00160] As used herein, "biologically compatible form suitable for administration in vivo" means a form of the substance to be administered in which any toxic effects are outweighed by the therapeutic effects. In various embodiments, the substances herein can be administered to living organisms including humans, and animals. Administration of a therapeutically active amount of the pharmaceutical compositions of the present disclosure is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result. For example, a therapeutically active amount of a substance can vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of inhibitor to elicit a desired response in the individual. Dosage regime can be adjusted to provide the optimum therapeutic response. For example, several divided doses can be administered daily or the dose can be proportionally reduced as indicated by the exigencies of the therapeutic situation.

[00161] In various embodiments, the active substance can be administered by, *e.g.*, injection (subcutaneous, intravenous, intramuscular, etc.), oral administration, inhalation, intranasal, transdermal or topical administration (such as topical cream or ointment, salve, paste or the like), pulmonary, buccal, subdermal, intradermal, transdermal or parenteral, rectal, subcutaneous, intravenous, intraurethral, intramuscular, ophthalmic or suppository administration. Depending on the route of administration, the active substance can, in certain embodiments, be coated in a material to protect the compound from the action of enzymes, acids and other natural conditions which could inactivate the compound.

[00162] In certain embodiments, the active substance can be formulated into delayed release formulations such that NR2F6 can be inhibited or activated for longer periods of time than a conventional formulation.

[00163] In certain embodiments, a method herein includes the following steps: (a) extraction of an amount of a patient's cellular material, including, but not limited to: blood, saliva, sweat, or any portion of a tumor known or believed to be in a diseased state; (b) isolating immune cells from the cellular material; (c) inhibiting or activating the NR2F6 target in the extracted immune cells; and (d)

re-administering the immune cells (for example, by injection) to the patient's body. This can have the effect of "reprogramming" the immune cells to attack tumors or other invasive cells.

[00164] In certain embodiments, other types of a patient's cellular material can also be extracted. These include, for example, any part of the blood (blood serum, red blood cells, white blood cells, plasma, platelets), any other material from the body that includes the patient's cells (for example, skin, hair, nails, saliva, cerebrospinal fluid, intracellular fluid, extracellular fluid, intravascular fluid, interstitial fluid, lymphatic fluid, transcellular fluid, exudates, lymph, sweat, sebum or serous fluid). In certain embodiments, the re-administering of the immune cells to the patient's body can be done by injection, introduction through the nose or mouth (for example, inhalation), skin or mucous membranes.

[00165] In certain embodiments, the present technology is directed to compounds alone or in combination with another medicament. As set forth herein, compounds herein include stereoisomers (including, *e.g.*, enantiomers, diastereomers, cis-trans and E-Z isomers, conformers and atropisomers), tautomers, solvates, prodrugs, metabolites, pharmaceutically acceptable salts and mixtures thereof. Compositions containing a compound herein can be prepared by conventional techniques, and can appear in conventional forms, for example, oral dosage forms; or any ingestible, inhalable (*e.g.*, through the mouth, nose or mucosa); or topical applications, *e.g.*, applicable to the skin, nails, eyes or the like. These can include, in various embodiments, capsules, tablets, pills, cachets, dispersible granules, lozenges, aerosols, solutions, powders, suspensions, emulsions, gels, mousses, foams, drops, lotions, creams, paste, dragees, suppositories and any application deliverable to the body of a user.

[00166] In various embodiments, dosages and compounds herein can be prepared and administered in a wide variety of oral, parenteral, and topical dosage forms, including, but not limited to, by injection (*e.g.*, intravenously, intramuscularly, intracutaneously, subcutaneously, intraduodenally, or intraperitoneally); by inhalation (*e.g.*. intranasally); or transdermally. In certain embodiments, multiple routes of administration can be used to optimize delivery of the compounds herein.

[00167] In various embodiments, the compositions described herein can be prepared by known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. On this basis, the compositions can include, albeit not exclusively, solutions of the substances in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

[00168] In certain embodiments, a powder or tablet according to a dosage form herein can contain about 5 to about 75%, about 10 to about 70%, or about 15 to about 65% of the active compound. Suitable carriers include, but are not limited to: magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like.

[00169] In various embodiments, carriers for certain dosages can include aqueous solutions of dextrose, saline, water, organic solvents including ethanol, glycerol, propylene glycol, oils including peanut oil or sesame oil; or polyoxyethylene-block polymers. Aqueous solutions or suspensions can be made by dispersing the finely divided active component in water or another solvent with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other suspending agents.

[00170] In various embodiments, the compounds or dosages herein can also be incorporated into liposomes or micelles, or administered via transdermal pumps or patches.

[00171] Some compounds may have limited solubility in water and therefore may require a surfactant or other appropriate co-solvent in the composition. Such co-solvents include: Polysorbate 20, 60, and 80; Pluronic F-68, F-84, and P-103; cyclodextrin; and polyoxyl 35 castor oil; and in various embodiments, are present in amounts of about 0.01 to about 10%, about 0.05 to about 5% or about 0.1 to about 3% by weight.

[00172] In certain embodiments, it may be desirable to increase the viscosity of the dosage forms herein for ease in dispensing or delivery. Such viscosity building agents include, for example,

polyvinyl alcohol, polyvinyl pyrrolidone, methyl cellulose, hydroxy propyl methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxy propyl cellulose, chondroitin sulfate and salts thereof, hyaluronic acid and salts thereof, and combinations of the foregoing; and in various embodiments, are present in amounts of about 0.01 to about 10%, about 0.05 to about 5% or about 0.1 to about 3% by weight.

[00173] The compositions herein can, in certain embodiments, additionally include components to provide sustained release or comfort. Such components include, but are not limited to, high molecular weight, anionic mucomimetic polymers, gelling polysaccharides, finely-divided drug carrier substrates, emollients, humectants, moisturizers, essential oils, oils, lipids, fatty acids, glycerides, extracts of natural ingredients, soaps and waxes.

Useful Compounds

[00174] The present disclosure includes various compounds that were found to be modulators of NR2F6 activity and NR2F6 utilizing compounds, and the immune modulation and modulation of cancer stem cell activity. Exemplary compounds and methods are shown in the attached FIGS. 1-58. These compounds were initially found to be modulators of NR2F6 activity and NR2F6 utilizing compounds, and the immune modulation and modulation of cancer stem cell activity. In various embodiments, the compounds comprise one or more of the following functional groups: a sulfonyl group, a sulfone group R–S(=O)2–R′ where R and R′ are any organic functional groups, a pyrazine group, any phenyl substituted with one or more halogens including chlorine or fluorine; or any composition comprising two or more phenyl constituents. In certain embodiments, a compound herein can be in amorphous form, crystalline form, or a mixture thereof; as well as any polymorph or amorphous form, a solvate, a hydrate or an unsolvated form.

EXAMPLE 1

[00175] The methodology for screening candidate compounds as NR2F6 agonists was as follows: For primary screening, hit criteria was ACT% > DMSO control + 5*SD (DSO control) at 10 μM, or any compound with S/B > 2. For rescreening, hit criteria was ACT% > DMSO control +

3*SD (DSO control) in each replicate at 10 μM. For counterscreening, hit criteria was mean ACT < DMSO control + 3*SD (DMSO control) with ERα transient transfection in duplicate at 10 μM.

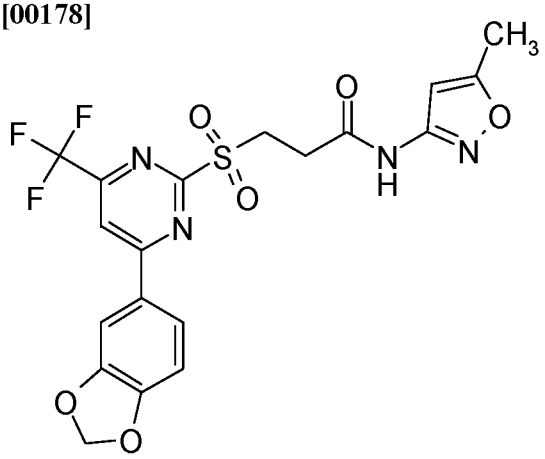
[00176] Table 1 shows screening results from a first set of compounds.

Compound I.D.	Firefly, cmpd/DMSO		Firefly_ERa, cmpd/DMSO		Renilla, cmpd/DMSO		Renilla_ERa, cmpd/DMSO	
	repeat 1	repeat 2	repeat 1	repeat 2	repeat 1	repeat 2	repeat 1	repeat 2
17	2.2	2.4	2.5	2.2	1.3	1.4	1.3	1.0
18	3.8	3.8	2.5	1.9	3.3	4.2	0.8	0.9
19	2.2	1.7	2.3	2.4	1.6	1.7	1.2	1.0
20	2.2	2.1	1.5	1.7	4.4	4.8	1.1	0.9
21	2.4	2.2	1.1	1.0	3.8	3.0	1.1	0.9
22	2.1	2.9	1.5	2.2	1.1	1.3	0.8	1.1
23	3.0	1.9	1.8	1.8	4.6	3.4	1.1	1.2
24	2.0	2.3	1.3	1.6	2.4	2.2	0.9	1.0
25	2.4	1.9	1.3	0.7	3.1	2.1	0.9	1.0
C1	3.4	4.1	1.0	1.0	4.1	1.5	1.3	1.2
C2	2.7	2.2	1.0	0.8	6.0	5.3	1.7	1.6
C3	2.0	2.0	1.8	1.9	1.6	1.2	1.1	1.2
C4	2.5	2.5	1.6	1.7	1.6	1.5	1.1	1.2
C5	2.2	1.7	1.6	2.0	1.3	1.4	1.2	1.0
C6	2.6	1.9	2.2	1.4	1.2	0.9	1.0	1.0
C7	2.1	2.1	0.8	0.5	1.1	1.4	0.8	0.8
C8	2.1	2.8	1.2	1.2	3.4	3.1	1.0	1.1
C9	2.7	1.9	2.5	2.4	2.4	2.5	0.9	1.0
C10	2.1	2.6	1.0	1.3	3.1	1.6	0.7	0.8
C11	13.5	12.6	1.7	1.7	3.8	3.3	1.3	1.2
C16	2.7	2.3	1.0	0.9	3.9	3.2	0.9	1.1

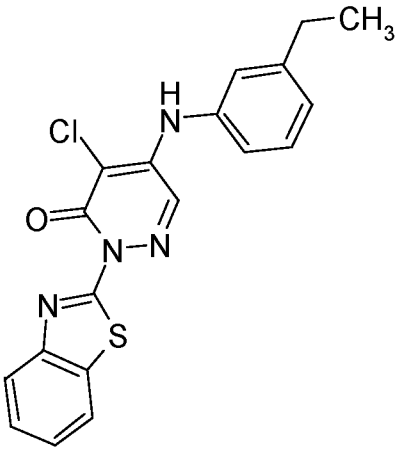
Table 1

[00177] C1, C7 and C11were found to have particularly good activity:

[00178]

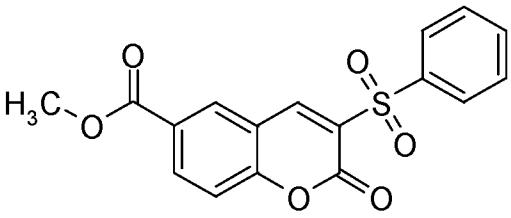


Compound C1

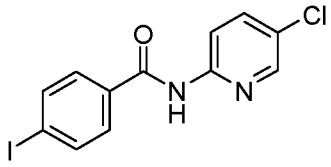


Compound C7

[00179]



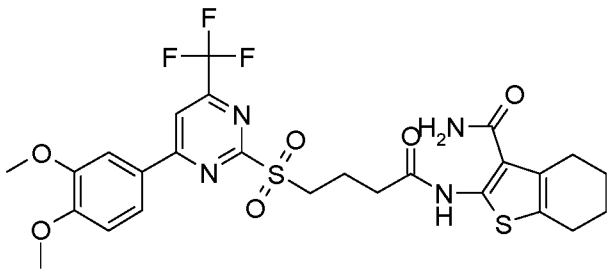
Compound C11



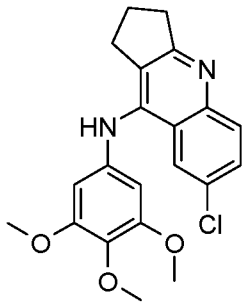
Compound 18

Additional compounds included the following:

[00180]

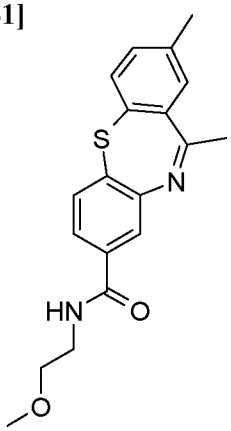


Compound C2

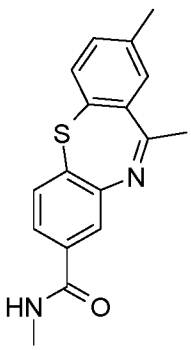


Compound C10

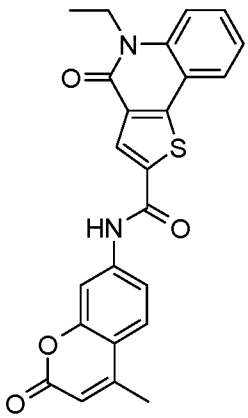
[00181]



Compound C8

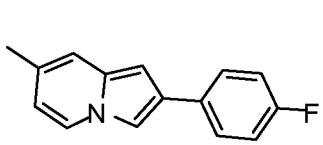


Compound C9

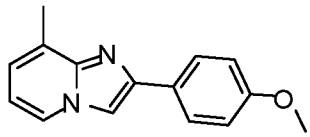


Compound C16

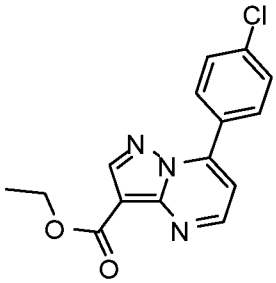
[00182] Yet additional compounds tested included Compounds 17, 19, 22 and C3-C6:



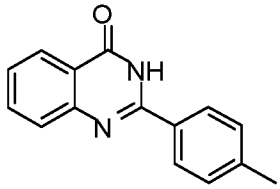
Compound 17



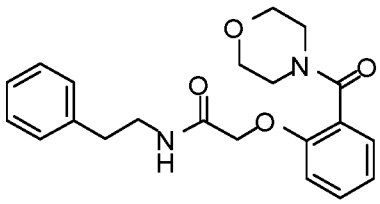
Compound 19



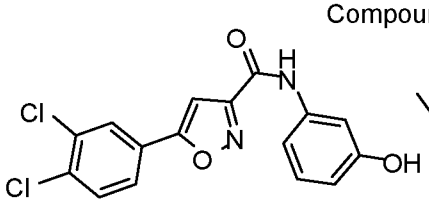
Compound C4



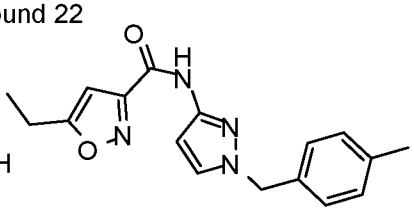
Compound C3



Compound 22



Compound C5



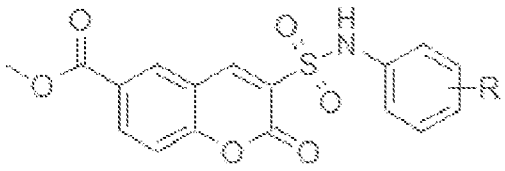
Compound C6

[00183] Compound C11 was found to be particularly promising. **FIGS. 16A** and **16B** show results of cytokins release by hPBMC and cytotox for Compound C11. For cytokines release and cytotox on hPBMCs, the Compound was tested at 1.25, 2.5, 5, 10, 25 and 50 uM in duplicates. For cytotox on HEK293, HEK293 pGL4 and HEK293 NR2F6 (full length) cmpd was tested from 50 uM with dilution step 3.16 in duplicates.

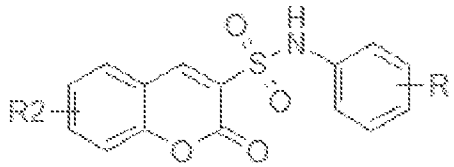
[00184] The human PBMC were activated by 10 ng/mL PMA + 500 ng/mL ionomycin. Data were normalized to controls with (100%) w/o compounds.

[00185] Compounds related to Compound C11 were further explored, and in particular, Compound C11 was substituted with various moieties to test how this affected its activity.

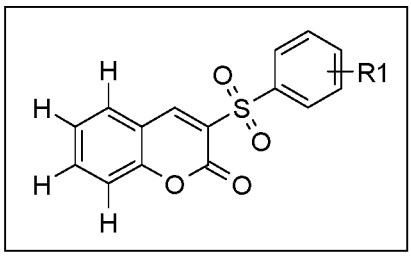
[00186] In certain embodiments, the present technology is directed to compounds of Formula (Ia), (Ib) or (Ic):



(Ia)



(Ib)



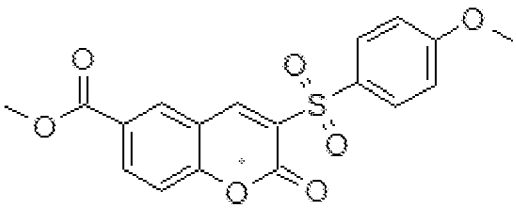
(Ic)

wherein any of R, R1 and R2 are C, H, N, O, S, a halogen, an alkyl group, a substituted alkyl group, a cyclic alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, an ester, an aldehyde, a ketone, a carboxylic acid, an amide, an amine, an ether, a thiol or a nitrile.

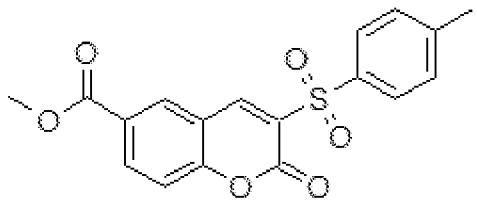
[00187] In certain embodiments of any of the Formulas (I through XVII) herein, any of R, RA, RB, R1-R8, X, Q, Q1, Q2, or A can be any of the following: Me, OMe, Br, N, H, Cl, F or NO_2. In certain embodiments, any of R, RA, RB, R1-R8, X, Q, Q1, Q2, or A can be any of the following: 4-Me, 4-OMe, 4-Br, 4-t-Bu, 3,4-di-Me, 4-Cl, 3,4-di-Cl, 3-Cl-4-F, 2-F, 3-Cl, 3-CH_3-4-F, 4-iPr, Ph, 4-MeO-C6H4, 4-tBu, 2, 4-diMe, 2-thienyl, 2-MeO-4-Cl, 4-Cl, 2-furayl, 4-F-C6H4, 2,4-diMeC6H3, 3-Me-4-F or 4-Cl-C6H4.

[00188] Compounds of Formulas I(a) and I(b) were rescreened in multiple assays to acquire statistical confidence in the results. Results were repeated in follow set screens from fresh powder. Analogs obtained are shown in **FIGS. 17B**.

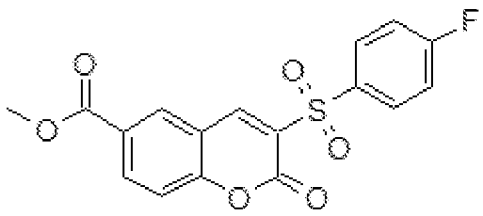
[00189] Four particularly useful compounds that were all based on C11 (Compounds C12 through C15) were identified as follows:



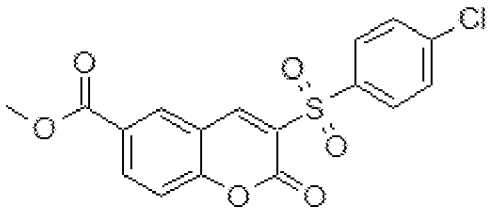
C12



C13



C14



C15

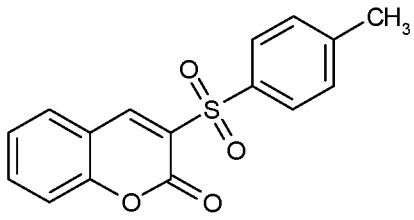
[00190] Activity of the above compounds (C12 through C15) is shown in Table 2:

IDNUMBER	Firefly, cmpd/DMSO		Renilla, cmpd/DMSO		Firefly_pGL, cmpd/DMSO	
	repeat 1	repeat 2	repeat 1	repeat 2	repeat 1	repeat 2
C12	20.0	16.7	4.5	9.4	2.6	2.9
C11	24.6	21.3	13.9	12.3	3.3	3.3
C13	14.0	12.9	6.4	8.2	3.2	3.2
C14	22.3	20.0	12.1	15.2	2.9	2.6
C15	0.9	1.7	5.8	10.7	1.2	1.7

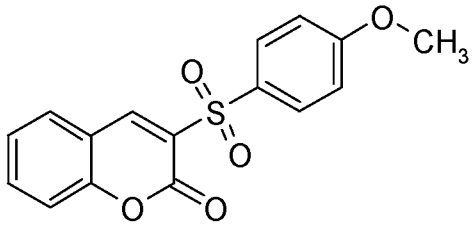
Table 2

[00191] Further compounds related to the Compound C11 and the compound of Formulas (Ia) (Ib) or (Ic) were tested. These include the following:

[00192]

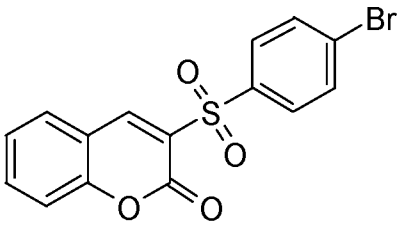


Compound C100

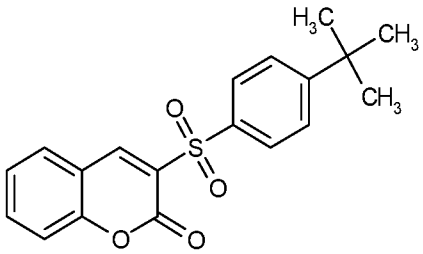


Compound C101

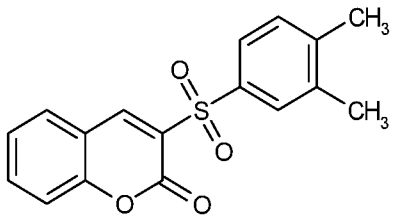
[00193]



Compound C102

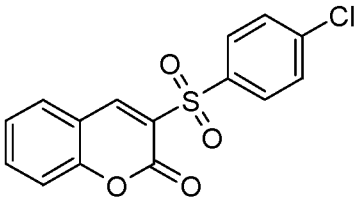


Compound C103

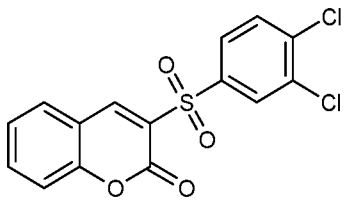


Compound C104

[00194]

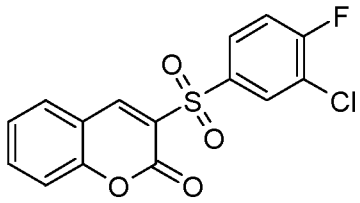


Compound C105



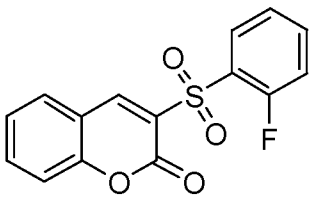
Compound C106

[00195]

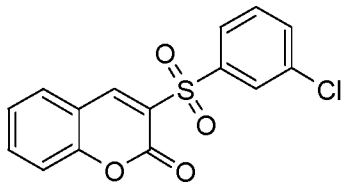


Compound C107

[00196]

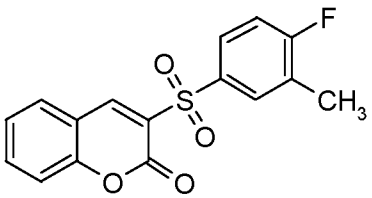


Compound C108

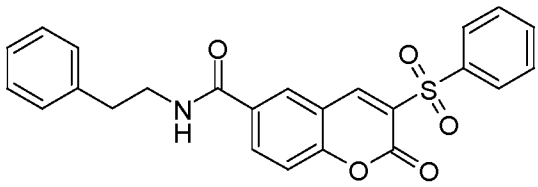


Compound C109

[00197]

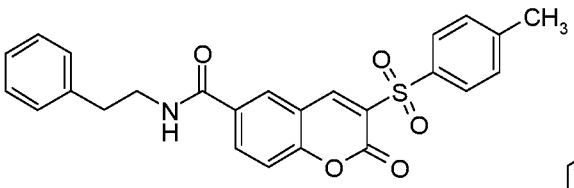


Compound C110

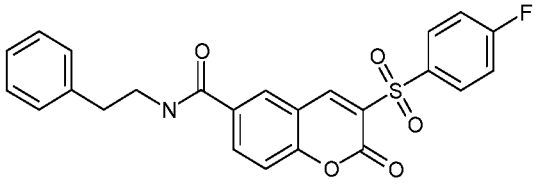


Compound C111

[00198]

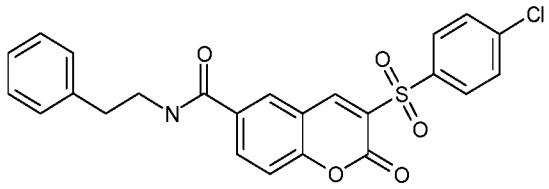


Compound C112

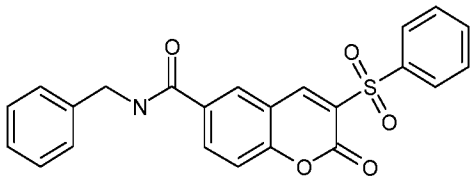


Compound C113

[00199]

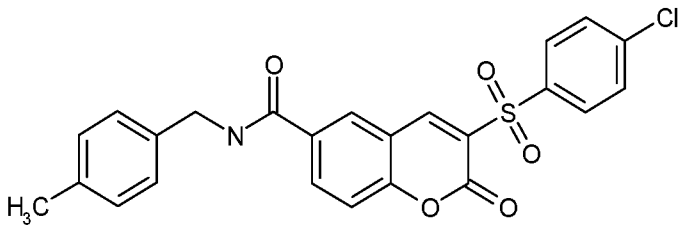


Compound C114



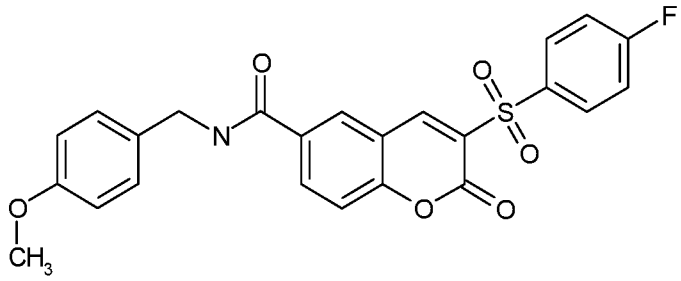
Compound C115

[00200]



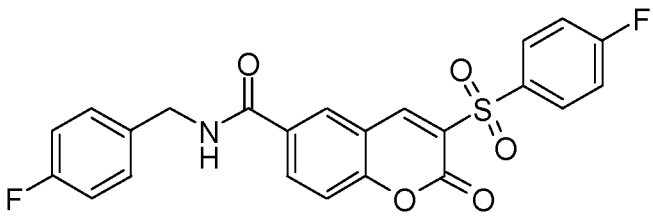
Compound C116

[00201]



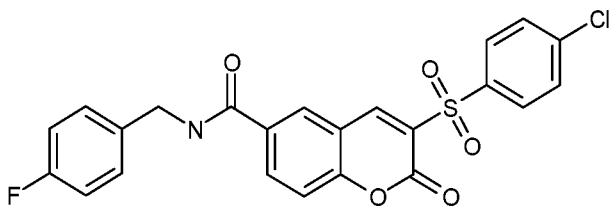
Compound C117

[00202]

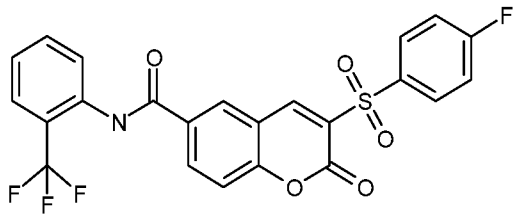


Compound C118

[00203]

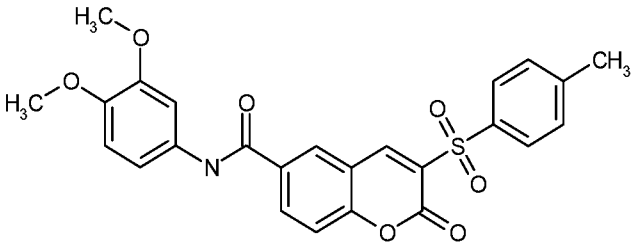


Compound C119

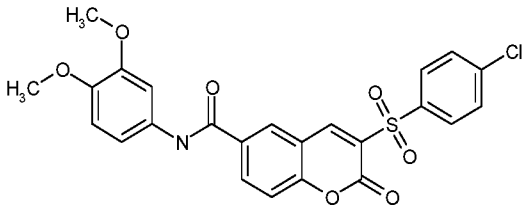


Compound C120

[00204]

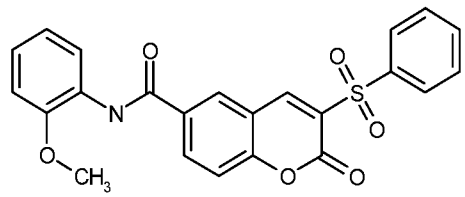


Compound C121

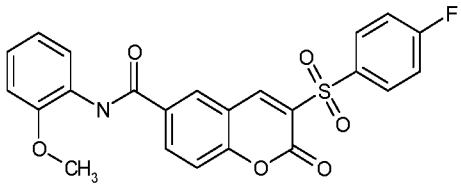


Compound C122

[00205]

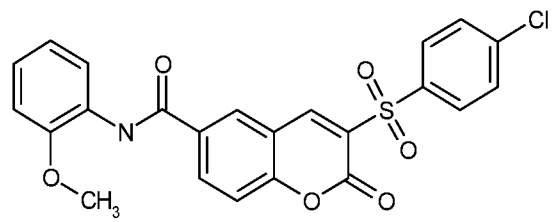


Compound C123

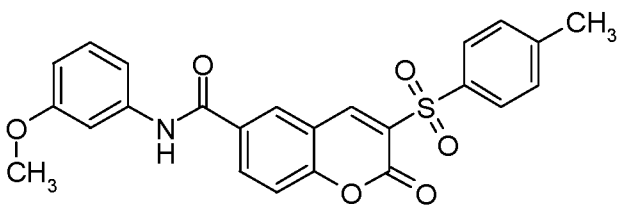


Compound C124

[00206]

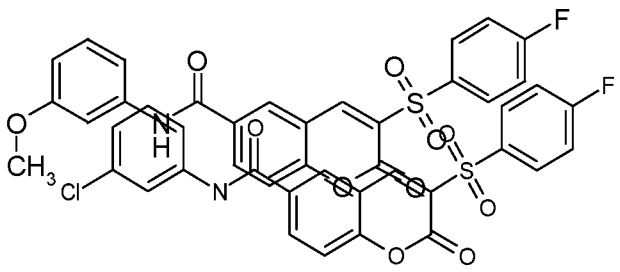


Compound C125



Compound C126

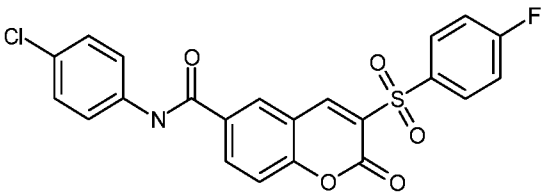
[00207]



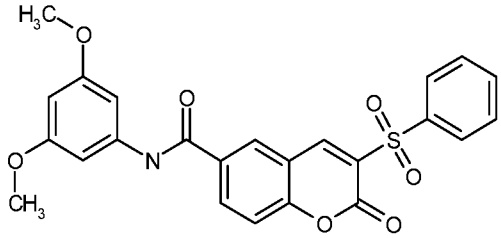
Compound C127

Compound C128

[00208]

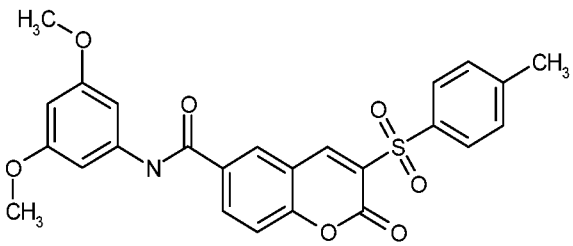


Compound C129

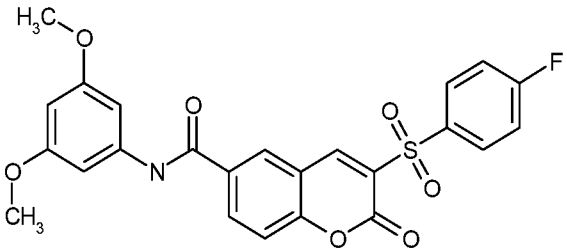


Compound C130

[00209]

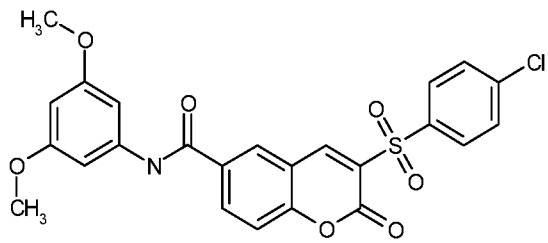


Compound C131

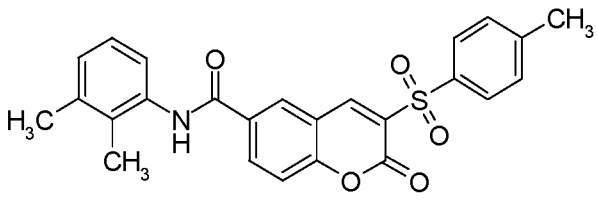


Compound C132

[00210]

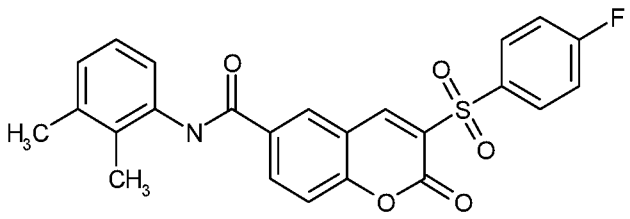


Compound C133



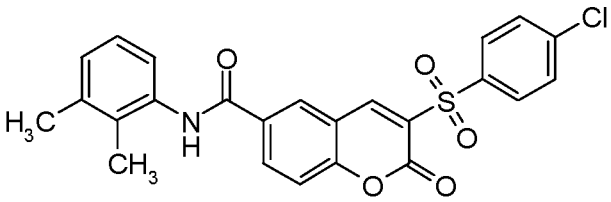
Compound C134

[00211]

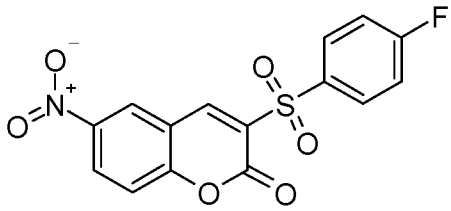


Compound C135

[00212]

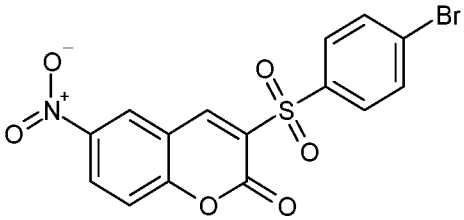


Compound C136

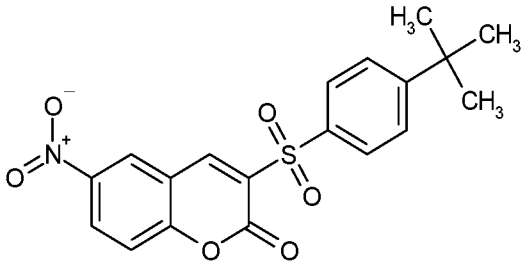


Compound C137

[00213]

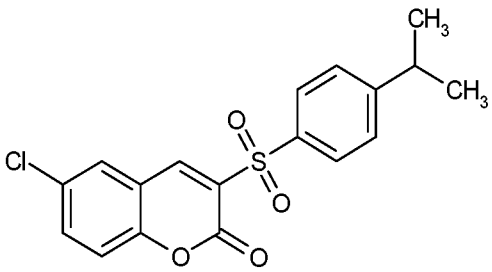


Compound C138

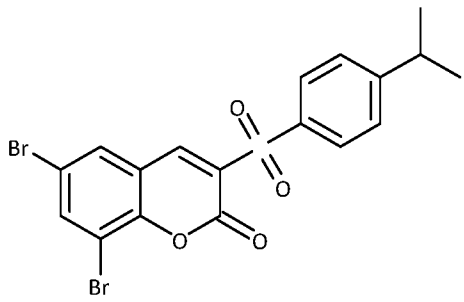


Compound C139

[00214]

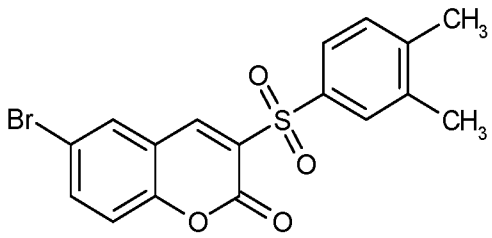


Compound C140

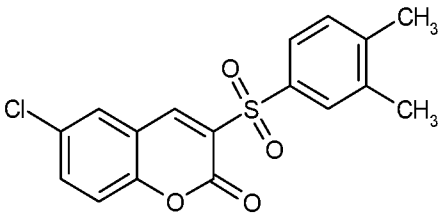


Compound C141

[00215]

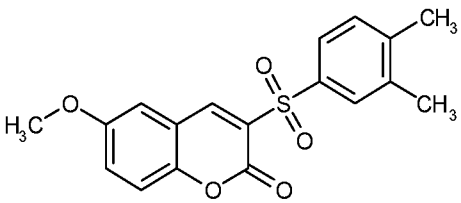


Compound C142

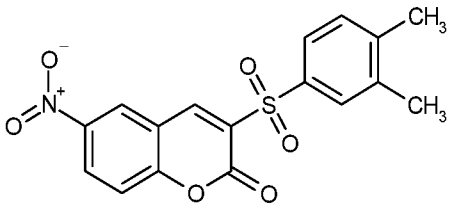


Compound C143

[00216]

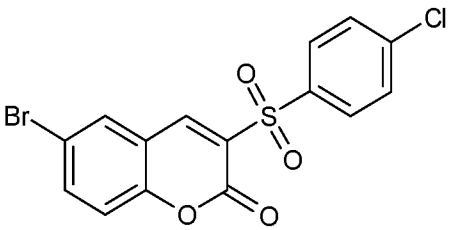


Compound C144

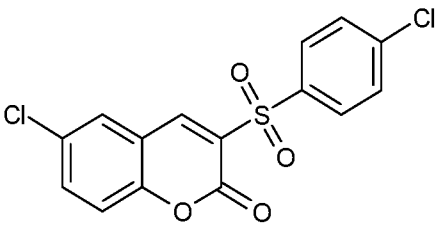


Compound C145

[00217]

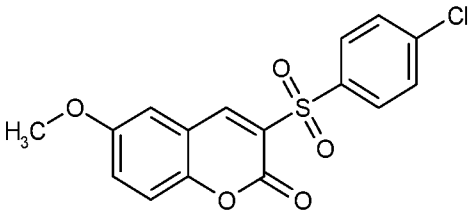


Compound C146

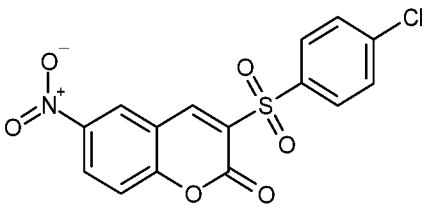


Compound C147

[00218]

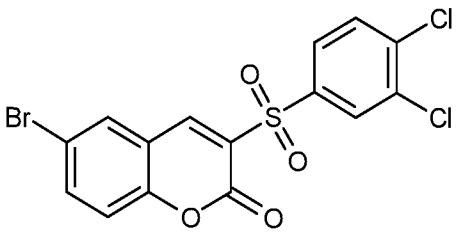


Compound C148

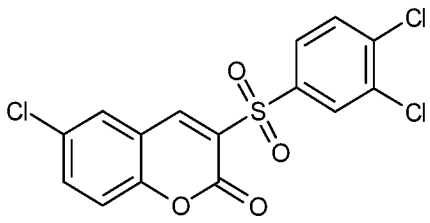


Compound C149

[00219]

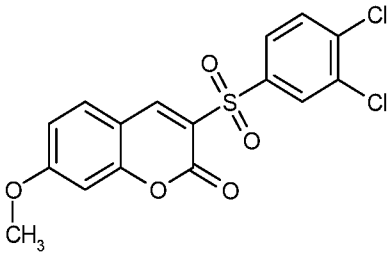


Compound C150

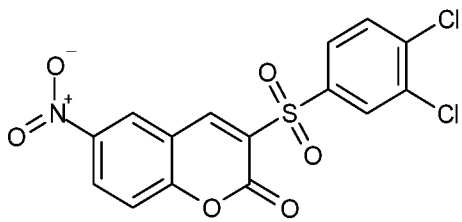


Compound C151

[00220]

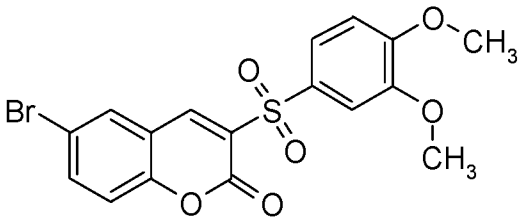


Compound C152

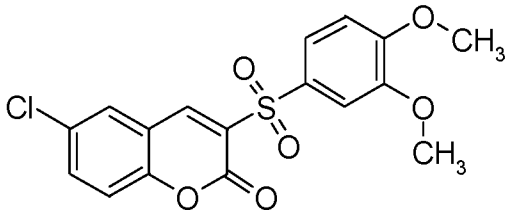


Compound C153

[00221]

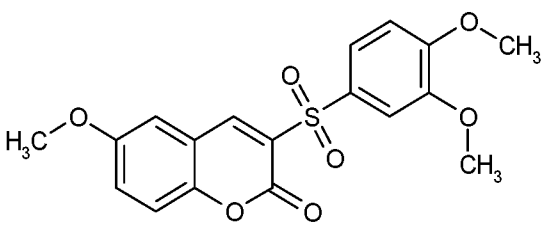


Compound C154

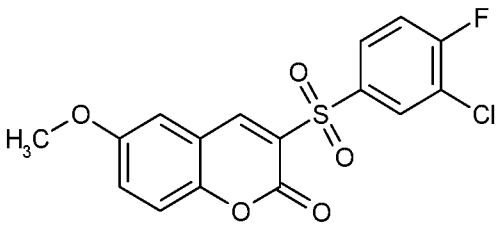


Compound C155

[00222]

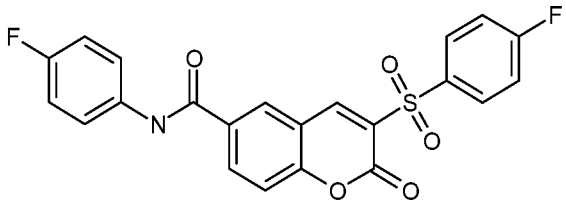


Compound C156

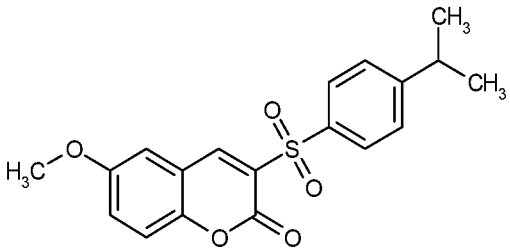


Compound C158

[00223]

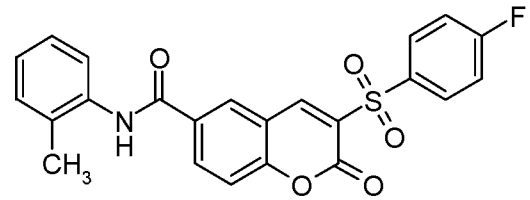


Compound C159

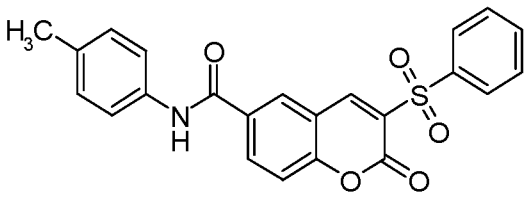


Compound C316

[00224]

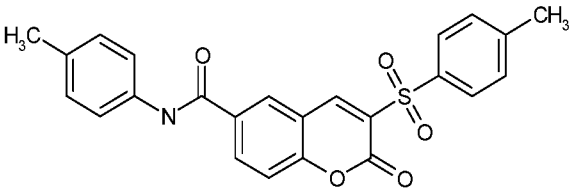


Compound C160

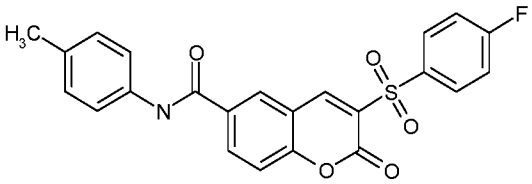


Compound C161

[00225]

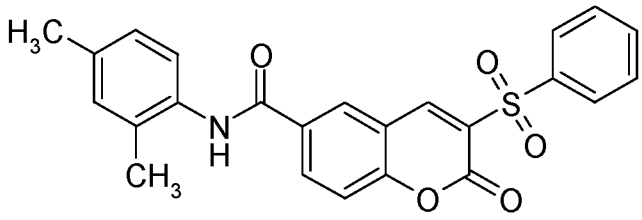


Compound C162



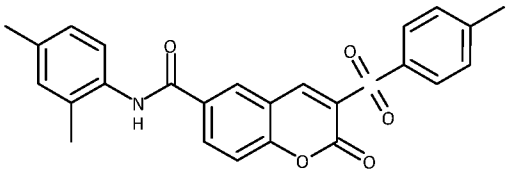
Compound C163

[00226]

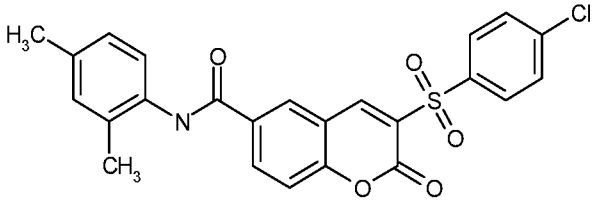


Compound C164

[00227]

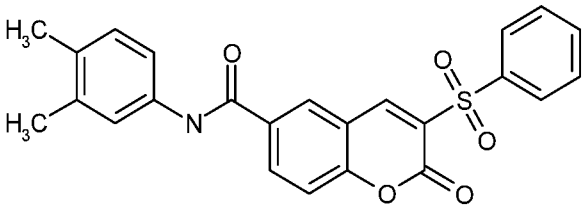


Compound C165

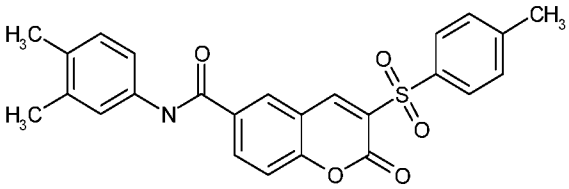


Compound C166

[00228]

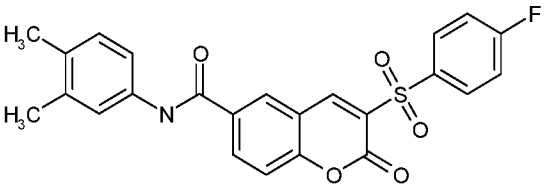


Compound C167

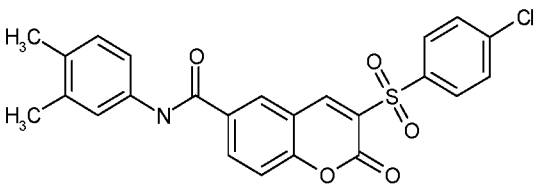


Compound C168

[00229]

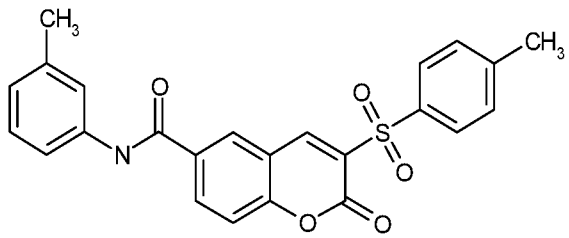


Compound C169

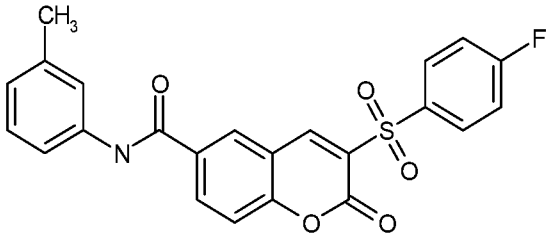


Compound C170

[00230]

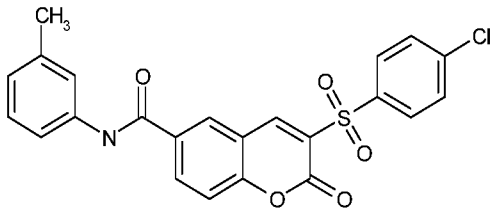


Compound C171

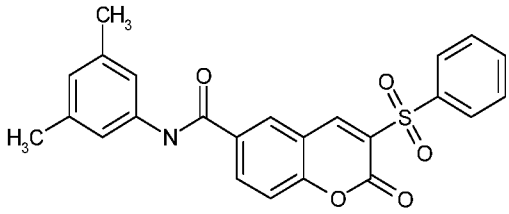


Compond C172

[00231]

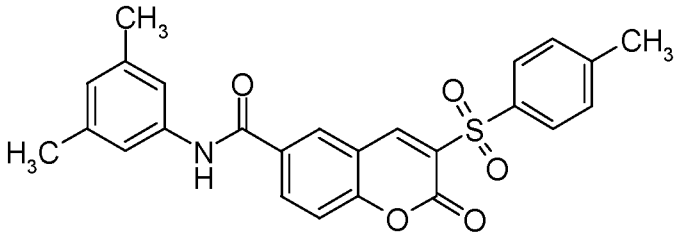


Compound C173



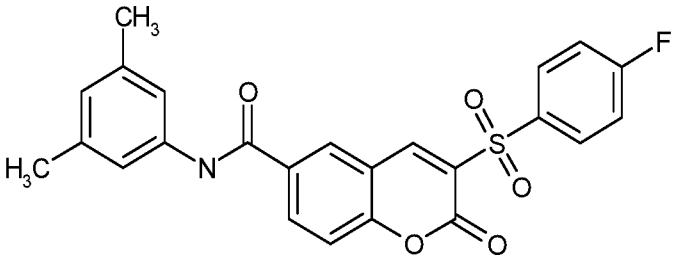
Compound C174

[00232]



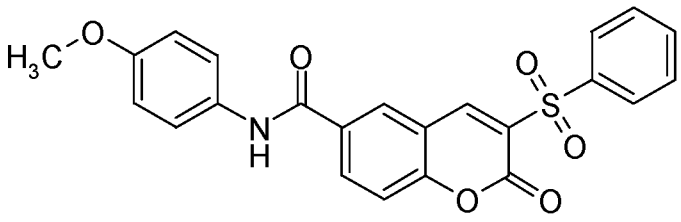
Compound C175

[00233]



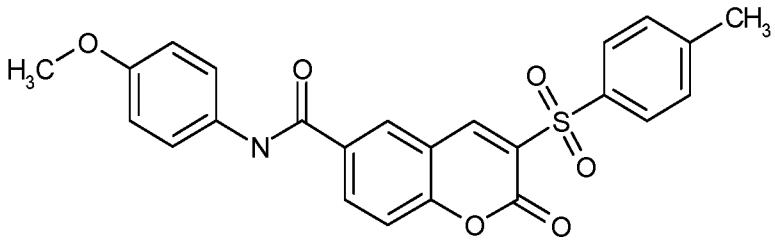
Compound C176

[00234]



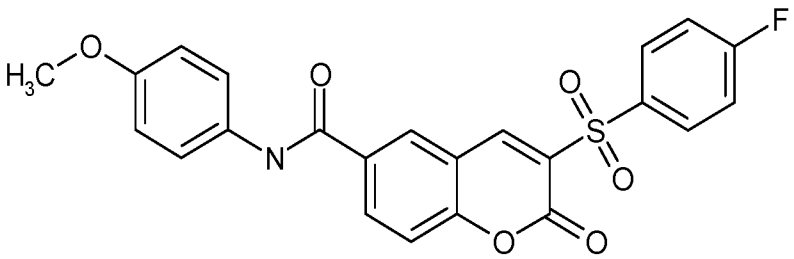
Compound C177

[00235]



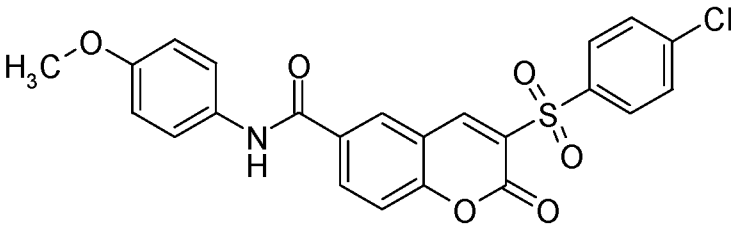
Compound C178

[00236]



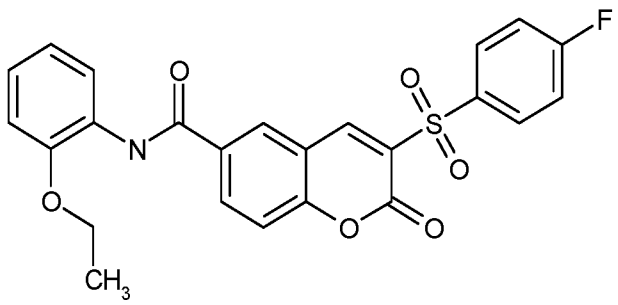
Compound C179

[00237]



Compound C180

[00238]



Compound C181

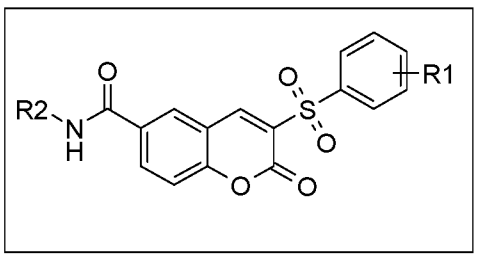
[00239] Further results of the testing of these compounds are shown in the tables below. Each compound shows results based on the different identities of the R1 moiety.

[00240] For example, compounds of Formula (Ia) and (Ib) were tested with different moieties as R1, and the results are shown below in Table 3:

ID NUMBER	R1	Firefly, cmpd/DMSO		Renilla, cmpd/DMSO		Firefly_pGL, cmpd/DMSO	
		repeat 1	repeat 2	repeat 1	repeat 2	repeat 1	repeat 2
C100	4-CH3	2.0	1.9	1.6	1.3	1.3	1.1
C101	4-OMe	4.5	4.7	1.8	2.0	1.2	1.3
C102	4-Br	13.0	15.3	5.8	6.1	2.3	2.5
C103	4-t-Bu	2.8	1.4	1.9	1.7	1.3	1.4
C104	3,4-di-Me	3.1	4.4	1.7	1.8	2.3	1.2
C105	4-Cl	5.7	4.8	1.7	2.3	1.5	1.8
C106	3,4-di-Cl	1.4	1.3	1.8	5.8	1.0	1.1
C107	3-Cl-4-F	11.0	11.0	8.7	6.5	1.7	2.1
C108	2-F	7.9	7.3	3.0	2.8	1.3	1.5
C109	3-Cl	9.2	10.3	3.4	3.3	3.5	3.4
C110	3-CH3-4-F	7.6	7.2	2.3	2.1	1.7	2.0

Table 3

[00241] In further embodiments, the present technology is directed to compounds of Formula (II):



(II)

wherein any of R1 and R2 are C, H, N, O, S, a halogen, an alkyl group, a substituted alkyl group, a cyclic alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, an ester, an aldehyde, a ketone, a carboxylic acid, an amide, an amine, an ether, a thiol or a nitrile.

[00242] In certain embodiments, any of R1 and R2 can be any of the moieties listed below in Tables 4 and 5. Various compounds of Formula (II) were tested with different moieties as R1 and R2, and the results are shown below in Tables 4 and 5:

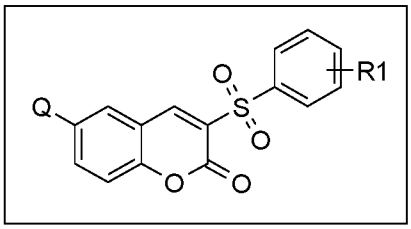
ID NUMBER	R2	R1	Firefly, cmpd/DMSO		Renilla, cmpd/DMSO		Firefly_pGL, cmpd/DMSO	
			repeat 1	repeat 2	repeat 1	repeat 2	repeat 1	repeat 2
C111	PhCH2CH2	H	29.8	35.9	29.4	30.8	3.7	3.9
C112	PhCH2CH2	4-CH3	11.7	10.1	5.6	5.1	2.5	2.8
C113	PhCH2CH2	4-F	5.8	7.0	10.2	10.8	2.2	3.2
C114	PhCH2Ch2	4-Cl	4.5	4.7	4.0	2.9	2.3	1.6
C115	PhCH2	H	6.8	21.9	4.8	10.9	6.2	7.2
C116	4-CH3C6H4CH2	4-Cl	8.6	9.0	9.8	9.9	2.1	2.0
C117	4-OMeC6H4CH2	4-F	17.3	24.1	28.1	35.6	3.9	4.5
C118	4-F-C6H4CH2	4-F	13.5	17.1	11.8	14.8	2.4	2.7
C119	4-F-C6H4CH2	4-Cl	18.4	16.2	27.8	27.6	2.0	2.2
C120	2-CF3C6H4	4-F	4.5	6.3	15.2	18.6	2.9	3.3
C121	3,4-di-MeO-C6H3	4-CH3	9.0	21.5	18.6	40.1	9.1	8.8
C122	3,4-di-MeO-C6H3	4-Cl	4.4	5.7	14.8	12.8	5.3	4.8
C123	2-MeO-C6H4	H	17.7	16.8	31.9	31.7	8.2	10.6
C124	2-MeO-C6H4	4-F	25.5	29.8	50.0	51.4	6.6	6.7
C125	2-MeO-C6H4	4-Cl	28.0	24.5	30.4	33.8	2.5	2.7
C126	3-MeO-C6H4	4-CH3	45.1	44.8	43.5	42.2	4.8	4.3
C127	3-MeO-C6H4	4-F	36.7	56.2	43.1	43.5	8.3	8.0
C128	3-Cl-C6H4	4-F	1.7	5.2	1.7	10.1	4.4	5.4
C129	4-Cl-C6H4	4-F	0.9	1.2	1.0	0.7	3.3	4.5
C130	3.5-di-MeO-C6H3	H	2.3	3.4	2.2	6.4	6.4	10.2
C131	3.5-di-MeO-C6H3	4-CH3	9.5	15.3	30.8	38.2	4.8	4.7
C132	3.5-di-MeO-C6H3	4-F	1.4	1.4	6.7	2.4	4.3	6.8
C133	3.5-di-MeO-C6H3	4-Cl	2.1	4.3	9.9	12.6	3.2	2.6
C134	2,3-di-MeC6H3	4-CH3	38.1	31.9	21.0	18.9	3.7	5.4
C135	2,3-di-MeC6H3	4-F	5.6	4.9	19.8	18.6	1.9	3.1
C136	2,3-di-MeC6H3	4-Cl	22.1	25.8	12.5	14.9	2.9	3.0

Table 4

ID NUMBER	R2	R1	Firefly, cmpd/DMSO		Renilla, cmpd/DMSO		Firefly_pGL, cmpd/DMSO	
			repeat 1	repeat 2	repeat 1	repeat 2	repeat 1	repeat 2
C159	4-F-C6H4	4-F	0.9	1.2	0.1	0.3	1.8	2.4
C160	2-me-C6H4	4-F	22.9	20.2	9.5	8.2	3.2	2.7
C161	4-Me-C6H4	H	35.4	39.0	28.6	27.7	4.0	5.1
C162	4-Me-C6H4	4-CH3	20.9	20.6	37.9	35.8	3.7	4.1
C163	4-Me-C6H4	4-F	2.0	5.2	6.9	15.8	5.5	5.0
C164	2,4-di-MeC6H3	H	29.3	19.6	13.8	8.9	3.1	2.8
C165	2,4-di-MeC6H3	4-CH3	4.2	4.0	4.7	4.6	1.4	1.4
C166	2,4-di-MeC6H3	4-Cl	3.4	4.2	2.9	2.9	1.4	1.7
C167	3,4-di-MeC6H3	H	1.0	0.7	0.2	0.2	0.9	0.9
C168	3,4-di-MeC6H3	4-CH3	11.8	11.4	14.8	14.0	2.4	1.6
C169	3,4-di-MeC6H3	4-F	8.8	10.6	9.9	10.9	2.0	1.9
C170	3,4-di-MeC6H3	4-Cl	1.7	1.6	0.2	0.3	0.7	1.0
C171	3-MeC6H4	4-CH3	6.4	4.2	15.4	9.8	2.0	2.3
C172	3-MeC6H4	4-F	30.4	32.8	44.9	37.0	3.5	4.7
C173	3-MeC6H4	4-Cl	9.8	6.9	19.8	19.5	2.3	2.4
C174	3,5-di-MeC6H3	H	5.8	14.3	16.0	28.3	4.3	6.0
C175	3,5-di-MeC6H3	4-CH3	13.2	11.9	10.5	12.0	2.5	1.9
C176	3,5-di-MeC6H3	4-F	3.7	9.6	19.0	29.1	3.9	3.8
C177	4-MeOC6H4	H	17.4	8.2	6.7	4.6	1.7	1.3
C178	4-MeOC6H4	4-CH3	15.2	16.9	27.7	28.2	3.5	4.4
C179	4-MeOC6H4	4-F	8.7	10.1	11.9	10.5	2.4	2.5
C180	4-MeOC6H4	4-Cl	34.0	34.5	30.9	30.0	2.5	2.7
C181	2-EtOC6H4	4-F	10.4	13.5	14.6	21.4	2.2	1.7

Table 5

[00243] In further embodiments, the present technology is direct to compounds of Formula (III):

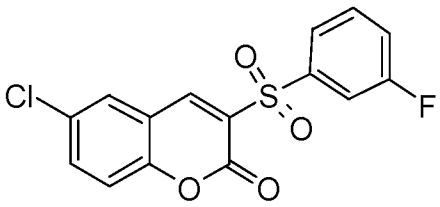


(III)

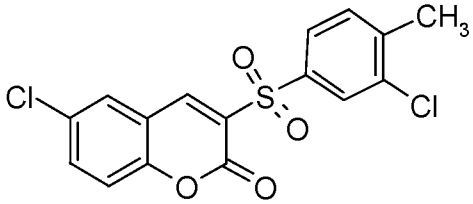
wherein any of Q and R1 are C, H, N, O, S, a halogen, an alkyl group, a substituted alkyl group, a cyclic alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, an ester, an aldehyde, a ketone, a carboxylic acid, an amide, an amine, an ether, a thiol or a nitrile.

[00244] Various compounds of Formula (III) were tested with different moieties as R1 and Q, and t . These include the following:

[00245]

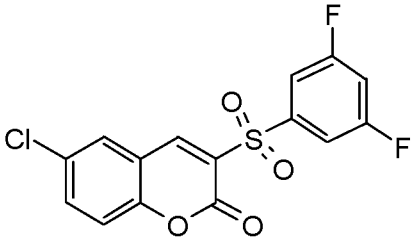


Compound C182



Compound C188

[00246]



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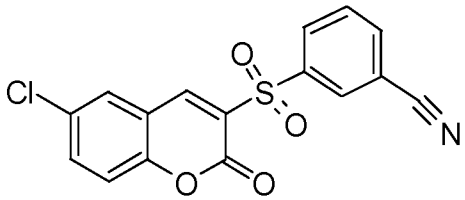

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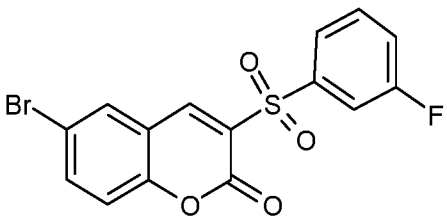
[00247]



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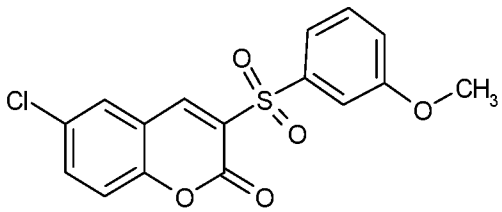
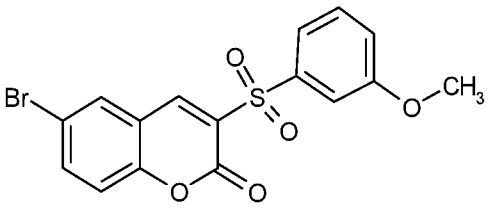


Compound 184

[00248]



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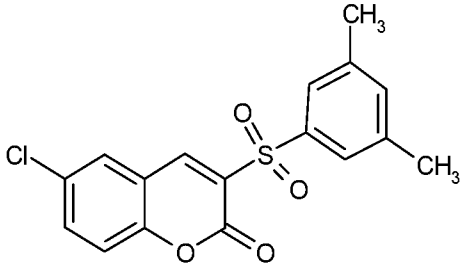

Compound C186

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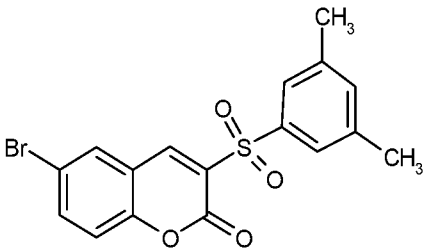

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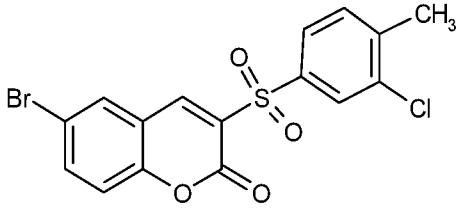

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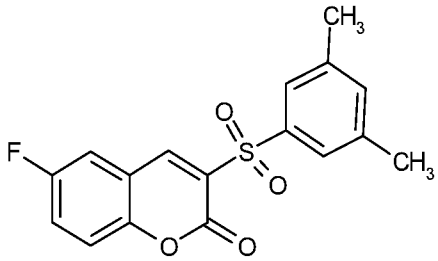

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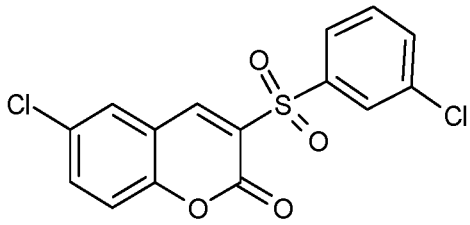

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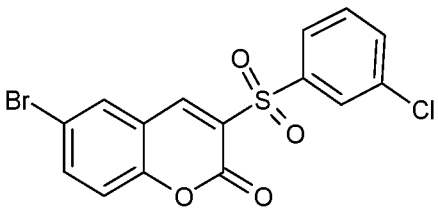
[00251]



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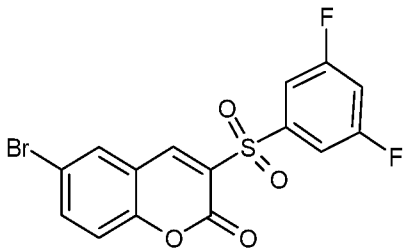
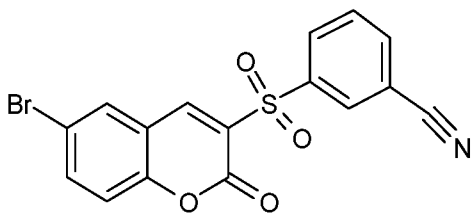


Compound C193

[00252]



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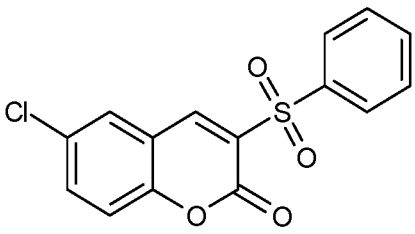
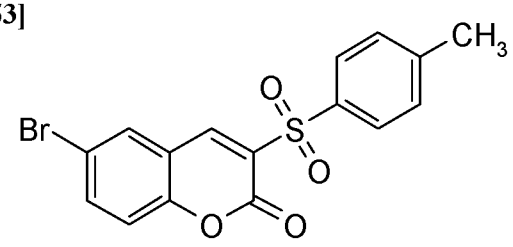


Compound C201

[00253]



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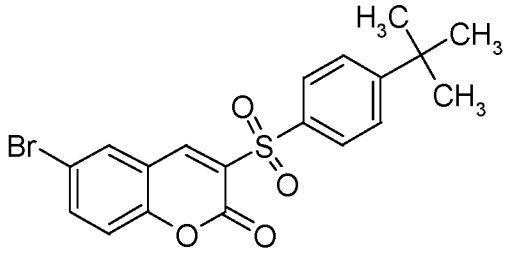

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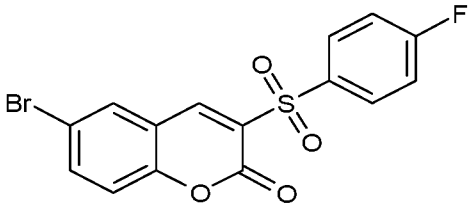
[00254]



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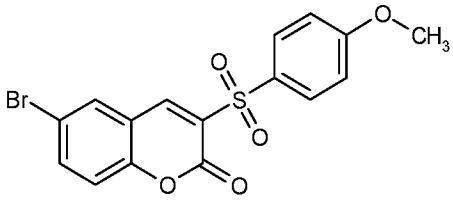
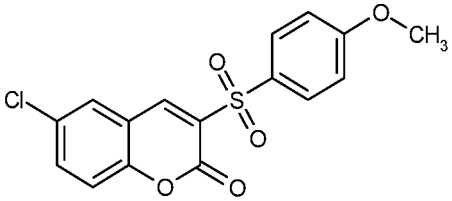


Compound C199

[00255]



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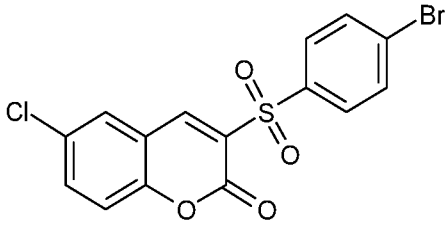

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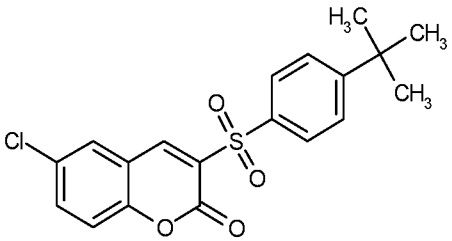
[00256]



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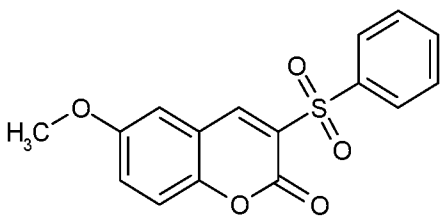
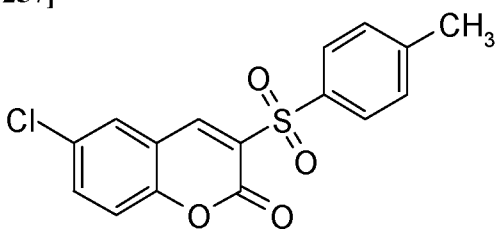


Compound C206

[00257]



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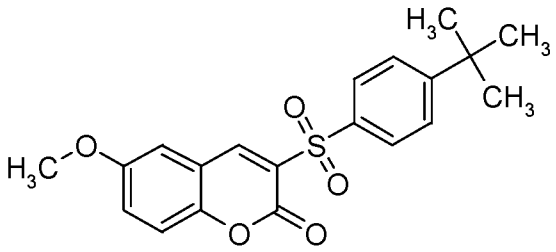

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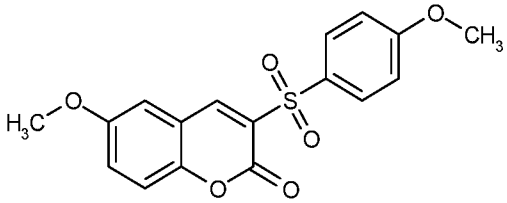

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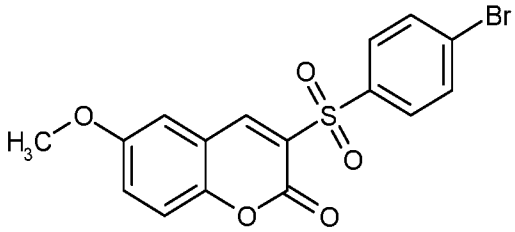

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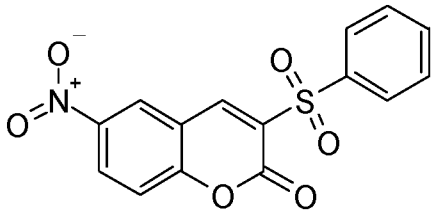
[00259]



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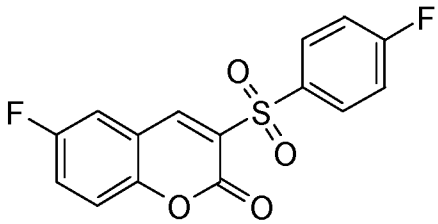
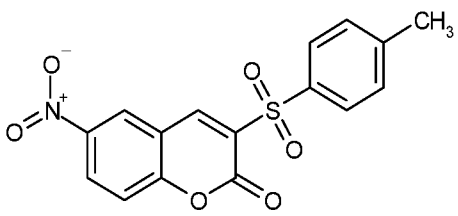


Compound C213

[00260]



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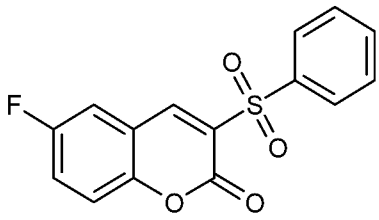

Compound C212

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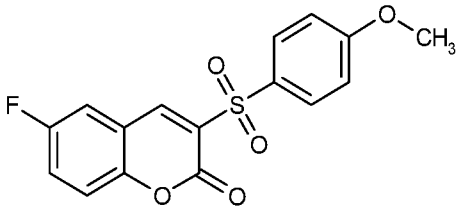

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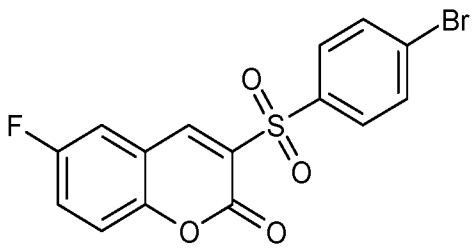
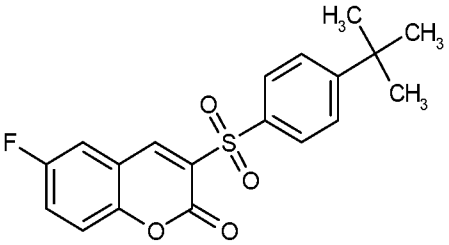


Compound C215

[00262]



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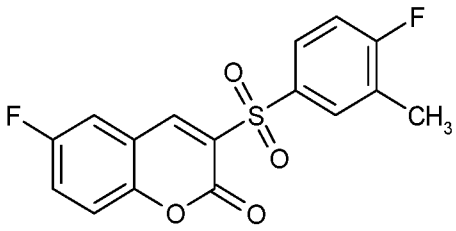

Compound C217

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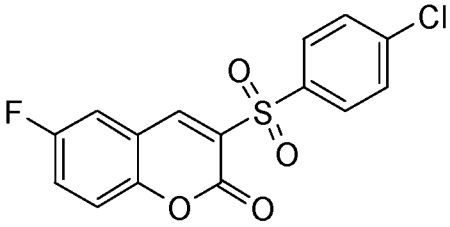
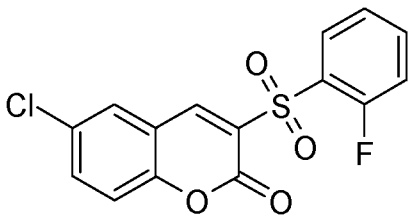
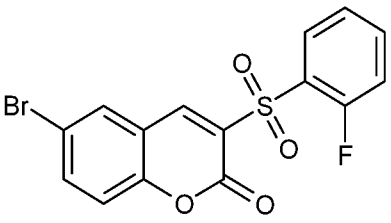


Compound C218



Compound C220

[00264]



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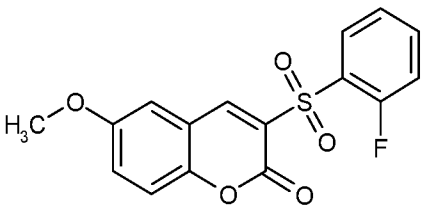
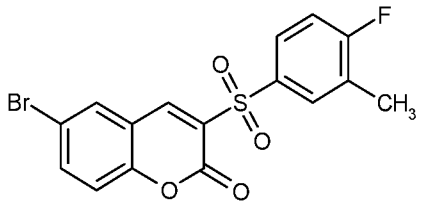
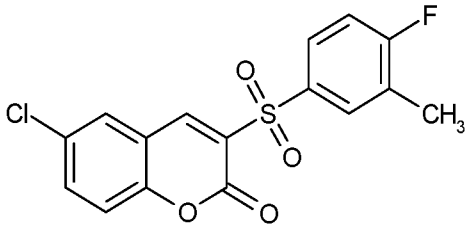


Compound C221

[00265]

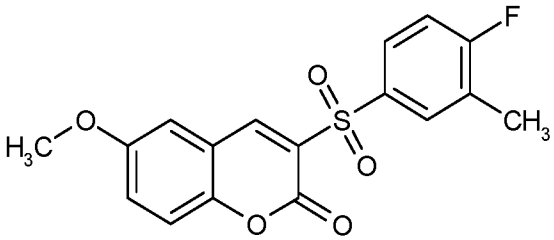


Compound C223

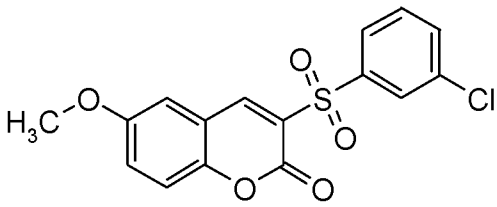


Compound C224

[00266]

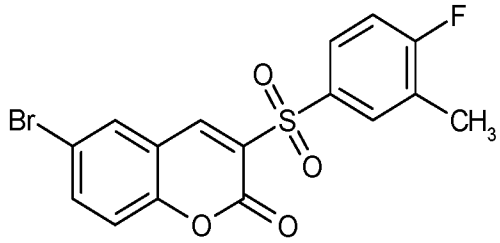


Compound C225



Compound C222

[00267]



Compound C223

[00268] The results of the tests are shown below in Tables 6-8:

ID NUMBER	Q	R1	Firefly, cmpd/DMSO		Renilla, cmpd/DMSO		Firefly_pGL, cmpd/DMSO	
			repeat 1	repeat 2	repeat 1	repeat 2	repeat 1	repeat 2
C182	Cl	3-F	16.2	17.3	15.1	13.2	2.0	2.1
C183	Br	3-F	1.2	1.2	3.1	2.4	1.1	1.2
C184	Cl	3-MeO	1.1	1.4	1.2	1.8	0.9	1.1
C185	Br	3-MeO	2.6	2.4	0.1	0.3	0.8	1.2
C186	Cl	3,5-diMe	1.0	6.9	0.4	18.8	1.2	1.7
C187	Br	3,5-diMe	0.8	0.7	1.0	0.8	0.9	1.1

C188	Cl	3-Cl-4-Me	9.4	8.7	7.6	6.9	1.8	1.8
C189	Br	3-Cl-4-Me	2.8	2.2	2.7	2.4	1.2	1.1
C190	F	3,5-diMe	2.1	2.5	1.4	1.4	1.3	1.1
C191	Cl	3-Cl	8.6	12.8	35.8	30.6	1.7	2.2
C192	Br	3-Cl	1.8	2.4	9.6	9.5	1.7	2.0
C193	Br	3,5-diF	1.8	1.9	6.5	14.6	1.5	1.8
C194	Cl	3,5-diF	10.6	6.3	7.2	6.2	1.3	1.3
C195	Cl	3-CN	38.7	29.4	22.8	10.1	3.7	5.1
C196	Br	3-CN	3.8	3.4	13.6	18.8	2.3	2.9
C197	Br	4-Me	17.6	14.8	16.1	13.7	2.6	2.5
C198	Br	4-F	1.0	0.8	8.8	5.3	1.2	1.1
C199	Br	4-MeO	19.3	16.1	25.9	23.2	2.2	2.9
C200	Br	4-t-Bu	11.0	8.1	5.8	10.4	1.6	1.9
C201	Cl	H	6.1	5.3	8.8	8.2	1.8	2.4
C202	Cl	4-Me	15.7	14.1	8.3	8.9	2.6	2.3
C203	Cl	4-MeO	9.5	15.5	20.2	22.8	2.1	2.7
C204	Cl	4-Br	5.0	5.8	28.9	27.9	1.5	1.8
C205	Cl	4-t-Bu	6.9	6.0	9.7	8.8	1.5	1.4
C206	MeO	H	2.1	1.8	1.1	1.1	1.2	1.4
C207	MeO	4-MeO	2.5	2.8	1.2	0.7	1.5	1.4
C208	MeO	4-Br	0.8	0.9	1.0	1.1	1.1	1.0
C209	MeO	4-t-Bu	3.1	4.2	0.9	1.1	1.3	1.1
C210	NO2	H	22.8	14.3	15.0	9.8	2.4	2.1
C211	NO2	4-Me	5.8	17.7	18.1	18.9	3.3	4.0

Table 6

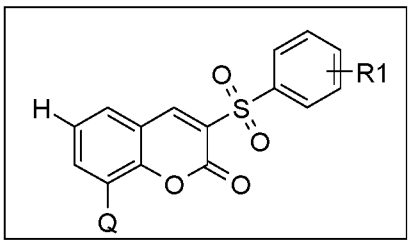
ID NUMBER	**Q**	**R1**	**Firefly, cmpd/DMSO**		**Renilla, cmpd/DMSO**		**Firefly_pGL, cmpd/DMSO**	
			repeat 1	repeat 2	repeat 1	repeat 2	repeat 1	repeat 2
C137	NO2	4-F	22.6	23.4	17.6	15.7	2.7	2.9
C138	NO2	4-Br	10.0	16.6	18.3	18.7	2.9	2.8
C139	NO2	4-t-Bu	6.6	6.4	7.0	6.7	1.4	1.8
C140	Cl	4-i-Pr	12.8	12.0	12.4	11.8	2.4	2.6
C141	MeO	4-i-Pr	1.6	1.6	1.2	1.3	1.4	1.1
C142	Br	3,4-diMe	1.2	1.7	1.4	9.8	1.1	1.6
C143	Cl	3,4-diMe	15.2	14.5	19.0	17.7	2.6	2.4
C144	MeO	3,4-diMe	0.9	0.8	3.4	1.1	1.0	0.9
C145	NO2	3,4-diMe	11.2	24.4	20.5	20.5	3.3	3.5
C146	Br	4-Cl	1.5	0.9	0.6	3.2	0.8	1.2
C147	Cl	4-Cl	2.6	2.9	1.8	5.0	1.5	1.4
C148	MeO	4-Cl	1.0	0.9	0.6	1.3	1.1	1.0
C149	NO2	4-Cl	7.8	15.3	15.3	19.7	2.7	2.3
C150	Br	3,4-diCl	8.5	7.9	12.2	11.5	1.7	1.7
C151	Cl	3,4-diCl	3.6	5.5	15.1	15.3	1.1	1.3
C152	OMe	3,4-di-Cl	4.5	3.1	4.4	4.2	1.3	1.1
C153	NO2	3,4-diCl	6.5	9.0	13.6	16.2	1.3	1.6
C154	Br	3,4-diMeO	35.4	35.4	25.6	19.5	4.0	3.8
C155	Cl	3,4-diMeO	37.3	33.3	19.3	15.4	3.5	3.0
C156	MeO	3,4-diMeO	1.0	1.0	0.9	1.0	1.0	1.0
C157	NO2	3,4-diMeO	24.9	20.4	6.9	6.0	2.3	2.1
C158	MeO	3-Cl-4-F	1.5	1.2	2.7	2.4	1.1	1.2

Table 7

ID NUMBER	Q	R1	Firefly, cmpd/DMSO		Renilla, cmpd/DMSO		Firefly_pGL, cmpd/DMSO	
			repeat 1	repeat 2	repeat 1	repeat 2	repeat 1	repeat 2
C212	F	H	1.7	1.9	1.7	1.7	1.0	1.4
C213	F	4-F	14.0	13.6	9.0	8.1	1.2	1.7
C214	F	4-MeO	1.9	1.9	1.7	1.7	1.0	1.4
C215	F	4-Br	11.6	13.8	14.8	15.1	2.1	2.2
C216	F	4-t-Bu	1.3	1.0	1.3	1.0	1.3	1.2
C217	F	3-CH3-4-F	1.8	1.4	1.8	1.5	0.9	1.4
C218	F	4-Cl	18.2	19.1	11.9	10.2	1.9	2.0
C219	Br	2-F	1.3	2.5	8.7	23.5	2.0	2.3
C220	Cl	2-F	10.4	6.5	8.1	6.7	1.3	1.5
C221	MeO	2-F	1.3	0.9	1.3	1.3	1.4	1.7
C222	MeO	3-Cl	4.5	3.7	2.3	1.8	1.0	1.3
C223	Br	3-CH3-4-F	4.5	5.1	3.7	5.9	1.9	1.4
C224	Cl	3-CH3-4-F	6.6	11.4	18.2	26.9	2.8	3.1
C225	MeO	3-CH3-4-F	1.5	3.1	1.2	1.4	1.3	1.5

Table 8

[00269] In further embodiments, the present technology is directed to compounds of Formula (IV):

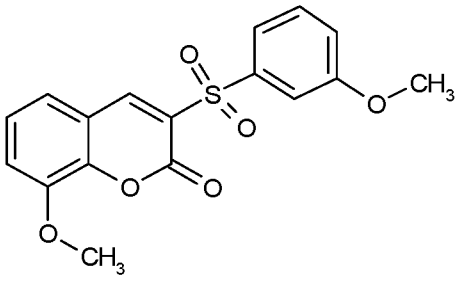


(IV)

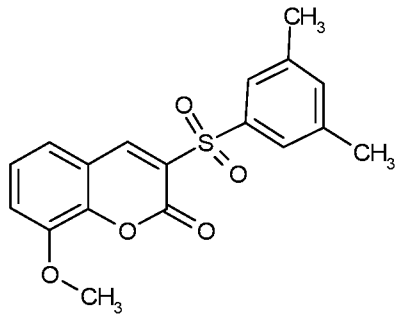
wherein any of Q and R1 are C, H, N, O, S, a halogen, an alkyl group, a substituted alkyl group, a cyclic alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, an ester, an aldehyde, a ketone, a carboxylic acid, an amide, an amine, an ether, a thiol or a nitrile.

[00270] Various compounds of Formula (IV) were tested with different moieties as Q and R1. These included the following:

[00271]

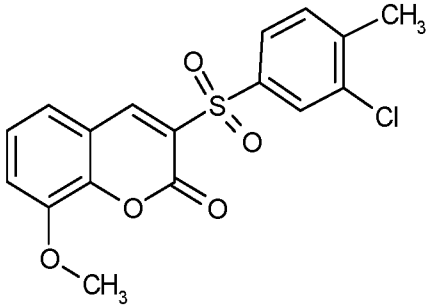


Compound C226



Compound C227

[00272]



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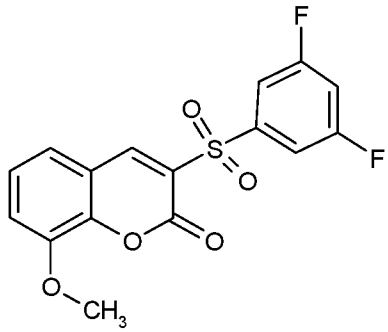
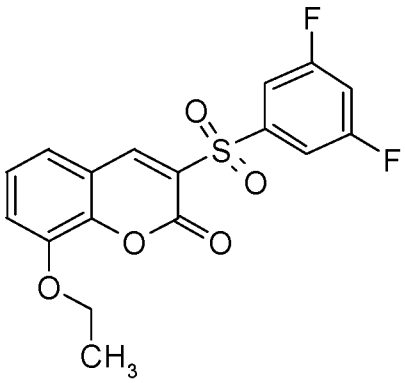
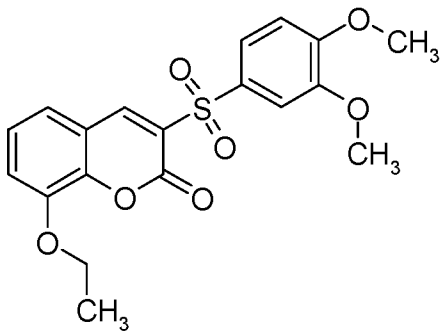


Compound C229

[00273]

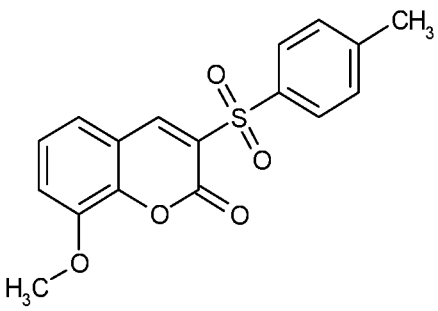


Compound C230



Compound C248

[00274]



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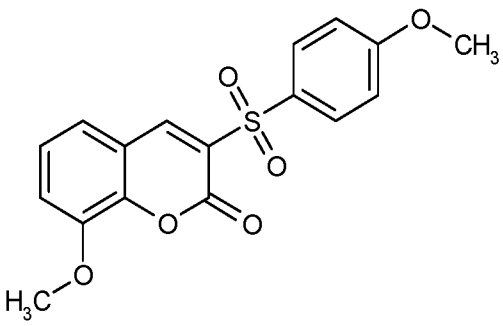
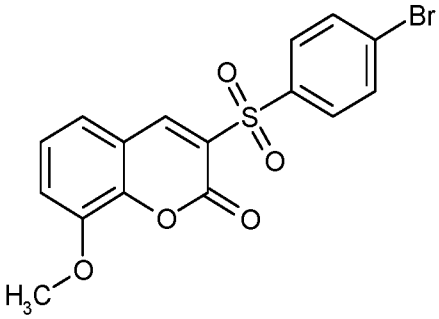


Compound C232

[00275]



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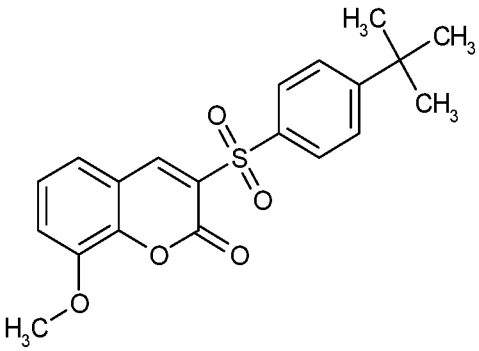
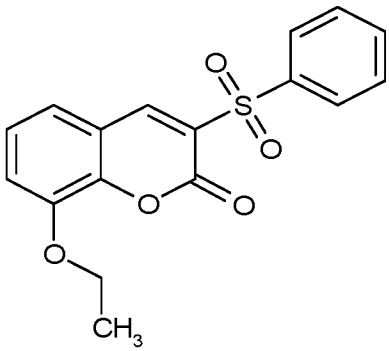
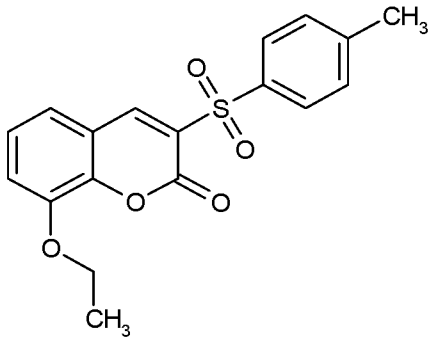


Compound C234

[00276]

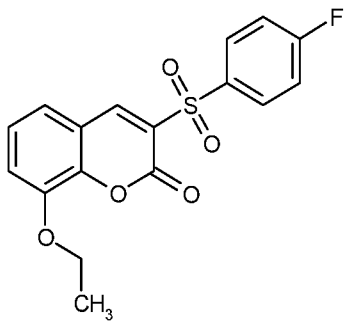


Compound C235

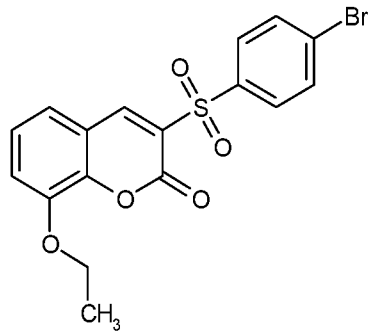


Compound C236

[00277]

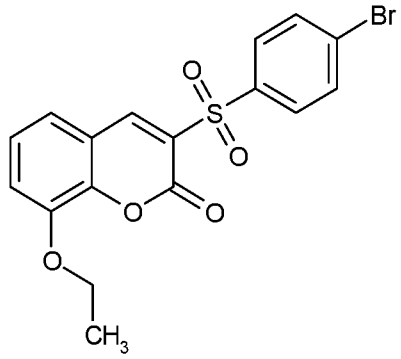


Compound C237

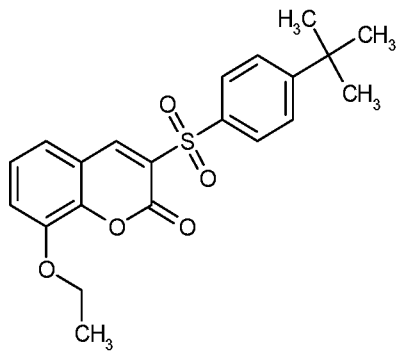


Compound C238

[00278]

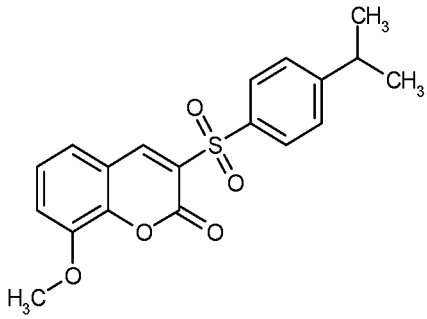


Compound C239

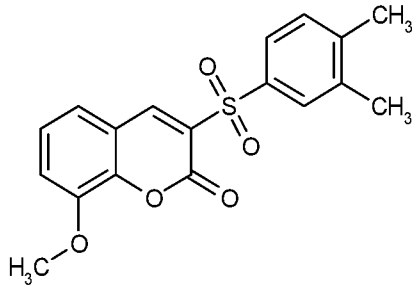


Compound C240

[00279]

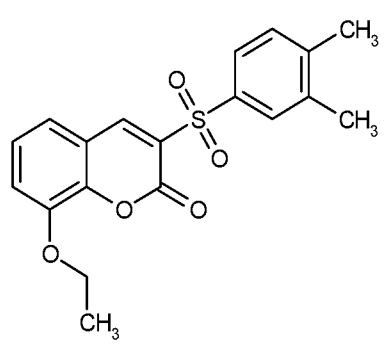


Compound C241

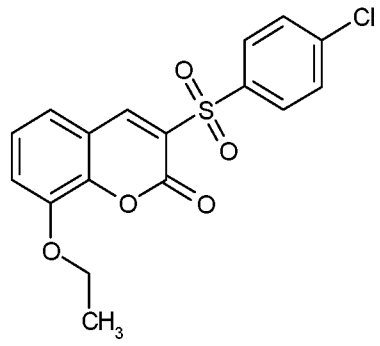


Compound C242

[00280]

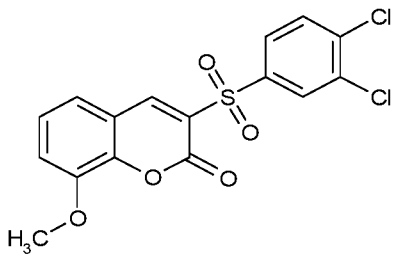


Compound C243

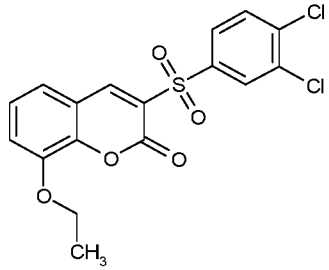


Compound C244

[00281]

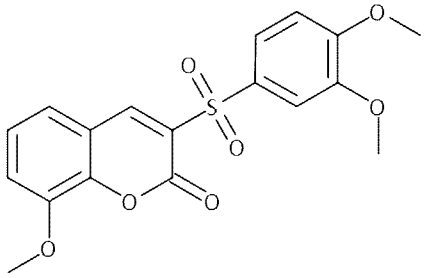


Compound C245

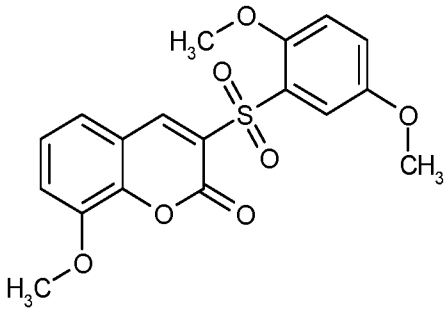


Compound C246

[00282]

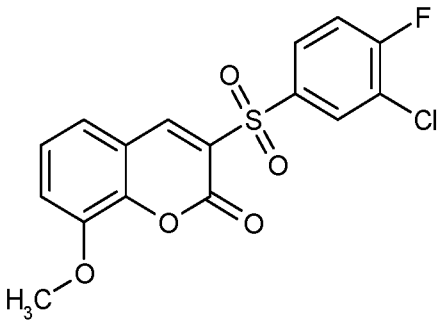


Compound C247

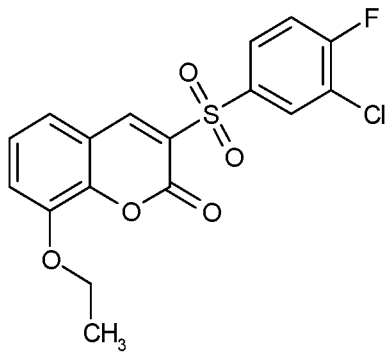


Compound C249

[00283]

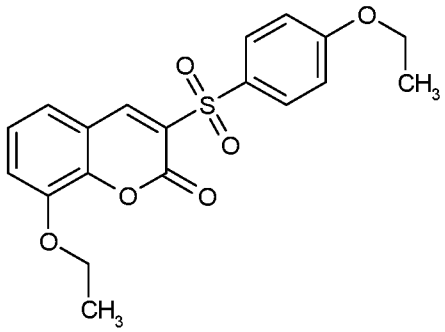


Compound C250

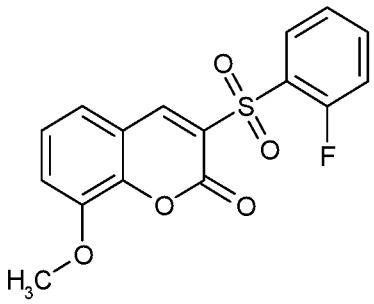


Compound C251

[00284]

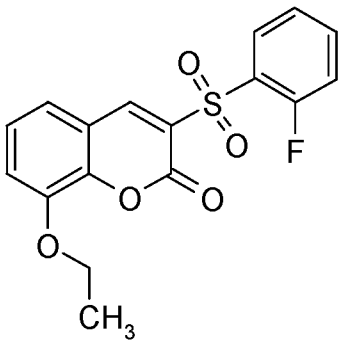


Compound C252



Compound C253

[00285]



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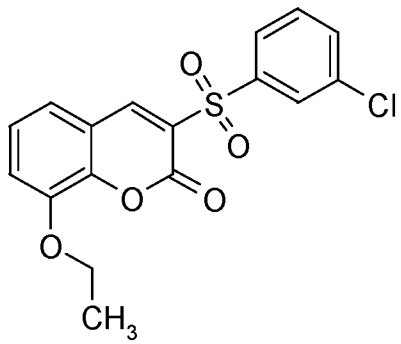
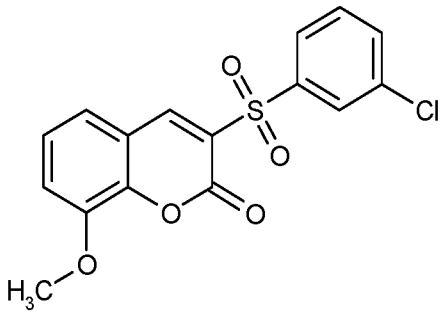
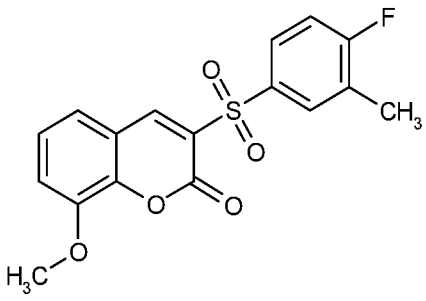


Compound C256

[00286]

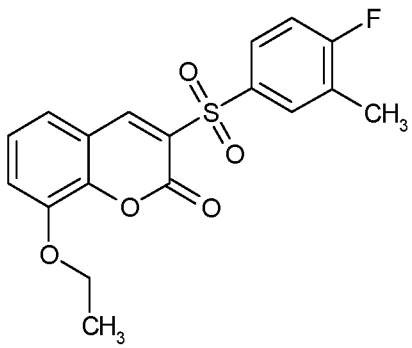


Compound C255



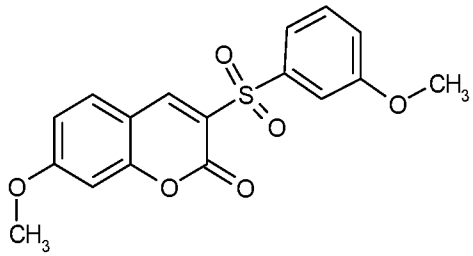
Compound C257

[00287]

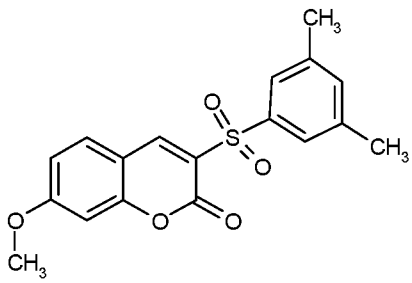


Compound C258

[00288]

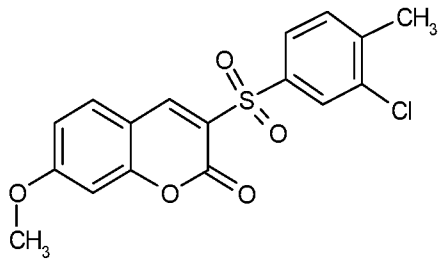


Compound C259

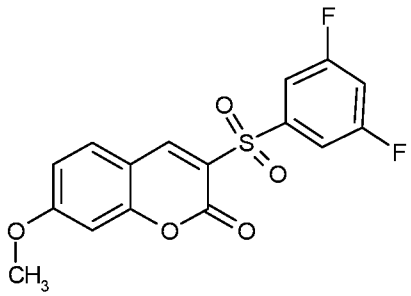


Compound C260

[00289]

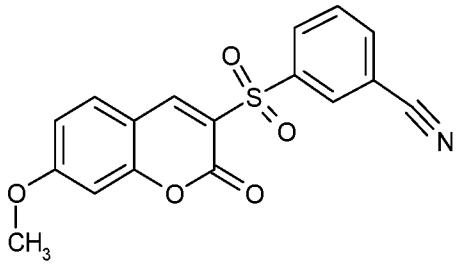


Compound C261

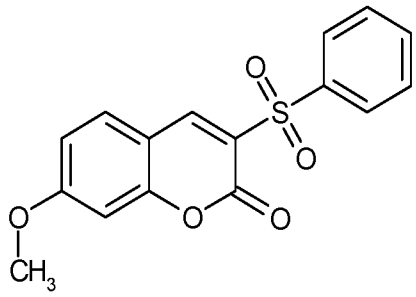


Compound C262

[00290]

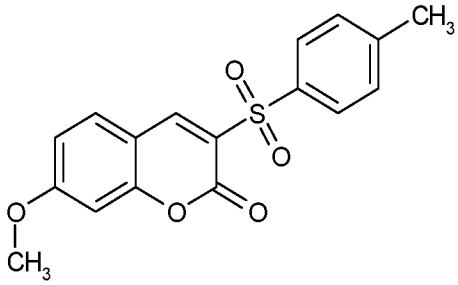


Compound C263

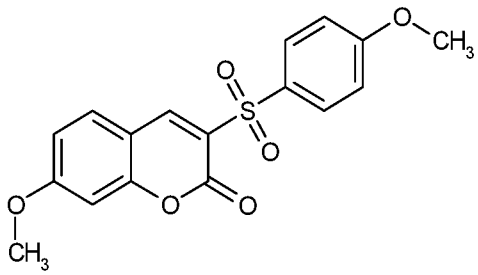


Compound C264

[00291]

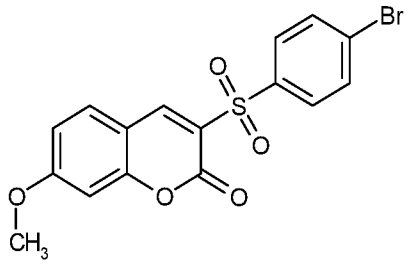


Compound C265

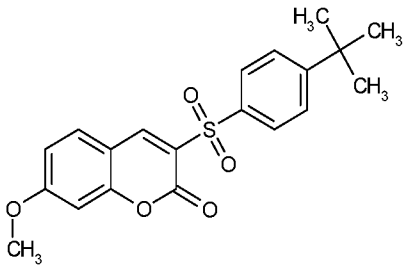


Compound C266

[00292]

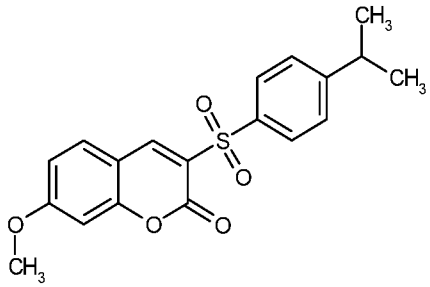


Compound C267

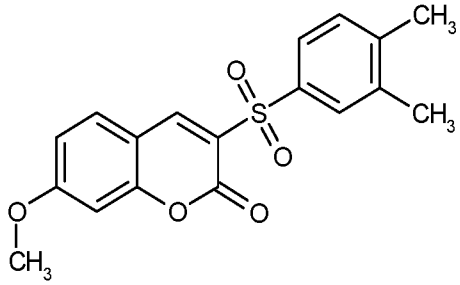


Compound C268

[00293]

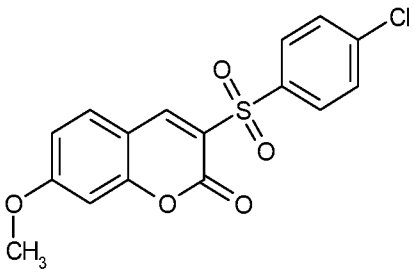


Compound C269

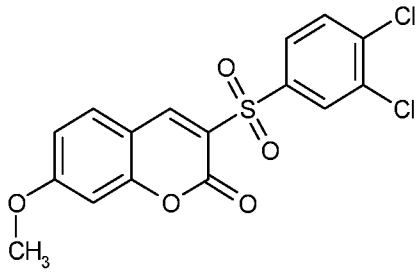


Compound C270

[00294]

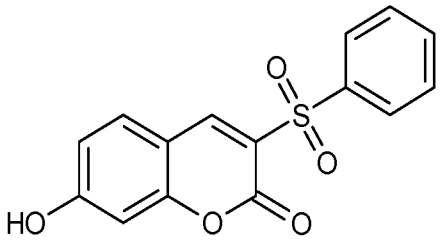


Compound C271

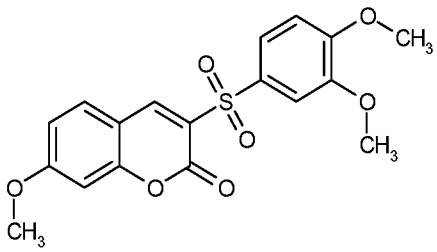


Compound C272

[00295]

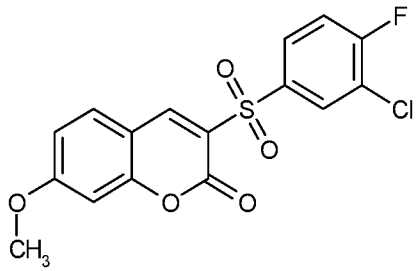


Compound C273

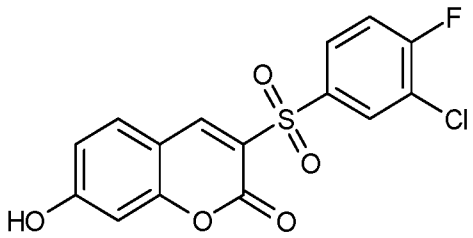


Compound C274

[00296]

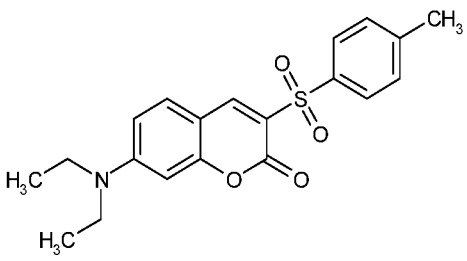


Compound C275

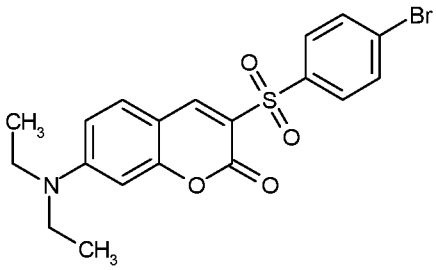


Compound C276

[00297]

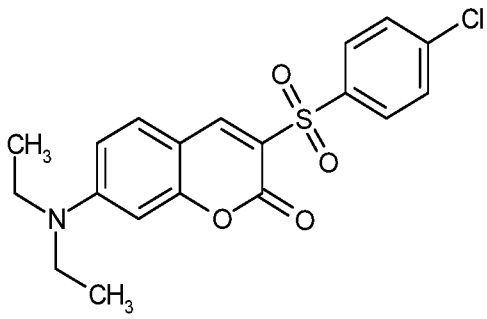


Compound C277



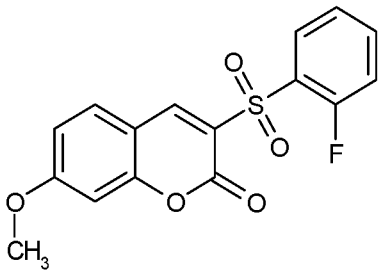
Compound C278

[00298]

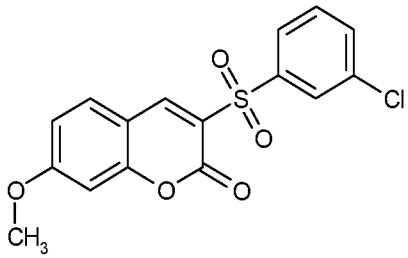


Compound C279

[00299]

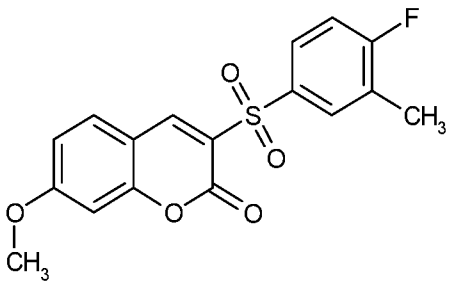


Compound C280



Compound C281

[00300]



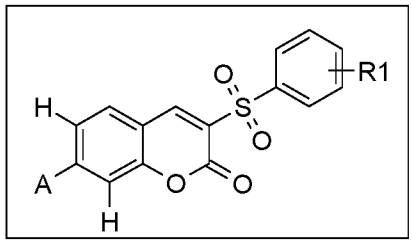
Compound C282

[00301] The results are shown below in Table 9:

IDNUMBER	Q	R1	Firefly, cmpd/DMSO		Renilla, cmpd/DMSO		Firefly_pGL, cmpd/DMSO	
			repeat 1	repeat 2	repeat 1	repeat 2	repeat 1	repeat 2
C226	OMe	3-OMe	1.1	1.0	0.5	1.1	1.2	1.3
C227	OMe	3,5-di-Me	1.0	1.3	1.1	1.1	1.0	1.1
C228	OMe	3-Cl-4-Me	1.0	1.3	2.2	2.1	1.1	1.0
C229	OMe	3,5-di-F	2.9	1.8	1.5	1.3	1.2	0.8
C230	OEt	3,5-di-F	12.5	8.3	4.5	3.8	1.8	1.5
C231	OMe	4-Me	0.7	0.9	1.3	1.1	1.1	1.0
C232	OMe	4-OMe	1.0	0.9	1.0	1.0	1.0	0.9
C233	OMe	4-Br	2.8	2.1	1.1	1.3	1.3	1.2
C234	OMe	4-t-Bu	2.2	1.8	4.3	2.7	1.0	1.1
C235	OMe	H	1.0	1.2	1.0	1.1	1.0	0.9
C236	OEt	CH3	4.4	4.9	3.1	3.2	1.6	1.6
C237	OEt	4-F	3.5	2.9	1.5	1.3	1.3	1.3
C238	OEt	4-OMe	1.3	1.4	1.5	1.0	1.3	1.2
C239	OEt	4-Br	1.4	2.2	8.4	17.2	1.2	1.1
C240	OEt	4-t-Bu	1.5	0.9	1.5	1.5	0.9	1.1
C241	OMe	4-i-Pr	2.1	2.7	1.5	1.9	1.2	1.8
C242	OMe	3,4-di-Me	1.1	1.1	0.9	1.1	1.1	1.1
C243	OEt	3,4-di-Me	2.0	3.3	2.3	2.3	1.4	1.9
C244	OEt	4-Cl	9.7	14.1	19.8	18.4	1.7	1.9
C245	OMe	3,4-di-Cl	1.9	1.3	1.7	1.8	1.1	1.3
C246	OEt	3,4-di-Cl	7.4	6.5	7.6	8.4	1.6	1.9
C247	OMe	3,4-di-OMe	2.2	1.6	1.7	1.5	1.3	1.1
C248	OEt	3,4-di-OMe	8.6	4.3	2.8	1.9	1.4	1.4
C249	OMe	2,5-di-OMe	1.4	1.5	1.0	1.1	1.1	1.3
C250	OMe	3-Cl-4-F	1.2	1.8	1.7	1.9	1.1	1.1
C251	OEt	3-Cl-4-F	2.1	1.9	2.4	2.0	1.0	0.9
C252	OEt	4-OEt	3.2	2.4	1.4	1.6	1.4	1.3
C253	OMe	2-F	2.5	2.7	1.5	1.2	1.3	1.2
C254	OEt	2-F	7.6	5.1	2.7	2.5	1.5	1.4
C255	OMe	3-Cl	1.5	1.5	1.2	1.1	1.3	1.0
C256	OEt	3-Cl	11.5	7.9	5.1	3.5	2.0	2.4
C257	OMe	3-Me-4-F	2.1	1.6	1.2	1.1	0.8	1.0
C258	OEt	3-Me-4-F	1.5	1.8	1.3	1.5	1.3	1.5

Table 9

[00302] In further embodiments, the present technology is directed to compounds of Formula (V):



(V)

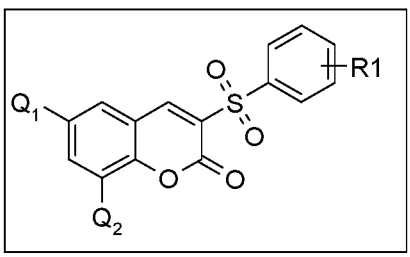
wherein any of A and R1 are C, H, N, O, S, a halogen, an alkyl group, a substituted alkyl group, a cyclic alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, an ester, an aldehyde, a ketone, a carboxylic acid, an amide, an amine, an ether, a thiol or a nitrile.

[00303] Various compounds of Formula (V) were tested with different moieties as A and R1, and the results are shown below in Table 10:

ID NUMBER	A	R	Firefly, cmpd/DMSO		Renilla, cmpd/DMSO		Firefly_pGL, cmpd/DMSO	
			repeat 1	repeat 2	repeat 1	repeat 2	repeat 1	repeat 2
C259	OMe	3-OMe	1.3	1.9	1.2	1.2	0.9	1.1
C260	OMe	3,5-di-Me	1.0	1.0	1.4	1.3	1.0	1.1
C261	OMe	3-Cl-4-CH3	2.0	1.4	1.7	1.5	0.9	1.2
C262	OMe	3,5-di-F	1.8	2.2	1.4	1.2	1.4	1.3
C263	OMe	3-CN	1.8	1.7	1.4	1.3	1.2	1.0
C264	OMe	H	1.1	0.8	1.2	1.2	0.9	1.0
C265	OMe	CH3	1.0	1.3	1.4	1.3	1.2	1.1
C266	OMe	OMe	0.9	1.2	1.4	1.3	1.0	1.0
C267	OMe	4-Br	1.4	1.7	2.5	1.2	1.2	1.6
C268	OMe	4-t-Bu	2.8	3.1	1.3	2.1	0.9	1.2
C269	OMe	4-i-Pr	3.0	3.0	1.5	1.4	1.2	1.0
C270	OMe	3,4-di-Me	3.2	3.1	2.2	1.8	1.3	1.2
C271	OMe	4-Cl	2.2	1.2	2.3	2.1	1.6	1.6
C272	OMe	3,4-di-Cl	4.5	3.1	4.4	4.2	1.3	1.1
C273	OH	H	2.0	1.5	1.6	2.0	1.0	1.2
C274	OMe	3,4-di-OMe	3.3	3.5	1.2	1.5	1.6	1.2
C275	OMe	3-Cl-4-F	2.6	2.5	2.3	1.9	1.2	1.3
C276	OH	3-Cl-4-F	1.2	1.3	2.3	1.9	0.9	1.0
C277	NEt2	4-Me	3.4	2.9	1.5	0.9	1.0	1.3
C278	NEt2	4-Br	2.4	3.0	1.5	1.7	1.0	1.2
C279	NEt2	4-Cl	3.9	2.1	1.4	1.6	0.9	0.9
C280	OMe	2-F	0.6	0.6	1.4	1.4	0.9	1.2
C281	OMe	3-Cl	3.0	4.4	1.8	1.9	1.4	1.3
C282	OMe	3-CH3-4-F	0.7	1.1	1.5	1.7	1.4	2.0

Table 10

[00304] In further embodiments, the present technology is directed to compounds of Formula (VI):

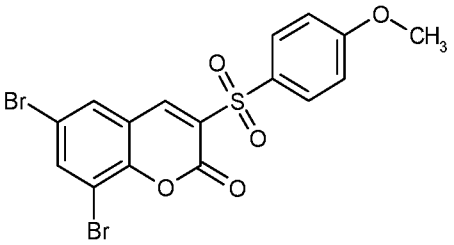


(VI)

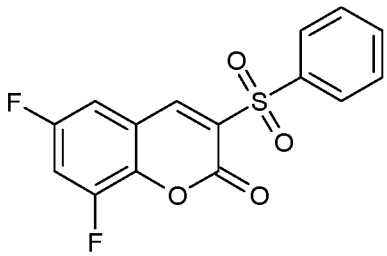
wherein any of Q1, Q2 and R1 are C, H, N, O, S, a halogen, an alkyl group, a substituted alkyl group, a cyclic alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, an ester, an aldehyde, a ketone, a carboxylic acid, an amide, an amine, an ether, a thiol or a nitrile.

[00305] Various compounds of Formula (VI) were tested with different moieties as Q1 and Q2. These included the following:

[00306]

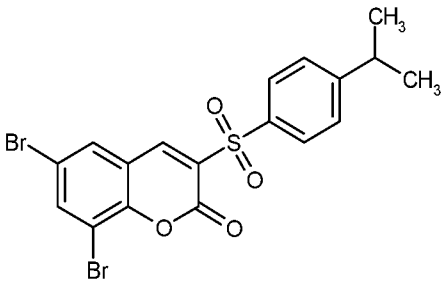


Compound C283

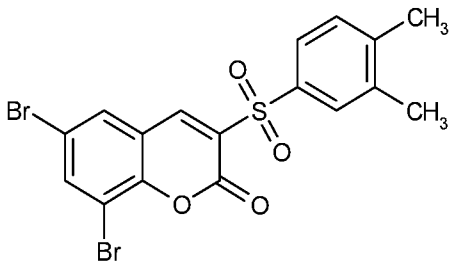


Compound C288

[00307]

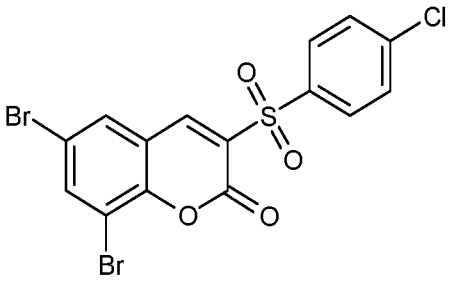


Compound C284

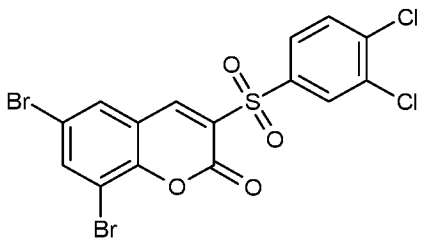


Compound C285

[00308]

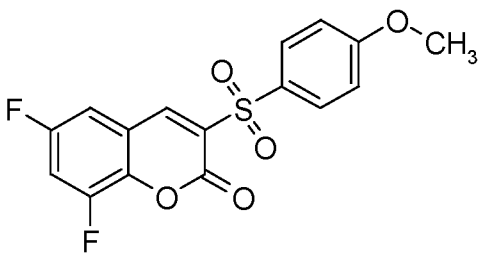


Compound C286

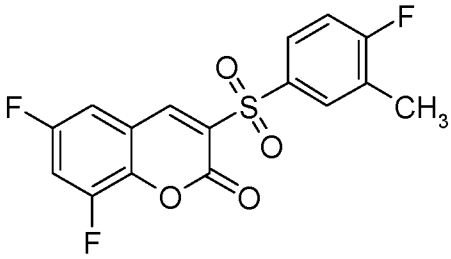


Compound C287

[00309]

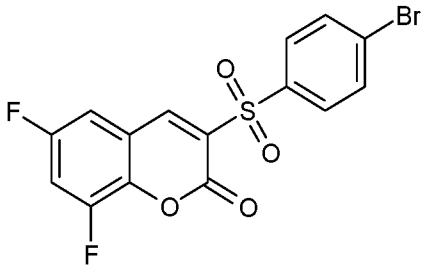


Compound C289

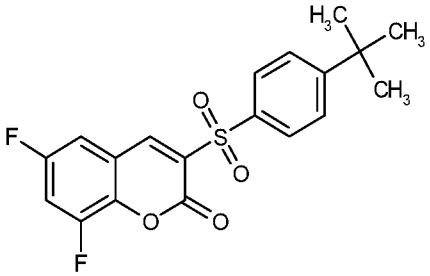


Compound C292

[00310]

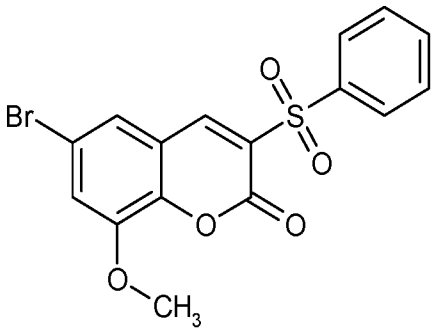


Compound C290

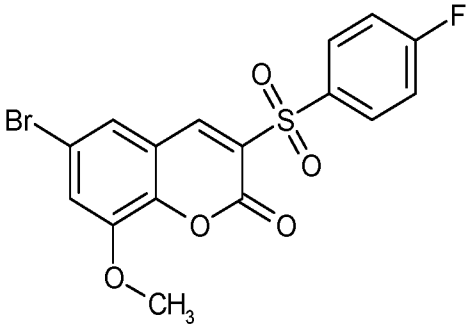


Compound C291

[00311]

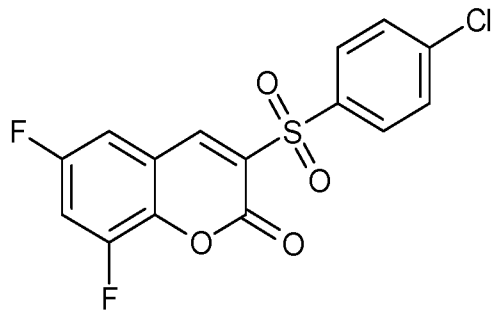


Compound C294

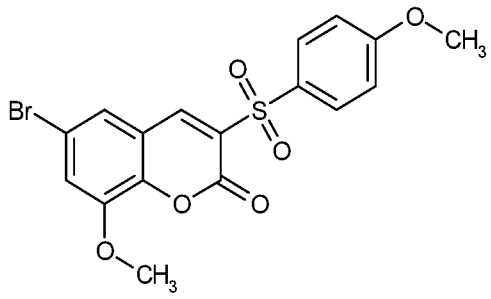


Compound C295

[00312]

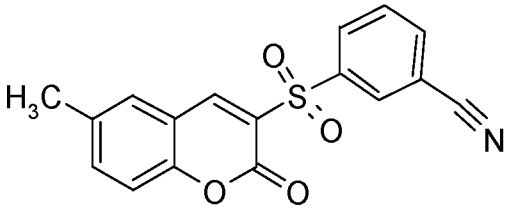


Compound C293

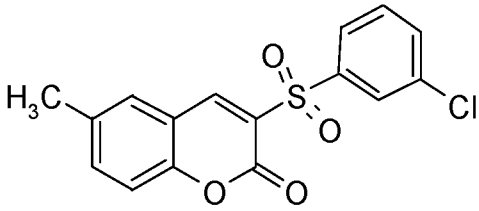


Compound C296

[00313]

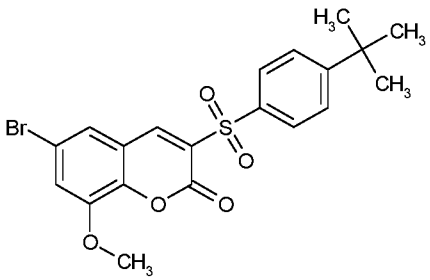


Compound C299

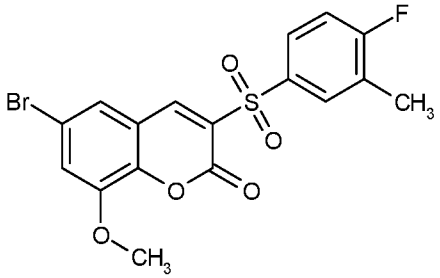


Compound C300

[00314]

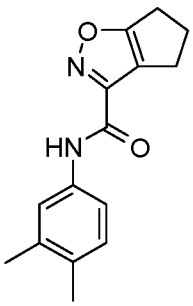


Compound C297

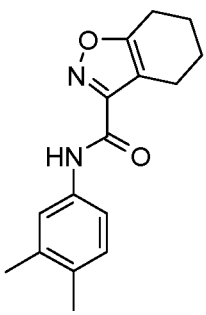


Compound C298

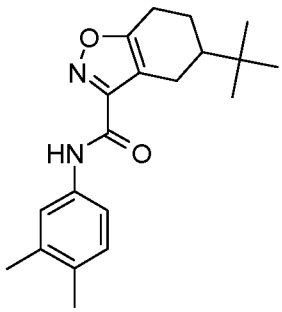
[00315]



Compound C301



Compound C302



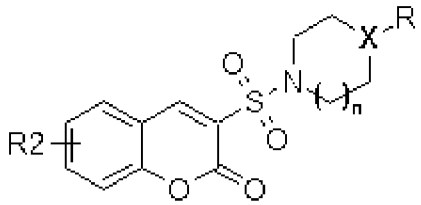
Compound C303

[00316] The results are shown below in Table 11:

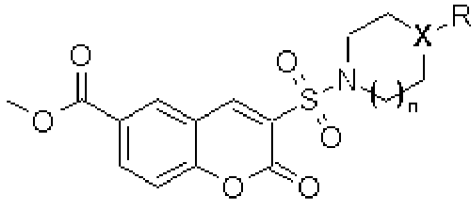
ID NUMBER	Q1	Q2	R1	Firefly, cmpd/DMSO		Renilla, cmpd/DMSO		Firefly_pGL, cmpd/DMSO	
				repeat 1	repeat 2	repeat 1	repeat 2	repeat 1	repeat 2
C283	Br	Br	OMe	1.0	1.3	0.1	0.2	1.0	0.9
C284	Br	Br	4-i-Pr	2.9	2.5	0.4	0.3	0.9	1.0
C285	Br	Br	3,4-di-Me	1.0	1.0	0.1	0.6	1.0	1.2
C286	Br	Br	4-Cl	2.3	2.4	0.3	0.9	0.8	1.1
C287	Br	Br	3,4-di-Cl	3.0	2.6	2.9	2.5	1.1	1.5
C288	F	F	H	6.8	3.3	4.4	3.3	1.6	1.4
C289	F	F	OMe	31.3	15.6	13.5	10.0	1.7	1.3
C290	F	F	4-Br	13.8	14.6	21.6	17.1	2.0	1.8
C291	F	F	4-t-Bu	0.9	0.8	0.1	0.2	0.7	0.7
C292	F	F	3-Me-4-F	18.3	14.8	15.6	13.4	1.7	1.7
C293	F	F	4-Cl	8.5	11.9	27.8	21.2	2.1	1.7
C294	Br	OMe	H	16.5	16.5	36.0	27.5	2.4	2.9
C295	Br	OMe	4-F	4.0	4.6	4.3	3.6	1.8	1.6
C296	Br	OMe	4-OMe	6.0	7.8	14.9	14.1	2.2	2.0
C297	Br	OMe	4-t-Bu	1.5	2.6	2.0	6.9	1.4	1.3
C298	Br	OMe	3-Me-4-F	2.2	3.2	1.9	1.9	1.3	1.4

Table 11

[00317] In certain embodiments, the technology is directed to compounds of Formulas (VII) or (VIII):



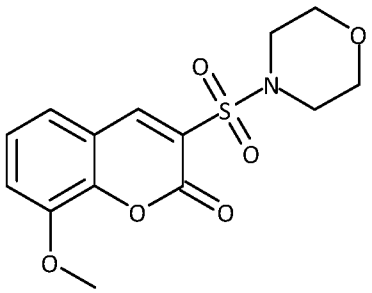
(VII)



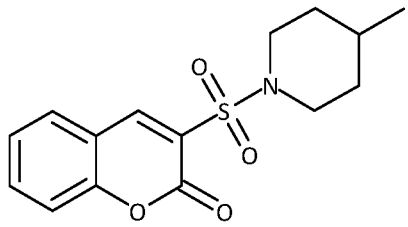
(VIII)

wherein any of X and R is: C, H, N, O, S, a halogen, an alkyl group, a substituted alkyl group, a cyclic alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, an ester, an aldehyde, a ketone, a carboxylic acid, and amide, an amine, an ether, a thiol or a nitrile; and wherein n is an integer 1, 2, 3, 4, 5 or 6. In certain embodiments, the X-R moiety represents a benzene ring fused to the n-membered ring containing the N substitution, to create a bicyclic functional group; *see*, *e.g.*, Compounds F41 through F47 below.

[00318] Exemplary compounds in accordance with these Formulas include the following:

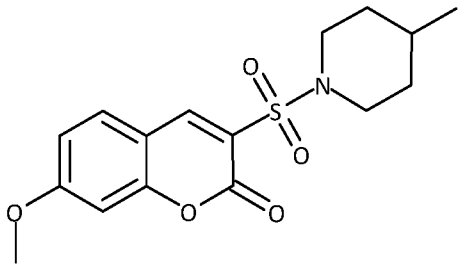


Compound C304

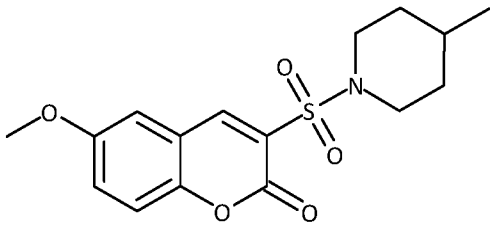


Compound C305

[00319]

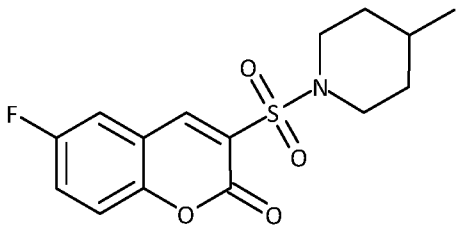


Compound C306

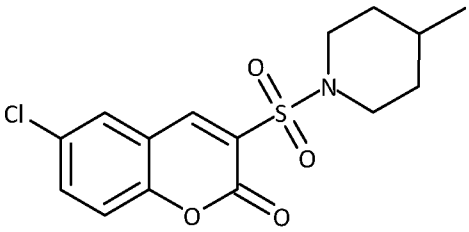


Compound C307

[00320]

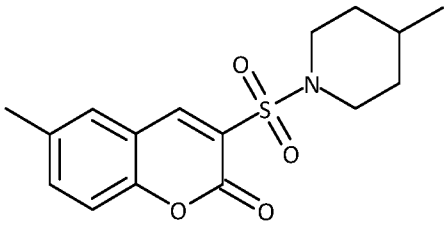


Compound C308

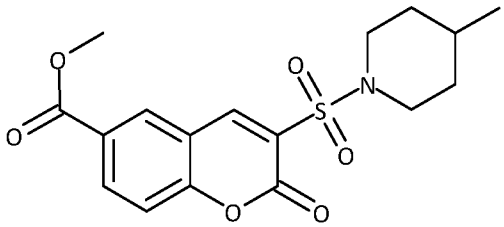


Compound C309

[00321]

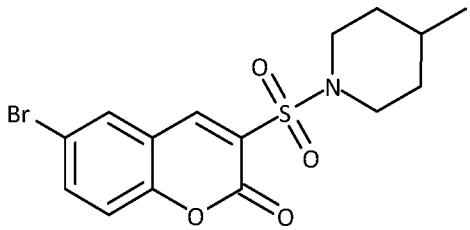


Compound C310

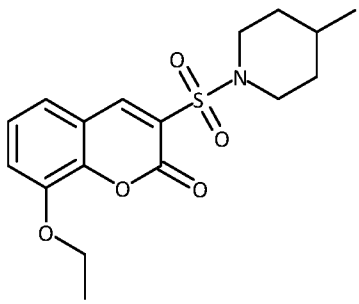


Compound C311

[00322]

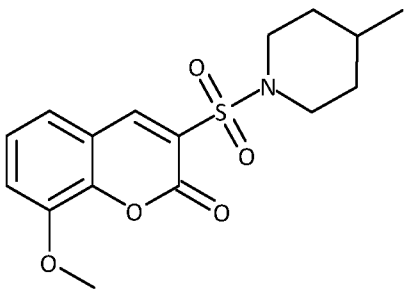


Compound C312

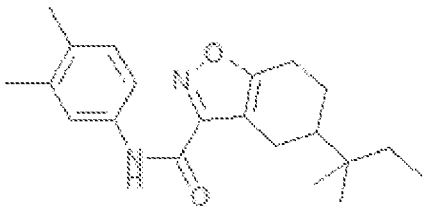


Compound C313

[00323]

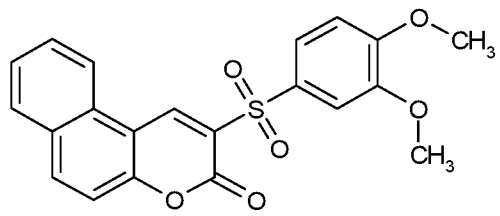


Compound C314

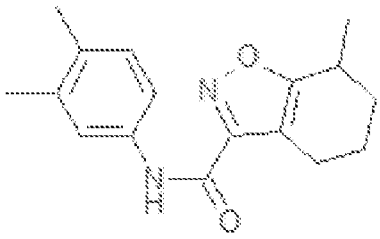


Compound Z170

[00324]

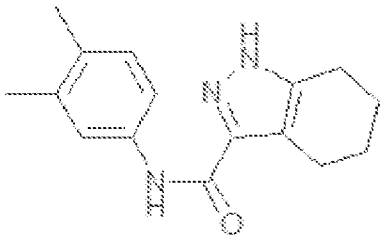


Compound C317

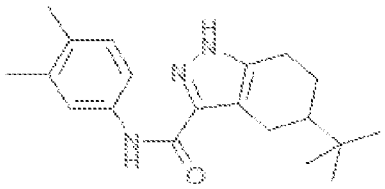


Compound Z171

[00325]

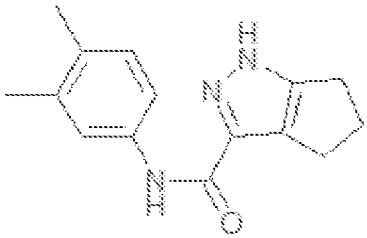


Compound Z172

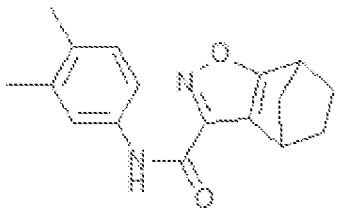


Compound Z173

[00326]

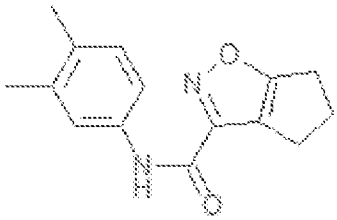


Compound Z174

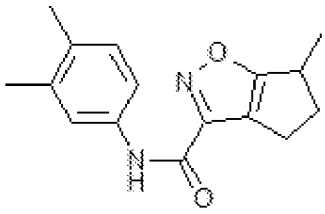


Compound Z175

[00327]

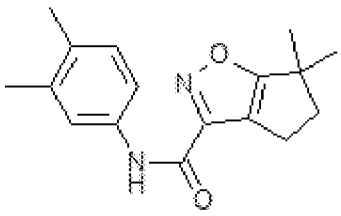


Compound Z176

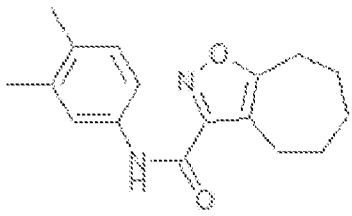


Compound Z177

[00328]

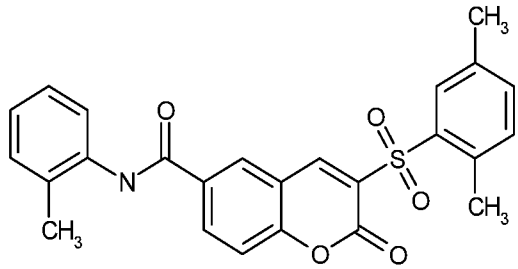


Compound Z178

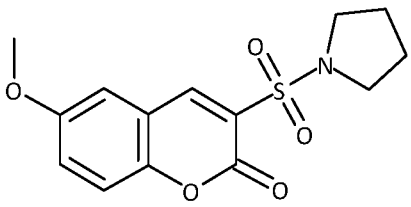


Compound Z179

[00329]



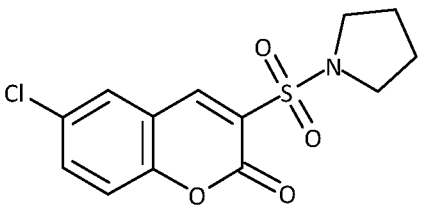
Compound C319



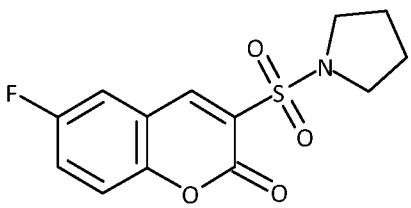
Compound F14

\

[00330]

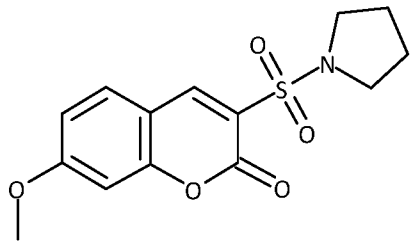


Compound F15

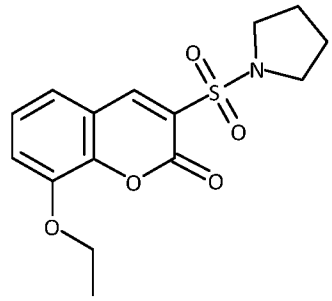


Compound F13

[00331]

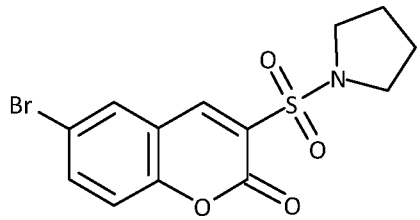


Compound F16

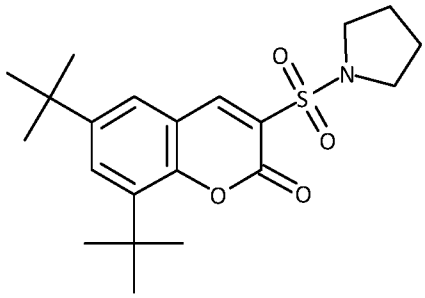


Compound F17

[00332]

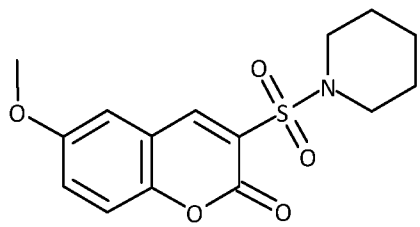


Compound F18

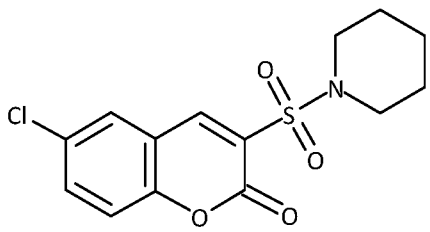


Compound F19

[00333]

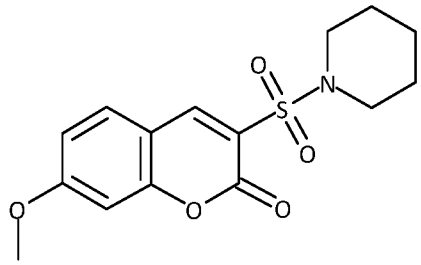


Compound F20

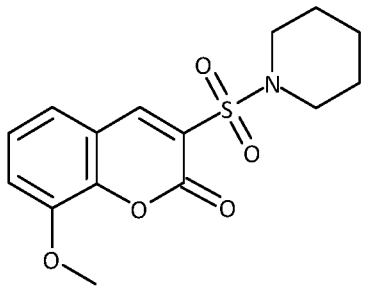


Compound F21

[00334]

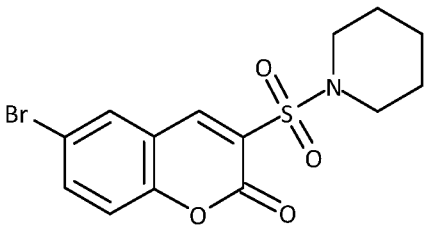


Compound F22

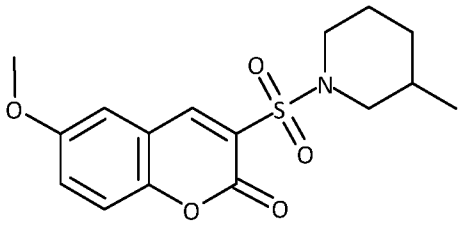


Compound F23

[00335]

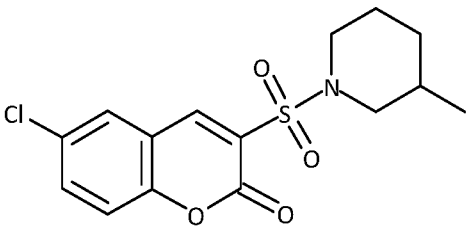


Compound F24

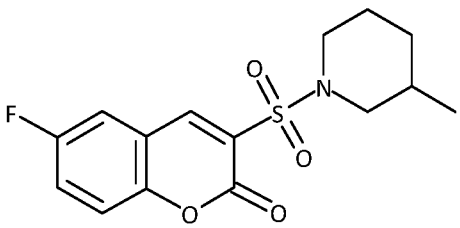


Compound F25

[00336]

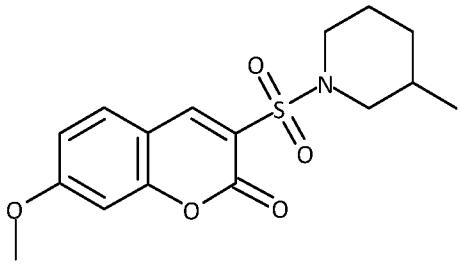


Compound F26

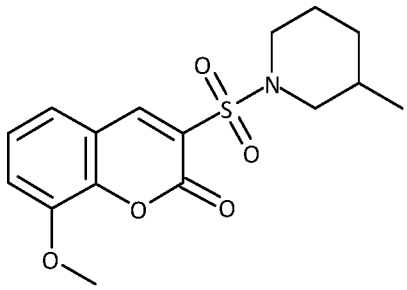


Compound F27

[00337]

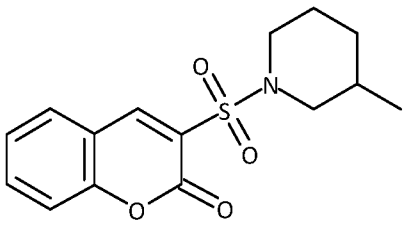


Compound F28

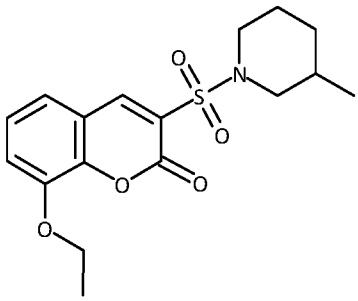


Compound F29

[00338]

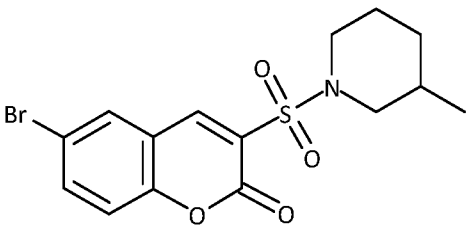


Compound F30

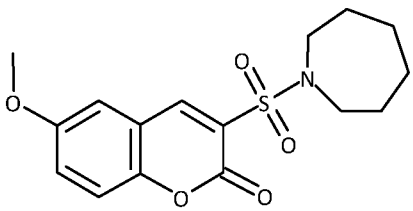


Compound F31

[00339]

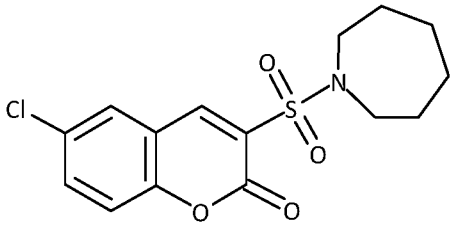


Compound F32

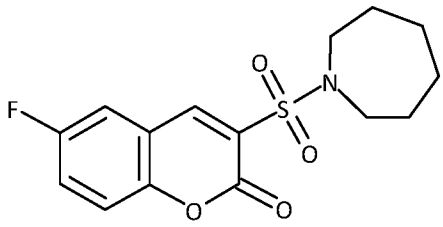


Compound F33

[00340]

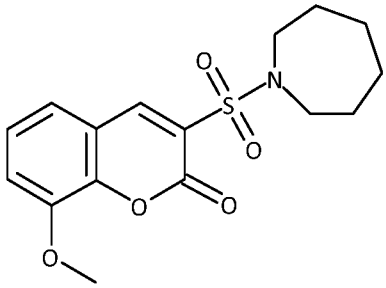


Compound F34

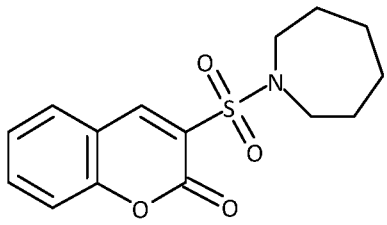


Compound F35

[00341]

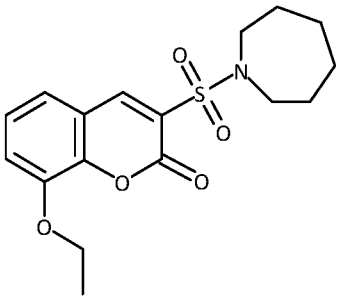


Compound F36

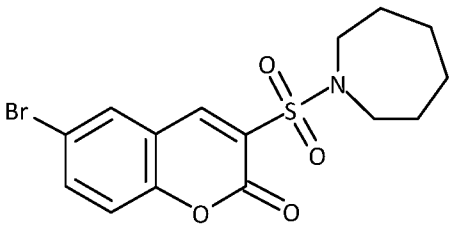


Compound F37

[00342]

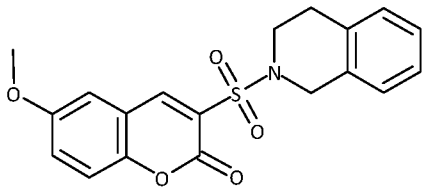


Compound F38

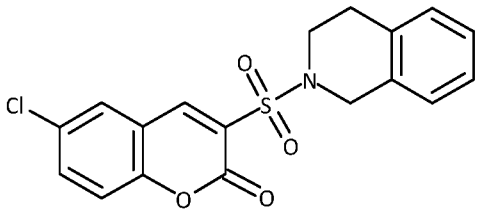


Compound F39

[00343]

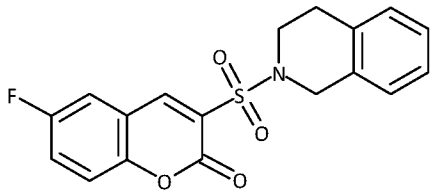


Compound F40

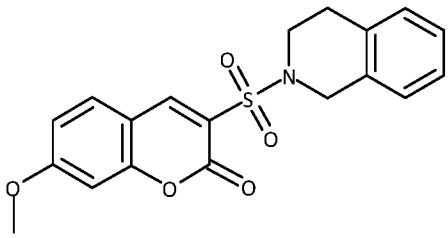


Compound F41

[00344]

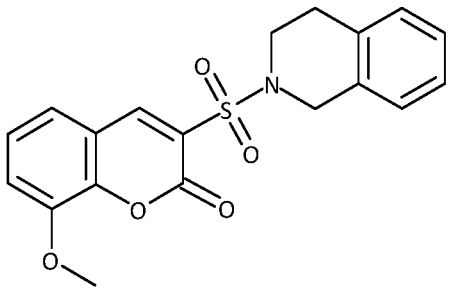


Compound F42

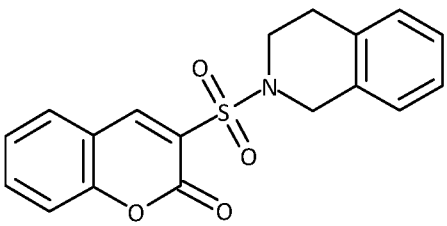


Compound F43

[00345]

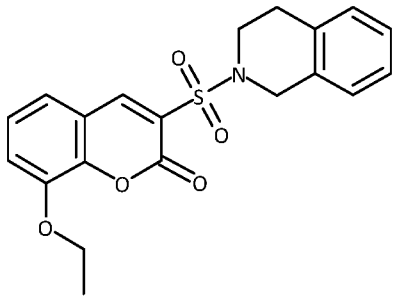


Compound F44

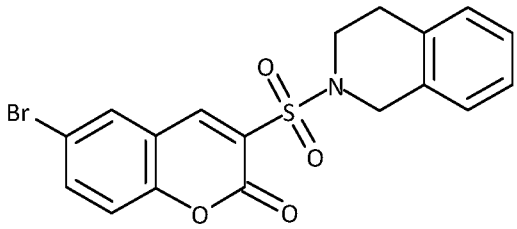


Compound F45

[00346]

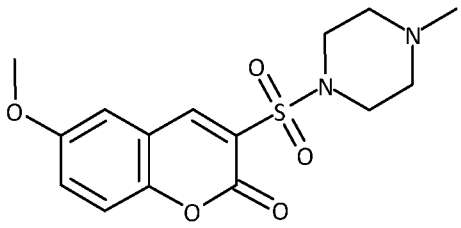


Compound F46

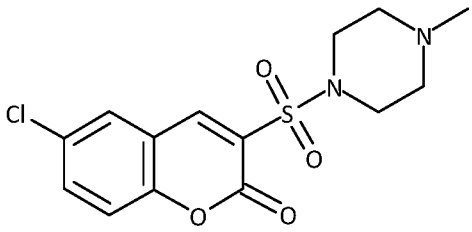


Compound F47

[00347]

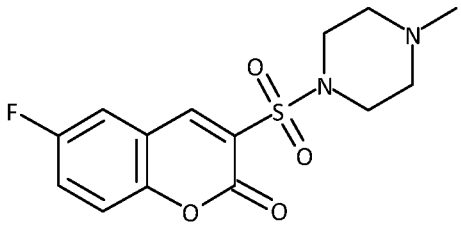


Compound F48

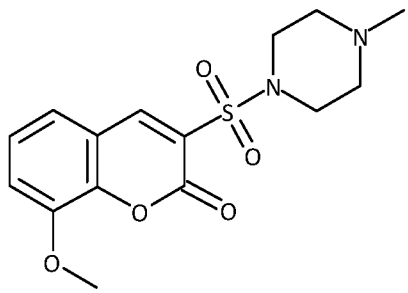


Compound F49

[00348]

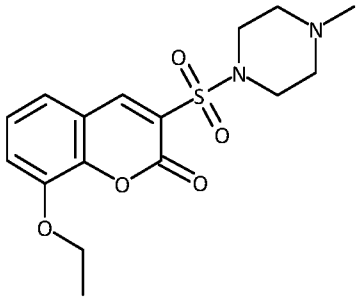


Compound F50

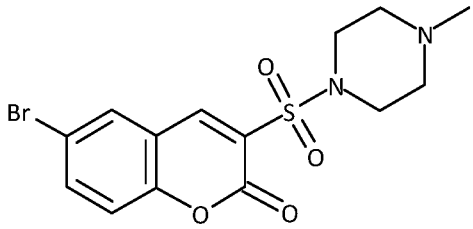


Compound F51

[00349]

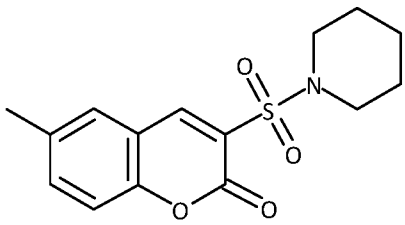


Compound F52

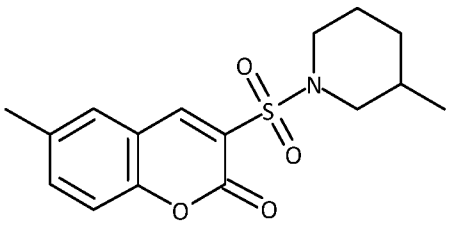


Compound F53

[00350]

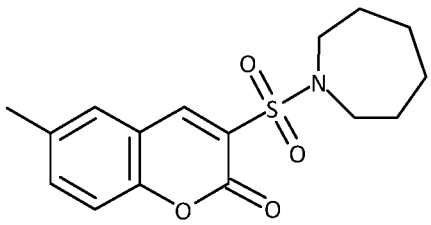


Compound F54

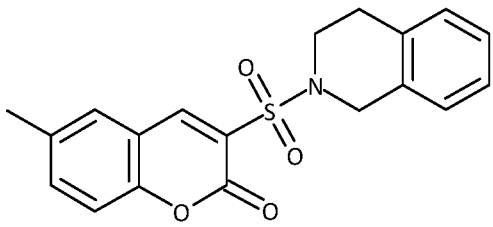


Compound F55

[00351]

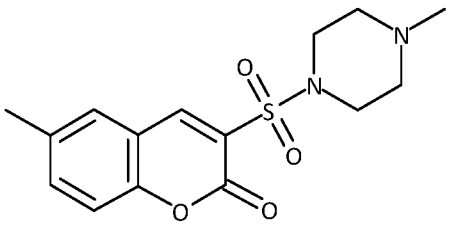


Compound F56

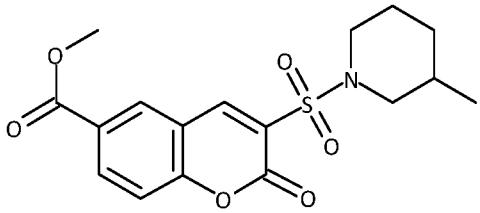


Compound F57

[00352]

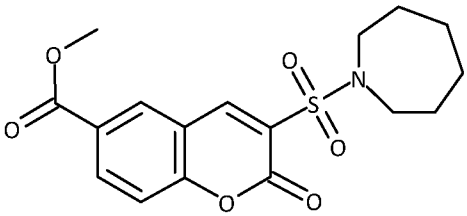


Compound F58

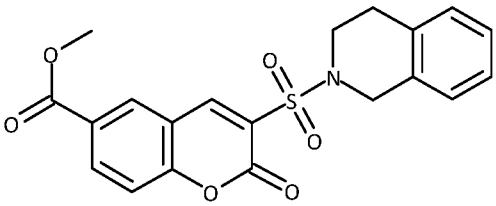


Compound F59

[00353]

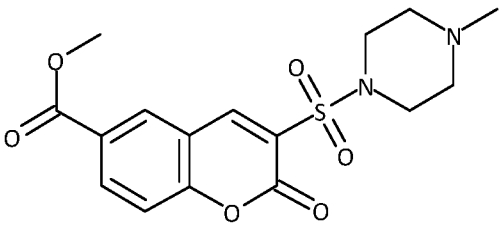


Compound F60

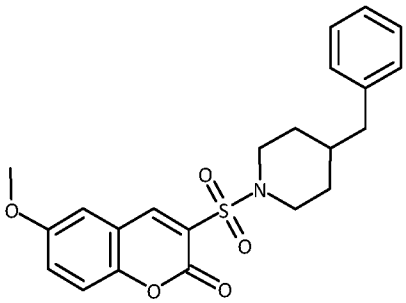


Compound F61

[00354]

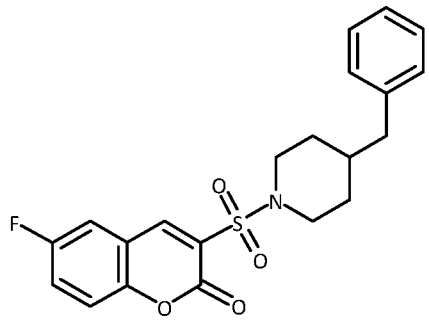


Compound F62

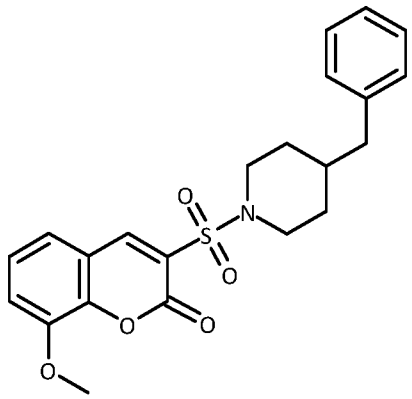


Compound F63

[00355]

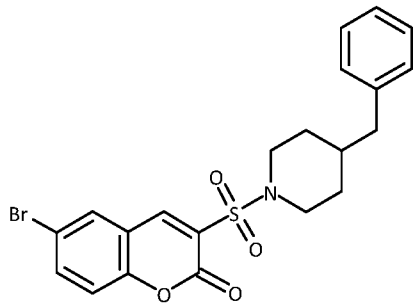


Compound F64

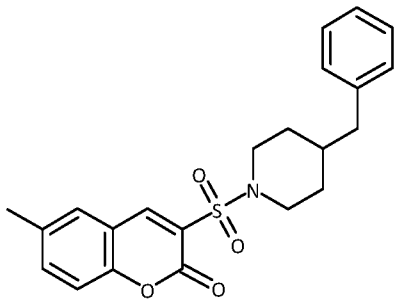


Compound F65

[00356]

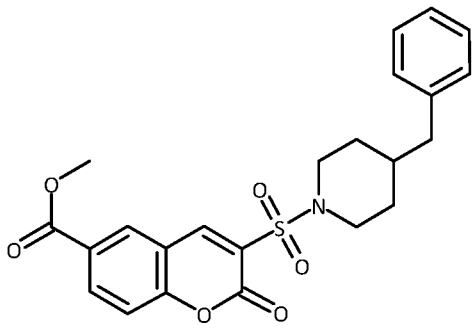


Compound F66



Compound F67

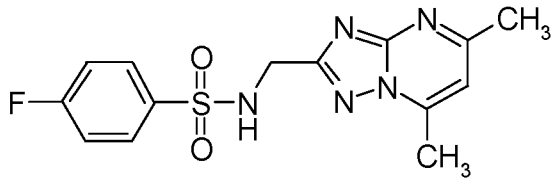
[00357]



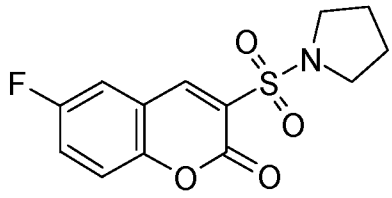
Compound F68

[00358] Further exemplary compounds tested in accordance with the various Formulas disclosed herein include the following:

[00359]

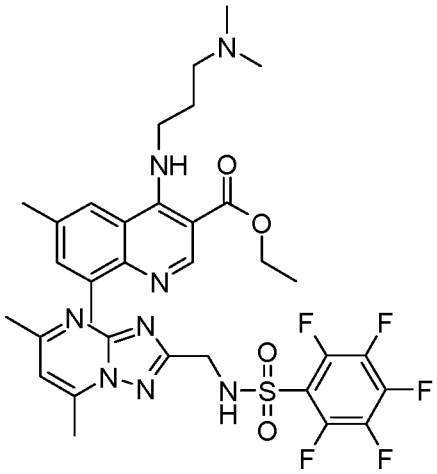


Compound E12

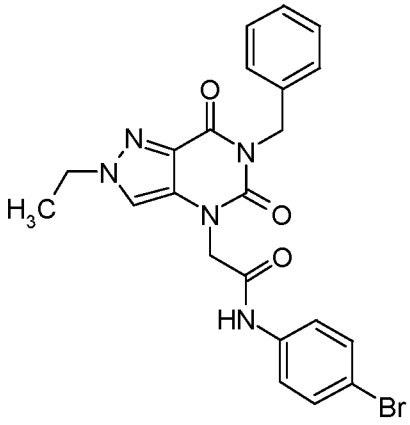


Compound F13

[00360]



Compound E22



Compound L1

[00361] Tables 12 and 13 show a summary of selected compounds for CRC analysis.

ID NUMBER	Firefly, cmpd/DMSO		Renilla, cmpd/DMSO		Firefly_pGL, cmpd/DMSO		Firefly/Renilla (NR2F6 stable,clone F4)	Firefly, NR2F6 stable (clone F4)/pGL4
	repeat 1	repeat 2	repeat 1	repeat 2	repeat 1	repeat 2		
C289	31.3	15.6	13.5	10.0	1.7	1.3	2.0	15.3
C155	37.3	33.3	19.3	15.4	3.5	3.0	2.0	10.9
C157	24.9	20.4	6.9	6.0	2.3	2.1	3.5	10.2
C299	21.5	15.2	5.5	3.8	1.8	1.8	4.0	10.1
C218	18.2	19.1	11.9	10.2	1.9	2.0	1.7	9.5
C177	17.4	8.2	6.7	4.6	1.7	1.3	2.3	8.5
C164	29.3	19.6	13.8	8.9	3.1	2.8	2.1	8.2
C136	22.1	25.8	12.5	14.9	2.9	3.0	1.7	8.1

C134	38.1	31.9	21.0	18.9	3.7	5.4	1.8	7.7
C195	38.7	29.4	22.8	10.1	3.7	5.1	2.1	7.7
C160	22.9	20.2	9.5	8.2	3.2	2.7	2.4	7.2
C11	24.6	21.3	13.9	12.3	3.3	3.3	1.8	7.0
C12	20.0	16.7	4.5	9.4	2.6	2.9	2.6	6.6
C230	12.5	8.3	4.5	3.8	1.8	1.5	2.5	6.5
C202	15.7	14.1	8.3	8.9	2.6	2.3	1.7	6.1
C102	13.0	15.3	5.8	6.1	2.3	2.5	2.4	5.9
C108	7.9	7.3	3.0	2.8	1.3	1.5	2.6	5.5
C248	8.6	4.3	2.8	1.9	1.4	1.4	2.8	4.6
C256	11.5	7.9	5.1	3.5	2.0	2.4	2.3	4.5
C254	7.6	5.1	2.7	2.5	1.5	1.4	2.4	4.4
C13	14.0	12.9	6.4	8.2	3.2	3.2	1.8	4.2
C112	11.7	10.1	5.6	5.1	2.5	2.8	2.0	4.1
C110	7.6	7.2	2.3	2.1	1.7	2.0	3.3	3.9
F312-0003	4.6	4.2	1.1	1.1	1.1	1.3	4.0	3.7
C101	4.5	4.7	1.8	2.0	1.2	1.3	2.4	3.6

Table 12

ID NUMBER	Firefly, cmpd/DMSO		Renilla, cmpd/DMSO		Firefly_pGL, cmpd/DMSO		Firefly/Renilla (NR2F6 stable,clone F4)	Firefly, NR2F6 stable (clone F4)/pGL4
	repeat 1	repeat 2	repeat 1	repeat 2	repeat 1	repeat 2		
C222	4.5	3.7	2.3	1.8	1.0	1.3	2.0	3.6
C105	5.7	4.8	1.7	2.3	1.5	1.8	2.6	3.1
C209	3.1	4.2	0.9	1.1	1.3	1.1	3.6	3.1
C109	9.2	10.3	3.4	3.3	3.5	3.4	2.9	2.9
C213	14.0	13.6	9.0	8.1	1.2	1.7	1.6	9.6
C300	9.8	7.4	6.2	4.7	1.7	2.3	1.6	4.3
C154	35.4	35.4	25.6	19.5	4.0	3.8	1.6	9.1
C14	22.3	20.0	12.1	15.2	2.9	2.6	1.5	7.6
C210	22.8	14.3	15.0	9.8	2.4	2.1	1.5	8.2
C107	11.0	11.0	8.7	6.5	1.7	2.1	1.4	5.8
C137	22.6	23.4	17.6	15.7	2.7	2.9	1.4	8.4
C161	35.4	39.0	28.6	27.7	4.0	5.1	1.3	8.1

C194	10.6	6.3	7.2	6.2	1.3	1.3	1.3	6.6
C188	9.4	8.7	7.6	6.9	1.8	1.8	1.2	5.0
C182	16.2	17.3	15.1	13.2	2.0	2.1	1.2	8.2
C200	11.0	8.1	5.8	10.4	1.6	1.9	1.2	5.4
C220	10.4	6.5	8.1	6.7	1.3	1.5	1.1	6.2
C118	13.5	17.1	11.8	14.8	2.4	2.7	1.1	5.9
C292	18.3	14.8	15.6	13.4	1.7	1.7	1.1	9.7
C180	34.0	34.5	30.9	30.0	2.5	2.7	1.1	13.1
C175	13.2	11.9	10.5	12.0	2.5	1.9	1.1	5.7
C111	29.8	35.9	29.4	30.8	3.7	3.9	1.1	8.7
C197	17.6	14.8	16.1	13.7	2.6	2.5	1.1	6.3
C127	36.7	56.2	43.1	43.5	8.3	8.0	1.1	5.7
C126	45.1	44.8	43.5	42.2	4.8	4.3	1.0	9.9

Table 13

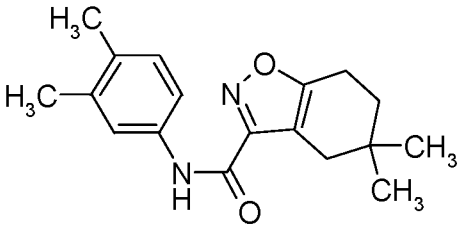
Compound ID	Firefly						Renilla		
	F4, cmpd/DMSO (mean)			pGL4, cmpd/DMSO (mean)			F4, cmpd/DMSO (mean)		
	40uM	10uM	2uM	40uM	10uM	2uM	40uM	10uM	2uM
17	1.3	1.2	1.9	1.3	1.6	1.7	1.0	1.0	0.8
19	0.5	1.0	1.7	0.9	1.3	1.9	0.6	1.0	1.1
22	0.6	1.4	1.3	1.5	1.0	1.6	1.0	1.1	0.8
C1	0.4	0.5	0.5	0.6	0.7	0.7	0.2	0.2	2.1
C3	0.9	1.1	1.5	1.0	1.3	1.1	0.9	0.9	1.0
C4	0.7	0.8	1.1	1.3	0.9	1.0	0.5	0.5	0.6
C5	0.8	0.8	0.9	1.0	1.2	1.3	1.1	0.5	0.7
C6	0.4	0.6	1.4	1.0	1.3	1.3	0.5	0.6	0.7
C301	1.0	1.0	1.2	1.1	1.6	2.4	1.0	0.7	1.1
C302	0.9	0.9	1.2	0.8	1.4	1.8	1.0	1.1	0.7
C303	0.9	0.9	1.2	1.1	1.1	1.1	1.0	0.8	1.0
C7	0.4	0.5	0.6	0.8	0.8	0.9	0.4	0.3	1.0
C11	0.4	1.6	1.7	0.8	1.7	1.1	0.2	1.2	1.1
E12	0.5	0.9	0.7	1.0	0.8	0.9	1.0	0.7	0.7
E53	1.4	1.1	1.0	1.6	1.4	1.5	1.1	0.8	0.9
L1	0.5	0.5	0.9	0.8	0.4	0.8	0.7	0.8	0.8
Z54	0.6	1.6	0.8	0.8	1.9	1.1	0.2	0.3	0.7
Z55	2.0	0.8	1.1	2.3	0.7	0.8	0.1	0.3	0.8
Z56	0.5	2.1	1.2	1.2	8.9	1.2	0.2	0.3	0.9
Z74	0.7	0.5	0.7	0.7	0.6	0.7	0.3	0.2	0.8
Z79	0.3	0.5	0.9	0.8	0.5	0.8	0.2	0.6	0.9
Z81	0.4	0.7	2.4	0.7	0.9	1.4	0.3	0.3	1.1

Z83	0.7	0.6	1.2	0.5	0.9	1.0	0.3	0.8	1.0
Z90	0.4	2.5	0.7	0.7	2.4	0.7	0.1	0.2	0.9
Z91	1.3	1.1	1.0	1.1	1.0	1.0	0.8	1.1	1.1

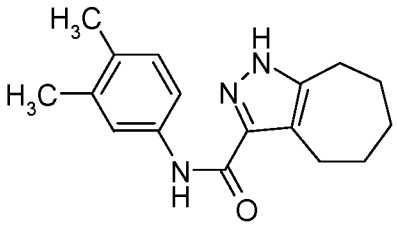
Table 14

[00362] Additional compounds were tested, including the following:

[00363]

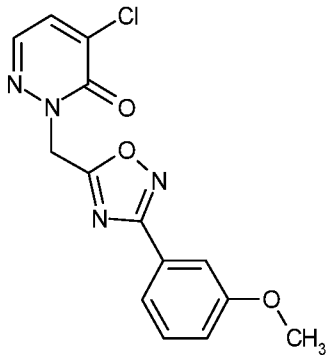


Compound Z1

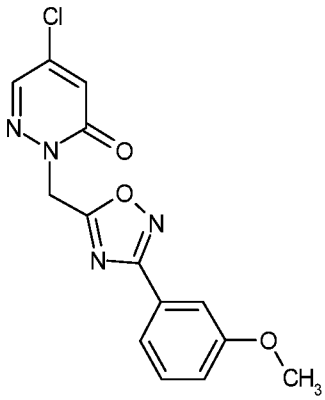


Compound Z2

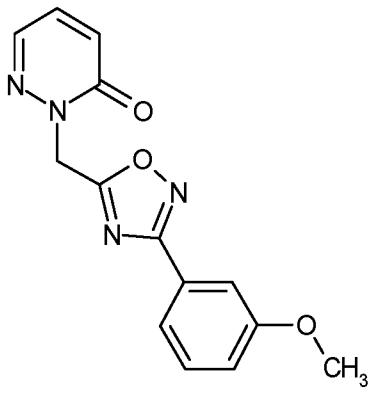
[00364]



Compound Z3

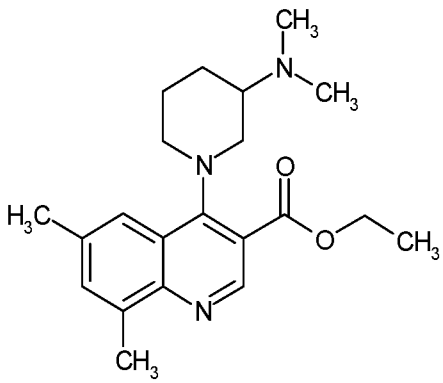


Compound Z4



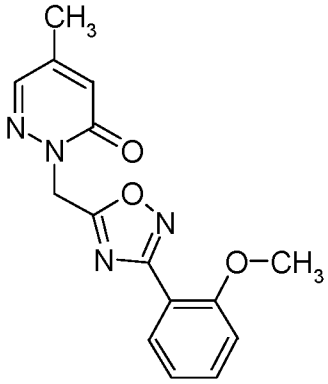
Compound Z5

[00365]

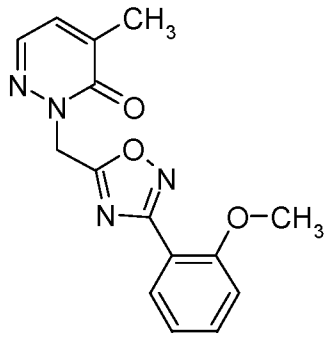


Compound Z27

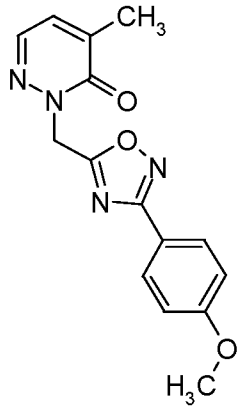
[00366]



Compound Z150

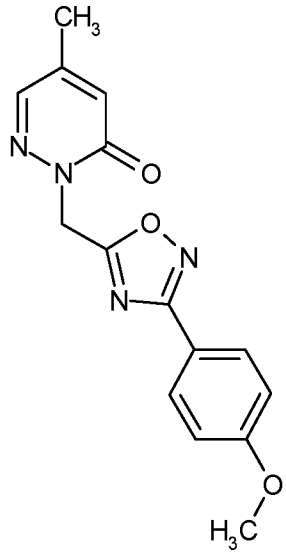


Compound Z151



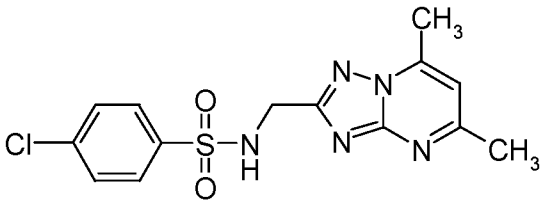
Compound Z153

[00367]

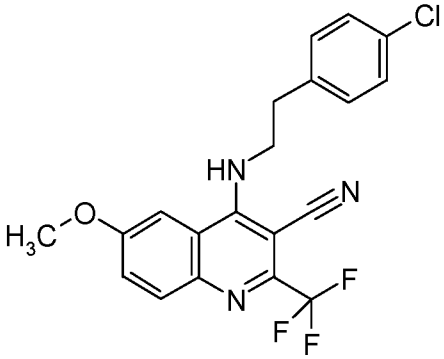


Compound Z152

[00368]

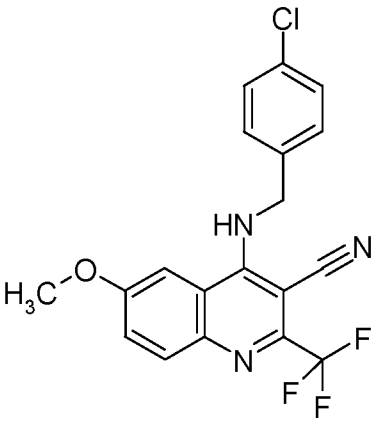


Compound Z91

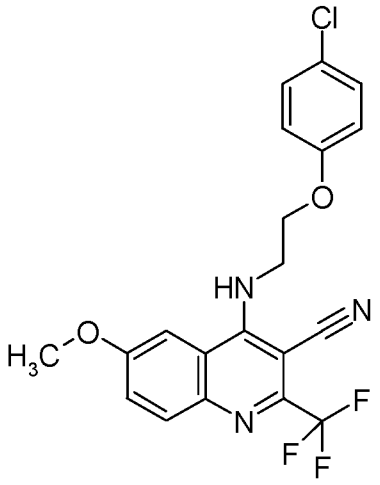


Compound Z39

[00369]

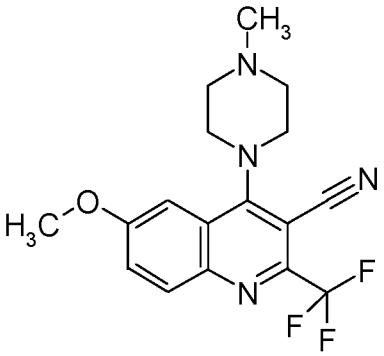


Compound Z38

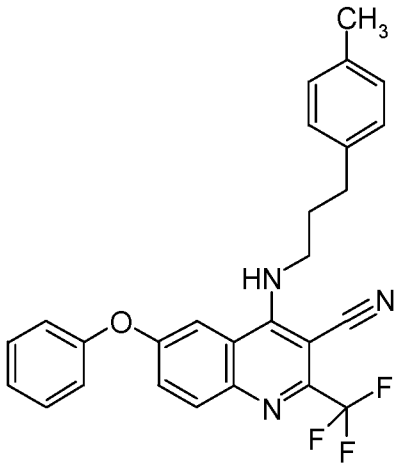


Compound Z40

[00370]

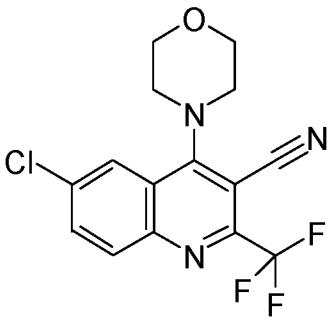


Compound Z41

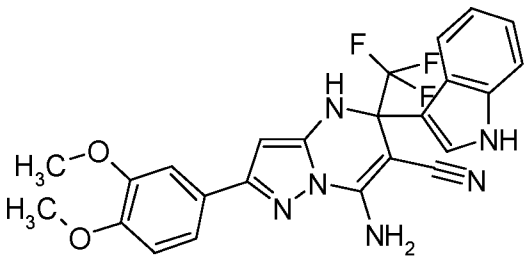


Compound Z42

[00371]

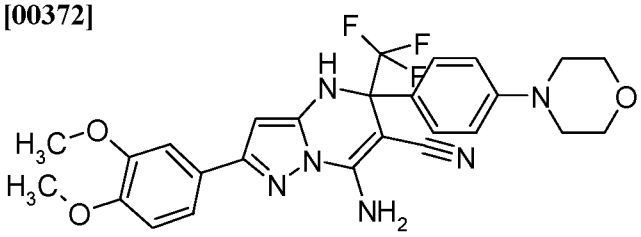


Compound Z43

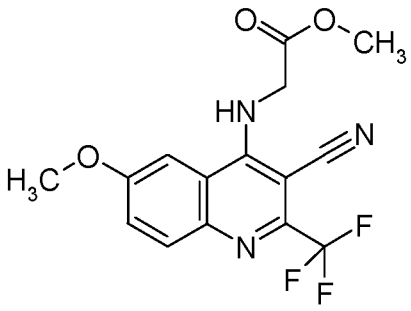


Compound Z44

[00372]

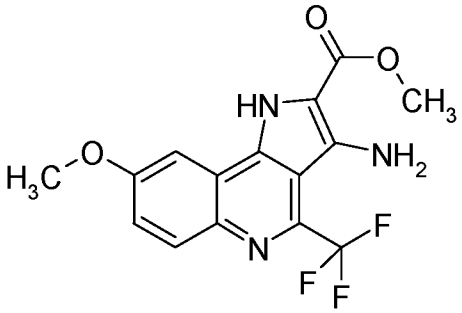


Compound Z45

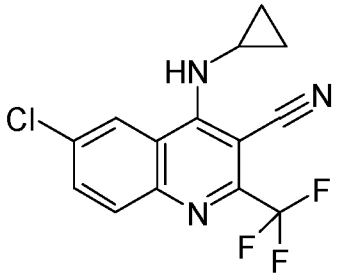


Compound Z46

[00373]

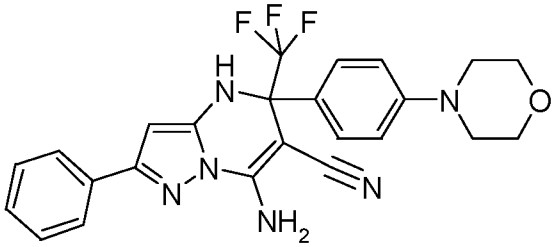


Compound Z47

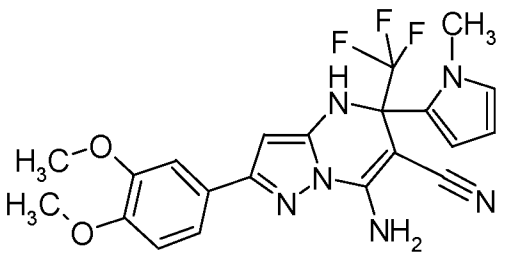


Compound Z48

[00374]

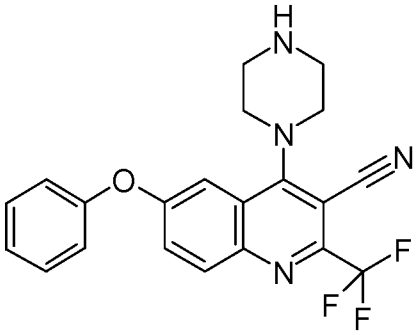


Compound Z49

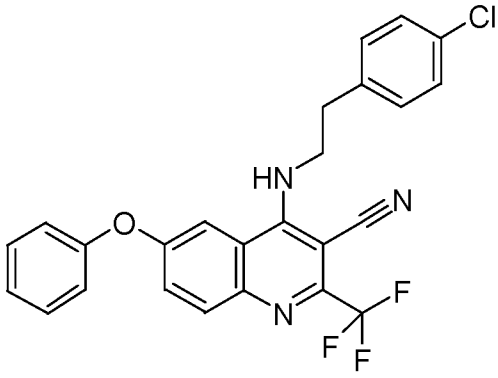


Compound Z50

[00375]

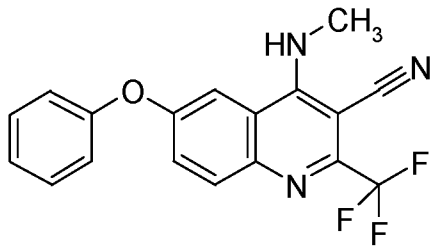


Compound Z51



Compound Z52

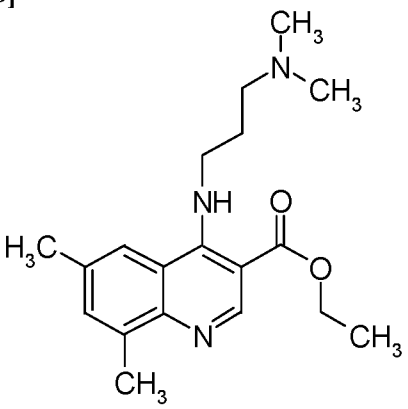
[00376]



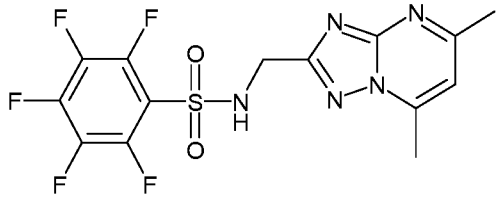
Compound Z53

[00377] Compounds Z54, Z55 and Z56 were found to have particularly good activity:

[00378]

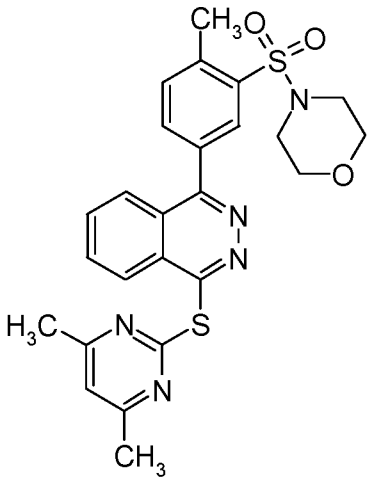


Compound Z54



Compound Z55

[00379]



Compound Z56

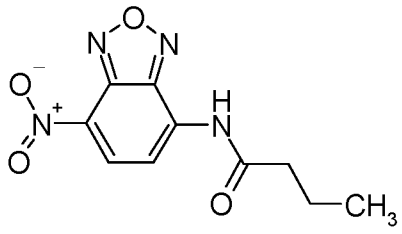
[00380] Table 15 shows screening results from another set of compounds.

Compound ID	Firefly						Renilla					
	F4, cmpd/DMSO (mean)			ERalpha transient, cmpd/DMSO (mean)			F4, cmpd/DMSO (mean)			ERalpha transient, cmpd/DMSO (mean)		
	40uM	10uM	2uM	40uM	10uM	2uM	40uM	10uM	2uM	40uM	10uM	2uM
Z1	0.8	1.8	1.9	0.9	2.2	2.4	0.8	1.6	1.3	0.7	1.0	1.1
Z2	0.6	1.9	1.4	0.7	1.1	1.6	1.2	1.8	1.2	1.0	1.1	1.2
Z3	1.6	1.1	1.5	1.7	2.6	2.2	0.9	1.4	1.5	0.9	1.0	1.1
Z4	0.8	1.2	1.5	0.8	1.4	1.5	1.4	1.6	1.1	0.8	1.1	1.2
Z5	1.2	0.9	1.5	2.4	2.5	2.4	1.2	0.9	1.0	1.0	1.0	1.0
Z6	1.1	1.4	1.4	1.1	2.2	2.0	0.9	1.1	1.1	0.9	1.1	1.1
Z7	1.0	1.2	0.9	1.5	2.0	1.3	1.1	1.1	1.3	1.0	1.1	1.1
Z8	0.7	4.0	2.6	0.4	2.4	2.2	0.2	9.1	2.7	0.1	1.4	1.4
Z9	1.2	6.7	2.5	0.5	2.7	2.2	0.3	4.9	2.1	0.2	0.8	1.3
Z10	0.7	1.6	5.8	0.5	1.5	4.2	0.3	3.3	3.4	0.2	0.8	1.3
Z11	0.5	1.2	3.5	0.6	1.6	2.1	0.3	2.6	2.9	0.2	0.8	1.4
Z12	0.4	1.8	3.9	0.4	1.3	2.4	0.4	2.7	2.7	0.2	0.7	1.3
Z13	1.6	0.9	1.0	1.1	1.7	1.2	0.4	0.7	0.8	0.4	0.6	0.8
Z14	1.2	0.8	1.2	1.0	1.2	1.0	1.0	0.9	0.8	1.0	1.1	1.1
Z15	0.9	0.5	1.0	1.1	1.1	0.9	1.0	1.1	0.8	0.9	1.0	1.0
Z58	0.8	4.5	0.9	0.8	6.5	1.5	0.8	1.2	1.3	0.7	1.1	1.2
Z17	0.6	6.5	1.5	2.9	9.5	1.7	0.6	1.1	1.2	0.6	1.1	1.3
Z61	0.8	2.5	1.7	0.7	9.1	3.0	0.6	0.5	1.3	0.7	0.8	1.4
Z19	0.7	6.3	1.3	0.7	12.7	1.9	0.7	0.9	1.1	0.6	1.0	1.5
Z67	0.7	0.5	1.3	0.5	1.4	1.4	0.6	1.0	1.2	0.6	1.0	1.1
Z68	1.2	1.1	1.5	0.7	1.5	1.1	0.6	1.0	0.9	0.6	0.9	1.1
Z70	1.0	1.0	1.1	1.5	1.1	0.9	1.3	1.3	0.8	1.2	1.0	1.1
Z71	0.8	1.0	0.8	0.4	1.0	1.4	0.4	0.9	1.1	0.5	1.0	1.0
Z75	0.8	1.2	0.9	0.9	1.9	1.1	0.8	1.0	1.1	0.7	0.9	1.0
Z76	0.8	1.8	0.8	0.7	1.9	1.3	0.5	0.8	1.0	0.4	0.8	1.1
Z78	1.6	1.5	1.4	1.1	2.4	1.5	0.8	1.0	1.1	0.6	0.9	1.0

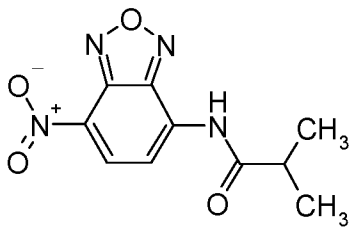
Table 15

[00381] Compounds Z8-Z12, Z17 and Z19 were found to have particularly good activity.

[00382]

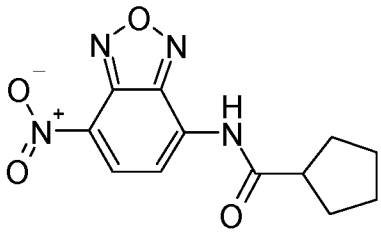


Compound Z8

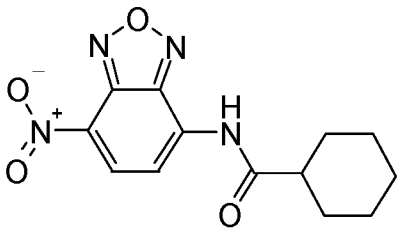


Compound Z9

[00383]

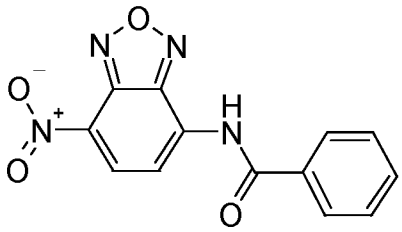


Compound Z10



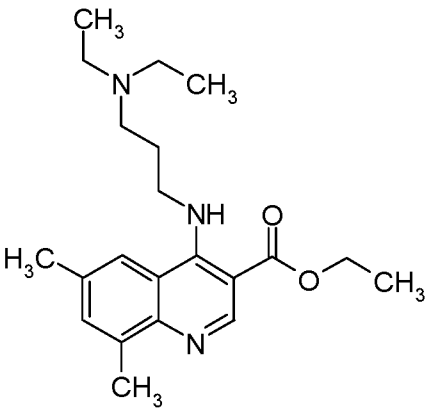
Compound Z11

[00384]

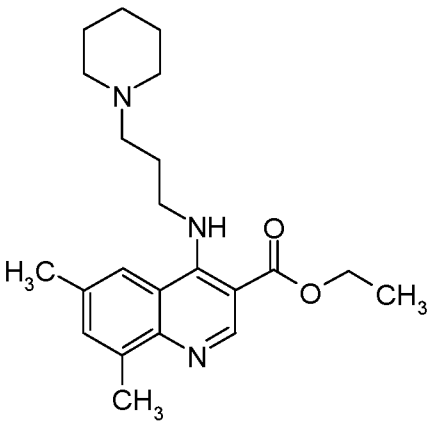


Compound Z12

[00385]



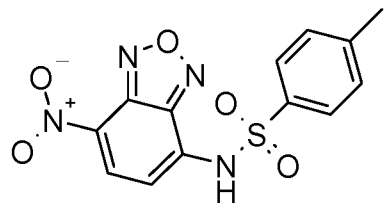
Compound Z17



Compound Z19

[00386] Dog's PMBC ELISA and cytotoxicity experiments were performed on Compound Z92, which also showed good activity. Results are shown in **FIGS. 19A** and **19B**.

[00387] Another useful compound is Compound Z95:



Compound Z95

[00388] Results of testing done on compound Z95 are shown in Table 16.

ID	NR2F6_full (stable, clone F4), cmpd/DMSO						NR2F6_full (transient), cmpd/DMSO						
	Firefly			Renilla				Firefly			Renilla		
	40uM	10uM	2uM	40uM	10uM	2uM		40uM	10uM	2uM	40uM	10uM	2uM
Z95	3.7	2.2	1.2	0.5	0.5	1.1		2.3	1.3	1.2	0.2	0.4	1.1

Table 16

[00389] **FIGS. 20A** and **20B** show further results of cytokines release by hPBMC and cytotox on Compound Z95.

[00390] Table 17 shows screening results from another set of compounds.

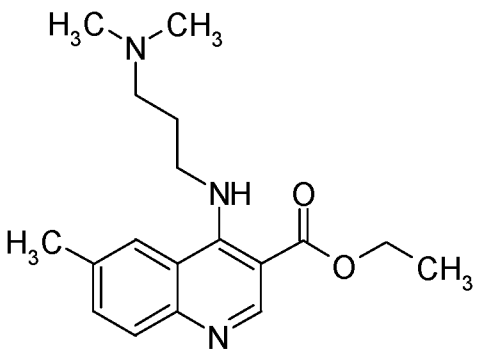
Compound ID	Firefly						Renilla					
	F4, cmpd/DMSO (mean)			ERalpha transient, cmpd/DMSO (mean)			F4, cmpd/DMSO (mean)			ERalpha transient, cmpd/DMSO (mean)		
	40uM	10uM	2uM	40uM	10uM	2uM	40uM	10uM	2uM	40uM	10uM	2uM
Z27	0.7	1.3	1.3	0.5	2.1	1.3	0.9	1.2	1.2	0.8	1.2	1.2
Z28	1.3	0.9	1.0	1.3	1.1	1.8	1.2	1.2	1.0	1.0	1.3	1.1
Z29	1.1	1.1	1.3	6.7	1.2	1.0	0.5	0.9	0.8	0.7	1.2	0.8
Z30	1.2	1.1	1.4	1.4	1.1	1.4	0.7	1.1	1.1	1.6	1.1	1.1
Z31	1.1	0.9	0.8	1.3	1.6	0.9	1.0	1.0	1.1	1.0	1.0	1.0

Z32	1.5	0.9	1.8	1.5	0.8	1.8	1.3	1.0	1.0	0.8	1.1	1.0
Z33	6.6	2.0	1.7	8.8	1.8	2.1	0.6	1.0	1.1	0.9	1.5	1.0
Z34	0.7	3.8	1.6	0.9	10.3	2.0	0.6	0.8	1.2	0.7	1.0	1.5
Z35	1.2	1.5	1.9	7.2	2.2	2.6	0.7	2.0	1.1	0.9	1.3	1.1
Z36	0.7	1.8	0.7	5.8	1.6	1.2	0.6	1.8	1.0	0.8	1.6	1.2
Z37	0.7	1.4	1.0	1.2	1.5	1.1	0.5	0.9	1.0	0.7	1.7	1.0
Z38	0.6	0.8	0.5	0.5	0.5	0.9	0.4	0.6	0.7	0.6	0.6	0.9
Z39	0.7	1.0	0.8	0.8	0.5	1.2	0.5	0.5	0.7	0.6	0.6	1.1
Z40	0.8	0.7	0.8	1.0	0.3	1.0	0.5	0.6	0.7	0.6	0.7	1.0
Z41	1.7	1.1	1.0	1.6	1.4	0.9	0.6	0.6	0.7	0.8	1.1	1.0
Z42	0.6	0.9	0.9	1.1	1.8	1.5	0.7	0.9	1.2	0.7	0.9	1.1
Z43	0.8	0.8	1.3	1.6	0.9	1.4	0.7	0.9	1.0	0.9	1.0	1.0
Z44	0.4	0.8	0.8	1.0	1.2	1.2	0.3	1.5	1.7	0.5	0.9	1.1
Z45	0.5	1.2	0.8	0.7	0.6	0.8	1.1	1.2	1.2	1.0	0.8	1.1
Z46	0.6	0.8	0.6	0.6	0.8	0.9	0.6	0.8	0.9	1.0	1.0	0.9
Z47	2.0	1.3	0.9	1.7	1.0	0.9	1.7	1.2	1.1	1.2	1.4	1.2
Z48	0.7	0.5	0.9	0.5	0.3	1.5	0.6	0.6	0.8	0.6	0.8	1.1
Z49	0.6	0.7	0.8	0.4	0.4	1.0	0.8	0.7	1.0	0.8	0.7	1.0
Z50	0.6	0.9	0.8	0.7	0.7	1.3	0.8	2.0	1.6	0.6	0.8	1.0
Z51	0.7	0.6	0.6	0.4	0.8	1.3	0.3	0.7	0.8	0.3	0.9	1.1
Z52	0.7	0.7	0.6	0.7	0.4	0.7	0.6	0.5	0.8	0.7	0.8	1.1
Z53	0.5	0.4	0.8	0.4	0.3	1.0	0.6	0.7	0.7	0.5	0.6	1.0

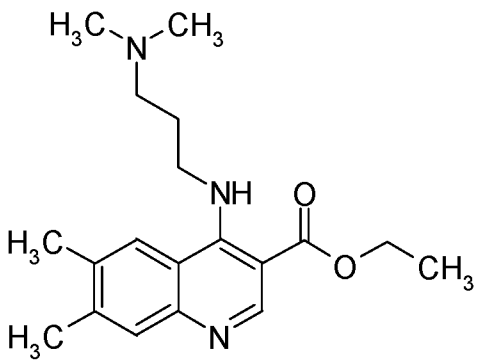
Table 17

[00391] Compounds Z33 and Z34 were found to have particularly good activity:

[00392]



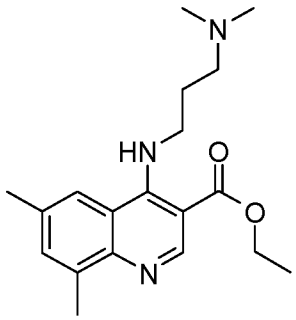
Compound Z33



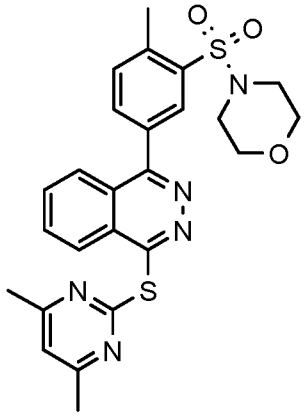
Compound Z34

[00393] Other useful compounds are Compound D28 and Compound F1:

[00394]



Compound D28



Compound F1

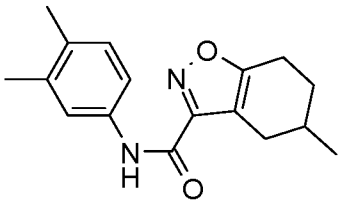
[00395] Compound D28 was tested in a cytokine release experiment – parent compound, dog's and human PMBC. Results are shown in **FIGS. 21A-21D**.

[00396] Compound Z17, previously mentioned above, was tested in a cytokine release experiment. Results are shown in **FIGS. 22A** and **22B**.

[00397] Compound Z33, previously mentioned above, was tested in a cytokine release experiment. Results are shown in **FIGS. 23A** and **23B**.

[00398] Another compound found to be useful is Compound E56:

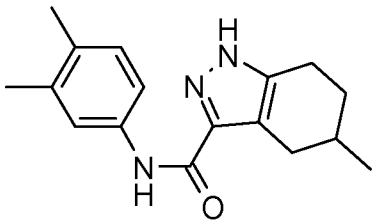
[00399]



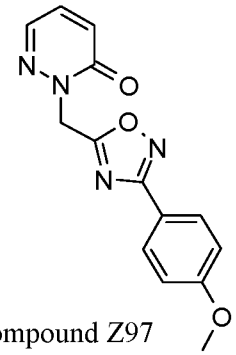
Compound E56

[00400] **FIG. 24** shows the results of testing done on Compound E56.

[00401] Additional compounds found to be useful are Compounds Z96 and Z97:



Compound Z96



Compound Z97

[00402] Results of testing done on compounds Z96 and Z97 are shown in Tables 18 and 19, respectively.

ID	NR2F6_full (stable, clone F4), cmpd/DMSO						NR2F6_full (transient), cmpd/DMSO						
	Firefly			Renilla				Firefly			Renilla		
	40uM	10uM	2uM	40uM	10uM	2uM	40uM		10uM	2uM	40uM	10uM	2uM
Z96	2.6	2.6	2.9	1.2	1.2	1.3		2.4	2.3	2.4	0.9	1.0	1.0

Table 18

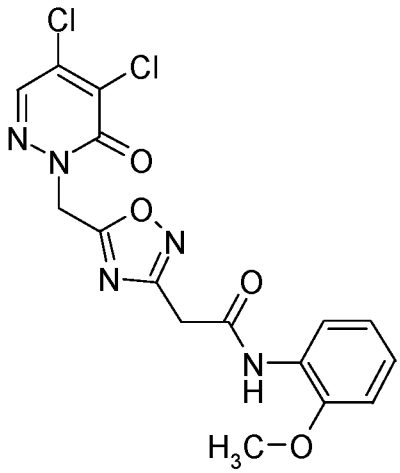
ID	NR2F6_full (stable, clone F4), cmpd/DMSO						NR2F6_full (transient), cmpd/DMSO						
	Firefly			Renilla				Firefly			Renilla		
	40uM	10uM	2uM	40uM	10uM	2uM	40uM		10uM	2uM	40uM	10uM	2uM
Z97	1.5	2.2	2.3	1.2	1.1	1.1		2.3	2.8	2.4	1.1	1.1	1.0

Table 19

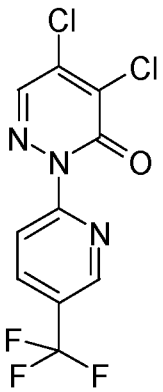
[00403] **FIGS. 25A** and **25B** show further results of testing done on Compound Z96. **FIGS. 26A** and **26B** show further results of testing done on Compound Z97.

[00404] Additional compounds found to be useful are Compounds Z93 and Z94:

[00405]



Compound Z93

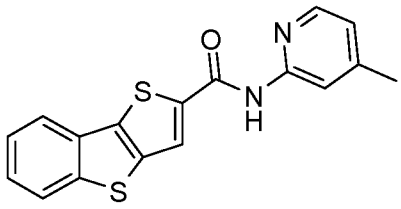


Compound Z94

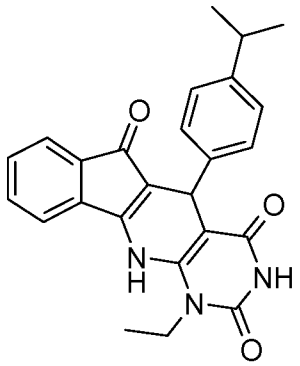
[00406] The results of dogs PBMC cytotox for Compounds Z93 and Z94 are included in **FIG. 19B**, which also shows results for the following Compounds: Z92, E54, E55 and E53.

[00407] Additional compounds tested included the following:

[00408]

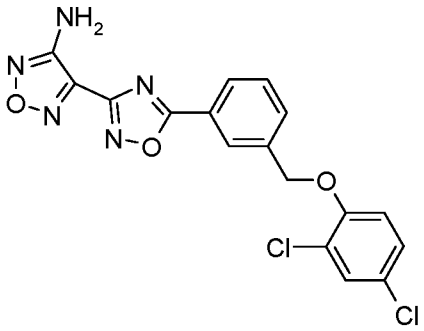


Compound 20

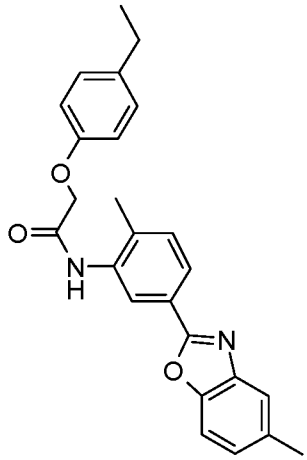


Compound 21

[00409]

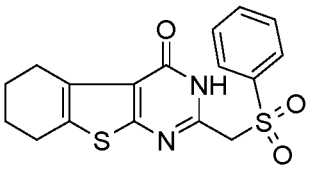


Compound 23

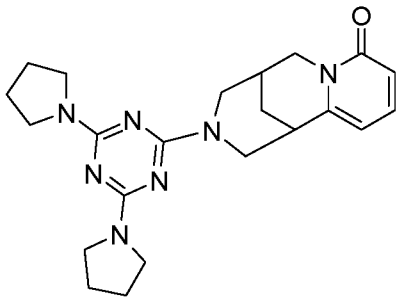


Compound 24

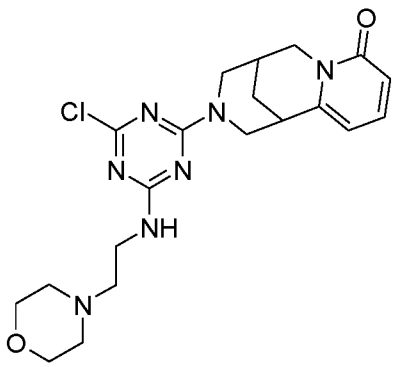
[00410]



Compound 25

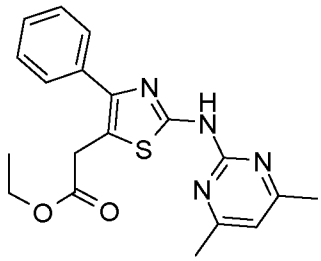


Compound 26

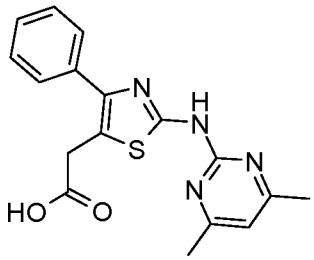


Compound 27

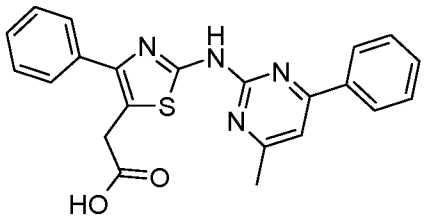
[00411]



Compound 28

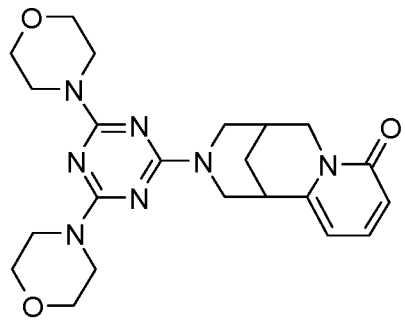


Compound 29

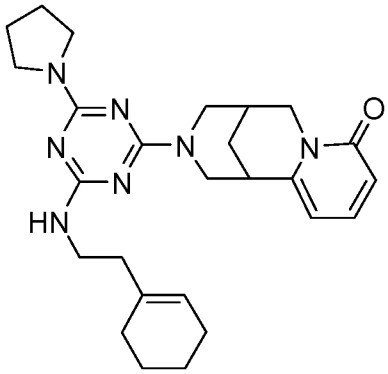


Compound 30

[00412]

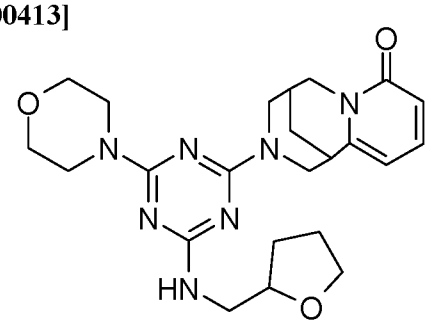


Compound 31

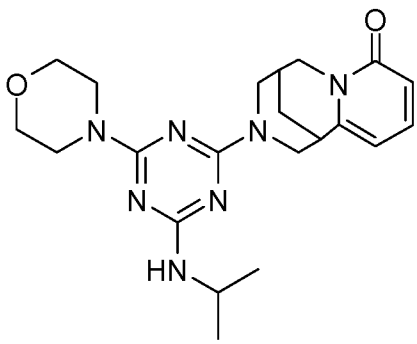


Compound 32

[00413]

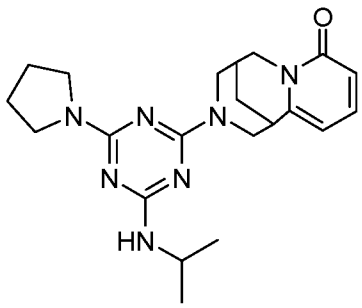


Compound 33

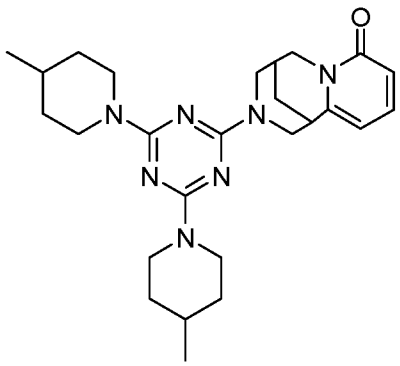


Compound 34

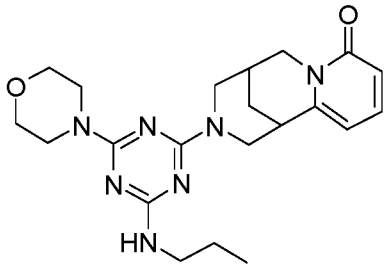
[00414]



Compound 35

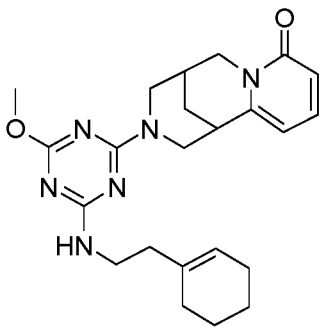


Compound 36

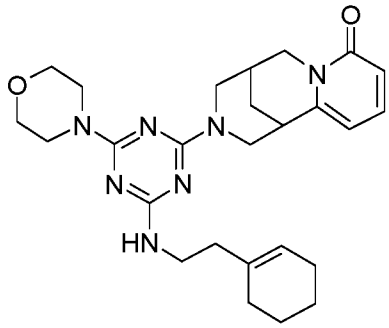


Compound 37

[00415]

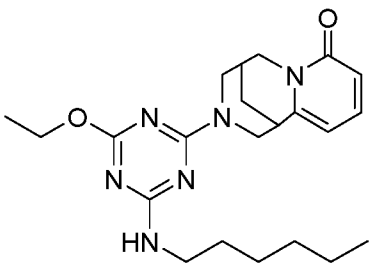


Compound 38

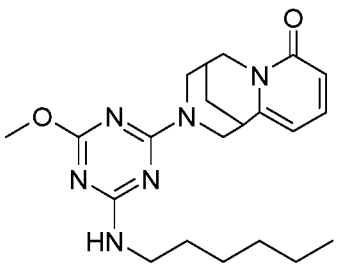


Compound 39

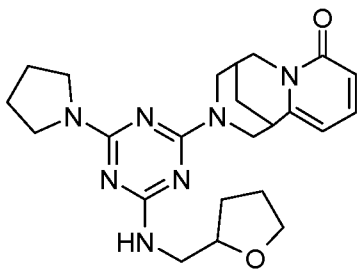
[00416]



Compound 40

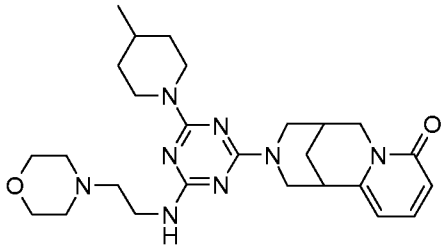


Compound 41

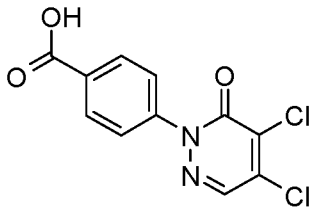


Compound 42

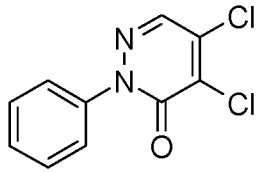
[00417]



Compound 43

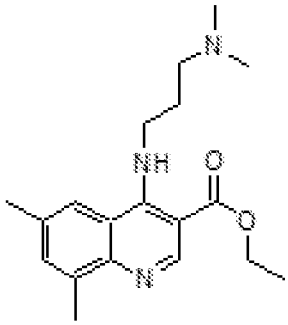


Compound 44



Compound 45

[00418]



Compound 47

[00419] Table 20 shows shows screening results from another set of compounds.

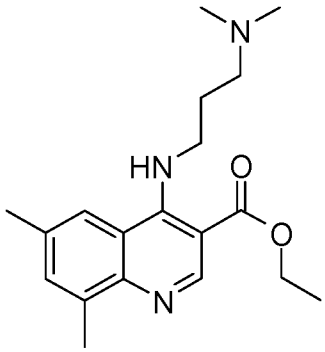
Compound ID	Firefly						Renilla					
	F4, cmpd/DMSO (mean)			ERalpha transient, cmpd/DMSO (mean)			F4, cmpd/DMSO (mean)			ERalpha transient, cmpd/DMSO (mean)		
	40uM	10uM	2uM	40uM	10uM	2uM	40uM	10uM	2uM	40uM	10uM	2uM
26	1.3	1.1	1.0	1.1	1.3	1.0	0.7	0.7	1.0	1.0	1.0	0.9
27	1.0	1.0	1.0	1.2	1.2	1.2	0.8	0.9	0.9	1.2	1.1	1.1
28	0.6	0.9	1.2	0.6	0.5	1.0	0.6	0.8	1.1	0.7	0.8	1.2
29	1.5	1.4	0.9	2.2	1.7	1.2	0.8	0.9	0.8	1.1	1.0	1.1
30	1.8	1.6	1.5	1.3	1.5	1.1	3.4	2.3	1.2	1.0	1.2	1.2
31	0.9	1.0	0.8	1.0	1.2	0.8	1.0	1.0	1.0	1.1	1.1	1.0
32	0.8	1.3	0.8	0.7	1.2	1.2	1.0	1.4	1.2	1.1	1.3	1.2
33	1.1	1.0	0.8	1.4	1.3	1.3	1.4	1.3	1.2	1.3	1.1	1.2
34	0.9	0.8	0.9	1.2	1.2	1.1	0.9	1.1	0.9	1.1	1.1	1.0
35	0.8	1.0	1.1	1.3	0.9	1.0	0.9	0.9	1.1	1.1	1.0	1.0
36	0.9	0.6	1.3	0.4	0.8	1.1	0.5	0.8	1.2	0.3	1.1	1.2
37	1.3	1.1	1.3	1.2	1.4	1.1	1.3	1.2	0.9	1.1	1.1	1.1
38	0.7	0.8	1.2	0.5	0.9	0.8	1.3	1.1	1.1	1.1	1.1	1.2

39	0.9	0.9	1.1	0.9	0.6	0.9	1.0	1.4	1.2	1.2	1.2	1.1
40	0.8	1.0	0.9	0.7	1.0	1.0	1.0	1.3	1.1	1.1	1.2	1.2
41	0.9	0.9	0.8	1.4	1.5	1.5	1.1	1.1	1.1	1.1	1.2	1.2
42	0.9	0.8	1.6	1.0	1.0	2.3	1.1	0.8	0.7	1.0	1.0	0.9
43	0.8	0.9	1.0	1.2	1.4	1.4	1.1	1.1	1.0	1.0	1.0	1.1
44	0.7	1.5	1.3	0.5	1.3	1.5	0.3	1.1	1.1	0.3	1.2	1.3
45	0.7	1.2	3.2	0.6	1.6	1.8	0.2	0.8	1.6	0.2	0.8	1.6

Table 20

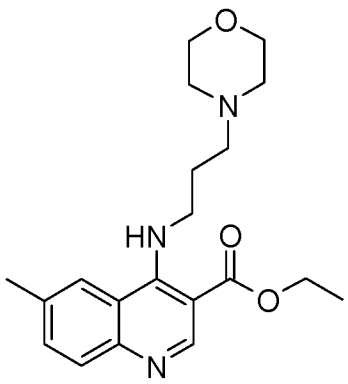
[00420] After several rounds of testing, the following compounds were found to be particularly optimal.

[00421]



Compound D28

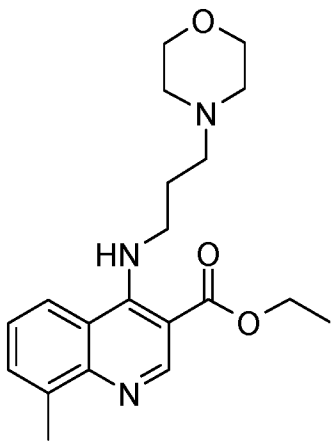
Activity/DMSO: 11/1.5



Compound D41

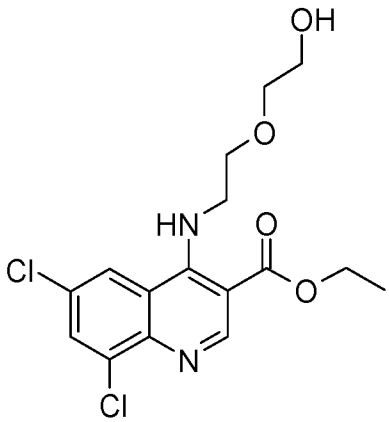
Activity/DMSO: 1.5

[00422]



Compound D84

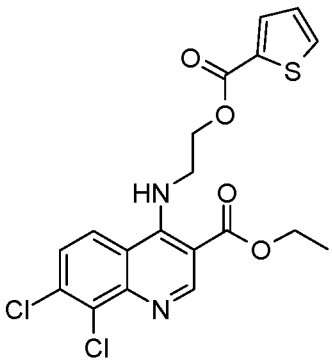
Activity/DMSO: 1.5



Compound D53

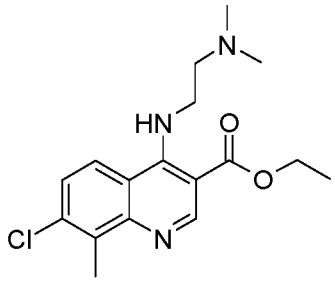
Activity/DMSO: 1.6

[00423]



Compound D18

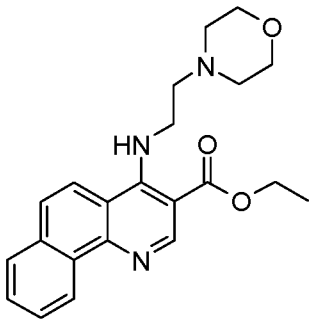
Activity/DMSO: 1.4



Compound D27

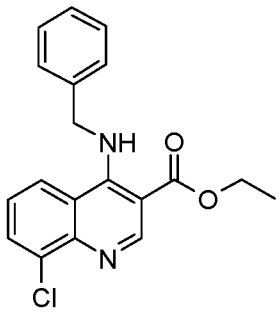
Activity/DMSO: 2.1/1.5

[00424]



Compound D93

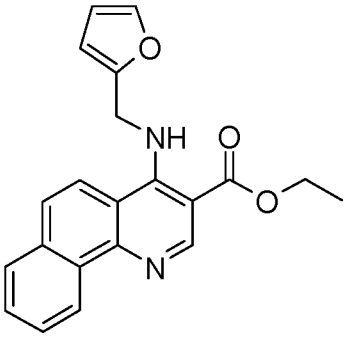
Activity/DMSO: 2.5



Compound D94

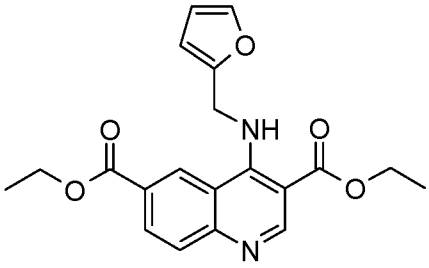
Activity/DMSO: 1.5

[00425]



Compound D89

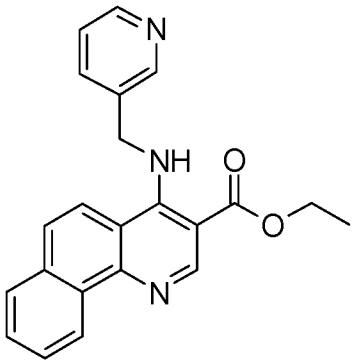
Activity/DMSO: 1.8



Compound D99

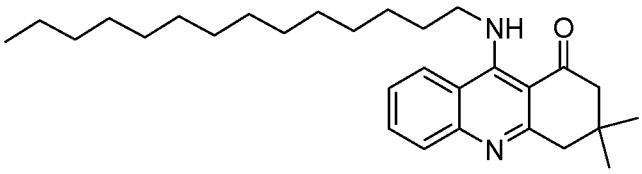
Activity/DMSO: 1.5

[00426]



Compound D83

Activity/DMSO: 1.6



Compound D101

Activity/DMSO: 1.4

[00427] Table 21 shows results of testing on Compound D28.

Firefly, cmpd/DMSO			Firefly, cmpd		Firefly, DMSO		Renilla, cmpd/DMSO		Renilla, DMSO	
repeat 1	repeat 2	mean	repeat 1	repeat 2	mean plate 1	mean plate 2	repeat 1	repeat 2	mean plate 1	mean plate 2
14.1	11.7	12.8	784	644	56	55	0.7	0.5	3237	2930

Table 21

[00428] Tables 21a and 21b show results of testing in various Compounds here:

Compound ID	Firefly					
	NR2F6 full length, cmpd/DMSO (mean)			F4, cmpd/DMSO (mean)		
Concentration, mM	40	10	2	40	10	2
Compound D93	0.7	1.9	1.6	0.4	1.0	1.6
Compound D41	0.9	0.9	1.3	0.3	0.9	0.9
Compound D53	0.5	0.9	1.3	0.2	0.4	0.8
Compound D84	1.1	1.2	0.9	0.5	1.1	1.1
Compound D18	0.8	0.9	1.2	1.1	0.6	1.3

Table 21a

[00429]

Compound ID	Firefly					
	NR2F6 full length, cmpd/DMSO (mean)			F4, cmpd/DMSO (mean)		
Concentration, mM	40	10	2	40	10	2
Compound D93	0.7	1.9	1.6	0.4	1.0	1.6
Compound D94	1.1	1.1	1.2	0.7	0.9	1.1
Compound D83	1.8	1.9	1.2	1.8	1.2	1.6
Compound D99	1.0	1.7	1.5	0.7	1.4	1.6

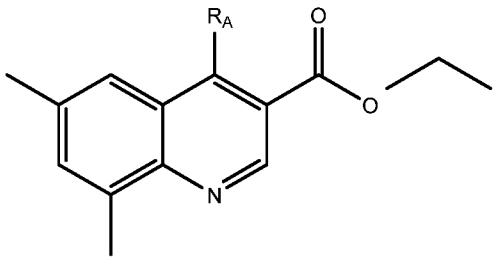
Table 21b

[00430] **FIGS. 27A** and **27B** show NR2F6 and LBD transient transfection, respectively, for Compound D28. Higher concentrations were excluded due to lower signal (tox effect). **FIGS. 27C** and **27D** show NR2F6 and LBD transient transfection at different concentrations for different compounds. 9 compounds were tested on LBD transfected cells (40, 10, 2 and 0.5 μM, 4 replicates). **FIGS. 27E** and **27F** show toxicity of Compound D28. 9 compounds were tested for cytotoxicity on LBD transfected cells (40, 10, 2 and 0.5 μM, 4 replicates). Tox effect was found to cause lower signal compared to DMSO. Cytotoxicity normalized to DMSO is shown in **FIG. 27F** (0% cytotoxicity corresponds to DMSO signal, 100% - zero signal).

[00431] **FIGS. 28A-D** show the results of a cytokind release experiment for dog and human PBMC. All compounds were tested at 5, 10, 25 and 50 uM in duplicates.

[00432] Dog PBMC (1 x 106 cells/mL) were activated by 10 ng/mL PMA + 500 ng/mL ionomycin. Data were normalized to controls with (100%) /without (0%) PMA + ionomycin activation.

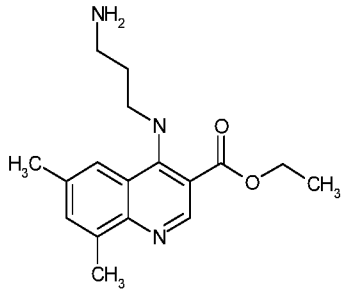
[00433] In further embodiments, the present technology is directed to compounds of Formula (IX):



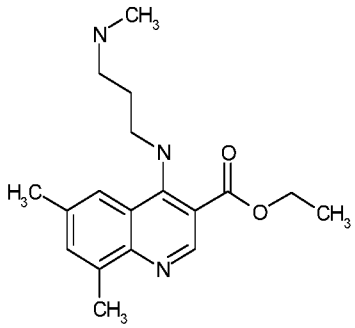
(IX)

wherein R_A is C, H, N, O, S, a halogen, an alkyl group, a substituted alkyl group, a cyclic alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, an ester, an aldehyde, a ketone, a carboxylic acid, an amide, an amine, an ether, a thiol or a nitrile. For example, exemplary but non-limiting compounds are shown below:

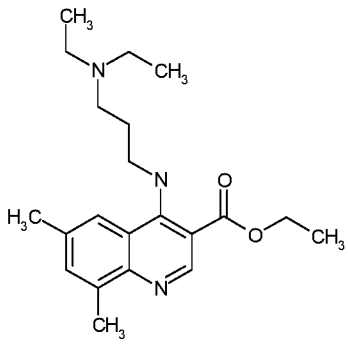
[00434]



Compound Z58

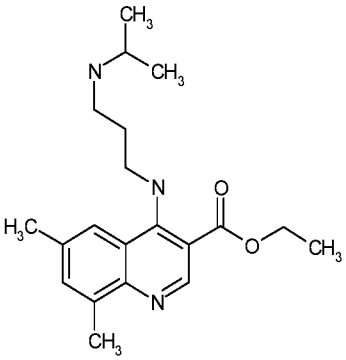


Compound Z59

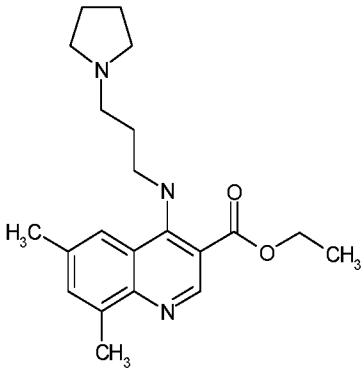


Compound Z17

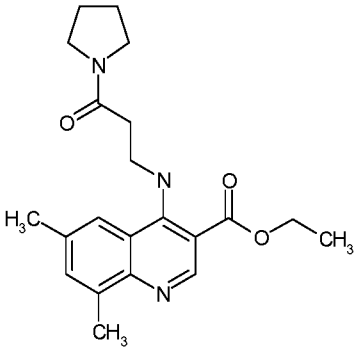
[00435]



Compound Z60

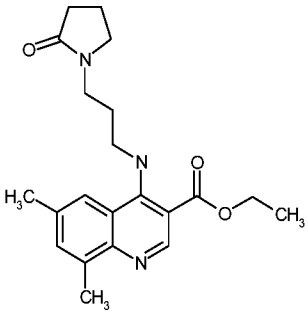


Compound Z61

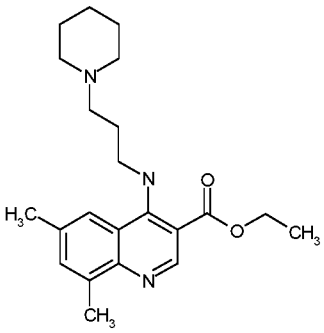


Compound Z62

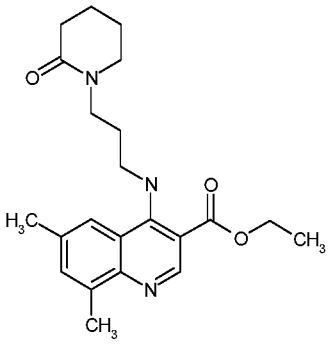
[00436]



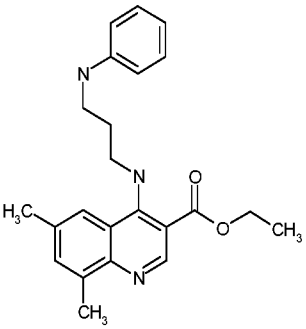
Compound Z63



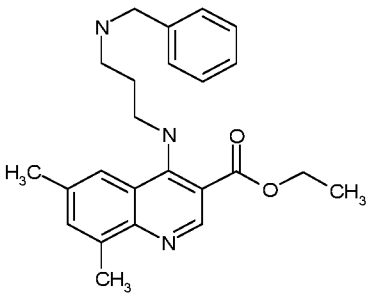
Compound Z19



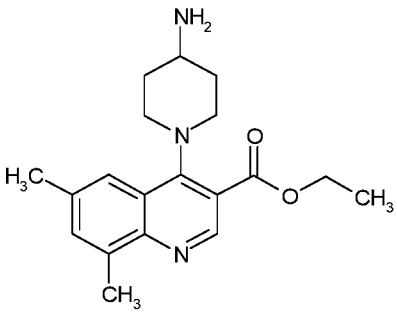
Compound Z64



Compound Z65

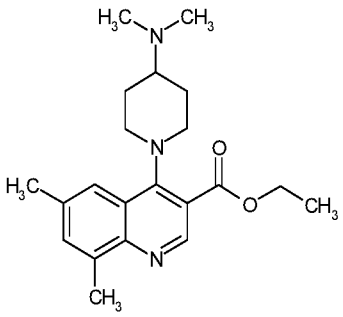


Compound Z66

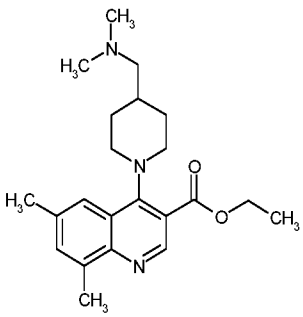


Compound Z67

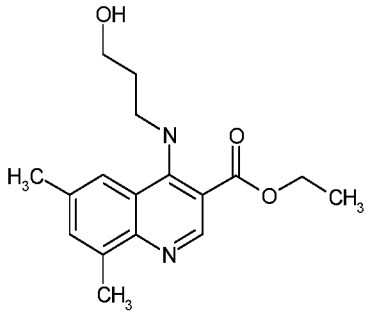
[00437]



Compound Z68

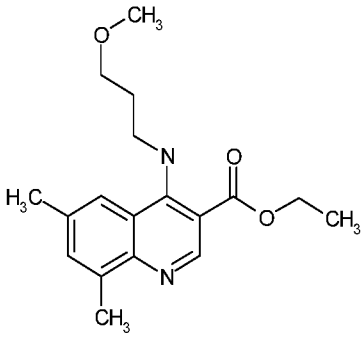


Compound Z69

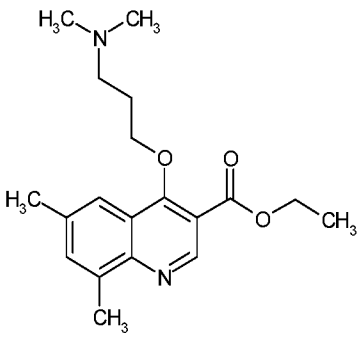


Compound Z70

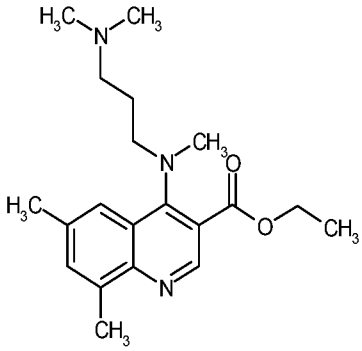
[00438]



Compound Z71

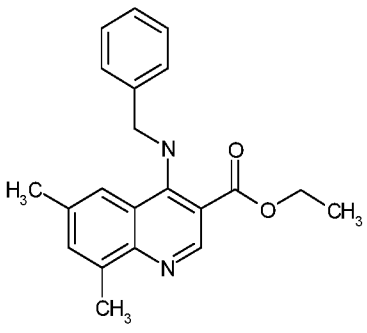


Compound Z72

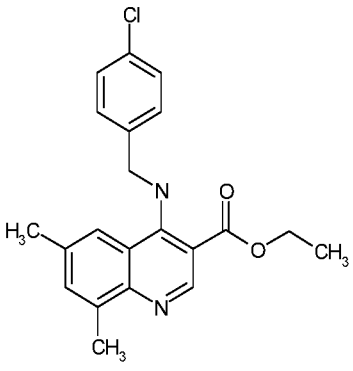


Compound Z73

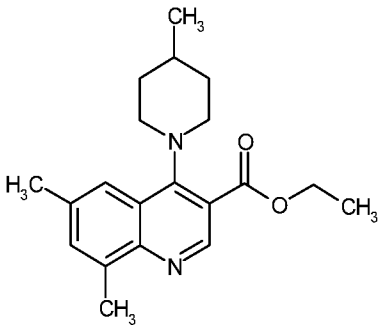
[00439]



Compound Z74

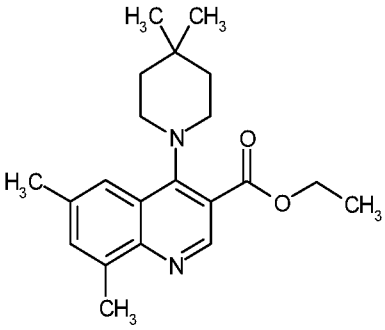


Compound Z75

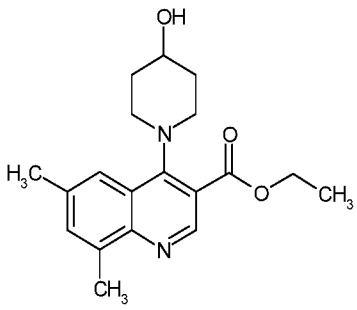


Compound Z76

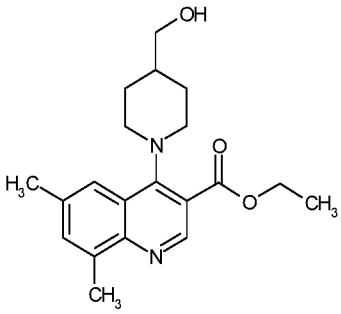
[00440]



Compound Z77

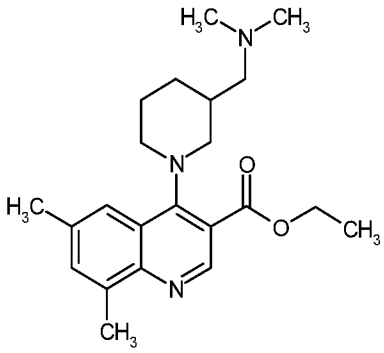


Compound Z78



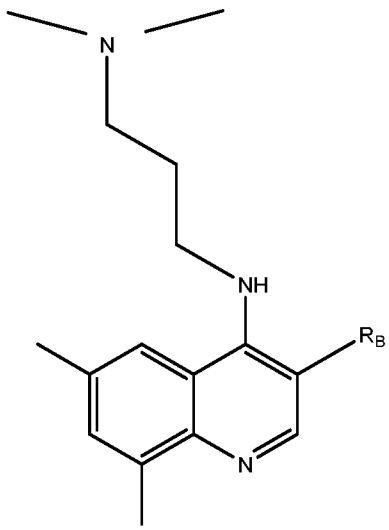
Compound Z79

[00441]



Compound Z80

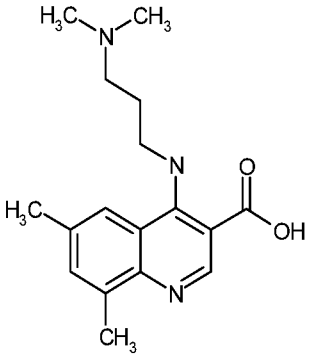
[00442] In further embodiments, the present technology is directed to compounds of Formula (X):



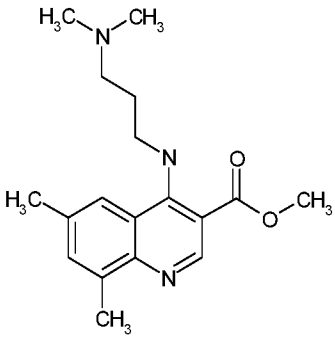
(X)

wherein R_B is C, H, N, O, S, a halogen, an alkyl group, a substituted alkyl group, a cyclic alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, an ester, an aldehyde, a ketone, a carboxylic acid, an amide, an amine, an ether, a thiol or a nitrile. For example, exemplary but non-limiting compounds are shown below:

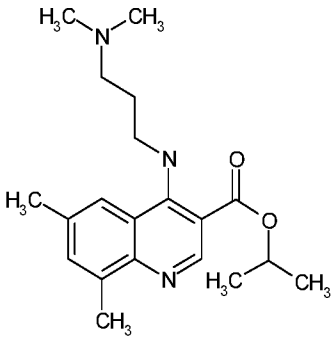
[00443]



Compound Z28

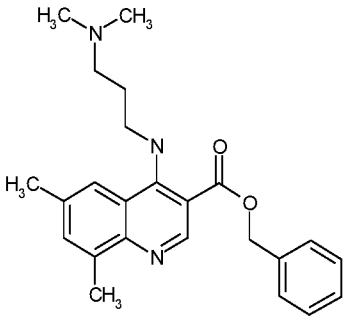


Compound Z81

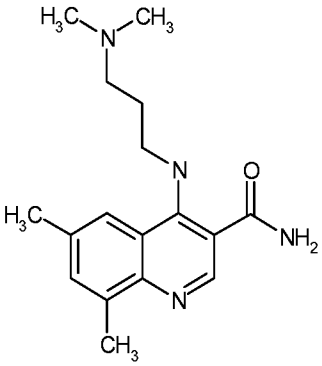


Compound Z29

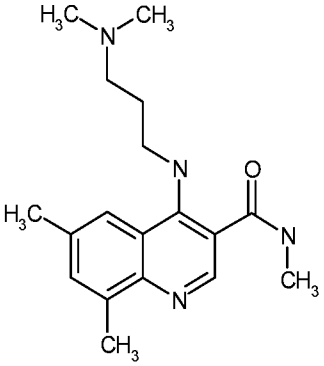
[00444]



Compound Z82

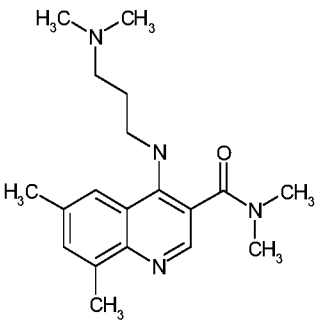


Compound Z83

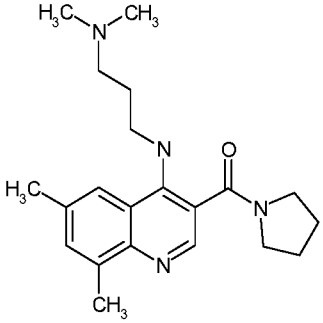


Compound Z30

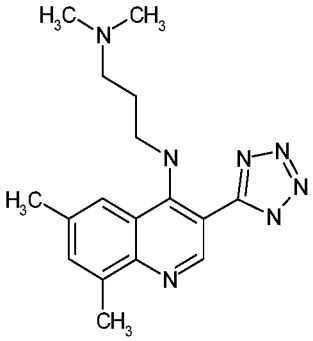
[00445]



Compound Z31

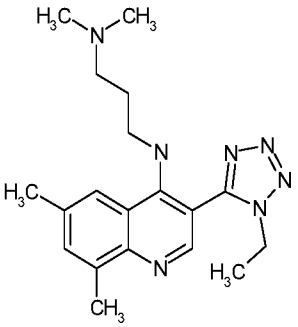


Compound Z32

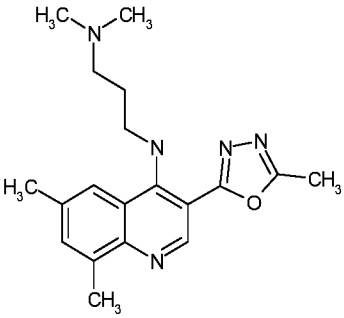


Compound Z84

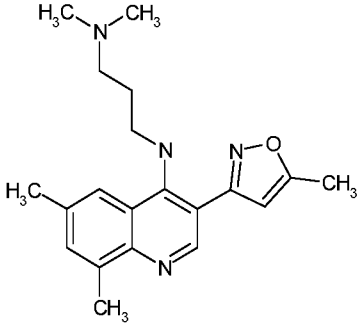
[00446]



Compound Z85

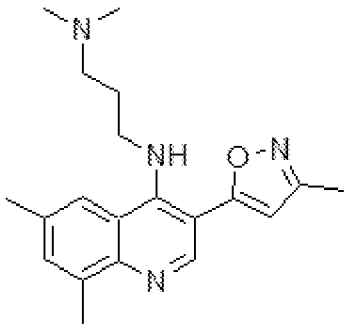


Compound Z86



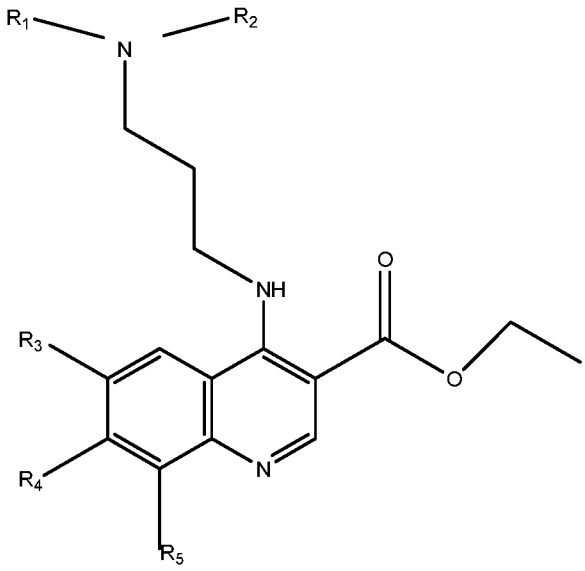
Compound Z87

[00447]



Compound Z88

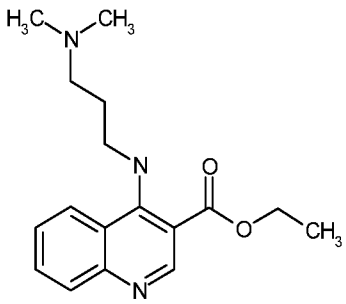
[00448] In further embodiments, the present technology is directed to compounds of Formula (XI):



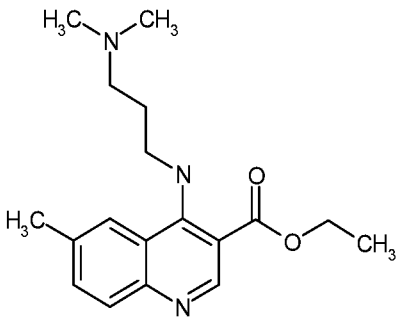
(XI)

wherein any of R_1-R_5 are C, H, N, O, S, a halogen, an alkyl group, a substituted alkyl group, a cyclic alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, an ester, an aldehyde, a ketone, a carboxylic acid, an amide, an amine, an ether, a thiol or a nitrile. For example, exemplary but non-limiting compounds are shown below:

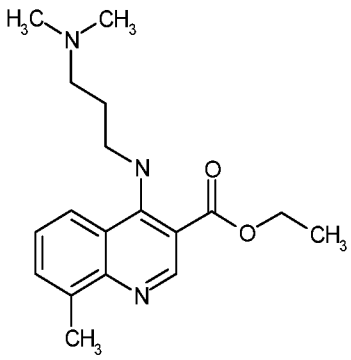
[00449]



Compound Z89

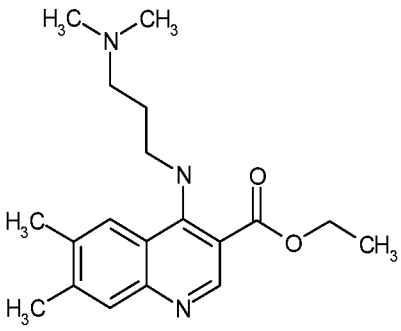


Compound Z33

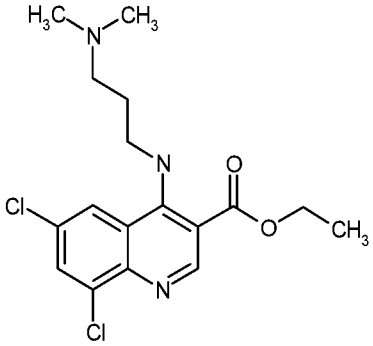


Compound Z90

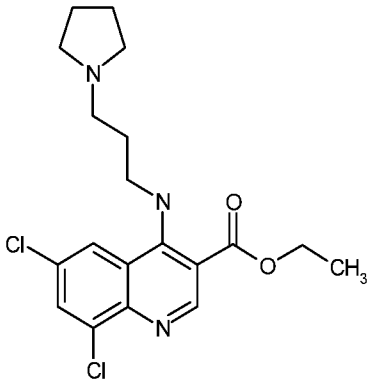
[00450]



Compound Z34

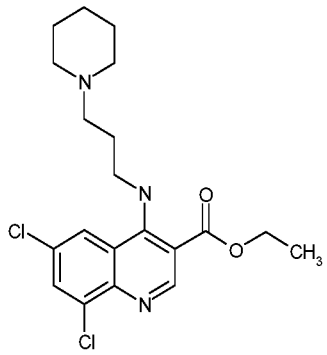


Compound Z35

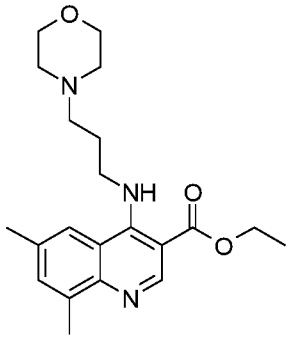


Compound Z36

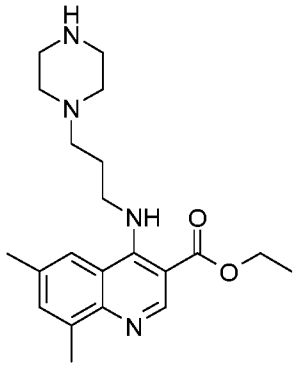
[00451]



Compound Z37

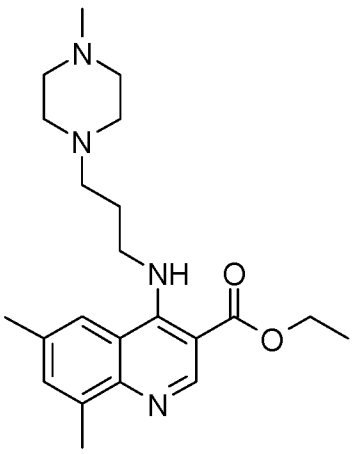


Compound Z98

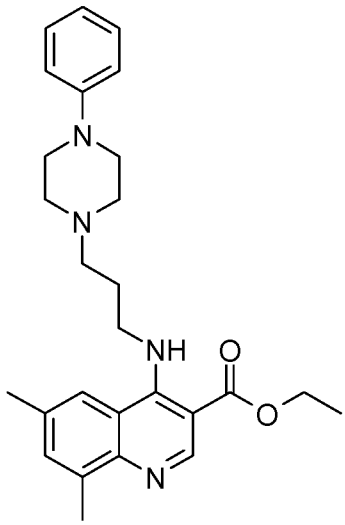


Compound Z99

[00452]



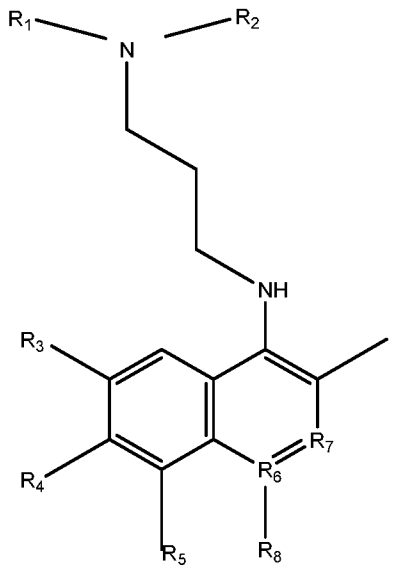
Compound Z100



Compound Z101

[00453] In various other embodiments, the structure of the Compounds found to be useful have other variations. For example, in certain embodiments, the present technology is directed to compounds of Formula (XII):

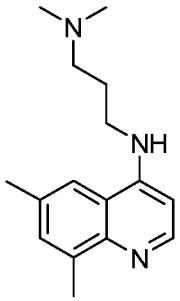
[00454]



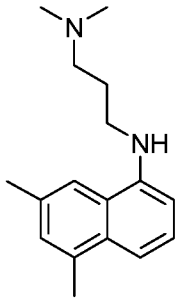
(XII)

wherein any of R_1 and R_8 is C, H, N, O, S, a halogen, an alkyl group, a substituted alkyl group, a cyclic alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, an ester, an aldehyde, a ketone, a carboxylic acid, an amide, an amine, an ether, or a thiol. Exemplary but non-limiting compounds are shown below:

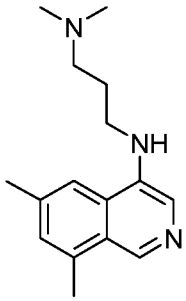
[00455]



Compound Z102

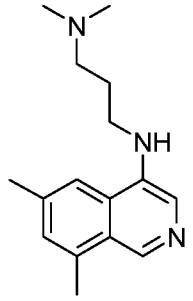


Compound Z103

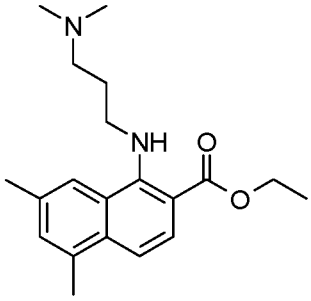


Compound Z104

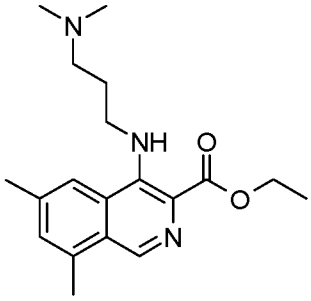
[00456]



Compound Z105

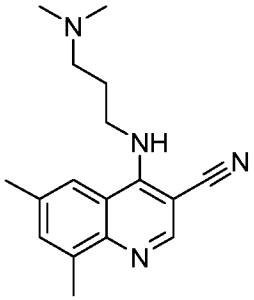


Compound Z106

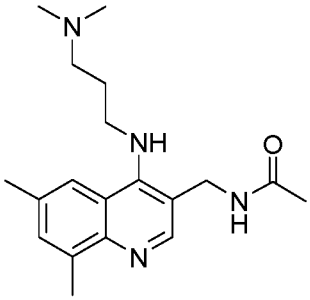


Compound Z107

[00457]



Compound Z108

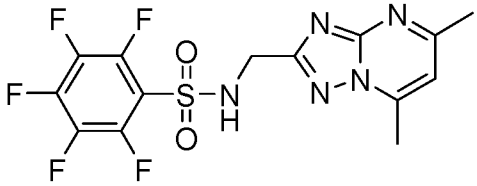


Compound Z109

[00458] **FIGS. 29-32** show exemplary methods of formulating the compounds that have been discussed herein.

[00459] **FIG. 31A** shows an exemplary synthesis of compounds including the following: Compound Z17, Compound Z61, Compound Z19, Compound Z70, Compound Z71, Compound Z67, Compound Z76, Compound Z75, Compound Z78, Compound Z68, Compound Z27, Compound Z79, Compound Z64, Compound Z69, Compound Z74, Compound Z154, Compound Z80, Compound Z155, Compound Z156, Compound Z157, Compound Z158, Compound Z159.

[00460] Another compound developed herein, and found to have desirable activity, is Compound E21:

[00461] 

[00462] Table 22 shows results of testing on Compound E21.

Firefly, cmpd/DMSO			Firefly, cmpd		Firefly, DMSO		Renilla, cmpd/DMSO		Renilla, DMSO	
repeat 1	repeat 2	mean	repeat 1	repeat 2	mean plate 1	mean plate 2	repeat 1	repeat 2	mean plate1	mean plate2
9.5	**5.6**	**7.3**	526	306	56	55	**0.6**	**0.8**	3237	2930

Table 22

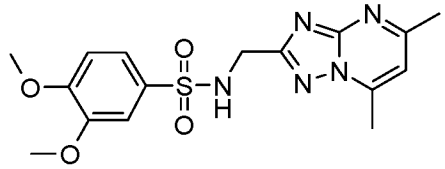
[00463] **FIG. 33A** and **33B** show NR2F6 and LBD transient transfection for Compound E21. Higher concentrations were excluded due to lower signal (tox) effect.

[00464] **FIGS. 34A** and **34B** show NR2F6 LBD transient transfection for Compound E21. 9 compounds were tested on LBD transfected cells (40, 10, 2 and 0.5 μM, 4 replicates). **FIGS. 34C** and **34D** show NR2F6 and LBD transient transfection at different concentrations for different compounds. 9 compounds were tested on LBD transfected cells (40, 10, 2 and 0.5 μM, 4 replicates). Tox effect was found to cause lower signal compared to DMSO. Cytotoxicity normalized to DMSO is shown in **FIG. 34D** (0% cytotoxicity corresponds to DMSO signal, 100% - zero signal). All compounds were tested at 5, 10, 25 and 50 uM in duplicates. Dog PBMC (1 x 106 cells/mL) were activated by 10 ng/mL PMA + 500 ng/mL ionomycin. Data were normalized to controls with (100%) /without (0%) PMA + ionomycin activation.

[00465] As shown in **FIG. 36**, related compounds were generated from Compound E21 and tested for activity.

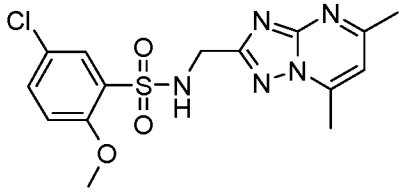
[00466] Additional related compounds found to have desirable activity include the following:

[00467]



Compound E8

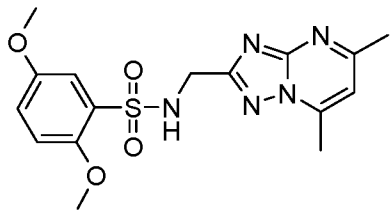
Activity/DMSO: 1.7



Compound E15

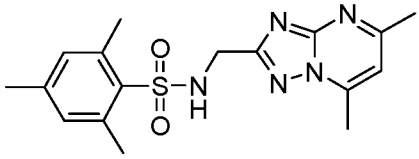
Activity/DMSO: 1.5

[00468]



Compound E4

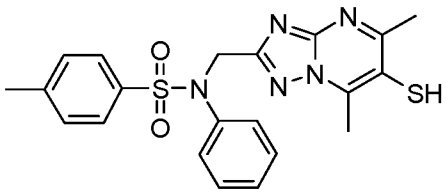
Activity/DMSO: 1.5



Compound E10

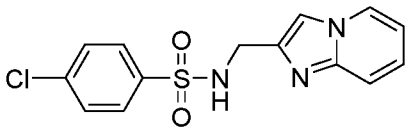
Activity/DMSO: 1.4

[00469]



Compound E57

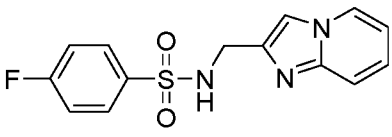
Activity/DMSO: 1.4



Compound E25

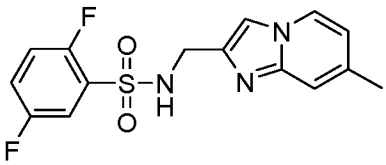
Activity/DMSO: 2.3/1.8

[00470]



Compound E26

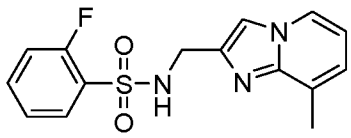
Activity/DMSO: 1.8



Compound E58

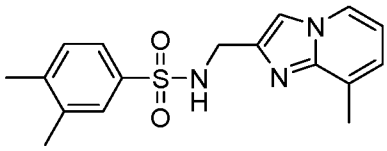
Activity/DMSO: 1.7

[00471]



Compound E52

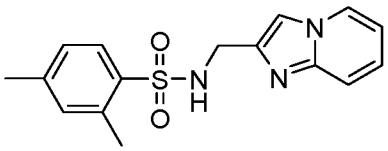
Activity/DMSO: 1.4



Compound E41

Activity/DMSO: 1.4

[00472]



Compound E24

Activity/DMSO: 1.4

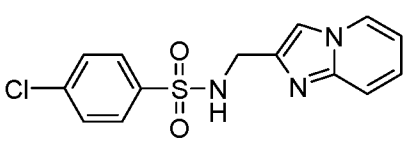
[00473] Table 23 shows the screening results of certain of the above compounds.

	Compound ID	Firefly					
		NR2F6 full length, cmpd/DMSO (mean)			F4, cmpd/DMSO (mean)		
	Concentration, mM	**40**	**10**	**2**	**40**	**10**	**2**
Screening Results	Compound E4	1.0	1.0	1.1	0.8	0.9	0.9
	Compound E8	0.9	1.2	1.2	0.7	1.0	1.0
	Compound E10	1.1	1.5	1.3	0.8	1.0	1.1
	Compound E15	1.1	0.8	1.2	0.8	1.1	1.3
	Compound E57	0.9	1.2	1.3	1.3	1.3	1.0
	Compound E24	1.1	1.1	1.0	0.7	0.9	1.0
	Compound E41	0.9	1.0	1.1	0.4	0.7	0.9
	Compound E52	1.4	1.3	1.0	0.7	0.9	0.7

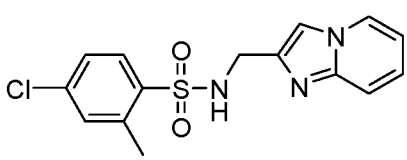
Table 23

[00474] Additional compounds synthesized and found to have desirable activity include the following:

[00475]

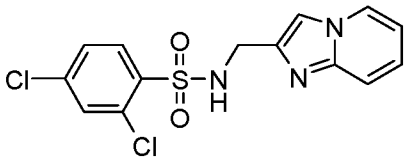


Compound E59

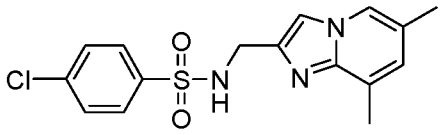


Compound E60

[00476]

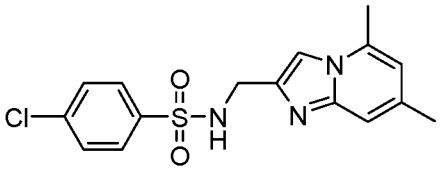


Compound E61

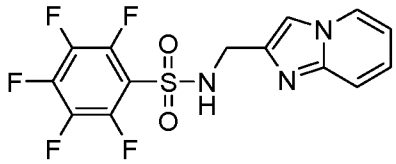


Compound E62

[00477]

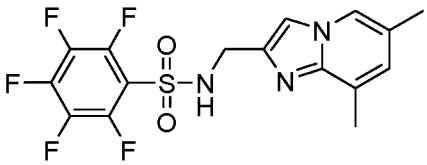


Compound E63

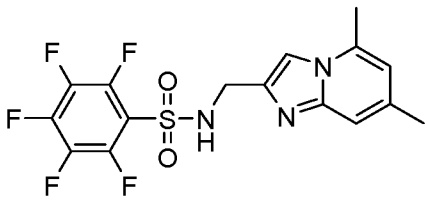


Compound E64

[00478]

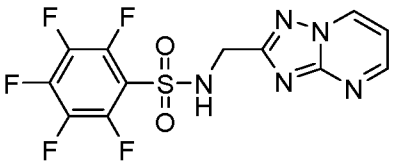


Compound E65

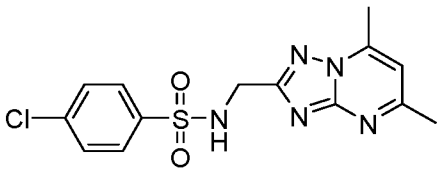


Compound E66

[00479]

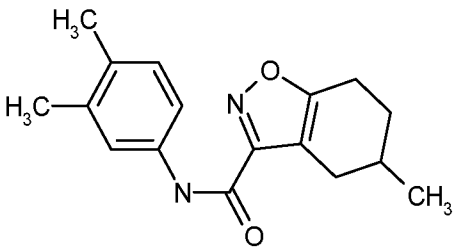


Compound E67

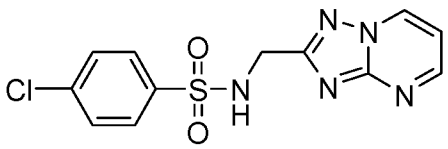


Compound E68

[00480]



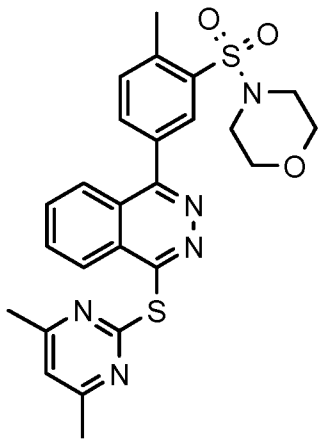
Compound E53



Compound E69

[00481] **FIG. 37** shows exemplary methods of formulating the compounds that have been discussed herein; specifically, exemplary synthesis of compounds including the following: Compound Z160, Compound Z161, Compound Z162, Compound Z163.

[00482] As discussed above, Compound F1 was of particular interest herein:



Compound F1

[00483] Table 24 shows results of testing on Compound F1.

Firefly, cmpd/DMSO			Firefly, cmpd		Firefly, DMSO		Renilla, cmpd/DMSO		Renilla, DMSO	
repeat 1	repeat 2	mean	repeat 1	repeat 2	mean plate1	mean plate2	repeat 1	repeat 2	mean plate 1	mean plate2
5.6	4.6	5.1	310	252	56	55	1.1	1.2	3237	2930

Table 24

[00484] **FIGS 38A** and **38B** show NR2F6 and LBD transient transfection, respectively, for Compound F1.

[00485] **FIGS. 39A** and **39B** show NR2F6 LBD transient transfection for Compound F1. 9 compounds were tested on LBD transfected cells (40, 10, 2 and 0.5 μM, 4 replicates). **FIGS. 39C** and **39D** show toxicity of NR2F6 LBD transient transfection. 9 compounds were tested for cytotoxicity on LBD transfected cells (40, 10, 2 and 0.5 μM, 4 replicates). Tox effect causes lower signal compared to DMSO. Cytotoxicity normalized to DMSO is shown in **FIG. 39D** (0% cytotoxicity corresponds to DMSO signal, 100% - zero signal).

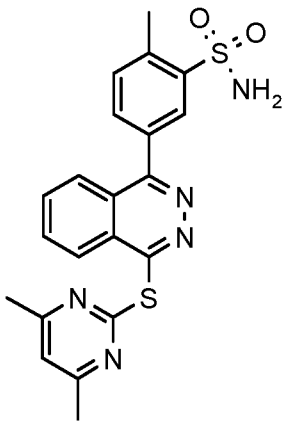
[00486] **FIGS. 40A-D** show the results of cytokine release experiment for dogs and human PBMC. All compounds were tested at 5, 10, 25 and 50 uM in duplicates.

[00487] Dog PBMC (1 x 106 cells/mL) were activated by 10 ng/mL PMA + 500 ng/mL ionomycin. Data were normalized to controls with (100%) /without (0%) PMA + ionomycin activation.

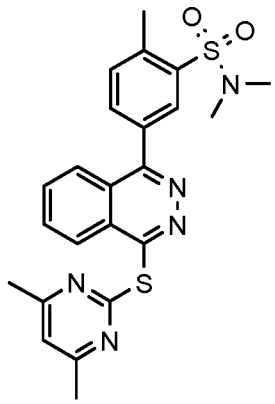
[00488] **FIG. 41** shows the general SAR strategy for testing Compound F1 and compounds related to it in structure. Formally, the active molecule was divided into four domains (Domains A through D). Each domain was evaluated independently to establish SAR trends. Combinations of optimized domains evaluated additive or synergistic effect. 4 related analogs were available.

[00489] For example, compounds were tested with varying values of Domain A. Exemplary compounds found to be useful are listed as follows:

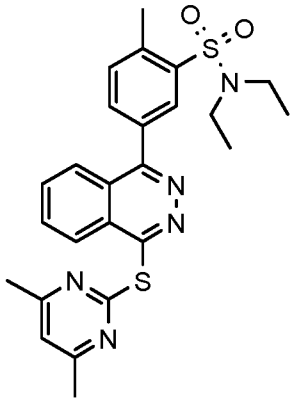
[00490]



Compound F2

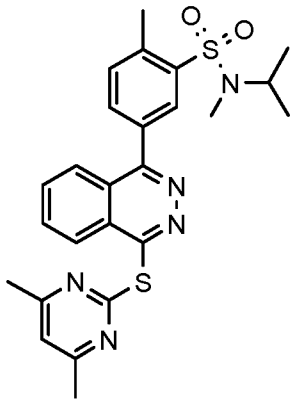


Compound F3

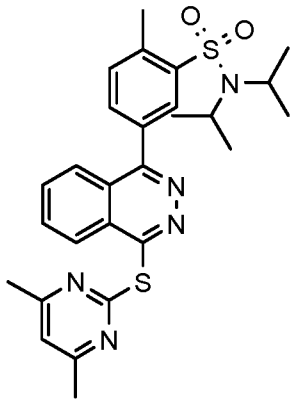


Compound F4

[00491]

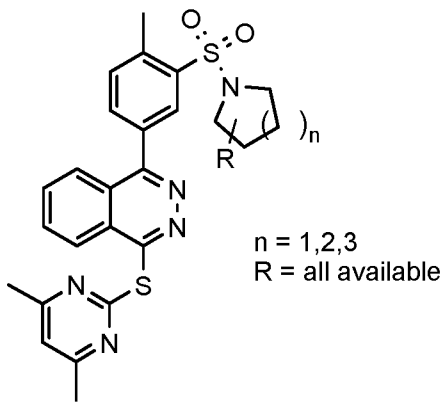


Compound F5



Compound F6

[00492] In certain embodiments, a compound herein has Formula (XIII):

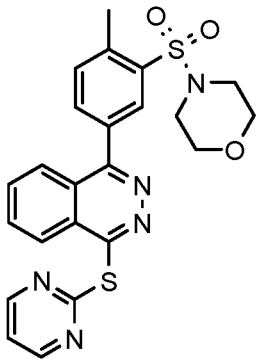


(XIII)

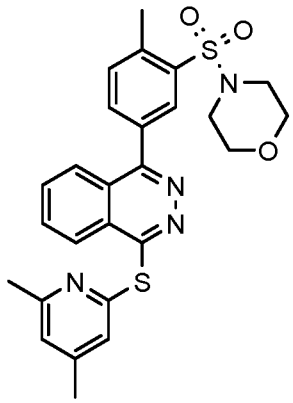
wherein n is an integer 1, 2, or 3, and R is any other moiety mentioned in the present disclosure (*e.g.*, C, H, N, O, S, a halogen, an alkyl group, a substituted alkyl group, a cyclic alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, an ester, an aldehyde, a ketone, a carboxylic acid, an amide, an amine, an ether, a thiol or a nitrile).

[00493] Similarly, compounds were tested with varying values of Domain C. Exemplary compounds found to be useful are listed as follows:

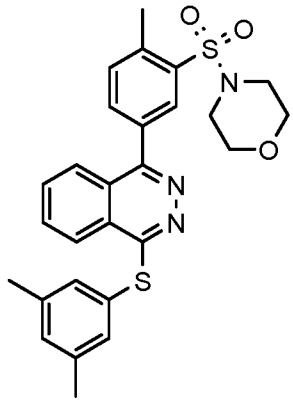
[00494]



Compound F7

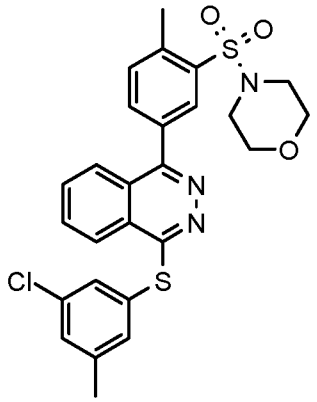


Compound F8

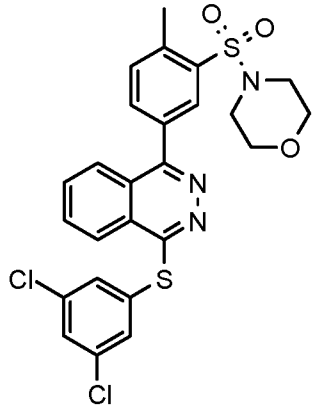


Compound F9

[00495]

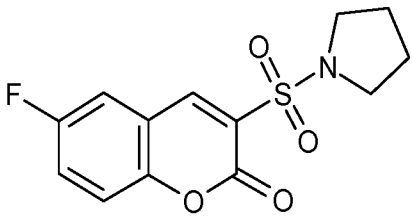


Compound F10



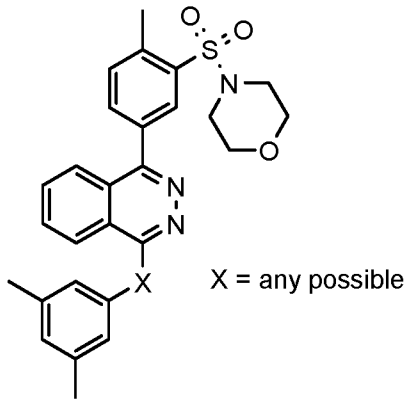
Compound F11

[00496]



Compound F13

[00497] In certain embodiments, a compound herein has Formula (XIV):



(XIV)

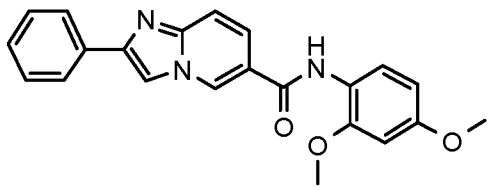
wherein X is C, H, N, O, S, a halogen, an alkyl group, a substituted alkyl group, a cyclic alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, an ester, an aldehyde, a ketone, a carboxylic acid, an amide, an amine, an ether, a thiol, a nitrile or any other moiety mentioned herein.

[00498] In other embodiments, the present technology is directed to compounds comprising a boronate, and synthesis of such compounds. For example, **FIG. 42** shows an exemplary synthesis of a boronate compound. In various embodiments, the synthesis achieved a yield of at least about 95%, at least about 90% and at least about 85%; with at least about 85% purity. In various embodiments, a regioisomer was present in the yield, in amounts of about 10 to about 20%, or about 12 to about 18%. In various embodiments, the regioisomers could be separated.

[00499] **FIG. 42** shows the results of other exemplary syntheses of compounds comprising boronate, and the relative proportions of resultant compounds.

[00500] Another compound found to have good activity is Compound P1:

[00501]



Compound P1

[00502] Table 25 shows results of testing on Compound P1.

Firefly, cmpd/DMSO			Firefly, cmpd		Firefly, DMSO		Renilla, cmpd/DMSO		Renilla, DMSO	
repeat 1	repeat 2	mean	repeat 1	repeat 2	mean plate 1	mean plate 2	repeat 1	repeat 2	mean plate 1	mean plate 2
4.0	3.0	3.5	236	166	59	56	1.0	0.9	3030	3118

Table 25

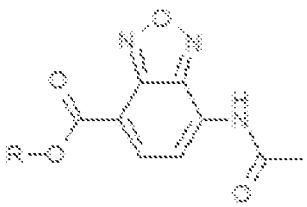
[00503] **FIGS. 44A** and **44B** show NRdF6 and LBD transient transfection of Compound P1.

[00504] **FIGS. 45A-D** show NR2F6 LBD transient transfection for 9 different compounds, including Compound P1.

[00505] **FIGS. 46A** and **46B** show the results of the cytokine release experiment with dogs PBMC.

[00506] **FIGS. 47A** and **47B** show NR2F6 agonist activity and NR2F6 agonist activity (Renilla signal) for 7 compounds along with a DMSO control. The conclusion is that Compound Z92 shown similar slight activity (~3 times firefly signal over DMSO level) at 10 uM and 50 uM. Compound E53 increases firefly activity in 6 times at 10 uM and appeared to show strong cytotox effect (great decreasing both renilla and firefly activity). Both Compounds Z92 and E53 will be tested on greater concentration range for confirmation on both cell line with double stable transfection (clone F1-pGL4) and cell line with transient transfection.

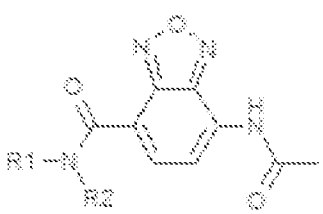
[00507] In certain embodiments, the present technology is directed to compounds of Formula (XV):



(XV)

wherein R is C, H, N, O, S, an alkyl group, a substituted alkyl group, a cyclic alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, an ester, an aldehyde, a ketone, a carboxylic acid, an amide, an amine, an ether, a thiol or a nitrile. In certain embodiments, R is H or an alkyl group.

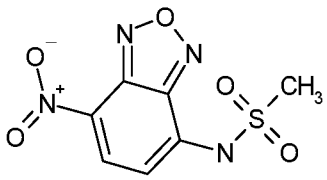
[00508] In certain embodiments, the present technology is directed to compounds of Formula (XVI):



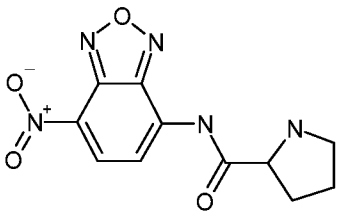
(XVI)

wherein any of R1 and R2 are C, H, N, O, S, a halogen, an alkyl group, a substituted alkyl group, a cyclic alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, an ester, an aldehyde, a ketone, a carboxylic acid, an amide, an amine, an ether, a thiol or a nitrile. In ertain embodiments, either or both of R1 and R2 are H, alkyl, phenyl, piperidine, or pyrrolidine.

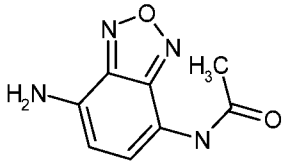
[00509] Exemplary compounds include the following:



Compound Z13

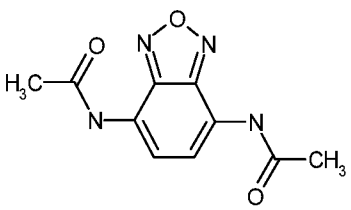


Compound Z102

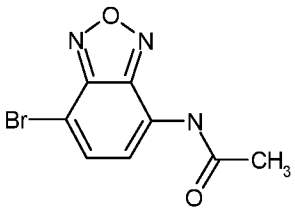


Compound Z111

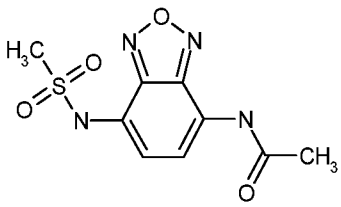
[00510]



Compound Z112

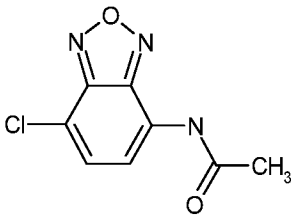


Compound Z14

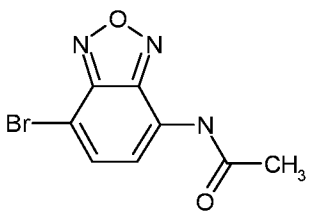


Compound Z15

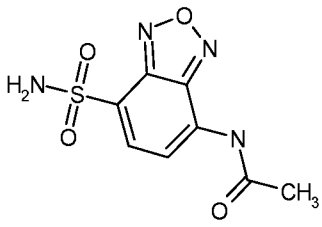
[00511]



Compound Z113

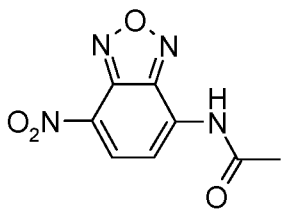


Compound Z114

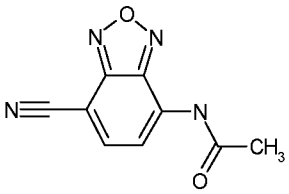


Compound Z115

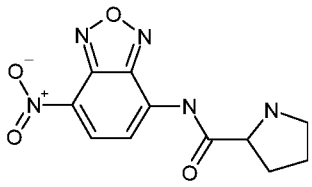
[00512]



Compound Z92



Compound Z116



Compound Z110

[00513] Table 26 shows activity results for two exemplary compounds, Compound Z95 and Compound Z113.

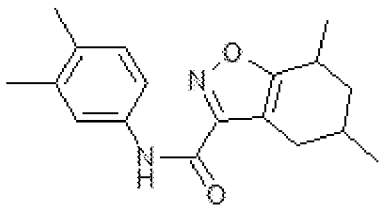
ID	Firefly, cmpd/DMSO (mean)			F4, cmpd/DMSO (mean)		
Concentration	40	10	2	40	10	2
Z95	2.3	1.3	1.2	3.7	2.2	1.2
Z113	0.9	1.1	1.3	1.2	1.5	1.2

Table 26

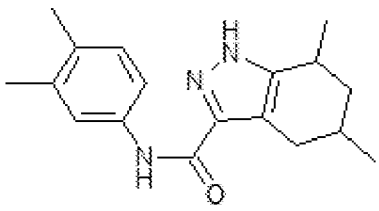
[00514] **FIGS. 48-51** show embodiments of a synthetic methods of formulating a compound according to the present technology.

[00515] Further compounds found to have good activity include the following:

[00516]

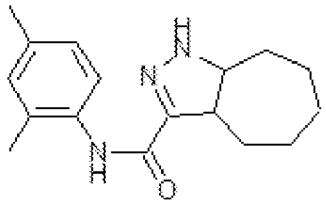


Compound Z117

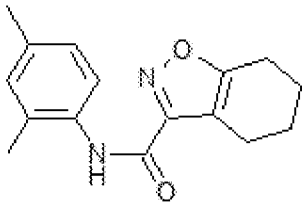


Compound Z118

[00517]

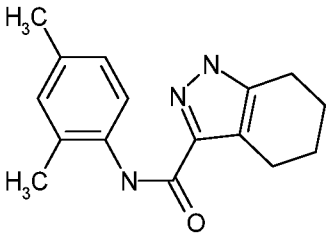


Compound Z119

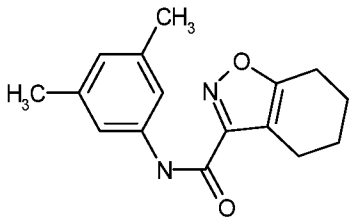


Compound Z120

[00518]

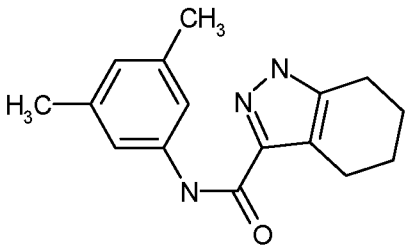


Compound Z121

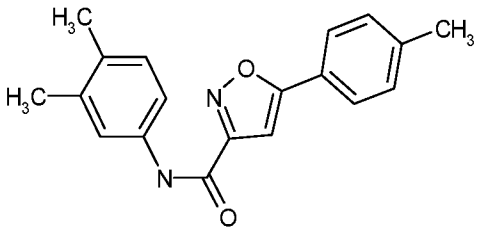


Compound Z122

[00519]

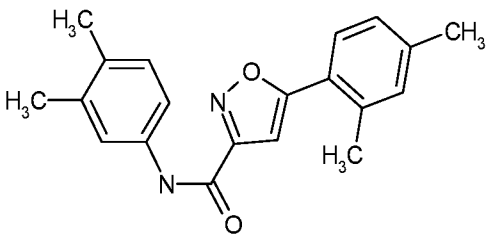


Compound Z123

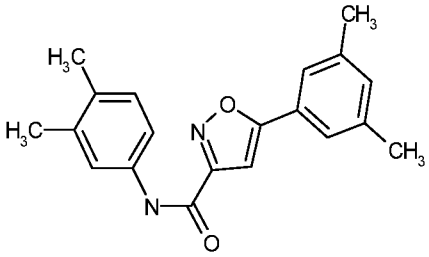


Compound Z124

[00520]



Compound Z125



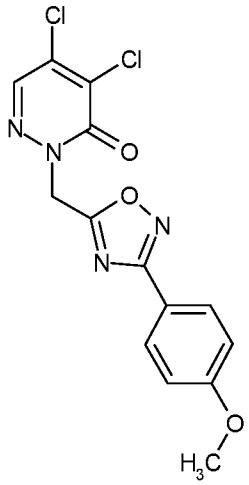
Compound Z126

[00521] **FIGS. 52-55** illustrate syntheses of various compounds discussed herein.

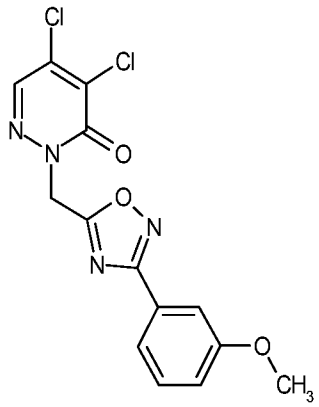
[00522] **FIGS. 56A** and **56B** show HTS activity confirmation for various compounds. Compound E53 shown similar slight activity (~3 times firefly signal over DMSO level) at 10 uM and 50 uM. 8010-3060 increase firefly activity in 6 times at 10 uM and It seems it shown strong cytotox effect (great decreasing both renilla and firefly activity). Both Compound Z92 and Compound E53 will be further tested on greater concentration range for confirmation on both cell line with double stable transfection (clone F1-pGL4) and cell line with transient transfection.

[00523] Further compounds found to have good activity include the following:

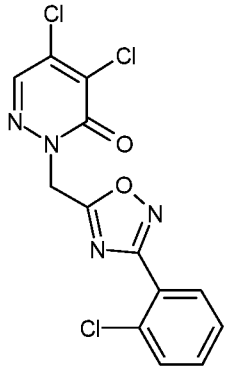
[00524]



Compound Z127

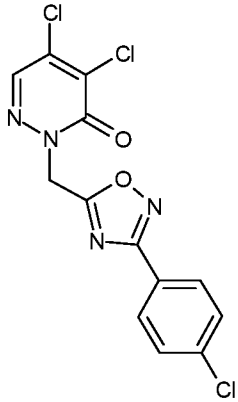


Compound Z128

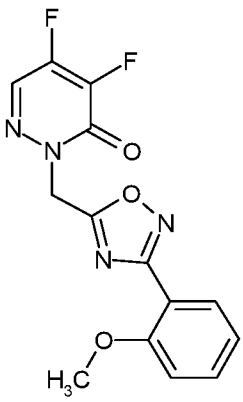


Compound Z129

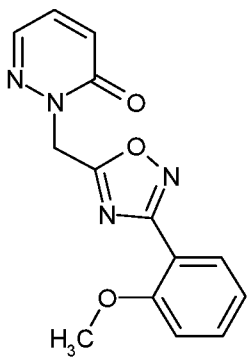
[00525]



Compound Z130

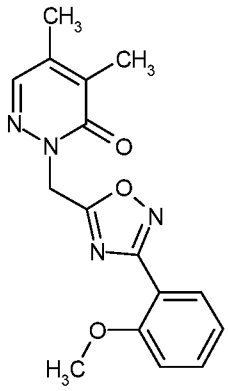


Compound Z131

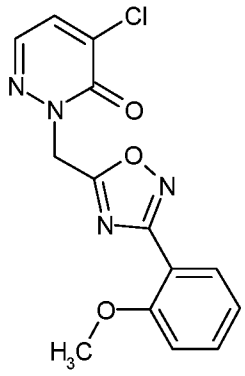


Compound Z132

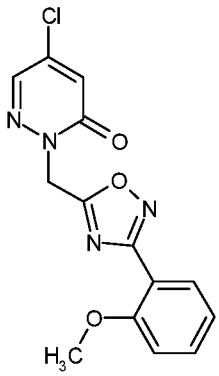
[00526]



Compound Z133

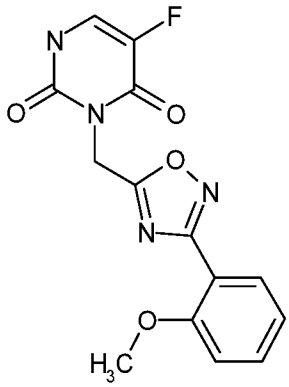


Compound Z134

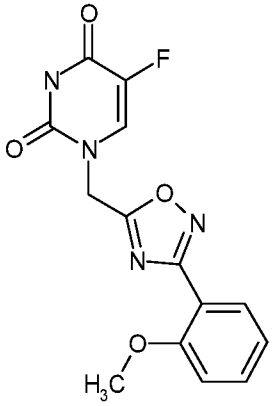


Compound Z135

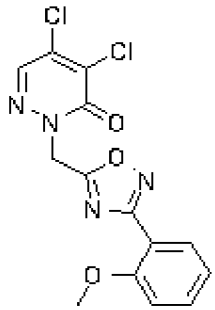
[00527]



Compound Z136

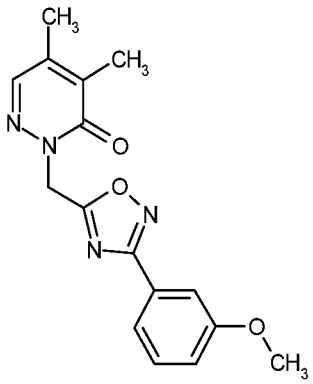


Compound Z137

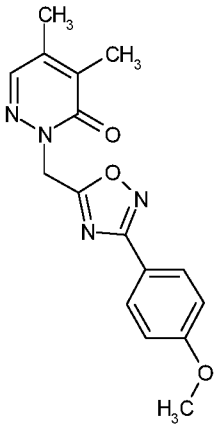


Compound Z138

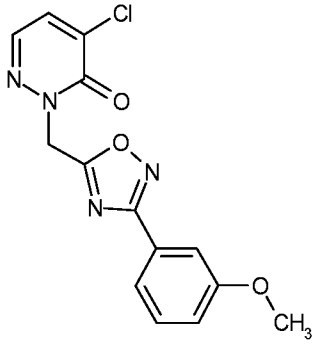
[00528]



Compound Z139

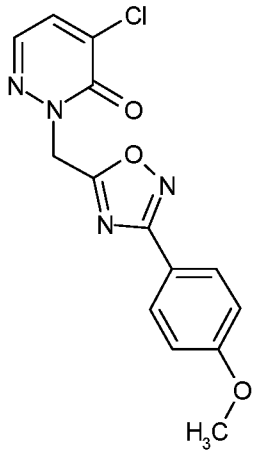


Compound Z140

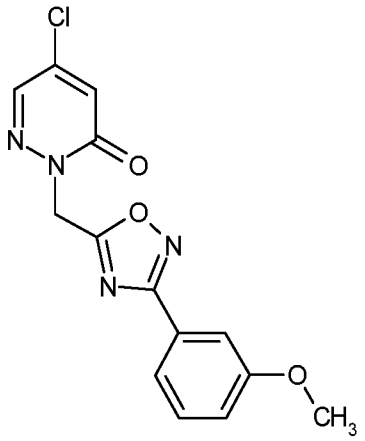


Compound Z23

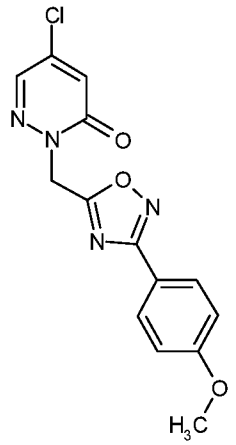
[00529]



Compound Z141

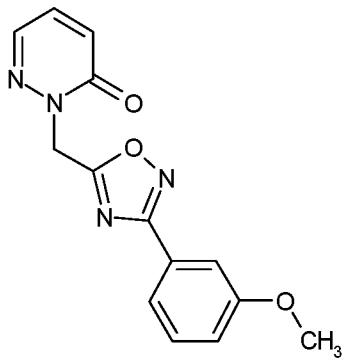


Compound Z24

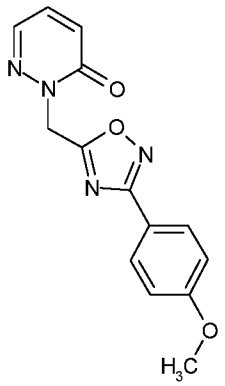


Compound Z142

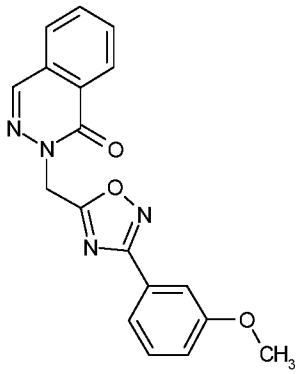
[00530]



Compound Z25

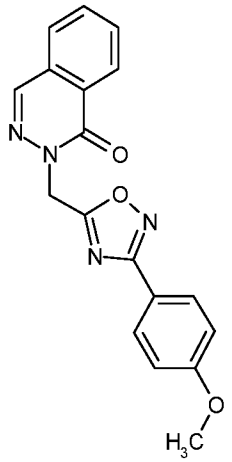


Compound Z97



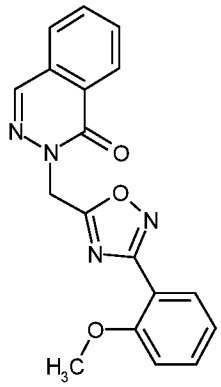
Compound Z6

[00531]

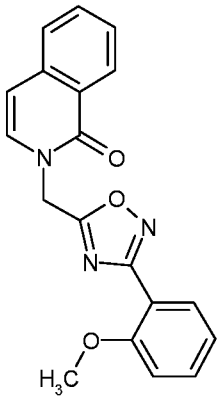


Compound Z7

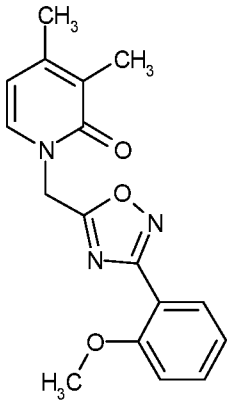
[00532]



Compound Z143

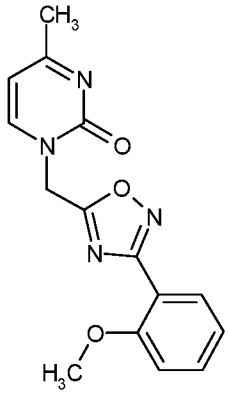


Compound Z144

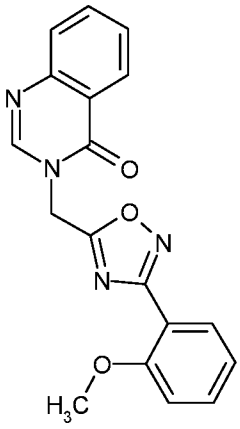


Compound Z145

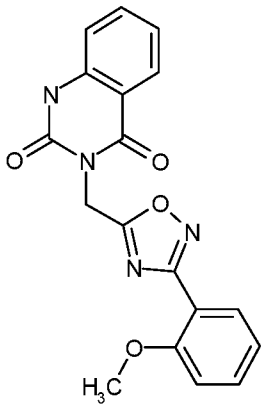
[00533]



Compound Z146

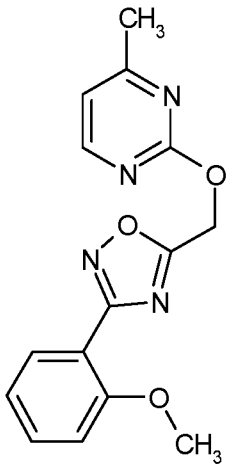


Compound Z147



Compound Z148

[00534]



Compound Z149

[00535] **FIGS. 57** and **58** show syntheses of exemplary compositions found to be useful.

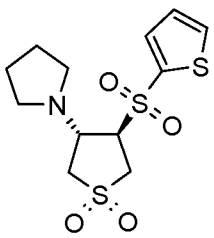
[00536] Table 27 shows the activity of various compounds discussed herein.

ID	Firefly, cmpd/DMSO (mean)	Renilla, cmpd/DMSO (mean)
Z134	1.1	0.9
Z135	1.0	1.0
Z152	1.6	1.0
Z153	2.0	1.1
Z150	1.0	0.9
Z151	1.1	1.0
Z149	1.5	1.0
Z139	1.5	1.1
Z140	1.2	1.0
Z141	1.8	0.9
Z142	1.1	0.9
Z97	2.1	1.0
Z113	1.0	0.9

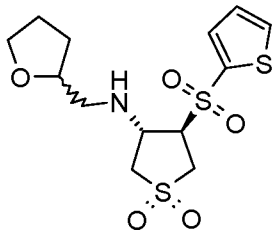
Table 27

[00537] Further compounds found to be useful include the following:

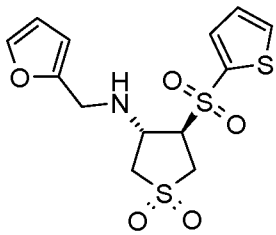
[00538]



Compound D104

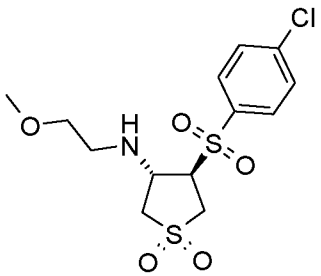


Compound D134

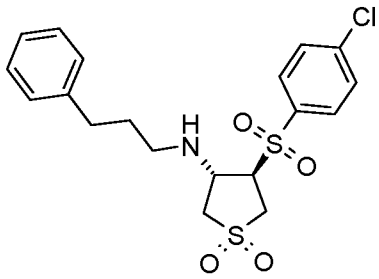


Compound D135

[00539]

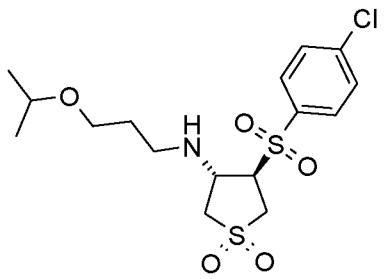


Compound D136

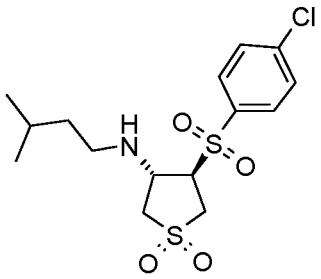


Compound D137

[00540]

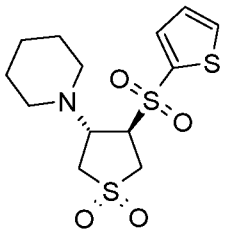


Compound D138

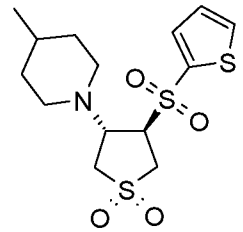


Compound D131

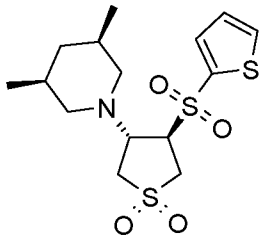
[00541]



Compound D105

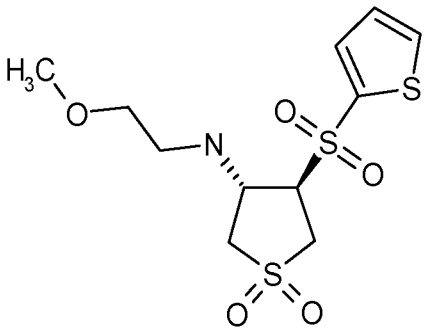


Compound D106

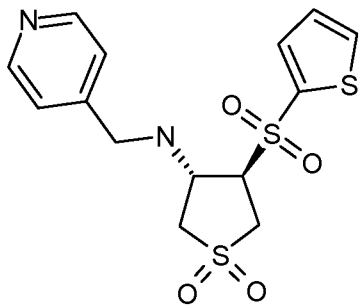


Compound D109

[00542]

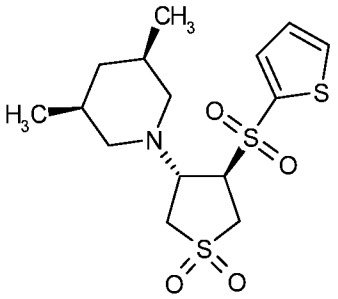


Compound D107

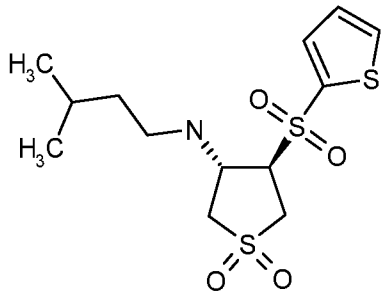


Compound D108

[00543]

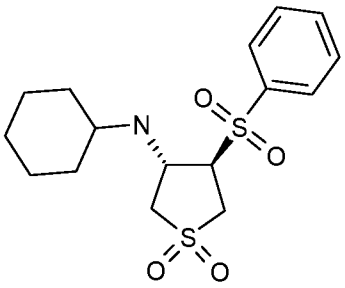


Compound D109

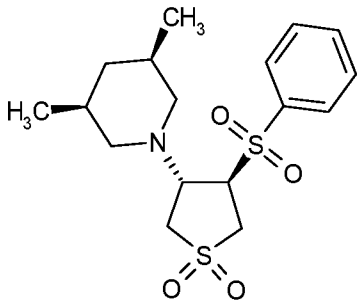


Compound D110

[00544]

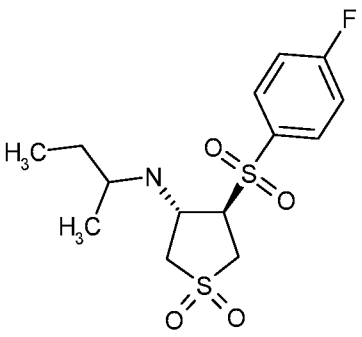


Compound D111

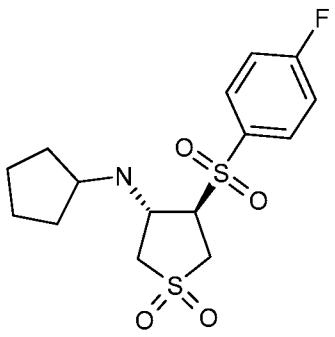


Compound D112

[00545]

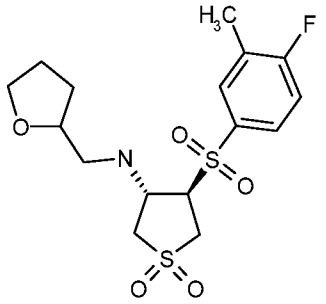


Compound D113

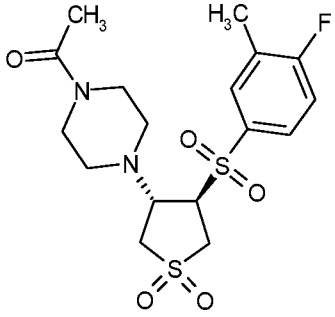


Compound D114

[00546]

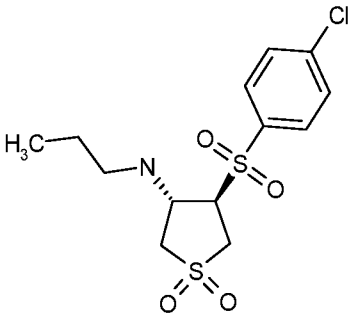


Compound D115

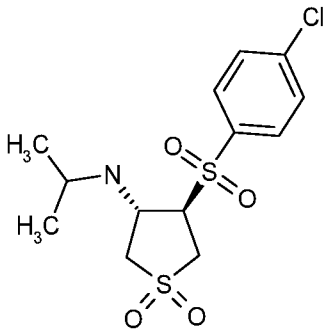


Compound D116

[00547]

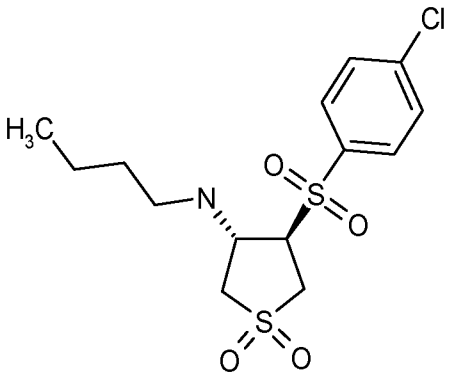


Compound D117

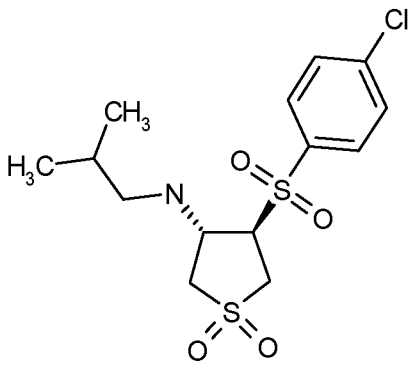


Compound D118

[00548]

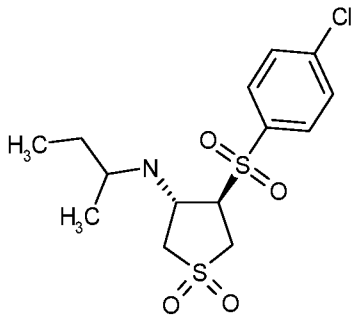


Compound D119

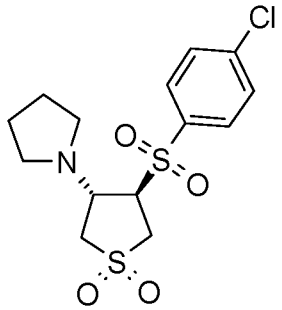


Compound D120

[00549]

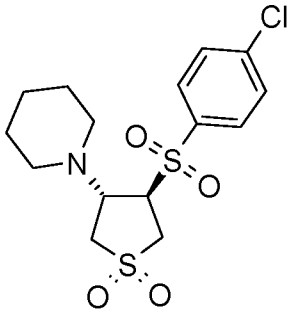


Compound D121

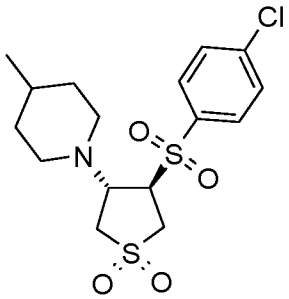


Compound D122

[00550]

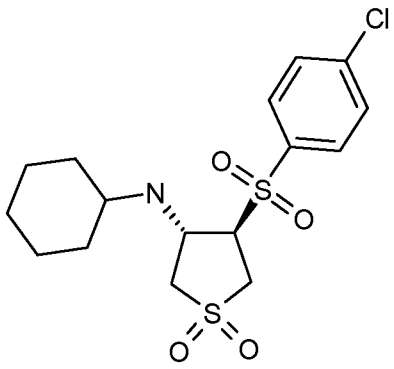


Compound D123



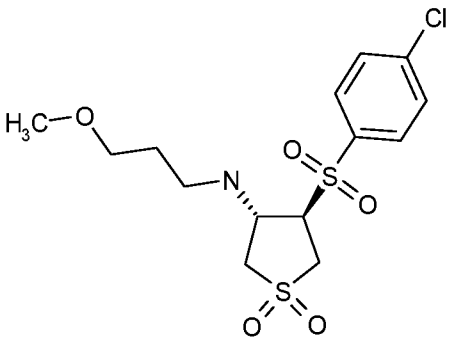
Compound D125

[00551]

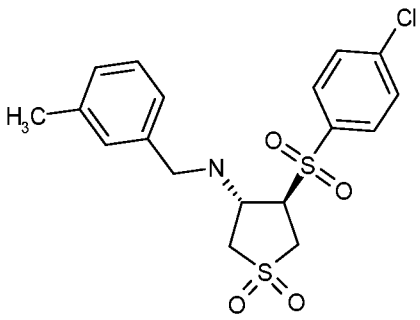


Compound D124

[00552]

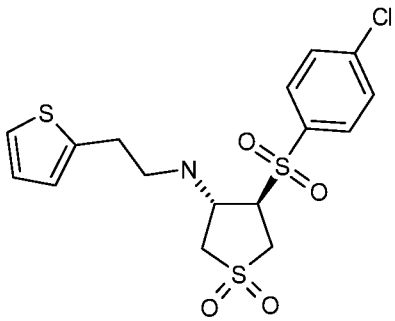


Compound D126

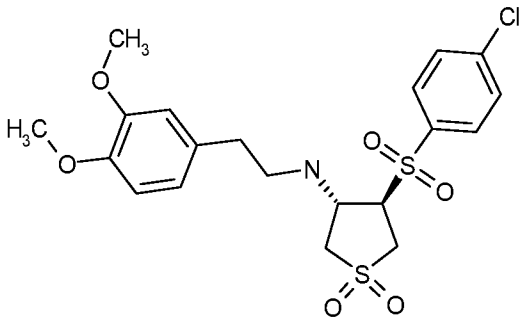


Compound D127

[00553]

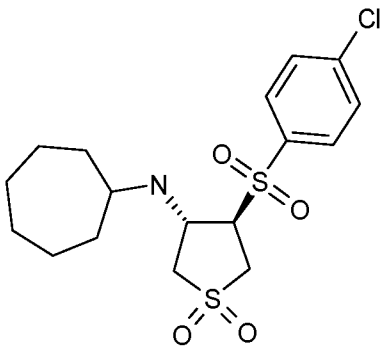


Compound D128

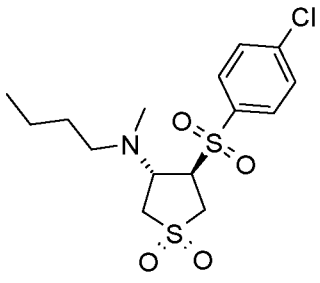


Compound D129

[00554]

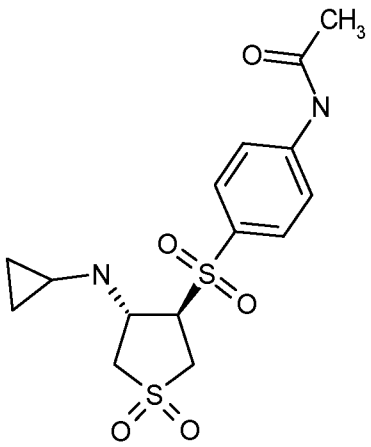


Compound D130



Compound D132

[00555]



Compound D133

[00556] Tables 28A and 28B, 29-29C, and 30 show the results of testing of these compounds.

[00557] For Tables 28A and 28B,

S/B > 5
S/B > 2
S/B <0.5

ID	Firefly											
	F4, cmpd/DMSO (mean)				LBD, cmpd/DMSO (mean)				pGL4, cmpd/DMSO (mean)			
	40uM	10uM	2uM	0.5uM	40uM	10uM	2uM	0.5uM	40uM	10uM	2uM	0.5uM
D104	0.8	6.2	2.4	1.7	0.6	3.3	1.7	1.3	0.7	4.0	1.4	1.3
D105	0.9	5.5	2.4	1.1	0.7	3.8	1.8	1.2	0.7	3.8	1.9	1.2
D106	0.8	5.4	2.3	1.5	0.7	4.0	1.7	1.2	0.7	3.6	1.6	1.1
D107	4.0	2.2	1.6	1.3	2.2	2.2	1.1	0.9	2.5	2.0	1.5	1.2
D108	1.9	1.3	1.3	1.0	2.1	1.3	1.4	1.0	1.5	1.2	1.0	0.9
D109	0.4	4.2	2.3	1.3	0.2	3.2	2.1	1.1	0.5	3.6	1.6	1.0
D110	1.4	3.5	1.9	1.7	0.8	3.0	1.4	1.1	0.7	3.3	1.4	1.2
D111	2.7	2.0	1.1	1.1	1.9	1.6	1.4	1.1	1.8	1.5	1.4	0.9
D112	2.6	2.8	1.9	1.2	1.9	1.8	1.2	1.1	2.2	2.2	1.2	1.0
D113	3.3	2.8	1.6	1.3	2.5	2.2	1.3	1.1	2.0	2.6	1.1	1.2
D114	3.6	2.4	1.6	1.6	2.2	1.9	1.3	1.1	2.0	2.0	1.3	1.1
D115	2.2	1.4	1.1	1.0	2.2	1.3	0.9	0.9	2.0	1.3	1.2	1.2
D116	1.2	1.4	1.0	1.5	1.2	1.1	1.2	0.9	1.4	1.1	1.2	1.0
D117	0.5	4.7	2.2	1.1	0.2	4.2	2.1	1.1	0.3	4.6	1.4	1.0
D118	2.4	6.2	1.6	1.6	0.4	3.8	1.8	1.2	0.4	4.8	1.5	1.2
D119	0.6	5.3	2.0	0.9	0.5	3.4	1.5	1.0	0.6	3.9	1.5	1.1
D120	2.2	5.1	1.5	1.3	0.8	4.1	1.7	1.1	0.8	3.7	1.5	1.4
D121	1.9	4.9	1.9	0.9	0.7	3.6	1.5	1.0	0.7	3.9	1.6	1.4
D122	0.7	6.8	3.3	1.6	0.6	3.1	2.5	1.2	0.6	2.3	2.4	1.3

D123	0.7	7.1	2.5	1.8	0.4	4.9	1.9	1.0	0.6	3.4	1.7	1.2
D124	2.1	4.6	1.3	1.6	0.5	3.6	1.6	1.4	0.7	3.1	1.5	1.4
D125	0.9	5.6	2.3	1.3	0.4	4.6	1.6	1.0	0.6	4.2	1.9	1.4
D126	2.3	4.5	1.8	1.0	2.1	2.9	1.5	1.6	0.8	3.0	1.2	1.2
D127	2.0	2.2	1.1	1.2	2.1	2.3	1.2	1.2	2.6	1.8	1.4	1.0
D128	3.5	2.7	1.7	1.1	2.6	2.4	1.1	1.0	1.5	2.1	1.6	1.2
D129	3.4	4.3	2.0	1.2	2.4	2.7	1.7	1.1	1.6	2.9	1.5	1.4
D130	1.6	4.0	2.2	1.4	0.7	3.1	1.7	1.1	0.6	3.4	1.8	1.2
D131	2.3	4.1	1.9	1.4	0.7	3.6	2.0	1.3	0.5	2.7	1.5	1.1
D132	1.1	5.5	1.9	1.5	0.4	4.3	2.1	1.1	0.5	4.9	1.6	1.6
D133	1.3	1.8	1.4	1.0	1.1	1.4	1.1	1.1	1.1	1.9	1.1	1.1

Table 28A

ID	**Renilla**							
	F4, cmpd/DMSO (mean)				**LBD, cmpd/DMSO (mean)**			
	40uM	**10uM**	**2uM**	**0.5uM**	**40uM**	**10uM**	**2uM**	**0.5uM**
D104	0.3	1.6	1.2	1.1	0.2	0.9	1.2	1.1
D105	0.7	1.7	1.2	1.1	0.4	0.9	1.1	1.0
D106	0.5	1.7	1.2	1.0	0.2	0.9	1.1	1.0
D107	1.5	1.2	1.0	1.1	0.7	1.1	1.1	1.1
D108	1.2	1.1	1.0	1.0	1.1	1.1	1.1	1.1
D109	0.5	1.6	1.2	1.1	0.3	0.9	1.0	1.0
D110	1.4	1.3	1.1	1.1	0.5	1.0	1.1	1.1
D111	1.4	1.1	1.0	1.0	0.8	1.1	1.1	1.1
D112	1.6	1.3	1.1	1.0	0.9	1.1	1.0	1.0
D113	1.4	1.3	1.0	1.1	0.7	1.1	1.0	1.1
D114	1.3	1.2	1.1	1.0	0.7	1.1	1.1	1.1
D115	1.1	1.0	1.0	1.0	1.1	1.1	1.1	1.1
D116	1.1	1.1	1.1	1.0	1.1	1.1	1.1	1.0
D117	0.4	1.7	1.3	1.0	0.1	0.9	1.1	1.0
D118	1.9	1.7	1.3	1.1	0.3	1.0	1.1	1.1
D119	0.2	1.6	1.2	1.2	0.1	0.9	1.1	1.1
D120	1.6	1.7	1.2	1.0	0.3	0.9	1.1	1.0
D121	1.7	1.5	1.1	1.2	0.4	1.1	1.0	1.1
D122	0.2	2.2	1.3	1.1	0.1	0.6	1.1	1.1
D123	0.3	1.9	1.4	1.1	0.2	0.9	1.1	1.1
D124	1.8	1.5	1.2	1.1	0.2	0.9	1.1	1.1
D125	1.3	1.8	1.3	1.0	0.4	1.0	1.1	1.1
D126	1.6	1.6	1.2	1.1	0.5	1.1	1.1	1.0
D127	1.3	1.2	1.0	1.0	0.9	1.1	1.1	1.2
D128	1.6	1.4	1.2	1.0	0.8	1.2	1.1	1.1
D129	1.6	1.4	1.2	1.1	0.6	1.0	1.1	1.0
D130	1.8	1.5	1.2	1.1	0.4	1.0	1.0	1.1
D131	1.8	1.6	1.2	1.1	0.4	1.1	1.1	1.1
D132	1.3	1.7	1.2	1.0	0.3	1.0	1.1	1.0
D133	1.1	1.1	1.0	1.0	1.0	1.1	1.1	1.0

Table 28B

[00558] For Tables 29A and 29B,

S/B > 10	S/B > 5	S/B > 3	S/B <0.5

Compound ID	Firefly															
	F4, 1% FBS, cmpd/DMSO (mean)				F4, 10% FBS, cmpd/DMSO (mean)				pGL4, 1% FBS, cmpd/DMSO (mean)				pGL4, 10% FBS, cmpd/DMSO (mean)			
	40uM	10uM	2uM	0.5uM	40uM	10uM	2uM	0.5uM	40uM	10uM	2uM	0.5uM	40uM	10uM	2uM	0.5uM
D104	0.7	8.5	1.9	0.9	1.4	11	1.4	0.8	0.6	1.5	1.0	1.0	0.5	2.7	1.7	1.1
D104	0.4	5.1	1.6	0.9	1.0	13	1.5	1.3	1.0	2.4	1.8	1.1	0.8	2.9	1.6	1.0
D105	0.3	4.9	1.7	1.5	3.6	8.9	1.5	1.0	1.1	2.6	1.2	1.4	1.7	2.6	1.6	1.3
D106	0.5	4.6	1.9	0.9	1.7	7.1	1.4	1.6	0.8	2.8	1.8	1.1	1.2	2.7	1.2	0.9
D107	3.8	1.4	0.8	1.5	6.1	1.5	1.4	0.8	1.4	1.8	1.4	1.3	2.3	1.7	1.3	1.1
D134	5.4	2.0	1.2	1.0	4.6	2.1	1.0	1.1	1.4	1.3	1.3	1.0	2.0	1.7	1.3	1.2
D135	3.3	1.1	1.6	1.2	3.7	1.8	0.7	1.0	1.1	0.8	1.0	1.0	1.6	1.7	1.1	0.9
D108	1.8	1.1	1.1	1.2	2.2	1.1	1.0	1.2	1.1	1.2	1.2	1.5	1.4	1.1	1.1	1.1
D109	0.2	5.5	1.6	1.5	3.1	6.5	1.4	1.3	1.1	2.1	1.3	1.2	1.1	2.2	1.2	1.4
D110	0.8	3.8	1.4	0.8	5.9	2.4	1.0	1.3	0.8	1.7	1.2	1.0	2.6	1.8	1.2	1.2
D111	2.1	1.6	1.1	1.0	3.6	1.1	1.6	1.6	1.2	1.2	1.2	1.0	1.3	1.3	1.2	1.2
D112	3.2	1.6	0.9	1.1	5.0	1.5	1.2	1.2	1.2	1.7	1.2	1.3	1.3	1.5	1.0	1.3
D113	2.5	2.8	1.1	1.4	5.8	2.1	1.5	1.7	1.6	1.8	1.2	1.0	2.5	1.7	1.2	1.1
D114	2.2	1.8	1.0	1.3	3.2	1.7	1.1	1.2	1.5	1.7	1.3	1.1	2.0	1.5	1.3	1.3
D115	1.8	1.1	0.8	1.0	1.3	0.7	1.3	1.5	1.6	1.2	1.2	1.1	2.1	1.6	1.2	1.2
D116	1.1	0.9	0.9	1.2	0.8	1.3	1.4	1.1	1.4	1.7	1.0	1.6	1.5	1.4	1.1	1.1
D117	0.1	5.9	2.1	0.9	2.9	4.1	1.2	0.9	0.6	1.9	1.4	1.0	1.2	2.2	1.6	1.1
D118	0.5	5.2	1.4	1.1	15	5.1	1.3	1.2	0.9	2.1	1.2	0.9	8.1	2.9	1.2	1.2
D119	0.2	4.4	1.8	1.5	1.6	7.3	1.1	1.5	0.7	2.4	1.4	1.2	0.5	2.2	1.2	1.1
D120	0.2	4.7	1.2	0.8	1.7	3.6	1.2	1.0	0.9	2.8	1.5	1.5	0.7	2.0	1.7	1.1
D121	0.1	3.9	1.2	1.5	3.4	3.3	1.4	1.2	0.8	3.2	1.1	1.1	1.2	2.4	1.4	1.1
D122	0.3	2.8	3.2	1.4	0.5	11	1.8	1.5	0.7	2.6	2.5	1.1	0.7	3.7	2.2	0.9
D123	0.2	4.4	2.1	1.3	0.8	7.5	2.0	1.1	0.8	2.7	1.8	1.1	0.8	3.0	1.6	1.2
D124	0.4	3.1	1.9	0.9	4.5	2.3	1.0	1.2	0.8	2.4	1.6	1.1	3.2	2.2	1.4	1.2
D127	0.5	4.8	1.8	1.0	0.6	5.5	1.4	1.2	0.6	2.7	1.9	1.3	0.6	2.7	1.7	1.2
D136	7.4	2.4	1.0	0.8	12	1.9	1.4	0.4	1.6	1.3	0.9	1.1	3.8	1.8	1.1	1.3
D126	0.6	3.1	1.5	0.7	8.3	2.9	1.0	1.4	1.6	2.0	1.8	1.4	3.9	1.8	1.1	1.1
D127	3.1	2.2	0.9	1.3	3.2	1.5	0.7	1.2	1.3	1.6	1.3	1.1	1.4	1.4	1.2	1.0
D128	2.0	2.0	1.5	1.2	3.3	1.4	0.9	0.8	1.5	1.9	1.3	1.1	1.7	1.5	1.1	0.8
D129	1.4	2.9	1.1	0.9	4.1	1.9	0.8	1.1	1.6	1.9	1.2	1.4	2.2	2.0	1.0	1.0
D137	3.4	3.9	2.2	1.2	13	4.6	1.4	0.9	1.5	1.5	1.0	1.0	4.3	2.1	1.1	0.7
D138	3.3	4.2	1.9	0.8	12	3.1	1.3	1.8	1.7	1.6	1.0	1.0	3.6	1.7	1.0	1.1
D130	0.6	3.6	1.2	0.7	5.0	3.4	1.2	1.1	0.9	2.0	2.0	1.2	2.5	2.5	1.6	1.0
D131	0.8	5.4	1.5	0.8	4.2	4.1	1.1	0.7	0.9	1.7	1.1	1.0	1.8	1.9	1.2	1.0
D131	0.7	3.6	0.9	0.9	6.2	2.3	1.1	1.5	1.1	2.4	1.6	1.2	2.9	2.4	1.5	1.2
D132	0.5	5.0	1.3	0.7	5.4	3.3	1.4	0.9	0.8	2.6	1.6	1.0	1.6	2.7	1.3	0.9
D133	0.9	1.2	0.8	0.8	1.0	1.0	0.9	1.5	1.0	1.3	1.1	1.3	1.2	1.3	0.9	0.9

Table 29A

Compound ID	Renilla							
	F4, 1% FBS, cmpd/DMSO (mean)				F4, 10% FBS, cmpd/DMSO (mean)			
	40uM	10uM	2uM	0.5uM	40uM	10uM	2uM	0.5uM
D104	1.0	2.4	1.4	1.1	2.2	2.5	1.3	1.0
D104	0.7	1.9	1.4	1.1	1.3	2.6	1.3	1.2
D105	1.6	2.0	1.2	1.1	2.6	2.1	1.2	1.1
D106	1.1	1.9	1.4	1.2	2.0	2.0	1.1	1.2
D107	1.8	1.3	1.2	0.9	2.1	1.3	1.0	1.0
D134	2.1	1.4	1.2	1.0	2.0	1.3	1.0	1.0
D135	1.7	1.2	1.3	1.0	1.9	1.1	1.1	1.0
D108	1.4	1.1	1.2	1.0	1.4	1.0	0.9	1.0
D109	1.1	1.7	1.3	1.0	2.4	1.9	1.2	1.0
D110	1.5	1.5	1.1	1.0	2.4	1.5	1.0	1.0
D111	1.7	1.3	1.1	1.1	1.8	1.2	1.1	1.1
D112	2.0	1.4	1.1	1.1	2.1	1.3	1.0	1.0
D113	1.8	1.6	1.2	1.3	1.8	1.2	1.0	1.0
D114	1.8	1.7	1.2	1.1	1.5	1.1	1.0	0.9
D115	1.5	1.2	1.0	1.1	1.3	1.1	1.0	1.0
D116	1.2	1.0	1.1	1.0	1.2	0.9	0.9	0.8
D117	0.8	1.7	1.4	1.0	2.4	1.8	1.0	1.0
D118	2.3	1.6	1.1	1.0	5.2	2.5	1.5	1.2
D119	0.4	1.8	1.1	1.1	1.3	2.7	1.5	1.4
D120	0.6	2.1	1.3	1.0	1.5	1.7	1.1	1.0
D121	1.3	1.7	1.4	1.1	2.6	1.4	1.1	1.0
D122	0.0	2.2	1.4	1.2	0.0	2.9	1.0	1.0
D123	0.1	1.6	1.4	1.1	0.2	1.9	1.1	0.9
D124	1.6	1.5	1.2	1.1	2.6	1.3	1.0	1.0
D127	1.5	1.8	1.2	1.1	0.4	1.7	1.1	1.0
D136	2.4	1.5	1.1	1.1	3.3	1.2	0.9	0.9
D126	1.8	1.5	1.2	1.2	2.8	1.7	1.1	1.0
D127	1.6	1.4	1.2	1.0	1.5	1.2	1.1	1.0
D128	1.7	1.6	1.1	1.2	1.5	1.1	1.0	0.9
D129	1.5	1.6	1.1	1.1	1.5	1.1	0.9	1.0
D137	3.5	2.1	1.3	1.0	4.0	2.1	1.4	1.2
D138	2.9	1.9	1.4	1.3	3.6	2.0	1.4	1.4
D130	1.9	1.6	1.2	1.1	2.1	1.3	0.9	0.8
D131	2.0	2.0	1.3	1.0	3.0	1.8	1.1	1.0
D131	1.8	1.7	1.2	1.1	2.5	1.3	1.0	0.9
D132	1.7	1.7	1.3	1.1	3.0	1.6	1.1	0.9
D133	1.1	1.0	1.0	1.0	1.0	1.0	0.9	0.9

Table 29B

[00559] For Table 29C, S/B > 4 and S/B > 2.

S/B > 4 | S/B > 2

Compound ID	Firefly, F4/pGL4 for S/B(F4)> 3								F4, Firefly/Renilla for S/B(F4)>3							
	1% FBS				10% FBS				1% FBS				10% FBS			
	40uM	10uM	2uM	0.5uM	40uM	10uM	2uM	0.5uM	40uM	10uM	2uM	0.5uM	40uM	10uM	2uM	0.5uM
D104		5.7				4.2				3.5				4.5		
D104		2.1				4.3				2.6				4.9		
D105		1.9			2.1	3.4				2.5			1.4	4.3		
D106		1.7				2.7				2.5				3.6		
D107	2.6				2.7				2.2				2.9			
D134	4.0				2.3				2.6				2.3			
D135	3.0				2.3				1.9				1.9			
D108																
D109		2.6			2.8	3.0				3.1			1.3	3.3		
D110		2.3			2.3					2.5			2.5			
D111					2.7								2.0			
D112	2.7				3.7				1.6				2.3			
D113					2.3								3.2			
D114					1.6								2.1			
D115																
D116																
D117		3.2				1.9				3.4				2.3		
D118		2.5			1.8	1.8				3.2			2.8	2.1		
D119		1.9				3.3				2.5				2.7		
D120		1.7				1.8				2.3				2.1		
D121		1.2			2.9	1.4				2.3			1.3	2.4		
D122			1.3			3.1					2.3			3.9		
D123		1.6				2.5				2.7				4.0		
D124		1.3			1.4					2.0			1.7			
D127		1.8				2.1				2.7				3.2		
D136	4.7				3.0				3.1				3.5			
D126		1.6			2.1					2.0			3.0			
D127	2.4				2.3				1.9				2.1			
D128					1.9								2.2			
D129					1.8								2.6			
D137	2.4	2.7			3.1	2.2			1.0	1.9			3.3	2.2		
D138	1.9	2.7			3.2	1.8			1.1	2.2			3.2	1.6		
D130		1.8			2.0	1.4				2.3			2.4	2.7		
D131		3.3			2.3	2.2				2.7			1.4	2.2		
D131		1.5			2.1					2.1			2.5			
D132		1.9			3.4	1.2				3.0			1.8	2.0		
D133																

Table 29C

[00560] Table 30 shows the results of further testing.

Compound ID	Firefly												Renilla							
	F4, cmpd/DMSO (mean)				LBD, cmpd/DMSO (mean)				pGL4, cmpd/DMSO (mean)				F4, cmpd/DMSO (mean)				LBD, cmpd/DMSO (mean)			
	40uM	10uM	2uM	0.5uM	40uM	10uM	2uM	0.5uM	40uM	10uM	2uM	0.5uM	40uM	10uM	2uM	0.5uM	40uM	10uM	2uM	0.5uM
D104	1.0	4.3	4.5	2.1	0.2	1.2	2.9	1.3	0.7	2.4	1.8	1.2	0.3	1.7	2.0	1.2	0.2	0.6	1.1	1.1
D134	2.8	2.1	2.3	1.5	1.8	1.9	1.5	1.5	1.7	1.6	1.3	1.0	1.5	1.3	1.2	1.1	0.7	1.0	0.9	0.9
D135	2.8	1.9	2.4	1.7	2.8	1.6	1.7	1.4	1.3	1.4	1.6	1.0	1.3	1.0	1.2	0.9	0.9	0.8	0.9	0.9
D136	5.2	2.7	1.1	1.5	2.9	2.6	1.5	1.0	2.6	1.8	1.0	1.1	1.7	1.4	1.1	0.9	0.6	1.0	1.0	1.1
D137	0.6	2.0	1.3	1.1	0.4	3.2	2.0	1.1	1.5	1.5	1.2	0.8	2.6	1.8	1.1	1.1	0.4	0.8	1.0	1.0
D138	1.7	2.6	1.1	1.0	0.4	3.7	1.8	1.3	1.5	1.5	0.9	0.9	2.3	1.2	1.0	0.8	0.3	0.9	1.1	0.9
D131	0.9	4.3	1.4	1.3	0.5	4.3	1.7	0.8	1.0	2.3	1.3	1.0	0.3	1.9	1.0	1.0	0.2	0.8	1.1	1.1

Table 30

For Table 30, S/B > 4 and S/B > 2.

S/B > 4
S/B > 2

[00561] Table 31 shows further results of testing.

[00562]

IDNUMBER	Activity, cmpd/DMSO	renilla, cmpd/DMSO
D104	7.1	2.2
D105	5.7	1.5
D106	5.2	2.2
D109	6.2	2.1
D122	6.6	2.0
D123	6.1	2.4
D125	7.3	2.5
D132	6.6	2.1

Table 31

[00563] For Table 31,

S/B > 5

[00564] **FIGS. 59A-D** show results of testing of Compounds D104, D118, D122 and D137. Compounds were tested at 10% FBS.

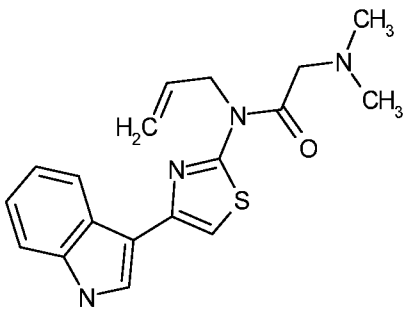
[00565] Table 30 shows the results of further testing on firefly and renilla. For Table 30,

S/B > 4
S/B > 2

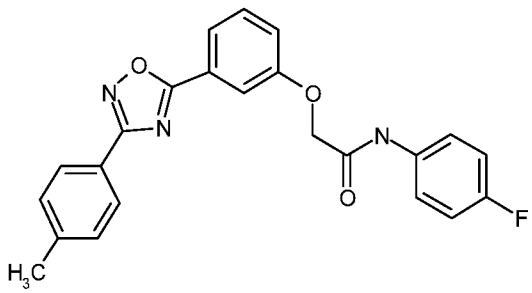
[00566] Compound D136 was further tested, and results are shown in **FIGS. 60A-C**. For cytokines release and cytotox on hPBMCs each compound was tested at 1.25, 2.5, 5, 10, 25 and 50 uM in duplicates. Human PBMC were activated by 10 ng/mL PMA + 500 ng/mL ionomycin. Data were normalized to controls with (100%) w/o cmpds.

[00567] **FIGS. 60B** and **60C** show comparisons with other compounds, including Compounds 140, 141, 142 and 166.

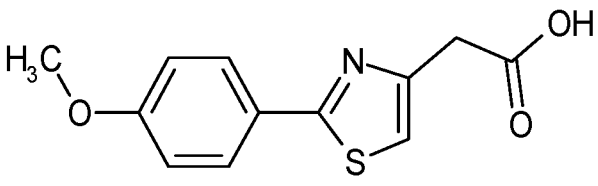
[00568]

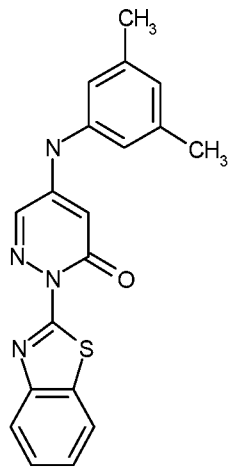


Compound D140



Compound D141





Compound D142 Compound Z166

[00569] **FIGS. 61A-61E** show further results of cytokine release by hPBMC for Compound D136. Each compound was tested at 1.25, 2.5, 5, 10, 25 and 50 uM in duplicates.

[00570] Human PBMC were activated by 10 ng/mL PMA + 500 ng/mL ionomycin. Data were normalized to controls with (100%) w/o cmpds.

[00571] No IL1b release activation by hPBMC was observed at high release activation of others cytokines.

[00572] **FIGS. 62A** and **62B** show stability in plasma (human plasma and rat plasma) and various pH solubility data for Compound D136.

[00573] Table 33 shows the pH test results for this compound.

ID	OD, nm	pH 2		pH 4		pH 7	
		solubility, mg/ml		solubility, mg/ml		solubility, mg/ml	
		value	SD	value	SD	value	SD
D136	250	0.66	0.01	0.22	0.06	0.19	0.01

Table 33

[00574] **FIGS. 63A** and **63B** show stability in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF), respectively, for Compound D136.

[00575] **FIGS. 64A** and **64B** show microsomal stability in human liver microsomes and rat liver microsomes, respectively, for Compound D136.

[00576] Tables 34 and 35 show CYP and PPB data for Compound D136.

CYP isoform	Test compound	Control inhibitor final concentration, uM	IC50 M	IC50 uM	
1A2-Phenacetin	D136	10-0.0098 uM	>1E-05	>10	no inhibition
2C19-Mephenytoin	D136	100-0.098 uM	>1E-05	>10	no inhibition
3A4-Midazolam	D136	100-0.098 uM	>1E-05	>10	no inhibition
3A4-Testosterone	D136	100-0.098 uM	>1E-05	>10	no inhibition

2D6-Dextromethorphan	D136	100-0.098 uM	>1E-05	>10	no inhibition
2C9-Tolbutamide	D136	100-0.098 uM	>1E-05	>10	no inhibition
2C8-Amodiaquine	D136	100-0.098 uM	>1E-05	>10	no inhibition

Table 34

[00577]

Species	Cmpd	Permeability, %	Recovery, %	%, Free (50%pl)	%, Free (recalc from 50%pl)	%, Bound	Conclusion
Human	D136	94	56.9	65.3	48.4	**51.6**	Low
Rat	D136	94	122	64.9	48.0	**52.0**	Low

Table 35

[00578] **FIGS. 65A** and **65B** show PK in rat plasma for Compound D136.

[00579] **FIGS. 66A** and **66B** show PK in mice plasma for Compound D136.

[00580] Tables 36, 37 and 38 show related data.

[00581]

Parameter	Units	Estimate
K_el	1/h	0.75
T_1/2	h	0.93
Tmax	h	0.5
Cmax	ng/ml	165
AUClast	h*ng/ml	197
AUCINF	h*ng/ml	208
Vz/F	ml/kg	66315
Cl/F	ml/h/kg	51948
MRTlast	h	1.13
MRTINF	h	1.33

Table 36

PO plasma	IV plasma	Fabs, %

AUCinf AUV last	Dose, mg/kg	AUCinf AUV last	Dose, mg/kg	
208	7.53	242	1.13	12.9%
197	7.53	239	1.13	12.3%

Table 37

[00582]

Parameter	**Units**	**Estimate**
K_el	1/h	2.11
T_1/2	h	0.33
Tmax	h	0.083
Cmax	ng/ml	524
AUClast	h*ng/ml	239
AUCINF	h*ng/ml	242
Vz/F	ml/kg	2263
Cl/F	ml/h/kg	4814
MRTlast	h	0.35
MRTINF	h	0.37

Table 38

[00583] Tables 39, 40 and 41 show related data.

Parameter	Units	Estimate
K_el	1/h	1.20
T_1/2	h	0.58
Tmax	h	0.25
Cmax	ng/ml	89.6
AUClast	h*ng/ml	116
AUCINF	h*ng/ml	117
Vz/F	ml/kg	22544
Cl/F	ml/h/kg	27092
MRTlast	h	1.04
MRTINF	h	1.08

Table 39

[00584]

PO plasma	**IV plasma**	**Fabs, %**

AUCinf AUV last	Dose, mg/kg	AUCinf AUV last	Dose, mg/kg	
117	3.18	124	1.12	33.2%
116	3.18	124	1.12	33.0%

Table 40

[00585]

Parameter	Units	Estimate
K_el	1/h	1.16
T_1/2	h	0.60
Tmax	h	0.083
Cmax	ng/ml	390
C0	ng/ml	770
AUClast	h*ng/ml	124
AUCINF	h*ng/ml	124
Vz	ml/kg	7743
Cl	ml/h/kg	9006
MRTlast	h	0.25
MRTINF	h	0.27
Vss	ml/kg	2413

Table 41

[00586] A Caco-2 permeability assay was performed on Compound D136. Results are shown in table 42.

ID	P_{app} A-B, 10^{-6} cm/s	P_{app} B-A, 10^{-6} cm/s	Asymmetry Index
Compound D136	33.6	47.9	
	36.0	54.6	
	33.1	50.9	
Mean	**34.2**	**51.1**	**1.5**
SD	**1.5**	**3.4**	
CV	**4.5**	**6.6**	

Table 42

[00587] Regarding Compound D136, the following were key characteristics:

[00588] 1. Activates full-length and ligand binding domain-only NR2F6 in cellular assays.

[00589] 2. Selectively inhibits IL-1, TNF-α, and IL-17a in human PBMC.

[00590] 3. Stable in human and rat plasma with low PPB.

[00591] 4. Extremely stable in the presence of human, rat and mouse liver microsomes and no inhibition of 7 major isoforms of hCyt P450.

[00592] 5. PK in rat, mouse and dog completed – orally bioavailable (~33% absorbance).

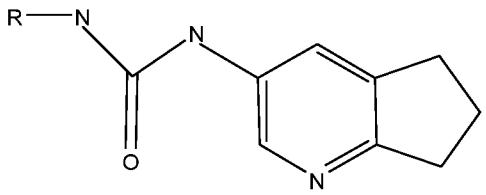
[00593] 6. MTD and acute toxicity in mice completed – no toxicity was observed.

[00594] 7. Solubility and pH stability studies completed – soluble at pH2, pH4 and pH7.4, extremely stable at pH2 and pH7.

[00595] 8. Caco-2 permeability and asymmetry – highly permeable and not a substrate for P-gp (MDR1).

[00596] 9. Considered a Class II biopharmaceutical.

[00597] In certain embodiments, useful compounds herein have the general Formula (XVIII):

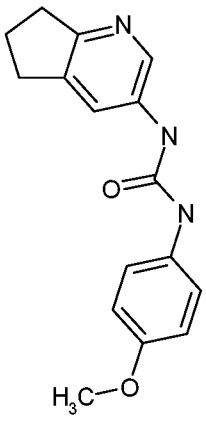


(XVIII)

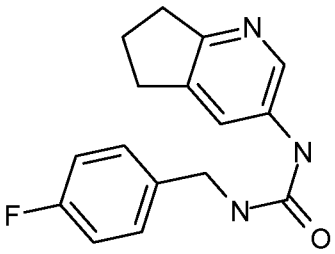
where R can be one or more of the following: C, H, N, O, S, a halogen, an alkyl group, a substituted alkyl group, a cyclic alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, an ester, an aldehyde, a ketone, a carboxylic acid, an amide, an amine, an ether, a thiol or a nitrile.

[00598] In certain embodiments, some compounds of Formula (XVIII) were found to be useful as NR2F6 inhibitors (antagonists). These include the following (Compounds T1 through T13):

[00599]

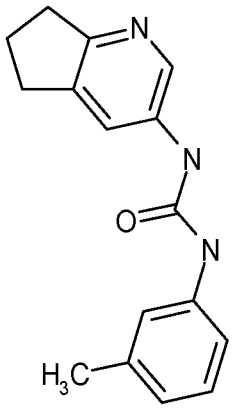


Compound T1

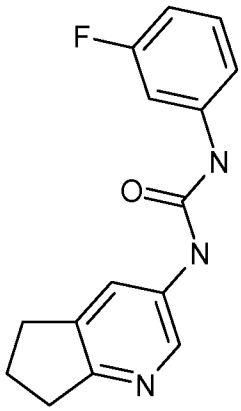


Compound T2

[00600]

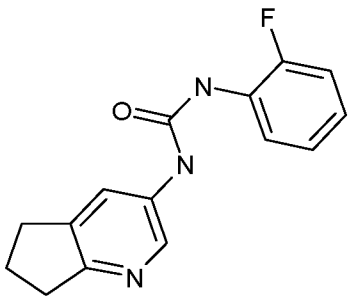


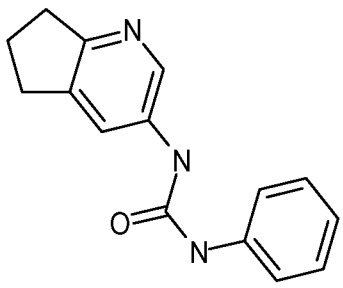
Compound T3



Compound T4

[00601]

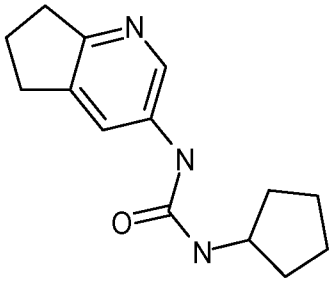


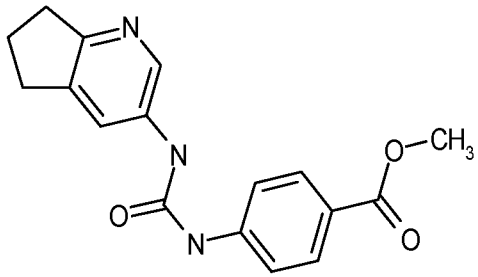


Compound T5

Compound T6

[00602]

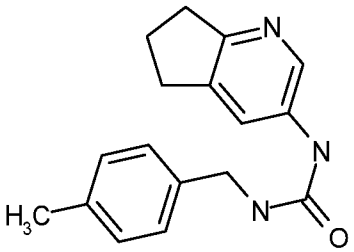


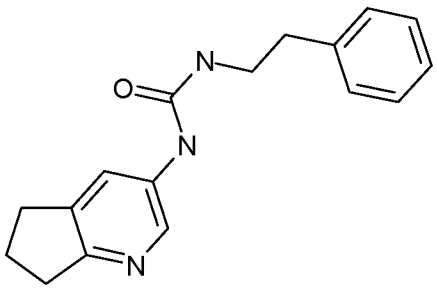


Compound T7

Compound T8

[00603]

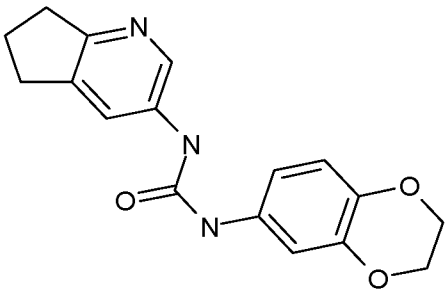


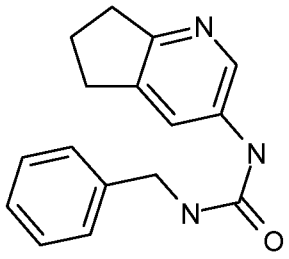


Compound T9

Compound T10

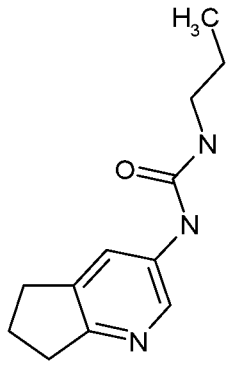
[00604]





Compound T11 Compound T12

[00605]



Compound T13

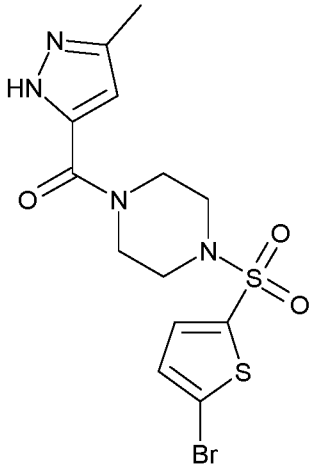
[00606] Table 43 shows the results of testing on these compounds.

Compound	Firefly activity, cmpd/DMSO (repeat 1)	Firefly activity, cmpd/DMSO (repeat 2)	Renilla activity, cmpd/DMSO (repeat 1)	Renilla activity, cmpd/DMSO (repeat 2)
T1	3.1	2.4	1.1	1.3
T2	1.5	1.2	1.0	0.8
T3	3.1	2.7	1.1	1.0
T4	2.2	2.5	1.2	0.9
T5	2.2	2.1	1.1	1.3
T6	2.5	5.6	1.0	1.1
T7	2.5	2.1	0.9	1.0
T8	3.0	2.5	1.8	0.9
T9	2.1	2.1	1.1	1.22.8
T10	2.8	3.0	1.0	1.3
T11	2.3	2.8	0.8	0.9

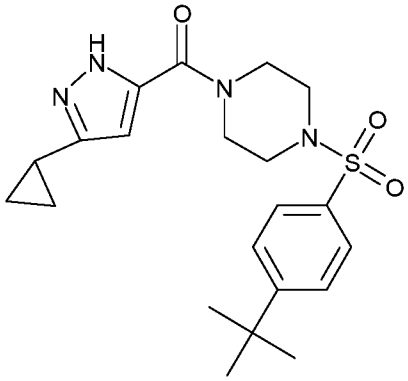
T12	2.3	2.3	1.1	0.8
T13	1.7	1.3	1.1	0.9

Table 43

[00607] In certain embodiments, the following compounds were found to be useful:

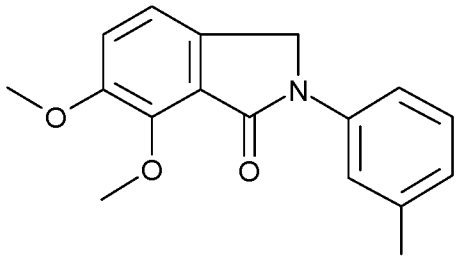


Compound E54

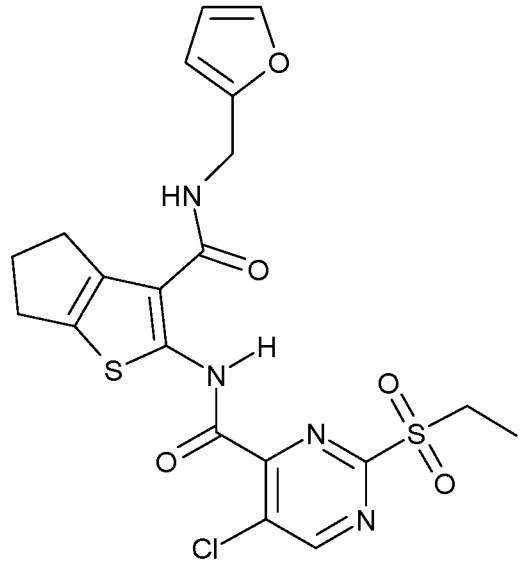


Compound E55

[00608]

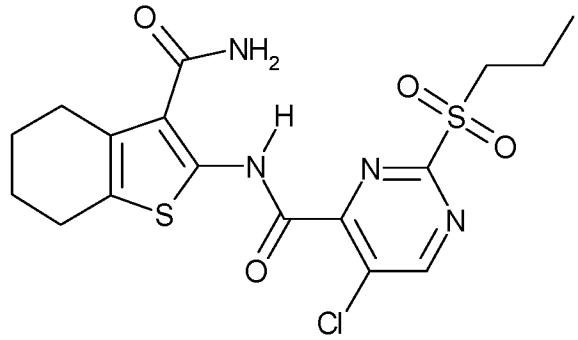


Compound 46

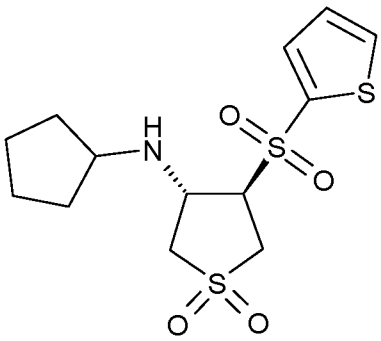


Compound D102

[00609]

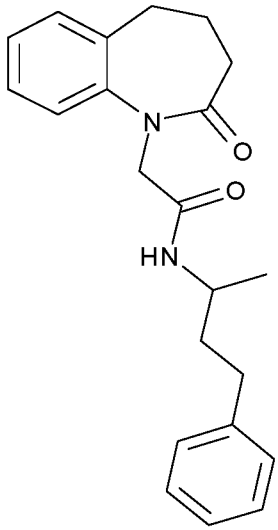


Compound D103

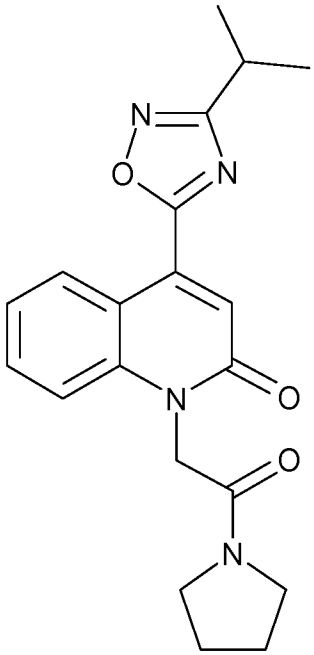


Compound D139

[00610]

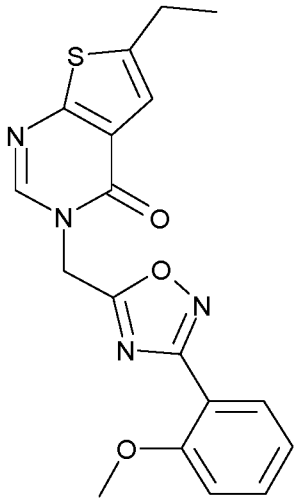


Compound Z164

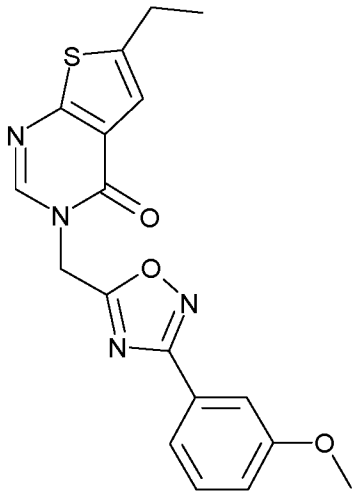


Compound Z165

[00611]

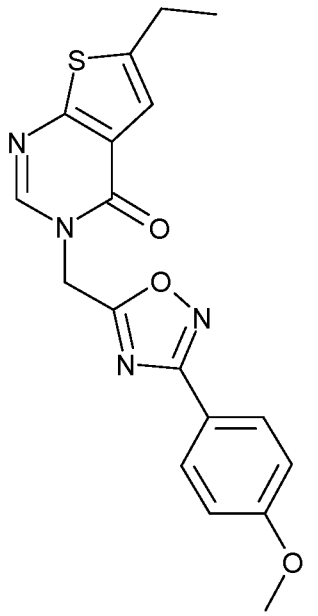


Compound Z167

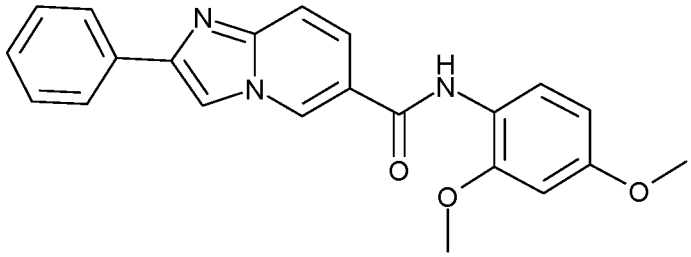


Compound Z168

[00612]

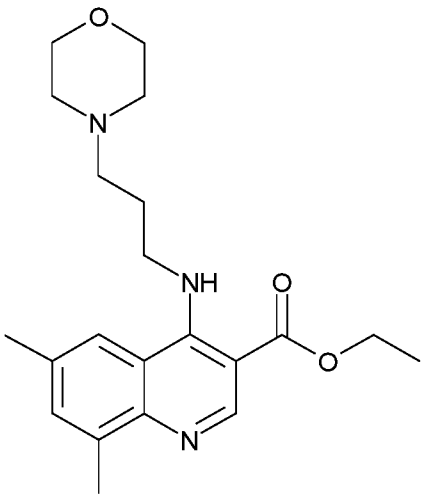


Compound Z169

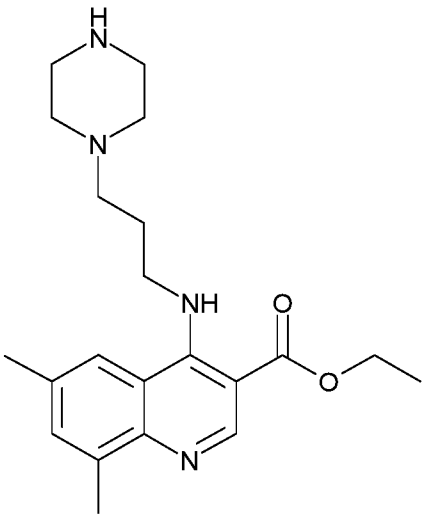


Compound Z57

[00613]

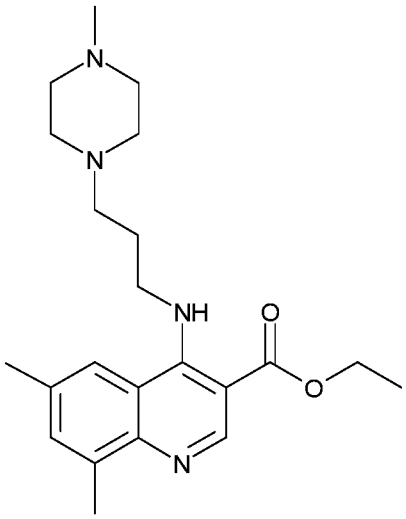


Compound Z154

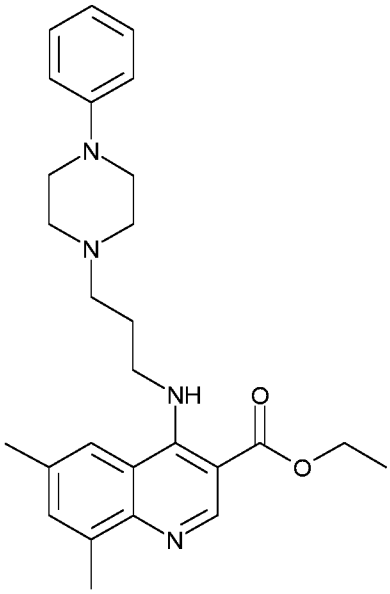


Compound Z155

[00614]

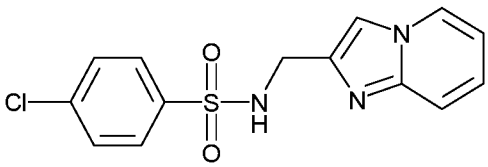


Compound Z156

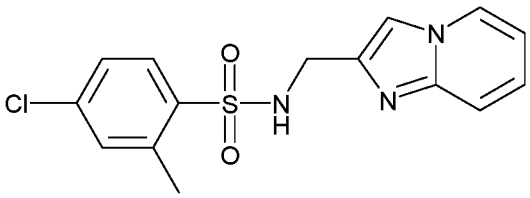


Compound Z157

[00615]

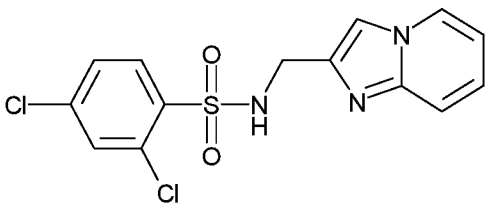


Compound Z160

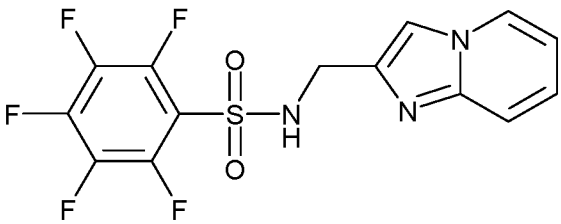


Compound Z161

[00616]

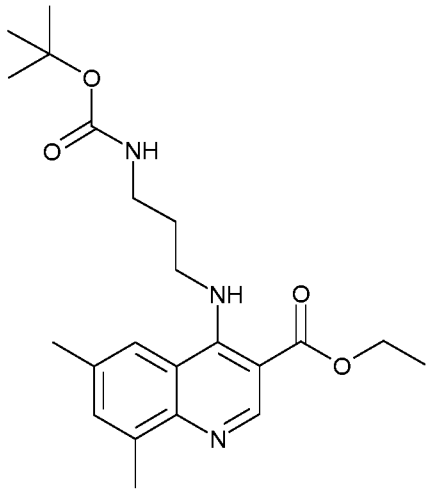


Compound Z162

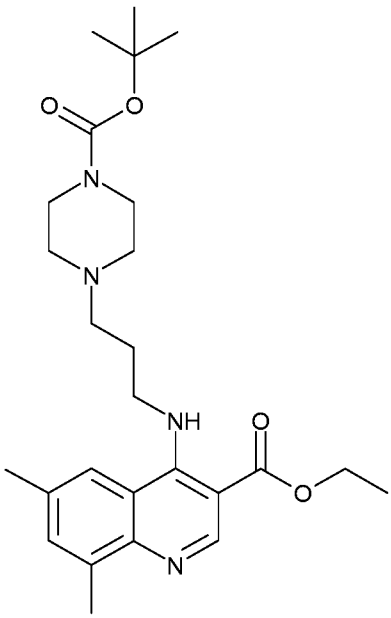


Compound Z163

[00617]



Compound Z158



Compound Z159

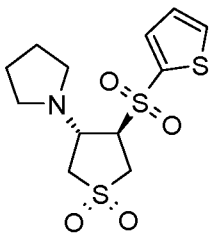
[00618] The present technology is directed to a composition according to any of the compounds decscribed herein, substantially free of impurities. As used herein, the term "substantially free of impurities" means that the compound contains no significant amount of extraneous matter, including starting materials, residual solvents, or any other impurities that may result from the preparation or isolation of the compounds herein. In various embodiments, at least about 95%, at least about 97% or at least about 98% by weight of a compound herein is present in a dosage form herein.

[00619] Although the present technology has been described in relation to particular embodiments thereof, these embodiments and examples are merely exemplary and not intended to be limiting. Many other variations and modifications and other uses will become apparent to those skilled in the art. The present technology should, therefore, not be limited by the specific disclosure herein, and can be embodied in other forms not explicitly described here, without departing from the spirit thereof.

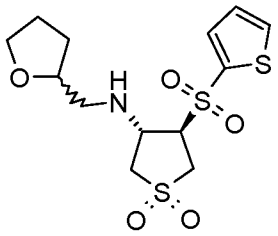
CLAIMS

We claim:

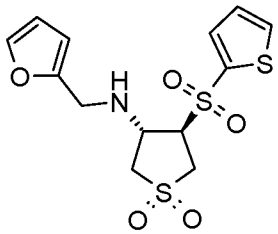
1. A veterinary compound having a structure of any of the following, or a pharmaceutically acceptable salt thereof:



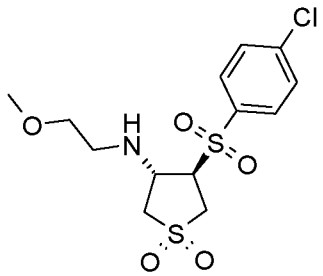
Compound D104



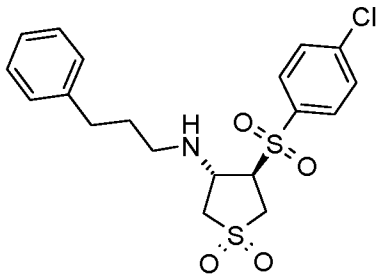
Compound D134



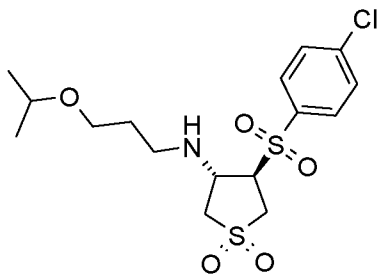
Compound D135



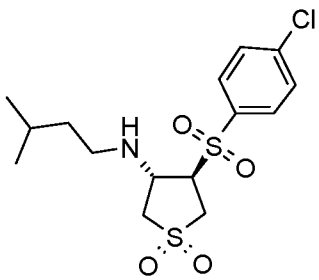
Compound D136



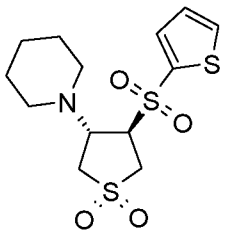
Compound D137



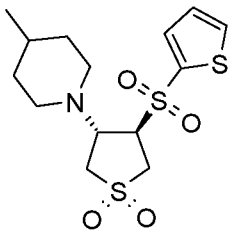
Compound D138



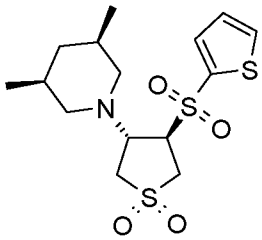
Compound D131



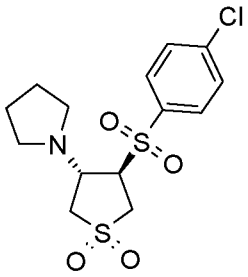
Compound D105



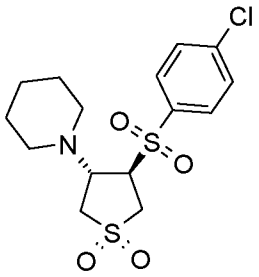
Compound D106



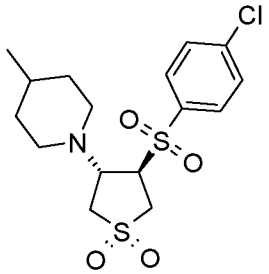
Compound D109



Compound D122

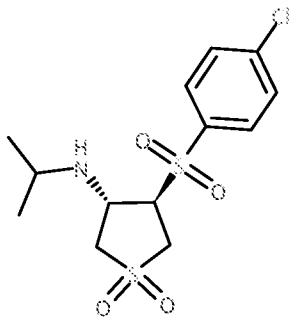


Compound D123

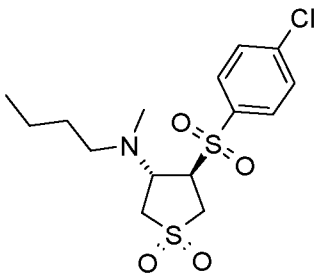


Compound D125

AND Enantiomer



Compound D118



Compound D132 .

2. A solid form of a compound of claim 1.

3. A veterinary pharmaceutical composition comprising the solid form of a compound of claim 1, with a pharmaceutically acceptable carrier or excipient.

4. A method of increasing activity of a cell in a non-human, comprising contacting a composition of claim 1 with the cell.

5. A method of reducing the size of a tumor in a non-human patient, comprising administering a compound of claim 1 to the tumor.

6. The method of claim 5, comprising the steps of:

(a) extracting an amount of the patient's cellular material;

(b) isolating immune cells from the cellular material;

(c) activating the NR2F6 target in the isolated immune cells by binding them with a compound of claim 1; and

(d) re-administering the isolated immune cells to the patient's body.

7. The method of claim 6, wherein the cellular material is blood.

8. The method of claim 6, wherein the re-administering step comprises injection of the immune cells into the patient's body.

9. The pharmaceutical composition of claim 3, in an oral dosage form.

10. A method of treating or reducing the effect of a reaction, disease or disorder, the method comprising activating the NR2F6 target in immune cells of a non-human by contacting them with a compound of claim

1.

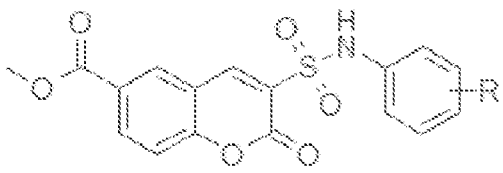
11. The method of claim 10, wherein the reaction, disease or disorder comprises an autoimmune response.

12. A method of treating a disorder in a non-human subject comprising administering to the subject an effective amount of a compound of claim 1 or a composition thereof.

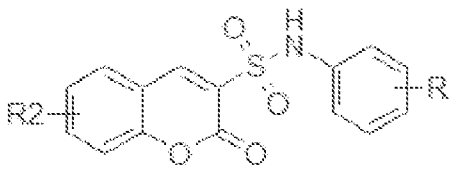
13. The method of claim 12, wherein the non-human subject is a mammal.

14. The method of claim 10, wherein the disorder is cancer.

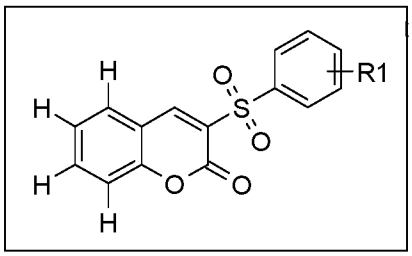
15. A compound having a structure of any of the following, or a pharmaceutically acceptable salt thereof:



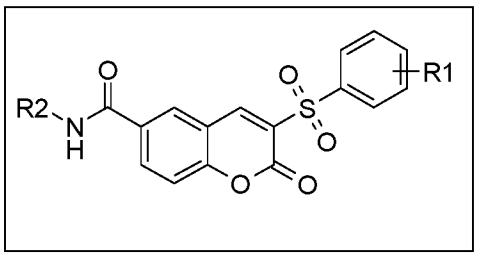
(Ia)



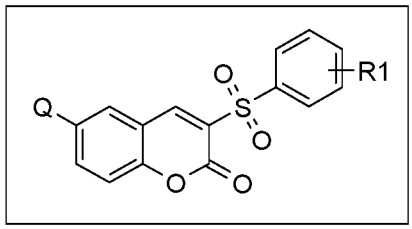
(Ib)



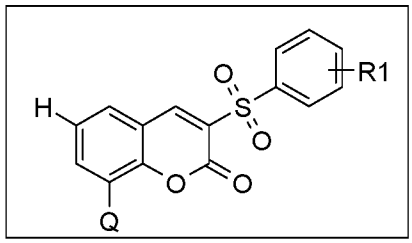
(Ic)



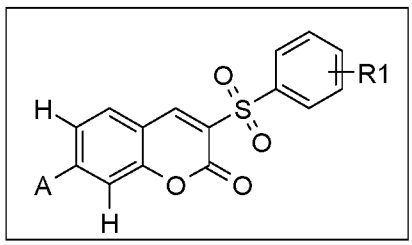
(II)



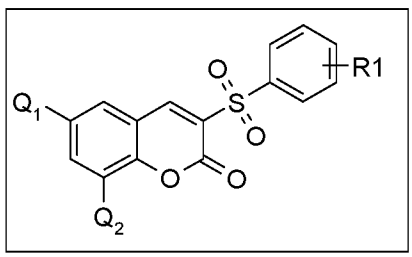
(III)



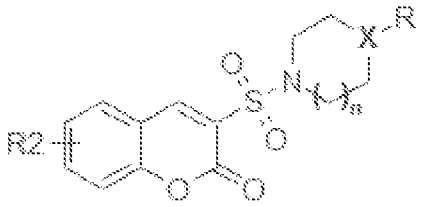
(IV)



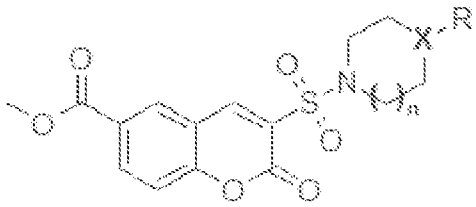
(V)



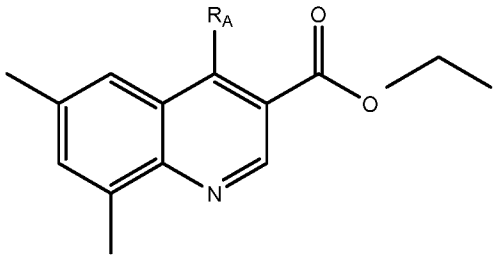
(VI)



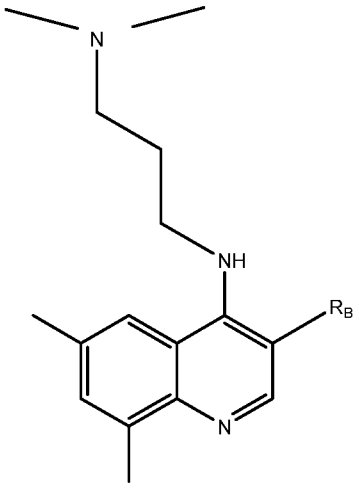
(VII)



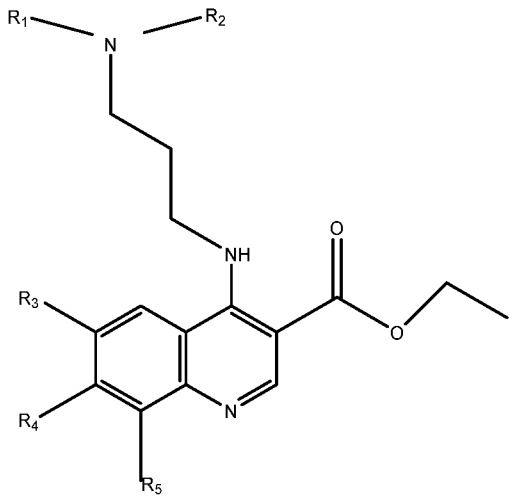
(VIII)



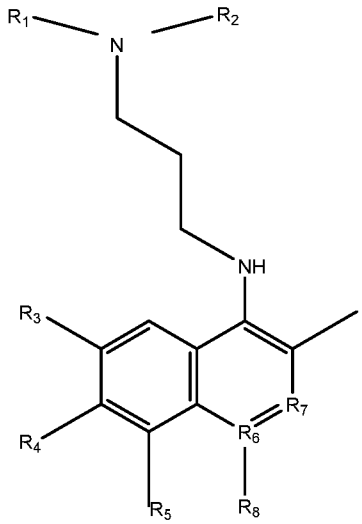
(IX)



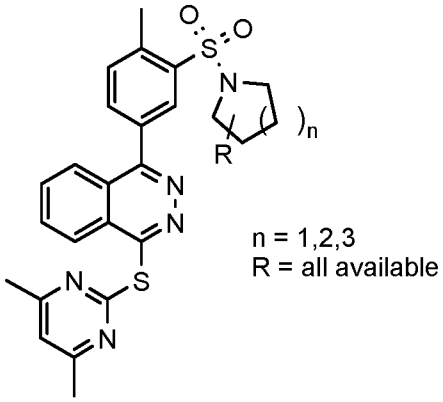
(X)



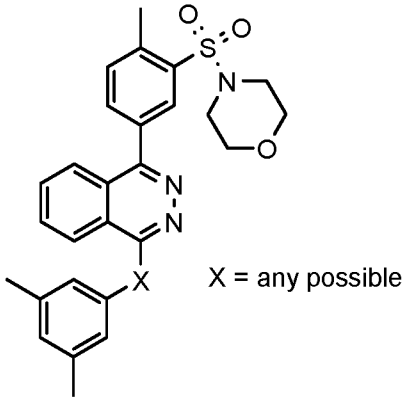
(XI)



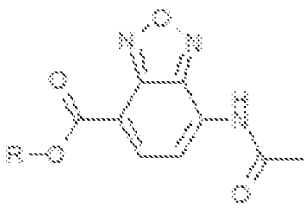
(XII)



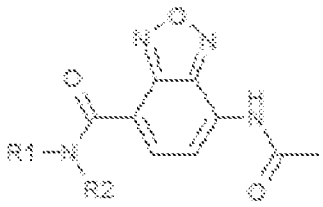
(XIII)



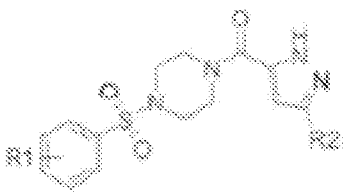
(XIV)



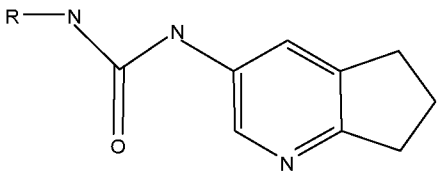
(XV)



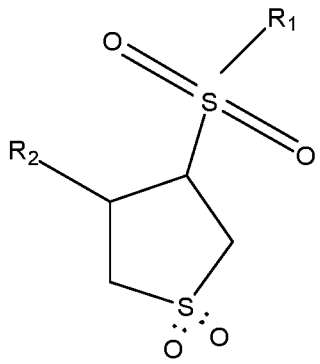
(XVI)



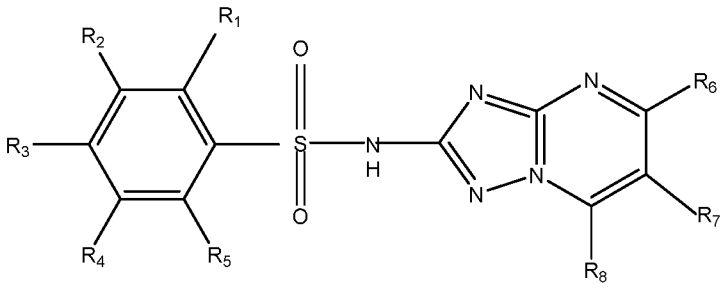
(XVII)



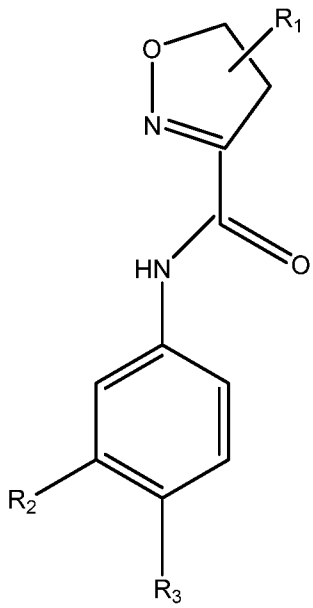
(XVIII)



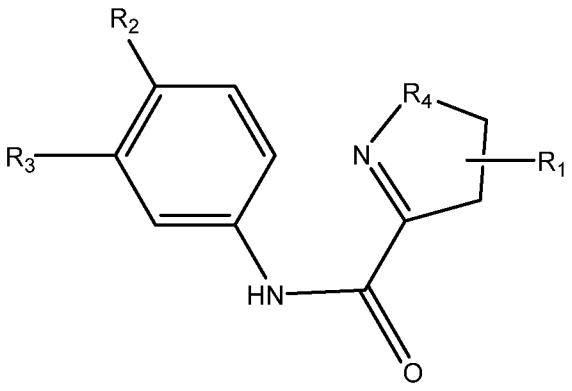
(XIX)



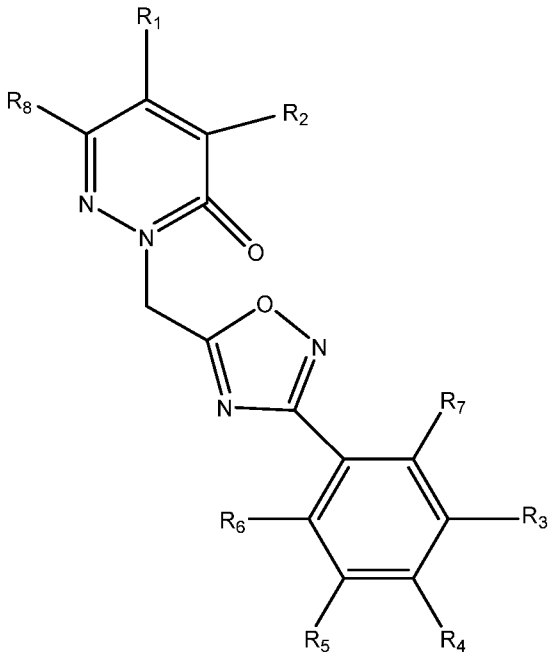
(XX)



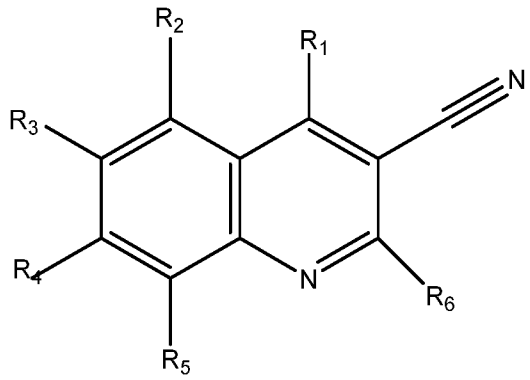
(XXI)



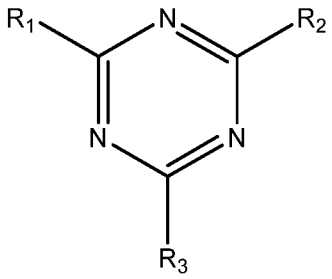
(XXII)



(XXIII)



(XXIV)



(XXV)

wherein in any of the above, R, RA, RB, R1-R8, X, Q, Q1, Q2, A is any of the following: C, H, N, O, S, a halogen, an alkyl group, a substituted alkyl group, a cyclic alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, an ester, an aldehyde, a ketone, a carboxylic acid, an amide, an amine, an ether, a thiol or a nitrile.

16. The compound of claim 15, wherein in any of the above, R, RA, RB, R1-R8, X, Q, Q1, Q2, A is any of the following: Me, OMe, Br, N, H, Cl, F or NO_2.

17. A solid form of a compound of claim 15.

18. A veterinary pharmaceutical composition comprising the solid form of a compound of claim 15, with a pharmaceutically acceptable carrier or excipient.

19. A method of increasing activity of a non-human cell, comprising contacting a composition of claim 15 with the cell.

20. A method of reducing the size of a tumor in a non-human subject, comprising administering a compound of claim 15 to the tumor.

21. A method of treating or reducing the effect of a reaction, disease or disorder in a non-human, the method comprising activating the NR2F6 target in immune cells by contacting them with a compound of claim 15.

22. The method of claim 21, wherein the reaction, disease or disorder comprises an autoimmune response.

23. A method of treating a disorder comprising administering to a non-human subject an effective amount of a compound of claim 15 or a composition thereof.

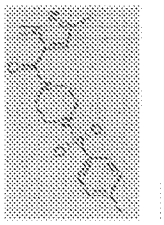


FIG. 1A

FIG. 1B

FIG. 1C

FIG. 1D

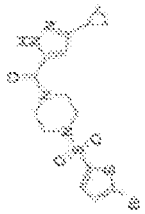


FIG. 1E

FIG. 1F

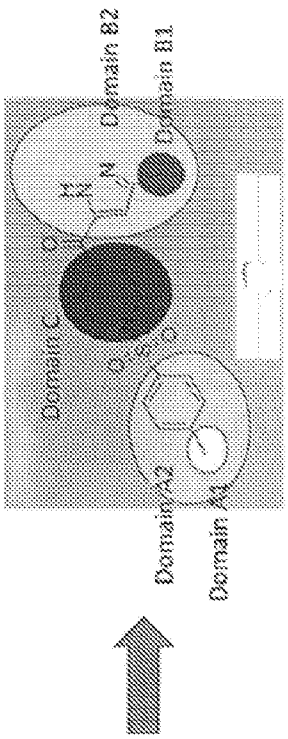


FIG. 2B

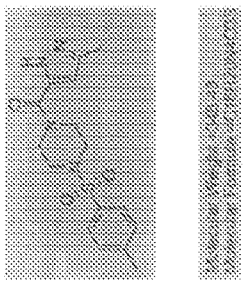


FIG. 2A

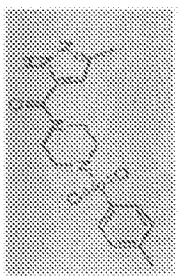


FIG. 3A

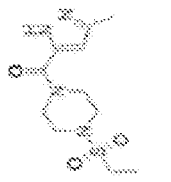


FIG. 3B

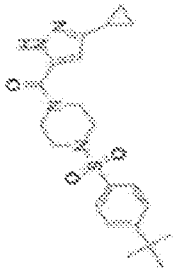


FIG. 3C

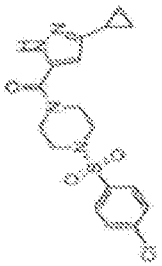


FIG. 3D

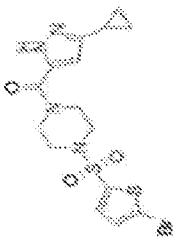


FIG. 3E

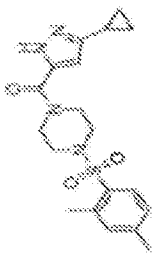


FIG. 3F

FIG. 4A

FIG. 4B

FIG. 4C

FIG. 4D

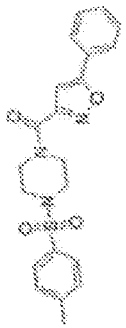


FIG. 4E

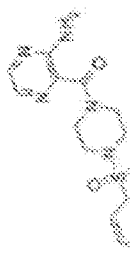


FIG. 4F

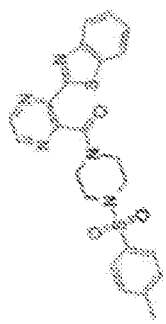


FIG. 4G

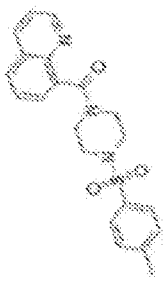


FIG. 4H

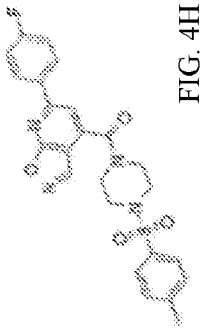


FIG. 4I

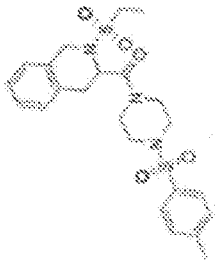


FIG. 4J

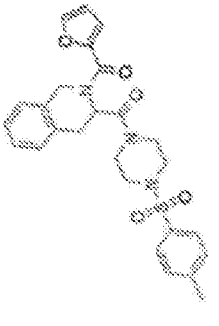


FIG. 4K

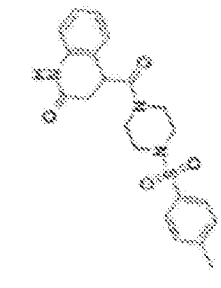
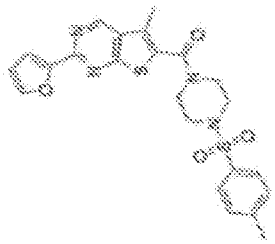


FIG. 4L



R1	R2
4-iPr	Ph
H	4-MeO-C_6H_4
4-tBu	4-MeO-C_6H_4
4-tBu	2-thienyl
2,4-diMe	2-thienyl
2-MeO-4-Cl	2-thienyl
4-Cl	2-furayl
2,4-diMe	Ph
2-MeO-4-Cl	4-F-C_6H_4
4-tBu	2,4-diMeC_6H_3
3-Me-4-F	2,4-diMeC_6H_3
2-MeO-4-Cl	2,4-diMeC_6H_3
4-Cl	4-Cl-C_6H_4

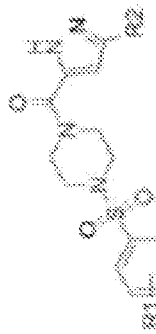


FIG. 5A

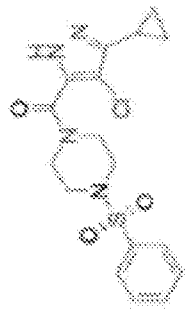


FIG. 5B

FIG. 6C

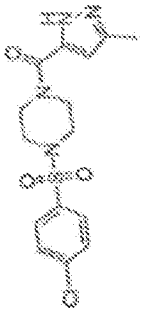


FIG. 6B

FIG. 6A

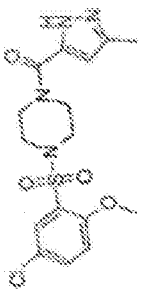


FIG. 6F

FIG. 6E

FIG. 6D

FIG. 7A 

FIG. 7B 

FIG. 7C 

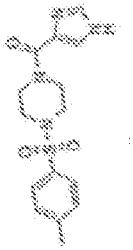
FIG. 7D 

FIG. 7E 

FIG. 7F 

FIG. 7G 

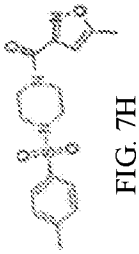
FIG. 7H 

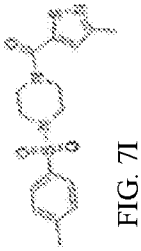
FIG. 7I 

FIG. 7J 

FIG. 7K

FIG. 7L

FIG. 7M

FIG. 7N

FIG. 7O

FIG. 8A

FIG. 8B

FIG. 8C

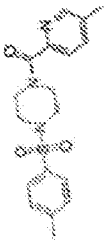


FIG. 8D

FIG. 8E

FIG. 8F

FIG. 8G

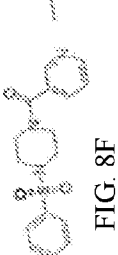


FIG. 8H

FIG. 8I

FIG. 8J

FIG. 8K

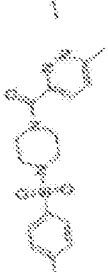


FIG. 8L

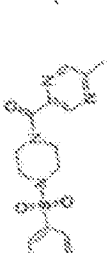


FIG. 8M

FIG. 9A

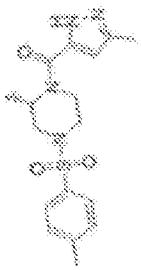


FIG. 9B

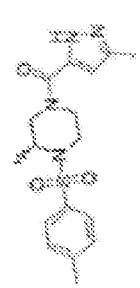


FIG. 9C

FIG. 9D

FIG. 9E

FIG. 9F

FIG. 9G

FIG. 9H

FIG. 9I

FIG. 9J

FIG. 10A 

FIG. 10B 

FIG. 10C 

FIG. 10D 

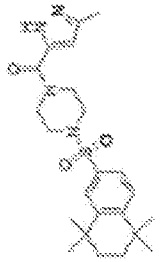
FIG. 10E 

FIG. 10F 

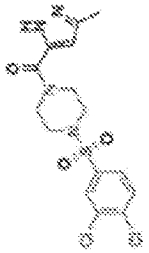
FIG. 10G 

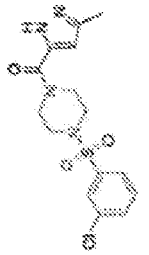
FIG. 10H 

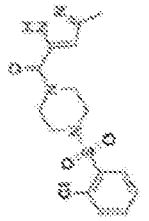
FIG. 10I 

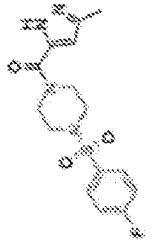
FIG. 10J 

FIG. 11A

FIG. 11B

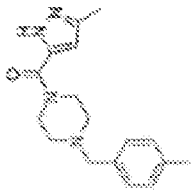


FIG. 11C

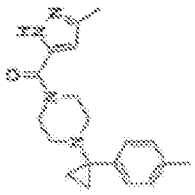


FIG. 11D

FIG. 11D

FIG. 11E

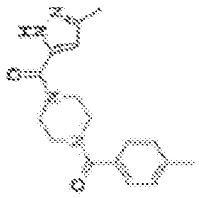


FIG. 11F

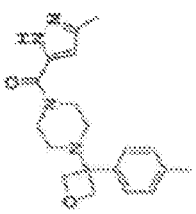
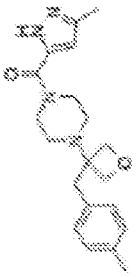


FIG. 11G



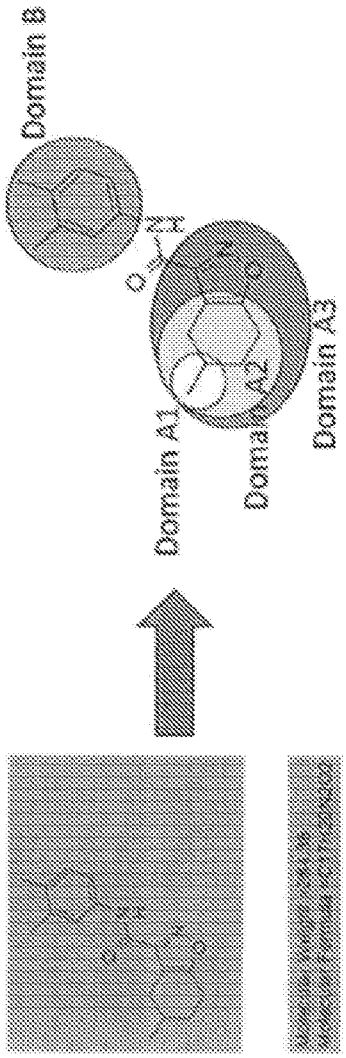


FIG. 12

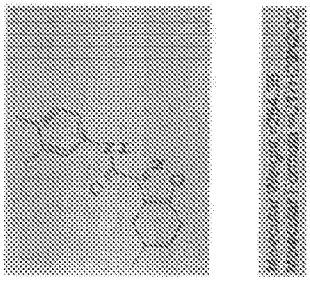


FIG. 13A

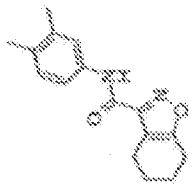


FIG. 13B

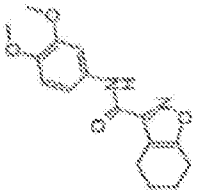


FIG. 13C

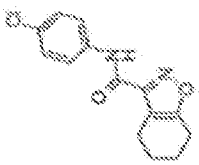


FIG. 13D

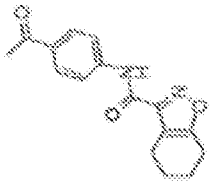


FIG. 13E

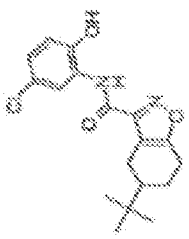


FIG. 13F

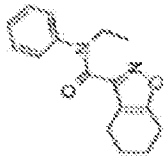


FIG. 13G

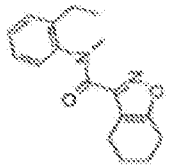


FIG. 13H

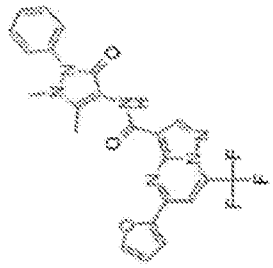


FIG. 14D

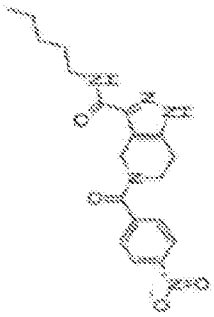


FIG. 14C

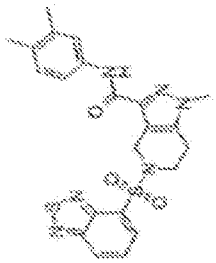


FIG. 14B

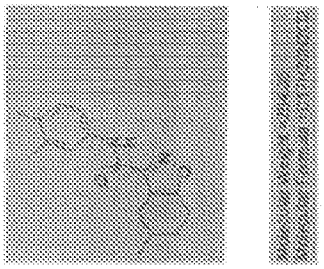


FIG. 14A

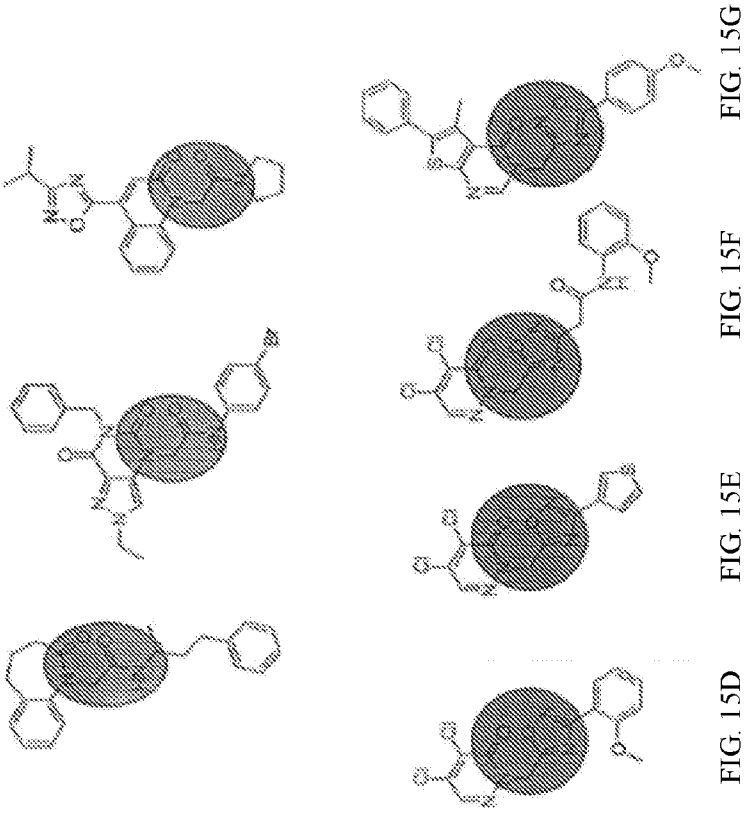
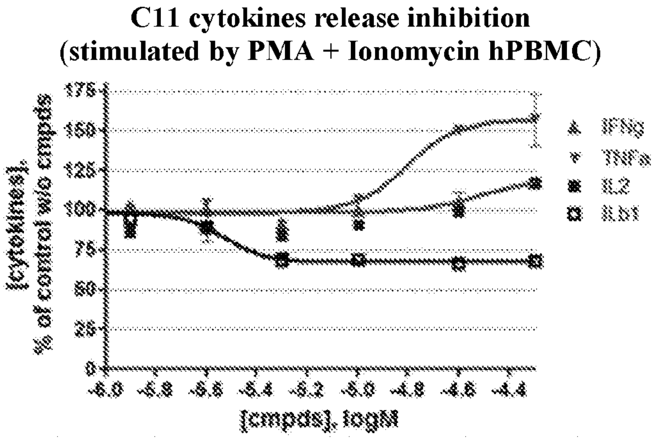


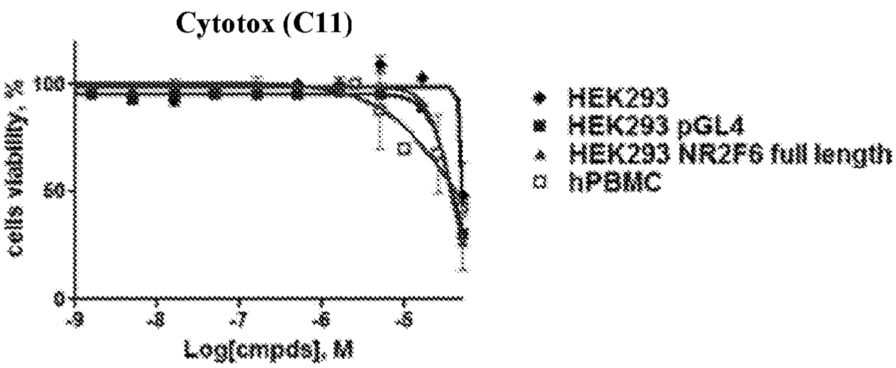
FIG. 15A FIG. 15B FIG. 15C FIG. 15D FIG. 15E FIG. 15F FIG. 15G

Cytokines Release by hPBMC and Cytotox Compound C11



	TNFa	IFNg	IL2	ILb1
EC50	1.550e-005	3.035e-005		2.922e-006

FIG. 16A



	Bottom	Top	LogEC50	HillSlope	EC50
HEK293	3.550e-007	99.21	~ -4.303	~ -19.23	~ 4.983e-005
HEK293 pGL4	-0.0000013	95.74	-4.416	-2.946	3.840e-005
HEK293 NR2F6 full length	-0.004884	98.96	-4.440	-3.064	3.635e-005
hPBMC	~ -5.000	101.0	-4.355	-0.9417	4.415e-005

FIG. 16B

FIG. 17: Analogs of Compound C11

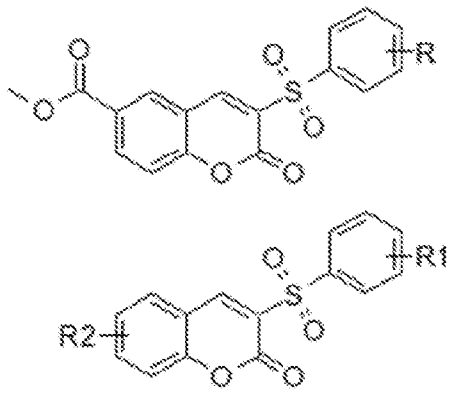


FIG. 17A

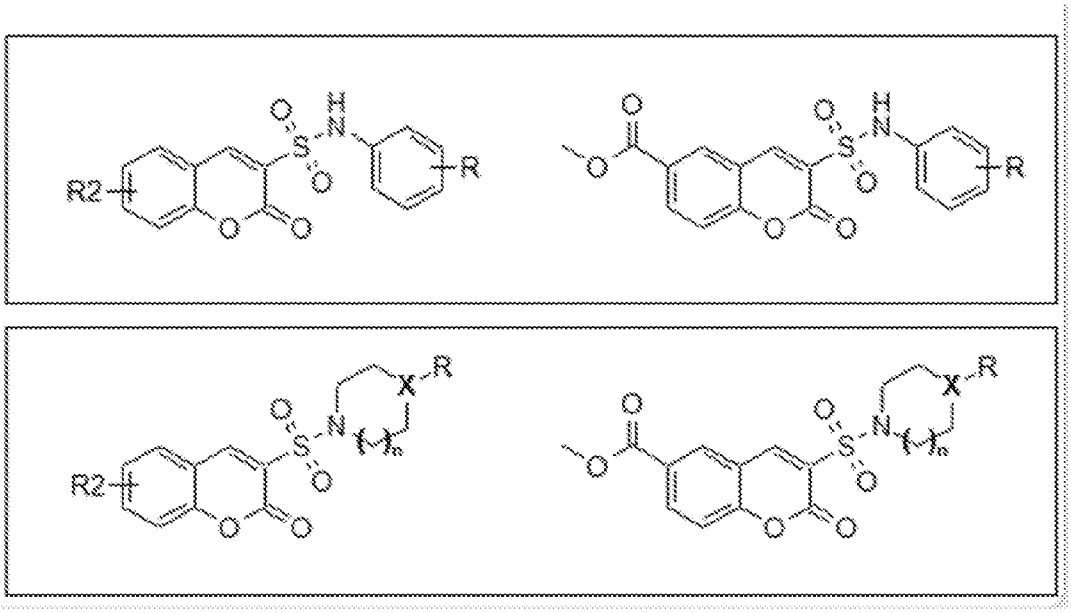


FIG. 17B

Compound 18 cytokines release inhibition (stimulated by PMA + Ionomycin hPBMC)

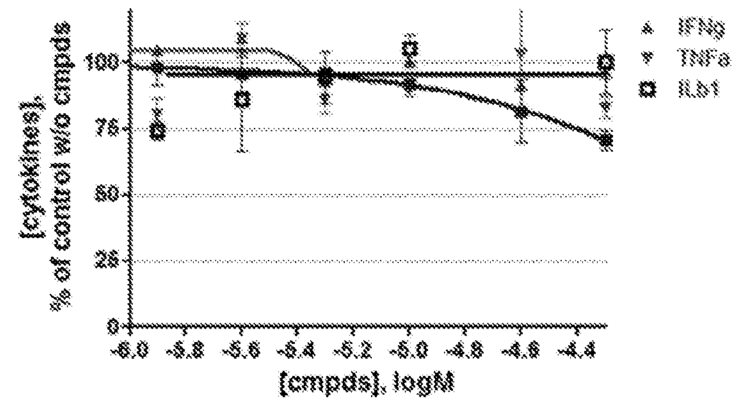
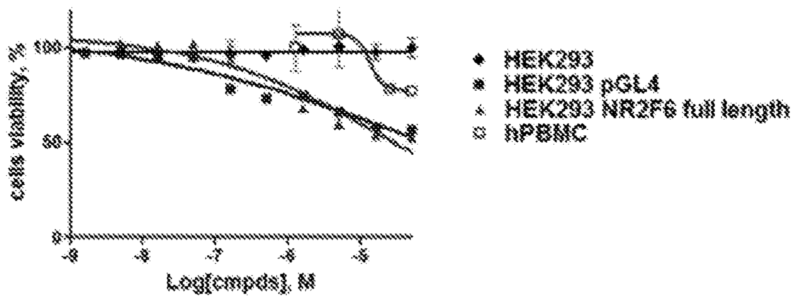


FIG. 18A

Cytotox (Compound 18)



	Bottom	Top	LogEC50	HillSlope	EC50
HEK293	1.644e-020	97.87	-0.5738	~ -20.03	0.2669
HEK293 pGL4	-3.390	108.9	-4.305	-0.2218	4.950e-005
HEK293 NR2F6 full length	0.4297	107.4	-4.720	-0.3517	1.906e-005
hPBMC	77.45	107.4	-4.878	-4.976	1.324e-005

FIG. 18B

Compound Z92 cytokines release inhibition (stimulated by PMA + Ionomycin hPBMC)

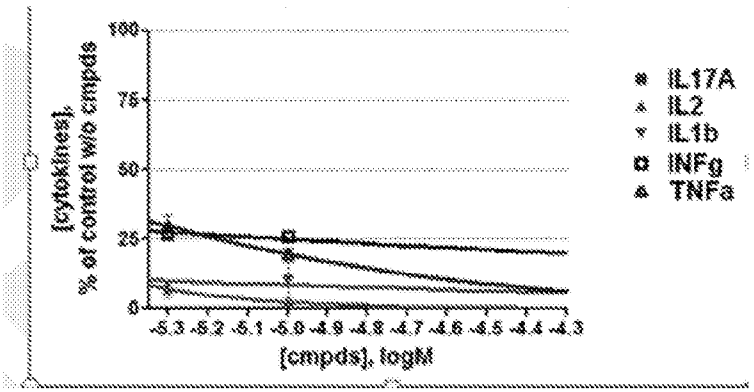
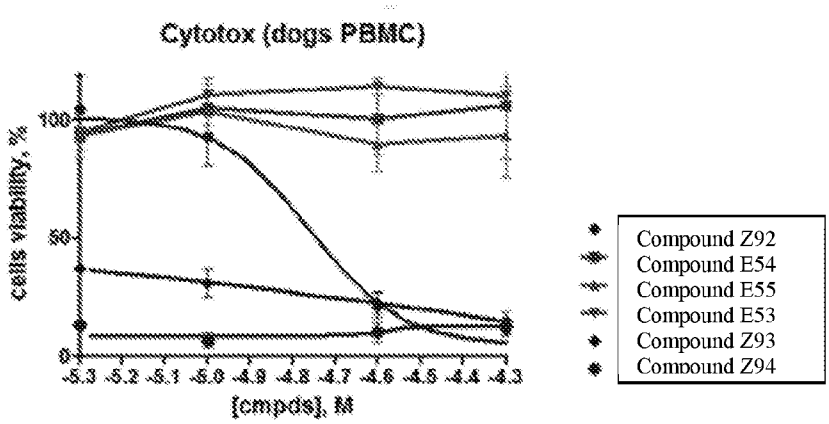


FIG. 19A



	EC50
Compound Z92	1.751e-005

FIG. 19B

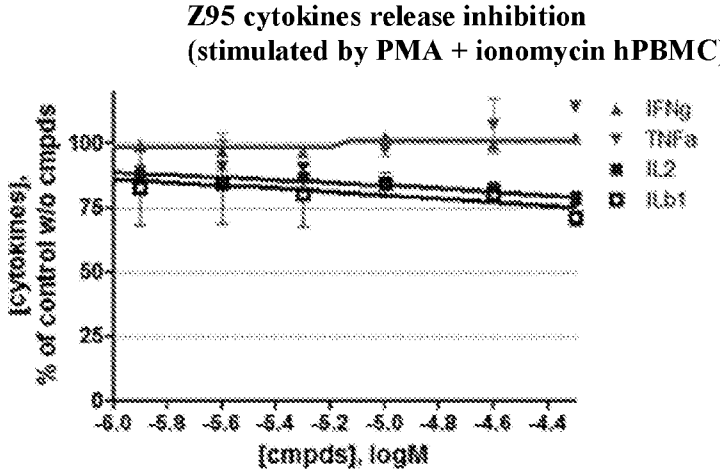
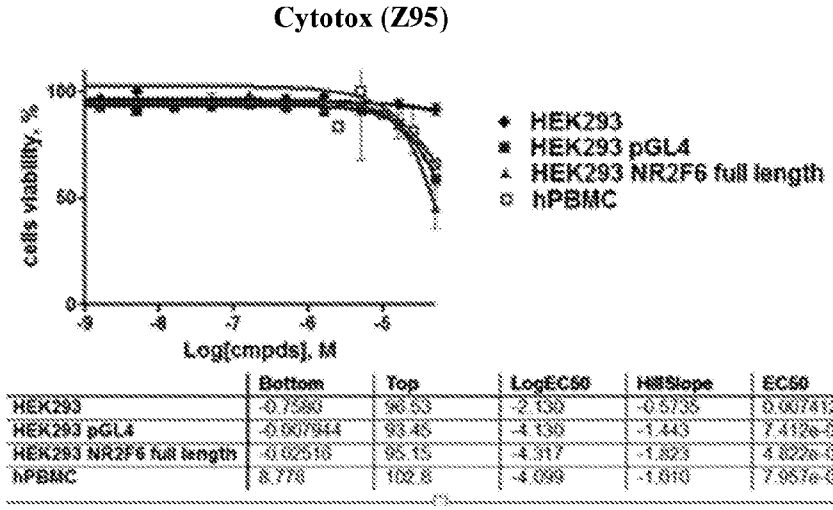


FIG. 20A



	Bottom	Top	LogEC50	HillSlope	EC50
HEK293	0.7580	96.53	-2.130	-0.6726	0.007412
HEK293 pGL4	-0.007844	93.45	-4.130	-1.443	7.412e-005
HEK293 NR2F6 full length	-0.02516	96.15	-4.317	-1.823	4.822e-005
hPBMC	8.778	102.8	-4.099	-1.010	7.967e-005

FIG. 20B

Compound D28 cytokines release inhibition (stimulated by PMA + Ionomycin hPBMC)

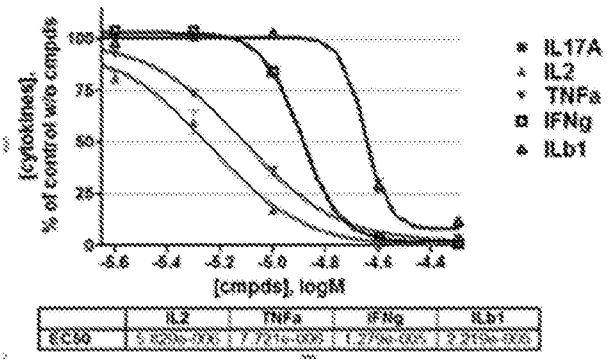
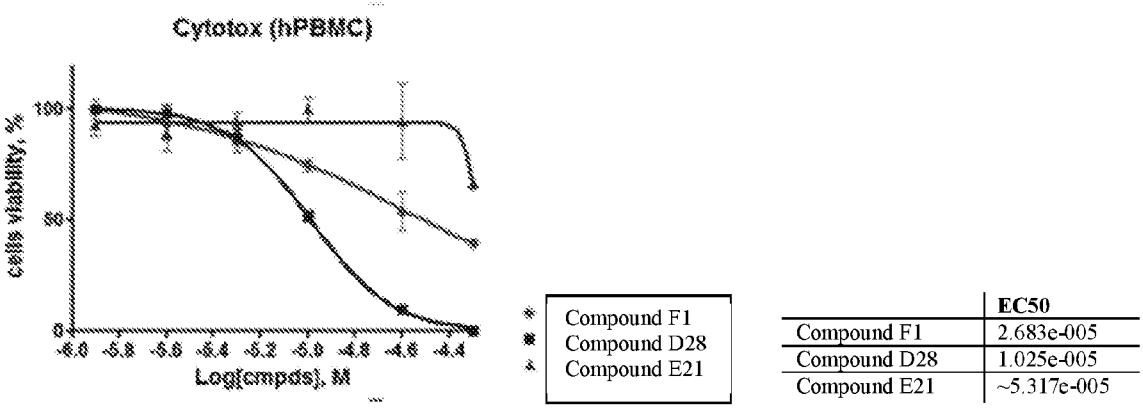


FIG. 21A



	EC50
Compound F1	2.683e-005
Compound D28	1.025e-005
Compound E21	~5.317e-005

FIG. 21B

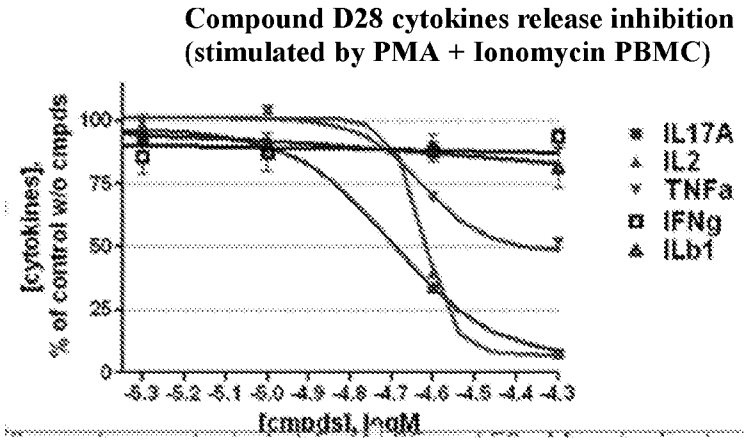


FIG. 21C

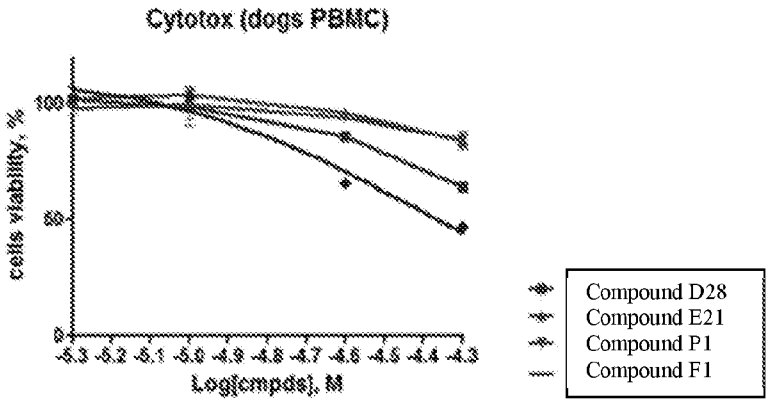
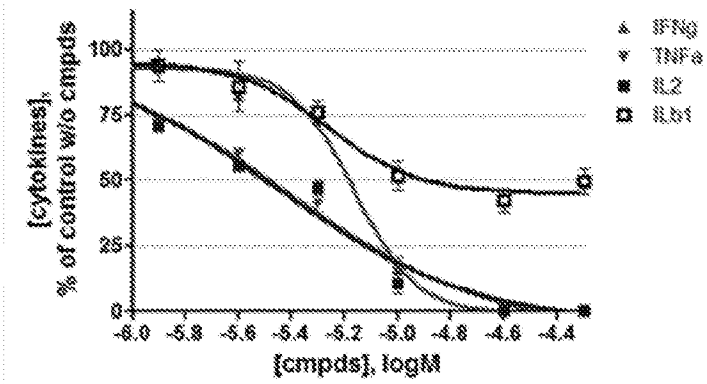


FIG. 21D

	EC50
Compound F1	3.614e-005

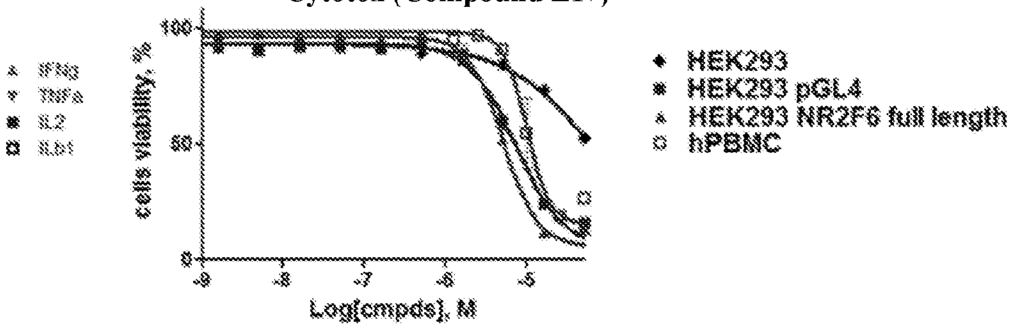
Compound Z17 cytokines release inhibition (stimulated by PMA + Ionomycin hPBMC)



	TNFa	IFNg	IL2	ILb1
EC50	3.853e-006	6.833e-006	3.553e-006	5.479e-006

FIG. 22A

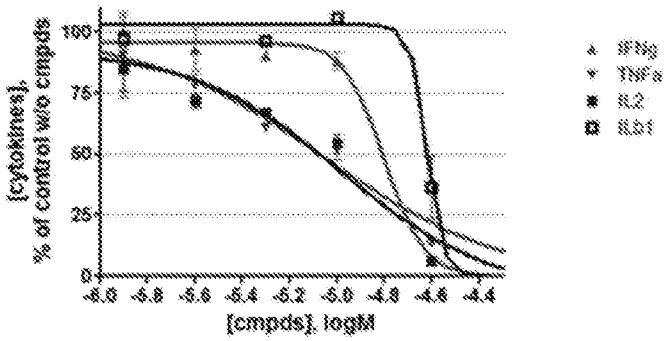
Cytotox (Compound Z17)



	Bottom	Top	LogEC50	HillSlope	EC50
HEK293	-0.09834	93.53	-4.144	-0.7946	7.173e-005
HEK293 pGL4	4.961	93.44	-5.141	-1.518	7.226e-006
HEK293 NR2F6 full length	5.068	96.58	-5.296	-2.984	5.054e-006
hPBMC	15.36	96.56	-5.012	-3.127	9.731e-006

FIG. 22B

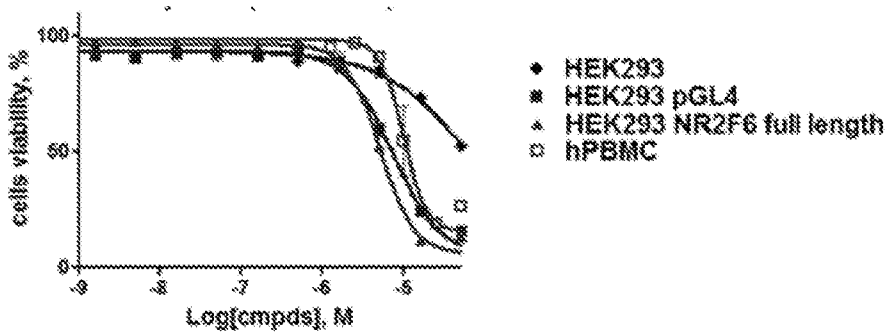
Compound Z33 cytokines release inhibition (stimulated by PMA + Ionomycin hPBMC)



	TNFa	IFNg	IL2	IL1b
EC50	8.77e-006	1.91e-005	1.03e-005	7.31e-006

FIG. 23A

Cyotox (Compound Z33)



	Bottom	Top	LogEC50	HillSlope	EC50
HEK293	-0.09604	93.53	-4.144	-0.7946	7.173e-005
HEK293 pGL4	4.961	93.44	-5.141	-1.518	7.220e-006
HEK293 NR2F6 full length	5.668	96.58	-5.296	-2.084	5.054e-006
hPBMC	15.36	98.56	-5.012	-3.127	9.731e-006

FIG. 23B

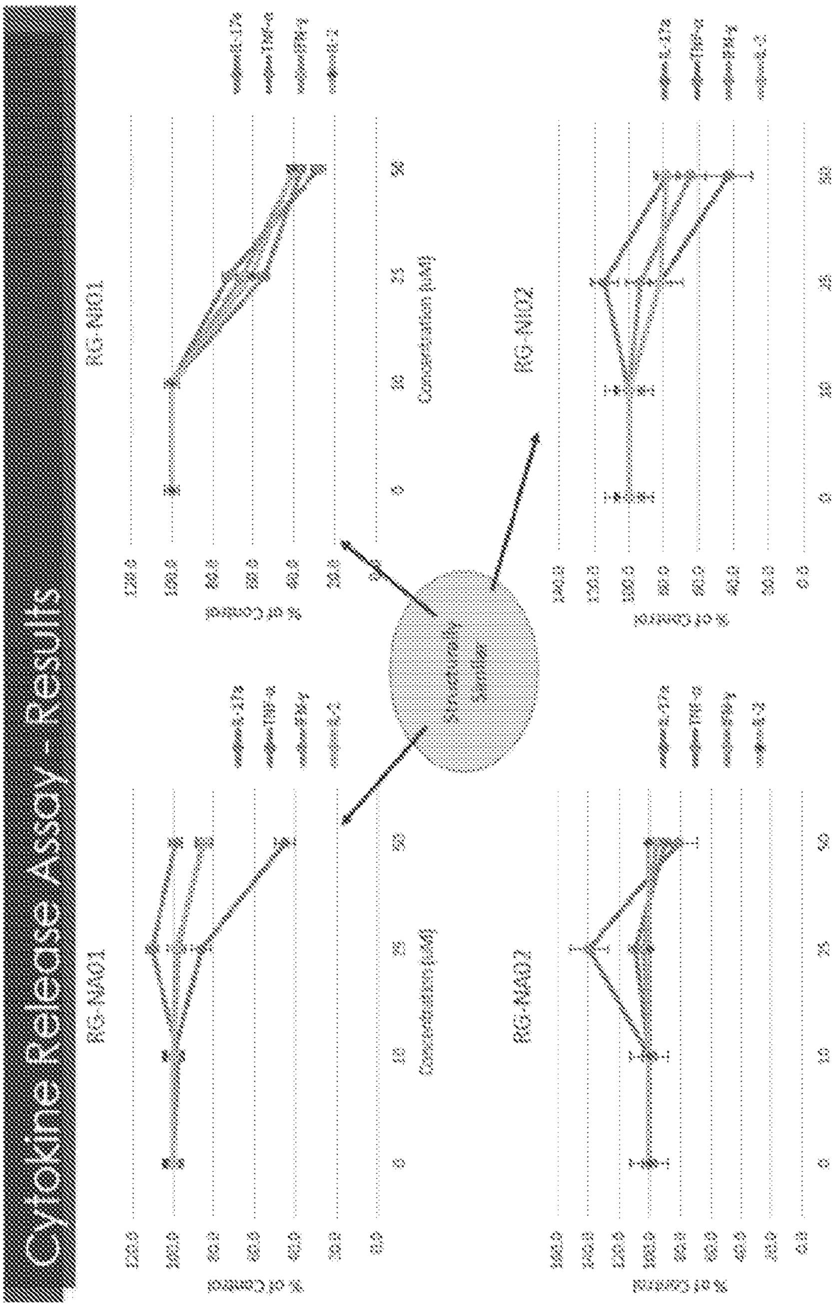


FIG. 24

Compound Z96 cytokines release inhibition (stimulated by PMA + Ionomycin hPBMC)

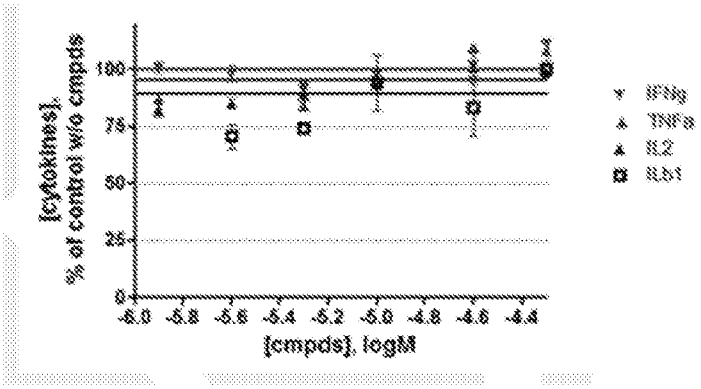
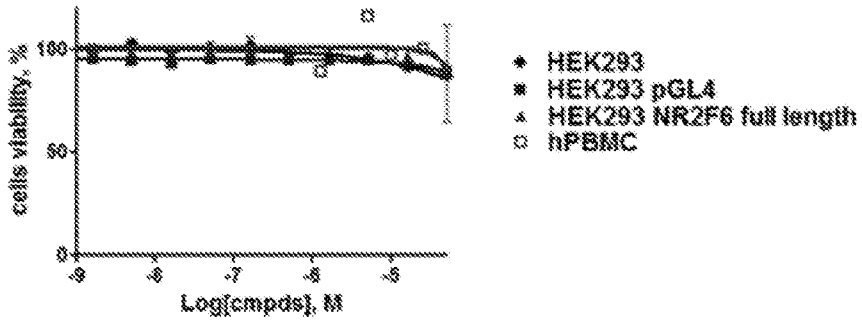


FIG. 25A

Cytotox (Compound Z96)



	Bottom	Top	LogEC50	HillSlope	EC50
HEK293	-0.3469	100.5	-2.663	-0.4618	0.002174
HEK293 pGL4	-0.08412	95.41	-2.772	-0.7619	0.001691
HEK293 NR2F6 full length	0.001506	101.3	-3.806	-2.031	0.0001564
hPBMC	13.22	100.6	-4.116	-4.290	7.655e-005

FIG. 25B

Compound Z97 cytokines release inhibition (stimulated by PMA + Ionomycin hPBMC)

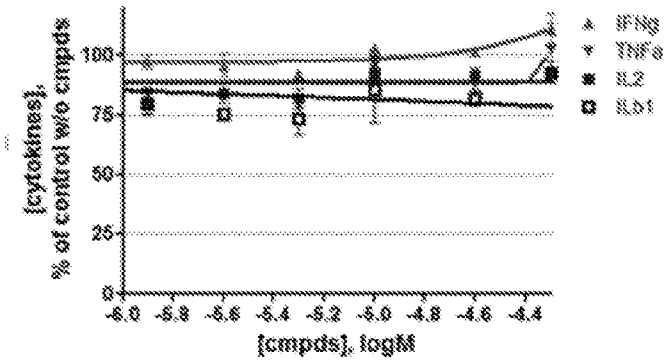
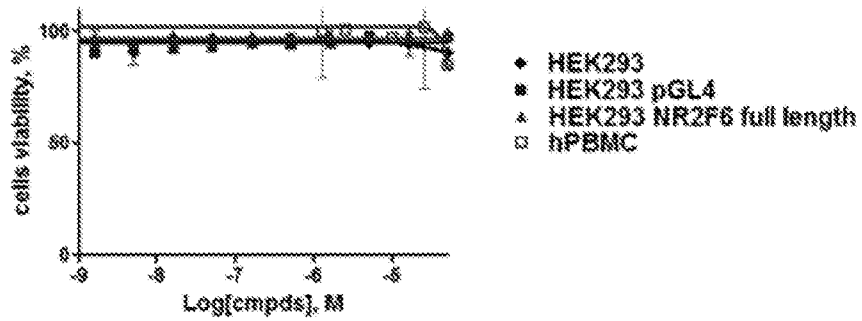


FIG. 26A

Cytotox (Compound Z97)



	Bottom	Top	LogEC50	HillSlope	EC50
HEK293	-0.01427	100.26	-2.932	-0.6972	0.001170
HEK293 pGL4	2.539e-008	94.77	~ -2.567	~ -10.92	~ 0.002711
HEK293 NR2F6 full length	7.003e-023	97.29	~ -3.208	~ -31.16	~ 0.0006187
hPBMC	10.92	102.3	-4.191	-5.750	6.443e-005

FIG. 26B

Testing of Compound D28

NR2F6 Transient Transfection

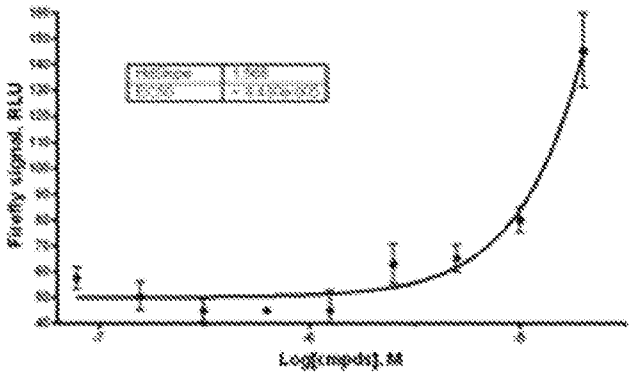


FIG. 27A

LBD Transient Transfection

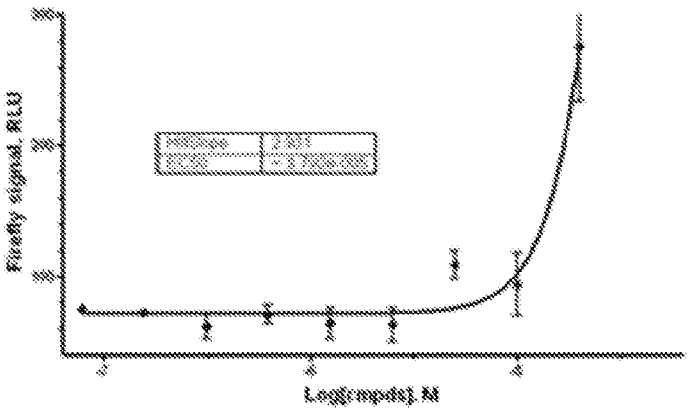


FIG. 27B

Compound D28 Activity: LBD Transfection

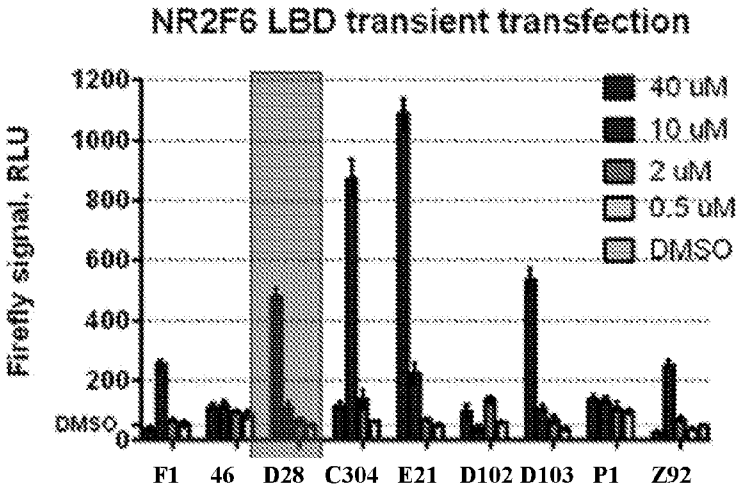


FIG. 27C

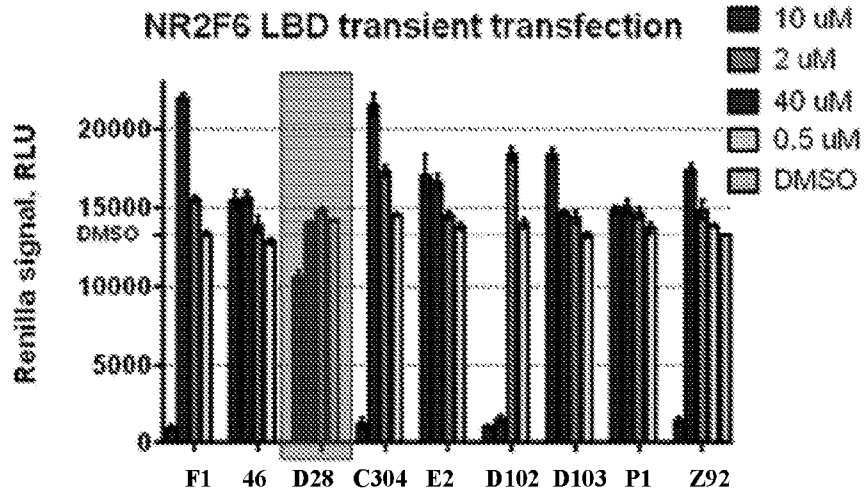


FIG. 27D

Compound D28 Toxicity

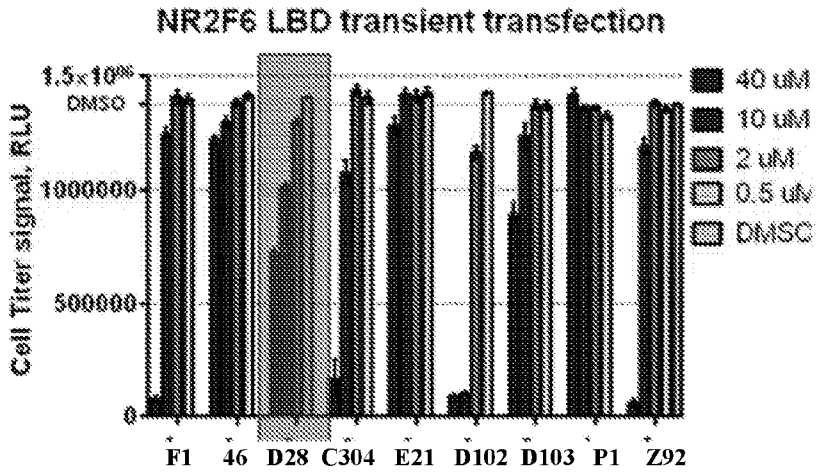


FIG. 27E

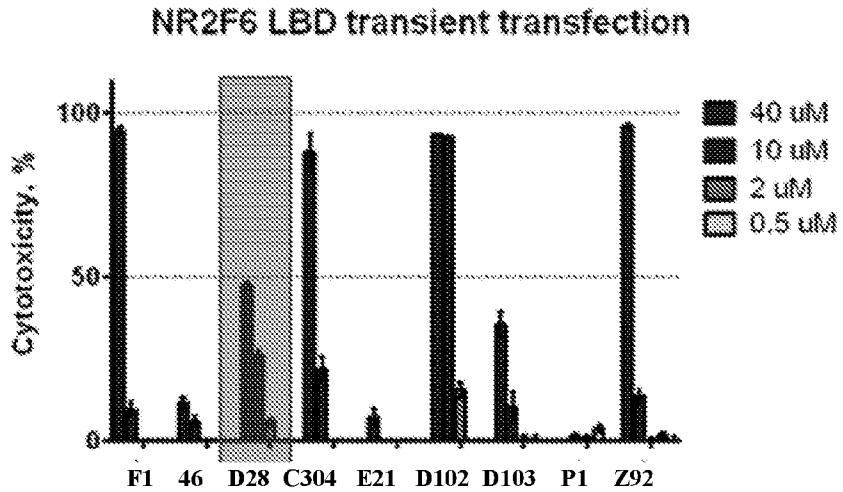


FIG. 27F

Compound D28 Cytokine Release Experiment
Dogs and Human PBMC

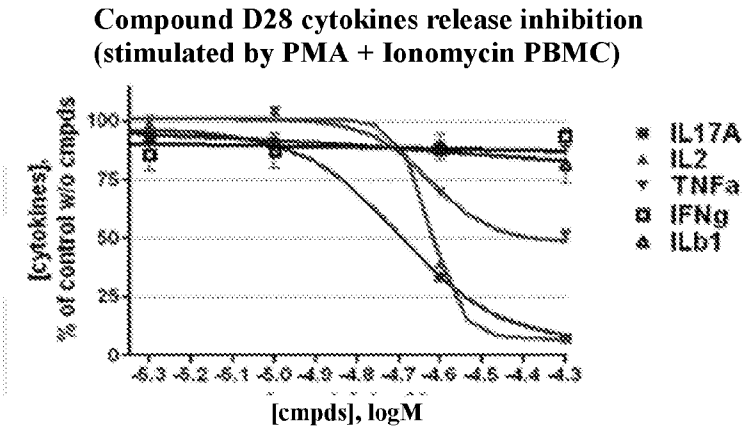


FIG. 28A

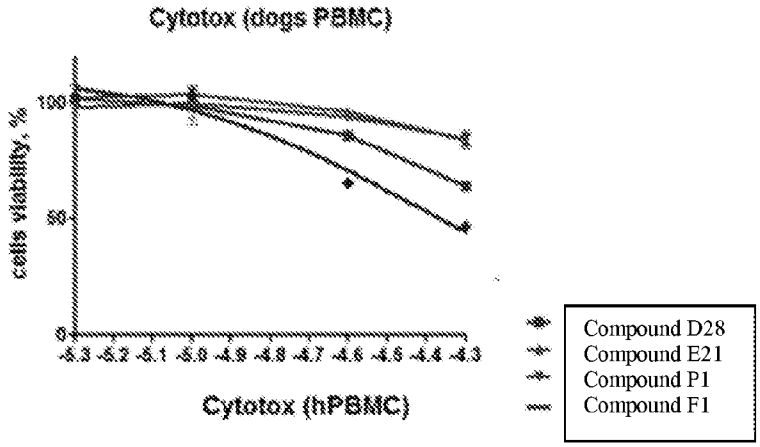


FIG. 28B

	EC50
Compound F1	3.614e-005

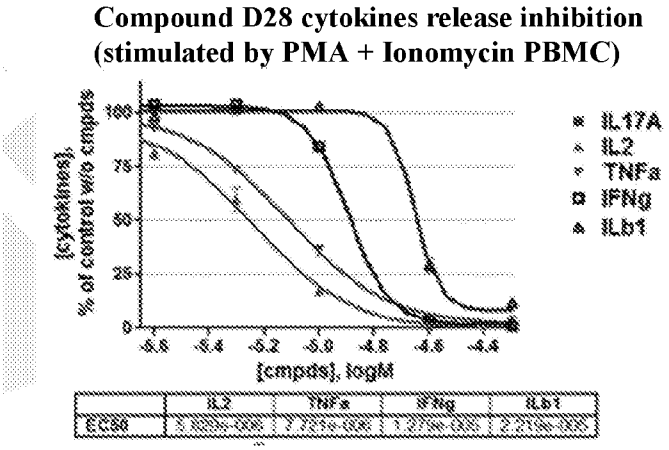


FIG. 28C

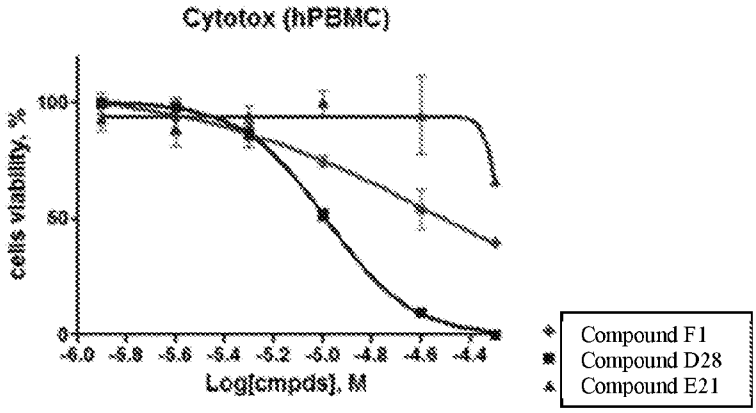


FIG. 28D

	EC50
Compound F1	2.683e-005
Compound D28	1.025e-005
Compound E21	~5.317e-005

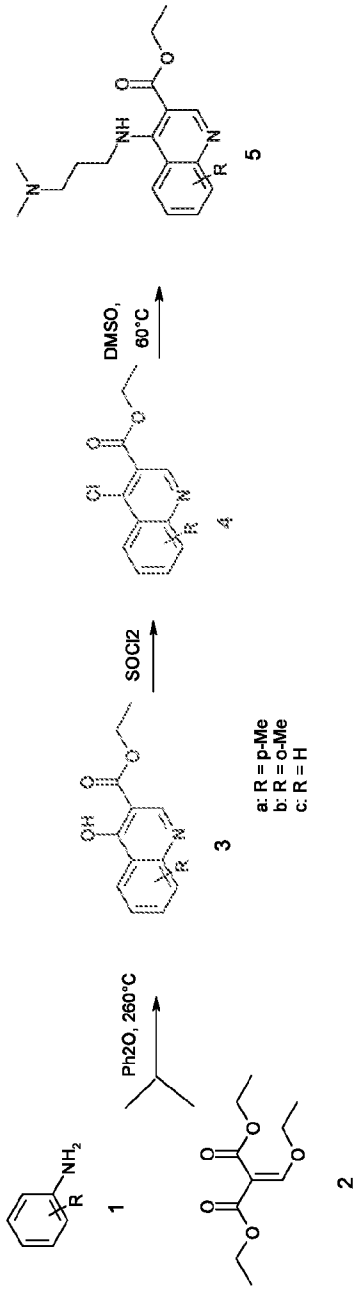


FIG. 29

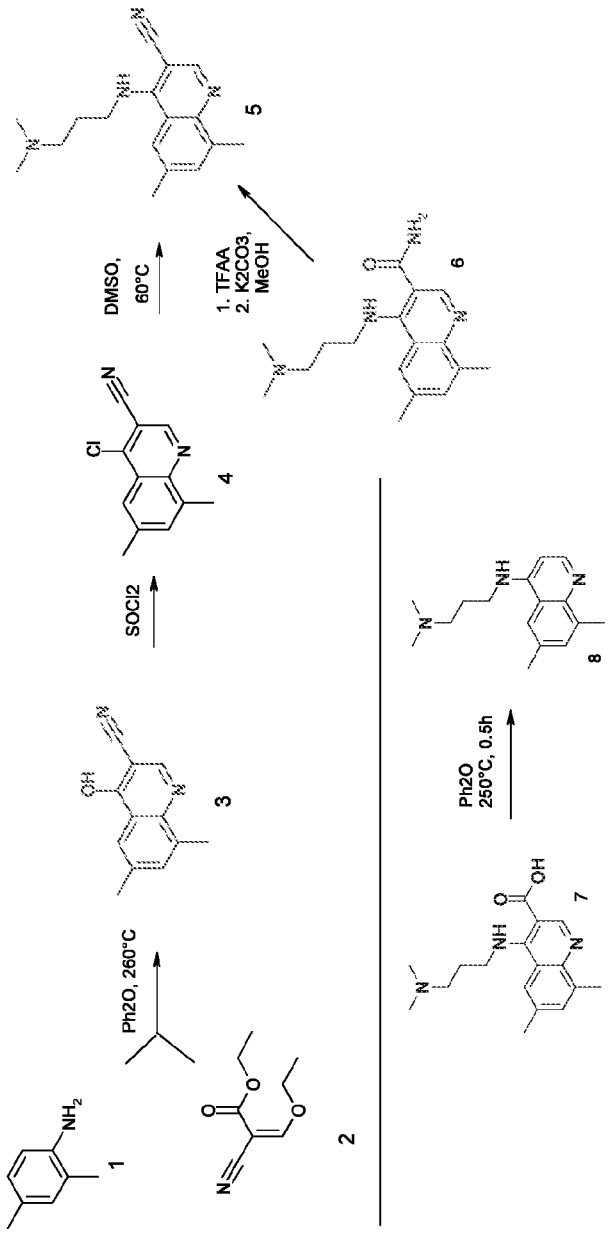

1
2
Ph2O, 260°C
3
SOCl2
4
DMSO, 60°C
5
1. TFAA
2. K2CO3, MeOH
6
Ph2O 250°C, 0.5h
7
8

FIG. 30

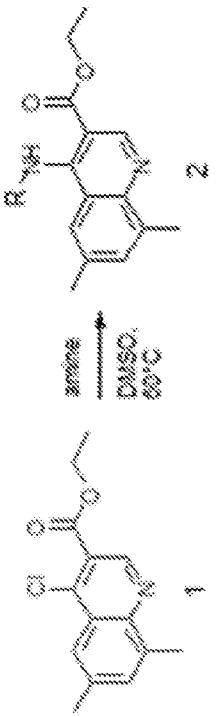


FIG. 31A

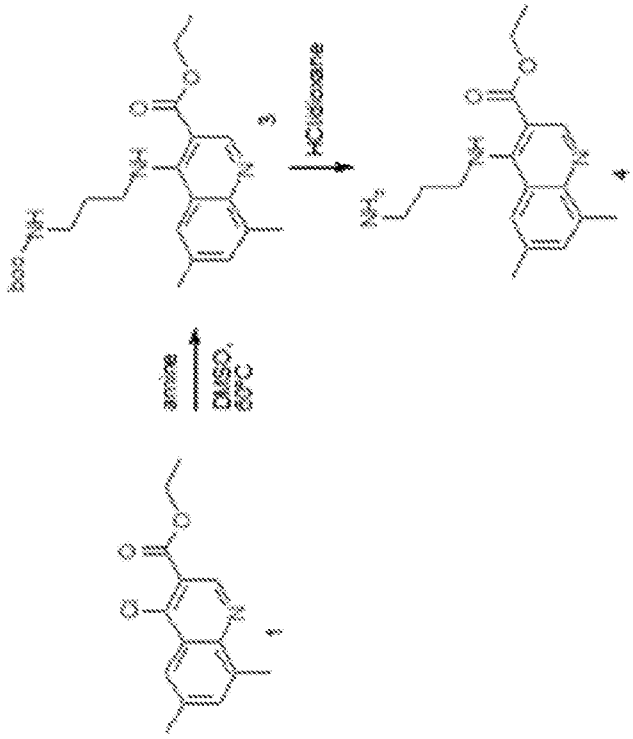


FIG. 31B

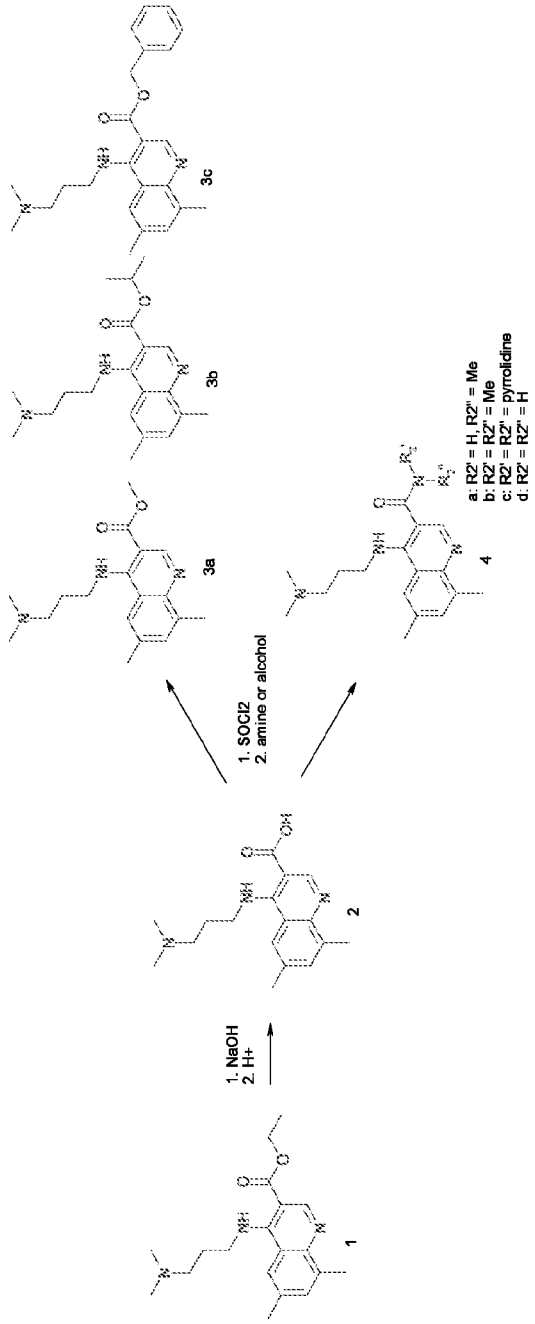

1. NaOH
2. H+
1. SOCl2
2. amine or alcohol
a: R2' = H, R2'' = Me
b: R2' = R2'' = Me
c: R2' = R2'' = pyrrolidine
d: R2' = R2'' = H

FIG. 32

Testing of Compound E21

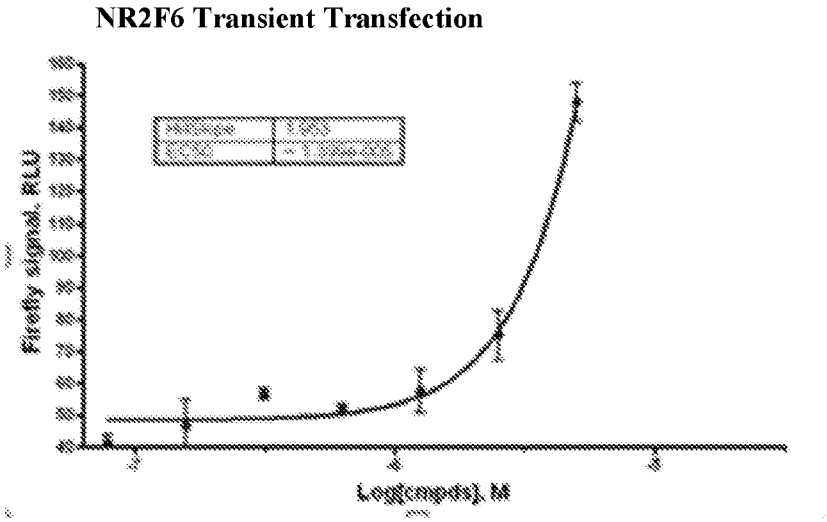


FIG. 33A

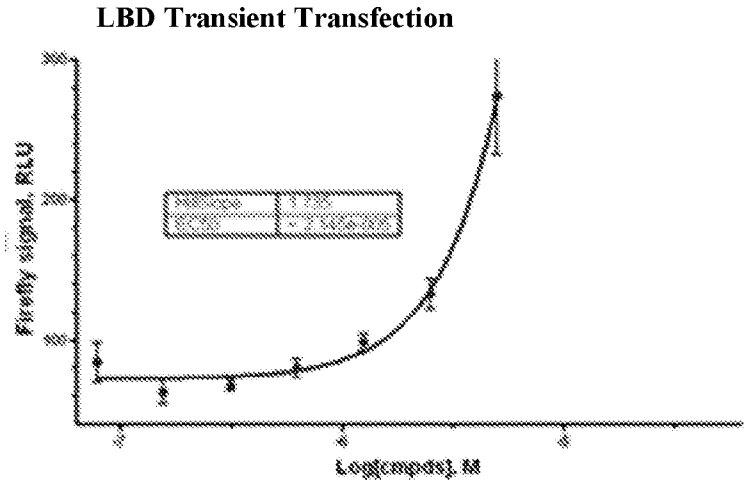


FIG. 33B

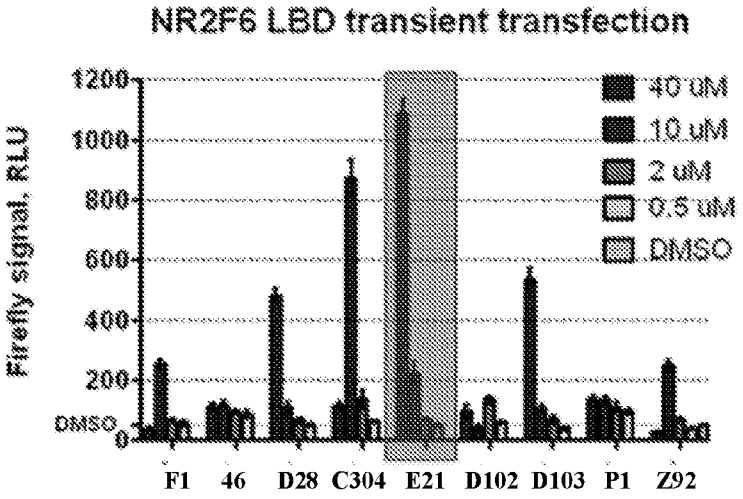


FIG. 34A

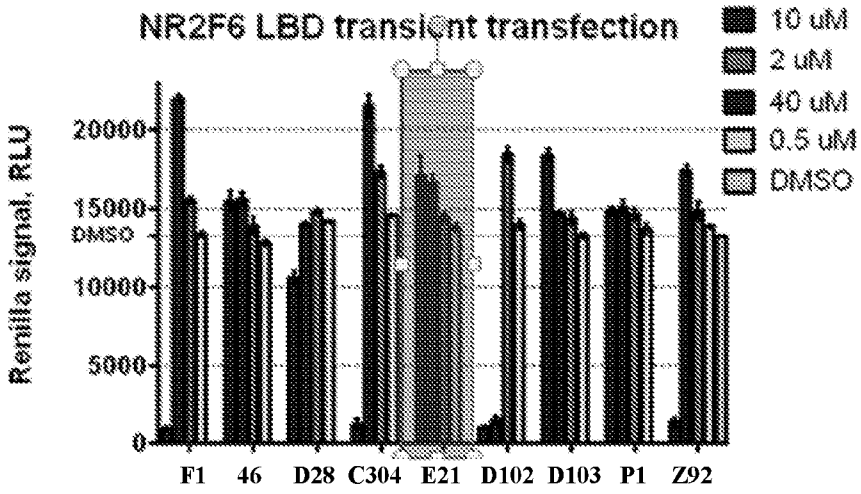


FIG. 34B

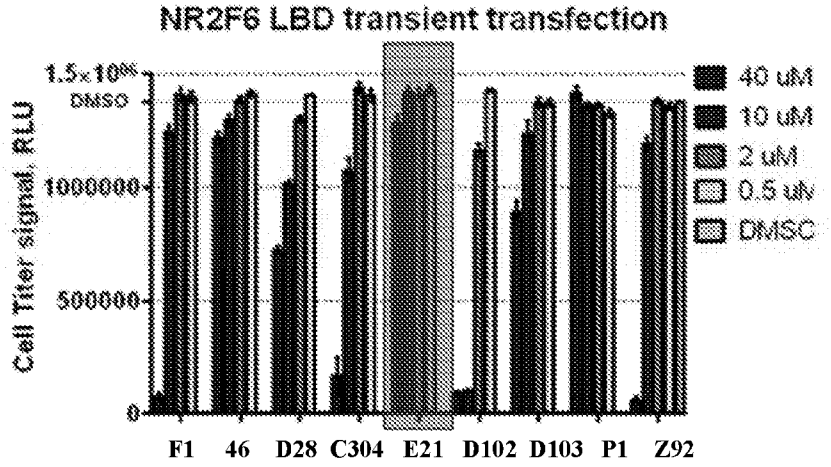


FIG. 34C

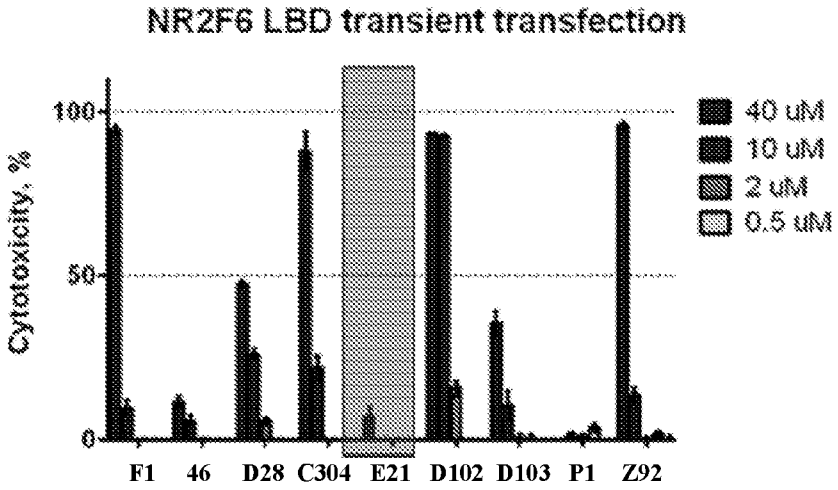


FIG. 34D

Compound E21 Cytokine Release Experiment
Dogs and Human PBMC

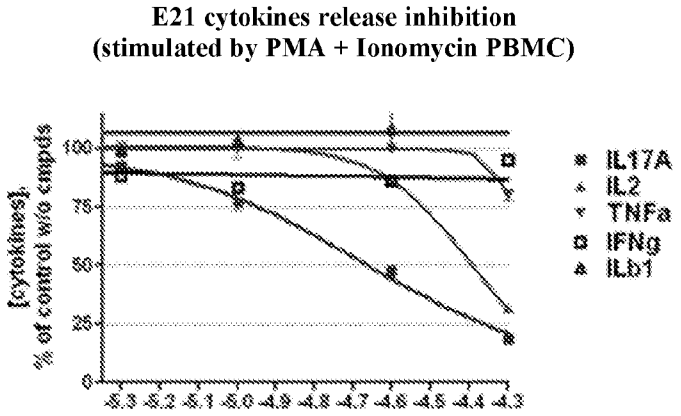
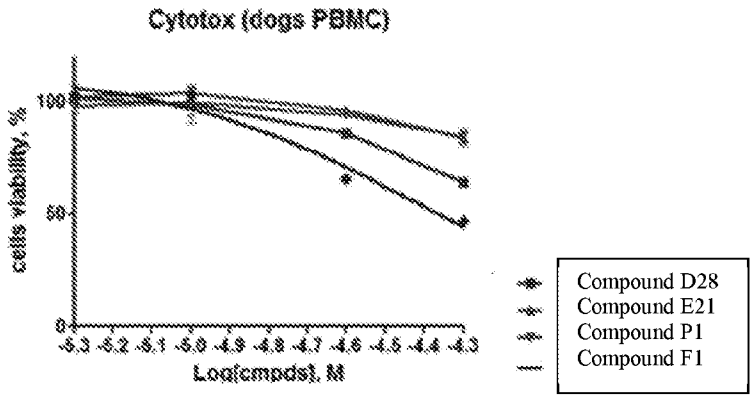


FIG. 35A



	EC50
Compound F1	3.614e-005

FIG. 35B

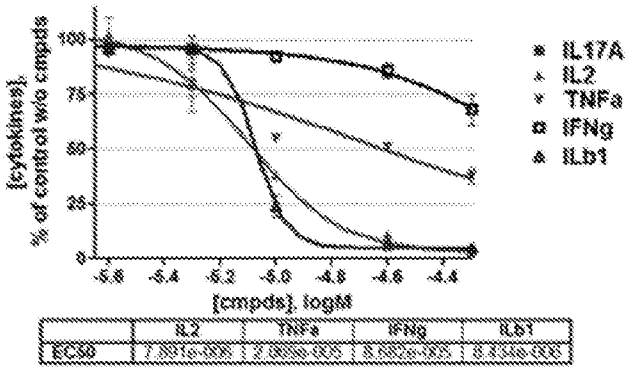


FIG. 35C

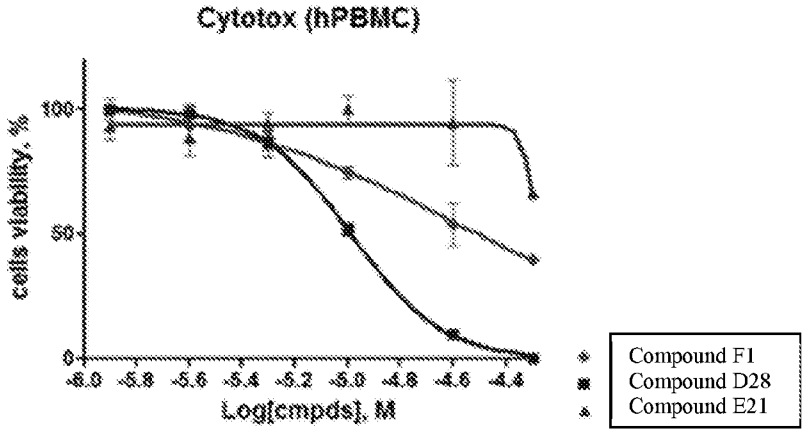
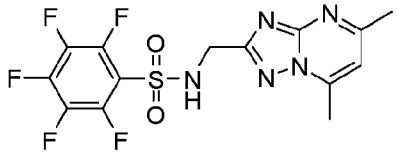


FIG. 35D

	EC50
Compound F1	2.683e-005
Compound D28	1.025e-005
Compound E21	~5.317e-005



Compound E21

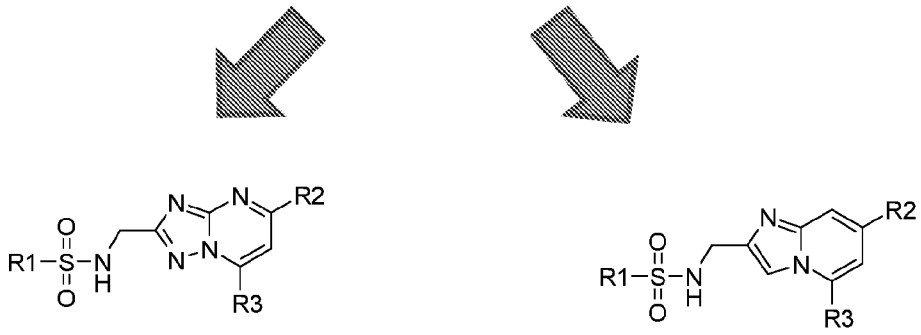


FIG. 36

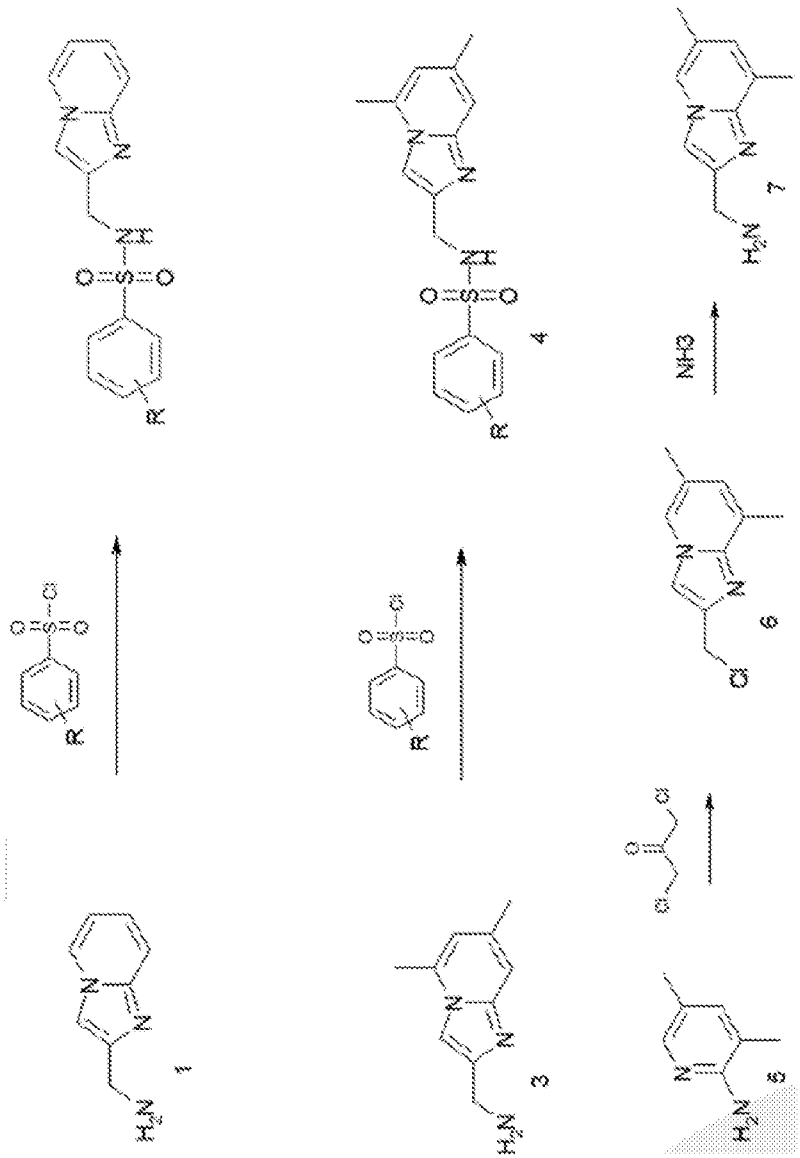


FIG. 37

Testing of Compound F1

NR2F6 Transient Transfection

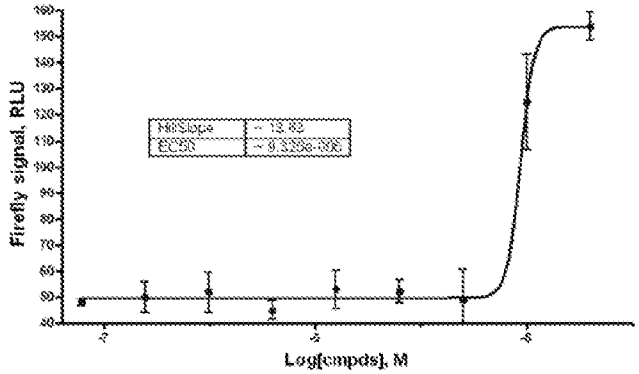


FIG. 38A

LBD Transient Transfection

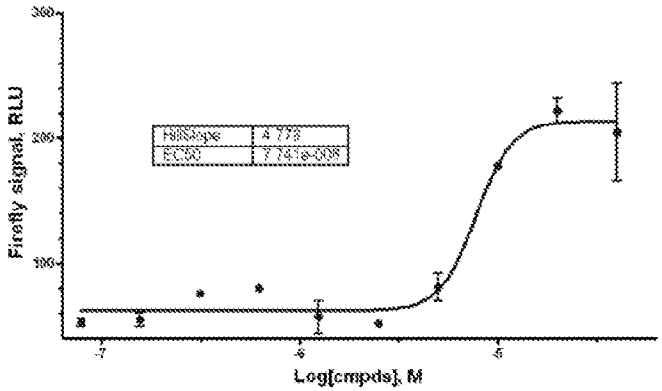


FIG. 38B

Testing of Compound F1

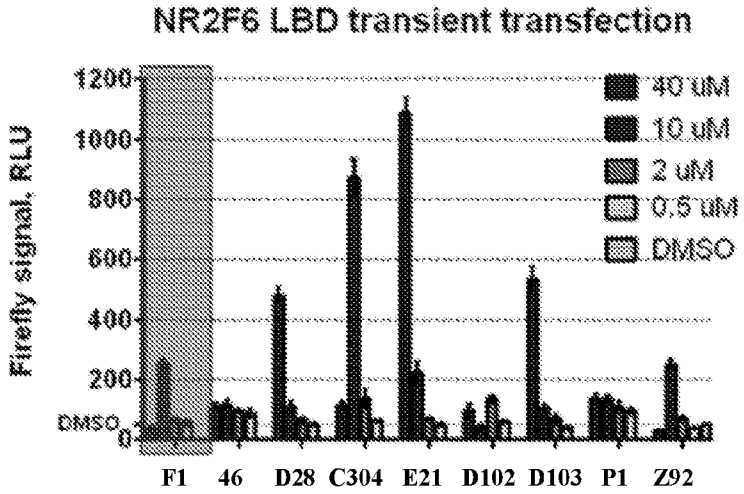


FIG. 39A

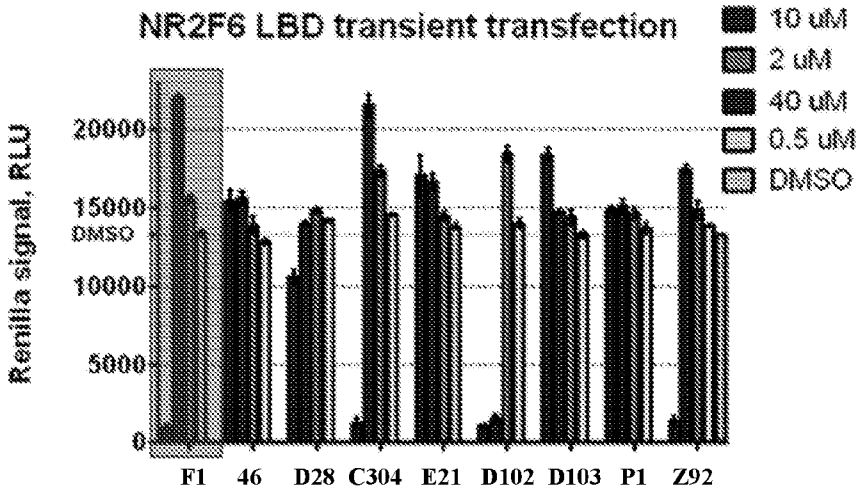


FIG. 39B

Testing of Compound F1

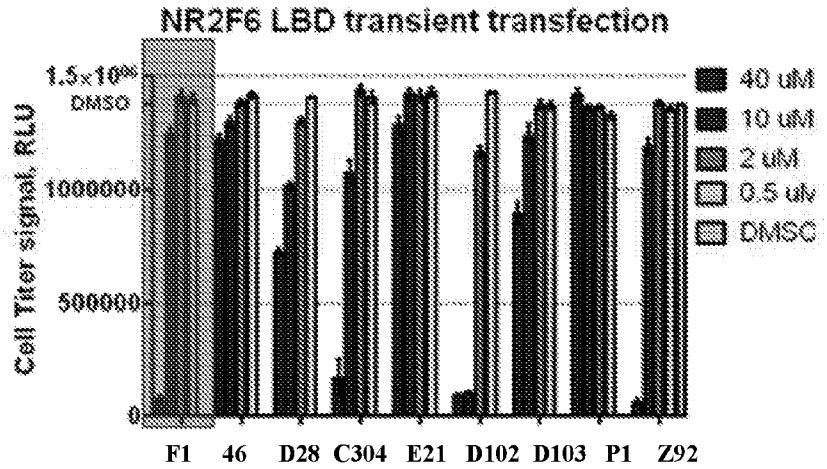


FIG. 39C

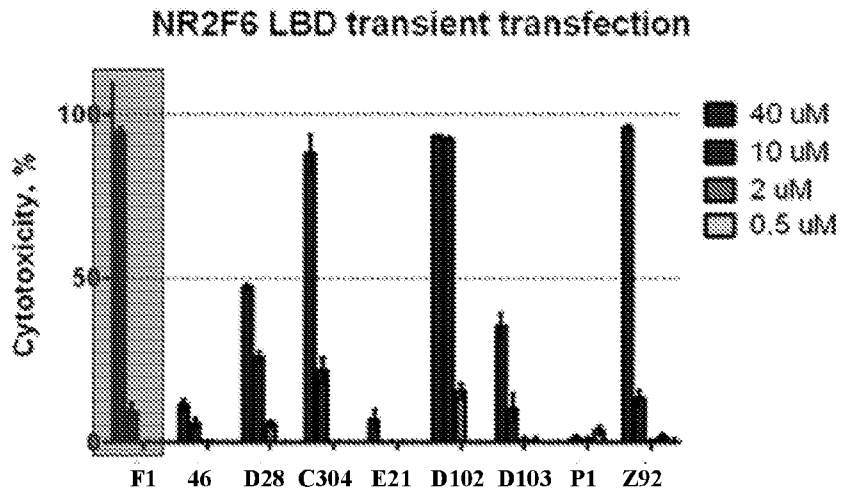


FIG. 39D

Testing of Compound F1

F1 cytokines release inhibition (stimulated by PMA + Ionomycin PBMC)

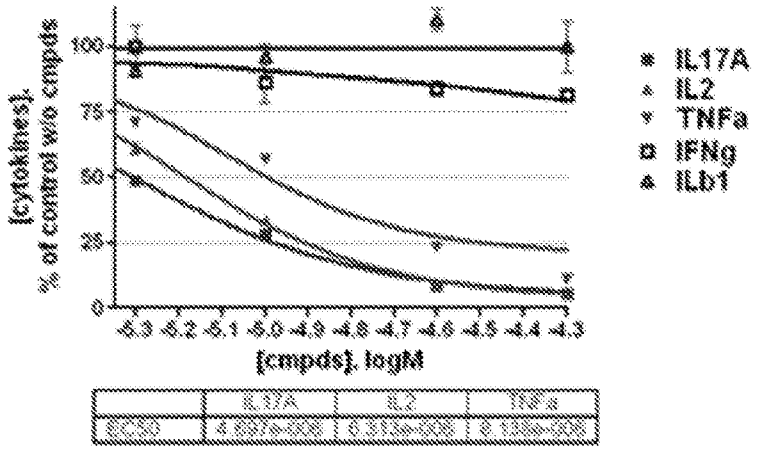
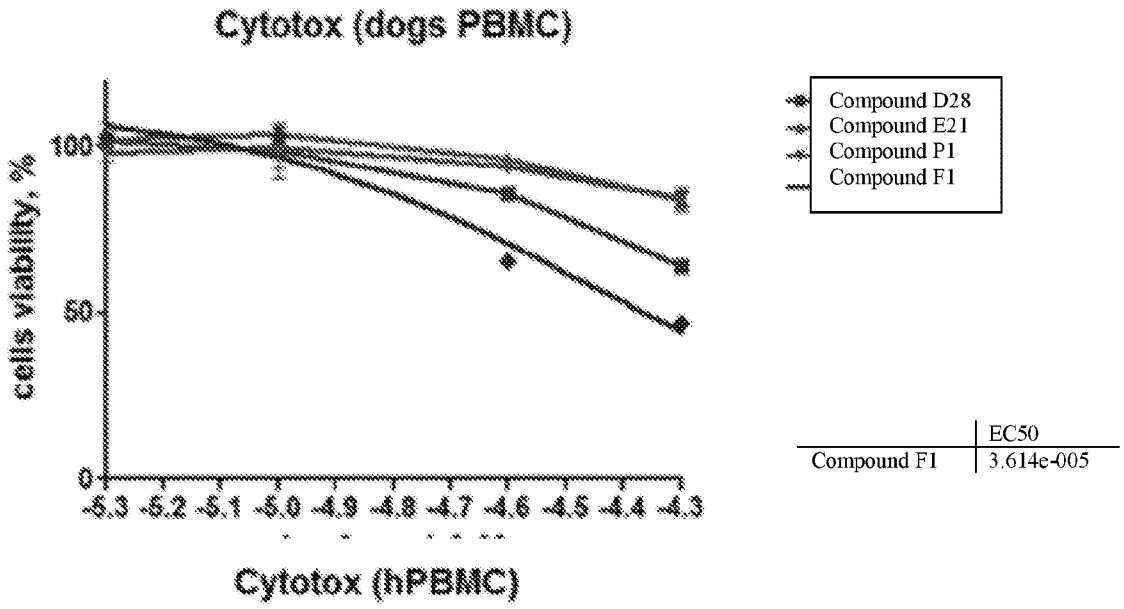


FIG. 40A

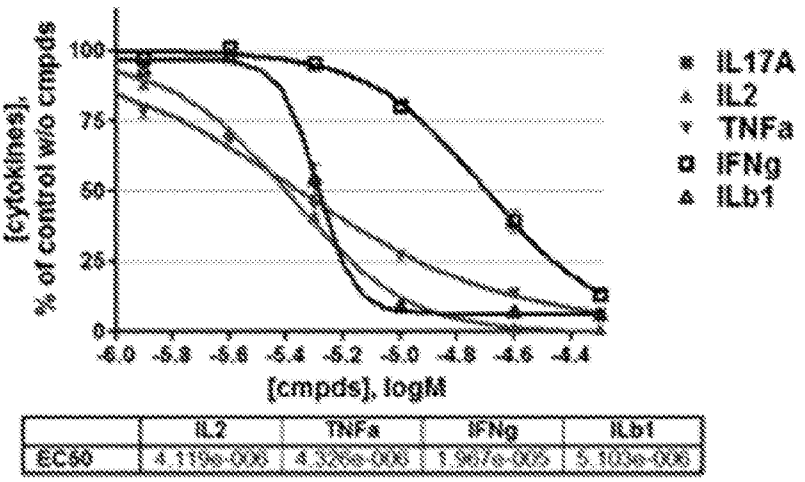


	EC50
Compound F1	3.614e-005

FIG. 40B

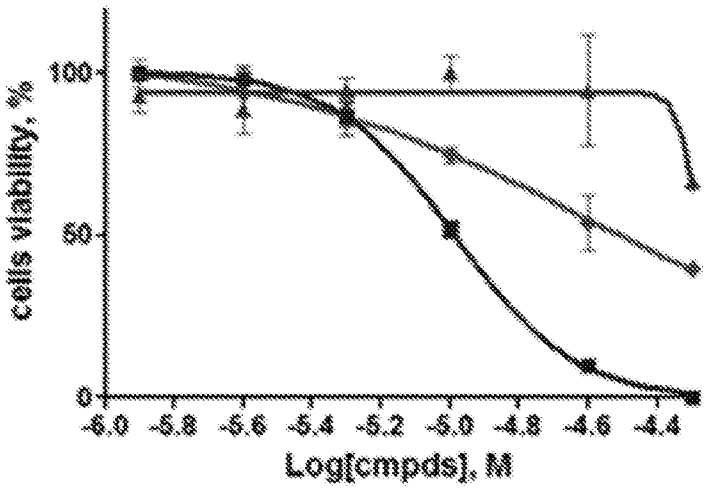
Testing of Compound F1

Z56 (F1) cytokines release inhibition (stimulated by PMA + Ionomycin hPBMC)



	IL2	TNFa	IFNg	IL1b1
EC50	4.110e-006	4.326e-006	1.367e-005	5.103e-006

FIG. 40C



	EC50
Compound F1	2.683e-005
Compound D28	1.025e-005
Compound E21	~5.317e-005

FIG. 40D

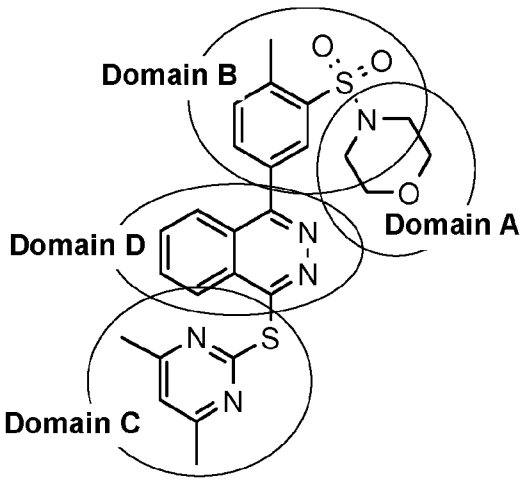


FIG. 41

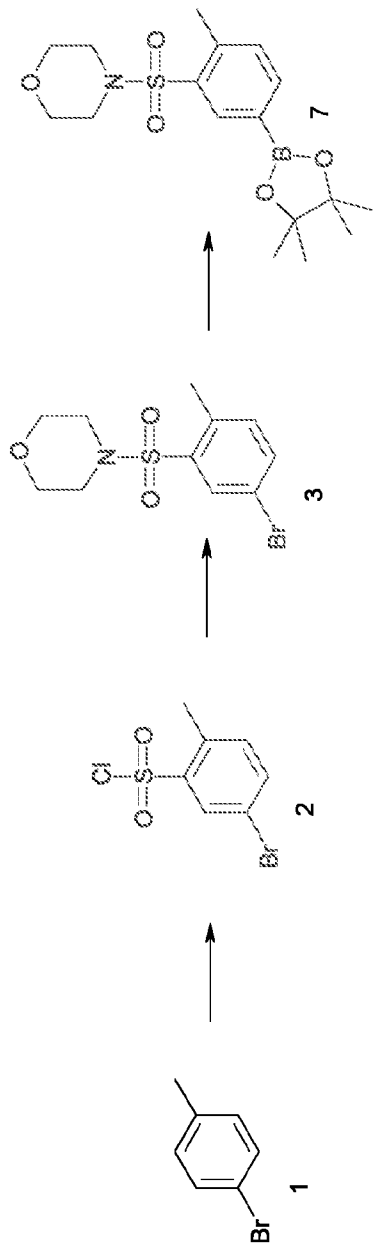


FIG. 42

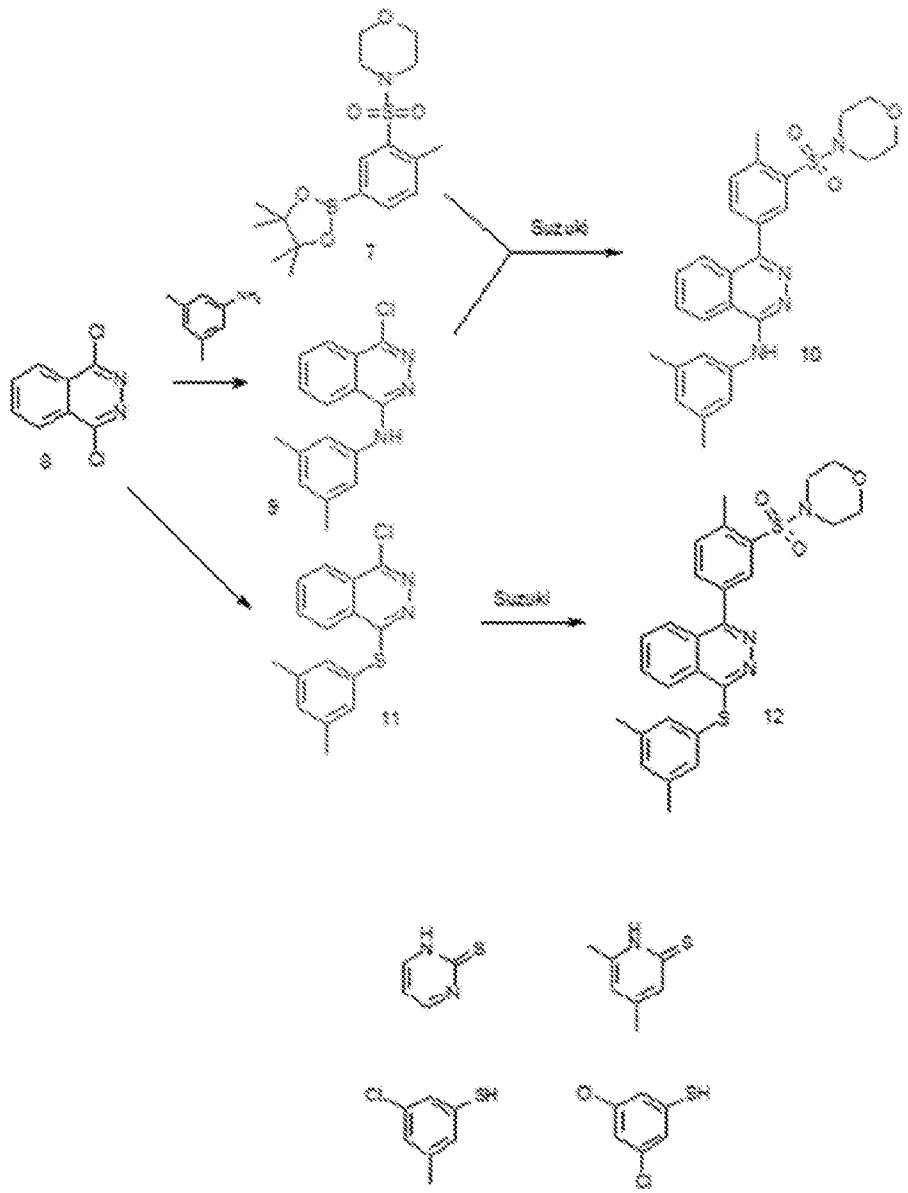


FIG. 43

Compound P1 –
NR2F6 (left) and LBD (right) transient transfection

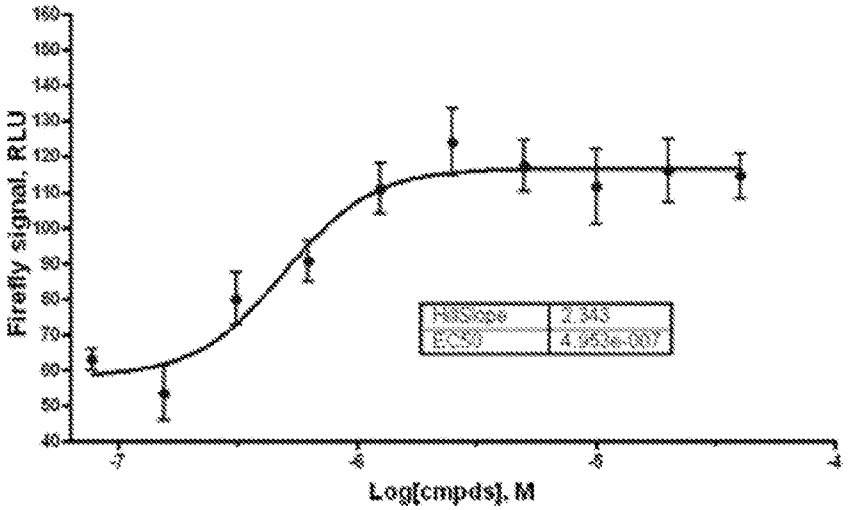


FIG. 44A

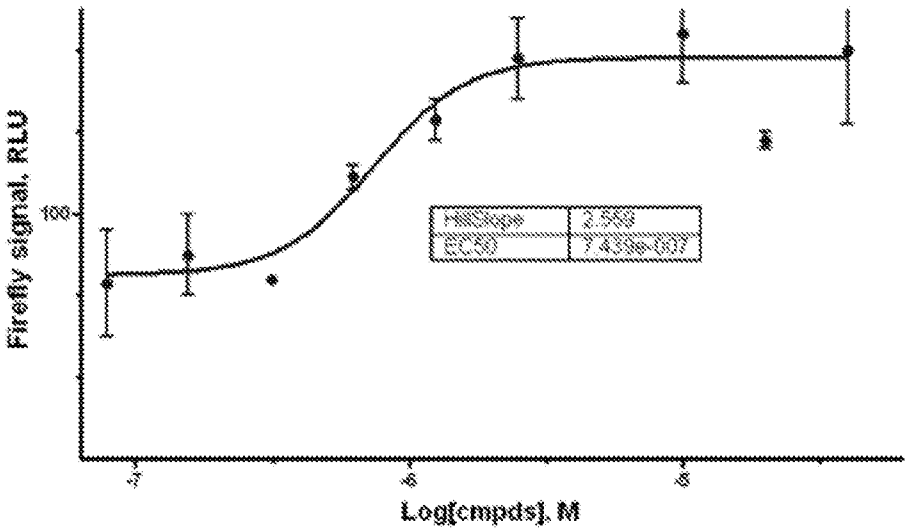


FIG. 44B

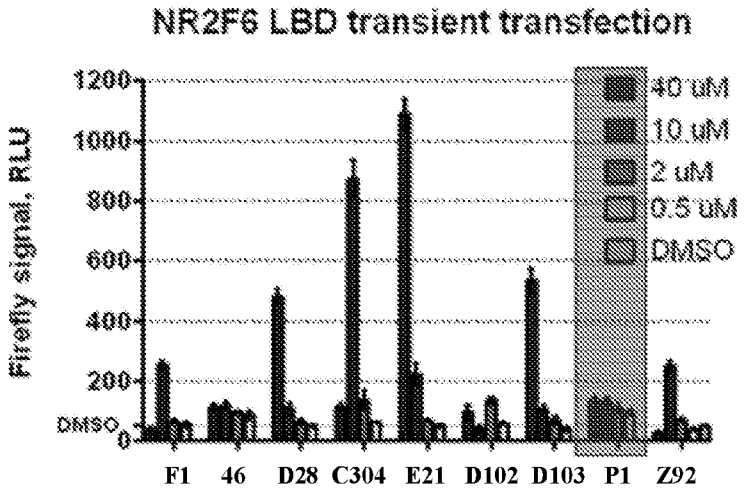


FIG. 45A

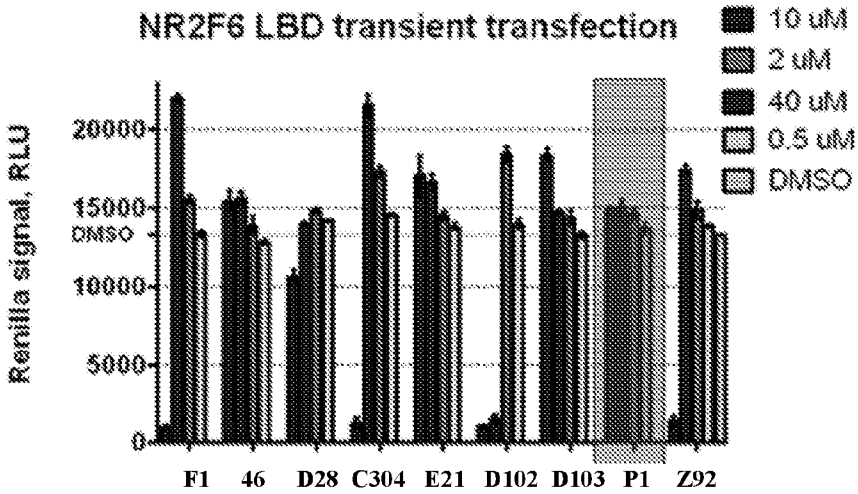


FIG. 45B

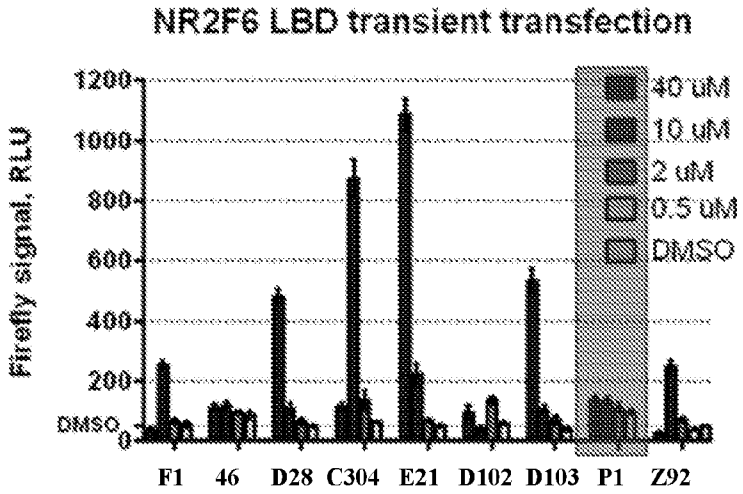


FIG.45C

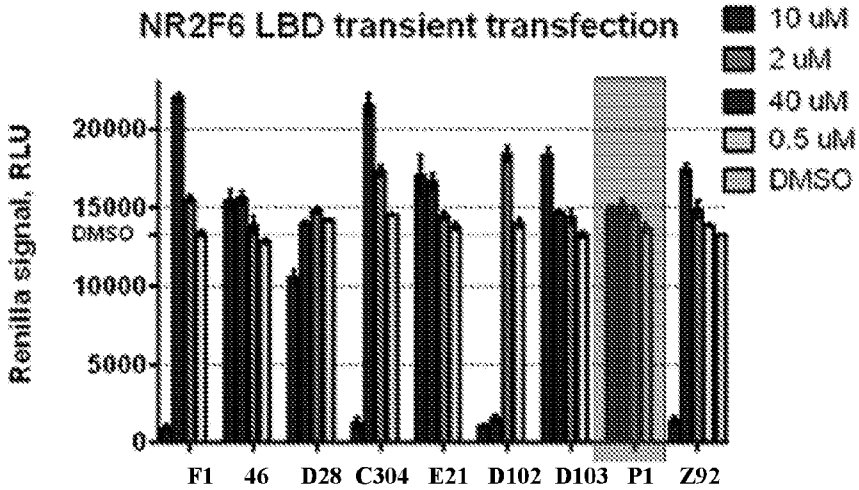


FIG. 45D

Compound P1 – Cytokine Release Experiment Dog's PBMC

Compound 1 cytokines release inhibition (simulated by PMA + lonomycin PMBC)

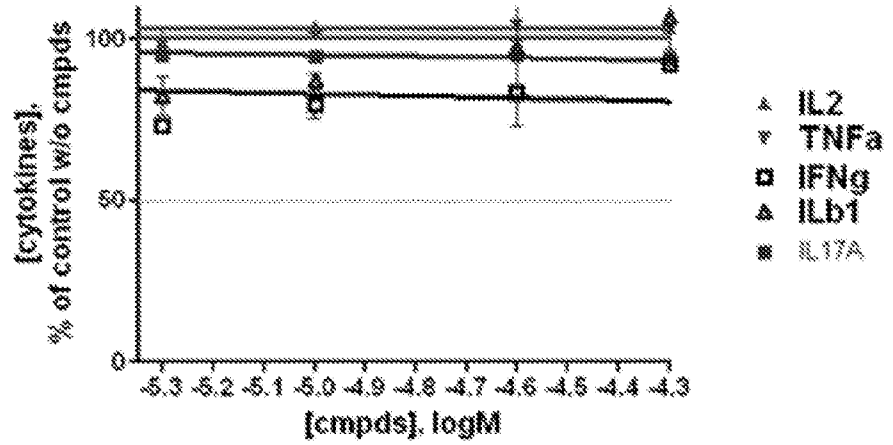
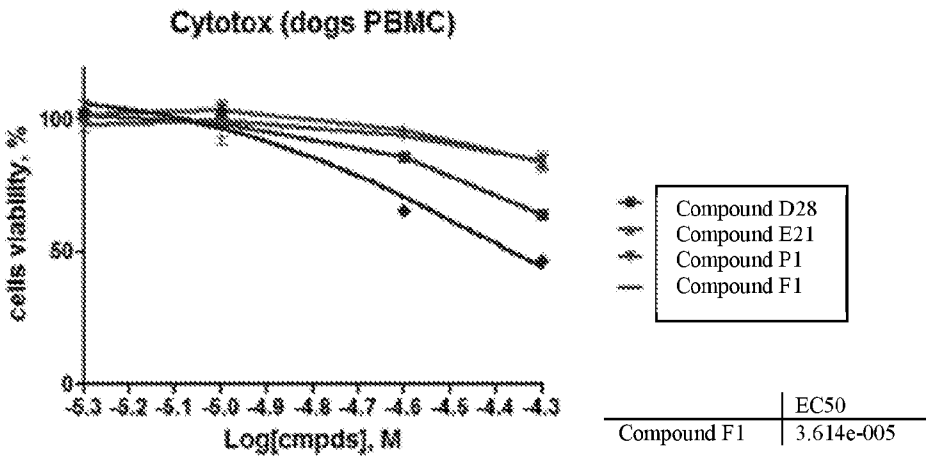


FIG. 46A



	EC50
Compound F1	3.614e-005

FIG. 46B

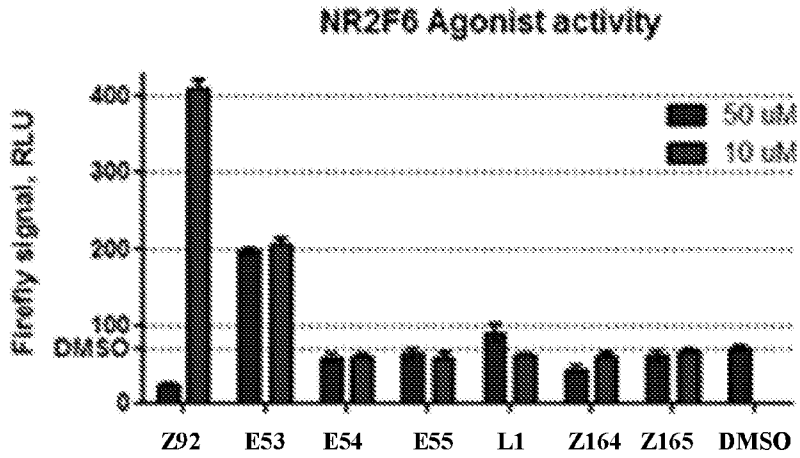


FIG. 47A

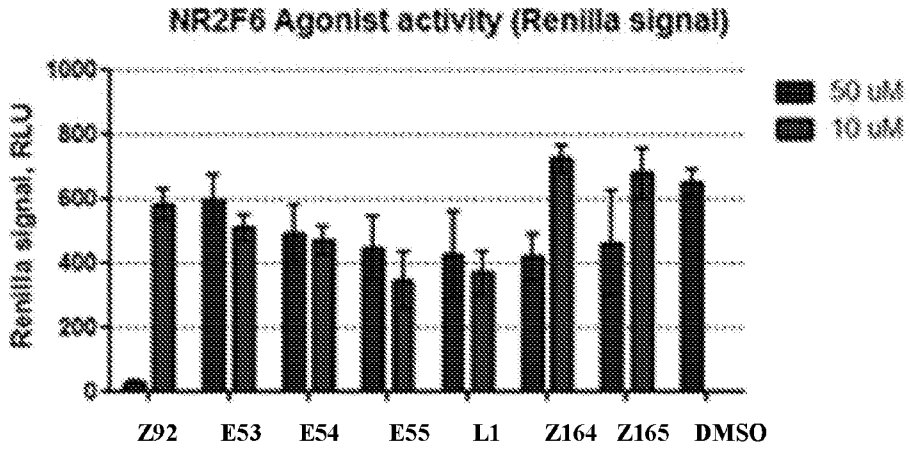


FIG. 47B

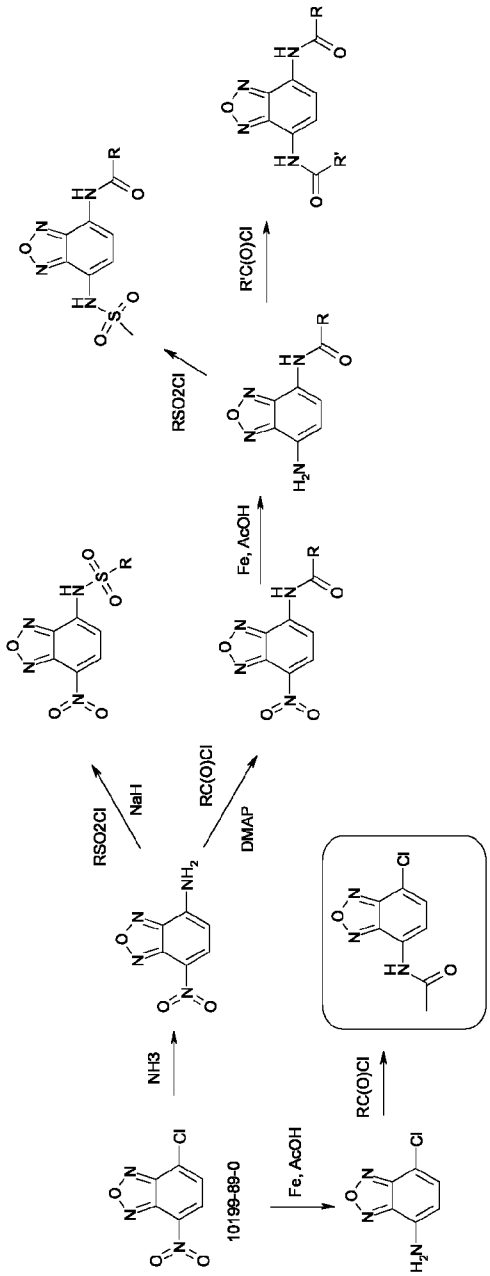

10199-89-0
NH3
Fe, AcOH
RC(O)Cl
RSO2Cl
NaH
DMAP
Fe, AcOH
RSO2Cl
R'C(O)Cl

FIG. 48

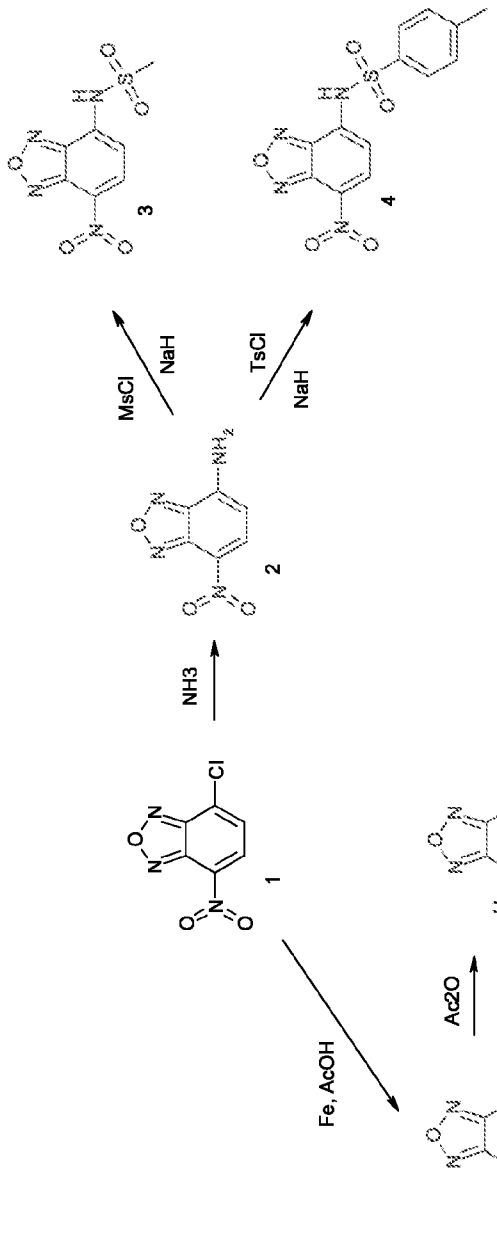


FIG. 49

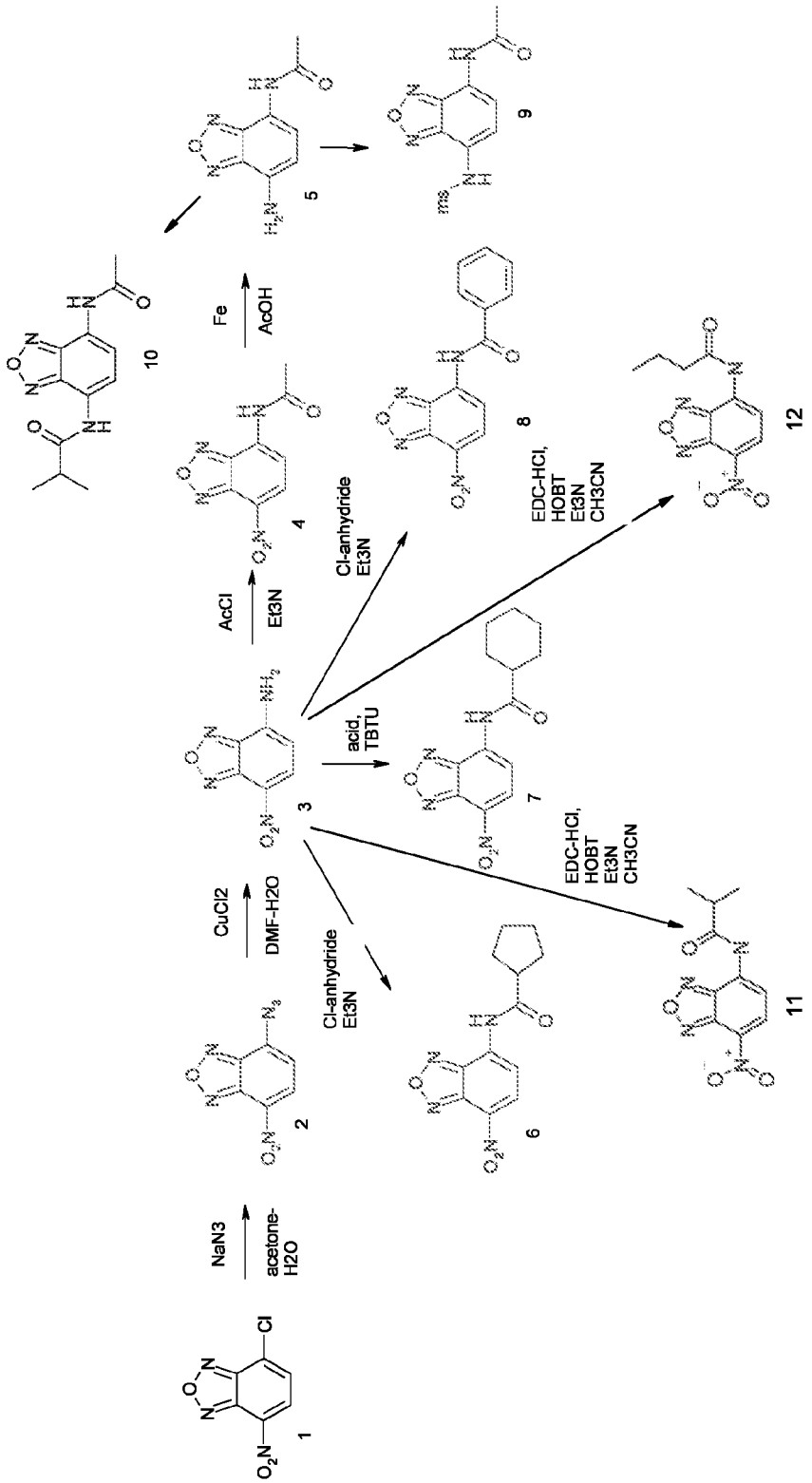

NaN3
acetone-H2O
CuCl2
DMF-H2O
Cl-anhydride
Et3N
acid, TBTU
EDC-HCl, HOBT Et3N CH3CN
AcCl
Et3N
Fe
AcOH
1
2
3
4
5
6
7
8
9
10
11
12

FIG. 50

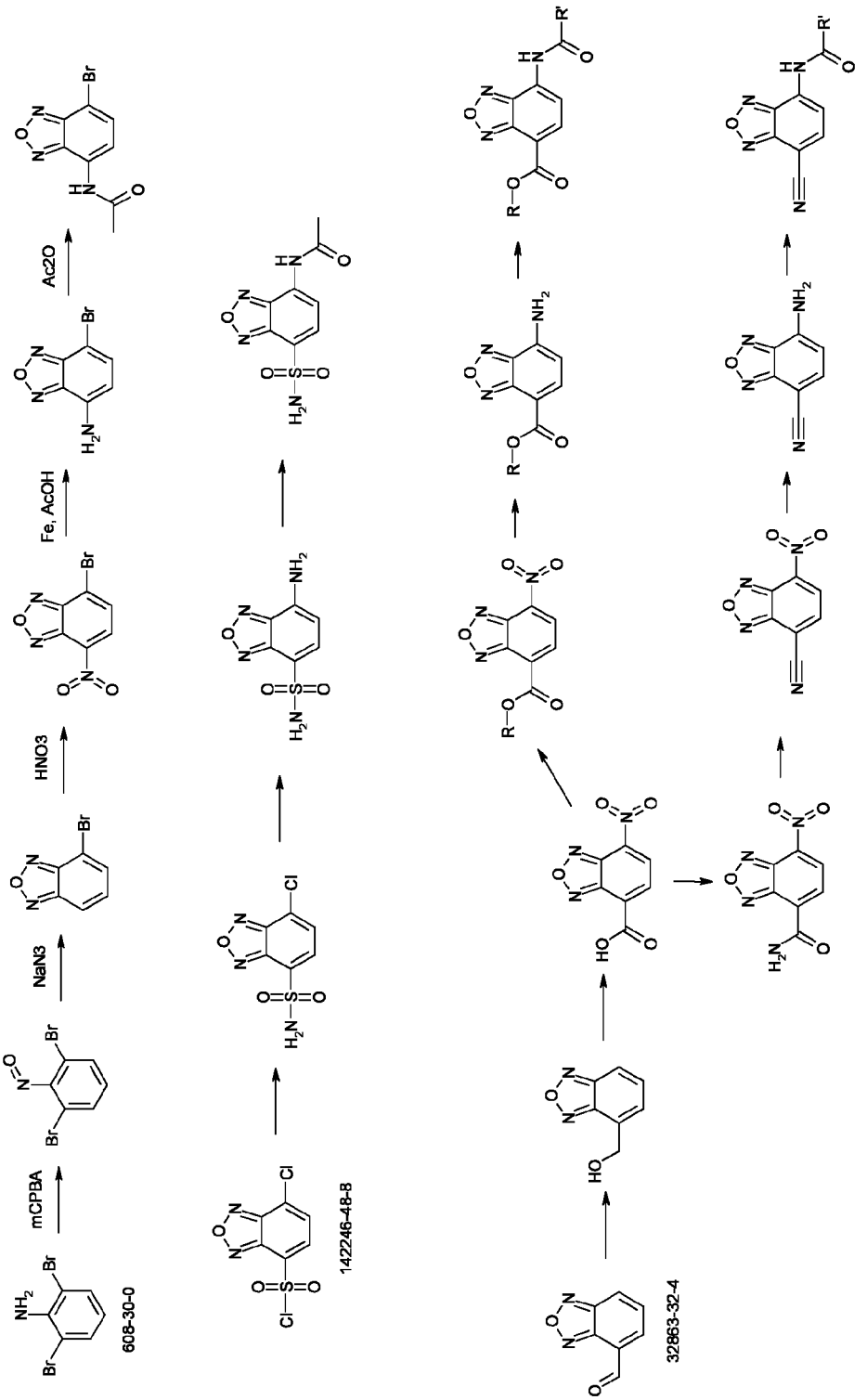


FIG. 51

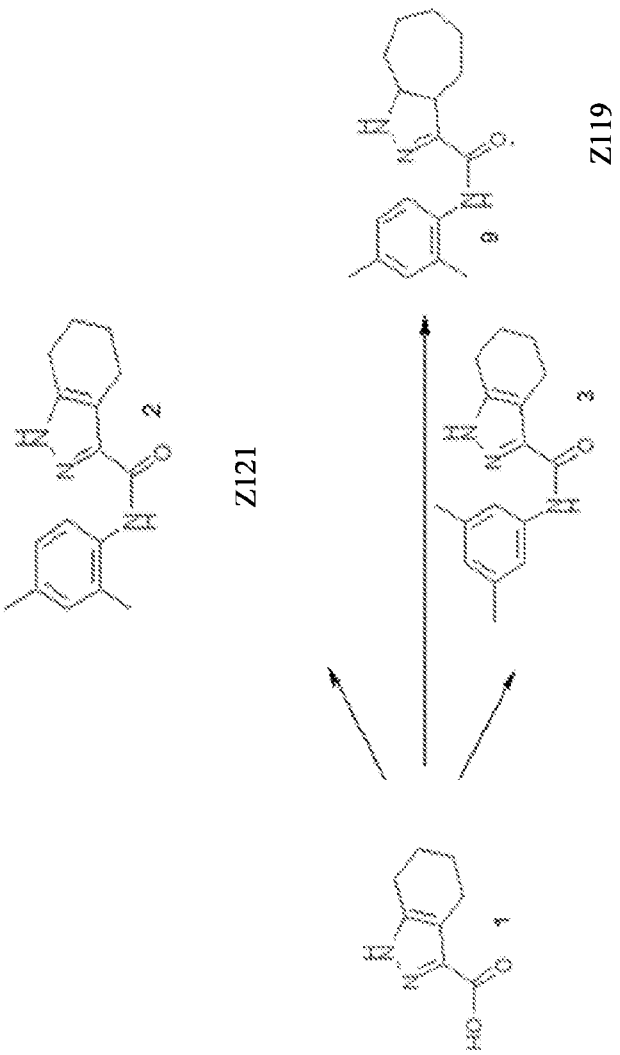


Fig. 52A

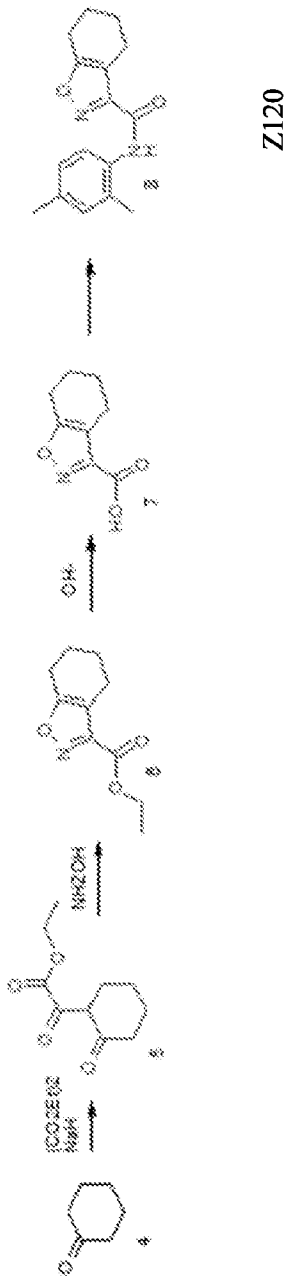


FIG. 52B

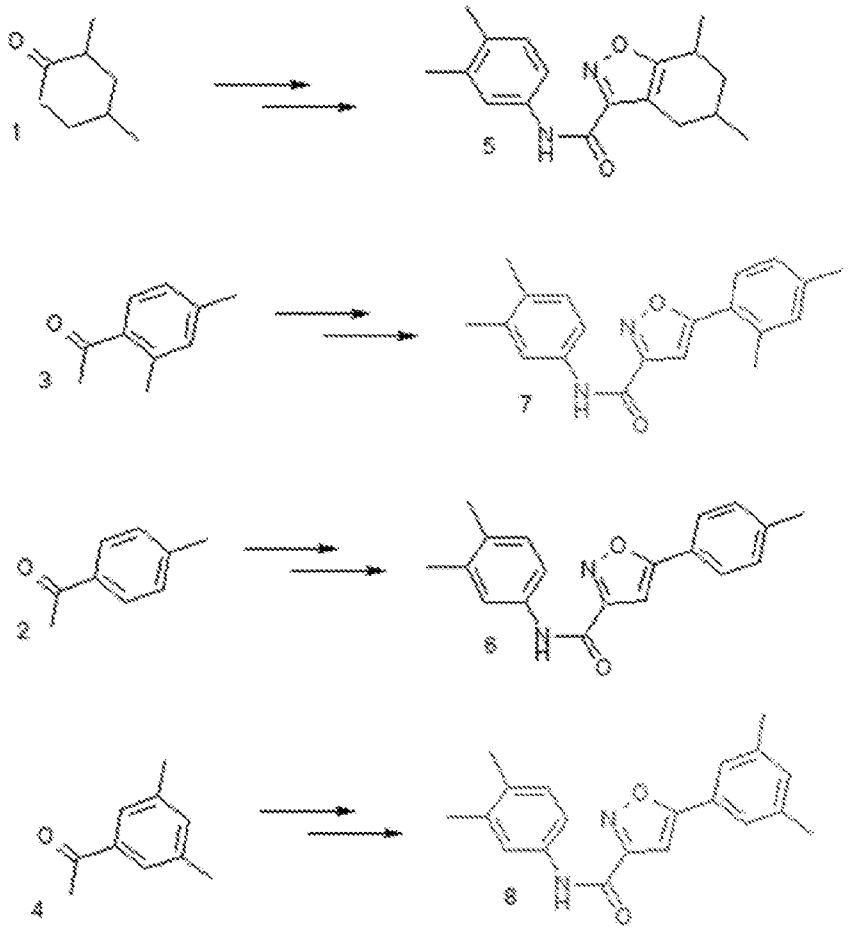


FIG. 53

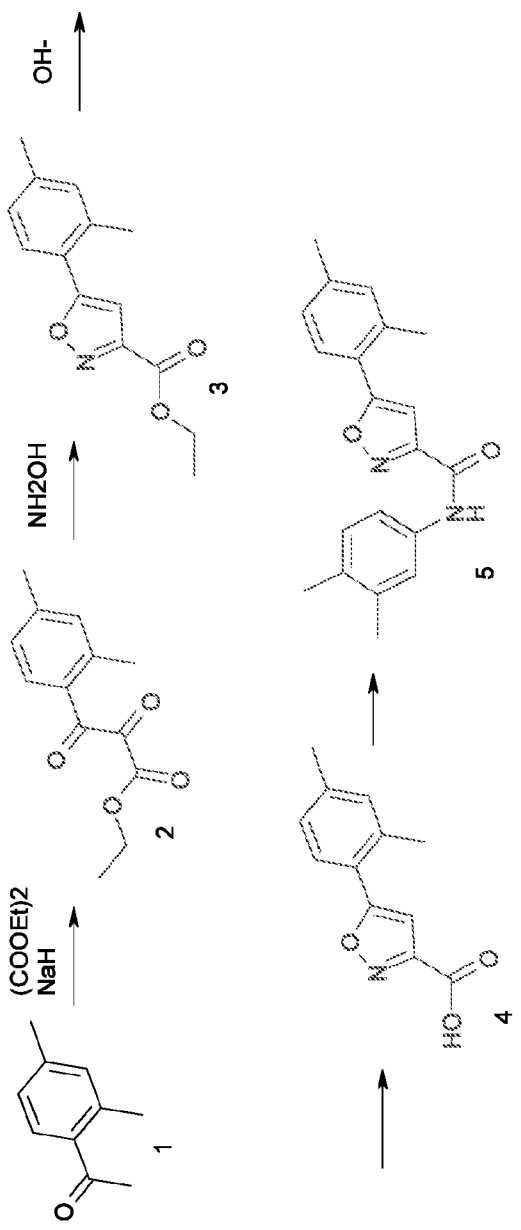


FIG. 54

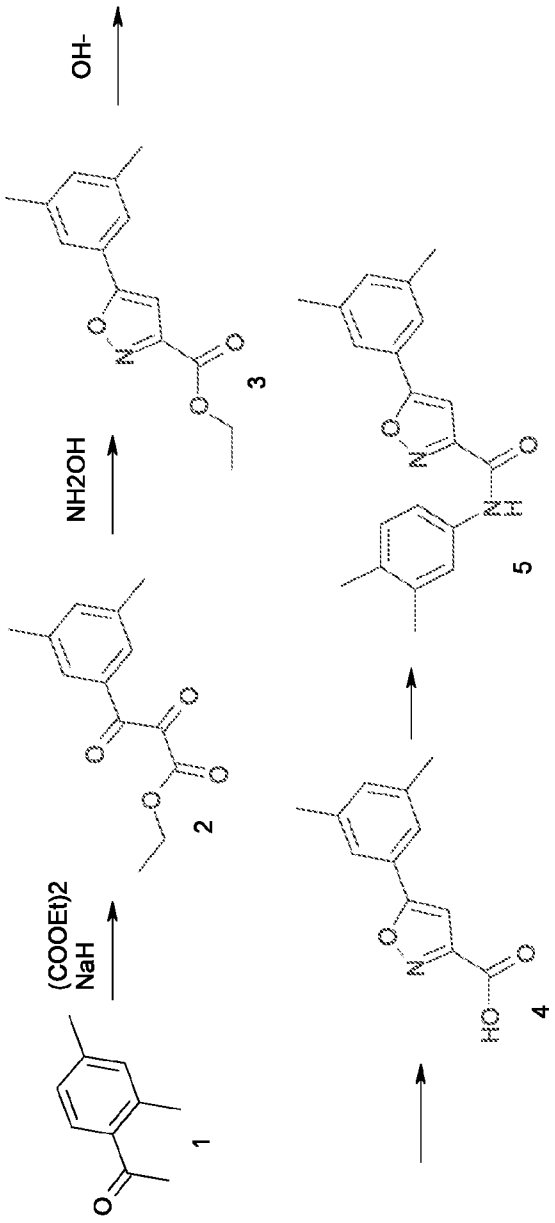


FIG. 55

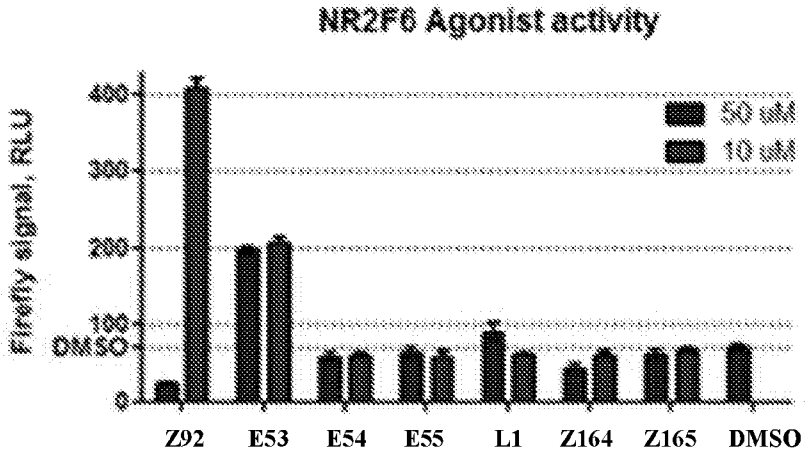


FIG. 56A

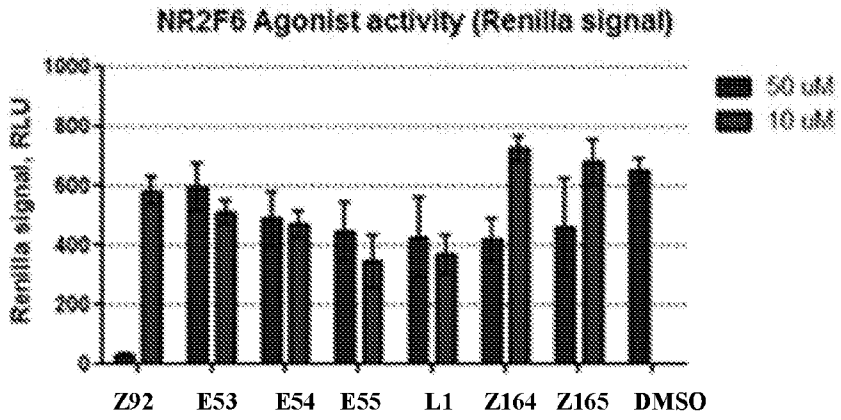


FIG. 56B

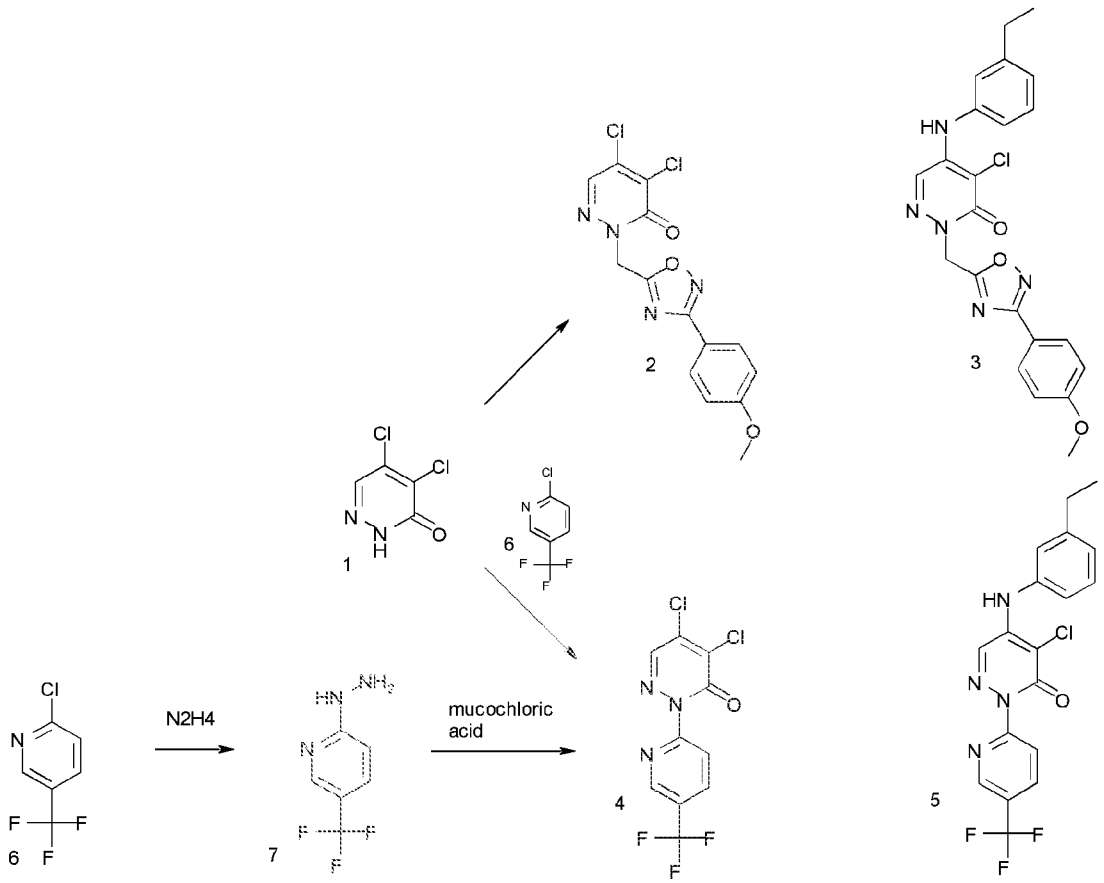


FIG. 57

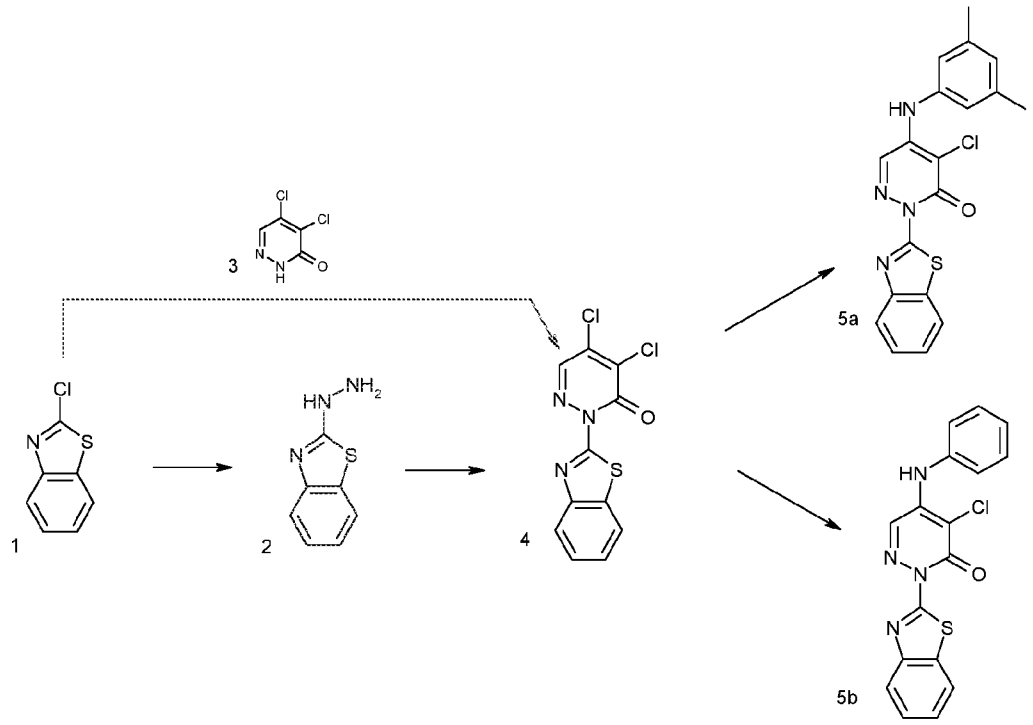

Cl
N
S
1
HN
NH2
N
S
2
3
Cl
Cl
N
N
H
O
Cl
Cl
N
N
O
N
S
4
HN
Cl
N
N
O
N
S
5a
HN
Cl
N
N
O
N
S
5b

FIG. 58

Compound D104

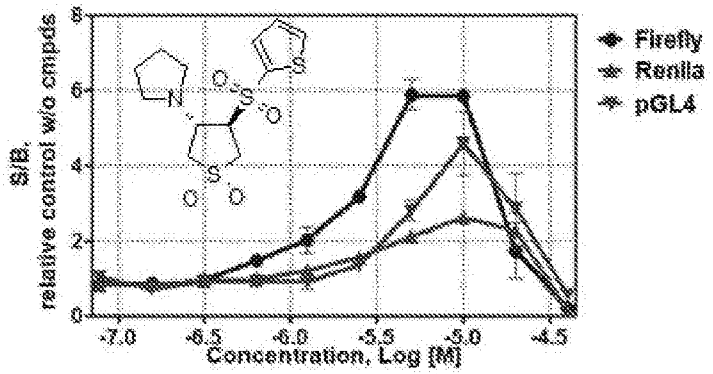


FIG. 59A

Compound D118

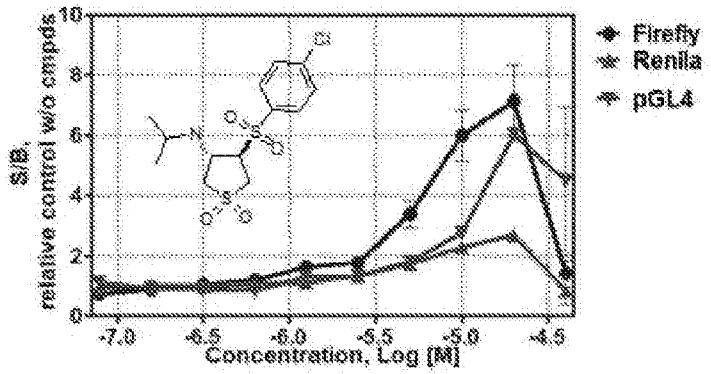


FIG. 59B

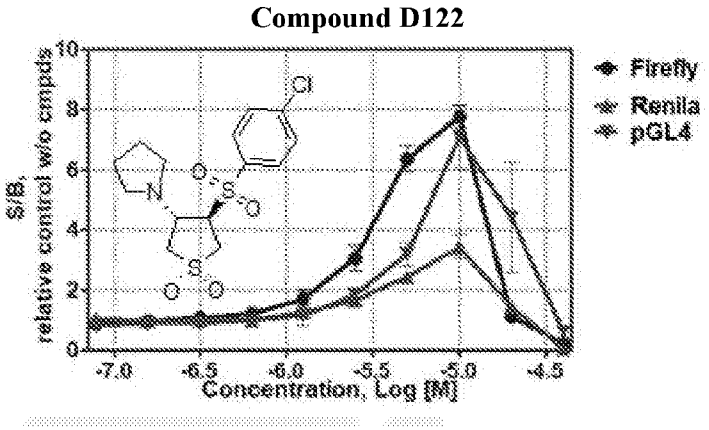


FIG. 59C

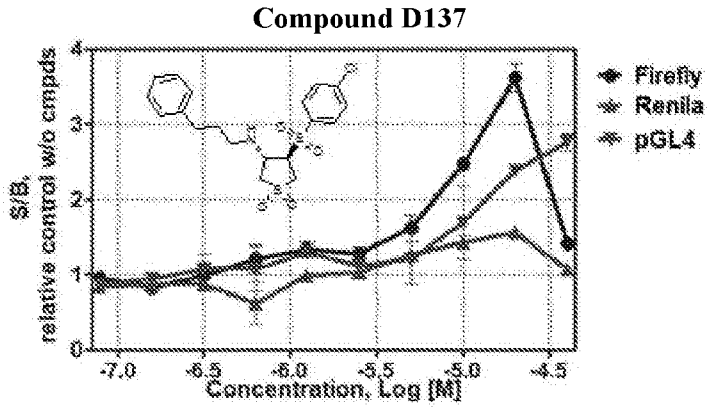
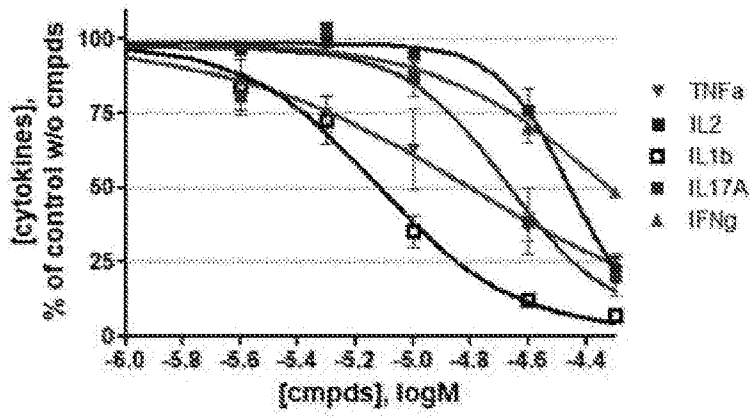


FIG. 59D

Compound D136 cytokines release inhibition (stimulated by PMA + Ionomycin hPBMC)



	IL2	IFNg	TNFa	IL1b	IL17A
EC50	3.540e-005	4.797e-005	1.577e-005	7.620e-006	2.123e-005

FIG. 60A

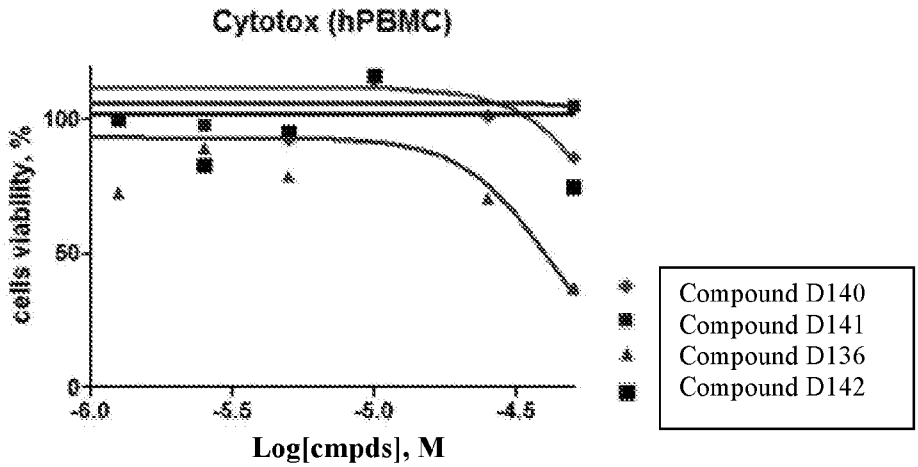
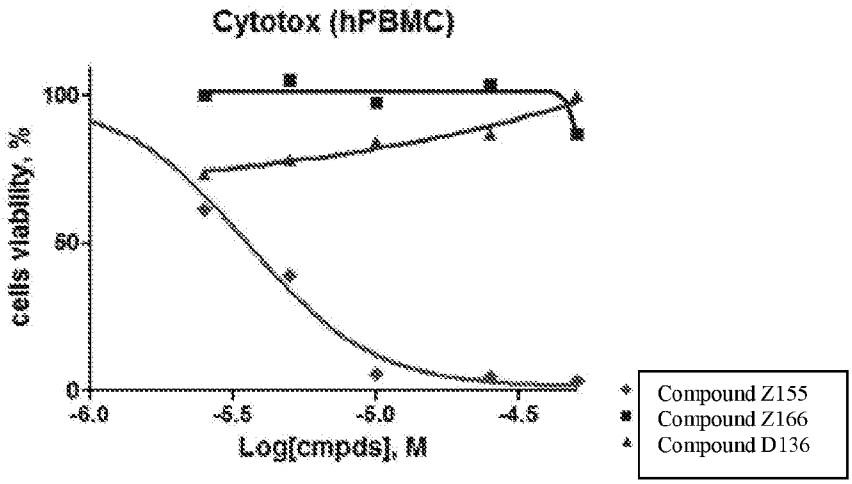


FIG. 60B

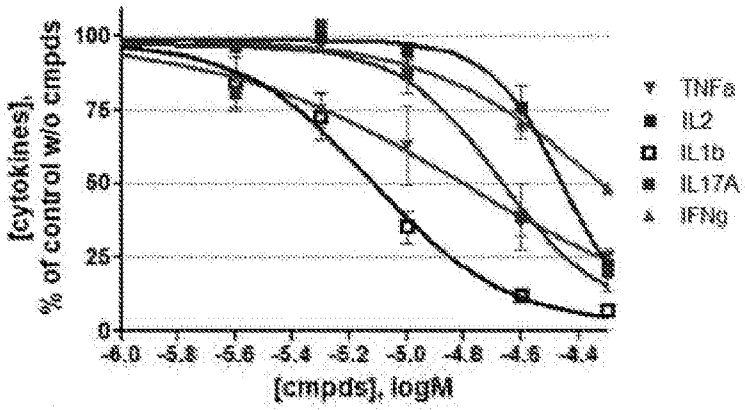
	EC50
Compound D140	7.553e-005
Compound D141	~0.0001379
Compound D136	4.190e-005
Compound D142	~2.937



	EC50
Compound Z155	3.560e-006
Compound Z166	~5.200e-005
Compound D136	~2.099

FIG. 60C

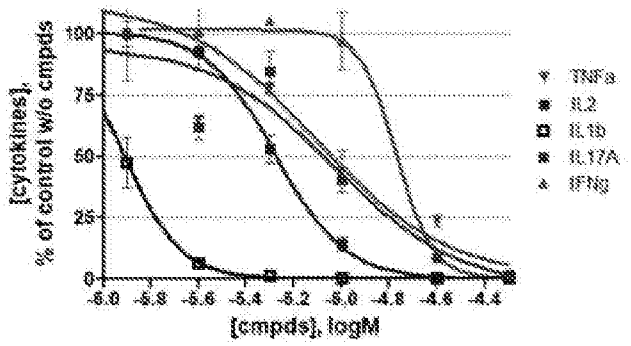
Compound D136 cytokines release inhibition
(stimulated by PMA + Ionomycin hPBMC)



	IL2	IFNg	TNFa	IL1b	IL17A
EC50	3.540e-005	4.797e-005	1.577e-005	7.620e-006	2.123e-005

FIG. 61A

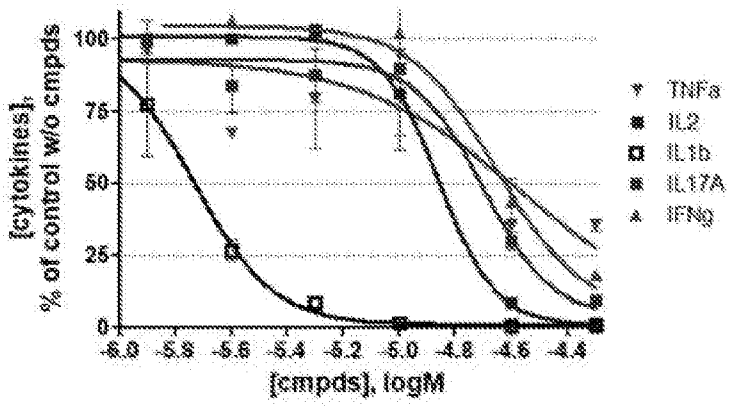
Compound D22 cytokines release inhibition
(stimulated by PMA + Ionomycin hPBMC)



	IL2	IFNg	TNFa	IL1b	IL17A
EC50	5.273e-006	1.699e-005	7.884e-006	1.223e-006	9.370e-006

FIG. 61B

Compound D136 cytokines release inhibition (stimulated by PMA + Ionomycin hPBMC)



	IL2	IFNg	TNFa	IL1b	IL17A
EC50	1.384e-005	2.225e-005	2.632e-005	1.825e-008	1.396e-005

FIG. 61C

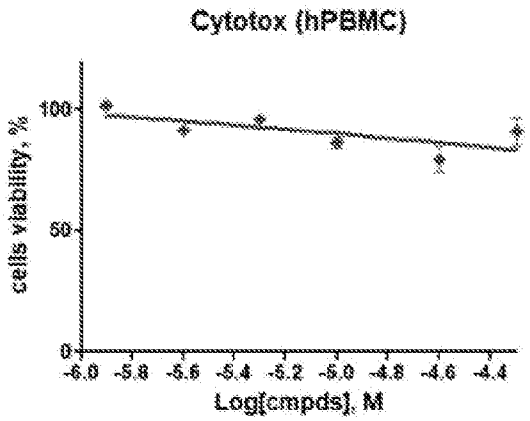
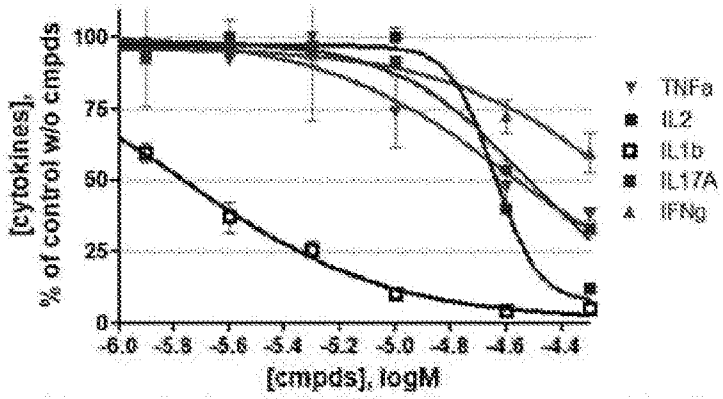


FIG. 61D

	EC50
Compound D137	~5.158e+024

Compound D137 cytokines release inhibition (stimulated by PMA + Ionomycin hPBMC)



	IL2	IFNg	TNFa	IL1b	IL17A
EC50	2.281e-005	6.824e-005	2.636e-005	1.684e-005	3.026e-005

FIG. 61E

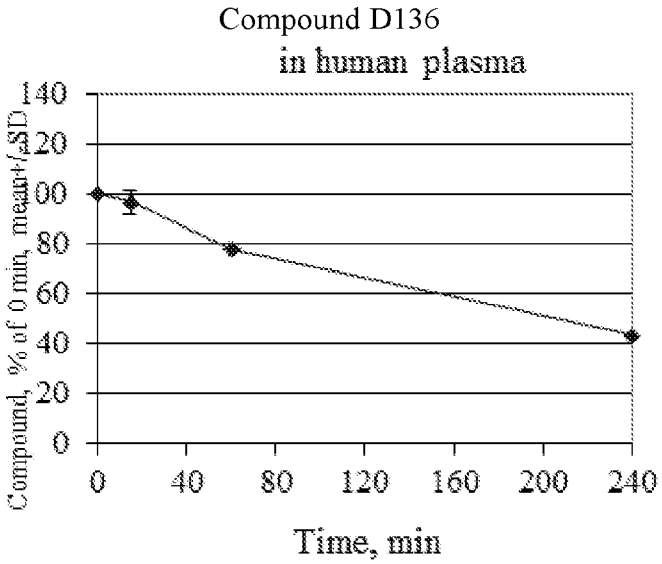


FIG. 62A

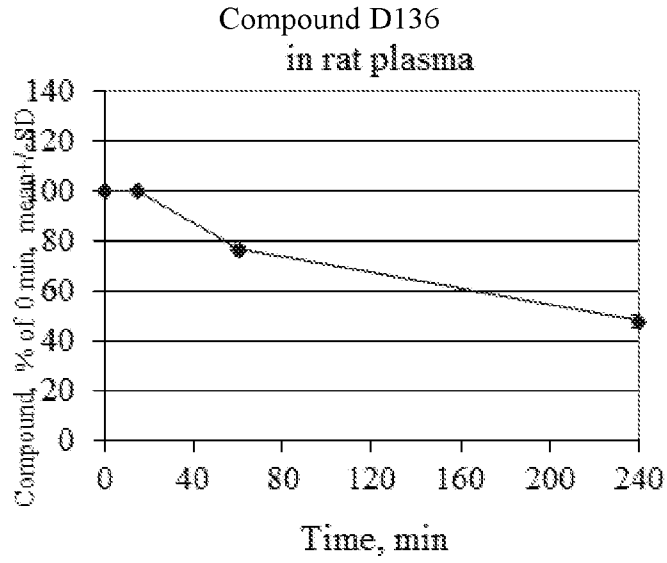


FIG. 62B

Compound D136 Stability in Simulated Gastric Fluid (SGF)

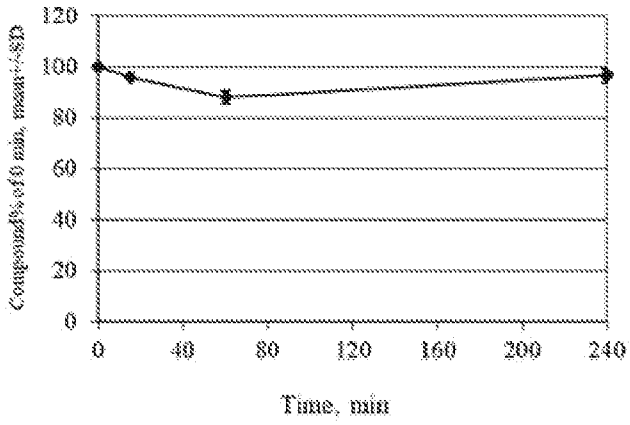


FIG. 63A

Compound D136 Stability in Simulated Intestinal Fluid (SIF)

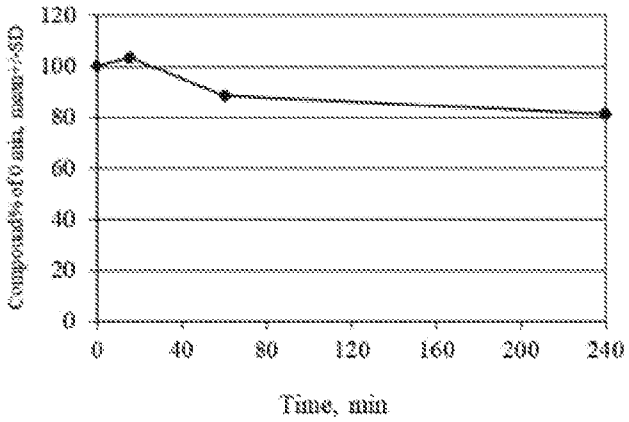


FIG. 63B

Compound D136 Microsomal Stability (Human Liver Microsomes)

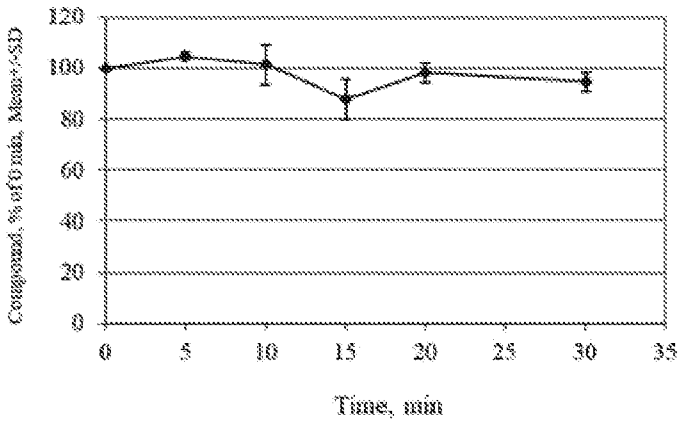


FIG. 64A

Compound D Microsomal Stability (Rat Liver Microsomes)

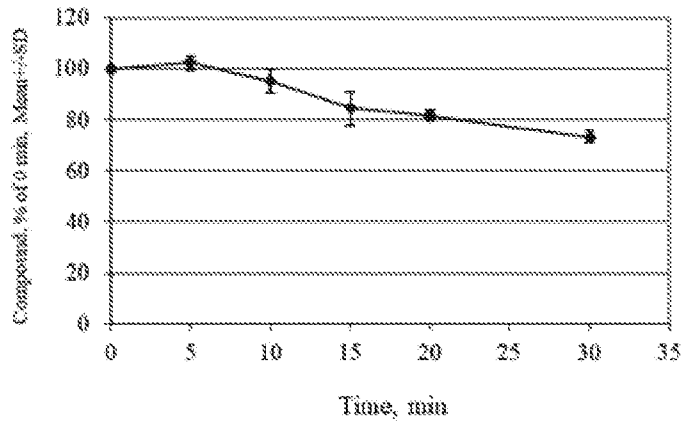


FIG. 64B

Compound D136
PK in rat plasma, PO 7.53 mg/kg
Mean +/- SE (n=3)

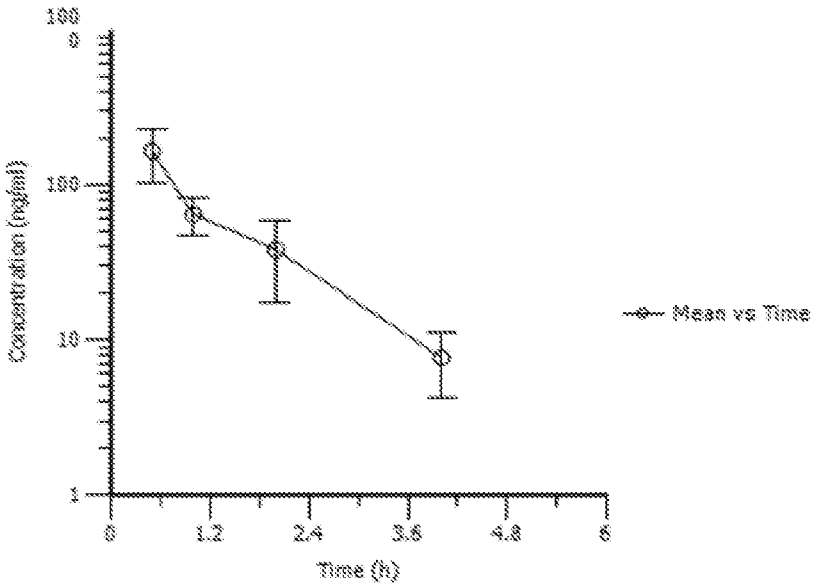


FIG. 65A

Compound D136
PK in rat plasma, IV, 1.13 mg/kg
Mean +/e SE (n=3)

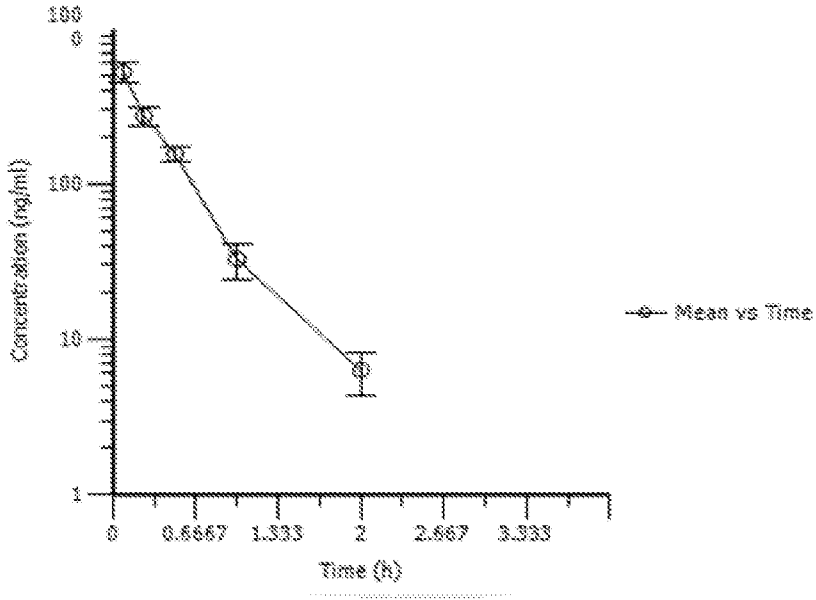


FIG. 65B

Compound D136
PK in mice plasma, PO, 3.18 mg/kg
Mean +/- SE (n=3)

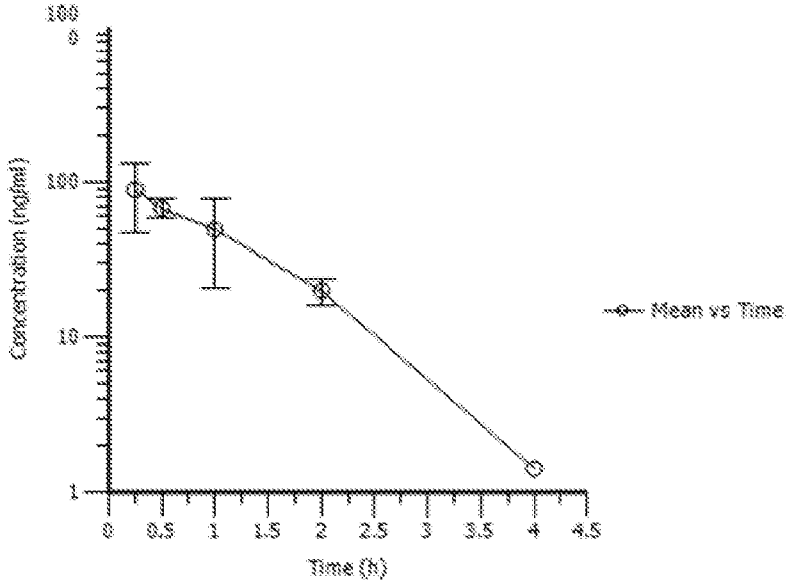


FIG. 66A

Compound D136
PK in mice plasma, IV, 1.12 mg/kg
Mean +/- SE (n=3)

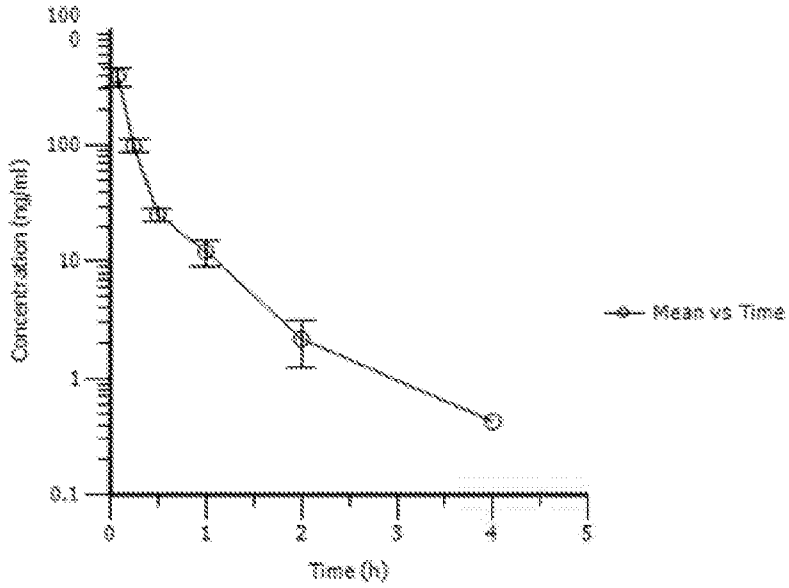


FIG. 66B

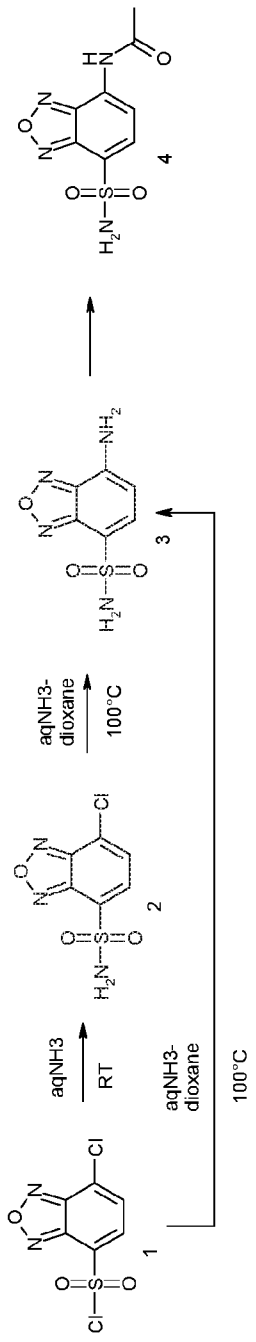


FIG. 67

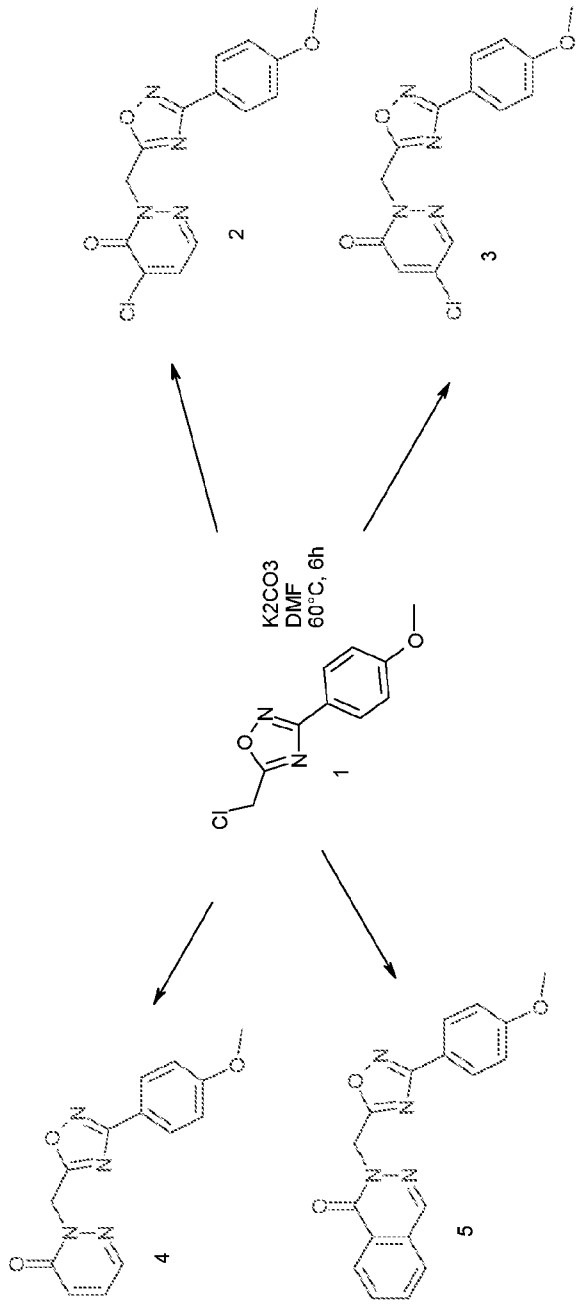


FIG. 68A

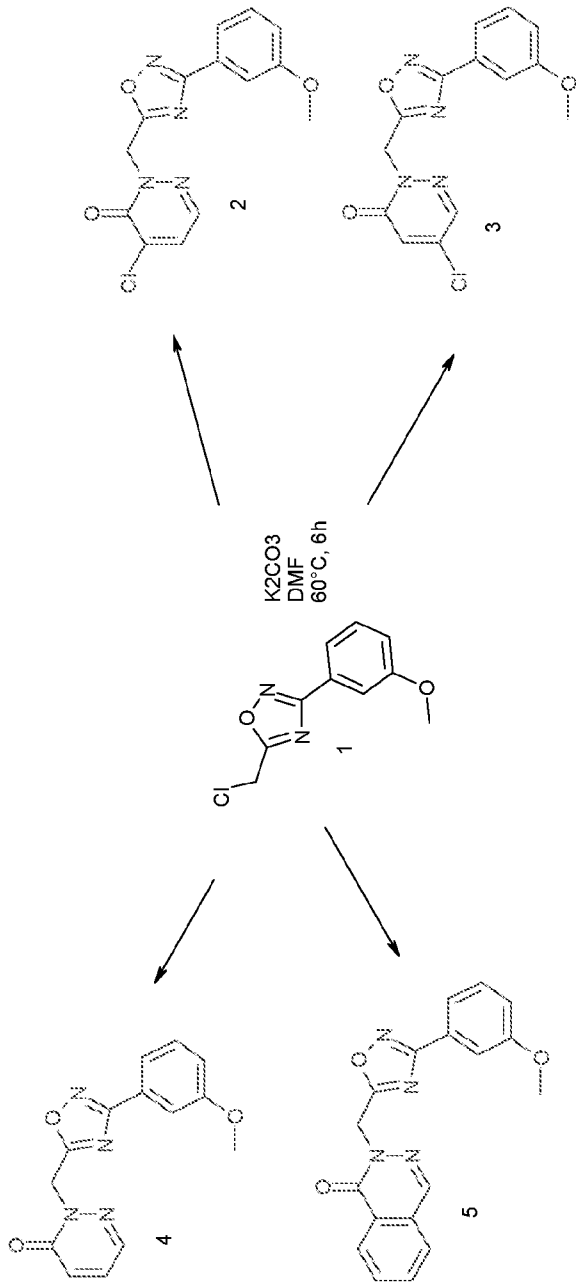


FIG. 68B

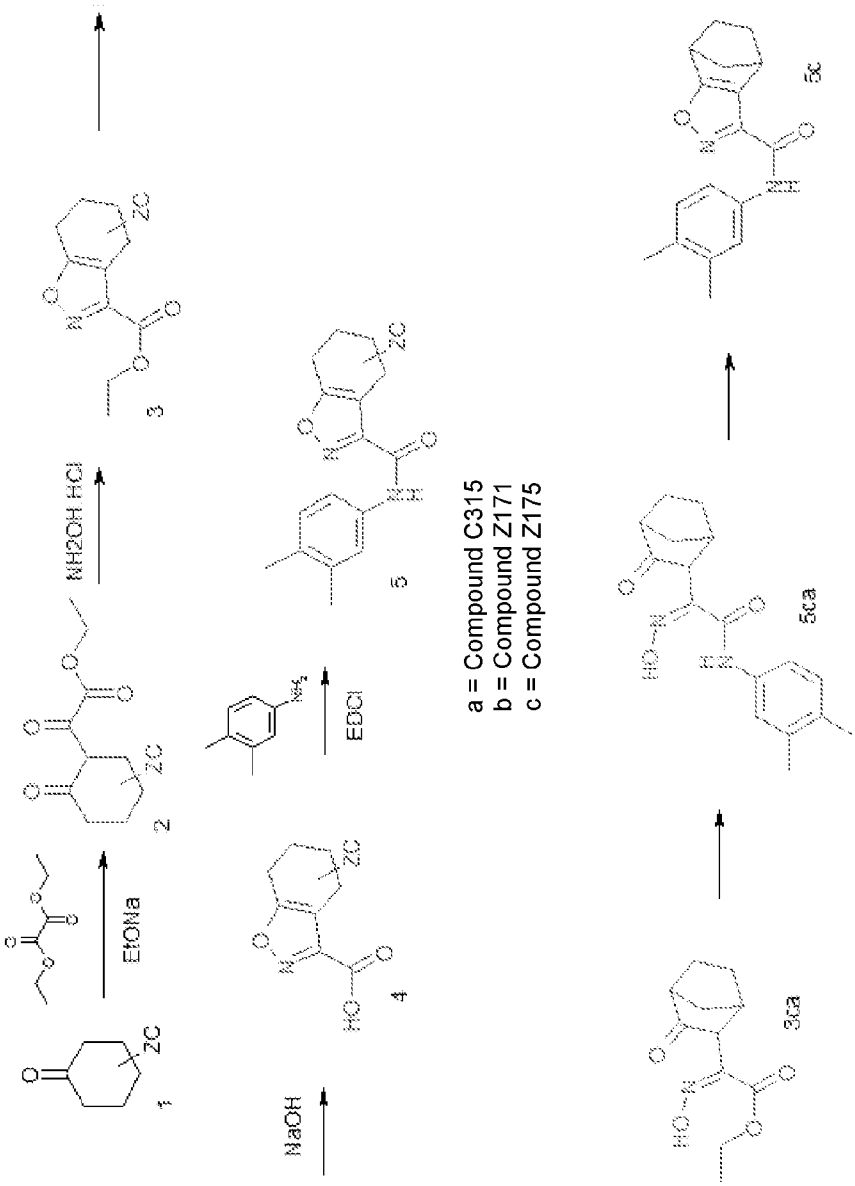

EtONa
NH2OH HCl
EDCI
NaOH
a = Compound C315
b = Compound Z171
c = Compound Z175

FIG. 69

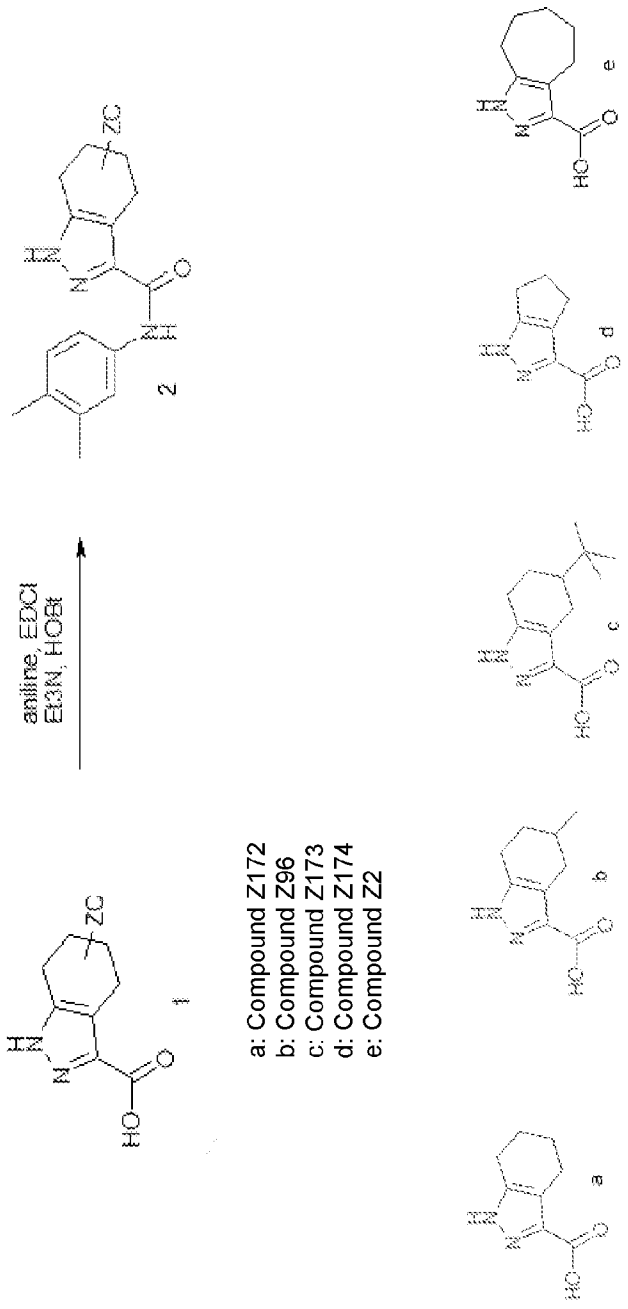

aniline, EDCI
Et3N, HOBt
a: Compound Z172
b: Compound Z96
c: Compound Z173
d: Compound Z174
e: Compound Z2

FIG. 70

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/062290

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/095; A61K 31/10; A61K 31/40; C07D 207/00; C07D 207/02; C07D 291/04 (2019.01)
CPC - A61K 31/095; A61K 31/10; A61K 31/40; C07D 207/02; C07D 291/04; C07D 295/00 (2019.02)

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
USPC - 514/183; 514/408; 514/430 (keyword delimited)

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 -	PUBCHEM, Substance Record for SID 106110021, Available Date: 22 February 2011 [retrieved on 06 March 2019]. Retrieved from the Internet: <URL: https://pubchem.ncbi.nlm.nih.gov/substance/106110021>. entire document	15
X -	PUBCHEM, Substance Record for SID 108177476, Available Date: 22 February 2011 [retrieved on 07 March 2019]. Retrieved from the Internet: <URL: https://pubchem.ncbi.nlm.nih.gov/substance/108177476>. entire document	15, 16
A	US 2016/0296633 A1 (IMMUNOMEDICS INC) 13 October 2016 (13.10.2016) entire document	1-3, 9, 15-18
A -	PUBCHEM, Substance Record for SID 61213508, Available Date: 28 May 2009 [retrieved on 17 January 2019]. Retrieved from the Internet: <URL: https://pubchem.ncbi.nlm.nih.gov/substance/61213508>. entire document	1-3, 9, 15-18

☐ 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
07 March 2019	25 MAR 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/062290

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.: 6-8
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 Extra Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying 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.:
1-3, 9, 15-18

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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/062290

Continued from Box No. III Observations where unity of invention is lacking

Claims 1-3, 9, and 15-18 have been analyzed subject to the restriction that the claims read on a veterinary compound having a structure of any of the following, or a pharmaceutically acceptable salt thereof, wherein the compound is Compound D104.

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. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-3, 9, and 15-18 are drawn to veterinary compounds having a structure of any of the following, or a pharmaceutically acceptable salt thereof, solids thereof, and veterinary pharmaceutical compositions thereof.

Group II: claims 4, 5, 10-14, and 19-23 are drawn to methods of increasing activity of a cell in a non-human, methods of reducing the size of a tumor in a non-human, and methods of treating or reducing the effect of a reaction, disease or disorder.

The first invention of Group I+ is restricted to a veterinary compound having a structure of any of the following, or a pharmaceutically acceptable salt thereof, wherein the compound is Compound D104; solids thereof; and veterinary pharmaceutical compositions thereof. It is believed that claims 1-3, 9, and 15-18 read on this first named invention and thus these claims will be searched without fee to the extent that they read on the above embodiment.

Applicant is invited to elect additional formula(e) for each additional compound to be searched in a specific combination by paying an additional fee for each set of election. Each additional elected formula(e) requires the selection of a single definition for each compound variable. An exemplary election would be a veterinary compound having a structure of any of the following, or a pharmaceutically acceptable salt thereof, wherein the compound is Compound D134; solids thereof; and veterinary pharmaceutical compositions thereof. Additional formula(e) will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on 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/examined.

The inventions listed in Groups I+ and II 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:

The special technical features of Group I drawn to veterinary compounds having a structure of any of the following, or a pharmaceutically acceptable salt thereof, solids thereof, and veterinary pharmaceutical compositions thereof, are not present in Group II; and the special technical features of Group II, methods of increasing activity of a cell in a non-human, methods of reducing the size of a tumor in a non-human, and methods of treating or reducing the effect of a reaction, disease or disorder, are not present in Group I+.

The Groups I+ and II formulae do not share a significant structural element requiring the selection of alternatives for the compound and compound variables R, RA, RB, R1, R2, R3, R4, R5, R6, R7, R8, X, Q, Q1, Q2, A, and n and accordingly these groups lack unity a priori. .

Additionally, even if Groups I+ and II were considered to share the technical features of a veterinary compound having a structure of any of the following structures shown in claim 1 and 15, or a pharmaceutically acceptable salt thereof, a solid form of a compound; a veterinary pharmaceutical composition comprising the solid form of a compound, with a pharmaceutically acceptable carrier or excipient, these shared technical features do not represent a contribution over the prior art as disclosed by Compound Summary for SID 61213508 to PubChem and US 2016/0296633 A1 to Immunomedics, Inc.

Specifically, Compound Summary for SID 61213508 to PubChem teaches of a compound having a structure of any of the following structures shown in claim 1, Compound D118 (Pg. 3;...see shown structure...) and 15, Formula (XIX), wherein R1 is a substituted aryl group; and R2 is an alkyl group (Pg. 3;...see shown structure...).

US 2016/0296633 A1 to Immunomedics, Inc. teach a veterinary compound having the core structure shown in claim 15, Formula (XXIV), (Para. [0197],...bosutinib...; Para. [0075],...veterinary applications...); a solid form of a compound (Para. [0197],...bosutinib...; Para. [0210],... solid dosage forms...; A compound present in a solid dosage form is inherently in a solid form.); a veterinary pharmaceutical composition (Para. [0207]; Para. [0075],...veterinary applications...) comprising the solid form of a compound (Para. [0197],... bosutinib...; Para. [0210],... solid dosage forms...), with a pharmaceutically acceptable carrier or excipient (Para. [0207],...suitable excipient...).

The inventions listed in Groups I+ and II therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.