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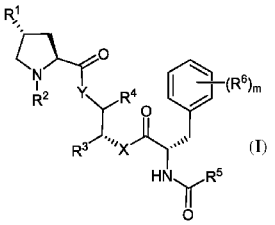
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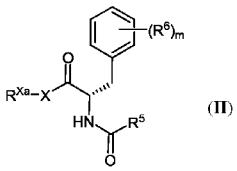
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(54) Title: ENOPEPTIN ANALOGAS AND METHODS OF USE THEREOF



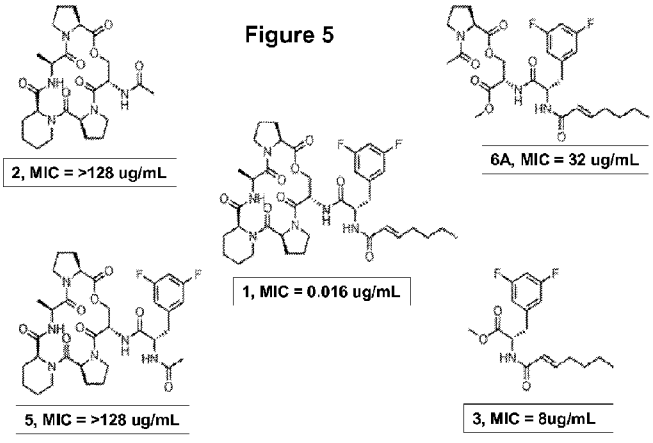
(I)



(II)

(57) Abstract: Described herein are enopeptin analogs of Formula (I) or (II): and pharmaceutically acceptable salts thereof, and pharmaceutical compositions thereof, wherein R^1, R^2, R^3, R^4, R^5, R^6, m, X, R^{Xa}, and Y are as defined herein. Such compounds have been found to exhibit antibacterial activity, to competatively interfere with the bacterial efflux pump, and/or to potentiate the activities of other antibacterial agents, such as an ADEP4, against bacteria that are not susceptible due to efflux pumps. Further provided are a methods of such compounds treatment and use.

Figure 5



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ENOPEPTIN ANALOGS AND METHODS OF USE THEREOF

BACKGROUND OF THE INVENTION

[0001] Infectious diseases are the third leading cause of death in developed countries and the second leading cause of death worldwide (see, *e.g.*, World Health Organization (WHO) Geneva, World Health Report (2002); Nathan, *Nature* (2004) *431*:899). The efficacy of many antibacterial drugs has been compromised by the emergence of drug resistant, pathogenic bacteria (see, *e.g.*, National Nosocomial Infections Surveillance (NNIS) System, *Am. J. Infect. Control* (2004) *32*:470). The *Infectious Disease Society of America* has recently outlined the deadly implications of a growing number of drug-resistant pathogens (see, *e.g.*, Boucher *et al., J. Clin. Infect. Dis.* (2009) 48:1). Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), and penicillin-resistant *Streptococcus epidermis* are especially worrisome in clinical settings. Unfortunately, the prevalence of multidrug-resistant bacteria has made antibiotics of last resort, like vancomycin, the first-line of therapy. The capacity of bacteria to routinely develop resistance to virtually any antibacterial agent necessitates a continuous search for new drugs. There is much evidence in the literature that natural products derived from microorganisms will continue to be a source of novel antibacterial drugs (see, *e.g.*, Clardy, *Nat. Biotechnol.* (2006) 24:1541). Although natural products often have chemical properties that are incompatible with chemotherapy, it is possible to use medicinal chemistry as a means to enhance their biological activity and/or pharmacological properties (see, *e.g.*, von Nussbaum *et al.*, *Angew. Chem. Int. Ed.* (2006) 45:5072).

[0002] A recent case where medicinal chemistry was used to improve the activity of a natural product was that of the enopeptins (see, *e.g.*, Hinzen *et al.*, *ChemMedChem (*2006) 1:689; U.S. 20050107288). The parent compounds were isolated from the soil-dwelling bacterium *Streptomyces* sp. RK-1051 and are defined by a 16-membered peptidolactone consisting of five L-amino acids to which a lipophilic polyene side chain is appended (see, *e.g.*, Osada *et al.*, *J. Antibiot.* (1991) 44:1463). A group of closely related compounds, called A54556 A and B, were isolated from *Streptomyces hawaiienesis* by a research group at Eli Lilly (U.S. Patent 4,492,650). The enopeptins attracted attention because of their potent activity against drug-resistant bacterial pathogens, including MRSA and VRE (see, *e.g.*, Brötz-Oesterhelt *et al.*, *Nat. Med.* (2005) 11:1082). The apparent lack of cross-resistance for all antibacterial agents on the market or those in clinical development has been ascribed to a

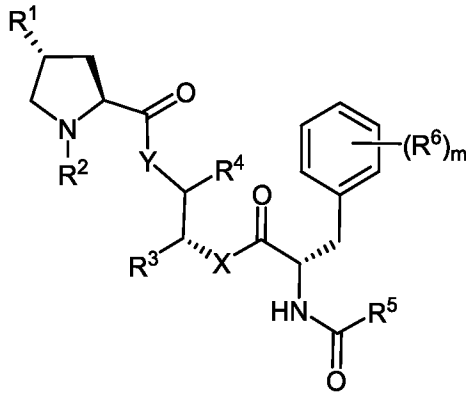
peculiar mechanism of action. The enopeptin antibiotics inhibit cell division and cause cell death by binding and deregulating the activity of the casein lytic protease (ClpP) (see, *e.g.*, Brötz-Oesterhelt, *supra*). Under normal conditions, this fourteen-subunit protease selectively degrades proteins through a physical and functional association with accessory ATPases that recognize and unfold its substrates (see, *e.g.*, Maurizi *et al.*, *Biochemistry* (1999) 37:7778; Singh *et al.*, *Proc. Natl. Acad. Sci., USA.* (2000) 97:8898; Baker *et al.*, *Trends Biochem. Sci.* (2006) 31:647; Hsiung *et al.*, *FEBS Lett.* (2007) 581: 3749). In the presence of the enopeptins, ClpP indiscriminately degrades folded cytoplasmic proteins, which ultimately causes cell death (see, *e.g.*, Brötz-Oesterhelt, *supra*). Recent structural studies indicate that the enopeptins bind the ClpP core structure and cause it to undergo a conformational change that exposes the enzymatic active sites of its subunits (see, *e.g.*, Lee *et al.*, *Nature Structural Biology* (2010) 17:471- 479).

SUMMARY OF THE INVENTION

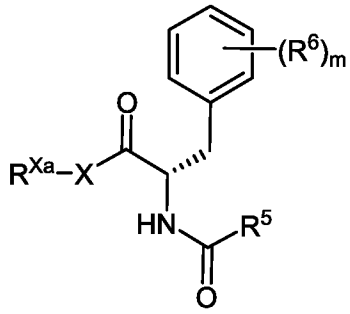
[0003] Although the enopeptin natural products have remarkable antibacterial activity *in vitro*, their chemical lability and poor solubility limit their efficacy *in vivo* (see, *e.g.*, Hinzen *et al.*, *ChemMedChem* (2006) 1:689). Therefore, there continues to be a need for the development and study of new and improved enopeptin analogs, such as smaller fragments of enopeptins, for use as the primary antibiotic agent and/or for use in combination with other antibiotic agents.

[0004] Described herein are fragments of the cyclic acyldepsipeptide (ADEP) scaffold which have been found to exhibit antibacterial activity, and have further been found, in some embodiments, to potentiate the activities of anti-bacterial agents, such as an ADEP4, against bacteria that are not susceptible due to efflux pumps *via* competitive interference. Further described herein are fragments of cyclic ADEP scaffold which do not display anti-bacterial activity, but have been found to suppress efflux pump-mediated ADEP-resistance by competitively interfering with ADEP efflux.

[0005] Exemplary fragments of cyclic ADEP scaffold include compounds of Formula (**I**) or (**II**):



(I)



(II)

or a pharmaceutically acceptable salt thereof;

wherein:

R^1 is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

R^2 is hydrogen, -OH, $-OR^{2A}$, $-C(=O)R^{2A}$, $-C(=O)OR^{2A}$, $-C(=O)NHR^{2A}$, $-C(=O)N(R^{2A})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, or a nitrogen protecting group;

R^3 is $-C(=O)R^{3A}$, $-C(=O)OR^{3A}$, $-C(=O)NHR^{3A}$, $-C(=O)N(R^{3A})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

each instance of R^{2A} and R^{3A} is independently optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl,

or two R^{2A} groups are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring,

or two R^{3A} groups are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring;

R^4 is hydrogen, –OH, –SH, $–NH_2$, substituted hydroxyl, substituted thiol, substituted amino, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

R^5 is optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, $–OR^{5A}$ or $–NHR^{5A}$, wherein each instance of R^{5A} is independently optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

each instance of R^6 is independently halogen, –OH, –SH, $–NH_2$, -CN, $-NO_2$, $-N_3$, $–SO_2H$, $–SO_3H$, substituted hydroxyl, substituted thiol, substituted amino, sulfonyl, sulfinyl, carbonyl, silyl, boronyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

m is 0 or an integer of between 1 and 5, inclusive;

Y is O, S, or NR^Y wherein R^Y is hydrogen, optionally substituted alkyl, or a nitrogen protecting group; and

X is O, S, or NR^{Xb},

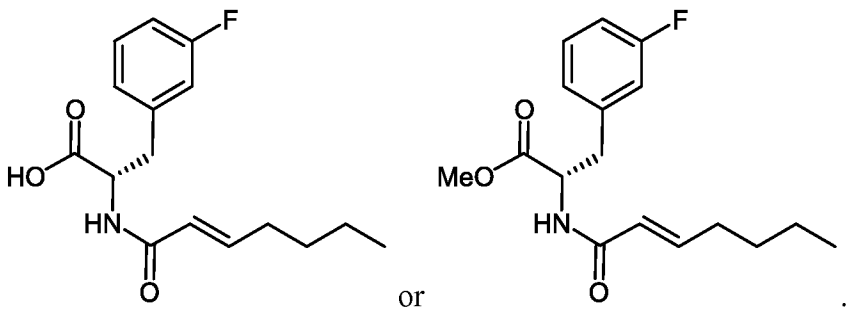
wherein:

R^{Xb} is hydrogen, optionally substituted alkyl, or a nitrogen protecting group; and

R^{Xa} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

or R^{Xa} and R^{Xb} are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring.

[0006] In certain embodiments, the compound of Formula (**II**) is not:



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[0007] In another aspect, provided is a pharmaceutical composition comprising an effective amount of a compound of Formula (**I**) or (**II**), or pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

[0008] In yet another aspect, provided is a method of treating a microbial infection in a subject comprising administering an effective amount of a compound of Formula (**I**) or (**II**), or pharmaceutically acceptable salt thereof, to the subject. In certain embodiments, the microbial infection is a bacterial infection. In certain embodiments, the bacterial infection is an *M. tuberculosis* infection. In certain embodiments, the method further comprises administering the compound in combination with an antibiotic.

[0009] In yet another aspect, provided is a method of treating microbial virulence comprising contacting an effective amount of a compound of Formula (**I**) or (**II**), or pharmaceutically acceptable salt thereof, to a microorganism. In certain embodiments, the compound blocks virulence factor production. In certain embodiments, the microbial virulence is bacterial virulence, and the microorganism is a bacterium.

[0010] In yet another aspect, provided is a method of potentiating the activity of an anti-bacterial agent against a bacterium comprising contacting the anti-bacterial agent in combination with a compound of Formula (**I**) or (**II**), or pharmaceutically acceptable salt thereof, with a bacterium. In certain embodiments, the ratio of the compound or a pharmaceutically acceptable salt thereof to the anti-bacterial agent (molar excess) is between about 1:1 and about 100:1, inclusive. In certain embodiments, the compound is of Formula (**II**) or a pharmaceutically acceptable salt thereof. In certain embodiments, the anti-bacterial agent is an enopeptin.

[0011] The details of one or more embodiments of the invention are set forth in the accompanying Figures and the Detailed Description. Other features, objects, and advantages of the invention will be apparent from the description and from the claims.

DEFINITIONS

Chemical definitions

[0012] Definitions of specific functional groups and chemical terms are described in more detail below. The chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed., inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in *Organic Chemistry*, Thomas Sorrell, University Science Books, Sausalito, 1999; Smith and March *March's Advanced Organic Chemistry*, 5th Edition, John Wiley & Sons, Inc., New York, 2001; Larock, *Comprehensive Organic Transformations*, VCH Publishers,

Inc., New York, 1989; and Carruthers, *Some Modern Methods of Organic Synthesis*, 3rd Edition, Cambridge University Press, Cambridge, 1987.

[0013] Compounds described herein can comprise one or more asymmetric centers, and thus can exist in various isomeric forms, *e.g.*, enantiomers and/or diastereomers. For example, the compounds described herein can be in the form of an individual enantiomer, diastereomer or geometric isomer, or can be in the form of a mixture of stereoisomers, including racemic mixtures and mixtures enriched in one or more stereoisomer. Isomers can be isolated from mixtures by methods known to those skilled in the art, including chiral high pressure liquid chromatography (HPLC) and the formation and crystallization of chiral salts; or preferred isomers can be prepared by asymmetric syntheses. See, for example, Jacques *et al.*, *Enantiomers, Racemates and Resolutions* (Wiley Interscience, New York, 1981); Wilen *et al.*, *Tetrahedron* 33:2725 (1977); Eliel, E.L. *Stereochemistry of Carbon Compounds* (McGraw–Hill, NY, 1962); and Wilen, S.H. *Tables of Resolving Agents and Optical Resolutions* p. 268 (E.L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, IN 1972). The invention additionally encompasses compounds as individual isomers substantially free of other isomers, and alternatively, as mixtures of various isomers.

[0014] When a range of values is listed, it is intended to encompass each value and sub–range within the range. For example "C_{1-6} alkyl" is intended to encompass, C_1, C_2, C_3, C_4, C_5, C_6, C_{1-6}, C_{1-5}, C_{1-4}, C_{1-3}, C_{1-2}, C_{2-6}, C_{2-5}, C_{2-4}, C_{2-3}, C_{3-6}, C_{3-5}, C_{3-4}, C_{4-6}, C_{4-5}, and C_{5-6} alkyl.

[0015] As used herein, "alkyl" refers to a radical of a straight–chain or branched saturated hydrocarbon group having from 1 to 30 carbon atoms ("C_{1-30} alkyl"). In some embodiments, an alkyl group has 1 to 20 carbon atoms ("C_{1-20} alkyl"). In some embodiments, an alkyl group has 1 to 10 carbon atoms ("C_{1-10} alkyl"). In some embodiments, an alkyl group has 1 to 9 carbon atoms ("C_{1-9} alkyl"). In some embodiments, an alkyl group has 1 to 8 carbon atoms ("C_{1-8} alkyl"). In some embodiments, an alkyl group has 1 to 7 carbon atoms ("C_{1-7} alkyl"). In some embodiments, an alkyl group has 1 to 6 carbon atoms ("C_{1-6} alkyl"). In some embodiments, an alkyl group has 1 to 5 carbon atoms ("C_{1-5} alkyl"). In some embodiments, an alkyl group has 1 to 4 carbon atoms ("C_{1-4} alkyl"). In some embodiments, an alkyl group has 1 to 3 carbon atoms ("C_{1-3} alkyl"). In some embodiments, an alkyl group has 1 to 2 carbon atoms ("C_{1-2} alkyl"). In some embodiments, an alkyl group has 1 carbon atom ("C_1 alkyl"). In some embodiments, an alkyl group has 2 to 6 carbon atoms ("C_{2-6} alkyl"). Examples of C_{1-6} alkyl groups include methyl (C_1), ethyl (C_2), n–propyl (C_3), isopropyl (C_3), n–butyl (C_4), tert–butyl (C_4), sec–butyl (C_4), iso–butyl (C_4), n–pentyl (C_5), 3–

pentanyl (C_5), amyl (C_5), neopentyl (C_5), 3–methyl–2–butanyl (C_5), tertiary amyl (C_5), and n–hexyl (C_6). Additional examples of alkyl groups include n–heptyl (C_7), n–octyl (C_8) and the like. Unless otherwise specified, each instance of an alkyl group is independently unsubstituted (an "unsubstituted alkyl") or substituted (a "substituted alkyl") with one or more substituents. In certain embodiments, the alkyl group is an unsubstituted $C_{1–10}$ alkyl (*e.g.*, $–CH_3$). In certain embodiments, the alkyl group is a substituted $C_{1–10}$ alkyl.

[0016] "Haloalkyl" is a substituted alkyl group as defined herein wherein at least one of the hydrogen atoms is independently replaced by a halogen, *e.g.,* fluoro, bromo, chloro, or iodo.

[0017] "Perhaloalkyl" is a substituted alkyl group as defined herein wherein all of the hydrogen atoms are independently replaced by a halogen, *e.g.,* fluoro, bromo, chloro, or iodo. In some embodiments, the alkyl moiety has 1 to 8 carbon atoms ("$C_{1–8}$ perhaloalkyl"). In some embodiments, the alkyl moiety has 1 to 6 carbon atoms ("$C_{1–6}$ perhaloalkyl"). In some embodiments, the alkyl moiety has 1 to 4 carbon atoms ("$C_{1–4}$ perhaloalkyl"). In some embodiments, the alkyl moiety has 1 to 3 carbon atoms ("$C_{1–3}$ perhaloalkyl"). In some embodiments, the alkyl moiety has 1 to 2 carbon atoms ("$C_{1–2}$ perhaloalkyl"). In some embodiments, all of the hydrogen atoms are replaced with fluoro. In some embodiments, all of the hydrogen atoms are replaced with chloro. Examples of perhaloalkyl groups include $–CF_3$, $–CF_2CF_3$, $–CF_2CF_2CF_3$, $–CCl_3$, $–CFCl_2$, $–CF_2Cl$, and the like.

[0018] As used herein, "heteroalkyl" refers to an alkyl group as defined herein which further includes at least one heteroatom (*e.g.,* 1, 2, 3, or 4 heteroatoms) selected from oxygen, nitrogen, or sulfur within (*i.e.,* inserted between adjacent carbon atoms of) and/or placed at one or more terminal position(s) of the parent chain. In certain embodiments, a heteroalkyl group refers to a saturated group having from 1 to 10 carbon atoms and 1 or more heteroatoms within the parent chain ("hetero$C_{1–10}$ alkyl"). In some embodiments, a heteroalkyl group is a saturated group having 1 to 9 carbon atoms and 1 or more heteroatoms within the parent chain ("hetero$C_{1–9}$ alkyl"). In some embodiments, a heteroalkyl group is a saturated group having 1 to 8 carbon atoms and 1 or more heteroatoms within the parent chain ("hetero$C_{1–8}$ alkyl"). In some embodiments, a heteroalkyl group is a saturated group having 1 to 7 carbon atoms and 1 or more heteroatoms within the parent chain ("hetero$C_{1–7}$ alkyl"). In some embodiments, a heteroalkyl group is a saturated group having 1 to 6 carbon atoms and 1 or more heteroatoms within the parent chain ("hetero$C_{1–6}$ alkyl"). In some embodiments, a heteroalkyl group is a saturated group having 1 to 5 carbon atoms and 1 or 2 heteroatoms within the parent chain ("hetero$C_{1–5}$ alkyl"). In some embodiments, a

heteroalkyl group is a saturated group having 1 to 4 carbon atoms and 1or 2 heteroatoms within the parent chain ("heteroC_{1-4} alkyl"). In some embodiments, a heteroalkyl group is a saturated group having 1 to 3 carbon atoms and 1 heteroatom within the parent chain ("heteroC_{1-3} alkyl"). In some embodiments, a heteroalkyl group is a saturated group having 1 to 2 carbon atoms and 1 heteroatom within the parent chain ("heteroC_{1-2} alkyl"). In some embodiments, a heteroalkyl group is a saturated group having 1 carbon atom and 1 heteroatom ("heteroC_1 alkyl"). In some embodiments, a heteroalkyl group is a saturated group having 2 to 6 carbon atoms and 1 or 2 heteroatoms within the parent chain ("heteroC_{2-6} alkyl"). Unless otherwise specified, each instance of a heteroalkyl group is independently unsubstituted (an "unsubstituted heteroalkyl") or substituted (a "substituted heteroalkyl") with one or more substituents. In certain embodiments, the heteroalkyl group is an unsubstituted heteroC_{1-10} alkyl. In certain embodiments, the heteroalkyl group is a substituted heteroC_{1-10} alkyl.

[0019] As used herein, "alkenyl" refers to a radical of a straight–chain or branched hydrocarbon group having from 2 to 30 carbon atoms and one or more carbon–carbon double bonds ("C_{2-30} alkenyl"). In some embodiments, an alkenyl group has 2 to 20 carbon atoms ("C_{2-20} alkenyl"). In some embodiments, an alkenyl group has 2 to 10 carbon atoms ("C_{2-10} alkenyl"). In some embodiments, an alkenyl group has 2 to 9 carbon atoms ("C_{2-9} alkenyl"). In some embodiments, an alkenyl group has 2 to 8 carbon atoms ("C_{2-8} alkenyl"). In some embodiments, an alkenyl group has 2 to 7 carbon atoms ("C_{2-7} alkenyl"). In some embodiments, an alkenyl group has 2 to 6 carbon atoms ("C_{2-6} alkenyl"). In some embodiments, an alkenyl group has 2 to 5 carbon atoms ("C_{2-5} alkenyl"). In some embodiments, an alkenyl group has 2 to 4 carbon atoms ("C_{2-4} alkenyl"). In some embodiments, an alkenyl group has 2 to 3 carbon atoms ("C_{2-3} alkenyl"). In some embodiments, an alkenyl group has 2 carbon atoms ("C_2 alkenyl"). The one or more carbon–carbon double bonds can be internal (such as in 2–butenyl) or terminal (such as in 1–butenyl). Examples of C_{2-4} alkenyl groups include ethenyl (C_2), 1–propenyl (C_3), 2–propenyl (C_3), 1–butenyl (C_4), 2–butenyl (C_4), butadienyl (C_4), and the like. Examples of C_{2-6} alkenyl groups include the aforementioned C_{2-4} alkenyl groups as well as pentenyl (C_5), pentadienyl (C_5), hexenyl (C_6), and the like. Additional examples of alkenyl include heptenyl (C_7), octenyl (C_8), octatrienyl (C_8), and the like. Unless otherwise specified, each instance of an alkenyl group is independently unsubstituted (an "unsubstituted alkenyl") or substituted (a "substituted alkenyl") with one or more substituents. In certain embodiments, the alkenyl

group is an unsubstituted C_{2-10} alkenyl. In certain embodiments, the alkenyl group is a substituted C_{2-10} alkenyl.

[0020] As used herein, "heteroalkenyl" refers to an alkenyl group as defined herein which further includes at least one heteroatom (*e.g.,* 1, 2, 3, or 4 heteroatoms) selected from oxygen, nitrogen, or sulfur within (*i.e.,* inserted between adjacent carbon atoms of) and/or placed at one or more terminal position(s) of the parent chain. In certain embodiments, a heteroalkenyl group refers to a group having from 2 to 10 carbon atoms, at least one double bond, and 1 or more heteroatoms within the parent chain ("heteroC_{2-10} alkenyl"). In some embodiments, a heteroalkenyl group has 2 to 9 carbon atoms at least one double bond, and 1 or more heteroatoms within the parent chain ("heteroC_{2-9} alkenyl"). In some embodiments, a heteroalkenyl group has 2 to 8 carbon atoms, at least one double bond, and 1 or more heteroatoms within the parent chain ("heteroC_{2-8} alkenyl"). In some embodiments, a heteroalkenyl group has 2 to 7 carbon atoms, at least one double bond, and 1 or more heteroatoms within the parent chain ("heteroC_{2-7} alkenyl"). In some embodiments, a heteroalkenyl group has 2 to 6 carbon atoms, at least one double bond, and 1 or more heteroatoms within the parent chain ("heteroC_{2-6} alkenyl"). In some embodiments, a heteroalkenyl group has 2 to 5 carbon atoms, at least one double bond, and 1 or 2 heteroatoms within the parent chain ("heteroC_{2-5} alkenyl"). In some embodiments, a heteroalkenyl group has 2 to 4 carbon atoms, at least one double bond, and 1or 2 heteroatoms within the parent chain ("heteroC_{2-4} alkenyl"). In some embodiments, a heteroalkenyl group has 2 to 3 carbon atoms, at least one double bond, and 1 heteroatom within the parent chain ("heteroC_{2-3} alkenyl"). In some embodiments, a heteroalkenyl group has 2 to 6 carbon atoms, at least one double bond, and 1 or 2 heteroatoms within the parent chain ("heteroC_{2-6} alkenyl"). Unless otherwise specified, each instance of a heteroalkenyl group is independently unsubstituted (an "unsubstituted heteroalkenyl") or substituted (a "substituted heteroalkenyl") with one or more substituents. In certain embodiments, the heteroalkenyl group is an unsubstituted heteroC_{2-10} alkenyl. In certain embodiments, the heteroalkenyl group is a substituted heteroC_{2-10} alkenyl.

[0021] As used herein, "alkynyl" refers to a radical of a straight–chain or branched hydrocarbon group having from 2 to 30 carbon atoms and one or more carbon–carbon triple bonds ("C_{2-30} alkynyl"). In certain embodiments, the alkynyl group optionally and additionally comprises one or more double bonds. In some embodiments, an alkynyl group has 2 to 20 carbon atoms ("C_{2-20} alkynyl"). In some embodiments, an alkynyl group has 2 to 10 carbon atoms ("C_{2-10} alkynyl"). In some embodiments, an alkynyl group has 2 to 9 carbon

atoms ("C_{2-9} alkynyl"). In some embodiments, an alkynyl group has 2 to 8 carbon atoms ("C_{2-8} alkynyl"). In some embodiments, an alkynyl group has 2 to 7 carbon atoms ("C_{2-7} alkynyl"). In some embodiments, an alkynyl group has 2 to 6 carbon atoms ("C_{2-6} alkynyl"). In some embodiments, an alkynyl group has 2 to 5 carbon atoms ("C_{2-5} alkynyl"). In some embodiments, an alkynyl group has 2 to 4 carbon atoms ("C_{2-4} alkynyl"). In some embodiments, an alkynyl group has 2 to 3 carbon atoms ("C_{2-3} alkynyl"). In some embodiments, an alkynyl group has 2 carbon atoms ("C_2 alkynyl"). The one or more carbon–carbon triple bonds can be internal (such as in 2–butynyl) or terminal (such as in 1–butynyl). Examples of C_{2-4} alkynyl groups include, without limitation, ethynyl (C_2), 1–propynyl (C_3), 2–propynyl (C_3), 1–butynyl (C_4), 2–butynyl (C_4), and the like. Examples of C_{2-6} alkenyl groups include the aforementioned C_{2-4} alkynyl groups as well as pentynyl (C_5), hexynyl (C_6), and the like. Additional examples of alkynyl include heptynyl (C_7), octynyl (C_8), and the like. Unless otherwise specified, each instance of an alkynyl group is independently unsubstituted (an "unsubstituted alkynyl") or substituted (a "substituted alkynyl") with one or more substituents. In certain embodiments, the alkynyl group is an unsubstituted C_{2-10} alkynyl. In certain embodiments, the alkynyl group is a substituted C_{2-10} alkynyl.

[0022] As used herein, "heteroalkynyl" refers to an alkynyl group as defined herein which further includes at least one heteroatom (*e.g.,* 1, 2, 3, or 4 heteroatoms) selected from oxygen, nitrogen, or sulfur within (*i.e.,* inserted between adjacent carbon atoms of) and/or placed at one or more terminal position(s) of the parent chain. In certain embodiments, a heteroalkynyl group refers to a group having from 2 to 10 carbon atoms, at least one triple bond, and 1 or more heteroatoms within the parent chain ("heteroC_{2-10} alkynyl"). In some embodiments, a heteroalkynyl group has 2 to 9 carbon atoms, at least one triple bond, and 1 or more heteroatoms within the parent chain ("heteroC_{2-9} alkynyl"). In some embodiments, a heteroalkynyl group has 2 to 8 carbon atoms, at least one triple bond, and 1 or more heteroatoms within the parent chain ("heteroC_{2-8} alkynyl"). In some embodiments, a heteroalkynyl group has 2 to 7 carbon atoms, at least one triple bond, and 1 or more heteroatoms within the parent chain ("heteroC_{2-7} alkynyl"). In some embodiments, a heteroalkynyl group has 2 to 6 carbon atoms, at least one triple bond, and 1 or more heteroatoms within the parent chain ("heteroC_{2-6} alkynyl"). In some embodiments, a heteroalkynyl group has 2 to 5 carbon atoms, at least one triple bond, and 1 or 2 heteroatoms within the parent chain ("heteroC_{2-5} alkynyl"). In some embodiments, a heteroalkynyl group has 2 to 4 carbon atoms, at least one triple bond, and 1or 2 heteroatoms within the parent chain ("heteroC_{2-4} alkynyl"). In some embodiments, a heteroalkynyl group has 2 to 3 carbon

atoms, at least one triple bond, and 1 heteroatom within the parent chain ("heteroC_{2-3} alkynyl"). In some embodiments, a heteroalkynyl group has 2 to 6 carbon atoms, at least one triple bond, and 1 or 2 heteroatoms within the parent chain ("heteroC_{2-6} alkynyl"). Unless otherwise specified, each instance of a heteroalkynyl group is independently unsubstituted (an "unsubstituted heteroalkynyl") or substituted (a "substituted heteroalkynyl") with one or more substituents. In certain embodiments, the heteroalkynyl group is an unsubstituted heteroC_{2-10} alkynyl. In certain embodiments, the heteroalkynyl group is a substituted heteroC_{2-10} alkynyl.

[0023] As used herein, "carbocyclyl" or "carbocyclic" refers to a radical of a non–aromatic cyclic hydrocarbon group having from 3 to 14 ring carbon atoms ("C_{3-14} carbocyclyl") and zero heteroatoms in the non–aromatic ring system. In some embodiments, a carbocyclyl group has 3 to 10 ring carbon atoms ("C_{3-10} carbocyclyl"). In some embodiments, a carbocyclyl group has 3 to 8 ring carbon atoms ("C_{3-8} carbocyclyl"). In some embodiments, a carbocyclyl group has 3 to 7 ring carbon atoms ("C_{3-7} carbocyclyl"). In some embodiments, a carbocyclyl group has 3 to 6 ring carbon atoms ("C_{3-6} carbocyclyl"). In some embodiments, a carbocyclyl group has 4 to 6 ring carbon atoms ("C_{4-6} carbocyclyl"). In some embodiments, a carbocyclyl group has 5 to 6 ring carbon atoms ("C_{5-6} carbocyclyl"). In some embodiments, a carbocyclyl group has 5 to 10 ring carbon atoms ("C_{5-10} carbocyclyl"). Exemplary C_{3-6} carbocyclyl groups include, without limitation, cyclopropyl (C_3), cyclopropenyl (C_3), cyclobutyl (C_4), cyclobutenyl (C_4), cyclopentyl (C_5), cyclopentenyl (C_5), cyclohexyl (C_6), cyclohexenyl (C_6), cyclohexadienyl (C_6), and the like. Exemplary C_{3-8} carbocyclyl groups include, without limitation, the aforementioned C_{3-6} carbocyclyl groups as well as cycloheptyl (C_7), cycloheptenyl (C_7), cycloheptadienyl (C_7), cycloheptatrienyl (C_7), cyclooctyl (C_8), cyclooctenyl (C_8), bicyclo[2.2.1]heptanyl (C_7), bicyclo[2.2.2]octanyl (C_8), and the like. Exemplary C_{3-10} carbocyclyl groups include, without limitation, the aforementioned C_{3-8} carbocyclyl groups as well as cyclononyl (C_9), cyclononenyl (C_9), cyclodecyl (C_{10}), cyclodecenyl (C_{10}), octahydro–1*H*–indenyl (C_9), decahydronaphthalenyl (C_{10}), spiro[4.5]decanyl (C_{10}), and the like. As the foregoing examples illustrate, in certain embodiments, the carbocyclyl group is either monocyclic ("monocyclic carbocyclyl") or polycyclic (*e.g.*, containing a fused, bridged or spiro ring system such as a bicyclic system ("bicyclic carbocyclyl") or tricyclic system ("tricyclic carbocyclyl")) and can be saturated or can contain one or more carbon–carbon double or triple bonds. "Carbocyclyl" also includes ring systems wherein the carbocyclyl ring, as defined above, is fused with one or more aryl or heteroaryl groups wherein the point of attachment is on the carbocyclyl ring, and in such

instances, the number of carbons continue to designate the number of carbons in the carbocyclic ring system. Unless otherwise specified, each instance of a carbocyclyl group is independently unsubstituted (an "unsubstituted carbocyclyl") or substituted (a "substituted carbocyclyl") with one or more substituents. In certain embodiments, the carbocyclyl group is an unsubstituted C_{3-14} carbocyclyl. In certain embodiments, the carbocyclyl group is a substituted C_{3-14} carbocyclyl.

[0024] In some embodiments, "carbocyclyl" is a monocyclic, saturated carbocyclyl group having from 3 to 14 ring carbon atoms ("C_{3-14} cycloalkyl"). In some embodiments, "carbocyclyl" is a monocyclic, saturated carbocyclyl group having from 3 to 10 ring carbon atoms ("C_{3-10} cycloalkyl"). In some embodiments, a cycloalkyl group has 3 to 8 ring carbon atoms ("C_{3-8} cycloalkyl"). In some embodiments, a cycloalkyl group has 3 to 6 ring carbon atoms ("C_{3-6} cycloalkyl"). In some embodiments, a cycloalkyl group has 4 to 6 ring carbon atoms ("C_{4-6} cycloalkyl"). In some embodiments, a cycloalkyl group has 5 to 6 ring carbon atoms ("C_{5-6} cycloalkyl"). In some embodiments, a cycloalkyl group has 5 to 10 ring carbon atoms ("C_{5-10} cycloalkyl"). Examples of C_{5-6} cycloalkyl groups include cyclopentyl (C_5) and cyclohexyl (C_5). Examples of C_{3-6} cycloalkyl groups include the aforementioned C_{5-6} cycloalkyl groups as well as cyclopropyl (C_3) and cyclobutyl (C_4). Examples of C_{3-8} cycloalkyl groups include the aforementioned C_{3-6} cycloalkyl groups as well as cycloheptyl (C_7) and cyclooctyl (C_8). Unless otherwise specified, each instance of a cycloalkyl group is independently unsubstituted (an "unsubstituted cycloalkyl") or substituted (a "substituted cycloalkyl") with one or more substituents. In certain embodiments, the cycloalkyl group is an unsubstituted C_{3-14} cycloalkyl. In certain embodiments, the cycloalkyl group is a substituted C_{3-14} cycloalkyl.

[0025] As used herein, "heterocyclyl" or "heterocyclic" refers to a radical of a 3– to 14–membered non–aromatic ring system having ring carbon atoms and 1 to 4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("3–14 membered heterocyclyl"). In heterocyclyl groups that contain one or more nitrogen atoms, the point of attachment can be a carbon or nitrogen atom, as valency permits. A heterocyclyl group can either be monocyclic ("monocyclic heterocyclyl") or polycyclic (*e.g.,* a fused, bridged or spiro ring system such as a bicyclic system ("bicyclic heterocyclyl") or tricyclic system ("tricyclic heterocyclyl")), and can be saturated or can contain one or more carbon–carbon double or triple bonds. Heterocyclyl polycyclic ring systems can include one or more heteroatoms in one or both rings. "Heterocyclyl" also includes ring systems wherein the heterocyclyl ring, as defined above, is fused with one or more carbocyclyl groups wherein the

point of attachment is either on the carbocyclyl or heterocyclyl ring, or ring systems wherein the heterocyclyl ring, as defined above, is fused with one or more aryl or heteroaryl groups, wherein the point of attachment is on the heterocyclyl ring, and in such instances, the number of ring members continue to designate the number of ring members in the heterocyclyl ring system. Unless otherwise specified, each instance of heterocyclyl is independently unsubstituted (an "unsubstituted heterocyclyl") or substituted (a "substituted heterocyclyl") with one or more substituents. In certain embodiments, the heterocyclyl group is an unsubstituted 3–14 membered heterocyclyl. In certain embodiments, the heterocyclyl group is a substituted 3–14 membered heterocyclyl.

[0026] In some embodiments, a heterocyclyl group is a 5–10 membered non–aromatic ring system having ring carbon atoms and 1–4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5–10 membered heterocyclyl"). In some embodiments, a heterocyclyl group is a 5–8 membered non–aromatic ring system having ring carbon atoms and 1–4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5–8 membered heterocyclyl"). In some embodiments, a heterocyclyl group is a 5–6 membered non–aromatic ring system having ring carbon atoms and 1–4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5–6 membered heterocyclyl"). In some embodiments, the 5–6 membered heterocyclyl has 1–3 ring heteroatoms selected from nitrogen, oxygen, and sulfur. In some embodiments, the 5–6 membered heterocyclyl has 1–2 ring heteroatoms selected from nitrogen, oxygen, and sulfur. In some embodiments, the 5–6 membered heterocyclyl has 1 ring heteroatom selected from nitrogen, oxygen, and sulfur.

[0027] Exemplary 3–membered heterocyclyl groups containing 1 heteroatom include, without limitation, azirdinyl, oxiranyl, and thiiranyl. Exemplary 4–membered heterocyclyl groups containing 1 heteroatom include, without limitation, azetidinyl, oxetanyl and thietanyl. Exemplary 5–membered heterocyclyl groups containing 1 heteroatom include, without limitation, tetrahydrofuranyl, dihydrofuranyl, tetrahydrothiophenyl, dihydrothiophenyl, pyrrolidinyl, dihydropyrrolyl and pyrrolyl–2,5–dione. Exemplary 5–membered heterocyclyl groups containing 2 heteroatoms include, without limitation, dioxolanyl, oxathiolanyl and dithiolanyl. Exemplary 5–membered heterocyclyl groups containing 3 heteroatoms include, without limitation, triazolinyl, oxadiazolinyl, and thiadiazolinyl. Exemplary 6–membered heterocyclyl groups containing 1 heteroatom include, without limitation, piperidinyl, tetrahydropyranyl, dihydropyridinyl, and thianyl. Exemplary 6–membered heterocyclyl groups containing 2 heteroatoms include, without

limitation, piperazinyl, morpholinyl, dithianyl, dioxanyl. Exemplary 6–membered heterocyclyl groups containing 3 heteroatoms include, without limitation, triazinanyl. Exemplary 7–membered heterocyclyl groups containing 1 heteroatom include, without limitation, azepanyl, oxepanyl and thiepanyl. Exemplary 8–membered heterocyclyl groups containing 1 heteroatom include, without limitation, azocanyl, oxecanyl and thiocanyl. Exemplary bicyclic heterocyclyl groups include, without limitation, indolinyl, isoindolinyl, dihydrobenzofuranyl, dihydrobenzothienyl, tetrahydrobenzothienyl, tetrahydrobenzofuranyl, tetrahydroindolyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, decahydroisoquinolinyl, octahydrochromenyl, octahydroisochromenyl, decahydronaphthyridinyl, decahydro–1,8–naphthyridinyl, octahydropyrrolo[3,2–b]pyrrole, indolinyl, phthalimidyl, naphthalimidyl, chromanyl, chromenyl, 1H–benzo[e][1,4]diazepinyl, 1,4,5,7–tetrahydropyrano[3,4–b]pyrrolyl, 5,6–dihydro–4H–furo[3,2–b]pyrrolyl, 6,7–dihydro–5H–furo[3,2–b]pyranyl, 5,7–dihydro–4H–thieno[2,3–c]pyranyl, 2,3–dihydro–1H–pyrrolo[2,3–b]pyridinyl, 2,3–dihydrofuro[2,3–b]pyridinyl, 4,5,6,7–tetrahydro–1H–pyrrolo-[2,3–b]pyridinyl, 4,5,6,7–tetrahydrofuro[3,2–c]pyridinyl, 4,5,6,7–tetrahydrothieno[3,2–b]pyridinyl, 1,2,3,4–tetrahydro–1,6–naphthyridinyl, and the like.

[0028] As used herein, "aryl" refers to a radical of a monocyclic or polycyclic (*e.g.,* bicyclic or tricyclic) 4n+2 aromatic ring system (*e.g.,* having 6, 10, or 14 π electrons shared in a cyclic array) having 6–14 ring carbon atoms and zero heteroatoms provided in the aromatic ring system ("C_{6-14} aryl"). In some embodiments, an aryl group has 6 ring carbon atoms ("C_6 aryl"; *e.g.,* phenyl). In some embodiments, an aryl group has 10 ring carbon atoms ("C_{10} aryl"; *e.g.,* naphthyl such as 1–naphthyl and 2–naphthyl). In some embodiments, an aryl group has 14 ring carbon atoms ("C_{14} aryl"; *e.g.,* anthracyl). "Aryl" also includes ring systems wherein the aryl ring, as defined above, is fused with one or more carbocyclyl or heterocyclyl groups wherein the radical or point of attachment is on the aryl ring, and in such instances, the number of carbon atoms continue to designate the number of carbon atoms in the aryl ring system. Unless otherwise specified, each instance of an aryl group is independently unsubstituted (an "unsubstituted aryl") or substituted (a "substituted aryl") with one or more substituents. In certain embodiments, the aryl group is an unsubstituted C_{6-14} aryl. In certain embodiments, the aryl group is a substituted C_{6-14} aryl.

[0029] "Aralkyl" is a subset of "alkyl" and refers to an alkyl group, as defined herein, substituted by an aryl group, as defined herein, wherein the point of attachment is on the alkyl moiety.

[0030] As used herein, "heteroaryl" refers to a radical of a 5–14 membered monocyclic or polycyclic (*e.g.,* bicyclic or tricyclic) 4n+2 aromatic ring system (*e.g.,* having 6, 10, or 14 π electrons shared in a cyclic array) having ring carbon atoms and 1–4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen and sulfur ("5–14 membered heteroaryl"). In heteroaryl groups that contain one or more nitrogen atoms, the point of attachment can be a carbon or nitrogen atom, as valency permits. Heteroaryl polycyclic ring systems can include one or more heteroatoms in one or both rings. "Heteroaryl" includes ring systems wherein the heteroaryl ring, as defined above, is fused with one or more carbocyclyl or heterocyclyl groups wherein the point of attachment is on the heteroaryl ring, and in such instances, the number of ring members continue to designate the number of ring members in the heteroaryl ring system. "Heteroaryl" also includes ring systems wherein the heteroaryl ring, as defined above, is fused with one or more aryl groups wherein the point of attachment is either on the aryl or heteroaryl ring, and in such instances, the number of ring members designates the number of ring members in the fused polycyclic (aryl/heteroaryl) ring system. Polycyclic heteroaryl groups wherein one ring does not contain a heteroatom (*e.g.,* indolyl, quinolinyl, carbazolyl, and the like) the point of attachment can be on either ring, *i.e.,* either the ring bearing a heteroatom (*e.g.,* 2–indolyl) or the ring that does not contain a heteroatom (*e.g.,* 5–indolyl).

[0031] In some embodiments, a heteroaryl group is a 5–10 membered aromatic ring system having ring carbon atoms and 1–4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5–10 membered heteroaryl"). In some embodiments, a heteroaryl group is a 5–8 membered aromatic ring system having ring carbon atoms and 1–4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5–8 membered heteroaryl"). In some embodiments, a heteroaryl group is a 5–6 membered aromatic ring system having ring carbon atoms and 1–4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5–6 membered heteroaryl"). In some embodiments, the 5–6 membered heteroaryl has 1–3 ring heteroatoms selected from nitrogen, oxygen, and sulfur. In some embodiments, the 5–6 membered heteroaryl has 1–2 ring heteroatoms selected from nitrogen, oxygen, and sulfur. In some embodiments, the 5–6 membered heteroaryl has 1 ring heteroatom selected from nitrogen, oxygen, and sulfur. Unless otherwise specified, each instance of a heteroaryl group is independently unsubstituted (an

"unsubstituted heteroaryl") or substituted (a "substituted heteroaryl") with one or more substituents. In certain embodiments, the heteroaryl group is an unsubstituted 5–14 membered heteroaryl. In certain embodiments, the heteroaryl group is a substituted 5–14 membered heteroaryl.

[0032] Exemplary 5–membered heteroaryl groups containing 1 heteroatom include, without limitation, pyrrolyl, furanyl and thiophenyl. Exemplary 5–membered heteroaryl groups containing 2 heteroatoms include, without limitation, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, and isothiazolyl. Exemplary 5–membered heteroaryl groups containing 3 heteroatoms include, without limitation, triazolyl, oxadiazolyl, and thiadiazolyl. Exemplary 5–membered heteroaryl groups containing 4 heteroatoms include, without limitation, tetrazolyl. Exemplary 6–membered heteroaryl groups containing 1 heteroatom include, without limitation, pyridinyl. Exemplary 6–membered heteroaryl groups containing 2 heteroatoms include, without limitation, pyridazinyl, pyrimidinyl, and pyrazinyl. Exemplary 6–membered heteroaryl groups containing 3 or 4 heteroatoms include, without limitation, triazinyl and tetrazinyl, respectively. Exemplary 7-membered heteroaryl groups containing 1 heteroatom include, without limitation, azepinyl, oxepinyl, and thiepinyl. Exemplary 5,6–bicyclic heteroaryl groups include, without limitation, indolyl, isoindolyl, indazolyl, benzotriazolyl, benzothiophenyl, isobenzothiophenyl, benzofuranyl, benzoisofuranyl, benzimidazolyl, benzoxazolyl, benzisoxazolyl, benzoxadiazolyl, benzthiazolyl, benzisothiazolyl, benzthiadiazolyl, indolizinyl, and purinyl. Exemplary 6,6–bicyclic heteroaryl groups include, without limitation, naphthyridinyl, pteridinyl, quinolinyl, isoquinolinyl, cinnolinyl, quinoxalinyl, phthalazinyl, and quinazolinyl. Exemplary tricyclic heteroaryl groups include, without limitation, phenanthridinyl, dibenzofuranyl, carbazolyl, acridinyl, phenothiazinyl, phenoxazinyl and phenazinyl.

[0033] "Heteroaralkyl" is a subset of "alkyl" and refers to an alkyl group, as defined herein, substituted by a heteroaryl group, as defined herein, wherein the point of attachment is on the alkyl moiety.

[0034] As used herein, the term "partially unsaturated" refers to a ring moiety that includes at least one double or triple bond. The term "partially unsaturated" is intended to encompass rings having multiple sites of unsaturation, but is not intended to include aromatic groups (*e.g.,* aryl or heteroaryl moieties) as herein defined.

[0035] As used herein, the term "saturated" refers to a ring moiety that does not contain a double or triple bond, *i.e.*, contains all single bonds.

[0036] Affixing the suffix "–ene" to a group indicates the group is a divalent moiety, *e.g.*, alkylene is the divalent moiety of alkyl, alkenylene is the divalent moiety of alkenyl, alkynylene is the divalent moiety of alkynyl, heteroalkylene is the divalent moiety of heteroalkyl, heteroalkenylene is the divalent moiety of heteroalkenyl, heteroalkynylene is the divalent moiety of heteroalkynyl, carbocyclylene is the divalent moiety of carbocyclyl, heterocyclylene is the divalent moiety of heterocyclyl, arylene is the divalent moiety of aryl, and heteroarylene is the divalent moiety of heteroaryl.

[0037] Alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl groups, as defined herein, are optionally substituted (*e.g.,* "substituted" or "unsubstituted" alkyl, "substituted" or "unsubstituted" alkenyl, "substituted" or "unsubstituted" alkynyl, "substituted" or "unsubstituted" carbocyclyl, "substituted" or "unsubstituted" heterocyclyl, "substituted" or "unsubstituted" aryl or "substituted" or "unsubstituted" heteroaryl group). In general, the term "substituted", whether preceded by the term "optionally" or not, means that at least one hydrogen present on a group (*e.g.,* a carbon or nitrogen atom) is replaced with a permissible substituent, *e.g.,* a substituent which upon substitution results in a stable compound, *e.g.,* a compound which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, or other reaction. Unless otherwise indicated, a "substituted" group has a substituent at one or more substitutable positions of the group, and when more than one position in any given structure is substituted, the substituent is either the same or different at each position. For purposes of this invention, heteroatoms such as nitrogen may have hydrogen substituents and/or any suitable substituent as described herein which satisfy the valencies of the heteroatoms and results in the formation of a stable moiety.

[0038] Exemplary substituents (*e.g.,* carbon atom substituents) include, but are not limited to, halogen, –CN, $–NO_2$, $–N_3$, $–SO_2H$, $–SO_3H$, –OH, $–OR^{aa}$, $–ON(R^{bb})_2$, $–N(R^{bb})_2$, $–N(R^{bb})_3^+X^-$, $–N(OR^{cc})R^{bb}$, –SH, $–SR^{aa}$, $–SSR^{cc}$, $–C(=O)R^{aa}$, $–CO_2H$, –CHO, $–C(OR^{cc})_2$, $–CO_2R^{aa}$, $–OC(=O)R^{aa}$, $–OCO_2R^{aa}$, $–C(=O)N(R^{bb})_2$, $–OC(=O)N(R^{bb})_2$, $–NR^{bb}C(=O)R^{aa}$, $–NR^{bb}CO_2R^{aa}$, $–NR^{bb}C(=O)N(R^{bb})_2$, $–C(=NR^{bb})R^{aa}$, $–C(=NR^{bb})OR^{aa}$, $–OC(=NR^{bb})R^{aa}$, $–OC(=NR^{bb})OR^{aa}$, $–C(=NR^{bb})N(R^{bb})_2$, $–OC(=NR^{bb})N(R^{bb})_2$, $–NR^{bb}C(=NR^{bb})N(R^{bb})_2$, $–C(=O)NR^{bb}SO_2R^{aa}$, $–NR^{bb}SO_2R^{aa}$, $–SO_2N(R^{bb})_2$, $–SO_2R^{aa}$, $–SO_2OR^{aa}$, $–OSO_2R^{aa}$, $–S(=O)R^{aa}$, $–OS(=O)R^{aa}$, $–Si(R^{aa})_3$, $–OSi(R^{aa})_3$ $–C(=S)N(R^{bb})_2$, $–C(=O)SR^{aa}$, $–C(=S)SR^{aa}$, $–SC(=S)SR^{aa}$, $–SC(=O)SR^{aa}$, $–OC(=O)SR^{aa}$, $–SC(=O)OR^{aa}$, $–SC(=O)R^{aa}$, $–P(=O)_2R^{aa}$, $–OP(=O)_2R^{aa}$, $–P(=O)(R^{aa})_2$, $–OP(=O)(R^{aa})_2$, $–OP(=O)(OR^{cc})_2$, $–P(=O)_2N(R^{bb})_2$, $–OP(=O)_2N(R^{bb})_2$, $–P(=O)(NR^{bb})_2$, $–OP(=O)(NR^{bb})_2$, $–NR^{bb}P(=O)(OR^{cc})_2$, $–NR^{bb}P(=O)(NR^{bb})_2$, $–P(R^{cc})_2$, –

$P(R^{cc})_3$, $-OP(R^{cc})_2$, $-OP(R^{cc})_3$, $-B(R^{aa})_2$, $-B(OR^{cc})_2$, $-BR^{aa}(OR^{cc})$, C_{1-10} alkyl, C_{1-10} perhaloalkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, C_{3-14} carbocyclyl, 3–14 membered heterocyclyl, C_{6-14} aryl, and 5–14 membered heteroaryl, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups;

or two geminal hydrogens on a carbon atom are replaced with the group =O, =S, $=NN(R^{bb})_2$, $=NNR^{bb}C(=O)R^{aa}$, $=NNR^{bb}C(=O)OR^{aa}$, $=NNR^{bb}S(=O)_2R^{aa}$, $=NR^{bb}$, or $=NOR^{cc}$;

each instance of R^{aa} is, independently, selected from C_{1-10} alkyl, C_{1-10} perhaloalkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, C_{3-10} carbocyclyl, 3–14 membered heterocyclyl, C_{6-14} aryl, and 5–14 membered heteroaryl, or two R^{aa} groups are joined to form a 3–14 membered heterocyclyl or 5–14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups;

each instance of R^{bb} is, independently, selected from hydrogen, –OH, $-OR^{aa}$, $-N(R^{cc})_2$, –CN, $-C(=O)R^{aa}$, $-C(=O)N(R^{cc})_2$, $-CO_2R^{aa}$, $-SO_2R^{aa}$, $-C(=NR^{cc})OR^{aa}$, $-C(=NR^{cc})N(R^{cc})_2$, $-SO_2N(R^{cc})_2$, $-SO_2R^{cc}$, $-SO_2OR^{cc}$, $-SOR^{aa}$, $-C(=S)N(R^{cc})_2$, $-C(=O)SR^{cc}$, $-C(=S)SR^{cc}$, $-P(=O)_2R^{aa}$, $-P(=O)(R^{aa})_2$, $-P(=O)_2N(R^{cc})_2$, $-P(=O)(NR^{cc})_2$, C_{1-10} alkyl, C_{1-10} perhaloalkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, C_{3-10} carbocyclyl, 3–14 membered heterocyclyl, C_{6-14} aryl, and 5–14 membered heteroaryl, or two R^{bb} groups are joined to form a 3–14 membered heterocyclyl or 5–14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups;

each instance of R^{cc} is, independently, selected from hydrogen, C_{1-10} alkyl, C_{1-10} perhaloalkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, C_{3-10} carbocyclyl, 3–14 membered heterocyclyl, C_{6-14} aryl, and 5–14 membered heteroaryl, or two R^{cc} groups are joined to form a 3–14 membered heterocyclyl or 5–14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups;

each instance of R^{dd} is, independently, selected from halogen, –CN, $-NO_2$, $-N_3$, $-SO_2H$, $-SO_3H$, –OH, $-OR^{ee}$, $-ON(R^{ff})_2$, $-N(R^{ff})_2$, $-N(R^{ff})_3{}^+X^-$, $-N(OR^{ee})R^{ff}$, –SH, $-SR^{ee}$, $-SSR^{ee}$, $-C(=O)R^{ee}$, $-CO_2H$, $-CO_2R^{ee}$, $-OC(=O)R^{ee}$, $-OCO_2R^{ee}$, $-C(=O)N(R^{ff})_2$, $-OC(=O)N(R^{ff})_2$, $-NR^{ff}C(=O)R^{ee}$, $-NR^{ff}CO_2R^{ee}$, $-NR^{ff}C(=O)N(R^{ff})_2$, $-C(=NR^{ff})OR^{ee}$, $-OC(=NR^{ff})R^{ee}$, $-OC(=NR^{ff})OR^{ee}$, $-C(=NR^{ff})N(R^{ff})_2$, $-OC(=NR^{ff})N(R^{ff})_2$, $-NR^{ff}C(=NR^{ff})N(R^{ff})_2$, $-NR^{ff}SO_2R^{ee}$, $-SO_2N(R^{ff})_2$, $-SO_2R^{ee}$, $-SO_2OR^{ee}$, $-OSO_2R^{ee}$, $-S(=O)R^{ee}$,

$-Si(R^{ee})_3$, $-OSi(R^{ee})_3$, $-C(=S)N(R^{ff})_2$, $-C(=O)SR^{ee}$, $-C(=S)SR^{ee}$, $-SC(=S)SR^{ee}$, $-P(=O)_2R^{ee}$, $-P(=O)(R^{ee})_2$, $-OP(=O)(R^{ee})_2$, $-OP(=O)(OR^{ee})_2$, C_{1-6} alkyl, C_{1-6} perhaloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} carbocyclyl, 3–10 membered heterocyclyl, C_{6-10} aryl, 5–10 membered heteroaryl, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{gg} groups, or two geminal R^{dd} substituents can be joined to form =O or =S;

each instance of R^{ee} is, independently, selected from C_{1-6} alkyl, C_{1-6} perhaloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} carbocyclyl, C_{6-10} aryl, 3–10 membered heterocyclyl, and 3–10 membered heteroaryl, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{gg} groups;

each instance of R^{ff} is, independently, selected from hydrogen, C_{1-6} alkyl, C_{1-6} perhaloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} carbocyclyl, 3–10 membered heterocyclyl, C_{6-10} aryl and 5–10 membered heteroaryl, or two R^{ff} groups are joined to form a 3–14 membered heterocyclyl or 5–14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{gg} groups; and

each instance of R^{gg} is, independently, halogen, $-CN$, $-NO_2$, $-N_3$, $-SO_2H$, $-SO_3H$, $-OH$, $-OC_{1-6}$ alkyl, $-ON(C_{1-6}$ alkyl$)_2$, $-N(C_{1-6}$ alkyl$)_2$, $-N(C_{1-6}$ alkyl$)_3{}^+X^-$, $-NH(C_{1-6}$ alkyl$)_2{}^+X^-$, $-NH_2(C_{1-6}$ alkyl$)\,{}^+X^-$, $-NH_3{}^+X^-$, $-N(OC_{1-6}$ alkyl$)(C_{1-6}$ alkyl$)$, $-N(OH)(C_{1-6}$ alkyl$)$, $-NH(OH)$, $-SH$, $-SC_{1-6}$ alkyl, $-SS(C_{1-6}$ alkyl$)$, $-C(=O)(C_{1-6}$ alkyl$)$, $-CO_2H$, $-CO_2(C_{1-6}$ alkyl$)$, $-OC(=O)(C_{1-6}$ alkyl$)$, $-OCO_2(C_{1-6}$ alkyl$)$, $-C(=O)NH_2$, $-C(=O)N(C_{1-6}$ alkyl$)_2$, $-OC(=O)NH(C_{1-6}$ alkyl$)$, $-NHC(=O)(\,C_{1-6}$ alkyl$)$, $-N(C_{1-6}$ alkyl$)C(=O)(\,C_{1-6}$ alkyl$)$, $-NHCO_2(C_{1-6}$ alkyl$)$, $-NHC(=O)N(C_{1-6}$ alkyl$)_2$, $-NHC(=O)NH(C_{1-6}$ alkyl$)$, $-NHC(=O)NH_2$, $-C(=NH)O(C_{1-6}$ alkyl$)$,$-OC(=NH)(C_{1-6}$ alkyl$)$, $-OC(=NH)OC_{1-6}$ alkyl, $-C(=NH)N(C_{1-6}$ alkyl$)_2$, $-C(=NH)NH(C_{1-6}$ alkyl$)$, $-C(=NH)NH_2$, $-OC(=NH)N(C_{1-6}$ alkyl$)_2$, $-OC(NH)NH(C_{1-6}$ alkyl$)$, $-OC(NH)NH_2$, $-NHC(NH)N(C_{1-6}$ alkyl$)_2$, $-NHC(=NH)NH_2$, $-NHSO_2(C_{1-6}$ alkyl$)$, $-SO_2N(C_{1-6}$ alkyl$)_2$, $-SO_2NH(C_{1-6}$ alkyl$)$, $-SO_2NH_2$,$-SO_2C_{1-6}$ alkyl, $-SO_2OC_{1-6}$ alkyl, $-OSO_2C_{1-6}$ alkyl, $-SOC_{1-6}$ alkyl, $-Si(C_{1-6}$ alkyl$)_3$, $-OSi(C_{1-6}$ alkyl$)_3$ $-C(=S)N(C_{1-6}$ alkyl$)_2$, $C(=S)NH(C_{1-6}$ alkyl$)$, $C(=S)NH_2$, $-C(=O)S(C_{1-6}$ alkyl$)$, $-C(=S)SC_{1-6}$ alkyl, $-SC(=S)SC_{1-6}$ alkyl, $-P(=O)_2(C_{1-6}$ alkyl$)$, $-P(=O)(C_{1-6}$ alkyl$)_2$, $-OP(=O)(C_{1-6}$ alkyl$)_2$, $-OP(=O)(OC_{1-6}$ alkyl$)_2$, C_{1-6} alkyl, C_{1-6} perhaloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, , C_{3-10} carbocyclyl, C_{6-10} aryl, 3–10 membered heterocyclyl, 5–10 membered heteroaryl; or two geminal R^{gg} substituents can be joined to form =O or =S; wherein X^- is a counterion.

[0039] As used herein, the term "hydroxyl" or "hydroxy" refers to the group –OH. The term "substituted hydroxy" or "substituted hydroxyl," by extension, refers to a hydroxyl group wherein the oxygen atom directly attached to the parent molecule is substituted with a group other than hydrogen, and includes groups selected from $–OR^{aa}$, $–ON(R^{bb})_2$, $–OC(=O)SR^{aa}$, $–OC(=O)R^{aa}$, $–OCO_2R^{aa}$, $–OC(=O)N(R^{bb})_2$, $–OC(=NR^{bb})R^{aa}$, $–OC(=NR^{bb})OR^{aa}$, $–OC(=NR^{bb})N(R^{bb})_2$, $–OS(=O)R^{aa}$, $–OSO_2R^{aa}$, $–OSi(R^{aa})_3$, $–OP(R^{cc})_2$, $–OP(R^{cc})_3$, $–OP(=O)_2R^{aa}$, $–OP(=O)(R^{aa})_2$, $–OP(=O)(OR^{cc})_2$, $–OP(=O)_2N(R^{bb})_2$, and $–OP(=O)(NR^{bb})_2$, wherein R^{aa}, R^{bb}, and R^{cc} are as defined herein.

[0040] As used herein, the term "thiol" or "thio" refers to the group –SH. The term "substituted thiol" or "substituted thio," by extension, refers to a thiol group wherein the sulfur atom directly attached to the parent molecule is substituted with a group other than hydrogen, and includes groups selected from $–SR^{aa}$, $–S=SR^{cc}$, $–SC(=S)SR^{aa}$, $–SC(=O)SR^{aa}$, $–SC(=O)OR^{aa}$, and $–SC(=O)R^{aa}$, wherein R^{aa} and R^{cc} are as defined herein.

[0041] As used herein, the term, "amino" refers to the group $–NH_2$. The term "substituted amino," by extension, refers to a monosubstituted amino, a disubstituted amino, or a trisubstituted amino, as defined herein.

[0042] As used herein, the term "monosubstituted amino" refers to an amino group wherein the nitrogen atom directly attached to the parent molecule is substituted with one hydrogen and one group other than hydrogen, and includes groups selected from $–NH(R^{bb})$, $–NHC(=O)R^{aa}$, $–NHCO_2R^{aa}$, $–NHC(=O)N(R^{bb})_2$, $–NHC(=NR^{bb})N(R^{bb})_2$, $–NHSO_2R^{aa}$, $–NHP(=O)(OR^{cc})_2$, and $–NHP(=O)(NR^{bb})_2$, wherein R^{aa}, R^{bb} and R^{cc} are as defined herein, and wherein R^{bb} of the group $–NH(R^{bb})$ is not hydrogen.

[0043] As used herein, the term "disubstituted amino" refers to an amino group wherein the nitrogen atom directly attached to the parent molecule is substituted with two groups other than hydrogen, and includes groups selected from $–N(R^{bb})_2$, $–NR^{bb}C(=O)R^{aa}$, $–NR^{bb}CO_2R^{aa}$, $–NR^{bb}C(=O)N(R^{bb})_2$, $–NR^{bb}C(=NR^{bb})N(R^{bb})_2$, $–NR^{bb}SO_2R^{aa}$, $–NR^{bb}P(=O)(OR^{cc})_2$, and $–NR^{bb}P(=O)(NR^{bb})_2$, wherein R^{aa}, R^{bb}, and R^{cc} are as defined herein, with the proviso that the nitrogen atom directly attached to the parent molecule is not substituted with hydrogen.

[0044] As used herein, the term "trisubstituted amino" refers to an amino group wherein the nitrogen atom directly attached to the parent molecule is substituted with three groups, and includes groups selected from $–N(R^{bb})_3$ and $–N(R^{bb})_3{}^+X^-$, wherein R^{bb} and X^- are as defined herein.

[0045] As used herein, the term "sulfonyl" refers to a group selected from $–SO_2N(R^{bb})_2$, $–SO_2R^{aa}$, and $–SO_2OR^{aa}$, wherein R^{aa} and R^{bb} are as defined herein.

[0046] As used herein, the term "sulfinyl" refers to the group $–S(=O)R^{aa}$, wherein R^{aa} is as defined herein.

[0047] As used herein, the term "acyl" or "carbonyl" refers a group wherein the carbon directly attached to the parent molecule is sp^2 hybridized, and is substituted with an oxygen, nitrogen or sulfur atom, *e.g.*, a group selected from ketones ($–C(=O)R^{aa}$), carboxylic acids ($–CO_2H$), aldehydes (–CHO), esters ($–CO_2R^{aa}$, $–C(=O)SR^{aa}$, $–C(=S)SR^{aa}$), amides ($–C(=O)N(R^{bb})_2$, $–C(=O)NR^{bb}SO_2R^{aa}$, $–C(=S)N(R^{bb})_2$), and imines ($–C(=NR^{bb})R^{aa}$, $–C(=NR^{bb})OR^{aa}$), $–C(=NR^{bb})N(R^{bb})_2$), wherein R^{aa} and R^{bb} are as defined herein.

[0048] As used herein, the term "silyl" refers to the group $–Si(R^{aa})_3$, wherein R^{aa} is as defined herein.

[0049] As used herein, the term "boronyl" refers to boranes, boronic acids, boronic esters, borinic acids, and borinic esters, *e.g.*, boronyl groups of Formula $–B(R^{aa})_2$, $–B(OR^{cc})_2$, and $–BR^{aa}(OR^{cc})$, wherein R^{aa} and R^{cc} are as defined herein.

[0050] As used herein, the term "halo" or "halogen" refers to fluorine (fluoro, –F), chlorine (chloro, –Cl), bromine (bromo, –Br), or iodine (iodo, –I).

[0051] As used herein, a "counterion" is a negatively charged group associated with a positively charged quarternary amine in order to maintain electronic neutrality. Exemplary counterions include halide ions (*e.g.,* $F^–$, $Cl^–$, $Br^–$, $I^–$), $NO_3^–$, $ClO_4^–$, $OH^–$, $H_2PO_4^–$, $HSO_4^–$, sulfonate ions (*e.g.,* methansulfonate, trifluoromethanesulfonate, p–toluenesulfonate, benzenesulfonate, 10–camphor sulfonate, naphthalene–2–sulfonate, naphthalene–1–sulfonic acid–5–sulfonate, ethan–1–sulfonic acid–2–sulfonate, and the like), and carboxylate ions (*e.g.,* acetate, ethanoate, propanoate, benzoate, glycerate, lactate, tartrate, glycolate, and the like).

[0052] Nitrogen atoms can be substituted or unsubstituted as valency permits, and include primary, secondary, tertiary, and quarternary nitrogen atoms. Exemplary nitrogen atom substitutents include, but are not limited to, hydrogen, –OH, $–OR^{aa}$, $–N(R^{cc})_2$, –CN, $–C(=O)R^{aa}$, $–C(=O)N(R^{cc})_2$, $–CO_2R^{aa}$, $–SO_2R^{aa}$, $–C(=NR^{bb})R^{aa}$, $–C(=NR^{cc})OR^{aa}$, $–C(=NR^{cc})N(R^{cc})_2$, $–SO_2N(R^{cc})_2$, $–SO_2R^{cc}$, $–SO_2OR^{cc}$, $–SOR^{aa}$, $–C(=S)N(R^{cc})_2$, $–C(=O)SR^{cc}$, $–C(=S)SR^{cc}$, $–P(=O)_2R^{aa}$, $–P(=O)(R^{aa})_2$, $–P(=O)_2N(R^{cc})_2$, $–P(=O)(NR^{cc})_2$, $C_{1–10}$ alkyl, $C_{1–10}$ perhaloalkyl, $C_{2–10}$ alkenyl, $C_{2–10}$ alkynyl, $C_{3–10}$ carbocyclyl, 3–14 membered heterocyclyl, $C_{6–14}$ aryl, and 5–14 membered heteroaryl, or two R^{cc} groups attached to an N atom are joined to form a 3–14 membered heterocyclyl or 5–14 membered heteroaryl ring, wherein each

alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups, and wherein R^{aa}, R^{bb}, R^{cc} and R^{dd} are as defined above.

[0053] In certain embodiments, the substituent present on the nitrogen atom is an amino protecting group (also referred to herein as a "nitrogen protecting group"). Amino protecting groups include, but are not limited to, –OH, $–OR^{aa}$, $–N(R^{cc})_2$, $–C(=O)R^{aa}$, $–C(=O)N(R^{cc})_2$, $–CO_2R^{aa}$, $–SO_2R^{aa}$, $–C(=NR^{cc})R^{aa}$, $–C(=NR^{cc})OR^{aa}$, $–C(=NR^{cc})N(R^{cc})_2$, $–SO_2N(R^{cc})_2$, $–SO_2R^{cc}$, $–SO_2OR^{cc}$, $–SOR^{aa}$, $–C(=S)N(R^{cc})_2$, $–C(=O)SR^{cc}$, $–C(=S)SR^{cc}$, $C_{1–10}$ alkyl (*e.g.,* aralkyl, heteroaralkyl), $C_{2–10}$ alkenyl, $C_{2–10}$ alkynyl, $C_{3–10}$ carbocyclyl, 3–14 membered heterocyclyl, $C_{6–14}$ aryl, and 5–14 membered heteroaryl groups, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aralkyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups, and wherein R^{aa}, R^{bb}, R^{cc} and R^{dd} are as defined herein. Amino protecting groups are well known in the art and include those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, incorporated herein by reference.

[0054] For example, amino protecting groups such as amide groups *(e.g.,* $–C(=O)R^{aa}$) include, but are not limited to, formamide, acetamide, chloroacetamide, trichloroacetamide, trifluoroacetamide, phenylacetamide, 3–phenylpropanamide, picolinamide, 3–pyridylcarboxamide, *N*–benzoylphenylalanyl derivative, benzamide, *p*–phenylbenzamide, *o*–nitophenylacetamide, *o*–nitrophenoxyacetamide, acetoacetamide, (*N'*–dithiobenzyloxyacylamino)acetamide, 3–(*p*–hydroxyphenyl)propanamide, 3–(*o*–nitrophenyl)propanamide, 2–methyl–2–(*o*–nitrophenoxy)propanamide, 2–methyl–2–(*o*–phenylazophenoxy)propanamide, 4–chlorobutanamide, 3–methyl–3–nitrobutanamide, *o*–nitrocinnamide, *N*–acetylmethionine derivative, *o*–nitrobenzamide and *o*–(benzoyloxymethyl)benzamide.

[0055] Amino protecting groups such as carbamate groups (*e.g.,* $–C(=O)OR^{aa}$) include, but are not limited to, methyl carbamate, ethyl carbamante, 9–fluorenylmethyl carbamate (Fmoc), 9–(2–sulfo)fluorenylmethyl carbamate, 9–(2,7–dibromo)fluoroenylmethyl carbamate, 2,7–di–*t*–butyl–[9–(10,10–dioxo–10,10,10,10–tetrahydrothioxanthyl)]methyl carbamate (DBD–Tmoc), 4–methoxyphenacyl carbamate (Phenoc), 2,2,2–trichloroethyl carbamate (Troc), 2–trimethylsilylethyl carbamate (Teoc), 2–phenylethyl carbamate (hZ), 1–(1–adamantyl)–1–methylethyl carbamate (Adpoc), 1,1–dimethyl–2–haloethyl carbamate, 1,1–dimethyl–2,2–dibromoethyl carbamate (DB–*t*–BOC), 1,1–dimethyl–2,2,2–trichloroethyl carbamate (TCBOC), 1–methyl–1–(4–biphenylyl)ethyl carbamate (Bpoc), 1–(3,5–di–*t*–butylphenyl)–1–

methylethyl carbamate (*t*–Bumeoc), 2–(2'– and 4'–pyridyl)ethyl carbamate (Pyoc), 2–(*N,N*–dicyclohexylcarboxamido)ethyl carbamate, *t*–butyl carbamate (BOC), 1–adamantyl carbamate (Adoc), vinyl carbamate (Voc), allyl carbamate (Alloc), 1–isopropylallyl carbamate (Ipaoc), cinnamyl carbamate (Coc), 4–nitrocinnamyl carbamate (Noc), 8–quinolyl carbamate, *N*–hydroxypiperidinyl carbamate, alkyldithio carbamate, benzyl carbamate (Cbz), *p*–methoxybenzyl carbamate (Moz), *p*–nitobenzyl carbamate, *p*–bromobenzyl carbamate, *p*–chlorobenzyl carbamate, 2,4–dichlorobenzyl carbamate, 4–methylsulfinylbenzyl carbamate (Msz), 9–anthrylmethyl carbamate, diphenylmethyl carbamate, 2–methylthioethyl carbamate, 2–methylsulfonylethyl carbamate, 2–(*p*–toluenesulfonyl)ethyl carbamate, [2–(1,3–dithianyl)]methyl carbamate (Dmoc), 4–methylthiophenyl carbamate (Mtpc), 2,4–dimethylthiophenyl carbamate (Bmpc), 2–phosphonioethyl carbamate (Peoc), 2–triphenylphosphonioisopropyl carbamate (Ppoc), 1,1–dimethyl–2–cyanoethyl carbamate, *m*–chloro–*p*–acyloxybenzyl carbamate, *p*–(dihydroxyboryl)benzyl carbamate, 5–benzisoxazolylmethyl carbamate, 2–(trifluoromethyl)–6–chromonylmethyl carbamate (Tcroc), *m*–nitrophenyl carbamate, 3,5–dimethoxybenzyl carbamate, *o*–nitrobenzyl carbamate, 3,4–dimethoxy–6–nitrobenzyl carbamate, phenyl(*o*–nitrophenyl)methyl carbamate, *t*–amyl carbamate, *S*–benzyl thiocarbamate, *p*–cyanobenzyl carbamate, cyclobutyl carbamate, cyclohexyl carbamate, cyclopentyl carbamate, cyclopropylmethyl carbamate, *p*–decyloxybenzyl carbamate, 2,2–dimethoxyacylvinyl carbamate, *o*–(*N,N*–dimethylcarboxamido)benzyl carbamate, 1,1–dimethyl–3–(*N,N*–dimethylcarboxamido)propyl carbamate, 1,1–dimethylpropynyl carbamate, di(2–pyridyl)methyl carbamate, 2–furanylmethyl carbamate, 2–iodoethyl carbamate, isoborynl carbamate, isobutyl carbamate, isonicotinyl carbamate, *p*–(*p'*–methoxyphenylazo)benzyl carbamate, 1–methylcyclobutyl carbamate, 1–methylcyclohexyl carbamate, 1–methyl–1–cyclopropylmethyl carbamate, 1–methyl–1–(3,5–dimethoxyphenyl)ethyl carbamate, 1–methyl–1–(*p*–phenylazophenyl)ethyl carbamate, 1–methyl–1–phenylethyl carbamate, 1–methyl–1–(4–pyridyl)ethyl carbamate, phenyl carbamate, *p*–(phenylazo)benzyl carbamate, 2,4,6–tri–*t*–butylphenyl carbamate, 4–(trimethylammonium)benzyl carbamate, and 2,4,6–trimethylbenzyl carbamate.

[0056] Amino protecting groups such as sulfonamide groups (*e.g.*, $–S(=O)_2R^{aa}$) include, but are not limited to, *p*–toluenesulfonamide (Ts), benzenesulfonamide, 2,3,6,–trimethyl–4–methoxybenzenesulfonamide (Mtr), 2,4,6–trimethoxybenzenesulfonamide (Mtb), 2,6–dimethyl–4–methoxybenzenesulfonamide (Pme), 2,3,5,6–tetramethyl–4–methoxybenzenesulfonamide (Mte), 4–methoxybenzenesulfonamide (Mbs), 2,4,6–trimethylbenzenesulfonamide (Mts), 2,6–dimethoxy–4–methylbenzenesulfonamide (iMds),

2,2,5,7,8–pentamethylchroman–6–sulfonamide (Pmc), methanesulfonamide (Ms), β–trimethylsilylethanesulfonamide (SES), 9–anthracenesulfonamide, 4–(4',8'–dimethoxynaphthylmethyl)benzenesulfonamide (DNMBS), benzylsulfonamide, trifluoromethylsulfonamide, and phenacylsulfonamide.

[0057] Other amino protecting groups include, but are not limited to, phenothiazinyl–(10)–acyl derivative, *N'–p*–toluenesulfonylaminoacyl derivative, *N'*–phenylaminothioacyl derivative, *N*–benzoylphenylalanyl derivative, *N*–acetylmethionine derivative, 4,5–diphenyl–3–oxazolin–2–one, *N*–phthalimide, *N*–dithiasuccinimide (Dts), *N*–2,3–diphenylmaleimide, *N*–2,5–dimethylpyrrole, *N*–1,1,4,4–tetramethyldisilylazacyclopentane adduct (STABASE), 5–substituted 1,3–dimethyl–1,3,5–triazacyclohexan–2–one, 5–substituted 1,3–dibenzyl–1,3,5–triazacyclohexan–2–one, 1–substituted 3,5–dinitro–4–pyridone, *N*–methylamine, *N*–allylamine, *N*–[2–(trimethylsilyl)ethoxy]methylamine (SEM), *N*–3–acetoxypropylamine, *N*–(1–isopropyl–4–nitro–2–oxo–3–pyroolin–3–yl)amine, quaternary ammonium salts, *N*–benzylamine, *N*–di(4–methoxyphenyl)methylamine, *N*–5–dibenzosuberylamine, *N*–triphenylmethylamine (Tr), *N*–[(4–methoxyphenyl)diphenylmethyl]amine (MMTr), *N*–9–phenylfluorenylamine (PhF), *N*–2,7–dichloro–9–fluorenylmethyleneamine, *N*–ferrocenylmethylamino (Fcm), *N*–2–picolylamino *N'*–oxide, *N*–1,1–dimethylthiomethyleneamine, *N*–benzylideneamine, *N–p*–methoxybenzylideneamine, *N*–diphenylmethyleneamine, *N*–[(2–pyridyl)mesityl]methyleneamine, *N*–(*N',N'*–dimethylaminomethylene)amine, *N,N'*–isopropylidenediamine, *N–p*–nitrobenzylideneamine, *N*–salicylideneamine, *N*–5–chlorosalicylideneamine, *N*–(5–chloro–2–hydroxyphenyl)phenylmethyleneamine, *N*–cyclohexylideneamine, *N*–(5,5–dimethyl–3–oxo–1–cyclohexenyl)amine, *N*–borane derivative, *N*–diphenylborinic acid derivative, *N*–[phenyl(pentaacylchromium– or tungsten)acyl]amine, *N*–copper chelate, *N*–zinc chelate, *N*–nitroamine, *N*–nitrosoamine, amine *N*–oxide, diphenylphosphinamide (Dpp), dimethylthiophosphinamide (Mpt), diphenylthiophosphinamide (Ppt), dialkyl phosphoramidates, dibenzyl phosphoramidate, diphenyl phosphoramidate, benzenesulfenamide, *o*–nitrobenzenesulfenamide (Nps), 2,4–dinitrobenzenesulfenamide, pentachlorobenzenesulfenamide, 2–nitro–4–methoxybenzenesulfenamide, triphenylmethylsulfenamide, and 3–nitropyridinesulfenamide (Npys).

[0058] In certain embodiments, the substituent present on an oxygen atom is a hydroxyl or thiol protecting group (also respectively referred to herein as an "oxygen protecting group" or "sulfur protecting group"). Hydroxyl and thiol protecting groups include, but are not limited to, $–R^{aa}$, $–N(R^{bb})_2$, $–C(=O)SR^{aa}$, $–C(=O)R^{aa}$, $–CO_2R^{aa}$, $–C(=O)N(R^{bb})_2$, $–C(=NR^{bb})R^{aa}$, –

$C(=NR^{bb})OR^{aa}$, $-C(=NR^{bb})N(R^{bb})_2$, $-S(=O)R^{aa}$, $-SO_2R^{aa}$, $-Si(R^{aa})_3$, $-P(R^{cc})_2$, $-P(R^{cc})_3$, $-P(=O)_2R^{aa}$, $-P(=O)(R^{aa})_2$, $-P(=O)(OR^{cc})_2$, $-P(=O)_2N(R^{bb})_2$, and $-P(=O)(NR^{bb})_2$, wherein R^{aa}, R^{bb}, and R^{cc} are as defined herein. Hydroxyl and thiol protecting groups are well known in the art and include those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, incorporated herein by reference.

[0059] Exemplary hydroxyl and thiol protecting groups include, but are not limited to, methyl, methoxylmethyl (MOM), methylthiomethyl (MTM), *t*-butylthiomethyl, (phenyldimethylsilyl)methoxymethyl (SMOM), benzyloxymethyl (BOM), *p*-methoxybenzyloxymethyl (PMBM), (4-methoxyphenoxy)methyl (*p*-AOM), guaiacolmethyl (GUM), *t*-butoxymethyl, 4-pentenyloxymethyl (POM), siloxymethyl, 2-methoxyethoxymethyl (MEM), 2,2,2-trichloroethoxymethyl, bis(2-chloroethoxy)methyl, 2-(trimethylsilyl)ethoxymethyl (SEMOR), tetrahydropyranyl (THP), 3-bromotetrahydropyranyl, tetrahydrothiopyranyl, 1-methoxycyclohexyl, 4-methoxytetrahydropyranyl (MTHP), 4-methoxytetrahydrothiopyranyl, 4-methoxytetrahydrothiopyranyl S,S-dioxide, 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl (CTMP), 1,4-dioxan-2-yl, tetrahydrofuranyl, tetrahydrothiofuranyl, 2,3,3a,4,5,6,7,7a-octahydro-7,8,8-trimethyl-4,7-methanobenzofuran-2-yl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 1-methyl-1-methoxyethyl, 1-methyl-1-benzyloxyethyl, 1-methyl-1-benzyloxy-2-fluoroethyl, 2,2,2-trichloroethyl, 2-trimethylsilylethyl, 2-(phenylselenyl)ethyl, *t*-butyl, allyl, *p*-chlorophenyl, *p*-methoxyphenyl, 2,4-dinitrophenyl, benzyl (Bn), *p*-methoxybenzyl, 3,4-dimethoxybenzyl, *o*-nitrobenzyl, *p*-nitrobenzyl, *p*-halobenzyl, 2,6-dichlorobenzyl, *p*-cyanobenzyl, *p*-phenylbenzyl, 2-picolyl, 4-picolyl, 3-methyl-2-picolyl *N*-oxido, diphenylmethyl, *p,p'*-dinitrobenzhydryl, 5-dibenzosuberyl, triphenylmethyl, α-naphthyldiphenylmethyl, *p*-methoxyphenyldiphenylmethyl, di(*p*-methoxyphenyl)phenylmethyl, tri(*p*-methoxyphenyl)methyl, 4-(4'-bromophenacyloxyphenyl)diphenylmethyl, 4,4′,4″-tris(4,5-dichlorophthalimidophenyl)methyl, 4,4′,4″-tris(levulinoyloxyphenyl)methyl, 4,4′,4″-tris(benzoyloxyphenyl)methyl, 3-(imidazol-1-yl)bis(4′,4″-dimethoxyphenyl)methyl, 1,1-bis(4-methoxyphenyl)-1′-pyrenylmethyl, 9-anthryl, 9-(9-phenyl)xanthenyl, 9-(9-phenyl-10-oxo)anthryl, 1,3-benzodithiolan-2-yl, benzisothiazolyl S,S-dioxido, trimethylsilyl (TMS), triethylsilyl (TES), triisopropylsilyl (TIPS), dimethylisopropylsilyl (IPDMS), diethylisopropylsilyl (DEIPS), dimethylthexylsilyl, *t*-butyldimethylsilyl (TBDMS), *t*-butyldiphenylsilyl (TBDPS), tribenzylsilyl, tri-*p*-xylylsilyl, triphenylsilyl,

diphenylmethylsilyl (DPMS), *t*-butylmethoxyphenylsilyl (TBMPS), formate, benzoylformate, acetate, chloroacetate, dichloroacetate, trichloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, phenoxyacetate, *p*-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate (levulinate), 4,4-(ethylenedithio)pentanoate (levulinoyldithioacetal), pivaloate, adamantoate, crotonate, 4-methoxycrotonate, benzoate, *p*-phenylbenzoate, 2,4,6-trimethylbenzoate (mesitoate), alkyl methyl carbonate, 9-fluorenylmethyl carbonate (Fmoc), alkyl ethyl carbonate, alkyl 2,2,2-trichloroethyl carbonate (Troc), 2-(trimethylsilyl)ethyl carbonate (TMSEC), 2-(phenylsulfonyl) ethyl carbonate (Psec), 2-(triphenylphosphonio) ethyl carbonate (Peoc), alkyl isobutyl carbonate, alkyl vinyl carbonate alkyl allyl carbonate, alkyl *p*-nitrophenyl carbonate, alkyl benzyl carbonate, alkyl *p*-methoxybenzyl carbonate, alkyl 3,4-dimethoxybenzyl carbonate, alkyl *o*-nitrobenzyl carbonate, alkyl *p*-nitrobenzyl carbonate, alkyl *S*-benzyl thiocarbonate, 4-ethoxy-1-napththyl carbonate, methyl dithiocarbonate, 2-iodobenzoate, 4-azidobutyrate, 4-nitro-4-methylpentanoate, *o*-(dibromomethyl)benzoate, 2-formylbenzenesulfonate, 2-(methylthiomethoxy)ethyl, 4-(methylthiomethoxy)butyrate, 2-(methylthiomethoxymethyl)benzoate, 2,6-dichloro-4-methylphenoxyacetate, 2,6-dichloro-4-(1,1,3,3-tetramethylbutyl)phenoxyacetate, 2,4-bis(1,1-dimethylpropyl)phenoxyacetate, chlorodiphenylacetate, isobutyrate, monosuccinoate, (*E*)-2-methyl-2-butenoate, *o*-(methoxyacyl)benzoate, α-naphthoate, nitrate, alkyl *N,N,N',N'*-tetramethylphosphorodiamidate, alkyl *N*-phenylcarbamate, borate, dimethylphosphinothioyl, alkyl 2,4-dinitrophenylsulfenate, sulfate, methanesulfonate (mesylate), benzylsulfonate, and tosylate (Ts).

[0060] These and other exemplary substituents are described in more detail in the Detailed Description, Examples, and claims. The invention is not intended to be limited in any manner by the above exemplary listing of substituents.

Other definitions

[0061] The term"salt" refers to any and all salts, including pharmaceutically acceptable salts.

[0062] As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, Berge *et al.*, describes pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences* (1977) 66:1–19.

Pharmaceutically acceptable salts include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2–hydroxy–ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2–naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3–phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p–toluenesulfonate, undecanoate, valerate salts, and the like. Salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and $N^+(C_{1-4}alkyl)_4$ salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate, and aryl sulfonate.

[0063] A "subject" to which administration is contemplated includes, but is not limited to, humans (*i.e.,* a male or female of any age group, *e.g.,* a pediatric subject (*e.g,* infant, child, adolescent) or adult subject (*e.g.,* young adult, middle–aged adult or senior adult)) and/or other non-human animals, for example mammals (*e.g.*, primates (*e.g.,* cynomolgus monkeys, rhesus monkeys); commercially relevant mammals such as cattle, pigs, horses, sheep, goats, cats, and/or dogs), birds (*e.g.*, commercially relevant birds such as chickens, ducks, geese, and/or turkeys), reptiles, amphibians, and fish. In certain embodiments, the non-human animal is a mammal. The non-human animal may be a male or female and at any stage of development. A non-human animal may be a transgenic animal.

[0064] As used herein, and unless otherwise specified, the terms "treat," "treating" and "treatment" contemplate an action that occurs while a subject is suffering from the specified infection which reduces the severity of the infection or retards or slows the progression of the infection ("therapeutic treatment"), and also contemplates an action that occurs before a

subject begins to suffer from the specified infection and which inhibits or reduces the severity of the infection ("prophylactic treatment").

[0065] In general, the "effective amount" of a compound refers to an amount sufficient to elicit the desired biological response, *i.e.*, treating an infection. As will be appreciated by those of ordinary skill in this art, the effective amount of a compound may vary depending on such factors as the desired biological endpoint, the pharmacokinetics of the compound, the condition being treated, the mode of administration, and the age, health, and condition of the subject. For example, the effective amount of a compound with anti-bacterial activity is the amount that results in a sufficient concentration to inhibit the growth, reduce, or kill bacteria, or make bacteria less virulent. An effective amount encompasses therapeutic and prophylactic treatment.

[0066] As used herein, and unless otherwise specified, a "therapeutically effective amount" of a compound is an amount sufficient to provide a therapeutic benefit in the treatment of a infection or to delay or minimize one or more symptoms associated with the infection. A therapeutically effective amount of a compound means an amount of therapeutic agent, alone or in combination with other therapies, which provides a therapeutic benefit in the treatment of the infection. The term "therapeutically effective amount" can encompass an amount that improves overall therapy, reduces or avoids symptoms or causes of infection, or enhances the therapeutic efficacy of another therapeutic agent.

[0067] As used herein, and unless otherwise specified, a "prophylactically effective amount" of a compound is an amount sufficient to prevent an infection, or one or more symptoms associated with the infection or prevent its recurrence. A prophylactically effective amount of a compound means an amount of a therapeutic agent, alone or in combination with other agents, which provides a prophylactic benefit in the prevention of the infection. The term "prophylactically effective amount" can encompass an amount that improves overall prophylaxis or enhances the prophylactic efficacy of another prophylactic agent.

[0068] As used herein "virulence" refers to the degree of pathogenicity within of a microorganism as indicated by case fatality rates and/or the ability of the organism to invade the issues of the subject; *e.g.*, the ability of the organism to cause an infection. A "reduction of virulence" refers to a reduction of this pathogenic capacity of the organism.

[0069] As used herein, use of the phrase "at least one instance" refers to one instance, but also encompasses a range, *e.g.*, for example, from one instance to four instances.

BRIEF DESCRIPTION OF THE DRAWINGS

[0070] ***Figure 1.*** Structures of a bioactive ADEP and synthetic fragments thereof. Dose-dependent effects of fragments on the minimal inhibitory concentration (MIC) of the ADEP against *S. coelicolor* grown on Difco nutrient agar is provided in Table 1A of Example 1. The MIC of the ADEP alone is 16 μg/mL.

[0071] ***Figure 2***. Assessment of analogs of compound **3**, a potentiator of ADEP activity. Concentrations indicate the ADEP MIC when it is combined with analogs at a 20:1 molar excess. Shown in parentheses is the fold-change in MIC relative to ADEP alone (degree of potentiation).

[0072] ***Figures 3A-3C***. Potentiation of ADEP activity in mycobacteria. *Figure 3A*: Potentiation of ADEP activity against *M. smegmatis* in solid growth media. *Figure 3B*: Potentiation of ADEP activity against *M. tuberculosis* H37Rv in liquid growth media. Compound **2** was used as a negative control. Figure 3C: Potentiation of ADEP4 (compound 1) activity in mycobacteria by compound (**14**).

[0073] ***Figure 4.*** Growth of *S. coelicolor* was assessed in the presence of either DMSO (WT) or compound (**14**) (WT+SC-14) in Tryptone Soya Broth (TSB). Initially, an equal amount of colony forming units (CFU) were added to 50mL TSB with or without compound (**14**) (50ug/mL) in an Erlenmeyer flask appropriately fitted with a spring to prevent clumping. These cultures were incubated at 30° C with constant agitation. 1 mL aliquots were removed from the TSB culture periodically and centrifuged. The supernatant was removed and the pellet washed twice with PBS. The resulting pellet was dried for 45 min and weighed. These experiments were performed in triplicate.

[0074] ***Figure 5.*** Fragments of a cyclic acyldepsipeptide have differential antibacterial activity against *B. subtilis*.

[0075] ***Figure 6.*** Structures and antibacterial activities of *N*-acyldifluorophenylalanine fragment analogs against *B. subtilis.*

[0076] ***Figures 7A-7B.*** ClpP binding of key *N*-acyldifluorophenylalanine fragment analogs. *Figure 7A*: Hydrolysis of a fluorogenic decapeptide substrate (15 μM) by *B. subtilis* ClpP (25 nM) was assayed in the presence of increasing concentrations of ADEP fragments, and activity was fit to a cooperative binding model (solid lines) to determine apparent binding constants (K_{app}) and Hill Coefficients. Error bars represent standard deviation among three replicates. *Figure 7B*: Calculated apparent dissociation constants (K_{app}) for ADEP fragment

binding to *B. subtilis* ClpP. Error bars represent the standard error of the binding model fit. Raw data for Figures 7A-7B is provided in Table 2A of Example 2.

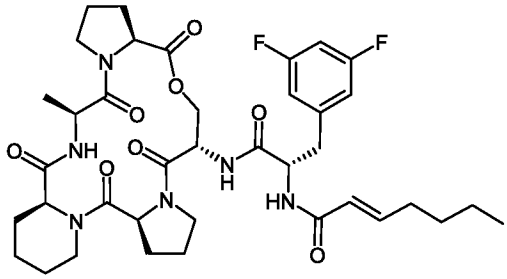
[0077] ***Figure 8.*** ClpP binding of ADEP (**1**): Hydrolysis of a fluorogenic decapeptide substrate (15 μM) by *B. subtilis* ClpP (1 nM) was assayed in the presence of increasing concentrations of ADEP, and activity was fit to a cooperative binding model (solid lines). Error bars represent standard deviation among three replicates. K*app* = 12±0.2 nM. Hill coefficient = 2.2±0.1.

[0078] ***Figures 9A-9B***. ADEP1 bound to *B. subtilis* ClpP. *Figure 9A*: Cartoon view of a heptameric face of ClpP shows ADEP1 bound between ClpP protomers. *Figure 9B*: The N-acylphenylalanine substituent lies in a hydrophobic pocket between protomers, while the serine-methylproline ester and the rest of the peptidolactone ring are largely exposed to solvent. Images rendered in PyMol from structure 3KTI. See, *e.g.*, Lee *et al., Nat. Struct. Mol. Biol.* (2010) *17:*471-478.

[0079] ***Figures 10A-10B***. *Figure 10A* depicts the effect of 200 μM of various compounds (as depicted in *Figure 10B*) on ClpX-ClpP1P2 protease activity. The ADEPs inhibit the capacity of the ClpX-ClpP1P2 from *M. tuberculosis* to degrade a protein. In contrast, certain fragments having antibacterial activity can enhance the capacity of the ClpX-ClpP1P2 from *M. tuberculosis* to degrade a protein. Fragments that enhance the enhance the capacity of the ClpX-ClpP1P2 from *M. tuberculosis* to degrade a protein can be of either Formula (I) or Formula (II). The fragments that do not markedly affect the capacity of of the ClpX-ClpP1P2 from *M. tuberculosis* to degrade a protein are able to potentiate the activty of the ADEP by competitively interfering with ADEP efflux.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS OF THE INVENTION

[0080] The discovery of new classes of antibacterial agents, particularly those with unique biological targets, is essential to keep pace with emerging drug resistance in pathogenic bacteria. Cyclic acyldepsipeptide (ADEP) antibiotics, also referred to as enopeptins, such as ADEP4 have garnered considerable attention because of their potent antibacterial activity against a broad range of bacterial pathogens.



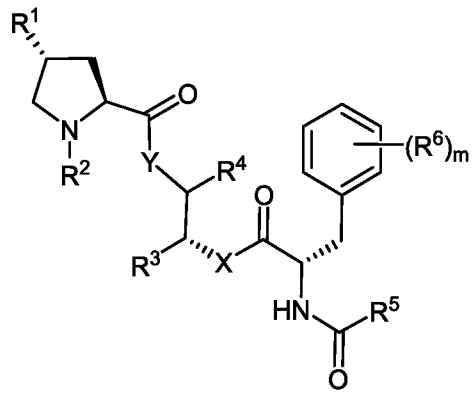
ADEP4 (compound1)

[0081] It has now been discovered certain fragments of the cyclic acyldepsipeptide (ADEP) scaffold exhibit antibacterial activity, particularly against *M. tuberculosis*.

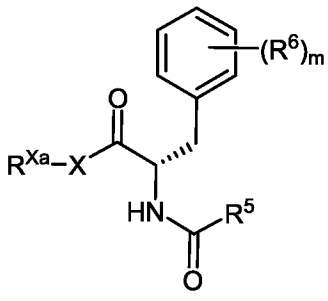
[0082] For example, it has been discovered that fragments of the ADEP exemplified by Formula (I) have antibacterial activity, in some cases that is superior to the intact ADEP. While such fragments have the same target as the intact ADEP, they can enhance the capacity of ClpP to degrade a protein rather than inhibit this capacity like the intact ADEPs.

[0083] It has also been discovered that some of these fragments retain the capacity to bind the ClpP enzyme from *M. tuberculosis*, but affect ClpP activity differently from the intact ADEPs. The intact ADEPs inhibit the capacity of ClpX-ClpP1P2 to degrade a protein substrate. In contrast, certain fragments enhance the capacity of ClpX-ClpP1P2 to degrade a protein substrate. It has further been established that fragments that minimally affect the the capacity of ClpX-ClpP1P2 to degrade a protein substrate can potentiate the activities of anti-bacterial agents, such as an ADEP4, against bacteria that are not susceptible due to efflux pumps by competitively interfering with ADEP efflux.

[0084] Enopeptin analogs contemplated herein comprise compounds of Formula (**I**) and (**II**):



(**I**)



(**II**)

or pharmaceutically acceptable salts thereof;

wherein:

R^1 is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

R^2 is hydrogen, -OH, -OR^{2A}, -C(=O)R^{2A}, -C(=O)OR^{2A}, -C(=O)NHR^{2A}, -C(=O)$N(R^{2A})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, or a nitrogen protecting group;

R^3 is -C(=O)R^{3A}, -C(=O)OR^{3A}, -C(=O)NHR^{3A}, -C(=O)$N(R^{3A})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

each instance of R^{2A} and R^{3A} is independently optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl,

or two R^{2A} groups are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring,

or two R^{3A} groups are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring;

R^4 is hydrogen, –OH, –SH, $–NH_2$, substituted hydroxyl, substituted thiol, substituted amino, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

R^5 is optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, -OR^{5A} or –NHR^{5A}, wherein each instance of R^{5A} is independently optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

each instance of R^6 is independently halogen, –OH, –SH, $–NH_2$, -CN, $-NO_2$, $-N_3$, $–SO_2H$, $–SO_3H$, substituted hydroxyl, substituted thiol, substituted amino, sulfonyl, sulfinyl, carbonyl, silyl, boronyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

m is 0 or an integer of between 1 and 5, inclusive;

Y is O, S, or NR^Y wherein R^Y is hydrogen, optionally substituted alkyl, or a nitrogen protecting group; and

X is O, S, or NR^{Xb},

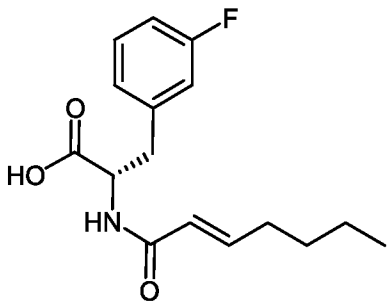
wherein:

R^{Xb} is hydrogen, optionally substituted alkyl, or a nitrogen protecting group; and

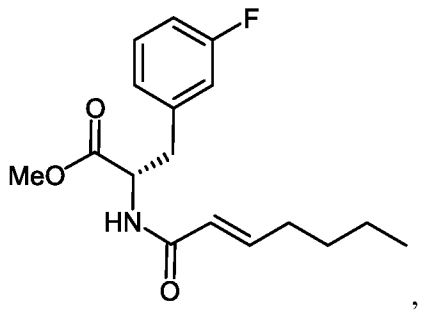
R^{Xa} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

or R^{Xa} and R^{Xb} are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring.

[0085] In certain embodiments, the compound of Formula (**II**) is not:



or



,

or a pharmaceutically acceptable salt thereof.

[0086] As generally described herein, R^1 is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl.

[0087] In certain embodiments, R^1 is hydrogen.

[0088] In certain embodiments, R^1 is optionally substituted alkyl, *e.g.,* optionally substituted C_{1-6}alkyl, optionally substituted C_{1-5}alkyl, optionally substituted C_{1-4}alkyl, optionally substituted C_{1-3}alkyl, optionally substituted C_{1-2}alkyl, or optionally substituted C_1alkyl. In certain embodiments wherein R^1 is alkyl, the alkyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^1 is alkyl, the alkyl group is unsubstituted.

[0089] In certain embodiments, R^1 is optionally substituted alkenyl, *e.g.,* optionally substituted C_{2-6}alkenyl, optionally substituted C_{2-5}alkenyl, optionally substituted C_{2-4}alkenyl,

optionally substituted C_{2-3}alkenyl, or optionally substituted C_2alkenyl. In certain embodiments wherein R^1 is alkenyl, the alkenyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^1 is alkenyl, the alkenyl group is unsubstituted.

[0090] In certain embodiments, R^1 is optionally substituted alkynyl, *e.g.,* optionally substituted C_{2-6}alkynyl, optionally substituted C_{2-5}alkynyl, optionally substituted C_{2-4}alkynyl, optionally substituted C_{2-3}alkynyl, or optionally substituted C_2alkynyl. In certain embodiments wherein R^1 is alkynyl, the alkynyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^1 is alkynyl, the alkynyl group is unsubstituted.

[0091] In certain embodiments, R^1 is optionally substituted carbocyclyl (*e.g.,* C_{3-6}carbocyclyl) or optionally substituted heterocyclyl (*e.g.,* a 3- to 6-membered heterocyclyl).

[0092] In certain embodiments, R^1 is optionally substituted aryl (*e.g.,* phenyl) or optionally substituted heteroaryl (*e.g.,* 5- to 6- membered heteroaryl).

[0093] In certain embodiments, R^1 is hydrogen or optionally substituted alkyl. In certain embodiments, R^1 is hydrogen. In certain embodiments, R^1 is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$.

[0094] As generally described herein, R^2 is hydrogen, -OH, $-OR^{2A}$, $-C(=O)R^{2A}$, $-C(=O)OR^{2A}$, $-C(=O)NHR^{2A}$, $-C(=O)N(R^{2A})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, or a nitrogen protecting group; R^3 is $-C(=O)R^{3A}$, $-C(=O)OR^{3A}$, $-C(=O)NHR^{3A}$, $-C(=O)N(R^{3A})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl; and each instance of R^{2A} and R^{3A} is independently optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl, or two R^{2A} groups are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring, or two R^{3A} groups are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring.

[0095] In certain embodiments, at least one instance of R^{2A} is optionally substituted alkyl, *e.g.,* optionally substituted C_{1-6}alkyl, optionally substituted C_{1-5}alkyl, optionally substituted

C_{1-4}alkyl, optionally substituted C_{1-3}alkyl, optionally substituted C_{1-2}alkyl, or optionally substituted C_1alkyl. In certain embodiments wherein R^{2A} is alkyl, the alkyl group is further substituted, *e.g.,* with halogen or substituted amino groups. However, in certain embodiments, wherein R^{2A} is alkyl, the alkyl group is unsubstituted.

[0096] In certain embodiments, at least one instance of R^{2A} is optionally substituted alkenyl, *e.g.,* optionally substituted C_{2-6}alkenyl, optionally substituted C_{2-5}alkenyl, optionally substituted C_{2-4}alkenyl, optionally substituted C_{2-3}alkenyl, or optionally substituted C_2alkenyl. In certain embodiments wherein R^{2A} is alkenyl, the alkenyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^{2A} is alkenyl, the alkenyl group is unsubstituted.

[0097] In certain embodiments, at least one instance of R^{2A} is optionally substituted alkynyl, *e.g.,* optionally substituted C_{2-6}alkynyl, optionally substituted C_{2-5}alkynyl, optionally substituted C_{2-4}alkynyl, optionally substituted C_{2-3}alkynyl, or optionally substituted C_2alkynyl. In certain embodiments wherein R^{2A} is alkynyl, the alkynyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^{2A} is alkynyl, the alkynyl group is unsubstituted.

[0098] In certain embodiments, at least one instance of R^{2A} is optionally substituted carbocyclyl (*e.g.,* C_{3-6}carbocyclyl) or optionally substituted heterocyclyl (*e.g.,* a 3- to 6-membered heterocyclyl).

[0099] In certain embodiments, at least one instance of R^{2A} is optionally substituted aryl (*e.g.,* phenyl) or optionally substituted heteroaryl (*e.g.,* 5- to 6- membered heteroaryl).

[00100] In certain embodiments, at least one instance of R^{3A} is optionally substituted alkyl, *e.g.,* optionally substituted C_{1-6}alkyl, optionally substituted C_{1-5}alkyl, optionally substituted C_{1-4}alkyl, optionally substituted C_{1-3}alkyl, optionally substituted C_{1-2}alkyl, or optionally substituted C_1alkyl. In certain embodiments wherein R^{3A} is alkyl, the alkyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^{3A} is alkyl, the alkyl group is unsubstituted.

[00101] In certain embodiments, at least one instance of R^{3A} is optionally substituted alkenyl, *e.g.,* optionally substituted C_{2-6}alkenyl, optionally substituted C_{2-5}alkenyl, optionally substituted C_{2-4}alkenyl, optionally substituted C_{2-3}alkenyl, or optionally substituted C_2alkenyl. In certain embodiments wherein R^{3A} is alkenyl, the alkenyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^{3A} is alkenyl, the alkenyl group is unsubstituted.

[00102] In certain embodiments, at least one instance of R^{3A} is optionally substituted alkynyl, *e.g.,* optionally substituted C_{2-6}alkynyl, optionally substituted C_{2-5}alkynyl, optionally substituted C_{2-4}alkynyl, optionally substituted C_{2-3}alkynyl, or optionally substituted C_2alkynyl. In certain embodiments wherein R^{3A} is alkynyl, the alkynyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^{3A} is alkynyl, the alkynyl group is unsubstituted.

[00103] In certain embodiments, at least one instance of R^{3A} is optionally substituted carbocyclyl (*e.g.,* C_{3-6}carbocyclyl) or optionally substituted heterocyclyl (*e.g.,* a 3- to 6-membered heterocyclyl).

[00104] In certain embodiments, at least one instance of R^{3A} is optionally substituted aryl (*e.g.,* phenyl) or optionally substituted heteroaryl (*e.g.,* 5- to 6- membered heteroaryl).

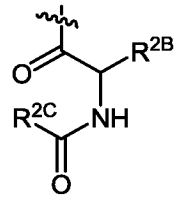
[00105] As generally described herein, R^2 is hydrogen, -OH, $-OR^{2A}$, $-C(=O)R^{2A}$, $-C(=O)OR^{2A}$, $-C(=O)NHR^{2A}$, $-C(=O)N(R^{2A})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, or a nitrogen protecting group; and wherein each instance of R^{2A} is independently optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl, or two R^{2A} groups are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring.

[00106] In certain embodiments, R^2 is hydrogen.

[00107] In certain embodiments, R^2 is –OH or $-OR^{2A}$ wherein R^{2A} is as described herein. In certain embodiments, R^2 is –OH. In certain embodiments, R^2 is $-OR^{2A}$ wherein R^{2A} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$.

[00108] In certain embodiments, R^2 is $-C(=O)R^{2A}$, wherein R^{2A} is as described herein. In certain embodiments, R^2 is $-C(=O)R^{2A}$ wherein R^{2A} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$.

[00109] In certain embodiments, R^2 is $-C(=O)R^{2A}$, R^{2A} is a substituted alkyl (*e.g.,* a C_1alkyl) group. In certain embodiments, the alkyl group is substituted with at least one substituted amino group. For example, in certain embodiments, R^2 is a group of formula:



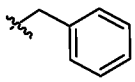
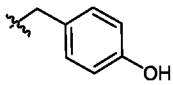
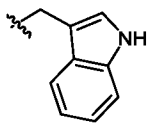
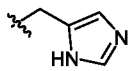
wherein:

R^{2B} is hydrogen and optionally substituted alkyl; and

R^{2C} is optionally substituted alkyl or $-OR^{2D}$ wherein R^{2D} is optionally substituted alkyl.

[00110] In certain embodiments, R^{2B} is hydrogen.

[00111] In certain embodiments, R^{2B} is optionally substituted alkyl, *e.g.*, straight chain or branched alkyl optionally substituted with hydroxyl, substituted hydroxyl, thiol, substituted thiol, amino, substituted amino, carbonyl, optionally substituted aryl, or optionally substituted heteroaryl groups. Such R^{2B} optionally substituted alkyl moieties encompass amino acid side chains of formula:

$-CH_3$	$-CH_2OH$	$-CH(CH_3)OH$	$-CH_2SH$
Alanine	Serine	Threonine	Cysteine
$-CH(CH_3)_2$	$-CH_2CH(CH_3)_2$	$-CH(CH_3)(CH_2CH_3)$	$-CH_2CH_2SCH_3$
Valine	Leucine	Isoleucine	Methionine
			
Phenylalanine	Tyrosine	Tryptophan	Histadine
$-CH_2CO_2H$	$-CH_2CH_2CO_2H$	$-CH_2C(=O)NH_2$	$-CH_2CH_2C(=O)NH_2$
Aspartic acid	Glutamic acid	Asparagine	Glutamine

$-CH_2CH_2CH_2CH_2NH_2$		$-CH_2CH_2CH_2NHC(=NH)NH_2$	
Lysine	or	Arginine	.

[00112] In certain embodiments, R^{2B} is an amino acid side chain selected from the group consisting of alanine, serine, threonine, cysteine, valine, leucine, isoleucine, methionine, and lysine side chains, as defined herein.

[00113] In certain embodiments, R^{2C} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$.

[00114] In certain embodiments, R^{2C} is $-OR^{2D}$ and R^{2D} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$.

[00115] In certain embodiments, R^2 is $-C(=O)OR^{2A}$, wherein R^{2A} is as described herein. In certain embodiments, R^2 is $-C(=O)OR^{2A}$ wherein R^{2A} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$.

[00116] In certain embodiments, R^2 is $-C(=O)NHR^{2A}$, wherein R^{2A} is as described herein. In certain embodiments, R^2 is $-C(=O)NHR^{2A}$ wherein R^{2A} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$.

[00117] In certain embodiments, R^2 is $-C(=O)N(R^{2A})_2$, wherein R^{2A} is as described herein. In certain embodiments, R^2 is $-C(=O)N(R^{2A})_2$ wherein at least one instance of R^{2A} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$, or two R^{2A} groups are joined to form an optionally substituted heterocyclic (*e.g.*, a 3- to 6-membered heterocyclic) ring or optionally substituted heteroaryl (*e.g.*, 5- to 6- membered heteroaryl) ring.

[00118] In certain embodiments, R^2 is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-6}alkyl, optionally substituted C_{1-5}alkyl, optionally substituted C_{1-4}alkyl, optionally substituted C_{1-3}alkyl, optionally substituted C_{1-2}alkyl, or optionally substituted C_1alkyl. In certain embodiments wherein R^2 is alkyl, the alkyl group is further substituted, *e.g.*, with halogen groups. However, in certain embodiments, wherein R^2 is alkyl, the alkyl group is unsubstituted.

[00119] In certain embodiments, R^2 is optionally substituted alkenyl, *e.g.*,optionally substituted C_{2-6}alkenyl, optionally substituted C_{2-5}alkenyl, optionally substituted C_{2-4}alkenyl, optionally substituted C_{2-3}alkenyl, or optionally substituted C_2alkenyl. In certain embodiments wherein R^2 is alkenyl, the alkenyl group is further substituted, *e.g.*, with halogen groups. However, in certain embodiments, wherein R^2 is alkenyl, the alkenyl group is unsubstituted.

[00120] In certain embodiments, R^2 is optionally substituted alkynyl, *e.g.*,optionally substituted C_{2-6}alkynyl, optionally substituted C_{2-5}alkynyl, optionally substituted C_{2-4}alkynyl, optionally substituted C_{2-3}alkynyl, or optionally substituted C_2alkynyl. In certain embodiments wherein R^2 is alkynyl, the alkynyl group is further substituted, *e.g.*, with halogen groups. However, in certain embodiments, wherein R^2 is alkynyl, the alkynyl group is unsubstituted.

[00121] In certain embodiments, R^2 is optionally substituted carbocyclyl (*e.g.*, C_{3-6}carbocyclyl) or optionally substituted heterocyclyl (*e.g.*, a 3- to 6-membered heterocyclyl).

[00122] In certain embodiments, R^2 is optionally substituted aryl (*e.g.,* phenyl) or optionally substituted heteroaryl (*e.g.,* 5- to 6- membered heteroaryl).

[00123] In certain embodiments, R^2 is hydrogen, $-C(=O)R^{2A}$, $-C(=O)OR^{2A}$, $-C(=O)NHR^{2A}$, or $-C(=O)N(R^{2A})_2$, wherein at least one instance of R^{2A} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$.

[00124] In certain embodiments, R^3 is $-C(=O)R^{3A}$, wherein R^{3A} is as described herein. In certain embodiments, R^3 is $-C(=O)R^{3A}$ wherein R^{3A} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$.

[00125] In certain embodiments, R^3 is $-C(=O)OR^{3A}$, wherein R^{3A} is as described herein. In certain embodiments, R^3 is $-C(=O)OR^{3A}$ wherein R^{3A} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$.

[00126] In certain embodiments, R^3 is $-C(=O)NHR^{3A}$, wherein R^{3A} is as described herein. In certain embodiments, R^3 is $-C(=O)NHR^{3A}$ wherein R^{3A} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$.

[00127] In certain embodiments, R^3 is $-C(=O)N(R^{3A})_2$, wherein R^{3A} is as described herein. In certain embodiments, R^3 is $-C(=O)N(R^{2A})_2$ wherein at least one instance of R^{3A} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$, or two R^{3A} groups are joined to form an optionally substituted heterocyclic (*e.g.,* a 3- to 6-membered heterocyclic) ring or optionally substituted heteroaryl (*e.g.,* 5- to 6- membered heteroaryl) ring.

[00128] In certain embodiments, R^3 is optionally substituted alkyl, *e.g.,* optionally substituted C_{1-6}alkyl, optionally substituted C_{1-5}alkyl, optionally substituted C_{1-4}alkyl, optionally substituted C_{1-3}alkyl, optionally substituted C_{1-2}alkyl, or optionally substituted C_1alkyl. In certain embodiments wherein R^3 is alkyl, the alkyl group is further substituted, *e.g.,* with halogen groups, hydroxyl, or substituted hydroxyl groups. However, in certain embodiments, wherein R^3 is alkyl, the alkyl group is unsubstituted.

[00129] In certain embodiments, R^3 is optionally substituted alkenyl, *e.g.,* optionally substituted C_{2-6}alkenyl, optionally substituted C_{2-5}alkenyl, optionally substituted C_{2-4}alkenyl, optionally substituted C_{2-3}alkenyl, or optionally substituted C_2alkenyl. In certain embodiments wherein R^3 is alkenyl, the alkenyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^3 is alkenyl, the alkenyl group is unsubstituted.

[00130] In certain embodiments, R^3 is optionally substituted alkynyl, *e.g.,* optionally substituted C_{2-6}alkynyl, optionally substituted C_{2-5}alkynyl, optionally substituted C_{2-4}alkynyl,

optionally substituted C_{2-3}alkynyl, or optionally substituted C_2alkynyl. In certain embodiments wherein R^3 is alkynyl, the alkynyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^3 is alkynyl, the alkynyl group is unsubstituted.

[00131] In certain embodiments, R^3 is optionally substituted carbocyclyl (*e.g.,* C_{3-6}carbocyclyl) or optionally substituted heterocyclyl (*e.g.,* a 3- to 6-membered heterocyclyl).

[00132] In certain embodiments, R^3 is optionally substituted aryl (*e.g.,* phenyl) or optionally substituted heteroaryl (*e.g.,* 5- to 6- membered heteroaryl).

[00133] In certain embodiments, R^3 is $-C(=O)R^{3A}$, $-C(=O)OR^{3A}$, $-C(=O)NHR^{3A}$, or $-C(=O)N(R^{3A})_2$,wherein at least one instance of R^{3A} is optionally substituted alkyl, *e.g.,* optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$, or R^3 is substituted C_{1-3}alkyl, wherein the alkyl group is further substituted with hydroxyl or substituted hydroxyl groups.

[00134] As generally described herein, R^4 is hydrogen, –OH, –SH, $–NH_2$, substituted hydroxyl, substituted thiol, substituted amino, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl.

[00135] In certain embodiments, the stereochemistry of the carbon substituted with R^4 may be (*R*) or (*S*) when R^4 is a non-hydrogen group. In certain embodiments when R^4 is a non-hydrogen group, the stereochemistry of the carbon substituted with R^4 is (*R*). In certain embodiments when R^4 is a non-hydrogen group, the stereochemistry of the carbon substituted with R^4 is (*S*).

[00136] In certain embodiments, R^4 is hydrogen.

[00137] In certain embodiments, R^4 is –OH, –SH, $–NH_2$, substituted hydroxyl, substituted thiol, or substituted amino, as defined herein.

[00138] In certain embodiments, R^4 is optionally substituted alkyl, *e.g.,* optionally substituted C_{1-6}alkyl, optionally substituted C_{1-5}alkyl, optionally substituted C_{1-4}alkyl, optionally substituted C_{1-3}alkyl, optionally substituted C_{1-2}alkyl, or optionally substituted C_1alkyl. In certain embodiments wherein R^4 is alkyl, the alkyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^4 is alkyl, the alkyl group is unsubstituted.

[00139] In certain embodiments, R^4 is optionally substituted alkenyl, *e.g.,* optionally substituted C_{2-6}alkenyl, optionally substituted C_{2-5}alkenyl, optionally substituted C_{2-4}alkenyl,

optionally substituted C_{2-3}alkenyl, or optionally substituted C_2alkenyl. In certain embodiments wherein R^4 is alkenyl, the alkenyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^4 is alkenyl, the alkenyl group is unsubstituted.

[00140] In certain embodiments, R^4 is optionally substituted alkynyl, *e.g.,* optionally substituted C_{2-6}alkynyl, optionally substituted C_{2-5}alkynyl, optionally substituted C_{2-4}alkynyl, optionally substituted C_{2-3}alkynyl, or optionally substituted C_2alkynyl. In certain embodiments wherein R^4 is alkynyl, the alkynyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^4 is alkynyl, the alkynyl group is unsubstituted.

[00141] In certain embodiments, R^4 is optionally substituted carbocyclyl (*e.g.,* C_{3-6}carbocyclyl) or optionally substituted heterocyclyl (*e.g.,* a 3- to 6-membered heterocyclyl).

[00142] In certain embodiments, R^4 is optionally substituted aryl (*e.g.,* phenyl) or optionally substituted heteroaryl (*e.g.,* 5- to 6- membered heteroaryl).

[00143] In certain embodiments, R^4 is hydrogen or optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$. In certain embodiments, R^4 is $-CH_3$. In certain embodiments when R^4 is optionally substituted alkyl, the stereochemistry of the carbon substituted with R^4 is (*R*). In certain embodiments when R^4 is optionally substituted alkyl, the stereochemistry of the carbon substituted with R^4 is (*S*).

[00144] As generally described herein, Y is O, S, or NR^Y wherein R^Y is hydrogen, optionally substituted alkyl, or a nitrogen protecting group. In certain embodiments, Y is O. In certain embodiments, Y is S. In certain embodiments, Y is NR^Y wherein R^Y is hydrogen or optionally substituted C_{1-3} alkyl, *e.g.,* $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$. In certain embodiments, Y is O or NR^Y wherein R^Y is hydrogen or $-CH_3$.

[00145] As generally described herein, X is O, S, or NR^{Xb}, wherein R^{Xb} is hydrogen, optionally substituted alkyl, or a nitrogen protecting group; and R^{Xa} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl; or R^{Xa} and R^{Xb} are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring.

[00146] In certain embodiments of Formula (**I**), X is O.

[00147] In certain embodiments of Formula (**I**), X is S.

[00148] In certain embodiments of Formula (**I**), X is NR^{Xb}, whereinR^{Xb} is hydrogen, optionally substituted alkyl (*e.g.,* $-CH_3$), or a nitrogen protecting group.

[00149] In certain embodiments of Formula (**II**), X is O, wherein R^{Xa} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl.

[00150] In certain embodiments of Formula (**II**), X is S, wherein R^{Xa} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl.

[00151] In certain embodiments of Formula (**II**), X is NR^{Xb}, wherein R^{Xb} is hydrogen, optionally substituted alkyl (*e.g.,* $-CH_3$), or a nitrogen protecting group, and R^{Xa} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl, or R^{Xa} and R^{Xb} are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring.

[00152] In any of the above embodiments wherein X is O, S, or NR^{Xb} in Formula (**II**), in certain embodiments, R^{Xa} is optionally substituted alkyl, *e.g.,* optionally substituted C_{1-6}alkyl, optionally substituted C_{1-5}alkyl, optionally substituted C_{1-4}alkyl, optionally substituted C_{1-3}alkyl, optionally substituted C_{1-2}alkyl, or optionally substituted C_1alkyl. In certain embodiments wherein R^{Xa} is alkyl, the alkyl group is further substituted, *e.g.,* with optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl groups. However, in certain embodiments, wherein R^{Xa} is alkyl, the alkyl group is unsubstituted.

[00153] In any of the above embodiments wherein X is O, S, or NR^{Xb} in Formula (**II**), in certain embodiments, R^{Xa} is optionally substituted alkenyl, *e.g.,* optionally substituted C_{2-6}alkenyl, optionally substituted C_{2-5}alkenyl, optionally substituted C_{2-4}alkenyl, optionally substituted C_{2-3}alkenyl, or optionally substituted C_2alkenyl. In certain embodiments wherein R^{Xa} is alkenyl, the alkenyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^{Xa} is alkenyl, the alkenyl group is unsubstituted.

[00154] In any of the above embodiments wherein X is O, S, or NR^{Xb} in Formula (**II**), in certain embodiments, R^{Xa} is optionally substituted alkynyl, *e.g.,* optionally substituted C_{2-6}alkynyl, optionally substituted C_{2-5}alkynyl, optionally substituted C_{2-4}alkynyl, optionally

substituted C_{2-3}alkynyl, or optionally substituted C_2alkynyl. In certain embodiments wherein R^{Xa} is alkynyl, the alkynyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^{Xa} is alkynyl, the alkynyl group is unsubstituted.

[00155] In any of the above embodiments wherein X is O, S, or NR^{Xb} in Formula (**II**), in certain embodiments, R^{Xa} is optionally substituted carbocyclyl (*e.g.,* C_{3-6}carbocyclyl) or optionally substituted heterocyclyl (*e.g.,* a 3- to 6-membered heterocyclyl).

[00156] In any of the above embodiments wherein X is O, S, or NR^{Xb} in Formula (**II**), in certain embodiments, R^{Xa} is optionally substituted aryl (*e.g.,* phenyl) or optionally substituted heteroaryl (*e.g.,* 5- to 6- membered heteroaryl).

[00157] In any of the above embodiments wherein X is NR^{Xb} in Formula (**II**), in certain embodiments, R^{Xa} and R^{Xb} are joined to form an optionally substituted heterocyclic (*e.g.,* optionally substituted 3- to 6- membered heterocyclic) ring or optionally substituted heteroaryl (*e.g.,* optionally substituted 5- to 6-membered heteroaryl) ring.

[00158] In certain embodiments of Formula (**I**), X is O or NR^{Xb}, wherein R^{Xb} is hydrogen or optionally substituted alkyl (*e.g.,* $-CH_3$).

[00159] In certain embodiments of Formula (**II**), X is O or NR^{Xb}, wherein R^{Xb} is hydrogen or optionally substituted alkyl (*e.g.,* $-CH_3$) and R^{Xa} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl.

[00160] In certain embodiments of Formula (**II**), X is O, wherein R^{Xa} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl.

[00161] In certain embodiments of Formula (**II**), X is O, wherein R^{Xa} is hydrogen.

[00162] In certain embodiments of Formula (**II**), X is O, wherein R^{Xa} is optionally substituted alkyl, *e.g.,* optionally substituted C_{1-3}alkyl. In certain embodiments, R^{Xa} is optionally substituted C_1alkyl (*e.g.,* $-CH_3$, or C_1aralkyl such as benzyl), optionally substituted C_2alkyl, or optionally substituted C_3alkyl.

[00163] In certain embodiments of Formula (**II**), X is O, wherein R^{Xa} is optionally substituted alkenyl, *e.g.,* optionally substituted C_{3-5}alkenyl. In certain embodiments, R^{Xa} is optionally substituted C_3alkenyl, optionally substituted C_4alkenyl, or optionally substituted C_5alkenyl.

[00164] In certain embodiments of Formula (**II**), X is O, wherein R^{Xa} is optionally substituted alkynyl, *e.g.,* optionally substituted C_{3-5}alkynyl. In certain embodiments, R^{Xa} is optionally substituted C_3alkynyl, optionally substituted C_4alkynyl, or optionally substituted C_5alkynyl.

[00165] In certain embodiments of Formula (**II**), X is NR^{Xb}, wherein R^{Xb} is hydrogen or optionally substituted alkyl (*e.g.*, $-CH_3$) and R^{Xa} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl.

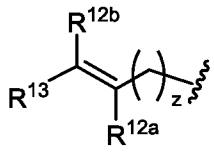
[00166] In certain embodiments of Formula (**II**), X is NR^{Xb}, wherein each of R^{Xa} and R^{Xb} is hydrogen.

[00167] In certain embodiments of Formula (**II**), X is NR^{Xb}, wherein R^{Xb} is hydrogen or optionally substituted alkyl (*e.g.*, $-CH_3$) and wherein R^{Xa} is optionally substituted C_1alkyl (*e.g.*, $-CH_3$, or C_1aralkyl such as benzyl), optionally substituted C_2alkyl, or optionally substituted C_3alkyl.

[00168] In certain embodiments of Formula (**II**), X is NR^{Xb}, wherein R^{Xb} is hydrogen or optionally substituted alkyl (*e.g.*, $-CH_3$) and wherein R^{Xa} is optionally substituted alkenyl, *e.g.*, optionally substituted C_{3-5}alkenyl. In certain embodiments, R^{Xa} is optionally substituted C_3alkenyl, optionally substituted C_4alkenyl, or optionally substituted C_5alkenyl.

[00169] In certain embodiments of Formula (**II**), X is NR^{Xb}, wherein R^{Xb} is hydrogen or optionally substituted alkyl (*e.g.*, $-CH_3$) and wherein R^{Xa} is optionally substituted alkynyl, *e.g.*, optionally substituted C_{3-5}alkynyl. In certain embodiments, R^{Xa} is optionally substituted C_3alkynyl, optionally substituted C_4alkynyl, or optionally substituted C_5alkynyl.

[00170] In certain embodiments of Formula (**II**), R^{Xa} is an optionally alkenyl group of formula (b):



(b)

wherein:

z is 1, 2, 3, or 4;

R^{12a} and R^{12b} are independently hydrogen or optionally substituted alkyl; and

R^{13} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl.

[00171] In certain embodiments, z is 1. In certain embodiments, z is 2. In certain embodiments, z is 3. In certain embodiments, z is 4.

[00172] In certain embodiments, at least one of R^{12a} and R^{12b} is hydrogen. In certain embodiments, at least one of R^{12a} and R^{12b} is optionally substituted alkyl (*e.g.*, $-CH_3$).

[00173] In certain embodiments, R^{12a} and R^{12b} are both hydrogen, *i.e.,* to provide a *trans* alkenyl group. In certain embodiments, R^{12a} and R^{13} are both hydrogen, *i.e.,* to provide a *cis* alkenyl group.

[00174] In certain embodiments, R^{13} is optionally substituted alkyl, *e.g.,* optionally substituted C_{1-6}alkyl, optionally substituted C_{1-5}alkyl, optionally substituted C_{1-4}alkyl, optionally substituted C_{1-3}alkyl, optionally substituted C_{1-2}alkyl, optionally substituted C_1alkyl, optionally substituted C_2alkyl, optionally substituted C_3alkyl, optionally substituted C_4alkyl, optionally substituted C_5alkyl, or optionally substituted C_6alkyl. In certain embodiments wherein R^{13} is alkyl, the alkyl group is further substituted, *e.g.,* with halogen groups, or branched (*e.g.,* with $-CH_3$ groups). However, in certain embodiments, wherein R^{13} is alkyl, the alkyl group is unsubstituted. In certain embodiments, wherein R^{13} is alkyl, the alkyl group is branched.

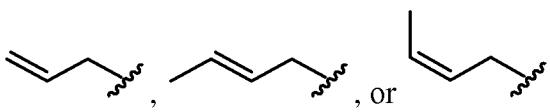
[00175] In certain embodiments, R^{13} is optionally substituted alkenyl, *e.g.,* optionally substituted C_{2-6}alkenyl, optionally substituted C_{2-5}alkenyl, optionally substituted C_{2-4}alkenyl, optionally substituted C_{2-3}alkenyl, or optionally substituted C_2alkenyl. In certain embodiments wherein R^{13} is alkenyl, the alkenyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^{13} is alkenyl, the alkenyl group is unsubstituted.

[00176] In certain embodiments, R^{13} is optionally substituted alkynyl, *e.g.,* optionally substituted C_{2-6}alkynyl, optionally substituted C_{2-5}alkynyl, optionally substituted C_{2-4}alkynyl, optionally substituted C_{2-3}alkynyl, or optionally substituted C_2alkynyl. In certain embodiments wherein R^{13} is alkynyl, the alkynyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^{13} is alkynyl, the alkynyl group is unsubstituted.

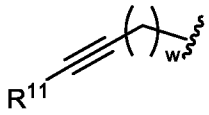
[00177] In certain embodiments, R^{13} is optionally substituted carbocyclyl (*e.g.,* C_3carbocyclyl, C_4carbocyclyl, C_5carbocyclyl, or C_6carbocyclyl,) or optionally substituted heterocyclyl (*e.g.,* a 3- to 6-membered heterocyclyl).

[00178] In certain embodiments, R^{13} is optionally substituted aryl (*e.g.,* phenyl) or optionally substituted heteroaryl (*e.g.,* 5- to 6- membered heteroaryl).

[00179] In certain embodiments, the alkenyl group of formula (b) is:



[00180] In certain embodiments of Formula (**II**), R^{Xa} is an optionally alkynyl group of formula (c):



(c)

wherein:

w is 1, 2, 3, or 4; and

R^{11} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl.

[00181] In certain embodiments, w is 1. In certain embodiments, w is 2. In certain embodiments, w is 3. In certain embodiments, w is 4.

[00182] In certain embodiments R^{11} is hydrogen.

[00183] In certain embodiments, R^{11} is optionally substituted alkyl, *e.g.,* optionally substituted C_{1-6}alkyl, optionally substituted C_{1-5}alkyl, optionally substituted C_{1-4}alkyl, optionally substituted C_{1-3}alkyl, optionally substituted C_{1-2}alkyl, optionally substituted C_1alkyl, optionally substituted C_2alkyl, optionally substituted C_3alkyl, optionally substituted C_4alkyl, optionally substituted C_5alkyl, or optionally substituted C_6alkyl. In certain embodiments wherein R^{11} is alkyl, the alkyl group is further substituted, *e.g.,* with halogen groups, or branched (*e.g.,* with $-CH_3$ groups). However, in certain embodiments, wherein R^{11} is alkyl, the alkyl group is unsubstituted. In certain embodiments, wherein R^{11} is alkyl, the alkyl group is branched.

[00184] In certain embodiments, R^{11} is optionally substituted alkenyl, *e.g.,* optionally substituted C_{2-6}alkenyl, optionally substituted C_{2-5}alkenyl, optionally substituted C_{2-4}alkenyl, optionally substituted C_{2-3}alkenyl, or optionally substituted C_2alkenyl. In certain embodiments wherein R^{11} is alkenyl, the alkenyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^{11} is alkenyl, the alkenyl group is unsubstituted.

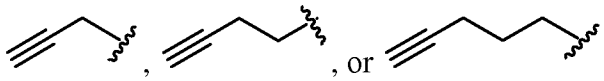
[00185] In certain embodiments, R^{11} is optionally substituted alkynyl, *e.g.,* optionally substituted C_{2-6}alkynyl, optionally substituted C_{2-5}alkynyl, optionally substituted C_{2-4}alkynyl, optionally substituted C_{2-3}alkynyl, or optionally substituted C_2alkynyl. In certain embodiments wherein R^{11} is alkynyl, the alkynyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^{11} is alkynyl, the alkynyl group is unsubstituted.

[00186] In certain embodiments, R^{11} is optionally substituted carbocyclyl (*e.g.*, C_3carbocyclyl, C_4carbocyclyl, C_5carbocyclyl, or C_6carbocyclyl,) or optionally substituted heterocyclyl (*e.g.*, a 3- to 6-membered heterocyclyl).

[00187] In certain embodiments, R^{11} is optionally substituted aryl (*e.g.*, phenyl) or optionally substituted heteroaryl (*e.g.*, 5- to 6- membered heteroaryl).

[00188] In certain embodiments, R^{11} is hydrogen, and w is 1, 2, or 3.

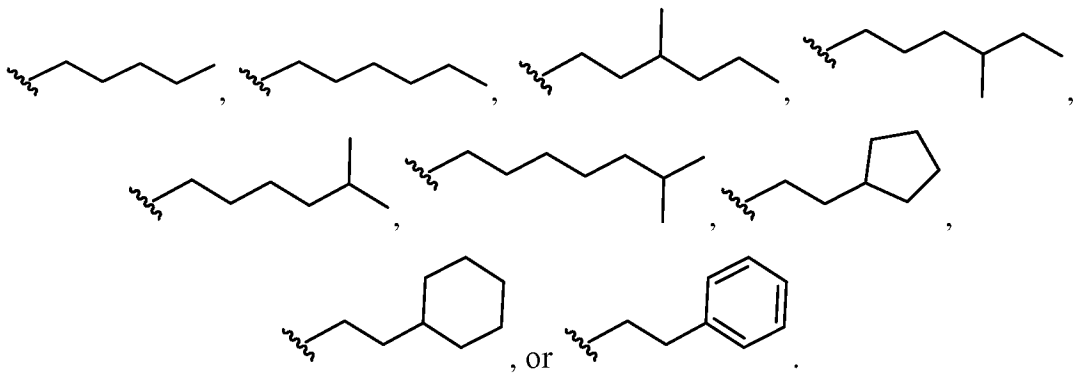
[00189] In certain embodiments, the alkynyl group of formula (c) is:



[00190] As generally described herein, R^5 is optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, $-OR^{5A}$ or $-NHR^{5A}$; wherein each instance of R^{5A} is independently optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl.

[00191] In certain embodiments, R^5 is optionally substituted alkyl. In certain embodiments, R^5 is unsubstituted alkyl. In certain embodiments, R^5 is substituted alkyl. In certain embodiments, R^5 is optionally substituted C_{1-30}alkyl. In certain embodiments, R^5 is optionally substituted C_{1-25}alkyl. In certain embodiments, R^5 is optionally substituted C_{1-15}alkyl. In certain embodiments, R^5 is optionally substituted C_{1-10}alkyl. In certain embodiments, R^5 is optionally substituted C_{2-10}alkyl. In certain embodiments, R^5 is optionally substituted C_{3-8}alkyl. In certain embodiments, R^5 is optionally substituted C_{5-15}alkyl.

[00192] In certain embodiments, R^5 is optionally substituted alkyl of formula:



[00193] In certain embodiments, R^5 is optionally substituted alkenyl. In certain embodiments, R^5 is an unsubstituted alkenyl. In certain embodiments, R^5 is a substituted

alkenyl. In certain embodiments, R^5 is optionally substituted C_{2-30} alkenyl. In certain embodiments, R^5 is optionally substituted C_{2-25} alkenyl. In certain embodiments, R^5 is optionally substituted C_{2-15} alkenyl. In certain embodiments, R^5 is optionally substituted C_{2-10} alkenyl. In certain embodiments, R^5 is optionally substituted C_{3-8} alkenyl. In certain embodiments, R^5 is optionally substituted C_{5-15} alkenyl. In certain embodiments, R^5 is optionally substituted C_5, C_6, C_7, C_8, C_9, or C_{10} alkenyl. In certain embodiments, R^5 is optionally substituted C_6 alkenyl. In certain embodiments, R^5 is unsubstituted C_6 alkenyl. In certain embodiments, R^5 is optionally substituted C_7 alkenyl. In certain embodiments, R^5 is unsubstituted C_7 alkenyl. In certain embodiments, R^5 is optionally substituted C_8 alkenyl. In certain embodiments, R^5 is unsubstituted C_8 alkenyl.

[00194] In certain embodiments, R^5 is a C_{7-30}alkyl or C_{7-30}alkenyl. In these instances, in certain embodiments, these groups may also collectively be referred to as "hydrocarbon tails." Hydrocarbon tails can be saturated and unsaturated, depending on whether or not the hydrocarbon tail comprises double bonds. The hydrocarbon tail can also comprise different lengths, often categorized as medium (*i.e.*, with tails between 7-12 carbons, *e.g.*, C_{7-12} alkyl or C_{7-12} alkenyl), long (*i.e.*, with tails greater than 12 carbons and up to 22 carbons, *e.g.*, C_{13-22} alkyl or C_{13-22} alkenyl), or very long (*i.e.*, with tails greater than 22 carbons, *e.g.*, C_{23-30} alkyl or C_{23-30} alkenyl).

[00195] Exemplary unsaturated hydrocarbon tails include, but are not limited to:

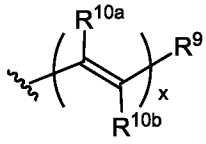
Myristoleic	$-(CH_2)_7CH=CH(CH_2)_3CH_3$,
Palmitoliec	$-(CH_2)_7CH=CH(CH_2)_5CH_3$,
Sapienic	$-(CH_2)_4CH=CH(CH_2)_8CH_3$,
Oleic	$-(CH_2)_7CH=CH(CH_2)_7CH_3$,
Linoleic	$-(CH_2)_7CH=CHCH_2CH=CH(CH_2)_4CH_3$,
α-Linolenic	$-(CH_2)_7CH=CHCH_2CH=CHCH_2CH=CHCH_2CH_3$,
Arachinodonic	$-(CH_2)_3CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_4CH_3$,
Eicosapentaenoic	$-(CH_2)_3CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH_3$,
Erucic	$-(CH_2)_{11}CH=CH(CH_2)_7CH_3$, and
Docosahexaenoic	$-(CH_2)_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH\text{-}CH_2CH_3$.

[00196] Exemplary saturated hydrocarbon tails include, but are not limited to:

Lauric	$-(CH_2)_{10}CH_3$,
Myristic	$-(CH_2)_{12}CH_3$,
Palmitic	$-(CH_2)_{14}CH_3$,

Stearic	$-(CH_2)_{16}CH_3$,
Arachidic	$-(CH_2)_{18}CH_3$,
Behenic	$-(CH_2)_{20}CH_3$,
Lignoceric	$-(CH_2)_{22}CH_3$, and
Cerotic	$-(CH_2)_{24}CH_3$.

[00197] In certain embodiments, R^5 is an optionally substituted alkenyl group of formula (a1):



(a1)

wherein:

x is an integer between 1 and 10, inclusive;

R^{10a} and R^{10b} are independently hydrogen or optionally substituted alkyl; and

R^9 is hydrogen, halogen, –OH, –SH, $–NH_2$, -CN, $-NO_2$, $-N_3$, $–SO_2H$, $–SO_3H$, substituted hydroxyl, substituted thiol, substituted amino, sulfonyl, sulfinyl, carbonyl, silyl, boronyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl.

[00198] In certain embodiments, x is an integer between 1 and 8, inclusive. In certain embodiments, x is an integer between 1 and 6, inclusive. In certain embodiments, x is an integer between 1 and 5, inclusive. In certain embodiments, x is an integer between 1 and 4, inclusive. In certain embodiments, x is an integer between 1 and 3, inclusive. In certain embodiments, x is an integer between 1 and 2, inclusive. In certain embodiments, x is 1. In certain embodiments, x is 2. In certain embodiments, x is 3. In certain embodiments, x is 4. In certain embodiments, x is 5.

[00199] In certain embodiments, R^{10a} and R^{10b} are both hydrogen. In certain embodiments, R^{10a} and R^{10b} are independently selected from hydrogen and $–CH_3$.

[00200] In certain embodiments, R^9 is optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl.

[00201] In certain embodiments, R^9 is optionally substituted alkyl, *e.g.,* optionally substituted C_{1-6}alkyl, optionally substituted C_{1-5}alkyl, optionally substituted C_{1-4}alkyl, optionally substituted C_{1-3}alkyl, optionally substituted C_{1-2}alkyl, optionally substituted C_1alkyl, optionally substituted C_2alkyl, optionally substituted C_3alkyl, optionally substituted

C_4alkyl, optionally substituted C_5alkyl, or optionally substituted C_6alkyl. In certain embodiments wherein R^9 is alkyl, the alkyl group is further substituted, *e.g.,* with aryl or halogen groups, or branched (*e.g.,* with $-CH_3$ groups). However, in certain embodiments, wherein R^9 is alkyl, the alkyl group is unsubstituted. In certain embodiments, wherein R^9 is alkyl, the alkyl group is branched.

[00202] In certain embodiments, R^9 is optionally substituted alkenyl, *e.g.,* optionally substituted C_{2-6}alkenyl, optionally substituted C_{2-5}alkenyl, optionally substituted C_{2-4}alkenyl, optionally substituted C_{2-3}alkenyl, or optionally substituted C_2alkenyl. In certain embodiments wherein R^9 is alkenyl, the alkenyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^9 is alkenyl, the alkenyl group is unsubstituted.

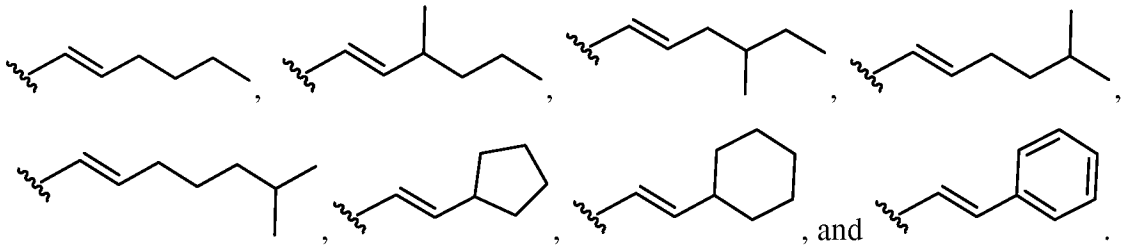
[00203] In certain embodiments, R^9 is optionally substituted alkynyl, *e.g.,* optionally substituted C_{2-6}alkynyl, optionally substituted C_{2-5}alkynyl, optionally substituted C_{2-4}alkynyl, optionally substituted C_{2-3}alkynyl, or optionally substituted C_2alkynyl. In certain embodiments wherein R^9 is alkynyl, the alkynyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^9 is alkynyl, the alkynyl group is unsubstituted.

[00204] In certain embodiments, R^9 is optionally substituted carbocyclyl (*e.g.,* C_3carbocyclyl, C_4carbocyclyl, C_5carbocyclyl, or C_6carbocyclyl,) or optionally substituted heterocyclyl (*e.g.,* a 3- to 6-membered heterocyclyl).

[00205] In certain embodiments, R^9 is optionally substituted aryl (*e.g.,* phenyl) or optionally substituted heteroaryl (*e.g.,* 5- to 6- membered heteroaryl).

[00206] In certain embodiments, R^5 is an optionally substituted alkenyl group of formula (a1) wherein x is 1, R^{10a} and R^{10b} are each hydrogen, and R^9 is optionally substituted C_{4-6}alkyl or optionally substituted C_{3-6}carbocyclyl, as described herein.

[00207] In certain embodiments, the optionally substituted alkenyl group of formula (a1) is selected from the group consisting of:



[00208] In certain embodiments, R^5 is optionally substituted alkynyl. In certain embodiments, R^5 is an unsubstituted alkynyl. In certain embodiments, R^5 is a substituted

alkynyl. In certain embodiments, R^5 is optionally substituted C_{2-30} alkynyl. In certain embodiments, R^5 is optionally substituted C_{2-25} alkynyl. In certain embodiments, R^5 is optionally substituted C_{2-15} alkynyl. In certain embodiments, R^5 is optionally substituted C_{2-10} alkynyl. In certain embodiments, R^5 is optionally substituted C_{3-8} alkynyl. In certain embodiments, R^5 is optionally substituted C_{5-15} alkynyl.

[00209] In certain embodiments, R^5 is optionally substituted carbocyclyl (*e.g.*, C_3carbocyclyl, C_4carbocyclyl, C_5carbocyclyl, or C_6carbocyclyl,) or optionally substituted heterocyclyl (*e.g.*, a 3- to 6-membered heterocyclyl).

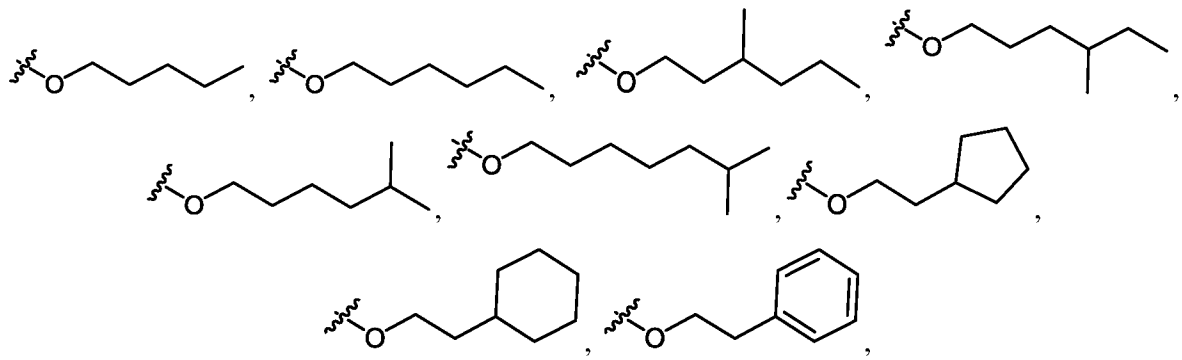
[00210] In certain embodiments, R^5 is optionally substituted aryl (*e.g.*, phenyl) or optionally substituted heteroaryl (*e.g.*, 5- to 6- membered heteroaryl).

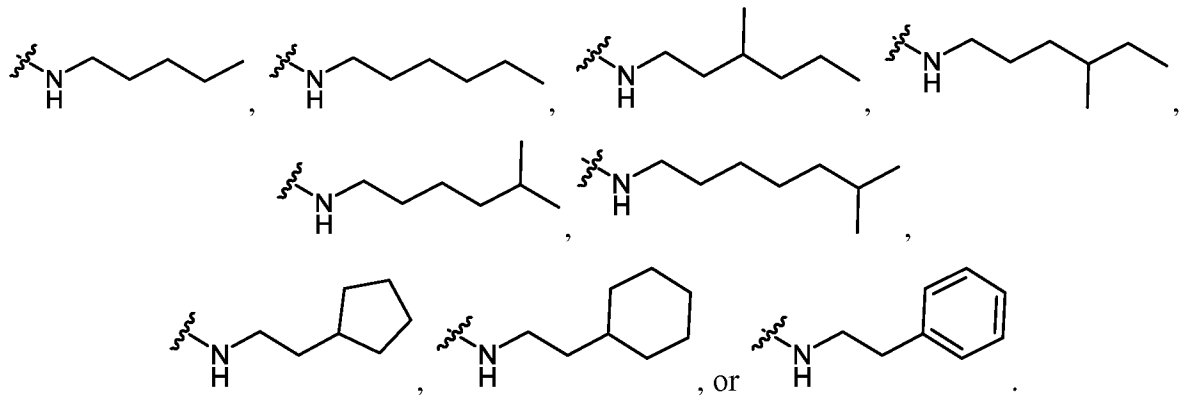
[00211] In certain embodiments, R^5 is $-OR^{5A}$ or $-NHR^{5A}$; wherein R^{5A} is optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl.

[00212] In certain embodiments, R^5 is $-OR^{5A}$. In certain embodiments, R^5 is$-NHR^{5A}$.

[00213] In certain embodiments, R^5 is $-OR^{5A}$ or $-NHR^{5A}$, and R^{5A} is optionally substituted alkyl. In certain embodiments, R^{5A} is unsubstituted alkyl. In certain embodiments, R^{5A} is substituted alkyl. In certain embodiments, R^{5A} is optionally substituted C_{1-30}alkyl. In certain embodiments, R^{5A} is optionally substituted C_{1-25}alkyl. In certain embodiments, R^{5A} is optionally substituted C_{1-15}alkyl. In certain embodiments, R^{5A} is optionally substituted C_{1-10}alkyl. In certain embodiments, R^{5A} is optionally substituted C_{2-10}alkyl. In certain embodiments, R^{5A} is optionally substituted C_{3-8}alkyl. In certain embodiments, R^{5A} is optionally substituted C_{5-15}alkyl.

[00214] In certain embodiments, wherein R^5 is $-OR^{5A}$ or $-NHR^{5A}$ and R^{5A} is an optionally substituted alkyl, provided is a group of formula:





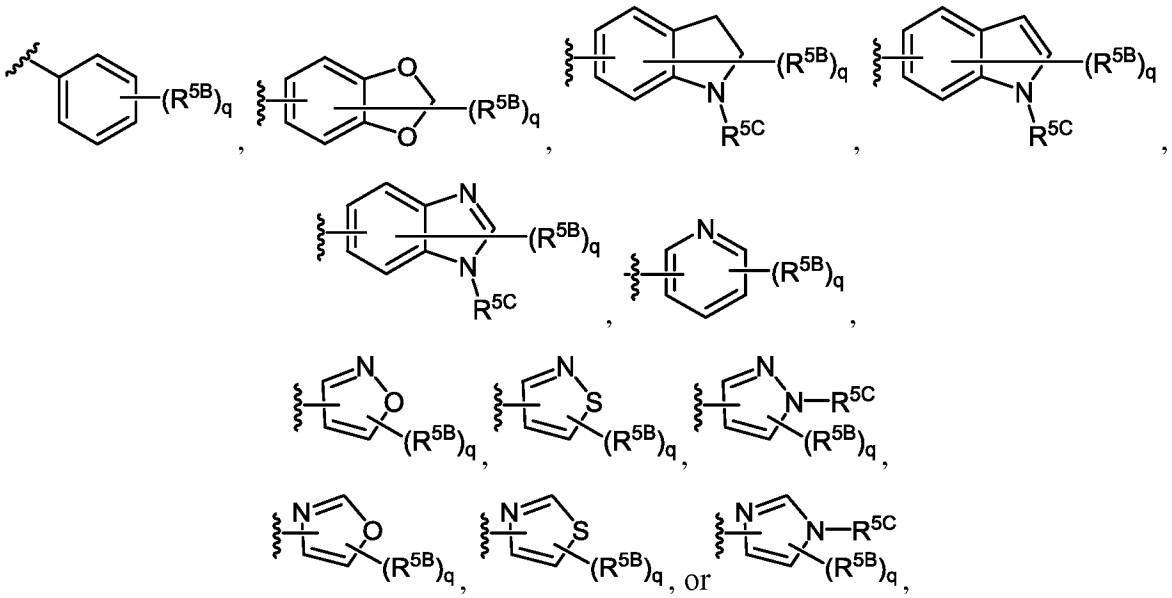
[00215] In certain embodiments, R^5 is $-OR^{5A}$ or $-NHR^{5A}$, and R^{5A} is optionally substituted alkenyl. In certain embodiments, R^{5A} is an unsubstituted alkenyl. In certain embodiments, R^{5A} is a substituted alkenyl. In certain embodiments, R^{5A} is optionally substituted C_{2-30} alkenyl. In certain embodiments, R^{5A} is optionally substituted C_{2-25} alkenyl. In certain embodiments, R^{5A} is optionally substituted C_{2-15} alkenyl. In certain embodiments, R^{5A} is optionally substituted C_{2-10} alkenyl. In certain embodiments, R^{5A} is optionally substituted C_{3-8} alkenyl. In certain embodiments, R^{5A} is optionally substituted C_{5-15} alkenyl. In certain embodiments, R^{5A} is optionally substituted C_5, C_6, C_7, C_8, C_9, or C_{10} alkenyl. In certain embodiments, R^{5A} is optionally substituted C_6 alkenyl. In certain embodiments, R^{5A} is unsubstituted C_6 alkenyl. In certain embodiments, R^{5A} is optionally substituted C_7 alkenyl. In certain embodiments, R^5 is unsubstituted C_7 alkenyl. In certain embodiments, R^{5A} is optionally substituted C_8 alkenyl. In certain embodiments, R^{5A} is unsubstituted C_8 alkenyl.

[00216] In certain embodiments, R^{5A} is optionally substituted alkynyl. In certain embodiments, R^{5A} is an unsubstituted alkynyl. In certain embodiments, R^5 is a substituted alkynyl. In certain embodiments, R^{5A} is optionally substituted C_{2-30} alkynyl. In certain embodiments, R^{5A} is optionally substituted C_{2-25} alkynyl. In certain embodiments, R^{5A} is optionally substituted C_{2-15} alkynyl. In certain embodiments, R^{5A} is optionally substituted C_{2-10} alkynyl. In certain embodiments, R^{5A} is optionally substituted C_{3-8} alkynyl. In certain embodiments, R^{5A} is optionally substituted C_{5-15} alkynyl.

[00217] In certain embodiments, R^{5A} is optionally substituted carbocyclyl (*e.g.*, C_3carbocyclyl, C_4carbocyclyl, C_5carbocyclyl, or C_6carbocyclyl,) or optionally substituted heterocyclyl (*e.g.*, a 3- to 6-membered heterocyclyl).

[00218] In certain embodiments, R^{5A} is optionally substituted aryl (*e.g.*, phenyl) or optionally substituted heteroaryl (*e.g.*, 5- to 6- membered heteroaryl).

[00219] For example, in certain embodiments, R^{5A} is an optionally substituted aryl or optionally substituted heteroaryl group of formulae:



wherein:

q is 0, 1, or 2;

each instance of R^{5B} is independently selected from the group consisting of halogen, C_{1-4}alkyl, C_{1-4}haloalkyl, $–OR^{5D}$, or $-NHR^{5D}$, wherein R^{5D} is C_{1-4}alkyl or C_{1-4}haloalkyl; and

R^{5C} is hydrogen or C_{1-4}alkyl.

[00220] In certain embodiments, q is 0. In certain embodiments, q is 1. In certain embodiments, q is 2.

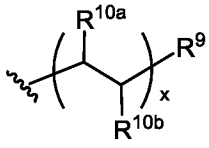
[00221] In certain embodiments, q is 1 or 2, and at least one instance of R^{5B} is halogen, *e.g.*, at least one instance of R^{5B} is -Cl.

[00222] In certain embodiments, q is 1 or 2, and at least one instance of R^{5B} is C_{1-4}alkyl or C_{1-4}haloalkyl, *e.g.*, at least one instance of R^{5B} is $–CH_3$, $-CH_2CH_3$, $-CH(CH_3)_2$, $-C(CH_3)_3$, or $–CF_3$.

[00223] In certain embodiments, q is 1 or 2, and at least one instance of R^{5B} is $–OR^{5D}$ wherein R^{5D} is C_{1-4}alkyl or C_{1-4}haloalkyl, *e.g.*, *e.g.*, at least one instance of R^{5B} is $–OCH_3$, $-OCH_2CH_3$, $-OCH(CH_3)_2$, $-OC(CH_3)_3$, or $–OCF_3$.

[00224] In certain embodiments, q is 1 or 2, and at least one instance of R^{5B} is $–NHR^{5D}$ wherein R^{5D} is C_{1-4}alkyl or C_{1-4}haloalkyl, *e.g.*, *e.g.*, at least one instance of R^{5B} is $–NHCH_3$, $-NHCH_2CH_3$, $-NHCH(CH_3)_2$, $-NHC(CH_3)_3$, or $–NHCF_3$.

[00225] In certain embodiments, R^5 is $–OR^{5A}$ or $–NHR^{5A}$ and R^{5A} is an optionally substituted group of formula (a2):



(a2)

wherein:

x is 0 or an integer between 1 and 10, inclusive;

R^{10a} and R^{10b} are independently hydrogen or optionally substituted alkyl; and

R^9 is hydrogen, halogen, –OH, –SH, $–NH_2$, -CN, $-NO_2$, $-N_3$, $–SO_2H$, $–SO_3H$, substituted hydroxyl, substituted thiol, substituted amino, sulfonyl, sulfinyl, carbonyl, silyl, boronyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl.

[00226] In certain embodiments, x is 0.

[00227] In certain embodiments, x is an integer between 1 and 8, inclusive. In certain embodiments, x is an integer between 1 and 6, inclusive. In certain embodiments, x is an integer between 1 and 5, inclusive. In certain embodiments, x is an integer between 1 and 4, inclusive. In certain embodiments, x is an integer between 1 and 3, inclusive. In certain embodiments, x is an integer between 1 and 2, inclusive. In certain embodiments, x is 1. In certain embodiments, x is 2. In certain embodiments, x is 3. In certain embodiments, x is 4. In certain embodiments, x is 5.

[00228] In certain embodiments, R^{10a} and R^{10b} are both hydrogen. In certain embodiments, R^{10a} and R^{10b} are independently selected from hydrogen and $–CH_3$.

[00229] In certain embodiments, R^9 is optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl.

[00230] In certain embodiments, R^9 is optionally substituted alkyl, *e.g.,* optionally substituted C_{1-6}alkyl, optionally substituted C_{1-5}alkyl, optionally substituted C_{1-4}alkyl, optionally substituted C_{1-3}alkyl, optionally substituted C_{1-2}alkyl, optionally substituted C_1alkyl, optionally substituted C_2alkyl, optionally substituted C_3alkyl, optionally substituted C_4alkyl, optionally substituted C_5alkyl, or optionally substituted C_6alkyl. In certain embodiments wherein R^9 is alkyl, the alkyl group is further substituted, *e.g.,* with aryl or halogen groups, or branched (*e.g.,* with $–CH_3$ groups). However, in certain embodiments, wherein R^9 is alkyl, the alkyl group is unsubstituted. In certain embodiments, wherein R^9 is alkyl, the alkyl group is branched.

[00231] In certain embodiments, R^9 is optionally substituted alkenyl, *e.g.,* optionally substituted C_{2-6}alkenyl, optionally substituted C_{2-5}alkenyl, optionally substituted C_{2-4}alkenyl, optionally substituted C_{2-3}alkenyl, or optionally substituted C_2alkenyl. In certain embodiments wherein R^9 is alkenyl, the alkenyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^9 is alkenyl, the alkenyl group is unsubstituted.

[00232] In certain embodiments, R^9 is optionally substituted alkynyl, *e.g.,* optionally substituted C_{2-6}alkynyl, optionally substituted C_{2-5}alkynyl, optionally substituted C_{2-4}alkynyl, optionally substituted C_{2-3}alkynyl, or optionally substituted C_2alkynyl. In certain embodiments wherein R^9 is alkynyl, the alkynyl group is further substituted, *e.g.,* with halogen groups. However, in certain embodiments, wherein R^9 is alkynyl, the alkynyl group is unsubstituted.

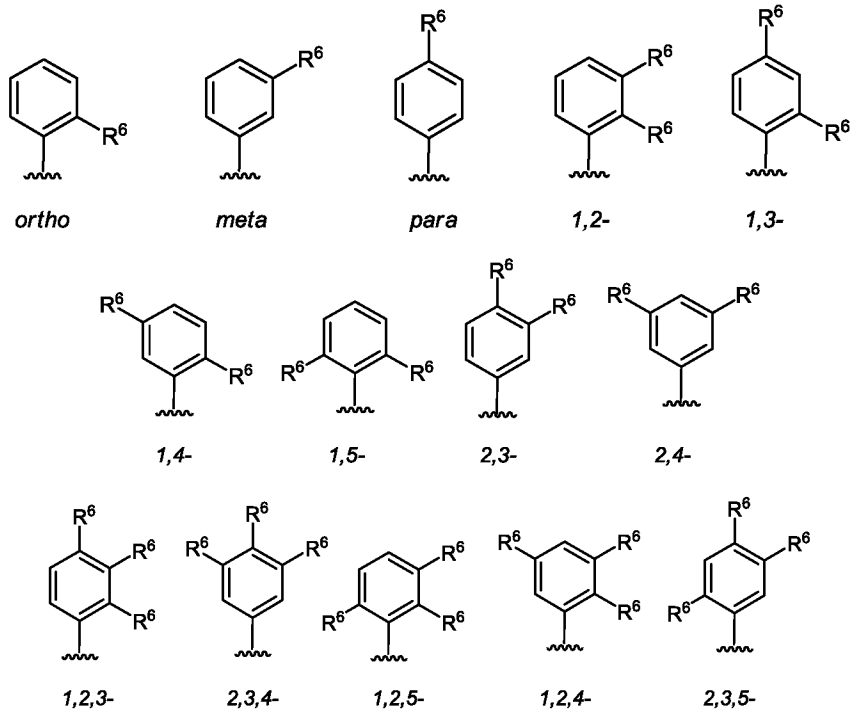
[00233] In certain embodiments, R^9 is optionally substituted carbocyclyl (*e.g.,* C_3carbocyclyl, C_4carbocyclyl, C_5carbocyclyl, or C_6carbocyclyl,) or optionally substituted heterocyclyl (*e.g.,* a 3- to 6-membered heterocyclyl).

[00234] In certain embodiments, R^9 is optionally substituted aryl (*e.g.,* phenyl) or optionally substituted heteroaryl (*e.g.,* 5- to 6- membered heteroaryl).

[00235] As generally described herein, each instance of R^6 is independently selected from the group consisting of halogen, –OH, –SH, $–NH_2$, -CN, $-NO_2$, $-N_3$, $–SO_2H$, $–SO_3H$, substituted hydroxyl, substituted thiol, substituted amino, sulfonyl, sulfinyl, carbonyl, silyl, boronyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, and optionally substituted heteroaryl; and m is 0 or an integer of between 1 and 5, inclusive.

[00236] In certain embodiments, m is 0. In certain embodiments, m is an integer of between 1 and 5, inclusive. In certain embodiments, m is an integer of between 1 and 4, inclusive. In certain embodiments, m is an integer of between 1 and 3, inclusive. In certain embodiments, m is an integer of between 1 and 2, inclusive. In certain embodiments, m is 1, *i.e.*, to provide a monosubstituted phenyl. In certain embodiments, m is 2, *i.e.*, to provide a disubstituted phenyl. In certain embodiments, m is 3, *i.e.*, to provide a trisubstituted phenyl. In certain embodiments, m is 4, *i.e.*, to provide a tetrasubstituted phenyl. In certain embodiments, m is 5, *i.e.*, to provide a pentasubstituted phenyl.

[00237] In certain preferred embodiments, m is 1, 2, or 3 to provide a monosubstituted, disubstituted, or trisubstituted phenyl. In this instance, all possible positional mono-, di-, and tri-substituted isomers are contemplated herein, *i.e.*:



[00238] For example, in certain embodiments, m is 1, *i.e.*, to provide a monosubstituted phenyl. In this instance, R^6 may be an *ortho*-, *meta*- or *para*-substituent relative to the point of attachment to the parent molecule. In certain embodiments, R^6 is an *ortho*-substituent. In certain embodiments, R^6 is a *meta*-substituent. In certain embodiments, R^6 is a *para*-substituent.

[00239] In certain embodiments, m is 2, *i.e.*, to provide a disubstituted phenyl. In this instance, the two R^6 substituents are 1,2-substituents, 1,3-substituents, 1,4-substituents, 1,5-substituents, 2,3-substituents, or 2,4-substituents, relative to the point of attachment to the parent molecule. In certain embodiments, the two R^6 substituents are1,2-substituents. In certain embodiments, the two R^6 substituents are 1,3-substituents. In certain embodiments, the two R^6 substituents are 1,4-substituents. In certain embodiments, the two R^6 substituents are 1,5-substituents. In certain embodiments, the two R^6 substituents are 2,3-substituents. In certain embodiments, the two R^6 substituents are 2,4-substituents.

[00240] In certain embodiments, m is 3, *i.e.*, to provide a trisubstituted phenyl. In this instance, the three R^6 substituents are 1,2,3-substituents, 2,3,4-substituents, 1,2,5-substituents, 1,2,4-substituents, 2,3,5-substituents, relative to the point of attachment to the parent molecule. In certain embodiments, the three R^6 substituents are 2,3,4-substituents.

[00241] In certain embodiments, m is 1, 2, or 3, optionally wherein at least one instance of R^6 is halogen (*e.g.*, fluoro (-F), chloro (-Cl), bromo (-Br), or iodo (-I)), –OH, –SH, $–NH_2$, $-NO_2$, carbonyl (*e.g.,* $-CO_2H$) or optionally substituted alkyl (*e.g.,* $-CH_3$). In certain embodiments, at least one instance of R^6 is halogen, –OH, –SH, or $–NH_2$. In certain embodiments, m is 1, 2, or 3 and at least one instance of R^6 is halogen, *e.g.,* fluoro. In certain embodiments, m is 1 and R^6 is fluoro. In certain embodiments, m is 1 and R^6 is a *meta* fluoro substituent. In certain embodiments, m is 2 and each R^6 is fluoro. In certain embodiments, m is 2 and the two R^6 groups are 2,4-fluoro substituents.

[00242] Various combinations of the above described embodiments are further described herein.

[00243] For example, in certain embodiments, a compound of Formula (**I**) and (**II**) or pharmaceutically acceptable salt thereto, may comprise a combination of two ore more of the below recited embodiments, *e.g.,* wherein:

a. R^1 is hydrogen or optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $–CH(CH_3)_2$;

b. R^2 is hydrogen, $-C(=O)R^{2A}$, $-C(=O)OR^{2A}$, $-C(=O)NHR^{2A}$, or $-C(=O)N(R^{2A})_2$, wherein at least one instance of R^{2A} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $–CH(CH_3)_2$;

c. R^3 is $-C(=O)R^{3A}$, $-C(=O)OR^{3A}$, $-C(=O)NHR^{3A}$, or $-C(=O)N(R^{3A})_2$,wherein at least one instance of R^{3A} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $–CH(CH_3)_2$, or R^3 is substituted C_{1-3}alkyl, wherein the alkyl group is further substituted with hydroxyl or substituted hydroxyl;

d. R^4 is hydrogen or optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $–CH(CH_3)_2$;

e. Y is O or NR^Y wherein R^Y is hydrogen or $–CH_3$;

f. for Formula (**I**), X is O or NR^{Xb}, wherein R^{Xb} is hydrogen or optionally substituted alkyl (*e.g.,* $-CH_3$);

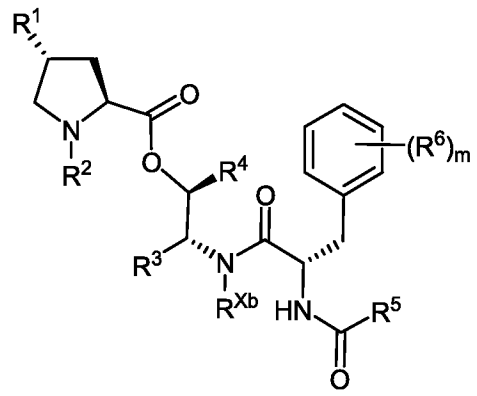
g. for Formula (**II**), X is O or or NR^{Xb}, wherein R^{Xb} is hydrogen or optionally substituted alkyl (*e.g.,* $-CH_3$) and R^{Xa} is hydrogen, optionally substituted alkyl (*e.g.,* $-CH_3$, or aralkyl such as benzyl), optionally substituted alkenyl (*e.g.,* of formula (b)), or optionally substituted alkynyl (*e.g.,* of formula (c));

h. R^5 is an optionally substituted alkenyl group of formula (a1), wherein x is 1, R^{10a} and R^{10b} are each hydrogen, and R^9 is optionally substituted C_{4-6}alkyl or optionally substituted C_{3-6}carbocyclyl;

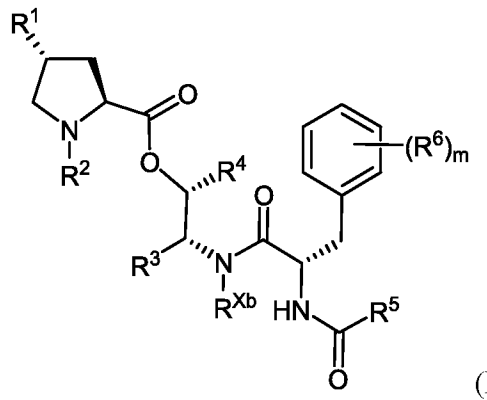
i. R^5 is $-OR^{5A}$ or $-NHR^{5A}$, wherein R^{5A} is optionally substituted alkyl, optionally substituted aryl, optionally substituted heteroaryl, or a group of formula (a2); and/or

j. m is 1, 2, or 3, optionally wherein at least one instance of R^6 is halogen (*e.g.*, fluoro (-F), chloro (-Cl), bromo (-Br), or iodo (-I)), –OH, –SH, $–NH_2$, $-NO_2$, carbonyl (*e.g.*, $-CO_2H$) or optionally substituted alkyl (*e.g.*, $-CH_3$); *e.g.*, for example, wherein m is 2 and the two R^6 groups are 2,4-fluoro substituents.

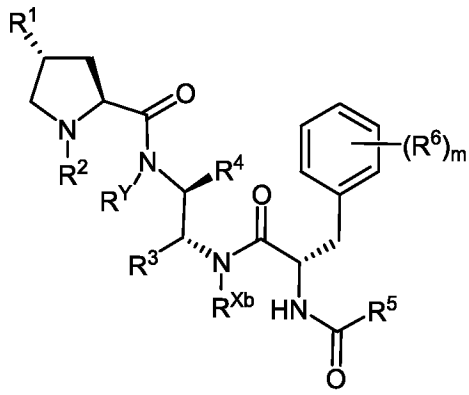
[00244] In certain embodiments of Formula (**I**), wherein Y is O or NR^Y, X is NR^{Xb} and R^4 (if a non-hydrogen group) has the following stereochemistry, provided is a compound of Formula:



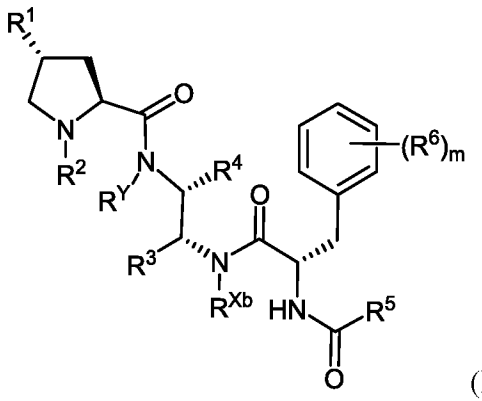
(**I-a1**)



(**I-b1**)



(**I-a2**)

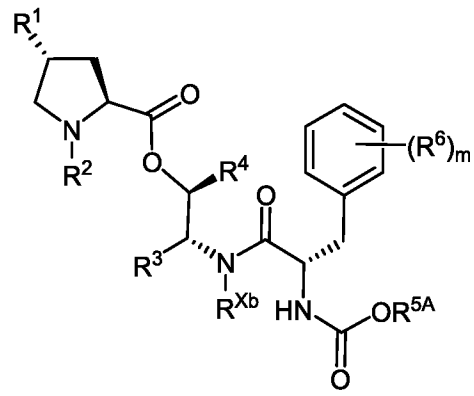


(**I-b2**)

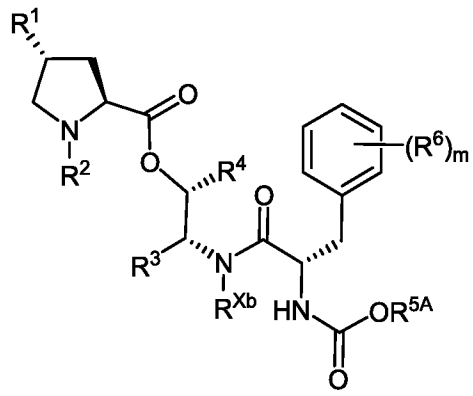
or a pharmaceutically acceptable salt thereof. In certain embodiments, R^1 is hydrogen or optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $–CH(CH_3)_2$. In certain embodiments, R^2 is hydrogen, $-C(=O)R^{2A}$, $-C(=O)OR^{2A}$, $-C(=O)NHR^{2A}$, or $-C(=O)N(R^{2A})_2$, wherein at least one instance of R^{2A} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, -

CH_2CH_3, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$. In certain embodiments, R^3 is $-C(=O)R^{3A}$, $-C(=O)OR^{3A}$, $-C(=O)NHR^{3A}$, or $-C(=O)N(R^{3A})_2$,wherein at least one instance of R^{3A} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$, or R^3 is substituted C_{1-3}alkyl, wherein the alkyl group is further substituted with hydroxyl or substituted hydroxyl group. In certain embodiments, R^4 is hydrogen or optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$. In certain embodiments, R^5 is an optionally substituted alkenyl group of formula (a1) wherein x is 1, R^{10a} and R^{10b} are each hydrogen, and R^9 is optionally substituted C_{4-6}alkyl or optionally substituted C_{3-6}carbocyclyl. In certain embodiments, R^5 is $-OR^{5A}$ or $-NHR^{5A}$, wherein R^{5A} is optionally substituted alkyl, optionally substituted aryl, optionally substituted heteroaryl. In certain embodiments, R^{5A} is a group of formula (a2). In certain embodiments, m is 1, 2, or 3, optionally wherein at least one instance of R^6 is halogen (*e.g.*, fluoro (-F), chloro (-Cl), bromo (-Br), or iodo (-I)), $-OH$, $-SH$, $-NH_2$, $-NO_2$, carbonyl (*e.g.*, $-CO_2H$) or optionally substituted alkyl (*e.g.*, $-CH_3$). In certain embodiments, m is 2 and the two R^6 groups are 2,4-fluoro substituents. In certain embodiments, R^{Xb} is hydrogen or optionally substituted alkyl (*e.g.*, $-CH_3$).

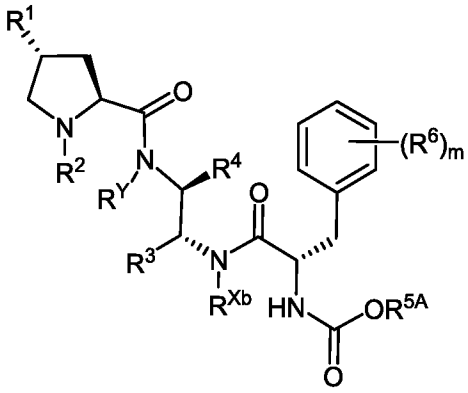
[00245] In certain embodiments of Formula (**I-a1**), (**I-a2**), (**I-b1**), and (**I-b2**), wherein R^5 is $-OR^{5A}$ or $-NHR^{5A}$, provided is a compound of Formula:



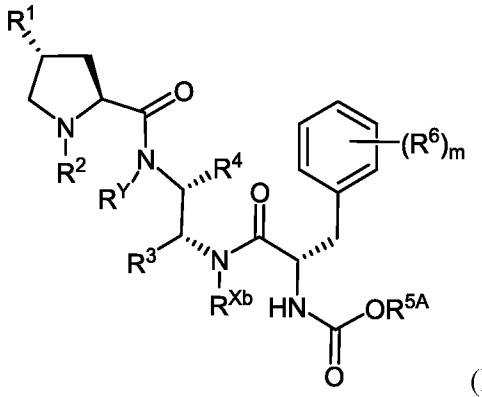
(**I-a1-a**)



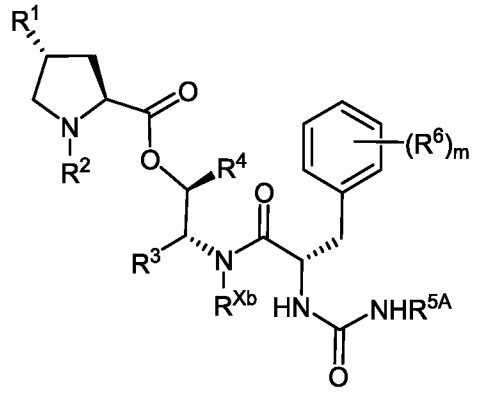
(**I-b1-a**)



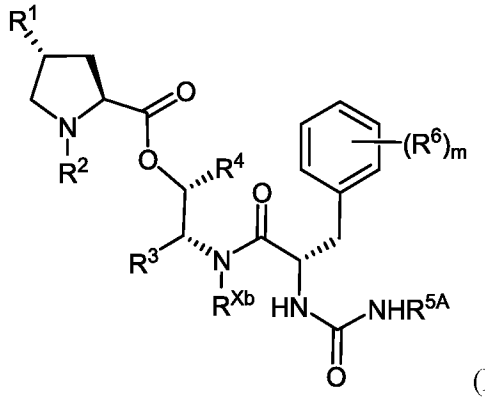
(**I-a2-a**)



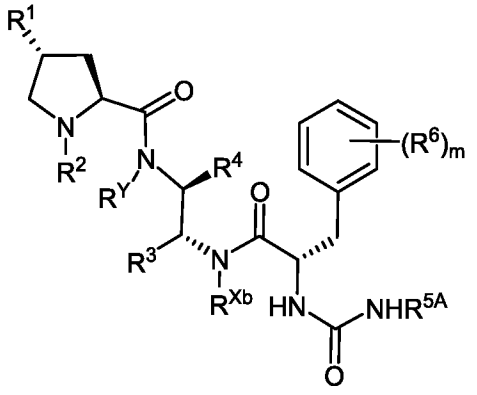
(**I-b2-a**)



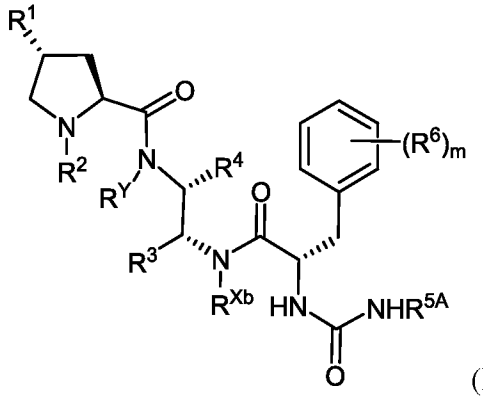
(I-a1-b)



(I-b1-b)



(I-a2-a)

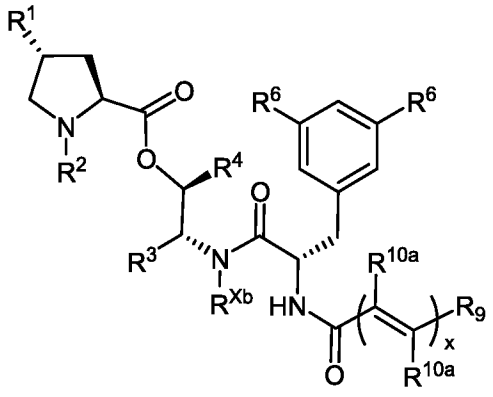


(I-b2-a)

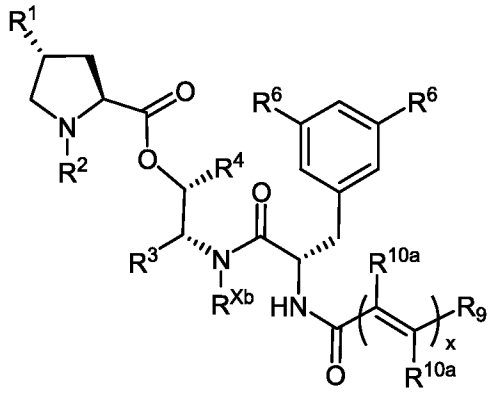
or a pharmaceutically acceptable salt thereof. In certain embodiments, R^1 is hydrogen or optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$. In certain embodiments, R^2 is hydrogen, $-C(=O)R^{2A}$, $-C(=O)OR^{2A}$, $-C(=O)NHR^{2A}$, or $-C(=O)N(R^{2A})_2$, wherein at least one instance of R^{2A} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$. In certain embodiments, R^3 is $-C(=O)R^{3A}$, $-C(=O)OR^{3A}$, $-C(=O)NHR^{3A}$, or $-C(=O)N(R^{3A})_2$,wherein at least one instance of R^{3A} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$, or R^3 is substituted C_{1-3}alkyl, wherein the alkyl group is further substituted with hydroxyl or substituted hydroxyl group. In certain embodiments, R^4 is hydrogen or optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$. In certain embodiments, R^{5A} is optionally substituted alkyl, optionally substituted aryl, or optionally substituted heteroaryl. In certain embodiments, R^{5A} is a group of formula (a2). In certain embodiments, m is 1, 2, or 3, optionally wherein at least one instance of R^6 is halogen (*e.g.*, fluoro (-F), chloro (-Cl), bromo (-Br), or iodo (-I)), –OH, –SH, $–NH_2$, $-NO_2$, carbonyl (*e.g.*, $-CO_2H$) or optionally substituted alkyl (*e.g.*, $-CH_3$). In certain embodiments, m is 2 and the two R^6 groups are 2,4-fluoro

substituents. In certain embodiments, R^{Xb} is hydrogen or optionally substituted alkyl (*e.g.*, -CH_3).

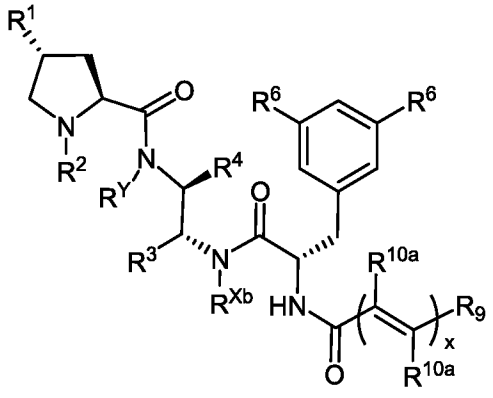
[00246] In certain embodiments of Formula (**I-a1**), (**I-a2**), (**I-b1**), and (**I-b2**), wherein R^5 is an optionally substituted alkenyl group of formula (a1), m is 2, and the two R^6 substituents are 2,4-substituents, provided is a compound of Formula:



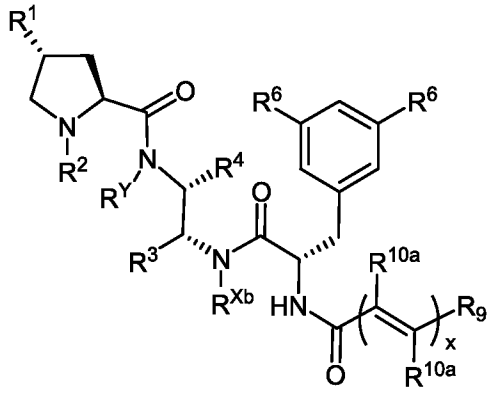
(**I-a1-1**)



(**I-b1-1**)



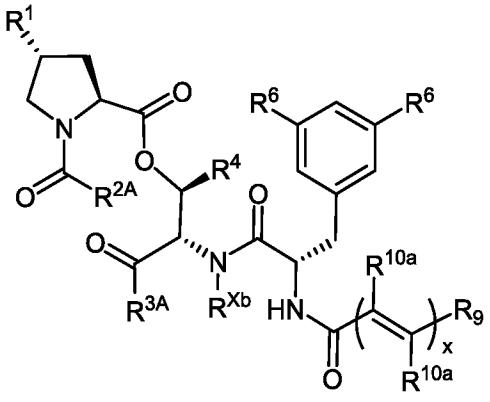
(**I-a2-1**)



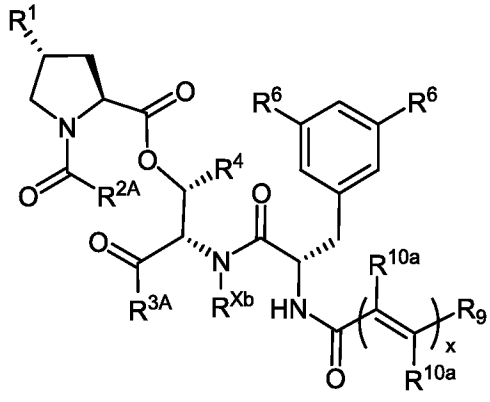
(**I-b2-1**)

or a pharmaceutically acceptable salt thereof. In certain embodiments, R^1 is hydrogen or optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$. In certain embodiments, R^2 is hydrogen, $-C(=O)R^{2A}$, $-C(=O)OR^{2A}$, $-C(=O)NHR^{2A}$, or $-C(=O)N(R^{2A})_2$, wherein at least one instance of R^{2A} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$. In certain embodiments, R^3 is $-C(=O)R^{3A}$, $-C(=O)OR^{3A}$, $-C(=O)NHR^{3A}$, or $-C(=O)N(R^{3A})_2$,wherein at least one instance of R^{3A} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$, or R^3 is substituted C_{1-3}alkyl, wherein the alkyl group is further substituted with hydroxyl or substituted hydroxyl group. In certain embodiments, R^4 is hydrogen or optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$. In certain embodiments, x is 1, R^{10a} and R^{10b} are each hydrogen, and R^9 is optionally substituted C_{4-6}alkyl or optionally substituted C_{3-6}carbocyclyl. In certain embodiments, at least one instance of R^6 is halogen (*e.g.*, fluoro (-F), chloro (-Cl), bromo (-Br), or iodo (-I)), –OH, –SH, $–NH_2$, $-NO_2$, carbonyl (*e.g.*, $-CO_2H$) or optionally substituted alkyl (*e.g.*, $-CH_3$). In certain embodiments, the two R^6 groups are 2,4-fluoro substituents. In certain embodiments, R^{Xb} is hydrogen or optionally substituted alkyl (*e.g.*, $-CH_3$).

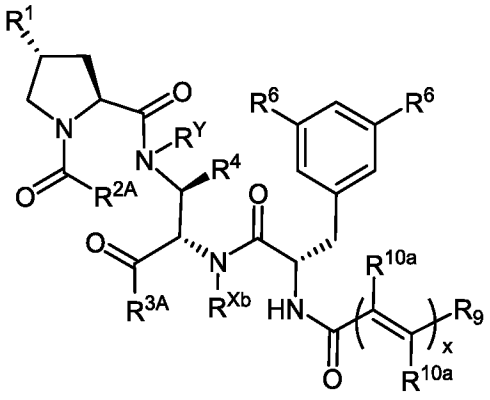
[00247] In certain embodiments of Formula (**I-a1-1**), (**I-a2-1**), (**I-b1-1**), and (**I-b2-1**), wherein R^2 is $–C(=O)R^{2A}$ and R^3 is $–C(=O)R^{3A}$, provided is a compound of Formula:



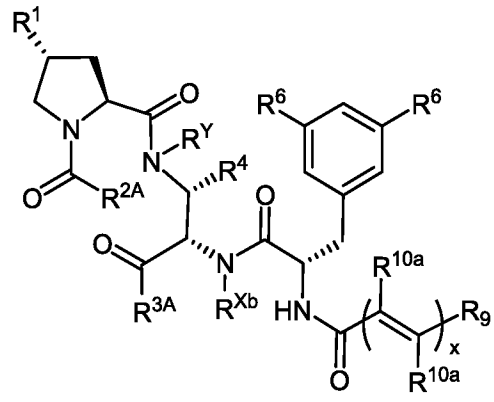
(I-a1-2)



(I-b1-2)



(I-a2-2)

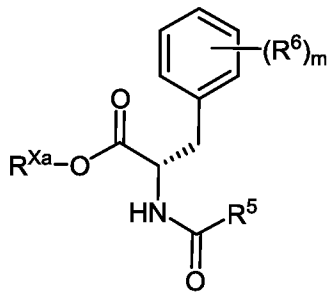


(I-b2-2)

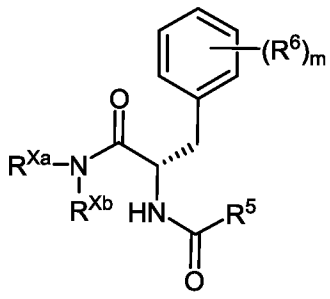
or a pharmaceutically acceptable salt thereof. In certain embodiments, R^1 is hydrogen or optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, -

CH_2CH_3, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$. In certain embodiments, R^{2A} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$. In certain embodiments, R^{3A} is optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$. In certain embodiments, R^4 is hydrogen or optionally substituted alkyl, *e.g.*, optionally substituted C_{1-3}alkyl, such as $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$. In certain embodiments, x is 1, R^{10a} and R^{10b} are each hydrogen, and R^9 is optionally substituted C_{4-6}alkyl or optionally substituted C_{3-6}carbocyclyl. In certain embodiments, at least one instance of R^6 is halogen (*e.g.*, fluoro (-F), chloro (-Cl), bromo (-Br), or iodo (-I)), –OH, –SH, $-NH_2$, $-NO_2$, carbonyl (*e.g.*, $-CO_2H$) or optionally substituted alkyl (*e.g.*, $-CH_3$). In certain embodiments, the two R^6 groups are 2,4-fluoro substituents. In certain embodiments, R^{Xb} is hydrogen or optionally substituted alkyl (*e.g.*, $-CH_3$).

[00248] In certain embodiments of Formula (**II**), wherein X is O or NR^{Xb}, provided is a compound of Formula:



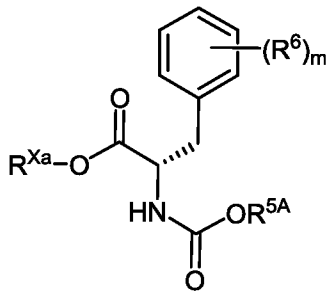
(**II-a1**)



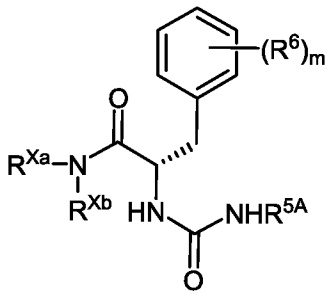
(**II-b1**)

or a pharmaceutically acceptable salt thereof. In certain embodiments, R^5 is an optionally substituted alkenyl group of formula (a1) wherein x is 1, R^{10a} and R^{10b} are each hydrogen, and R^9 is optionally substituted C_{4-6}alkyl or optionally substituted C_{3-6}carbocyclyl. In certain embodiments, R^5 is $-OR^{5A}$ or $-NHR^{5A}$, wherein R^{5A} is optionally substituted alkyl, optionally substituted aryl, or optionally substituted heteroaryl. In certain embodiments, R^{5A} is a group of formula (a2). In certain embodiments, m is 1, 2, or 3, optionally wherein at least one instance of R^6 is halogen (*e.g.*, fluoro (-F), chloro (-Cl), bromo (-Br), or iodo (-I)), –OH, –SH, $-NH_2$, $-NO_2$, carbonyl (*e.g.*, $-CO_2H$) or optionally substituted alkyl (*e.g.*, $-CH_3$). In certain embodiments, m is 2 and the two R^6 groups are 2,4-fluoro substituents. In certain embodiments, R^{Xb} is hydrogen or optionally substituted alkyl (*e.g.*, $-CH_3$). In certain embodiments, R^{Xa} is hydrogen, optionally substituted alkyl (*e.g.*, $-CH_3$, or aralkyl such as benzyl), optionally substituted alkenyl (*e.g.*, of formula (b)), or optionally substituted alkynyl (*e.g.*, of formula (c)).

[00249] For example, in certain embodiments of Formula (**II-a1**) and (**II-b1**), wherein R^5 is $-OR^{5A}$ or $-NHR^{5A}$, provided is a compound of Formula:



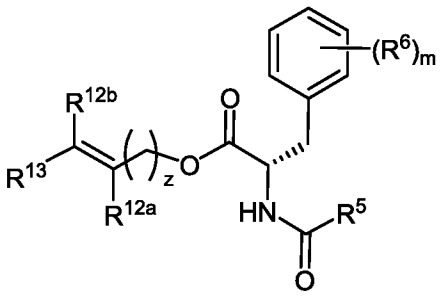
(**II-a1-a**)



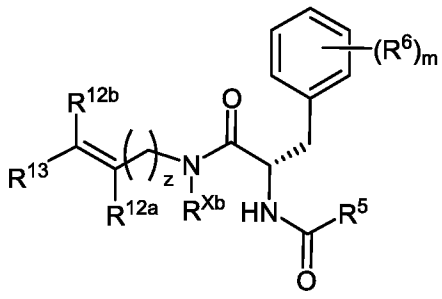
(**II-b1-b**)

or a pharmaceutically acceptable salt thereof. In certain embodiments, R^5 is an optionally substituted alkenyl group of formula (a1) wherein x is 1, R^{10a} and R^{10b} are each hydrogen, and R^9 is optionally substituted C_{4-6}alkyl or optionally substituted C_{3-6}carbocyclyl. In certain embodiments, R^{5A} is optionally substituted alkyl, optionally substituted aryl, or optionally substituted heteroaryl. In certain embodiments, R^{5A} is a group of formula (a2). In certain embodiments, m is 1, 2, or 3, optionally wherein at least one instance of R^6 is halogen (*e.g.*, fluoro (-F), chloro (-Cl), bromo (-Br), or iodo (-I)), –OH, –SH, $-NH_2$, $-NO_2$, carbonyl (*e.g.*, $-CO_2H$) or optionally substituted alkyl (*e.g.*, $-CH_3$). In certain embodiments, m is 2 and the two R^6 groups are 2,4-fluoro substituents. In certain embodiments, R^{Xb} is hydrogen or optionally substituted alkyl (*e.g.*, $-CH_3$). In certain embodiments, R^{Xa} is hydrogen, optionally substituted alkyl (*e.g.*, $-CH_3$, or aralkyl such as benzyl), optionally substituted alkenyl (*e.g.*, of formula (b)), or optionally substituted alkynyl (*e.g.*, of formula (c)).

[00250] In certain embodiments of Formula (**II-a1**) and (**II-b1**), wherein R^{Xa} is an optionally substituted alkenyl group of formula (b), provided is a compound of Formula:



(**II-a2**)

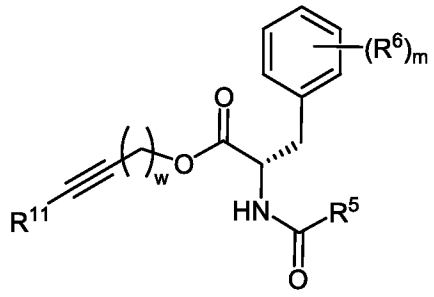


(**II-b2**)

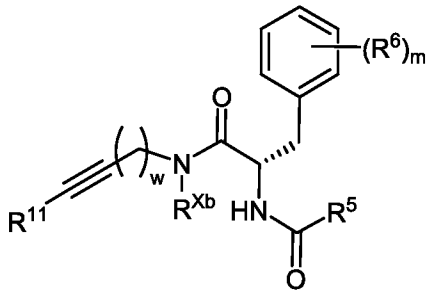
or a pharmaceutically acceptable salt thereof. In certain embodiments, R^5 is an optionally substituted alkenyl group of formula (a1) wherein x is 1, R^{10a} and R^{10b} are each hydrogen, and R^9 is optionally substituted C_{4-6}alkyl or optionally substituted C_{3-6}carbocyclyl. In certain embodiments, R^5 is $-OR^{5A}$ or $-NHR^{5A}$, wherein R^{5A} is optionally substituted alkyl, optionally substituted aryl, or optionally substituted heteroaryl. In certain embodiments, R^{5A} is a group of formula (a2). In certain embodiments, m is 1, 2, or 3, optionally wherein at least one

instance of R^6 is halogen (*e.g.*, fluoro (-F), chloro (-Cl), bromo (-Br), or iodo (-I)), –OH, –SH, $–NH_2$, $-NO_2$, carbonyl (*e.g.*, $-CO_2H$) or optionally substituted alkyl (*e.g.*, $-CH_3$). In certain embodiments, m is 2 and the two R^6 groups are 2,4-fluoro substituents. In certain embodiments, R^{Xb} is hydrogen or optionally substituted alkyl (*e.g.*, $-CH_3$).

[00251] In certain embodiments of Formula (**II-a1**) and (**II-b1**), wherein R^{Xa} is an optionally substituted alkynyl group of formula (c), provided is a compound of Formula:



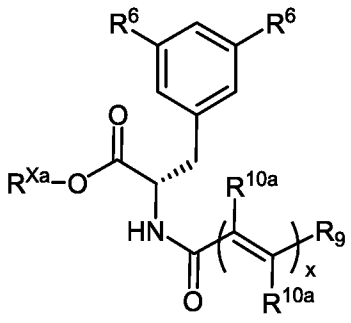
(**II-a3**)



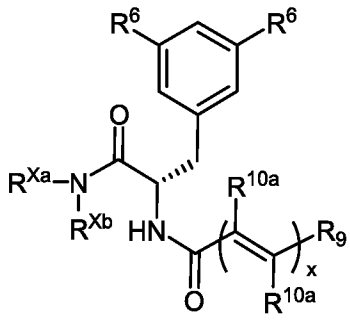
(**II-b3**)

or a pharmaceutically acceptable salt thereof. In certain embodiments, R^5 is an optionally substituted alkenyl group of formula (a1) wherein x is 1, R^{10a} and R^{10b} are each hydrogen, and R^9 is optionally substituted C_{4-6}alkyl or optionally substituted C_{3-6}carbocyclyl. In certain embodiments, R^5 is $–OR^{5A}$ or $–NHR^{5A}$, wherein R^{5A} is optionally substituted alkyl, ptionally substituted aryl, or optionally substituted heteroaryl. In certain embodiments, R^{5A} is a group of formula (a2). In certain embodiments, m is 1, 2, or 3, optionally wherein at least one instance of R^6 is halogen (*e.g.*, fluoro (-F), chloro (-Cl), bromo (-Br), or iodo (-I)), –OH, –SH, $–NH_2$, $-NO_2$, carbonyl (*e.g.*, $-CO_2H$) or optionally substituted alkyl (*e.g.*, $-CH_3$). In certain embodiments, m is 2 and the two R^6 groups are 2,4-fluoro substituents. In certain embodiments, R^{Xb} is hydrogen or optionally substituted alkyl (*e.g.*, $-CH_3$).

[00252] In certain embodiments of Formula (**II-a1**) and (**II-b1**), wherein R^5 is an optionally substituted alkenyl group of formula (a1), m is 2, and the two R^6 substituents are 2,4-substituents, provided is a compound of Formula:



(**II-a4**)

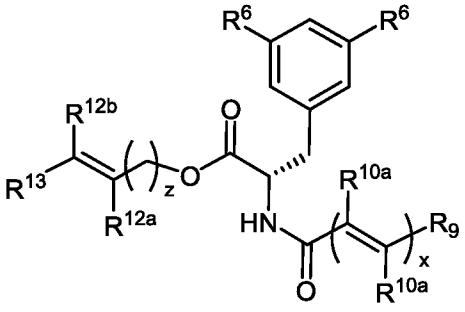


(**II-b4**)

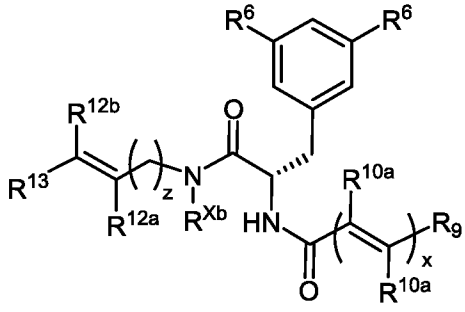
or a pharmaceutically acceptable salt thereof. In certain embodiments, x is 1, R^{10a} and R^{10b} are each hydrogen, and R^9 is optionally substituted C_{4-6}alkyl or optionally substituted C_{3-6}carbocyclyl. In certain embodiments, at least one instance of R^6 is halogen (*e.g.*, fluoro (-F),

chloro (-Cl), bromo (-Br), or iodo (-I)), –OH, –SH, –NH_2, -NO_2, carbonyl (*e.g.*, -CO_2H) or optionally substituted alkyl (*e.g.*, -CH_3). In certain embodiments, the two R^6 groups are 2,4-fluoro substituents. In certain embodiments, R^{Xb} is hydrogen or optionally substituted alkyl (*e.g.*, -CH_3). In certain embodiments, R^{Xa} is hydrogen, optionally substituted alkyl (*e.g.*, -CH_3, or aralkyl such as benzyl), optionally substituted alkenyl (*e.g.*, of formula (b)), or optionally substituted alkynyl (*e.g.*, of formula (c)).

[00253] For example, in certain embodiments of Formula (**II-a4**) and (**II-b4**), wherein R^{Xa} is an optionally substituted alkenyl group of formula (b), provided is a compound of Formula:



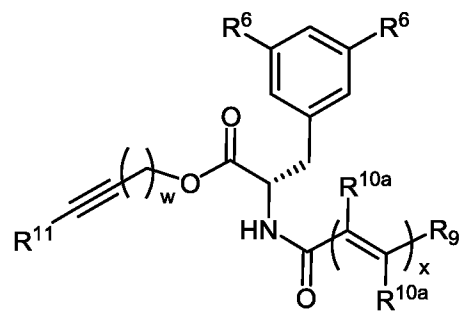
(**II-a5**)



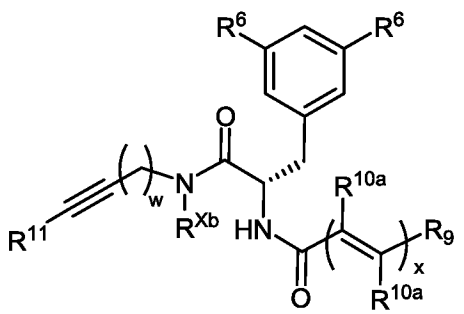
(**II-b5**)

or a pharmaceutically acceptable salt thereof. In certain embodiments, x is 1, R^{10a} and R^{10b} are each hydrogen, and R^9 is optionally substituted C_{4-6}alkyl or optionally substituted C_{3-6}carbocyclyl. In certain embodiments, at least one instance of R^6 is halogen (*e.g.*, fluoro (-F), chloro (-Cl), bromo (-Br), or iodo (-I)), –OH, –SH, –NH_2, -NO_2, carbonyl (*e.g.*, -CO_2H) or optionally substituted alkyl (*e.g.*, -CH_3). In certain embodiments, the two R^6 groups are 2,4-fluoro substituents. In certain embodiments, R^{Xb} is hydrogen or optionally substituted alkyl (*e.g.*, -CH_3).

[00254] In certain embodiments of Formula (**II-a4**) and (**II-b4**), wherein R^{Xa} is an optionally substituted alkynyl group of formula (c), provided is a compound of Formula:



(**II-a6**)

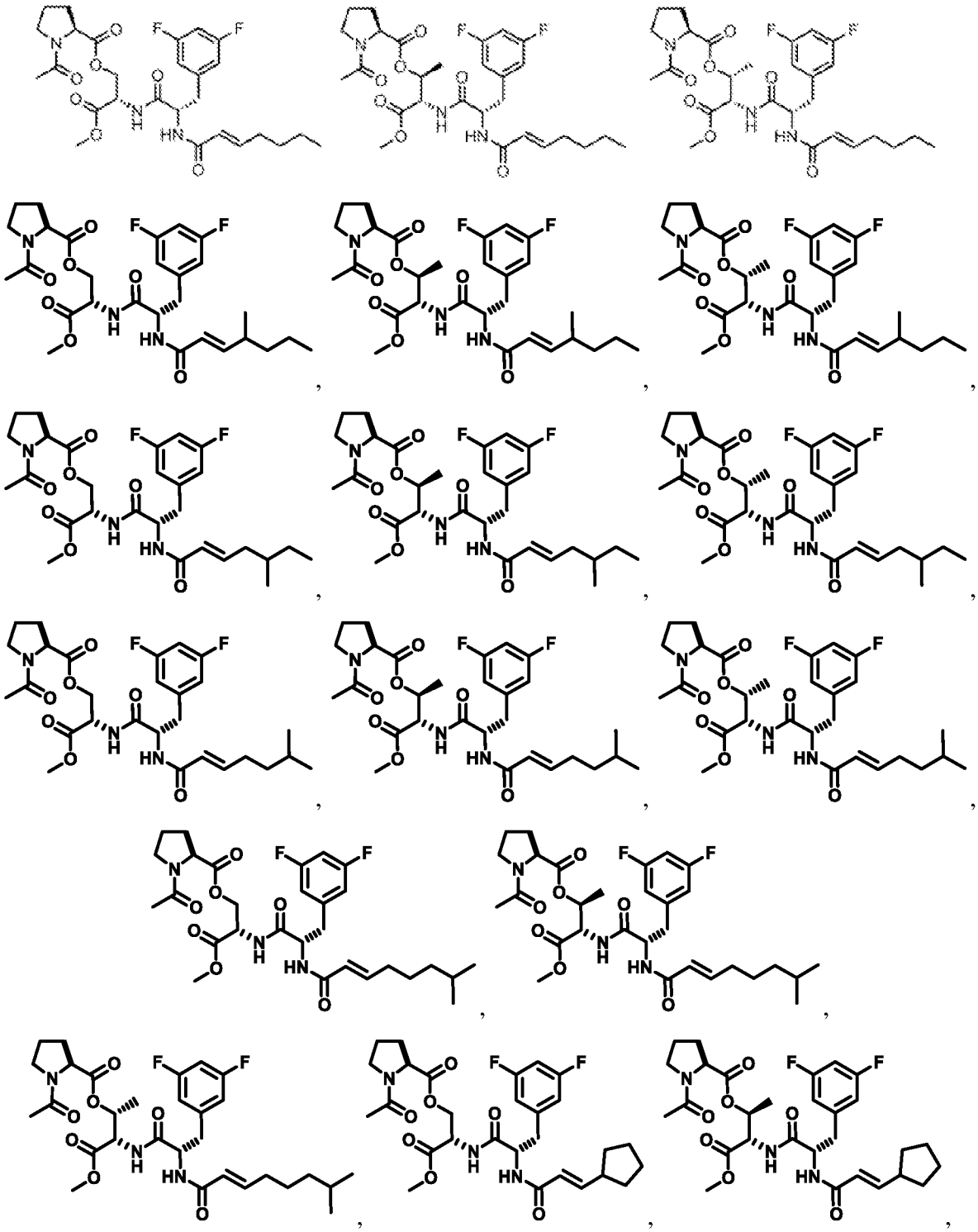


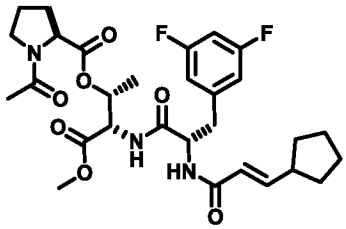
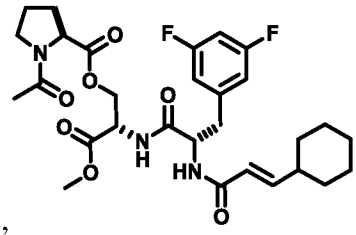
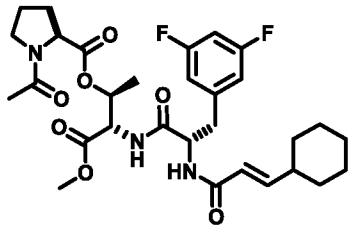
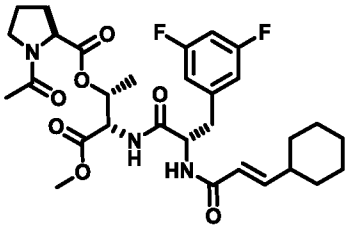
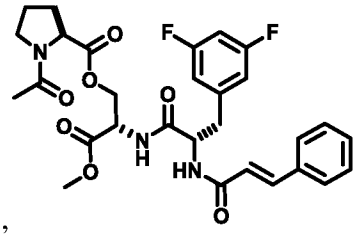
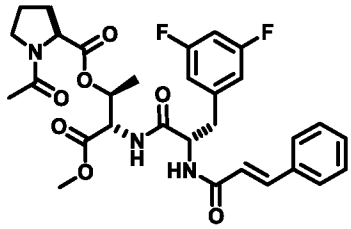
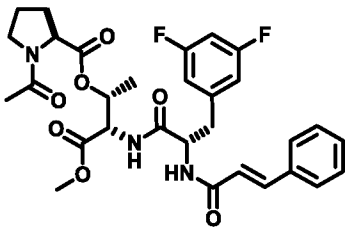
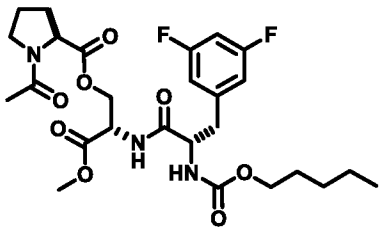
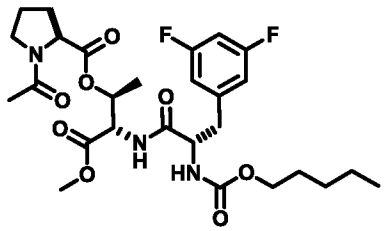
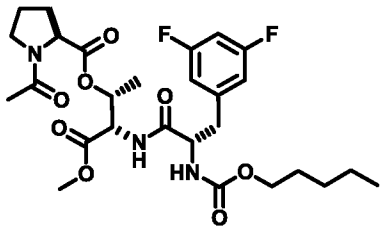
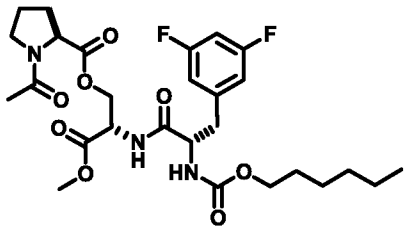
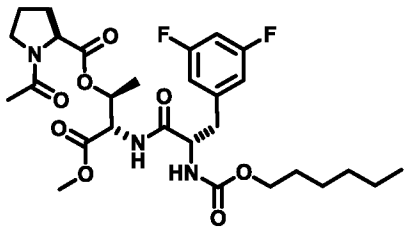
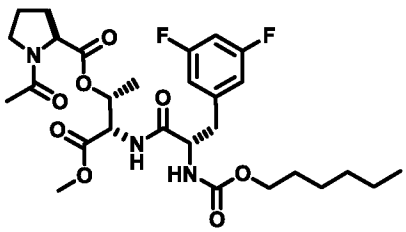
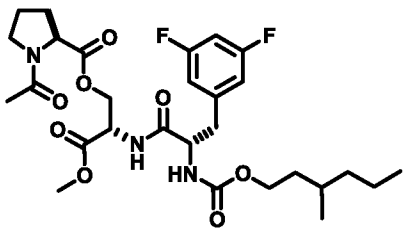
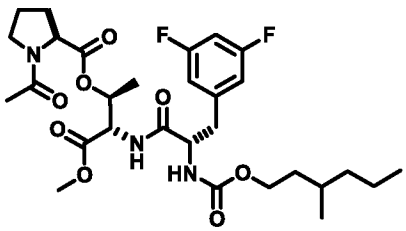
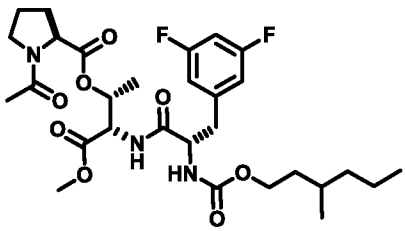
(**II-b6**)

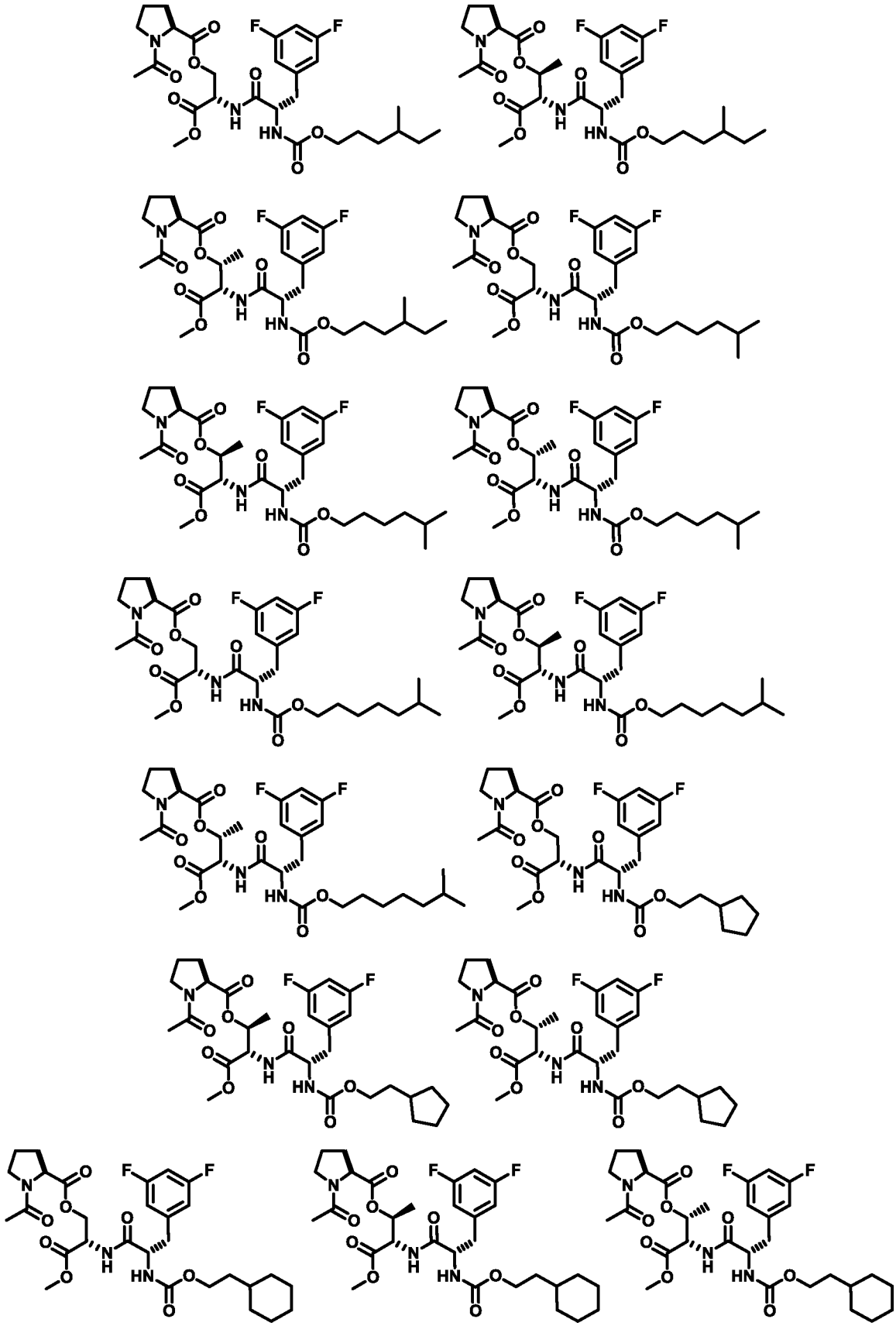
or a pharmaceutically acceptable salt thereof. In certain embodiments, x is 1, R^{10a} and R^{10b} are each hydrogen, and R^9 is optionally substituted C_{4-6}alkyl or optionally substituted C_{3-6}carbocyclyl. In certain embodiments, at least one instance of R^6 is halogen (*e.g.*, fluoro (-F), chloro (-Cl), bromo (-Br), or iodo (-I)), –OH, –SH, –NH_2, -NO_2, carbonyl (*e.g.*, -CO_2H) or

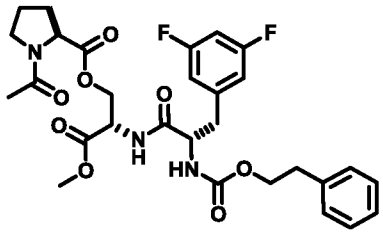
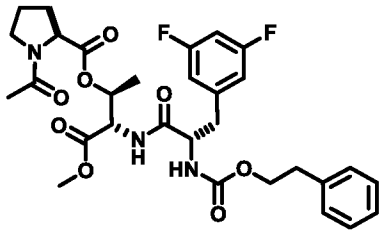
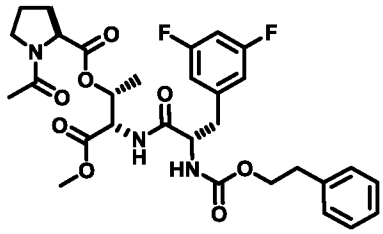
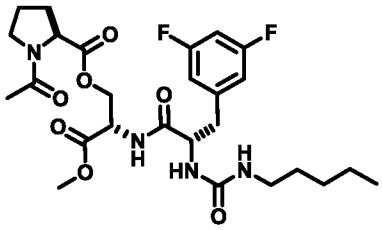
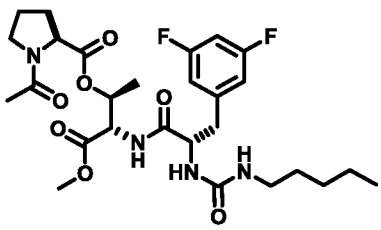
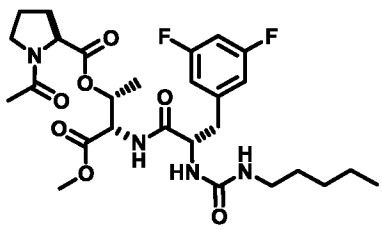
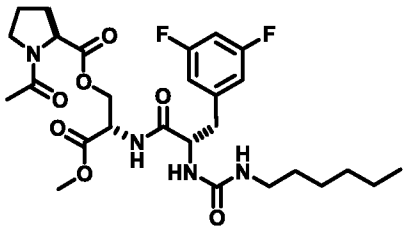
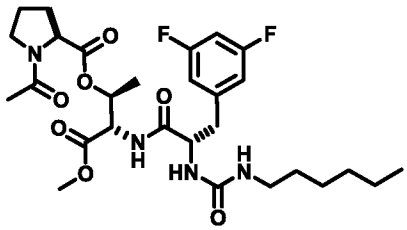
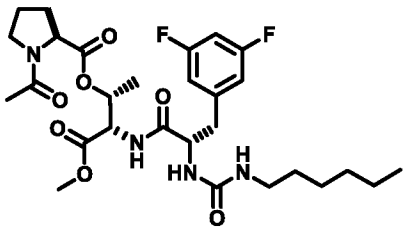
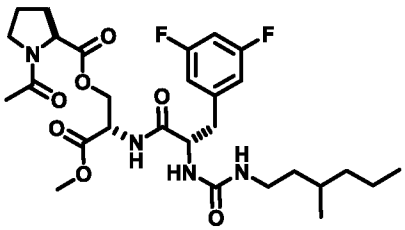
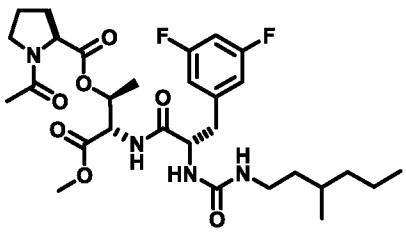
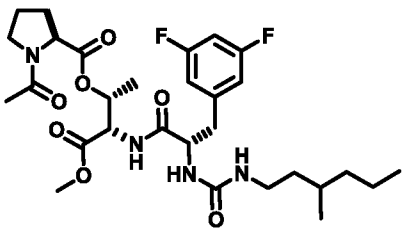
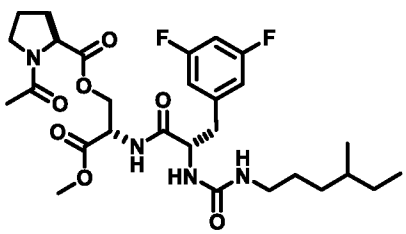
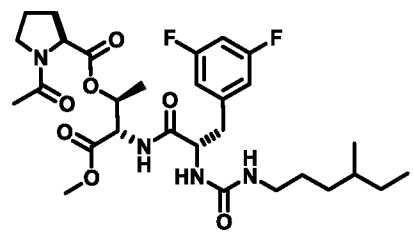
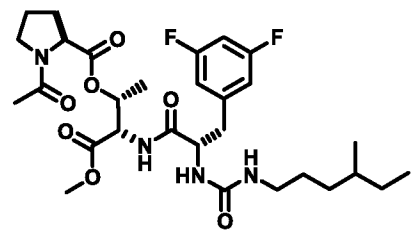
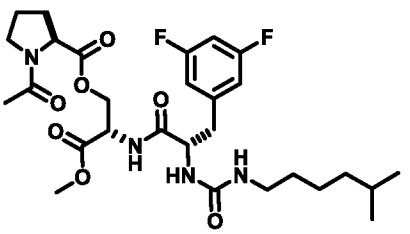
optionally substituted alkyl (*e.g.*, $-CH_3$). In certain embodiments, the two R^6 groups are 2,4-fluoro substituents. In certain embodiments, R^{Xb} is hydrogen or optionally substituted alkyl (*e.g.*, $-CH_3$).

[00255] Exemplary compounds of Formula (**I**) include, but are not limited to:

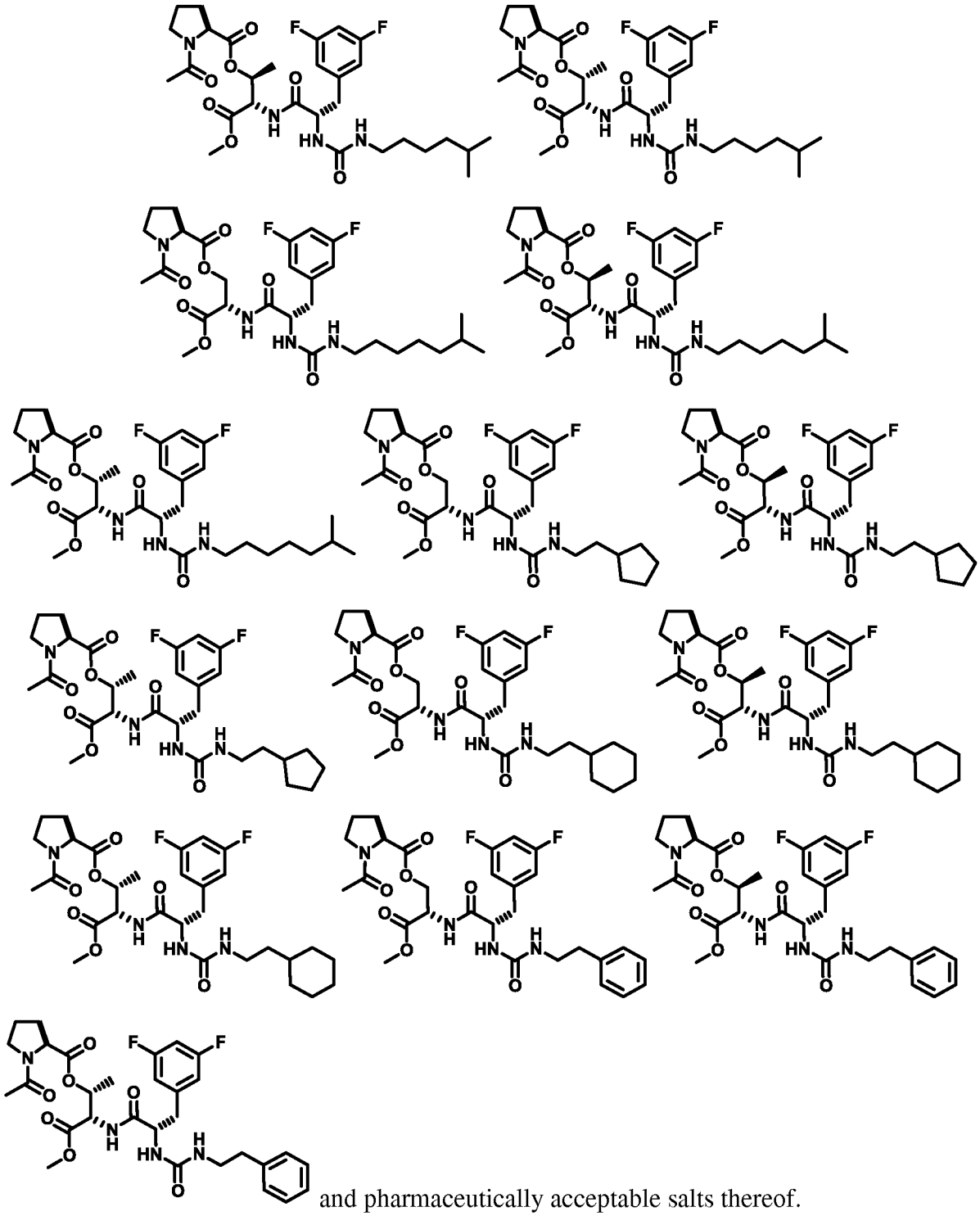



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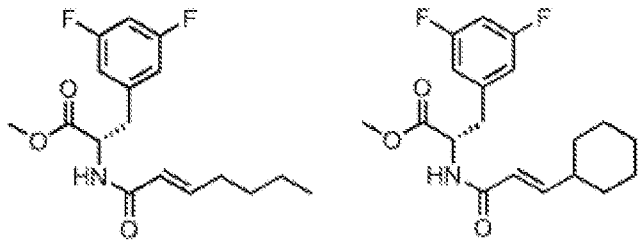




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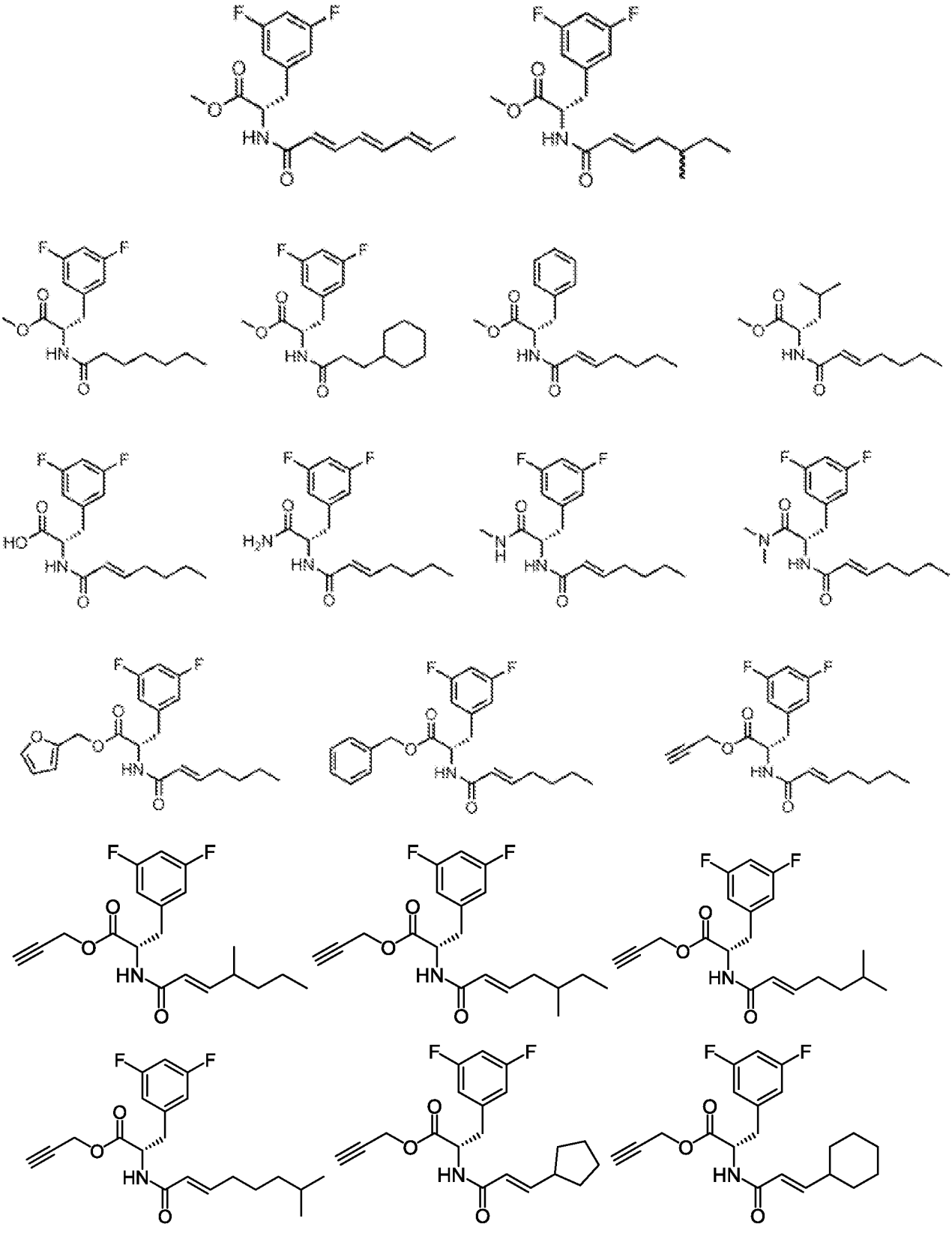


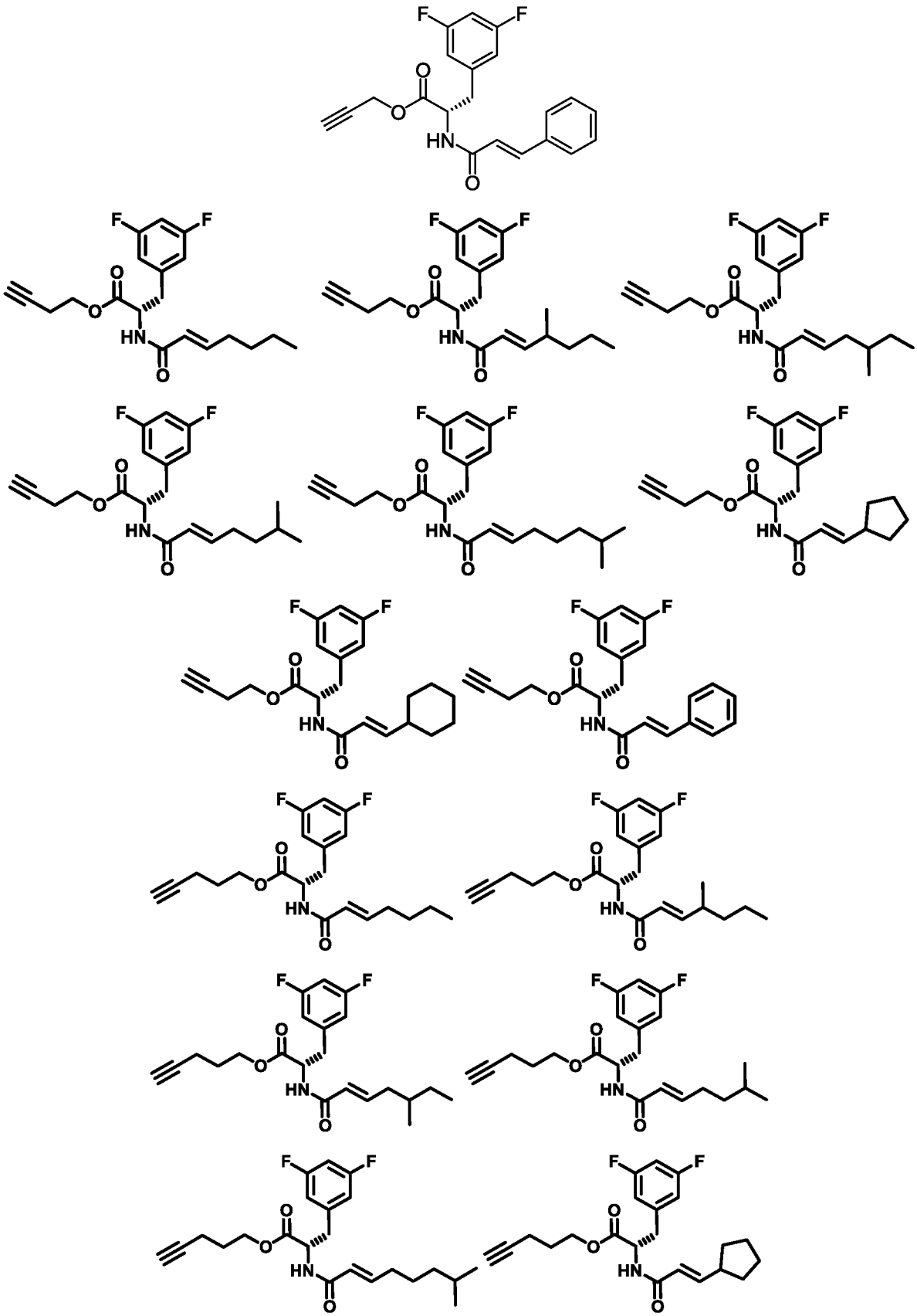


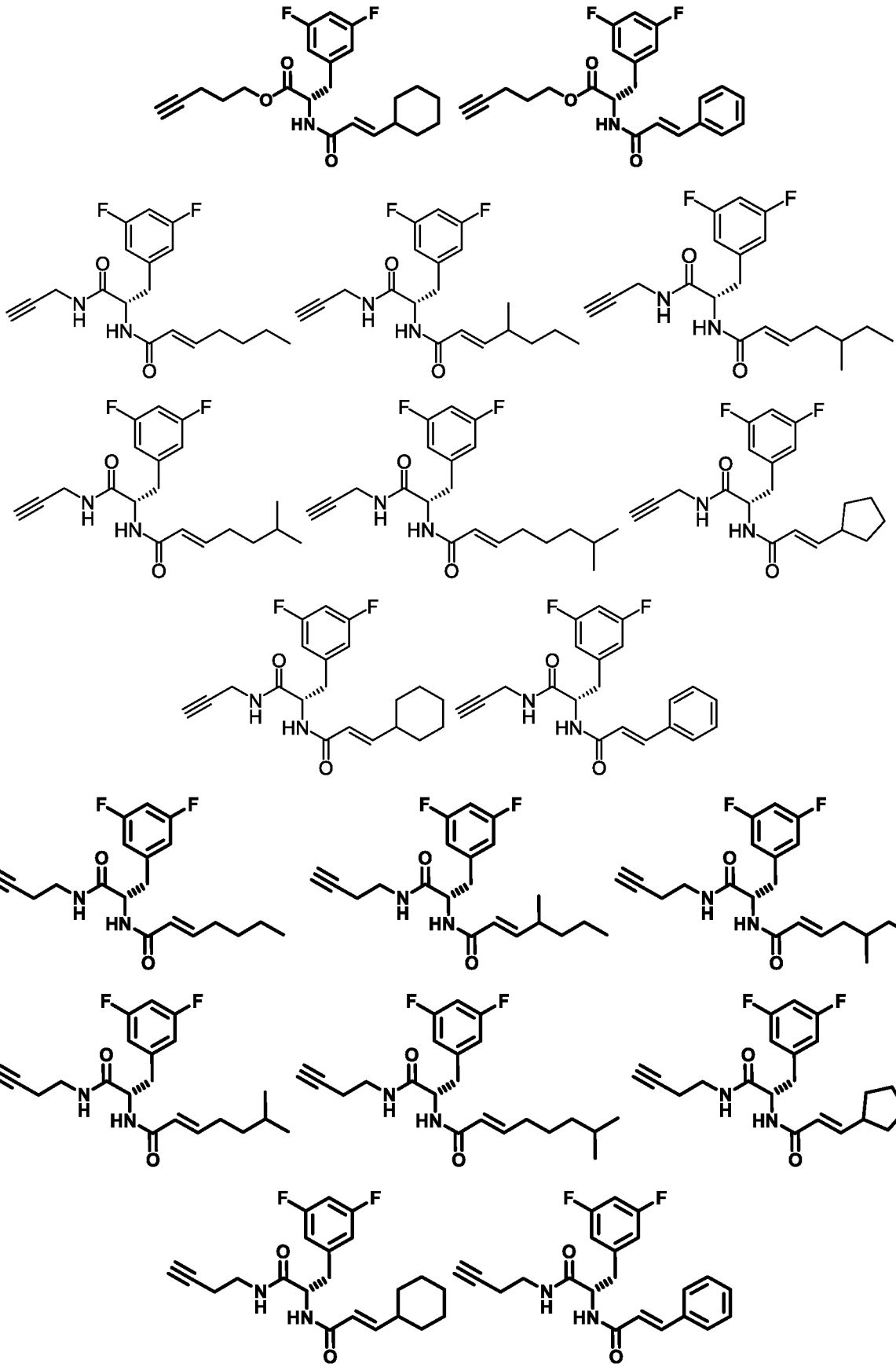
and pharmaceutically acceptable salts thereof.

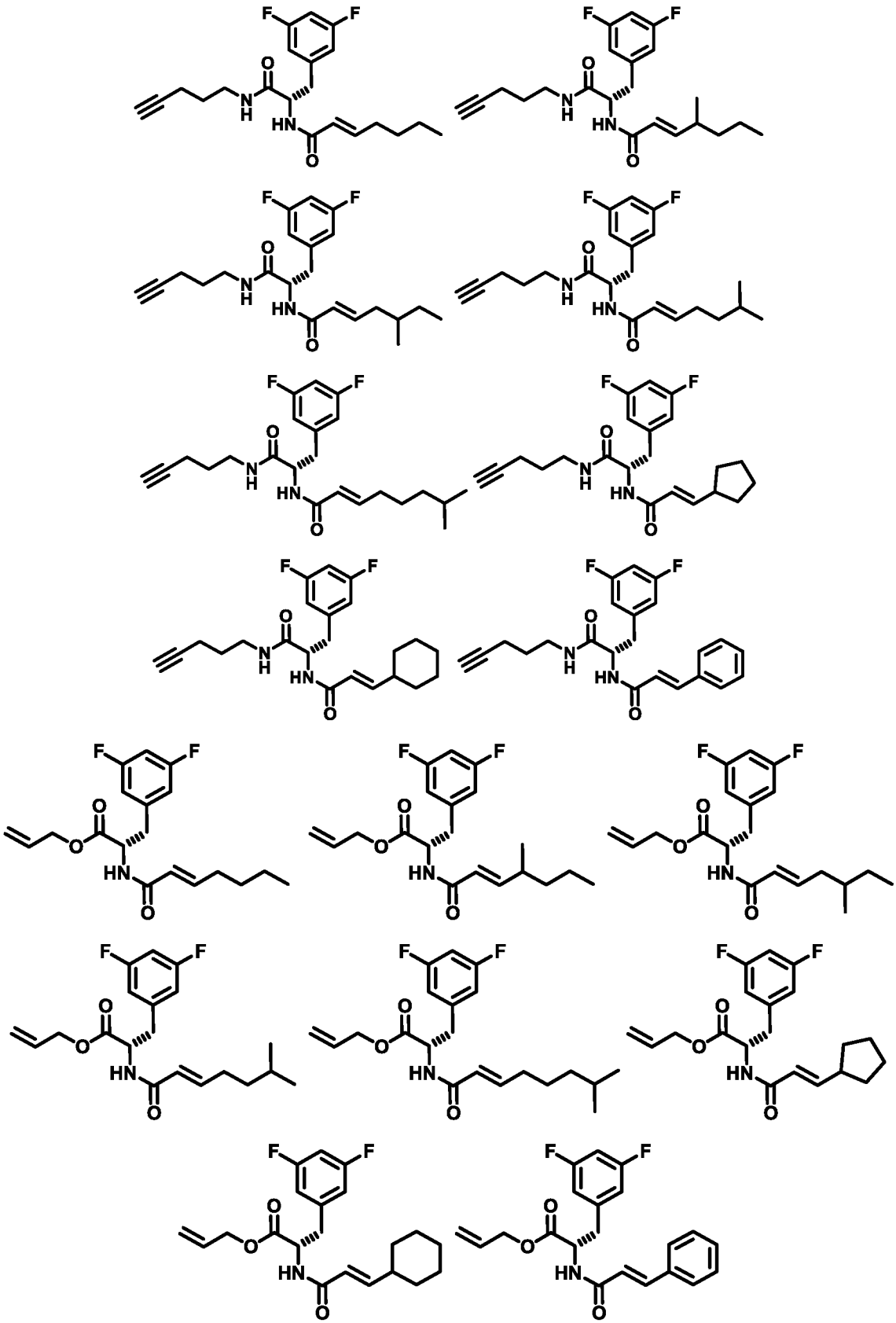
[00256] Exemplary compounds of Formula (**II**) include, but are not limited to:

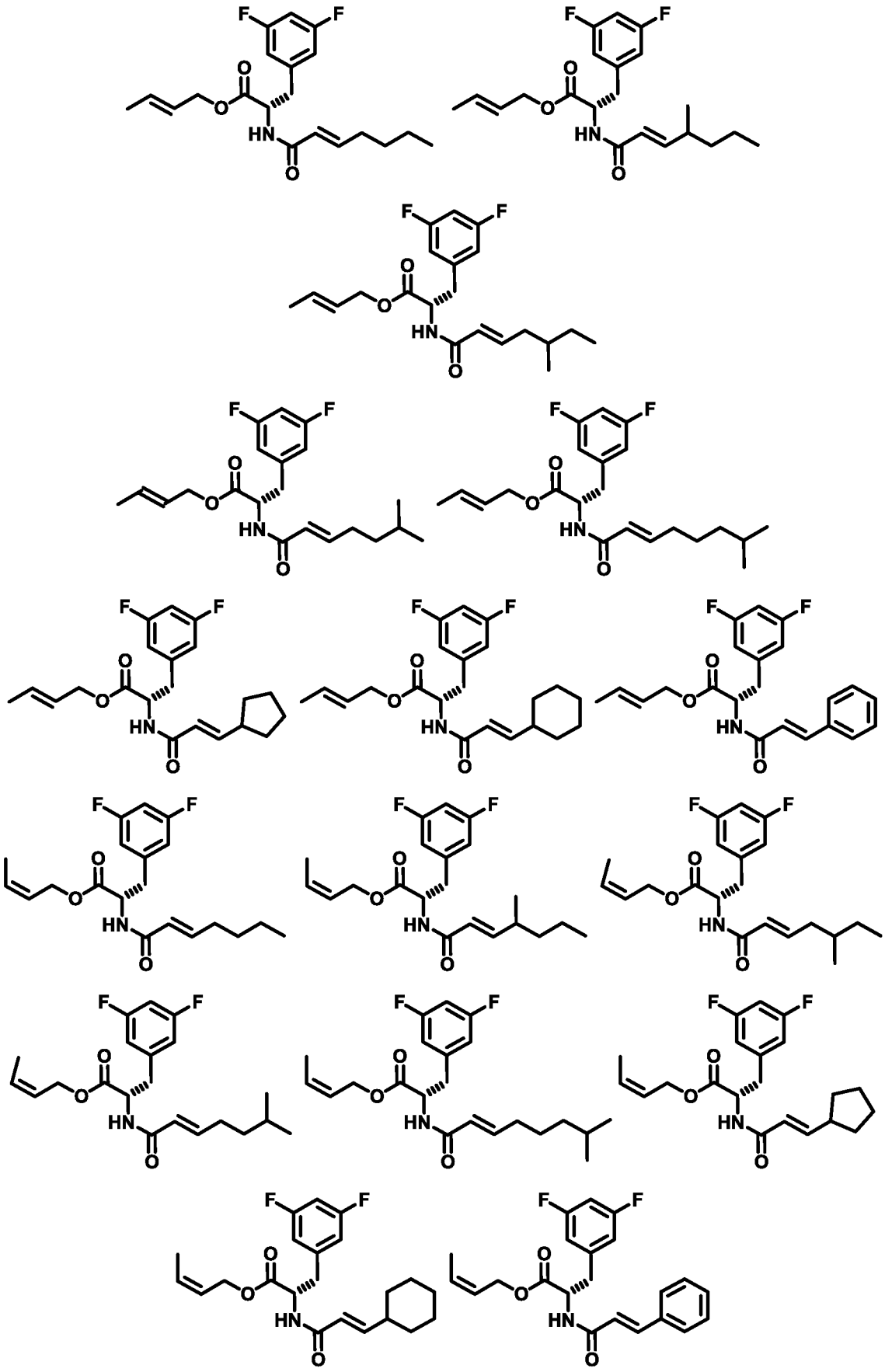


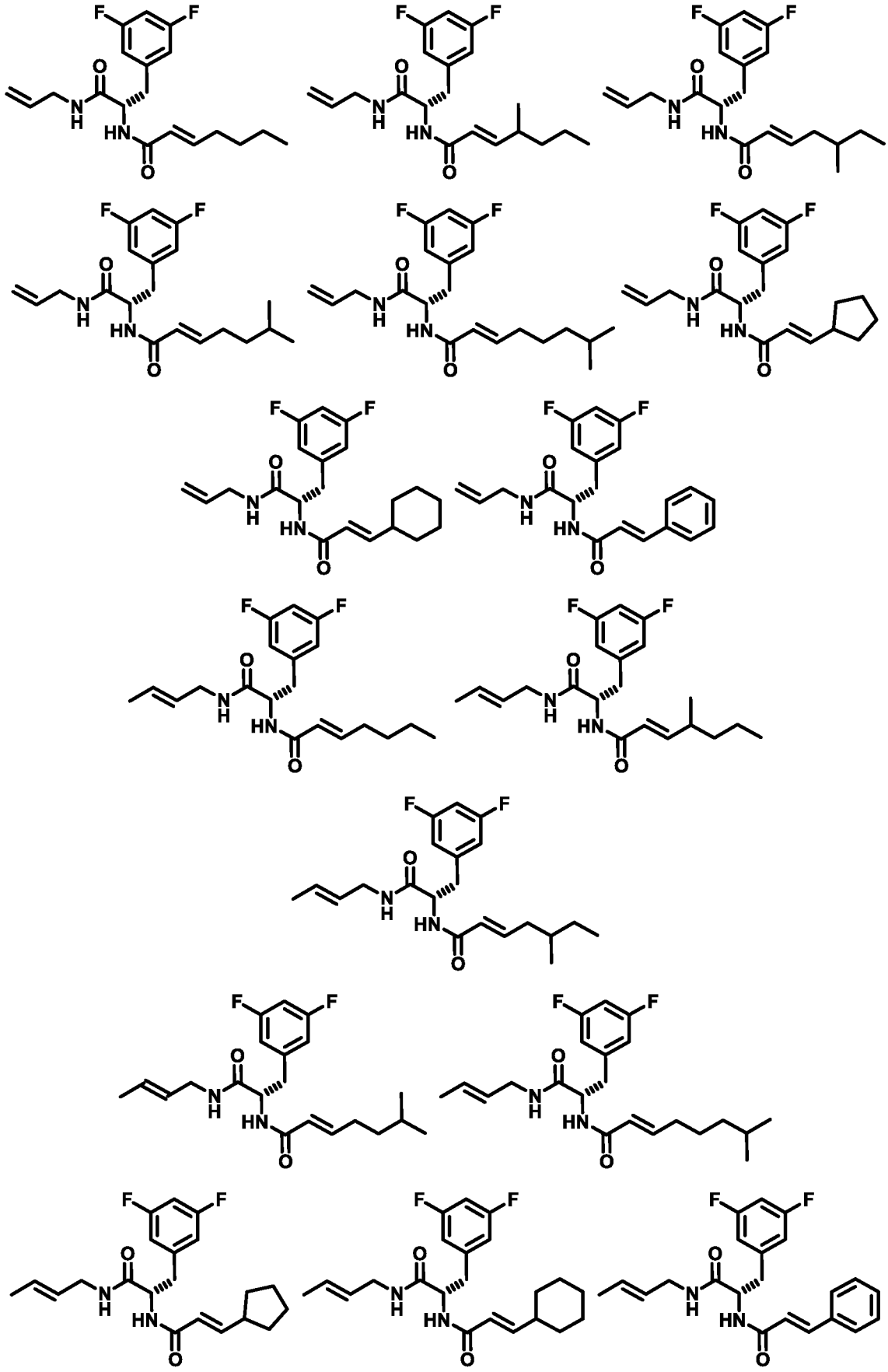


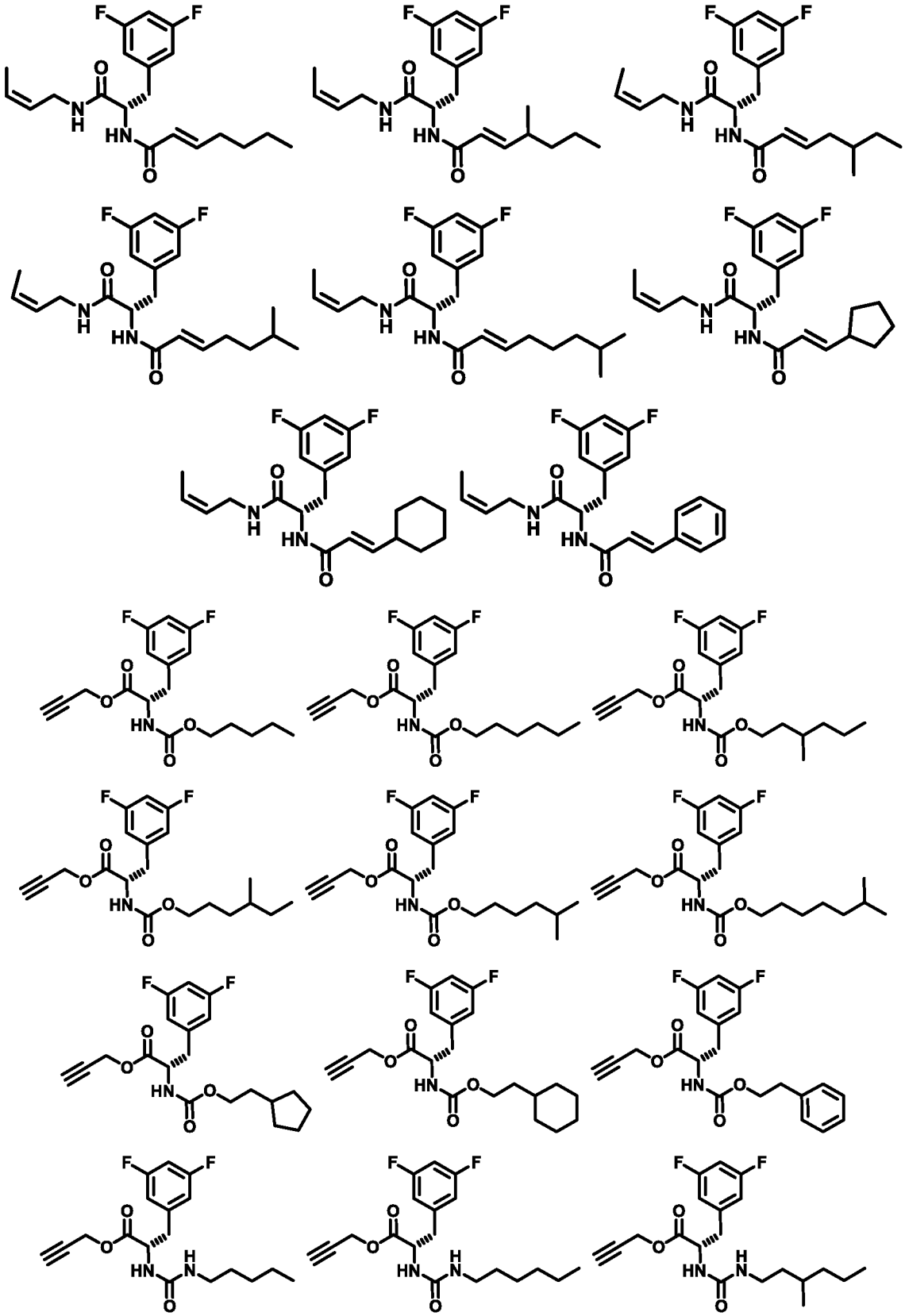


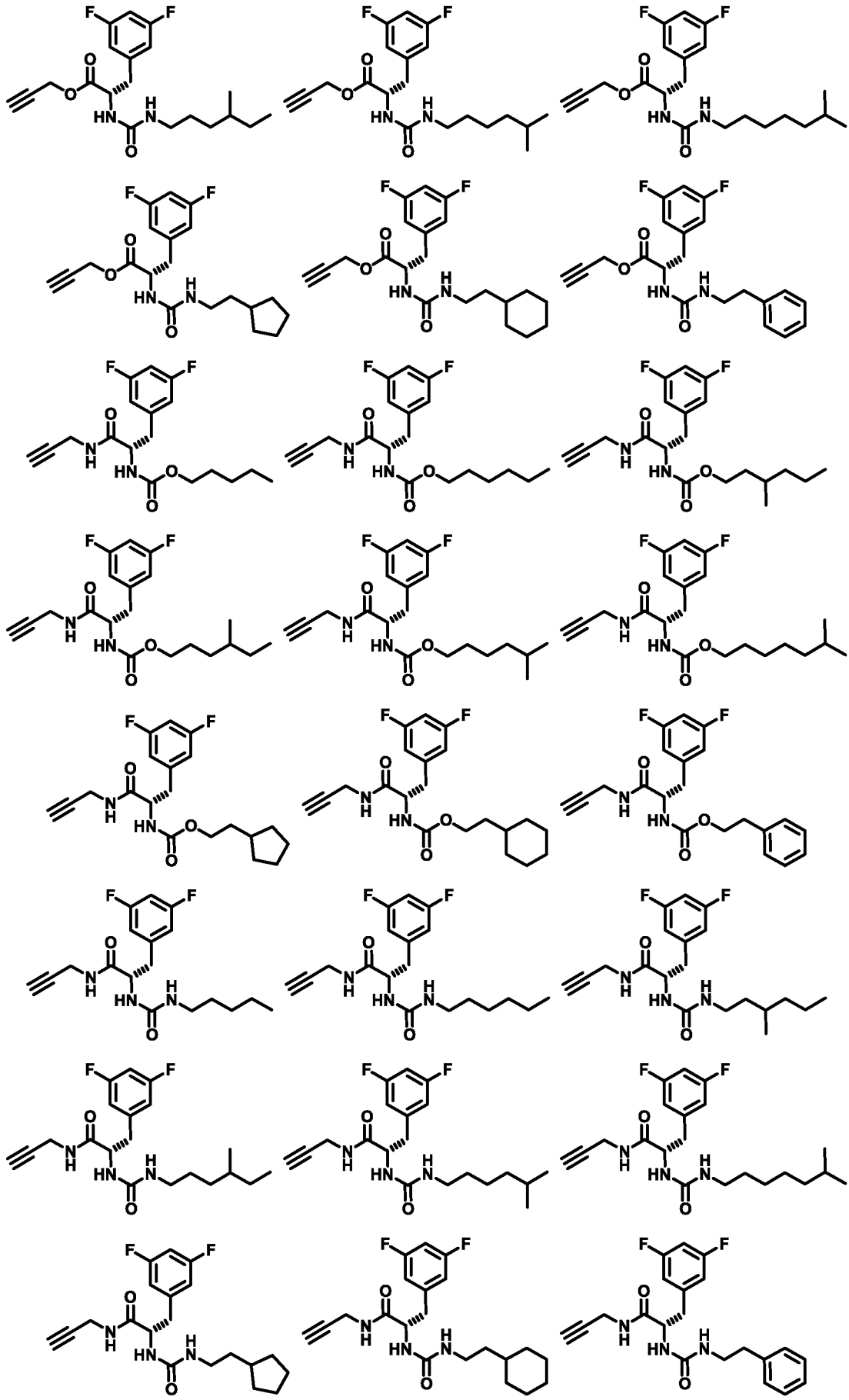


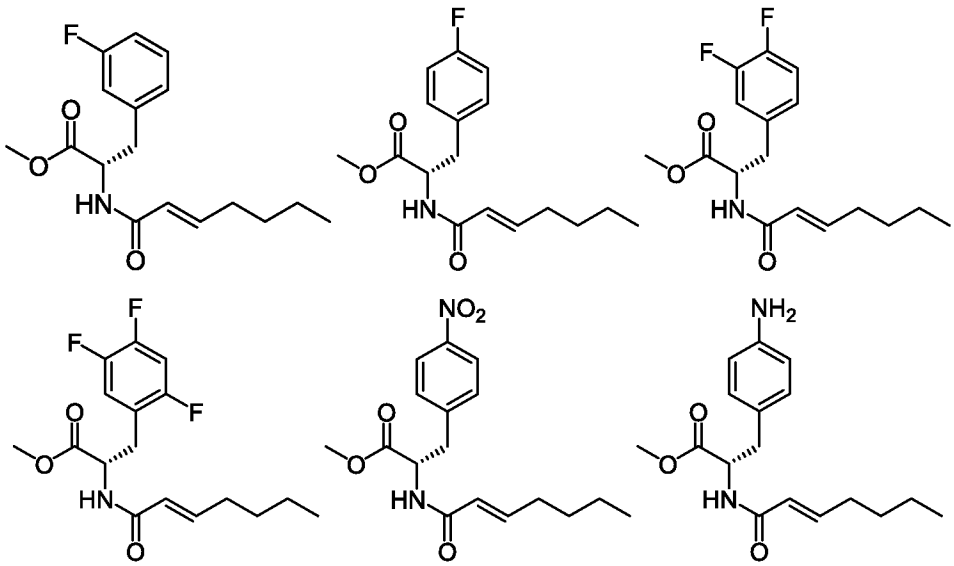






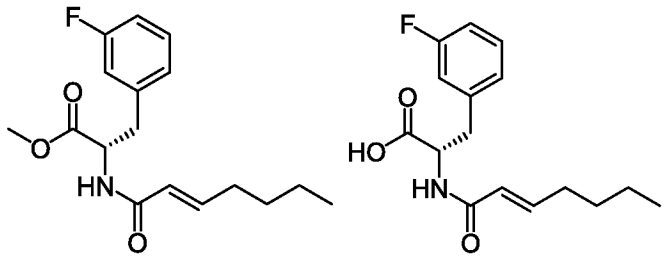






and pharmaceutically acceptable salts thereof.

[00257] However, in certain embodiments, the compound is not:



or a pharmaceutically acceptable salt thereof.

Labeled Compounds of Formula (I) and (II)

[00258] Compounds of Formula (**I**) and (**II**) may further be substituted with a label, optionally tethered to the compound via a divalent linker moiety, such as an optionally substituted alkylene, optionally substituted alkenylene, optionally substituted alkynylene, optionally substituted heteroalkylene, optionally substituted heteroalkenylene, optionally substituted heteroalkynylene, optionally substituted carbocyclylene, optionally substituted heterocycylene, optionally substituted arylene, optionally substituted heteroarylene divalent moiety, or a divalent moiety comprising a combination of two or more of such groups sequentially linked together. A "label," as used herein, refers to a detectable moiety, *i.e.,* a moiety which provides a detectable (*e.g.,* visually detectable) analytical signal. Various detectable moieties are contemplated herein, and include, but are not limited to, (a) a molecule which contains isotopic moieties, *e.g.,* for use in radioimmunoassays, which may be radioactive and/or isotopic and include, but not limited to, ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{31}P, ^{32}P, ^{35}S, ^{67}Ga, ^{99}mTc (Tc-^{99}m), ^{111}In, ^{123}I, ^{125}I, ^{169}Yb, and ^{186}Re), (b) an enzyme or coenzyme; (c) a

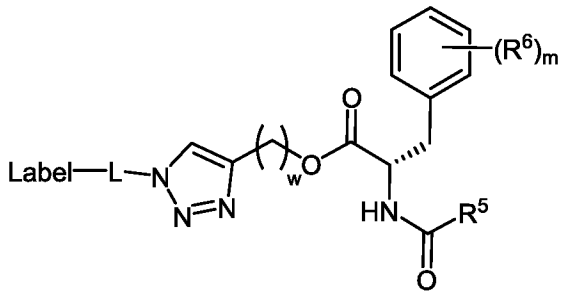
molecule which is colored (chromophore), fluorescent, phosphorescent, or chemiluminescent dyes; (d) a molecule which has one or more photo affinity moieties; (e) a molecule which has a ligand moiety with one or more known binding partners (haptens such as biotin, dinitrophenol (DNP), and digoxigenin); (f) latex and magnetic particles; (g) gold, silver, and selenium colloidal particles; (h) metal chelates; and (i) oligonucleotides. A wide variety of detectable moieties can be used, with the choice of label depending on the sensitivity required, stability requirements, and available instrumentation and disposal provisions. All of above can be used as label, and by attaching to the compound, they can be located with different detection method, *e.g.,* for example, using Halo tag.

[00259] In certain embodiments, the label is biotin. The biotin moiety allows the compound of Formula (**I**) or (**II**) to bind to certain surface such as, for example, beads, glass, or metal surface.

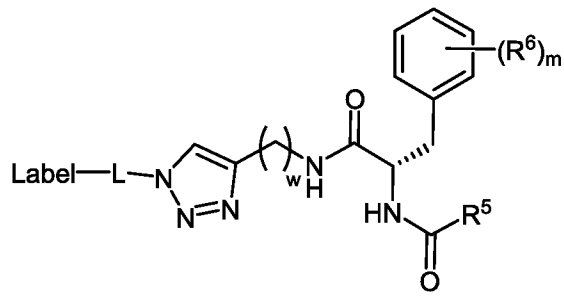
[00260] In certain embodiments, the label is a fluorescent moiety. A "fluorescent moiety" or label as used herein refer to a moiety that absorbs light energy at a defined excitation wavelength and emit light energy at a different wavelength. Examples of fluorescent labels include, but are not limited to: Alexa Fluor dyes (Alexa Fluor 350, Alexa Fluor 488, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 633, Alexa Fluor 660 and Alexa Fluor 680), AMCA, AMCA-S, BODIPY dyes (BODIPY FL, BODIPY R6G, BODIPY TMR, BODIPY TR, BODIPY 493/503, BODIPY 530/550, BODIPY 558/568, BODIPY 564/570, BODIPY 576/589, BODIPY 581/591, BODIPY 630/650, BODIPY 650/665), Carboxyrhodamine 6G, carboxy-X-rhodamine (ROX), Cascade Blue, Cascade Yellow, Coumarin 343, Cyanine dyes (Cy3, Cy5, Cy3.5, Cy5.5), Dansyl, Dapoxyl, Dialkylaminocoumarin, 4',5'-Dichloro-2',7'-dimethoxy-fluorescein, DM-NERF, Eosin, Erythrosin, Fluorescein, FAM, Hydroxycoumarin, IRDyes (IRD40, IRD 700, IRD 800), JOE, Lissamine rhodamine B, Marina Blue, Methoxycoumarin, Naphthofluorescein, Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, PyMPO, Pyrene, Rhodamine B, Rhodamine 6G, Rhodamine Green, Rhodamine Red, Rhodol Green, 2',4',5',7'-Tetra-bromosulfone-fluorescein, Tetramethyl-rhodamine (TMR), Carboxytetramethylrhodamine (TAMRA), Texas Red, Texas Red-X, 5(6)-Carboxyfluorescein, 2,7-Dichlorofluorescein, N,N-Bis(2,4,6-trimethylphenyl)-3,4:9,10-perylenebis(dicarboximide, HPTS, Ethyl Eosin, DY-490XL MegaStokes, DY-485XL MegaStokes, Adirondack Green 520, ATTO 465, ATTO 488, ATTO 495, YOYO-1,5-FAM, BCECF, dichlorofluorescein, rhodamine 110, rhodamine 123, YO-PRO-1, SYTOX Green, Sodium Green, SYBR Green I, Alexa Fluor 500, FITC, Fluo-3, Fluo-4, fluoro-emerald, YoYo-1 ssDNA, YoYo-1 dsDNA, YoYo-1, SYTO

RNASelect, Diversa Green-FP, Dragon Green, EvaGreen, Surf Green EX, Spectrum Green, NeuroTrace 500525, NBD-X, MitoTracker Green FM, LysoTracker Green DND-26, CBQCA, PA-GFP (post-activation), WEGFP (post-activation), FlASH-CCXXCC, Azami Green monomeric, Azami Green, green fluorescent protein (GFP), EGFP (Campbell Tsien 2003), EGFP (Patterson 2001), Kaede Green, 7-Benzylamino-4-Nitrobenz-2-Oxa-1,3-Diazole, Bexl, Doxorubicin, Lumio Green, and SuperGlo GFP, green fluorescent protein (GFP), phycoerythrin, hydroxycoumarin, aminocoumarin, methoxycoumarin, cascade blue, pacific blue, pacific orange, Lucifer yellow, NBD, R–Phycoerythrin (PE), PE–Cy5, PE–Cy7, Red 613, PerCP, TruRed, FluorX, fluorescein, fluorescein isothiocyanate (FITC), X–Rhodamine, Lissamine Rhodamine B, Texas red, tetrarhodamine isothiocynate (TRITC), BODIPY–FL, Cy2, Cy3, Cy3B, Cy3.5, Cy5, Cy5.5, Cy7, Allophycocyanin (APC), APC–Cy7, ATTO655, Alexa Fluor 405, Alexa Fluor 568, and Alexa Fluor 647. See also Lavis *et al., ACS Chem. Biol.* (2008) 142-155 disclosing various florescent labels. In certain embodiments, the label is fluorescein isothiocyanate (FITC).

[00261] Exemplary labeled compounds include compounds of Formula:



(**II-a1-label**)



(**II-b1-label**)

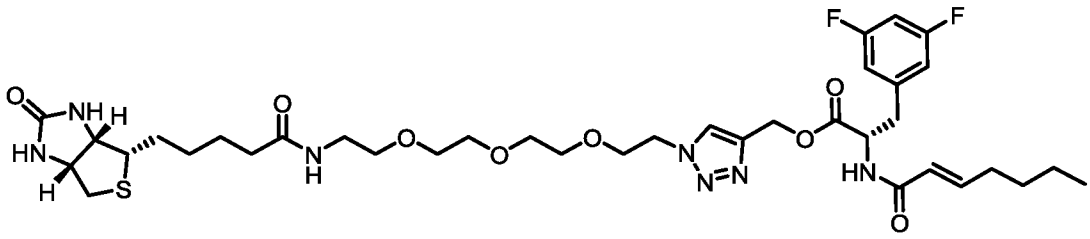
wherein:

w is 1, 2, 3, or 4;

L is a divalent linker moiety as defined herein; and

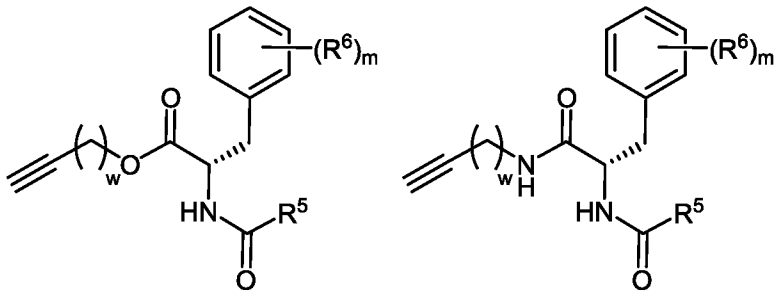
and Label is a lable moiety as defined herein.

[00262] Exemplary labeled compounds of Formula (**II-a1-label**) include, but are not limited to, the below biotin-labled compound:



or a pharmaceutically acceptable salt thereof.

[00263] Such compounds may be synthesized, for example, from "click chemistry" coupling of the corresponding alkynyl compound of Formula:



and an azido moiety of formula Label—L—N_3.

Pharmaceutical Compositions

[00264] In certain embodiments, provided are pharmaceutical compositions comprising a compound of Formula (**I**) or (**II**) or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient. In certain embodiments, the compound is provided in an effective amount in the pharmaceutical composition, *e.g.*, a therapeutically effective amount and/or a prophylactically effective amount, depending upon the intended method of treatment.

[00265] Pharmaceutically acceptable excipients include any and all solvents, diluents or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. General considerations in formulation and/or manufacture of pharmaceutical compositions agents can be found, for example, in *Remington's Pharmaceutical Sciences*, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980), and *Remington: The Science and Practice of Pharmacy*, 21st Edition (Lippincott Williams & Wilkins, 2005).

[00266] Pharmaceutical compositions described herein can be prepared by any method known in the art of pharmacology. In general, such preparatory methods include the steps of bringing the compound of Formula (**I**) or (**II**) or pharmaceutically acceptable salt thereof (the

"active ingredient") into association with a carrier and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single– or multi–dose unit.

[00267] Pharmaceutical compositions can be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one–half or one–third of such a dosage.

[00268] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the pharmaceutical composition may comprise between 0.1% and 100% (w/w) active ingredient.

[00269] Pharmaceutically acceptable excipients used in the manufacture of provided pharmaceutical compositions include inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and/or oils. Excipients such as cocoa butter and suppository waxes, coloring agents, coating agents, sweetening, flavoring, and perfuming agents may also be present in the composition.

[00270] Exemplary diluents include calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, cornstarch, powdered sugar, *etc.*, and combinations thereof.

[00271] Exemplary granulating and/or dispersing agents include potato starch, corn starch, tapioca starch, sodium starch glycolate, clays, alginic acid, guar gum, citrus pulp, agar, bentonite, cellulose and wood products, natural sponge, cation–exchange resins, calcium carbonate, silicates, sodium carbonate, cross–linked poly(vinyl–pyrrolidone) (crospovidone), sodium carboxymethyl starch (sodium starch glycolate), carboxymethyl cellulose, cross–linked sodium carboxymethyl cellulose (croscarmellose), methylcellulose, pregelatinized starch (starch 1500), microcrystalline starch, water insoluble starch, calcium carboxymethyl

cellulose, magnesium aluminum silicate (Veegum), sodium lauryl sulfate, quaternary ammonium compounds, *etc.*, and combinations thereof.

[00272] Exemplary surface active agents and/or emulsifiers include natural emulsifiers (*e.g.* acacia, agar, alginic acid, sodium alginate, tragacanth, chondrux, cholesterol, xanthan, pectin, gelatin, egg yolk, casein, wool fat, cholesterol, wax, and lecithin), colloidal clays (*e.g.* bentonite [aluminum silicate] and Veegum [magnesium aluminum silicate]), long chain amino acid derivatives, high molecular weight alcohols (*e.g.* stearyl alcohol, cetyl alcohol, oleyl alcohol, triacetin monostearate, ethylene glycol distearate, glyceryl monostearate, and propylene glycol monostearate, polyvinyl alcohol), carbomers (*e.g.* carboxy polymethylene, polyacrylic acid, acrylic acid polymer, and carboxyvinyl polymer), carrageenan, cellulosic derivatives (*e.g.* carboxymethylcellulose sodium, powdered cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose), sorbitan fatty acid esters (*e.g.* polyoxyethylene sorbitan monolaurate (Tween 20), polyoxyethylene sorbitan (Tween 60), polyoxyethylene sorbitan monooleate (Tween 80), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span 60), sorbitan tristearate (Span 65), glyceryl monooleate, sorbitan monooleate (Span 80)), polyoxyethylene esters (*e.g.* polyoxyethylene monostearate (Myrj 45), polyoxyethylene hydrogenated castor oil, polyethoxylated castor oil, polyoxymethylene stearate, and Solutol), sucrose fatty acid esters, polyethylene glycol fatty acid esters (*e.g.* Cremophor), polyoxyethylene ethers, (*e.g.* polyoxyethylene lauryl ether (Brij 30)), poly(vinyl–pyrrolidone), diethylene glycol monolaurate, triethanolamine oleate, sodium oleate, potassium oleate, ethyl oleate, oleic acid, ethyl laurate, sodium lauryl sulfate, Pluronic F-68, Poloxamer P188, cetrimonium bromide, cetylpyridinium chloride, benzalkonium chloride, docusate sodium, *etc.* and/or combinations thereof.

[00273] Exemplary binding agents include starch (*e.g.* cornstarch and starch paste), gelatin, sugars (*e.g.* sucrose, glucose, dextrose, dextrin, molasses, lactose, lactitol, mannitol, *etc.*), natural and synthetic gums (*e.g.* acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, cellulose acetate, poly(vinyl–pyrrolidone), magnesium aluminum silicate (Veegum), and larch arabogalactan), alginates, polyethylene oxide, polyethylene glycol, inorganic calcium salts, silicic acid, polymethacrylates, waxes, water, alcohol, *etc.*, and/or combinations thereof.

[00274] Exemplary preservatives include antioxidants, chelating agents, antimicrobial preservatives, antifungal preservatives, alcohol preservatives, acidic preservatives, and other preservatives.

[00275] Exemplary antioxidants include alpha tocopherol, ascorbic acid, acorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, monothioglycerol, potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabisulfite, and sodium sulfite.

[00276] Exemplary chelating agents include ethylenediaminetetraacetic acid (EDTA) and salts and hydrates thereof (*e.g.*, sodium edetate, disodium edetate, trisodium edetate, calcium disodium edetate, dipotassium edetate, and the like), citric acid and salts and hydrates thereof (*e.g.*, citric acid monohydrate), fumaric acid and salts and hydrates thereof, malic acid and salts and hydrates thereof, phosphoric acid and salts and hydrates thereof, and tartaric acid and salts and hydrates thereof. Exemplary antimicrobial preservatives include benzalkonium chloride, benzethonium chloride, benzyl alcohol, bronopol, cetrimide, cetylpyridinium chloride, chlorhexidine, chlorobutanol, chlorocresol, chloroxylenol, cresol, ethyl alcohol, glycerin, hexetidine, imidurea, phenol, phenoxyethanol, phenylethyl alcohol, phenylmercuric nitrate, propylene glycol, and thimerosal.

[00277] Exemplary antifungal preservatives include butyl paraben, methyl paraben, ethyl paraben, propyl paraben, benzoic acid, hydroxybenzoic acid, potassium benzoate, potassium sorbate, sodium benzoate, sodium propionate, and sorbic acid.

[00278] Exemplary alcohol preservatives include ethanol, polyethylene glycol, phenol, phenolic compounds, bisphenol, chlorobutanol, hydroxybenzoate, and phenylethyl alcohol.

[00279] Exemplary acidic preservatives include vitamin A, vitamin C, vitamin E, beta–carotene, citric acid, acetic acid, dehydroacetic acid, ascorbic acid, sorbic acid, and phytic acid.

[00280] Other preservatives include tocopherol, tocopherol acetate, deteroxime mesylate, cetrimide, butylated hydroxyanisol (BHA), butylated hydroxytoluened (BHT), ethylenediamine, sodium lauryl sulfate (SLS), sodium lauryl ether sulfate (SLES), sodium bisulfite, sodium metabisulfite, potassium sulfite, potassium metabisulfite, Glydant Plus, Phenonip, methylparaben, Germall 115, Germaben II, Neolone, Kathon, and Euxyl. In certain embodiments, the preservative is an anti–oxidant. In other embodiments, the preservative is a chelating agent.

[00281] Exemplary buffering agents include citrate buffer solutions, acetate buffer solutions, phosphate buffer solutions, ammonium chloride, calcium carbonate, calcium

chloride, calcium citrate, calcium glubionate, calcium gluceptate, calcium gluconate, D–gluconic acid, calcium glycerophosphate, calcium lactate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen–free water, isotonic saline, Ringer's solution, ethyl alcohol, *etc.*, and combinations thereof.

[00282] Exemplary lubricating agents include magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, glyceryl behanate, hydrogenated vegetable oils, polyethylene glycol, sodium benzoate, sodium acetate, sodium chloride, leucine, magnesium lauryl sulfate, sodium lauryl sulfate, *etc.*, and combinations thereof.

[00283] Exemplary natural oils include almond, apricot kernel, avocado, babassu, bergamot, black current seed, borage, cade, camomile, canola, caraway, carnauba, castor, cinnamon, cocoa butter, coconut, cod liver, coffee, corn, cotton seed, emu, eucalyptus, evening primrose, fish, flaxseed, geraniol, gourd, grape seed, hazel nut, hyssop, isopropyl myristate, jojoba, kukui nut, lavandin, lavender, lemon, litsea cubeba, macademia nut, mallow, mango seed, meadowfoam seed, mink, nutmeg, olive, orange, orange roughy, palm, palm kernel, peach kernel, peanut, poppy seed, pumpkin seed, rapeseed, rice bran, rosemary, safflower, sandalwood, sasquana, savoury, sea buckthorn, sesame, shea butter, silicone, soybean, sunflower, tea tree, thistle, tsubaki, vetiver, walnut, and wheat germ oils. Exemplary synthetic oils include, but are not limited to, butyl stearate, caprylic triglyceride, capric triglyceride, cyclomethicone, diethyl sebacate, dimethicone 360, isopropyl myristate, mineral oil, octyldodecanol, oleyl alcohol, silicone oil, and combinations thereof.

[00284] Liquid dosage forms for oral and parenteral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredients, the liquid dosage forms may comprise 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, dimethylformamide, oils (*e.g.*, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and

mixtures thereof. Besides inert diluents, the oral compositions can include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents. In certain embodiments for parenteral administration, the active ingredient is mixed with solubilizing agents such as Cremophor, alcohols, oils, modified oils, glycols, polysorbates, cyclodextrins, polymers, and combinations thereof.

[00285] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions can be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation can be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3–butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono– or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

[00286] The injectable formulations can be sterilized, for example, by filtration through a bacterial–retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[00287] In order to prolong the effect of a drug, it is often desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This can be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

[00288] Compositions for rectal or vaginal administration are typically suppositories which can be prepared by mixing the active ingredient with suitable non–irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active ingredient.

[00289] Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active ingredient is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium

phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may comprise buffering agents.

[00290] Solid compositions of a similar type can be employed as fillers in soft and hard–filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally comprise opacifying agents and can be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. Solid compositions of a similar type can be employed as fillers in soft and hard–filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polethylene glycols and the like.

[00291] The active ingredients can be in micro–encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active ingredient can be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may comprise, as is normal practice, additional substances other than inert diluents, *e.g.*, tableting lubricants and other tableting aids such a magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may comprise buffering agents. They may optionally comprise opacifying agents and can be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

[00292] Dosage forms for topical and/or transdermal administration of the active ingredient may include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants and/or patches. Generally, the active ingredient is admixed under sterile conditions with a pharmaceutically acceptable carrier and/or any needed preservatives and/or buffers as can be required. Additionally, the transdermal patches are contemplated, which often have the added advantage of providing controlled delivery of an active ingredient to the body. Such dosage forms can be prepared, for example, by dissolving and/or dispensing the active ingredient in the proper medium. Alternatively or additionally, the rate can be controlled by either providing a rate controlling membrane and/or by dispersing the active ingredient in a polymer matrix and/or gel.

[00293] Suitable devices for use in delivering intradermal pharmaceutical compositions described herein include short needle devices such as those described in U.S. Patents 4,886,499; 5,190,521; 5,328,483; 5,527,288; 4,270,537; 5,015,235; 5,141,496; and 5,417,662. Intradermal compositions can be administered by devices which limit the effective penetration length of a needle into the skin, such as those described in PCT publication WO 99/34850 and functional equivalents thereof. Jet injection devices which deliver liquid vaccines to the dermis via a liquid jet injector and/or via a needle which pierces the stratum corneum and produces a jet which reaches the dermis are suitable. Jet injection devices are described, for example, in U.S. Patents 5,480,381; 5,599,302; 5,334,144; 5,993,412; 5,649,912; 5,569,189; 5,704,911; 5,383,851; 5,893,397; 5,466,220; 5,339,163; 5,312,335; 5,503,627; 5,064,413; 5,520,639; 4,596,556; 4,790,824; 4,941,880; 4,940,460; and PCT publications WO 97/37705 and WO 97/13537. Ballistic powder/particle delivery devices which use compressed gas to accelerate vaccine in powder form through the outer layers of the skin to the dermis are suitable. Alternatively or additionally, conventional syringes can be used in the classical mantoux method of intradermal administration.

[00294] Formulations suitable for topical administration include, but are not limited to, liquid and/or semi liquid preparations such as liniments, lotions, oil in water and/or water in oil emulsions such as creams, ointments and/or pastes, and/or solutions and/or suspensions. Topically–administrable formulations may, for example, comprise from about 1% to about 10% (w/w) active ingredient, although the concentration of the active ingredient can be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

[00295] A pharmaceutical composition can be prepared, packaged, and/or sold in a formulation suitable for pulmonary administration via the buccal cavity. Such a formulation may comprise dry particles which comprise the active ingredient and which have a diameter in the range from about 0.5 to about 7 nanometers or from about 1 to about 6 nanometers. Such compositions are conveniently in the form of dry powders for administration using a device comprising a dry powder reservoir to which a stream of propellant can be directed to disperse the powder and/or using a self propelling solvent/powder dispensing container such as a device comprising the active ingredient dissolved and/or suspended in a low–boiling propellant in a sealed container. Such powders comprise particles wherein at least 98% of the particles by weight have a diameter greater than 0.5 nanometers and at least 95% of the particles by number have a diameter less than 7 nanometers. Alternatively, at least 95% of the particles by weight have a diameter greater than 1 nanometer and at least 90% of the particles by number have a diameter less than 6 nanometers. Dry powder compositions may include a solid fine powder diluent such as sugar and are conveniently provided in a unit dose form.

[00296] Low boiling propellants generally include liquid propellants having a boiling point of below 65 °F at atmospheric pressure. Generally the propellant may constitute 50 to 99.9% (w/w) of the composition, and the active ingredient may constitute 0.1 to 20% (w/w) of the composition. The propellant may further comprise additional ingredients such as a liquid non–ionic and/or solid anionic surfactant and/or a solid diluent (which may have a particle size of the same order as particles comprising the active ingredient).

[00297] Pharmaceutical compositions formulated for pulmonary delivery may provide the active ingredient in the form of droplets of a solution and/or suspension. Such formulations can be prepared, packaged, and/or sold as aqueous and/or dilute alcoholic solutions and/or suspensions, optionally sterile, comprising the active ingredient, and may conveniently be administered using any nebulization and/or atomization device. Such formulations may further comprise one or more additional ingredients including, but not limited to, a flavoring agent such as saccharin sodium, a volatile oil, a buffering agent, a surface active agent, and/or a preservative such as methylhydroxybenzoate. The droplets provided by this route of administration may have an average diameter in the range from about 0.1 to about 200 nanometers.

[00298] Formulations described herein as being useful for pulmonary delivery are useful for intranasal delivery of a pharmaceutical composition. Another formulation suitable for intranasal administration is a coarse powder comprising the active ingredient and having an

average particle from about 0.2 to 500 micrometers. Such a formulation is administered by rapid inhalation through the nasal passage from a container of the powder held close to the nares.

[00299] Formulations for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of the active ingredient, and may comprise one or more of the additional ingredients described herein. A pharmaceutical composition can be prepared, packaged, and/or sold in a formulation for buccal administration. Such formulations may, for example, be in the form of tablets and/or lozenges made using conventional methods, and may contain, for example, 0.1 to 20% (w/w) active ingredient, the balance comprising an orally dissolvable and/or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations for buccal administration may comprise a powder and/or an aerosolized and/or atomized solution and/or suspension comprising the active ingredient. Such powdered, aerosolized, and/or aerosolized formulations, when dispersed, may have an average particle and/or droplet size in the range from about 0.1 to about 200 nanometers, and may further comprise one or more of the additional ingredients described herein.

[00300] A pharmaceutical composition can be prepared, packaged, and/or sold in a formulation for ophthalmic administration. Such formulations may, for example, be in the form of eye drops including, for example, a 0.1/1.0% (w/w) solution and/or suspension of the active ingredient in an aqueous or oily liquid carrier. Such drops may further comprise buffering agents, salts, and/or one or more other of the additional ingredients described herein. Other opthalmically–administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form and/or in a liposomal preparation. Ear drops and/or eye drops are contemplated as being within the scope of this disclosure.

[00301] Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with ordinary experimentation.

[00302] Still further contemplated are kits (*e.g.*, pharmaceutical packs). The kits provided may comprise a pharmaceutical composition or compound of Formula (**I**) or (**II**) or pharmaceutically acceptable salt thereof and a container (*e.g.*, a vial, ampule, bottle, syringe, and/or dispenser package, or other suitable container). In some embodiments, provided kits may optionally further include a second container comprising a pharmaceutical excipient for dilution or suspension of a pharmaceutical composition or compound of Formula (**I**) or (**II**) or pharmaceutically acceptable salt thereof. In some embodiments, the pharmaceutical composition or compound of Formula (**I**) or (**II**) or pharmaceutically acceptable salt thereof provided in the container and the second container are combined to form one unit dosage form.

[00303] Optionally, a single container may comprise one or more compartments for containing a pharmaceutical composition or compound of Formula (**I**) or (**II**) or pharmaceutically acceptable salt thereof, and/or a pharmaceutically acceptable excipient for suspension or dilution. In some embodiments, a single container can be appropriate for modification such that the container may receive a physical modification so as to allow combination of compartments and/or components of individual compartments. For example, a foil or plastic bag may comprise two or more compartments separated by a perforated seal which can be broken so as to allow combination of contents of two individual compartments once the signal to break the seal is generated. A kit may thus comprise such multi-compartment containers providing a pharmaceutical composition or compound of Formula (**I**) or (**II**) or pharmaceutically acceptable salt thereof and one or more pharmaceutically acceptable excipients.

[00304] Optionally, instructions for use are additionally provided in such kits. Such instructions may provide, generally, for example, instructions for dosage and administration. In other embodiments, instructions may further provide additional detail relating to specialized instructions for particular containers and/or systems for administration. Still further, instructions may provide specialized instructions for use in conjunction and/or in combination with an additional therapeutic agent.

Methods of Use and Treatment

[00305] Further provided are methods of using a compound of Formula (**I**) or (**II**) or a pharmaceutically acceptable salt thereof, as described herein.

[00306] For example, in one aspect, provided are methods of treating a microbial infection in a subject, comprising administering an effective amount of a compound of Formula (**I**) or (**II**) or a pharmaceutically acceptable salt thereof to the subject in need thereof.

[00307] In another aspect, provided is a method of treating microbial virulence. Such a method can be conducted by contacting the compound of Formula (**I**) or (**II**) or a pharmaceutically acceptable salt thereof *in vivo* (*i.e.*, by administration to a subject) or *in vitro* (*e.g.*, upon contact with the microorganism in a cell culture).

[00308] For example, in certain embodiments, provided is a method of treating microbial virulence comprising contacting an effective amount of the a compound of Formula (**I**) or (**II**) or a pharmaceutically acceptable salt thereof with a microorganism. In certain embodiments, provided is an *in vitro* method of treating microbial virulence comprising contacting an effective amount of a compound of Formula (**I**) or (**II**) or a pharmaceutically acceptable salt thereof with a microorganism in a cell culture. In certain embodiments, provided is an *in vivo* method of treating microbial virulence comprising administering an effective amount of a compound of Formula (**I**) or (**II**) or a pharmaceutically acceptable salt thereof to a subject with a microbial infection. In certain embodiments, compound of Formula (**I**) or (**II**) or a pharmaceutically acceptable salt thereof blocks virulence factor production. In certain embodiments, the microbial virulence is bacterial virulence, and the microorganism is a bacterium.

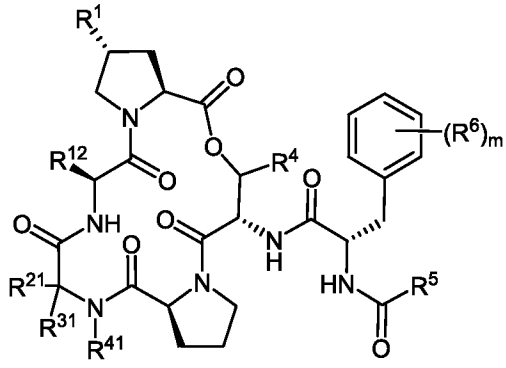
[00309] In yet another aspect, provided is a method of potentiating the anti-bacterial activity of an anti-bacterial agent against a bacterium comprising delivering the anti-bacterial agent in combination with a compound of Formula (**I**) or (**II**) or a pharmaceutically acceptable salt thereof *in vivo* (*i.e.*, by administration to a subject) or *in vitro* (*e.g.*, upon contact with the microorganism in a cell culture) to the bacterium.

[00310] For example, in certain embodiments, provided is a method of potentiating the activity of an anti-bacterial agent against a bacterium comprising contacting the anti-bacterial agent in combination with a compound of Formula (**I**) or (**II**) or a pharmaceutically acceptable salt thereof with a bacterium. In certain embodiments, provided is an *in vitro* method of potentiating the activity of an anti-bacterial agent against a bacterium comprising contacting the anti-bacterial agent in combination with a compound of Formula (**I**) or (**II**) or a pharmaceutically acceptable salt thereof with a bacterium in a cell culture. In certain

embodiments, provided is an *in vivo* method of potentiating the activity of an anti-bacterial agent against a bacterium comprising adminstering the anti-bacterial agent in combination with a compound of Formula (**I**) or (**II**) or a pharmaceutically acceptable salt thereof to a subject with a bacterial infection. In certain embodiments, the fragment used in the potentiation therapy is a compound of Formula (**II**) or a pharmaceutically acceptable salt thereof.

[00311] As used herein, "potentiate" or "potentiating" refers to the ability of the compound of Formula (**I**) or (**II**) to improve (*i.e.,* increase) the effectiveness of the anti-bacterial agent against a bacterium. In certain embodiments, the potentiation is synergistic (*i.e.,* the combination results in a non-linear increase in effectiveness). In certain embodiments, the potentiation is additive. In certain embodiments, the effectiveness is measured by a reduction of MIC of the anti-bacterial agent, *e.g.,* by about 1 ug/mL and about 50 ug/mL, inclusive, compared to the MIC of the anti-bacterial agent administered alone. In certain embodiments, the potentiation is a result of the compound of Formula (**I**) or (**II**) or a pharmaceutically acceptable salt thereof competitively interfering with the efflux pumps of the bacterium. In certain embodiments, the ratio of the compound of Formula (**I**) or (**II**) or a pharmaceutically acceptable salt thereof to the anti-bacterial agent (molar excess) is between about 1:1 and about 100:1, inclusive; *e.g.,* between about 1:1 and about 90:1, between about 1:1 and about 80:1, between about 1:1 and about 70:1, between about 1:1 and about 60:1, between about 1:1 and about 50:1, between about 1:1 and about 40:1, between about 1:1 and about 30:1, between about 1:1 and about 20:1, between about 1:1 and about 10:1, between about 2:1 and about 50:1, between about 2:1 and about 40:1 between about 2:1 and about 30:1, between about 2:1 and about 20:1, between about 5:1 and about 50:1, between about 5:1 and about 40:1, between about 5:1 and about 30:1, between about 5:1 and about 20:1, between about 5:1 and about 10:1, between about 10:1 and about 50:1, between about 10:1 and about 40:1, between about 10:1 and about 30:1, or between about 10:1 and about 20:1, inclusive.

[00312] In certain embodiments, the anti-bacterial agent is any one of the agents described herein as additional therapeutically effective anti-bacterial agents. See *infra*. For example, in certain embodiments, the anti-baterial agent used in combination with a compound of Formula (**I**) or (**II**) or pharmaceutically acceptable salt thereof is reserpine. In certain embodiments, the anti-bacterial agent used in combination with a compound of Formula (**I**) or (**II**) or pharmaceutically acceptable salt thereof is an enopeptin (ADEP) compound, *e.g.,* as described in PCT Publication Application No. WO/2012/135615. In certain embodiments, the anti-bacterial agent is an enopeptin (ADEP) compound, *e.g.,* of Formula (**A**):



(A)

or a pharmaceutically acceptable salt thereof; wherein R^1, R^4, R^5, R^6, and m are as defined herein; further wherein:

R^{21} is hydrogen, and R^{31} and R^{41} are joined to form an optionally substituted heterocyclyl; or

R^{21} and R^{31} are joined to form a spiro-fused optionally substituted carbocyclyl or spiro-fused optionally substituted heterocyclyl, and R^{41} is hydrogen, optionally substituted alkyl, or an amino protecting group;

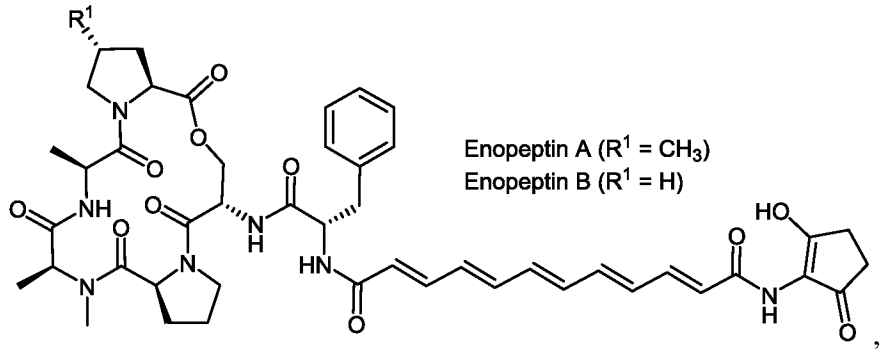
or

R^{21} and R^{31} are independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl, provided that R^{21} and R^{31} are not both hydrogen; and R^{41} is hydrogen, optionally substituted alkyl, or an amino protecting group;

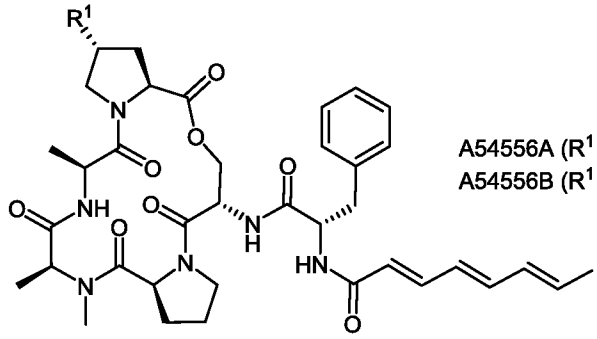
and

R^{12} is hydrogen, –OH, –SH, $–NH_2$, substituted hydroxyl, substituted thiol, substituted amino, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl.

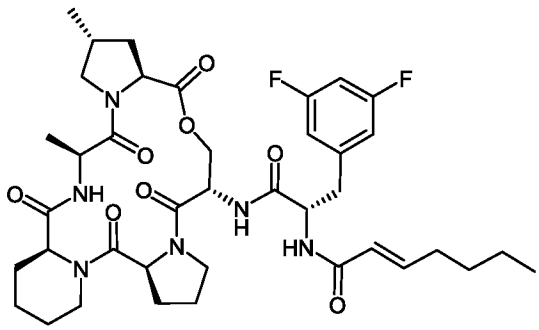
[00313] Exemplary ADEPs of Formula (**A**) contemplated useful in and potentiated by the combination with a compound of Formula (**I**) or (**II**) include, but are not limited to:



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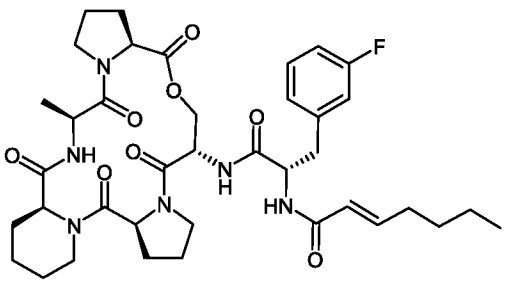


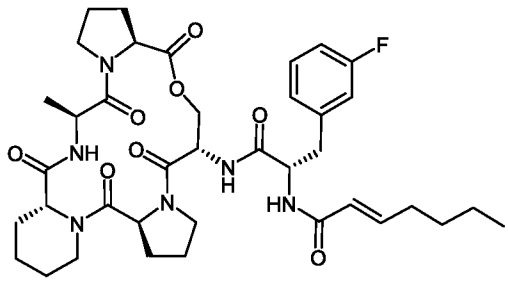
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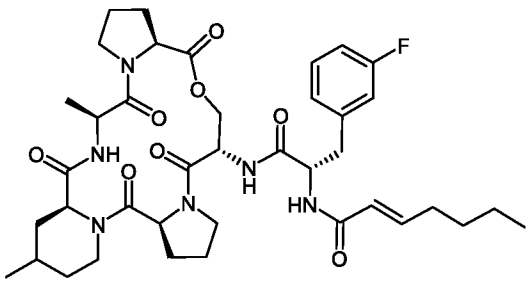


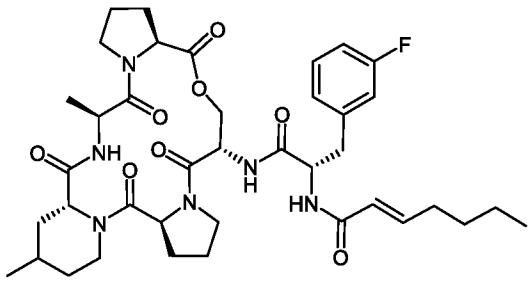
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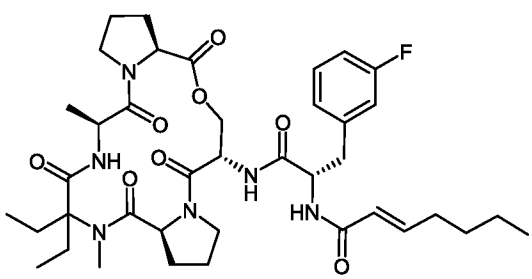
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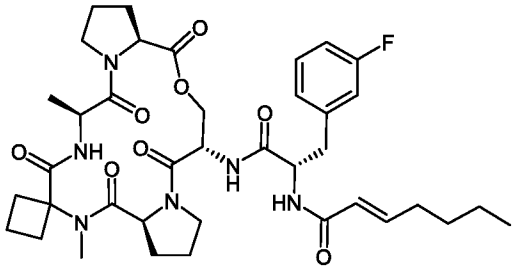


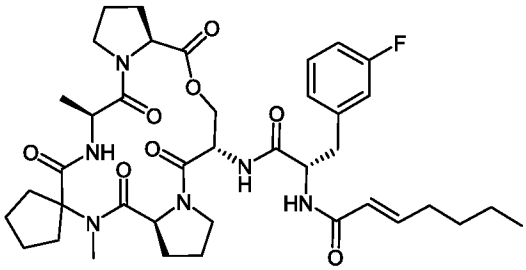


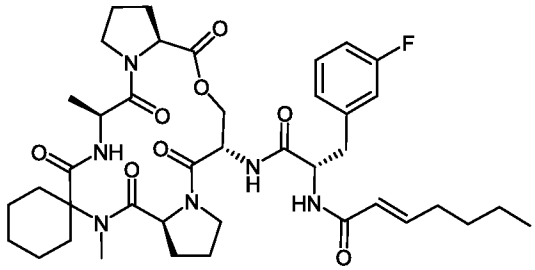


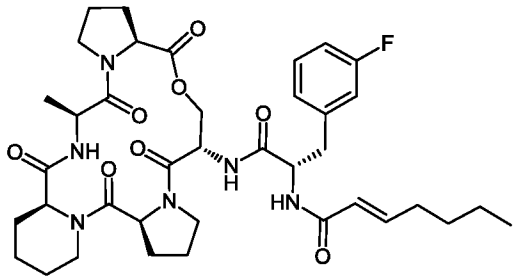


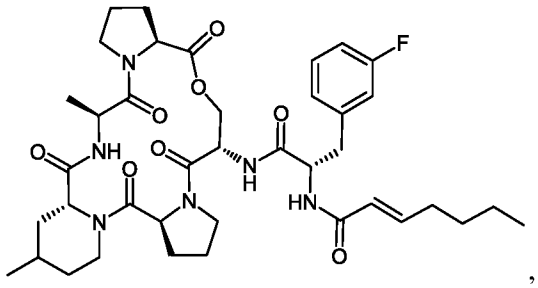


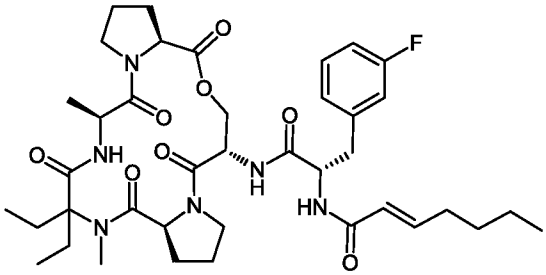


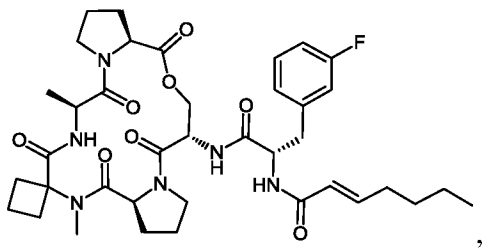


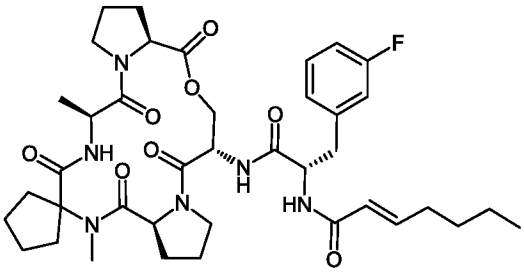


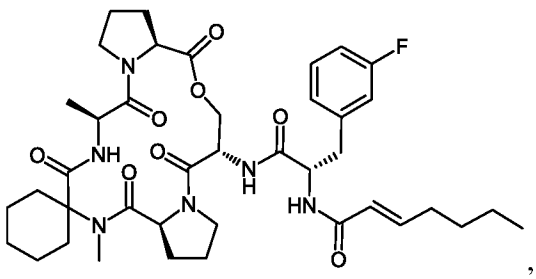
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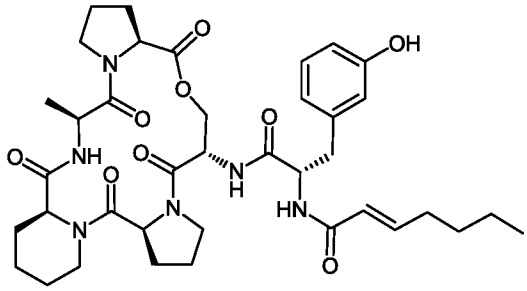
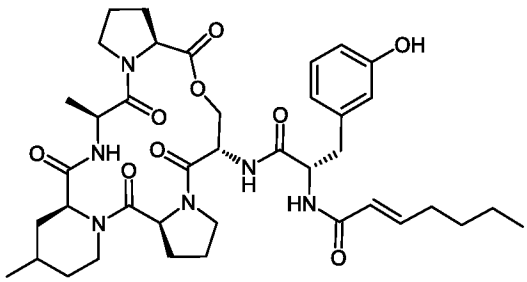
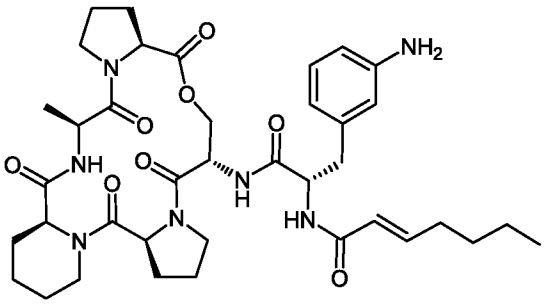
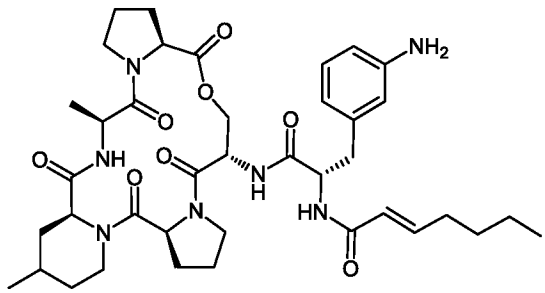
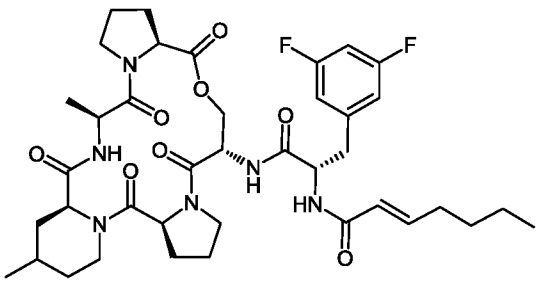
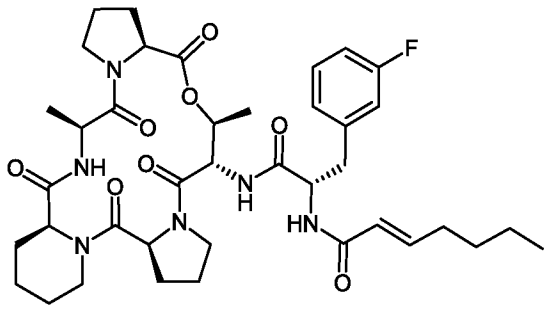
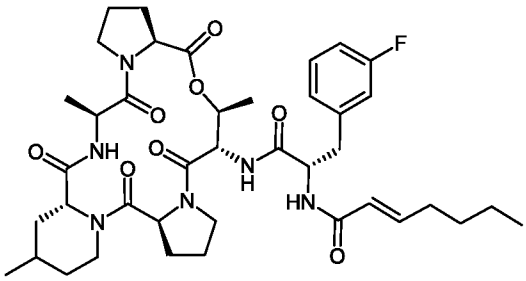
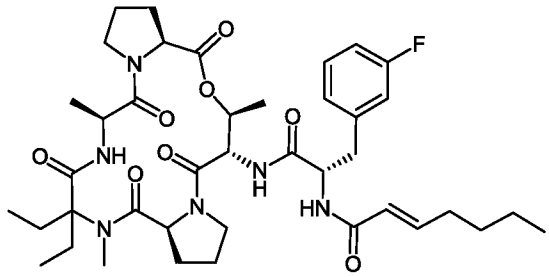
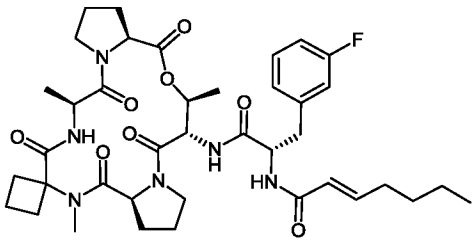
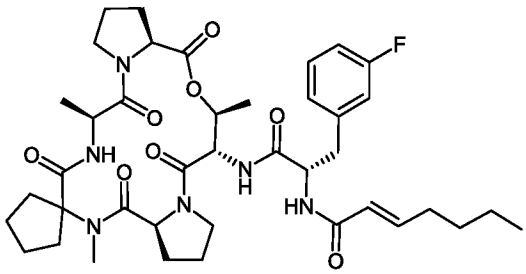
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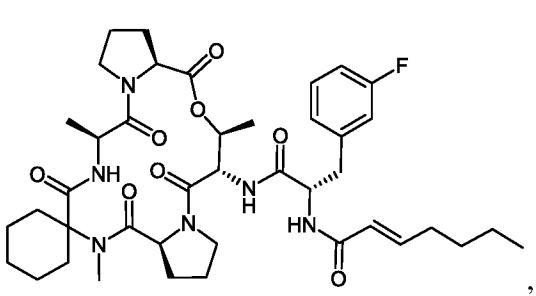
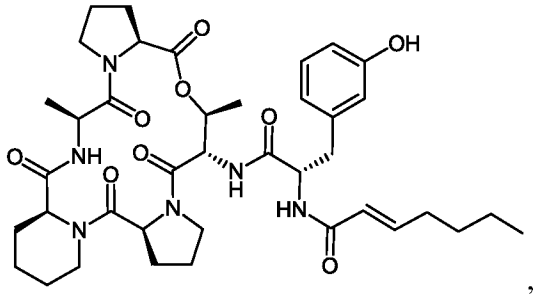
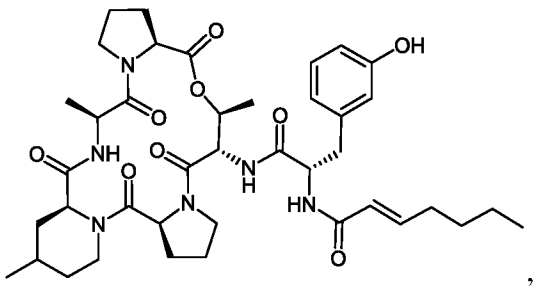
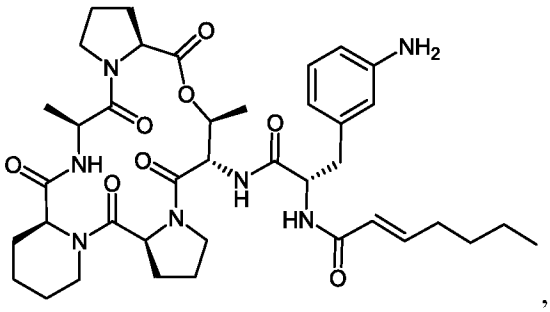
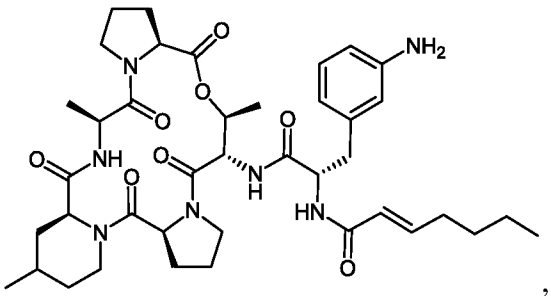
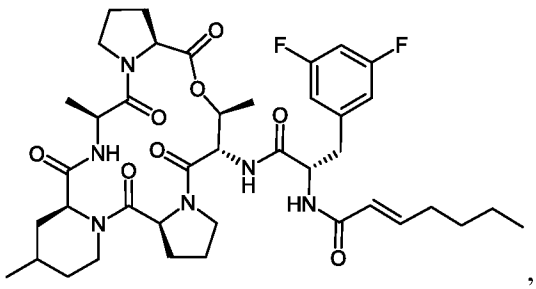
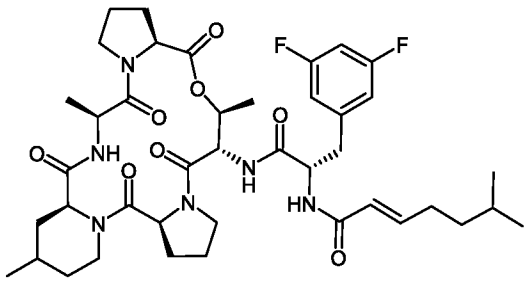
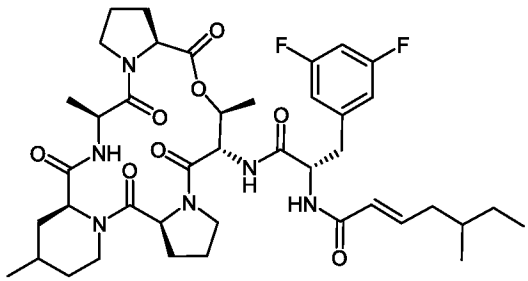
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and pharmaceutically acceptable salts thereof.

[00314] As used herein, a "microbial infection" refers to an infection with a microorganism, such as a fungus, bacteria or virus. In certain embodiments, the microbial infection is an infection with a fungus, *i.e.*, a fungal infection. In certain embodiments, the microbial infection is an infection with a virus, *i.e.*, a viral infection. In certain embodiments, the microbial infection is an infection with a bacteria, *i.e.*, a bacterial infection. Various microbial infections include, but are not limited to, skin infections, GI infections, urinary tract infections, genito-urinary infections, sepsis, blood infections, and systemic infections.

[00315] In certain embodiments, the microbial infection is an infection with a bacteria, *i.e.*, a bacterial infection. In certain embodiments, a compound of Formula (**I**) or (**II**) or a pharmaceutically acceptable salt thereof exhibits anti-bacterial activity. For example, in certain embodiments, a compound of Formula (**I**) or (**II**) or a pharmaceutically acceptable salt thereof has a mean inhibitory concentration (MIC), with respect to a particular bacterium, of less than 50 μg/mL, less than 25 μg/mL, 1ess than 10 μg/mL, less than 5 μg/mL, 1ess than 3 μg/mL, or less than 1 μg/mL.

[00316] Exemplary bacterial infections include, but are not limited to, infections with a gram positive bacteria (*e.g.*, of the phylum *Actinobacteria,* phylum *Firmicutes*, or phylum *Tenericutes*); gram negative bacteria (*e.g.*, of the phylum *Aquificae*, phylum *Deinococcus-Thermus*, phylum *Fibrobacteres/Chlorobi/Bacteroidetes* (FCB), phylum *Fusobacteria*, phylum *Gemmatimonadest*, phylum *Ntrospirae*, phylum *Planctomycetes/Verrucomicrobia/Chlamydiae* (PVC), phylum *Proteobacteria*, phylum *Spirochaetes*, or phylum *Synergistetes*); or other bacteria (*e.g.*, of the phylum *Acidobacteria*, phylum *Chlroflexi*, phylum *Chrystiogenetes*, phylum *Cyanobacteria*, phylum *Deferrubacteres*, phylum *Dictyoglomi*, phylum *Thermodesulfobacteria*, or phylum *Thermotogae*).

[00317] In certain embodiments, the bacterial infection is an infection with a gram positive bacteria.

[00318] In certain embodiments, the gram positive bacteria is a bacteria of the phylum *Actinobacteria.*

[00319] In certain embodiments, the bacteria is a member of the phylum *Actinobacteria* and the genus *Mycobacteriium*, *i.e.*, the bacterial infection is a *Mycobacteriium* infection. Exemplary *Mycobacteriium* bacteria include, but are not limited to, *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. canetti*, *M. caprae*, *M. microti*, and *M. pinnipedii*. In certain embodiments, the *Mycobacteriium* infection is an *M. tuberculosis* infection.

[00320] In certain embodiments, the bacteria is a member of the phylum *Actinobacteria* and the genus *Streptomyces, i.e.*, the bacterial infection is a *Streptomyces* infection. Exemplary *Streptomyces* bacteria include, but are not limited to, *S. coelicolor.*

[00321] In certain embodiments, the gram positive bacteria is a bacteria of the phylum *Firmicutes*.

[00322] In certain embodiments, the bacteria is a member of the phylum *Firmicutes* and the genus *Enterococcus*, *i.e.*, the bacterial infection is an *Enterococcus* infection. Exemplary *Enterococci* bacteria include, but are not limited to, *E. avium*, *E. durans*, *E. faecalis*, *E.*

faecium, *E. gallinarum*, *E. solitarius*, *E. casseliflavus*, and *E. raffinosus*. In certain embodiments, the *Enterococcus* infection is an *E. faecalis* infection. In certain embodiments, the *Enterococcus* infection is an *E. faecium* infection.

[00323] In certain embodiments, the bacteria is a member of the phylum *Firmicutes* and the genus *Staphylococcus*, *i.e.*, the bacterial infection is a *Staphylococcus* infection. Exemplary *Staphylococci* bacteria include, but are not limited to, *S. arlettae, S. aureus, S. auricularis, S. capitis, S. caprae, S. carnous, S. chromogenes, S. cohii, S. condimenti, S. croceolyticus, S. delphini, S. devriesei, S. epidermis, S. equorum, S. felis, S. fluroettii, S. gallinarum, S. haemolyticus, S. hominis, S. hyicus, S. intermedius, S. kloosii, S. leei, S. lenus, S. lugdunesis, S. lutrae, S. lyticans, S. massiliensis, S. microti, S. muscae, S. nepalensis, S. pasteuri, S. penttenkoferi, S. piscifermentans, S. psuedointermedius, S. psudolugdensis, S. pulvereri, S. rostri, S. saccharolyticus, S. saprophyticus, S. schleiferi, S. sciuri, S. simiae, S. simulans, S. stepanovicii, S. succinus, S. vitulinus, S. warneri,* and *S. xylosus*. In certain embodiments, the *Staphylococcus* infection is an *S. aureus* infection. In certain embodiments, the *Staphylococcus* infection is an *S. epidermis* infection.

[00324] In certain embodiments, the bacteria is a member of the phylum *Firmicutes* and the genus *Bacillus*, *i.e.*, the bacterial infection is a *Bacillus* infection. Exemplary *Bacillus* bacteria include, but are not limited to, *B. alcalophilus*, *B. alvei*, *B. aminovorans*, *B. amyloliquefaciens*, *B. aneurinolyticus*, *B. anthracis*, *B. aquaemaris*, *B. atrophaeus*, *B. boroniphilus*, *B. brevis*, *B. caldolyticus*, *B. centrosporus*, *B. cereus*, *B. circulans*, *B. coagulans*, *B. firmus*, *B. flavothermus*, *B. fusiformis*, *B. globigii*, *B. infernus*, *B. larvae*, *B. laterosporus*, *B. lentus*, *B. licheniformis*, *B. megaterium*, *B. mesentericus*, *B. mucilaginosus*, *B. mycoides*, *B. natto*, *B. pantothenticus*, *B. polymyxa*, *B. pseudoanthracis*, *B. pumilus*, *B. schlegelii*, *B. sphaericus*, *B. sporothermodurans*, *B. stearothermophilus*, *B. subtilis*, *B. thermoglucosidasius*, *B. thuringiensis*, *B. vulgatis*, and *B. weihenstephanensis*. In certain embodiments, the *Bacillus* infection is a *B. subtilis* infection.

[00325] In certain embodiments, the bacteria is a member of the phylum *Firmicutes* and the genus *Strepococcus*, *i.e.*, the bacterial infection is a *Strepococcus* infection. Exemplary *Strepococcus* bacteria include, but are not limited to, *S. agalactiae*, *S. anginosus*, *S. bovis*, *S. canis*, *S. constellatus*, *S. dysgalactiae*, *S. equinus*, *S. iniae*, *S. intermedius*, *S. mitis*, *S. mutans*, *S. oralis*, *S. parasanguinis*, *S. peroris*, *S. pneumoniae*, *S. pyogenes*, *S. ratti*, *S. salivarius*, *S. thermophilus*, *S. sanguinis*, *S. sobrinus*, *S. suis*, *S. uberis*, *S. vestibularis*, *S. viridans*, and *S. zooepidemicus*. In certain embodiments, the *Strepococcus* infection is an *S. pyogenes* infection. In certain embodiments, the *Strepococcus* infection is an *S. pneumoniae* infection.

[00326] In certain embodiments, the bacterial infection is an infection with a Gram negative bacteria.

[00327] In certain embodiments, the Gram negtive bacteria is a bacteria of the phylum *Proteobacteria* and the genus *Escherichia. i.e.,* the bacterial infection is an *Escherichia* infection. Exemplary *Escherichia* bacteria include, but are not limited to, *E. albertii*, *E. blattae*, *E. coli*, *E. fergusonii*, *E. hermannii*, and *E. vulneris.* In certain embodiments, the *Escherichia* infection is an *E. coli* infection.

[00328] In certain embodiments, the Gram negtive bacteria is a bacteria of the phylum *Proteobacteria* and the genus *Haemophilus. i.e.,* the bacterial infection is an *Haemophilus* infection. Exemplary *Haemophilus* bacteria include, but are not limited to, *H. aegyptius*, *H. aphrophilus*, *H. avium*, *H. ducreyi*, *H. felis*, *H. haemolyticus*, *H. influenzae*, *H. parainfluenzae*, *H. paracuniculus*, *H. parahaemolyticus*, *H. pittmaniae*, *Haemophilus segnis*, and *H. somnus.* In certain embodiments, the *Escherichia* infection is an *H. influenzae* infection.

[00329] In certain embodiments, the bacterial infection is resistant to other antibiotic therapy. For example, in certain embodiments, the bacterial infection is vancomycin resistant (VR). In certain embodiments, the bacterial infection is a methicillin-resistant (MR). In certain embodiments, the bacterial infection is a methicillin-resistant *S. aureus* (MRSA) infection.

[00330] In yet another aspect, provided is a method of treating bacterial infection and/or virulence including the treatment of bacteria or infection caused by bacteria that are resistent to other treatments, are multi-drug tolerant or resistent and/or that neither grow nor die in the presence of or as a result of other treatments. Such a method can be conducted *in vivo* (*i.e.*, by administration to a subject) or *in vitro* (*e.g.*, upon contact with bacteria in a cell culture). For example, in certain embodiments, provided is a method of treating bacterial virulence comprising administering an effective amount of a compound of Formula (**I**) or (**II**) or a pharmaceutically acceptable salt thereof, to a subject with a bacterial infection. In certain embodiments, the compound blocks virulence factor production.

[00331] In another aspect, a compound of Formula (**I**) or (**II**) or pharmaceutically acceptable salt thereof may inhibit the growth of or kill rapidly dividing cells such as stimulated inflammatory cells. Thus, further contemplated herein are methods of treating a disease, disorder, or condition associated with abnormal cellular proliferation, such as cancer, autoimmune diseases, inflammatory diseases, and diabetic retinopathy.

[00332] Thus, in one aspect, provided is a method of treating cancer comprising administering an effective amount of a compound of Formula (**I**) or (**II**) or pharmaceutically acceptable salt thereof to a subject.

[00333] In another aspect, provided is a method of treating an autoimmune disease comprising administering an effective amount of a compound of Formula (**I**) or (**II**) or pharmaceutically acceptable salt thereof to a subject.

[00334] In yet another aspect, provided is a method of treating an inflammatory disease comprising administering a compound of Formula (**I**) or (**II**) or pharmaceutically acceptable salt thereof to a subject.

[00335] In yet another aspect, provided is a method of treating diabetic retinopathy comprising administering an effective amount of a compound of Formula (**I**) or (**II**) or pharmaceutically acceptable salt thereof to a subject.

[00336] Compounds provided herein are typically formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of the compositions will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject or organism will depend upon a variety of factors including the disease, disorder, or condition being treated and the severity of the disorder; the activity of the specific active ingredient employed; the specific composition employed; the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific active ingredient employed; the duration of the treatment; drugs used in combination or coincidental with the specific active ingredient employed; and like factors well known in the medical arts.

[00337] The compounds and compositions provided herein can be administered by any route, including enteral (*e.g.*, oral), parenteral, intravenous, intramuscular, intra–arterial, intramedullary, intrathecal, subcutaneous, intraventricular, transdermal, interdermal, rectal, intravaginal, intraperitoneal, topical (as by powders, ointments, creams, and/or drops), mucosal, nasal, bucal, sublingual; by intratracheal instillation, bronchial instillation, and/or inhalation; and/or as an oral spray, nasal spray, and/or aerosol. Specifically contemplated routes are oral administration, intravenous administration (*e.g.*, systemic intravenous injection), regional administration via blood and/or lymph supply, and/or direct administration to an affected site. In general the most appropriate route of administration will depend upon a variety of factors including the nature of the agent (*e.g.*, its stability in the

environment of the gastrointestinal tract), the condition of the subject (*e.g.*, whether the subject is able to tolerate oral administration), *etc.*

[00338] The exact amount of a compound required to achieve an effective amount will vary from subject to subject, depending, for example, on species, age, and general condition of a subject, severity of the side effects or disorder, identity of the particular compound(s), mode of administration, and the like. The desired dosage can be delivered three times a day, two times a day, once a day, every other day, every third day, every week, every two weeks, every three weeks, or every four weeks. In certain embodiments, the desired dosage can be delivered using multiple administrations (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations).

[00339] In certain embodiments, an effective amount of a compound for administration one or more times a day to a 70 kg adult human may comprise about 0.0001 mg to about 3000 mg, about 0.0001 mg to about 2000 mg, about 0.0001 mg to about 1000 mg, about 0.001 mg to about 1000 mg, about 0.01 mg to about 1000 mg, about 0.1 mg to about 1000 mg, about 1 mg to about 1000 mg, about 1 mg to about 100 mg, about 10 mg to about 1000 mg, or about 100 mg to about 1000 mg, of a compound per unit dosage form.

[00340] In certain embodiments, the compound may be administered orally or parenterally at dosage levels sufficient to deliver from about 0.001 mg/kg to about 100 mg/kg, from about 0.01 mg/kg to about 50 mg/kg, preferably from about 0.1 mg/kg to about 40 mg/kg, preferably from about 0.5 mg/kg to about 30 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, and more preferably from about 1 mg/kg to about 25 mg/kg, of subject body weight per day, one or more times a day, to obtain the desired therapeutic effect.

[00341] It will be appreciated that dose ranges as described herein provide guidance for the administration of provided pharmaceutical compositions to an adult. The amount to be administered to, for example, a child or an adolescent can be determined by a medical practitioner or person skilled in the art and can be lower or the same as that administered to an adult.

[00342] It will be also appreciated that a compound of Formula (**I**) or (**II**) or pharmaceutically acceptable salt thereof or composition, as described herein, can be administered in combination with one or more additional therapeutically active agents. The compounds or compositions can be administered in combination with additional therapeutically active agents that improve and/or potentiate their bioavailability, reduce and/or modify their metabolism, inhibit their excretion, and/or modify their distribution

within the body. It will also be appreciated that the therapy employed may achieve a desired effect for the same disorder, and/or it may achieve different effects.

[00343] The compound of Formula (**I**) or (**II**) or pharmaceutically acceptable salt thereof or composition can be administered concurrently with, prior to, or subsequent to, one or more additional therapeutically active agents. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent. In will further be appreciated that the additional therapeutically active agent utilized in this combination can be administered together in a single composition or administered separately in different compositions. The particular combination to employ in a regimen will take into account compatibility of the active ingredient with the additional therapeutically active agent and/or the desired therapeutic effect to be achieved. In general, it is expected that additional therapeutically active agents utilized in combination be utilized at levels that do not exceed the levels at which they are utilized individually. In some embodiments, the levels utilized in combination will be lower than those utilized individually.

[00344] Exemplary additional therapeutically active agents include, but are not limited to, antibiotics, anti–viral agents, anesthetics, anti–coagulants, inhibitors of an enzyme, steroidal agents, steroidal or non–steroidal anti–inflammatory agents, antihistamine, immunosuppressant agents, antigens, vaccines, antibodies, decongestant, sedatives, opioids, pain–relieving agents, analgesics, anti–pyretics, hormones, and prostaglandins, *etc.* Therapeutically active agents include small organic molecules such as drug compounds (*e.g.*, compounds approved by the US Food and Drug Administration as provided in the Code of Federal Regulations (CFR)), peptides, proteins, carbohydrates, monosaccharides, oligosaccharides, polysaccharides, nucleoproteins, mucoproteins, lipoproteins, synthetic polypeptides or proteins, small molecules linked to proteins, glycoproteins, steroids, nucleic acids, DNAs, RNAs, nucleotides, nucleosides, oligonucleotides, antisense oligonucleotides, lipids, hormones, vitamins and cells.

[00345] In certain embodiments, the additional therapeutically agent is an anti-bacterial agent (an antibiotic). Exemplary antibiotics include, but are not limited to, penicillins (*e.g.*, penicillin, amoxicillin), cephalosporins (*e.g.*, cephalexin), macrolides (*e.g.*, erythromycin, clarithormycin, azithromycin, troleandomycin), fluoroquinolones (*e.g.*, ciprofloxacin, levofloxacin, ofloxacin), sulfonamides (*e.g.*, co-trimoxazole, trimethoprim), tetracyclines (*e.g.*, tetracycline, chlortetracycline, oxytetracycline, demeclocycline, methacycline, sancycline, doxycline, aureomycin, terramycin, minocycline, 6-deoxytetracycline, lymecycline, meclocycline, methacycline, rolitetracycline, and glycylcycline antibiotics (*e.g.*,

tigecycline)), aminoglycosides (*e.g.*, gentamicin, tobramycin, paromomycin), aminocyclitol (*e.g.*, spectinomycin), chloramphenicol, sparsomycin, quinupristin/dalfoprisin (Syndercid™), reserpine, or an enopeptin (*e.g.*, ADEP4).

[00346] In certain embodiments, the antibiotic is a ribosome-targeting antibiotic.

[00347] Antibiotics target ribosomes at distinct locations within functionally relevant sites. They exert their inhibitory action by diverse modes, including competing with substrate binding, interfering with ribosomal dynamics, minimizing ribosomal mobility, facilitating miscoding, hampering the progression of the mRNA chain, and blocking the nascent protein exit tunnel. Examples of antibiotics that reveal novel ribosomal properties or enforced otherwise observed findings include the following: decoding (paromomycin); mRNA progression (spectinomycin); A-site binding to the small (tetracycline antibiotic) and the large (chloramphenicol) subunits; PTC mobility (sparsomycin); tRNA rotatory motion (quinupristin/dalfoprisin), and tunnel gating (troleandomycin); see, *e.g.*, Yonath, *Annu. Rev. Biochem.* (2005) 74:649-679.

[00348] In certain embodiments, the ribosome-targeting antibiotic is a tetracycline antibiotic. Exemplary tetracycline antibiotics include, but are not limited to, tetracycline, chlortetracycline, oxytetracycline, demeclocycline, methacycline, sancycline, doxycline, aureomycin, terramycin, minocycline, 6-deoxytetracycline, lymecycline, meclocycline, methacycline, rolitetracycline, and glycylcycline antibiotics (*e.g.*, tigecycline).

EXAMPLES

[00349] In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting this invention in any manner.

Example 1. A Fragment-Based Strategy for Investigating and Suppressing the Efflux of Bioactive Small Molecules

[00350] Organisms from all kingdoms of life exhibit some measure of resistance to toxic compounds due to the presence and activity of efflux pumps. These membrane-bound proteins are problematic in medicine because they can compromise the efficacies of therapeutic small molecules. For example, P-glycoprotein and other multidrug resistant (MDR)-efflux pumps are linked to the poor responses of some tumors to anticancer drugs.[1] Likewise, the treatment of infectious diseases is becoming more challenging because nearly all classes of antimicrobial drugs are known to be acted upon by efflux pumps.[2-10] Indeed,

drug resistance phenotypes of pathogenic microorganisms like *Candida albicans*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Mycobacterium tuberculosis* can be ascribed to genes encoding these proteins.[3, 4, 8, 9, 11] Remarkably, some efflux pumps exhibit high specificity for a particular molecule, while others act upon molecules of disparate structural classes.[1, 5, 7, 12-16] Those in the latter category underly a worrisome phenomenon wherein the acquisition of a single gene renders a tumor or pathogen multi-drug resistant.

[00351] Recently, insights into the mechanistic bases of small molecule recognition and export have been gleaned from the few available structures of efflux pumps in complex with their ligands.[17-20] It is anticipated that these structures can guide the design of either efflux inhibitors of various mechanistic classes[2, 11, 20, 22-31] or drugs that are recalcitrant to export.[21] However, structure-based design is not a trivial proposition for many reasons. For example, the identities of efflux pumps underlying a drug resistance phenotype are not always known. Even when the identity of an efflux pump of interest is known, resolution of its structure and the means by which it recognizes its ligand are likely to be necessary and arduous. A further complication of structure-based design in this context is that the structures of drugs can rarely be altered in ways that preclude efflux without negatively affecting bioactivity.[21, 28] To date, a more fruitful approach to the discovery of efflux inhibitors has been high-throughput screens for small molecules that potentiate drug activity against organisms harboring efflux pumps.[2, 7, 24, 26, 31] Nevertheless, screening suffers from its reliance on serendipity and has yet to yield a clinically used efflux inhibitor.

[00352] Given the challenges of structure-based design and the low "hit" rates of high-throughput screening, we have been exploring an alternative, rational strategy for the development of compounds that perturb drug efflux. Our premise is that efflux is contingent on a molecular recognition event, wherein the pump binds either the entire molecule or a substructure thereof. In the latter scenario, a fragment of an exported molecule containing the moiety recognized by the pump could competitively interfere with efflux. Accordingly, we predict that such a fragment would potentiate the activity of the full bioactive molecule when co-administered in a molar excess. The key advantages of this approach are that it does not require knowledge of the identity, structure, or mechanism of the efflux pump acting upon the bioactive molecule. Equally important, evidence of fragment-based drug potentiation would reveal insights into how an efflux pump recognizes a bioactive compound, which are notoriously difficult to acquire and could be valuable in designing efflux-resistant drugs.

[00353] To assess the viability of the fragment-based strategy for competitively interfering with drug efflux, we applied it to the discovery of small molecules that suppress efflux of the cyclic acyldepsipeptides (ADEPs), a promising group of antibacterial drug leads.[32-37] These natural products were extensively optimized via medicinal chemistry efforts,[33, 35, 36] and the resulting analogs have demonstrable activity in animal models of bacterial infection.[32] Importantly, the ADEPs' mechanism of action is distinct from all clinically used antibacterial agents. They bind and dysregulate the catalytic activity of the ClpP peptidase, triggering cell death via the indiscriminate degradation of cellular proteins.[38-40] Although the ADEPs exhibit impressive activity against a wide range of Gram-positive bacterial pathogens like *S. aureus, Streptococcus pneumoniae,* and the *Enterococci,*[33, 34, 36] they are only weakly active against *M. tuberculosis*, the etiological agent of tuberculosis. Parish and co-workers' observations that ADEP activity against *M. tuberculosis* could be potentiated by efflux pump inhibitors (*i.e.*, reserpine and verapamil) indicated that efflux was the basis of the bacterium's resistance.[41] The identities of the efflux pump(s) in *M. tuberculosis* and the means by which they recognize the ADEPs have yet to be reported. Their identification and characterization are likely to be challenging because the bacterium has dozens of putative efflux pump genes.[42-44] The apparent efflux-mediated resistance of these actinobacteria to the ADEPs and the ease with which ADEP fragments could be synthesized made this an ideal case for proof-of-principle experiments.

[00354] Initially, we synthesized a collection of molecules (**2-5**) that were sub-structures of a bioactive ADEP (**1**). See Figure 1. These fragments were evaluated in ADEP potentiation experiments with *S. coelicolor,* wherein the bacterium was grown on solid media titrated with solutions containing the ADEP with 2.5, 5, 10, or 20-fold molar excesses of the fragments. See Table 1A.

Table 1A.

	ADEP MIC [ug/mL]			
Frag:ADEP	**2.5:1**	**5:1**	**10:1**	**20:1**
2	16	16	16	16
3	13	13	10	9
4	16	16	16	16
5	16	16	16	16

[00355] *S. coelicolor* was selected because it is a non-pathogenic relative of *M. tuberculosis* and the ADEP resistance of streptomycetes has likewise been linked to efflux pumps.[46] *N*-E-2-heptenoyldifluorophenylalanine methyl ester (**3**) (*i.e.*, the ADEP side chain moiety) potentiated the activity of ADEP against *S. coelicolor* in a dose-dependent fashion. In contrast, ADEP fragments such as the peptidolactone (**2**), the peptidolactone with a truncated side chain (**5**), or simply the *N*-acetyl difluorophenylalanine methyl ester (**4**) did not display any efficacy as ADEP potentiators. These results provide the first evidence that the as of yet identified pump(s) recognize the ADEPs primarily by the side chain appended to their peptidolactone.

[00356] Through a positional scanning analysis of compound **3**, we set out to define the structural requirements for its ADEP potentiation and by extension those for ADEP recognition by the pump(s). First, we prepared compounds in which the α-amino group of difluorophenylalanine was coupled to various acyl moieties, including an acetyl group, those with saturated and unsaturated straight chains, and one with a cyclic structure (**3, 6-10**). See Figures 1A and 2. In the ADEP potentiation assays with *S. coelicolor*, we found that none of the compounds were superior to the parent compound **3**. We were intrigued to find that a fragment with the polyunsaturated acyl moiety of a naturally occurring ADEP exhibited no potentiation activity (compound **10**, Figure 2).[35] From the perspective of evolution, one might anticipate that an efflux pump conferring ADEP resistance would be biased to recognize and act on a fragment of a natural product rather than a synthetic compound. However, an alternative explanation for the weak ADEP potentiation of compound **10** could be its lability via inter- or intramolecular reactions. Next, we sought to define the significance of the amino acid residue's identity in ADEP potentiation. A compound with a phenylalanine (**11**) in the place of difluorophenylalanine was an active potentiator, but was less effective than **3** (Figure 2). Again, these results are interesting in the context of efflux pump evolution because the ADEP natural products have phenylalanine. In contrast, substitution of the difluorophenylalanine with leucine (**12**) completely abolished potentiation activity. Lastly, five different functionalities were installed in place of the methyl ester of compound **3**, including a carboxylic acid, an ester, and three amides (**13-17**, Figure 2). Only the compounds with amides (**14-16**) were superior to compound **3** as ADEP potentiators, which is interesting because the ADEPs have an amide bond at the analogous position. Compound **14** was the most effective of the potentiators at fragment:ADEP ratios lower than 20:1 and was also a better than reserpine when the compounds were used at the same molar ratios

(3.2- vs. 1.8-fold). Interestingly, despite strong congruence between the structure-activity relationships of the fragments for potentiation and those of the ADEPs for antibacterial activity,[35] the potentiating fragments do not inhibit growth of *S. coelicolor* at concentrations sufficient for potentiation (see supporting information) nor as high as 200 μg/mL.

[00357] The efficacy of compound **14** as a potentiator of ADEP activity against *S. coelicolor* motivated us to test it in the same fashion against phylogenetically related *Mycobacteria*. Initially, we assessed the dose-dependence of ADEP potentiation using *Mycobacterium smegmatis* MC2155, a non-pathogenic surrogate for *M. tuberculosis* (Figure 3A). Compound **14** was a four-fold potentiator of ADEP activity at 50 ug/mL on solid growth media. Based on the success of the experiments with *M. smegmatis*, we investigated the capacity of compound **14** to potentiate ADEP activity against a virulent strain of *M. tuberculosis* (Figures 3B, 3C). Remarkably, the highest degree of ADEP potentiation by compound **14** (*i.e.*, five-fold) was greater in *M. tuberculosis* than those observed in both *M. smegmatis* and *S. coelicolor*. It should also be noted that reserpine was a 1.25-fold potentiator of ADEP activity at the same concentration (50 μg/mL). While we do note that the apparent degree of ADEP potentiation against *M. tuberculosis* by reserpine was lower than that reported by Parrish and co-workers (2.5-fold),[34] the design principle that yielded compound **14** and its superior efficacy as a potentiator of ADEP activity are truly noteworthy and bode well for the use of ADEPs in treating tuberculosis.

[00358] The apparent potentiation of ADEP activity against resistant actinobacteria by compound **14** and related structures is consistent with our proposal that a fragment can competitively interfere with efflux of the cognate bioactive compound. Nevertheless, we carried out additional experiments to rule out other mechanisms of potentiation. First, we confirmed that compound **14** did not present any antibacterial activity in and above the concentration ranges at which it potentiated the ADEP. Consistent with the lack of antibacterial activity, we have found that the compound does not activate a model ClpP peptidase *in vitro*.[48] To further exclude the possibility that compound **14** was non-specifically interfering with efflux, we assessed its ability to potentiate the activities of chloramphenicol and spiramycin against *S. coelicolor*. These experiments were chosen because *S. coelicolor* is known to have specific efflux pumps that confer resistance to these antibiotics.[46, 47] We found that compound **14** did not potentiate the activities of either antibacterial agent, which is consistent with the competitive interference mechanism. Although there is strong evidence for competitive interference, we do note the requirement of the potentiator in molar excesses

as high as 20-fold indicates that compound **14** is either an imperfect or incomplete mimic of the ADEP sub-structure recognized by the efflux pump.

[00359] In conclusion, we present a fundamentally new strategy for suppressing efflux of a bioactive compound. It is distinct from other counter-efflux approaches such as the use of small molecules to occlude a pump's outer-membrane channel, to perturb pump assembly, or to inhibit the energy-consuming mechanism that mediates efflux.[7, 8, 10, 11, 20, 22-26, 28-31, 49] The use of fragments to competitively interfere with efflux of therapeutic agents does not require any knowledge of the pump's identity, structure, or its mechanism of action. In fact, we show how it can be used to reveal new insights into the structural basis by which efflux occurs. Although it is not a panacea for multidrug efflux, this approach could be generalized to any small molecule therapeutics that are acted upon by efflux pumps. The structural complexity of many bioactive molecules presents technical challenges for further demonstrations of this fragment-based strategy, but such efforts are now underway in these laboratories.

General Methods

[00360] **Strains, Media and Culture Conditions**. *Streptomyces coelicolor* M145 was grown at 30 °C on mannitol soya flour medium (SFM), Difco nutrient agar medium (DNA), Tryptone Soya Broth (TSB), yeast extract-malt extract medium (YEME), or minimal liquid medium (NMMP).(44) *Mycobacterium smegmatis* MC2155 was grown in Luria–Bertani medium supplemented with 0.2% (v/v) glycerol at 37 °C. *Mycobacterium tuberculosis* H37Rv was maintained in Middlebrook 7H9 broth supplemented with 0.2% (v/v) glycerol, 0.05% Tween 80, and 10% (v/v) oleic acid-albumin-dextrose-catalase.

[00361] **MIC assays.** *S. coelicolor* M145 MIC assays were performed on Difco nutrient agar medium supplemented with the indicated concentrations of compound. Growth was assessed after incubation at 30°C for 48 h. The lowest drug concentration that inhibited ≥90% growth was considered to be the MIC. *All experiments were performed in triplicate.*

[00362] *Mycobacterium smegmatis* MIC assays were performed on Difco nutrient agar medium supplemented with 0.2% glycerol and the indicated concentrations of compound. Growth was assessed after incubation at 30°C for 72 h. The lowest drug concentration that inhibited ≥90% growth was considered to be the MIC. *All experiments were performed in triplicate.*

[00363] To determine the MICs of compounds for *M. tuberculosis*, OD600-based assays were used. Bacteria were grown to midlog phase and plated in 96-well plates at OD600 = 0.025 in the presence of small molecule inhibitors for indicated time periods, and growth was

assessed by reading OD600. The MIC value was determined as the lowest concentration that inhibited growth by >90% relative to the DMSO control. *All experiments were performed in triplicate.*

Table 1B. ADEP Potentiator Optimization					
	ADEP MIC (μM)[a]				
Pot. : ADEP Molar Ratio	**0 : 1**	**2.5 : 1**	**5 : 1**	**10 : 1**	**20 : 1**
3	22	17	17	14	12
6	22	22	22	22	22
7	22	22	22	22	22
8	22	22	22	22	22
9	22	22	22	22	22
10	22	22	22	22	22
11	22	22	22	17	17
12	22	22	22	22	22
13	22	22	22	22	22
14	22	17	12	9	7
15	22	22	22	22	22
16	22	22	14	9	7
17	22	22	22	22	22
Reserpine	22	22	22	14	12

[a]ADEP Minimal Inhibitory Concentrations when compound is applied in molar excess over ADEP.

Table 1C. Potentiation is Specific to ADEP[a]		
Compound	**MIC (μg/mL)**	
	- Cmpd. 14	**+ Cmpd. 14**
ADEP	16	5
Chloramphenicol	80	80
Spiramycin	225	225

[a]Potentiation of chloramphenicol and spiramycin against *S. coelicolor* in solid growth media

References Cited

1. Chen C, Chin J, Ueda K, Clark D, Pastan I, Gottesman M, Roninson I. *Cell.* **1986**;*47*(3), 381-389.
2. a) Poole K. *J Antimicrob Chemother.* **2005**;56(1):20-51. b) Renau T, Léger R, Flamme E, Sangalang J, She M, Yen R, Gannon C, Griffith D, Chamberland S, Lomovskaya O, Hecker S, Lee V, Ohta T, Nakayama K. *J Med Chem.* **1999**;42(24):4928-31.
3. Adams K, Takaki K, Connolly L, Wiedenhoft H, Winglee K, Humbert O, Edelstein P, Cosma C, Ramakrishnan L. *Cell.* **2011**;145(1):39-53.
4. Balganesh M, Dinesh N, Sharma S, Kuruppath S, Nair AV, Sharma U. *Antimicrob Agents Chemother.* **2012**;56(5):2643-51.
5. Borges-Walmsley M, McKeegan K, Walmsley A. *J Biochem.* **2003**;376:313-38.
6. Machado D, Couto I, Perdigão J, Rodrigues L, Portugal I, Baptista P, Veigas B, Amaral L, Viveiros M. *PloS one.* **2012**;7(4):e34538.
7. Nikaido H, Pagès J. *FEMS Microbiol Rev.* **2012**;36(2):340-63.
8. Rouveix B. *J Antimicrob Chemother.* **2007**;59(6):1208-9.
9. Webber M, Piddock L. *J Antimicrob Chemother.* **2003**;51(1):9-11.
10. Li X, Nikaido H. *Science.* **1994**;264(5157):382-8.
11. Lynch AS. *Biochem Pharmacol.* **2006**;71(7):949-56.
12. Bachas S, Eginton C, Gunio D, Wade H. *Proc Natl Acad Sci.* **2011**;108(27):11046-51.
13. Gottesman M, Pastan I. *Annu Rev Biochem.* **1993**;62(1):385-427.
14. Gutmann D, Ward A, Urbatsch I, Chang G, Van Veen H. *Trends Biochem Sci.* **2010**;35(1):36-42.
15. Piddock L. *Nat Rev Microbiol.* **2006**;4(8):629-36.
16. Wong K, Ma J, Rothnie A, Biggin P, Kerr I. *Trends Biochem Science.* **2014**;39(1):8-16.
17. Yu E, Aires J, Nikaido H. *J Bacteriol.* **2003**;185(19):5657-64.
18. Yu E, McDermott G, Zgurskaya H, Nikaido H, Koshland D. *Science.* **2003**;300(5621):976-80.
19. Murakami S, Nakashima R, Yamashita E, Matsumoto T, Yamaguchi A. *Nature.* **2006**;443(7108):173-9.

20. Nakashima R, Sakurai K, Yamasaki S, Hayashi K, Nagata C, Hoshino K, Onodera Y, Nishino K, Yamaguchi A. *Nature*. **2013**;500(7460):102-6.

21. Lee R, Hurdle J, Liu J, Bruhn D, Matt T, Scherman M, Vaddady P, Zheng Z, Qi J, Akbergenov R, Das S, Madhura D, Rathi C, Trivedi A, Villellas C, Lee R, Rakesh, Waidyarachchi S, Sun D, McNeil M, Ainsa J, Boshoff H, Gonzalez-Juarrero M, Meibohm B, Böttger E, Lenaerts A. *Nat Med.* **2014**;20(2):152-8

22. Lomovskaya O, Bosian K. *Biochem Pharma.* **2006**;71(7):910-8.

23. Lomovskaya O, Zgurskaya H, Totrov M, Watkins W. *Nat Rev Drug Disc.* **2007**;6(1):56-65.

24. Lomovskaya O, Warren M, Lee A, Galazzo J, Fronko R, Lee M, Blais J, Cho D, Chamberland S, Renau T, Leger R, Hecker S, Watkins W, Hoshino K, Ishida H, Lee V. *Antimicrob Agents Chemother.* **2001**;45(1):105-16.

25. Marquez B. *Biochimie.* **2005**;87(12):1137-47.

26. Nelson M, Park B, Levy S. *J Med Chem.* **1994**;37(9):1355-61.

27. Okandeji B, Greenwald D, Wroten J, Sello J. *Bioorg Med Chem.* **2011**;19(24):7679-89.

28. Pages J, Amaral L. *Biochimica et Biophysica Acta - Prot Proteom.* **2009**;1794(5):826-33.

29. Renau T, Léger R, Flamme E, She M, Gannon C, Mathias K, Lomovskaya O, Chamberland S, Lee V, Ohta T, Nakayama K, Ishida Y. *Bioorg Med Chem Lett.* **2001**;11(5):663-667.

30. Stavri M, Piddock LJV, Gibbons S. *J Antimicrob Chemother.* **2007**;59(6):1247-60.

31. Viveiros M, Martins M, Rodrigues L, Machado D, Couto I, Ainsa J, Amaral L. **2012**. 983-998.

32. Brötz-Oesterhelt H, Beyer D, Kroll H, Endermann R, Ladel C, Schroeder W, Hinzen B, Raddatz S, Paulsen H, Henninger K, Bandow J, Sahl H, Labischinski H. *Nat Med.* **2005**;11(10):1082-7.

33. Carney D, Schmitz K, Truong J, Sauer R, Sello J. *J Am Chem Soc.* **2014**;136(5):1922-9

34. Conlon B, Nakayasu E, Fleck L, LaFleur M, Isabella V, Coleman K, Leonard S, Smith R, Adkins J, Lewis K. *Nature.* **2013**;503(7476):365-70.

35. Hinzen B, Raddatz S, Paulsen H, Lampe T, Schumacher A, Häbich D, Hellwig V, Benet-Buchholz J, Endermann R, Labischinski H, Brötz-Oesterhelt H. *ChemMedChem.* **2006**;1(7):689-93.

36. Socha A, Tan N, LaPlante K, Sello J. *Bioorg Med Chem.* **2010**;18(20):7193-202.
37. Koshino H, Osada H, Yano T, Uzawa J, Isono K. *Tett Lett.* **1991**;32(52):7707-10.
38. Lee B, Park E, Lee K, Jeon H, Sung K, Paulsen H, Rübsamen-Schaeff H, Brötz-Oesterhelt H, Song H. *Nat Struc Mol Biol.* **2010**;17:471-8.
39. Li D, Chung Y, Gloyd M, Joseph E, Ghirlando R, Wright G, Cheng Y, Maurizi M, Guarne A, Ortega *J. Chem Bio.* **2010**;17(9):959-69.
40. Sass P, Josten M, Famulla K, Schiffer G, Sahl H, Hamoen L, Brötz-Oesterhelt H. *Proc Natl Acad Sci.* **2011**;108(42):17474-9.
41. Ollinger J, O'Malley T, Kesicki E, Odingo J, Parish T. *J Bacteriol.* **2012**;194(3):663-668.
42. Cole S, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon S, Eiglmeier K, Gas S, Barry C, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Krogh A, McLean J, Moule S, Murphy L, Oliver K, Osborne J, Quail M, Rajandream M, Rogers J, Rutter S, Seeger K, Skelton J, Squares R, Squares S, Sulston J, Taylor K, Whitehead S, Barrell B. *Nature.* **1998**;393(6685):537-44.
43. Sassetti C, Rubin E. *Proc Natl Acad Sci.* **2003**;100(22):12989-94.
44. Gupta A, Katoch V, Chauhan D, Sharma R, Singh M, Venkatesan K, Sharma V. *Microbial Drug Resist.* **2010**;16(1):21-8.
45. Kieser T, Bibb M, Buttner M, Chater K, Hopwood D. The John Innes Foundation; **2000**.
46. Gominet M, Seghezzi N, Mazodier P. *Microbiol.* **2011**;157(8):2226-34.
47. Vecchione, J. V., Aleander, B., Jr., Sello, J. K. 2009; *Antimicrob Agents Chemother.* 2009; 53, 4673-4677.
48. Carney, D. W., Compton, C. L., Schmitz, K. R., Saur, R. T. Sello, J. K. *ChemBioChem*, **2014**, in press.
49. Mahamoud A, Chevalier J, Alibert-Franco S, Kern WV, Pagès J. *J Anti Chemother.* **2007**;59(6):1223-9.

Example 2. A Simple Fragment of the Cyclic Acyldepsipeptides is Necessary and Sufficient for ClpP Activation and Antibacterial Activity

[00364] Recently, the cyclic acyldepsipeptide (ADEP) antibiotics have garnered considerable attention because of their potent antibacterial activity against a broad range of Gram-positive bacterial pathogens and their efficacy in animal models of bacterial

infection.[S1] The cellular target of the ADEPs is ClpP, a component of the highly conserved Clp proteolytic complexes in bacteria.[S1c, S2] ClpP is a self- compartmentalized, tetradecameric serine peptidase that functions in conjunction with accessory AAA+ partners (ATPases associated with diverse cellular activities), which themselves play important roles in substrate recognition and regulation of ClpP proteolytic activity.[S2d, S3] Clp proteolytic complexes degrade a variety of misfolded and native target proteins, including those involved regulating stress responses and virulence-factor production.[S4] ADEPs bind ClpP and dysregulate its activity by opening its axial pores, yielding a peptidase that indiscriminately degrades large peptides and unstructured proteins without the involvement of AAA+ partners, ultimately leading to inhibition of cell division and cell death.[S1c, S2a]

[00365] In crystal structures of the B. subtilis ClpP tetradecamer in complex with ADEPs, the small molecules bind with exquisite specificity at the intra-subunit sites to which AAA+ partners, like ClpX and ClpA, also bind.[S2b, S2c, S5] Intriguingly, it has been proposed that the *N*-acylphenylalanine side chain appended to the ADEP peptidolactone mimics the highly conserved IGF or LGF tripeptide motifs of ClpX and ClpA that mediate interactions with ClpP.[S2b, S5] We find this proposal to be thought-provoking in light of our recent finding that rigidification of the peptidolactone via incorporation of conformationally constrained amino acids can improve ClpP activation *in vitro* by as much as 7-fold.[6] These observations motivated us to carry out experiments to define the relative contributions of the ADEP peptidolactone and its side chain to ClpP binding and activation.

[00366] For this analysis, we prepared fragments of the ADEP structure (**1**) including an ADEP peptidolactone with an *N*-acetylated serine residue (**2**), an *N*-acetyldifluorophenylalanyl peptidolactone (**5**), a serine-prolyl ester coupled to *N*- E-2-heptenoyldifluoro-phenylalanine (**6A**), and an *N*-E-2-heptenoyldifluorophenylalanine methyl ester (**3**). See Figure 4. The bioactivities of the fragments were assessed in growth inhibition assays using wild-type *B. subtilis* AG174, a highly ADEP-susceptible bacterium. Only, fragments **6A** and **3** exhibited antibacterial activity, albeit with much lower potency than intact ADEP (Figure 4). Clearly, the *N*-acyldifluorophenylalanine moiety is necessary and sufficient for antibacterial activity. Interestingly, fragment **6A**, which has an acyclic portion of the ADEP peptidolactone, is less active than fragment **3**, which is composed only of the *N*-acyldifluorophenylalanine moiety.

[00367] We have also found that fragment **6A** kills *Mycobacterium tuberculosis* (Mtb) with an MIC of about 25 ug/mL. This compares quite favorably to ADEP (compound **1**) which has an Mtb MIC of about 150 ug/mL.

[00368] Based on our recent report that conformational flexibility of the ADEP peptidolactones strongly influences ClpP binding,[S6] we predicted that substitution of the serine in **6A** with either *allo*-threonine (**6B**) or threonine (**6C**) (Figure 6) would yield more rigid and bioactive fragments. However, both compounds exhibited the same antibacterial activity as fragment **6A** (MIC = 32 μg/mL), suggesting that the *allo*-threonine and threonine residues do not significantly or favorably restrict the conformations of the acyclic fragments.

[00369] A positional scanning approach was used to define the minimal structural requirements for the bioactivity of fragment **3**. First, we synthesized analogs with varied acyl chains (**7, 10, 18**; Figure 6). Fragment **7** bears an acyl chain that is common to previously reported synthetic ADEPs[S1c] and fragment **10** bears the acyl chain of the A54556 ADEP natural products.[S1a] Fragment **18** is an analog that we synthesized to test the effects of acyl group carbon-branching. Fragments **3**, **7**, and **18** were equally potent (MIC = 8 μg/mL), whereas fragment **10** exhibited attenuated activity (MIC = 32 μg/mL). This result is consistent with the literature, wherein analogs with polyunsaturated side chains are reportedly less active compared to analogs with α,β-unsaturation.[S1d] Fragments **8** and **9** (Figure 6) with saturated acyl groups had reduced antibacterial activity like their ADEP counterparts.[S1d] We next studied a pair of analogs in which the difluorophenylalanine was replaced by either phenylalanine (**11**) or leucine (**12**). The relatively modest activity of the fragment with the phenylalanine residue (**11**, MIC = 64 μg/mL) is consistent with the proposal that the fluorine atoms of difluorophenylalnine are engaged in hydrogen bonds with backbone amides of ClpP.[S1d] In contrast, the analog containing leucine (**12**) was completely inactive. Collectively, the results are consistent with published studies of ADEP structure activity relationships.[S1d]

[00370] Next, we explored the effects of several C-terminal manipulations on the antibacterial activity of fragment **3**. Interestingly, the methyl ester (**3**) was more potent that than either the carboxylic acid or amide analogs thereof (**13, 19, 14, 16**). The low potencies of the carboxamides were surprising, because the *N*-acyldifluorophenylalanine moiety in the ADEPs is linked to the peptidolactone via an amide bond. In any case, three additional ester analogs (**20, 17, 21**) were prepared. Only the propargyl ester **21** (MIC = 2 μg/mL) was more potent than **3**. The potency of the intact ADEPs and the findings that modification of the carboxy-terminus of **3** can improve potency suggest that the structural context in which the bioactive *N*-acyldifluorophenylalanine moiety is presented is important and can be optimized.

[00371] We tested our operating assumption that ADEPs and *N*-acyldifluorophenylalanine fragments have the same mechanism of killing bacteria in a series of genetic and biochemical

experiments. In initial genetic experiments, an ADEP (**1**) and fragments **6A** and **3** were tested for antibacterial activity against two engineered strains of *B. subtilis*- a *spx* null strain (AG 1927 *spx*::*neo*) and a strain lacking both *spx* and *clpP* (AG 1246 *spx*::*neo* and *clpP*::*cat*).[S7] The *clpP-spx* null strain was selected because the *spx* null mutation suppresses the slow growth defect exhibited by a *B. subtilis* strain lacking *clpP*.[S7] The *spx* null and wild-type strains of *B. subtilis*, both of which contain a functional ClpP, were equally susceptible to each of the three compounds. Importantly, neither intact ADEP nor fragments **6A** and **3** were toxic to the *spx-clpP* null strain (MIC > 128 μg/mL). The essentiality of a functional *clpP* gene for the toxicity of both compounds indicates that the fragments share the same mechanism as the ADEPs.

[00372] We also tested for cross-resistance by selecting for spontaneously resistant mutants to either **1** or **3** in the *spx* null strain. Mutants with resistance to the intact ADEP and fragment **3** were observed at frequencies of 3×10^{-6} colony forming units (cfu) and 7×10^{-5} cfu, respectively. As expected, all mutants resistant to **1** were resistant to **3** and *vice-versa* (MICs >300 μg/mL). By sequencing the *clpP* locus in the mutants, we determined that resistance was highly correlated with mutations in the promoter of the *clpP* gene or with mis-sense or frameshift mutations in the *clpP* open-reading frame.

[00373] To biochemically validate the proposal that ADEP fragments activate ClpP-mediated peptidolysis, they were tested for their capacity to stimulate hydrolysis of a fluorogenic decapeptide substrate by *B. subtilis* ClpP (Figure 7A-7B and Table 2A). All fragments exhibited concentration-dependent activation of ClpP decapeptidase activity and exhibited apparent activation constants (K_{app}) ranging from 3.9 – 7.9 μM. Since the binding affinities fall into a narrow range, the large differences in bioactvities of the compounds can be primarily atributed to their *in vivo* stability and/or cell-permeability. Nevertheless, the fragment with the most potent antibacterial activity (**21**) was also the tightest ClpP binder. In any case, fragment binding to and activation of ClpP were much weaker than those of ADEP (**1**) (K_{app} = 12 nM, Hill coefficient 2.02±0.08). However, the ADEP (**1**) and all fragments tested exhibited modest positive cooperativity in ClpP binding (*i.e.*, Hill coefficients >1), suggesting that the ADEP and fragments bind in the same general fashion.

Table 2A.

Compound	ClpP K_{app} (μM)	Hill Coefficient
6A	6.4±0.1	1.4±0.04
3	6.3±0.2	1.6±0.07

Table 2A.		
Compound	**ClpP K_{app} (μM)**	**Hill Coefficient**
6	7.9±0.4	1.5±0.08
7	11±0.3	1.6±0.06
8	7.3±0.3	1.2±0.05
21	3.9±0.1	1.6±0.04

[00374] To complement our finding that fragments activate ClpP *in vitro*, we used chemical proteomics experiments utilizing fragment-derived, affinity reagent.[S8] The *N*-acyldifluorophenylalanine propargyl ester (**21**) was coupled to a biotin-azide conjugate via a Cu(I)-catalyzed Click reaction.[S9] After the binding to avidin–agarose beads, the resulting affinity matrix was used to capture proteins from cell lysates of *B. subtilis*. The specifically bound proteins were eluted using free fragment **3** and identified using MASCOT proteomic analysis. We were gratified to find ClpP among the seventeen unique proteins that were positively identified (Table 2B). Although we cannot definitively rule out off-target binding, an intriguing possibility is that some of the additional proteins that were captured are substrates of the fragment-activated ClpP. Indeed, FtsZ, one of the captured proteins, is a *bona fide* substrate of ADEP-activated ClpP.[S10]

Table 2B. Proteins captured from *B. subtilis* cell lysate[a]	
Acetyl CoA carboxylase	Lipoamide acyltransferase
ATP synthase	Manganese transport system
cellobiose phosphotransferase	Nucleoside diphosphate kinase
ClpP	3-oxoacyl synthase
Clp proteinase-like protein	Peptidase T
CTP synthetase	Phosphate acetyltransferase
Cysteine protease, yraA	RNA polymerase
Enoyl-CoA hydratase	Rod shape determining protein
FtsZ	Surfactin synthetase
Glyoxal reductases	

[a] Seventeen proteins positively identified by MASCOT Proteomic Analysis as specifically bound to *N*-E-2-heptenoyldifluorophenylalanine affinity matrix. ClpP and FtsZ are known substrates of activated ClpP.

[00375] In conclusion, a truly remarkable example of perturbation of protein-protein interactions by a small molecule underlies the antibacterial activities of the ADEPs. Their binding to ClpP induces significant changes in the quaternary structure[S2b, S2c] of the enzyme, which enhance off-target activity and precludes interaction with AAA+ partners.[S2a] It has been proposed that binding and activation of ClpP are predicated on the mimicry of IGF and LGF motifs of the AAA+ partners by the ADEP side chain. Here, we report that only the *N*-acyldifluorophenylalanine moiety of the ADEPs is required for their bioactivities. These results are especially notable in light of reports that an IGF tripeptide alone does not activate ClpP or interfere with the binding of ClpP to ClpX.[S11] We believe that the essentiality of the N-acyldifluorophenylalanine moiety for both ClpP activation and antibacterial activity can be reconciled with our recently reported finding that restriction of the peptidolactone dyanamics improves activity. Specifically, the strengthened trans-annular hydrogen bonding between the macrocycle and the side chain could enhance cell-permeability and lock the side chain in a conformation for optimized ClpP binding.

[00376] Our definition of the ADEPs' simple pharmacophore has important implications. Firstly, the ease of fragment synthesis, relative to the ADEPs, makes ClpP activators more accessible to the growing number of groups interested in their mechanism of killing bacteria. In addition, the reported fragments are generally stable and can be stored at room temperature for weeks without any detectable degradation with two exceptions (**10**: light sensitive; **20**: acid sensitive). Secondly, our findings provide a starting point for fragment-based design of non-peptide activators of ClpP. In this ligand-design strategy, weakly active fragments are structurally elaborated into higher affinity ligands. [S12] Although fragment-based drug design is a relatively new, it has become widely appreciated as a powerful tool in drug discovery.[S12d-f] The fragment-based design strategy is a viable alternative to screening libraries of compounds in the search for non-peptide ClpP activators.[S13] Such efforts are motivated by concerns that the ADEP peptidolactone backbone could have pharmacological liabilities that are often associated with peptides, [S14] despite the fact that structurally optimized ADEPs effectively cure bacterial infections in mice and rats.[S1c, S1d, S1g] Conveniently, the *N*-acyldifluorophenylalanine moiety is highly amenable to structural elaboration as it possesses a reactive carboxylate functionality that can be easily coupled to a wide array of scaffolds and rapidly diversified. Work to develop more potent antibacterial agents using this strategy is underway in these laboratories.

General Methods

[00377] Analysis of the *clpP* loci in spontaneously resistant *B. subtilis* mutants. *B. subtillis* was grown in the presence of toxic amounts of either ADEP (compound **1**) or the bioactive fragment (compound **3**) in order to select for spontaneously resistant mutants. The frequencies of spontaneous resistance were similar for **1** (1 in 3 x 10^6 CFU) and **3** (1 in 7 x 10^5 CFU). Genomic DNA was isolated from several of these resistant mutants and their *clpP* loci were amplified using standard PCR techniques. Several of the mutations that render *B. subtillis* resistant to **1** (3 mutants) and **3** (5 mutants), were single nucleotide insertions or deletions resulting in frame shift mutations that confer loss of ClpP function. One resistant causing mutation resulted in alteration of the conserved Gly127 to valine. Gly127 is located in the equatorial interface between ClpP heptamers and is adjacent to the substrate-binding site. Mutation to valine may result in loss of ClpP function by decreasing oligomer stability and may sterically interfere with substrate binding. Finally, two of the mutants resistant to the ADEP and one of the mutants resistant to compound **3** did not harbor mutations in their *clpP* loci. It is possible that these resistant strains harbor mutations in proteins that perturb *clpP* expression.

Table 2C. *B.subtilis* Resistance Conferring Mutations	
Compound 1	**Compound 3**
585 - insertion of A	-7 - insertion of G
160 - Insertion of C	50 - Deletion of C
393 - Insertion of T	9 - Insertion of T
380 - Change of G to T Results in AA127 G-V	31 - Deletion of A
	334 – Deletion of C

Table 2D. Strains	
AG174	*B. subtilis* wild type
JK10	*E. coli clpP::cat, Δlon, slyD::kan*, λDE3
BOSE 1227	AG174, *spx::neo*
BOSE 1246	AG174, *spx::neo* and *clpP::cat*

Table 2E. Plasmids	
B.suclpP F (-250)	G A A C G T C A G C A C A A C T A A T T A T A C G (SEQ ID NO: 1)
BsuClpP_NdeI_F	A A T C A A C A T A T G A A T T T A A T A C C T A C A (SEQ ID NO: 2)
BsuClpP_XhoI_R	T C C T G A C T C G A G C T T T T T G T C T T C T G T G T G (SEQ ID NO: 3)
B.suClpP R (+250)	G A T G T T T C C C C T G C T G A T T G C (SEQ ID NO: 4)
T7 sequencing	T A A T A C G A C T C A C T A T A G G G (SEQ ID NO: 5)
T3 sequencing	A T T A A C C C T C A C T A A A G G G A (SEQ ID NO: 6)

Minimum inhibitory concentration determinations

[00378] Compound MICs against *B. subtilis* were determined using standard agar dilution techniques. Liquid cultures (L Broth, 3 mL) inoculated from a fresh single colony were grown for ~6 hours (OD600 ~1.9, WT ~$6x10^7$ cfu/mL, Δ*spx* ~$2x10^6$ cfu/mL, Δ*clpP* ~ $9x10^6$ cfu/mL at 37°C). LB Agar plates supplemented with varying concentrations of test compound were inoculated with 5 μL of the liquid culture and then incubated at 37°C for 48 hours, after which the agar plates were inspected for growth. Because of the relatively high rate of spontaneous resistance observed, the MIC was assessed as the lowest concentration of compound able to inhibit *B. subtilis* growth to less than 10 single colonies.

Isolation, cloning, and sequencing of *clpP* loci from spontaneously fragment-resistant *B. subtillis* mutants

[00379] To determine frequency and cause of resistant of spontaneously resistant *B. subtillis* mutants, 5 mL liquid cultures (LB broth) were inoculated with a single colony of *B. subtillis*. After 4 hours of growth (OD_{600} ~ 0.4) 100μL of culture were plated on LB Agar plates containing either compound **1** (1 μg/mL) or compound **17** (100 μg/mL). A dilution series was also carried out to determine colony-forming unit count. After 24 hours of growth, the resistant colonies were placed into fresh LB Broth maintaining selection for resistance by adding compound **1** or compound **17** at the same compound concentration. After 5 hours of growth, 3 mL fresh LB Broth cultures containing double the concentration of compound were inoculated with 1% of 5 h culture. After 3 hours of growth, total genomic DNA (gDNA) was harvested using the Qiagen blood and tissue purification kit following manufacturer's protocol for bacterial cells. DNA concentrations were measured using a NanoDrop ND-1000 spectrophotometer

[00380] The isolated gDNA was subjected to PCR to amplify the *clpP* locus with 250 base pairs (bp) upstream and 250 bp downstream. PCRs were performed with Pfu polymerase enzyme according to the manufacturer's protocol. An equal quantity of total gDNA was employed in all PCRs. A 1094 bp band corresponding to the *clpP* locus was obtained. The PCR program used to amplify the *clpP* locus was 94°C for 2 min, 30 cycles of 94 °C for 45 s, 57 °C for 45 s, and 72 °C for 60 s, and a final elongation at 72 °C for 10 min.

[00381] The amplified *clpP* loci were cloned into pBluescript KS+ and sequenced from both T7 and T3 primers by Davis Sequencing (Davis, CA).

Protein expression and purification

[00382] The ClpP coding region was amplified by PCR from *Bacillus subtilis* strain 168 genomic DNA (ATCC) and cloned into plasmid pET-22b (EMD Millipore) in frame with a C-terminal 6xHis tag. ClpP was overexpressed in the *clpP*$^-$ *E. coli* strain JK10 (J. Kenniston, T. A. Baker, R. Sauer. *PNAS*, **2005**, 102, 1390-1395) at room temperature for 5 h, following induction with 0.5 mM isopropyl β-D-1-thiogalactopyranoside. ClpP was purified from clarified lysates by metal affinity (Ni-NTA; Thermo), anion exchange (MonoQ; GE Healthcare) and size exclusion (Superdex 200; GE Healthcare) chromatography. As reported previously (B. Lee, E. Y. Park, K. Lee, H. Jeon, K. H. Sung, H. Paulsen, H. Rubsamen-Schaeff, H. Brotz-Oesterhelt, H. K. Song, *Nat. Struct. Mol. Biol.* **2010**, *17,* 471-478), recombinantly overexpressed ClpP eluted as two populations during anion exchange and size exclusion chromatography. The majority species eluted as tetradecamer by size exclusion chromatography, and was purified to homogeneity. Purified ClpP was spin concentrated to 150 μM tetradecamer in storage buffer (25 mM HEPES pH 7.5, 150 mM NaCl, 10% glycerol).

ClpP activation assays

[00383] Activation assays were performed at 30°C in 25 mM HEPES pH 7.5, 5 mM $MgCl_2$, 100 mM KCl, 10% glycerol, 1 mM DTT, 0.1 mM EDTA. ClpP was incubated with various concentrations of ADEP or fragment and an internally quenched fluorogenic peptide substrate incorporating a 2-aminobenzoic acid (Abz) fluorophore and 3-nitrotyrosine (Y^{NO2}) quencher. To assay ADEP activation, 1 nM ClpP was used along with the substrate peptide Abz-KASPVSLGY^{NO2}D (SEQ ID NO: 7). Fragment activation assays incorporated 25 nM ClpP and the substrate Abz-DFAPKMALVPY^{NO2} (SEQ ID NO: 8). Hydrolysis of

fluorogenic peptides was followed by the increase in 420 nm fluorescence upon 320 nm excitation in a SpectraMax M5 microplate reader (Molecular Devices).

Chemical Proteomics: Identification of the ADEP Fragment Binding Proteins

[00384] Shaken liquid cultures (25 mL) of either wild-type *Bacillus subtilis* or the strain *clpP-spx null strain* were grown to stationary phase and harvested by centrifugation at 4,000 rpm for 10 min. The resulting bacterial cell pellets were washed twice with 5 mL of phosphate buffer saline (PBS) to remove secreted proteins, and resuspended in 500 mL Lysis Buffer. The cell suspensions were then lysed by treatment with lysozyme at a final concentration of 1 mg enzyme/mL of culture for 1 hr at 37°C and clarified. In a separate container, the *N*-acyldifluorophenylalanine – Biotin conjugate was mixed with avidin-agarose beads for 30 minutes. Following incubation, the agarose beads with N-acyldifluorophenylalanine bound were added to the cell lysates and incubated on a nutating shaker for 1h at RT. Controls in which no *N*-acyldifluorophenylalanine – Biotin conjugate was added were carried out in parallel. The beads were washed 3x with PBS and the supernatant was combined and saved. Next, the beads were washed twice with free *N*-acyldifluorophenylalanine in PBS and the supernatant collected and saved.

[00385] To denature the pooled protein solutions they were heated to ~95°C for 5 minutes and then rapidly cooled it on ice. Next, disulfides were reduced by addition of dithiothreitol (DTT) to a final concentration of 20 mM and incubated at 56°C for 45 minutes. After cooling the solution to room temperature, alkylation of the sample was performed by incubating with *freshly prepared* iodoacetamide (IAA; 55 mM final concentration) by incubating for 30 minutes at room temperature *in the dark*. Lastly, trypsin was added at a ration of 1:50 (wt:wt) and the samples were incubated overnight at 37°C.

Protein identification via mass spectrometry and bioinformatics

[00386] Following in-solution tryptic digestion, peptides were fractionated on a 12 cm × 75 μm I.D. C18 reversed phase column (3 μm Monitor resin; Orochem Technologies, Naperville, IL., USA) and eluted with a linear gradient starting with 100% solvent A (0.1 M acetic acid in water) to 70% solvent B (0.1 M acetic acid in acetonitrile) over 60 min using an Agilent 1200 HPLC (Agilent Technologies, Paolo Alto, CA, USA). Eluted peptides were introduced onto a LTQ Velos Orbitrap mass spectrometer (Thermo Electron Corporation, San Jose, CA, USA) with a 1.8 kV electrospray voltage. Full MS scans in the m/z range of 300 –

1700 in the Orbitrap (60,000 nominal resolution) were followed by data dependent acquisition of MS/MS spectra for the ten most abundant ions in the LTQ ion trap, using a 30-second dynamic exclusion time. Peptide spectrum matching was performed against a *B. subtilis* specific database that was downloaded from NCBI from www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome using MASCOT v. 2.4 (Matrix Science, Ltd, London W1U 7GB UK). A concatenated database containing *25,091* "target" and "decoy" sequences was employed to estimate the false discovery rate (FDR), and protein identifications were derived from the peptide matches after filtering the peptide identifications to 1% FDR. In the bioinformatic identifications of the peptide fragments, trypsin specificity with two missed cleavage sites was allowed and the MS mass tolerance was 7 ppm while the MS-MS tolerance was 0.5 Daltons. Identifications were contingent on at least one unique peptide spectrum match (PSM) in the molecular weight search with a protein score cut-off of 32.8. Proteins that were nonspecifically eluted from the beads with PBS washes and proteins that were eluted from the no *N*-acyldifluorophenylalanine controls were subtracted from the compound washes, resulting in a list of specific binders.

Table 2F.*

All Positively Identified Proteins Isolated from Wild-type *B. subtillis*	**All Positively Identified Proteins from the *clpP-spx* null strain of *B. subtillis***
2-succinyl-6-hydroxy-2,4-cyclohexadiene-1- carboxylate synthase	2-isopropylmalate synthase
3-ketoacyl-ACP reductase	2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate synthase
Alpha-galactosidase	3-ketoacyl-ACP reductase
Aminoglycoside	Acetoin catabolism protein AcuA
6-adenylyltransferase	Acetoin utilization protein
AnsB	AroD
Aspartokinase II alpha subunit	Bacillorin synthetase A
ATP-dependent nuclease	Carboxypeptidase
Bacillomycin synthetase A	Cell division ATP-binding protein
Cell division ATP-binding protein	ComEB
Chemotaxis protein	Deoxyribodipyrimidine photolyase
ClpP	DNA helicase
Deoxyribodipyrimidine photolyase	DNA-polymerase I
DNA polymerase I	DnaB
DnaB	Excinuclease ABC subunit B
DnaI	Fengycin synthetase
Fengycin synthetase	Flagellar switch protein
Flagellar switch protein	Formamidopyrimidine-DNA glycosidase
Formamidopyrimidine-DNA glycosidase	GerC2
Fructose-1-phosphate kinase	GerC3
GerC3	Gln synthetase
GltC	GltC
Gluconate kinase	Gluconate kinase

Table 2F.*	
All Positively Identified Proteins Isolated from Wild-type *B. subtillis*	**All Positively Identified Proteins from the *clpP-spx* null strain of *B. subtillis***
Gramicidin S sythetase	Glycine betain/L-proline transmembrane protein
Heptaprenyl diphosphate synthase	Gramicidin S sythetase
L-aspartase	Heptaprenyl diphosphate synthase
Lysine decarboxylase	Lysine decarboxylase
MinC	MafA
Molybdopterin converting factor	MinC
RapJ	PEP synthase
Ribosomal protein B	Peroxidase
RNA polymerase	Phosphoribosyl aminoidazole
sigma-54 factor	succinocarboxamide synthetase
SHCHC synthase	Phosphoribosyl aminoimidazole carboxylase II
SinR	
Spore coat protein	Phosphoribosylformyl glycinamidine synthetase
SpoVS	II
SrfAA surfactin synthetase	Protx
SubA	PTS sucrose-specific enzyme IIBC component
Sucrase	Pyrimidine nucleoside phosphorylase
Superoxide dismutase	Pyruvate water dikinase/PEP synthase-like
TreP	protein
YcgC	RecR
YcgT	Replication initiation protein
YckG	Ribosomal protein B
YdiB	RNA polymerase sigma-54 factor
YdiE	SecY
YdiG	SHCHC synthase
YdiH	
YdjI	
YfhQ	
YfjR	
YitU	
YpsB	
YqcF	
YqeU	
YqfL	
YqfN	
YqhY	
YqiD	
YqiW	
YqjS	
YtbJ	
YtcG	
YtdI	
YtlQ	
YtmP	
YtpS	
YtsP	
YxeH	

*For the proteomic analyses, the *B. subtilis* strain cultivations and protein isolations were performed in duplicate. Both sets of samples were subjected to tryptic digests, peptide mass fingerprinting, and bioinformatics analyses in parallel.

References Cited

[S1] a) K. H. Michel, R. E. Kastner, U.S. Patent No. 4492650 A **1985**. b) H. Osada, T. Yano, H. Koshino, K. Isono, *J. Antibiot.* **1991**, *44,* 1463-1466. c) H. Brotz-Oesterhelt, D. Beyer, H. Kroll, R. Endermann, C. Ladel, W. Schroeder, B. Hinzen, S. Raddatz, H. Paulsen, K. Henninger, J. Bandow, H. Sahl, H. Labischinski, *Nat. Med.* **2005**, *11,* 1082-1087. d) B. Hinzen, S. Raddatz, H. Paulsen, T. Lampe, A. Schumacher, D. Häbich, V. Hellwig, J. Benet-Buchholz, R. Endermann, H. Labischinski, H. Brötz-Oesterhelt, *ChemMedChem* **2006**, *1,* 689-693. e) A. M. Socha, N. Y. Tan, K. L. LaPlante, J. K. Sello, *Bioorg. Med. Chem.* **2010**, *18,* 7193-7202. f) J. Ollinger, T. O'Malley, E. A. Kesicki, J. Odingo, T. Parish, *J. Bacteriol.* **2012**, *194,* 663-668. g) B. Conlon, E. Nakayasu, L. Fleck, M. LaFleur, V. Isabella, K. Coleman, S. Leonard, R. Smith, J. Adkins, K. Lewis, *Nature* **2013**, *503,* 365-370.

[S2] a) J. Kirstein, A. Hoffmann, H. Lilie, R. Schmidt, H. Rübsamen-Waigmann, H. Brötz-Oesterhelt, A. Mogk, K. Turgay, *EMBO Mol. Med.* **2009**, *1,* 37-49. b) D. H. S. Li, Y. S. Chung, M. Gloyd, E. Joseph, R. Ghirlando, G. D. Wright, Y. Cheng, M. R. Maurizi, A. Guarne, J. Ortega, *Chem. Biol.* **2010**, *17,* 959-969. c) B. Lee, E. Y. Park, K. Lee, H. Jeon, K. H. Sung, H. Paulsen, H. Rubsamen-Schaeff, H. Brotz-Oesterhelt, H. K. Song, *Nat. Struct. Mol. Biol.* **2010**, *17,* 471-478. d) J. A. Alexopoulos, A. Guarné, J. Ortega, *J. Struct. Biol.* **2012**, 176, 202-210.

[S3] a) S. A. Joshi, G. L. Hersch, T. A. Baker, R. T. Sauer, *Nat. Struct. Mol. Boil.* **2004**, *11,* 404-411. b) A. Y. H. Yu, W. A. Houry, *FEBS Lett.* **2007**, *581,* 3749-3757.c) M. E. Lee, T. A. Baker, R. T. Sauer, *J. Mol. Biol.* **2010**, *399,* 707-718.

[S4] a) S. Gottesman, S. Wickner, M. R. Maurizi, C. R. Beals, N. A. Clipstone, S. N. Ho, G. R. Crabtree, I. Zamir, J. Zhang, M. A. Lazar, *Genes Dev.* **1997**. b) S. Gottesman, E. Roche, Y. Zhou, R. T. Sauer, *Genes Dev.* **1998**, *12,* 1338-1347. c) O. Gaillot, E. Pellegrini, S. Bregenholt, S. Nair, P. Berche, *Mol. Microbiol.* **2000**, *35,* 1286-1294. d) G. T. Robertson, W. Ng, J. Foley, R. Gilmour, M. E. Winkler, *J. Bacteriol.* **2002**, *184,* 3508-3520. e) D. Frees, S. N. A. Qazi, P. J. Hill, H. Ingmer, *Mol. Microbiol.* **2003**, *48,* 1565-1578. f) H. Kwon, S. Kim, M. Choi, A. D. Ogunniyi, J. C. Paton, S. Park, S. Pyo, D. Rhee, *Infect. Immun.* **2003**, *71,* 3757-3765. g) S. Gottesman, *Annu. Rev. Cell Dev. Biol.* **2003**, *19,* 565-587. h) H. Kwon, A. D. Ogunniyi, M. Choi, S. Pyo, D. Rhee,

J. C. Paton, *Infect. Immun.* **2004**, *72,* 5646-5653. i) D. Frees, K. Sorensen, H. Ingmer, *Infect. Immun.* **2005**, *73,* 8100-8108. j) R. T. Sauer, T. A. Baker, *Annu. Rev. Biochem.* **2011**, *80,* 587-612. k) D. Frees, U. Gerth, H. Ingmer, *Int. J. Med. Microbiol.* **2013**, 304, 142-149.

[S5] a) Y. Kim, I. Levchenko, K. Fraczkowska, R. V. Woodruff, R. T. Sauer, T. A. Baker, *Nat. Struct. Mol Biol* **2001**, *8,* 230-233. b) A. Martin, T. A. Baker, R. T. Sauer, *Mol. Cell* **2007**, *27,* 41-52.

[S6] D. W. Carney, K. R. Schmitz, J. V. Truong, R. T. Sauer, J. K. Sello, *J. Am. Chem. Soc.* **2014**, *136,* 1922-1929.

[S7] M. M. Nakano, F. Hajarizadeh, Y. Zhu, P. Zuber, *Mol. Microbiol.* **2001**, *42,* 383-394.

[S8] U. Rix, G. Superti-Furga, *Nat. Chem. Biol.* **2009**, *5,* 616-624.

[S9] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2002**, *114,* 2708-2711.

[S10] P. Sass, M. Josten, K. Famulla, G. Schiffer, H. Sahl, L. Hamoen, H. Brötz-Oesterhelt, *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108,* 17474-17479.

[S11] S. A. Joshi, G. L. Hersch, T. A. Baker, R. T. Sauer, *Nat. Struct. Mol. Biol.* **2004**, *11,* 404-411.

[S12] a) D. A. Erlanson, R. S. McDowell, T. O'Brien, *J. Med. Chem.* **2004**, *47,* 3463-3482. b) P. J. Hajduk, J. Greer, *Nat. Rev. Drug Disc.* **2007**, *6,* 211-219. c) M. Congreve, G. Chessari, D. Tisi, A. J. Woodhead, *J. Med. Chem.* **2008**, *51,* 3661-3680. d) A. W. Hung, A. Ramek, Y. Wang, J. A. Wilson, P. A. Clemons, D. W. Young, *Proc. Natl. Acad. Sci., USA.* **2011**, 108, 6799-6804. e) E. Scott, A. G. Coyne, S. A. Hudson, C. Abell, *Biochemistry.* **2012**, *51,* 4990-5003. f) D. J-McCarthy, A. J. Campbell, G. Kern, D. Moustakas, *J. Chem. Inf. Model.* **2014**, 54, 693-704.

[S13] E. Leung, A. Datti, M. Cossette, J. Goodreid, S. E. McCaw, M. Mah, A. Nakhamchik, K. Ogata, M. El Bakkouri, Y. Cheng, *Chem. Biol.* **2011**, *18,* 1167-1178.

[S14] a) C. M. Edwards, M. A. Cohen, S. R. Bloom, *QJM* **1999,** *92,* 1-4. b) A. Loffet, *J. Pept. Sci.* **2002,** *8,* 1-7. c) P. Vlieghe, V. Lisowski, J. Martinez, M. Khrestchatisky, *Drug Discov. Today.* **2010,** *15,* 40-56.

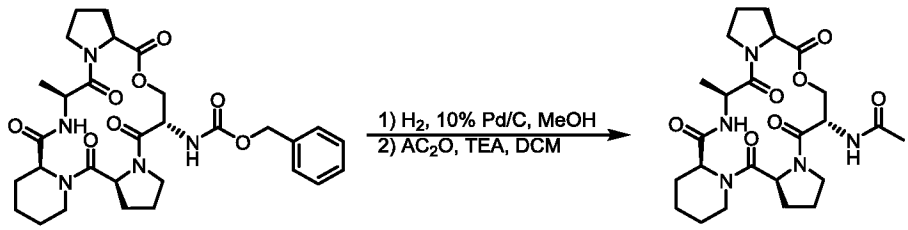
Chemical synthesis of ADEP fragments

[00387] All reagents were purchased from Sigma-Aldrich or VWR. Column chromatography was performed using 60 Å (230–400 mesh ASTM) silica gel. NMR analyses were performed on a Bruker Advance Ultrashield Spectrometer (400 or 300 MHz).

Chemical synthesis

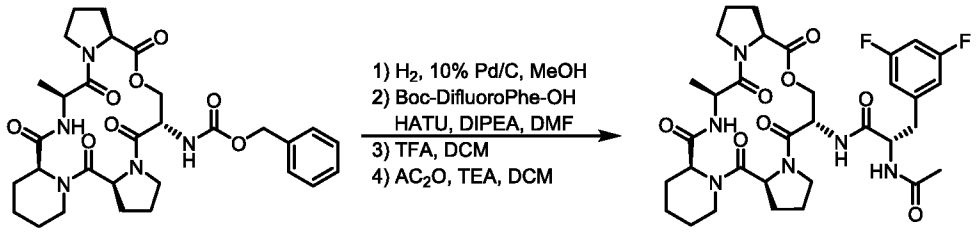
[00388] **Synthesis General:** All commercially available reagents were used without further purification. All reactions were conducted in oven-dried glassware, under ambient atmosphere, using dry solvents unless otherwise stated. NMR chemical shifts were referenced to residual solvent peaks: $CDCl_3$ (δ = 7.27 ppm for ^{1}H-NMR and 77.00 ppm for ^{13}C-NMR), Acetone-d6 (δ = 2.05 for ^{1}H-NMR and δ = 29.92 for ^{13}C-NMR).

Synthesis of *N*-acetyl ADEP peptidolactone:



[00389] Cbz ADEP peptidolactone (58 mg, 0.10 mmol) was dissolved in MeOH (1 mL) and treated with 10% Pd/C (13 mg) and 1 M HCl (0.10 mL). The reaction flask was sealed with a septum through which H_2 was delivered from a balloon. Upon complete conversion of the starting material, the reaction was filtered through celite, rinsing with methanol. The crude product was then concentrated and subsequently dissolved in DCM (0.33 mL) and treated with acetic anhydride (20 µL, 0.20 mmol) and triethylamine (28 µL, .20 mmol). The acetylation reaction was allowed to proceed for 3 hours after which the reaction solution was transferred directly to a silica gel column and chromatographed with 80% acetone in ethyl acetate. The product was isolated as a white solid: 39 mg, 78% yield. HRMS (ESI) predicted for $[C_{24}H_{35}N_5O_7 + H]^+$: 506.2615, found:506.2607. ^{1}H NMR (600MHz, CDCl3) δ = 8.62 (d, J = 9.5 Hz, 1 H), 6.44 (d, J = 9.5 Hz, 1 H), 5.22 (dd, J = 3.1, 9.0 Hz, 1 H), 4.99 (qd, J = 6.6, 9.5 Hz, 1 H), 4.80 (dd, J = 1.5, 11.7 Hz, 1 H), 4.73 - 4.64 (m, 1 H), 4.62 - 4.55 (m, 1 H), 4.55 - 4.45 (m, 2 H), 3.78 (ddd, J = 5.1, 8.2, 11.6 Hz, 1 H), 3.72 - 3.61 (m, 2 H), 3.58 - 3.45 (m, 2 H), 2.73 (d, J = 11.7 Hz, 1 H), 2.63 (dt, J = 2.6, 13.3 Hz, 1 H), 2.41 - 2.29 (m, 1 H), 2.26 - 2.10 (m, 2 H), 2.04 - 1.99 (m, 1 H), 1.97 (s, 3 H), 1.96 - 1.92 (m, 3 H), 1.78 - 1.71 (m, 2 H), 1.62 (d, J = 14.3 Hz, 1 H), 1.53 - 1.42 (m, 2 H), 1.41 (d, J = 6.6 Hz, 3 H), 1.38 - 1.29 (m, 1 H). ^{13}C NMR (151MHz CDCl3-d) δ = 173.5, 171.2, 170.1, 169.8, 168.6, 166.4, 65.5, 58.7, 57.2, 56.5, 51.4, 48.1, 46.9, 46.4, 41.1, 31.0, 30.6, 28.4, 24.8, 23.1, 23.0, 21.3, 21.3, 17.9.

Synthesis of N-acetyl-3,5-difluorophenylalanyl ADEP peptidolactone:

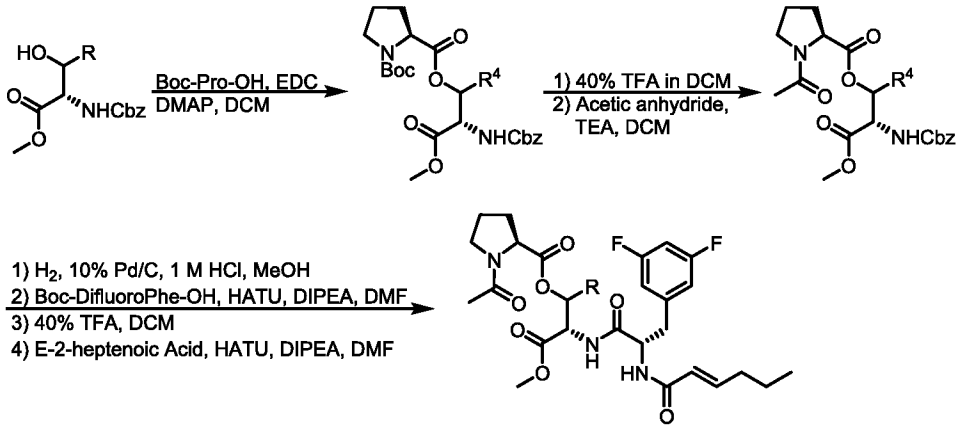


[00390] Cbz ADEP peptidolactone (58 mg, 0.10 mmol) was dissolved in MeOH (1 mL) and treated with 10% Pd/C (13 mg) and 1 M HCl (0.10 mL). The reaction flask was sealed with a septum through which H_2 was delivered from a balloon. Upon complete conversion of the starting material, the reaction was filtered through celite, rinsing with methanol. The crude product was then concentrated and subsequently dissolved in DMF (0.33 mL) and combined with Boc-3,5-difluorophenylalanine. Once homogeneous, the reaction solution was treated with HATU (38 mg, 0.10 mmol) and DIPEA (35 µL, 0.20 mmol) and allowed to react over night. The reaction was then diluted with ethyl acetate (3 mL) and extracted: 3x 1M HCl, 3x sat $NaHCO_3$, 1x brine, and then dried over sodium sulfate. After removal of the solvent, the crude Boc-3,5-difluorophenylalanyl ADEP peptidolactone was used without further purification.

[00391] The crude Boc-3,5-difluorophenylalanyl ADEP peptidolactone was deprotected by treatment with 40% TFA in DCM (0.5 mL). Upon complete conversion of the starting material, the reaction was concentrated first by blowing with a stream of nitrogen and then by placing the residue under high vacuum. Once dry, the Boc- deprotected 3,5-difluorophenylalanyl ADEP peptidolactone was dissolved in DCM (0.5 mL) and treated with acetic anhydride (16 µL, 0.17 mmol) and triethylamine (24 µL, 0.17 mmol). The reaction was allowed to proceed for 3 hours, after which the reaction solution was added directly to a silica gel column and chromatographed with 50-100% acetone in ethyl acetate. The final product was isolated as a white solid: 41 mg, 59% over 4 steps. HRMS (ESI) predicted for $[C_{33}H_{42}F_2N_6O_8 + H]^+$: 689.3110, found: 689.3097. ^{1}H NMR (600MHz, $CDCl_3$) δ = 8.54 (d, *J* = 9.5 Hz, 1 H), 7.51 (d, *J* = 9.5 Hz, 1 H), 6.99 (d, *J* = 8.1 Hz, 1 H), 6.76 - 6.68 (m, 2 H), 6.64 (tt, *J* = 2.3, 9.0 Hz, 1 H), 5.16 (dd, *J* = 2.9, 8.8 Hz, 1 H), 5.01 - 4.91 (m, 1 H), 4.79 (dd, *J* = 1.7, 11.6 Hz, 1 H), 4.74 (dt, *J* = 5.1, 7.9 Hz, 1 H), 4.71 - 4.66 (m, 2 H), 4.56 - 4.47 (m, 2 H), 3.77 (ddd, *J* = 5.5, 8.1, 11.7 Hz, 1 H), 3.71 - 3.59 (m, 2 H), 3.56 (dd, *J* = 10.1, 11.6 Hz, 1 H), 3.41 - 3.32 (m, 1 H), 2.95 (dd, *J* = 8.4, 13.2 Hz, 1 H), 2.88 (dd, *J* = 4.8, 13.6 Hz, 1 H), 2.71 (d, *J* = 10.3 Hz, 1 H), 2.65 - 2.57 (m, 1 H), 2.38 (dt, *J* = 5.1, 8.8 Hz, 1 H), 2.26 - 2.16 (m, 2 H), 2.15 (s, 3 H), 2.06 - 1.90 (m, 5 H), 1.79 - 1.74 (m, 1 H), 1.65 (d, *J* = 12.5 Hz, 1 H), 1.53 -

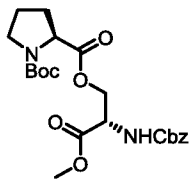
1.44 (m, 2 H), 1.44 - 1.36 (m, 1 H), 1.33 (d, J = 6.6 Hz, 3 H). ^{13}C NMR (151MHz, $CDCl_3$) δ = 172.5, 171.1, 170.8, 170.5, 169.8, 169.4, 165.0, 163.6, 162.0, 140.2, 112.6, 112.6, 112.4, 102.4, 102.3, 65.1, 59.0, 57.1, 56.9, 54.2, 51.2, 47.8, 46.8, 46.5, 41.0, 38.5, 30.8, 30.4, 29.2, 27.9, 25.0, 23.1, 22.8, 21.3, 21.2, 17.9.

Synthesis of Boc-Proline Esters General Procedure:



[00392] An *N*-Cbz amino acid methyl ester (1 equivalent) and Boc proline (1.1 equivalent) were dissolved in DCM at a concentration of 0.2 M with respect to the N-Cbz amino acid. The solution was treated with DMAP (0.1 equivalents) and EDC·HCl (1.5-2.0 equivalents), which was added in portions until the staring material was completely consumed (monitoring with TLC 1:1 EtOAc/Hexanes). After 24 hours the reaction was concentrated *in vacuo* and then diluted with ethyl acetate (50 mL). The ethyl acetate solution was extracted 3 x 1M HCl_{aq}, 3 x saturated $NaHCO_{3aq}$, and 1 x Brine then dried over sodium sulfate. The proline ester products were isolated by silica gel chromatography using a 30-50% ethyl acetate in hexanes solvent gradient.

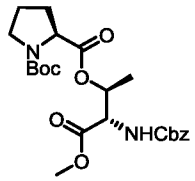
***N*-Cbz-O-Boc-prolyl-serine methyl ester:**



[00393] Product isolated as a white solid 97% yield. HRMS (FAB) Predicted for $[C_{22}H_{30}N_2O_8 + Na]^+$: 473.18999, Found: 473.1885. 1:1 Mixture of amide rotamers ^{1}H NMR (600MHz, $CDCl_3$) d = 7.42 - 7.27 (m, 10 H), 5.97 (d, J = 8.4 Hz, 1 H), 5.54 (d, J = 8.1 Hz, 1 H), 5.20 - 5.02 (m, 4 H), 4.69 - 4.56 (m, 3 H), 4.52 - 4.38 (m, 3 H), 4.28 (dd, J = 4.0, 8.4 Hz, 1 H), 4.20 (dd, J = 3.9, 8.6 Hz, 1 H), 3.76 (s, 6 H), 3.52 (ddd, J = 5.1, 7.4, 10.5 Hz, 1 H), 3.47

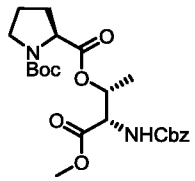
- 3.40 (m, 2 H), 3.40 - 3.34 (m, 1 H), 2.25 - 2.11 (m, 2 H), 1.98 - 1.81 (m, 6 H), 1.43 (s, 9 H), 1.38 (s, 9 H). ^{13}C NMR (151MHz, $CDCl_3$) d = 172.7, 172.3, 169.9, 169.6, 156.0, 155.6, 154.6, 153.5, 136.2, 135.9, 128.5, 128.4, 128.2, 128.1, 128.0, 80.1, 80.0, 67.2, 67.0, 64.3, 64.2, 58.9, 58.8, 53.3, 52.8, 52.7, 46.5, 46.2, 30.8, 29.9, 28.3, 28.2, 24.3, 23.4.

_N_-Cbz-O-Boc-prolyl-*allo*-threonine methyl ester:



[00394] Product isolated as a colorless oil 79% yield. HRMS (ESI) Predicted for $C_{22}H_{30}N_2O_8$ + H]$^+$: 465.2236, Found: 465.2237. 1:1 Mixture of amide rotamers. ^{1}H NMR (600MHz, $CDCl_3$) δ = 7.39 - 7.30 (m, 10 H), 5.97 (d, *J* = 9.2 Hz, 1 H), 5.55 (d, *J* = 8.8 Hz, 1 H), 5.31 - 5.21 (m, 2 H), 5.16 - 5.07 (m, 4 H), 4.64 (dd, *J* = 3.5, 8.6 Hz, 1 H), 4.61 (dd, *J* = 2.9, 9.2 Hz, 1 H), 4.25 (dd, *J* = 4.2, 8.6 Hz, 1 H), 4.15 (dd, *J* = 3.9, 8.6 Hz, 1 H), 3.78 (s, 3 H), 3.76 (s, 3 H), 3.58 - 3.50 (m, 1 H), 3.50 - 3.35 (m, 3 H), 2.19 - 2.08 (m, 2 H), 2.03 - 1.87 (m, 4 H), 1.87 - 1.79 (m, 2 H), 1.45 (s, 9 H), 1.40 (s, 9 H), 1.33 (d, *J* = 6.6 Hz, 3 H), 1.31 (d, *J* = 6.6 Hz, 3 H). ^{13}C NMR (151MHz, $CDCl_3$) δ = 172.4, 172.0, 169.5, 155.9, 155.7, 154.7, 153.7, 136.2, 136.0, 128.9, 128.5, 128.4, 128.2, 128.1, 128.0, 128.0, 127.7, 80.0, 79.8, 71.3, 70.8, 67.2, 66.9, 59.0, 58.8, 57.2, 57.1, 52.7, 52.3, 46.6, 46.3, 30.6, 29.8, 28.4, 28.3, 24.3, 23.5, 16.3, 16.1.

_N_-Cbz-O-Boc-prolyl-threonine methyl ester:



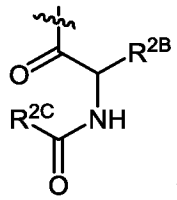
[00395] Product isolated as a colorless oil, 96% yield. HRMS (ESI) Predicted for $C_{22}H_{30}N_2O_8$ + H]$^+$: 465.2236, Found: 465.2239. 1:1 Mixture of amide rotamers. ^{1}H NMR (600MHz, $CDCl_3$) δ = 7.39 - 7.31 (m, 10 H), 5.85 (d, *J* = 9.5 Hz, 1 H), 5.44 - 5.29 (m, 3 H), 5.17 - 5.11 (m, 4 H), 4.52 (dd, *J* = 2.6, 9.5 Hz, 1 H), 4.48 (dd, *J* = 2.6, 9.5 Hz, 1 H), 4.24 - 4.17 (m, 2 H), 3.73 (s, 3 H), 3.72 (s, 3 H), 3.53 - 3.47 (m, 1 H), 3.47 - 3.40 (m, 2 H), 3.39 - 3.31 (m, 1 H), 2.25 - 2.15 (m, 1 H), 2.15 - 2.06 (m, 1 H), 1.95 - 1.79 (m, 6 H), 1.49 - 1.35 (m, 18 H), 1.34 - 1.30 (m, 6 H). ^{13}C NMR (151MHz, $CDCl_3$) δ = 172.0, 171.7, 170.3, 170.1, 156.7, 156.4, 154.2, 153.6, 136.1, 135.9, 128.5, 128.4, 128.4, 128.2, 128.1, 128.1, 128.0,

80.0, 79.9, 71.1, 70.9, 67.3, 67.1, 67.0, 66.5, 59.2, 59.1, 59.0, 58.8, 57.6, 57.4, 52.6, 52.4, 46.4, 46.3, 46.2, 30.9, 30.8, 29.6, 28.4, 28.2, 28.2, 24.4, 23.5, 23.3, 19.9, 17.1, 16.8.

Proline Deprotection and Acetylation General Procedure:

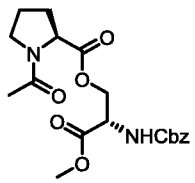
[00396] Boc-proline esters (1 equivalent) were deprotected by treatment with 40% TFA in DCM. Upon complete conversion of the starting material, the reaction was concentrated first by blowing with a stream of nitrogen and then by placing the residue under high vacuum. Once dry, the residue was dissolved in DCM to a concentration of 0.2 M and treated with triethylamine (2 equivalents) and acetic anhydride (2 equivalents). The reactions were allowed to proceed for 16 hours after which the solvent was removed in vacuo. The N-acetyl proline esters were isolated by silica gel chromatography 100% ethyl acetate.

[00397] This general acetylation procedure may be modified to provide other acylated compounds contemplated herein, for example, wherein R^2 is a group of the following formula:



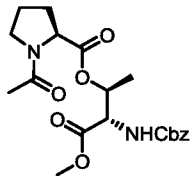
wherein R^{2B} and R^{2C} are as defined herein.

(S)-2-(((benzyloxy)carbonyl)amino)-3-methoxy-3-oxopropyl acetyl-L-prolinate:



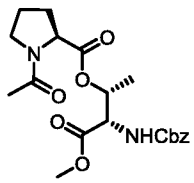
[00398] Product isolated as a colorless oil 85%. HRMS (FAB) Predicted for: $[C_{19}H_{24}N_2O_7 + Na]^+$: 415.1481, Found 415.1472. ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.42 - 7.28 (m, 5 H), 6.10 (d, *J* = 8.5 Hz, 1 H), 5.14 (d, *J* = 2.8 Hz, 2 H), 4.68 (dd, *J* = 3.3, 11.3 Hz, 1 H), 4.61 (td, *J* = 3.3, 8.7 Hz, 1 H), 4.45 - 4.39 (m, 1 H), 4.35 (dd, *J* = 3.6, 11.2 Hz, 1 H), 3.77 (s, 3 H), 3.62 - 3.52 (m, 1 H), 3.52 - 3.43 (m, 1 H), 2.28 - 2.11 (m, 1 H), 2.07 (s, 3 H), 2.02 - 1.91 (m, 3 H). ^{13}C NMR (101MHz $CDCl_3$) δ = 171.5, 170.1, 169.9, 156.1, 136.3, 128.5, 128.4, 128.2, 127.9, 127.9, 66.9, 64.2, 58.7, 53.4, 52.7, 47.7, 29.2, 24.8, 22.1.

(2S,3S)-3-(((benzyloxy)carbonyl)amino)-4-methoxy-4-oxobutan-2-yl acetyl-L-prolinate:



[00399] Product isolated as a white foam, 52 % yield. HRMS (ESI) Predicted for $[C_{20}H_{26}N_2O_7 + H]^+$: 407.1818, Found: 407.1816. ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.38 - 7.27 (m, 5 H), 6.11 (d, *J* = 9.3 Hz, 1 H), 5.21 (dq, *J* = 3.1, 6.7 Hz, 1 H), 5.11 (s, 2 H), 4.55 (dd, *J* = 3.1, 9.2 Hz, 1 H), 4.37 (dd, *J* = 4.0, 8.3 Hz, 1 H), 3.75 (s, 3 H), 3.60 - 3.52 (m, 1 H), 3.52 - 3.45 (m, 1 H), 2.20 - 2.13 (m, 1 H), 2.10 (s, 3 H), 2.02 - 1.91 (m, 3 H), 1.32 (d, *J* = 6.8 Hz, 3 H). ^{13}C NMR (151MHz, $CDCl_3$) δ = 171.3, 170.3, 169.6, 156.1, 136.5, 129.0, 128.5, 128.4, 128.1, 127.9, 127.8, 127.6, 71.3, 66.8, 58.9, 57.2, 52.3, 47.8, 29.2, 24.8, 22.2, 16.4.

(2R,3S)-3-(((benzyloxy)carbonyl)amino)-4-methoxy-4-oxobutan-2-yl acetyl-L-prolinate:



[00400] Product isolated as a white foam, 91 % yield. Predicted for $[C_{20}H_{26}N_2O_7 + H]^+$: 407.1818, Found: 407.1806. ~2:1 mixture of amide rotamers. ^{1}H NMR (600MHz, $CDCl_3$) δ = 7.42 - 7.28 (m, 5 H), 5.69 (d, *J* = 9.2 Hz, 1 H), 5.50 - 5.36 (m, 1 H), 5.18 - 5.09 (m, 2 H), 4.57 - 4.46 (m, 1 H), 4.36 (dd, *J* = 4.2, 8.6 Hz, 1 H), 3.77 (s, 2 H), 3.71 (s, 1 H), 3.59 (qd, *J* = 4.9, 7.7 Hz, 1 H), 3.53 - 3.41 (m, 1 H), 2.21 - 2.10 (m, 1 H), 2.05 (s, 3 H), 2.01 - 1.83 (m, 3 H), 1.34 - 1.23 (m, 3 H). ^{13}C NMR (151MHz, $CDCl_3$) δ = 171.1, 171.0, 170.0, 169.3, 169.2, 156.5, 156.3, 136.1, 135.8, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 71.4, 71.1, 67.4, 67.1, 66.6, 59.9, 58.5, 58.3, 57.5, 57.2, 52.8, 52.7, 47.6, 46.2, 31.4, 29.3, 29.1, 24.7, 22.6, 22.1, 22.0, 16.8, 16.7.

Side Chain Synthesis General Procedure:

[00401] *Cbz removal:* The CBZ protected proline ester was dissolved in methanol at a concentration of 0.14 M. Once the solution was homogeneous, 10% Pd/C (130 mg / mmol peptidolactone) was added followed by 1M HCl (1 equivalent with respect to proline ester). With the reaction flask septum sealed, hydrogen gas was delivered to the reaction by a balloon affixed to a syringe. The reaction was allowed to proceed for 90 minutes after which

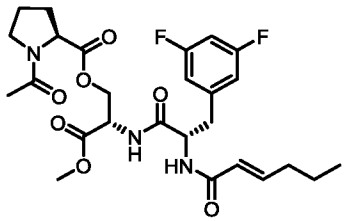
it was filtered through celite in order to remove the Pd/C. Removal of solvent revealed the proline hydrochloride salt as an off white foam, which is used without further purification.

[00402] *Boc-DifluoroPhe acylation:* The proline ester hydrochloride salt was dissolved in DMF at a concentration of 0.25 M. Once the solution was homogeneous, Boc-difluorophenylalanine (1.1 equivalents) was added followed by HATU (1.1 equivalents). The reaction was initiated by the addition of DIPEA (2.2 equivalents) and allowed to proceed for 2 hours. After the reaction, the DMF solution was diluted ~10 x the reaction volume with ethyl acetate, and extracted 3x 1M HCl, 3x saturated $NaHCO_3$, 1x brine, then dried over sodium sulfate. The Boc-difluorophenylalanine acylated proline esters were isolated by silica gel chromatography using an ethyl acetate / acetone solvent gradient. Final removal of the solvent revealed a fine white powder, which was used without further purification.

[00403] *Boc group removal:* The Boc-difluorophenylalanyl proline esters were treated with 40% TFA in DCM at a concentration of 0.3 M. Upon complete conversion of the starting material, the reaction was concentrated by blowing with a stream of nitrogen then by evaporation under high vacuum. The deprotected product was used without further purification.

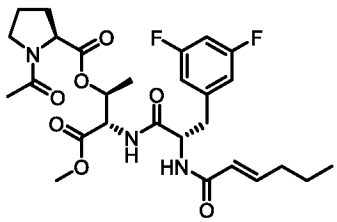
[00404] *E-2-Heptenoate Coupling:* The difluorophenylalanyl proline ester TFA salts were dissolved in DMF at a concentration of 0.2 M. Once the solution was homogeneous, E-2-heptenoic acid (1.1 equivalents) was dissolved in DMF at a concentration of 0.2 M. The acid was then treated with HATU (1.1 equivalents) and DIPEA (1.1 equivalents) and allowed to stir for 15 minutes. The preactivated acid was then added to a solution of the difluorophenylalanyl proline ester TFA salts followed by additional DIPEA (1.1 equivalents). The reaction was allowed to proceed for 2 hours, after which it was diluted ~10x with ethyl acetate and extracted 3x 1M HCl, 3x saturated $NaHCO_3$, 1x brine, then dried over sodium sulfate. The final products were purified by silica gel chromatography using an ethyl acetate / acetone solvent gradient. Final removal of the solvent revealed the products as white foams.

(S)-2-((S)-3-(3,5-difluorophenyl)-2-((E)-hex-2-enamido)propanamido)-3-methoxy-3-oxopropyl acetyl-L-prolinate:



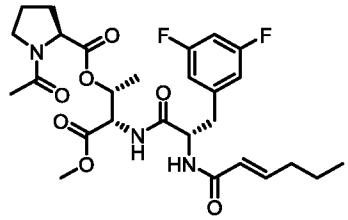
[00405] 0.46 mmol scale, yield: 185 mg, 72% over 4 steps. HRMS (FAB) Predicted for $[C_{27}H_{35}F_2N_3O_7 + Na]^+$:574.2341, Found: 574.2358. ^{1}H NMR (600MHz, $CDCl_3$) δ = 7.29 (d, *J* = 8.8 Hz, 1 H), 7.24 (d, *J* = 7.7 Hz, 1 H), 6.83 (td, *J* = 7.0, 15.4 Hz, 1 H), 6.79 (dd, *J* = 2.2, 8.1 Hz, 2 H), 6.62 (tt, *J* = 2.3, 9.0 Hz, 1 H), 5.87 (td, *J* = 1.5, 15.3 Hz, 1 H), 5.03 - 4.92 (m, 1 H), 4.89 - 4.78 (m, 2 H), 4.32 (dd, *J* = 5.0, 8.6 Hz, 1 H), 4.27 - 4.21 (m, 1 H), 3.77 (s, 3 H), 3.60 - 3.51 (m, 2 H), 3.26 (dd, *J* = 5.3, 14.1 Hz, 1 H), 3.06 (dd, *J* = 8.1, 13.9 Hz, 1 H), 2.31 - 2.21 (m, 1 H), 2.20 - 2.13 (m, 2 H), 2.13 (s, 3 H), 2.07 - 1.95 (m, 3 H), 1.46 - 1.38 (m, 2 H), 1.36 - 1.29 (m, 2 H), 0.90 (t, *J* = 7.3 Hz, 3 H). ^{13}C NMR (151MHz, $CDCl_3$) δ = 171.2, 170.6, 170.4, 169.4, 165.9, 163.6, 163.5, 161.9, 161.9, 145.4, 141.3, 141.3, 141.2, 123.2, 112.5, 112.5, 112.4, 112.4, 102.1, 101.9, 101.7, 63.0, 59.2, 53.6, 52.7, 51.7, 48.0, 37.6, 31.8, 30.2, 29.2, 25.0, 22.3, 22.2, 13.8.

(2S,3S)-3-((S)-3-(3,5-difluorophenyl)-2-((E)-hex-2-enamido)propanamido)-4-methoxy-4-oxobutan-2-yl acetyl-L-prolinate:



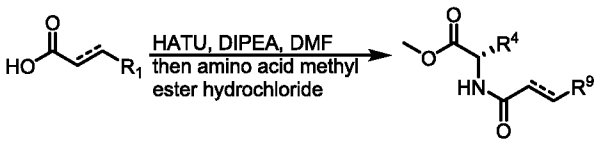
[00406] 0.52 mmol scale, yield: 212 mg, 72% over 4 steps. HRMS (ESI) Predicted for $[C_{28}H_{37}F_2N_3O_7 + H]^+$: 566.2679, Found: 566.2659. ^{1}H NMR (600MHz, $CDCl_3$) δ = 7.39 - 7.29 (m, 2 H), 6.82 (td, *J* = 7.0, 15.4 Hz, 1 H), 6.79 - 6.74 (m, 2 H), 6.63 (tt, *J* = 2.3, 9.0 Hz, 1 H), 5.90 (td, *J* = 1.7, 15.4 Hz, 1 H), 5.33 (dq, *J* = 2.9, 6.7 Hz, 1 H), 4.92 (dt, *J* = 5.1, 8.8 Hz, 1 H), 4.66 (dd, *J* = 2.9, 8.4 Hz, 1 H), 4.40 (dd, *J* = 4.4, 8.8 Hz, 1 H), 3.76 (s, 3 H), 3.61 - 3.51 (m, 2 H), 3.24 (dd, *J* = 5.1, 14.3 Hz, 1 H), 3.00 (dd, *J* = 8.6, 14.1 Hz, 1 H), 2.33 - 2.23 (m, 1 H), 2.19 (s, 3 H), 2.17 - 2.13 (m, 2 H), 2.07 - 1.93 (m, 3 H), 1.46 - 1.37 (m, 2 H), 1.36 - 1.26 (m, 5 H), 0.89 (t, *J* = 7.2 Hz, 3 H). ^{13}C NMR (151MHz, $CDCl_3$) δ = 171.1, 170.7, 170.3, 168.7, 165.8, 163.6, 163.6, 162.0, 161.9, 145.4, 141.3, 141.2, 141.1, 123.2, 112.3, 112.3, 112.2, 112.2, 102.2, 102.0, 101.9, 70.4, 59.5, 55.3, 53.9, 52.2, 48.0, 38.0, 31.7, 30.2, 29.4, 24.9, 22.4, 22.2, 16.9, 13.8.

(2R,3S)-3-((S)-3-(3,5-difluorophenyl)-2-((E)-hex-2-enamido)propanamido)-4-methoxy-4-oxobutan-2-yl acetyl-L-prolinate:



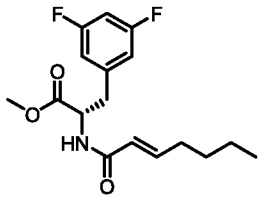
[00407] 0.50 mmol scale, Yield: 222 mg 79% over 4 steps. HRMS (ESI) Predicted for $[C_{28}H_{37}F_2N_3O_7+H]+$: 566.2679, Found: 566.2667. ^{1}H NMR (400MHz CDCl3-d) δ = 7.28 (d, *J* = 8.8 Hz, 1 H), 7.13 (d, *J* = 8.8 Hz, 1 H), 6.87 - 6.75 (m, 3 H), 6.60 (tt, *J* = 2.3, 9.0 Hz, 1 H), 5.84 (td, *J* = 1.5, 15.3 Hz, 1 H), 5.18 (quin, *J* = 6.5 Hz, 1 H), 5.01 (ddd, *J* = 5.4, 7.2, 8.8 Hz, 1 H), 4.67 (dd, *J* = 6.5, 8.5 Hz, 1 H), 4.12 (dd, *J* = 5.0, 8.0 Hz, 1 H), 3.74 (s, 3 H), 3.68 - 3.59 (m, 1 H), 3.57 - 3.47 (m, 1 H), 3.23 (dd, *J* = 5.4, 13.9 Hz, 1 H), 3.13 (dd, *J* = 7.2, 13.9 Hz, 1 H), 2.26 - 2.11 (m, 4 H), 2.10 (s, 3 H), 2.03 - 1.91 (m, 2 H), 1.45 - 1.37 (m, 2 H), 1.36 - 1.27 (m, 5 H), 0.92 - 0.87 (m, 3 H). ^{13}C NMR (151MHz CDCl3-d) δ = 171.7, 171.1, 169.7, 169.4, 166.3, 163.5, 163.4, 161.9, 161.8, 145.6, 141.3, 141.2, 123.0, 112.8, 112.8, 112.6, 102.0, 101.8, 101.6, 70.2, 58.6, 56.2, 53.4, 52.7, 48.0, 37.1, 31.8, 30.2, 29.1, 25.0, 22.2, 18.0, 13.8.

Synthesis of ADEP side chain methyl esters general procedure:



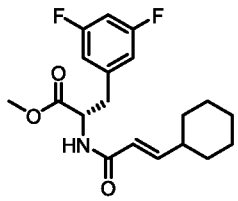
[00408] The carboxylic acid (1 equivalent) was dissolved in DMF at a concentration of 0.2 M. The acid was then treated with HATU (1.1 equivalents) and DIPEA (2.2 equivalents) and allowed to stir for 15 minutes after which, an amino acid methyl ester hydrochloride was added. The coupling reaction was allowed to proceed for 3 hours, after which the reaction solution was diluted to 5x original volume with ethyl acetate and extracted 3x 1M HCl, 3x sat NaHCO3, 1x Brine. The organic layer was then dried over sodium sulfate and purified by silica gel flash chromatography using a hexanes/ethyl acetate solvent gradient.

Methyl (S,E)-3-(3,5-difluorophenyl)-2-(hept-2-enamido)propanoate:



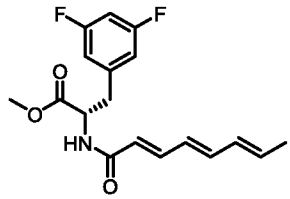
[00409] Compound isolated as white solid: 80% yield. HRMS (ESI) predicted for $[C_{17}H_{21}F_2NO_3 + H]^+$: 326.1568, found:326.1559. 1H NMR (400MHz, $CDCl_3$) δ = 6.87 (td, J = 7.0, 15.3 Hz, 1 H), 6.73 - 6.66 (m, 1 H), 6.66 - 6.60 (m, 2 H), 6.02 (d, J = 6.8 Hz, 1 H), 5.80 (td, J = 1.5, 15.3 Hz, 1 H), 4.95 (td, J = 5.7, 7.4 Hz, 1 H), 3.79 - 3.73 (m, 3 H), 3.19 (dd, J = 5.8, 13.8 Hz, 1 H), 3.11 (dd, J = 5.3, 13.8 Hz, 1 H), 2.19 (dq, J = 1.4, 7.2 Hz, 2 H), 1.49 - 1.39 (m, 2 H), 1.38 - 1.29 (m, 2 H), 0.91 (t, J = 7.3 Hz, 3 H). 13C NMR (75MHz, $CDCl_3$) δ = 171.6, 165.5, 164.6, 164.5, 161.3, 161.2, 146.4, 140.0, 139.9, 139.7, 122.6, 112.4, 112.3, 112.1, 112.0, 103.0, 102.6, 102.3, 52.8, 52.5, 37.6, 31.7, 30.2, 22.2, 13.8.

Methyl (S,E)-2-(3-cyclohexylacrylamido)-3-(3,5-difluorophenyl)propanoate:



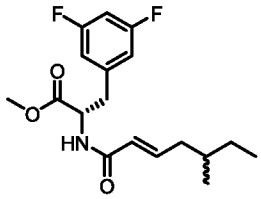
[00410] Compound isolated as white solid: 83% yield. HRMS (ESI) predicted for $[C_{19}H_{23}F_2NO_3 + H]^+$: 352.1724, found:352.1716. ^{1}H NMR (400MHz, $CDCl_3$) δ = 6.83 (dd, *J* = 6.8, 15.3 Hz, 1 H), 6.70 (tt, *J* = 2.1, 9.0 Hz, 1 H), 6.67 - 6.60 (m, 2 H), 6.00 (d, *J* = 7.0 Hz, 1 H), 5.74 (dd, *J* = 1.3, 15.3 Hz, 1 H), 5.00 - 4.91 (m, 1 H), 3.76 (s, 3 H), 3.19 (dd, *J* = 5.8, 13.8 Hz, 1 H), 3.12 (dd, *J* = 5.3, 13.8 Hz, 1 H), 2.20 - 2.06 (m, 1 H), 1.80-1.72 (m, 4 H), 1.68 (d, *J* = 12.5 Hz, 1 H), 1.37 - 1.24 (m, 2 H), 1.24 - 1.07 (m, 3 H). ^{13}C NMR (101MHz, $CDCl_3$) δ = 171.6, 165.8, 164.2, 164.1, 161.7, 161.6, 151.4, 139.9, 139.9, 120.2, 112.3, 112.3, 112.2, 112.1, 102.9, 102.7, 102.4, 52.9, 52.5, 40.3, 37.6, 31.8, 25.9, 25.7

Methyl (S)-3-(3,5-difluorophenyl)-2-((2E,4E,6E)-octa-2,4,6-trienamido)propanoate:



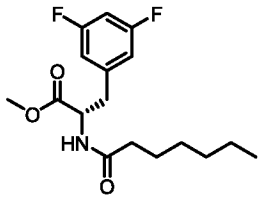
[00411] Compound isolated as white solid: 77% yield. HRMS (ESI) predicted for $[C_{18}H_{19}F_2NO_3 + H]^+$: 336.1411, found:336.1405. ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.33 - 7.22 (m, 1 H), 6.71 (t, *J* = 9.0 Hz, 1 H), 6.68 - 6.62 (m, 2 H), 6.53 (dd, *J* = 10.8, 14.8 Hz, 1 H), 6.24 - 6.11 (m, 2 H), 6.09 (d, *J* = 7.5 Hz, 1 H), 6.02 - 5.89 (m, 1 H), 5.86 (d, *J* = 15.1 Hz, 1 H), 5.07 - 4.90 (m, 1 H), 3.77 (s, 3 H), 3.21 (dd, *J* = 5.8, 13.8 Hz, 1 H), 3.13 (dd, *J* = 5.3, 13.8 Hz, 1 H), 1.84 (d, *J* = 6.5 Hz, 3 H). ^{13}C NMR (101MHz $CDCl_3$) δ = 171.6, 165.7, 164.2, 164.1, 161.7, 161.6, 142.4, 140.7, 139.8, 134.7, 131.2, 127.3, 121.4, 112.3, 112.3, 112.1, 112.1, 102.9, 102.7, 102.4, 52.9, 52.5, 37.6, 18.5.

Methyl (2S)-3-(3,5-difluorophenyl)-2-((E)-5-methylhept-2-enamido)propanoate:



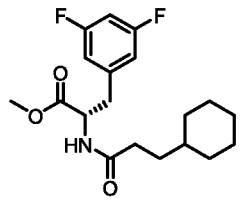
[00412] Compound isolated as white solid: 78% yield. HRMS (ESI) predicted for $[C_{18}H_{23}F_2NO_3 + H]^+$: 340.1724, found:340.1715. ^{1}H NMR (400MHz $CDCl_3$) δ = 6.87 (td, *J* = 7.5, 15.1 Hz, 1 H), 6.70 (tt, *J* = 2.3, 9.0 Hz, 1 H), 6.67 - 6.59 (m, 2 H), 5.99 (d, *J* = 7.0 Hz, 1 H), 5.80 (d, *J* = 15.6 Hz, 1 H), 5.00 - 4.89 (m, 1 H), 3.76 (s, 3 H), 3.21 (dd, *J* = 5.8, 13.8 Hz, 1 H), 3.12 (dd, *J* = 5.3, 13.8 Hz, 1 H), 2.25 - 2.15 (m, 1 H), 2.08 - 1.98 (m, 1 H), 1.59-1.49 (m, 1 H), 1.44 - 1.32 (m, 1 H), 1.23 - 1.13 (m, 1 H), 0.92 - 0.86 (m, 6 H). ^{13}C NMR (101MHz $CDCl_3$) δ = 171.6, 165.4, 164.2, 164.1, 161.7, 161.6, 145.4, 139.9, 139.8, 123.7, 112.3, 112.3, 112.2, 112.1, 102.9, 102.7, 102.4, 52.8, 52.6, 39.2, 37.6, 34.2, 29.2, 29.1, 19.1, 11.3.

Methyl (S,E)-3-(3,5-difluorophenyl)-2-(hept-2-enamido)propanoate:



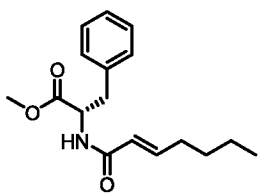
[00413] Compound isolated as white solid: 80% yield. HRMS (ESI) predicted for $[C_{17}H_{21}F_2NO_3 + H]^+$: 326.1568, found:326.1559. 1H NMR (400MHz, $CDCl_3$) δ = 6.87 (td, J = 7.0, 15.3 Hz, 1 H), 6.73 - 6.66 (m, 1 H), 6.66 - 6.60 (m, 2 H), 6.02 (d, J = 6.8 Hz, 1 H), 5.80 (td, J = 1.5, 15.3 Hz, 1 H), 4.95 (td, J = 5.7, 7.4 Hz, 1 H), 3.79 - 3.73 (m, 3 H), 3.19 (dd, J = 5.8, 13.8 Hz, 1 H), 3.11 (dd, J = 5.3, 13.8 Hz, 1 H), 2.19 (dq, J = 1.4, 7.2 Hz, 2 H), 1.49 - 1.39 (m, 2 H), 1.38 - 1.29 (m, 2 H), 0.91 (t, J = 7.3 Hz, 3 H). 13C NMR (75MHz, $CDCl_3$) δ = 171.6, 165.5, 164.6, 164.5, 161.3, 161.2, 146.4, 140.0, 139.9, 139.7, 122.6, 112.4, 112.3, 112.1, 112.0, 103.0, 102.6, 102.3, 52.8, 52.5, 37.6, 31.7, 30.2, 22.2, 13.8.

Methyl (S)-2-(3-cyclohexylpropanamido)-3-(3,5-difluorophenyl)propanoate:



[00414] Compound isolated as white solid: 84% yield. HRMS (ESI) predicted for $[C_{19}H_{25}F_2NO_3 + H]^+$: 354.1881, found:354.1873. ^{1}H NMR (400MHz, $CDCl_3$) δ = 6.74 - 6.68 (m, 1 H), 6.66 - 6.60 (m, 2 H), 5.95 (d, *J* = 6.8 Hz, 1 H), 4.92 - 4.86 (m, 1 H), 3.77 (s, 3 H), 3.17 (dd, *J* = 5.8, 13.9 Hz, 1 H), 3.07 (dd, *J* = 5.4, 13.8 Hz, 1 H), 2.26 - 2.19 (m, 2 H), 1.68 (s, 3 H), 1.71 (s, 2 H), 1.55 - 1.47 (m, 2 H), 1.28 - 1.11 (m, 4 H), 0.89 (q, *J* = 11.4 Hz, 2 H). ^{13}C NMR (101MHz, $CDCl_3$) δ = 173.0, 171.7, 164.2, 161.7, 139.9, 139.8, 112.3, 112.2, 112.1, 112.1, 102.9, 102.7, 102.4, 52.6, 52.6, 37.6, 37.2, 34.0, 33.0, 32.9, 26.5, 26.1.

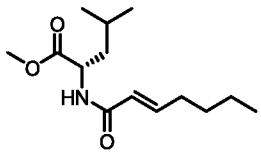
Methyl (E)-hept-2-enoyl-L-phenylalaninate:



[00415] Compound isolated as a white solid: 97% yield. HRMS (ESI) predicted for [C17H23NO3 + H]+: 290.1756, found:290.1750. ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.37 - 7.19

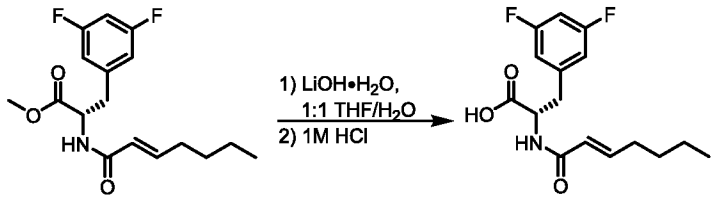
(m, 3 H), 7.14 - 7.05 (m, 2 H), 6.86 (td, J = 7.0, 15.1 Hz, 1 H), 5.96 (d, J = 7.5 Hz, 1 H), 5.77 (td, J = 1.5, 15.1 Hz, 1 H), 4.97 (td, J = 5.6, 7.8 Hz, 1 H), 3.73 (s, 3 H), 3.24 - 3.09 (m, 2 H), 2.25 - 2.10 (m, 2 H), 1.48 - 1.38 (m, 2 H), 1.38 - 1.28 (m, 2 H), 0.90 (t, J = 7.0 Hz, 3 H). ^{13}C NMR (101MHz, $CDCl_3$) δ = 172.1, 165.4, 145.9, 135.8, 129.2, 128.5, 127.0, 122.8, 53.0, 52.3, 37.8, 31.7, 30.2, 22.2, 13.8.

Methyl (E)-hept-2-enoyl-L-leucinate:



[00416] Compound isolated as a colorless oil: 95% yield. HRMS (ESI) predicted for $[C_{14}H_{25}NO_3 + H]^+$: 256.1913, found:256.1904. ^{1}H NMR (600MHz, $CDCl_3$) δ = 6.87 (td, J = 7.0, 15.4 Hz, 1 H), 5.91 - 5.83 (m, 1 H), 5.81 (td, J = 1.5, 15.3 Hz, 1 H), 4.73 (dt, J = 4.8, 8.6 Hz, 1 H), 3.74 (s, 3 H), 2.19 (dq, J = 1.5, 7.2 Hz, 2 H), 1.70 - 1.65 (m, 2 H), 1.60 - 1.53 (m, 1 H), 1.47 - 1.39 (m, 2 H), 1.39 - 1.30 (m, 2 H), 0.96 (d, J = 5.9 Hz, 3 H), 0.94 (d, J = 6.2 Hz, 3 H), 0.91 (t, J = 7.2 Hz, 3 H). ^{13}C NMR (151MHz, $CDCl_3$) δ = 173.8, 165.7, 145.9, 122.9, 52.3, 50.5, 41.9, 31.7, 30.2, 24.8, 22.8, 22.2, 22.0, 13.8.

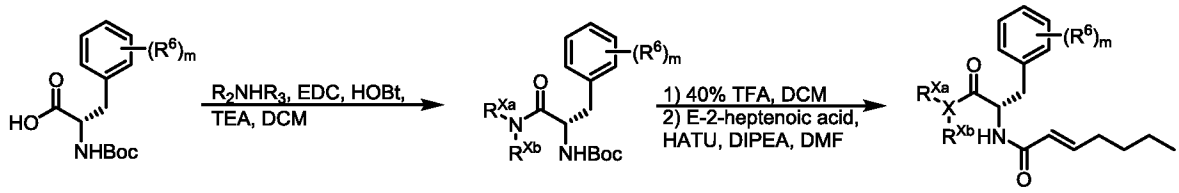
Synthesis of ADEP Side Chain Free Acid:



[00417] Methyl (S,E)-3-(3,5-difluorophenyl)-2-(hept-2-enamido)propanoate (50 mg, 0.15 mmol) was dissolved in 1:1 THF/H_2O (0.5 mL) and treated with lithium hydroxide monohydrate (17 mg, 0.41 mmol). The reaction was allowed to proceed for 45 minutes, after which 1 M HCl (0.62 mL) was added. The reaction solution was then transferred to a separatory funnel and extracted 3x DCM. The DCM extracts were combined and dried over sodium sulfate. After removal of solvent, the ADEP side chain free acid was revealed as a white solid and used without further purification: 43 mg, 93% yield. HRMS (ESI) predicted for $[C_{16}H_{19}F_2NO_3 + H]^+$: 311.1411, found:312.1402. ^{1}H NMR (600MHz, $CDCl_3$) δ = 10.12 (br. s., 1 H), 6.89 (td, J = 7.0, 15.4 Hz, 1 H), 6.77 - 6.58 (m, 3 H), 6.27 (d, J = 7.0 Hz, 1 H), 5.82 (td, J = 1.5, 15.3 Hz, 1 H), 4.93 (td, J = 5.7, 7.3 Hz, 1 H), 3.26 (dd, J = 5.7, 14.1 Hz, 1 H), 3.15 (dd, J = 5.7, 14.1 Hz, 1 H), 2.24 - 2.11 (m, 2 H), 1.48 - 1.37 (m, 2 H), 1.37 - 1.24 (m,

2 H), 0.90 (t, J = 7.3 Hz, 3 H). ^{13}C NMR (151MHz, $CDCl_3$) δ = 173.6, 166.7, 163.8, 163.7, 162.1, 162.0, 147.6, 139.7, 139.6, 122.1, 112.4, 112.4, 112.3, 112.3, 102.9, 102.7, 102.5, 53.1, 37.0, 31.8, 30.1, 22.2, 13.8.

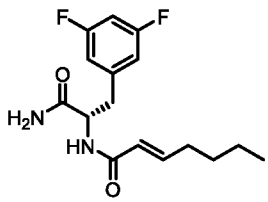
Synthesis of ADEP side chain methyl amides general procedure:



[00418] A Boc-amino acid (1 equivalent) and either 7 M ammonia in methanol (1 equivalent), 2 M methyl amine in methanol (1 equivalent), or dimethylamine hydrochloride (1 equivalent) were dissolved in dichloromethane at a concentration of 0.1 M with respect to the Boc-amino acid. The solution was then treated with EDC (1.1 equivalents), HOBt (1.1 equivalents), and TEA (1.1 equicalents for free base amines, 2.1 equivalents for dimethyl amine HCl). The coupling reactions were allowed to run over night after which solvent was removed and the residue was diluted in ethyl acetate to twice the original reaction volume. The ethyl acetate solution was extracted 3x 1M HCl, 3x sat NaHCO3, 1x brine, and then dried over sodium sulfate. The concentrated crude Boc-amino amides were advanced without further purification.

[00419] Crude Boc-amino amides were deprotected by treatment with 40% TFA in DCM. Upon complete conversion of the starting material, the reaction was concentrated first by blowing with a stream of nitrogen and then by placing the residue under high vacuum. Once dry, the free base amino amides were subjected to HATU mediated coupling with a carboxylic acid and purified as described in the general procedure for the synthesis of side chain methyl esters.

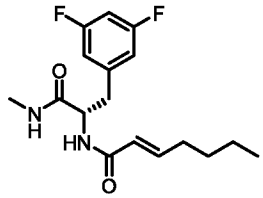
(S,E)-N-(1-amino-3-(3,5-difluorophenyl)-1-oxopropan-2-yl)hept-2-enamide:



[00420] Compound isolated as white solid: 69% yield over 3 steps. HRMS (ESI) predicted for $[C_{16}H_{20}F_2N_2O_2 + H]^+$: 31.1571, found:311.1562. ^{1}H NMR (400MHz, DMSO-d_6) δ = 8.12 (d, J = 9.0 Hz, 1 H), 7.50 (s, 1 H), 7.11 (s, 1 H), 7.04 (tt, J = 2.4, 9.4 Hz, 1 H), 7.00 - 6.90 (m,

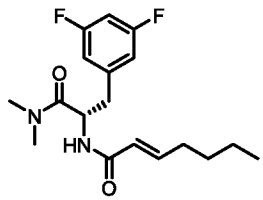
2 H), 6.54 (td, *J* = 7.0, 15.1 Hz, 1 H), 6.03 - 5.83 (m, 1 H), 4.61 - 4.42 (m, 1 H), 3.02 (dd, *J* = 4.5, 13.6 Hz, 1 H), 2.78 (dd, *J* = 10.0, 14.1 Hz, 1 H), 2.17 - 2.06 (m, 2 H), 1.40 - 1.31 (m, 2 H), 1.31 - 1.22 (m, 2 H), 0.86 (t, *J* = 7.3 Hz, 3 H). ^{13}C NMR (101MHz, DMSO-d_6) δ = 172.7, 164.8, 163.3, 163.2, 160.9, 160.7, 143.0, 142.9, 142.8, 142.7, 124.1, 112.4, 112.3, 112.2, 112.1, 102.0, 101.8, 101.5, 53.3, 37.3, 30.8, 29.9, 21.6, 13.7.

(S,E)-N-(3-(3,5-difluorophenyl)-1-(methylamino)-1-oxopropan-2-yl)hept-2-enamide:



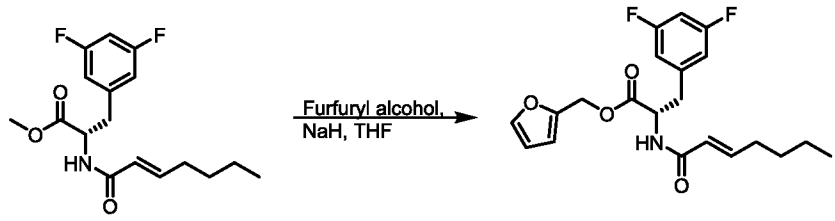
[00421] Compound isolated as white solid: 72% yield over 3 steps. HRMS (ESI) predicted for [$C_{17}H_{22}F_2N_2O_2$ + H]$^+$: 325.1727, found:325.1719. ^{1}H NMR (400MHz, $CDCl_3$) δ = 6.89 - 6.78 (m, 1 H), 6.76 (d, *J* = 6.3 Hz, 2 H), 6.67 (dt, *J* = 2.4, 9.0 Hz, 1 H), 6.55 (d, *J* = 7.5 Hz, 2 H), 5.79 (d, *J* = 15.3 Hz, 1 H), 4.77 (q, *J* = 7.5 Hz, 1 H), 3.15 - 2.99 (m, 2 H), 2.74 (d, *J* = 4.8 Hz, 3 H), 2.18 (q, *J* = 6.9 Hz, 2 H), 1.48 - 1.38 (m, 2 H), 1.37 - 1.28 (m, 2 H), 0.90 (t, *J* = 7.3 Hz, 3 H). ^{13}C NMR (101MHz, $CDCl_3$) δ = 171.3, 166.0, 146.3, 140.7, 140.6, 122.7, 112.3, 112.0, 102.4, 54.1, 38.1, 31.7, 30.2, 26.2, 22.2, 13.8.

(S,E)-N-(3-(3,5-difluorophenyl)-1-(dimethylamino)-1-oxopropan-2-yl)hept-2-enamide:



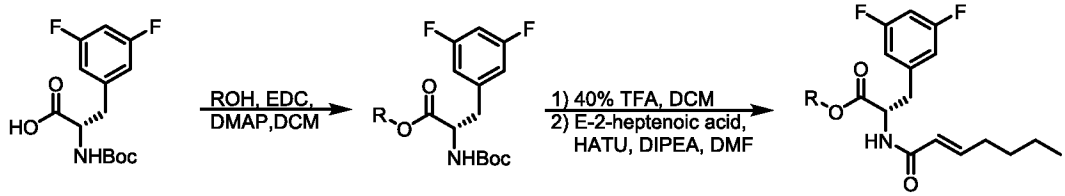
[00422] Compound isolated as white solid: 82% yield over 3 steps. HRMS (ESI) predicted for [$C_{18}H_{24}F_2N_2O_2$ + H]$^+$: 339.1884, found:339.1874. ^{1}H NMR (400MHz, $CDCl_3$) δ = 6.92 - 6.79 (m, 1 H), 6.77 - 6.65 (m, 3 H), 6.47 (d, *J* = 8.0 Hz, 1 H), 5.80 (d, *J* = 15.1 Hz, 1 H), 5.22 (dt, *J* = 6.0, 7.8 Hz, 1 H), 3.01 (t, *J* = 6.3 Hz, 2 H), 2.92 (s, 3 H), 2.86 - 2.79 (m, 3 H), 3.07-2.95 (m, 2 H), 1.49 - 1.40 (m, 2 H), 1.38 - 1.30 (m, 2 H), 0.91 (t, *J* = 7.3 Hz, 4 H). ^{13}C NMR (101MHz $CDCl_3$) δ = 170.7, 165.3, 164.2, 164.0, 161.7, 161.6, 145.8, 140.3, 140.2, 123.0, 112.4, 112.3, 112.2, 112.1, 102.8, 102.5, 102.3, 49.6, 39.1, 37.0, 35.6, 31.7, 30.2, 22.2, 13.8.

Synthesis of ADEP side chain furfuryl ester:



[00423] Furfuryl alcohol (26 μL, 0.3 mmol) was dissolved in THF (2 mL) and treated with NaH 60% in mineral oil (1 mg, 0.03 mmol) and allowed to react for 5 minutes before the addition of SC1 (65 mg, 0.2 mmol). After reacting for 6 hours, the reaction was concentrated and the crude product residue was loaded directly onto a silica gel column and chromatographed with 20% ethyl acetate in hexanes. The product was isolated as a white solid: 29 mg, 37% yield. HRMS (ESI) predicted for $[C_{21}H_{23}F_2NO_4 + H]^+$: 391.1673, found:392.1669. ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.47 (s, 1 H), 6.96 - 6.81 (m, 1 H), 6.67 (tt, *J* = 2.3, 9.0 Hz, 1 H), 6.59 - 6.48 (m, 2 H), 6.45 (d, *J* = 3.0 Hz, 1 H), 6.42 - 6.36 (m, 1 H), 5.98 (d, *J* = 7.5 Hz, 1 H), 5.80 (d, *J* = 15.6 Hz, 1 H), 5.25 (d, *J* = 13.0 Hz, 1 H), 5.04 (d, *J* = 13.1 Hz, 1 H), 5.00 - 4.89 (m, 1 H), 3.25 - 3.06 (m, 2 H), 2.20 (q, *J* = 7.2 Hz, 2 H), 1.50 - 1.40 (m, 2 H), 1.40 - 1.31 (m, 2 H), 0.91 (t, *J* = 7.0 Hz, 3 H). ^{13}C NMR (101MHz, $CDCl_3$) δ = 170.8, 165.4, 164.1, 164.0, 161.6, 161.5, 148.3, 146.5, 143.7, 139.7, 139.6, 139.5, 122.7, 112.5, 112.4, 112.3, 112.2, 111.7, 110.7, 102.9, 102.6, 102.4, 59.0, 52.7, 37.5, 31.7, 30.2, 22.2, 13.8.

Synthesis of side chain benzyl and propargyl esters:



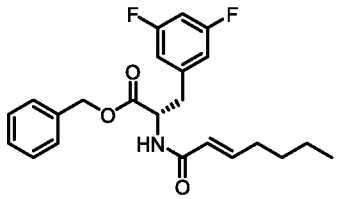
[00424] Boc-3,5-Difluorophenylalanine (1 equivalent) and an alcohol (1 equivalent) were dissolved in DCM at a concentration of 0.2 M. The solution was treated with EDC and DMAP and allowed to react for 3 hours. after which solvent was removed and the residue was diluted in ethyl acetate to twice the original reaction volume. The ethyl acetate solution was extracted 3x 1M HCl, 3x sat NaHCO3, 1x brine, and then dried over sodium sulfate. The concentrated crude Boc-amino acid esters were advanced without further purification.

[00425] Crude Boc-amino acid esters were deprotected by treatment with 40% TFA in DCM. Upon complete conversion of the starting material, the reaction was concentrated first

by blowing with a stream of nitrogen and then by placing the residue under high vacuum. Once dry, the free base amino amides were subjected to HATU mediated coupling with a carboxylic acid and purified as described in the general procedure for the synthesis of side chain methyl esters.

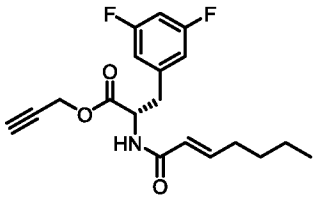
[00426] Synthesis of the corresponding urea compounds, *e.g.,* wherein R^5 is –C(=O)NHR^{5A}, may be accomplished by reacting C-capped phenylalanine analogs with isocyanates of formula C≡NR^{5A} in a non-nucleophilic solvent, such as dimethylformamide (DMF) or tetrahydrofuran (THF). Synthesis of the corresponding carbamate compounds, *e.g.,* wherein R^5 is –C(=O)OR^{5A}, may be accomplished under similar conditions by reacting C-capped phenylalanine analogs with activated compounds of formula XC(=O)OR^{5A}, wherein X is a leaving group. A "leaving group" is an art–understood term referring to a molecular fragment that departs with a pair of electrons in heterolytic bond cleavage, wherein the molecular fragment is an anion or neutral molecule. See, for example, Smith, March *Advanced Organic Chemistry* 6th ed. (501–502). Exemplary leaving groups include, but are not limited to, halo (*e.g.*, chloro, bromo, iodo) and sulfonyl substituted hydroxyl groups (*e.g.*, tosyl, mesyl, besyl). Exemplary activated compounds include chloroformates of formula ClC(=O)OR^{5A}.

Benzyl (S,E)-3-(3,5-difluorophenyl)-2-(hept-2-enamido)propanoate:



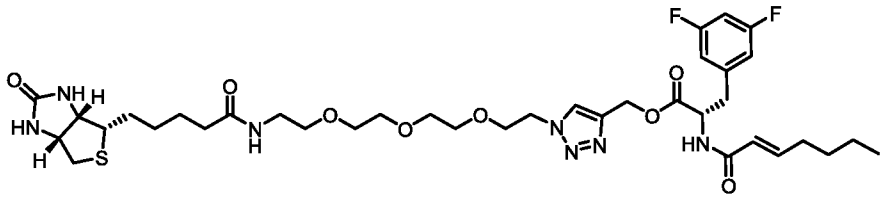
[00427] Compound isolated as a white solid: 55% yield over 3 steps. HRMS (ESI) predicted for $[C_{23}H_{25}F_2NO_3 + H]^+$: 401.1881, found:401.1870. ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.43 - 7.36 (m, 3 H), 7.36 - 7.30 (m, 2 H), 6.88 (td, *J* = 7.0, 15.6 Hz, 1 H), 6.66 (tt, *J* = 2.3, 9.0 Hz, 1 H), 6.58 - 6.46 (m, 2 H), 6.01 (d, *J* = 7.0 Hz, 1 H), 5.80 (td, *J* = 1.5, 15.1 Hz, 1 H), 5.21 (d, *J* = 12.0 Hz, 1 H), 5.13 (d, *J* = 12.0 Hz, 1 H), 4.98 (td, *J* = 5.4, 7.3 Hz, 1 H), 3.17 (dd, *J* = 6.0, 14.1 Hz, 1 H), 3.10 (dd, *J* = 5.0, 14.1 Hz, 1 H), 2.26 - 2.11 (m, 2 H), 1.52 - 1.39 (m, 2 H), 1.39 - 1.29 (m, 2 H), 0.91 (t, *J* = 7.2 Hz, 3 H). ^{13}C NMR (101MHz CDCl3-d) δ = 171.0, 165.4, 164.1, 164.0, 161.6, 161.5, 146.4, 139.8, 139.7, 134.7, 128.8, 128.7, 128.7, 122.7, 112.4, 112.3, 112.2, 112.2, 102.8, 102.6, 102.3, 67.6, 52.8, 37.5, 31.7, 30.2, 22.2, 13.8.

Prop-2-yn-1-yl (S,E)-3-(3,5-difluorophenyl)-2-(hept-2-enamido)propanoate:



[00428] Compound isolated as a white solid 36% yield over 3 steps. HRMS (ESI) predicted for $[C_{19}H_{21}F_2NO_3 + H]^+$: 350.1568, found: 350.1560. ^{1}H NMR (400MHz, $CDCl_3$) δ = 6.88 (td, *J* = 7.0, 15.6 Hz, 1 H), 6.80 - 6.60 (m, 3 H), 6.01 (d, *J* = 7.5 Hz, 1 H), 5.80 (td, *J* = 1.5, 15.1 Hz, 1 H), 4.99 (td, *J* = 5.5, 7.5 Hz, 1 H), 4.91 - 4.78 (m, 1 H), 4.67 (dd, *J* = 2.5, 15.6 Hz, 1 H), 3.25 - 3.12 (m, 2 H), 2.56 (t, *J* = 2.5 Hz, 1 H), 2.25 - 2.14 (m, 2 H), 1.49 - 1.39 (m, 2 H), 1.39 - 1.29 (m, 2 H), 0.93 - 0.87 (m, 3 H). ^{13}C NMR (101MHz, $CDCl_3$) δ = 170.4, 165.5, 164.2, 164.1, 161.7, 161.6, 146.6, 139.5, 139.4, 139.3, 122.5, 112.6, 112.5, 112.4, 112.3, 103.0, 102.7, 102.5, 75.9, 52.9, 52.6, 37.4, 31.7, 30.2, 22.2, 13.8.

***N*-E-2-Heptenoylldifluorophenylalanine – Biotin Conjugate:**



[00429] Prop-2-yn-1-yl (S,E)-3-(3,5-difluorophenyl)-2-(hept-2-enamido)propanoate (17 mg, 0.05 mmol) and commercially available Azido-Biotin Conjugate (SigmaAldrich) (16 mg, 0.04 mmol) were dissolved in 1:1 water / ethanol (1 mL) and treated with Cu(II) sulfate (1 mg, 0.006 mmol), TCEP (2 mg, 0.007 mmol), and 2,6-lutidine (50 µL, 0.43 mmol). The reaction solution turned blue/green after the addition of 2,6-lutidine, an indication of Cu(I) formation. The reaction was allowed to proceed over night, over the course of which, a blue/green precipitate had fomed. The reaction solution was filtered and diluted with 1:1 water/ethanol to a final theoretical concentration of 8.2 mM (4.8 mL) with respect to the N-acyldifluorophenylalanine – biotin conjugate. This solution was used directly in chemical proteomics experiments without further purification. Formation of the conjugate was confirmed by ESI MS analysis.

Other Embodiments

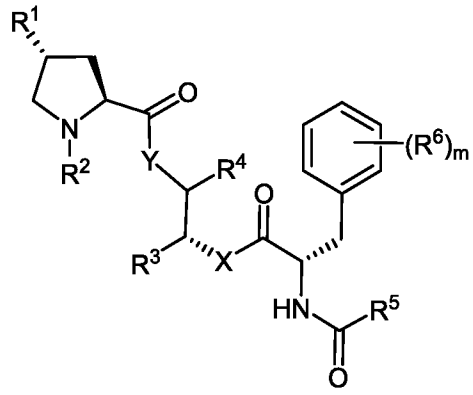
[00430] All patents, patent applications, and literature references cited herein are incorporated herein by reference.

[00431] The foregoing has been a description of certain non–limiting embodiments of the invention. Those of ordinary skill in the art will appreciate that various changes and modifications to this description may be made without departing from the spirit or scope of the present invention, as defined in the following claims.

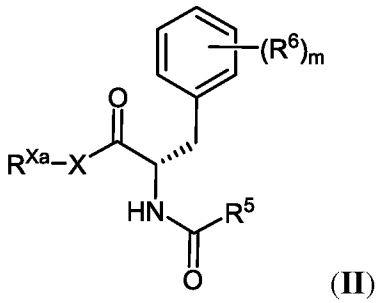
CLAIMS

What is claimed is:

1. A compound of Formula (**I**) or (**II**):



(**I**)



(**II**)

or a pharmaceutically acceptable salt thereof;

wherein:

R^1 is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

R^2 is hydrogen, -OH, $-OR^{2A}$, $-C(=O)R^{2A}$, $-C(=O)OR^{2A}$, $-C(=O)NHR^{2A}$, $-C(=O)N(R^{2A})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, or a nitrogen protecting group;

R^3 is $-C(=O)R^{3A}$, $-C(=O)OR^{3A}$, $-C(=O)NHR^{3A}$, $-C(=O)N(R^{3A})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

each instance of R^{2A} and R^{3A} is independently optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl,

or two R^{2A} groups are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring,

or two R^{3A} groups are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring;

R^4 is hydrogen, –OH, –SH, $–NH_2$, substituted hydroxyl, substituted thiol, substituted amino, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

R^5 is optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, $–OR^{5A}$ or $–NHR^{5A}$; wherein each instance of R^{5A} is independently optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

each instance of R^6 is independently halogen, –OH, –SH, $–NH_2$, -CN, $-NO_2$, $-N_3$, $–SO_2H$, $–SO_3H$, substituted hydroxyl, substituted thiol, substituted amino, sulfonyl, sulfinyl, carbonyl, silyl, boronyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

m is 0 or an integer of between 1 and 5, inclusive;

Y is O, S, or NR^Y wherein R^Y is hydrogen, optionally substituted alkyl, or a nitrogen protecting group; and

X is O, S, or NR^{Xb},

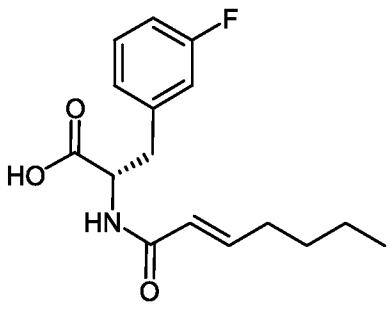
wherein:

R^{Xb} is hydrogen, optionally substituted alkyl, or a nitrogen protecting group; and

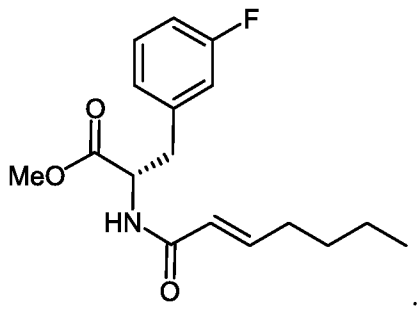
R^{Xa} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

or R^{Xa} and R^{Xb} are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring;

provided the compound of Formula (**II**) is not:



or



.

2. The compound of claim 1, wherein R^1 is hydrogen or optionally substituted alkyl.

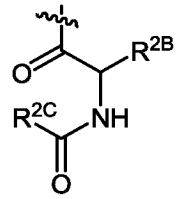
3. The compound of claim 2, wherein R^1 is optionally substituted $C_{1\text{-}3}$alkyl.

4. The compound of any one of claims 1-3, wherein R^1 is $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$.

5. The compound of any one of claims 1-4, wherein R^2 is hydrogen, $-C(=O)R^{2A}$, $-C(=O)OR^{2A}$, $-C(=O)NHR^{2A}$, or $-C(=O)N(R^{2A})_2$, wherein at least one instance of R^{2A} is optionally substituted alkyl.

6. The compound of claim 5, wherein R^2 is $-C(=O)R^{2A}$ wherein R^{2A} is optionally substituted alkyl.

7. The compound of claim 6, wherein R^2 is a group of the formula:



wherein:

R^{2B} is hydrogen and optionally substituted alkyl; and

R^{2C} is optionally substituted alkyl or $-OR^{2D}$ wherein R^{2D} is optionally substituted alkyl.

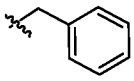
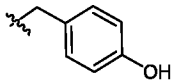
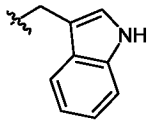
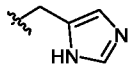
8. The compound of claim 7, wherein R^{2C} is optionally substituted alkyl.

9. The compound of claim 7, wherein R^{2C} is $-OR^{2D}$ wherein R^{2D} is optionally

substituted alkyl.

10. The compound of any one of claims 7-9, wherein R^{2B} is optionally substituted alkyl.

11. The compound of claim 10, wherein R^{2B} is an optionally substituted alkyl of formula:

$-CH_3$	$-CH_2OH$	$-CH(CH_3)OH$	$-CH_2SH$
$-CH(CH_3)_2$	$-CH_2CH(CH_3)_2$	$-CH(CH_3)(CH_2CH_3)$	$-CH_2CH_2SCH_3$
			
$-CH_2CO_2H$	$-CH_2CH_2CO_2H$	$-CH_2C(=O)NH_2$	$-CH_2CH_2C(=O)NH_2$
$-CH_2CH_2CH_2CH_2NH_2$	Or	$-CH_2CH_2CH_2NHC(=NH)NH_2$	.

12. The compound of claim 5, wherein at least one instance of R^{2A} is optionally substituted $C_{1\text{-}3}$alkyl.

13. The compound of claim 12, wherein at least one instance of R^{2A} is $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$.

14. The compound of any one of claims 1-13, wherein R^3 is $-C(=O)R^{3A}$, $-C(=O)OR^{3A}$, $-C(=O)NHR^{3A}$, or $-C(=O)N(R^{3A})_2$,wherein at least one instance of R^{3A} is optionally substituted alkyl.

15. The compound of claim 14, wherein at least one instance of R^{3A} is $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$.

16. The compound of any one of claims 1-15, wherein R^3 is optionally substituted alkyl.

17. The compound of claim 16, wherein R^3 is $C_{1\text{-}3}$alkyl substituted with hydroxyl or substituted hydroxyl.

18. The compound of any one of claims 1-17, wherein R^4 is hydrogen or optionally substituted alkyl.

19. The compound of claim 18, wherein R^4 is optionally substituted $C_{1\text{-}3}$alkyl.

20. The compound of claim 19, wherein R^4 is $-CH_3$, $-CF_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, or $-CH(CH_3)_2$.

21. The compound of any one of claims 1-20, wherein Y is O.

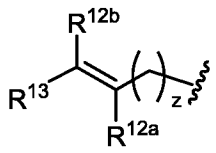
22. The compound of any one of claims 1-20, wherein Y is NR^Y, and R^Y is hydrogen or $-CH_3$.

23. The compound of any one of claims 1-22, wherein X is O or NR^{Xb}, and R^{Xb} is hydrogen or optionally substituted alkyl.

24. The compound of any one of claims 1-23, wherein R^{Xa} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl.

25. The compound of claim 24, wherein R^{Xa} is optionally substituted C_1alkyl, optionally substituted C_2alkyl, or optionally substituted C_3alkyl.

26. The compound of claim 24, wherein R^{Xa} is an optionally substituted alkenyl of formula (b):



(b)

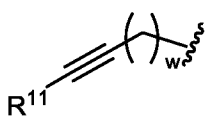
wherein:

z is 1, 2, 3, or 4;

R^{12a} and R^{12b} are independently hydrogen or optionally substituted alkyl; and

R^{13} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl.

27. The compound of claim 22, wherein R^{Xa} is an optionally substituted alkynyl of formula (c):



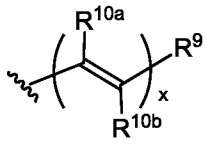
(c)

wherein:

w is 1, 2, 3, or 4; and

R^{11} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl.

28. The compound of any one of claims 1-27, wherein R^5 is an optionally substituted alkenyl group of Formula (a1):



(a1)

wherein:

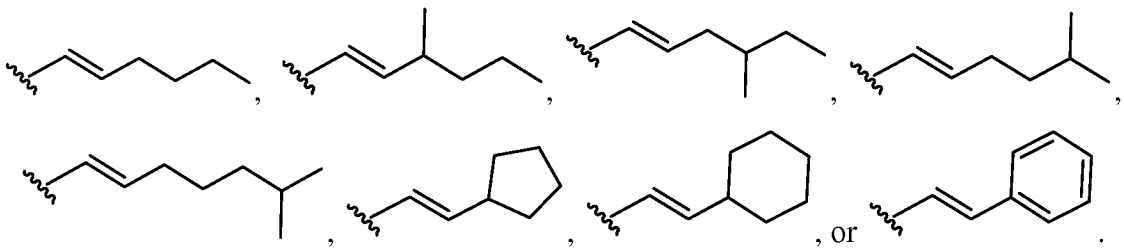
x is an integer between 1 and 10, inclusive;

R^{10a} and R^{10b} are independently hydrogen or optionally substituted alkyl; and

R^9 is hydrogen, halogen, –OH, –SH, $–NH_2$, -CN, $-NO_2$, $-N_3$, $–SO_2H$, $–SO_3H$, substituted hydroxyl, substituted thiol, substituted amino, sulfonyl, sulfinyl, carbonyl, silyl, boronyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl.

29. The compound of claim 28, wherein x is 1, R^{10a} and R^{10b} are each hydrogen, and R^9 is optionally substituted C_{4-6}alkyl or optionally substituted C_{3-6}carbocyclyl.

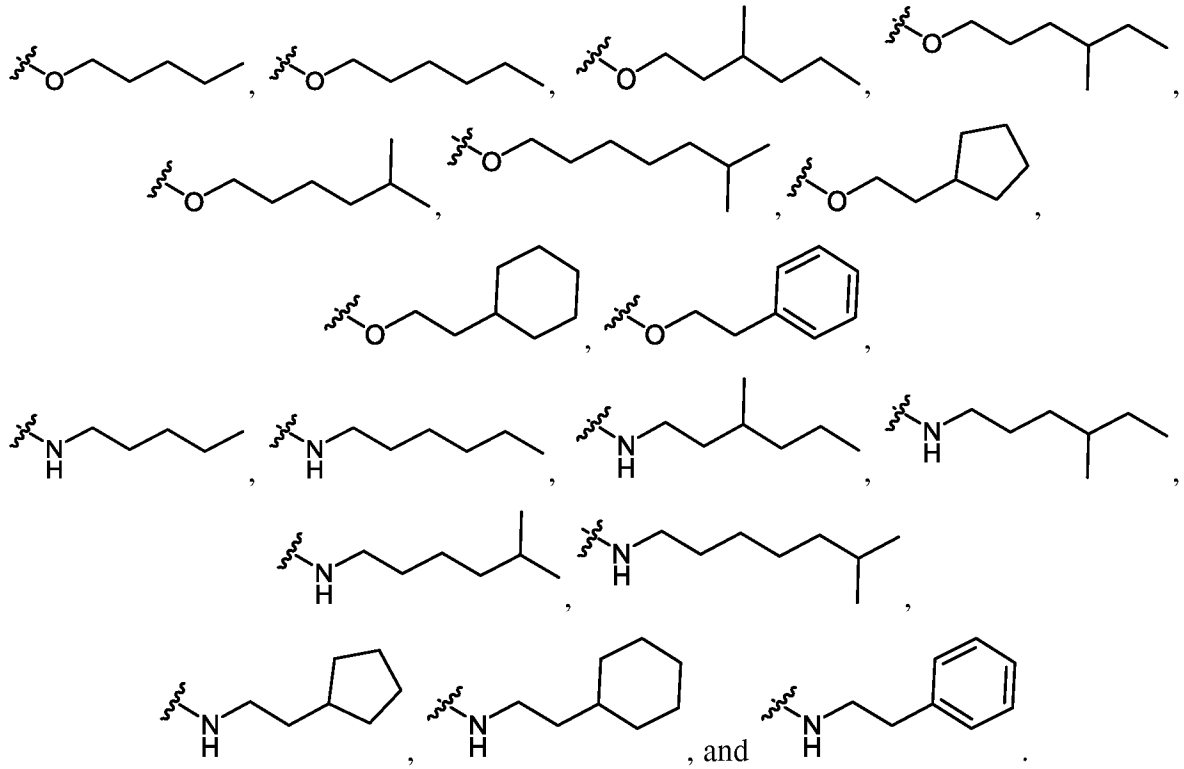
30. The compound of claim 28, wherein the alkenyl group of formula (a1) is selected from the group consisting of:


, or .

31. The compound of claim 1, wherein R^5 is $–OR^{5A}$ or $–NHR^{5A}$.

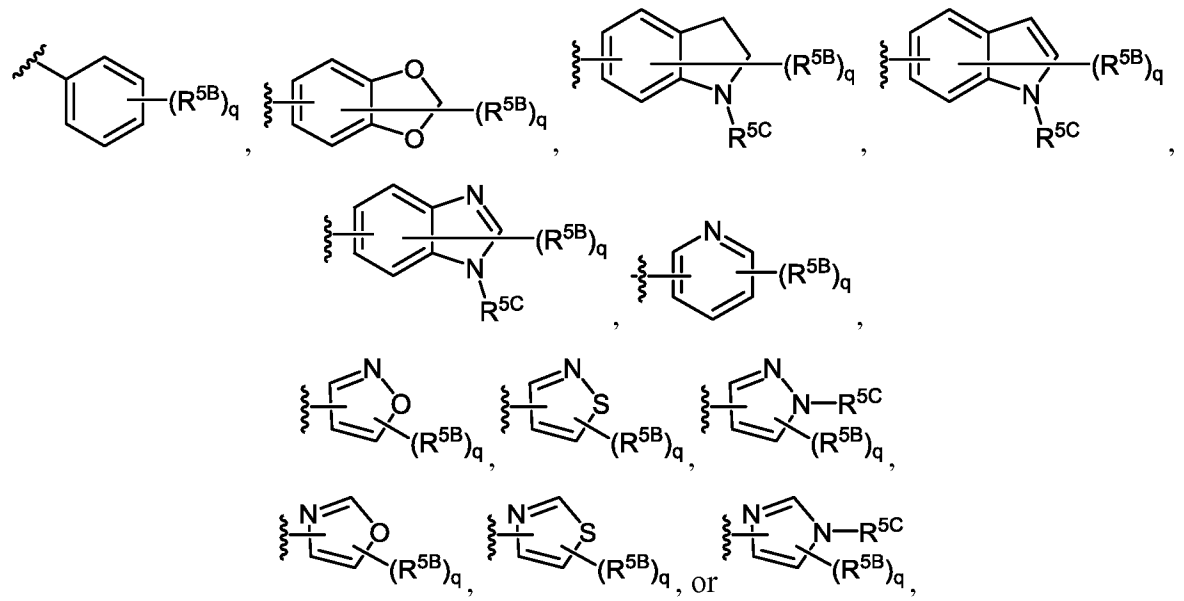
32. The compound of claim 31, wherein R^{5A} is optionally substituted alkyl.

33. The compound of claim 31, wherein R^5 is selected from the group consisting of:



34. The compound of claim 31, wherein R^{5A} is optionally substituted aryl or optionally substituted heteroaryl.

35. The compound of claim 34, wherein R^{5A} is an optionally substituted aryl or optionally substituted heteroaryl group of formulae:



wherein:

q is 0, 1, or 2;

each instance of R^{5B} is independently selected from the group consisting of halogen, C_{1-4}alkyl, C_{1-4}haloalkyl, $–OR^{5D}$, or $-NHR^{5D}$, wherein R^{5D} is C_{1-4}alkyl or C_{1-4}haloalkyl; and

R^{5C} is hydrogen or C_{1-4}alkyl.

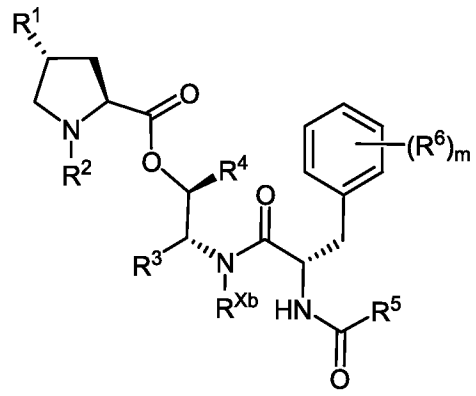
36. The compound of any one of claims 1-35, wherein m is 1, 2, or 3.

37. The compound of any one of claims 1-36, wherein at least one instance of R^6 is halogen, –OH, –SH, $–NH_2$, $-NO_2$, carbonyl, or optionally substituted alkyl.

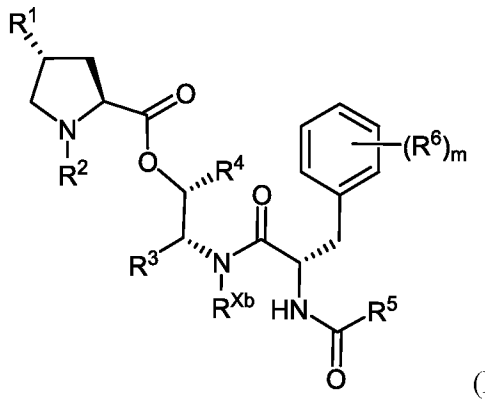
38. The compound of claim 37, wherein at least one instance of R^6 is halogen.

39. The compound of claim 38, wherein at least one instance of R^6 is fluoro.

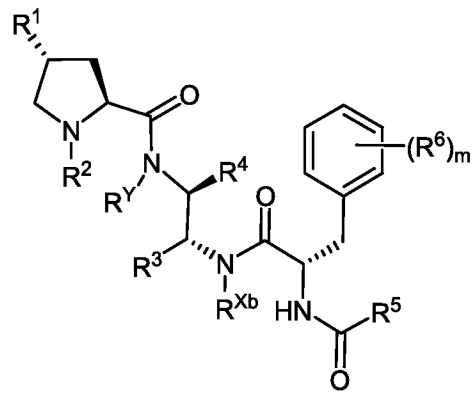
40. The compound of claim 1, wherein the compound of Formula (**I**) is of formula:



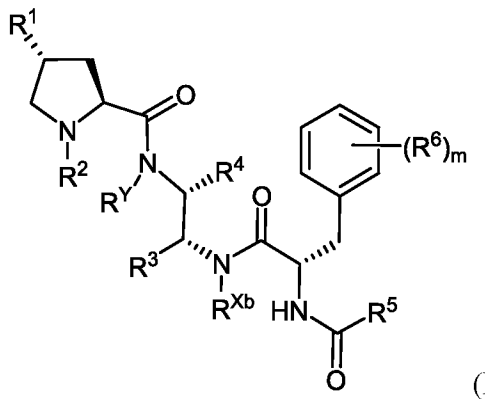
(**I-a1**)



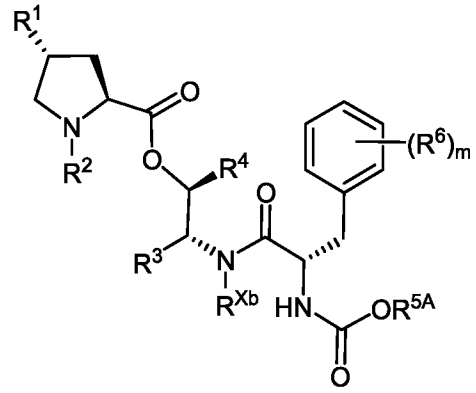
(**I-b1**)



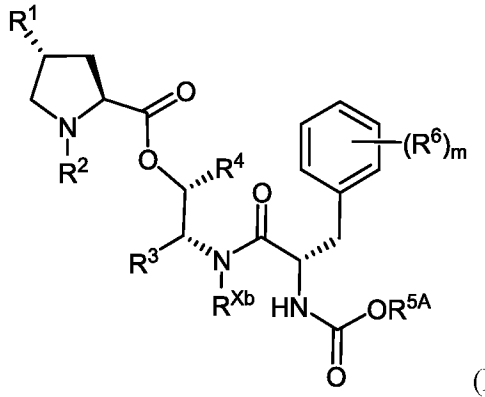
(**I-a2**)



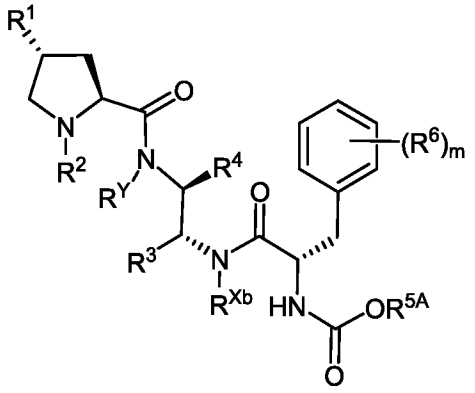
(**I-b2**)



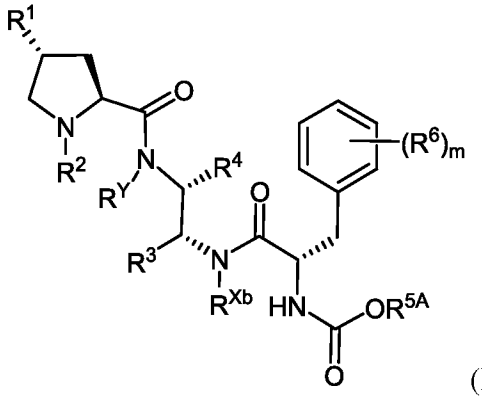
(I-a1-a)



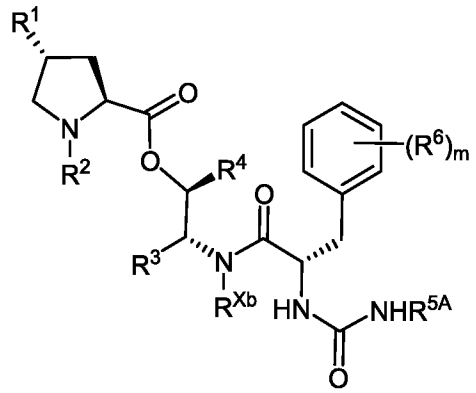
(I-b1-a)



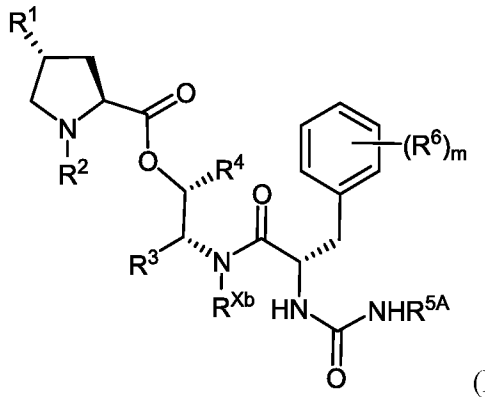
(I-a2-a)



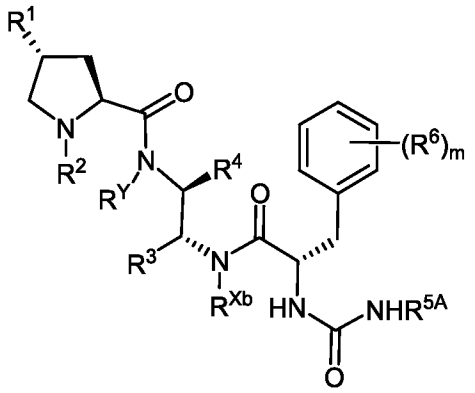
(I-b2-a)



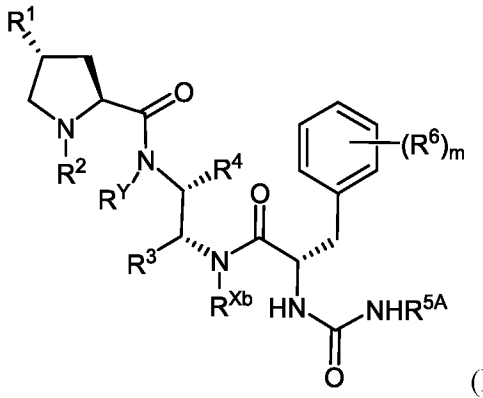
(I-a1-b)



(I-b1-b)



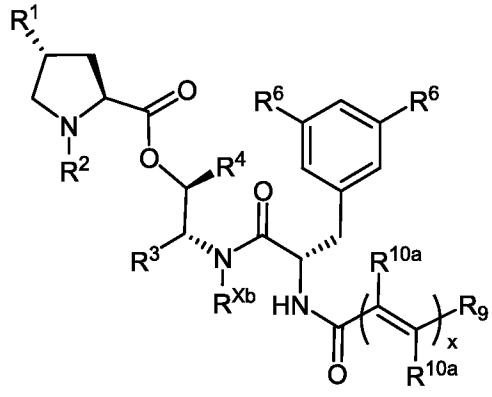
(I-a2-a)



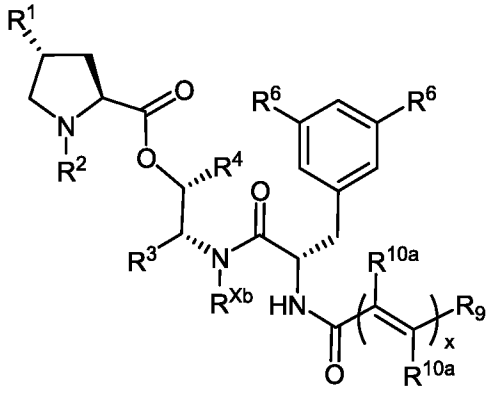
(I-b2-a)

or a pharmaceutically acceptable salt thereof.

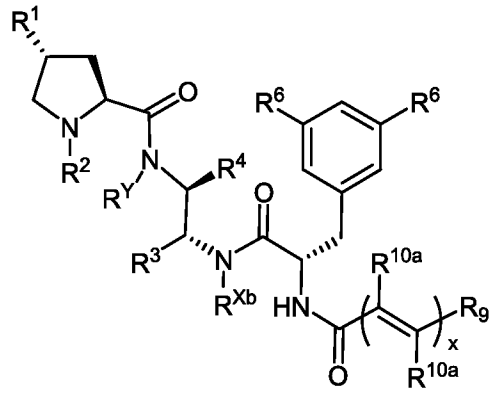
41. The compound of claim 1, wherein the compound of Formula (**I**) is of formula:



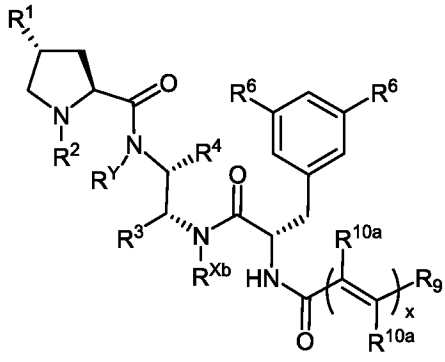
(I-a1-1)



(I-b1-1)



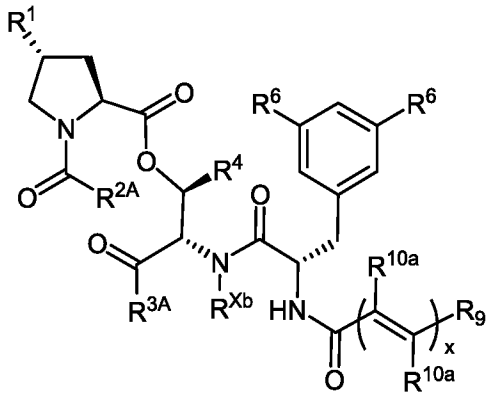
(I-a2-1)



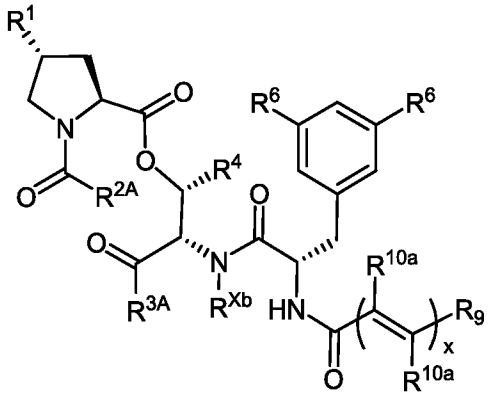
(I-b2-1)

or a pharmaceutically acceptable salt thereof.

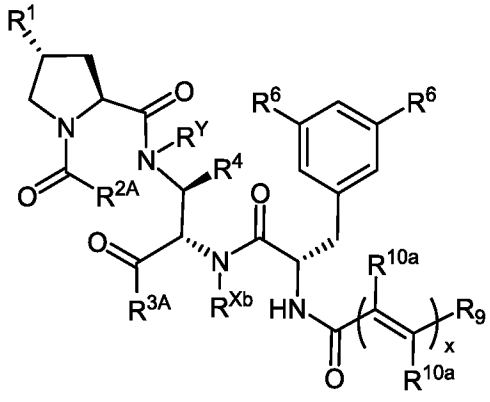
42. The compound of claim 1, wherein the compound of Formula (**I**) is of formula:



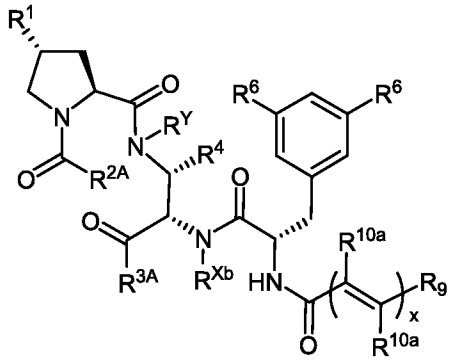
(I-a1-2)



(I-b1-2)



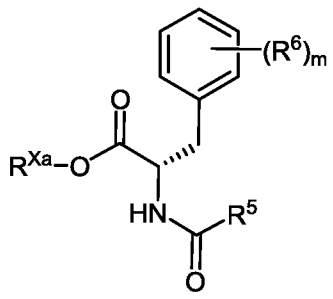
(I-a2-2)



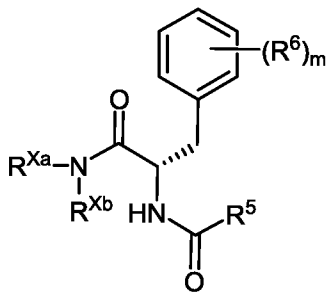
(I-b2-2)

or a pharmaceutically acceptable salt thereof.

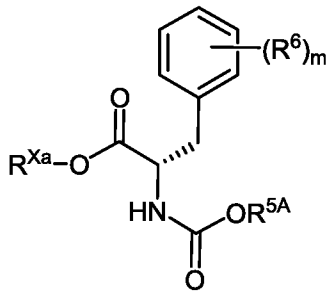
43. The compound of claim 1, wherein the compound of Formula (**II**) is of formula:



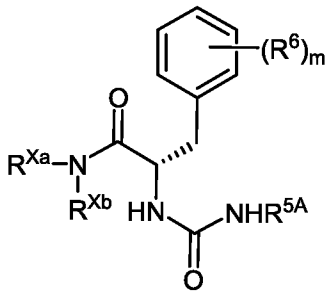
(II-a1)



(II-b1)



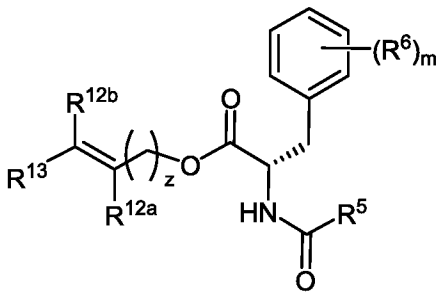
(**II-a1-a**)



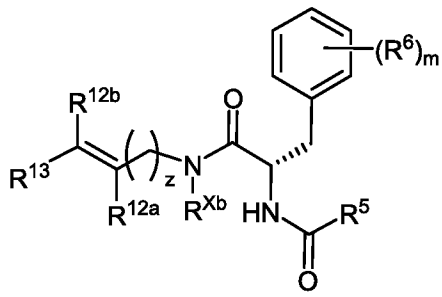
(**II-b1-b**)

or a pharmaceutically acceptable salt thereof.

44. The compound of claim 1, wherein the compound of Formula (**II**) is of formula:



(**II-a2**) or



(**II-b2**)

or a pharmaceutically acceptable salt thereof;

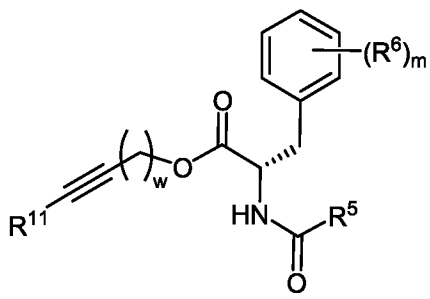
wherein:

z is 1, 2, 3, or 4;

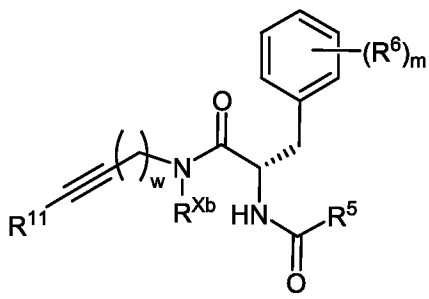
R^{12a} and R^{12b} are independently hydrogen or optionally substituted alkyl; and

R^{13} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl.

45. The compound of claim 1, wherein the compound of Formula (**II**) is of formula:



(**II-a3**) or



(**II-b3**)

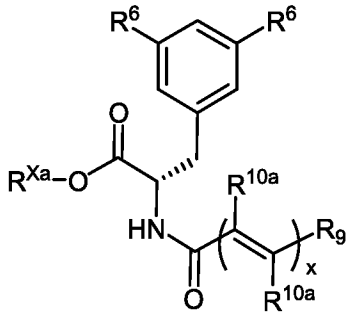
or a pharmaceutically acceptable salt thereof;

wherein:

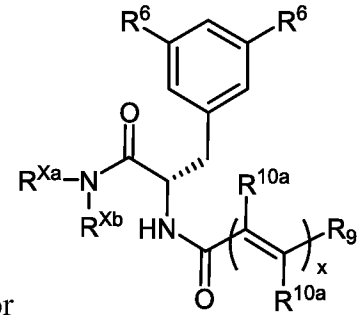
w is 1, 2, 3, or 4; and

R^{11} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl.

46. The compound of claim 1, wherein the compound of Formula (**II**) is of formula:



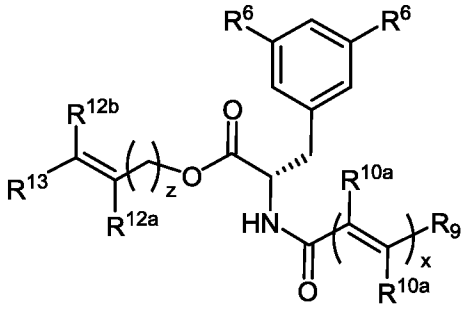
(**II-a4**) or



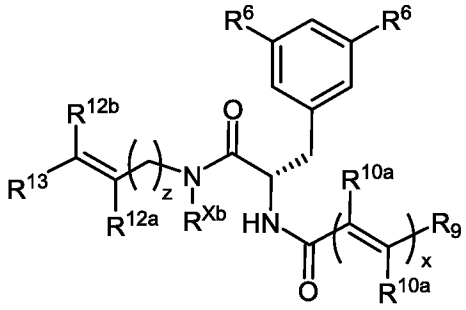
(**II-b4**)

or a pharmaceutically acceptable salt thereof.

47. The compound of claim 1, wherein the compound of Formula (**II**) is of formula:



(**II-a5**) or



(**II-b5**)

or a pharmaceutically acceptable salt thereof;

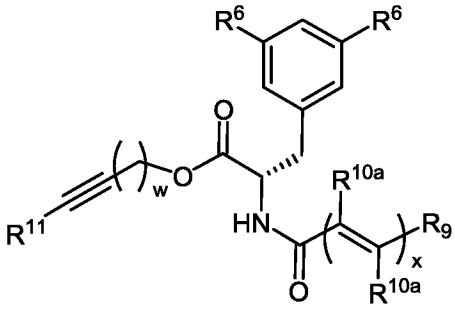
wherein:

z is 1, 2, 3, or 4;

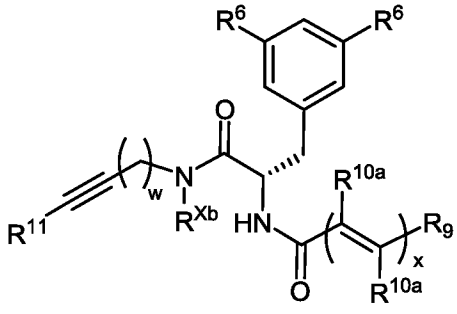
R^{12a} and R^{12b} are independently hydrogen or optionally substituted alkyl; and

R^{13} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl.

48. The compound of claim 1, wherein the compound of Formula (**II**) is of formula:



(**II-a6**) or



(**II-b6**)

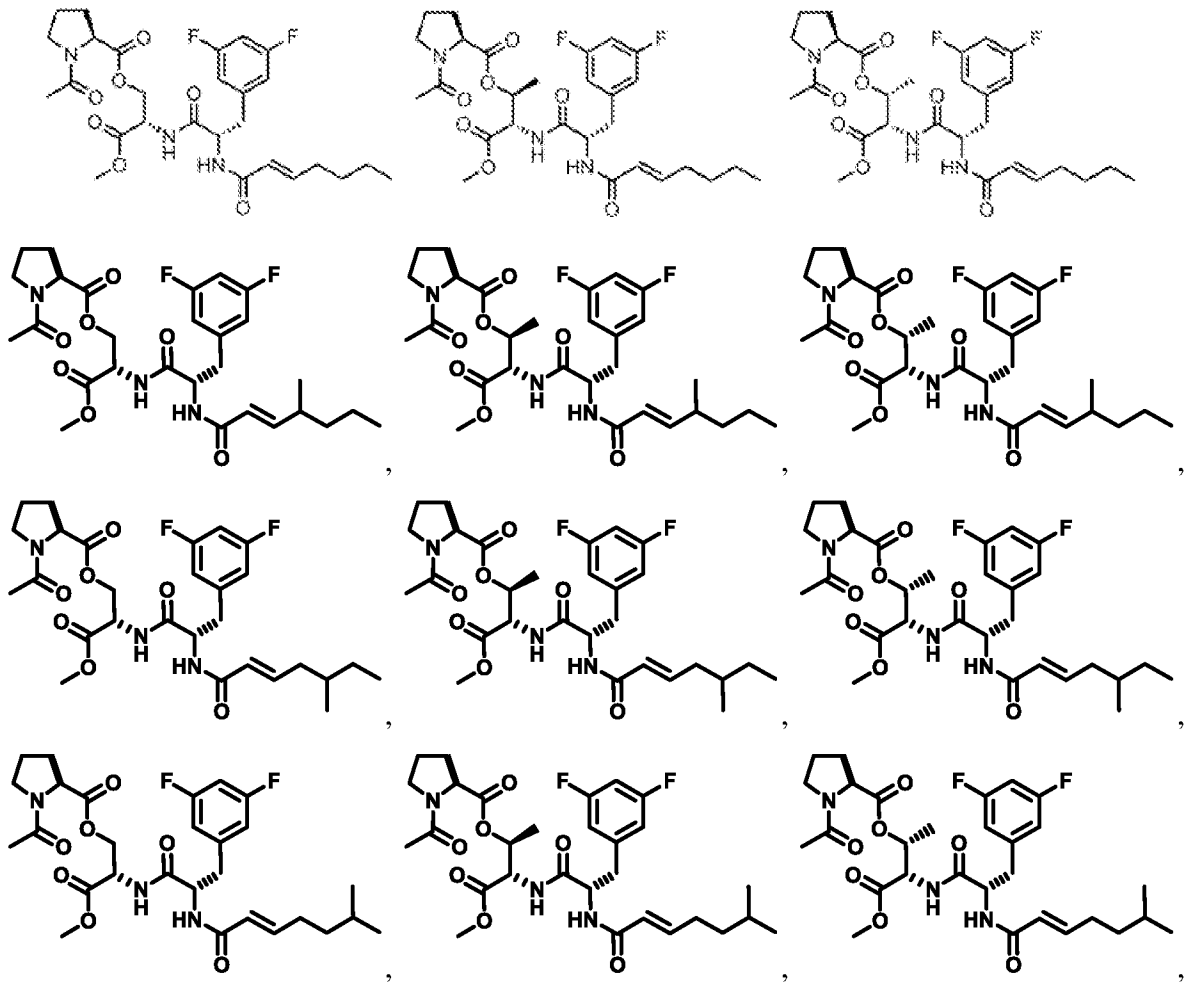
or a pharmaceutically acceptable salt thereof;

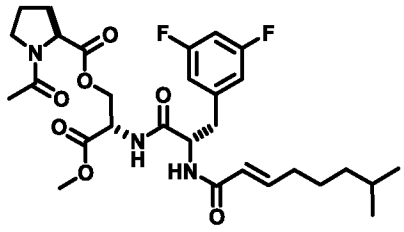
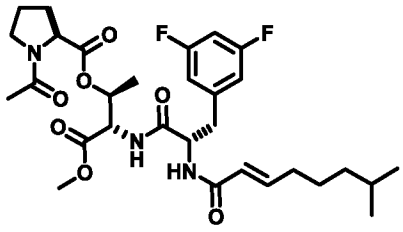
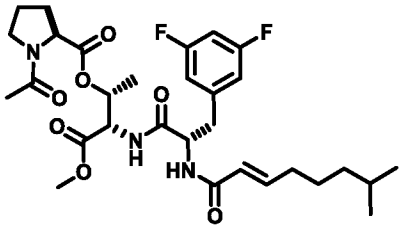
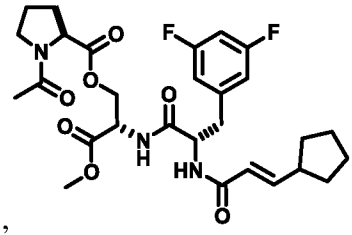
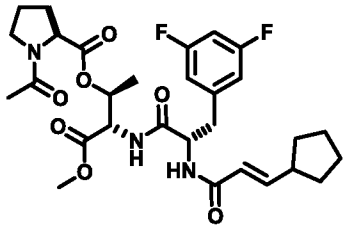
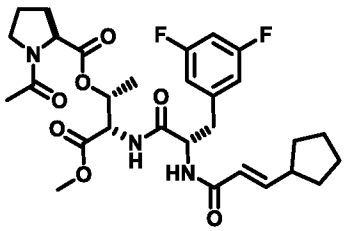
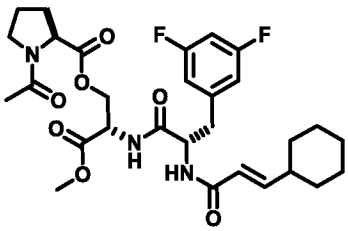
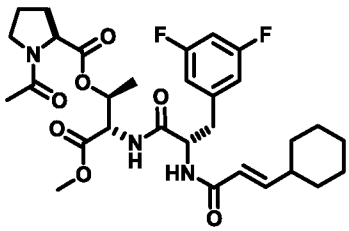
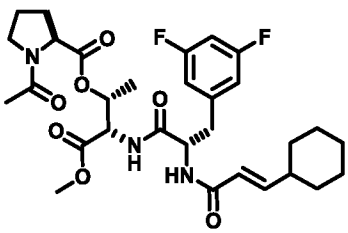
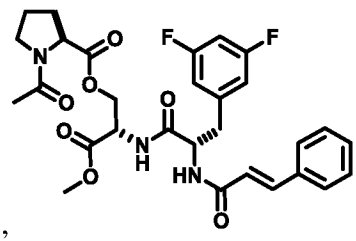
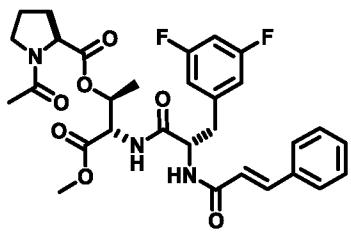
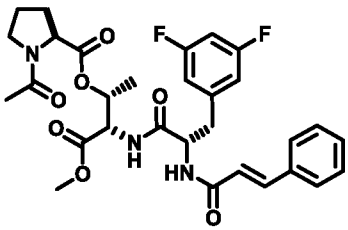
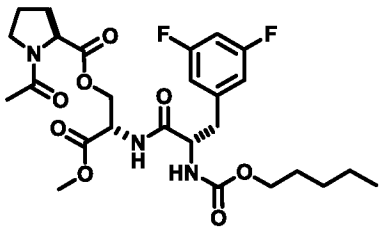
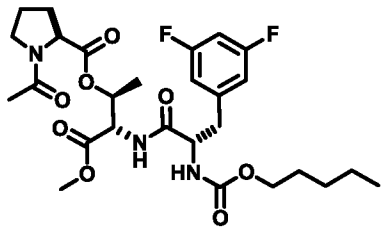
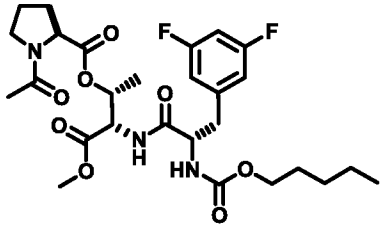
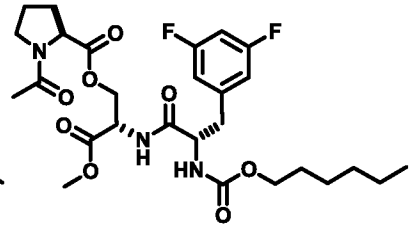
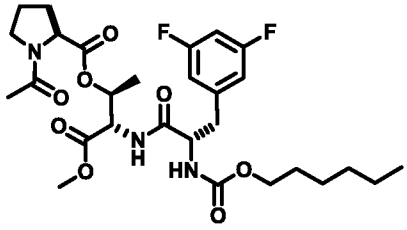
wherein:

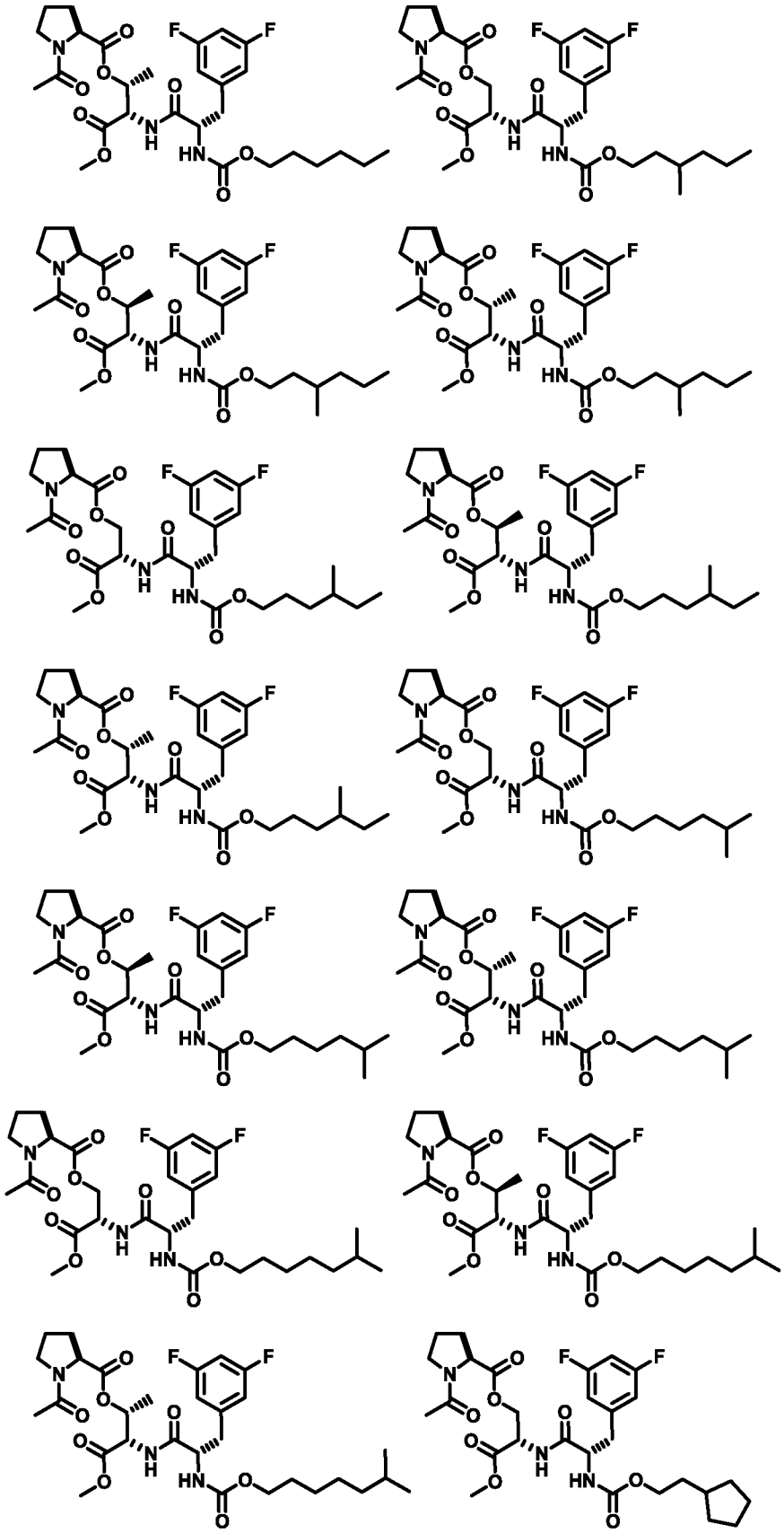
w is 1, 2, 3, or 4; and

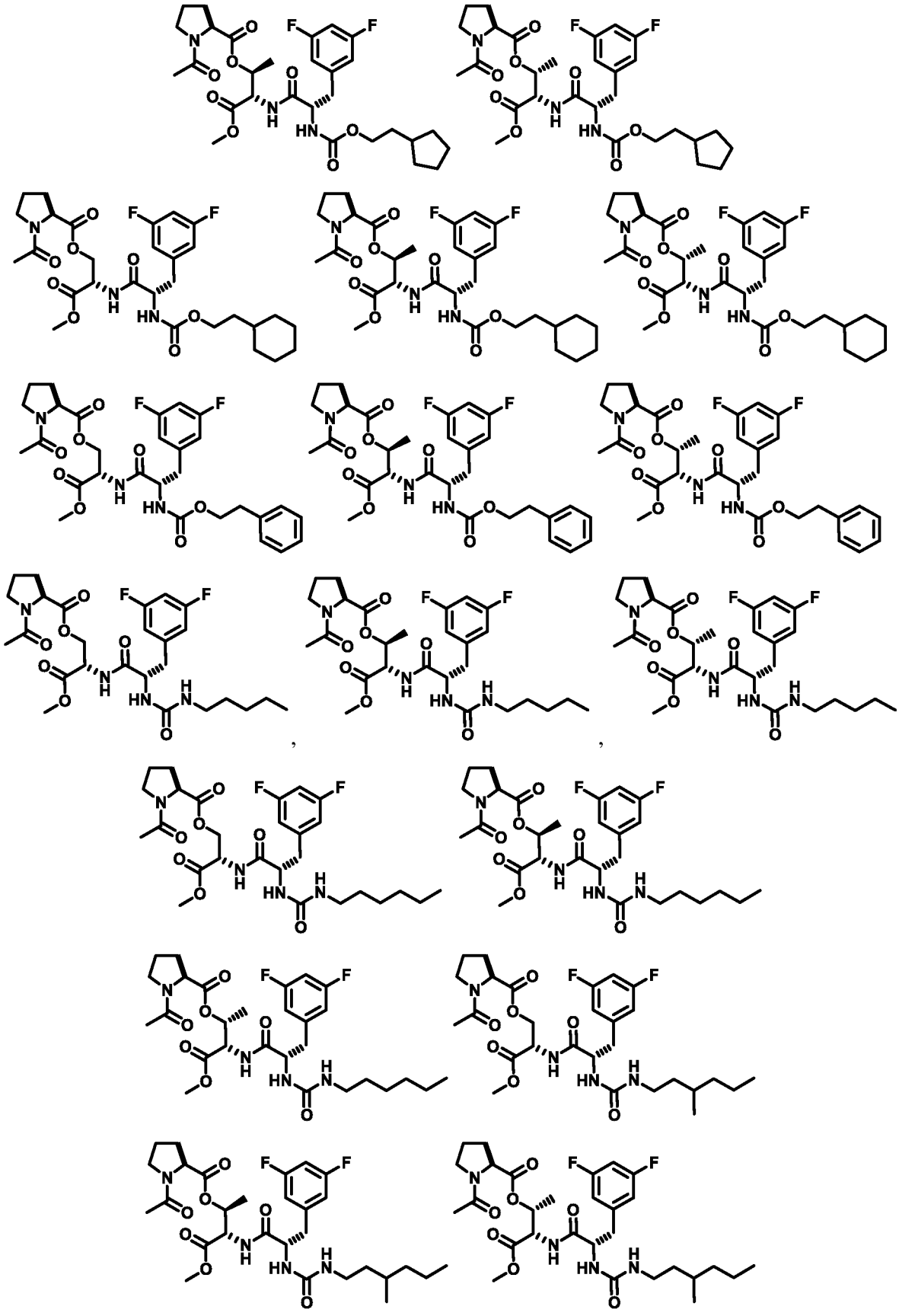
R^{11} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl.

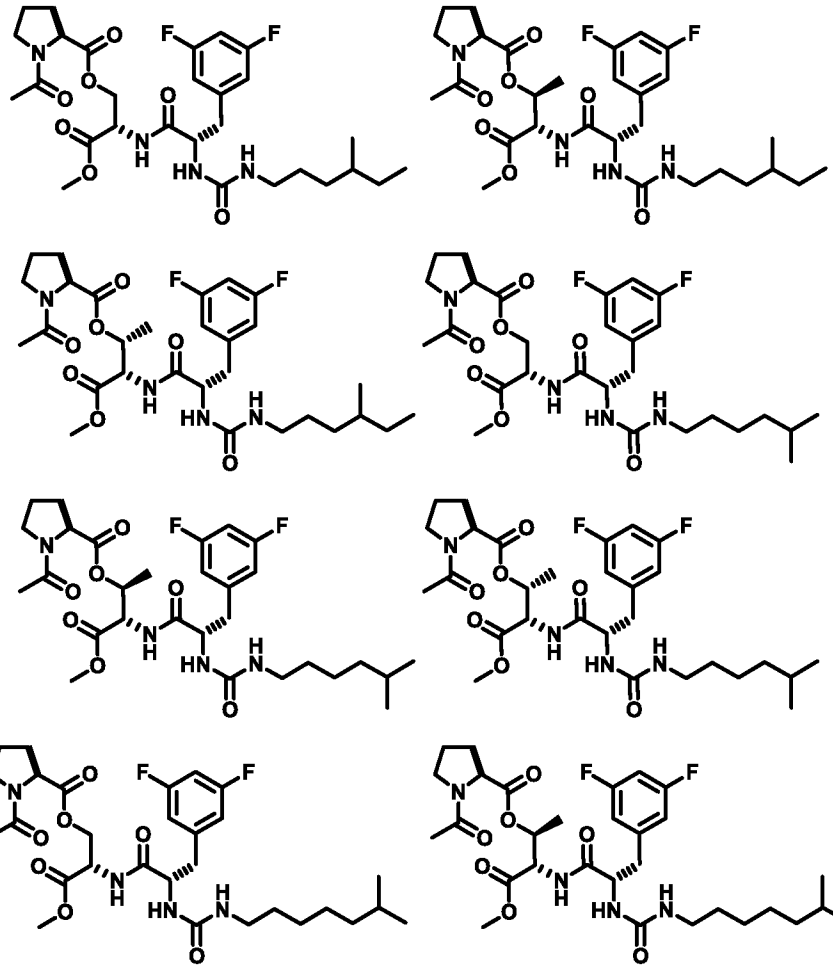
49. The compound of claim 1, selected from the group consisting of:

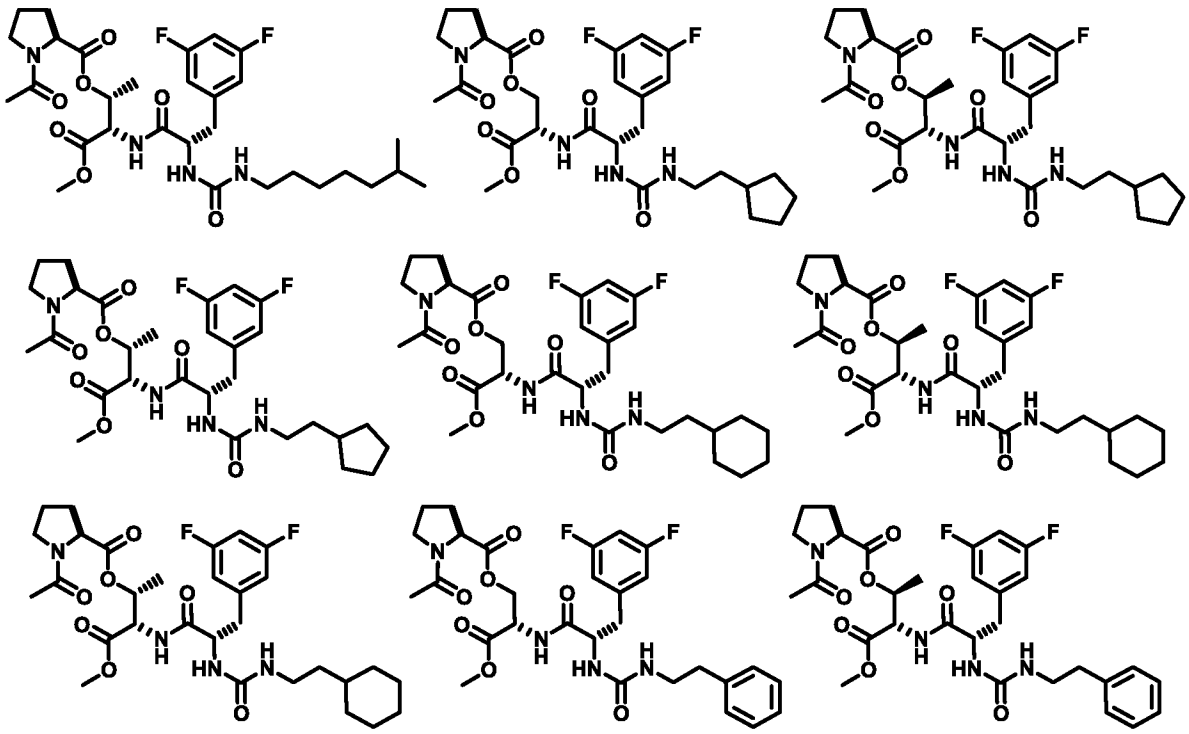


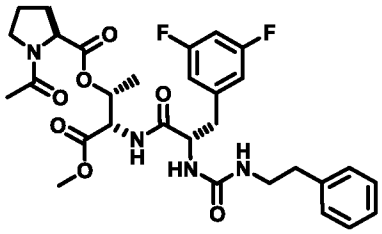

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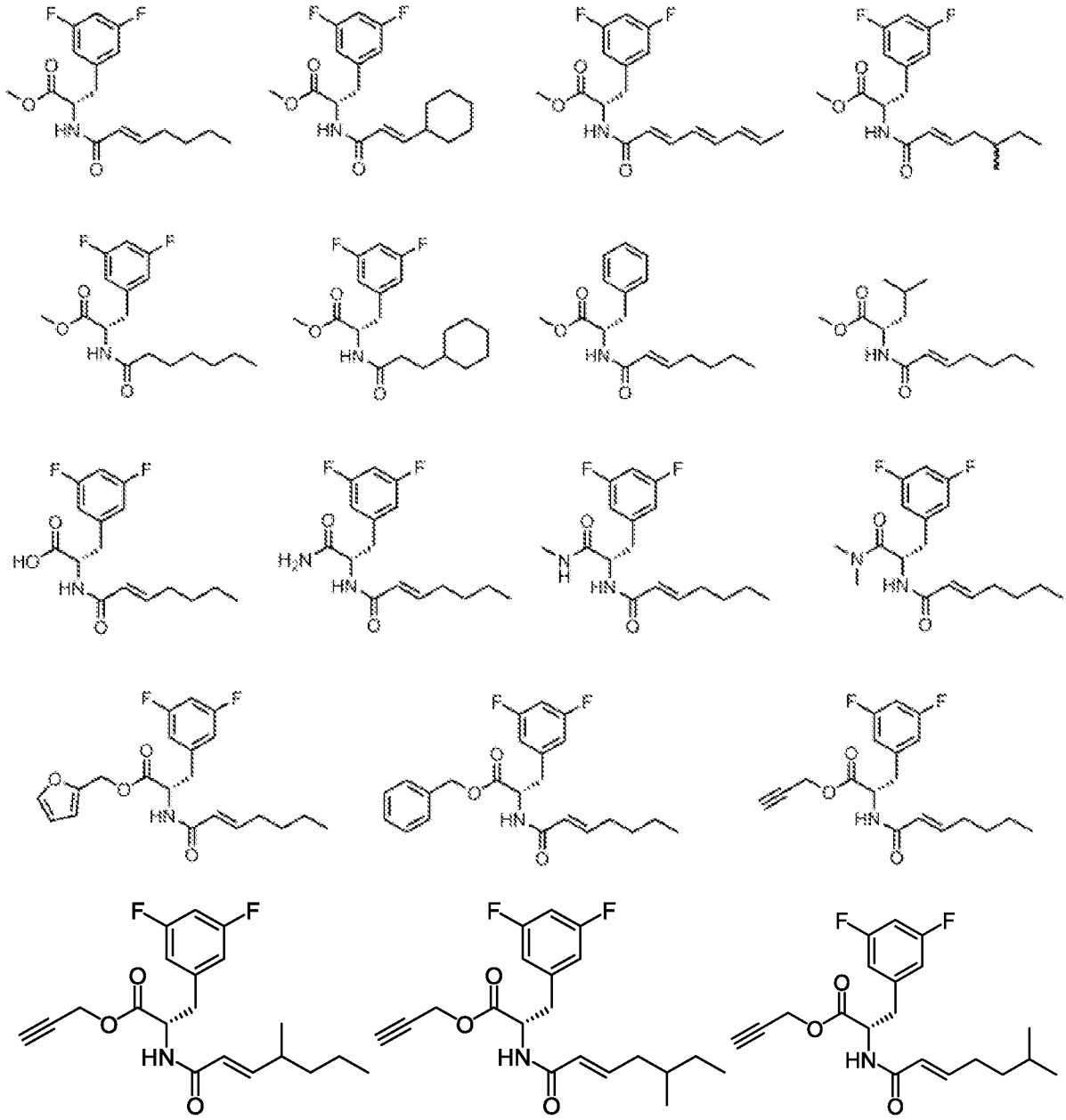


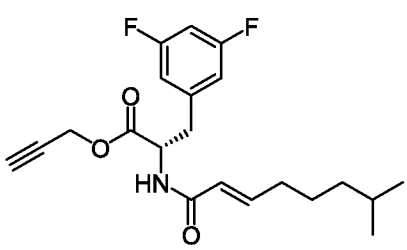
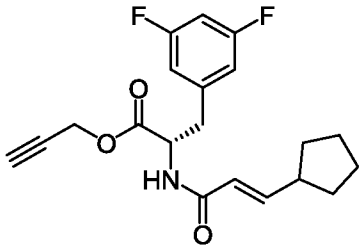
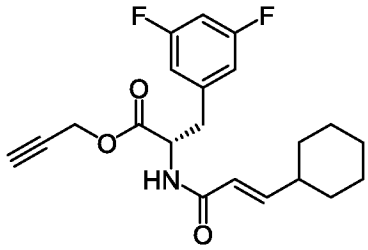
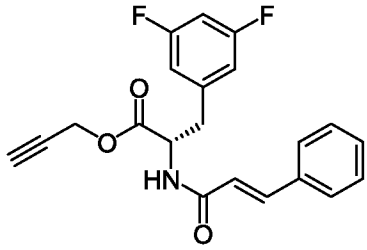
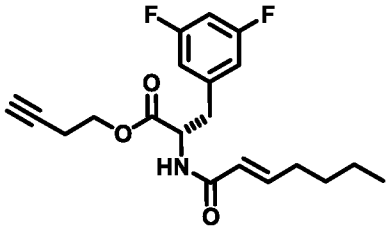
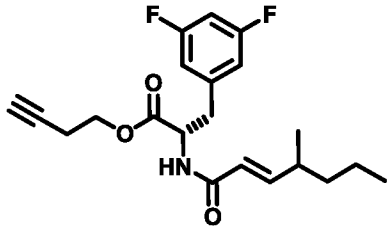
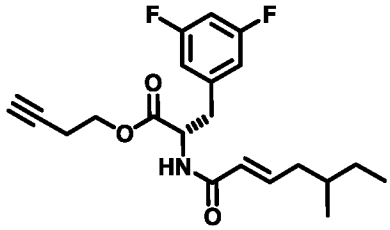
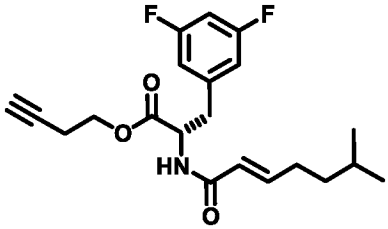
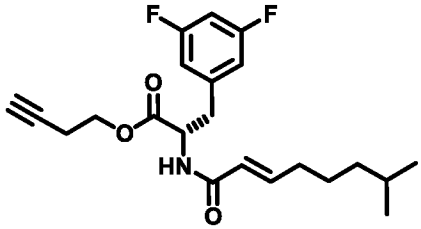
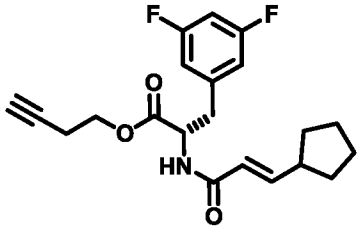
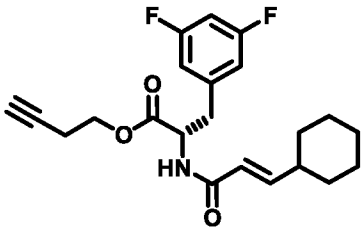
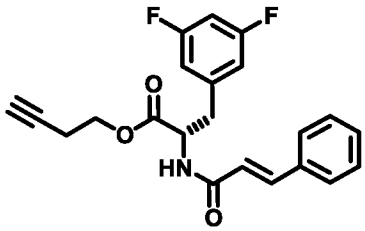
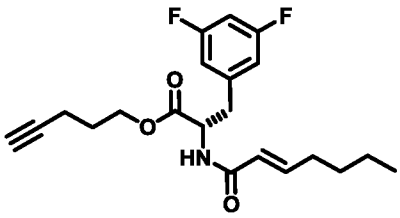
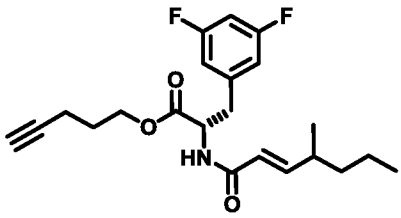
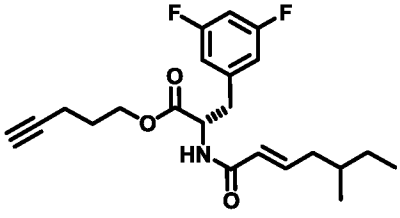
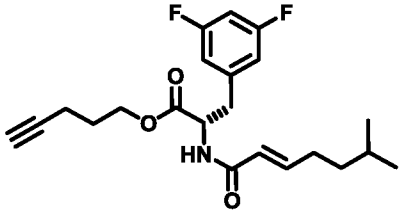


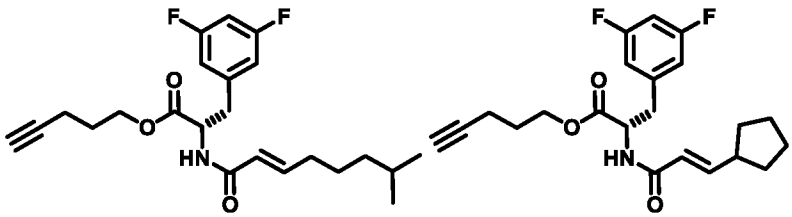
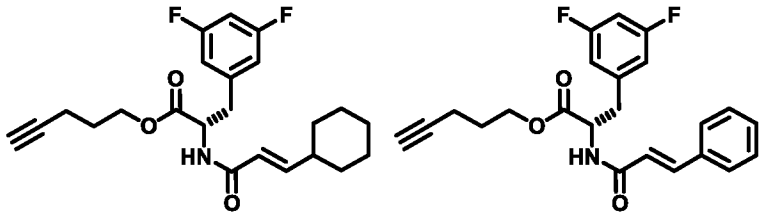
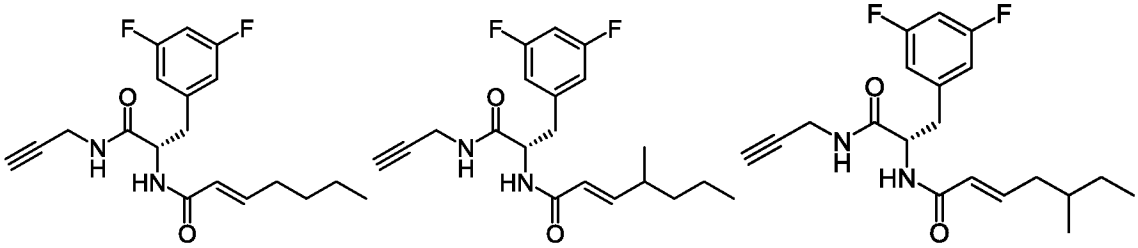
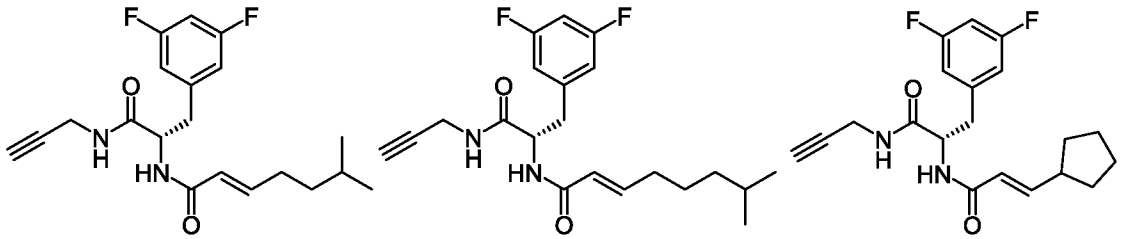
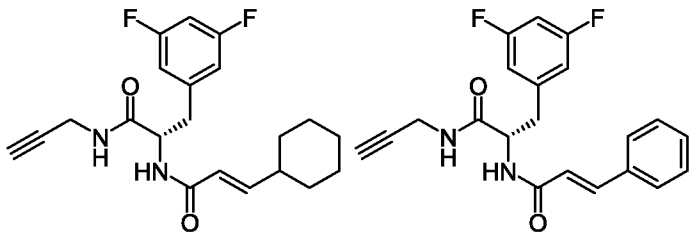
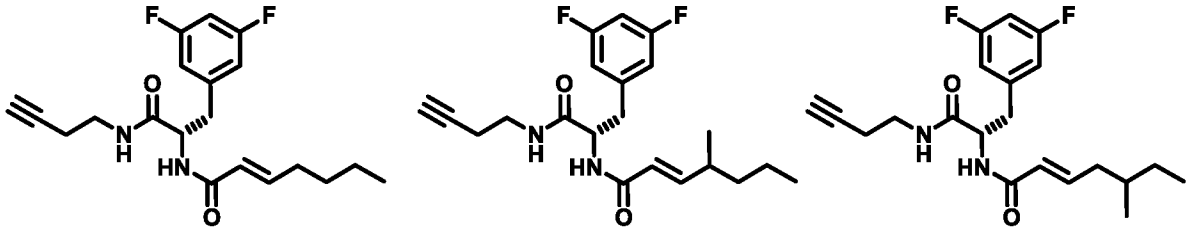
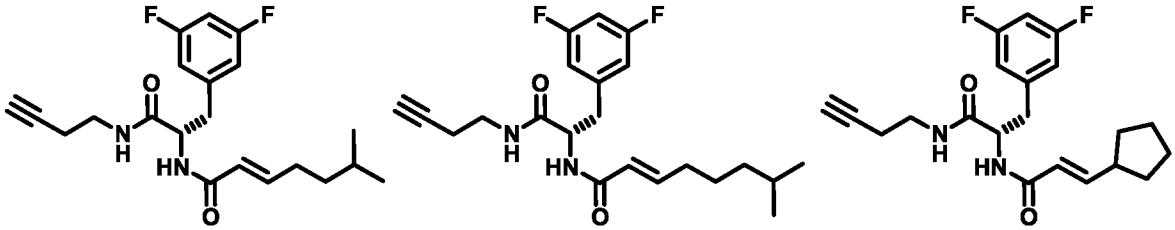


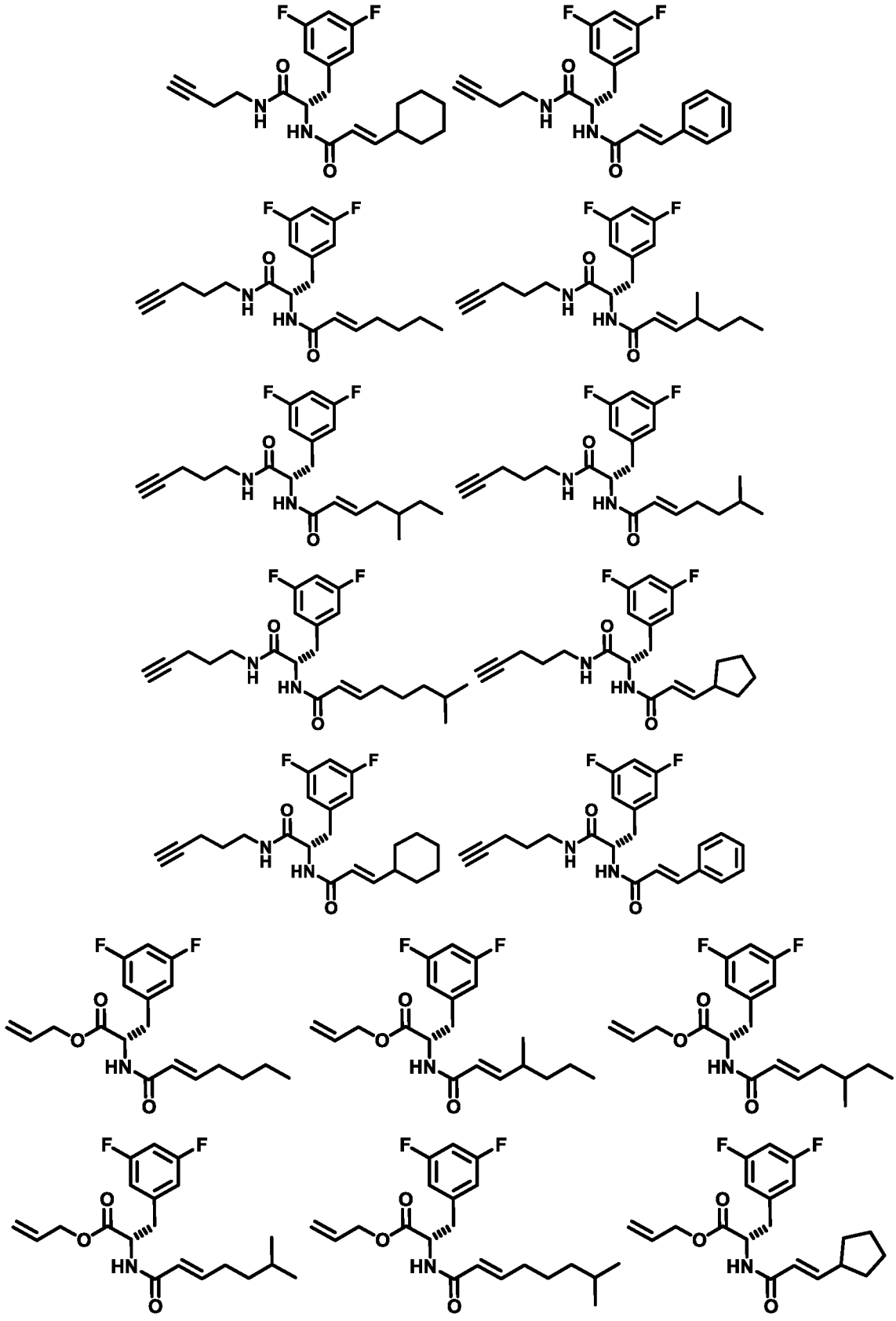

and pharmaceutically acceptable salts thereof.

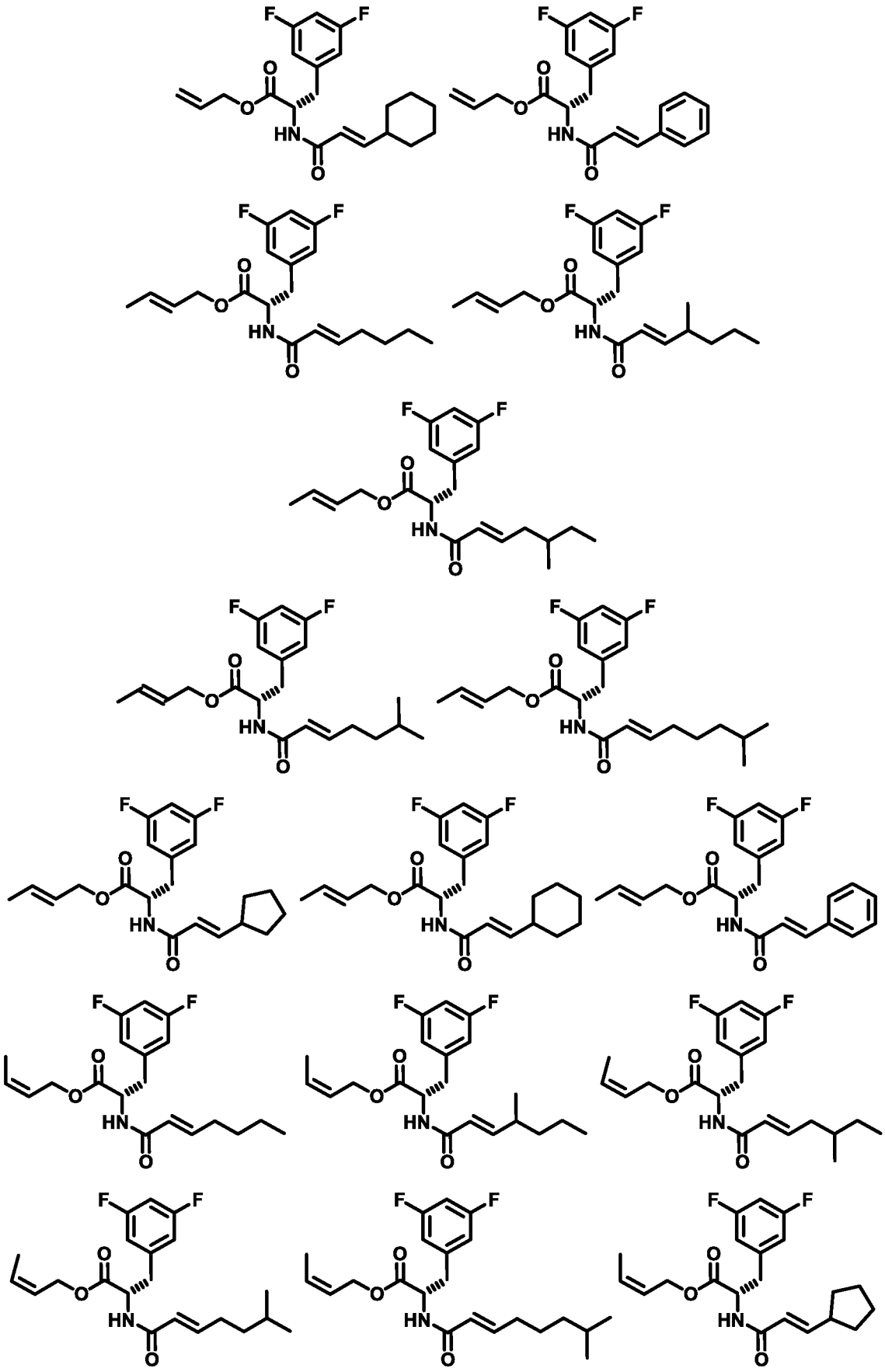
50. The compound of claim 1, selected from the group consisting of:

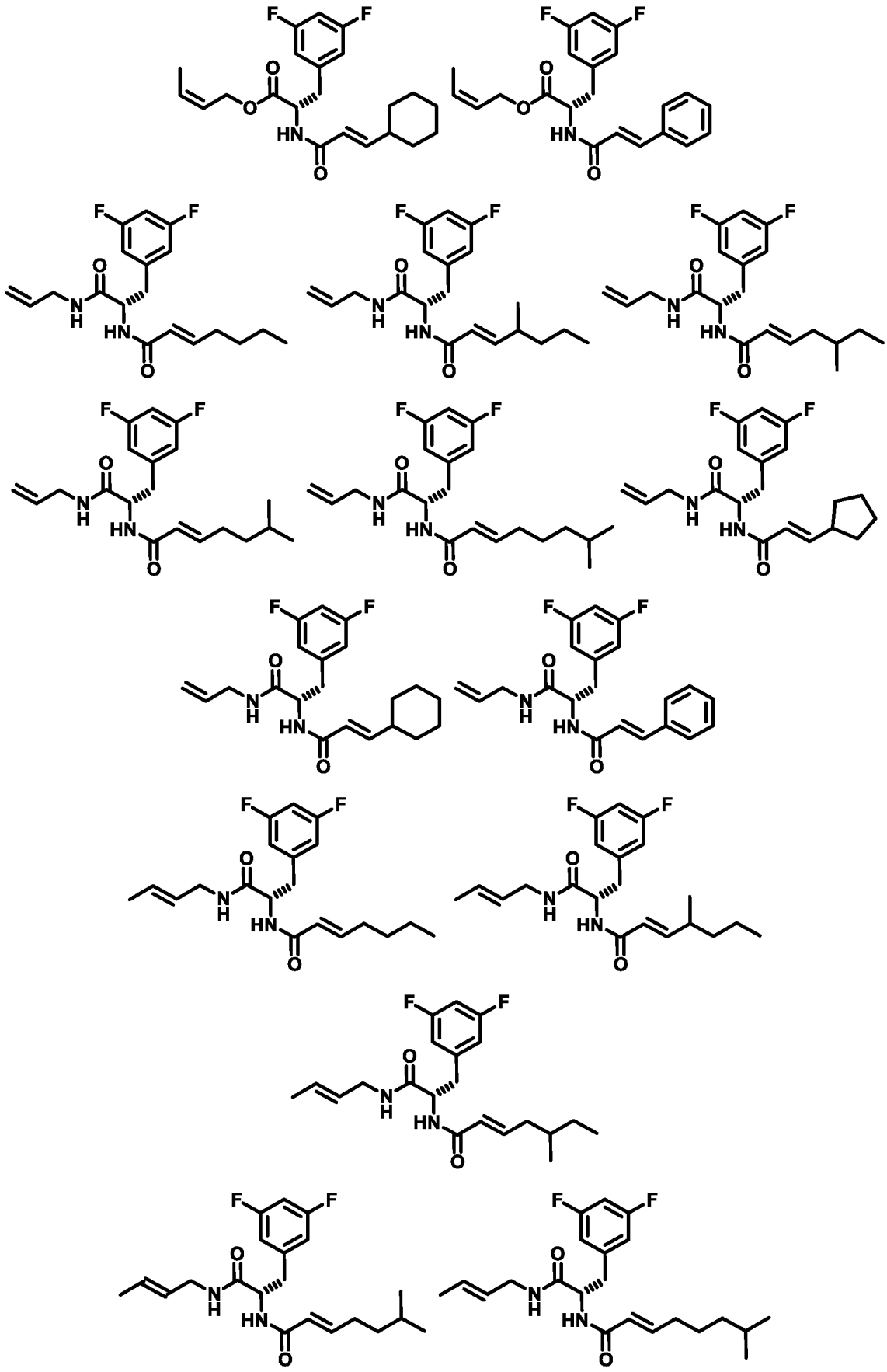


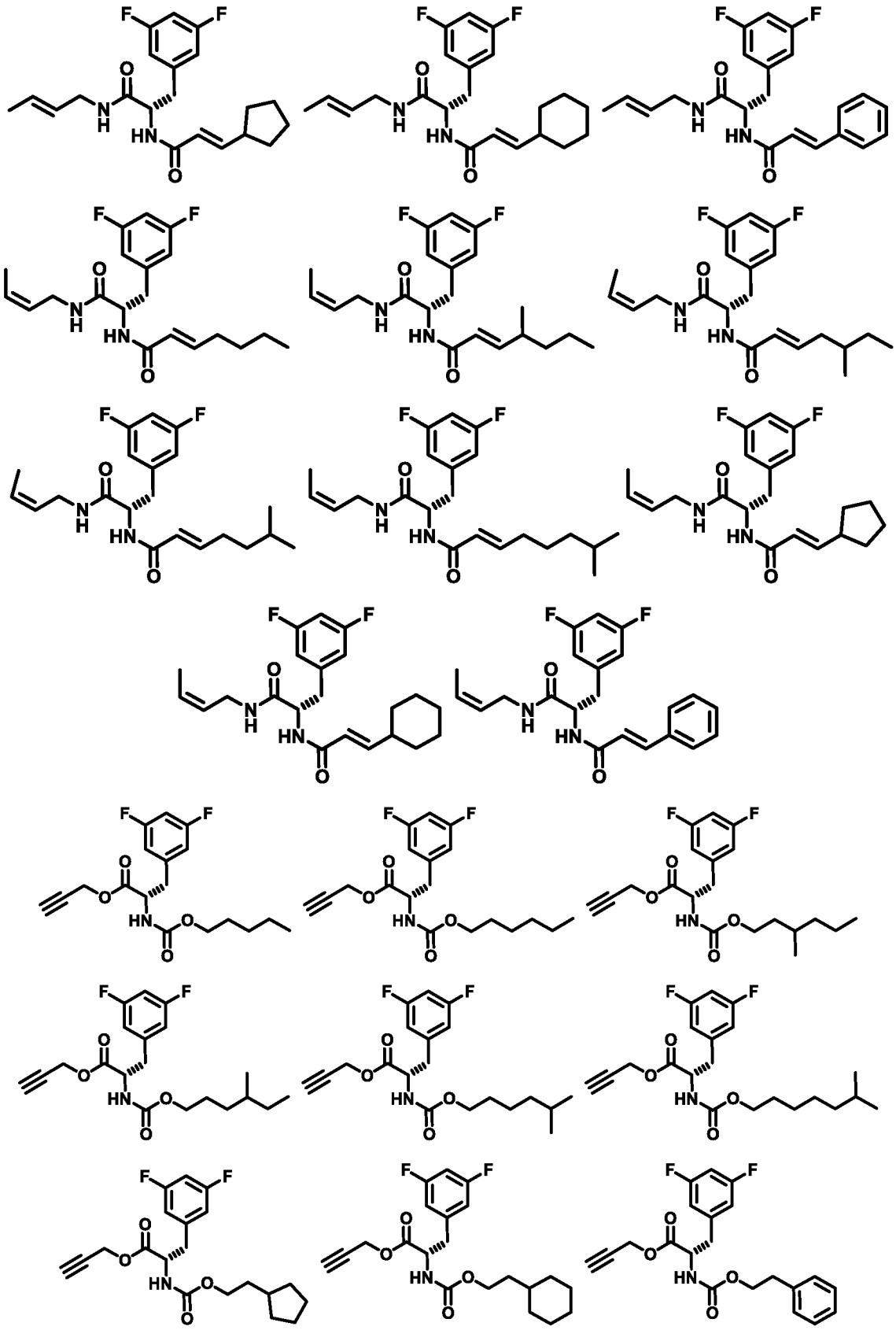

















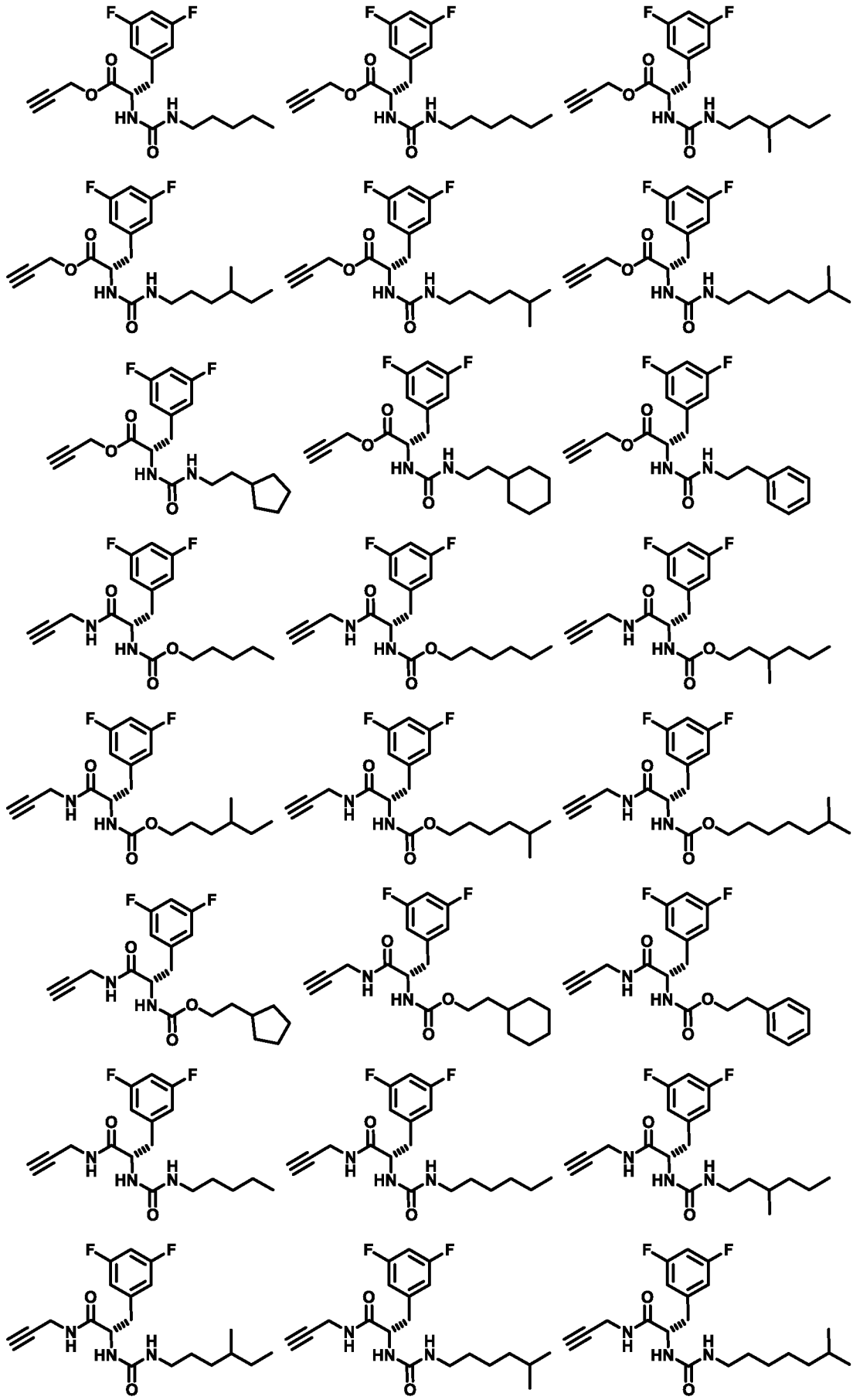








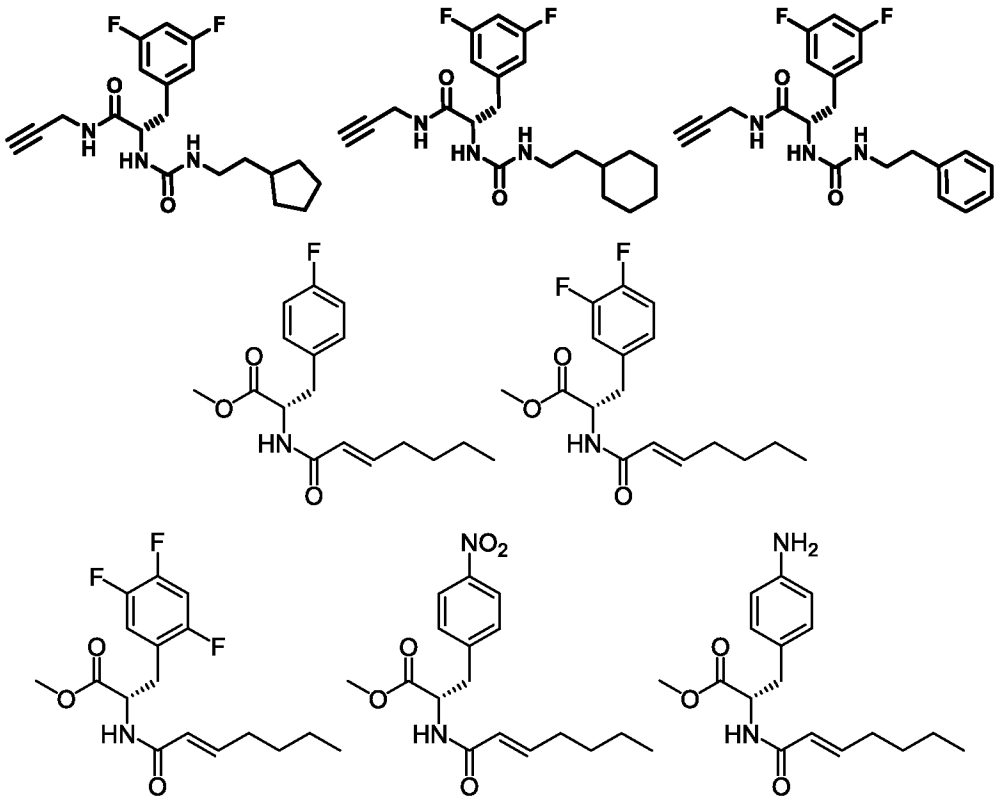






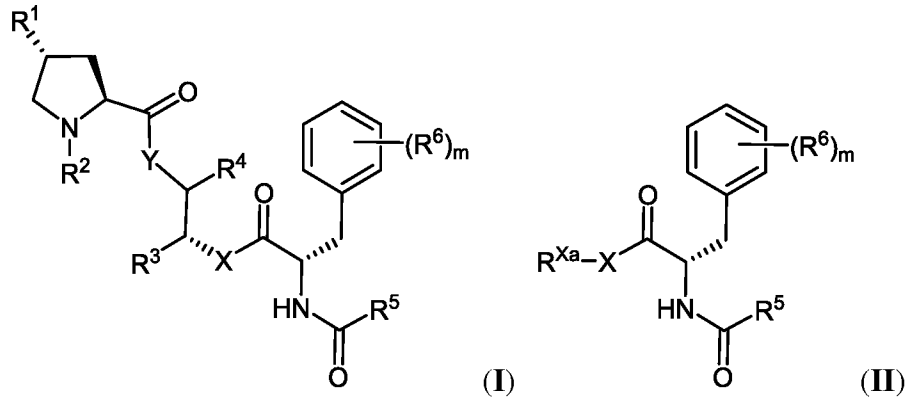






and pharmaceutically acceptable salts thereof.

51. A pharmaceutical composition comprising an effective amount of a compound of Formula (**I**) or (**II**):



(**I**) (**II**)

or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient; wherein:

R^1 is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

R^2 is hydrogen, -OH, $-OR^{2A}$, $-C(=O)R^{2A}$, $-C(=O)OR^{2A}$, $-C(=O)NHR^{2A}$, $-C(=O)N(R^{2A})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally

substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, or a nitrogen protecting group;

R^3 is $-C(=O)R^{3A}$, $-C(=O)OR^{3A}$, $-C(=O)NHR^{3A}$, $-C(=O)N(R^{3A})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

each instance of R^{2A} and R^{3A} is independently optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl,

or two R^{2A} groups are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring,

or two R^{3A} groups are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring;

R^4 is hydrogen, –OH, –SH, $–NH_2$, substituted hydroxyl, substituted thiol, substituted amino, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

R^5 is optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, $–OR^{5A}$ or $–NHR^{5A}$; wherein each instance of R^{5A} is independently optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

each instance of R^6 is independently halogen, –OH, –SH, $–NH_2$, -CN, $-NO_2$, $-N_3$, $–SO_2H$, $–SO_3H$, substituted hydroxyl, substituted thiol, substituted amino, sulfonyl, sulfinyl, carbonyl, silyl, boronyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

m is 0 or an integer of between 1 and 5, inclusive;

Y is O, S, or NR^Y wherein R^Y is hydrogen, optionally substituted alkyl, or a nitrogen protecting group; and

X is O, S, or NR^{Xb},

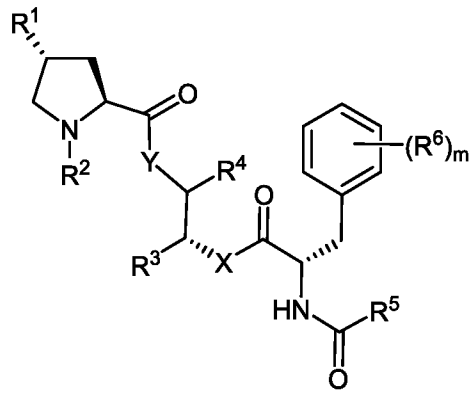
wherein:

R^{Xb} is hydrogen, optionally substituted alkyl, or a nitrogen protecting group; and

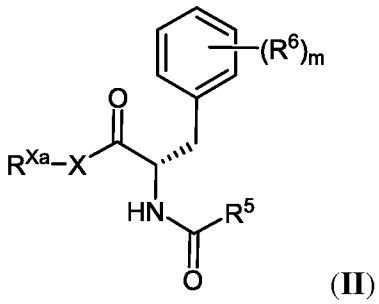
R^{Xa} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

or R^{Xa} and R^{Xb} are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring.

52. A method of treating a microbial infection in a subject comprising administering an effective amount of a compound of Formula (**I**) or (**II**):



(**I**)



(**II**)

or a pharmaceutically acceptable salt thereof, to the subject;

wherein:

R^1 is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

R^2 is hydrogen, -OH, -OR^{2A}, -C(=O)R^{2A}, -C(=O)OR^{2A}, -C(=O)NHR^{2A}, -C(=O)$N(R^{2A})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, or a nitrogen protecting group;

R^3 is -C(=O)R^{3A}, -C(=O)OR^{3A}, -C(=O)NHR^{3A}, -C(=O)$N(R^{3A})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

each instance of R^{2A} and R^{3A} is independently optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl,

optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl,

or two R^{2A} groups are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring,

or two R^{3A} groups are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring;

R^4 is hydrogen, –OH, –SH, $–NH_2$, substituted hydroxyl, substituted thiol, substituted amino, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

R^5 is optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, $–OR^{5A}$ or $–NHR^{5A}$; wherein each instance of R^{5A} is independently optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

each instance of R^6 is independently halogen, –OH, –SH, $–NH_2$, -CN, $-NO_2$, $-N_3$, $–SO_2H$, $–SO_3H$, substituted hydroxyl, substituted thiol, substituted amino, sulfonyl, sulfinyl, carbonyl, silyl, boronyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

m is 0 or an integer of between 1 and 5, inclusive;

Y is O, S, or NR^Y wherein R^Y is hydrogen, optionally substituted alkyl, or a nitrogen protecting group; and

X is O, S, or NR^{Xb},

wherein:

R^{Xb} is hydrogen, optionally substituted alkyl, or a nitrogen protecting group; and

R^{Xa} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

or R^{Xa} and R^{Xb} are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring.

53. The method of claim 52, wherein the microbial infection is a bacterial infection.

54. The method of claim 52, wherein the bacterial infection is an *M. tuberculosis* infection.

55. The method of claim 52, further comprising administering the compound in combination with an antibiotic.

56. The method of claim 55, wherein the antibiotic is a ribosome-targeting antibiotic.

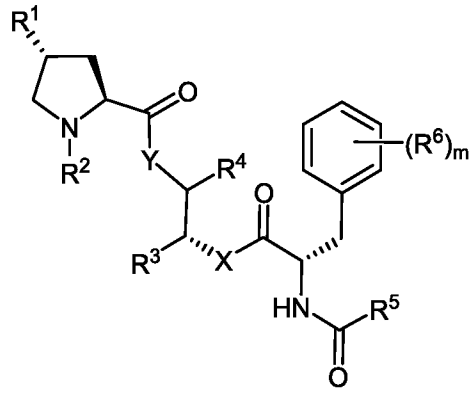
57. The method of claim 52, wherein the bacterial infection is resistant to other treatments.

58. The method of claim 52, wherein the bacterial infection is multi-drug tolerant.

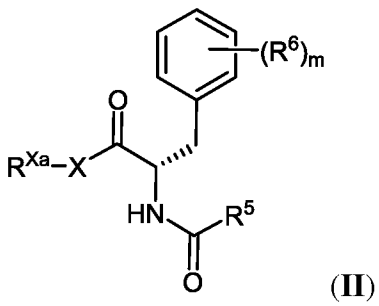
59. The method of claim 52, wherein the bacterial infection is multi-drug resistant.

60. The method of claim 52, wherein the bacterium causing the bacterial infection neither grow nor die in the presence of or as a result of other treatments.

61. A method of treating microbial virulence comprising contacting an effective amount of a compound of Formula (**I**) or (**II**):



(**I**)



(**II**)

or a pharmaceutically acceptable salt thereof, to a microorganism;

wherein:

R^1 is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

R^2 is hydrogen, -OH, -OR^{2A}, -C(=O)R^{2A}, -C(=O)OR^{2A}, -C(=O)NHR^{2A}, -C(=O)$N(R^{2A})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, or a nitrogen protecting group;

R^3 is -C(=O)R^{3A}, -C(=O)OR^{3A}, -C(=O)NHR^{3A}, -C(=O)$N(R^{3A})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

each instance of R^{2A} and R^{3A} is independently optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl,

or two R^{2A} groups are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring,

or two R^{3A} groups are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring;

R^4 is hydrogen, –OH, –SH, $–NH_2$, substituted hydroxyl, substituted thiol, substituted amino, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

R^5 is optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, –OR^{5A} or –NHR^{5A}; wherein each instance of R^{5A} is independently optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

each instance of R^6 is independently halogen, –OH, –SH, $–NH_2$, -CN, $-NO_2$, $-N_3$, $–SO_2H$, $–SO_3H$, substituted hydroxyl, substituted thiol, substituted amino, sulfonyl, sulfinyl, carbonyl, silyl, boronyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

m is 0 or an integer of between 1 and 5, inclusive;

Y is O, S, or NR^Y wherein R^Y is hydrogen, optionally substituted alkyl, or a nitrogen protecting group; and

X is O, S, or NR^{Xb},

wherein:

R^{Xb} is hydrogen, optionally substituted alkyl, or a nitrogen protecting group; and

R^{Xa} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

or R^{Xa} and R^{Xb} are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring.

62. The method of claim 61, wherein the compound blocks virulence factor production.

63. The method of 61, wherein the microbial virulence is bacterial virulence, and the microorganism is a bacterium.

64. The method of claim 63, wherein the bacterium is *M. tuberculosis*.

65. The method of claim 61, wherein the method is an *in vivo* or *in vitro* method.

66. The method of claim 63, wherein the bacterium is resistant to other treatments.

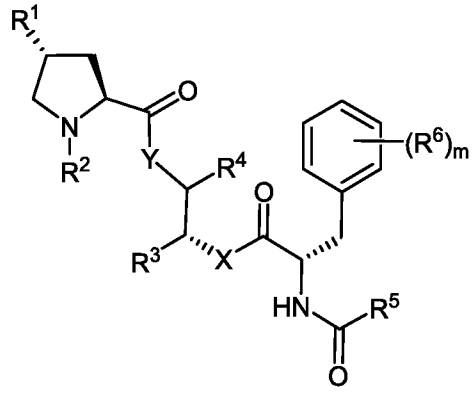
67. The method of claim 63, wherein the bacterium is multi-drug tolerant.

68. The method of claim 63, wherein the bacterium is multi-drug resistant.

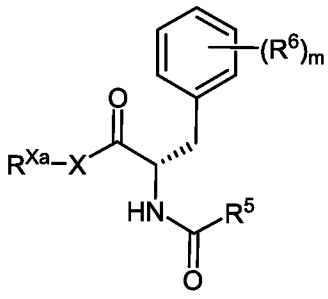
69. The method of claim 63, wherein the bacterium neither grows nor dies in the presence of or as a result of other treatments.

70. A method of potentiating the activity of an anti-bacterial agent against a bacterium comprising contacting the bacterium with an anti-bacterial agent and a compound of Formula

(**I**) or (**II**):



(**I**)



(**II**)

or a pharmaceutically acceptable salt thereof;

wherein:

R^1 is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

R^2 is hydrogen, -OH, $-OR^{2A}$, $-C(=O)R^{2A}$, $-C(=O)OR^{2A}$, $-C(=O)NHR^{2A}$, $-C(=O)N(R^{2A})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, or a nitrogen protecting group;

R^3 is $-C(=O)R^{3A}$, $-C(=O)OR^{3A}$, $-C(=O)NHR^{3A}$, $-C(=O)N(R^{3A})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

each instance of R^{2A} and R^{3A} is independently optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl,

or two R^{2A} groups are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring,

or two R^{3A} groups are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring;

R^4 is hydrogen, –OH, –SH, $–NH_2$, substituted hydroxyl, substituted thiol, substituted amino, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

R^5 is optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl, $–OR^{5A}$ or $–NHR^{5A}$; wherein each instance of R^{5A} is independently optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

each instance of R^6 is independently halogen, –OH, –SH, $–NH_2$, -CN, $-NO_2$, $-N_3$, $–SO_2H$, $–SO_3H$, substituted hydroxyl, substituted thiol, substituted amino, sulfonyl, sulfinyl, carbonyl, silyl, boronyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

m is 0 or an integer of between 1 and 5, inclusive;

Y is O, S, or NR^Y wherein R^Y is hydrogen, optionally substituted alkyl, or a nitrogen protecting group; and

X is O, S, or NR^{Xb},

wherein:

R^{Xb} is hydrogen, optionally substituted alkyl, or a nitrogen protecting group; and

R^{Xa} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

or R^{Xa} and R^{Xb} are joined to form an optionally substituted heterocyclic ring or optionally substituted heteroaryl ring.

71. The method of claim 70, wherein the bacterium is of the phylum *Actinobacteria*.

72. The method of claim 71, the bacterium is a member of the phylum *Actinobacteria* and the genus *Mycobacteriium*.

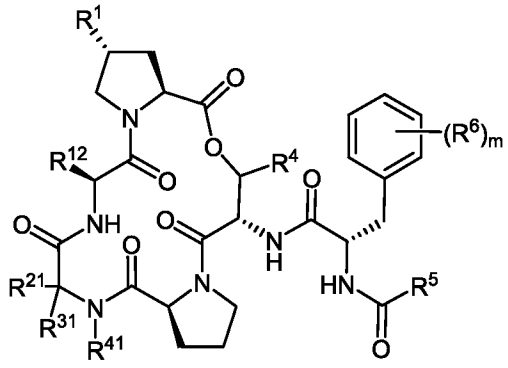
73. The method of claim 72, wherein the bacterium is *M. tuberculosis*.

74. The method of claim 70, wherein the ratio of the compound or a pharmaceutically acceptable salt thereof to the anti-bacterial agent (molar excess) is between about 1:1 and about 100:1, inclusive.

75. The method of claim 70, wherein the compound is of Formula (**II**) or a pharmaceutically acceptable salt thereof.

76. The method of claim 70, wherein the anti-bacterial agent is an enopeptin.

77. The method of claim 76, wherein the enopeptin is of Formula (**A**):



(**A**)

or a pharmaceutically acceptable salt thereof;

wherein:

R^{21} is hydrogen, and R^{31} and R^{41} are joined to form an optionally substituted heterocyclyl; or

R^{21} and R^{31} are joined to form a spiro-fused optionally substituted carbocyclyl or spiro-fused optionally substituted heterocyclyl, and R^{41} is hydrogen, optionally substituted alkyl, or an amino protecting group; or

R^{21} and R^{31} are independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl, provided that R^{21} and R^{31} are not both hydrogen; and R^{41} is hydrogen, optionally substituted alkyl, or an amino protecting group;

R^{12} is hydrogen, –OH, –SH, $–NH_2$, substituted hydroxyl, substituted thiol, substituted amino, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

R^4 is hydrogen, –OH, –SH, $–NH_2$, substituted hydroxyl, substituted thiol, substituted amino, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted

alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

R^5 is optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

each instance of R^6 is independently halogen, –OH, –SH, $–NH_2$, -CN, $-NO_2$, $-N_3$, $–SO_2H$, $–SO_3H$, substituted hydroxyl, substituted thiol, substituted amino, sulfonyl, sulfinyl, carbonyl, silyl, boronyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl; and

m is 0 or an integer of between 1 and 5, inclusive.

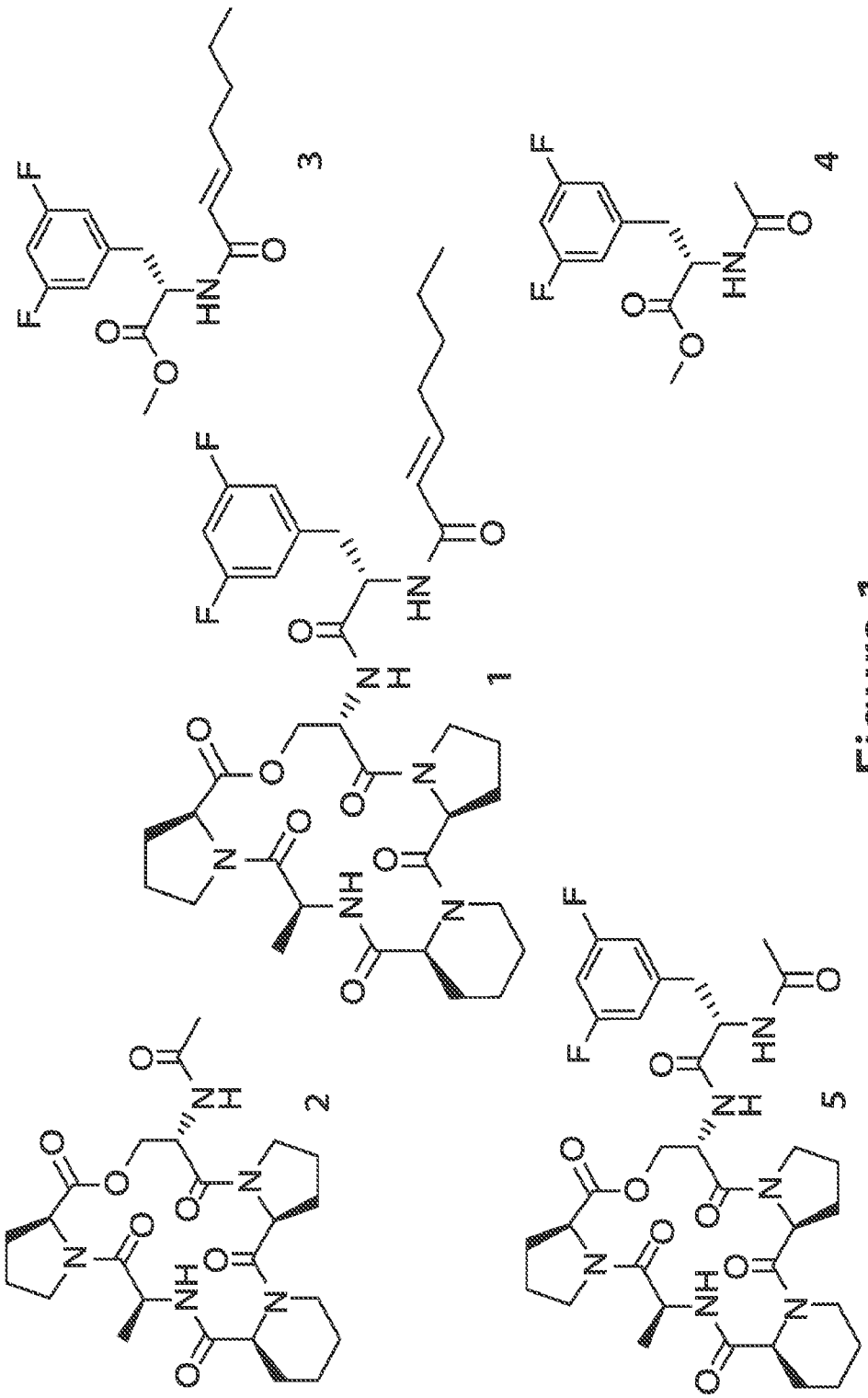


Figure 1

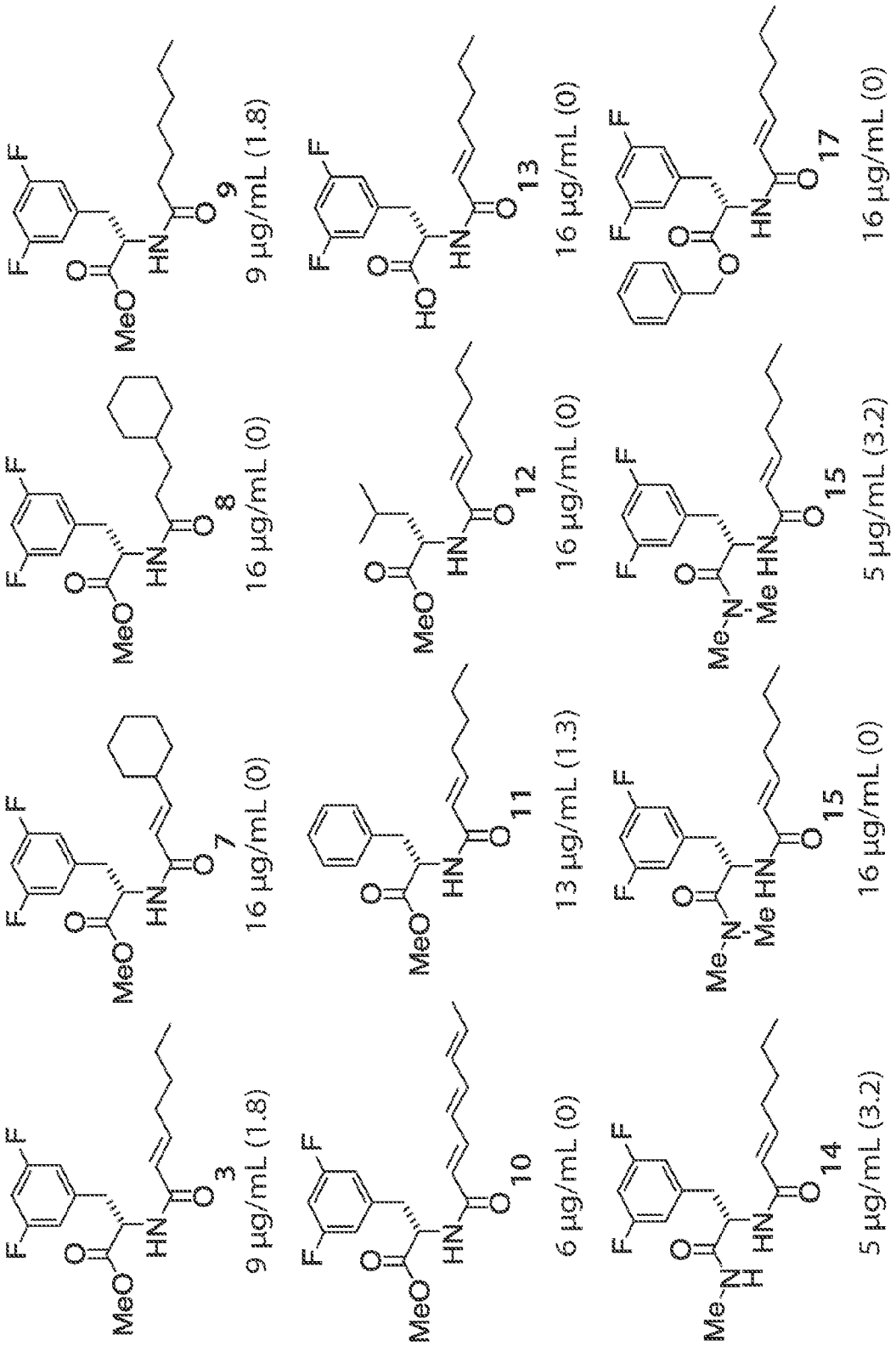

3
9 µg/mL (1.8)
7
16 µg/mL (0)
8
16 µg/mL (0)
9
9 µg/mL (1.8)
10
6 µg/mL (0)
11
13 µg/mL (1.3)
12
16 µg/mL (0)
13
16 µg/mL (0)
14
5 µg/mL (3.2)
15
16 µg/mL (0)
15
5 µg/mL (3.2)
17
16 µg/mL (0)

Figure 2

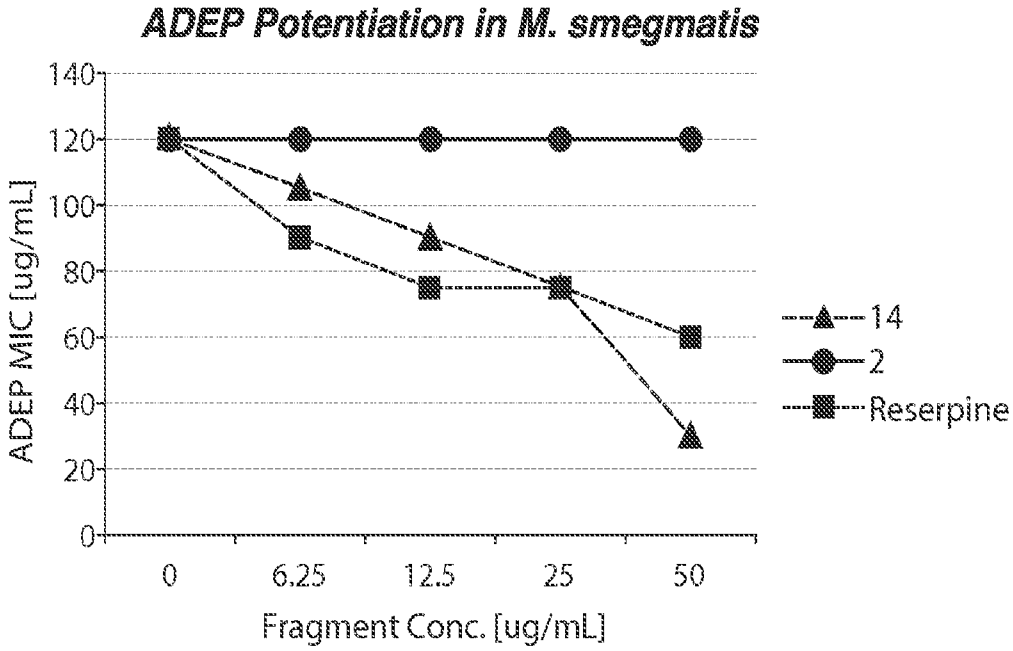


Figure 3A

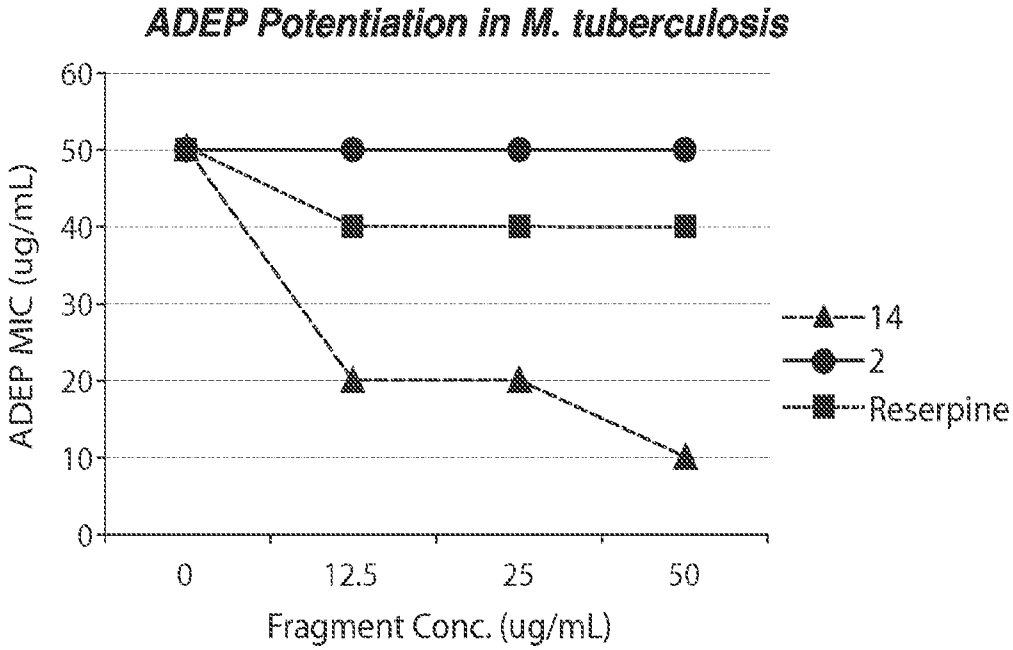


Figure 3B

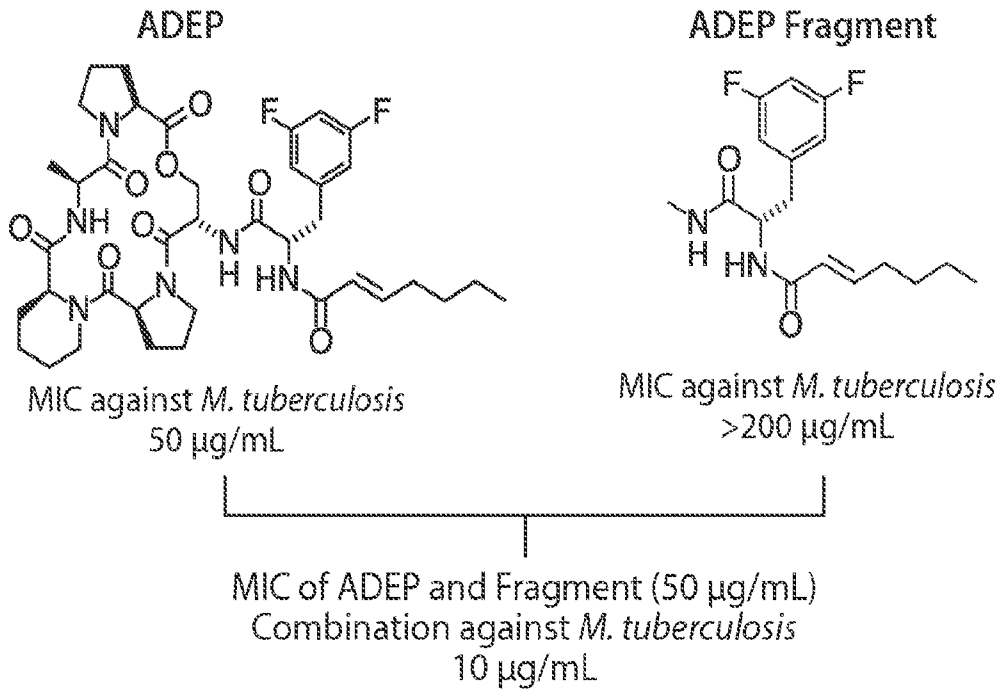

ADEP
ADEP Fragment
MIC against M. tuberculosis
50 µg/mL
MIC against M. tuberculosis
>200 µg/mL
MIC of ADEP and Fragment (50 µg/mL)
Combination against M. tuberculosis
10 µg/mL

Figure 3C

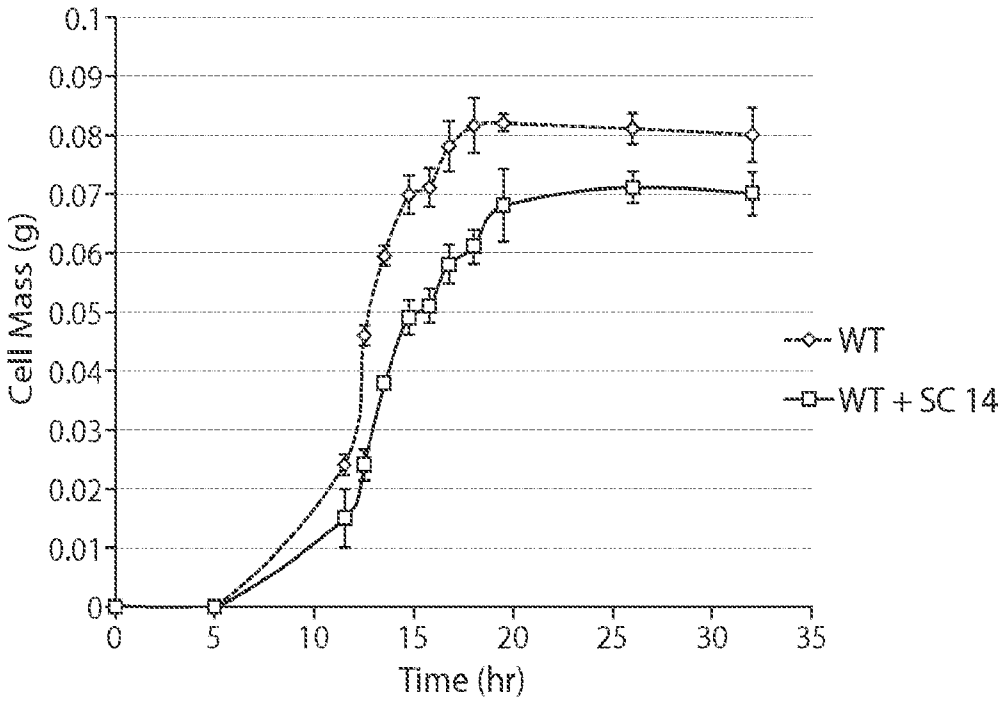

0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Cell Mass (g)
0
5
10
15
20
25
30
35
Time (hr)
WT
WT + SC 14

Figure 4

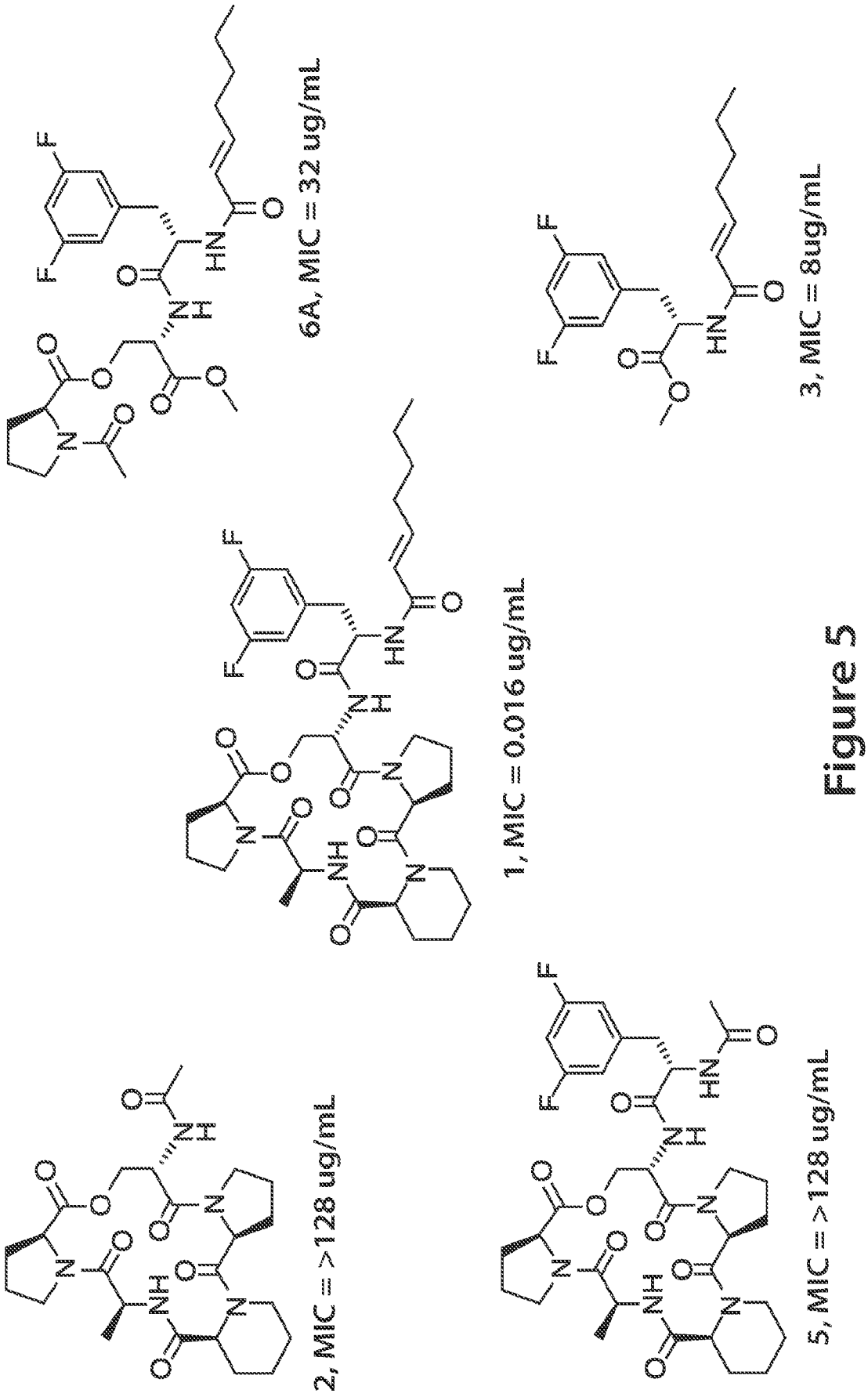

6A, MIC = 32 ug/mL
3, MIC = 8ug/mL
1, MIC = 0.016 ug/mL
2, MIC = >128 ug/mL
5, MIC = >128 ug/mL

Figure 5

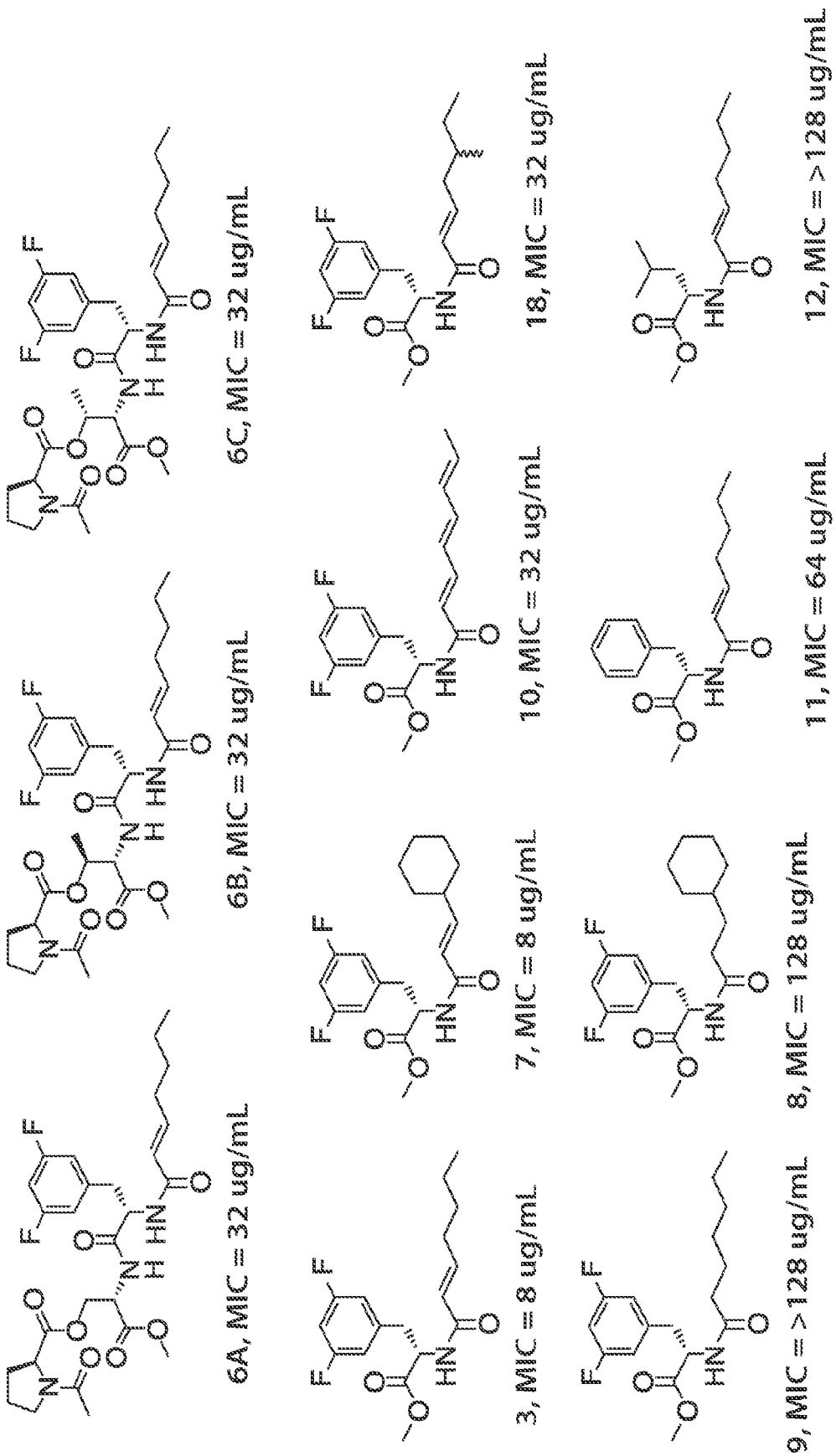

6A, MIC = 32 ug/mL
6B, MIC = 32 ug/mL
6C, MIC = 32 ug/mL
3, MIC = 8 ug/mL
7, MIC = 8 ug/mL
10, MIC = 32 ug/mL
18, MIC = 32 ug/mL
9, MIC = >128 ug/mL
8, MIC = 128 ug/mL
11, MIC = 64 ug/mL
12, MIC = >128 ug/mL

Figure 6

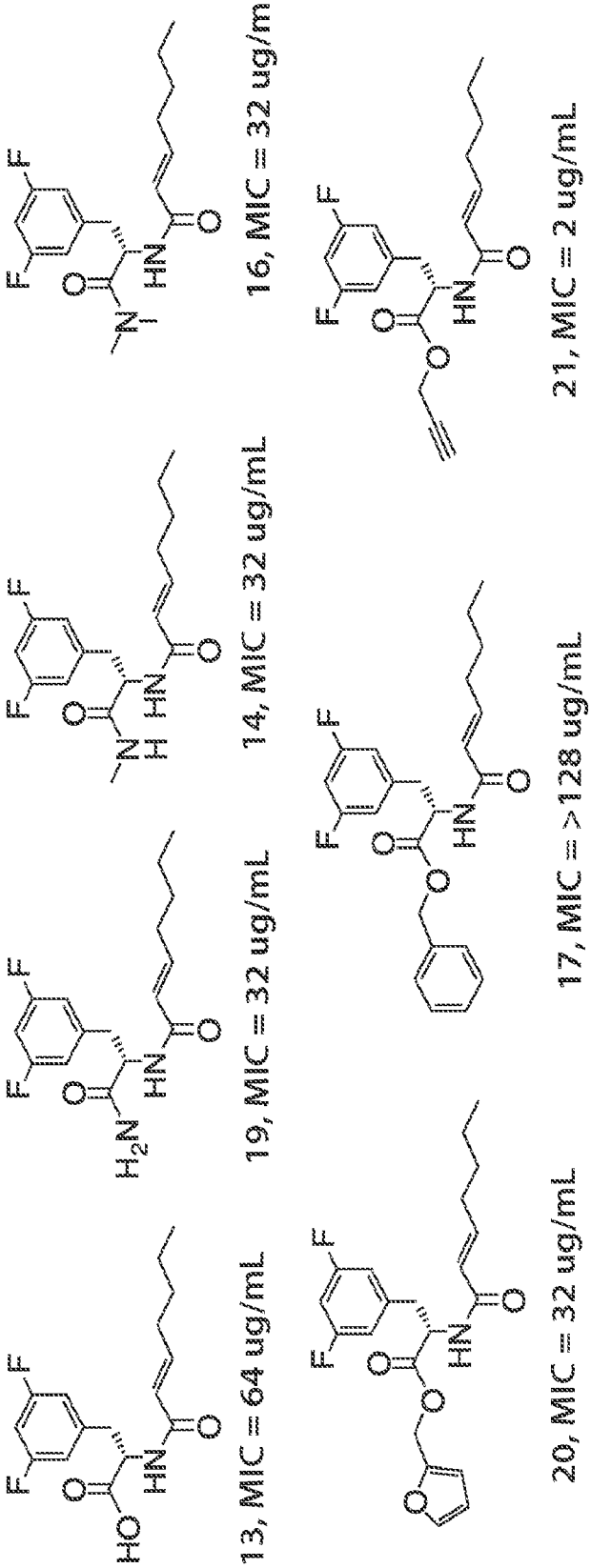


Figure 6 (continued)

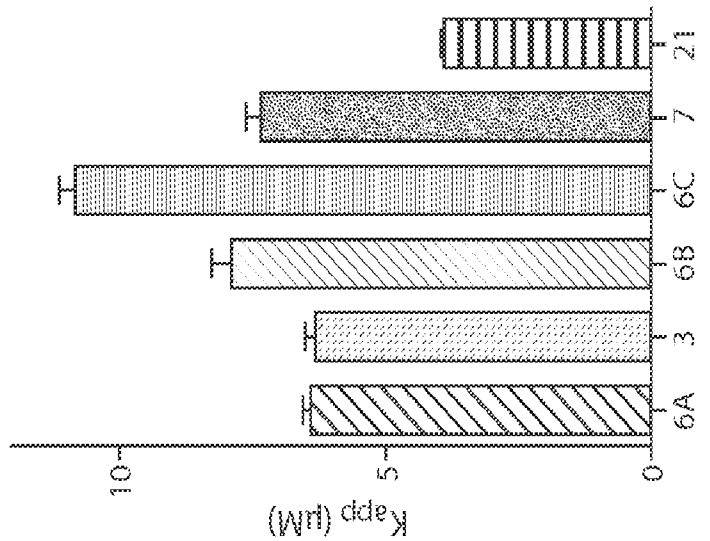


Figure 7B

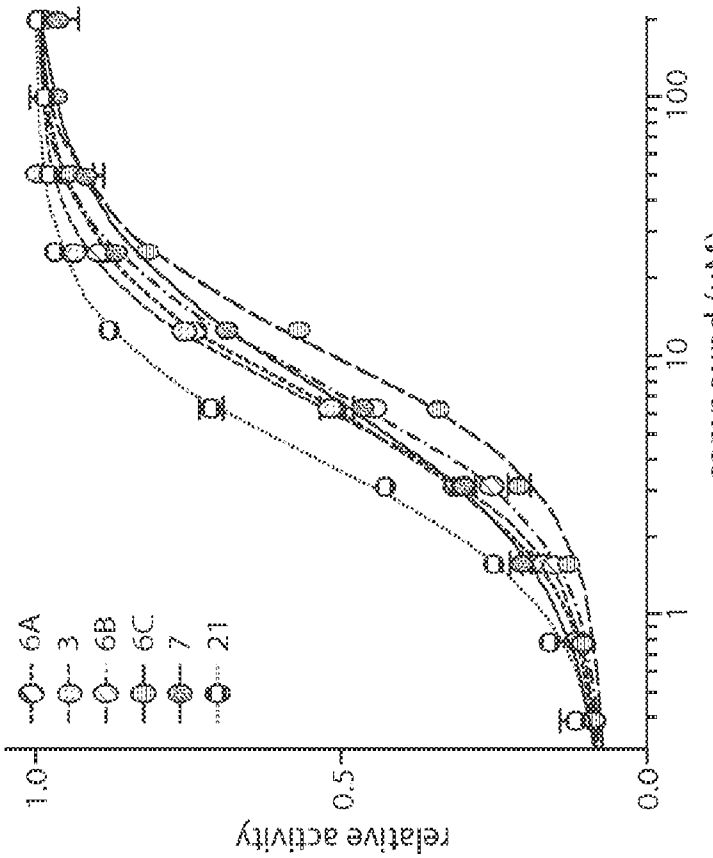


Figure 7A

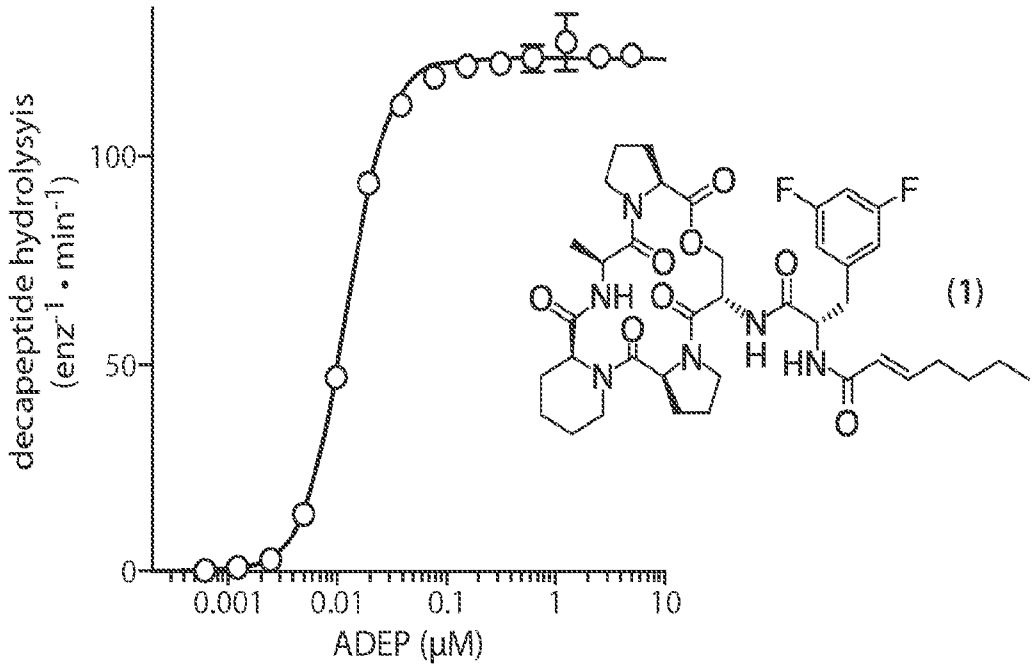

decapeptide hydrolysyis
(enz-1 • min-1)
100
50
0
0.001
0.01
0.1
1
10
ADEP (μM)
F
F
NH
N
H
HN
O
(1)

Figure 8

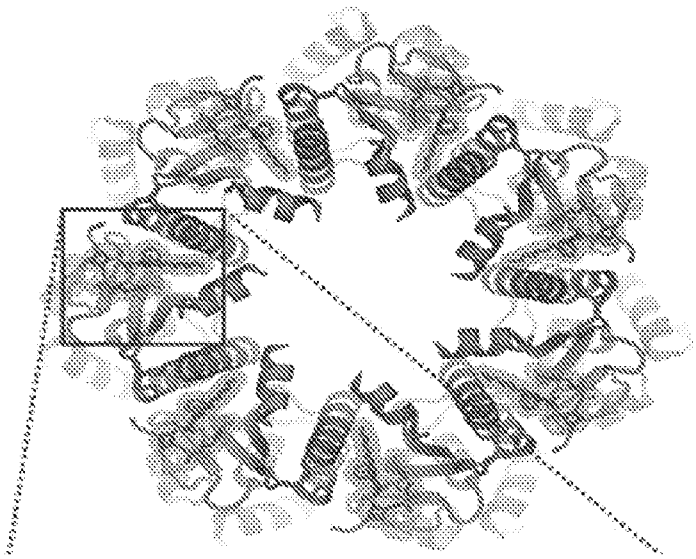


Figure 9A

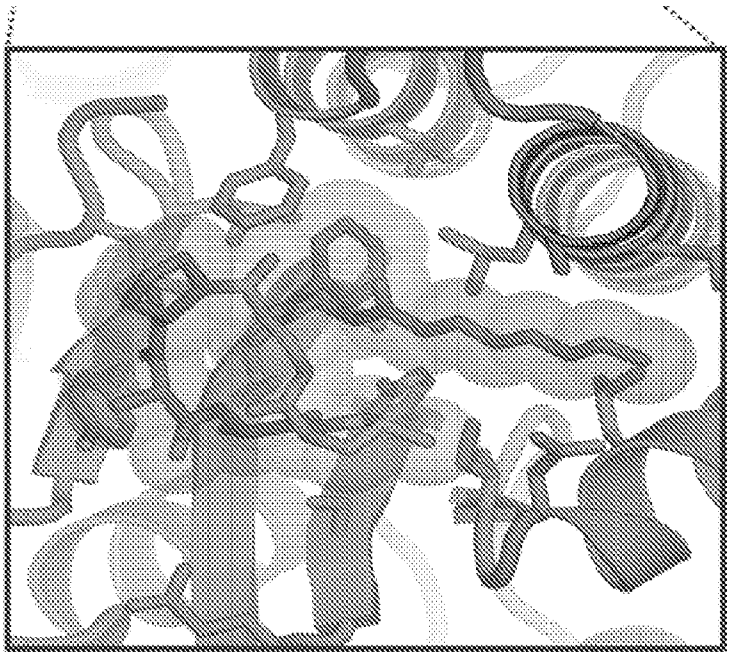


Figure 9B

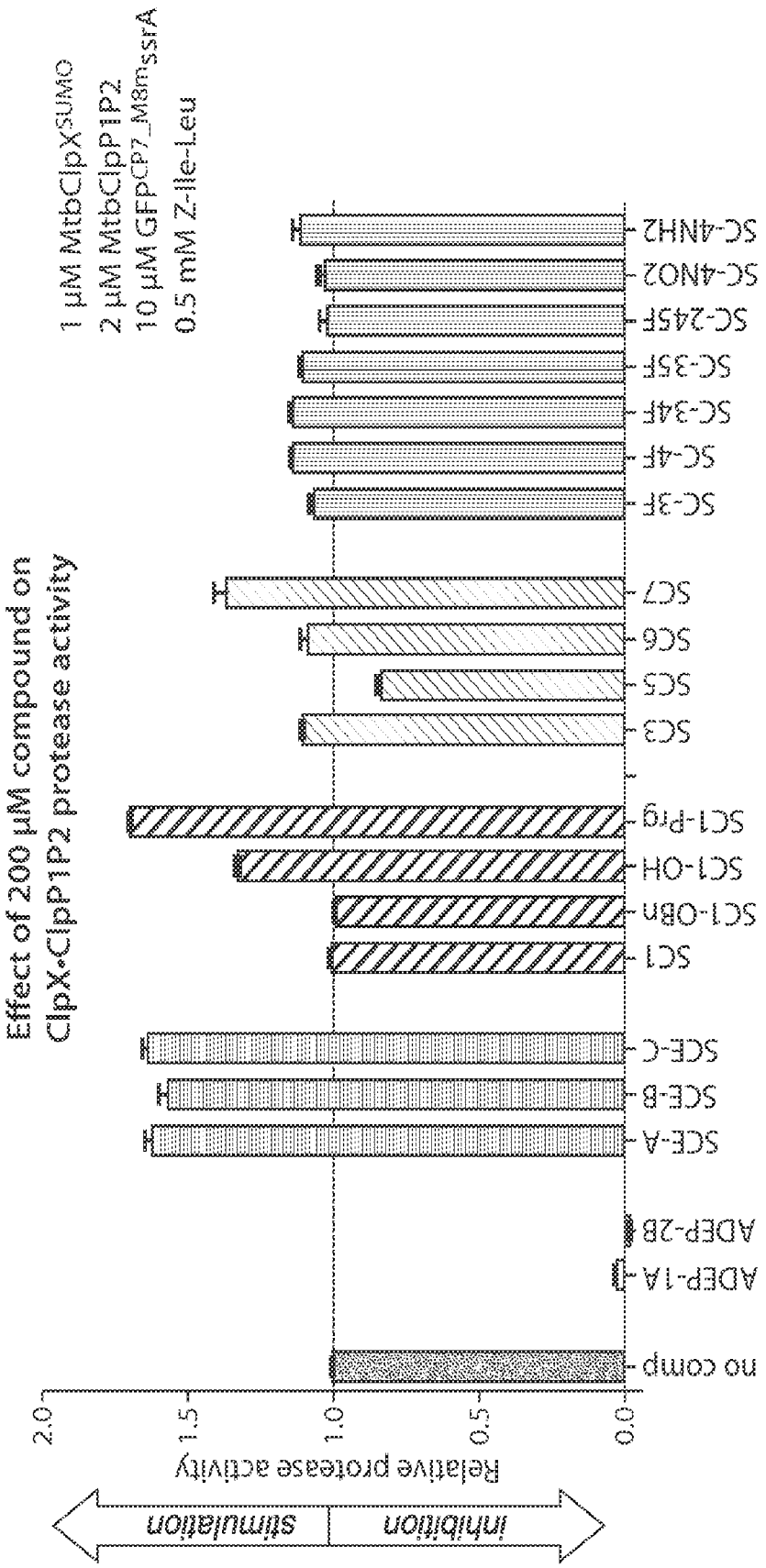

Effect of 200 µM compound on
ClpX•ClpP1P2 protease activity
1 µM MtbClpX^SUMO
2 µM MtbClpP1P2
10 µM GFP^CP7_M8m_ssrA
0.5 mM Z-Ile-Leu
Relative protease activity
2.0
1.5
1.0
0.5
0.0
inhibition
stimulation
no comp
ADEP-1A
ADEP-2B
SCE-A
SCE-B
SCE-C
SC1
SC1-OBn
SC1-OH
SC1-Prg
SC3
SC5
SC6
SC7
SC-3F
SC-4F
SC-34F
SC-35F
SC-245F
SC-4NO2
SC-4NH2

Figure 10A

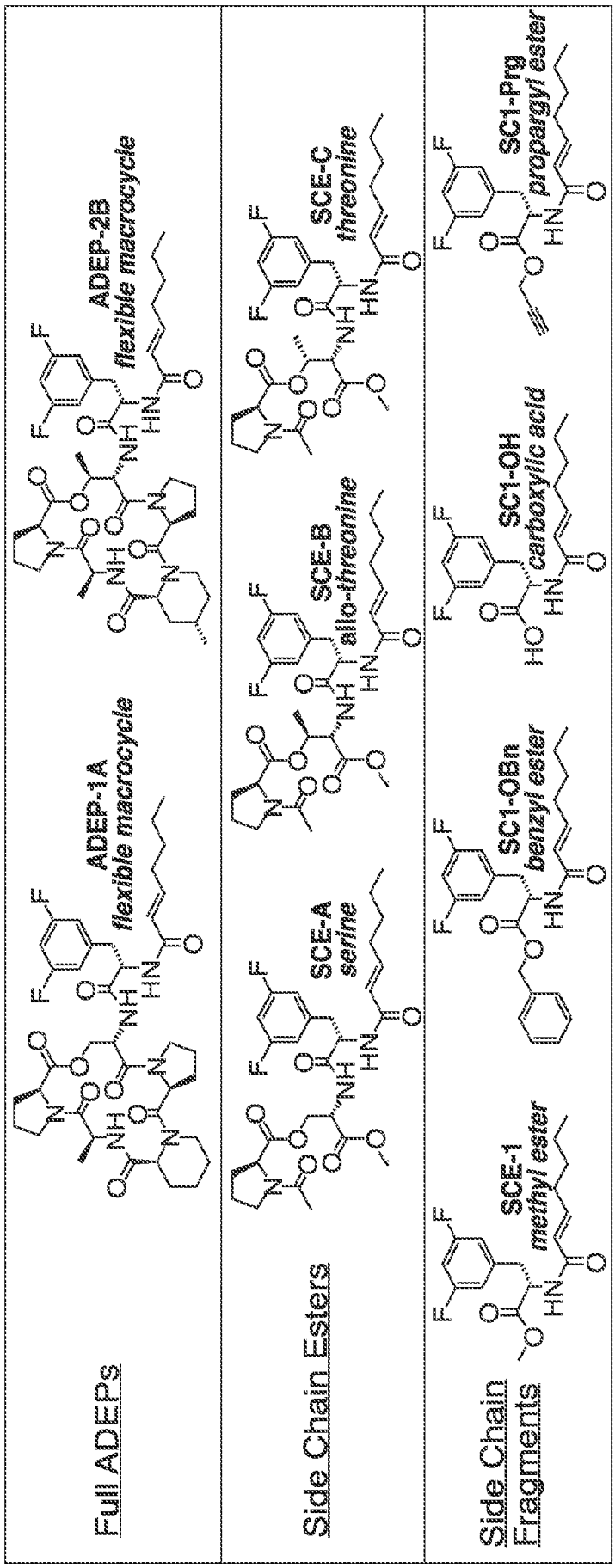

Full ADEPs
ADEP-1A
flexible macrocycle
ADEP-2B
flexible macrocycle
Side Chain Esters
SCE-A
serine
SCE-B
allo-threonine
SCE-C
threonine
Side Chain Fragments
SC1-1
methyl ester
SC1-OBn
benzyl ester
SC1-OH
carboxylic acid
SC1-Prg
propargyl ester

Figure 10B

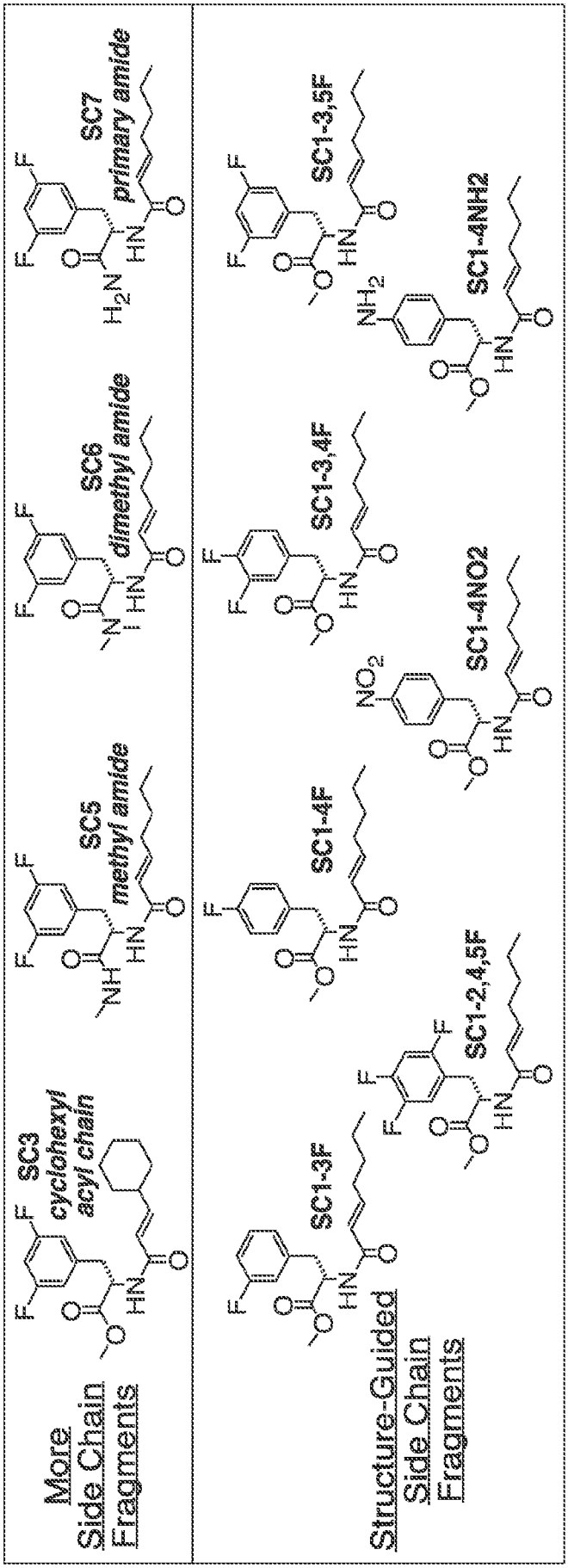


Figure 10B (continued)