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(54) Title: FATTY ACID CYSTEAMINE CONJUGATES OF CFTR MODULATORS AND THEIR USE IN TREATING MEDICAL DISORDERS

(57) Abstract: The invention relates to fatty acid cysteamine conjugates of a CFTR modulator, compositions comprising a fatty acid cysteamine conjugate of a CFTR modulator, and methods for using such conjugates and compositions to treat disease, such as a disease caused by dysregulation of autophagy.

FATTY ACID CYSTEAMINE CONJUGATES OF CFTR MODULATORS AND THEIR USE IN TREATING MEDICAL DISORDERS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 62/084,764, filed November 26, 2014, the entire disclosure of which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

[0002] The invention relates to fatty acid cysteamine conjugates of a CFTR modulator, compositions comprising a fatty acid cysteamine conjugate of a CFTR modulator, and methods for using such conjugates and compositions to treat disease, such as a disease caused by dysregulation of autophagy.

BACKGROUND

[0003] Autophagy is an evolutionarily conserved lysosomal degradation pathway to essentially self-digest some cellular components (see, Levine and Kroemer (2008) CELL, 132, p. 27-42). This self-digestion process helps cells remove extraneous or damaged organelles, defective or mis-folded proteins, and even invading microorganisms. It has been speculated that autophagy is down-regulated in a number of diseases, for example, cystic fibrosis (Luciani *et al.* (2011) AUTOPHAGY, 7, p. 104-106).

[0004] Cystic fibrosis (CF) has been described as one of the most common, life-shortening autosomal recessive hereditary diseases in the Caucasian population. It is an orphan disease that affects approximately 30,000 children and adults in the U.S. (70,000 worldwide); and about 1,000 new cases are diagnosed each year. The disease is characterized by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), which results in either loss or impaired ability to transport chloride ions by various secretory and absorptive epithelial cells in

the lung, pancreas, liver, and intestine (*see*, for example, Derichs (2013) EUR. RESP. REV, 22, p. 58-65). The resulting decrease in anion transport and imbalance in fluid homeostasis produce thick and viscous mucus in the lungs, which can obstruct airways, causing chronic inflammation and infection. This leads to a progressive decline in lung function and a limited life expectancy in patients with the more severe form of the disease.

[0005] The CFTR is a cAMP-activated ATP-gated ion channel composed of approximately 1,480 amino acids. The protein consists of 5 domains: two transmembrane domains, each containing 6 spans of alpha helices. Each transmembrane domain is connected to a nucleotide binding domain (NBD). The first NBD is connected to the second transmembrane domain by a regulatory "R" domain. The gene encoding CFTR was reported in year 1989 (*see*, Rommens *et al.* (1989) SCIENCE, 245, p. 1059-1065). Since then, over 1900 sequence variations in the CFTR gene have been identified, the majority of which fall into one of the following 6 classes: Class I mutations result from non-sense and frame shift mutations, which reduce the quantity of the CFTR; Class II mutations have folding defects which result in premature degradation; Class III mutations result in limited channel gating; Class IV mutations have conductance defects; Class V mutations have a transcriptional defect that results in a reduced quantity of the CFTR being produced; Class VI mutations have a high turnover of the CFTR at the channel surface (*see*, for example, Rowntree and Harris (2003) ANN. HUM. GENET., 67, p. 471-485; Zielenski (2000) RESPIRATION, 67, p. 117-133; and MacDonald *et al.* (2007) PAEDIATRIC DRUGS, 9, p. 1-10).

[0006] To manifest the debilitating CF disease, an individual inherits two defective CFTR alleles, one from each parent. Of the over 1900 sequence variations in the CFTR that have been identified, the following 4 mutations have a worldwide prevalence of around 1-3% each: G551D, W1282X, G542X and N1303K. The most prevalent CFTR mutation, with an allelic frequency of about 90% worldwide, is the ΔF508 mutation (a Class II mutation, deletion of a phenylalanine which causes protein mis-folding and premature degradation). The ΔF508 deletion mutation can be manifested in either homozygous or heterozygous form.

[0007] Research on therapeutic interventions has identified several anti-inflammatory and anti-infective therapies useful in controlling certain debilitating symptoms of CF (*see*, for example, Nichols *et al.* (2008) CLINIC REV. ALLERG. IMMUNOL., 35, p. 135-153). More

recently, disease-modifying therapies have been introduced to address the defective CFTR. CFTR "potentiators" were designed to increase the open probability of CFTR channels that are available at the membrane but have gating (Class III) and conductance (Class IV) mutations. Ivacaftor (VX-770) is a CFTR potentiator that received FDA approval for the treatment of CF patients with gating mutations that included G551D, G178R, S549N, S549R, G551S, G124E, S1251N, S1255P, and G1349D (*see*, for example, Van Goor *et al.* (2009) PNAS, 106, p. 18825-18830). However, patients with these gating mutations represent only a small percentage of CF patients worldwide.

[0008] In addition to CFTR potentiators, clinical developments have been reported evaluating the potential of a CFTR "corrector" to increase the amount of CFTR that can be delivered to the cell membrane. VX-809 (Lumacaftor) is a CFTR corrector that has recently been approved by the FDA, when used in combination with Ivacaftor, in CF patients with homozygous ΔF508 mutation (*see*, for example, Van Goor *et al.* (2011) PNAS, 108, p. 18843-18848; and Ren *et al.* (2013) MOL. BIOL. CELL, 24, p. 3016-3024).

[0009] Other ways of potentially "correcting" the mis-folded ΔF508 CFTR protein is to use a molecular chaperone (Chanoux and Rubenstein (2012) FRONTIERS IN PHARMACOLOGY, 3; doi. 10.3389/fpharm. 2012.00137) or a small molecule inhibitor of the enzyme S-nitrosoglutathione reductase (GSNOR). GSNOR inhibitors have been shown to be efficacious in pre-clinical animal models of asthma, chronic obstructive pulmonary disease and inflammatory bowel disease (Green *et al.* (2012) BIOCHEMISTRY, 51, p. 2157-2168). GSNOR inhibitors have recently been shown to have a modest effect in rescuing the mis-folded ΔF508 CFTR protein when used in combination with VX-809 (Angers *et al.* (2014) "Pharmacological correction and acute inhibition of GSNOR results in improved in vitro CFTR function" Abstracts from the 28th Annual N. America CF Conference, Atlanta, GA).

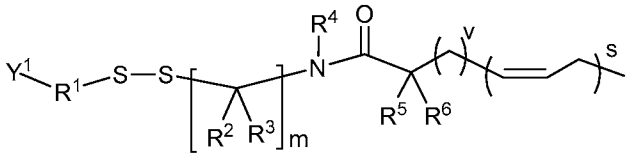
[0010] Despite the efforts made to date, there is still an ongoing need for additional compositions and methods for treating CF, and in particular certain forms of CF associated with mutations that are difficult to treat using existing therapies.

SUMMARY

[0011] The invention provides methods and compositions for activating autophagy and treating various medical diseases associated with dysregulation of autophagy, for example, disorders where autophagy is reduced relative to subjects without the disorder. The invention is based, in part, upon the discovery that fatty acid cysteamine conjugates of a CFTR modulator are useful in activating autophagy, and that the conjugate can be used treat a variety of human diseases such as CF. Fatty acid cysteamine conjugates of a CFTR modulator described herein have therapeutic effects that cannot be achieved by administering the CFTR modulator, cysteamine, or a fatty acid separately or as a combination of individual components. The covalent linkage of the CFTR modulator, cysteamine, and a fatty acid, for example, an omega-3 fatty acid, allows the simultaneous delivery of the three components to an intracellular location, whereupon the individual components are released by cleavage (e.g., enzymatic cleavage) at the location and at the same time.

[0012] One benefit of the invention is that administration of the fatty acid cysteamine conjugate of a CFTR modulator results in a greater lever of activation of autophagy than can be achieved by administering the components individually. Furthermore, administration of a fatty acid cysteamine conjugate of a CFTR modulator can cause a synergistic decrease in inflammation and an increase in CFTR function at a much lower concentration than the individual components, or in combination with the three individual components. Thus, the fatty acid cysteamine conjugates of a CFTR modulator provide multiple benefits that cannot be achieved by separate administration of individual components (separately or co-administered) that are conjugated to produce the fatty acid cysteamine conjugate of a CFTR modulator.

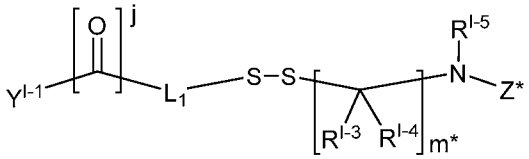
[0013] Exemplary fatty acid cysteamine conjugates of a CFTR modulator are described herein using generic and specific chemical formulae. For example, in one aspect, the invention provides a family of fatty acid cysteamine conjugates of a CFTR modulator embraced by Formula A:



(A)

or a pharmaceutically acceptable salt or solvate thereof; wherein the variables are as defined in the detailed description.

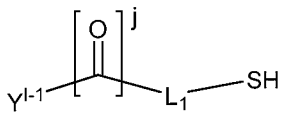
[0014] In another aspect, the invention provides a family of fatty acid cysteamine conjugates of a CFTR modulator embraced by Formula I:



(I)

or a pharmaceutically acceptable salt or solvate thereof; wherein the variables are as defined in the detailed description.

[0015] In another aspect, the invention provides a family of cysteamine conjugates of a CFTR modulator embraced by Formula II:



(II)

wherein the variables are as defined in the detailed description.

[0016] Additional generic formulae and specific fatty acid cysteamine conjugates of a CFTR modulator and cysteamine conjugates of a CFTR modulator are described in the detailed description and examples.

[0017] Another aspect of the invention provides a method of treating a disease described herein, such as CF, idiopathic pulmonary fibrosis (IPF), a neurodegenerative disease, inflammatory disease, liver disease, muscle disease, infection, mitochondria disease or immune disease. The method comprises administering to a subject in need thereof a therapeutically effective amount of a fatty acid cysteamine conjugate of a CFTR modulator, such as a compound of Formula A or Formula I, to treat the disease. Exemplary neurodegenerative diseases include Huntington's disease, Parkinson's disease, Alzheimer's disease, and transmissible spongiform encephalopathies. In certain embodiments, the disease to be treated is CF.

[0018] Another aspect of the invention provides a method of activating autophagy in a patient. The method comprises administering to a subject in need thereof an effective amount of a fatty acid cysteamine conjugate of a CFTR modulator described herein, such as a compound of Formula A or Formula I, to activate autophagy in the subject. In certain embodiments, the subject suffers from CF, a neurodegenerative disease, or inflammatory disease.

[0019] Pharmaceutical compositions that comprise a fatty acid cysteamine conjugate of a CFTR modulator (for example, the conjugate of Formula A or Formula I) and a pharmaceutically acceptable carrier are provided. The compositions are useful for treating a disease by activating autophagy.

[0020] Various aspects and embodiments of the invention are described in more detail below. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, illustrative methods and materials are now described. Other features, objects, and advantages of the invention will be apparent from the description and from the claims. In the specification and the appended claims, the singular forms also include the plural unless the context clearly dictates otherwise.

BRIEF DESCRIPTION OF THE FIGURES

[0021] **Figure 1A** is an immunoblot of HT-29 cells when treated with either 12.5 μM or 25 μM of compound **I-2** for 24 hours. **Figures 1B** and **1C** are bar charts showing the level of CFTR and an autophagy marker, as indicated by the ratio of LC3-II to LC3-I.

[0022] **Figure 2A** is an immunoblot of HT-29 cells when treated with 50 μM of compound **I-2** and VX-809. **Figure 2B** is a bar chart showing biotinylation results reflecting the increased level of cell surface CFTR.

[0023] **Figure 3** is a bar chart showing the activity of compound **I-2** in the assay described in Example 3, where HT-29 cells were treated with 50 μM each of compound **I-2**, or the individual components as recited in the bar chart (i.e., 4-PBA, cystamine, and EPA), or the three different combinations of the individual components (i.e., the combination of cystamine, EPA, and 4-PBA; the combination consisting of EPA and 4-PBA; and the combination consisting of cystamine and 4-PBA).

[0024] **Figure 4A** is a bar chart showing the ratio of LC3-II/LC3-I when HT-29 cells were treated with compounds **I-3**, **I-11**, **I-14** and **I-15** (all test compounds were evaluated at 25 μM). **Figure 4B** is a bar chart showing the corresponding level of cell surface CFTR when HT-29 cells were treated with **I-3**, **I-11**, **I-14** and **I-15**.

[0025] **Figure 5A** is a bar chart showing the ratio of LC3-II/LC3-I when HT-29 cells were treated with compounds **I-25** (25 μM) and **I-26** (25 μM). **Figure 5B** is a bar chart showing the corresponding level of cell surface CFTR when HT-29 cells were treated with **I-25** (25 μM) and **I-26** (25 μM).

[0026] **Figure 6** is a bar chart showing the increase in the cell surface CFTR when HT-29 cells were incubated with compound **I-49** (25 μM).

[0027] **Figure 7A** is a bar chart showing the CFTR band C level when primary CF cells (homozygous ΔF508) were incubated with 1) vehicle + VX-770; 2) VX-809 + VX-770; 3) compound **I-34** (25 μM) + VX-809 + VX-770; 4) compound **I-41**(25 μM) + VX-809 + VX-770. **Figure 7B** is a bar chart showing the CFTR band C level when primary CF cells (homozygous ΔF508) were incubated with 1) vehicle + VX-770; 2) VX-809 + VX-770; 3) compound **I-25** (25 μM) + VX-809 + VX-770; 4) compound **I-28** (25 μM) + VX-809 + VX-770.

[0028] **Figure 8A** is a graph showing a short circuit current (I_{SC}) trace generated when FRT cells were treated for 24 hours with: 1) vehicle + VX-770; 2) compound **I-25** (10 μM)+ VX-

770; 3) the positive control group,VX-809 + VX-770; 4) compound **I-25** (10 μM) + VX-809 + VX-770. Short circuit currents were generated in an Ussing chamber assay. **Figure 8B** shows the quantification of the steady state response of the traces shown in **Figure 8A** upon the addition of Forskolin, as measured by ΔI_{SC} ($\mu A/cm^2$); **Figure 8C** is a bar chart showing the quantification of the steady state response of the traces shown in **Figure 8A** upon the addition of Forskolin, expressed as % of control; **Figure 8D** is a bar chart showing the quantification of steady state response of the traces shown in **Figure 8A** upon the addition of the $CFTR_{inh}$ -172, as measured by ΔI_{SC} ($\mu A/cm^2$); and **Figure 8E** is a bar chart showing the quantification of steady state response of the traces shown in **Figure 8A** upon the addition of the $CFTR_{inh}$ -172, expressed as % of control.

[0029] **Figure 9A** is a graph showing a short circuit current (I_{ISC}) trace generated when primary CF cells (homozygous ΔF508) were incubated for 24 hours with: 1) vehicle + VX-770; 2) positive control group, VX-809 + VX-770; 3) compound **I-28** (1 μM) + VX-809 + VX-770. Short circuit currents were generated in an Ussing chamber assay. **Figure 9B** is a bar chart showing the quantification of the steady state response of the traces shown in Figure 9A upon Forskolin addition, as measured by ΔI_{SC} ($\mu A/cm^2$); **Figure 9C** is a bar chart showing the quantification of the overall response of the traces shown in **Figure 9A**, as measured by the area under the curve (AUC) and expressed as % of control; **Figure 9D** is a bar chart showing the quantification of the steady state response upon Forskolin addition, as measured by ΔI_{SC} ($\mu A/cm^2$), for the following treatment groups: 1) vehicle + VX-770; 2) compound **I-34** (1 μM) + VX-809 + VX-770; 3) compound **I-41** (1 μM) + VX-809 + VX-770; and **Figure 9E** is a bar chart showing the quantification of the response shown in Figure 9D, as measured by the area under the curve (AUC) and expressed as % of control.

[0030] **Figure 10A** is a bar chart showing the stability data for compound **I-25** in mouse, rat, beagle and human plasma; **Figure 10B** is a bar chart showing the stability data for compound **I-28** in mouse, rat, beagle and human plasma; and **Figure 10C** is a bar chart showing the stability data for compound **I-29** in mouse, rat, beagle and human plasma.

[0031] **Figure 11A** is a bar chart showing the mRNA level of Collagen 1a1 (COL1a1) when normal human lung fibroblasts (NLF) or idiopathic pulmonary fibrosis cells (LL29 and LL79A) were treated with compound **I-25** (25 μM) or **I-28** (25 μM) under either PBS or TGFβ

stimulation; **Figure 11B** is a bar chart showing the mRNA level of MMP-2 when normal human lung fibroblasts (NLF) or idiopathic pulmonary fibrosis cells (LL29 and LL79A) were treated with compound **I-25** (25 μM) or **I-28** (25 μM) under either PBS or TGFβ stimulation; and **Figure 11C** is a bar chart showing the mRNA level of TIMP-2 when normal human lung fibroblasts (NLF) or idiopathic pulmonary fibrosis cells (LL29 and LL79A) were treated with compound **I-25** (25 μM) or **I-28** (25 μM) under either PBS or TGFβ stimulation.

[0032] **Figure 12A** is a bar chart showing the basal level of TIMP-2 (PBS treatment) when NLF, LL29 or LL97A cells were treated with either the vehicle or compound **I-28** (25 μM); and **Figure 12B** is a bar chart showing the level of TIMP-2 when NLF, LL29 or LL97A cells were treated with either the vehicle or compound **I-28** (25 μM) under TGFβ stimulation.

[0033] **Figure 13A** is a bar chart showing the basal level of MMP-2 (PBS treatment) when NLF, LL29 or LL97A cells were treated with either the vehicle or compound **I-28** (25 μM); and **Figure 13B** is a bar chart showing the level of MMP-2 when NLF, LL29 or LL97A cells were treated with either the vehicle or compound **I-28** (25 μM) under TGFβ stimulation.

[0034] **Figure 14A** is a bar chart showing the quantification of the steady state response upon the addition of Forskolin, as measured by ΔI_{SC} ($\mu A/cm^2$), for the following treatment groups: 1) vehicle + VX-770; 2) VX-809 + VX-770; 3) **II-1** (10 μM) + VX-770. In this assay, compound **II-1** was as functionally active as the well-known CFTR corrector VX-809. Figure 14A shows that when VX-809 was replaced with compound **II-1**, the level of functional activity was essentially the same for the two treatment groups. **Figure 14B** is a bar chart showing the quantification of the steady state response upon the addition of Forskolin, as measured by ΔI_{SC} ($\mu A/cm^2$), for the following treatment groups: 1) vehicle + VX-770; 2) VX-809 + VX-770; 3) compound **II-4** (10 μM) + VX-809 + VX-770.

[0035] **Figure 15** is a bar chart showing the level of CFTR band C that could be rescued when primary CF cells (homozygous ΔF508) were incubated with the following treatment groups: 1) vehicle + VX-770; 2) VX-809 + VX-770; 2) compound **II-1** (25 μM) + VX-770; 3) compound **II-4** (25 μM) + VX-770.

DETAILED DESCRIPTION

[0036] The invention provides methods and compositions for activating autophagy and treating various medical diseases, in particular diseases associated with autophagy dysregulation. The invention is based, in part, upon the discovery that fatty acid cysteamine conjugates of a CFTR modulator are useful in activating autophagy, and can be used to treat a variety of human diseases, for example, CF. Fatty acid cysteamine conjugates of a CFTR modulator described herein have therapeutic effects that cannot be achieved by administering the three individual components separately or in combination. The covalent linkage of a CFTR modulator, cysteamine, and an omega-3 fatty acid allows the simultaneous delivery of the three components to an intracellular location, whereupon the individual components are released by cleavage (e.g., enzymatic cleavage) at the location and at the same time. A benefit of the invention is that administration of the fatty acid cysteamine conjugate of a CFTR modulator results in a greater lever of activation of autophagy than can be achieved by administering the components individually. Furthermore, administration of the fatty acid cysteamine conjugate of a CFTR modulator can cause a synergistic decrease in inflammation and an increase in CFTR function at a much lower concentration than the three individual components administered alone, or in combination with the fatty acid. As a result, fatty acid cysteamine conjugates of a CFTR modulator provide multiple benefits that cannot be achieved by separate administration of individual components (either separately or co-administered) that make up fatty acid cysteamine conjugate of a CFTR modulator. The fatty acid cysteamine conjugates of a CFTR modulator and the therapeutic methods described herein are contemplated to have particular advantages in treating CF.

[0037] CF is an orphan disease that affects some 30,000 patients in the U.S. It is a debilitating disease that is associated with a genetic mutation that leads to a defective CFTR, an ion channel that transports chloride ions across epithelial cell membranes. Patients with CF have been shown to have a defective and decreased level of autophagy, an evolutionarily conserved lysosomal degradation pathway that serves as a means to help cells remove extraneous or damaged organelles, defective or mis-folded proteins and even invading microorganisms. Activating autophagy has been shown to be potentially useful in restoring function to a defective CFTR.

[0038] It is contemplated that the activation of autophagy is also useful for the treatment of a variety of diseases other than CF, for example, diseases associated with reduced autophagy in cells, tissues, organelles, organs. Such diseases include, for example, idiopathic pulmonary fibrosis (IPF), pulmonary hypertension (PH), neurodegenerative diseases, liver diseases, muscle diseases, cardiac diseases, metabolic diseases, infection, immunity and inflammatory diseases. Pulmonary hypertension includes pulmonary arterial hypertension (WHO group I, idiopathic, heritable and drug/toxin-induced PH), pulmonary hypertension due to systolic or diastolic dysfunction, valvular heart disease (WHO group II) and pulmonary hypertension of other classifications that include those from WHO group III-V. Liver diseases include non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), NASH cirrhosis and hepatocellular carcinoma (HCC). An example of a metabolic disease that can be treated with a fatty acid cysteamine conjugate includes type 2 diabetes, which is commonly observed among CF patients. Neurodegenerative diseases include Huntington's disease, Parkinson's disease, Alzheimer's disease, and transmissible spongiform encephalopathies. Autophagy restoration therapy could also be useful for diseases such as Vici syndrome, sarcopenia and muscular dystrophy. There are multiple forms of muscular dystrophy and these include Duchenne muscular dystrophy, which is most common. Other forms of muscular dystrophy include Becker, limb-girdle, congenital, facioscapulohumeral, myotonic, oculopharyngeal, distal and Emery-Dreifuss muscular dystrophy. Other diseases that have defective autophagy include age-related macular degeneration, Danon disease, X-linked myopathy, infantile autophagic vacuolar myopathy, adult onset vacuolar myopathy, Pompe disease, sporadic inclusion body myositis, limb girdle muscular dystrophy type 2B, and Miyoshi myopathy. Fatty acid cysteamine conjugates may also useful for the treatment of mitochondrial diseases such as Leigh Syndrome, Diabetes Mellitus and Deafness (DAD), Leber's hereditary optic neuropathy, Neuropathy-ataxia-retinis pigmentosa and ptosis (NARP), myoneurogenic gastrointestinal encephalopathy (MNGIE), myoclonic epilepsy with ragged red fibers (MERRF), and mitochondrial myopathy-encephalomyopathy-lactic acidosis-stroke like symptoms (MELAS). Since cysteamine is being released intracellularly, the compounds of the invention may also be used to treat the lysosomal disorder nephropathic cystinosis.

[0039] Unless otherwise indicated, the practice of the present invention employs conventional techniques of organic chemistry, cell biology, biochemistry, pharmacology,

formulation and drug delivery. Various aspects of the invention are set forth below in sections for clarity; however, it is understood that aspects of the invention described in one particular section are not to be limited to any particular section.

I. DEFINITIONS

[0040] To facilitate an understanding of the present invention, a number of terms and phrases are defined below.

[0041] The articles "a" and "an" are used in this disclosure to refer to one or more than one (*i.e.*, to at least one) of the grammatical object of the article, unless the context is inappropriate. By way of example, "an element" means one element or more than one element.

[0042] The term "and/or" is used in this disclosure to mean either "and" or "or" unless indicated otherwise.

[0043] The term "alkyl" as used herein refers to a saturated straight or branched hydrocarbon, such as a straight or branched group of 1-12, 1-10, or 1-6 carbon atoms, referred to herein as C_1-C_{12}alkyl, C_1-C_{10}alkyl, and C_1-C_6alkyl, respectively. Exemplary alkyl groups include, but are not limited to, methyl, ethyl, propyl, isopropyl, 2-methyl-1-propyl, 2-methyl-2-propyl, 2-methyl-1-butyl, 3-methyl-1-butyl, 2-methyl-3-butyl, 2,2-dimethyl-1-propyl, 2-methyl-1-pentyl, 3-methyl-1-pentyl, 4-methyl-1-pentyl, 2-methyl-2-pentyl, 3-methyl-2-pentyl, 4-methyl-2-pentyl, 2,2-dimethyl-1-butyl, 3,3-dimethyl-1-butyl, 2-ethyl-1-butyl, butyl, isobutyl, t-butyl, pentyl, isopentyl, neopentyl, hexyl, heptyl, octyl, etc.

[0044] The term "C_1-C_3 alkyl" refers to a straight or branched chain saturated hydrocarbon containing 1-3 carbon atoms. Examples of a C_1-C_3 alkyl group include, but are not limited to, methyl, ethyl, propyl and isopropyl. The term "C_1-C_4 alkyl" refers to a straight or branched chain saturated hydrocarbon containing 1-4 carbon atoms. Examples of a C_1-C_4 alkyl group include, but are not limited to, methyl, ethyl, propyl, butyl, isopropyl, isobutyl, *sec*-butyl and *tert*-butyl. The term "C_1-C_5 alkyl" refers to a straight or branched chain saturated hydrocarbon containing 1-5 carbon atoms. Examples of a C_1-C_5 alkyl group include, but are not limited to, methyl, ethyl, propyl, butyl, pentyl, isopropyl, isobutyl, *sec*-butyl and *tert*-butyl, isopentyl and neopentyl. The term "C_1-C_6 alkyl" refers to a straight or branched chain saturated hydrocarbon

containing 1-6 carbon atoms. Examples of a C_1-C_6 alkyl group include, but are not limited to, methyl, ethyl, propyl, butyl, pentyl, hexyl, isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, isopentyl, and neopentyl.

[0045] The term "hydroxyalkyl" refers to an alkyl group that is substituted with at least one hydroxyl group. In certain embodiments, the hydroxyalkyl group is an alkyl group that is substituted with one hydroxyl group. In certain other embodiments, the hydroxyalkyl group is an alkyl group that is substituted with two or three hydroxyl groups.

[0046] The term "alkylene" refers to a diradical of an alkyl group. Exemplary alkylene groups include $-CH_2-$, $-CH_2CH_2-$, and $-CH_2C(H)(CH_3)CH_2-$.

[0047] The term "cycloalkyl" refers to a cyclic, saturated hydrocarbon, such as one containing 3-6 carbon atoms. The cycloalkyl may contain 3-12, 3-8, 4-8, or 4-6 ring carbon atoms, referred to herein, e.g., as "C_{4-8}cycloalkyl". Examples of a cycloalkyl group include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl. Unless specified otherwise, it is understood that any of the substitutable hydrogens on a cycloalkyl can be substituted with halogen, C_1-C_3 alkyl, hydroxyl, alkoxy and cyano groups. In certain embodiments, the cycloalkyl is not substituted.

[0048] Unless indicated otherwise, the term "aryl" refers to carbocyclic, aromatic hydrocarbon group having 1 to 2 aromatic rings, including monocyclic or bicyclic groups such as phenyl, biphenyl or naphthyl. Where containing two aromatic rings (bicyclic, *etc.*), the aromatic rings of the aryl group may be joined at a single point (*e.g.*, biphenyl), or fused (*e.g.*, naphthyl). The aryl group may be optionally substituted by one or more substituents, *e.g.*, 1 to 5 substituents, at any point of attachment, such substituents include, for example, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino, nitro, sulfhydryl, imino, amido, carboxylic acid, -C(O)alkyl, $-CO_2$alkyl, carbonyl, carboxyl, alkylthio, sulfonyl, sulfonamido, sulfonamide, ketone, aldehyde, ester, heterocyclyl, aryl or heteroaryl moieties, $-CF_3$, -CN, or the like. In certain other embodiments, the aromatic ring is not substituted, i.e., it is unsubstituted. In certain embodiments, the aryl group is a 6-10 membered ring structure. In certain embodiments, the aryl group is a 6-10 membered carbocyclic ring structure.

[0049] The term "aralkyl" refers to an alkyl group substituted with an aryl group.

[0050] The terms "heterocyclyl" and "heterocyclic group" are art-recognized and refer to saturated, partially unsaturated, or aromatic 3- to 10-membered ring structures, alternatively 3- to 7-membered rings, whose ring structures include one to four heteroatoms, such as nitrogen, oxygen, and sulfur. The number of ring atoms in the heterocyclyl group can be specified using C_x-C_x nomenclature where x is an integer specifying the number of ring atoms. For example, a C_3-C_7heterocyclyl group refers to a saturated or partially unsaturated 3- to 7-membered ring structure containing one to four heteroatoms, such as nitrogen, oxygen, and sulfur. The designation "C_3-C_7" indicates that the heterocyclic ring contains a total of from 3 to 7 ring atoms, inclusive of any heteroatoms that occupy a ring atom position. One example of a C_3heterocyclyl is aziridinyl. Heterocycles may also be mono-, bi-, or other multi-cyclic ring systems. A heterocycle may be fused to one or more aryl, partially unsaturated, or saturated rings. Heterocyclyl groups include, for example, biotinyl, chromenyl, dihydrofuryl, dihydroindolyl, dihydropyranyl, dihydrothienyl, dithiazolyl, homopiperidinyl, imidazolidinyl, isoquinolyl, isothiazolidinyl, isooxazolidinyl, morpholinyl, oxolanyl, oxazolidinyl, phenoxanthenyl, piperazinyl, piperidinyl, pyranyl, pyrazolidinyl, pyrazolinyl, pyridyl, pyrimidinyl, pyrrolidinyl, pyrrolidin-2-onyl, pyrrolinyl, tetrahydrofuryl, tetrahydroisoquinolyl, tetrahydropyranyl, tetrahydroquinolyl, thiazolidinyl, thiolanyl, thiomorpholinyl, thiopyranyl, xanthenyl, lactones, lactams such as azetidinones and pyrrolidinones, sultams, sultones, and the like. Unless specified otherwise, the heterocyclic ring is optionally substituted at one or more positions with substituents such as alkanoyl, alkoxy, alkyl, alkenyl, alkynyl, amido, amidino, amino, aryl, arylalkyl, azido, carbamate, carbonate, carboxy, cyano, cycloalkyl, ester, ether, formyl, halogen, haloalkyl, heteroaryl, heterocyclyl, hydroxyl, imino, ketone, nitro, oxo, phosphate, phosphonato, phosphinato, sulfate, sulfide, sulfonamido, sulfonyl and thiocarbonyl. In certain embodiments, the heterocyclyl group is not substituted, i.e., it is unsubstituted.

[0051] The term "heteroaryl" is art-recognized and refers to aromatic groups that include at least one ring heteroatom. In certain instances, a heteroaryl group contains 1, 2, 3, or 4 ring heteroatoms. Representative examples of heteroaryl groups include pyrrolyl, furanyl, thiophenyl, imidazolyl, oxazolyl, thiazolyl, triazolyl, pyrazolyl, pyridinyl, pyrazinyl, pyridazinyl and pyrimidinyl, and the like. Unless specified otherwise, the heteroaryl ring may

be substituted at one or more ring positions with, for example, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino, nitro, sulfhydryl, imino, amido, carboxylic acid, -C(O)alkyl, -CO_2alkyl, carbonyl, carboxyl, alkylthio, sulfonyl, sulfonamido, sulfonamide, ketone, aldehyde, ester, heterocyclyl, aryl or heteroaryl moieties, -CF_3, -CN, or the like. The term "heteroaryl" also includes polycyclic ring systems having two or more rings in which two or more carbons are common to two adjoining rings (the rings are "fused rings") wherein at least one of the rings is heteroaromatic, e.g., the other cyclic rings may be cycloalkyls, cycloalkenyls, cycloalkynyls, and/or aryls. In certain embodiments, the heteroaryl ring is substituted at one or more ring positions with halogen, alkyl, hydroxyl, or alkoxyl. In certain other embodiments, the heteroaryl ring is not substituted, i.e., it is unsubstituted. In certain embodiments, the heteroaryl group is a 5- to 10-membered ring structure, alternatively a 5- to 6-membered ring structure, whose ring structure includes 1, 2, 3, or 4 heteroatoms, such as nitrogen, oxygen, and sulfur.

[0052] The term "alkenyl" as used herein refers to an unsaturated straight or branched hydrocarbon having at least one carbon-carbon double bond, such as a straight or branched group of 2-12, 2-10, or 2-6 carbon atoms, referred to herein as C_2-C_{12}alkenyl, C_2-C_{10}alkenyl, and C_2-C_6alkenyl, respectively. Exemplary alkenyl groups include vinyl, allyl, butenyl, pentenyl, hexenyl, butadienyl, pentadienyl, hexadienyl, 2-ethylhexenyl, 2-propyl-2-butenyl, 4-(2-methyl-3-butene)-pentenyl, and the like.

[0053] The term "alkynyl" as used herein refers to an unsaturated straight or branched hydrocarbon having at least one carbon-carbon triple bond, such as a straight or branched group of 2-12, 2-10, or 2-6 carbon atoms, referred to herein as C_2-C_{12}alkynyl, C_2-C_{10}alkynyl, and C_2-C_6alkynyl, respectively. Exemplary alkynyl groups include ethynyl, prop-1-yn-1-yl, and but-1-yn-1-yl.

[0054] The terms "amine" and "amino" are art-recognized and refer to both unsubstituted and substituted amines, e.g., a moiety represented by the general formula –$N(R^{50})(R^{51})$, wherein R^{50} and R^{51} each independently represent hydrogen, alkyl, cycloalkyl, heterocyclyl, alkenyl, aryl, aralkyl, or -$(CH_2)_m$-R^{61}; or R^{50} and R^{51}, taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure; R^{61} represents an aryl, a cycloalkyl, a cycloalkenyl, a heterocycle or a polycycle; and m is zero or

an integer in the range of 1 to 8. In certain embodiments, R^{50} and R^{51} each independently represent hydrogen, alkyl, alkenyl, or $-(CH_2)_m-R^{61}$.

[0055] The terms "alkoxyl" or "alkoxy" are art-recognized and refer to an alkyl group, as defined above, having an oxygen radical attached thereto. Representative alkoxyl groups include methoxy, ethoxy, propyloxy, tert-butoxy and the like. An "ether" is two hydrocarbons covalently linked by an oxygen. Accordingly, the substituent of an alkyl that renders that alkyl an ether is or resembles an alkoxyl, such as may be represented by one of -O-alkyl, -O-alkenyl, -O-alkynyl, $-O-(CH_2)_m-R_{61}$, where m and R_{61} are described above.

[0056] The term "carbamate" as used herein refers to a radical of the form $-R_gOC(O)N(R_h)-$, $-R_gOC(O)N(R_h)R_i-$, or $-OC(O)NR_hR_i$, wherein R_g, R_h and R_i are each independently alkoxy, aryloxy, alkyl, alkenyl, alkynyl, amide, amino, aryl, arylalkyl, carboxy, cyano, cycloalkyl, ester, ether, formyl, halogen, haloalkyl, heteroaryl, heterocyclyl, hydroxyl, ketone, nitro, sulfide, sulfonyl, or sulfonamide. Exemplary carbamates include arylcarbamates and heteroaryl carbamates, e.g., wherein at least one of R_g, R_h and R_i are independently aryl or heteroaryl, such as phenyl and pyridinyl.

[0057] The symbol "〰" indicates a point of attachment.

[0058] The compounds of the disclosure may contain one or more chiral centers and/or double bonds and, therefore, exist as stereoisomers, such as geometric isomers, enantiomers or diastereomers. The term "stereoisomers" when used herein consist of all geometric isomers, enantiomers or diastereomers. These compounds may be designated by the symbols "R" or "S," depending on the configuration of substituents around the stereogenic carbon atom. The present invention encompasses various stereoisomers of these compounds and mixtures thereof. Stereoisomers include enantiomers and diastereomers. Mixtures of enantiomers or diastereomers may be designated "(±)" in nomenclature, but the skilled artisan will recognize that a structure may denote a chiral center implicitly. It is understood that graphical depictions of chemical structures, e.g., generic chemical structures, encompass all stereoisomeric forms of the specified compounds, unless indicated otherwise.

[0059] Individual stereoisomers of compounds of the present invention can be prepared synthetically from commercially available starting materials that contain asymmetric or stereogenic centers, or by preparation of racemic mixtures followed by resolution methods well known to those of ordinary skill in the art. These methods of resolution are exemplified by (1) attachment of a mixture of enantiomers to a chiral auxiliary, separation of the resulting mixture of diastereomers by recrystallization or chromatography and liberation of the optically pure product from the auxiliary, (2) salt formation employing an optically active resolving agent, or (3) direct separation of the mixture of optical enantiomers on chiral chromatographic columns. Stereoisomeric mixtures can also be resolved into their component stereoisomers by well-known methods, such as chiral-phase gas chromatography, chiral-phase high performance liquid chromatography, crystallizing the compound as a chiral salt complex, or crystallizing the compound in a chiral solvent. Further, stereoisomers can be obtained from stereomerically-pure intermediates, reagents, and catalysts by well-known asymmetric synthetic methods.

[0060] Geometric isomers can also exist in the compounds of the present invention. The symbol $\overset{\cdots\cdots}{=}$ denotes a bond that may be a single, double or triple bond as described herein. The present invention encompasses the various geometric isomers and mixtures thereof resulting from the arrangement of substituents around a carbon-carbon double bond or arrangement of substituents around a carbocyclic ring. Substituents around a carbon-carbon double bond are designated as being in the "*Z*" or "*E*" configuration wherein the terms "*Z*" and "*E*" are used in accordance with IUPAC standards. Unless otherwise specified, structures depicting double bonds encompass both the "*E*" and "*Z*" isomers.

[0061] Substituents around a carbon-carbon double bond alternatively can be referred to as "cis" or "trans," where "cis" represents substituents on the same side of the double bond and "trans" represents substituents on opposite sides of the double bond. The arrangement of substituents around a carbocyclic ring are designated as "cis" or "trans." The term "cis" represents substituents on the same side of the plane of the ring and the term "trans" represents substituents on opposite sides of the plane of the ring. Mixtures of compounds wherein the substituents are disposed on both the same and opposite sides of plane of the ring are designated "cis/trans."

[0062] The invention also embraces isotopically labeled compounds of the invention which are identical to those recited herein, except that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, fluorine and chlorine, such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F, and ^{36}Cl, respectively.

[0063] Certain isotopically-labeled disclosed compounds (*e.g.,* those labeled with ^{3}H and ^{14}C) are useful in compound and/or substrate tissue distribution assays. Tritiated (*i.e.,* ^{3}H) and carbon-14 (*i.e.,* ^{14}C) isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium (*i.e.,* ^{2}H) may afford certain therapeutic advantages resulting from greater metabolic stability (*e.g.,* increased *in vivo* half-life or reduced dosage requirements) and hence may be preferred in some circumstances. Isotopically labeled compounds of the invention can generally be prepared by following procedures analogous to those disclosed in, e.g., the Examples herein by substituting an isotopically labeled reagent for a non-isotopically labeled reagent.

[0064] The term "fatty acid cysteamine conjugate of a CFTR modulator" includes any and all possible isomers, stereoisomers, enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, and prodrugs of the fatty acid cysteamine conjugates of a CFTR modulator described herein.

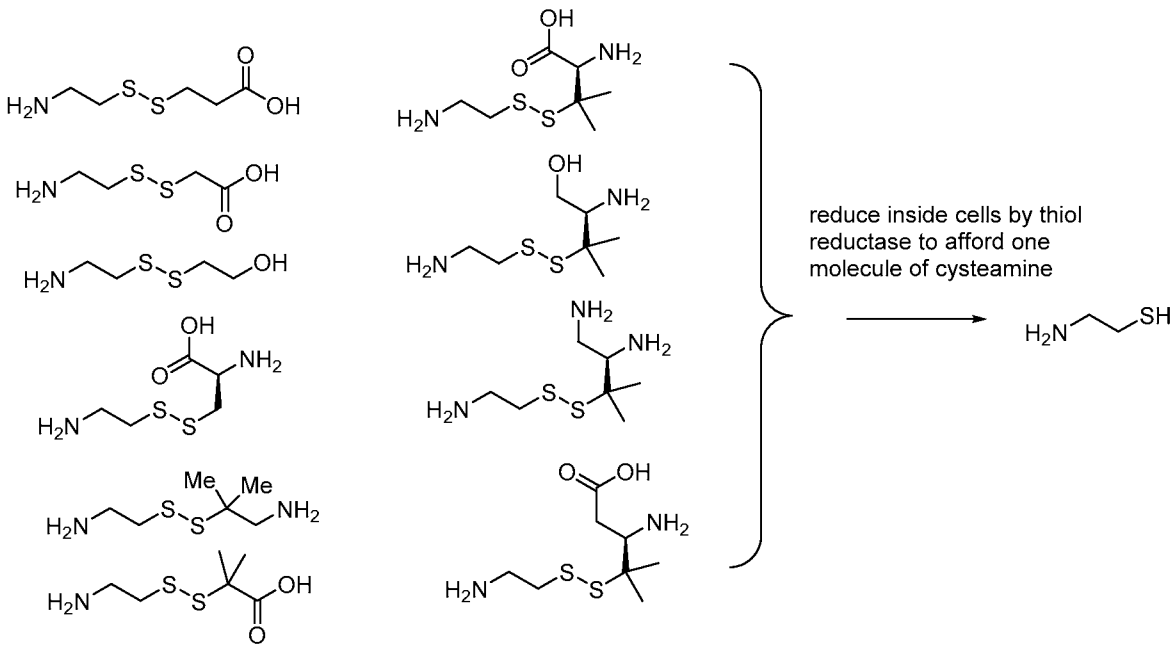
[0065] The term "any side chain of a naturally occurring amino acid" refer to a side chain of any one of the following amino acids: Isoleucine, Alanine, Leucine, Asparagine, Lysine, Aspartate, Methionine, Cysteine, Phenylalanine, Glutamate, Threonine, Glutamine, Tryptophan, Glycine, Valine, Proline, Arginine, Serine, Histidine, and Tyrosine.

[0066] The term "fatty acid" as used herein means an omega-3 fatty acid and fatty acids that are metabolized *in vivo* to omega-3 fatty acids. Non-limiting examples of fatty acids are *all-cis*-7,10,13-hexadecatrienoic acid, α-linolenic acid (ALA or *all-cis*-9,12,15-octadecatrienoic acid), stearidonic acid (STD or *all-cis*-6,9,12,15-octadecatetraenoic acid), eicosatrienoic acid (ETE or *all-cis*-11,14,17-eicosatrienoic acid), eicosatetraenoic acid (ETA or *all-cis*-8,11,14,17-eicosatetraenoic acid), eicosapentaenoic acid (EPA or *all-cis*-5,8,11,14,17-eicosapentaenoic

acid), docosapentaenoic acid (DPA, clupanodonic acid or *all-cis*-7,10,13,16,19-docosapentaenoic acid), docosahexaenoic acid (DHA or *all-cis*-4,7,10,13,16,19-docosahexaenoic acid), tetracosapentaenoic acid (*all-cis*-9,12,15,18,21-docosahexaenoic acid), or tetracosahexaenoic acid (nisinic acid or *all-cis*-6,9,12,15,18,21-tetracosenoic acid).

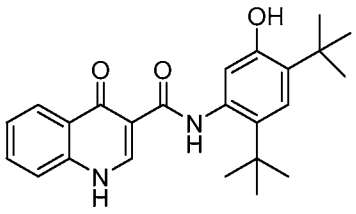
[0067] The term, "cysteamine" refers to a molecule having a formula  (also known as 2-aminoethane-1-thiol), which can be derived from a cystamine. A cystamine is the disulfide form of a thiol containing compound cysteamine, also known as 2-aminoethane-1-thiol. When the disulfide form cystamine is taken up inside cells, it is reduced to the thiol compound cysteamine by the action of thiol reductase (*see,* Arunachalam *et al.* (2000) PNAS, 97, p. 745-750). The thiol compound cysteamine is considered to be the active component of cystamine in cells. Non-limiting examples of cystamine molecules that can deliver the active thiol compound cysteamine inside cells are listed in Scheme A below.

Scheme A

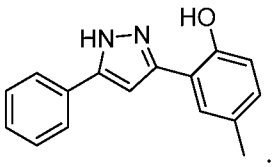


[0068] A "CFTR modulator" refers to a small molecule that can increase the function of a defective CFTR. A small molecule can increase the function of a CFTR by being either a

CFTR potentiator or a CFTR corrector. A CFTR potentiator is useful for CFTR channels that have either gating or conductance mutations (class III or class IV CF mutations). Non-limiting examples of a CFTR potentiator include:



and



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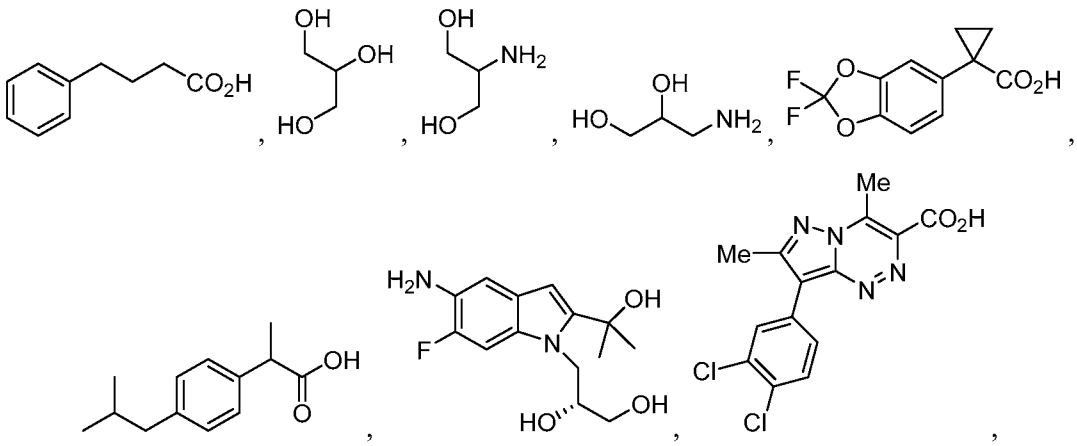
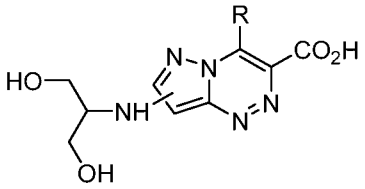
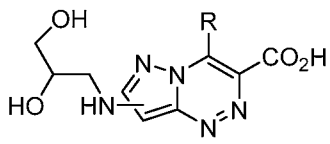
[0069] A CFTR corrector is a small molecule that can increase the amount of functioning CFTR protein to the cell membrane. Although the precise mechanism is not known in most cases, a CFTR corrector can accomplish in a number of ways, which may include 1) correcting the mis-folding protein, 2) allowing the trafficking of the defective CFTR protein to the membrane by interacting with various chaperone proteins, 3) stabilizing the defective CFTR protein at the cell membrane, 4) inhibiting S-nitrosoglutathione reductase, and/or 5) activating autophagy. Non-limiting examples of a CFTR corrector include:




,

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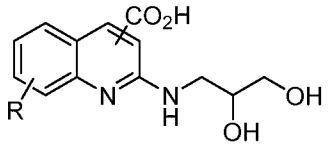
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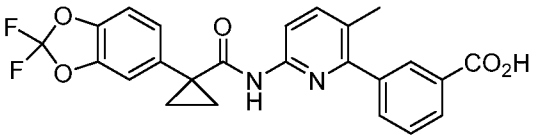
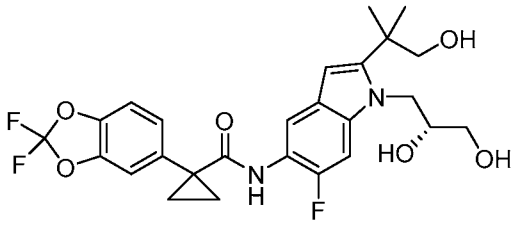

, and

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[0070] Additional non-limiting examples of a CFTR corrector can be found in WO 2007/021982, WO 2009/064959, WO 2010/053471, WO 2012/154880A1, WO 2013/112706A1, and WO 2014/210159A1.

[0071] As used herein, the terms "patient" and "subject" refer to an organism to be treated by the methods and compositions of the present invention. Such organisms are preferably mammals (*e.g.*, human, mouse, rat, guinea pig, dog, cat, horse, cow, pig, or non-human primate, such as a monkey, chimpanzee, baboon, rhesus, and the like), and more preferably humans.

[0072] As used herein, the term "effective amount" refers to the amount of a compound (*e.g.*, a compound of the present invention) sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages and is not intended to be limited to a particular formulation or administration route.

[0073] As used herein, the term "pharmaceutical composition" refers to the combination of an active agent with a carrier, inert or active, making the composition especially suitable for diagnostic or therapeutic use *in vivo* or *ex vivo*.

[0074] As used herein, the term "pharmaceutically acceptable carrier" refers to any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions (*e.g.*, such as an oil/water or water/oil emulsions), and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers,

stabilizers and adjuvants, *see* Martin, Remington's Pharmaceutical Sciences, 15th Ed., Mack Publ. Co., Easton, PA (1975).

[0075] As used herein, the term "pharmaceutically acceptable salt" refers to any pharmaceutically acceptable salt (*e.g.*, acid or base) of a compound of the present invention which, upon administration to a patient, is capable of providing a compound of this invention or an active metabolite or residue thereof. As is known to those of skill in the art, "salts" of the compounds of the present invention may be derived from inorganic or organic acids and bases. Examples of acids include, but are not limited to, hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycolic, lactic, salicylic, succinic, toluene-p-sulfonic, tartaric, acetic, citric, methanesulfonic, ethanesulfonic, formic, benzoic, malonic, naphthalene-2-sulfonic, benzenesulfonic acid, and the like. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts.

[0076] Examples of bases include, but are not limited to, alkali metal (*e.g.*, sodium) hydroxides, alkaline earth metal (*e.g.*, magnesium) hydroxides, ammonia, and compounds of formula NW_4^+, wherein W is C_{1-4} alkyl, and the like.

[0077] Examples of salts include, but are not limited to: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, flucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, palmoate, pectinate, persulfate, phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, undecanoate, and the like. Other examples of salts include anions of the compounds of the present invention compounded with a suitable cation such as Na^+, NH_4^+, and NW_4^+ (wherein W is a C_{1-4} alkyl group), and the like. For therapeutic use, salts of the compounds of the present invention are contemplated as being pharmaceutically acceptable. However, salts of acids and bases that are non-pharmaceutically acceptable may also find use, for example, in the preparation or purification of a pharmaceutically acceptable compound.

[0078] The term "cystic fibrosis" or "CF" refers to disorders, diseases and syndromes involving a defective CFTR. There are >1900 mutations that could lead to CF. These mutations are further divided into 6 different classes (Class I-VI). CF can refer to any of the possible mutations that could be present in any of the 6 different classes.

[0079] The term "carrier" refers to excipients and diluents, and means a material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a pharmaceutical agent from one organ, or portion of the body, to another organ, or portion of the body.

[0080] As used herein, the term "treating" includes any effect, *e.g.,* lessening, reducing, modulating, ameliorating or eliminating, that results in the improvement of the condition, disease, disorder, and the like, or ameliorating a symptom thereof. Treating can be curing, improving, or at least partially ameliorating the disorder. In certain embodiments, treating is curing the disease.

[0081] The term "disorder" refers to and is used interchangeably with, the terms disease, condition, or illness, unless otherwise indicated.

[0082] The term "prodrug" refers to a compound which is convertible *in vivo* by metabolic means (*e.g.*, by hydrolysis) to a fatty acid cystamine conjugate.

[0083] The following abbreviations are used herein and have the indicated definitions: Boc and BOC are *tert*-butoxycarbonyl, Boc_2O is di-*tert*-butyl dicarbonate, BSA is bovine serum albumin, CDI is 1,1'-carbonyldiimidazole, DCC is *N,N'*-dicyclohexylcarbodiimide, DIEA is *N,N*-diisopropylethylamine, DMAP is 4-dimethylaminopyridine, DMEM is Dulbecco's Modified Eagle Medium, DMF is *N,N*-dimethylformamide, DOSS is sodium dioctyl sulfosuccinate, EDC and EDCI are 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, ELISA is enzyme-linked immunosorbent assay, EtOAc is ethyl acetate, FBS is fetal bovine serum, h is hour, HATU is 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HIV is human immunodeficiency virus, HPMC is hydroxypropyl methylcellulose, oxone is potassium peroxymonosulfate, Pd/C is palladium on carbon, TFA is trifluoroacetic acid, TGPS is tocopherol propylene glycol succinate, THF is tetrahydrofuran; HBTU is *N,N,N',N'*-tetramethyl-O-(1*H*-benzotriazol-1-yl)uronium

hexafluorophosphate; and VX-809 is the compound having the chemical name 3-{6-{[1-(2,2-difluoro-1,3-benzodioxol-5-yl)cyclopropanecarbonyl] amino}-3-methylpyridin-2-yl}benzoic acid.

[0084] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[0085] Throughout the description, where compositions and kits are described as having, including, or comprising specific components, or where processes and methods are described as having, including, or comprising specific steps, it is contemplated that, additionally, there are compositions and kits of the present invention that consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present invention that consist essentially of, or consist of, the recited processing steps.

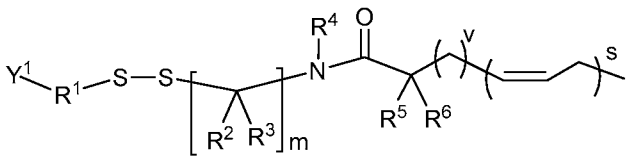
[0086] As a general matter, compositions specifying a percentage are by weight unless otherwise specified. Further, if a variable is not accompanied by a definition, then the previous definition of the variable controls.

II. FATTY ACID CYSTEAMINE CONJUGATES OF A CFTR MODULATOR

[0087] Exemplary fatty acid cysteamine conjugates of a CFTR modulator for use in the therapeutic applications and pharmaceutical compositions are described below.

Formula A

[0088] One aspect of the invention provides a compound of Formula A represented by



(A)

or a pharmaceutically acceptable salt or solvate thereof; wherein:

R^1 is C_1-C_6 alkylene optionally substituted by -C(O)N(R^2)(hydroxyalkyl), -N(R^2)C(O)-(hydroxyalkyl), -O-(hydroxyalkyl), -N(R^2)-(hydroxyalkyl), -OC(O)N(R^2)(hydroxyalkyl), -C(O)N(R^2)(R^8), -N(R^2)C(O)R^9, or -CO_2R^9;

R^2, R^3, R^4, and R^7 each represent independently for each occurrence hydrogen or C_1-C_3 alkyl;

R^5 and R^6 are independently hydrogen, C_1-C_4 alkyl, or halogen;

R^8 is C_1-C_6 alkyl, phenyl, benzyl, C_1-C_6 alkylene-CO_2R^7, or C_1-C_6 alkylene-C(O)N(R^7)$_2$;

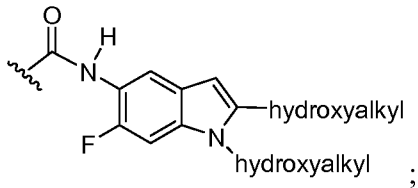
R^9 represents independently for each occurrence hydrogen, C_1-C_6 alkyl, phenyl, benzyl, C_1-C_6 alkylene-CO_2R^7, or C_1-C_6 alkylene-C(O)N(R^7)$_2$;

m is 2 or 3;

s is 3, 5, or 6;

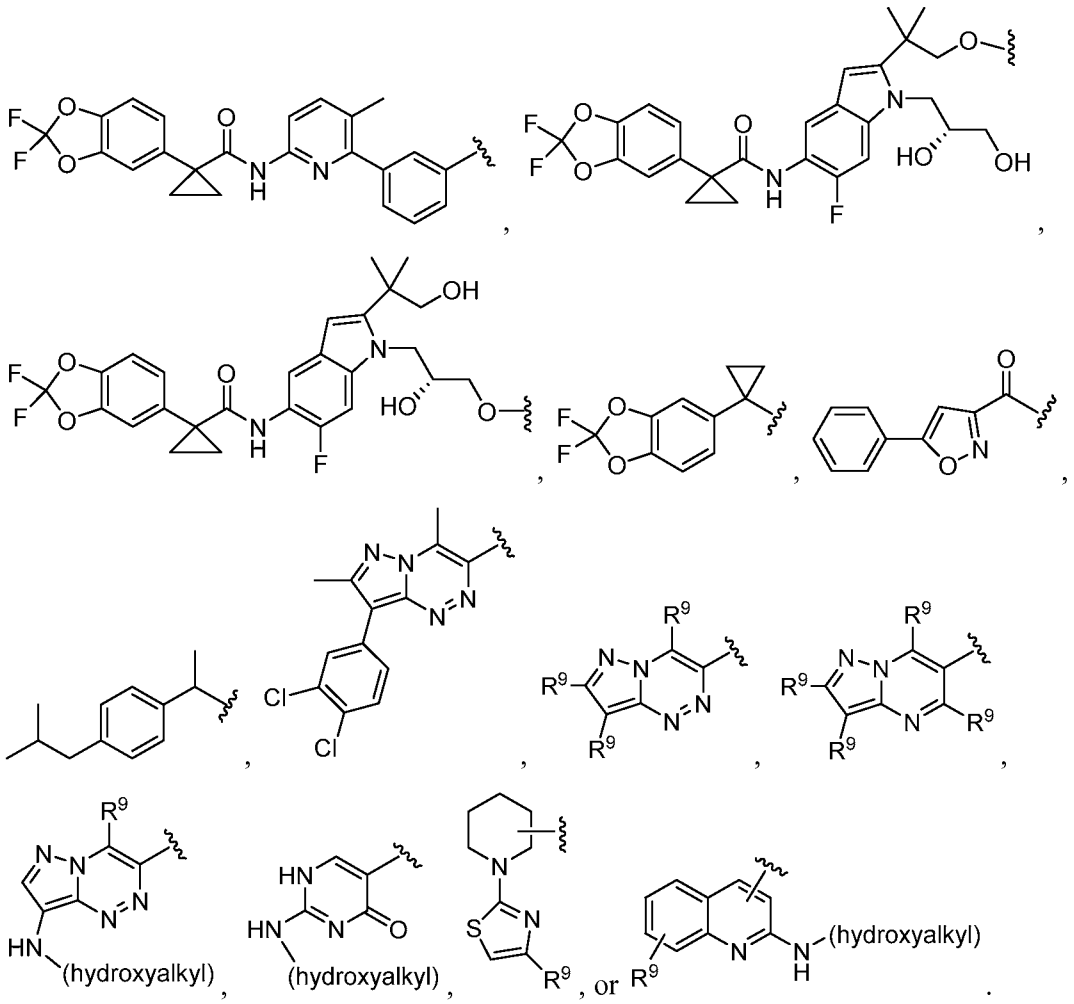
v is 1, 2, or 6;

Y^1 is an amide or carbamate selected from the group consisting of -N(R^7)C(O)-aralkyl, -N(R^7)C(O)-(hydroxyalkyl), -C(O)N(R^7)(hydroxyalkyl), -N(R^7)CO_2-(hydroxyalkyl), -OC(O)N(R^7)(hydroxyalkyl), -N(R^7)C(O)-Z^1, and



; and

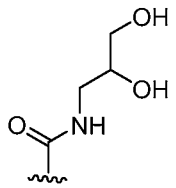
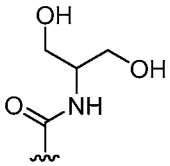
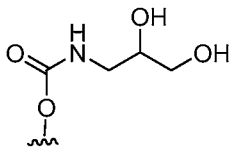
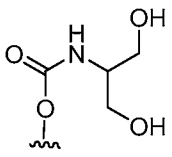
Z^1 is one of the following:



[0089] Definitions of the variables in Formula A above encompass multiple chemical groups. The application contemplates embodiments where, for example, i) the definition of a variable is a single chemical group selected from those chemical groups set forth above, ii) the definition is a collection of two or more of the chemical groups selected from those set forth above, and iii) the compound is defined by a combination of variables in which the variables are defined by (i) or (ii).

[0090] In certain embodiments, the compound is a compound of Formula A or a pharmaceutically acceptable salt thereof.

[0091] In certain embodiments, R^1 is C_1-C_6 alkylene. In certain embodiments, R^1 is ethylene. In certain embodiments, R^1 is C_1-C_6 alkylene substituted by -$C(O)N(R^2)$(hydroxyalkyl), -$N(R^2)C(O)$(hydroxyalkyl), -O-(hydroxyalkyl), -$N(R^2)$-(hydroxyalkyl) or -$OC(O)N(R^2)$(hydroxyalkyl). In certain embodiments, R^1 is C_2-C_4 alkylene substituted by one of the following:

. In certain embodiments, the substituent on the C_1-C_6 alkylene is attached to the same carbon atom of the C_1-C_6 alkylene as the Y^1 group.

[0092] In certain embodiments, R^2, R^3, and R^4 are hydrogen.

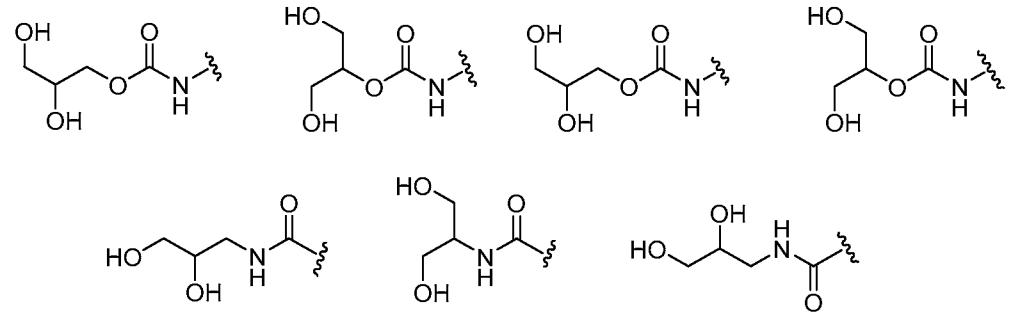
[0093] In certain embodiments, R^5 and R^6 are hydrogen.

[0094] In certain embodiments, R^7 is hydrogen.

[0095] In certain embodiments, m is 2.

[0096] In certain embodiments, s is 5. In certain embodiments, s is 6. In certain embodiments, v is 1. In certain embodiments, v is 2. In certain embodiments, v is 1, and s is 6. In certain embodiments, v is 2, and s is 5.

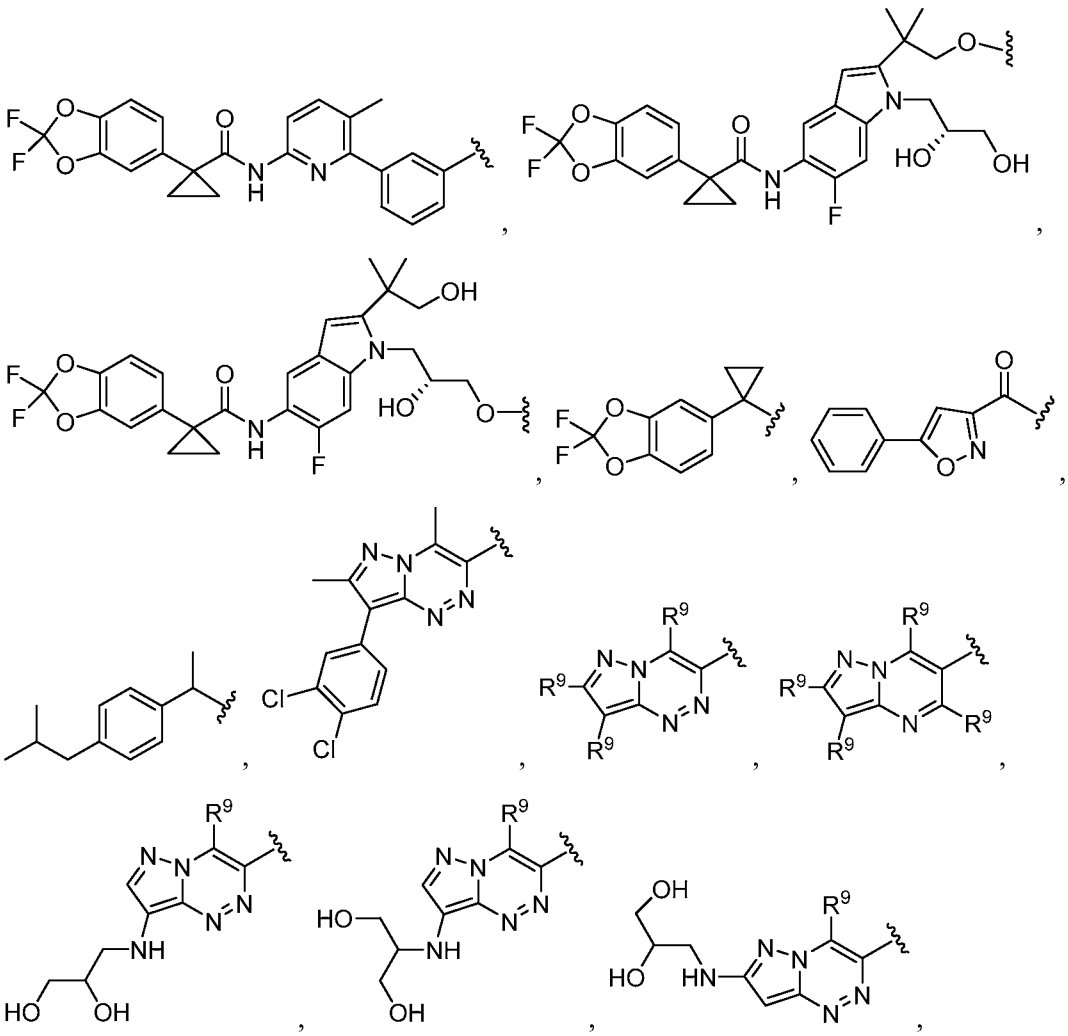
[0097] In certain embodiments, Y^1 is -$N(R^7)C(O)$-aralkyl. In certain embodiments, Y^1 is -$N(R^7)C(O)$-$(CH_2)_3$-phenyl. In certain embodiments, Y^1 is -$N(R^7)C(O)$-(hydroxyalkyl), -$C(O)N(R^7)$(hydroxyalkyl), -$N(R^7)CO_2$-(hydroxyalkyl), or -$OC(O)N(R^7)$(hydroxyalkyl). In certain embodiments, Y^1 is one of the following:



.

[0098] In certain embodiments, Y^1 is $-N(R^7)C(O)-Z^1$.

[0099] In certain embodiments, Z^1 is



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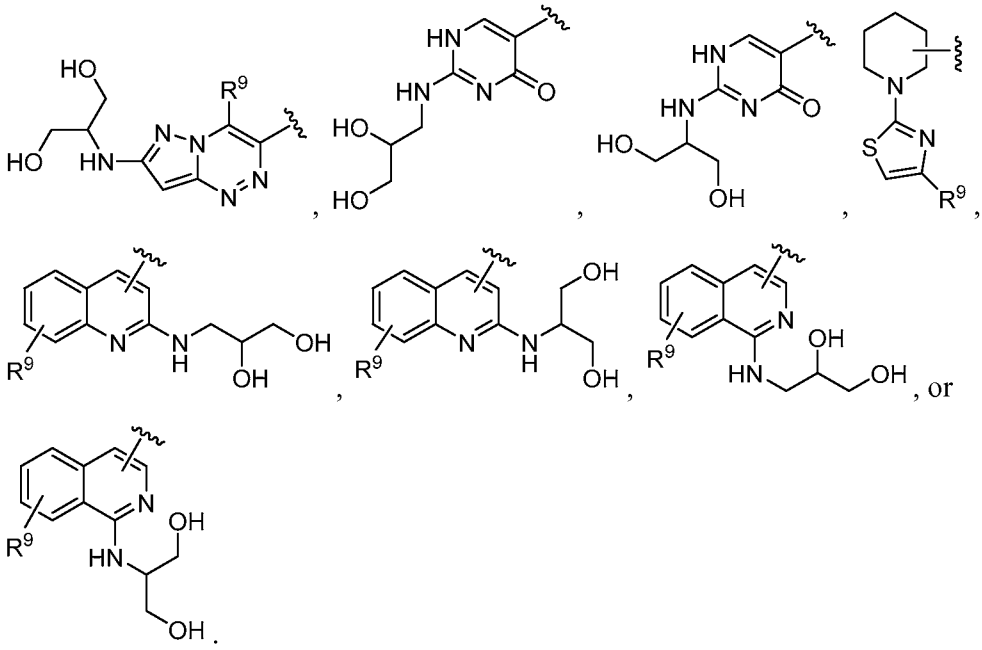
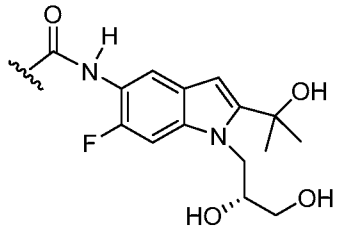
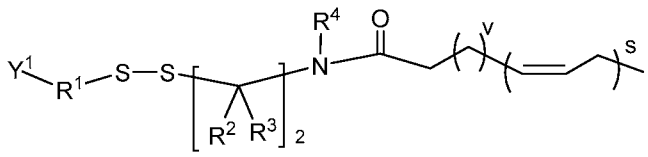




[00100] In certain embodiments, Y^1 is .

[00101] The description above describes multiple embodiments relating to compounds of Formula A. The invention specifically contemplates all combinations of the embodiments.

[00102] Another aspect of the invention provides a compound of Formula A-1:



(A-1)

or a pharmaceutically acceptable salt thereof; wherein:

R^1 is C_1-C_6 alkylene optionally substituted by -C(O)N(R^2)(hydroxyalkyl), -N(R^2)C(O)-(hydroxyalkyl), -O-(hydroxyalkyl), -N(R^2)-(hydroxyalkyl), -OC(O)N(R^2)(hydroxyalkyl), -C(O)N(R^2)(R^8), -N(R^2)C(O)R^9, or -CO_2R^9;

R^2, R^3, R^4, and R^7 each represent independently for each occurrence hydrogen or methyl;

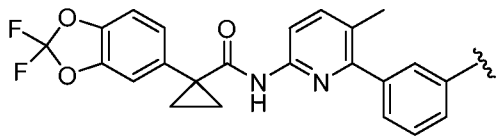
R^8 is C_1-C_6 alkyl, phenyl, benzyl, C_1-C_6 alkylene-CO_2R^7, or C_1-C_6 alkylene-C(O)N(R^7)$_2$;

R^9 represents independently for each occurrence hydrogen or C_1-C_6 alkyl;

s is 5 or 6;

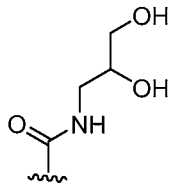
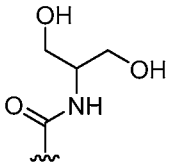
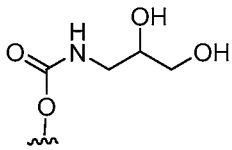
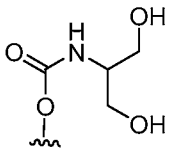
v is 1 or 2;

Y^1 is -N(R^7)C(O)-(C_1-C_6 alkylene)-phenyl, -N(R^7)C(O)-(hydroxyalkyl), -C(O)N(R^7)(hydroxyalkyl), -N(R^7)CO_2-(hydroxyalkyl), -OC(O)N(R^7)(hydroxyalkyl), or -N(R^7)C(O)-Z^1; and

Z^1 is  .

[00103] Definitions of the variables in Formula A-1 above encompass multiple chemical groups. The application contemplates embodiments where, for example, i) the definition of a variable is a single chemical group selected from those chemical groups set forth above, ii) the definition is a collection of two or more of the chemical groups selected from those set forth above, and iii) the compound is defined by a combination of variables in which the variables are defined by (i) or (ii).

[00104] In certain embodiments, R^1 is C_1-C_6 alkylene. In certain embodiments, R^1 is ethylene. In certain embodiments, R^1 is C_1-C_6 alkylene substituted by -C(O)N(R^2)(hydroxyalkyl), or -OC(O)N(R^2)(hydroxyalkyl). In certain embodiments, R^1 is C_2-C_4 alkylene substituted by one of the following:





. In certain embodiments, the substituent on the C_1-C_6 alkylene is attached to the same carbon atom of the C_1-C_6 alkylene as the Y^1 group.

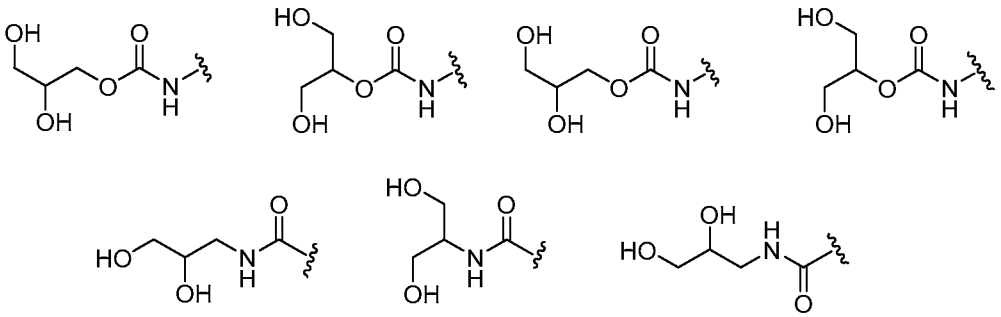
[00105] In certain embodiments, R^2, R^3, and R^4 are hydrogen.

[00106] In certain embodiments, R^5 and R^6 are hydrogen.

[00107] In certain embodiments, R^7 is hydrogen.

[00108] In certain embodiments, v is 1, and s is 6. In certain embodiments, v is 2, and s is 5.

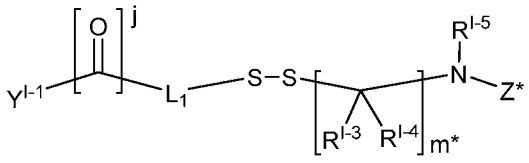
[00109] In certain embodiments, Y^1 is $-N(R^7)C(O)-(CH_2)_3$-phenyl. In certain embodiments, Y^1 is $-N(R^7)C(O)-(C_1$-C_6 alkylene)-phenyl, $-N(R^7)C(O)-$(hydroxyalkyl), $-C(O)N(R^7)$(hydroxyalkyl), $-N(R^7)CO_2-$(hydroxyalkyl), $-OC(O)N(R^7)$(hydroxyalkyl). In certain embodiments, Y^1 is one of the following:



[00110] The description above describes multiple embodiments relating to compounds of Formula A-1. The invention specifically contemplates all combinations of the embodiments.

Formula I

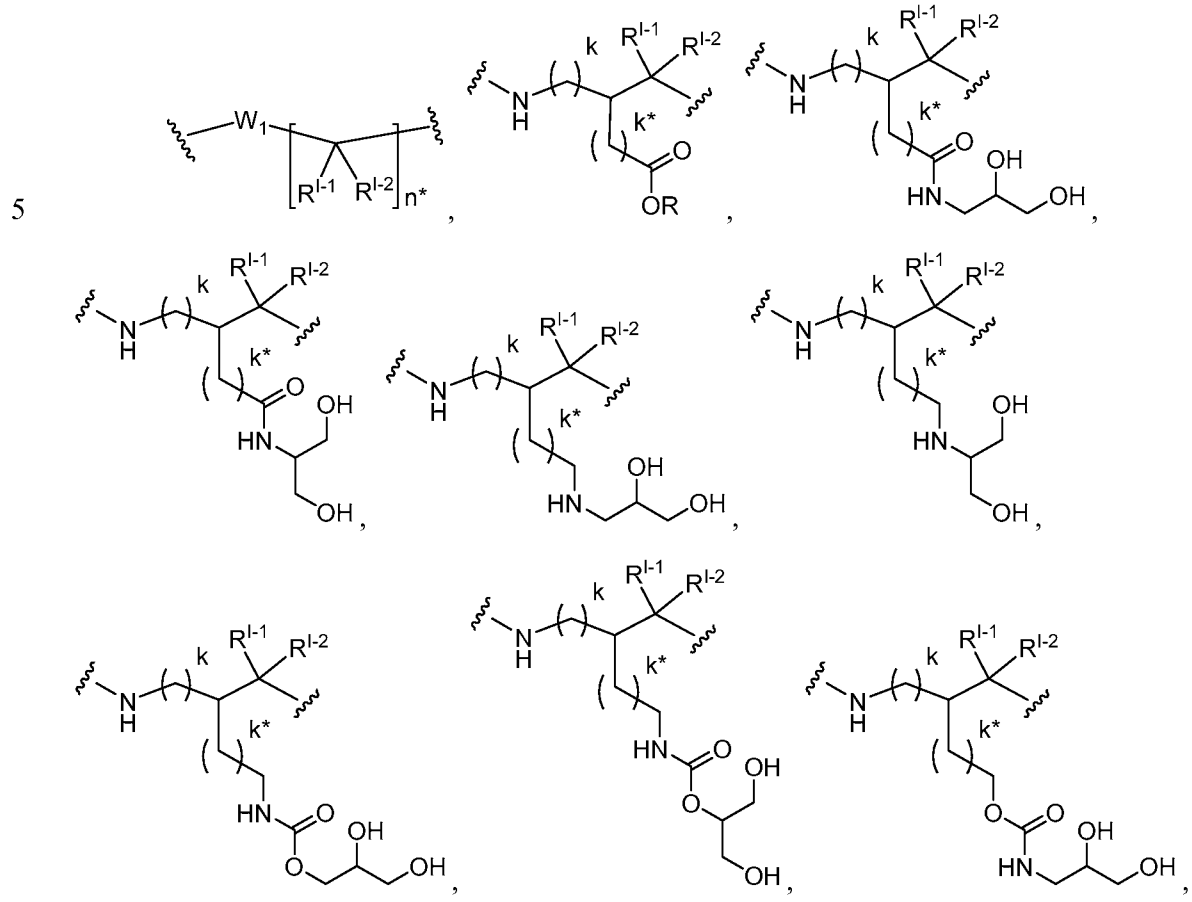
[00111] Another aspect of the invention provides a compound of Formula I represented by:

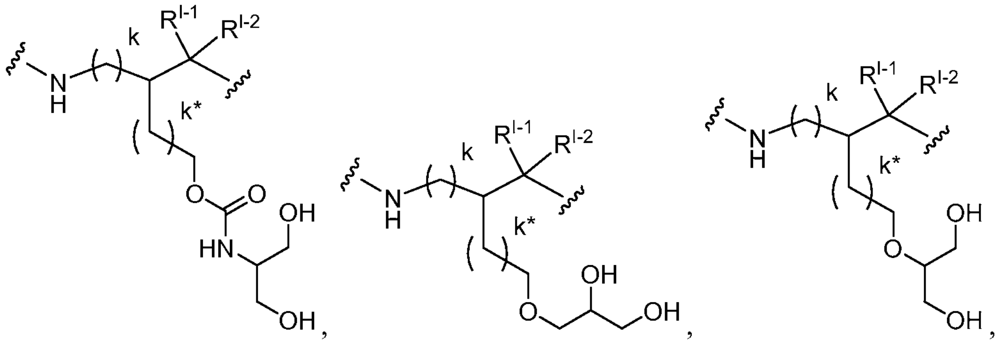


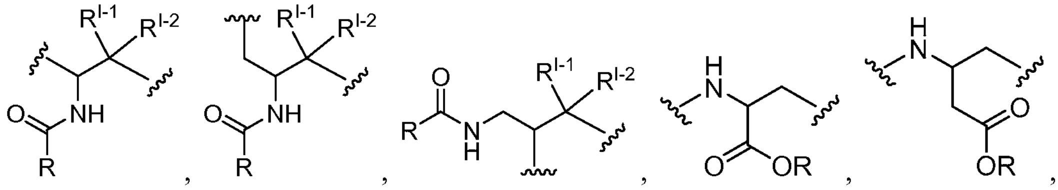
(I)

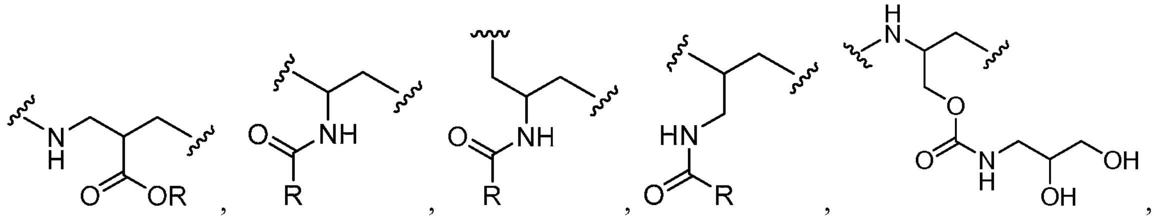
or a pharmaceutically acceptable salt or solvate thereof; wherein:

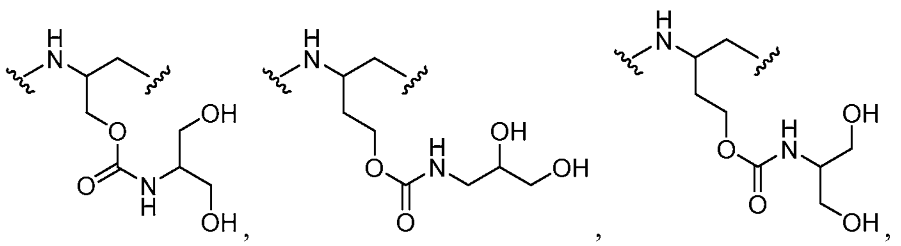
L_1 is independently

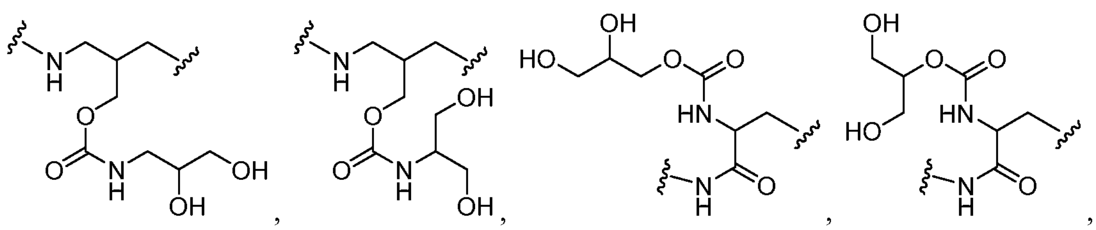


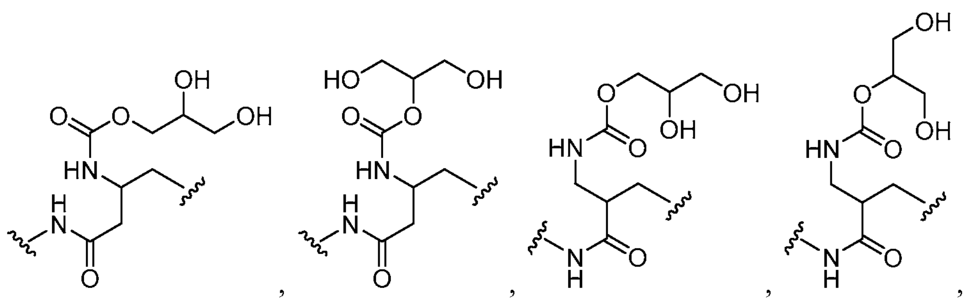


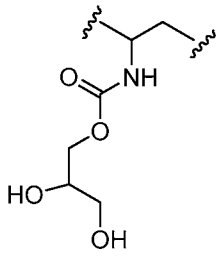
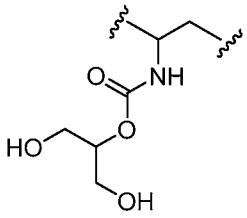
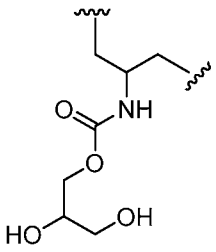
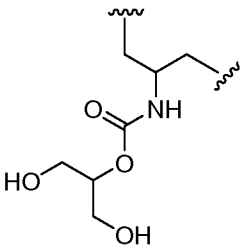
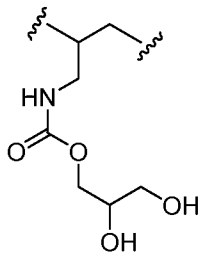
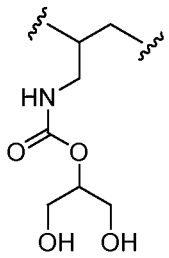










, or ;

wherein the representation of L is limited directionally left to right as is depicted, with the right hand side connected to one of the two S groups shown in Formula I;

R^{I-1}, R^{I-2}, R^{I-3}, R^{I-4}, and R^{I-5} each represent independently for each occurrence hydrogen or C_1-C_3 alkyl;

W_1 is independently a bond, O, or $N(R^{I-1})$;

j is 0 or 1;

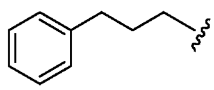
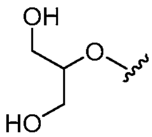
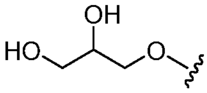
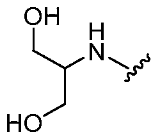
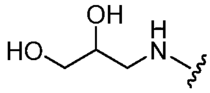
k and k* is independently 0 or 1;

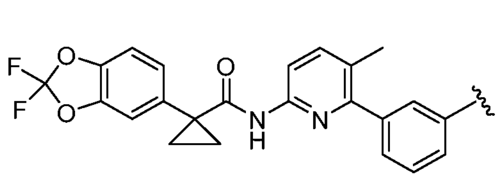
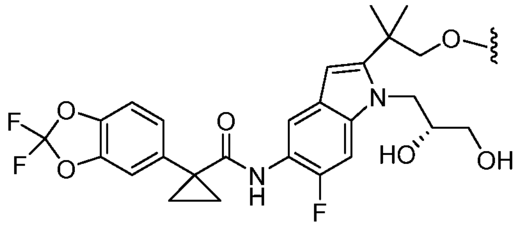
n* is independently 1, 2, or 3, with the proviso that when n* = 1 then W_1 cannot be O or NR^{I-1};

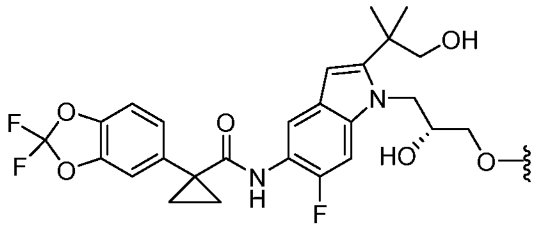
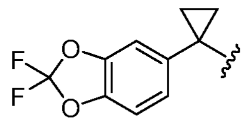
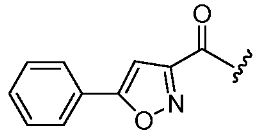
m* is 2 or 3;

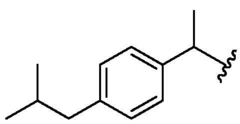
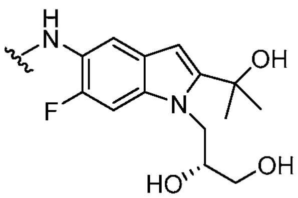
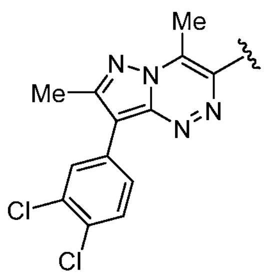
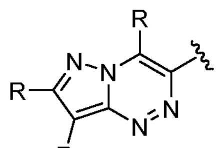
each R is independently -H, -C_1-C_6 alkyl, phenyl, benzyl, $-CH_2CO_2R^{I-1}$, $-CH_2CON(R^{I-1})(R^{I-1})$;

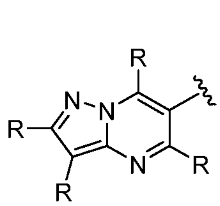
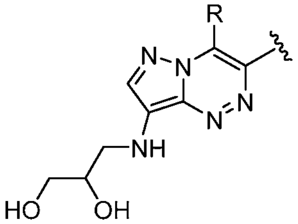
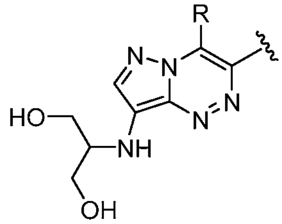
$Y^{I\text{-}1}$ is independently selected from

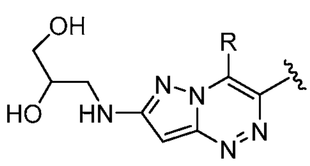
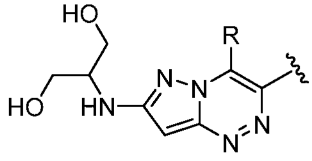
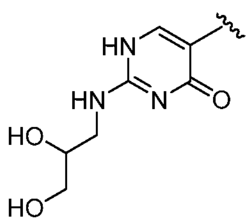
 ,  ,  ,  ,  ,

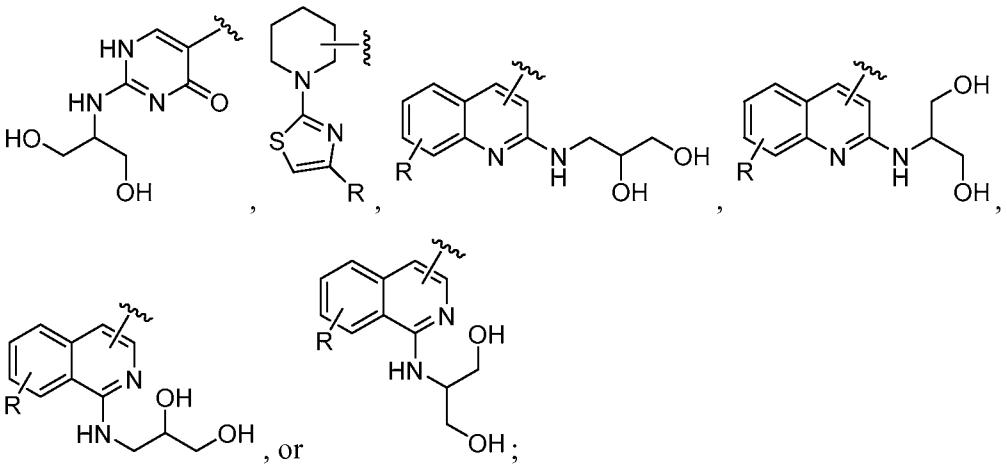
 ,  ,

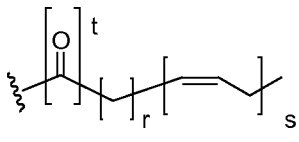
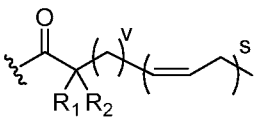
 ,  ,  ,

 ,  ,  ,  ,

 ,  ,  ,

 ,  ,  ,



Z* is  or  wherein:

R_1 and R_2 independently are hydrogen, C_1-C_4 alkyl, or halogen;

r is 2, 3, or 7;

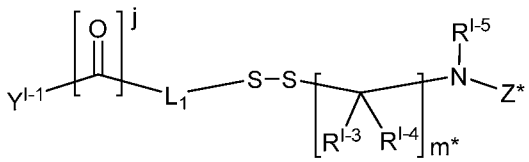
s is 3, 5, or 6;

t is 0 or 1; and

v is 1, 2, or 6.

Formula I-A

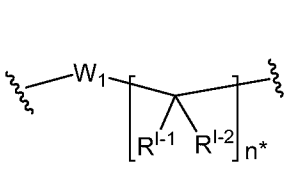
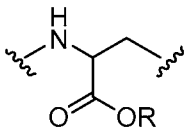
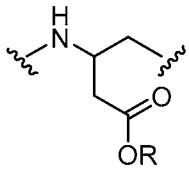
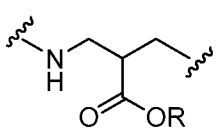
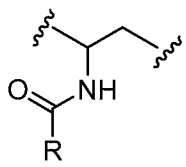
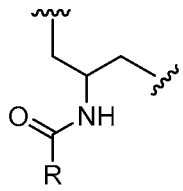
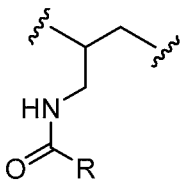
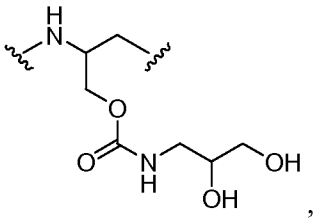
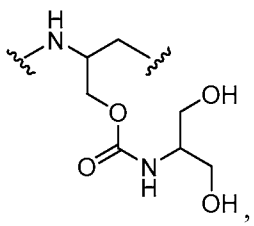
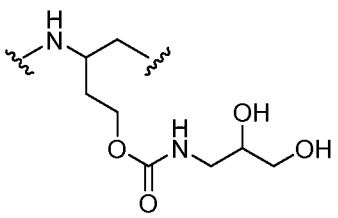
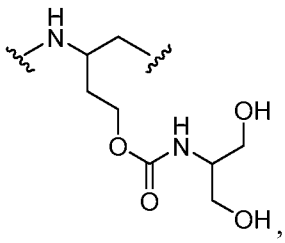
[00112] Another aspect of the invention provides a compound of Formula I-A represented by:



(I-A)

or a pharmaceutically acceptable salt or solvate thereof; wherein:

L_1 is independently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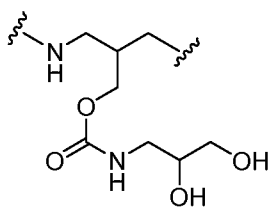
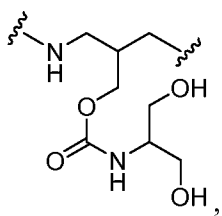
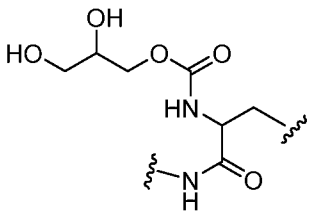
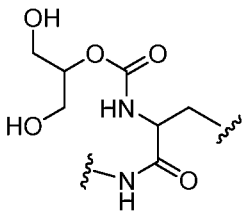
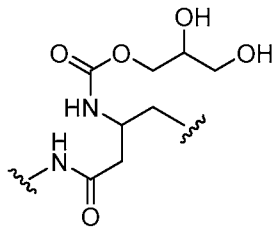
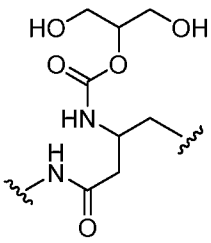
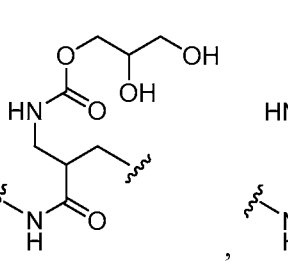
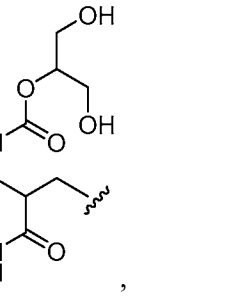
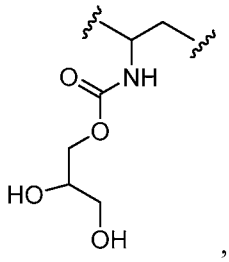
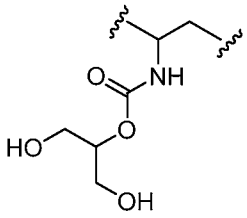
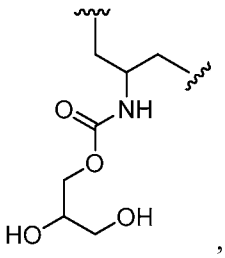
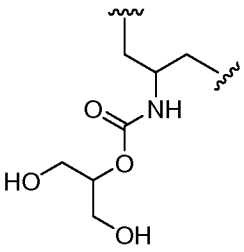
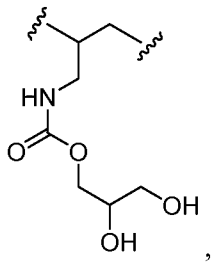
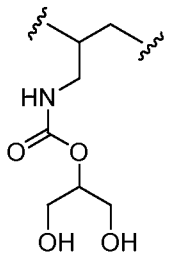









wherein the representation of L is limited directionally left to right as is depicted, with the right hand side connected to one of the two S groups shown in Formula I-A;

R^{I-1}, R^{I-2}, R^{I-3}, R^{I-4}, and R^{I-5} each represent independently for each occurrence hydrogen or C_1-C_3 alkyl;

W_1 is independently a bond, O, or $N(R^{I-1})$;

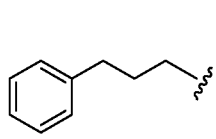
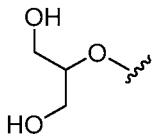
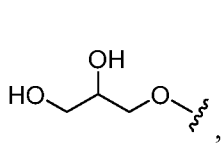
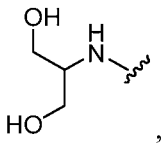
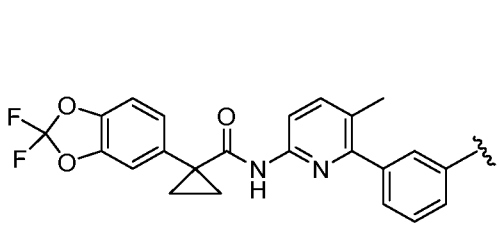
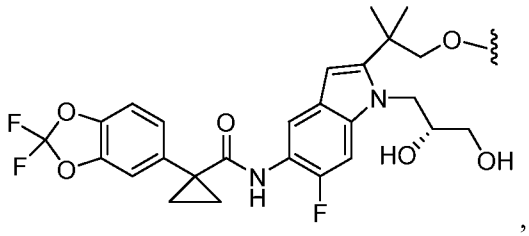
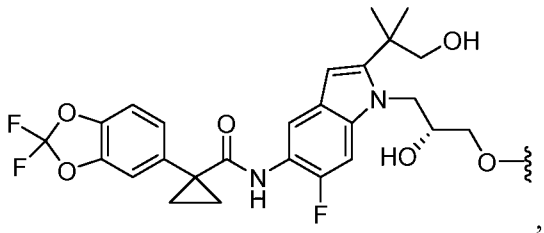
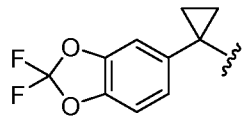
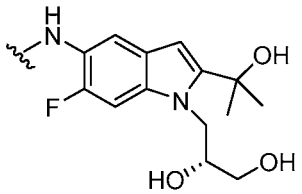
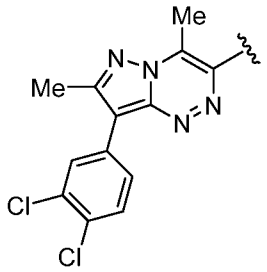
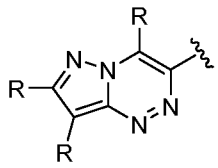
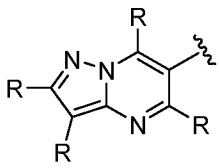
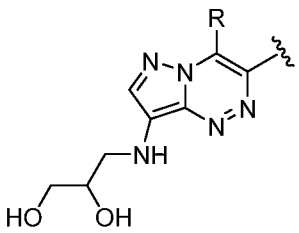
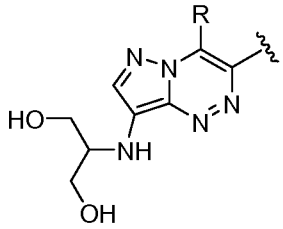
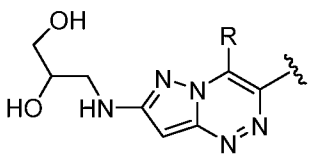
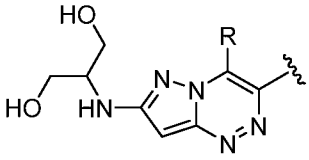
j is 0 or 1;

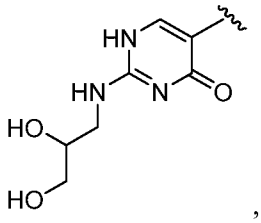
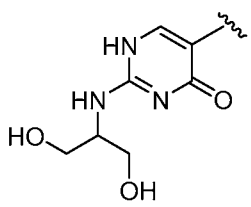
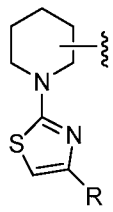
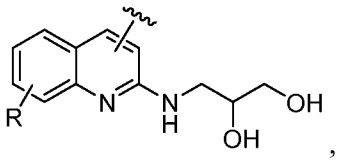
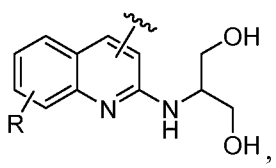
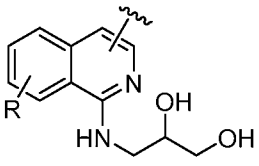
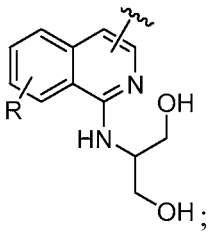
n* is independently 1, 2, or 3, with the proviso that when n* = 1 then W_1 cannot be O or NR^{I-1};

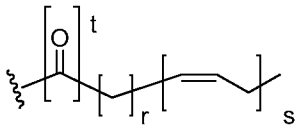
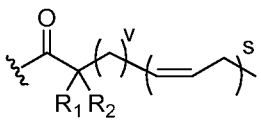
m* is 2 or 3;

each R is independently -H, -C_1-C_6 alkyl, phenyl, benzyl, $-CH_2CO_2R^{I-1}$, $-CH_2CON(R^{I-1})(R^{I-1})$;

Y^{I-1} is independently selected from

Z* is  or  wherein:

R_1 and R_2 independently are hydrogen, C_1-C_4 alkyl, or halogen;

r is 2, 3, or 7;

s is 3, 5, or 6;

t is 0 or 1; and

v is 1, 2, or 6.

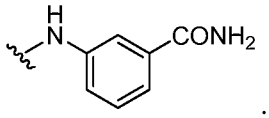
[00113] Definitions of the variables in Formula I-A above encompass multiple chemical groups. The application contemplates embodiments where, for example, i) the definition of a variable is a single chemical group selected from those chemical groups set forth above, ii) the definition is a collection of two or more of the chemical groups selected from those set forth above, and iii) the compound is defined by a combination of variables in which the variables are defined by (i) or (ii).

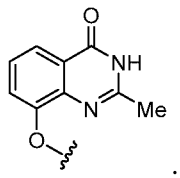
[00114] In certain embodiments, the compound is a compound of (i) Formula I or a pharmaceutically acceptable salt thereof or (ii) Formula I-A or a pharmaceutically acceptable salt thereof.

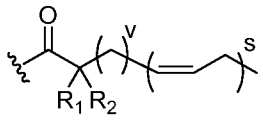
[00115] For the compounds of each of Formula I or Formula IA, the compounds can have one or more of the embodiments set forth below.

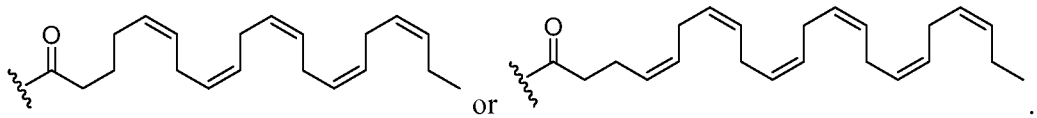
[00116] For example, in certain embodiments, R^{I-1}, R^{I-2}, R^{I-3}, R^{I-4}, and R^{I-5} each represent independently for each occurrence hydrogen or methyl. In certain embodiments, R^{I-1}, R^{I-2}, R^{I-3}, R^{I-4}, and R^{I-5} are hydrogen.

[00117] In certain embodiments, n* is 2. In certain embodiments, m* is 2. In certain embodiments, n* is 2, and m* is 2.

[00118] In certain embodiments, Y^{I-1} is  .

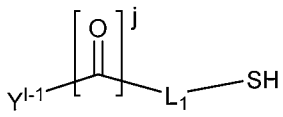
[00119] In certain embodiments, Y^{I-1} is  .

[00120] In certain embodiments, Z* is  wherein R_1 and R_2 are hydrogen or methyl. In certain embodiments, R_1 and R_2 are hydrogen. In certain embodiments, Z* is one of the following:



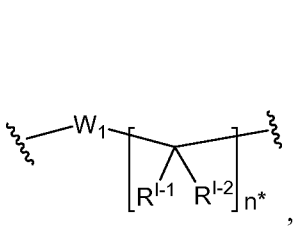
[00121] The description above describes multiple embodiments relating to compounds of Formula I or Formula I-A. The invention specifically contemplates all combinations of the foregoing embodiments.

[00122] In another aspect, the invention provides a compound of Formula II represented by:



(II) , wherein

L_1 is independently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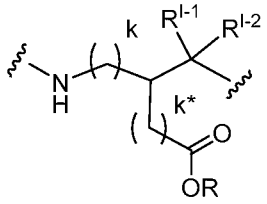
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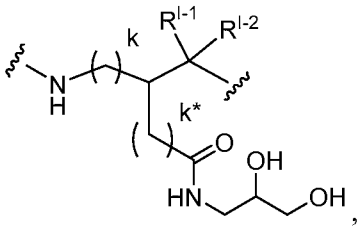
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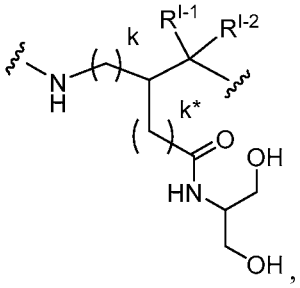
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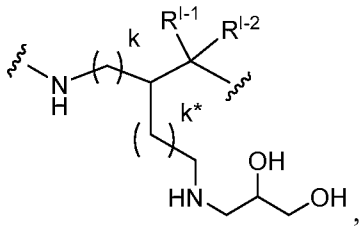
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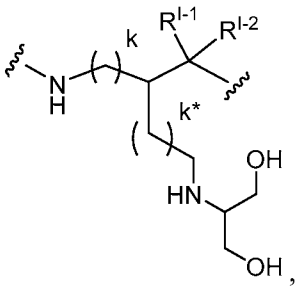
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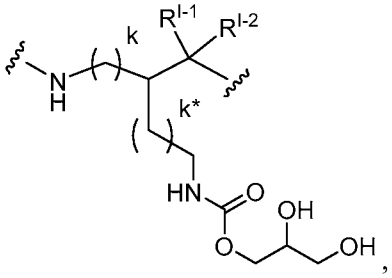
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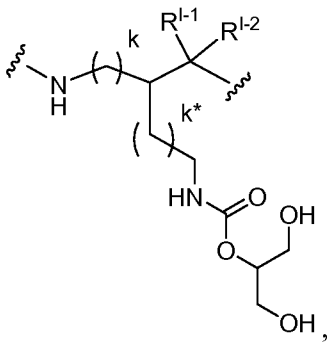
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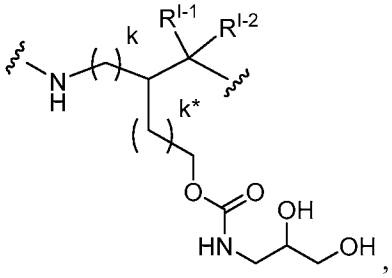
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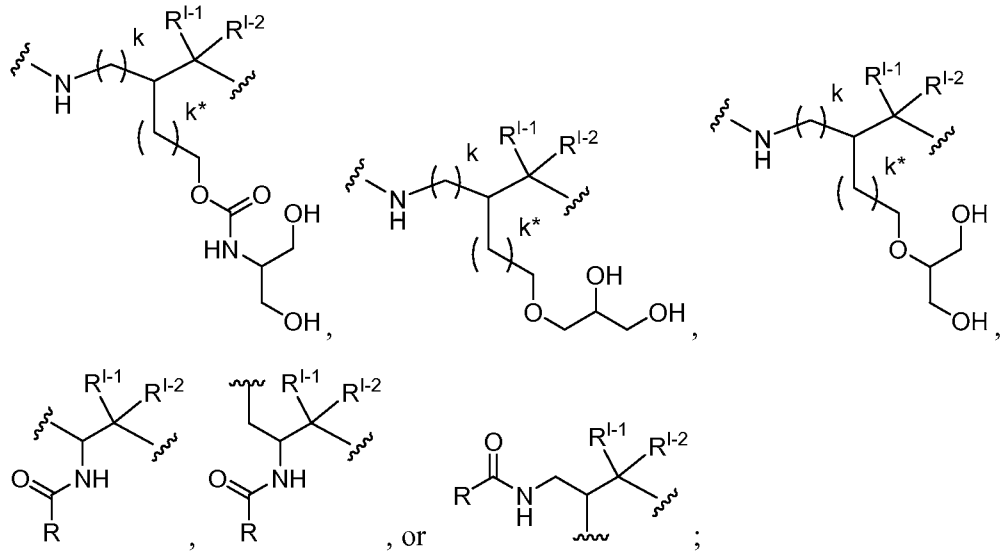


wherein the representation of L is limited directionally left to right as is depicted, with the right hand side connected to one of the two S groups shown in Formula II;

R^{I-1} and R^{I-2}, each represent independently for each occurrence hydrogen or C_1-C_3 alkyl;

W_1 is independently a bond, O, or $N(R^{I-1})$;

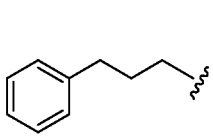
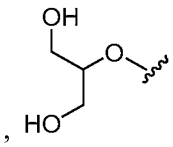
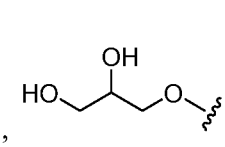
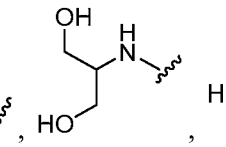
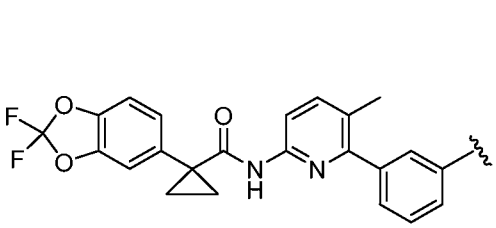
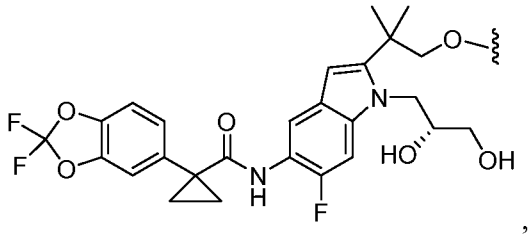
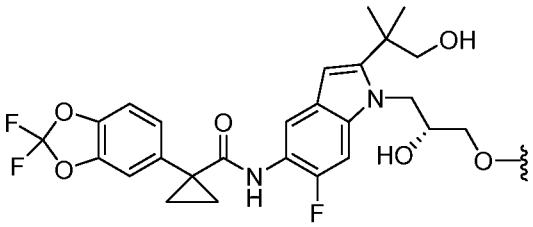
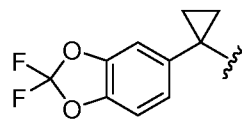
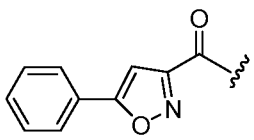
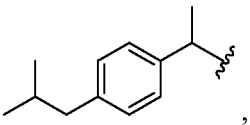
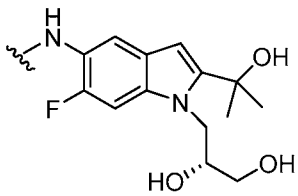
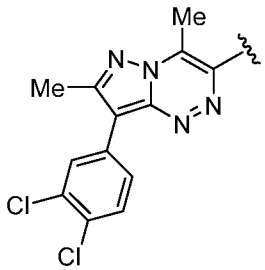
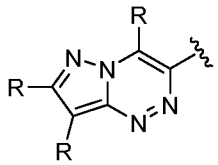
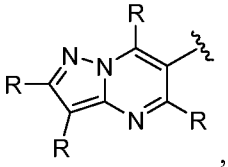
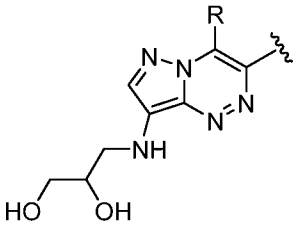
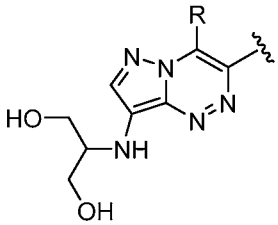
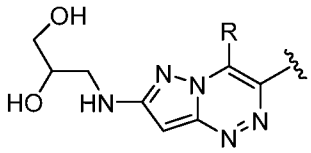
j is 0 or 1;

k and k* is independently 0 or 1;

n* is independently 1, 2, or 3, with the proviso that when n* = 1 then W_1 cannot be O or NR^{I-1};

each R is independently -H, -C_1-C_6 alkyl, phenyl, benzyl, $-CH_2CO_2R^{I-1}$, $-CH_2CON(R^{I-1})(R^{I-1})$;

Y^{I-1} is independently selected from

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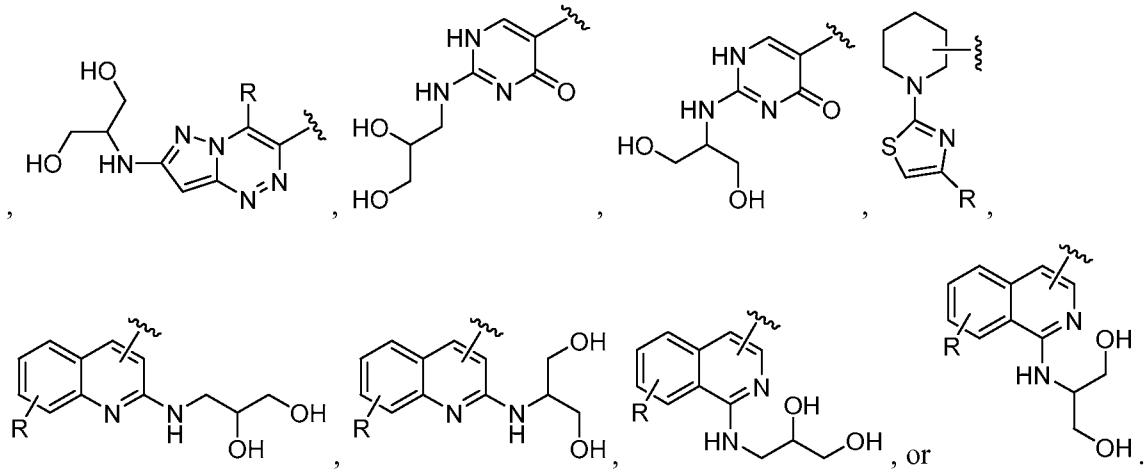
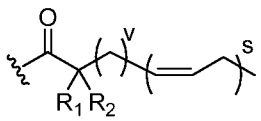
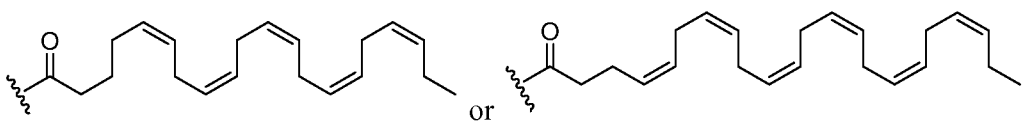


[00123] Definitions of the variables in Formula II above encompass multiple chemical groups. The application contemplates embodiments where, for example, i) the definition of a variable is a single chemical group selected from those chemical groups set forth above, ii) the definition is a collection of two or more of the chemical groups selected from those set forth above, and iii) the compound is defined by a combination of variables in which the variables are defined by (i) or (ii).

[00124] In certain embodiments, R^{I-1}, R^{I-2}, R^{I-3}, R^{I-4}, and R^{I-5} each represent independently for each occurrence hydrogen or methyl. In certain embodiments, R^{I-1}, R^{I-2}, R^{I-3}, R^{I-4}, and R^{I-5} are hydrogen.

[00125] In certain embodiments, n* is 2. In certain embodiments, m* is 2. In certain embodiments, n* is 2, and m* is 2.

[00126] In certain embodiments, Z* is  wherein R_1 and R_2 are hydrogen or methyl. In certain embodiments, R_1 and R_2 are hydrogen. In certain embodiments, Z* is one of the following:



[00127] The description above describes multiple embodiments relating to compounds of Formula II. The invention specifically contemplates all combinations of the embodiments.

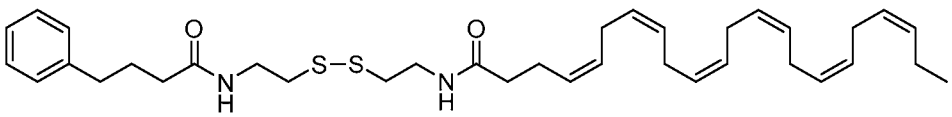
Additional Fatty Acid Cysteamine Conjugates of a CFTR Modulator

[00128] Another aspect of the invention provides a molecular conjugate comprising a CFTR modulator and cysteamine covalently linked via a linker to a fatty acid, wherein the fatty acid is selected from the group consisting of omega-3 fatty acids and fatty acids that are metabolized *in vivo* to omega-3 fatty acids. The conjugate is capable of intracellular hydrolysis to produce free cysteamine and free fatty acid.

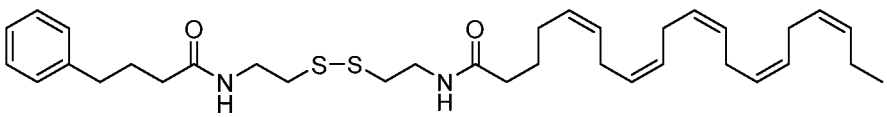
[00129] In certain embodiments, the fatty acid is selected from the group consisting of *all-cis*-7,10,13-hexadecatrienoic acid, α-linolenic acid, stearidonic acid, eicosatrienoic acid, eicosatetraenoic acid, eicosapentaenoic acid (EPA), docosapentaenoic acid, docosahexaenoic acid (DHA), tetracosapentaenoic acid and tetracosahexaenoic acid. In other embodiments, the fatty acid is selected from eicosapentaenoic acid and docosahexaenoic acid. In other embodiments, the fatty acid is selected from eicosapentaenoic acid and docosahexaenoic acid. In some embodiments, the fatty acid is eicosapentaenoic acid (EPA). In other embodiments, the fatty acid is docosahexaenoic acid (DHA). In some embodiments, the hydrolysis is enzymatic.

Exemplary Specific Compounds

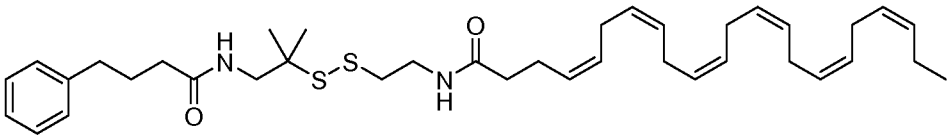
[00130] In certain embodiments, the compound is one of the following or a pharmaceutically acceptable salt thereof:



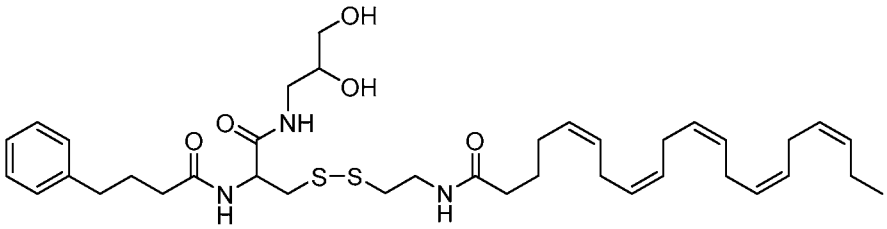
(4Z,7Z,10Z,13Z,16Z,19Z)-*N*-(2-((2-(4-phenylbutanamido)ethyl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-1**);



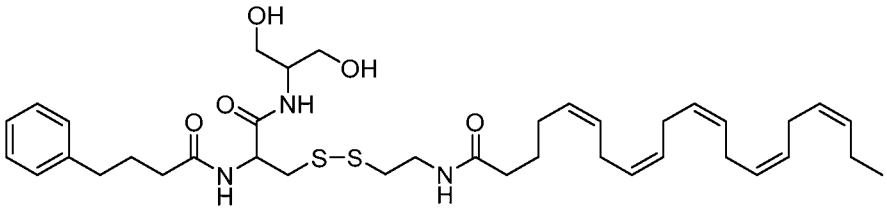
(5Z,8Z,11Z,14Z,17Z)-*N*-(2-((2-(4-phenylbutanamido)ethyl)disulfanyl)ethyl)icosa-5,8,11,14,17-pentaenamide (**I-2**);



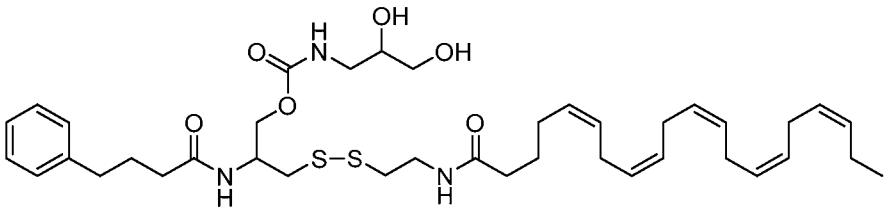
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-((2-methyl-1-(4-phenylbutanamido)propan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-3**);



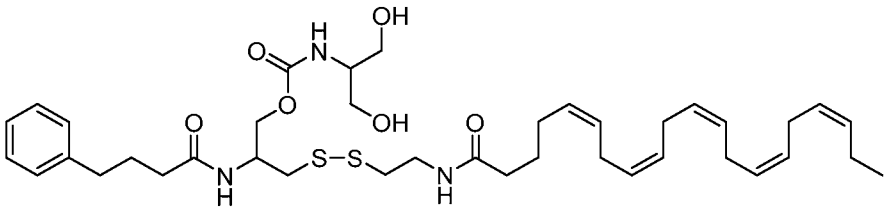
(5Z,8Z,11Z,14Z,17Z)-*N*-(2-((3-((2,3-dihydroxypropyl)amino)-3-oxo-2-(4-phenylbutanamido)propyl)disulfanyl)ethyl)icosa-5,8,11,14,17-pentaenamide (**I-4**);



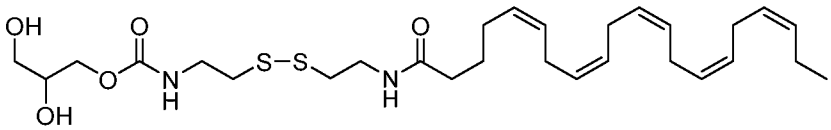
(5Z,8Z,11Z,14Z,17Z)-*N*-(2-((3-((1,3-dihydroxypropan-2-yl)amino)-3-oxo-2-(4-phenylbutanamido)propyl)disulfanyl)ethyl)icosa-5,8,11,14,17-pentaenamide (**I-5**);



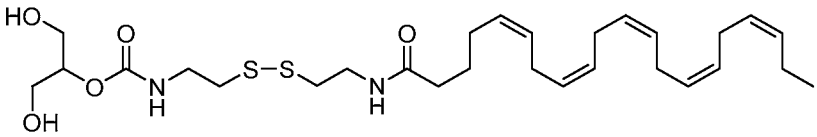
3-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)-2-(4-phenylbutanamido)propyl (2,3-dihydroxypropyl)carbamate (**I-6**);



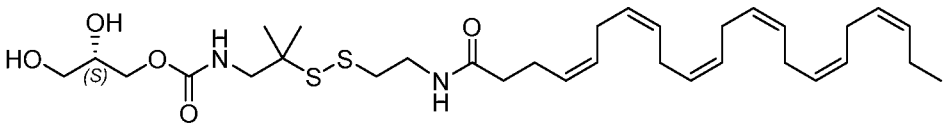
3-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)-2-(4-phenylbutanamido)propyl (1,3-dihydroxypropan-2-yl)carbamate (**I-7**);



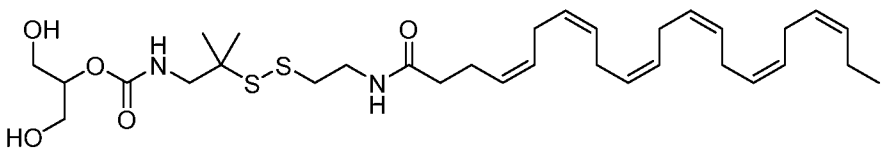
2,3-dihydroxypropyl (2-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)ethyl)carbamate (**I-8**);



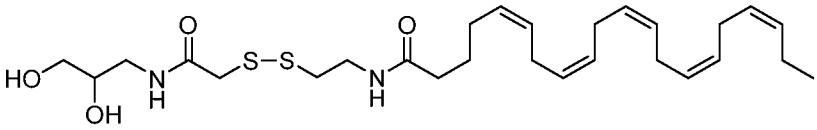
1,3-dihydroxypropan-2-yl (2-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)ethyl)carbamate (**I-9**);



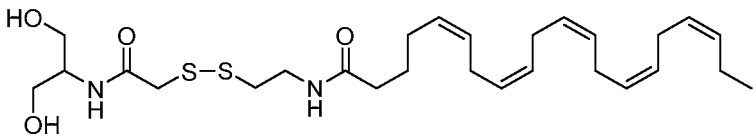
(S)-2,3-dihydroxypropyl (2-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-2-methylpropyl)carbamate (**I-10**);



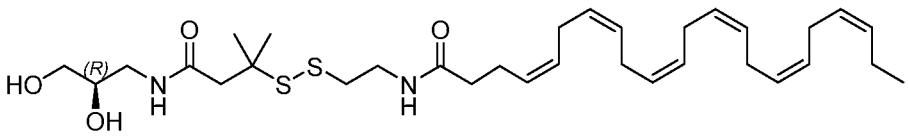
1,3-dihydroxypropan-2-yl (2-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-2-methylpropyl)carbamate (**I-11**);



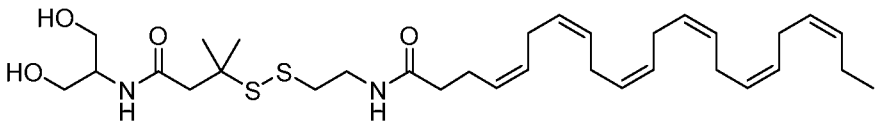
(5Z,8Z,11Z,14Z,17Z)-*N*-(2-((2-((2,3-dihydroxypropyl)amino)-2-oxoethyl)disulfanyl)ethyl)icosa-5,8,11,14,17-pentaenamide (**I-12**);



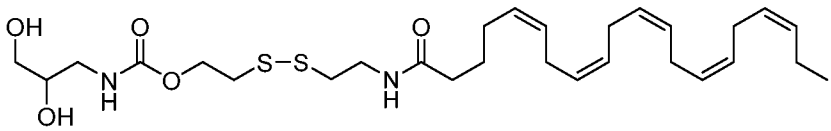
(5Z,8Z,11Z,14Z,17Z)-*N*-(2-((2-((1,3-dihydroxypropan-2-yl)amino)-2-oxoethyl)disulfanyl)ethyl)icosa-5,8,11,14,17-pentaenamide (**I-13**);



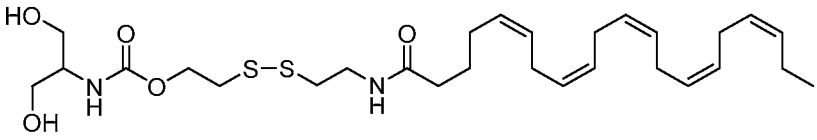
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-((4-(((R)-2,3-dihydroxypropyl)amino)-2-methyl-4-oxobutan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-14**);



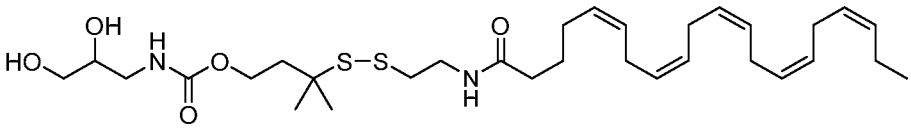
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-((4-((1,3-dihydroxypropan-2-yl)amino)-2-methyl-4-oxobutan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-15**);



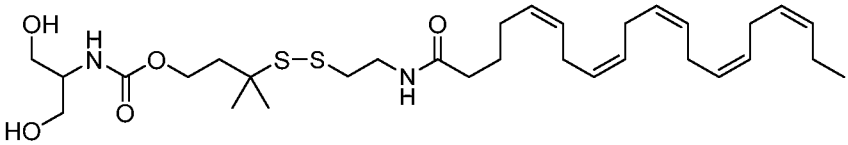
2-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)ethyl (2,3-dihydroxypropyl)carbamate (**I-16**);



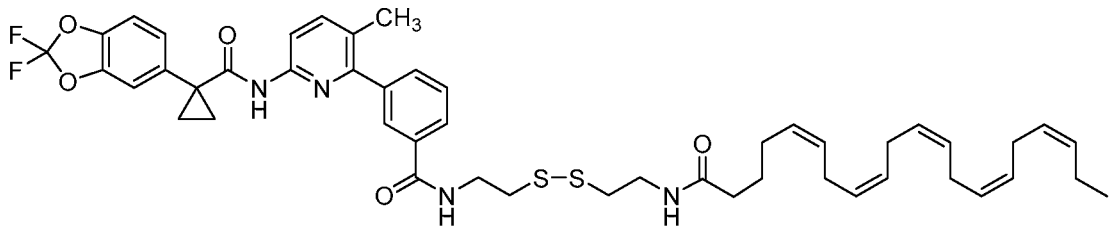
2-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)ethyl (1,3-dihydroxypropan-2-yl)carbamate (**I-17**);



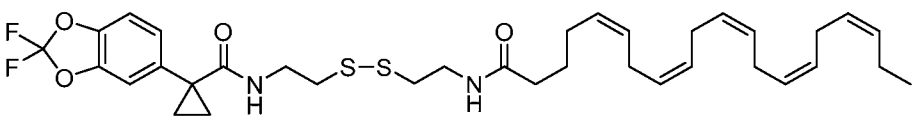
3-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)-3-methylbutyl (2,3-dihydroxypropyl)carbamate (**I-18**);



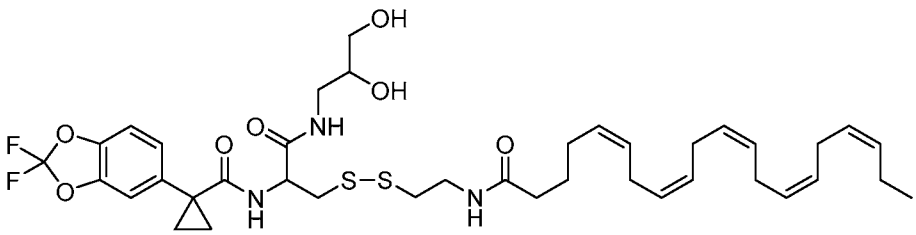
3-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)-3-methylbutyl (1,3-dihydroxypropan-2-yl)carbamate (**I-19**);



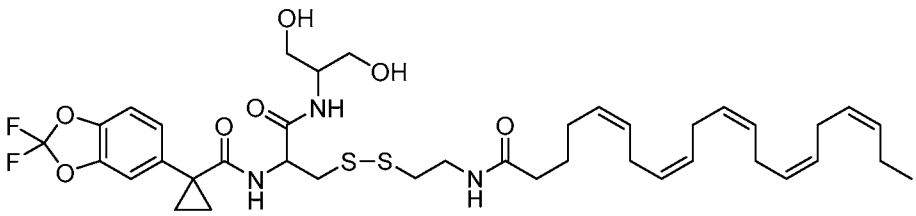
3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropane-1-carboxamido)-3-methylpyridin-2-yl)-*N*-(2-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)ethyl)benzamide (**I-20**);



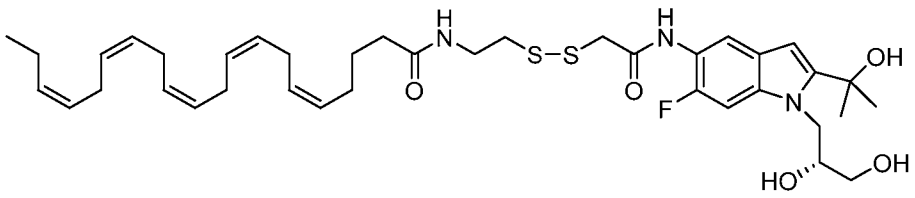
1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-*N*-(2-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)ethyl)cyclopropane-1-carboxamide (**I-21**);



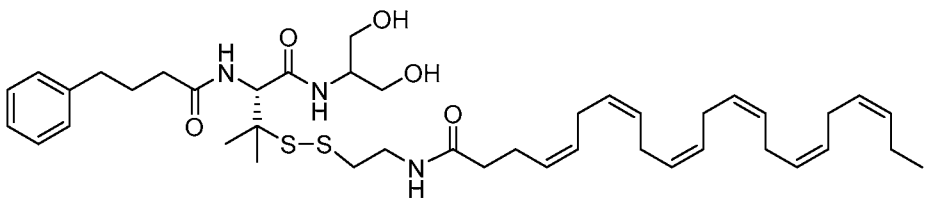
1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-*N*-(1-((2,3-dihydroxypropyl)amino)-3-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)-1-oxopropan-2-yl)cyclopropane-1-carboxamide (**I-22**);



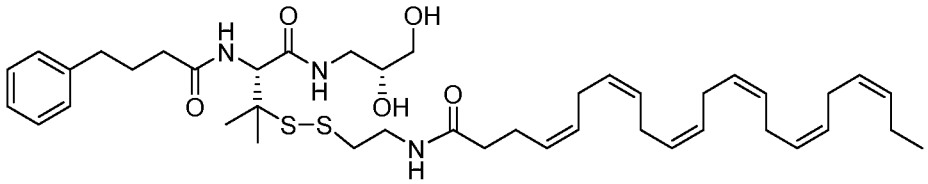
1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-*N*-(1-((1,3-dihydroxypropan-2-yl)amino)-3-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)-1-oxopropan-2-yl)cyclopropane-1-carboxamide (**I-23**);



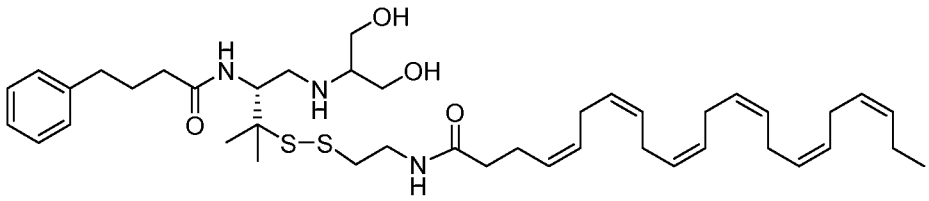
(5Z,8Z,11Z,14Z,17Z)-*N*-(2-((2-((1-((R)-2,3-dihydroxypropyl)-6-fluoro-2-(2-hydroxypropan-2-yl)-1H-indol-5-yl)amino)-2-oxoethyl)disulfanyl)ethyl)icosa-5,8,11,14,17-pentaenamide (**I-24**);



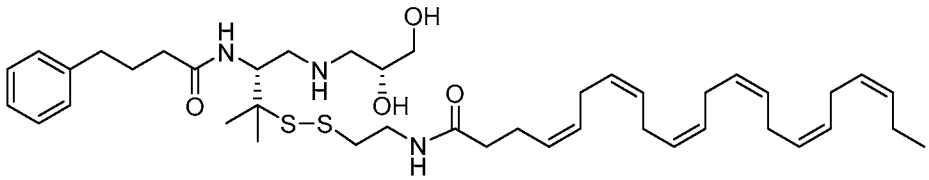
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-((1,3-dihydroxypropan-2-yl)amino)-2-methyl-4-oxo-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-25**);



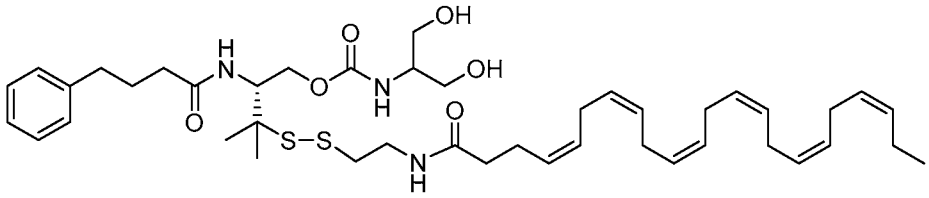
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-(((R)-2,3-dihydroxypropyl)amino)-2-methyl-4-oxo-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-26**);



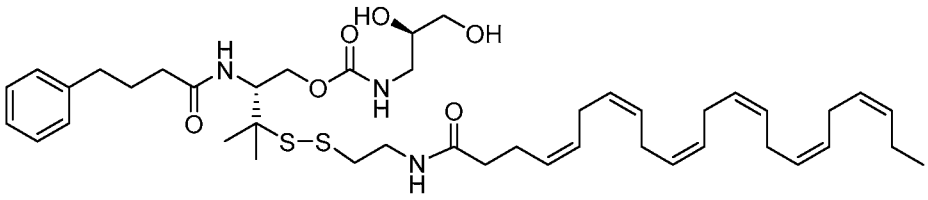
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-((1,3-dihydroxypropan-2-yl)amino)-2-methyl-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-27**);



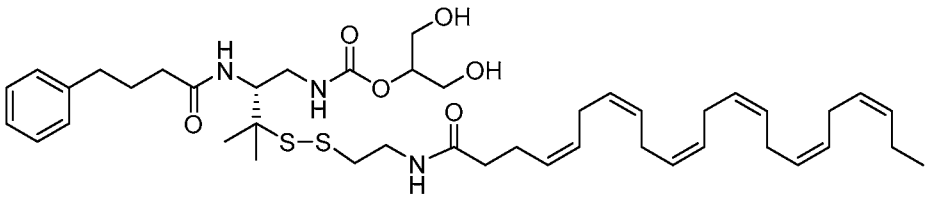
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-(((R)-2,3-dihydroxypropyl)amino)-2-methyl-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-28**);



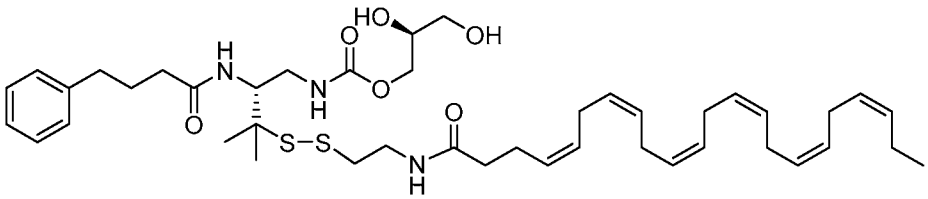
(R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-2-(4-phenylbutanamido)butyl (1,3-dihydroxypropan-2-yl)carbamate (**I-29**);



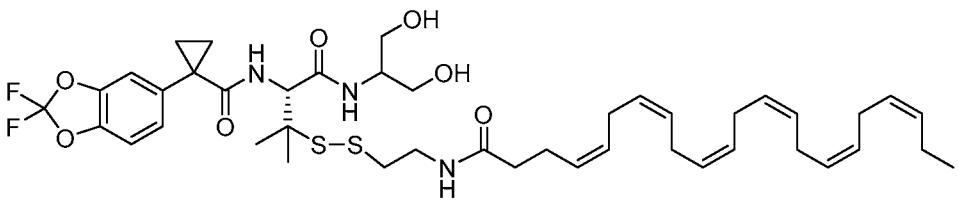
(R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-2-(4-phenylbutanamido)butyl ((R)-2,3-dihydroxypropyl)carbamate (**I-30**);



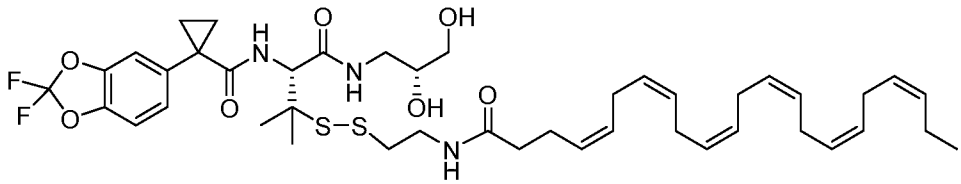
1,3-dihydroxypropan-2-yl ((R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-2-(4-phenylbutanamido)butyl)carbamate (**I-31**);



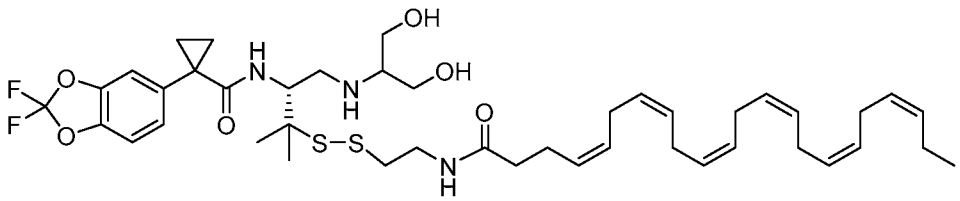
(S)-2,3-dihydroxypropyl ((R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-2-(4-phenylbutanamido)butyl)carbamate (**I-32**);



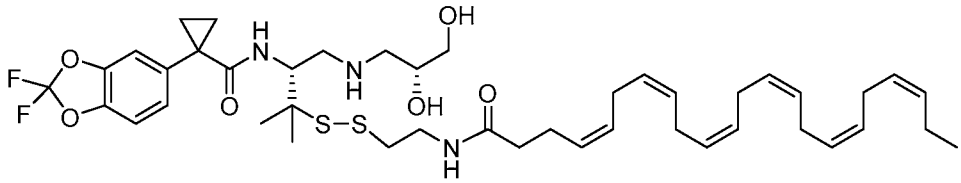
1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-((R)-1-((1,3-dihydroxypropan-2-yl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-1-oxobutan-2-yl)cyclopropane-1-carboxamide (**I-33**);



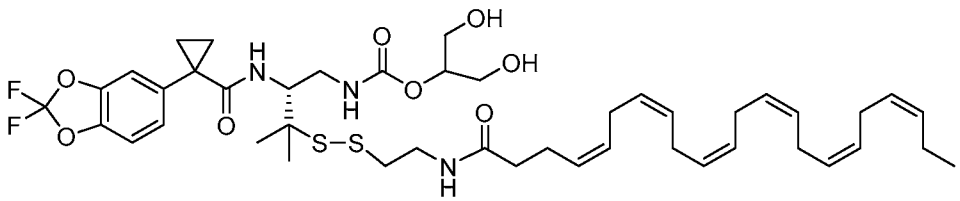
1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-((R)-1-(((R)-2,3-dihydroxypropyl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-1-oxobutan-2-yl)cyclopropane-1-carboxamide (**I-34**);



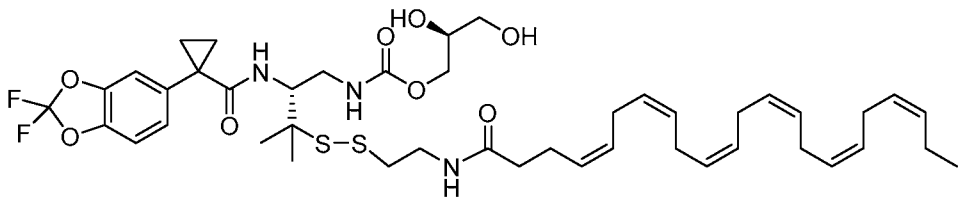
1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-((R)-1-((1,3-dihydroxypropan-2-yl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methylbutan-2-yl)cyclopropane-1-carboxamide (**I-35**);



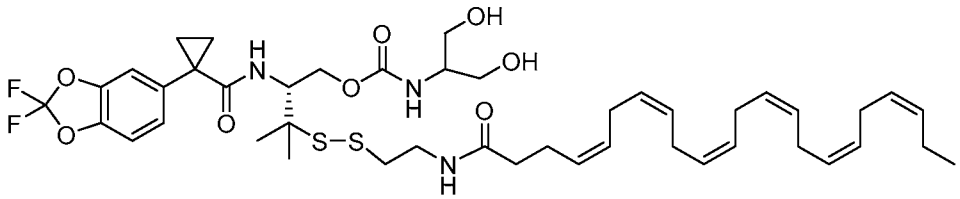
1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-((R)-1-(((R)-2,3-dihydroxypropyl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methylbutan-2-yl)cyclopropane-1-carboxamide (**I-36**);



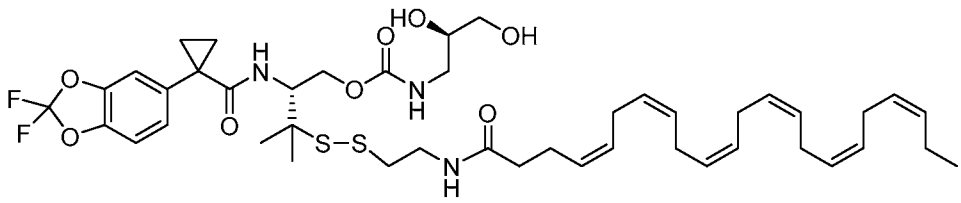
1,3-dihydroxypropan-2-yl ((R)-2-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropane-1-carboxamido)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methylbutyl)carbamate (**I-37**);



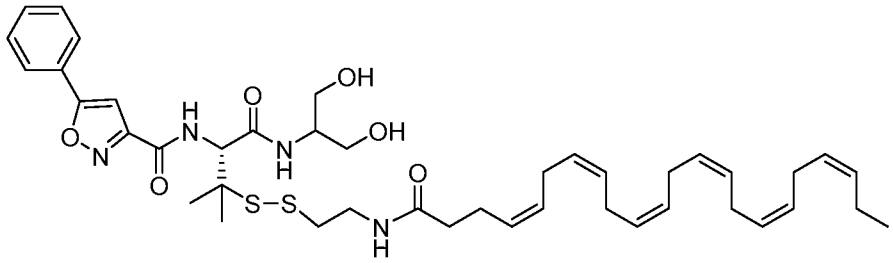
(S)-2,3-dihydroxypropyl ((R)-2-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropane-1-carboxamido)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methylbutyl)carbamate (**I-38**);



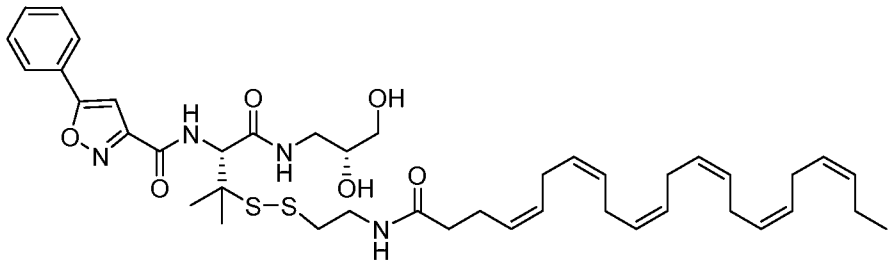
(R)-2-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropane-1-carboxamido)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methylbutyl (1,3-dihydroxypropan-2-yl)carbamate (**I-39**);



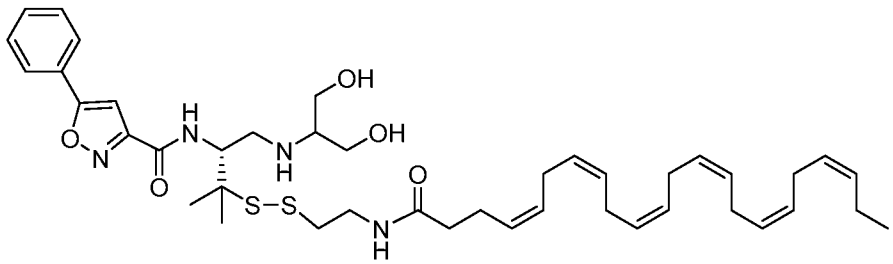
(R)-2-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropane-1-carboxamido)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methylbutyl ((R)-2,3-dihydroxypropyl)carbamate (**I-40**);



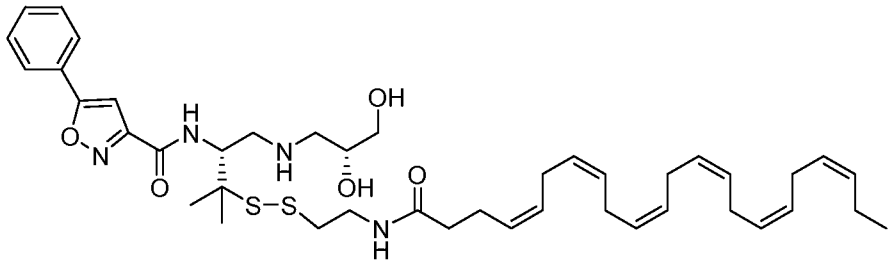
N-((R)-1-((1,3-dihydroxypropan-2-yl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-1-oxobutan-2-yl)-5-phenylisoxazole-3-carboxamide (**I-41**);



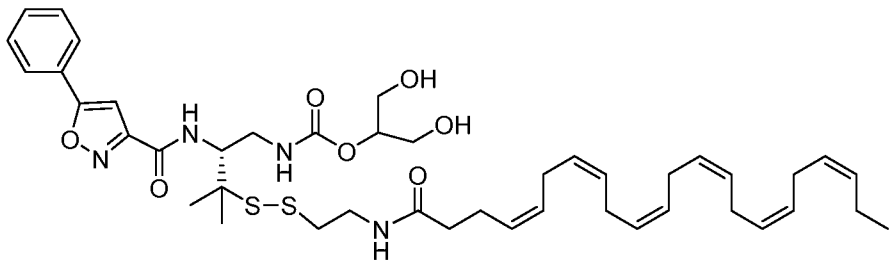
N-((R)-1-(((R)-2,3-dihydroxypropyl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-1-oxobutan-2-yl)-5-phenylisoxazole-3-carboxamide (**I-42**);



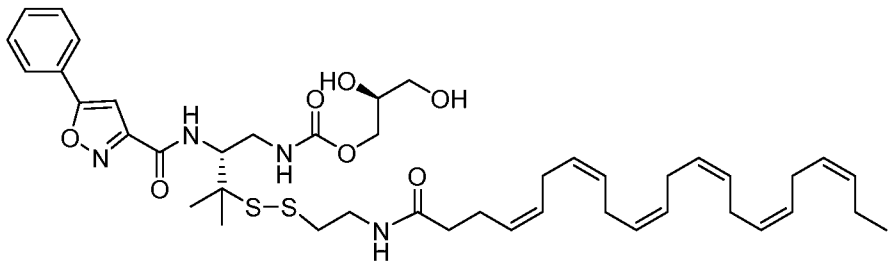
N-((R)-1-((1,3-dihydroxypropan-2-yl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methylbutan-2-yl)-5-phenylisoxazole-3-carboxamide (**I-43**);



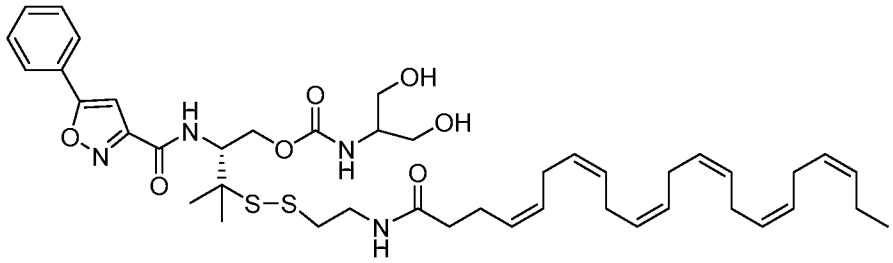
N-((R)-1-(((R)-2,3-dihydroxypropyl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methylbutan-2-yl)-5-phenylisoxazole-3-carboxamide (**I-44**);



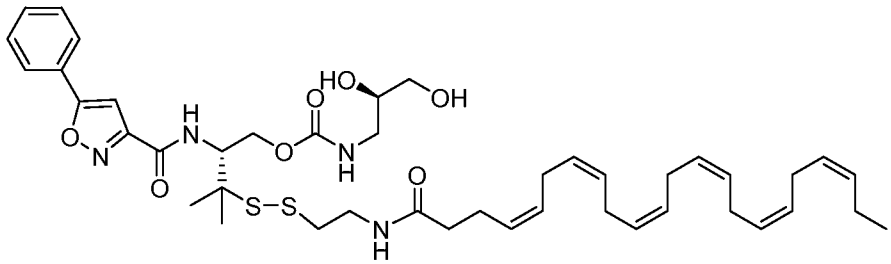
1,3-dihydroxypropan-2-yl ((R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-2-(5-phenylisoxazole-3-carboxamido)butyl)carbamate (**I-45**);



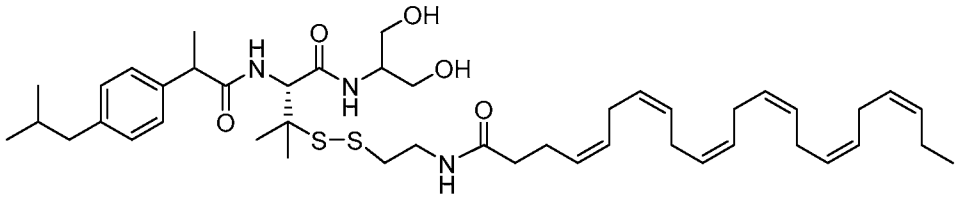
(S)-2,3-dihydroxypropyl ((R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-2-(5-phenylisoxazole-3-carboxamido)butyl)carbamate (**I-46**);



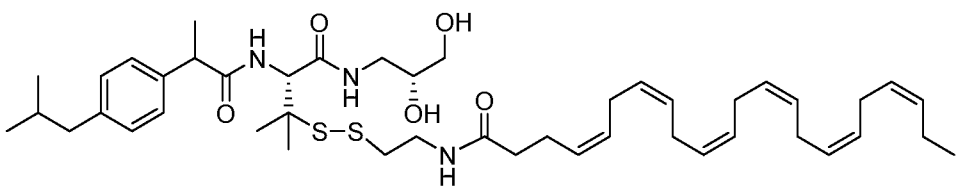
(R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-2-(5-phenylisoxazole-3-carboxamido)butyl (1,3-dihydroxypropan-2-yl)carbamate (**I-47**);



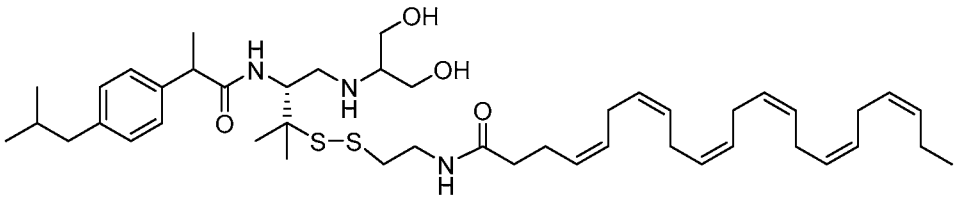
(R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-2-(5-phenylisoxazole-3-carboxamido)butyl ((R)-2,3-dihydroxypropyl)carbamate (**I-48**);



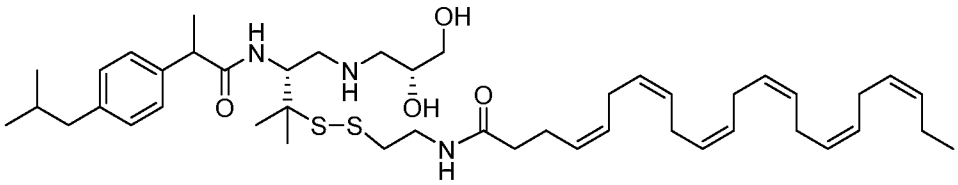
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((3R)-4-((1,3-dihydroxypropan-2-yl)amino)-3-(2-(4-isobutylphenyl)propanamido)-2-methyl-4-oxobutan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-49**);



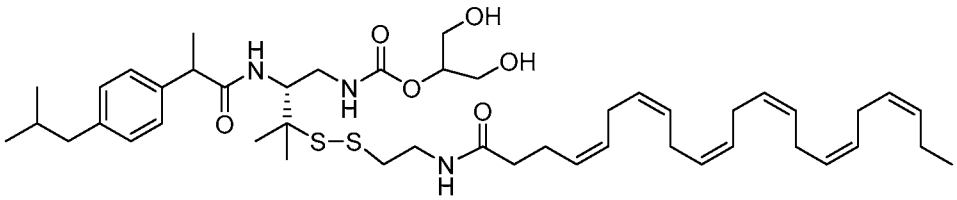
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((3R)-4-(((R)-2,3-dihydroxypropyl)amino)-3-(2-(4-isobutylphenyl)propanamido)-2-methyl-4-oxobutan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-50**);



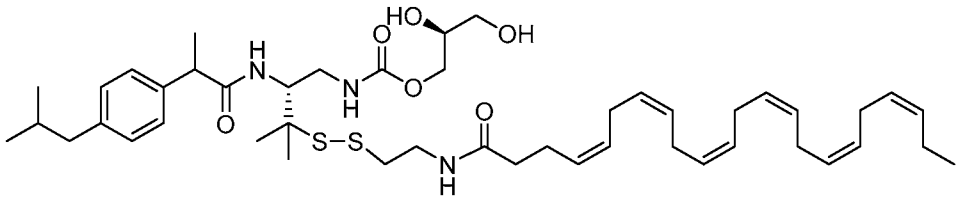
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((3R)-4-((1,3-dihydroxypropan-2-yl)amino)-3-(2-(4-isobutylphenyl)propanamido)-2-methylbutan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-51**);



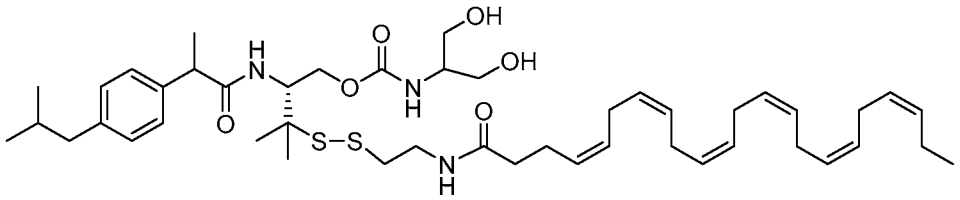
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((3R)-4-(((R)-2,3-dihydroxypropyl)amino)-3-(2-(4-isobutylphenyl)propanamido)-2-methylbutan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-52**);



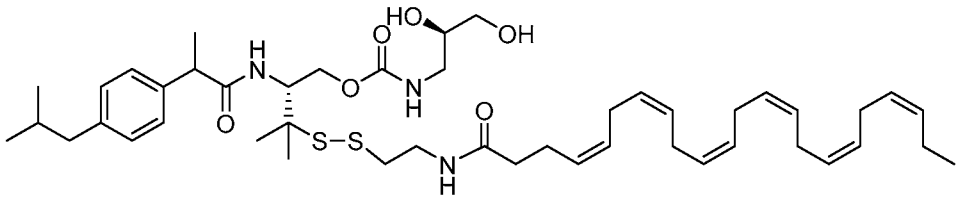
1,3-dihydroxypropan-2-yl ((2R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-2-(2-(4-isobutylphenyl)propanamido)-3-methylbutyl)carbamate (**I-53**);



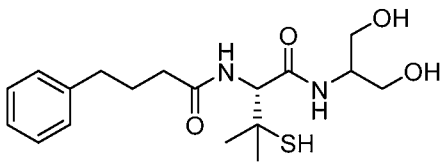
(S)-2,3-dihydroxypropyl ((2R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-2-(2-(4-isobutylphenyl)propanamido)-3-methylbutyl)carbamate (**I-54**);



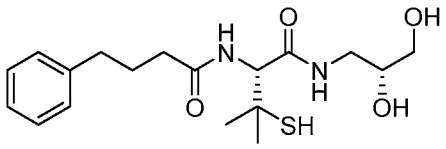
(2R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-2-(2-(4-isobutylphenyl)propanamido)-3-methylbutyl (1,3-dihydroxypropan-2-yl)carbamate (**I-55**);



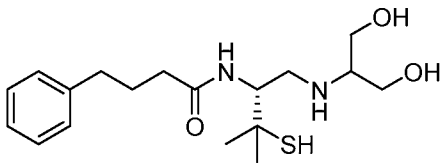
(2R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-2-(2-(4-isobutylphenyl)propanamido)-3-methylbutyl ((R)-2,3-dihydroxypropyl)carbamate (**I-56**);



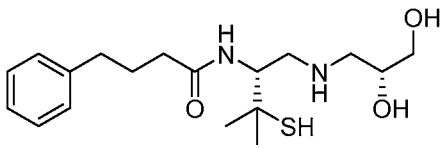
(R)-N-(1,3-dihydroxypropan-2-yl)-3-mercapto-3-methyl-2-(4-phenylbutanamido)butanamide (**II-1**);



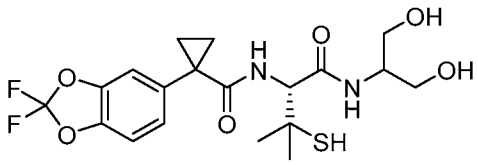
(R)-N-((R)-2,3-dihydroxypropyl)-3-mercapto-3-methyl-2-(4-phenylbutanamido)butanamide (**II-2**);



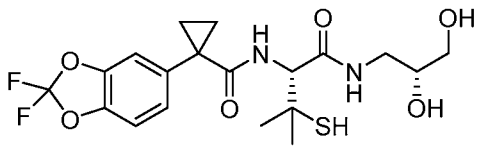
(R)-N-(1-((1,3-dihydroxypropan-2-yl)amino)-3-mercapto-3-methylbutan-2-yl)-4-phenylbutanamide (**II-3**);



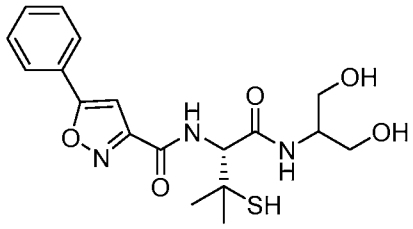
N-((R)-1-(((R)-2,3-dihydroxypropyl)amino)-3-mercapto-3-methylbutan-2-yl)-4-phenylbutanamide (**II-4**);



(R)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(1-((1,3-dihydroxypropan-2-yl)amino)-3-mercapto-3-methyl-1-oxobutan-2-yl)cyclopropane-1-carboxamide (**II-5**);

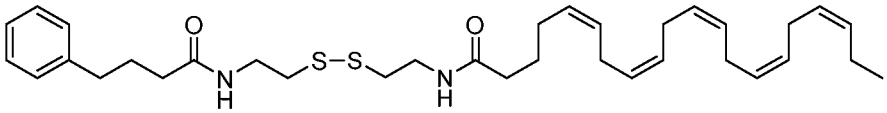


1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-((R)-1-(((R)-2,3-dihydroxypropyl)amino)-3-mercapto-3-methyl-1-oxobutan-2-yl)cyclopropane-1-carboxamide (**II-6**); or



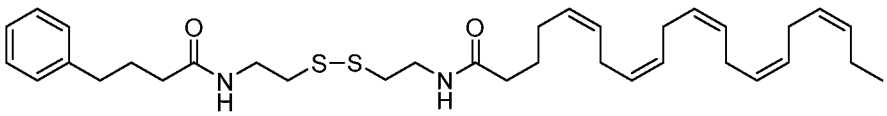
(R)-N-(1-((1,3-dihydroxypropan-2-yl)amino)-3-mercapto-3-methyl-1-oxobutan-2-yl)-5-phenylisoxazole-3-carboxamide (**II-7**).

[00131] In certain embodiments, the compound is the following or a pharmaceutically acceptable salt thereof:



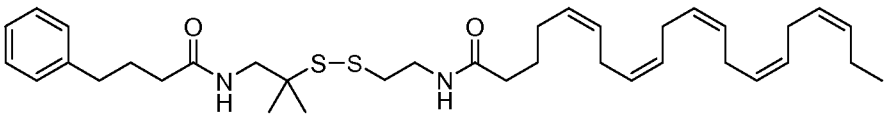
(5Z,8Z,11Z,14Z,17Z)-*N*-(2-((2-(4-phenylbutanamido)ethyl)disulfanyl)ethyl)icosa-5,8,11,14,17-pentaenamide (**I-2**).

[00132] In certain embodiments, the compound is:



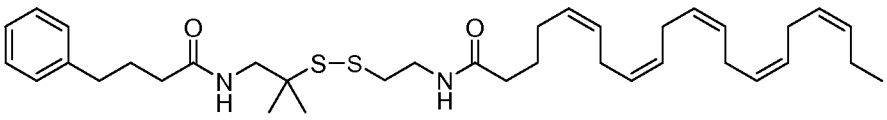
(5Z,8Z,11Z,14Z,17Z)-*N*-(2-((2-(4-phenylbutanamido)ethyl)disulfanyl)ethyl)icosa-5,8,11,14,17-pentaenamide (**I-2**).

[00133] In certain embodiments, the compound is the following or a pharmaceutically acceptable salt thereof:



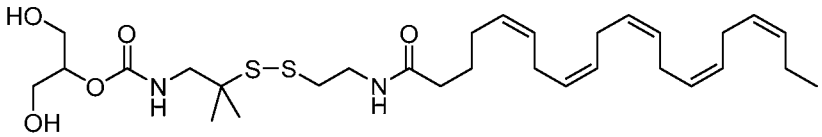
(5Z,8Z,11Z,14Z,17Z)-*N*-(2-((2-methyl-1-(4-phenylbutanamido)propan-2-yl)disulfanyl)ethyl)icosa-5,8,11,14,17-pentaenamide (**I-3**).

[00134] In certain embodiments, the compound is:



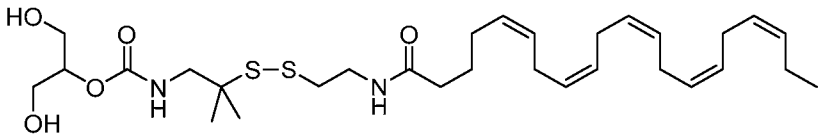
(5Z,8Z,11Z,14Z,17Z)-*N*-(2-((2-methyl-1-(4-phenylbutanamido)propan-2-yl)disulfanyl)ethyl)icosa-5,8,11,14,17-pentaenamide (**I-3**).

[00135] In certain embodiments, the compound is the following or a pharmaceutically acceptable salt thereof:



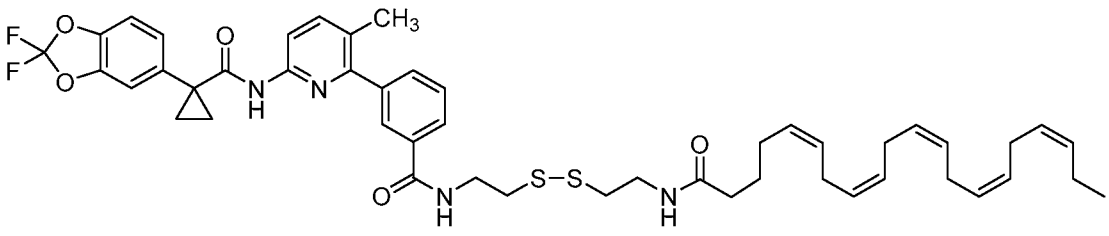
1,3-dihydroxypropan-2-yl (2-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)-2-methylpropyl)carbamate (**I-11**).

[00136] In certain embodiments, the compound is:



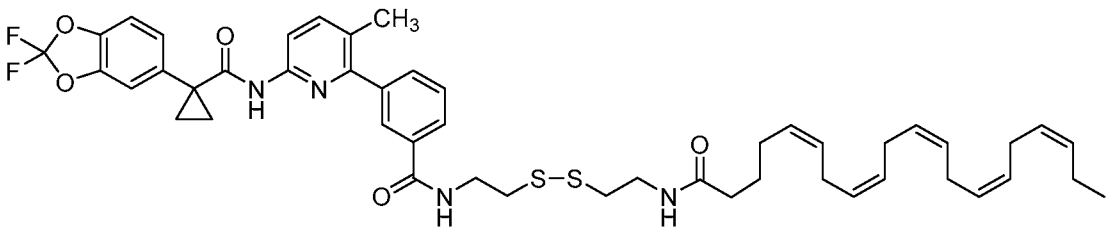
1,3-dihydroxypropan-2-yl (2-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)-2-methylpropyl)carbamate (**I-11**).

[00137] In certain embodiments, the compound is the following or a pharmaceutically acceptable salt thereof:



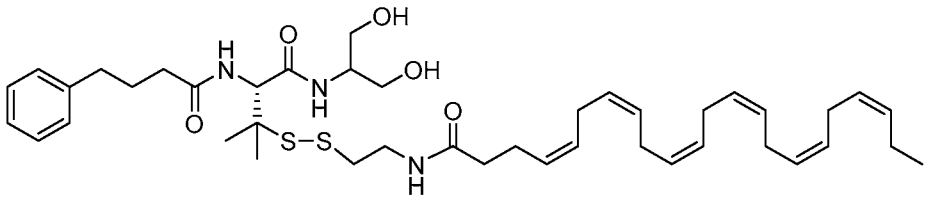
3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropane-1-carboxamido)-3-methylpyridin-2-yl)-*N*-(2-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)ethyl)benzamide (**I-20**).

[00138] In certain embodiments, the compound is:



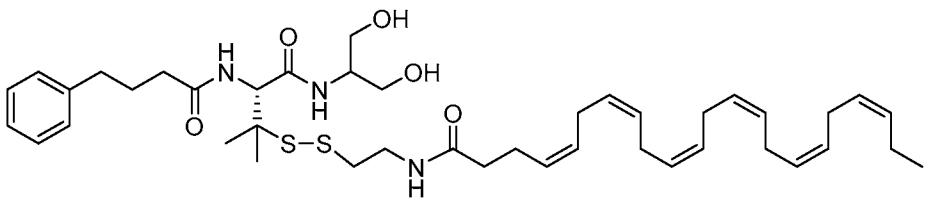
3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropane-1-carboxamido)-3-methylpyridin-2-yl)-*N*-(2-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)ethyl)benzamide (**I-20**).

[00139] In certain embodiments, the compound is the following or a pharmaceutically acceptable salt thereof:



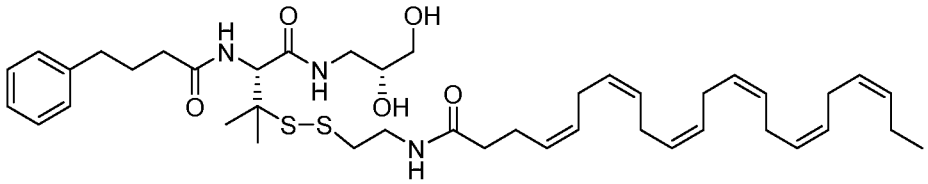
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-((1,3-dihydroxypropan-2-yl)amino)-2-methyl-4-oxo-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-25**).

[00140] In certain embodiments, the compound is:



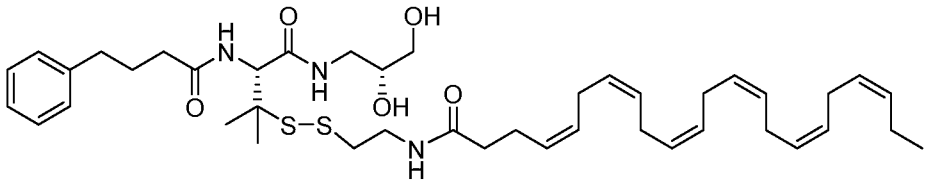
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-((1,3-dihydroxypropan-2-yl)amino)-2-methyl-4-oxo-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-25**).

[00141] In certain embodiments, the compound is the following or a pharmaceutically acceptable salt thereof:



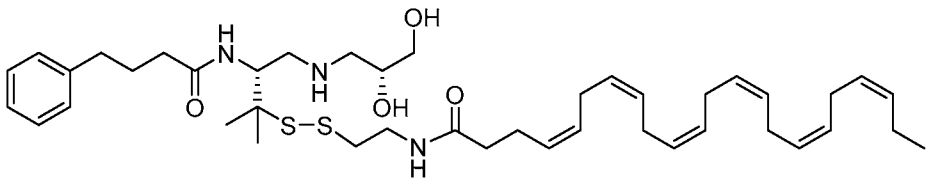
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-(((R)-2,3-dihydroxypropyl)amino)-2-methyl-4-oxo-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-26**).

[00142] In certain embodiments, the compound is:



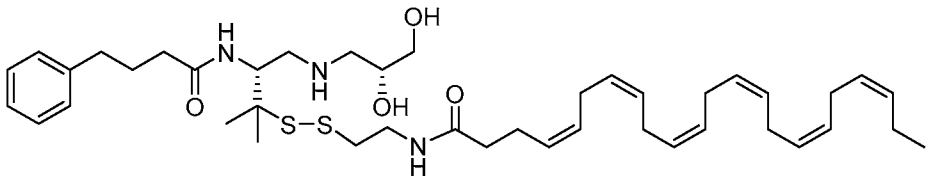
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-(((R)-2,3-dihydroxypropyl)amino)-2-methyl-4-oxo-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-26**).

[00143] In certain embodiments, the compound is the following or a pharmaceutically acceptable salt thereof:



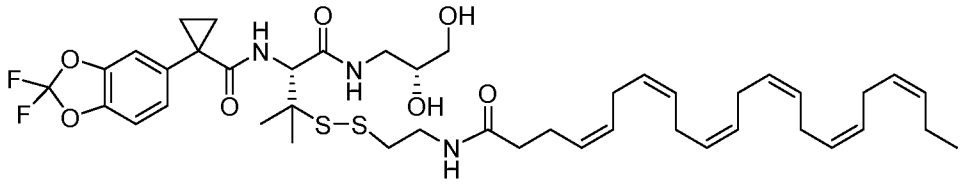
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-(((R)-2,3-dihydroxypropyl)amino)-2-methyl-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-28**).

[00144] In certain embodiments, the compound is:



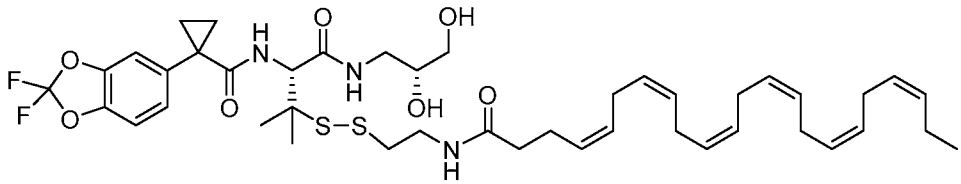
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-(((R)-2,3-dihydroxypropyl)amino)-2-methyl-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-28**).

[00145] In certain embodiments, the compound is the following or a pharmaceutically acceptable salt thereof:



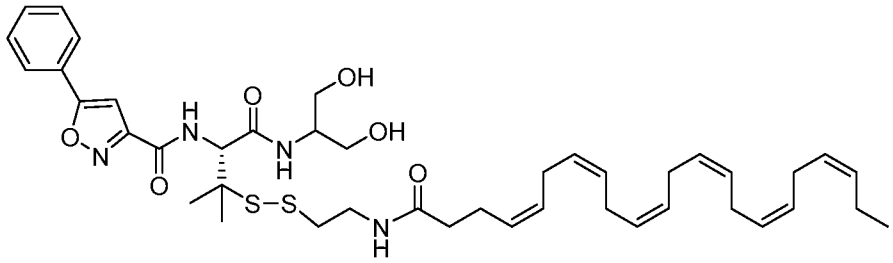
1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-((R)-1-(((R)-2,3-dihydroxypropyl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-1-oxobutan-2-yl)cyclopropane-1-carboxamide (**I-34**).

[00146] In certain embodiments, the compound is:



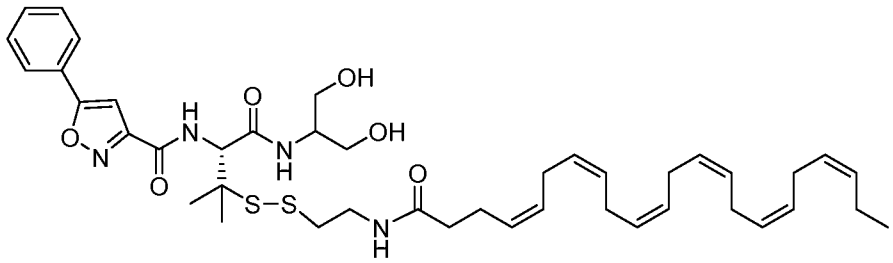
1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-((R)-1-(((R)-2,3-dihydroxypropyl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-1-oxobutan-2-yl)cyclopropane-1-carboxamide (**I-34**).

[00147] In certain embodiments, the compound is the following or a pharmaceutically acceptable salt thereof:



N-((R)-1-((1,3-dihydroxypropan-2-yl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-1-oxobutan-2-yl)-5-phenylisoxazole-3-carboxamide (**I-41**).

[00148] In certain embodiments, the compound is:



N-((R)-1-((1,3-dihydroxypropan-2-yl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-1-oxobutan-2-yl)-5-phenylisoxazole-3-carboxamide (**I-41**).

[00149] As indicated above, the invention provides a pharmaceutical composition comprising a compound described herein and a pharmaceutically acceptable carrier. It is contemplated that the compounds of the invention can be synthesized using conventional reagents and methodologies, for example, by using exemplary reagents and exemplary methods and protocols set forth in the Examples.

III. THERAPEUTIC APPLICATIONS OF FATTY ACID CYSTEAMINE CONJUGATES OF A CFTR MODULATOR

[00150] As indicated above, the invention is based, in part, upon the discovery that fatty acid cysteamine conjugates of a CFTR modulator are useful in activating autophagy. The fatty acid cysteamine conjugates of a CFTR modulator of the invention have therapeutic effects that

cannot be achieved by administering the three individual components separately or as a combination of individual components, and offer a superior way of activating autophagy to treat CF in a way that cannot be replicated by administering the individual components or the combination of the individual components. The covalent linkage of a CFTR modulator and an omega-3 fatty acid to cysteamine allows the simultaneous delivery of the three components to a intracellular location, whereupon the individual components are released by cleavage, for example, enzymatic cleavage, at the location and at the same time. Exemplary therapeutic methods and additional features of the therapeutic applications are described below.

Exemplary Therapeutic Methods

[00151] One aspect of the invention provides a method of treating a disease described herein (*e.g.*, a disease selected from the group consisting of CF, a neurodegenerative disease, inflammatory disease, a liver disease, muscle disease, infection, and an immune disease). The method comprises administering to a patient in need thereof a therapeutically effective amount of a fatty acid cysteamine conjugate of a CFTR modulator described herein, such as a compound of Formula A, A-1, I, or I-A, to treat the disease. In certain embodiments, the compound is a compound of Formula A. In certain embodiments, the compound is a compound of Formula A-1. In certain embodiments, the compound is a compound of Formula I. In certain embodiments, the compound is a compound of Formula I-A.

[00152] In certain embodiments, the disease is CF. In certain embodiments, the disease is a neurodegenerative disease (e.g., Huntington's disease, Alzheimer's disease, or Parkinson's disease). In certain embodiments, the disease is an inflammatory disease. In certain embodiments, the disease a neurodegenerative disease, liver disease, muscle disease, infection, immunity, or inflammatory disease. Neurodegenerative diseases include Huntington's disease, Parkinson's disease, Alzheimer's disease, and transmissible spongiform encephalopathies. In certain embodiments, the disease is idiopathic pulmonary fibrosis. In certain embodiments, the disease is age-related macular degeneration. In yet other embodiments, the disease is a cardiac disease.

[00153] In certain embodiments, in the method of treating the disease, the administration of the compound of Formula A increases autophagy in a subject by at least 5%, 10%, 25%, 50%,

or 100%. In certain embodiments, in the method of treating the disease, the administration of the compound of Formula A-1 increases autophagy in a subject by at least 5%, 10%, 25%, 50%, or 100%. In certain embodiments, in the method of treating the disease, the administration of the compound of Formula I increases autophagy in a subject by at least 5%, 10%, 25%, 50%, or 100%. In certain embodiments, in the method of treating the disease, the administration of the compound of Formula I-A increases autophagy in a subject by at least 5%, 10%, 25%, 50%, or 100%.

[00154] Additional diseases contemplated for treatment using methods described herein include, for example, the following diseases that are understood to have defective autophagy: Danon disease, X-linked myopathy, infantile autophagic vacuolar myopathy, adult onset vacuolar myopathy, Pompe disease, sporadic inclusion body myositis, limb girdle muscular dystrophy type 2B, and Miyoshi myopathy.

[00155] The fatty acid cysteamine conjugates of a CFTR modulator described herein may also useful for the treatment of mitochondrial diseases such as Leigh Syndrome, Diabetes Mellitus and Deafness (DAD), Leber's hereditary optic neuropathy, Neuropathy-ataxia-retinis pigmentosa and ptosis (NARP), myoneurogenic gastrointestinal encephalopathy (MNGIE), myoclonic epilepsy with ragged red fibers (MERRF), and mitochondrial myopathy-encephalomyopathy-lactic acidosis-stroke like symptoms (MELAS), Keam-Sayre syndrome, subacute necrotizing encephalopathy (Leigh's Syndrome), and mitochondrial cardiomyopathies and othersyndromes due to multiplemitochondrial DNA deletions. Additional mitochondrial diseases include neurogenic muscle weakness, progressive external opthalmoplegia (PEO), and Complex I disease, Complex II disease, Complex III disease, Complex IV disease and Complex V disease, which relates to dysfunction ofthe OXPHOS complexes, and MEGDEL syndrome (3-methylglutaconic aciduria type IV with sensorineural deafness, encephalopathy and Leigh-like syndrome.

[00156] In certain embodiments, the patient is a human.

[00157] Another aspect of the invention provides a method of activating autophagy in a patient. The method comprises administering to a patient in need thereof an effective amount of a fatty acid cystamine conjugate of a CFTR modulator described herein, such as a compound

of Formula A, A-1, I, or I-A, to activate autophagy in the patient. In certain embodiments, the patient suffers from CF, a neurodegenerative disease, or an inflammatory disease.

[00158] In certain embodiments, in the method of treating the disease, the administration of a compound for example, Formula A increases autophagy in a subject by at least 5%, 10%, 25%, 50% or least 100%. In certain embodiments, in the method of treating the disease, the administration of a compound for example, Formula A-1 increases autophagy in a subject by at least 5%, 10%, 25%, 50% or least 100%. In certain embodiments, in the method of treating the disease, the administration of a compound for example, Formula I increases autophagy in a subject by at least 5%, 10%, 25%, 50% or least 100%. In certain embodiments, in the method of treating the disease, the administration of a compound for example, Formula I-A increases autophagy in a subject by at least 5%, 10%, 25%, 50% or least 100%.

[00159] In certain embodiments, activation of autophagy can be characterized according to changes in the amount of certain biomarkers. One exemplary biomarker is microtubule-associated protein 1A/1B-light chain 3 (LC3), which is a soluble protein with a molecular mass of approximately 17 kDa that occurs throughout many mammalian tissues and cultured cells. In cells, a cytosolic form of LC3 (LC3-I) becomes conjugated to phosphatidylethanolamine to form LC3-phosphatidylethanolamine conjugate (LC3-II). See, for example, Tanida *et al.* (2008) Methods Mol. Biol., vol 445, p. 77-88. The amount of LC3-II relative to LC3-I can be used to analyze changes in the amount of autophagy. Accordingly, in certain embodiments, the administration of one or more of the foregoing compounds increases the ratio of LC3-II to LC3-I in the subject, such as at least about 10%, 25%, 50%, 75%, or 100% increase the ratio of LC3-II to LC3-I in the subject.

[00160] Another exemplary biomarker is p62 protein, also called sequestosome 1 (SQSTM1), which is a ubiquitin-binding scaffold protein that has been reported to colocalize with ubiquitinated protein aggregates. See, for example, Bjorkoy *et al.* (2009) METHODS ENZYMOL., vol. 452, p. 181-197. Accordingly, in certain embodiments, the administration of one or more of the foregoing compounds decreases the amount of p62 protein in the subject, such as by at least about 1%, 5%, 10%, 15%, 25%, 50%, 75%, or 90% w/w reduction in the amount of p62 protein in the subject.

[00161] In certain embodiments, in the method for increasing autophagy, the subject has been diagnosed as having CF. In certain embodiments, in the method for increasing autophagy, the subject has been diagnosed as having a neurodegenerative disease.

[00162] Further, and more generally, another aspect of the invention provides a method of increasing autophagy, wherein the method comprises administering to a subject in need thereof an effective amount of a molecular conjugate comprising a CFTR modulator and a fatty acid covalently linked to cysteamine, wherein the fatty acid is selected from the group consisting of omega-3 fatty acids and fatty acids that are metabolized *in vivo* to omega-3 fatty acids.

[00163] Another aspect of the invention provides a method of treating a disease susceptible to treatment with a fatty acid cysteamine conjugate of a CFTR modulator in a subject in need thereof by administering to the patient an effective amount of a fatty acid cysteamine conjugate of a CFTR modulator.

[00164] Another aspect of the invention provides a method of treating a metabolic disease by administering to a subject in need thereof an effective amount of a fatty acid cystamine conjugate of a CFTR modulator.

Additional Features of Autophagy, CFTR Modulator, Cysteamine, and Conjugates

[00165] Autophagy is an evolutionarily conserved lysosomal degradation pathway to essentially self-digest some of the cellular components (*see*, Levine and Kroemer (2008) CELL, 132, p. 27-42). This self-digestion process serves as a means to help cells remove extraneous or damaged organelles, defective or mis-folded proteins and even invading microorganisms. It is known that autophagy is down-regulated in CF patients (Luciani *et al.* (2011) AUTOPHAGY, 7, p. 104-106). Autophagy also represents an important cellular mechanism for removing pathogens such as *Pseudomonas aegurinosa* from infected tissues such as lungs. Activation of autophagy can potentially help CF patients clear out *Pseudomonas aegurinosa* from their chronically infected lungs (Junkins *et al.* (2013) PLOS ONE, 8, e72263).

[00166] In CF, the defective CFTR causes an up-regulation of reactive oxygen species, which increases the activity of tissue transglutaminase (TG2), an enzyme that facilitates the cross linking between proteins. The increased TG2 activity induces the cross-linking of Beclin-1, a

key protein in regulating autophagy. The cross-linking process of Beclin-1 displaces it from the endoplasmic reticulum, down-regulates autophagy and consequently causes an accumulation of p62 (also referred to SQSTM1). The increased p62 can sequester the mis-folded CFTR into aggresomes, which are then targeted for degradation by proteasomes. It has been observed that when human epithelial cells from CF patients with homozygous ΔF508 mutation were treated with a high concentration of cystamine (250 μM), there was an up-regulation of autophagy and a restoration of the CFTR to the plasma membrane (Luciani *et al.* (2012) AUTOPHAGY, 8, p. 1657-1672; Luciani *et al.* (2010) NAT. CELL BIOL., 12, p. 863-875). The rationale was that cystamine can inhibit TG2 activity, which decreases the cross-linking of BECN1. This process causes a reduction in the level of p62, which then allows the mis-folded CFTR to escape sequestration into the aggresomes and to localize in the Golgi for transport to the membrane. Though promising, this method of restoring activity to the defective CFTR has one major drawback, namely the high concentration of cystamine that is needed to induce autophagy in various epithelial cell lines (250 μM). It is contemplated that this high concentration of cystamine would require a human dose that may be impractical as well as potentially non-compliant to the patients since it is known that cystamine/cysteamine can induce a significant level of GI discomfort at high doses (Kan *et al.* (1984) BRIT. J. EXP. PATHOLOGY, 65, p. 759-765).

[00167] The fatty acid cysteamine conjugates of a CFTR modulator have been designed to bring together a CFTR modulator, a cysteamine and an omega-3 fatty acid into a single molecular conjugate. The activity of the fatty acid cysteamine conjugates of a CFTR modulator is substantially greater than the sum of the individual components of the molecular conjugate, suggesting that the activity induced by the fatty acid cysteamine conjugate of a CFTR modulator is synergistic. Another benefit of the fatty acid cysteamine conjugates of a CFTR modulator of the invention is that they demonstrate very low or no peripheral toxicity.

IV. PHARMACEUTICAL COMPOSITIONS

[00168] The invention provides pharmaceutical compositions comprising a fatty acid cysteamine conjugate of a CFTR modulator, such as a compound of Formula A, A-1, I, or I-A. In certain embodiments, the compound is a compound of Formula A. In certain embodiments, the compound is a compound of Formula A-1. In certain embodiments, the compound is a

compound of Formula I. In certain embodiments, the compound is a compound of Formula I-A. In certain embodiments, the pharmaceutical compositions preferably comprise a therapeutically-effective amount of one or more of the fatty acid cysteamine conjugates of a CFTR modulator described above, formulated together with one or more pharmaceutically acceptable carriers. As described in detail below, the pharmaceutical compositions of the present invention may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets (e.g., those targeted for buccal, sublingual, and/or systemic absorption), boluses, powders, granules, pastes for application to the tongue; (2) parenteral administration by, for example, subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; (3) topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin; (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; (5) sublingually; (6) ocularly; (7) transdermally; or (8) nasally.

[00169] The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[00170] Formulations of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration.

[00171] The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl

cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be formulated for rapid release, e.g., freeze-dried. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

[00172] Liquid dosage forms for oral administration of the compounds of the invention include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

[00173] Administration of the fatty acid cysteamine conjugates of a CFTR modulator can be accomplished via any suitable mode of administration for therapeutic agents. These modes include systemic or local administration such as oral, nasal, parenteral, transdermal, subcutaneous, vaginal, buccal, rectal or topical administration modes.

[00174] Depending on the intended mode of administration, the compositions can be in solid, semi-solid or liquid dosage form, such as, for example, injectables, tablets, suppositories, pills, time-release capsules, elixirs, tinctures, emulsions, syrups, powders, liquids, suspensions, or the like, sometimes in unit dosages and consistent with conventional pharmaceutical practices. Likewise, they can also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous or intramuscular form, all using forms well known to those skilled in the pharmaceutical arts.

[00175] Illustrative pharmaceutical compositions are tablets and gelatin capsules comprising a fatty acid cysteamine conjugate and a pharmaceutically acceptable carrier, such as: a) a diluent, *e.g.*, purified water, triglyceride oils, such as hydrogenated or partially hydrogenated vegetable oil, or mixtures thereof, corn oil, olive oil, sunflower oil, safflower oil, fish oils, such as EPA or DHA, or their esters or triglycerides or mixtures thereof; b) a lubricant, *e.g.*, silica, talcum, stearic acid, its magnesium or calcium salt, sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and/or polyethylene glycol; for tablets also; c) a binder, *e.g.*, magnesium aluminum silicate, starch paste, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, magnesium carbonate, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, waxes and/or polyvinylpyrrolidone, if desired; d) a disintegrant, *e.g.*, starches, agar, methyl cellulose, bentonite, xanthan gum, alginic acid or its sodium salt, or effervescent mixtures; e) absorbent, colorant, flavorant and sweetener; f) an emulsifier or dispersing agent, such as Tween 80, Labrasol, HPMC, DOSS, caproyl 909, labrafac, labrafil, peceol, transcutol, capmul MCM, capmul PG-12, captex 355, gelucire, vitamin E TGPS or other acceptable emulsifier; and/or g) an agent that enhances absorption of the compound such as cyclodextrin, hydroxypropyl–cyclodextrin, PEG400, PEG200.

[00176] Liquid, particularly injectable, compositions can, for example, be prepared by dissolution, dispersion, *etc.* For example, the fatty acid cysteamine conjugate is dissolved in or mixed with a pharmaceutically acceptable solvent such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form an injectable isotonic solution or suspension. Proteins such as albumin, chylomicron particles, or serum proteins can be used to solubilize the fatty acid cysteamine conjugates.

[00177] The fatty acid cysteamine conjugates of a CFTR modulator can be also formulated as a suppository that can be prepared from fatty emulsions or suspensions; using polyalkylene glycols such as propylene glycol, as the carrier.

[00178] The fatty acid cysteamine conjugates of a CFTR modulator can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, containing cholesterol, stearylamine or phosphatidylcholines. In some embodiments, a film of

lipid components is hydrated with an aqueous solution of drug to a form lipid layer encapsulating the drug, as described in United States Patent No. 5,262,564.

[00179] Parenteral injectable administration is generally used for subcutaneous, intramuscular or intravenous injections and infusions. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions or solid forms suitable for dissolving in liquid prior to injection.

[00180] Compositions can be prepared according to conventional mixing, granulating or coating methods, respectively, and the present pharmaceutical compositions can contain from about 0.1 % to about 80 %, from about 5 % to about 60 %, or from about 1 % to about 20 % of the fatty acid cystamine conjugate by weight or volume.

[00181] The dosage regimen utilizing the fatty acid cysteamine conjugates of a CFTR modulator is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal or hepatic function of the patient; and the particular fatty acid cysteamine conjugate of CFTR modulator employed. A physician or veterinarian of ordinary skill in the art can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition.

[00182] Effective dosage amounts of the present invention, when used for the indicated effects, range from about 20 mg to about 5,000 mg of the fatty acid cysteamine conjugate of a CFTR modulator conjugate per day. Compositions for *in vivo* or *in vitro* use can contain about 20, 50, 75, 100, 150, 250, 500, 750, 1,000, 1,250, 2,500, 3,500, or 5,000 mg of the fatty acid cystamine conjugate of a CFTR modulator. In one embodiment, the compositions are in the form of a tablet that can be scored. Effective plasma levels of the fatty acid cysteamine conjugates of a CFTR modulator can range from about 5 ng/mL to 5000 ng/mL per day. Appropriate dosages of the fatty acid cysteamine conjugate of a CFTR modulator can be determined as set forth in Goodman, L. S.; Gilman, A. (1975) THE PHARMACOLOGICAL BASIS OF THERAPEUTICS, 5th ed.; MacMillan: New York, pp. 201-226.

[00183] Fatty acid cysteamine conjugates of a CFTR modulator can be administered in a single daily dose, or the total daily dosage can be administered in divided doses of two, three or

four times daily. Furthermore, fatty acid cysteamine conjugates of a CFTR modulator can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration can be continuous rather than intermittent throughout the dosage regimen. Other illustrative topical preparations include creams, ointments, lotions, aerosol sprays and gels, wherein the concentration of the fatty acid cysteamine conjugate of a CFTR modulator conjugate ranges from about 0.1 % to about 15 %, w/w or w/v.

Combination therapies

[00184] Fatty acid cysteamine conjugates may also be administered with other therapeutic agents such as CFTR modulators, epithelial sodium channel (ENaC) inhibitors, anti-inflammatory agents, anti-fibrotic agents and antibacterial agents. In some embodiments, the other therapeutic agent is a CFTR modulator. Non-limiting examples of a CFTR modulator include Ivacaftor (VX-770), Lumacaftor (VX-809), VX-661, Orkambi (the combination of VX-770 and VX-809), the combination of VX-661 and VX-770, VX-152, VX-440, the combination of VX-152/VX-809 and VX-770, the combination of VX-440/VX-809 and VX-770, P-1037, Riociguat, N91115, QBW251, QR-010, GLPG1837, GLPG2222, GLP2665, genistein, baicalein, epigallocatechin gallate (EGCG), trimethylangelicin and Ataluren.

[00185] In some embodiments, the other therapeutic agent is an anti-inflammatory agent. Non-limiting examples of an anti-inflammatory agent include ibuprofen, prednisolone, dexamethasone, hydrocortisone, methylprednisolone, beclometasone, budesonide, fluticasone, mometasone, Seretide (fluticasone plus salmeterol), Symbicort (budesonide plus formoterol) and N91115.

[00186] In some embodiments, the other therapeutic agent is an anti-bacterial agent. Non-limiting examples of an anti-bacterial agent include azithromycin, tobramycin, aztreonam lysine, colistin, aminoglycosides, vancomycin, ciprofloxacin, levofloxacin and sulfonamides.

[00187] In some embodiments, the other therapeutic agent is an epithelial sodium channel (ENaC) inhibitor. Non-limiting examples of ENaC inhibitors include amiloride, BA-39-9437, GS-9411 and P-1037.

[00188] In some embodiments, the other therapeutic agent is an anti-fibrotic agent. Non-limiting examples of anti-fibrotic agents include pirfenidone, nintedanib, INT-767, STX-100, AM152, pentoxyphilline, FG-3019, CNTO 888, Tralokinumab, SAR156597, GS-6624, BMS-986020, Lebrikizumab, GSK2126458, ACT-064992, vismodegib, PRM-151, IW001 and Fresolimumab.

V. KITS FOR USE IN MEDICAL APPLICATIONS

[00189] Another aspect of the invention provides a kit for treating a disorder. The kit comprises: i) instructions for treating a medical disorder, such as CF; and ii) a fatty acid cysteamine conjugate of a CFTR modulator described herein. The kit may comprise one or more unit dosage forms containing an amount of a fatty acid cysteamine conjugate of a CFTR modulator described herein.

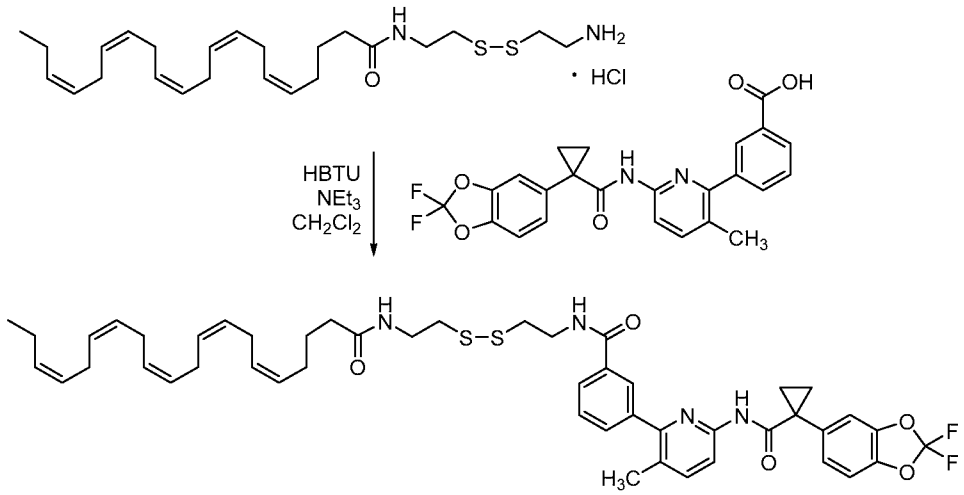
[00190] The description above describes multiple aspects and embodiments of the invention, including a fatty acid cysteamine conjugate of a CFTR modulator, compositions comprising a fatty acid cysteamine conjugate of a CFTR modulator, methods of using the fatty acid cysteamine conjugate of a CFTR modulator, and kits. The invention specifically contemplates all combinations and permutations of the aspects and embodiments.

EXAMPLES

[00191] The disclosure is further illustrated by the following examples, which are not to be construed as limiting this disclosure in scope or spirit to the specific procedures herein described. It is to be understood that the examples are provided to illustrate certain embodiments and that no limitation to the scope of the disclosure is intended thereby.

Example 1

Preparation of 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropane-1-carboxamido)-3-methylpyridin-2-yl)-*N*-(2-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)ethyl)benzamide (I-20)



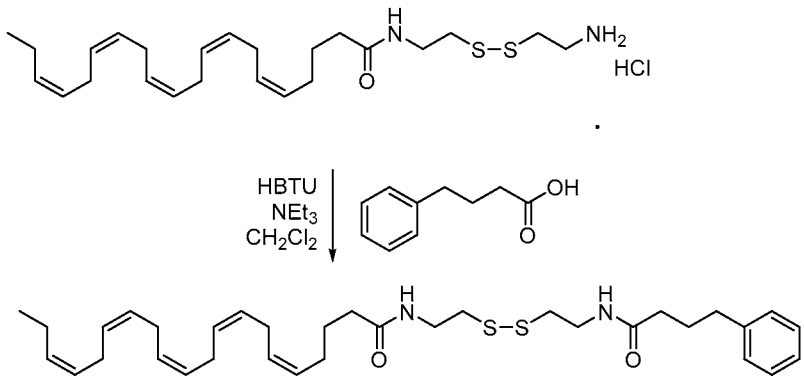
[00192] tert-Butyl (2-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)ethyl)carbamate (5 g, 9.3 mmol, prepared according to the procedures outlined in WO 2012/115695) was dissolved in tetrahydrofuran (5 mL). 4N HCl in dioxane (9.3 mL, 37.2 mmol) was added and the reaction stirred at room temperature overnight. Then, the solvent was azeotroped with CH_3OH/EtOAc and dried under high vacuum to generate (5Z,8Z,11Z,14Z,17Z)-N-(2-((2-aminoethyl)disulfanyl)ethyl)icosa-5,8,11,14,17-pentaenamide as the HCl salt. The product was carried on to the next step without further purification.

[00193] (5Z,8Z,11Z,14Z,17Z)-*N*-(2-((2-Aminoethyl)disulfanyl)ethyl)icosa-5,8,11,14,17-pentaenamide hydrochloride salt (80 mg, 0.184 mmol) was dissolved in dichloromethane (2 mL). 3-(6-(1-(2,2-Difluorobenzo[d][1,3]dioxol-5-yl)cyclopropane-1-carboxamido)-3-methylpyridin-2-yl)benzoic acid (100 mg, 0.221 mmol), HBTU (105 mg, 0.276 mmol) and triethylamine (0.077 mL, 0.552 mmol) were added successively. The resulting reaction mixture stirred at room temperature overnight. Then, the solution was diluted with dichlormethane (2 mL) and washed with ½ saturated NH_4Cl and brine. The organic layer was

dried over sodium sulfate and the solvent was evaporated under reduced pressure. The resulting residue was purified via silica gel chromatography with a gradient of 0-60% ethyl acetate in pentane to generate the title compound as a white gel (42 mg). MS (EI) calc'd for $C_{48}H_{56}F_2N_4O_5S_2$: 870.37; Found: 871 $[M+H]^+$.

Example 2

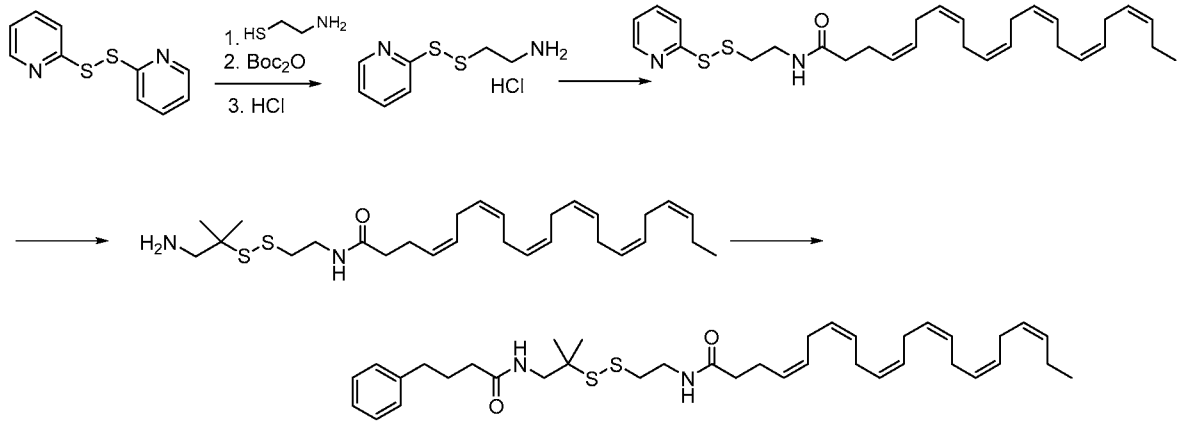
Preparation of (5Z,8Z,11Z,14Z,17Z)-*N*-(2-((2-(4-phenylbutanamido)ethyl) disulfanyl)ethyl)icosa-5,8,11,14,17-pentaenamide (I-2)



[00194] (5Z,8Z,11Z,14Z,17Z)-*N*-(2-((2-Aminoethyl)disulfanyl)ethyl)icosa-5,8,11,14,17-pentaenamide hydrochloride salt (1.9 g, 4.35 mmol, prepared according to the procedures outlined in WO 2012/115695) was dissolved in dichloromethane (15 mL). 4-Phenylbutyric acid (1.07 g, 6.52 mmol), HBTU (2.48 g, 6.52 mmol), and triethylamine (1.8 mL, 13.0 mmol) were added in succession. The resulting reaction mixture stirred at room temperature overnight. Then, the solution was diluted with dichlormethane (15 mL) and washed with ½ saturated NH_4Cl and brine. The organic layer was dried over sodium sulfate and the solvent was evaporated under reduced pressure. The resulting residue was purified via silica gel chromatography with a gradient of 0-12% CH_3OH in CH_2Cl_2 to generate the title compound as an off-white waxy solid (895 mg). MS (EI) calc'd for $C_{34}H_{50}N_2O_2S_2$: 582.33; Found: 584 $[M+H]^+$.

Example 3

Preparation of (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-((2-methyl-1-(4-phenylbutanamido)propan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (I-3):



[00195] A solution containing cysteamine hydrochloride (7.87 g, 69.3 mmol) in methanol (100 mL) was added dropwise to a solution of 2,2'-dithiopyridine (25.0 g, 113.6 mmol) in methanol (300 mL). The resulting reaction mixture was stirred at room temperature for 18 hours. Di-*tert*-butyl dicarbonate (15.1 g, 69.3 mmol) and aqueous sodium hydroxide (5*M* , 30 mL) were added slowly. The reaction mixture was stirred at room temperature for an additional 4 hr. The mixture was then extracted with ethyl acetate (300 mL×2). The combined organic layers were washed with brine (300 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (hexanes/ethyl acetate = 10:1 to 5:1) to afford *tert*-butyl 2-(pyridin-2-yldisulfanyl)ethylcarbamate (8.3 g, 42.3% yield) as a yellow oil.

[00196] *tert*-Butyl 2-(pyridin-2-yldisulfanyl)ethylcarbamate (5.8 g, 25.6 mmol) was dissolved in 1,4-dioxane (30 mL) and the solution was cooled to 0°C. A solution of HCl in 1,4-dioxane (5M, 20 mL) was then added dropwise. The resulting reaction mixture was stirred for 2 hours and then concentrated under reduced pressure to afford 2-(pyridin-2-yldisulfanyl)ethanamine (5.6 g, 100% yield, HCl salt).

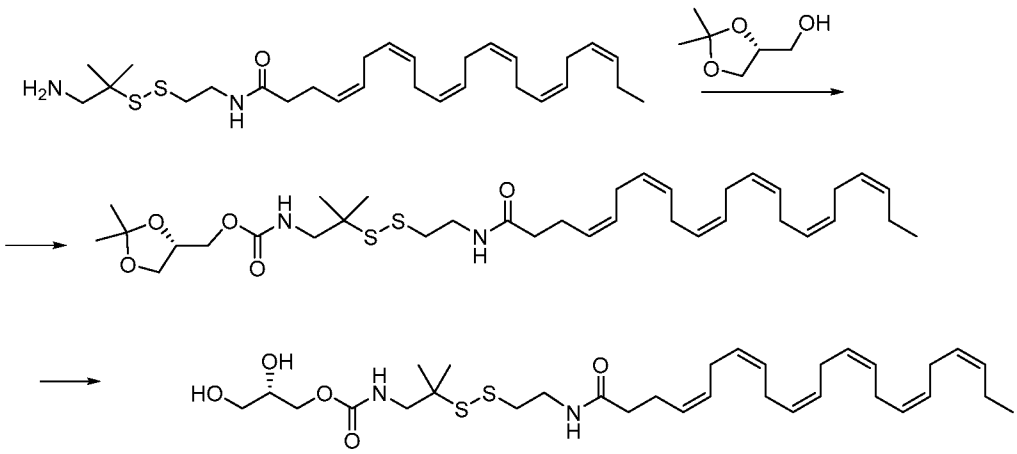
[00197] A mixture of 2-(pyridin-2-yldisulfanyl)ethanamine (12.0 g, 64.5 mmol), DHA (51.6 mmol) and HATU (29.3 g, 77 mmol) in DCM (150 mL) was cooled to 0°C and Hunig's base (25 g, 190 mmol) was added. The resulting reaction mixture was allowed to warm to room temperature and stirred for 18 hours. Saturated aqueous NH_4Cl (200 mL) was added to quench the reaction and the resulting mixture was extracted with CH_2Cl_2 (300 mL×2). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (methanol/CH_2Cl_2 = 0.5% to 2.0%) to afford (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(pyridin-2-yldisulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide as a yellow oil.

[00198] A solution containing (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(pyridin-2-yldisulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (21.3 mmol, 1.0 eq) in 10 mL of 1:1 MeOH/DMF was added slowly, dropwise, to a solution containing 1-amino-2-methylpropane-2-thiol (21.3 mmol, 1.0 eq) in 20 mL ofMeOH/DMF (1: 1). The resulting reaction mixture was stirred at room temperature for 18 hours. The mixture was diluted with water and extracted with EtOAc. The combined organic layers were washed with water (2 × 100 mL), brine (100 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (CH_2Cl_2/MeOH) to afford (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-((1-amino-2-methylpropan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide.

[00199] A mixture containing (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-((1-amino-2-methylpropan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (5 mmol), 4-phenylbutanoic acid (5 mmol), HATU (6.1 mmol) and Hunig's base (7.5 mmol) in 10 mL of CH_2Cl_2 was stirred at room temperature for 8 hours. The reaction mixture was then diluted with saturated aqueous NH_4Cl and the two layers were separated. The organic layer was washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. Purification by chromatography (CH_2Cl_2/MeOH) afforded (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-((2-methyl-1-(4-phenylbutanamido)propan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide. MS (EI) calc'd for $C_{38}H_{56}N_2O_2S_2$ 636.38; found 637 $[M+H]^+$.

Example 4

Preparation of (S)-2,3-dihydroxypropyl (2-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-2-methylpropyl)carbamate (I-10):

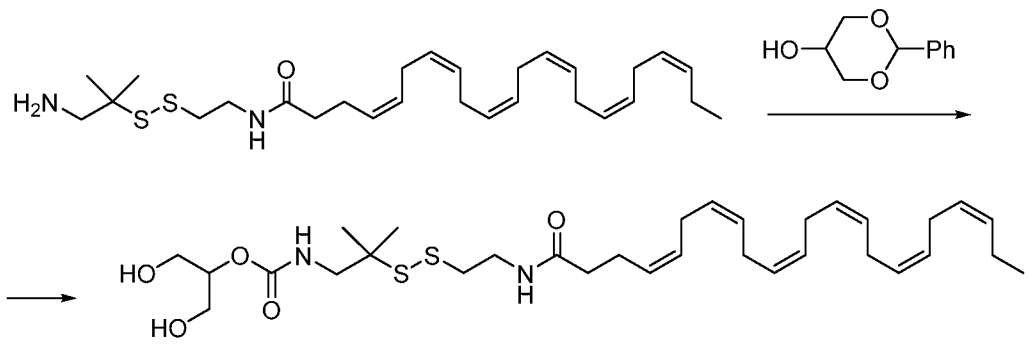


[00200] Triphosgene (4.4 g, 15 mmol) and pyridine (880 mg, 12.3 mmol) in benzene (10 mL) was added to the solution containing (R)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (2 g, 15 mmol) in CH_2Cl_2 (20 mL). The resulting reaction mixture was stirred at room temperature for 2 hours and concentrated under reduced pressure. The resulting residue, along with (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-((1-amino-2-methylpropan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (4 g, 8.2 mmol) (prepared essentially as described in Example 3) were taken up in CH_2Cl_2 (20 mL). Triethylamine (2.27 g, 22.5 mmol) was then added slowly. The resulting reaction mixture was stirred at room temperature for 18 hours and then concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (pentanes/EtOAc) to afford 3.4 g of ((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl (2-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-2-methylpropyl)carbamate (34% yield) as a colorless oil. This material (3.1 g, 4.78 mmol) was dissolved in 30 mL of THF and 2 N HCl (5 mL) was added. The resulting reaction mixture was stirred at room temperature for 18 hours. Enough $NaHCO_3$ was then added to neutralize the reaction mixture. The resulting aqueous mixture was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. Purification by silica gel chromatography (pentanes/EtOAc) afford 1.76 g of (S)-2,3-dihydroxypropyl (2-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-

docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-2-methylpropyl)carbamate (59% yield) as a light brown oil. MS (EI) calc'd for $C_{32}H_{52}N_2O_5S_2$ 608.33; found 609 $[M+H]^+$.

Example 5

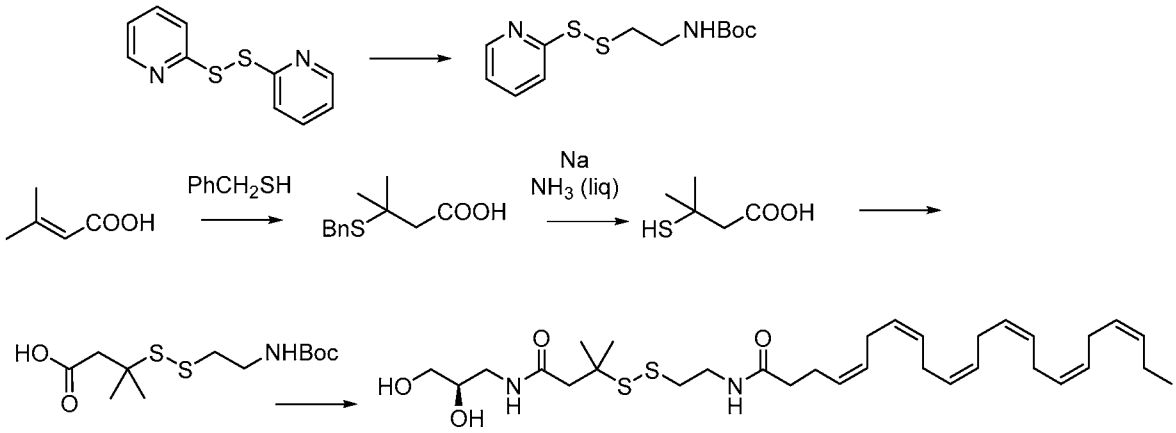
Preparation of 1,3-dihydroxypropan-2-yl (2-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-2-methylpropyl)carbamate (I-11):



[00201] The same experimental procedure outlined in the preparation of (S)-2,3-dihydroxypropyl (2-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-2-methylpropyl)carbamate (*see*, Example 4) was used, except that 2-phenyl-1,3-dioxan-5-ol was substituted as the appropriate starting material. MS (EI) calc'd for $C_{32}H_{52}N_2O_5S_2$ 608.33; found 609 $[M+H]^+$.

Example 6

Preparation of (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-((4-(((R)-2,3-dihydroxypropyl)amino)-2-methyl-4-oxobutan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (I-14):



[00202] A solution of cysteamine hydrochloride (7.87 g, 69.3 mmol) in methanol (100 mL) was added dropwise to a solution of 2,2'-dithiopyridine (25.0 g, 113.6 mmol) in methanol (300 mL). The resulting reaction mixture was stirred at room temperature for 18 hr. Di-tert-butyl dicarbonate (15.1 g, 69.3 mmol) and aqueous sodium hydroxide (5M, 30 mL) were then added slowly. The resulting reaction mixture was stirred at room temperature for 4 hours and then extracted with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (hexane/EtOAc) to afford 8.3 g of tert-butyl (2-(pyridin-2-yldisulfanyl)ethyl)carbamate with 1,2-di(pyridin-2-yl)disulfane (42% yield) as a yellow oil.

[00203] Separately, a mixture of 3-methylbut-2-enoic acid (20.0 g, 0.2 mol) and benzyl mercaptan (25.0 g, 0.2 mol) in piperidine (40 mL) was stirred under reflux for 18 hours. The reaction mixture was cooled in an ice-bath, then acidified with 6M aqueous HCl and then extracted with EtOAc. The combined organic layers were washed with brine dried (Na_2SO_4) and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (hexane/EtOAc) to afford 30 g of 3-(benzylthio)-3-methylbutanoic acid (67% yield) as a yellow oil. This material (15.0 g, 66.9 mmol) was added in one portion to liquid ammonia (100 mL) at -60°C. Sodium (3.1 g, 134.7 mmol) was then added in small pieces over a 30 minute period. The resulting reaction mixture was stirred for 3 hours at -60°C and then slowly warmed to room temperature. A stream of nitrogen was passed through the mixture to remove an excess of ammonia. The residue was dissolved in dilute aqueous hydrochloric acid and then extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried

(Na_2SO_4) and concentrated under reduced pressure to afford 8.3 g of 3-mercapto-3-methylbutanoic acid (92% crude yield).

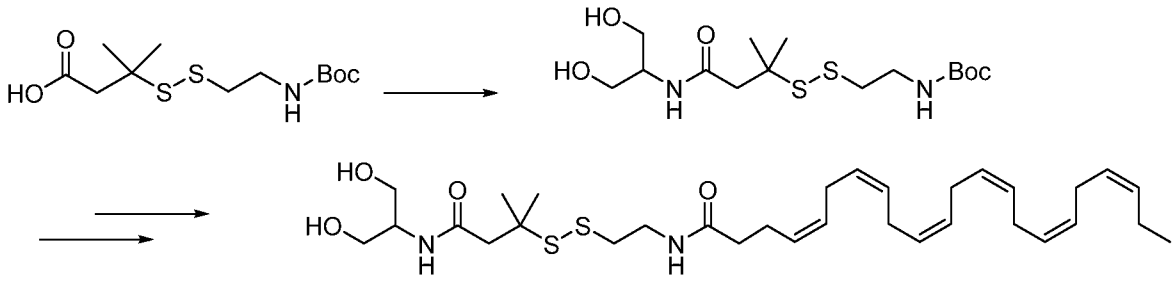
[00204] A solution of containing 3-mercapto-3-methylbutanoic acid (4.7 g, 35.1 mmol) in MeOH (50 mL) was added dropwise to a solution of tert-butyl (2-(pyridin-2-yldisulfanyl)ethyl)carbamate (10.0 g, 34.9 mmol) in MeOH (100 mL). The resulting reaction mixture was stirred at room temperature for 18 hours and then extracted with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (hexane/EtOAc) to afford 3-((2-((tert-butoxycarbonyl)amino)ethyl)disulfanyl)-3-methylbutanoic acid (6.2 g) as a yellow oil.

[00205] A mixture containing 3-((2-((tert-butoxycarbonyl)amino)ethyl)disulfanyl)-3-methylbutanoic acid (2.8 g, 9.0 mmol), (R)-3-aminopropane- 1,2-diol (0.83 g, 9.0 mmol) and HATU (4.1 g, 10.8 mmol) in CH_2Cl_2 (50 mL) was cooled to 0°C and Hunig's base (3.5 g, 27.1 mmol) was added. The resulting reaction mixture was allowed to warm to room temperature and stirred for 18 hours. Saturated NH_4Cl (30 mL) was added and the resulting mixture was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (CH_2Cl_2/MeOH) to afford 2.3 g of tert-butyl (R)-(2-((4-((2,3-dihydroxypropyl)amino)-2-methyl-4-oxobutan-2-yl)disulfanyl)ethyl)carbamate (66% yield) as a yellow oil. This material (2.3 g, 6.0 mmol) was dissolved in 1,4-dioxane (8 mL) and the solution was then cooled to 0 °C while 5M HCl/1,4-dioxane (20 mL) was added drop wise. The resulting reaction mixture was stirred at 0 °C for 2 hours, then concentrated under reduced pressure to give (R)-3-((2-aminoethyl)disulfanyl)-N-(2,3-dihydroxypropyl)-3-methylbutanamide. A mixture containing (R)-3-((2-aminoethyl)disulfanyl)-N-(2,3-dihydroxypropyl)-3-methylbutanamide (HCl salt, 1.9 g, 6.0 mmol), DHA (1.58 g, 4.8 mmol) and HATU (2.75 g, 7.2 mmol) in CH_2Cl_2 (50 mL) was cooled to 0°C Hunig's base (2.3 g, 18.0 mmol) was added. The reaction mixture was allowed to warm to room temperature and stirred for 18 hours. Saturated NH_4Cl (30 mL) was added and the resulting mixture was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduce pressure. Purification by silica gel chromatography

(CH_2Cl_2/MeOH) afforded 2.0 g of (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-((4-(((R)-2,3-dihydroxypropyl)amino)-2-methyl-4-oxobutan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (70% yield). MS (EI) calc'd for $C_{32}H_{52}N_2O_4S_2$ 592.34; found 593 $[M+H]^+$.

Example 7

Preparation of (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-((4-((1,3-dihydroxypropan-2-yl)amino)-2-methyl-4-oxobutan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (I-15):

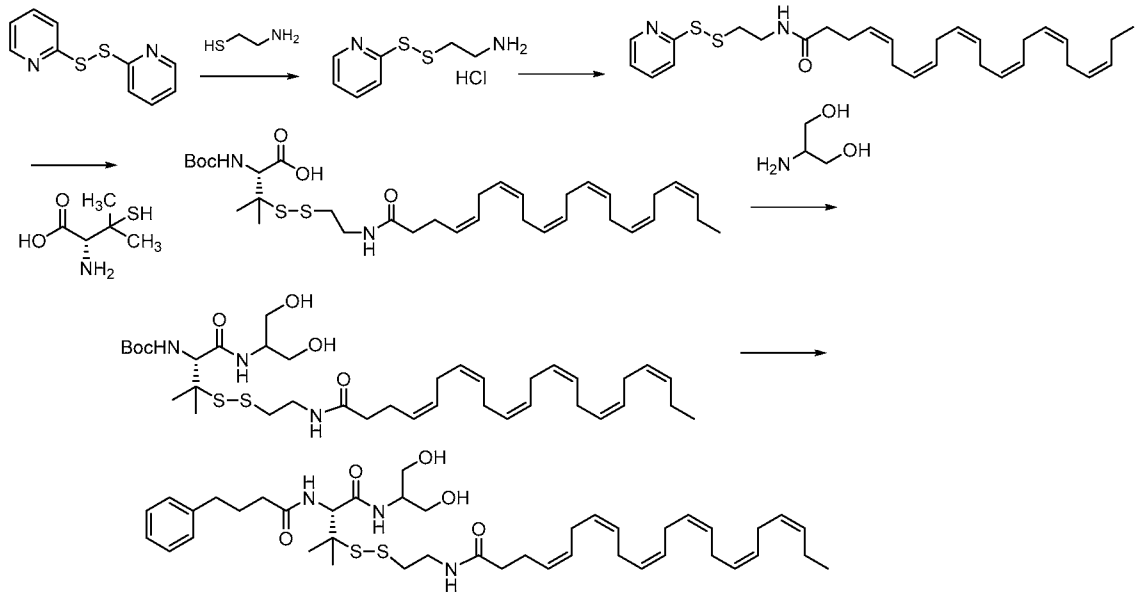


[00206] The same experimental procedure outlined in the synthesis of (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-((4-(((R)-2,3-dihydroxypropyl)amino)-2-methyl-4-oxobutan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide was used (*see*, Example 6), substituting 2-aminopropane-1,3-diol as the appropriate starting material. MS (EI) calc'd for $C_{32}H_{52}N_2O_4S_2$ 592.34; found 593 $[M+H]^+$.

Example 8

Preparation of (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-((1,3-dihydroxypropan-2-yl)amino)-2-methyl-4-oxo-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-

4,7,10,13,16,19-hexaenamide (I-25):



[00207] A solution containing cysteamine hydrochloride (7.87 g, 69.3 mmol) in methanol (100 mL) was added dropwise to a solution of 2,2'-dithiopyridine (25.0 g, 113.6 mmol) in methanol (300 mL). The resulting reaction mixture was stirred at room temperature for 18 hours. Di-*tert*-butyl dicarbonate (15.1 g, 69.3 mmol) and aqueous sodium hydroxide (5*M* , 30 mL) were added slowly. The reaction mixture was stirred at room temperature for an additional 4 hours. The mixture was then extracted with ethyl acetate (300 mL×2). The combined organic layers were washed with brine (300 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (hexanes/ethyl acetate = 10:1 to 5:1) to afford *tert*-butyl 2-(pyridin-2-yldisulfanyl)ethylcarbamate (8.3 g, 42.3% yield) as a yellow oil.

[00208] *tert*-Butyl 2-(pyridin-2-yldisulfanyl)ethylcarbamate (5.8 g, 25.6 mmol) was dissolved in 1,4-dioxane (30 mL) and the solution was cooled to 0°C. A solution of HCl in 1,4-dioxane (5M, 20 mL) was then added dropwise. The resulting reaction mixture was stirred for 2 hours and then concentrated under reduced pressure to afford 2-(pyridin-2-yldisulfanyl)ethanamine (5.6 g, 100% yield, HCl salt).

[00209] A mixture of 2-(pyridin-2-yldisulfanyl)ethanamine (12.0 g, 64.5 mmol), DHA (51.6 mmol) and HATU (29.3 g, 77 mmol) in CH_2Cl_2 (150 mL) was cooled to 0°C and Hunig's base

(25 g, 190 mmol) was added. The resulting reaction mixture was allowed to warm to room temperature and stirred for 18 hours. Saturated aqueous NH_4Cl (200 mL) was added to quench the reaction and the resulting mixture was extracted with CH_2Cl_2 (300 mL×2). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (methanol/CH_2Cl_2 = 0.5% to 2.0%) to afford (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(pyridin-2-yldisulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide as a yellow oil.

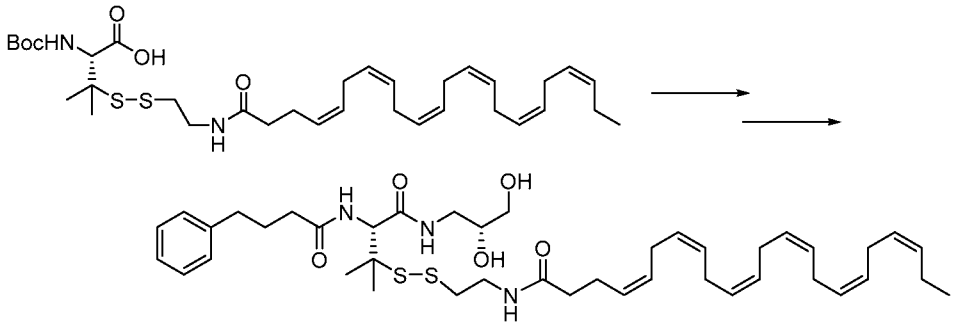
[00210] A mixture of (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(pyridin-2-yldisulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (4.8 g, 9.6 mmol) and (R)-2-amino-3-mercapto-3-methyl butanoic acid (1.44 g, 9.6 mol) in MeOH (100 mL) were stirred at room temperature for 18 hours. Di-tert-butyl dicarbonate (2.1 g, 9.6 mmol) was then added, followed by the slow addition of 3 M aqueous sodium hydroxide solution (30 mL). The resulting reaction mixture was stirred at room temperature for 4 hours and then extracted with EtOAc (100 mL×2). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (methanol/CH_2Cl_2 = 0% to 1.5%) to afford (R)-2-((tert-butoxycarbonyl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methylbutanoic acid (5.4 g, 88.2% yield) as a yellow oil.

[00211] A mixture of 2-aminopropane-1,3-diol (0.17 g, 1.9 mmol), (R)-2-((tert-butoxycarbonyl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methylbutanoic acid (1.2 g, 1.9 mmol) and HATU (0.86 g, 2.2 mmol) in CH_2Cl_2 (20 mL) was cooled to 0°C and Hunig's base (0.73 g, 5.6 mmol) was added. The reaction mixture was allowed to warm to room temperature and stirred for 18 hours. Saturated aqueous NH_4Cl (20 mL) was added and the resulting mixture was extracted with CH_2Cl_2 (50 mL×2). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (CH_2Cl_2/MeOH to afford tert-butyl ((R)-1-((1,3-dihydroxypropan-2-yl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-1-oxobutan-2-yl)carbamate as a yellow oil.

[00212] A mixture containing tert-butyl ((R)-1-((1,3-dihydroxypropan-2-yl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-1-oxobutan-2-yl)carbamate (1.5 mmol) in 1,4-dioxane (5 mL) was cooled to 0 °C and a solution containing 5M HCl in 1,4-dioxane (8 mL) was added dropwise. The resulting reaction was stirred for 2 hours then concentrated under reduced pressure to afford the HCl salt of (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-3-amino-4-((1,3-dihydroxypropan-2-yl)amino)-2-methyl-4-oxobutan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide. This material (1 mmol) was taken up in 10 mL of CH_2Cl_2 along with 4-phenylbutyric acid (1 mmol), HATU (1.2 mmol) and Hunig's base (1.5 mmol). The resulting reaction mixture was stirred at room temperature for 8 hours, diluted with saturated aqueous NH_4Cl and extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (CH_2Cl_2/MeOH) to afford (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-((1,3-dihydroxypropan-2-yl)amino)-2-methyl-4-oxo-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide. MS (EI) calc'd for $C_{42}H_{63}N_3O_5S_2$ 753.42; found 754 $[M+H]^+$.

Example 9

Preparation of (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-(((R)-2,3-dihydroxypropyl)amino)-2-methyl-4-oxo-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (I-26):

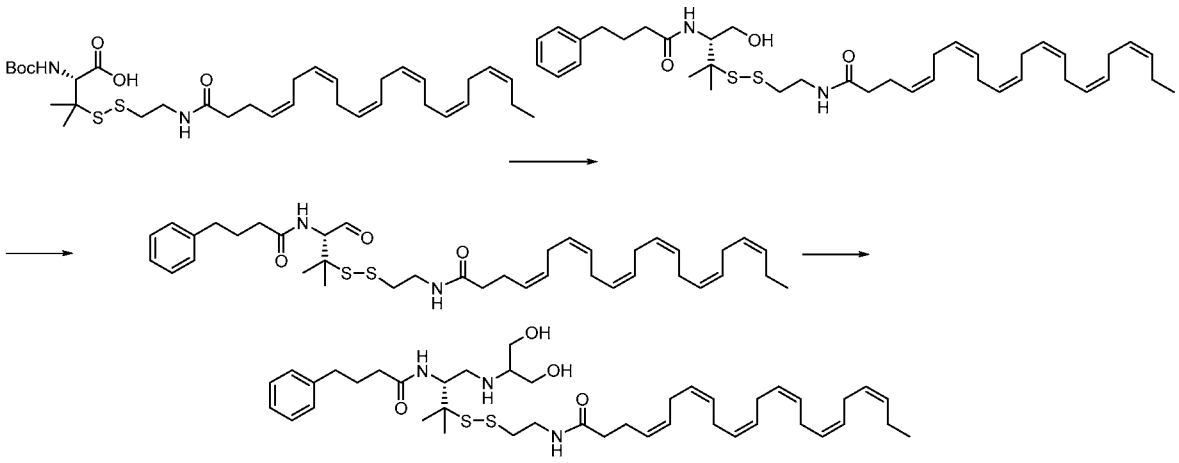


[00213] The same experimental procedures outlined in the synthesis of (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-((1,3-dihydroxypropan-2-yl)amino)-2-methyl-4-oxo-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (*see*,

Example 8) was used, substituting (R)-3-aminopropane- 1,2-diol as the appropriate starting material. MS (EI) calc'd for $C_{42}H_{63}N_3O_5S_2$ 753.42; found 754 $[M+H]^+$.

Example 10

Preparation of (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-((1,3-dihydroxypropan-2-yl)amino)-2-methyl-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (I-27):



[00214] (R)-2-((tert-Butoxycarbonyl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methylbutanoic acid (8.0 g, 12.6 mmol (prepared as described in Example 8) was dissolved in THF (100 ml) and the solution was then cooled to -15°C while N-methylmorpholine (1.3 g, 13 mmol) was added followed by isobutyl carbonochloridate (1.8 g, 13 mmol). The resulting reaction mixture was stirred for 30 minutes. It was then warmed to room temperature and filtered. The filtrate was cooled to -20°C, a suspension of sodium borohydride (0.96 g, 25 mmol) in water (2 mL) was added carefully. The resulting reaction mixture was stirred for 2 h and then quenched with water (200 mL). The resulting mixture was extracted with CH_2Cl_2 (200 mL×4). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (methanol/CH_2Cl_2 = 0.5% to 1.2%) to afford tert-butyl ((R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-1-hydroxy-3-methylbutan-2-yl)carbamate (5.6 g, 71.8% yield) as a yellow oil. This material (5.6 g, 9.03 mmol) was dissolved in 1,4-dioxane (10 mL) and the

solution was cooled to 0 °C while 5M HCl/1,4-dioxane (15 mL) was added dropwise. The resulting reaction mixture was stirred at room temperature for 30 minutes and then concentrated under reduced pressure to afford the HCl salt of (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-3-amino-4-hydroxy-2-methylbutan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide.

[00215] The HCl salt of (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-3-amino-4-hydroxy-2-methylbutan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (5.6 g, 9.03 mmol) was taken up in CH_2Cl_2 (10 mL) along with 4-phenylbutyric acid (1.5 g, 9 mmol) and HATU (4.1 g, 10.8 mmol). The mixture was cooled to 0°C and Hunig's base (1.5 g, 27 mmol) was added. The resulting reaction mixture was allowed to warm to room temperature and stirred for 2 hours. Saturated NH_4Cl (30 mL) was added and the resulting mixture was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (CH_2Cl_2/MeOH) to afford 3.5 g of (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-hydroxy-2-methyl-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (58% yield) as a yellow oil.

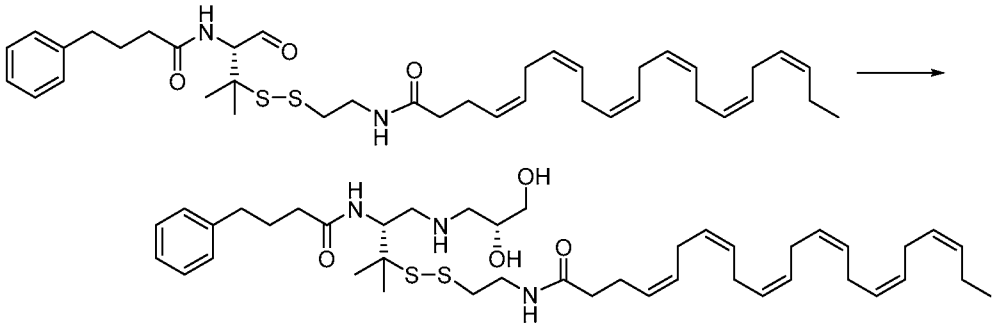
[00216] N-((R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-1-hydroxy-3-methylbutan-2-yl)nicotinamide (2.0g, 3.0 mmol) was dissolved in CH_2Cl_2 (50 mL) and the solution was then cooled to 0°C while Dess-Martin periodinane (1.9 g, 4.5 mmol) was added. The reaction was allowed to warm to room temperature and stirred for 2 hours. The resulting mixture was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure to afford 2.1 g of the crude intermediate aldehyde, namely (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-hydroxy-2-methyl-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide.

[00217] 2-Aminopropane-1,3-diol (1.0 mmol) and (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-hydroxy-2-methyl-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (1.0 mmol) in methanol (30 mL) was stirred for 1 hour, and sodium cyanoborohydride (2.0 mmol) was added. The resulting reaction mixture was allowed to warm to room temperature and stirred for overnight. Saturated aqueous NH_4Cl (20 mL) is added and

the resulting mixture was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers are washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH) to afford (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-((1,3-dihydroxypropan-2-yl)amino)-2-methyl-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide. MS (EI) calc'd for $C_{42}H_{65}N_3O_4S_2$ 739.44; found 740 $[M+H]^+$.

Example 11

Preparation of (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-(((R)-2,3-dihydroxypropyl)amino)-2-methyl-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (I-28)

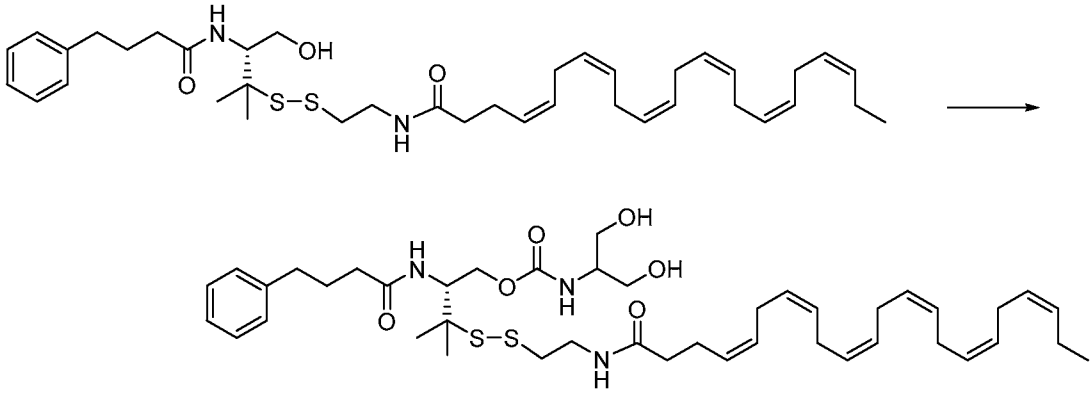


[00218] The same experimental procedure outlined in the preparation of (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-((1,3-dihydroxypropan-2-yl)amino)-2-methyl-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (*see*, Example 10) was used, except that (R)-3-aminopropane-1,2-diol was substituted as the appropriate starting material. MS (EI) calc'd for $C_{42}H_{65}N_3O_4S_2$ 739.44; found 740 $[M+H]^+$.

Example 12

Preparation of (R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-2-(4-phenylbutanamido)butyl (1,3-

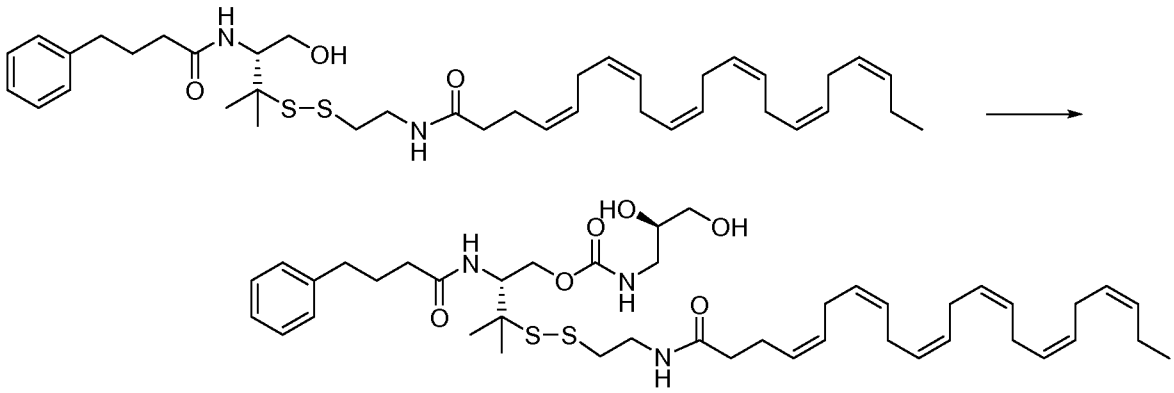
dihydroxypropan-2-yl)carbamate (I-29):



[00219] (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-Hydroxy-2-methyl-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (3.0 g, 4.5 mmol) (*see*, Example 10) was dissolved in THF (50 mL) and the solution was then cooled to 0°C while pyridine (1.4 g, 18 mmol) and 4-nitrophenyl chloroformate (1.36 g, 6.75 mmol) were added. The resulting reaction mixture was allowed to warm to room temperature and stirred for 18 hours. It was then extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (CH_2Cl_2/MeOH) to afford (R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-2-(4-phenylbutanamido)butyl (4-nitrophenyl) carbonate (2.8 g, 74.8% yield) as a yellow oil. A mixture of 2-aminopropane-1, 3-diol (0.16 g, 1.8 mmol), (R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-2-(4-phenylbutanamido)butyl (4-nitrophenyl) carbonate (1.4 g, 1.7 mmol) in DMF (20 mL) was cooled to 0°C and Hunig's base (0.44 g, 3.4 mmol) was added. The reaction mixture was allowed to warm to room temperature and stirred for 18 hours. Saturated NH_4Cl (20 mL) was added and the resulting mixture was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (CH_2Cl_2/MeOH) to afford 0.75 g of (R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-2-(4-phenylbutanamido)butyl (1,3-dihydroxypropan-2-yl)carbamate (56% yield) as a yellow oil. MS (EI) calc'd for $C_{43}H_{65}N_3O_6S_2$ 783.43; found 784 $[M+H]^+$.

Example 13

Preparation of (R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-2-(4-phenylbutanamido)butyl ((R)-2,3-dihydroxypropyl)carbamate (I-30):

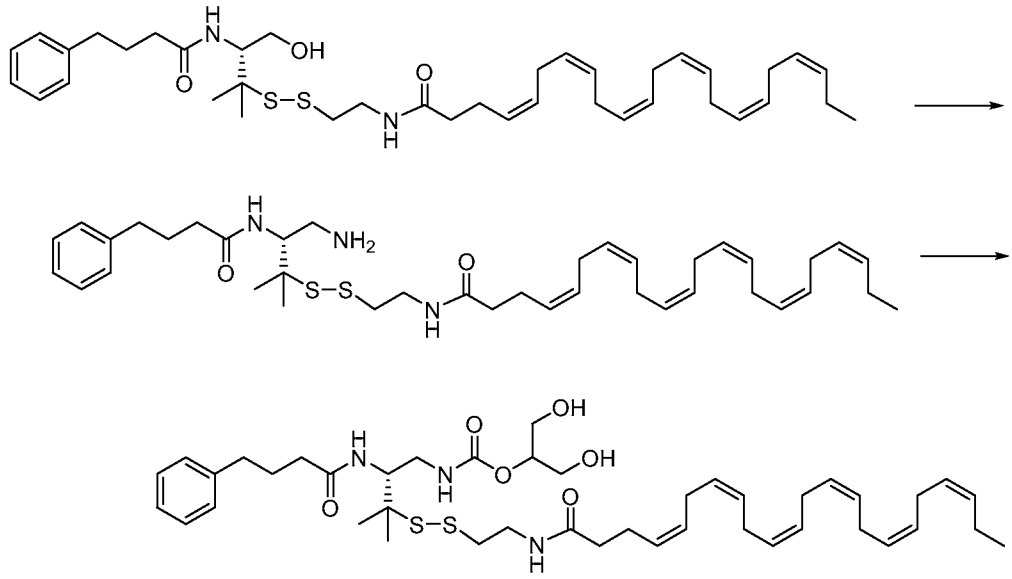


[00220] The same experimental procedure outlined in the preparation of (R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-2-(4-phenylbutanamido)butyl (1,3-dihydroxypropan-2-yl)carbamate was used (*see*, Example 12), except that (R)-3-aminopropane-1,2-diol was substituted as the appropriate starting material. MS (EI) calc'd for $C_{43}H_{65}N_3O_6S_2$ 783.43; found 784 $[M+H]^+$.

Example 14

Preparation of 1,3-dihydroxypropan-2-yl ((R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-2-(4-

phenylbutanamido)butyl)carbamate (I-31):



[00221] A mixture containing (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-hydroxy-2-methyl-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (2.5 g, 3.6 mmol) (*see*, Example 10), phthalimide (0.78 g, 5.3 mmol) and triphenylphoshpine (1.4 g, 5.3 mmol) in THF (50 mL) was cooled to 0°C and diisopropylazodicarboxylate (DIAD, 1.1 g, 17 mmol) was added. The reaction mixture was allowed to warm to room temperature and stirred for 2 hours. Saturated NH_4Cl (20 mL) was added and the resulting mixture was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (CH_2Cl_2/MeOH) to afford the corresponding phthalimide.

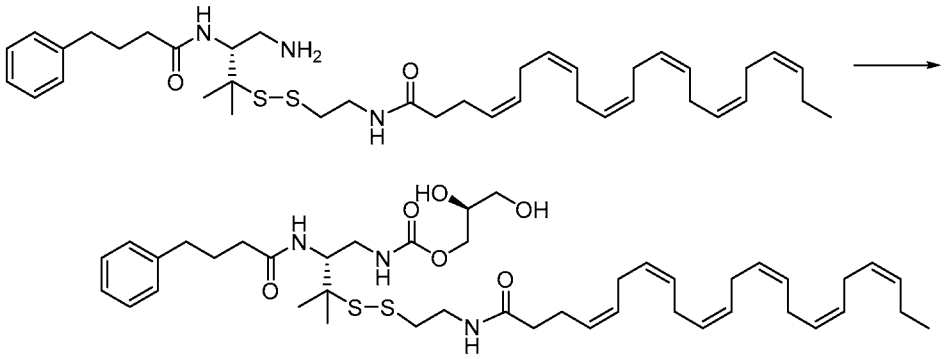
[00222] This phthalimide (2.0 g, 2.5 mmol) was dissolved in ethanol (20 mL) and NH_2-NH_2.H_2O (85%, 6 mL) was added. The resulting reaction mixture was stirred for 30 minutes. The resulting reaction mixture was quenched with water (60 mL) and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The resulting residue was purified by silica gel (methanol/CH_2Cl_2) to afford (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-amino-2-methyl-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide.

[00223] 2-Phenyl-1, 3-dioxan-5-ol (0.5 g, 2.7 mmol) was dissolved in THF (50 mL) and cooled to 0°C while pyridine (0.44 g, 5.4 mmol) and 4-nitrophenyl chloroformate (0.84 g, 4.1 mmol) were added. The resulting reaction mixture was allowed to warm to room temperature and stirred for 2 hours. The resulting mixture was extracted with EtOAc (2 × 30 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The resulting residue wasa purified by silica gel chromatography (hexanes/EtOAc) to afford 4-nitrophenyl (2-phenyl-1,3-dioxan-5-yl) carbonate. This material (0.42 g, 1.2 mmol) and (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-amino-2-methyl-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (0.8 g, 1.2 mmol) were taken up in DMF (20 mL). The mixture was cooled to 0°C and Hunig's base (0.31 g, 2.4 mmol) was added. The resulting reaction mixture was allowed to warm to room temperature and stirred for 18 hours. Saturated aqueous NH_4Cl (20 mL) was added and the resulting mixture was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The resulting residue was treated with 3N HCl-dioxane (5 mL) and MeOH (1 mL) at room temperature for 2 hours and then concentrated under reduced pressure. Purification by silica gel chromatography ($MeOH/CH_2Cl_2$) afforded 1,3-dihydroxypropan-2-yl ((R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-2-(4-phenylbutanamido)butyl)carbamate. MS (EI) calc'd for $C_{43}H_{65}N_3O_6S_2$ 783.43; found 784 $[M+H]^+$.

Example 15

Preparation of (S)-2,3-dihydroxypropyl ((R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-2-(4-

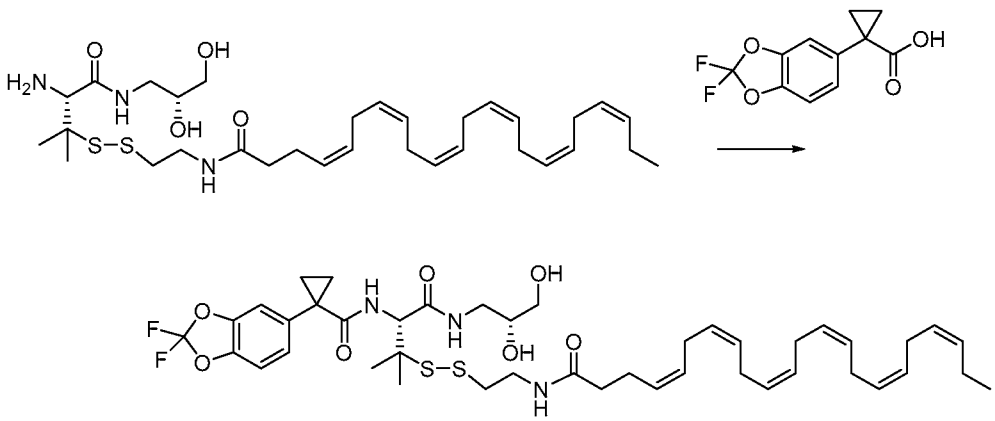
phenylbutanamido)butyl)carbamate (I-32):



[00224] The same experimental procedure outlined in the preparation of 1,3-dihydroxypropan-2-yl ((R)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-2-(4-phenylbutanamido)butyl)carbamate (*see*, Example 14) was used, except that (R)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol was substituted as the appropriate starting material. MS (EI) calc'd for $C_{43}H_{65}N_3O_6S_2$ 783.43; found 784 $[M+H]^+$.

Example 16

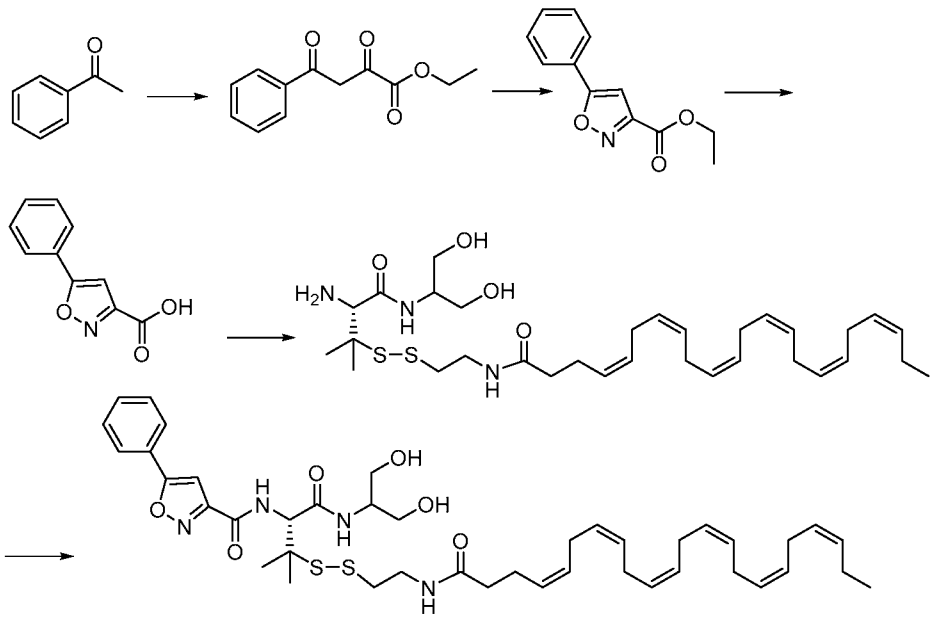
Preparation of 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-((R)-1-(((R)-2,3-dihydroxypropyl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-1-oxobutan-2-yl)cyclopropane-1-carboxamide (I-34):



[00225] 1-(2,2-Difluorobenzo[d][1,3]dioxol-5-yl)-N-((R)-1-(((R)-2,3-dihydroxypropyl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-1-oxobutan-2-yl)cyclopropane-1-carboxamide was prepared using the same general amide coupling procedure described in Example 8. 1-(2,2-Difluorobenzo[d][1,3]dioxol-5-yl)cyclopropane-1-carboxylic acid was commercially available. (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-3-Amino-4-(((R)-2,3-dihydroxypropyl)amino)-2-methyl-4-oxobutan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide was prepared using the procedure outlined in the synthesis of (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-3-amino-4-((1,3-dihydroxypropan-2-yl)amino)-2-methyl-4-oxobutan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (*see*, Example 8), except that (R)-3-aminopropane-1,2-diol was substituted as the appropriate starting material. MS (EI) calc'd for $C_{49}H_{59}F_2N_3O_7S_2$ 831.38; found 832 $[M+H]^+$.

Example 17

Preparation of N-((R)-1-((1,3-dihydroxypropan-2-yl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-1-oxobutan-2-yl)-5-phenylisoxazole-3-carboxamide (I-41):

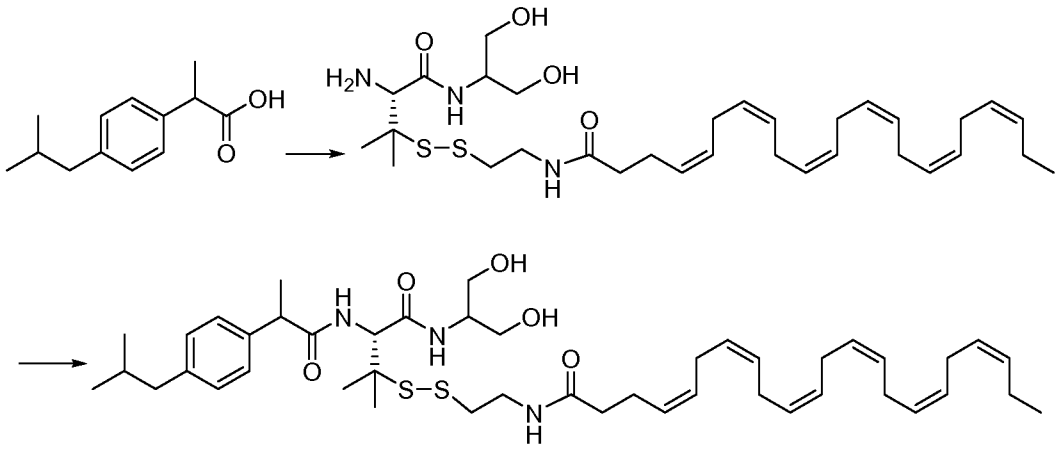


[00226] Acetophenone (3 g, 25 mmol) was taken up in 30 mL of dry toluene and NaH (780 mg, 32 mmol) was then added. The resulting reaction mixture was stirred at room temperature

for 60 minutes. A solution of diethyl oxalate (5.5 g, 37.5 mmol) in dry toluene (25 mL) was then added drop wise and stirred at room temperature for 1 hour. The reaction mixture was concentrated under reduced pressure and the resulting residue was diluted with ice water. The precipitated solids were collected by filtration and dried to afford 2.85 g of ethyl 2,4-dioxo-4-phenylbutanoate (52%yield) as a yellow solid. This material (2.85 g, 12.9 mmol) was taken up in EtOH (25 mL) along with $NH_2OH.HCl$ (1.16 g, 16.8 mmol) and then stirred under reflux for 3 hours. The reaction mixture was concentrated under reduced pressure. The resulting residue was diluted with water and extracted with EtOAc. The combined organic layers were washed with H_2O, dried (Na_2SO_4) and concentrated under reduced pressure. Purification by silica gel chromatography (pentanes/EtOAc) afforded ethyl 5-phenylisoxazole-3-carboxylate (2.53 g, 90% yield) as a white solid. This material (2.53 g, 11.6 mmol) was taken up in THF/H_2O (45 mL/5 mL) along with $LiOH.H_2O$ (1.0 g, 23.3 mmol) and the resulting reaction mixture was stirred at room temperature for 2 hours. The reaction mixture was then concentrated under reduced pressure. Sufficient 1 N HCl was added to the resulting residue to bring the pH to about 5. The resulting solids were collected by filtration and dried under high vacuum to afford 1.5 g of 5-phenylisoxazole-3-carboxylic acid (69%) as a white solid. 5-Phenylisoxazole-3-carboxylic acid was then coupled with (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-3-amino-4-((1,3-dihydroxypropan-2-yl)amino)-2-methyl-4-oxobutan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide using the same general amide coupling procedure described earlier (see example 8) to obtain N-((R)-1-((1,3-dihydroxypropan-2-yl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-1-oxobutan-2-yl)-5-phenylisoxazole-3-carboxamide. MS (EI) calc'd for $C_{49}H_{58}N_4O_6S_2$ 778.38; found 779 $[M+H]^+$.

Example 18

Preparation of (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((3R)-4-((1,3-dihydroxypropan-2-yl)amino)-3-(2-(4-isobutylphenyl)propanamido)-2-methyl-4-oxobutan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (I-49):



[00227] (4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((3R)-4-((1,3-Dihydroxypropan-2-yl)amino)-3-(2-(4-isobutylphenyl)propanamido)-2-methyl-4-oxobutan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide was prepared according to the procedure outlined in the preparation of N-((R)-1-((1,3-dihydroxypropan-2-yl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-1-oxobutan-2-yl)-5-phenylisoxazole-3-carboxamide (*see*, Example 17), except that ibuprofen was substituted as the appropriate starting material. MS (EI) calc'd for $C_{45}H_{69}N_3O_5S_2$ 795.47; found 796 $[M+H]^+$.

Example 19

Effect of Compounds on Autophagy, CFTR in Huh-7, HT-29 or THP-1 Cells

[00228] The compounds of the invention were evaluated for their effect on autophagy and CFTR trafficking using the following procedure.

[00229] *Compound Preparation*: Test compounds were first solubilized in 100% DMSO as 50 mM solution, and then diluted 1 to 100 in FBS as a 10 X stock solution of 500 μm.

[00230] *Immunoblotting*: Huh-7 or HT-29 or THP-1 cells were seeded in 10% FBS DMEM overnight. The cells media were replaced with drug diluted 1 to 10 in DMEM (final

concentration 50 μm in 10% FBS DMEM). 24 hours after the drug addition, cells were lysed in RIPA buffer. Cell lysates were analyzed by immunoblotting with anti-LC3A/B antibodies (Cell signaling 12741) and anti-CFTR antibodies (Cell signaling 2269). Data of LC3 are presented as LC3-II/LC3-I ratio compared to vehicle treated samples. Data of CFTR were normalized with actin and represented as ratio compared to the vehicle treated samples.

[00231] *Confocal Microscopy*: Huh-7 cells were seeded on cover slips overnight. Cells were infected with GFP-LC3 BacMan (Life Technology) for 24 hours and treated with test compound. Four hours later, cells were fixed in 2% paraformaldehyde and mounted with anti-Fade with DAPI (Life Technology). Images were taken with Zeiss LSM 510, with 40X lens.

[00232] *Cell Surface Biotinylation*: HT-29 cells were seeded at 2.0 $X10^6$ cells in 10 mm^2 plates in 10% FBS DMEM overnight. The cells media were replaced with drug diluted 1 to 10 in DMEM (final concentration 50 μm in 10% FBS DMEM). 24 hours after the drug addition, cells were processed for cell surface biotinylation using Pierce Cell Surface Protein Isolation Kit (Thermo Scientific 89881). Briefly, cells were washed once with cold PBS and incubated with Sulfo-NHS-SS-Biotin for 30 minutes at 4°C and reaction was stopped by adding Quenching solution. Cells were scraped and lysed and centrifuged at 10,000 x g. Cell suspension were incubated with NeutrAvidin Agarose for 60 minutes at room temperature. Cell pellet (intracellular) were lysed in RIPA buffer. Protein bound to NeutrAvidin Agarose (cell surface) were eluted by SDS-PAGE sample buffer containing 50 mM DTT. Both cell surface and intracellular parts were analyzed by immunoblotting with anti-CFTR antibodies.

Results:

[00233] Compound **I-2** was evaluated in this assay using HT-29 cells. Figure 1 shows the immunoblot for HT-29 cells when treated with 12.5 μM, 25 μM, or 50 μM of compound **I-2**, after a 24 hours incubation. As shown in Figure 1C, compound **I-2** effectively activated autophagy at a concentration of 25 μM and 50 μM, as indicated by the increase in the ratio of LC3-II to LC3-I. This increase in autophagy also resulted in a corresponding increase in the CFTR at the cell membrane (*see*, Figure 1B). The results shown represented the average of three separate measurements.

[00234] Figure 2A shows the immunoblot for HT-29 cells when treated with either 50 μM of compound **I-2** or VX-809. Figure 2B confirms the increased level of cell surface CFTR by cell surface biotinylation. Under the conditions used, compound **I-2** has comparable activity to the CFTR corrector VX-809, a positive control. The results shown represented the average of three separate measurements.

[00235] In a synergy experiment, compound **I-2** was evaluated in HT-29 cells at a concentration of 50 μM in the same manner as described above for the cell surface biotinylation against 50 μM each of the individual components, i.e., 4-phenylbutyric acid (4-PBA), cystamine (Cys), EPA, or a combination of the individual components (i.e., the combination consisting of 50 μM each of cystamine, EPA, and 4-PBA; the combination consisting of 50 μM each of EPA and 4-PBA; and the combination consisting of 50 μM each of cystamine and 4-PBA). The results are shown in Figure 3, which illustrate that compound **I-2** provides enhanced activity in trafficking CFTR that could not be achieved using the individual components that make up compound **I-2** or combinations of such individual components tested in this assay. The results shown represented the average of three separate measurements.

[00236] Compounds **I-3**, **I-11**, **I-14** and **I-15** were evaluated in the HT-29 cells for autophagy activation and for the CFTR trafficking activity. As shown in Figure 4A, compounds **I-3**, **I-11**, **I-14** and **I-15** all activated autophagy in HT-29 cells, as noted by the increase in the ratio of LC3-II/LC3-I, when compared with the vehicle control group. All test compounds were incubated in HT-29 cells for 24 hours at a concentration of 25 μM. This increase in autophagy also led to a corresponding increase in the trafficking of the CFTR to the cell surface, as shown in Figure 4B. The results shown represented the average of three separate measurements.

[00237] Compounds **I-25** and **I-26** were evaluated in the HT-29 cells (each at 25 μM, 24 hour incubation) for autophagy activation and for the CFTR trafficking activity. As shown in Figure 5A, compounds **I-25** and **I-26** activated autophagy in HT-29 cells, as noted by the increase in the ratio of LC3-II/LC3-I, when compared with the vehicle control group for this particular run. This increase in autophagy also led to a corresponding increase in the trafficking of the CFTR to the cell surface, as shown in Figure 5B. The results shown represented the average of three separate measurements.

[00238] Compound **I-49** contains an ibuprofen molecule that is covalently joined to a fatty acid cysteamine conjugate. When 25 μM of compound **I-49** was incubated in HT-29 cells for 24 hours, an increase in the cell surface CFTR was observed, when compared to the vehicle control group (*see*, Figure 6). The results shown represented the average of three separate measurements.

Example 20

Effect of Compounds of the Invention on Primary CF Human Bronchial Epithelial Cells, Homozygous for ΔF508 CFTR Deletion Mutation: Immunoblot Analysis and Immunoprecipitation

[00239] It is contemplated that compounds of the invention may be useful in treating CF given their ability to activate autophagy. The compounds of the invention were evaluated in the following cellular assay to determine their ability to traffic the defective, mutant CFTR to the cell membrane. Primary cells from homozygous ΔF508 CF patients were obtained from either Asterand Bioscience (Detroit, MI) or ChanTest, a Charles River Company (Cleveland, OH). Cells were then treated at various concentrations to determine the compound's ability to restore the defective CFTR. As reviewed in Derichs (2013) EUR. RESP. REV., 22, p. 58-65, a successful detection of the mutant CFTR band C by immunoblot indicates that the defective CFTR can be rescued to the cell membrane.

[00240] *Compound preparation*: Compounds of the invention were first solubilized in 100% DMSO as 50 mM solution, and then diluted 1 to 100 in FBS as a 10 X stock solution of 500 μM.

[00241] *Immunoblotting*: Primary CF cells (homozygous ΔF508, source: ChanTest, KKCFFT004I) were prepared and grown on Snapwell™ filter inserts according to the procedures outlined in Amaral, M.D. and Kunzelmann, K. (Eds) (2011) CYSTIC FIBROSIS, METHODS IN MOLECULAR BIOLOGY 741, DOI 10.1007/978-1-61779-117-8_4 Springer Science+Business Media, LLC). Primary CF cells were kept in differentiation media consisting of Dulbecco's MEM (DMEM)/F12, Ultroser-G (2.0%; Pall, Catalog # 15950-017), fetal clone II (2%), insulin (2.5 μg/ml), bovine brain extract (0.25%; Lonza, Kit #CC-4133, component # CC-4092C), hydrocortisone (20 nM), triiodothyronine (500 nM), transferrin (2.5

μg/ml: Invitrogen, Catalog # 0030124SA), ethanolamine (250 nM), epinephrine (1.5 μM), phosphoethanolamine (250 nM), and retinoic acid (10 nM). The test compounds, solubilized in FBS according to the procedure outlined above and diluted to the desired concentration, were then added to the individua Snapwell™ filter inserts in the differentiation media at 37 °C. Twenty-four hours after the drug addition, cells were snap frozen and later lysed in RIPA buffer. The amounts of proteins were determined by Bio-Rad protein assay. Fifty μg of total cell lysates were analyzed by immunoblotting with anti-CFTR, anti-Beclin-1, anti-p62 and anti-LC3 antibodies. The immuno-activity was normalized with actin as the loading control. Data were presented as CFTR-band-C/actin, Beclin-1/actin, p62/actin and LC3-II/LC3-I ratio compared to vehicle treated samples. Antibodies against CFTR clone M3A7 (Cell Signaling Technology, 2269), LC3A/B antibodies (Cell signaling, 12741), Beclin-1 (Cell Signaling Technology, 3495), p62 (Cell Signaling Technology, 5114) and β-actin (Cell Signaling Technology, 4970) were used as primary antibodies. Compounds **I-34** (25 μM) and **I-41** (25 μM) were evaluated in this manner and the results are shown in Figure 7A. Both compounds were able to increase the level of the ΔF508 CFTR when used in combination with VX-809 (3 μM) and VX-770 (100 nM). Likewise, compounds **I-25** (25 μM) and **I-28** (25 μM) were evaluated in the same manner, in combination with VX-809 (3 μM) and VX-770 (100 nM). As shown in Figure 7B, both **I-25** and **I-28** were able to increase the level of the ΔF508 CFTR.

Example 21

Evaluation of Compounds in the Fisher Rat Thyroid Epithelial Cells Ussing Chamber for Functional Rescue of CFTR Ion Channel Activity.

[00242] The most prevalent disease causing mutation of the CF transmembrane conductance regulator (CFTR) chloride channel is deletion of phenylalanine at position 508 in the primary sequence of CFTR (ΔF508-CFTR). This mutation causes a trafficking defect resulting in a severe reduction of ΔF508-CFTR protein at the cell surface. The trafficking defect can be corrected by incubation at low temperature (27 °C overnight) or pharmacologically by small molecules and CFTR correctors. Chloride transport function of Fisher Rat Thyroid (FRT) epithelial cells overexpressing ΔF508-CFTR in monolayers grown on Snapwell™ filter inserts were monitored as the CFTR agonist evoked short circuit (I_{SC}) current output of an Ussing epithelial voltage clamp

apparatus. An objective of this study was to measure the ability of test compounds to restore function to defective ΔF508-CFTR in FRT epithelial cell monolayers.

[00243] Measurement of corrector efficacy was divided into two phases. The initial phase was incubation of epithelia with the test compounds for a period of time (that can range from 2 hours to one or two days) in a 37 °C incubator and the second phase was measurement of epithelial ΔF508-CFTR chloride channel current with an epithelial voltage clamp (Ussing assay). The short circuit current (I_{SC}) was measured under short circuit conditions (0 mV transepithelial potential). The I_{SC} magnitude is an index of corrector efficacy and is compared to vehicle and positive control.

[00244] Cryopreserved FRT cells stably transfected with ΔF508-CFTR cDNA (Pedemonte *et al.* (2005) J. CLIN. INVEST., 115, p. 2564-2571) were expanded and plated on Snapwell™ filters for measurement of short circuit current in an Ussing apparatus (Physiologic Instruments, Inc., Sand Diego, CA). Cells were grown in Coon's modification of Ham's F-12 media supplemented with zeocin and G-418.

[00245] To conduct the assay, a compound of the invention was solubilized as follows:

1) Prepare 25 mM stock solution in 100% DMSO.
2) Dilute 12 μL of 25 mM DMSO stock in 1.20 mL of FBS to make 250 μM intermediate dilution 10X stock (1% DMSO, 99% FBS), and gently vortex until they become clear.
3) Prepare final dilution of 10 μM in 10 mL of Coon's media per well (4 wells x 2 mL per well bottom + 0.2 mL per insert top = 8.8 mL and 1.2 mL reserve for handling losses) by addition of 400 μL of 10X stock and 600 μL of 10X carrier (1% DMSO, 99% FBS) to 9000 μL of Coon's media.

[00246] For the following example, the test compound was solubilized and added to the appropriate inserts of Ussing chambers (n = 4 for each test compound, final test concentration of 10 μM). A DMSO vehicle control and a positive control (VX-809 at 3 μM) were also used. For this particular example, all the test articles, including the positive control, were incubated with the cells for a period of 4 hours. The FRT cell monolayers grown on Snapwell™ filter inserts were transferred to Physiologic Instruments Ussing recording chambers (Physiologic Instruments, Inc., San Diego, CA) and superfused with HB-PS on the basolateral side and

78CF-PS on the apical side. One or more 6-channel or 8-channel Physiologic Instruments VCC MC6 or VCC MC8 epithelial voltage clamps were then used in combination to record the short circuit current (I_{SC}) during the entire run. To initiate the I_{SC} measurement, amphotericin (100 μM) was added to the basolateral side of the Snapwell™ filter insert to permeabilize the epithelial cells for 15 min. Forskolin (10 μM), IBMX (100 μM), Genistein (20 μM) and the $CFTR_{inh}$-172 (20 μM) were added sequentially after the following incubation periods (15 min, 20 min, 10 min, 15 min and 15 min respectively). Data acquisition and analyses were performed using iWorx data acquisition hardware and Labscribe 2 software (iWorx, Dover, NH). Comparison of agonist evoked I_{SC} among both corrector positive control, negative control and test article treated epithelia was obtained with one-way ANOVA followed by Dunnett's multiple comparison test and Student's t-test when appropriate. Significant correction was defined at the level of $P<0.05$.

[00247] In this assay, the positive control VX-809 was able to functionally rescue the defective CFTR under the test conditions when the cells were treated sequentially with Forskolin, IBMX and then with the CFTR potentiator Genistein. To test for CFTR specificity, the commercially available inhibitor $CFTR_{inh}$-172 (which has chemical name (*E*)-4-((4-oxo-2-thioxo-3-(3-(trifluoromethyl)phenyl)thiazolidin-5-ylidene)methyl)benzoic acid (CAS no. 307510-92-5)) was added near the completion of the run to bring the short circuit current down to the baseline.

[00248] An alternative protocol to this assay involved the chronic pre-incubation of compounds of the invention along with VX-770 or the combination of VX-809 and VX-770. With this protocol, the compounds of the invention were pre-incubated with either VX-770 or a combination of VX-770 and VX-809 for 24 hours using the same protocols outlined above. Amphotericin (100 μM) was first added to permeabilize the cell membrane. Fifteen minutes after the addition of amphotericin, Forskolin (20 μM) was added. Twenty minutes after the addition of Forskolin, the commercially available inhibitor $CFTR_{inh}$-172 was added. The reaction was then terminated 15 minutes after the addition of the $CFTR_{inh}$-172. A representative trace of the short circuit currents was then obtained from this type of experiment. The functional activity of the compounds of the invention was assessed when comparison was made between the vehicle group and the positive control group. For all the Ussing chamber

experiments, the positive control was a combination of the CFTR corrector VX-809 (3 μM) and the CFTR potentiator VX-770 (100 nM). Quantification of the short circuit currents was carried out to determine the ΔI_{SC} at two different time points, first upon the addition of Forskolin and later upon the addition of the $CFTR_{inh}$-172 (For a more comprehensive description of the assay, please refer to Van Goor *et al.* (2011) PNAS, 108, no. 46, p. 18843-18848).

[00249] Figures 8A, 8B, 8C, 8D and 8E summarize the data when the FRT cells were incubated for 24 hours with the following treatment groups: 1) vehicle + VX-770 (100 nM); 2) compound **I-25** (10 μM) + VX-770 (100 nM); 3) the positive control group,VX-809 (3 μM) + VX-770 (100 nM); 4) compound **I-25** (10 μM) + VX-809 (3 μM) + VX-770 (100 nM). Each treatment group was evaluated with 4 separate inserts. As shown in Figure 8A, compound **I-25** was functionally active in this assay, as noted by the increase in the short circuit current; the effect was most pronounced with the combination of **I-25** + VX-809 + VX-770. Figure 8B shows the quantification of the steady state response upon the addition of Forskolin, as measured by ΔI_{SC} ($\mu A/cm^2$); whereas Figure 8C shows the same response, expressed as % of control (the positive control VX-809 + VX-770 was expressed as 100%). The combination of **I-25** (10 μM) + VX-770 produced a modest response, when compared with the vehicle + VX-770 group. However, the combination consisting of **I-25** (10 μM) + VX-809 + VX-770 produced a significant 150.1% increase over the positive control. Figure 8D shows the quantification of steady state response upon the addition of the $CFTR_{inh}$ -172, as measured by ΔI_{SC} ($\mu A/cm^2$); whereas Figure 8E shows the same response, expressed as % of control (the positive control VX-809 + VX-770 was expressed as 100%). Again, at this time point, the combination consisting of **I-25** (10 μM) + VX-809 + VX-770 produced a significant 147.5% increase over the positive control group.

Example 22

Evaluation of Compounds in the Primary CF Bronchial Epithelial Cells (homozygous F508) Ussing Chamber for Functional Rescue of CFTR Ion Channel Activity

[00250] Primary CF cells (homozygous ΔF508, source: ChanTest, KKCFFT004I) were prepared and grown on Snapwell™ filter inserts according to the procedures outlined in

Amaral, M.D. and Kunzelmann, K. (Eds) (2011) CYSTIC FIBROSIS, METHODS IN MOLECULAR BIOLOGY 741, DOI 10.1007/978-1-61779-117-8_4 Springer Science+Business Media, LLC). Primary CF cells were kept in differentiation media consisting of Dulbecco's MEM (DMEM)/F12, Ultroser-G (2.0%; Pall, Catalog # 15950-017), fetal clone II (2%), insulin (2.5 μg/ml), bovine brain extract (0.25%; Lonza, Kit #CC-4133, component # CC-4092C), hydrocortisone (20 nM), triiodothyronine (500 nM), transferrin (2.5 μg/ml: Invitrogen, Catalog # 0030124SA), ethanolamine (250 nM), epinephrine (1.5 μM), phosphoethanolamine (250 nM), and retinoic acid (10 nM). The test compounds were solubilized in FBS as follows: 100 μL of a 25 mM DMSO stock solution of the test compound was diluted in 10.0 mL of FBS in a centrifuge tube to prepare an intermediate 250 μM intermediate dilution 10× stock (1% DMSO, 99% FBS). This solution was allowed to sit in the centrifuge tube at room temperature for 1 hour and then discarded; a new 250 μM 10× stock was then prepared in the now conditioned centrifuge tube. This 10× stock solution was used to prepare the subsequent test article concentrations. For instance, the 25 μM concentration in 10 mL of differentiation media was prepared by adding 1000 μL of the 10× stock solution to 9000 μL of differentiation media. This 25 μM solution was allowed to sit in the centrifuge tube at room temperature for 1 hour and then discarded; and a new 25 μM solution was then prepared in the now conditioned centrifuge tube. The 10 μM concentration in 10 mL of differentiation media was prepared by adding 400 μL of the 10× stock solution to 9000 μL of differentiation media and 600 μL of 1% DMSO, 99% FBS solution. The subsequent 3 and 1 μM concentrations in 10 mL of differentiation media were prepared in the same manner by adding the appropriate volume of the 10× stock solution to the differentiation media and 1% DMSO, 99% FBS solution. The same conditioning step described above was used in all the dilution steps.

[00251] To run the Ussing chamber assay, the test compounds (solubilized in FBS according to the procedure outlined above, and diluted to the desired concentration) were then added to the individual Snapwell™ filter inserts in the differentiation media at 37 °C. Twenty-four hours after the drug addition, the inserts were transferred to Physiologic Instruments Ussing recording chambers (Physiologic Instruments, Inc.; San Diego, CA) and maintained in both the apical and basolateral chambers with a HEPES buffered physiological saline (HB-PS) with composition (in mM): NaCl, 137; KCl, 4.0; $CaCl_2$, 1.8; $MgCl_2$, 1; HEPES, 10; Glucose, 10; pH adjusted to 7.4 with NaOH. One or more 6-channel or 8-channel Physiologic Instruments VCC

MC6 or VCC MC8 epithelial voltage clamps were then used in combination to record the short circuit current (I_{SC}) during the entire run. The short circuit I_{SC} measurements were conducted at 27 °C. To initiate the run, amiloride (30 μM) was added to the apical side of the Snapwell™ filter inserts to block epithelial Na channels (ENaC). Fifteen minutes later, Forskolin (10 μM) was added to activate the CFTR. Sixty minutes later, the experiment was terminated by the addition of the $CFTR_{inh}$-172 (20 μM). Data acquisition and analyses were performed using iWorx data acquisition hardware and Labscribe 2 software (iWorx, Dover, NH). Comparison of agonist evoked I_{SC} among both corrector positive control, negative control and test article treated epithelia was obtained with one-way ANOVA followed by Dunnett's multiple comparison test and Student's t-test when appropriate. Significant correction was defined at the level of $P<0.05$.

[00252] Figures 9A, 9B and 9C summarize the data when primary CF cells (homozygous ΔF508) were incubated for 24 hours with the following treatment groups: 1) vehicle + VX-770 (100 nM); 2) positive control group, VX-809 (3 μM) + VX-770 (100 nM); 3) compound **I-28** (1 μM) + VX-809 (3 μM) + VX-770 (100 nM). Figure 9A shows the traces of the short circuit current (I_{SC}) measured during the assay. The traces shown in Figure 9A indicate that the combination consisting of compound **I-28** (1 μM) + VX-809 + VX-770 was functionally more active than the positive control, the combination consisting of VX-809 + VX-770. Figure 9B shows the quantification of the steady state response upon Forskolin addition, as measured by ΔI_{SC} ($\mu A/cm^2$). The combination consisting of **I-28** (1 μM) + VX-809 + VX-770 was functionally more active than the positive control group (i.e. VX-809 +VX-770), as noted by the larger increase in the ΔI_{SC}. Figure 9C shows the quantification of the overall response, as measured by the area under the curve (AUC) and expressed as % of control (wherein the positive control was expressed as 100%). As shown in Figure 9C, the combination consisting of **I-28** (1 μM) + VX-809 + VX-770 showed a 327.8% increase in the AUC, when compared with the positive control (i.e. VX-809 + VX-770).

[00253] Compounds **I-34** and **I-41** were also evaluated in the same assay using the same protocols. Figure 9D shows the quantification of the steady state response upon Forskolin addition, as measured by ΔI_{SC} ($\mu A/cm^2$), for the following treatment groups: 1) vehicle + VX-770 (100 nM); 2) **I-34** (1 μM) + VX-809 (3 μM) + VX-770 (100 nM); 3) **I-41** (1 μM) + VX-

809 (3 μM) + VX-770 (100 nM). Both **I-34** and **I-41**, when used in combination with VX-809/VX-770 showed a statistically significant increase in functional activity, as expressed by the ΔI_{SC} ($\mu A/cm^2$) values. Figure 9E shows the quantification of the overall response shown in Figure 9D, as measured by the area under the curve (AUC) and expressed as % of control (wherein the positive control was expressed as 100%). As shown in Figure 9D, the combination consisting of **I-28** (1 μM) + VX-809 + VX-770 showed a 138.8% increase in the AUC, when compared with the vehicle +VX-770 control. Likewise, the combination consisting of **I-41** (1 μM) + VX-809 + VX-770 showed a 128.5% increase in the AUC, when compared with the vehicle +VX-770 control.

Example 23

***In Vitro* Bacterial Clearance Assay Using Human Bronchial Epithelial Cells**

[00254] In this assay, normal 16HBE cells are cultured and seeded at 2×10^5 cells per well using a 48-well plate. The resulting plates are incubated at 37 °C with 5% CO_2 until ~90% confluency. Cells are then treated with the vehicle control, the desired test compound and a positive control (Cytochalasin-D) for 24 hours and then infected with *Pseudomona aeruginosa* strain Xen05 at a multiplicity of infection (MOI) of 1:50 (i.e. ratio of cells:bacteria) for 2 hours. Cells then are incubated with 500 μL of a mixture consisting non-permeable antibiotic (50 U/mL each of pencillin and streptomycin, mixed with 200 μg/mL gentamicin) for 3 hr to remove the extracellular bacteria. Afterwards, cells are lysed and a bacteria count is carried out to determine the remaining intracellular bacteria load.

Example 24

Assay to Assess Plasma Stability of Fatty Acid Cysteamine Conjugates

[00255] The *in vitro* stability of the test compounds was studied in human, mouse, beagle and rat plasma (Plasma was purchased from Bioreclamation). Plasma was diluted to 50% with PBS (pH 7.4). Test compounds were dissolved in DMSO to a final concentration of 10 mM and then diluted to 1 mM in MeOH. Incubations were carried out at a test compound concentration of 5 μM with a final DMSO concentration of 2.5%. Plasma (198 μL) was added to 96- well plate and incubated at 37°C for 30 minutes before the addition of 2 μL of the test compound. The

resulting mixture was then incubated at 37 °C for 2 hours. At appropriate time intervals (0, 30, 60 and 120 min), aliquots (50 µL) were removed and reactions were terminated by adding 200 µL of acetonitrile with an internal standard. Simultaneously, plasma samples containing Benflourex or Procaine (control compound) were terminated by adding 200 µL of acetonitrile internal standard. The sample plate was centrifuged at 3500 rpm for 45 minutes at 4 °C and the supernatant was transferred to a new plate for analysis by LC/MS-MS (Agilent Model No: HPLC: 1200, MS: 6410). Chromatographic separation was achieved with a Phenomenex C6-phenyl (5u) column. A binary gradient consisting of 0.1% formic acid in water and 0.1% formic acid in methanol was used for analyte elution.

[00256] Figures 10A, 10B and 10C showed the plasma stability data for compounds **I-25**, **I-28** and **I-29**. All three compounds showed complete plasma stability across the 4 species tested.

Example 25

Evaluation of Fatty Acid Cysteamine Conjugate in an Oral Cannulated Rat PK Study

[00257] The compounds of the invention were solubilized in a mixture of excipients consisting 40%Tween, 50% Peceol, 10% PEG400 and diluted with water to form a self-emulsifying aqueous mixture for oral administration to animals. For this study, Sprague Dawley rats that had been surgically implanted with indwelling jugular vein cannula (JVC) and portal vein cannula (PVC) were used (Agilux, Worcester, MA). This approach using double-cannulated rats allows the measurement of the drug concentration that was delivered in the portal vein as well as the drug concentration that was present in the peripheral. For the PK study, serial blood collection was carried out at both the portal and jugular vein at the following time points: 10, 20, 40 minutes and 1, 2, 4 and 6 hours post dose. The bioanalytical portion of the PK study was carried out using an LC/MS/MS system (Agilent Model No: HPLC: 1200, MS: 6410) and analyzed with the appropriate software (WinNonlin Phoenix 64 6.3.0 395).

[00258] Compound **I-25** was evaluated in this oral cannulated rat PK experiment. The portal C_{max} for compound **I-25** was 58.0 ± 29.3 ng/mL, along with an AUC_{last} of 74.2 ± 32.8 Hr*ng/mL. In the systemic circulation, the peripheral C_{max} of compound **I-25** was 33.1 ± 8.55 ng/mL, along with an AUC_{last} of 37.6 ± 5.3 Hr*ng/mL.

Example 26

***In Vivo* Determination of Autophagy Activation**

[00259] In order to evaluate for *in vivo* autophagy activation, naïve male C57BL/6 mice are dosed orally with the desired test compound (100 mg/kg, BID, 3.5 days). One hour after the last dose, lung tissues and plasma are collected to analyze for drug concentration and autophagy biomarkers. As discussed in earlier examples, the ratio of LC3-II to LC3-I, obtained from lung tissues, is used as autophagy biomarker.

Example 27

Assessment of Fatty Acid Cysteamine Conjugate in a Model of Murine Lung Infection With *Pseudomoma aeruginosa*

[00260] In this model of murine lung infection with *Pseudomona aeruginosa*, female BALB/c mice, aged 6-7 weeks, are allowed to acclimate for one week in five groups of 10 animals per cage. From 3.5 days prior to the infection, animals are treated with the test compound (formulated as described above) at 100 mg/kg po, BID; animals were then kept on the same compound treatment for the duration of the study. Four other treatment groups are used in this study, including the vehicle control and the positive control groups: Group 1) vehicle, po (BID from day -3.5) and s.c. (BID from 8 hours post infection); Group 2) test compound, po (BID, 100 mg/kg from day -3.5) plus vehicle s.c. (BID from 8 hours post infection); Group hours hours r post infection), plus vehicle p.o. (BID from day -3.5); Group 4) Ciprofloxacin, 5 mg/kg s.c. (BID from 8 hours post infection), plus test compound po (BID, 100 mg/kg, from day -3.5); Group 5) Ciprofloxacin, positive control, 20 mg/kg s.c. (BID from 8 hours post infection).

[00261] Animals are weighed prior to treatment and daily thereafter until the termination of the study. Once infected with *Pseudomona aeruginosa*, animals are observed regularly for signs of ill-health and body temperatures were monitored. Animals reaching humane endpoints are terminated and time of death recorded. At termination, 24/48 hours post infection, lungs are removed and signs of gross pathology scored and photographed. Lung, spleen, and kidney

are removed, weighed and transferred into PBS, homogenized and serial dilutions plated out to determine the bacterial load.

Example 28

Anti-fibrotic Cell Assays

Cells

[00262] Normal human lung fibroblasts (ScienCell Research Laboratory 3420), lung fibroblasts from idiopathic pulmonary fibrosis (IPF) patients LL29 (AnHa) (ATCC) and LL97A (ALMy) (ATCC) were maintained in DMEM F12 (Gibco 10565) supplemented with 15% fetal bovine serum (FBS) (Gibco 10437-028) plus Pen-Strep (1%) (Gibco 15140-122). Cells were split every 3 to 4 days at 1:2/1:3 dilution each time. The day before the experiment, cells were trypsinized using Trypsin-EDTA (0.05%) (Gibco 25300-054) and plated on 24-well fish at 1×10^5 cells per well.

[00263] THP-1 cells were obtained from ATCC® TIB202. THP-1 cells were maintained in RPMI1640 (Gibco® RPMI 1640) supplemented with 10% fetal bovine serum. DMEM (#11095) and fetal bovine serum (low endotoxin grade) (#10437) was obtained from Invitrogen.

Drug treatment

[00264] Compounds **I-25** and **I-28** were first solubilized in 100% DMSO as 50 mM solution, and then diluted 1 to 200 in 1% BSA as a 10 X stock solution of 250 μm, and series stock dilution (1 to 2 dilution) were carried out as needed. The 10 X stock solution were added to the cell media and cells were incubated for 24 hours at 37°C. For LPS stimulation in THP-1 cells, compounds were added to the cell media for 6 hours at and at the end of 4 hours, 50 μg/ml final concentration of LPS (Sigma L3024) was added and cells were incubated for 2 hours. Normal human lung fibroblasts (NLF) or idiopathic pulmonary fibrosis cells (LL29 and LL79A cells, ATCC) were incubated with compound **I-25** (25 μM) and **I-28** (25 μM) for 24 hours in the presence of TGFβ (Abcam ab50036, 50 ng/mL) or in the absence of TGFβ (referred to as PBS treatment group). The test compounds were added to cells 30 min prior to TGFβ addition.

The total RNA was harvested, and the relative mRNA expression levels were assessed via RT-PCR with HPRT as the internal control. Data are represented as the mean ΔmRNA/HPRT, error bars represent the standard error of the mean (SEM). Significance was determined by student's t-test in comparison to control.

ELISA

[00265] Conditioned media were collected at the end of the experiment. The levels of Matrix Metalloproteinase 2 (MMP-2) (R&D System MMP200) and human Tissue Inhibitor of Metalloproteinase 2 (TIMP-2) (R&D System DTM200) were determined following the manufacturer's instruction. A 100-fold dilution of conditioned media was used in these assays. The ELISA was measured on a Victor X5 multilabel plate reader (PerkinElmer) at an absorbance of 450nm with background correction at 550nm. Standard curves were generated and levels of TIMP-2 and MMP-2 were calculated according to the standard curves. Conditioned media were collected and the levels of Matrix Metalloproteinase 2 (MMP-2) and human Tissue Inhibitor of Metalloproteinase 2 (TIMP-2) were determined. Data are represented as the mean fold change over the control and error bars represent the standard error of the mean (SEM). Significance was determined by student's t-test in comparison to control.

RT-PCR

[00266] Total RNA was collected using RNeasy Plus Mini Kit (Qiagen #74136) and cDNA generated using SuperScriptIII (Invitrogen #18080-044) with random hexamers following the manufacturer's protocol. Relative mRNA expression levels were determined using TaqMan probes (Applied Biosystems, using the recommended best primer pairs) with HPRT (hypoxanthine phosphoryltransferase) as the internal control. All PCR probes were purchased from Invitrogen. TNFα (HS 01113624), IL1β (HS 01555410), CCL2 (HS 00234140), Collagen 1a1 (HS 00164004), FN1 (Fibronectin 1, HS 00365052), TIMP-2 (HS 00234278), MMP-2 (HS 01548727). Collagen 1a1 (COL1a1), FN1, TIMP-2, and MMP-2 are well-known markers for fibrosis (*see*, Selman *et al.* (2000) AM. J. PHYS. LUNG CELL MOL. PHYSIOL., 279, L562-L574).

Results

[00267] Compounds **I-25** and **I-28** were evaluated in both normal human lung fibroblasts (NLF) and lung fibroblasts from idiopathic pulmonary fibrosis (IPF) patients (LL29 and LL97A). The results are summarized in Figures 11A-C.

[00268] Figure 11A shows the mRNA level of Collagen 1a1 (COL1a1) when these 3 different types of cells were treated with compound **I-25** (25 μM) or **I-28** (25 μM) under either PBS or TGFβ stimulation. Cells, either normal lung fibroblasts (NLF) or IPF cells (LL29 or LL97A), that have been treated with TGFβ showed a significantly enhanced level of Collagen 1a1, which was suppressed upon treatment with either compound **I-25** (25 μM) or **I-28** (25 μM). Figures 11B and 11C show the corresponding mRNA level of MMP-2 and TIMP-2 when these 3 different types of cells were treated with compound **I-25** (25 μM) or **I-28** (25 μM) under either PBS or TGFβ stimulation. NLF, LL29 or LL97A cells that have been treated with TGFβ showed a significantly enhanced level of MMP-2 and TIMP-2, which were suppressed upon treatment with either compound **I-25** (25 μM) or **I-28** (25 μM). COL1a1, MMP-2and TIMP-2 are well-known markers of fibrosis; and suppression of these markers indicated anti-fibrotic activity for compounds **I-25** and **28**.

[00269] The level of MMP-2, a known mediator of matrix degradation, and its natural inhibitor TIMP-2 were also evaluated in the conditioned media. As shown in Figure 12A-B and Figure 13A-B, the level of MMP2 and TIMP-2 was both elevated in the disease lung fibroblasts, when compared to normal lung. This imbalance between MMP-2 and TIMP-2 has been reported to cause the accumulation of the extracellular matrix (ECM) in fibrogenesis (*see*, Selman *et al.* (2000) AM. J. PHYS. LUNG CELL MOL. PHYSIOL., 279, L562-L574). Accordingly, a greater increase in the level of TIMP-2 than that of MMP-2 in IPF lung tissues was reported and such an imbalance would favor the enhanced deposition of ECM proteins. Figure 12A shows the basal level of TIMP-2 (PBS treatment) when NLF, LL29 or LL97A cells were treated with either the vehicle or compound **I-28** (25 μM). Figure 12B shows the level of TIMP-2 when NLF, LL29 or LL97A cells were treated with either the vehicle or compound **I-28** (25 μM) under TGFβ stimulation. Treatment with compound **I-28** resulted in a marked reduction of TIMP-2 level, in the presence of TGFβ. Figures 13A and 13B show the corresponding basal (PBS treatment) and TGFβ-stimulated level of MMP-2 for NLF, LL29 and

LL97A cells upon treatment with either the vehicle or compound **I-28** (25 μM). Again, treatment with compound **I-28** (25 μM) resulted in a marked reduction of MMP-2 level, in the presence of TGFβ.

Example 29

Evaluation of Thiol Metabolites Corresponding to the Compounds of the Formula II

[00270] When compounds of the Formula I are dosed orally, the corresponding thiol metabolites of the Formula II are generated. For example, when compound **I-25** and **I-28** are dosed orally, the corresponding thiol metabolite **II-1** and **II-4** are generated. The thiol metabolites of the Formula II are biologically active, as demonstrated by their functional activity in the Ussing chamber assay and by their ability to rescue the CFTR band C in primary CF cells (homozygous ΔF508).

[00271] The thiol metabolites **II-1** and **II-4** were generated according to the following general procedures: In order to form **II-1**, compound **I-25** (0.15 mmol) was dissolved in EtOH (2 mL). Racemic-dithiothreitol (0.175 mmol) was subsequently added to this ethanolic solution, followed by 250 μL of 1N NaOH to bring the pH of the reaction mixture to approximately 8.5 - 9.0. The resulting reaction mixture was stirred at room temperature for 40 minutes and then concentrated under reduced pressure. Purification by silica gel chromatography (CH_2Cl_2/MeOH) afforded compound **II-1**. In the same fashion, compound **I-28** was used to prepare the corresponding thiol metabolite **II-4**.

[00272] The FRT cells Ussing chamber was carried out using the protocols outlined in Example 21. As described earlier, the short circuit currents were used as a measure of functional activity.

[00273] Figure 14A shows the quantification of the steady state response upon the addition of Forskolin, as measured by ΔI_{SC} ($\mu A/cm^2$). In this Ussing chamber run, the treatment group consisted of: 1) vehicle + VX-770 (100 nM); 2) VX-809 (3 μM) + VX-770 (100 nM); 3) **II-1** (10 μM) + VX-770 (100 nM). In this assay, compound **II-1** was as functionally active as the well-known CFTR corrector VX-809. Figure 14A shows that when VX-809 was replaced with compound **II-1**, the level of functional activity was essentially the same for the two treatment

groups. Figure 14B shows another FRT cells Ussing chamber study using the following treatment groups: 1) vehicle + VX-770 (100 nM); 2) VX-809 (3 μM) + VX-770 (100 nM); 3) compound **II-4** (10 μM) + VX-809 (3 μM) + VX-770 (100 nM). In this particular assay, compound **II-4** showed an additive effect when used in combination with VX-809 + VX-770. As shown in Figure 14B, the increase in the ΔI_{SC} was statistically significant ($p < 0.05$).

[00274] The thiol metabolites of the Formula II were also evaluated in the primary CF cells (homozygous ΔF508) assay according to the procedures outlined in Example 20. Figure 15 shows the result for the incubation experiment wherein primary CF cells (homozygous ΔF508) were incubated with the following treatment groups: 1) vehicle + VX-770 (100 nM); 2) VX-809 (3 μM) + VX-770 (100 nM); 2) compound **II-1** (25 μM) + VX-770 (100 nM); 3) compound **II-4** (25 μM) + VX-770 (100 nM). Both the thiol metabolites **II-1**and **II-4**, in combination with VX-770, were more effective in increasing the mature CFTR band C than the combination consisting of VX-809 + VX-770.

[00275] The thiol metabolites of the Formula II were generated when compounds of the Formula I were dosed orally. In the following example, compound **I-25** was dosed orally to Sprague Dawley rats in a PK study; and the biologically active metabolite **II-1** was detected in the plasma. The PK study was conducted according to the protocols outlined in Example 25 using Sprague Dawley rats that have been surgically implanted with indwelling jugular vein cannula (JVC) and portal vein cannula (PVC) (Agilux, Worcester, MA). The following concentration of the parent compound **I-25** was detected in the portal and peripheral circulations: portal C_{max} = 58.0 ± 29.3 ng/mL and peripheral C_{max} = 33.1 ± 8.55 ng/mL. The following concentration of the biologically active metabolite **II-1** was detected in the portal and peripheral circulations: portal C_{max} = 6638 ± 2723 ng/mL and peripheral C_{max} = 2642 ± 872 ng/mL.

Example 30

Assessment of a Fatty Acid Cysteamine Conjugate in A Bleomycin Mouse Model of Fibrosis

[00276] Specific pathogen-free 7 weeks old female C57BL/6J mice are used for the experiment. On day 0, 40 mice are induced to develop pulmonary fibrosis by a single

intratracheal administration of bleomycin sulfate (BLM) in saline at a dose of 3 mg/kg using Microsprayer® (Penn-Century, USA). Animals then are randomized into 4 groups of 10 mice, based on the body weight on the day before the start of the treatment. Individual body weight will be measured daily during the duration of the study. Survival, clinical signs and behavior of mice are monitored daily. The compounds of the invention are administered orally using the formulation described in earlier examples. The 4 treatment groups of the study consist of: Group 1) vehicle; Group 2) the test compound, dosed po, BID at 30 mg/kg daily from day 0 to 20; Group 3) the test compound, dosed po, BID at 100 mg/kg daily from day 0 to 20; Group 4) dexamethasone control group, dosed orally at 0.25 mg/kg. On day 21, mice in all groups are terminated. For the biochemical analysis, the lung hydroxyproline will be quantified by a hydrolysis method. For the histological analysis of lung sections, Masson's Trichome staining and estimation of Ashcroft score will be carried out using known protocols (for a review, *see* Schaefer *et al.* (2011) EUR. RESP. REV., 20:120, p. 85-97). Statistical tests can be performed using Bonferroni Multiple Comparison Test. P values < 0.05 are considered to be statistically significant.

INCORPORATION BY REFERENCE

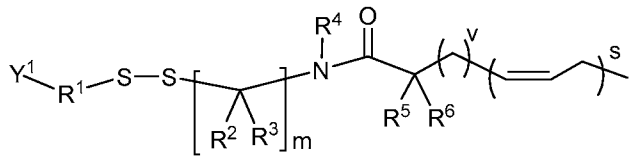
[00277] The entire disclosure of each of the patent and scientific documents referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

[00278] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

What is claimed is:

1. A compound of Formula A:



(A)

or a pharmaceutically acceptable salt or solvate thereof; wherein:

R^1 is C_1-C_6 alkylene optionally substituted by -C(O)N(R^2)(hydroxyalkyl), -N(R^2)C(O)-(hydroxyalkyl), -O-(hydroxyalkyl), -N(R^2)-(hydroxyalkyl), -OC(O)N(R^2)(hydroxyalkyl), -C(O)N(R^2)(R^8), -N(R^2)C(O)R^9, or -CO_2R^9;

R^2, R^3, R^4, and R^7 each represent independently for each occurrence hydrogen or C_1-C_3 alkyl;

R^5 and R^6 are independently hydrogen, C_1-C_4 alkyl, or halogen;

R^8 is C_1-C_6 alkyl, phenyl, benzyl, C_1-C_6 alkylene-CO_2R^7, or C_1-C_6 alkylene-C(O)N(R^7)$_2$;

R^9 represents independently for each occurrence hydrogen, C_1-C_6 alkyl, phenyl, benzyl, C_1-C_6 alkylene-CO_2R^7, or C_1-C_6 alkylene-C(O)N(R^7)$_2$;

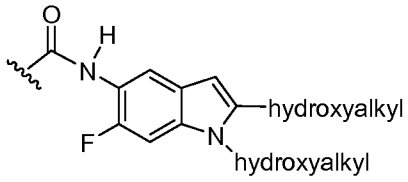
m is 2 or 3;

s is 3, 5, or 6;

v is 1, 2, or 6;

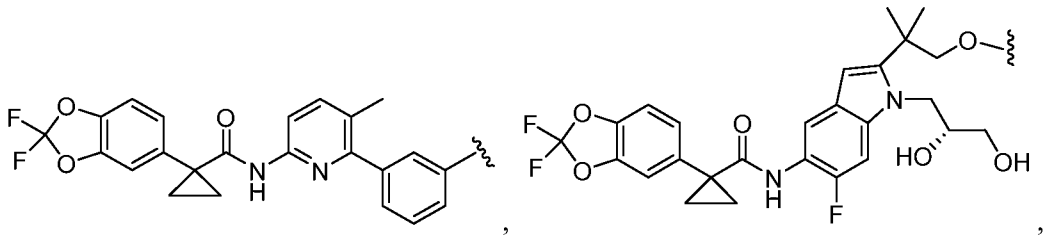
Y^1 is an amide or carbamate selected from the group consisting of -N(R^7)C(O)-aralkyl, -N(R^7)C(O)-(hydroxyalkyl), -C(O)N(R^7)(hydroxyalkyl), -N(R^7)CO_2-

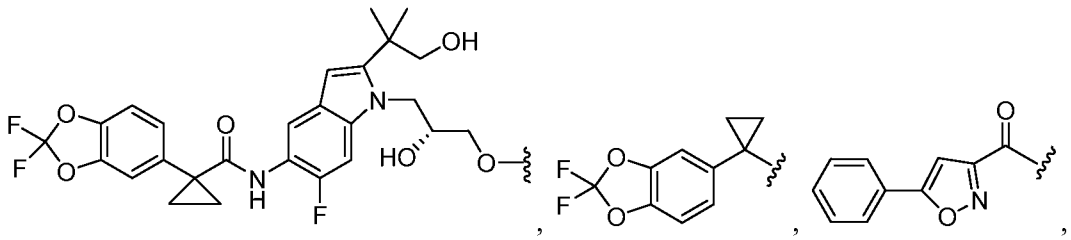
(hydroxyalkyl), -OC(O)N(R^7)(hydroxyalkyl), -N(R^7)C(O)-Z^1, and

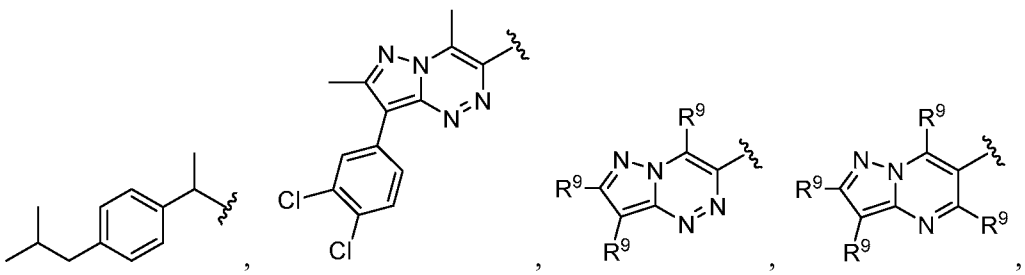


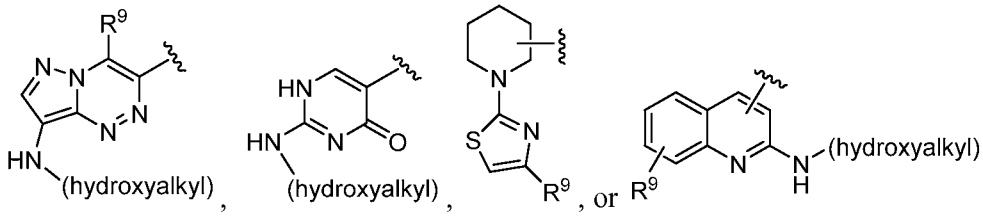
; and

Z^1 is one of the following:









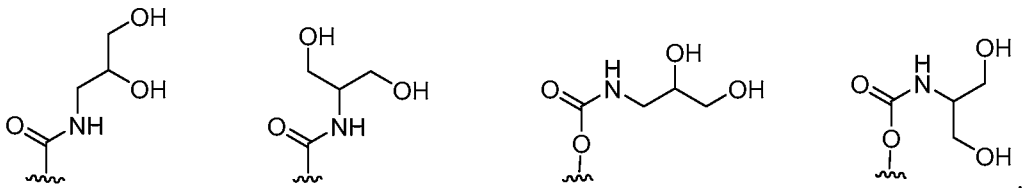
, or .

1 2. The compound of claim 1, wherein the compound is a compound of Formula A or a
2 pharmaceutically acceptable salt thereof.

1 3. The compound of claim 1 or 2, wherein R^1 is C_1-C_6 alkylene.

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

5. The compound of claim 1 or 2, wherein R^1 is C_1-C_6 alkylene substituted by -C(O)N(R^2)(hydroxyalkyl), -N(R^2)C(O)(hydroxyalkyl), -O-(hydroxyalkyl), -N(R^2)-(hydroxyalkyl) or -OC(O)N(R^2)(hydroxyalkyl).

6. The compound of claim 1 or 2, wherein R^1 is C_2-C_4 alkylene substituted by one of the following:  .

7. The compound of any one of claims 1-6, wherein R^2, R^3, and R^4 are hydrogen.

8. The compound of any one of claims 1-7, wherein R^5 and R^6 are hydrogen.

9. The compound of any one of claims 1-8, wherein R^7 is hydrogen.

10. The compound of any one of claims 1-9, wherein m is 2.

11. The compound of any one of claims 1-10, wherein v is 1, and s is 6.

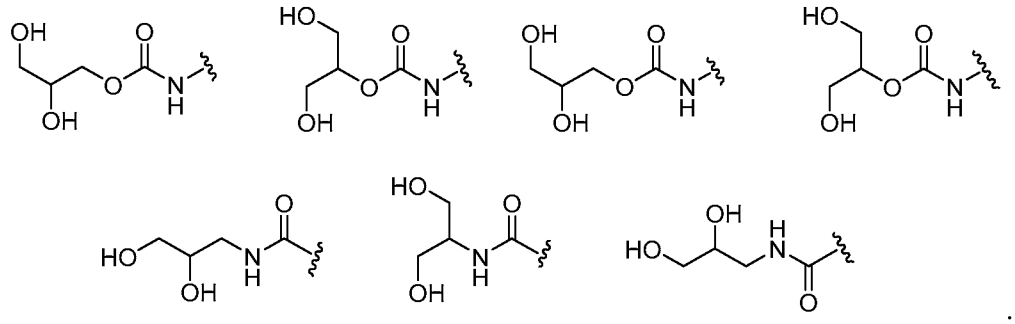
12. The compound of any one of claims 1-10, wherein v is 2, and s is 5.

13. The compound of any one of claims 1-12, wherein Y^1 is -N(R^7)C(O)-aralkyl.

14. The compound of any one of claims 1-12, wherein Y^1 is -N(R^7)C(O)-$(CH_2)_3$-phenyl.

15. The compound of any one of claims 1-12, wherein Y^1 is -N(R^7)C(O)-(hydroxyalkyl), -C(O)N(R^7)(hydroxyalkyl), -N(R^7)CO_2-(hydroxyalkyl), or -OC(O)N(R^7)(hydroxyalkyl).

16. The compound of any one of claims 1-12, wherein Y^1 is one of the following:



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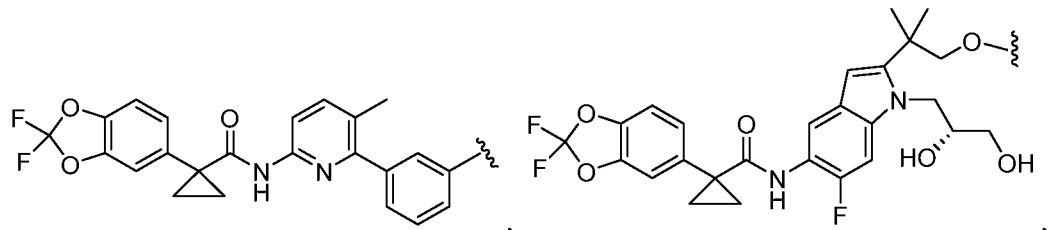
17. The compound of any one of claims 1-12, wherein Y^1 is $-N(R^7)C(O)-Z^1$.

18. The compound of any one of claims 1-12 or 17, wherein Z^1 is



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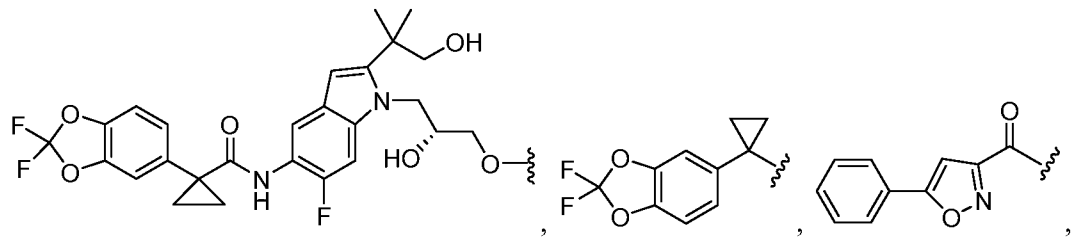

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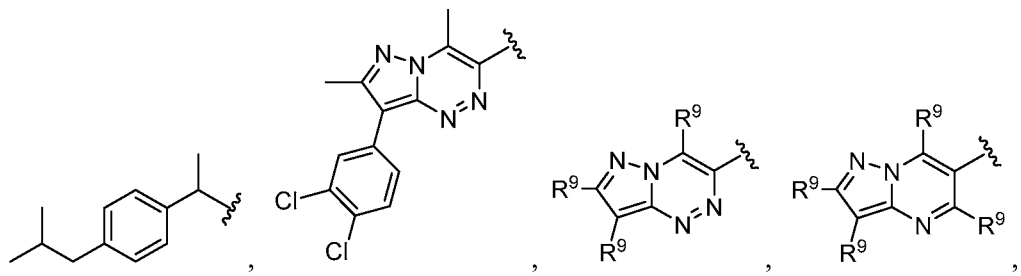

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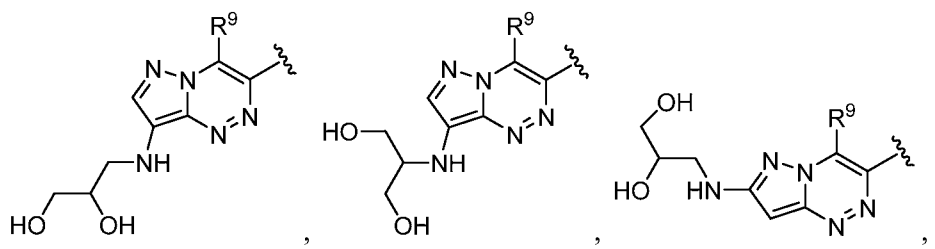

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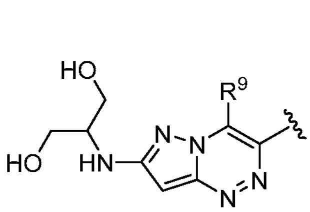
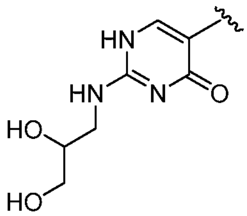
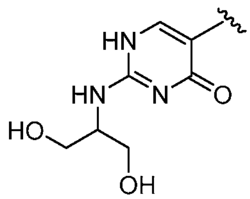
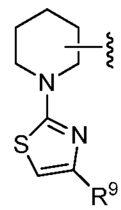
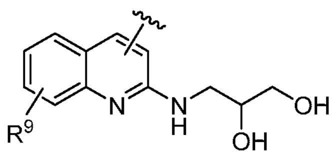
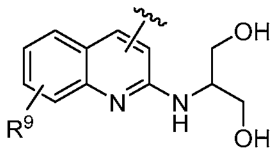
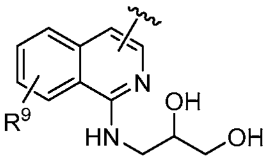
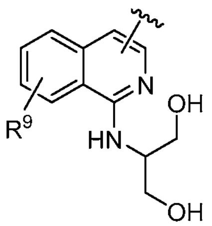
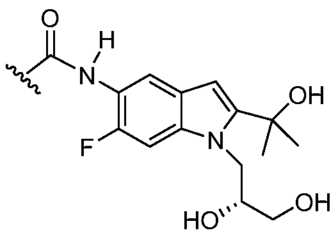
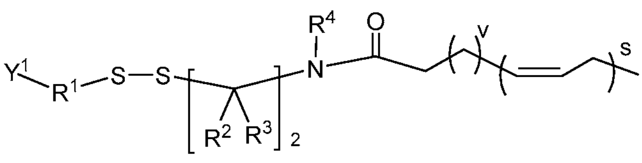
 ,  ,  ,  ,

 ,  ,  , or

 .

19. The compound of any one of claims 1-12, wherein Y^1 is  .

20. The compound of claim 1, wherein the compound is a compound of Formula A-1:



(A-1)

or a pharmaceutically acceptable salt thereof; wherein:

R^1 is C_1-C_6 alkylene optionally substituted by -C(O)N(R^2)(hydroxyalkyl), -N(R^2)C(O)-(hydroxyalkyl), -O-(hydroxyalkyl), -N(R^2)-(hydroxyalkyl), -OC(O)N(R^2)(hydroxyalkyl), -C(O)N(R^2)(R^8), -N(R^2)C(O)R^9, or -CO_2R^9;

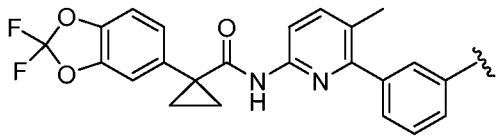
R^2, R^3, R^4, and R^7 each represent independently for each occurrence hydrogen or methyl;

R^9 represents independently for each occurrence hydrogen or C_1-C_6 alkyl;

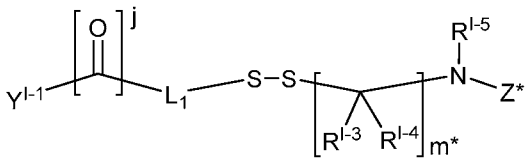
s is 5 or 6;

v is 1 or 2;

Y^1 is -N(R^7)C(O)-(C_1-C_6 alkylene)-phenyl, -N(R^7)C(O)-(hydroxyalkyl), -C(O)N(R^7)(hydroxyalkyl), -N(R^7)CO_2-(hydroxyalkyl), -OC(O)N(R^7)(hydroxyalkyl), or -N(R^7)C(O)-Z^1; and

Z^1 is  .

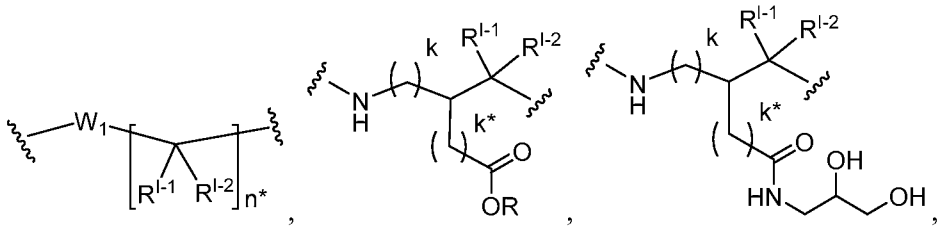
21. A compound of Formula I:

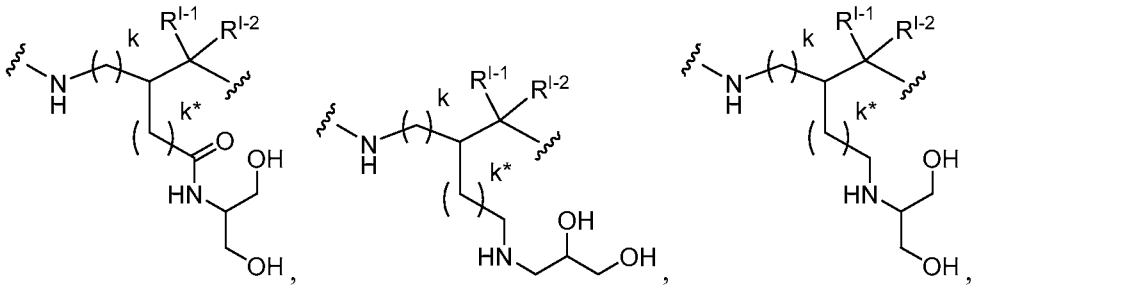


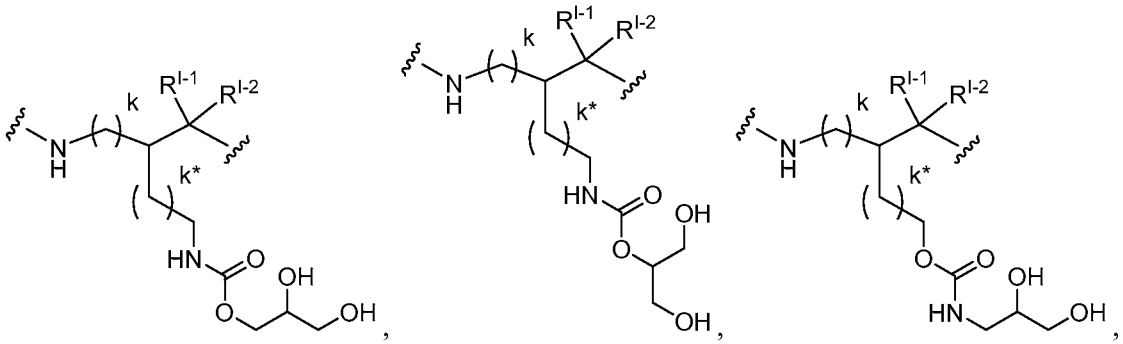
(I)

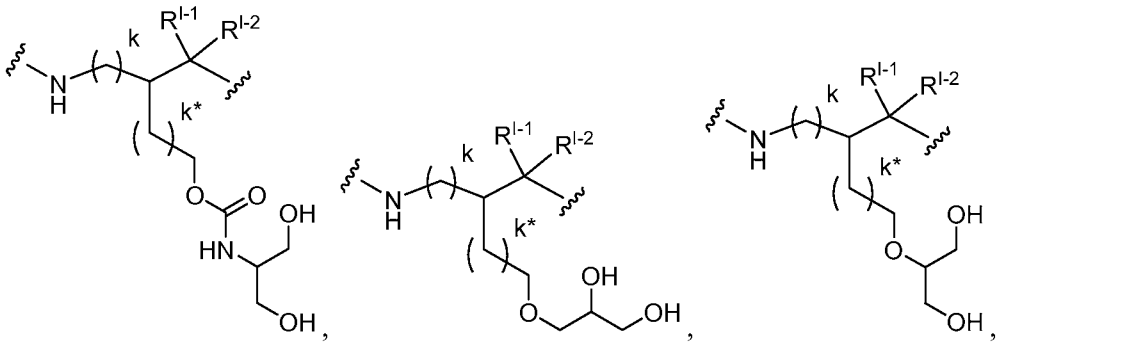
or a pharmaceutically acceptable salt or solvate thereof; wherein:

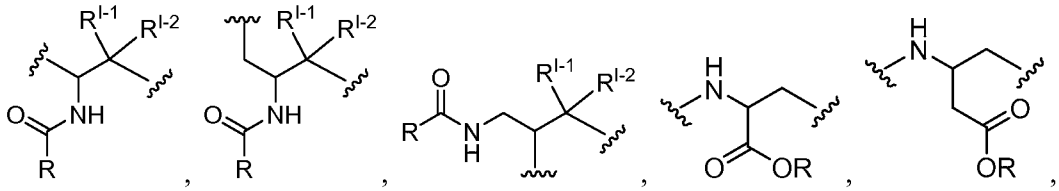
L_1 is independently

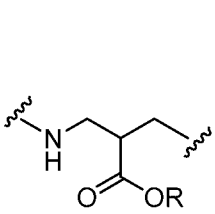
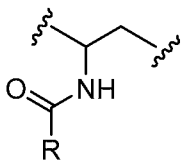
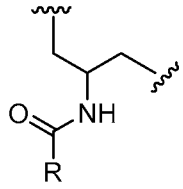
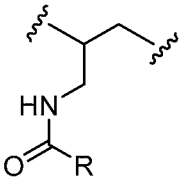
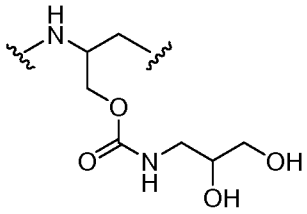
6 

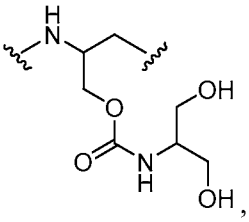
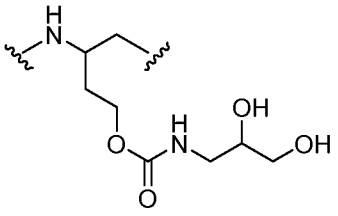
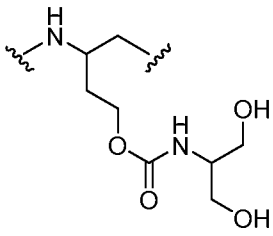
7 

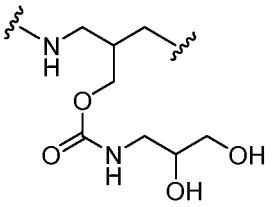
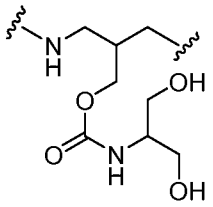
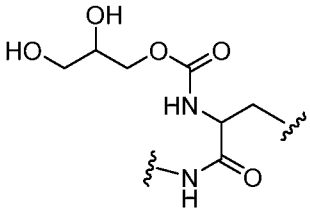
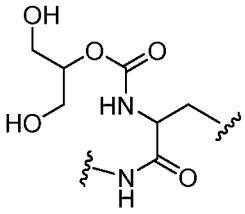
8 

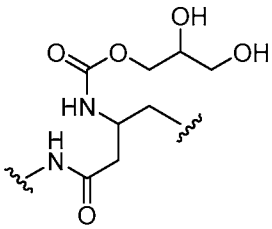
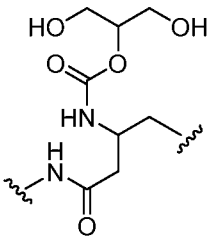
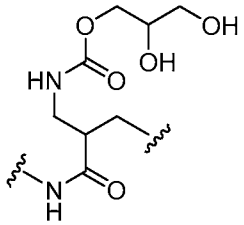
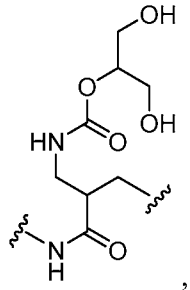
9 

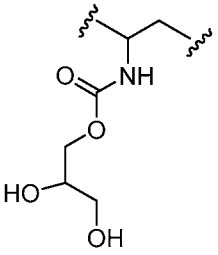
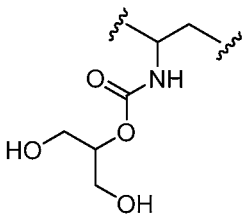
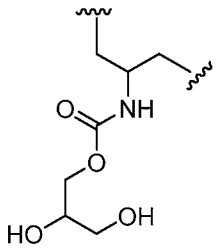
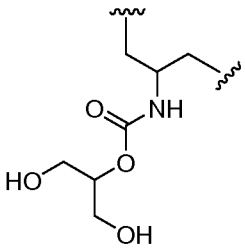
10 

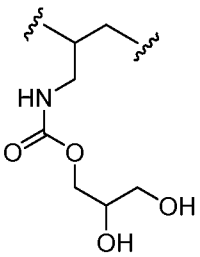
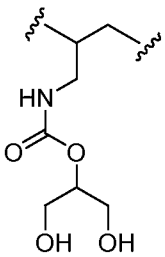
11  ,  ,  ,  ,  ,

12  ,  ,  ,

13  ,  ,  ,  ,

14  ,  ,  ,  ,

15  ,  ,  ,  ,

16  , or  ;

wherein the representation of L is limited directionally left to right as is depicted, with the right hand side connected to one of the two S groups shown in Formula I;

R^{I-1}, R^{I-2}, R^{I-3}, R^{I-4}, and R^{I-5} each represent independently for each occurrence hydrogen or C_1-C_3 alkyl;

W_1 is independently a bond, O, or $N(R^{I-1})$;

j is 0 or 1;

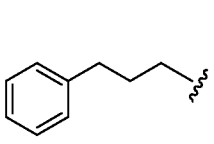
k and k* is independently 0 or 1;

n* is independently 1, 2, or 3, with the proviso that when n* = 1 then W_1 cannot be O or NR^{I-1};

m* is 2 or 3;

each R is independently -H, -C_1-C_6 alkyl, phenyl, benzyl, -$CH_2CO_2R^{I-1}$, -$CH_2CON(R^{I-1})(R^{I-1})$;

Y^{I-1} is independently selected from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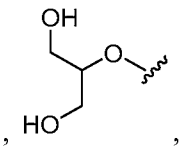
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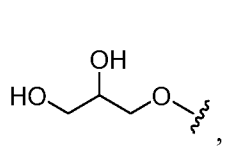
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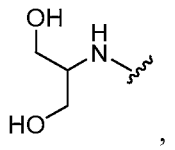
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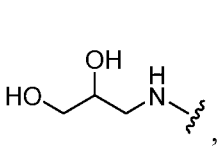
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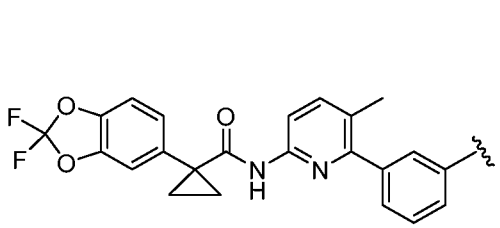
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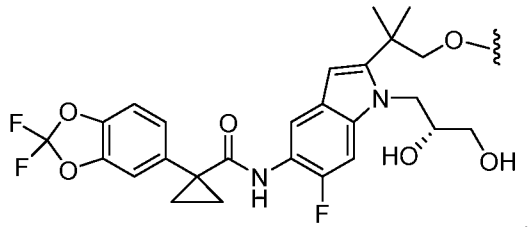
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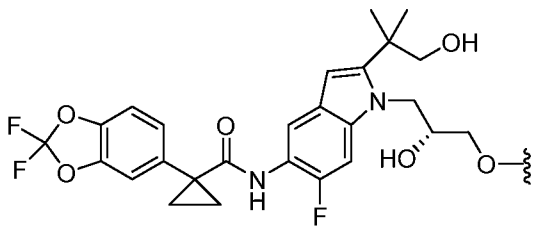
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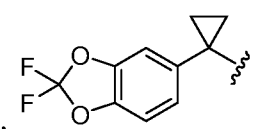
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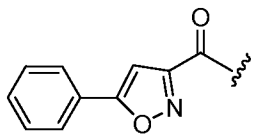
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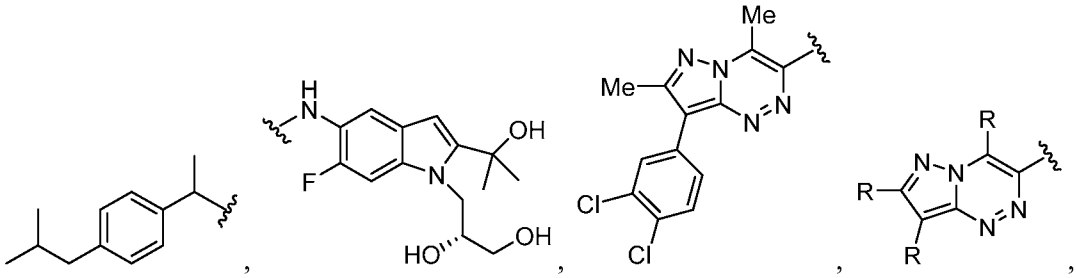
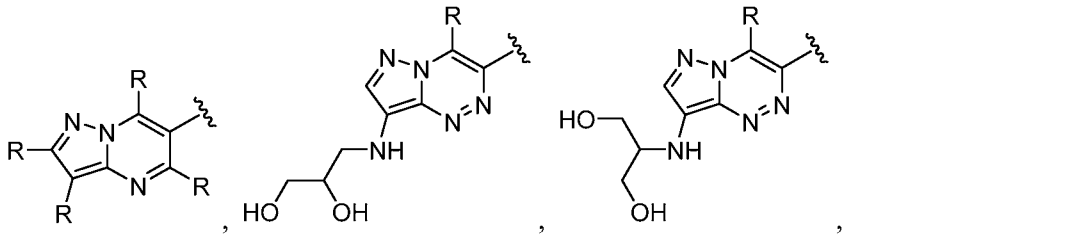
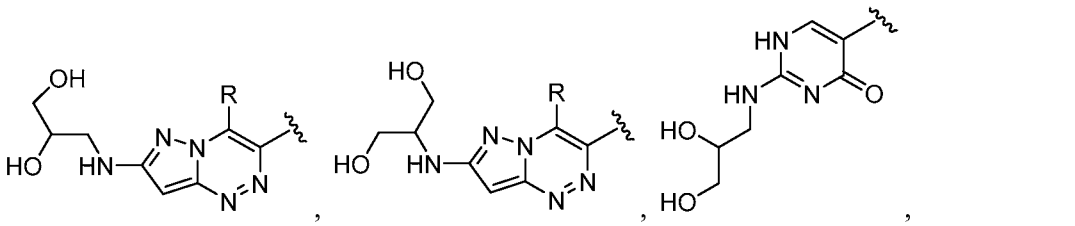
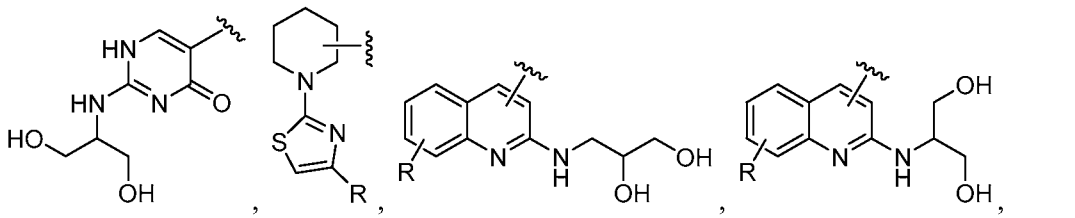
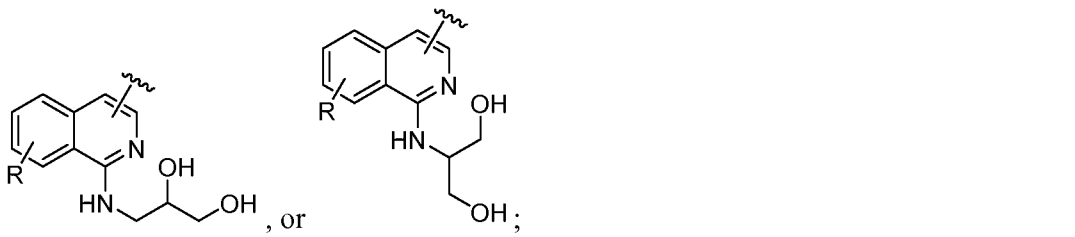
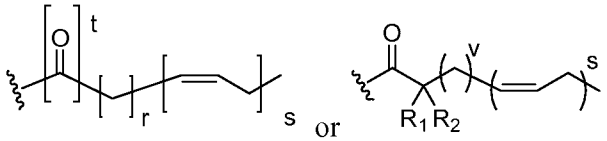
33 

34 

35 

36 

37 

38 Z* is  wherein:

39 R_1 and R_2 independently are hydrogen, C_1-C_4 alkyl, or halogen;

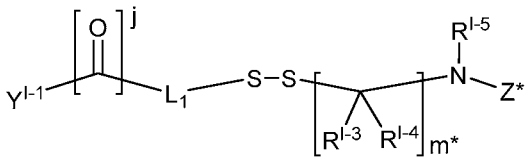
40 r is 2, 3, or 7;

s is 3, 5, or 6;

t is 0 or 1; and

v is 1, 2, or 6.

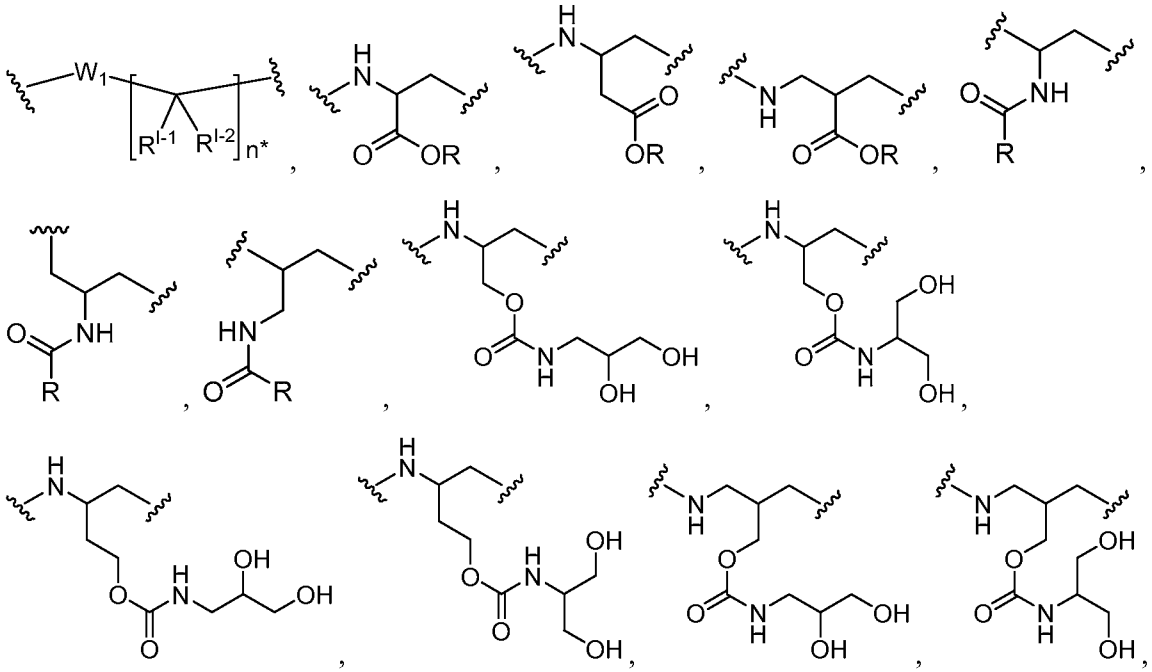
22. The compound of claim 21, wherein the compound is a compound of Formula I-A:

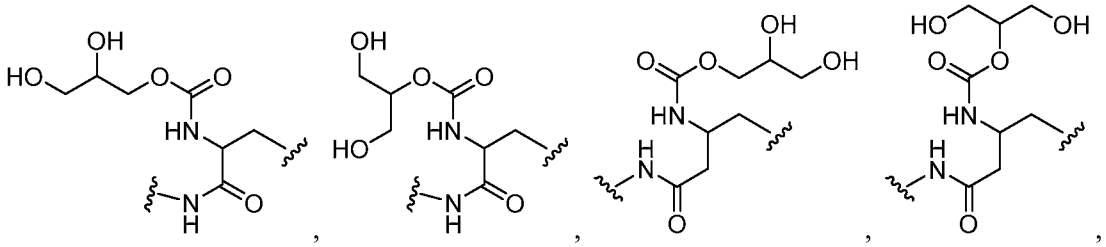
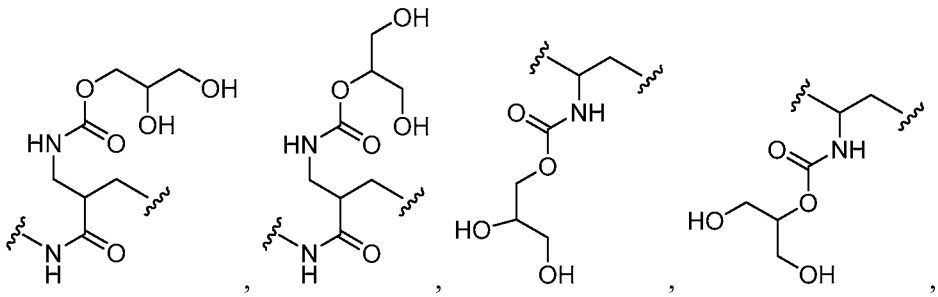
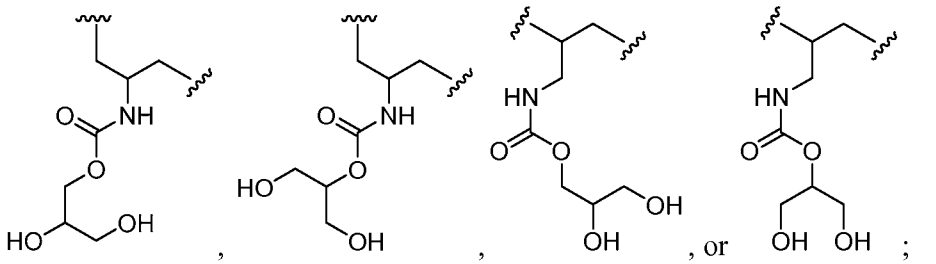


(I-A)

or a pharmaceutically acceptable salt or solvate thereof; wherein:

L_1 is independently



wherein the representation of L is limited directionally left to right as is depicted, with the right hand side connected to one of the two S groups shown in Formula I-A;

R^{I-1}, R^{I-2}, R^{I-3}, R^{I-4}, and R^{I-5} each represent independently for each occurrence hydrogen or C_1-C_3 alkyl;

W_1 is independently a bond, O, or $N(R^{I-1})$;

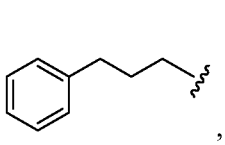
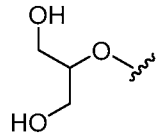
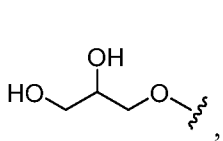
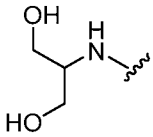
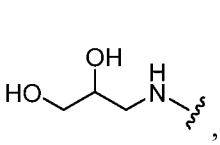
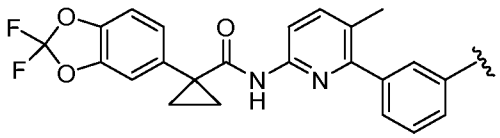
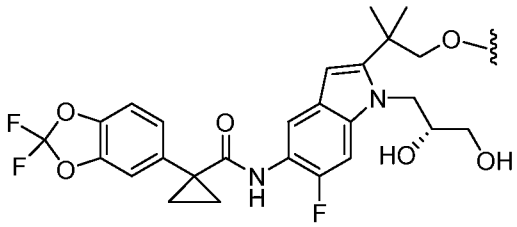
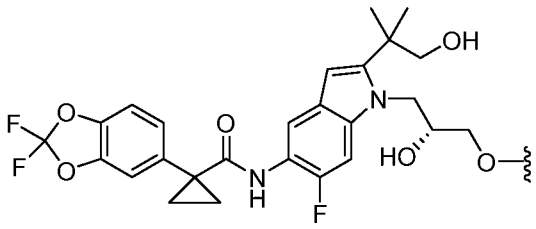
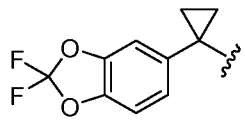
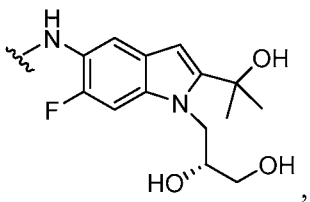
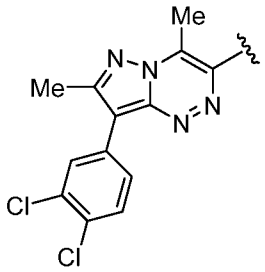
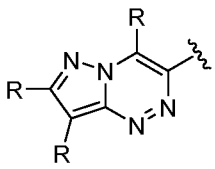
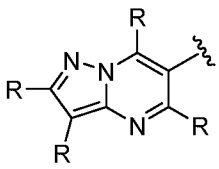
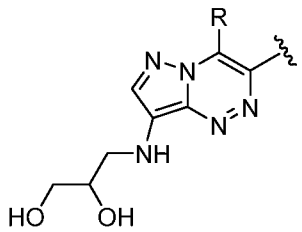
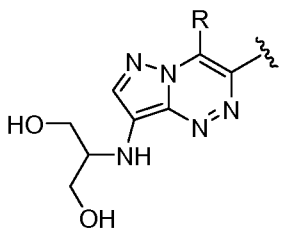
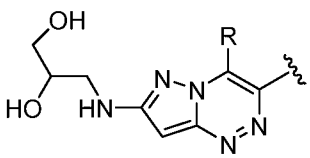
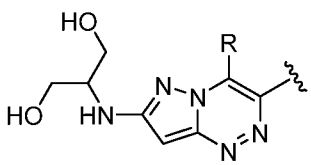
j is 0 or 1;

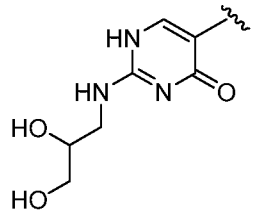
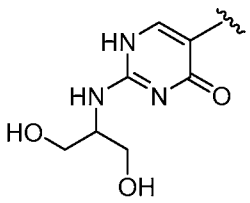
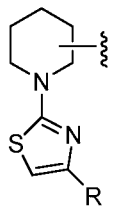
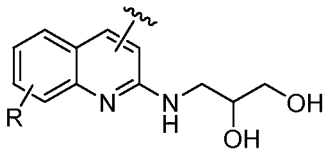
n* is independently 1, 2, or 3, with the proviso that when n* = 1 then W_1 cannot be O or NR^{I-1};

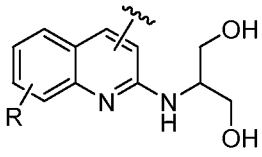
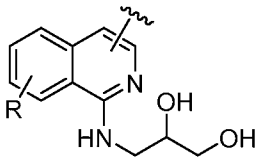
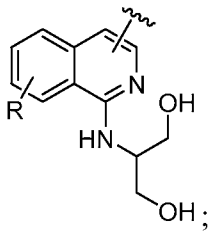
m* is 2 or 3;

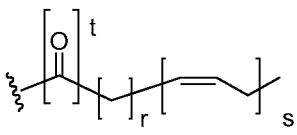
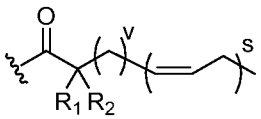
each R is independently -H, -C_1-C_6 alkyl, phenyl, benzyl, -$CH_2CO_2R^{I-1}$, -$CH_2CON(R^{I-1})(R^{I-1})$;

Y^{I-1} is independently selected from

29  ,  ,  ,  ,

30  ,  , or  ;

31 Z* is  or  wherein:

32 R_1 and R_2 independently are hydrogen, C_1-C_4 alkyl, or halogen;

33 r is 2, 3, or 7;

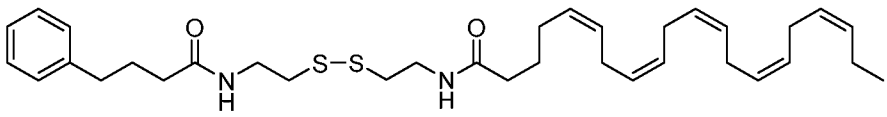
34 s is 3, 5, or 6;

35 t is 0 or 1; and

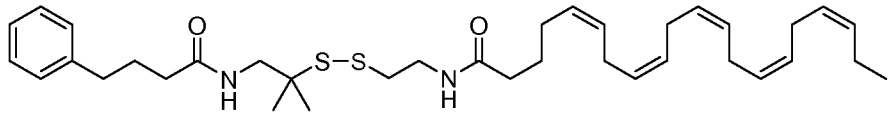
36 v is 1, 2, or 6.

1 23. The compound of claim 1, 20, 21 or 22, wherein the compound is a compound of
2 Formula A-1 or I-A or pharmaceutically acceptable salt thereof.

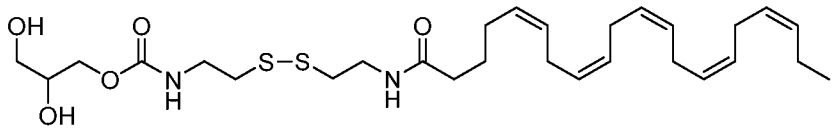
1 24. The compound of claim 1, 21, or 22, wherein the compound is one of the following or a
2 pharmaceutically acceptable salt thereof:

3 

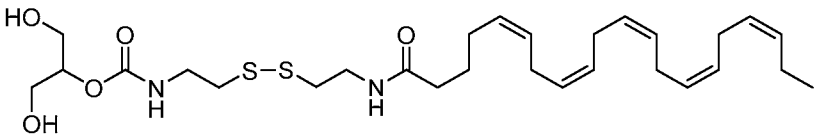
(5Z,8Z,11Z,14Z,17Z)-N-(2-((2-(4-phenylbutanamido)ethyl)disulfanyl)ethyl)icosa-5,8,11,14,17-pentaenamide (**I-2**);



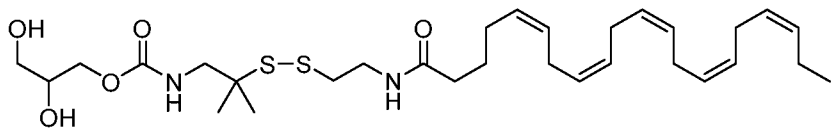
(5Z,8Z,11Z,14Z,17Z)-N-(2-((2-methyl-1-(4-phenylbutanamido)propan-2-yl)disulfanyl)ethyl)icosa-5,8,11,14,17-pentaenamide (**I-3**);



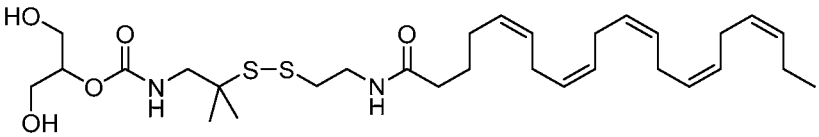
2,3-dihydroxypropyl (2-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)ethyl)carbamate (**I-8**);



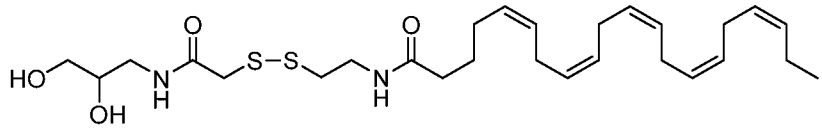
1,3-dihydroxypropan-2-yl (2-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)ethyl)carbamate (**I-9**);



2,3-dihydroxypropyl (2-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)-2-methylpropyl)carbamate (**I-10**);

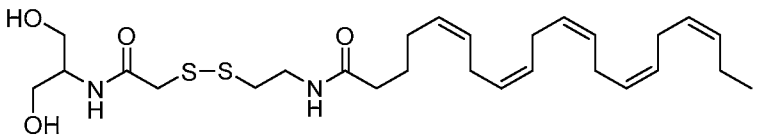


1,3-dihydroxypropan-2-yl (2-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)-2-methylpropyl)carbamate (**I-11**);

21 

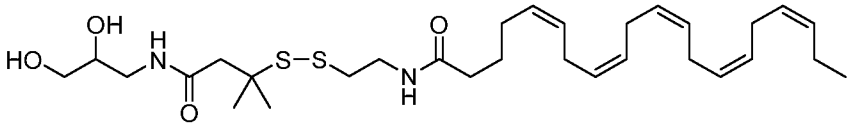
22 (5Z,8Z,11Z,14Z,17Z)-N-(2-((2-((2,3-dihydroxypropyl)amino)-2-

23 oxoethyl)disulfanyl)ethyl)icosa-5,8,11,14,17-pentaenamide (**I-12**);

24 

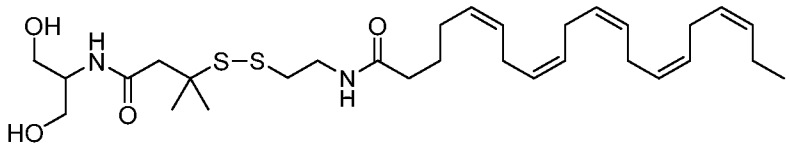
25 (5Z,8Z,11Z,14Z,17Z)-N-(2-((2-((1,3-dihydroxypropan-2-yl)amino)-2-

26 oxoethyl)disulfanyl)ethyl)icosa-5,8,11,14,17-pentaenamide (**I-13**);

27 

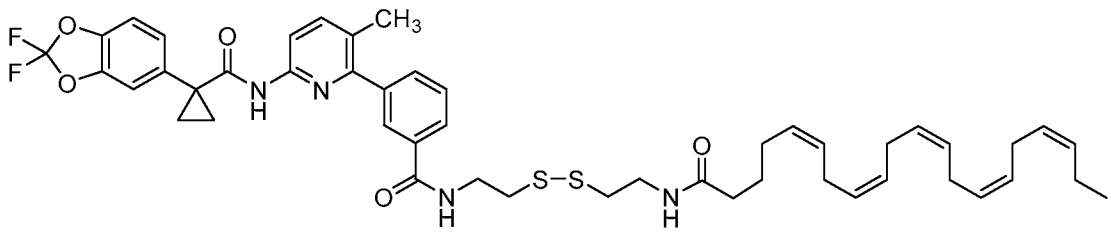
28 (5Z,8Z,11Z,14Z,17Z)-N-(2-((4-((2,3-dihydroxypropyl)amino)-2-methyl-4-oxobutan-2-

29 yl)disulfanyl)ethyl)icosa-5,8,11,14,17-pentaenamide (**I-14**);

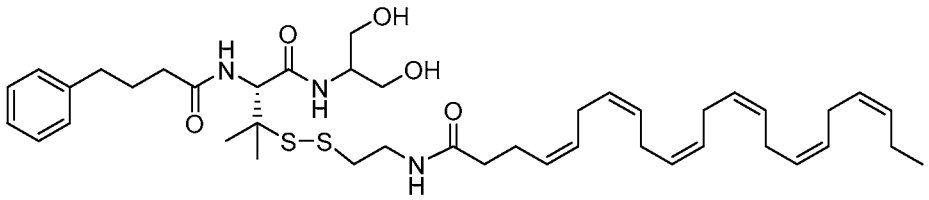
30 

31 (5Z,8Z,11Z,14Z,17Z)-N-(2-((4-((1,3-dihydroxypropan-2-yl)amino)-2-methyl-4-oxobutan-2-

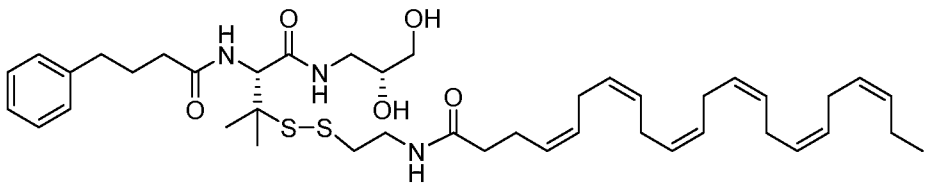
32 yl)disulfanyl)ethyl)icosa-5,8,11,14,17-pentaenamide (**I-15**);

33 

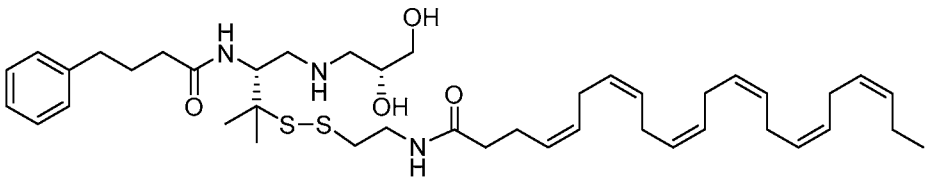
3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropane-1-carboxamido)-3-methylpyridin-2-yl)-N-(2-((2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenamido)ethyl)disulfanyl)ethyl)benzamide (**I-20**);



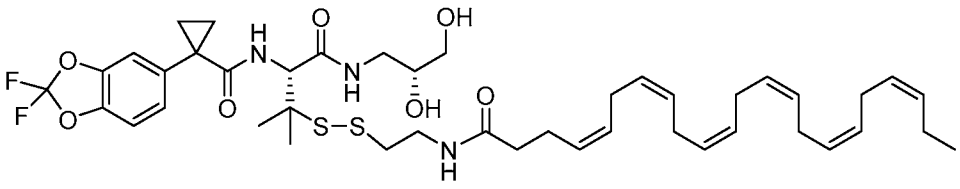
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-((1,3-dihydroxypropan-2-yl)amino)-2-methyl-4-oxo-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-25**);



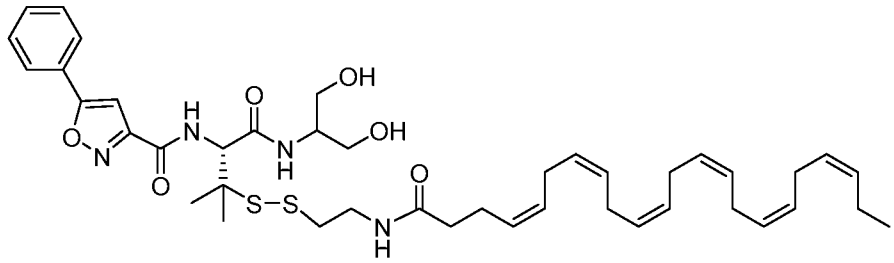
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-(((R)-2,3-dihydroxypropyl)amino)-2-methyl-4-oxo-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-26**);



(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-(((R)-2,3-dihydroxypropyl)amino)-2-methyl-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-28**);

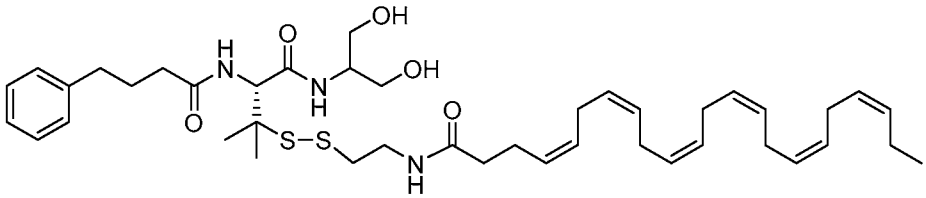


1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-((R)-1-(((R)-2,3-dihydroxypropyl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-1-oxobutan-2-yl)cyclopropane-1-carboxamide (**I-34**); or



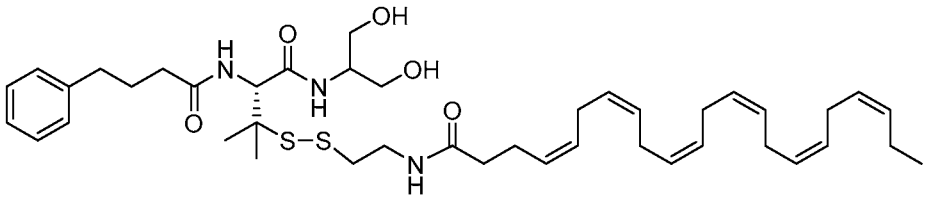
N-((R)-1-((1,3-dihydroxypropan-2-yl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-1-oxobutan-2-yl)-5-phenylisoxazole-3-carboxamide (**I-41**).

25. The compound of claim 1, 21, or 22, wherein the compound is the following or a pharmaceutically acceptable salt thereof:



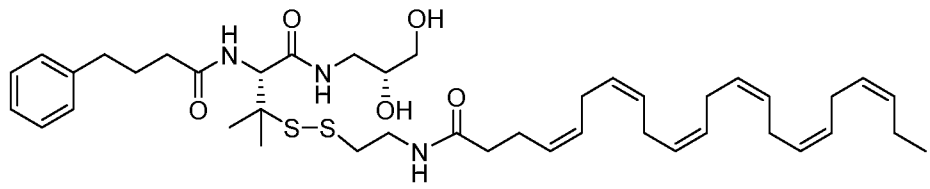
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-((1,3-dihydroxypropan-2-yl)amino)-2-methyl-4-oxo-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-25**).

26. The compound of claim 1, 21, or 22, wherein the compound is:



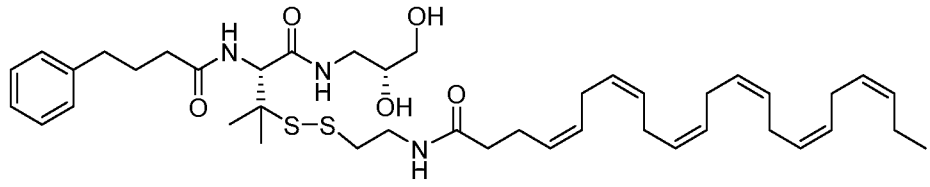
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-((1,3-dihydroxypropan-2-yl)amino)-2-methyl-4-oxo-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-25**).

27. The compound of claim 1, 21, or 22, wherein the compound is the following or a pharmaceutically acceptable salt thereof:



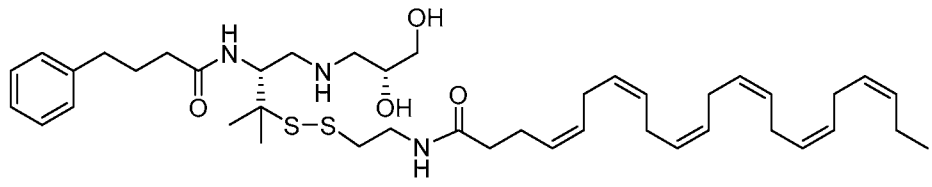
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-(((R)-2,3-dihydroxypropyl)amino)-2-methyl-4-oxo-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-26**).

28. The compound of claim 1, 21, or 22, wherein the compound is:



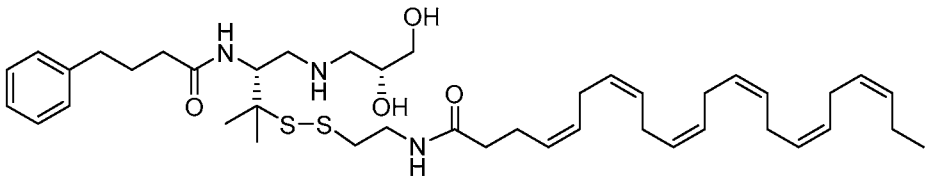
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-(((R)-2,3-dihydroxypropyl)amino)-2-methyl-4-oxo-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-26**).

29. The compound of claim 21, wherein the compound is the following or a pharmaceutically acceptable salt thereof:



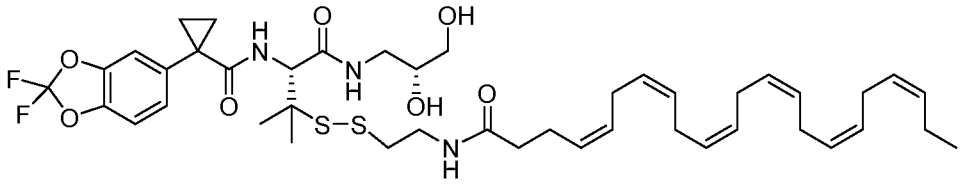
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-(((R)-2,3-dihydroxypropyl)amino)-2-methyl-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-28**).

30. The compound of claim 1, 21, or 22, wherein the compound is:



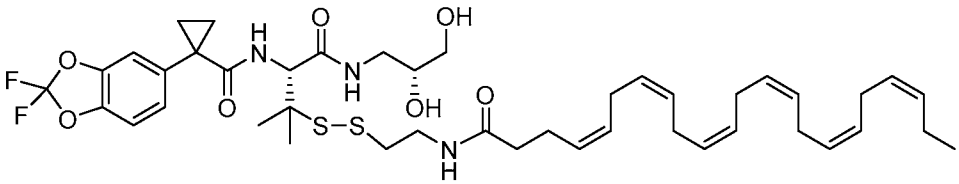
(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(((R)-4-(((R)-2,3-dihydroxypropyl)amino)-2-methyl-3-(4-phenylbutanamido)butan-2-yl)disulfanyl)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**I-28**).

31. The compound of claim 1, 21, or 22, wherein the compound is the following or a pharmaceutically acceptable salt thereof:



1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-((R)-1-(((R)-2,3-dihydroxypropyl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-1-oxobutan-2-yl)cyclopropane-1-carboxamide (**I-34**).

32. The compound of claim 1, 21, or 22, wherein the compound is:



1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-((R)-1-(((R)-2,3-dihydroxypropyl)amino)-3-((2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)ethyl)disulfanyl)-3-methyl-1-oxobutan-2-yl)cyclopropane-1-carboxamide (**I-34**).

33. A pharmaceutical composition comprising a compound of any one of claims 1-32 and a pharmaceutically acceptable carrier.

34. A method of treating a disease selected from the group consisting of CF, a neurodegenerative disease, inflammatory disease, liver disease, muscle disease, infection, and immune disease in a patient, the method comprising administering to a subject in need thereof a therapeutically effective amount of a compound of any one of claims 1-32 to treat the disease.

35. The method of claim 34, wherein the disease is CF.

36. The method of claim 34, wherein the disease is a neurodegenerative disease.

37. The method of claim 36, wherein the neurodegenerative disease is Huntington's disease, Alzheimer's disease, or Parkinson's disease.

38. A method of claim 35, wherein the method further comprises administering a second therapeutic agent selected from the group consisting of Ivacaftor (VX-770), Lumacaftor (VX-809), VX-661, Orkambi (a combination of VX-770 and VX-809), and a combination of VX-661 and VX-770.

39. The method of claim 34, wherein the disease is idiopathic pulmonary fibrosis.

40. A method of activating autophagy in a subject, the method comprising administering to a subject in need thereof an effective amount of a compound of any one of claims 1-32 to activate autophagy in the subject.

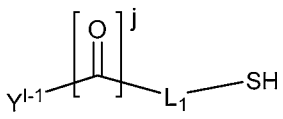
41. The method of claim 40, wherein the administering increases the ratio of LC3-II to LC3-I in the subject by at least 10%.

42. The method of claim 40 or 41, wherein the administering decreases the amount of p62 protein in the subject by at least 1% w/w.

43. The method of any one of claims 34-42, wherein the patient has been diagnosed as having CF or a neurodegenerative disease or idiopathic pulmonary fibrosis.

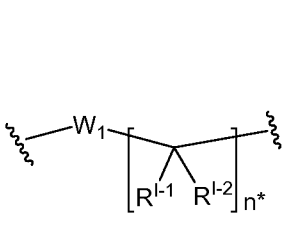
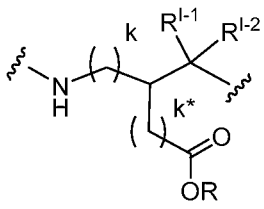
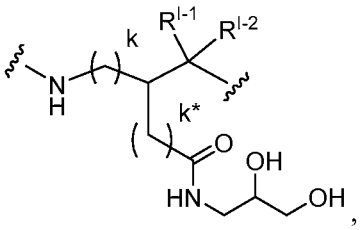
44. The method of any one of claims 34-43, wherein the patient is a human.

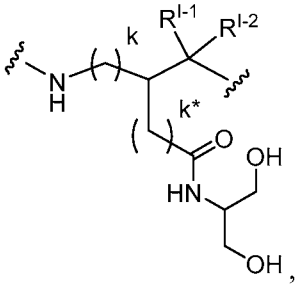
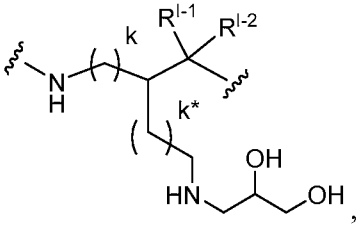
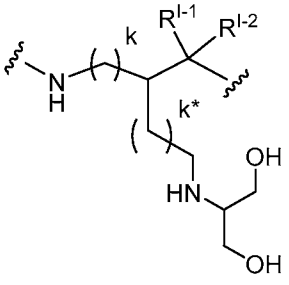
45. A compound of Formula II:

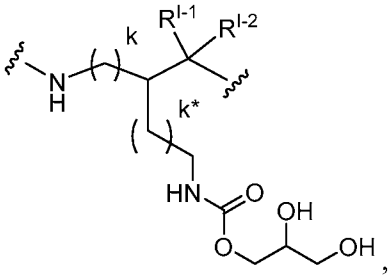
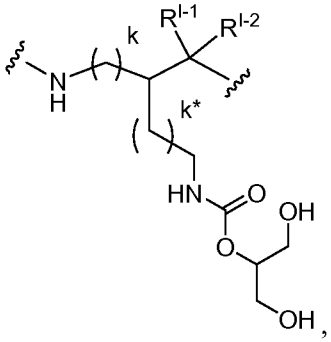
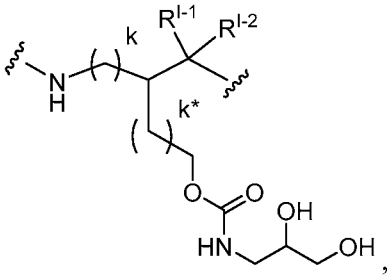


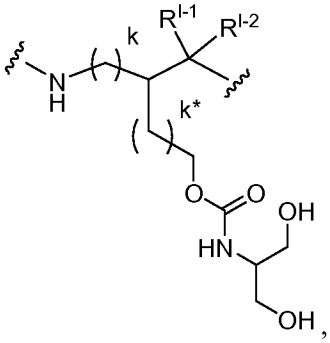
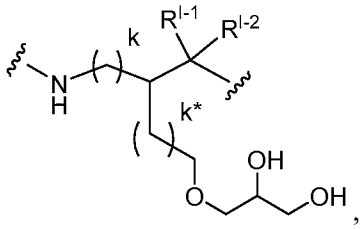
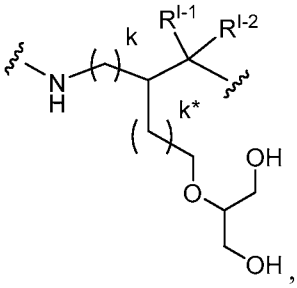
(II) , wherein

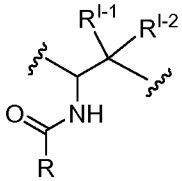
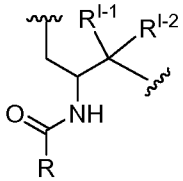
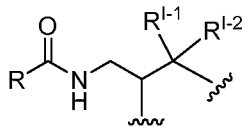
L_1 is independently



, or

;

wherein the representation of L is limited directionally left to right as is depicted, with the right hand side connected to one of the two S groups shown in Formula II;

R^{I-1} and R^{I-2}, each represent independently for each occurrence hydrogen or C_1-C_3 alkyl;

W_1 is independently a bond, O, or $N(R^{I-1})$;

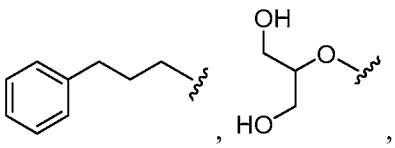
j is 0 or 1;

k and k* is independently 0 or 1;

n* is independently 1, 2, or 3, with the proviso that when n* = 1 then W_1 cannot be O or NR^{I-1};

each R is independently -H, -C_1-C_6 alkyl, phenyl, benzyl, -$CH_2CO_2R^{I-1}$, -$CH_2CON(R^{I-1})(R^{I-1})$;

Y^{I-1} is independently selected from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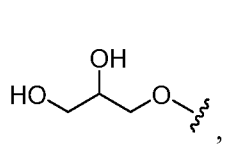
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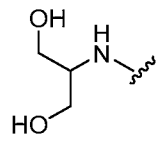
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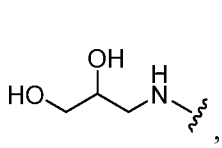
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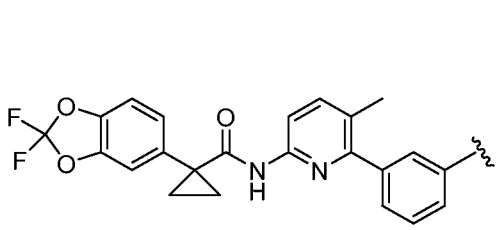
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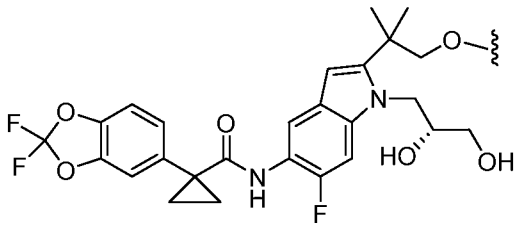
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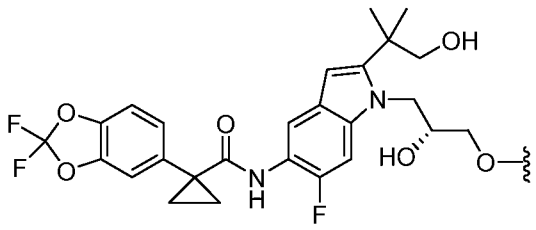
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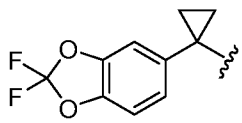
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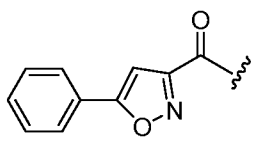
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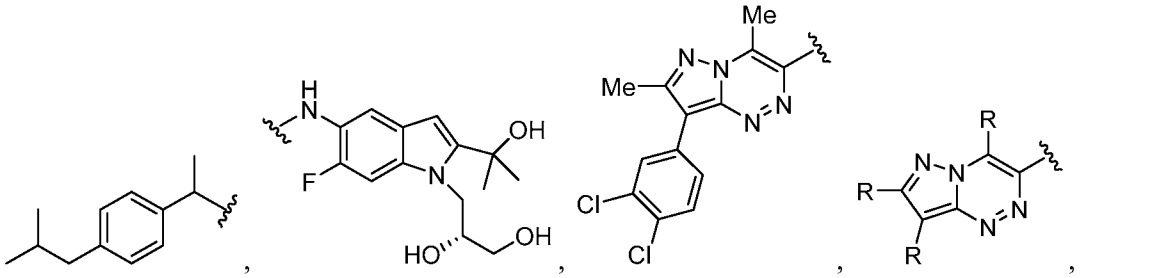
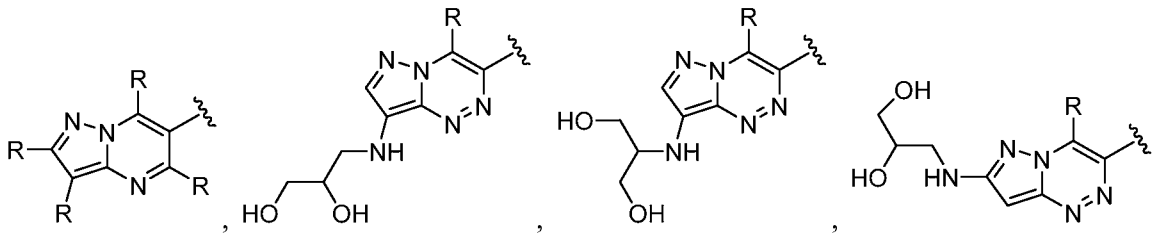
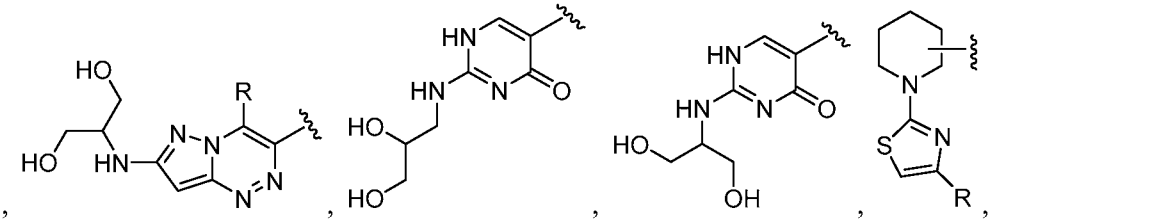
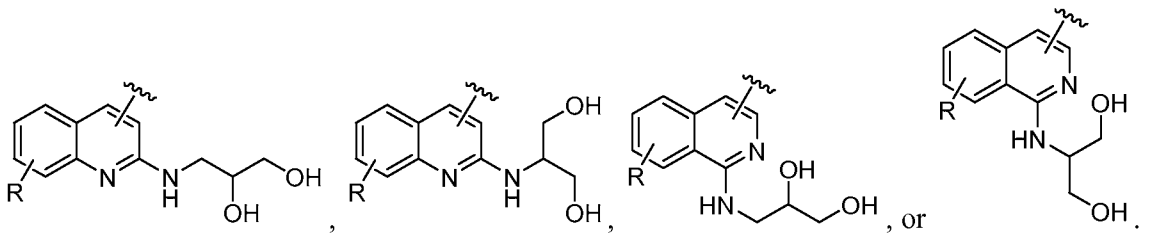
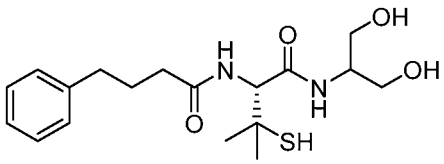
25 

26 

27 

28 

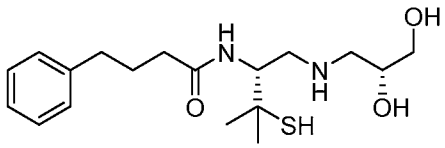
1 46. The compound of claim 45, wherein the compound is

2 

3 (R)-N-(1,3-dihydroxypropan-2-yl)-3-mercapto-3-methyl-2-(4-phenylbutanamido)butanamide

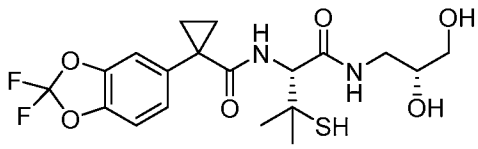
4 (**II-1**).

1 47. The compound of claim 45, wherein the compound is



N-((R)-1-(((R)-2,3-dihydroxypropyl)amino)-3-mercapto-3-methylbutan-2-yl)-4-phenylbutanamide (**II-4**).

48. The compound of claim 45, wherein the compound is



1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-((R)-1-(((R)-2,3-dihydroxypropyl)amino)-3-mercapto-3-methyl-1-oxobutan-2-yl)cyclopropane-1-carboxamide (**II-6**).

49. A method of treating a disease selected from the group consisting of CF, a neurodegenerative disease, inflammatory disease, liver disease, muscle disease, infection, and immune disease in a subject, comprising providing to, or creating in, a subject in need thereof a therapeutically effective amount of a compound of claims 45-48 to treat the disease.

50. The method of claim 49, wherein the disease is CF.

Fig. 1A

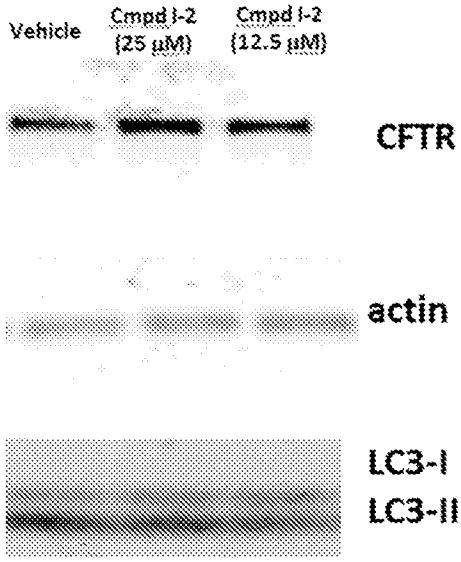


Fig. 1B

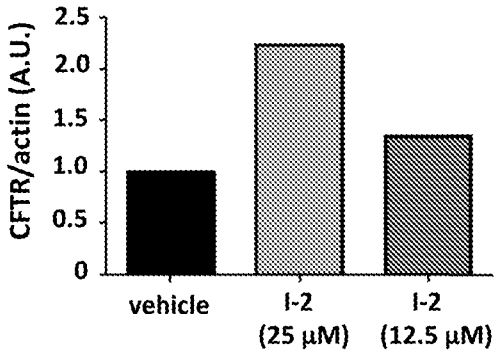


Fig. 1C

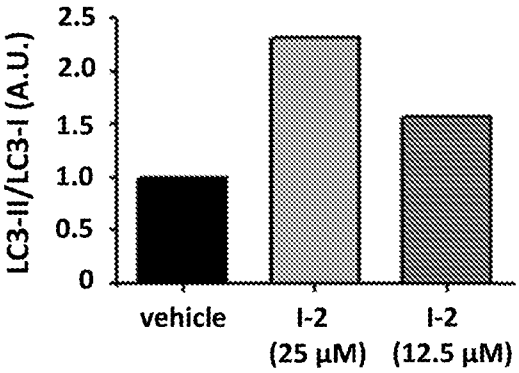


Fig. 2A

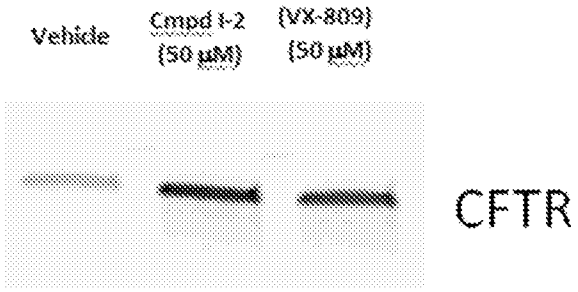

Vehicle
Cmpd I-2 (50 μM)
(VX-809) (50 μM)
CFTR

Fig. 2B

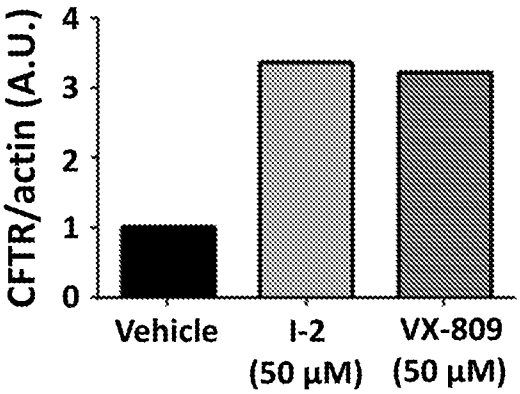

CFTR/actin (A.U.)
4
3
2
1
0
Vehicle
I-2 (50 μM)
VX-809 (50 μM)

Fig. 3

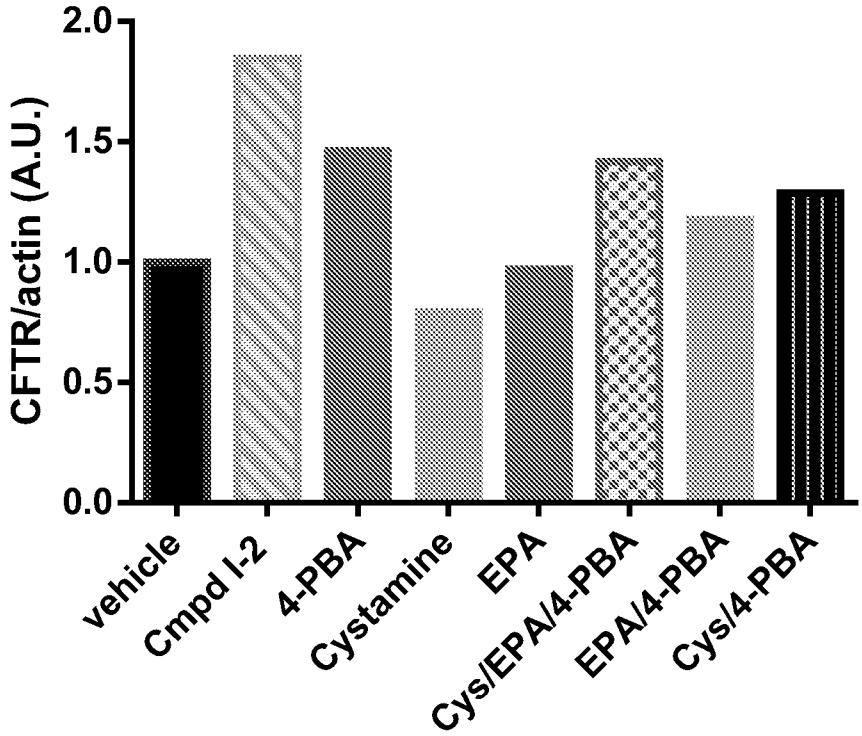


Fig. 4A

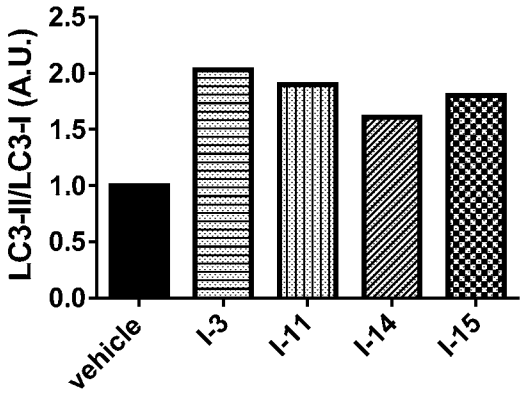


Fig. 4B

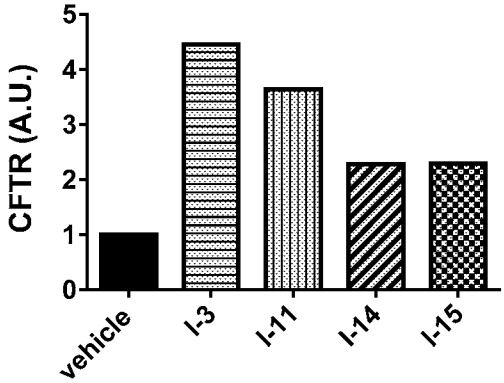


Fig. 5A

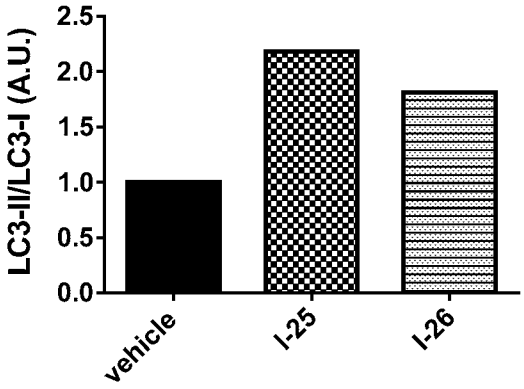


Fig. 5B

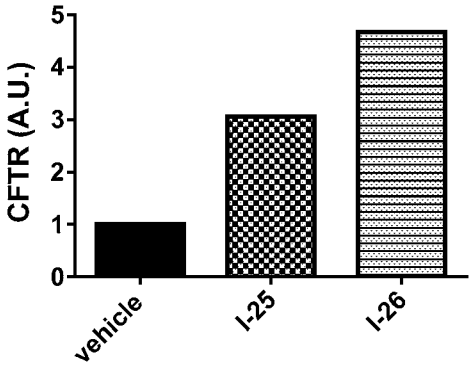


Fig. 6

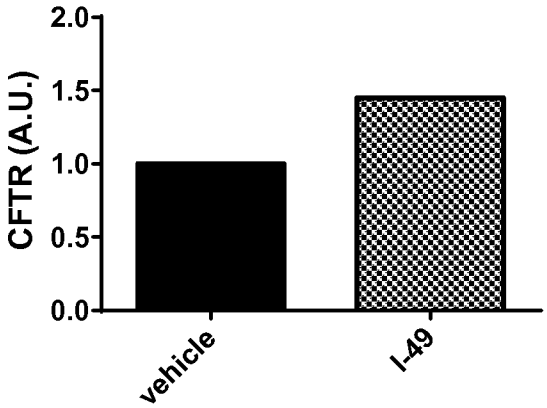


Fig. 7A

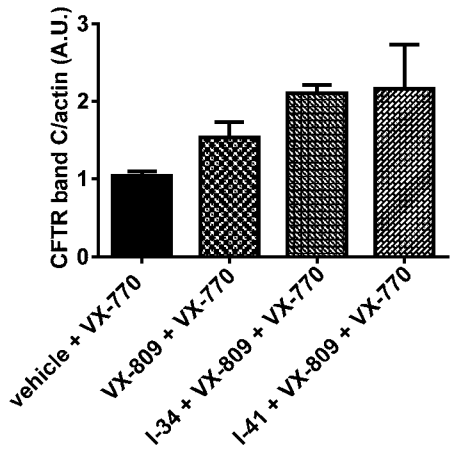


Fig. 7B

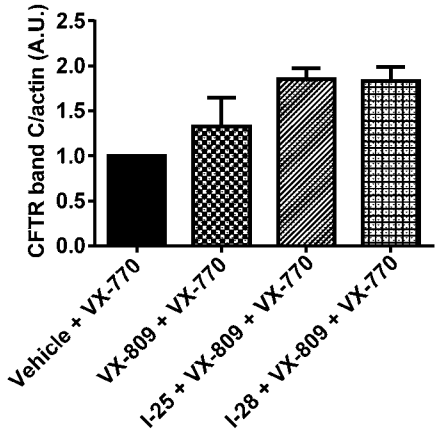


Fig. 8A

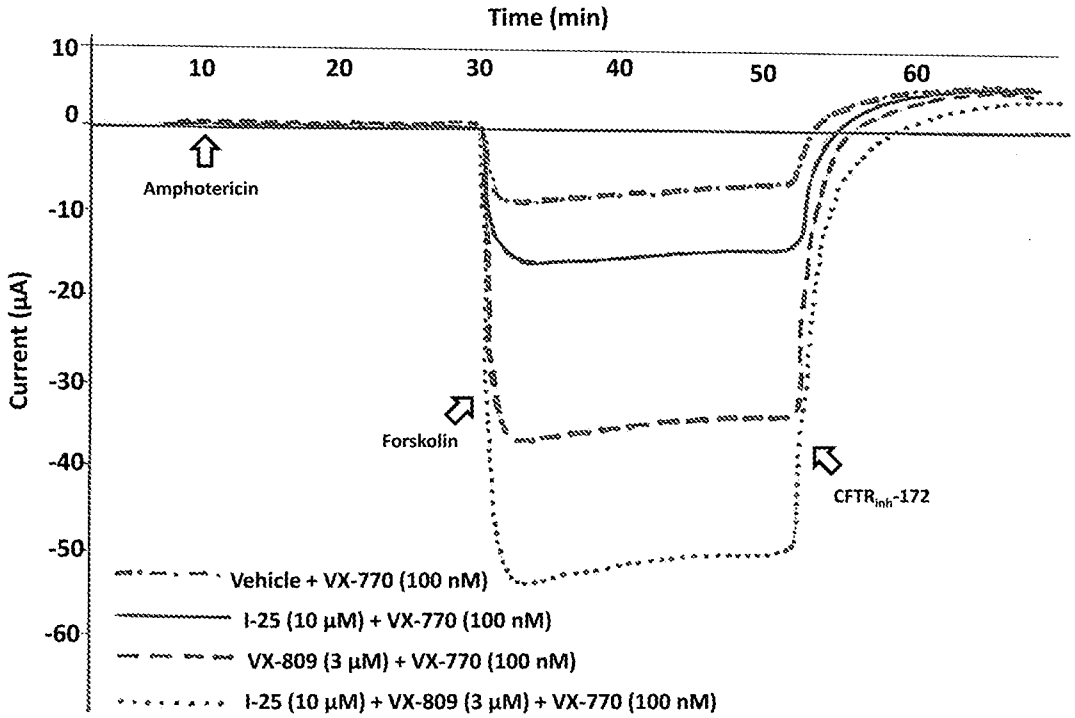


Fig. 8B

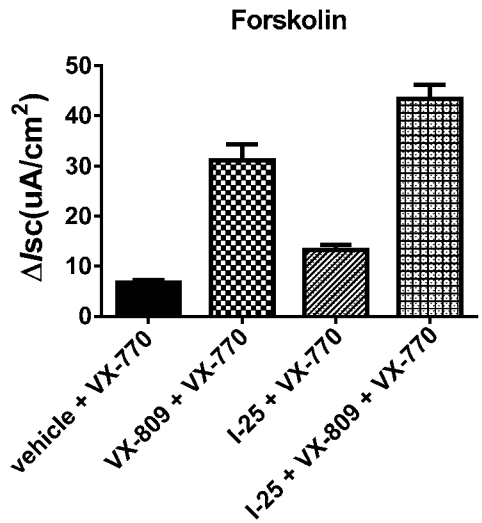


Fig. 8C

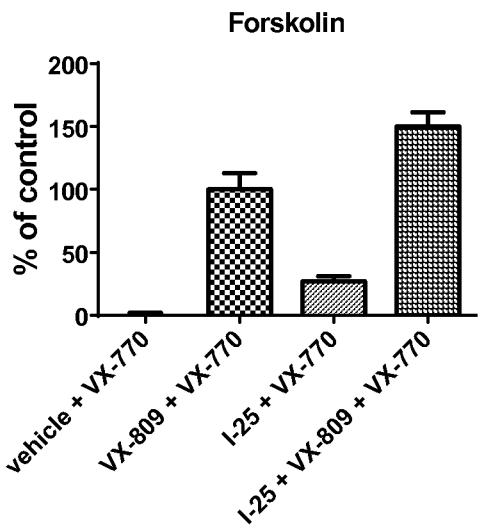


Fig. 8D

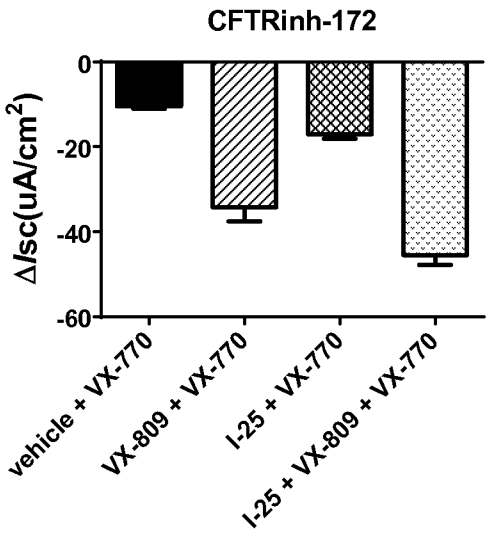

CFTRinh-172
ΔIsc(uA/cm2)
0
-20
-40
-60
vehicle + VX-770
VX-809 + VX-770
I-25 + VX-770
I-25 + VX-809 + VX-770

Fig. 8E

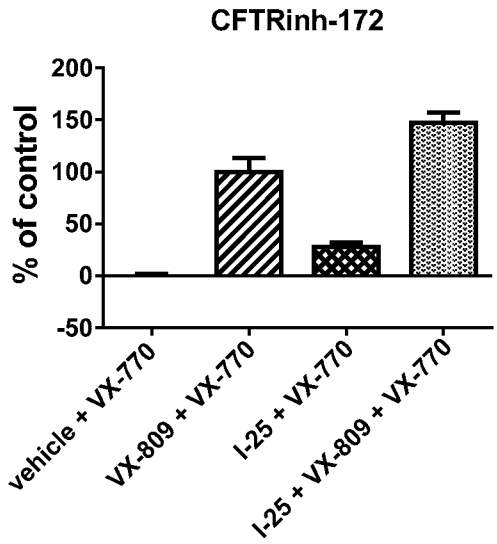

CFTRinh-172
% of control
200
150
100
50
0
-50
vehicle + VX-770
VX-809 + VX-770
I-25 + VX-770
I-25 + VX-809 + VX-770

Fig. 9A

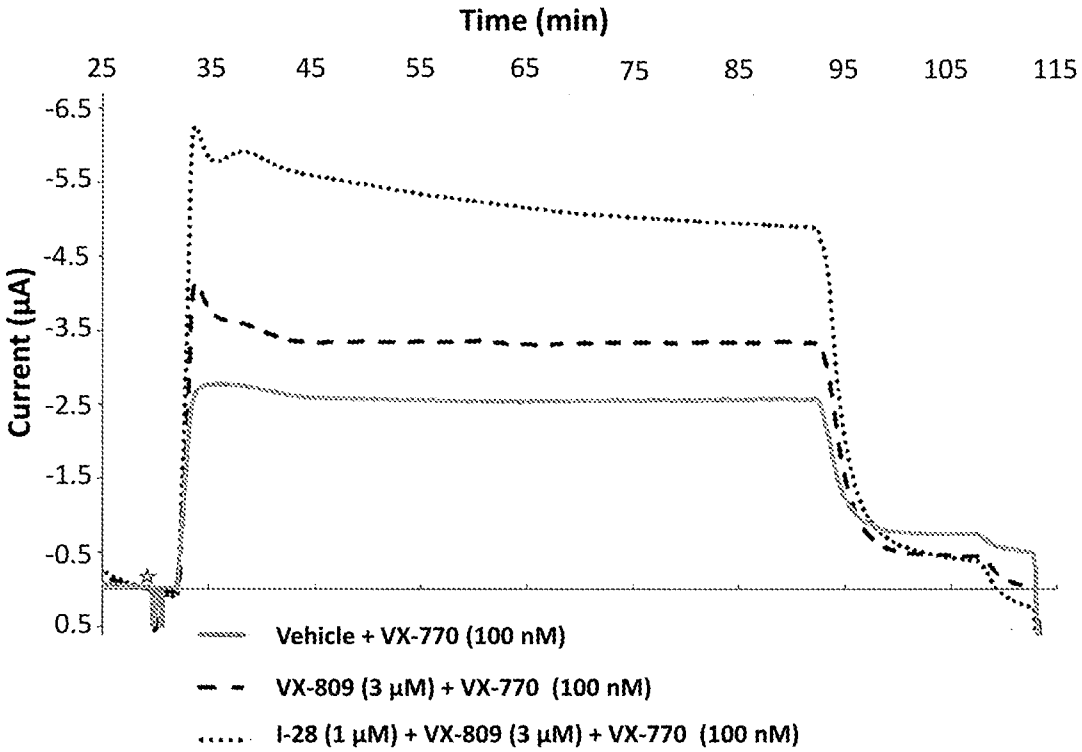


Fig. 9B

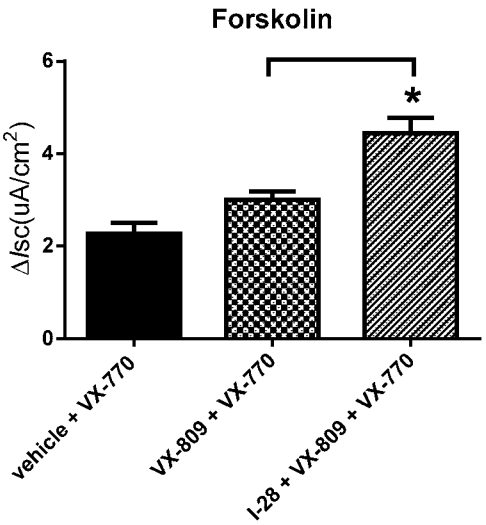


Fig. 9C

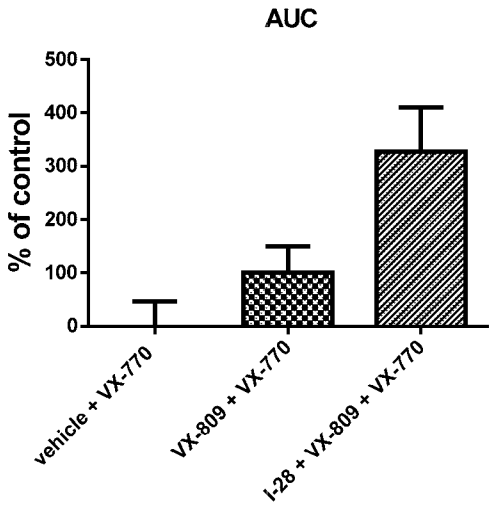


Fig. 9D

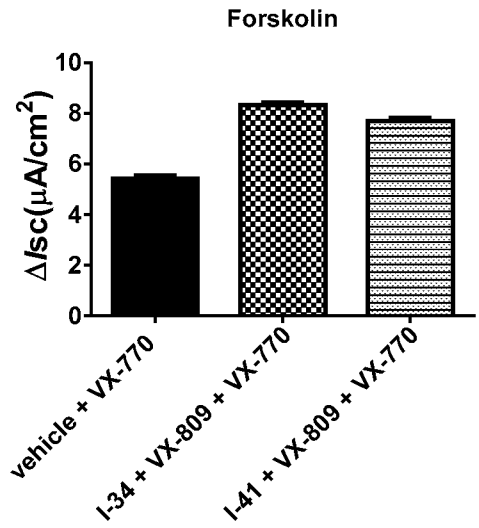

Forskolin
ΔIsc(μA/cm²)
10
8
6
4
2
0
vehicle + VX-770
I-34 + VX-809 + VX-770
I-41 + VX-809 + VX-770

Fig. 9E

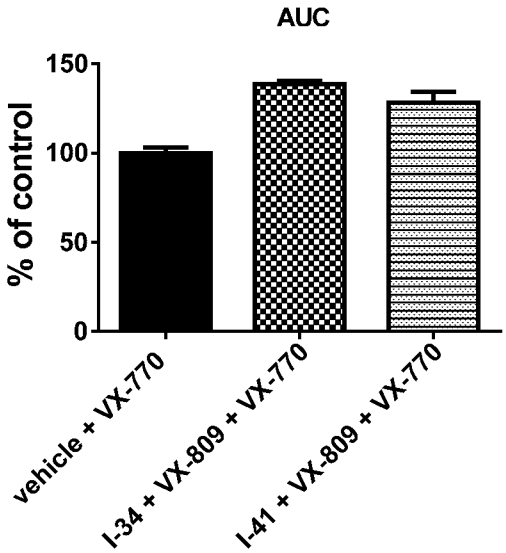

AUC
% of control
150
100
50
0
vehicle + VX-770
I-34 + VX-809 + VX-770
I-41 + VX-809 + VX-770

Fig. 10A

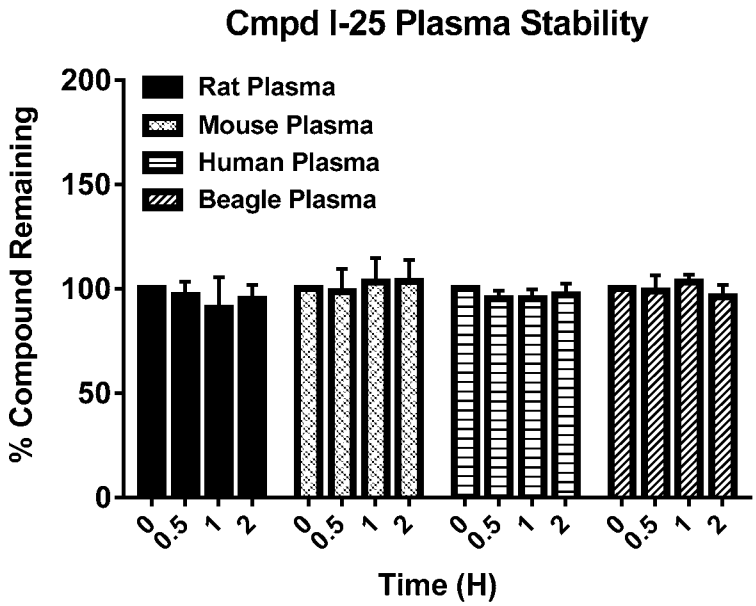


Fig. 10B

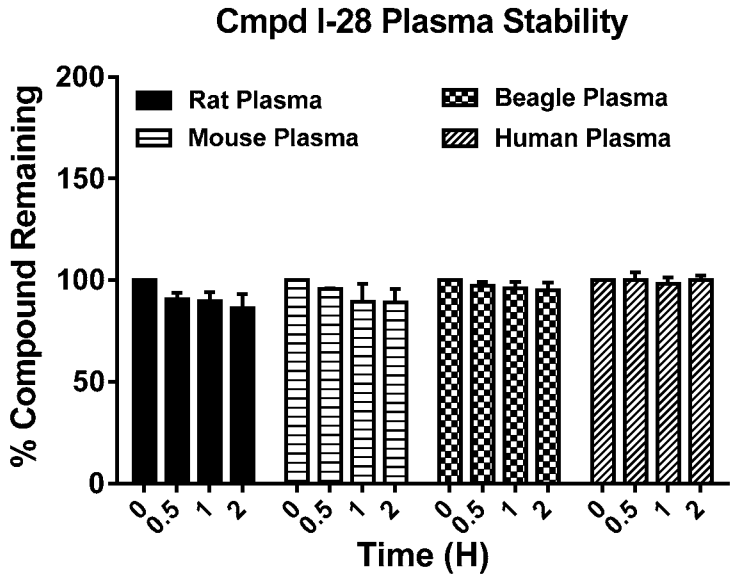


Fig. 10C

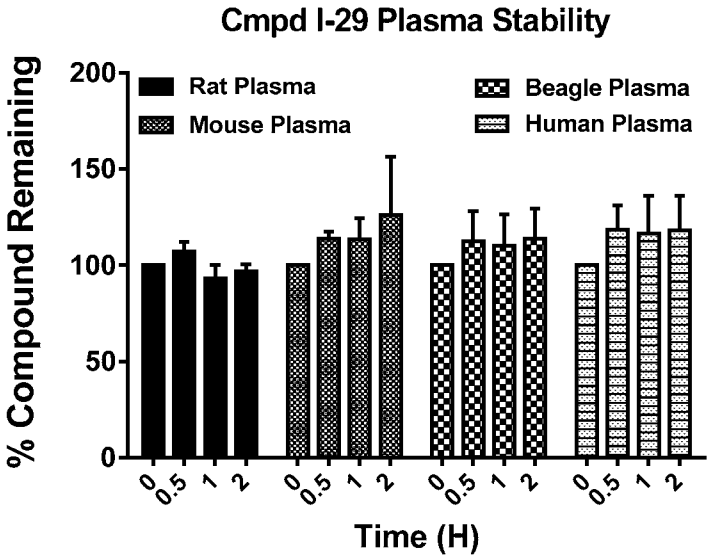

Cmpd I-29 Plasma Stability
% Compound Remaining
200
150
100
50
0
Rat Plasma
Mouse Plasma
Beagle Plasma
Human Plasma
0
0.5
1
2
Time (H)

Fig. 11A

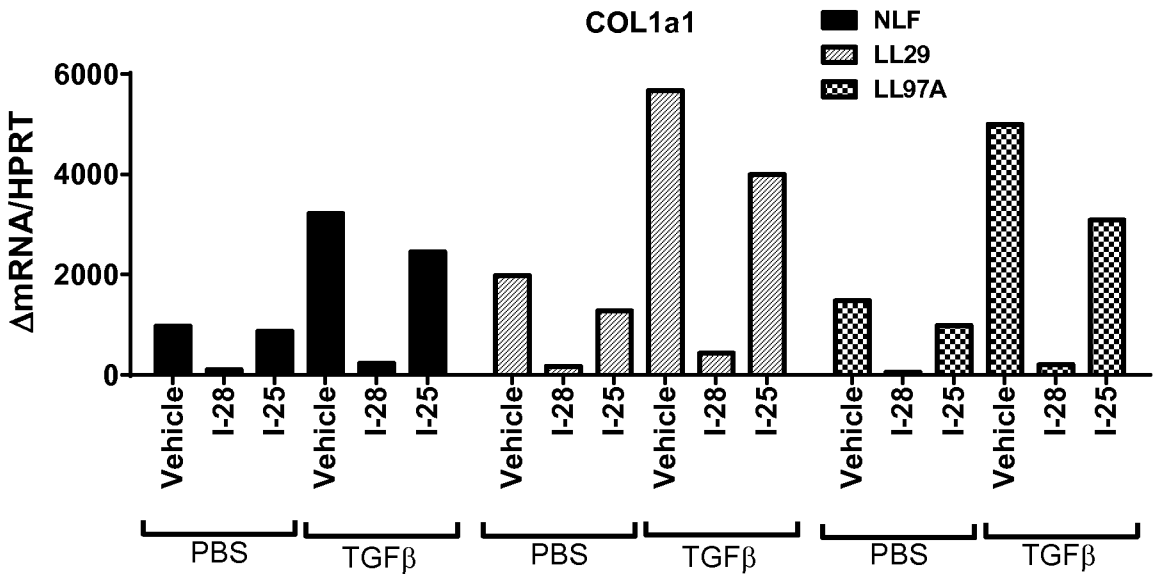


Fig. 11B

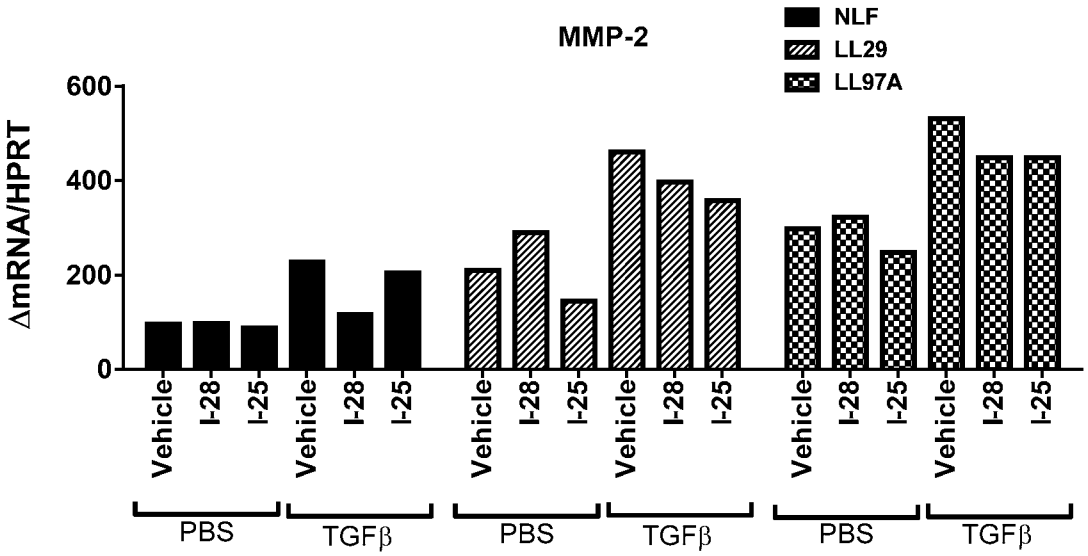


Fig. 11C

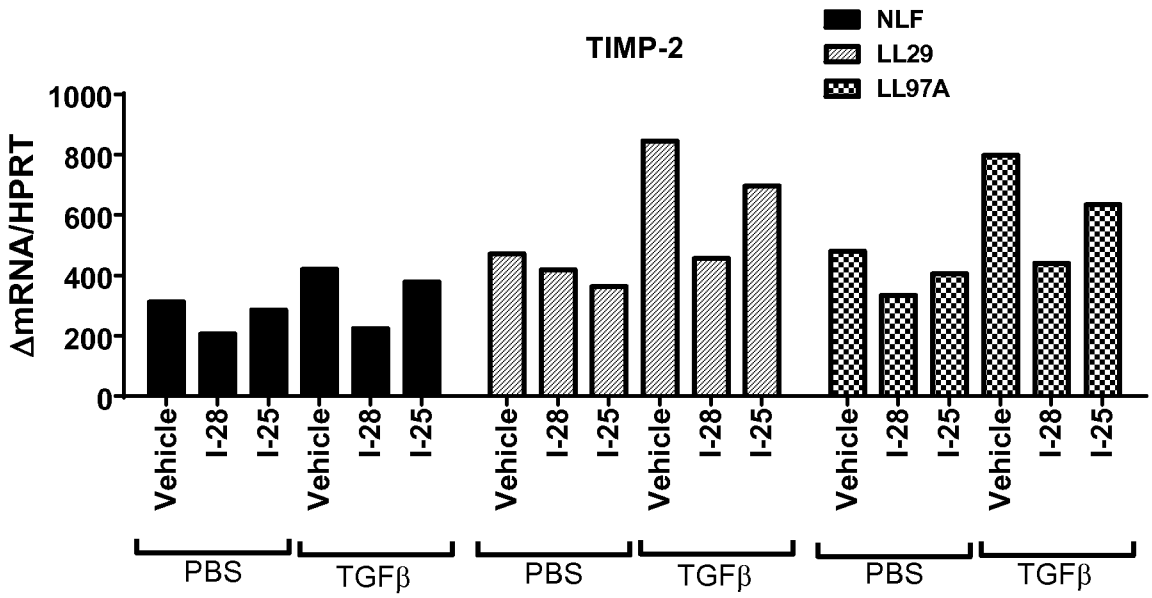


Fig. 12A

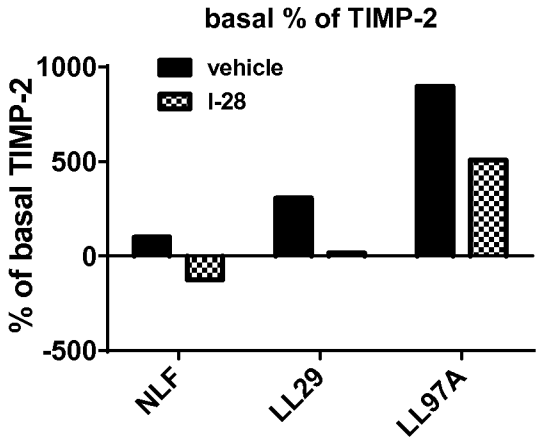


Fig. 12B

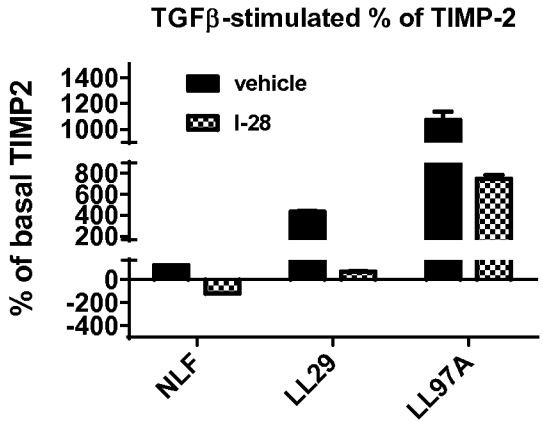


Fig. 13A

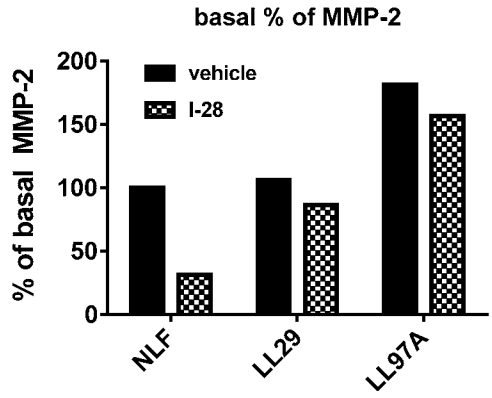


Fig. 13B

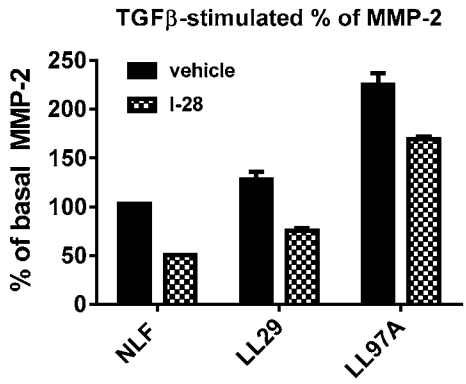


Fig. 14A

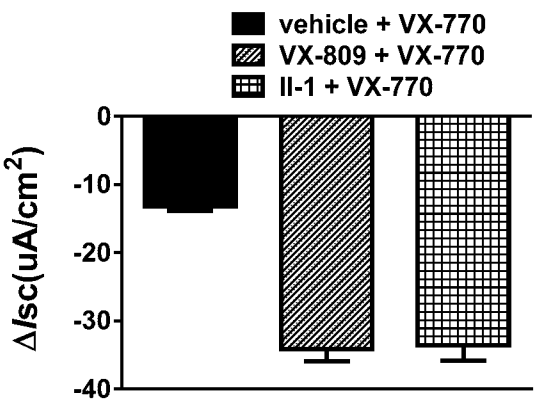


Fig. 14B

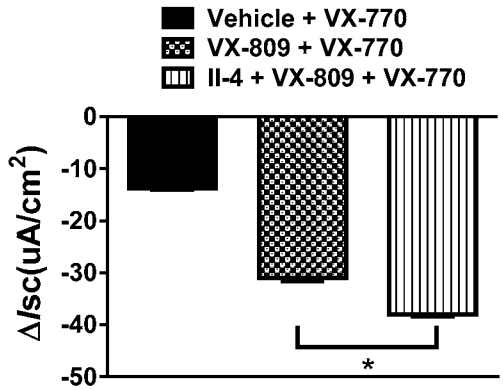
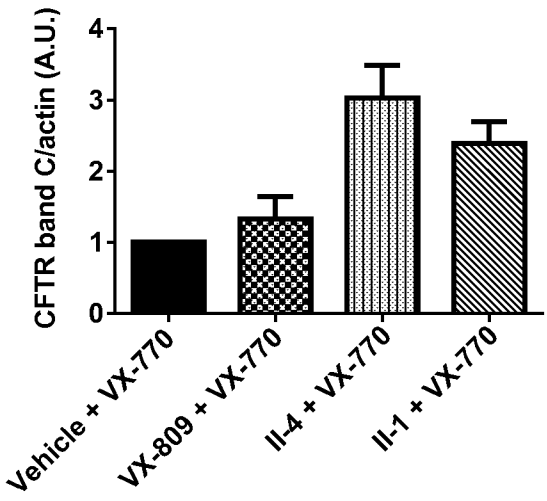


Fig. 15



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/062688

A. CLASSIFICATION OF SUBJECT MATTER

[See Supplemental Sheet]

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)

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

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

REGISTRY, CA: Structure search based on Formula A and I and a structure search based on Formula II

CA, BIOSIS, BIOTECH ABS, MEDLINE: Keyword search using cystic fibrosis, CFTR, modulate, potentiate, corrector, cysteamine, cystamine, fatty acid, conjugate and like terms

Applicant & Inventors names searched in Espacenet and internal databases provided by IP Australia

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] 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
17 March 2016	17 March 2016

Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Kate Bryce AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262223682

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/US2015/062688**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 2013/033602 A2 (MILNE ET AL.) 07 March 2013 See paras 5-6, 11, 17, 102 & 103; page 33, 3rd compound; page 34, 1st and 3rd compounds; and page 35, 2nd compound. See paras 5-6, 11, 102, 103; pages 6-7; & pages 33-34, compounds I-3 & I-10	1-4, 7-12, 15, 20, 23, 33-37, 43 & 44 1-4, 7-24, 33-38, 40-45, 49 & 50
X	GALANAKIS, D. et al., 'Synthesis and pharmacological evaluation of amide conjugates of NSAIDs with L-cysteine ethyl ester, combining potent antiinflammatory and antioxidant properties with significantly reduced gastrointestinal toxicity', Bioorg. Med. Chem. Lett., 2004, Vol. 14, pages 3639-3643. See page 3640, Table 1, compound 5 & pages 3640-3641	45 & 49
X Y	KOUROUNAKIS, A. et al., 'Synthesis and Pharmacological Evaluation of Novel Derivatives of Anti-Inflammatory Drugs with Increased Antioxidant and Anti-Inflammatory Activities', Drug Development Research, 1999, Vol. 47, pages 9-16. See abstract, page 12, Fig.1, compound 4 & page 15 See abstract, page 12, Fig.1, compound 4 & page 15	45 & 49 1-4, 7-13, 17, 18, 20, 21, 23, 33, 34, 40-42, 44, 45 & 49
X	ALLEGRETTI, M. et al., '2-Arylpropionic CXC Chemokine Receptor 1 (CXCR1) Ligands as Novel Noncompetitive CXCL8 Inhibitors', J. Med. Chem., 2005, Vol. 48, pages 4312-4331. See page 4318, Table 4, compound 49	45
X	US 2006/0036223 A1 (BALDWIN et al.) 16 February 2006 See para 52	45
X	US 2009/0325838 A1 (COHEN et al.) 31 December 2009 See para 18	45
X	CAS RN 1565260-47-0, STN Entry Date 10 March 2014 See compound	45
X	CAS RN 1535425-09-2, STN Entry Date 2 February 2014 See compound	45
X	CAS RN 1503820-06-1, STN Entry Date 26 December 2013 See compound	45
X	CAS RN 1478258-76-2, STN Entry Date 21 November 2013 See compound	45
X	CAS RN 1027369-31-8, STN Entry Date 11 June 2008 See compound	45
X	CAS RN 750524-64-2, STN Entry Date 24 September 2004 See compound	45
Y	EP 2664326 A1 (I.E.R.F.C. European Institute for Cystic Fibrosis Research) 20 November 2013 See abstract; page 2, para 6; page 3, para 11; & page 4, para 15	1-4, 7-24, 33-38, 40-45, 49 & 50

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation).	DOCUMENTS CONSIDERED TO BE RELEVANT	**PCT/US2015/062688**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE STEFANO, D. et al., 'Restoration of CFTR function in patients with cystic fibrosis carrying the F508del-CFTR mutation', Autophagy, 2014, Vol. 10, No. 11, pages 2053-2074, published online 27 October 2014. See abstract	

INTERNATIONAL SEARCH REPORT	International application No. PCT/US2015/062688

Supplemental Box – IPC Marks

C07C 323/41 (2006.01)

A61K 31/16 (2006.01)

A61K 31/164 (2006.01)

A61K 31/27 (2006.01)

A61K 31/36 (2006.01)

A61K 31/42 (2006.01)

A61K 31/443 (2006.01)

A61P 1/16 (2006.01)

A61P 21/00 (2006.01)

A61P 25/28 (2006.01)

A61P 29/00 (2006.01)

A61P 31/00 (2006.01)

A61P 37/00 (2006.01)

C07C 323/43 (2006.01)

C07C 323/60 (2006.01)

C07D 209/12 (2006.01)

C07D 261/18 (2006.01)

C07D 317/60 (2006.01)

C07D 405/02 (2006.01)

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2015/062688**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2013/033602 A2	07 March 2013	WO 2013033602 A2	07 Mar 2013
		AU 2012344700 A1	19 Jun 2014
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		CN 104662151 A	27 May 2015
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		US 2015147310 A1	28 May 2015
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		WO 2013078564 A2	06 Jun 2013
		WO 2013185235 A1	19 Dec 2013

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2015/062688**

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Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
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		US 7265257 B2	04 Sep 2007
		AU 2005278010 A1	02 Mar 2006
		AU 2005278010 B2	04 Nov 2010
		BR PI0514031 A	27 May 2008
		CN 101001650 A	18 Jul 2007
		CN 101001650 B	02 Nov 2011
		EP 1791574 A1	06 Jun 2007
		EP 1791574 B1	11 Nov 2009
		KR 20070040390 A	16 Apr 2007
		KR 101245903 B1	20 Mar 2013
		MX 2007001587 A	02 Apr 2007
		WO 2006022960 A1	02 Mar 2006
US 2009/0325838 A1	31 December 2009	US 2009325838 A1	31 Dec 2009
		US 7924142 B2	12 Apr 2011
		AR 072173 A1	11 Aug 2010
		WO 2010001294 A2	07 Jan 2010
EP 2664326 A1	20 November 2013	EP 2664326 A1	20 Nov 2013
		US 2013310329 A1	21 Nov 2013
		US 9132123 B2	15 Sep 2015
		US 2015328203 A1	19 Nov 2015

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2015/062688**

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Publication Number	**Publication Date**	**Publication Number**	**Publication Date**

End of Annex

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