(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 March 2017 (16.03.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/044592 A1

(51) International Patent Classification:
C07D 207/06 (2006.01) *C07K 5/06* (2006.01)

(21) International Application Number:
PCT/US2016/050720

(22) International Filing Date:
8 September 2016 (08.09.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/215,551 8 September 2015 (08.09.2015) US

(71) Applicant: **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 1111 Franklin Street, Twelfth Floor, Oakland, CA 94607-5200 (US).

(72) Inventors: **RUCHALA, Piotr**; c/o The Regents of The University of California, 1111 Franklin Street, Twelfth Floor, Oakland, CA 94607-5200 (US). **MICEWICZ, Ewa, D.**; c/o The Regents of The University of California, 1111 Franklin Street, Twelfth Floor, Oakland, CA 94607-5200 (US).

(74) Agent: **BARRETT, Tamsen, L.**; NORTON ROSE FULBRIGHT US LLP, 98 San Jacinto Blvd., Suite 1100, Austin, TX 78701 (US).

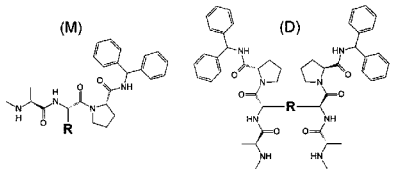
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU,

[Continued on next page]

(54) Title: CONJUGATED ANTICANCER SMAC ANALOGS

(57) Abstract: Lipid-conjugated monovalent and bivalent secondary mitochondria derived activator of caspases (Smac) derivatives were synthesized and examined for in vitro and in vivo anticancer activity. Methods for treating cancer with these compounds are also disclosed.



Compound	R	EC_{50} [μM] MDA-MB-231
M1	Ser^{OH}	NA
M2	Cys^{NH}	NA
M3	$Cys^{S\text{-}Nle}$	49.9 ± 0.7
M4	Lys	NA
M5	Lys^{Glu}	NA
M6	$Lys^{NH\text{-}DNFB}$	44.7 ± 15.0
M7	$Lys^{NH\text{-}Fmoc}$	19.0 ± 1.6
M8	$Lys^{NH\text{-}Pal}$	6.1 ± 0.1
M9	$Lys^{NH\text{-}Lig}$	NA
M10	$Lys^{NH\text{-}Chol}$	9.7 ± 0.5
M11	$Cys^{S\text{-}Nle}$	4.4 ± 0.1
D1	$Lys^{NH\text{-}Urea}$	NA
D2	$Lys^{NH\text{-}Sub}$	NA
D3	$Lys^{NH\text{-}DFDNB}$	3.3 ± 0.2
D4	$Lys^{NH\text{-}PDI}$	34.8 ± 2.7
D5	$Lys^{NH\text{-}OPI}$	14.4 ± 1.4
D6	$Lys^{NH\text{-}Ida}$	NA
D7	$Lys^{NH\text{-}Ida\text{-}Pal}$	4.2 ± 0.1
D8	$Lys^{NH\text{-}Ida\text{-}N\text{-}EtO\text{-}Pal}$	4.3 ± 0.1
D9	$Cys^{aS\text{-}DVS}$	52.4 ± 4.3
D10	$Cys^{S\text{-}CAEDA}$	NA
D11	$Cys^{S\text{-}pBMB}$	9.6 ± 0.6
D12	$Cys^{S\text{-}mBMB}$	7.4 ± 0.4
D13	$Cys^{S\text{-}Bip}$	4.0 ± 0.1
D14	$Cys^{S\text{-}CMPB}$	18.4 ± 1.1
D15	$Cys^{S\text{-}BMBB}$	NA
D16	$Cys^{S\text{-}mBMPB}$	11.3 ± 0.6

FIG. 1

WO 2017/044592 A1

TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

CONJUGATED ANTICANCER SMAC ANALOGS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of U.S. Provisional Patent Application No. 62/215,551, filed September 8, 2015, which is hereby incorporated by reference in its entirety.

STATEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made with government support under grant number 5U19AI067769 awarded by the National Institutes of Health/National Institute of Allergy and Infectious Diseases. The government has certain rights in this invention.

BACKGROUND OF THE INVENTION

A. Field of the Invention

[0003] Embodiments of this invention are directed generally to chemistry, microbiology, and medicine. In certain aspects the invention is directed to the treatment of cancer.

B. Description of Related Art

[0004] Apoptosis, also called programmed cell death (PCD) is an important mechanism controlling a variety of physiological processes including: host defense, development, homeostasis, and suppression of oncogenesis with implications in human pathologies spanning from cancer to inflammation and neurodegeneration. Regulation of apoptosis depends on Inhibitors of Apoptosis Proteins (IAPs). Structurally, IAPs contain one or more of Baculovirus IAP Repeat (BIR) domains, which are capable of binding to and inhibition of various caspases, enzymes belonging to cysteine–aspartyl proteases family, which are crucial for the apoptotic process. To date, eight mammalian IAPs have been identified: neuronal IAP (NIAP), cellular IAP1 (cIAP1), cellular IAP2 (cIAP2), X chromosome-linked IAP (XIAP), survivin, ubiquitin-conjugating BIR domain enzyme apollon, melanoma IAP (ML-IAP) and IAP-like protein 2, with the most potent caspase inhibitor family member being XIAP15,16, which simultaneously inhibits caspases -3, -7, and -9. Only cIAP1, cIAP2, and ML-IAP, were shown to play a direct role in the regulation of apoptosis by inhibiting caspases' activity or their activation. Anti-apoptotic activity of IAPs is in turn regulated by the second mitochondria derived activator of caspases (Smac), also called direct IAP binding protein with low pI (DIABLO), which acts as their endogenous

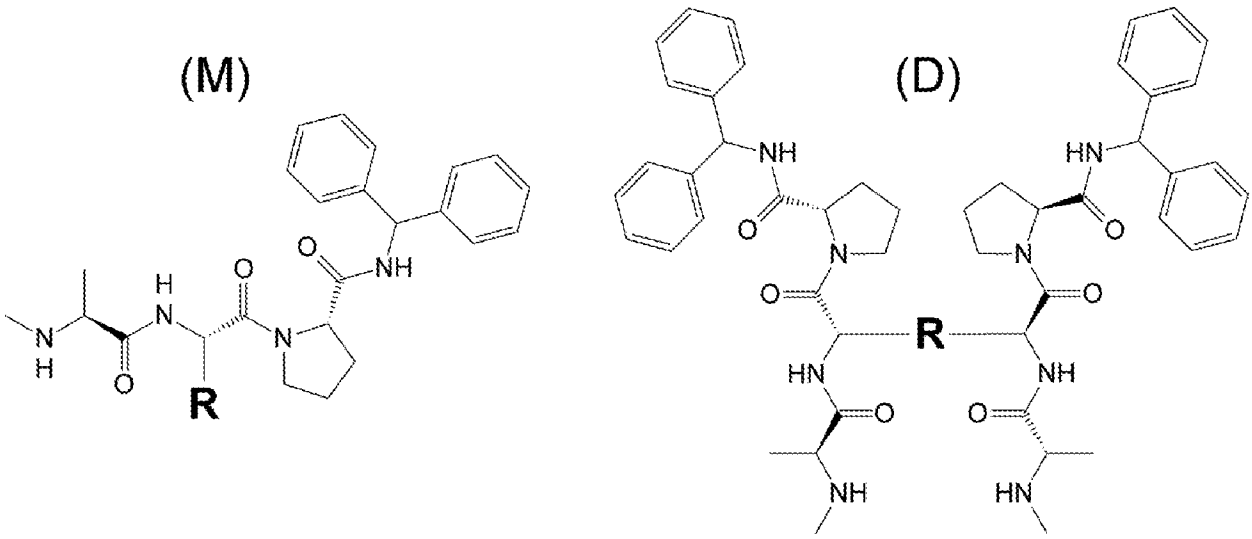
pro-apoptotic antagonist promoting programmed cell death. Structurally, N-terminal tetrapeptide AVPI (Ala-Val-Pro-Ile), the so called binding motif, is responsible for pro-apoptotic effects of mature Smac. In the case of XIAP, the homodimeric form of Smac is capable of binding to both BIR2 and BIR3 domains of the protein abrogating its inhibition of caspases-3, -7, and -9. For cIAP1 and cIAP2, only the BIR3 domain is targeted by a single AVPI binding motif.

[0005] Over the past decade, Smac mimics have become a promising therapeutic modalities in anti-cancer treatment, with several compounds advancing into clinical trials. Among these, bivalent Smac analogues containing two AVPI mimics tethered with a linker and capable of binding to both BIR2 and BIR3 XIAP domains became the focus of investigation due to their high potency. Monovalent Smac mimics are also desirable due to their favorable pharmaceutical properties, including low molecular weight, favorable pharmacokinetics and potential oral bioavailability. There remains a need to develop lipid-derivatized Smac mimics for treatments effective in treating cancer and tumors.

SUMMARY OF THE INVENTION

[0006] A library of monovalent and bivalent Smac mimics was synthesized based on Smac monomers with the general structure NMeAla-Xaa-Pro-BHA (Xaa=Cys or Lys). Position 2 of the compounds was derivatized to dimerize two types of monomers employing various bis-reactive linkers, as well as to modify selected compounds with lipids. The resulting library was screened *in vitro* against metastatic human breast cancer cell line MDA-MB-231, and the two most active compounds selected for *in vivo* studies. The most active lipid-conjugated analogue M11, showed *in vivo* activity while administered both subcutaneously and orally. The findings demonstrate that lipidation is a viable approach in the development of novel Smac-based therapeutic leads. Therefore, embodiments concern compounds, pharmaceutical compositions, methods of making such compounds and compositions, and methods of using these compounds and compositions, including methods of treating cancer or treating a tumor in a patient. In certain embodiments, the patient is a human patient.

[0007] In some embodiments, a method of treating cancer in a subject is provided, the method comprising administering to the subject an effective amount of a 2-lysine- or 2-cysteine-functionalized Smac derivative is provided. In some aspects, the 2-lysine- or 2-cysteine-functionalized Smac derivative is a compound of formula M or D,



wherein R is SerOH, CysSH, CysS-StBu, Lys, LysGu, LysNH-DNFB, LysNH-Fmoc, LysNH-Pal, LysNH-Lig, LysNH-Chol, CysS-Ste, LysNH-Urea, LysNH-Sub, LysNH-DFDNB, LysNH-PDI, LysNH-OPI, LysNH-Ida, LysNH-Ida-Pal, LysNH-Ida-N-EtO-Pal, CysS-DVS, CysS-CAEDA, CysS-pBMB, CysS-mBMB, CysS-Bip, CysS-CMPB, CysS-BMBB, or CysS-mBMPB, or a salt, prodrug, enantiomer, or diastereomer thereof.

[0008] In some embodiments, R is an amino acid moiety of a Smac derivative. In other words, R includes a side chain, an amine group, a carbonyl group, and a chiral carbon bound to the side chain, amine, and carbonyl groups. In some embodiments, the R amino acid is bound to N-methyl alanine through its amine moiety and to proline through its carbonyl moiety. Therefore, in some embodiments, R does not represent an amino acid side chain, but rather R represents an amino acid which includes a side chain.

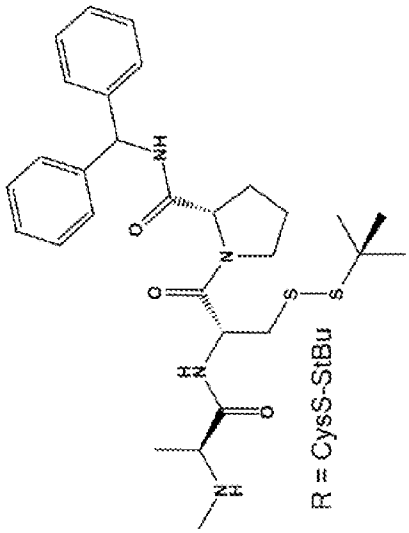
[0009] Some aspects are directed towards delaying the growth of a tumor comprising administering to the subject an effective amount of a 2-lysine- or 2-cysteine-functionalized Smac derivative. In some embodiments, a 2-lysine- or 2-cysteine-functionalized Smac derivative is administered to a subject to treat cancer. In some embodiments, the cancer is melanoma, carcinoma, lymphoma, blastoma, sarcoma, leukemia or lymphoid malignancies, breast cancer, colon cancer, rectal cancer, colorectal cancer, kidney or renal cancer, lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, squamous cell cancer (*e.g.* epithelial squamous cell cancer), cervical cancer, ovarian cancer, prostate cancer, liver cancer, bladder cancer, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, head and neck cancer, glioblastoma,

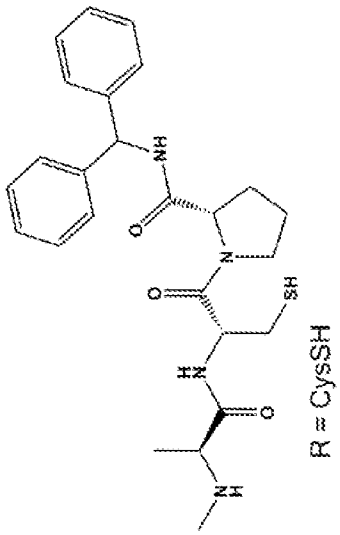
retinoblastoma, astrocytoma, thecomas, arrhenoblastomas, hepatoma, hematologic malignancies including non-Hodgkins lymphoma (NHL), multiple myeloma and acute hematologic malignancies, endometrial or uterine carcinoma, endometriosis, fibrosarcomas, choriocarcinoma, salivary gland carcinoma, vulval cancer, thyroid cancer, esophageal carcinomas, hepatic carcinoma, anal carcinoma, penile carcinoma, nasopharyngeal carcinoma, laryngeal carcinomas, Kaposi's sarcoma, melanoma, skin carcinomas, Schwannoma, oligodendroglioma, neuroblastomas, rhabdomyosarcoma, osteogenic sarcoma, leiomyosarcomas, urinary tract carcinomas, thyroid carcinomas, Wilm's tumor, as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome. In particular embodiments, the cancer is human breast cancer.

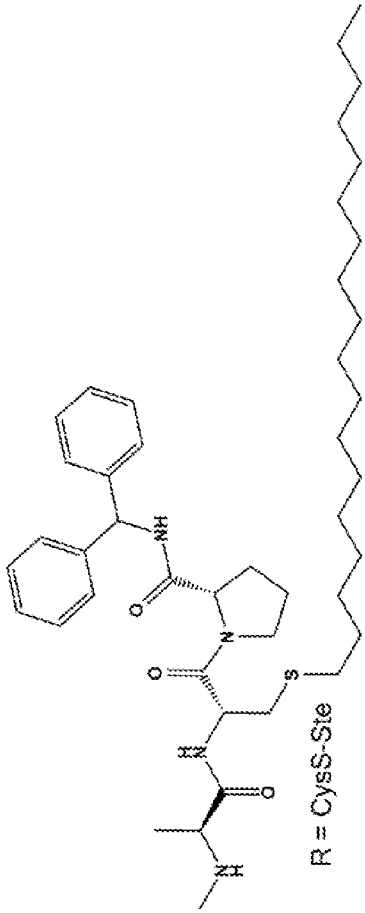
[0010] In some embodiments, the subject is further administered a distinct cancer therapy. The distinct cancer therapy may comprise surgery, radiotherapy, chemotherapy, toxin therapy, immunotherapy, cryotherapy or gene therapy. In some embodiments, the cancer is a chemotherapy or radio-resistant cancer. In some aspects, the 2-lysine- or 2-cysteine-functionalized Smac derivative is a monovalent 2-lysine- or 2-cysteine-functionalized Smac derivative. In some embodiments, the monovalent 2-lysine- or 2-cysteine-functionalized Smac derivative is functionalized with a lipophilic moiety. The lipophilic moiety may be a substituted or unsubstituted hydrocarbon comprising at least 6 carbon atoms. In some embodiments, the lipophilic moiety is selected from the group consisting of palmitoyl, lignoceroyl, stearyl, and cholesteryl.

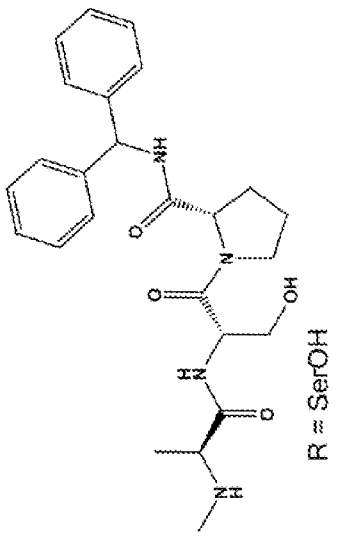
[0011] In some embodiments, the 2-lysine- or 2-cysteine-functionalized Smac derivative is a bivalent 2-lysine- or 2-cysteine-functionalized Smac derivative. In some aspects, the bivalent 2-lysine- or 2-cysteine-functionalized Smac derivative comprises two Smac moieties conjugated to each other through the 2-positions. In some embodiments, the bivalent Smac derivative comprises a lipophilic linker conjugating the two Smac moieties.

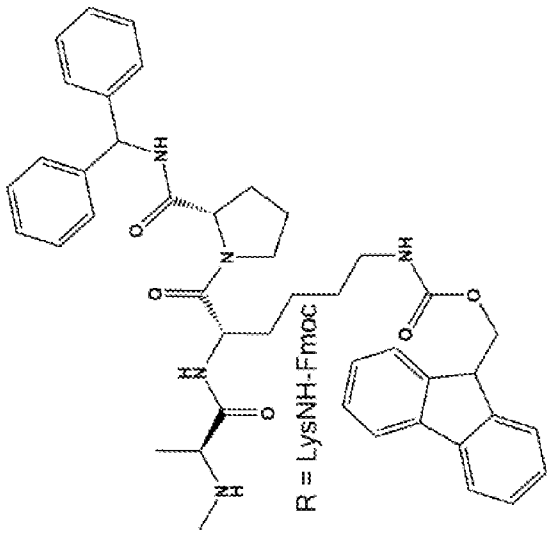
[0012] In some aspects, a method of treating cancer in a subject comprises administering to the subject an effective amount of any one of the following compounds:

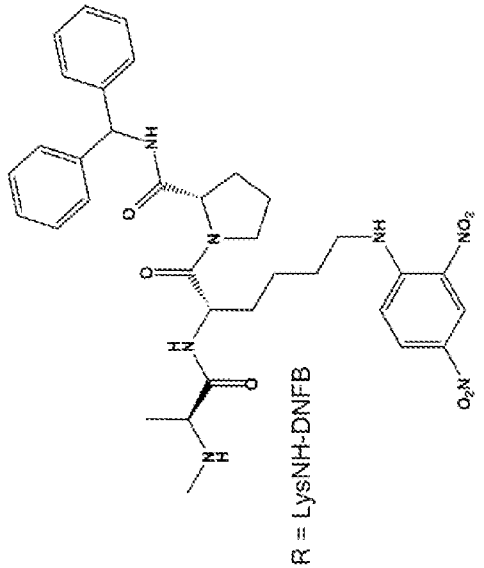

R = CysS-StBu

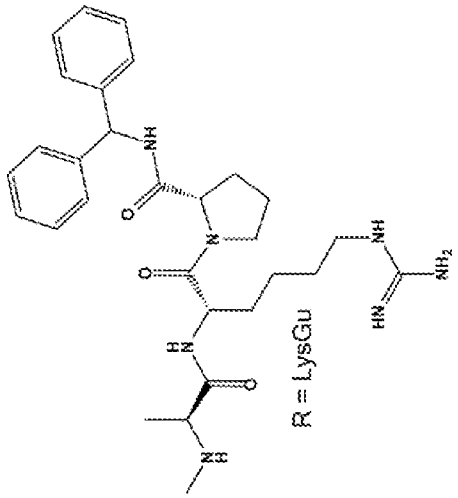

R = CysSH

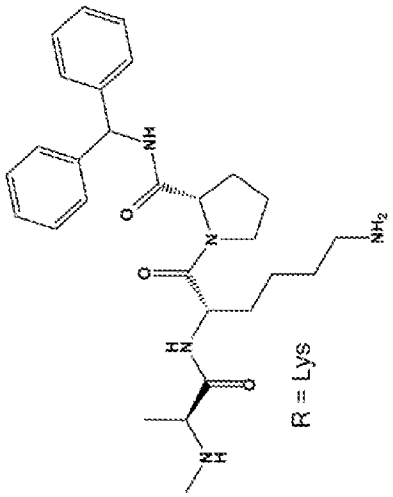

R = CysS-Ste

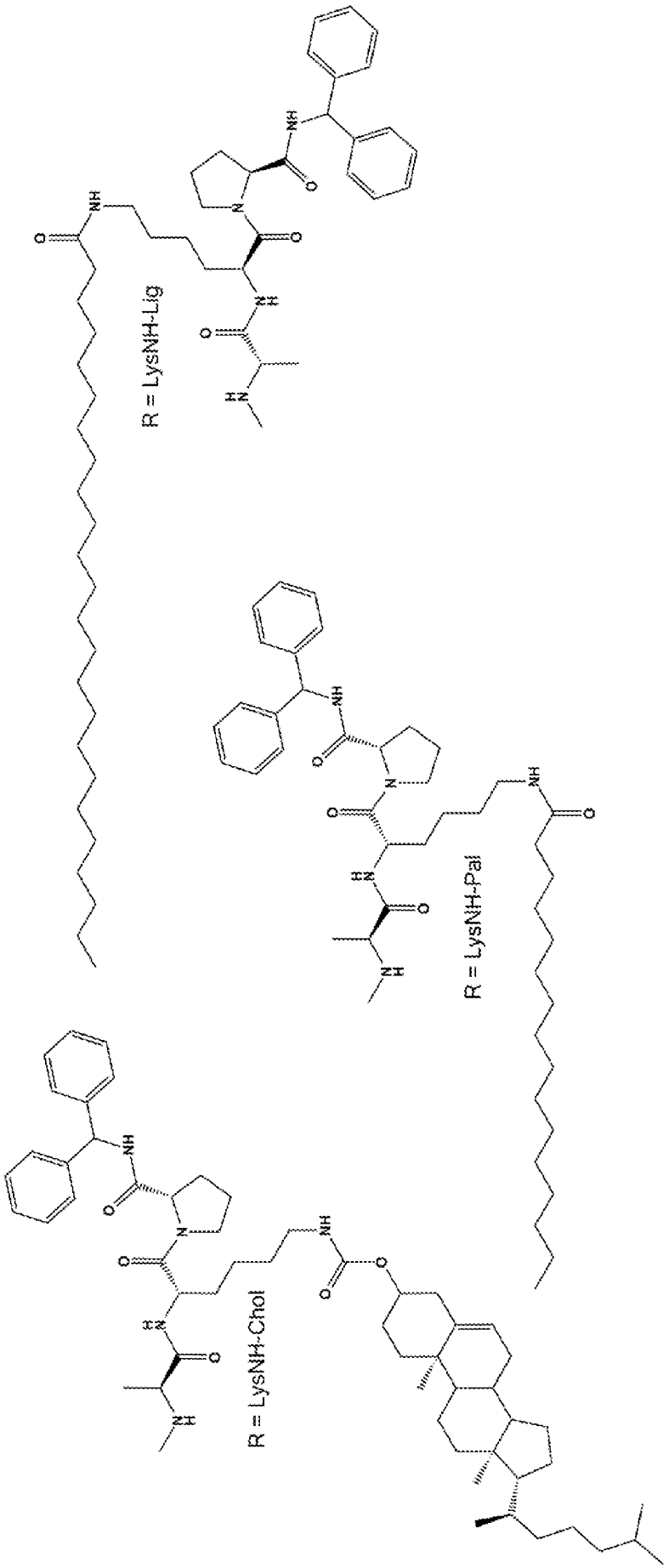

R = SerOH

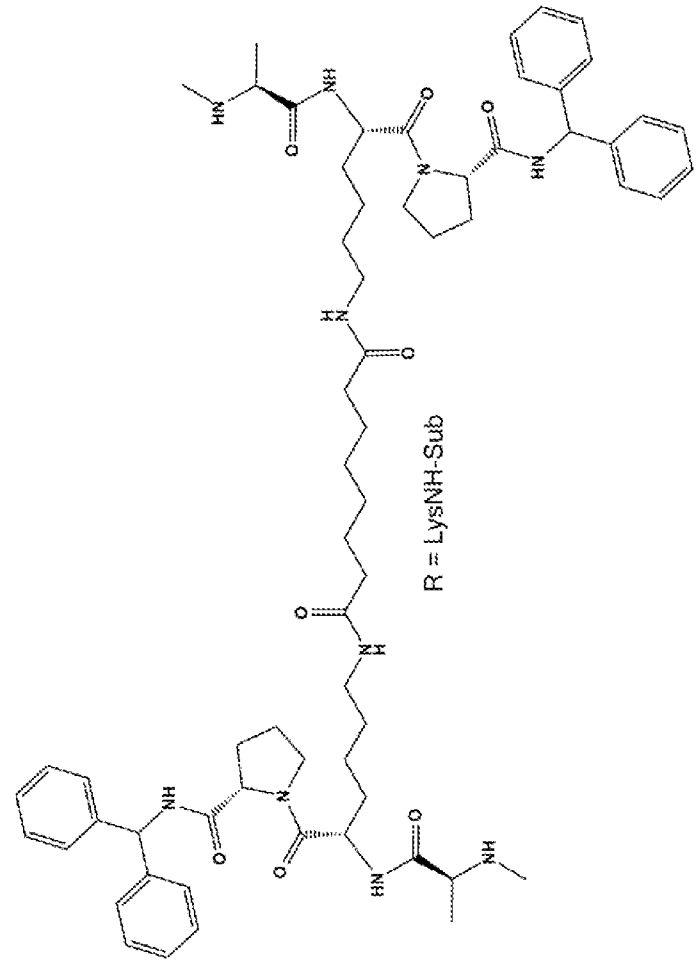

R = LysNH-Fmoc

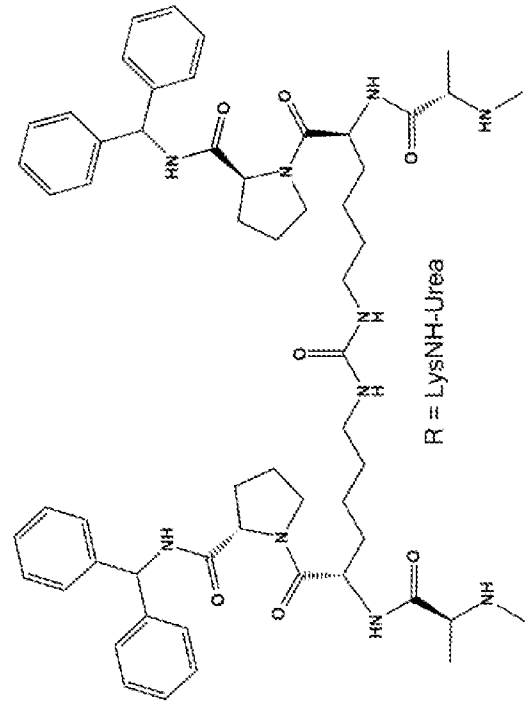

R = LysNH-DNFB

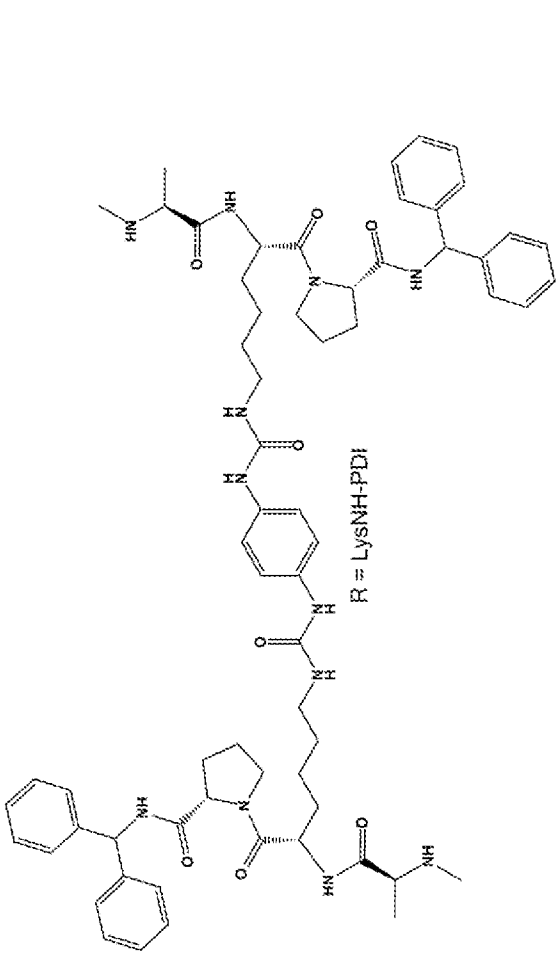

R = LysGu

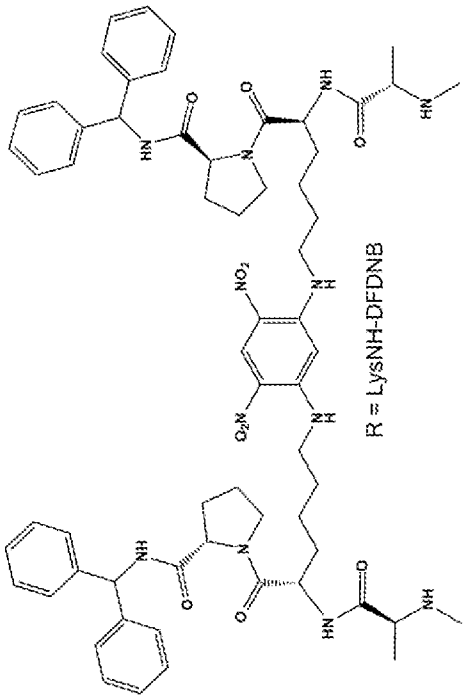

R = Lys

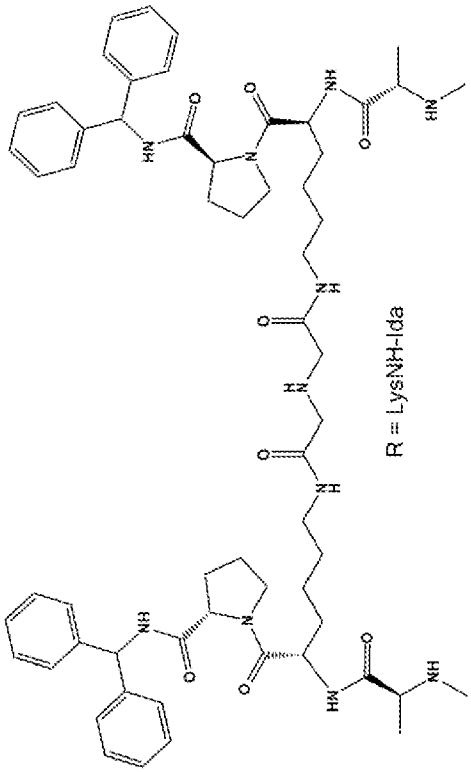

R = LysNH-Lig
R = LysNH-Pal
R = LysNH-Chol

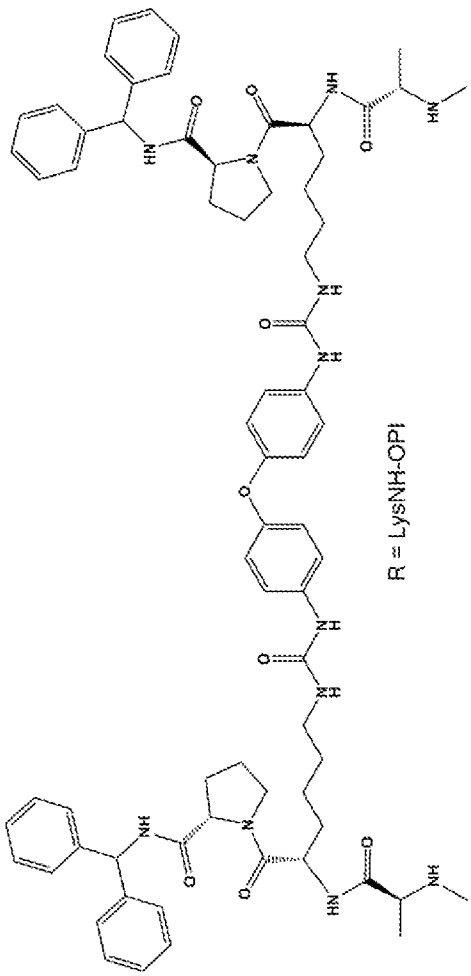

R = LysNH-Sub

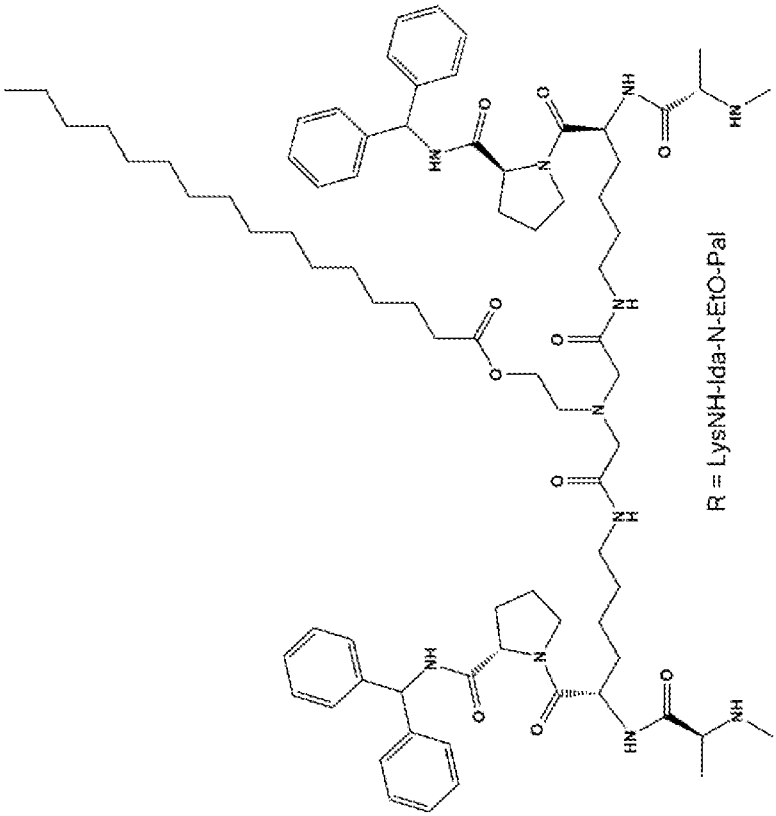

R = LysNH-Urea

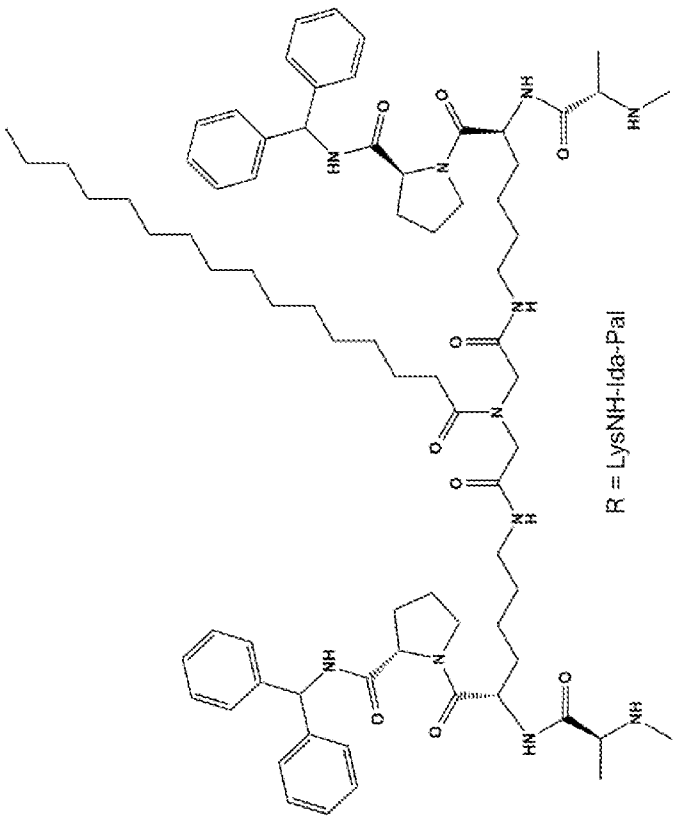

R = LysNH-PDI

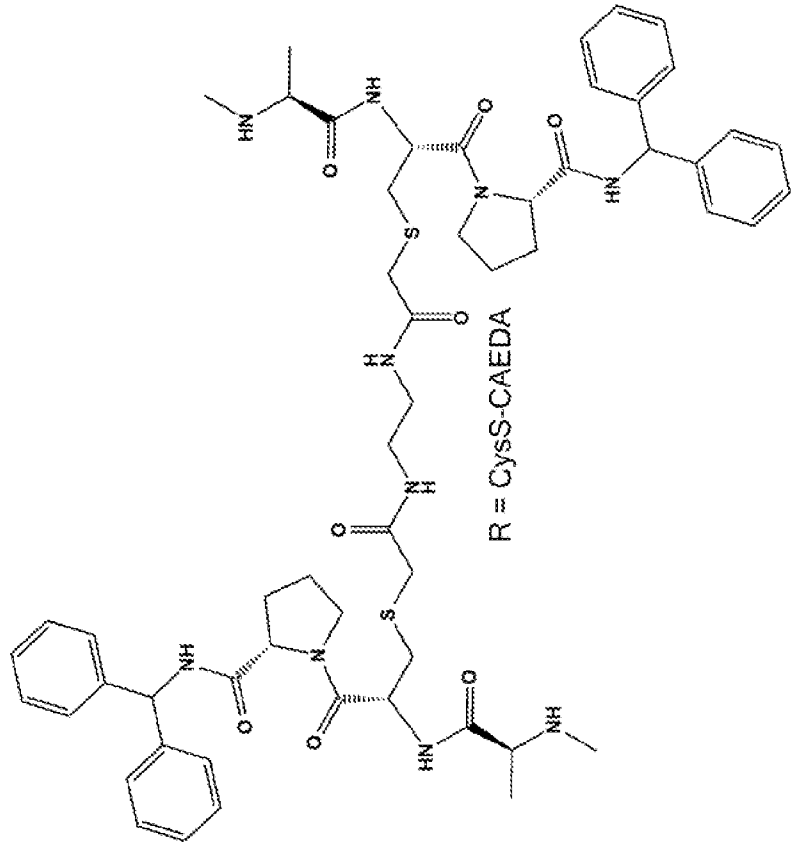

R = LysNH-DFDNB

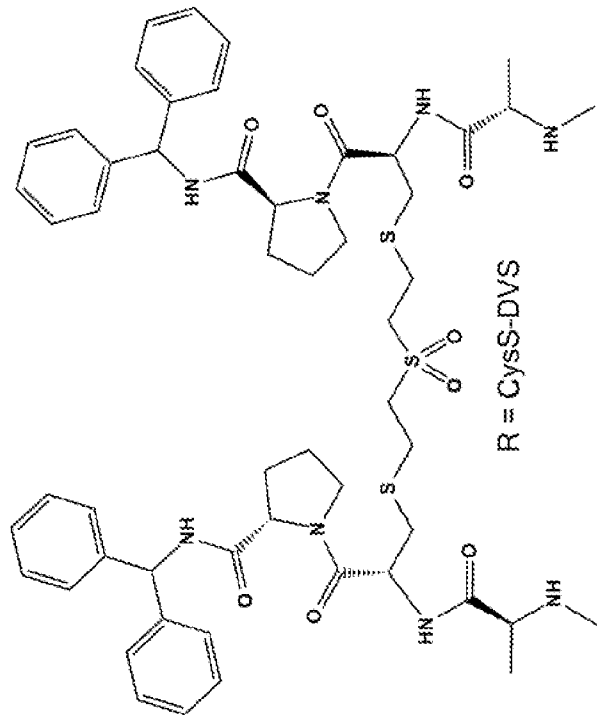

R = LysNH-Ida

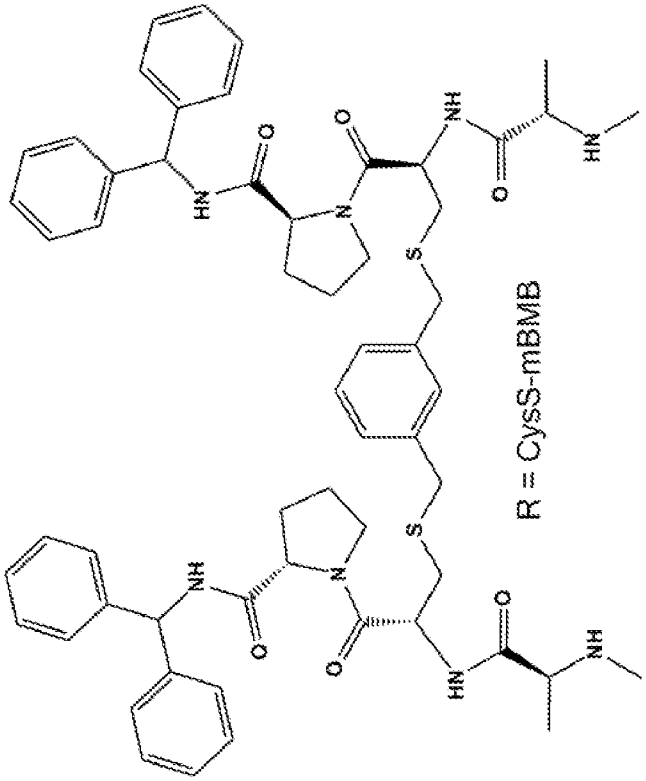

R = LysNH-OPI

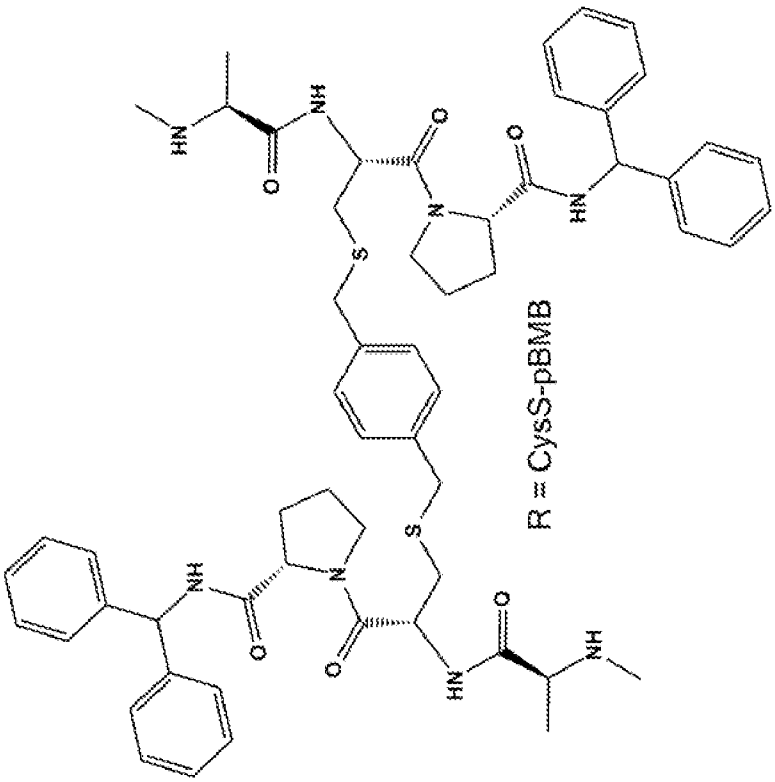

R = LysNH-Ida-N-EtO-Pal

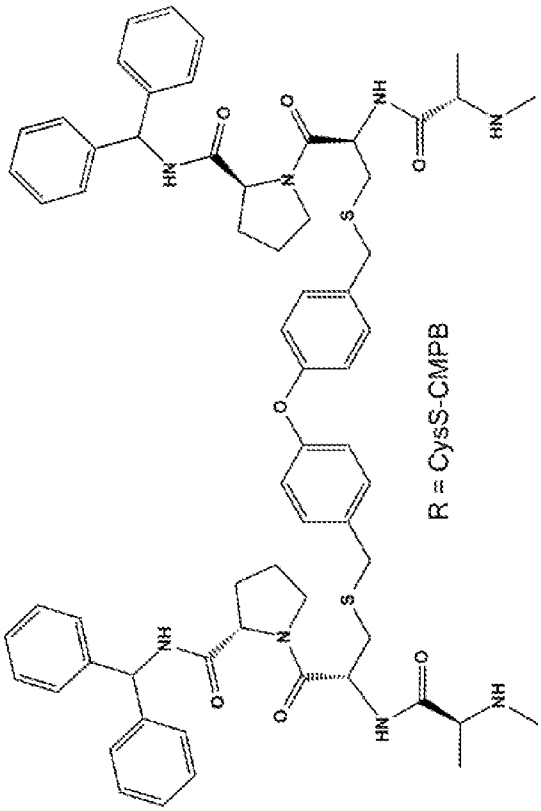

R = LysNH-Ida-Pal

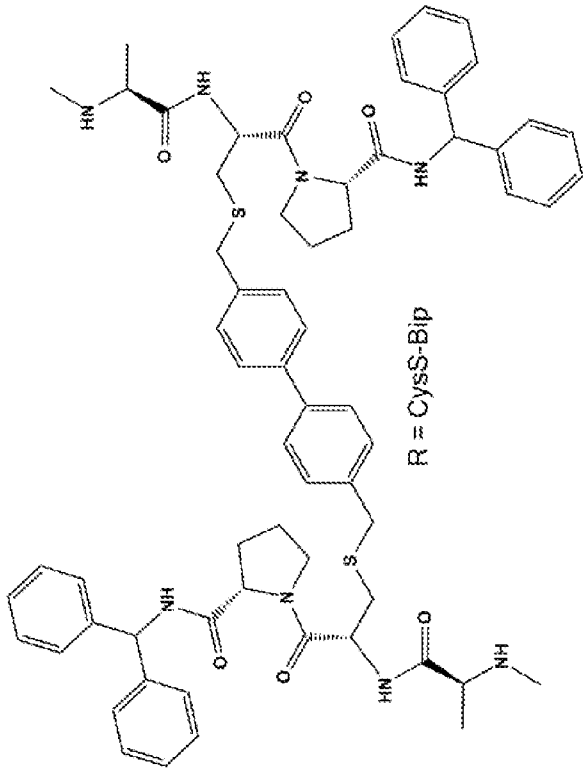

R = CysS-CAEDA

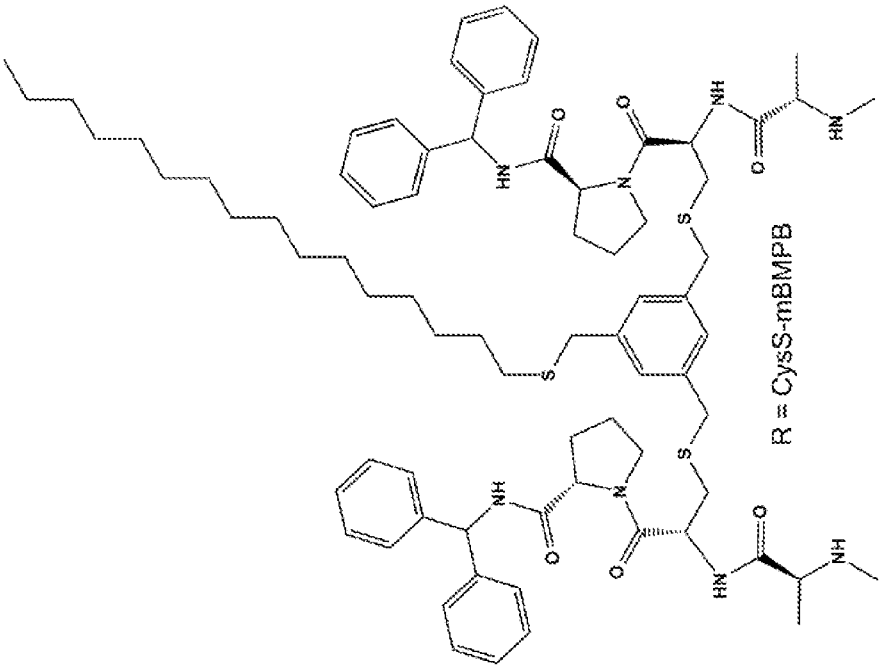

R = CysS-DVS

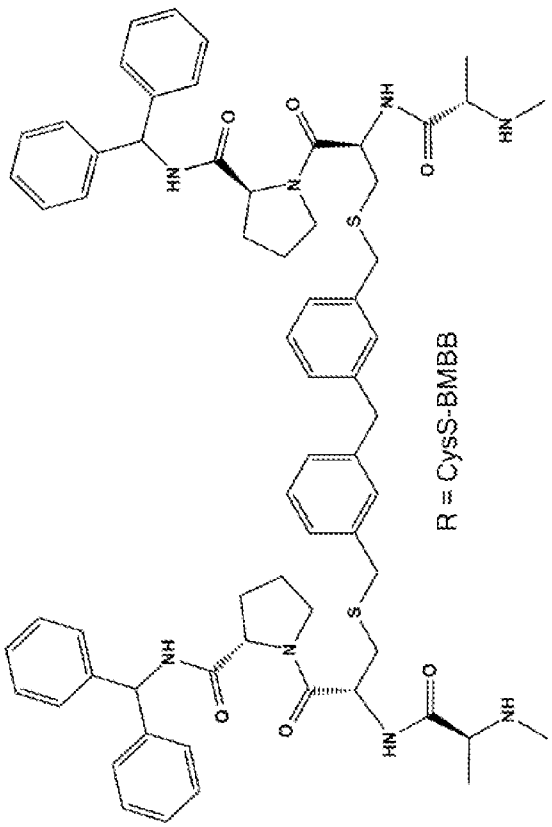

R = CysS-mBMB


R = CysS-pBMB

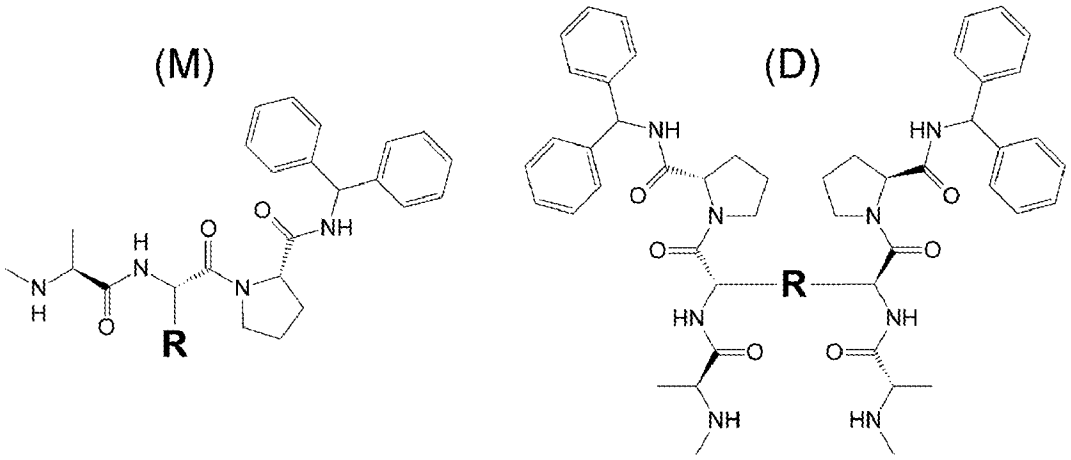

R = CysS-CMPB


R = CysS-Bip


R = CysS-mBMPB


R = CysS-BMBB

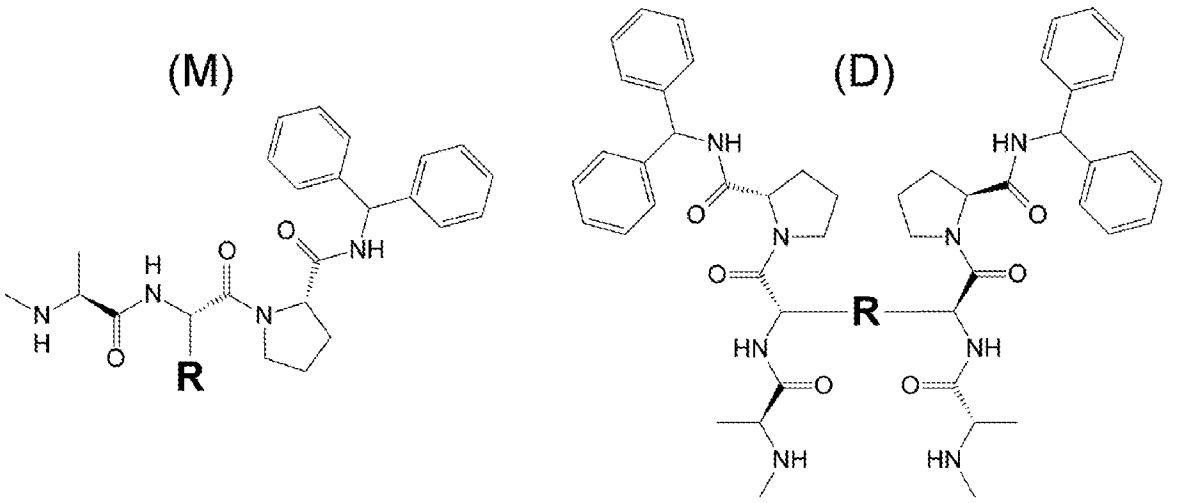
[0013] Some aspects of the invention are directed towards a compound of the formula M or D:



wherein R is SerOH, CysSH, CysS-StBu, Lys, LysGu, LysNH-DNFB, LysNH-Fmoc, LysNH-Pal, LysNH-Lig, LysNH-Chol, CysS-Ste, LysNH-Urea, LysNH-Sub, LysNH-DFDNB, LysNH-PDI, LysNH-OPI, LysNH-Ida, LysNH-Ida-Pal, LysNH-Ida-N-EtO-Pal, CysS-DVS, CysS-CAEDA, CysS-pBMB, CysS-mBMB, CysS-Bip, CysS-CMPB, CysS-BMBB, or CysS-mBMPB.

[0014] In some embodiments, the compound is a monovalent 2-lysine- or 2-cysteine-functionalized Smac derivative. In further embodiments, the monovalent 2-lysine- or 2-cysteine-functionalized Smac derivative is functionalized with a lipophilic moiety. The lipophilic moiety may be a substituted or unsubstituted hydrocarbon comprising at least 6 carbon atoms. In some embodiments, the lipophilic moiety is selected from the group consisting of palmitoyl, lignoceroyl, stearyl, and cholesteryl.In other embodiments, the 2-lysine- or 2-cysteine-functionalized Smac derivative is a bivalent 2-lysine- or 2-cysteine-functionalized Smac derivative. In further embodiments, the bivalent 2-lysine- or 2-cysteine-functionalized Smac derivative comprises two Smac moieties conjugated to each other through the 2-positions. The bivalent Smac derivative may comprise a linker conjugating the two Smac moieties. In some aspects, the linker is a lipophilic linker.

[0015] Some aspects are directed towards a method of killing or inhibiting the growth of cells comprising contacting the cells with a composition comprising an amount of a 2-lysine- or 2-cysteine-functionalized Smac derivative effective to kill or inhibit the growth of the cells. In some aspects, the method comprises contacting the cells with a 2-lysine- or 2-cysteine-functionalized Smac derivative is a compound of formula M or D:



,

wherein R is wherein R is SerOH, CysSH, CysS-StBu, Lys, LysGu, LysNH-DNFB, LysNH-Fmoc, LysNH-Pal, LysNH-Lig, LysNH-Chol, CysS-Ste, LysNH-Urea, LysNH-Sub, LysNH-DFDNB, LysNH-PDI, LysNH-OPI, LysNH-Ida, LysNH-Ida-Pal, LysNH-Ida-N-EtO-Pal, CysS-DVS, CysS-CAEDA, CysS-pBMB, CysS-mBMB, CysS-Bip, CysS-CMPB, CysS-BMBB, or CysS-mBMPB, or a salt, prodrug, enantiomer or diastereomer thereof. In some embodiments, the cells are in a patient's body. In further embodiments, the cells are cancer cells. In some embodiments, the cancer cells are in a tumor. In other embodiments, the cancer cells are human breast cancer cells. In other embodiments, the cells are in cell culture.

[0016] Certain embodiments are directed to pharmaceutical compositions comprising any of the compounds or Smac derivatives disclosed herein, or a pharmaceutically acceptable salt, prodrug, enantiomer, or diastereomer thereof, and an excipient. The compositions may be administered in any appropriate manner. In some embodiments, the composition is administered orally, intraadiposally, intraarterially, intraarticularly, intracranially, intradermally, intralesionally, intramuscularly, intranasally, intraocularally, intrapericardially, intraperitoneally, intrapleurally, intraprostaticaly, intrarectally, intrathecally, intratracheally, intratumorally, intraumbilically, intravaginally, intravenously, intravesicularlly, intravitreally, liposomally, locally, mucosally, orally, parenterally, rectally, subconjunctival, subcutaneously, sublingually, topically, transbuccally, transdermally, vaginally, in cremes, in lipid compositions, via a catheter, via a lavage, via continuous infusion, via infusion, via inhalation, via injection, via local delivery, via localized perfusion, bathing target cells directly, or any combination thereof. In some embodiments, the administration is topical.

[0017] Methods may involve administering a composition containing about, at least about, or at most about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4,

0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7. 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, 15.5, 16.0, 16.5, 17.0, 17.5, 18.0, 18.5, 19.0. 19.5, 20.0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 410, 420, 425, 430, 440, 441, 450, 460, 470, 475, 480, 490, 500, 510, 520, 525, 530, 540, 550, 560, 570, 575, 580, 590, 600, 610, 620, 625, 630, 640, 650, 660, 670, 675, 680, 690, 700, 710, 720, 725, 730, 740, 750, 760, 770, 775, 780, 790, 800, 810, 820, 825, 830, 840, 850, 860, 870, 875, 880, 890, 900, 910, 920, 925, 930, 940, 950, 960, 970, 975, 980, 990, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000, 6000, 7000, 8000, 9000, 10000 nanograms (ng), micrograms (mcg), milligrams (mg), or grams of an Smac derivative, or any range derivable therein.

[0018] Alternatively, embodiments may involve providing or administering to the patient or to cells or tissue of the patient about, at least about, or at most about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7. 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, 15.5, 16.0, 16.5, 17.0, 17.5, 18.0, 18.5, 19.0. 19.5, 20.0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67,

68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 410, 420, 425, 430, 440, 441, 450, 460, 470, 475, 480, 490, 500, 510, 520, 525, 530, 540, 550, 560, 570, 575, 580, 590, 600, 610, 620, 625, 630, 640, 650, 660, 670, 675, 680, 690, 700, 710, 720, 725, 730, 740, 750, 760, 770, 775, 780, 790, 800, 810, 820, 825, 830, 840, 850, 860, 870, 875, 880, 890, 900, 910, 920, 925, 930, 940, 950, 960, 970, 975, 980, 990, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000, 6000, 7000, 8000, 9000, 10000 nanograms (ng), micrograms (mcg), milligrams (mg), or grams of Smac derivative, or any range derivable therein, in one dose or collectively in multiple doses. In some embodiments, the composition comprises between about 0.1 ng and about 2.0 g of Smac derivative.

[0019] Alternatively, the composition may have a concentration of Smac derivative that is 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7. 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, 15.5, 16.0, 16.5, 17.0, 17.5, 18.0, 18.5, 19.0. 19.5, 20.0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 410, 420, 425, 430, 440, 441, 450, 460, 470, 475, 480, 490, 500, 510, 520, 525, 530, 540, 550, 560, 570, 575, 580, 590, 600, 610, 620, 625, 630, 640, 650, 660, 670, 675, 680, 690, 700, 710, 720, 725, 730, 740, 750, 760, 770, 775, 780, 790, 800, 810, 820, 825, 830, 840, 850, 860, 870,

875, 880, 890, 900, 910, 920, 925, 930, 940, 950, 960, 970, 975, 980, 990, 1000 micrograms/ml or mg/ml, or any range derivable therein.

[0020] If a liquid, gel, or semi-solid composition, the volume of the composition that is administered to the patient may be about, at least about, or at most about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7. 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, 15.5, 16.0, 16.5, 17.0, 17.5, 18.0, 18.5, 19.0. 19.5, 20.0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 microliters (µl) or milliliters (ml), or any range derivable therein. In certain embodiments, the patient is administered up to about 10 ml of the composition.

[0021] The amount of Smac derivative that is administered or taken by the patient may be based on the patient's weight (in kilograms). Therefore, in some embodiments, the patient is administered or takes a dose or multiple doses amounting to about, at least about, or at most about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7. 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, 15.5, 16.0, 16.5, 17.0, 17.5, 18.0, 18.5, 19.0. 19.5, 20.0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 410,

420, 425, 430, 440, 441, 450, 460, 470, 475, 480, 490, 500, 510, 520, 525, 530, 540, 550, 560, 570, 575, 580, 590, 600, 610, 620, 625, 630, 640, 650, 660, 670, 675, 680, 690, 700, 710, 720, 725, 730, 740, 750, 760, 770, 775, 780, 790, 800, 810, 820, 825, 830, 840, 850, 860, 870, 875, 880, 890, 900, 910, 920, 925, 930, 940, 950, 960, 970, 975, 980, 990, 1000 micrograms/kilogram (kg) or mg/kg, or any range derivable therein.

[0022] The composition may be administered to (or taken by) the patient 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more times, or any range derivable therein, and they may be administered every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 hours, or 1, 2, 3, 4, 5, 6, 7 days, or 1, 2, 3, 4, 5 weeks, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, or any range derivable therein. It is specifically contemplated that the composition may be administered once daily, twice daily, three times daily, four times daily, five times daily, or six times daily (or any range derivable therein) and/or as needed to the patient. Alternatively, the composition may be administered every 2, 4, 6, 8, 12 or 24 hours (or any range derivable therein) to or by the patient.

[0023] "Treatment" or "treating" includes (1) inhibiting a disease in a subject or patient experiencing or displaying the pathology or symptomatology of the disease (e.g., arresting further development of the pathology and/or symptomatology), (2) ameliorating a disease in a subject or patient that is experiencing or displaying the pathology or symptomatology of the disease (e.g., reversing the pathology and/or symptomatology), and/or (3) effecting any measurable decrease in a disease in a subject or patient that is experiencing or displaying the pathology or symptomatology of the disease.

[0024] Tumor," as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms "cancer," "cancerous," "cell proliferative disorder," "proliferative disorder," and "tumor" are not mutually exclusive as referred to herein.

[0025] The cancers amendable for treatment by the present invention include, but are not limited to, melanoma, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include breast cancer, colon cancer, rectal cancer, colorectal cancer, kidney or renal cancer, lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, squamous cell cancer (*e.g.* epithelial squamous cell cancer), cervical cancer, ovarian cancer, prostate cancer, liver cancer, bladder cancer, cancer of the

peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, head and neck cancer, glioblastoma, retinoblastoma, astrocytoma, thecomas, arrhenoblastomas, hepatoma, hematologic malignancies including non-Hodgkins lymphoma (NHL), multiple myeloma and acute hematologic malignancies, endometrial or uterine carcinoma, endometriosis, fibrosarcomas, choriocarcinoma, salivary gland carcinoma, vulval cancer, thyroid cancer, esophageal carcinomas, hepatic carcinoma, anal carcinoma, penile carcinoma, nasopharyngeal carcinoma, laryngeal carcinomas, Kaposi's sarcoma, melanoma, skin carcinomas, Schwannoma, oligodendroglioma, neuroblastomas, rhabdomyosarcoma, osteogenic sarcoma, leiomyosarcomas, urinary tract carcinomas, thyroid carcinomas, Wilm's tumor, as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome. The cancerous conditions amendible for treatment of the invention include metastatic cancers.

[0026] "Effective amount" or "therapeutically effective amount" or "pharmaceutically effective amount" means that amount which, when administered to a subject or patient for treating a disease, is sufficient to effect such treatment for the disease. In some embodiments, the subject is administered at least about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 mg/kg (or any range derivable therein).

[0027] "Pharmaceutically acceptable" means that which is useful in preparing a pharmaceutical composition that is generally safe, non-toxic and neither biologically nor otherwise undesirable and includes that which is acceptable for veterinary use as well as human pharmaceutical use.

[0028] "Pharmaceutically acceptable salts" means salts of compounds of the present invention which are pharmaceutically acceptable, as defined above, and which possess the desired pharmacological activity. Such salts include acid addition salts formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or with organic acids such as 1,2-ethanedisulfonic acid, 2-hydroxyethanesulfonic acid, 2-naphthalenesulfonic acid, 3-phenylpropionic acid, 4,4'-methylenebis(3-hydroxy-2-ene-1-carboxylic acid), 4-methylbicyclo[2.2.2]oct-2-ene-1-carboxylic acid, acetic acid, aliphatic mono- and dicarboxylicacids, aliphatic sulfuric acids, aromatic sulfuric acids, benzenesulfonic acid, benzoic acid, camphorsulfonic acid, carbonic acid, cinnamic acid, citric acid, cyclopentanepropionic acid, ethanesulfonic acid, fumaric acid, glucoheptonic acid, gluconic acid, glutamic acid, glycolic acid, heptanoic acid, hexanoic

acid, hydroxynaphthoic acid, lactic acid, laurylsulfuric acid, maleic acid, malic acid, malonic acid, mandelic acid, methanesulfonic acid, muconic acid, o-(4-hydroxybenzoyl)benzoic acid, oxalic acid, p-chlorobenzenesulfonic acid, phenyl-substituted alkanoic acids, propionic acid, p-toluenesulfonic acid, pyruvic acid, salicylic acid, stearic acid, succinic acid, tartaric acid, tertiarybutylacetic acid, trimethylacetic acid, and the like. Pharmaceutically acceptable salts also include base addition salts which may be formed when acidic protons present are capable of reacting with inorganic or organic bases. Acceptable inorganic bases include sodium hydroxide, sodium carbonate, potassium hydroxide, aluminum hydroxide and calcium hydroxide. Acceptable organic bases include ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine and the like. It should be recognized that the particular anion or cation forming a part of any salt of this invention is not critical, so long as the salt, as a whole, is pharmacologically acceptable. Additional examples of pharmaceutically acceptable salts and their methods of preparation and use are presented in Handbook of Pharmaceutical Salts: Properties, and Use (*P. H. Stahl & C. G. Wermuth eds., Verlag Helvetica Chimica Acta*, 2002).

[0029] Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the measurement or quantitation method.

[0030] The use of the word "a" or "an" when used in conjunction with the term "comprising" may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

[0031] The words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[0032] The compositions and methods for their use can "comprise," "consist essentially of," or "consist of" any of the ingredients or steps disclosed throughout the specification. Compositions and methods "consisting essentially of" any of the ingredients or steps disclosed limits the scope of the claim to the specified materials or steps which do not materially affect the basic and novel characteristic of the claimed invention.

[0033] It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the invention, and vice versa. Furthermore, compositions of the invention can be used to achieve methods of the invention.

[0034] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description. Note that simply because a particular compound is ascribed to one particular generic formula doesn't mean that it cannot also belong to another generic formula.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0036] **FIG. 1** depicts the general structures of synthesized monomeric (M) and dimeric (D) Smac derivatives and *in vitro* activity in cell growth inhibition assay, using MDA-MB-231 human metastatic breast cancer cell line.

[0037] **FIG. 2** is a reaction scheme depicting synthetic routes employed for the synthesis of monomeric Smac derivatives.

[0038] **FIG. 3** is a reaction scheme depicting synthetic routes employed for the synthesis of additional monomeric Smac derivatives.

[0039] **FIGS. 4A-F** FIG. 4A is a graph depicting cell viability curves obtained for MDA-MB-231 human metastatic breast cancer cell line treated with lipidated compounds M11 and D7. FIG. 4B is a graph depicting increases in enzymatic activity of caspases-3/7 and -9 in MDA-MB-231 cells treated with peptides: M11, D3, D7, and D13. FIG. 4C is a dose-response curve of increases in enzymatic activity of caspases-3/7 and -9 in response to different doses of peptides D7 and M11. FIG. 4D is a graph depicting peptide pharmacokinetics for subcutaneous (SC) administration. FIG. 4E is a graph depicting peptide pharmacokinetics for oral (OR) administration. FIG. 4F is a graph depicting plasma stabilities of M11 and D7 analogues.

[0040] **FIG. 5** is a graph depicting anticancer effects of M11 and D7 treatment in a xenograft mouse model.

[0041] **FIGS. 6A-B** is a drawing depicting structures of synthesized dimeric Smac derivatives containing the amino acid lysine in position 2.

[0042] **FIGS. 7A-B** is a drawing depicting structures of synthesized dimeric Smac derivatives containing the amino acid cysteine in position 2.

[0043] **FIGS. 8A-B** FIG. 8A is a representative analytical RP-HPLC profile and corresponding MS-spectra obtained for bivalent lipidated analog D7. FIG. 8B is a graph depicting overlaid FTIR spectras obtained for analogs (A) M11 self-film, (B) M11 in POPC, (C) D7 self-film, and (D) D7 in 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC).

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0044] The synthesis and biological evaluation of these novel Smac derivatives was examined. To ascertain whether lipidation can be a useful approach in the development of Smac mimics, a small library of analogues with structures summarized in **FIG. 1** was synthesized and analyzed. The overall hydrophobicity of the Smac mimics may be important for their biological activity, most likely promoting cell permeability and increasing intracellular concentration of analogues, resulting in more potent therapeutic effects. In this context, modification of Smac derivatives by lipidation was examined.

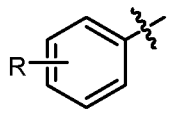
A. Definitions

[0045] When used in the context of a chemical group, "hydrogen" means −H; "hydroxy" means −OH; "oxo" means =O; "halo" means independently −F, −Cl, −Br or −I; "amino" means $-NH_2$; "hydroxyamino" means −NHOH; "nitro" means $-NO_2$; imino means =NH; "cyano" means −CN; "isocyanate" means −N=C=O; "azido" means $-N_3$; in a monovalent context "phosphate" means $-OP(O)(OH)_2$ or a deprotonated form thereof; in a divalent context "phosphate" means −OP(O)(OH)O− or a deprotonated form thereof; "mercapto" means −SH; "thio" means =S; "sulfonyl" means $-S(O)_2-$; and "sulfinyl" means −S(O)−.

[0046] In the context of chemical formulas, the symbol "−" means a single bond, "=" means a double bond; and "≡" means triple bond. The symbol "----" represents an optional bond, which if present is either single or double. The symbol "≡≡≡" represents a single bond

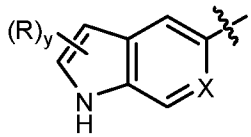
or a double bond. Thus, for example, the structure  includes the structures , , ,  and . As will be understood by a person of skill in the art, no one such ring atom forms part of more than one double bond. The symbol "", when drawn perpendicularly across a bond indicates a point of attachment of the group. It is noted that the point of attachment is typically only identified in this manner for larger groups in order to assist the reader in rapidly and unambiguously identifying a point of attachment. The symbol "" means a single bond where the group attached to the thick end of the wedge is "out of the page." The symbol "" means a single bond where the group attached to the thick end of the wedge is "into the page". The symbol "" means a single bond where the conformation (*e.g.*, either *R* or *S*) or the geometry is undefined (*e.g.*, either *E* or *Z*).

[0047] Any undefined valency on an atom of a structure shown in this application implicitly represents a hydrogen atom bonded to the atom. When a group "R" is depicted as a "floating group" on a ring system, for example, in the formula:



,

[0048] then R may replace any hydrogen atom attached to any of the ring atoms, including a depicted, implied, or expressly defined hydrogen, so long as a stable structure is formed. When a group "R" is depicted as a "floating group" on a fused ring system, as for example in the formula:



,

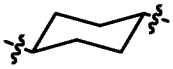
[0049] then R may replace any hydrogen attached to any of the ring atoms of either of the fused rings unless specified otherwise. Replaceable hydrogens include depicted hydrogens (*e.g.*, the hydrogen attached to the nitrogen in the formula above), implied hydrogens (*e.g.*, a hydrogen of the formula above that is not shown but understood to be present), expressly defined hydrogens, and optional hydrogens whose presence depends on the identity of a ring atom (*e.g.*, a hydrogen attached to group X, when X equals −CH−), so long as a stable structure is formed. In the example depicted, R may reside on either the 5-

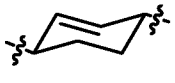
membered or the 6-membered ring of the fused ring system. In the formula above, the subscript letter "y" immediately following the group "R" enclosed in parentheses, represents a numeric variable. Unless specified otherwise, this variable can be 0, 1, 2, or any integer greater than 2, only limited by the maximum number of replaceable hydrogen atoms of the ring or ring system.

[0050] For the groups and classes below, the following parenthetical subscripts further define the group/class as follows: "(Cn)" defines the exact number (n) of carbon atoms in the group/class. "(C≤n)" defines the maximum number (n) of carbon atoms that can be in the group/class, with the minimum number as small as possible for the group in question, *e.g.*, it is understood that the minimum number of carbon atoms in the group "alkenyl$_{(C\leq 8)}$" or the class "alkene$_{(C\leq 8)}$" is two. For example, "alkoxy$_{(C\leq 10)}$" designates those alkoxy groups having from 1 to 10 carbon atoms (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, or any range derivable therein (*e.g.*, 3 to 10 carbon atoms). (Cn-n′) defines both the minimum (n) and maximum number (n′) of carbon atoms in the group. Similarly, "alkyl$_{(C2\text{-}10)}$" designates those alkyl groups having from 2 to 10 carbon atoms (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10, or any range derivable therein (*e.g.*, 3 to 10 carbon atoms)).

[0051] The term "saturated" as used herein means the compound or group so modified has no carbon-carbon double and no carbon-carbon triple bonds, except as noted below. The term does not preclude carbon-heteroatom multiple bonds, for example a carbon oxygen double bond or a carbon nitrogen double bond. Moreover, it does not preclude a carbon-carbon double bond that may occur as part of keto-enol tautomerism or imine/enamine tautomerism.

[0052] The term "aliphatic" when used without the "substituted" modifier signifies that the compound/group so modified is an acyclic or cyclic, but non-aromatic hydrocarbon compound or group. In aliphatic compounds/groups, the carbon atoms can be joined together in straight chains, branched chains, or non-aromatic rings (alicyclic). Aliphatic compounds/groups can be saturated, that is joined by single bonds (alkanes/alkyl), or unsaturated, with one or more double bonds (alkenes/alkenyl) or with one or more triple bonds (alkynes/alkynyl). When the term "aliphatic" is used without the "substituted" modifier only carbon and hydrogen atoms are present. When the term is used with the "substituted" modifier one or more hydrogen atom has been independently replaced by −OH, −F, −Cl, −Br, −I, $-NH_2$, $-NO_2$, $-CO_2H$, $-CO_2CH_3$, −CN, −SH, $-OCH_3$, $-OCH_2CH_3$, $-C(O)CH_3$, $-N(CH_3)_2$, $-C(O)NH_2$, $-OC(O)CH_3$, or $-S(O)_2NH_2$.

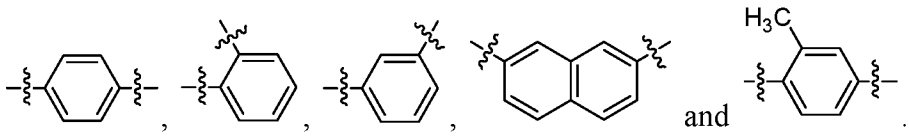
[0053] The term "alkyl" when used without the "substituted" modifier refers to a monovalent saturated aliphatic group with a carbon atom as the point of attachment, a linear or branched, cyclo, cyclic or acyclic structure, and no atoms other than carbon and hydrogen. Thus, as used herein cycloalkyl is a subset of alkyl. The groups $-CH_3$ (Me), $-CH_2CH_3$ (Et), $-CH_2CH_2CH_3$ (*n*-Pr), $-CH(CH_3)_2$ (*iso*-Pr), $-CH(CH_2)_2$ (cyclopropyl), $-CH_2CH_2CH_2CH_3$ (*n*-Bu), $-CH(CH_3)CH_2CH_3$ (*sec*-butyl), $-CH_2CH(CH_3)_2$ (*iso*-butyl), $-C(CH_3)_3$ (*tert*-butyl), $-CH_2C(CH_3)_3$ (*neo*-pentyl), cyclobutyl, cyclopentyl, cyclohexyl, and cyclohexylmethyl are non-limiting examples of alkyl groups. The term "alkanediyl" when used without the "substituted" modifier refers to a divalent saturated aliphatic group, with one or two saturated carbon atom(s) as the point(s) of attachment, a linear or branched, cyclo, cyclic or acyclic structure, no carbon-carbon double or triple bonds, and no atoms other than carbon and hydrogen. The groups, $-CH_2-$ (methylene), $-CH_2CH_2-$, $-CH_2C(CH_3)_2CH_2-$, $-CH_2CH_2CH_2-$, and , are non-limiting examples of alkanediyl groups. The term "alkylidene" when used without the "substituted" modifier refers to the divalent group $=CRR'$ in which R and R′ are independently hydrogen, alkyl, or R and R′ are taken together to represent an alkanediyl having at least two carbon atoms. Non-limiting examples of alkylidene groups include: $=CH_2$, $=CH(CH_2CH_3)$, and $=C(CH_3)_2$. When any of these terms is used with the "substituted" modifier one or more hydrogen atom has been independently replaced by $-OH$, $-F$, $-Cl$, $-Br$, $-I$, $-NH_2$, $-NO_2$, $-CO_2H$, $-CO_2CH_3$, $-CN$, $-SH$, $-OCH_3$, $-OCH_2CH_3$, $-C(O)CH_3$, $-N(CH_3)_2$, $-C(O)NH_2$, $-OC(O)CH_3$, or $-S(O)_2NH_2$. The following groups are non-limiting examples of substituted alkyl groups: $-CH_2OH$, $-CH_2Cl$, $-CF_3$, $-CH_2CN$, $-CH_2C(O)OH$, $-CH_2C(O)OCH_3$, $-CH_2C(O)NH_2$, $-CH_2C(O)CH_3$, $-CH_2OCH_3$, $-CH_2OC(O)CH_3$, $-CH_2NH_2$, $-CH_2N(CH_3)_2$, and $-CH_2CH_2Cl$. The term "haloalkyl" is a subset of substituted alkyl, in which one or more hydrogen atoms has been substituted with a halo group and no other atoms aside from carbon, hydrogen and halogen are present. The group, $-CH_2Cl$ is a non-limiting examples of a haloalkyl. An "alkane" refers to the compound H–R, wherein R is alkyl. The term "fluoroalkyl" is a subset of substituted alkyl, in which one or more hydrogen has been substituted with a fluoro group and no other atoms aside from carbon, hydrogen and fluorine are present. The groups, $-CH_2F$, $-CF_3$, and $-CH_2CF_3$ are non-limiting examples of fluoroalkyl groups. An "alkane" refers to the compound H–R, wherein R is alkyl.

[0054] The term "alkenyl" when used without the "substituted" modifier refers to an monovalent unsaturated aliphatic group with a carbon atom as the point of attachment, a linear or branched, cyclo, cyclic or acyclic structure, at least one nonaromatic carbon-carbon double bond, no carbon-carbon triple bonds, and no atoms other than carbon and hydrogen. Non-limiting examples of alkenyl groups include: $-CH=CH_2$ (vinyl), $-CH=CHCH_3$, $-CH=CHCH_2CH_3$, $-CH_2CH=CH_2$ (allyl), $-CH_2CH=CHCH_3$, and $-CH=CH-C_6H_5$. The term "alkenediyl" when used without the "substituted" modifier refers to a divalent unsaturated aliphatic group, with two carbon atoms as points of attachment, a linear or branched, cyclo, cyclic or acyclic structure, at least one nonaromatic carbon-carbon double bond, no carbon-carbon triple bonds, and no atoms other than carbon and hydrogen. The groups, $-CH=CH-$, $-CH=C(CH_3)CH_2-$, $-CH=CHCH_2-$, and , are non-limiting examples of alkenediyl groups. When these terms are used with the "substituted" modifier one or more hydrogen atom has been independently replaced by $-OH$, $-F$, $-Cl$, $-Br$, $-I$, $-NH_2$, $-NO_2$, $-CO_2H$, $-CO_2CH_3$, $-CN$, $-SH$, $-OCH_3$, $-OCH_2CH_3$, $-C(O)CH_3$, $-N(CH_3)_2$, $-C(O)NH_2$, $-OC(O)CH_3$, or $-S(O)_2NH_2$. The groups, $-CH=CHF$, $-CH=CHCl$ and $-CH=CHBr$, are non-limiting examples of substituted alkenyl groups. An "alkene" refers to the compound H–R, wherein R is alkenyl.

[0055] The term "alkynyl" when used without the "substituted" modifier refers to an monovalent unsaturated aliphatic group with a carbon atom as the point of attachment, a linear or branched, cyclo, cyclic or acyclic structure, at least one carbon-carbon triple bond, and no atoms other than carbon and hydrogen. As used herein, the term alkynyl does not preclude the presence of one or more non-aromatic carbon-carbon double bonds. The groups, $-C\equiv CH$, $-C\equiv CCH_3$, and $-CH_2C\equiv CCH_3$, are non-limiting examples of alkynyl groups. When alkynyl is used with the "substituted" modifier one or more hydrogen atom has been independently replaced by $-OH$, $-F$, $-Cl$, $-Br$, $-I$, $-NH_2$, $-NO_2$, $-CO_2H$, $-CO_2CH_3$, $-CN$, $-SH$, $-OCH_3$, $-OCH_2CH_3$, $-C(O)CH_3$, $-N(CH_3)_2$, $-C(O)NH_2$, $-OC(O)CH_3$, or $-S(O)_2NH_2$. An "alkyne" refers to the compound H–R, wherein R is alkynyl.

[0056] The term "aryl" when used without the "substituted" modifier refers to a monovalent unsaturated aromatic group with an aromatic carbon atom as the point of attachment, said carbon atom forming part of a one or more six-membered aromatic ring structure, wherein the ring atoms are all carbon, and wherein the group consists of no atoms other than carbon and hydrogen. If more than one ring is present, the rings may be fused or

unfused. As used herein, the term does not preclude the presence of one or more alkyl group (carbon number limitation permitting) attached to the first aromatic ring or any additional aromatic ring present. Non-limiting examples of aryl groups include phenyl (Ph), methylphenyl, (dimethyl)phenyl, $-C_6H_4CH_2CH_3$ (ethylphenyl), naphthyl, and the monovalent group derived from biphenyl. The term "arenediyl" when used without the "substituted" modifier refers to a divalent aromatic group, with two aromatic carbon atoms as points of attachment, said carbon atoms forming part of one or more six-membered aromatic ring structure(s) wherein the ring atoms are all carbon, and wherein the monovalent group consists of no atoms other than carbon and hydrogen. As used herein, the term does not preclude the presence of one or more alkyl group (carbon number limitation permitting) attached to the first aromatic ring or any additional aromatic ring present. If more than one ring is present, the rings may be fused or unfused. Non-limiting examples of arenediyl groups include:

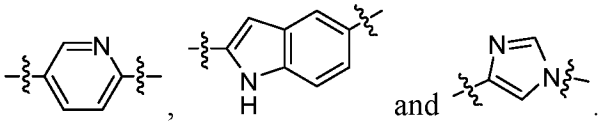


[0057] When these terms are used with the "substituted" modifier one or more hydrogen atom has been independently replaced by $-OH$, $-F$, $-Cl$, $-Br$, $-I$, $-NH_2$, $-NO_2$, $-CO_2H$, $-CO_2CH_3$, $-CN$, $-SH$, $-OCH_3$, $-OCH_2CH_3$, $-C(O)CH_3$, $-N(CH_3)_2$, $-C(O)NH_2$, $-OC(O)CH_3$, or $-S(O)_2NH_2$. An "arene" refers to the compound H–R, wherein R is aryl.

[0058] The term "aralkyl" when used without the "substituted" modifier refers to the monovalent group –alkanediyl–aryl, in which the terms alkanediyl and aryl are each used in a manner consistent with the definitions provided above. Non-limiting examples of aralkyls are: phenylmethyl (benzyl, Bn) and 2-phenyl-ethyl. When the term is used with the "substituted" modifier one or more hydrogen atom from the alkanediyl and/or the aryl has been independently replaced by $-OH$, $-F$, $-Cl$, $-Br$, $-I$, $-NH_2$, $-NO_2$, $-CO_2H$, $-CO_2CH_3$, $-CN$, $-SH$, $-OCH_3$, $-OCH_2CH_3$, $-C(O)CH_3$, $-N(CH_3)_2$, $-C(O)NH_2$, $-OC(O)CH_3$, or $-S(O)_2NH_2$. Non-limiting examples of substituted aralkyls are: (3-chlorophenyl)-methyl, and 2-chloro-2-phenyl-eth-1-yl.

[0059] The term "heteroaryl" when used without the "substituted" modifier refers to a monovalent aromatic group with an aromatic carbon atom or nitrogen atom as the point of attachment, said carbon atom or nitrogen atom forming part of one or more aromatic ring structures wherein at least one of the ring atoms is nitrogen, oxygen or sulfur, and wherein the heteroaryl group consists of no atoms other than carbon, hydrogen, aromatic nitrogen,

aromatic oxygen and aromatic sulfur. As used herein, the term does not preclude the presence of one or more alkyl, aryl, and/or aralkyl groups (carbon number limitation permitting) attached to the aromatic ring or aromatic ring system. If more than one ring is present, the rings may be fused or unfused. Non-limiting examples of heteroaryl groups include furanyl, imidazolyl, indolyl, indazolyl (Im), isoxazolyl, methylpyridinyl, oxazolyl, phenylpyridinyl, pyridinyl, pyrrolyl, pyrimidinyl, pyrazinyl, quinolyl, quinazolyl, quinoxalinyl, triazinyl, tetrazolyl, thiazolyl, thienyl, and triazolyl. The term "heteroarenediyl" when used without the "substituted" modifier refers to an divalent aromatic group, with two aromatic carbon atoms, two aromatic nitrogen atoms, or one aromatic carbon atom and one aromatic nitrogen atom as the two points of attachment, said atoms forming part of one or more aromatic ring structure(s) wherein at least one of the ring atoms is nitrogen, oxygen or sulfur, and wherein the divalent group consists of no atoms other than carbon, hydrogen, aromatic nitrogen, aromatic oxygen and aromatic sulfur. As used herein, the term does not preclude the presence of one or more alkyl, aryl, and/or aralkyl groups (carbon number limitation permitting) attached to the aromatic ring or aromatic ring system. If more than one ring is present, the rings may be fused or unfused. Non-limiting examples of heteroarenediyl groups include:



[0060] When these terms are used with the "substituted" modifier one or more hydrogen atom has been independently replaced by $-OH$, $-F$, $-Cl$, $-Br$, $-I$, $-NH_2$, $-NO_2$, $-CO_2H$, $-CO_2CH_3$, $-CN$, $-SH$, $-OCH_3$, $-OCH_2CH_3$, $-C(O)CH_3$, $-N(CH_3)_2$, $-C(O)NH_2$, $-OC(O)CH_3$, or $-S(O)_2NH_2$.

[0061] The term "heterocycloalkyl" when used without the "substituted" modifier refers to a monovalent non-aromatic group with a carbon atom or nitrogen atom as the point of attachment, said carbon atom or nitrogen atom forming part of one or more non-aromatic ring structures wherein at least one of the ring atoms is nitrogen, oxygen or sulfur, and wherein the heterocycloalkyl group consists of no atoms other than carbon, hydrogen, nitrogen, oxygen and sulfur. As used herein, the term does not preclude the presence of one or more alkyl groups (carbon number limitation permitting) attached to the ring or ring system. If more than one ring is present, the rings may be fused or unfused. Non-limiting examples of heterocycloalkyl groups include aziridinyl, azetidinyl, pyrrolidinyl, piperidinyl,

piperazinyl, morpholinyl, thiomorpholinyl, tetrahydrofuranyl, tetrahydrothiofuranyl, tetrahydropyranyl, and pyranyl. When the term "heterocycloalkyl" used with the "substituted" modifier one or more hydrogen atom has been independently replaced by −OH, −F, −Cl, −Br, −I, $-NH_2$, $-NO_2$, $-CO_2H$, $-CO_2CH_3$, −CN, −SH, $-OCH_3$, $-OCH_2CH_3$, $-C(O)CH_3$, $-N(CH_3)_2$, $-C(O)NH_2$, $-OC(O)CH_3$, or $-S(O)_2NH_2$.

[0062] The term "acyl" when used without the "substituted" modifier refers to the group −C(O)R, in which R is a hydrogen, alkyl, aryl, aralkyl or heteroaryl, as those terms are defined above. The groups, −CHO, $-C(O)CH_3$ (acetyl, Ac), $-C(O)CH_2CH_3$, $-C(O)CH_2CH_2CH_3$, $-C(O)CH(CH_3)_2$, $-C(O)CH(CH_2)_2$, $-C(O)C_6H_5$, $-C(O)C_6H_4CH_3$, $-C(O)CH_2C_6H_5$, −C(O)(imidazolyl) are non-limiting examples of acyl groups. A "thioacyl" is defined in an analogous manner, except that the oxygen atom of the group −C(O)R has been replaced with a sulfur atom, −C(S)R. When either of these terms are used with the "substituted" modifier one or more hydrogen atom (including the hydrogen atom directly attached the carbonyl or thiocarbonyl group) has been independently replaced by−OH, −F, −Cl, −Br, −I, $-NH_2$, $-NO_2$, $-CO_2H$, $-CO_2CH_3$, −CN, −SH, $-OCH_3$, $-OCH_2CH_3$, $-C(O)CH_3$, $-N(CH_3)_2$, $-C(O)NH_2$, $-OC(O)CH_3$, or $-S(O)_2NH_2$. The groups, $-C(O)CH_2CF_3$, $-CO_2H$ (carboxyl), $-CO_2CH_3$ (methylcarboxyl), $-CO_2CH_2CH_3$, $-C(O)NH_2$ (carbamoyl), and $-CON(CH_3)_2$, are non-limiting examples of substituted acyl groups.

[0063] The term "alkoxy" when used without the "substituted" modifier refers to the group −OR, in which R is an alkyl, as that term is defined above. Non-limiting examples of alkoxy groups include: $-OCH_3$ (methoxy), $-OCH_2CH_3$ (ethoxy), $-OCH_2CH_2CH_3$, $-OCH(CH_3)_2$ (isopropoxy), $-OCH(CH_2)_2$, −O−cyclopentyl, and −O−cyclohexyl. The terms "alkenyloxy", "alkynyloxy", "aryloxy", "aralkoxy", "heteroaryloxy", and "acyloxy", when used without the "substituted" modifier, refers to groups, defined as −OR, in which R is alkenyl, alkynyl, aryl, aralkyl, heteroaryl, and acyl, respectively. The term "alkoxydiyl" refers to the divalent group −O−alkanediyl−, −O−alkanediyl−O−, or −alkanediyl−O−alkanediyl−. The term "alkylthio" and "acylthio" when used without the "substituted" modifier refers to the group −SR, in which R is an alkyl and acyl, respectively. When any of these terms is used with the "substituted" modifier one or more hydrogen atom has been independently replaced by −OH, −F, −Cl, −Br, −I, $-NH_2$, $-NO_2$, $-CO_2H$, $-CO_2CH_3$, −CN, −SH, $-OCH_3$, $-OCH_2CH_3$, $-C(O)CH_3$, $-N(CH_3)_2$, $-C(O)NH_2$, $-OC(O)CH_3$, or $-S(O)_2NH_2$. The term "alcohol" corresponds to an alkane, as defined above, wherein at least one of the hydrogen atoms has been replaced with a hydroxy group.

[0064] The term "alkylamino" when used without the "substituted" modifier refers to the group −NHR, in which R is an alkyl, as that term is defined above. Non-limiting examples of alkylamino groups include: $-NHCH_3$ and $-NHCH_2CH_3$. The term "dialkylamino" when used without the "substituted" modifier refers to the group −NRR′, in which R and R′ can be the same or different alkyl groups, or R and R′ can be taken together to represent an alkanediyl. Non-limiting examples of dialkylamino groups include: $-N(CH_3)_2$, $-N(CH_3)(CH_2CH_3)$, and *N*-pyrrolidinyl. The terms "alkoxyamino", "alkenylamino", "alkynylamino", "arylamino", "aralkylamino", "heteroarylamino", and "alkylsulfonylamino" when used without the "substituted" modifier, refers to groups, defined as −NHR, in which R is alkoxy, alkenyl, alkynyl, aryl, aralkyl, heteroaryl, and alkylsulfonyl, respectively. A non-limiting example of an arylamino group is $-NHC_6H_5$. The term "amido" (acylamino), when used without the "substituted" modifier, refers to the group −NHR, in which R is acyl, as that term is defined above. A non-limiting example of an amido group is $-NHC(O)CH_3$. The term "alkylimino" when used without the "substituted" modifier refers to the divalent group =NR, in which R is an alkyl, as that term is defined above. The term "alkylaminodiyl" refers to the divalent group −NH−alkanediyl−, −NH−alkanediyl−NH−, or −alkanediyl−NH−alkanediyl−. When any of these terms is used with the "substituted" modifier one or more hydrogen atom has been independently replaced by −OH, −F, −Cl, −Br, −I, $-NH_2$, $-NO_2$, $-CO_2H$, $-CO_2CH_3$, −CN, −SH, $-OCH_3$, $-OCH_2CH_3$, $-C(O)CH_3$, $-N(CH_3)_2$, $-C(O)NH_2$, $-OC(O)CH_3$, or $-S(O)_2NH_2$. The groups $-NHC(O)OCH_3$ and $-NHC(O)NHCH_3$ are non-limiting examples of substituted amido groups.

[0065] The terms "alkylsulfonyl" and "alkylsulfinyl" when used without the "substituted" modifier refers to the groups $-S(O)_2R$ and −S(O)R, respectively, in which R is an alkyl, as that term is defined above. The terms "alkenylsulfonyl", "alkynylsulfonyl", "arylsulfonyl", "aralkylsulfonyl", and "heteroarylsulfonyl", are defined in an analogous manner. When any of these terms is used with the "substituted" modifier one or more hydrogen atom has been independently replaced by −OH, −F, −Cl, −Br, −I, $-NH_2$, $-NO_2$, $-CO_2H$, $-CO_2CH_3$, −CN, −SH, $-OCH_3$, $-OCH_2CH_3$, $-C(O)CH_3$, $-N(CH_3)_2$, $-C(O)NH_2$, $-OC(O)CH_3$, or $-S(O)_2NH_2$.

[0066] Abbreviations used herein include: Bip - 4,4′-bis(bromomethyl)biphenyl; BMBB - 1-(bromo-methyl)-3-[3-(bromomethyl)benzyl]benzene; CAEDA - N,N'-bis(2-chloro-acetylo)ethylenediamine; CMPB - 1-(chloromethyl)-4-[4-(chloromethyl)-phenoxy]benzene; Chol - cholesterol; DFDNB - 1,5-difluoro-2,4-dinitrobenzene; DNFB - 1-

fluoro-2,4-dinitrobenzene; Fmoc - fluorenylmethyloxy-carbonyl; Gu - guanidine; Ida - iminodiacetyl; Lig - lignoceryl; mBMB - 1,3-bis(bromomethyl)benzene; mBMPB - 3,5-bis(bromomethyl)-1-(methyl-S-palmityl)-benzene; OPI - 4,4'-Oxybis(phenyl isocyanate); Pal - palmityl; pBMB - 1,4-bis(bromomethyl)benzene; PDI - 1,4-phenylene diisocyanate; StBu - S-*tert*-butylthio; Ste - stearyl; Sub - suberoyl; and NA - not active. In some aspects, the compound names include names of the compounds used during the respective syntheses. For example, DNFB is 1-fluoro-2,4-dinitrobenzene, however, the LysNH-DNFB Smac derivative does not include a fluoro group. 1-fluoro-2,4-dinitrobenzene was used to incorporate the 2,4-dinitrobenzene moiety into the LysNH-DNFB Smac derivative.

[0067] As used herein, a "chiral auxiliary" refers to a removable chiral group that is capable of influencing the stereoselectivity of a reaction. Persons of skill in the art are familiar with such compounds, and many are commercially available.

[0068] The term "effective," as that term is used in the specification and/or claims, means adequate to accomplish a desired, expected, or intended result.

[0069] The term "hydrate" when used as a modifier to a compound means that the compound has less than one (*e.g.*, hemihydrate), one (*e.g.*, monohydrate), or more than one (*e.g.*, dihydrate) water molecules associated with each compound molecule, such as in solid forms of the compound.

[0070] As used herein, the term "IC_{50}" refers to an inhibitory dose which is 50% of the maximum response obtained. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological, biochemical or chemical process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half.

[0071] An "isomer" of a first compound is a separate compound in which each molecule contains the same constituent atoms as the first compound, but where the configuration of those atoms in three dimensions differs.

[0072] As used herein, the term "patient" or "subject" refers to a living mammalian organism, such as a human, monkey, cow, sheep, goat, dog, cat, mouse, rat, guinea pig, or transgenic species thereof. In certain embodiments, the patient or subject is a primate. Non-limiting examples of human subjects are adults, juveniles, infants and fetuses.

[0073] As generally used herein "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of

sound medical judgment, suitable for use in contact with the tissues, organs, and/or bodily fluids of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio.

[0074] "Pharmaceutically acceptable salts" means salts of compounds of the present invention which are pharmaceutically acceptable, as defined above, and which possess the desired pharmacological activity. Such salts include acid addition salts formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or with organic acids such as 1,2-ethanedisulfonic acid, 2-hydroxyethanesulfonic acid, 2-naphthalenesulfonic acid, 3-phenylpropionic acid, 4,4'-methylenebis(3-hydroxy-2-ene-1-carboxylic acid), 4-methylbicyclo[2.2.2]oct-2-ene-1-carboxylic acid, acetic acid, aliphatic mono- and dicarboxylic acids, aliphatic sulfuric acids, aromatic sulfuric acids, benzenesulfonic acid, benzoic acid, camphorsulfonic acid, carbonic acid, cinnamic acid, citric acid, cyclopentanepropionic acid, ethanesulfonic acid, fumaric acid, glucoheptonic acid, gluconic acid, glutamic acid, glycolic acid, heptanoic acid, hexanoic acid, hydroxynaphthoic acid, lactic acid, laurylsulfuric acid, maleic acid, malic acid, malonic acid, mandelic acid, methanesulfonic acid, muconic acid, *o*-(4-hydroxybenzoyl)benzoic acid, oxalic acid, *p*-chlorobenzenesulfonic acid, phenyl-substituted alkanoic acids, propionic acid, *p*-toluenesulfonic acid, pyruvic acid, salicylic acid, stearic acid, succinic acid, tartaric acid, tertiarybutylacetic acid, trimethylacetic acid, and the like. Pharmaceutically acceptable salts also include base addition salts which may be formed when acidic protons present are capable of reacting with inorganic or organic bases. Acceptable inorganic bases include sodium hydroxide, sodium carbonate, potassium hydroxide, aluminum hydroxide and calcium hydroxide. Acceptable organic bases include ethanolamine, diethanolamine, triethanolamine, tromethamine, *N*-methylglucamine and the like. It should be recognized that the particular anion or cation forming a part of any salt of this invention is not critical, so long as the salt, as a whole, is pharmacologically acceptable. Additional examples of pharmaceutically acceptable salts and their methods of preparation and use are presented in *Handbook of Pharmaceutical Salts: Properties, and Use* (2002).

[0075] The term "pharmaceutically acceptable carrier," as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a chemical agent.

[0076] "Prevention" or "preventing" includes: (1) inhibiting the onset of a disease in a subject or patient which may be at risk and/or predisposed to the disease but does not yet experience or display any or all of the pathology or symptomatology of the disease, and/or (2) slowing the onset of the pathology or symptomatology of a disease in a subject or patient which may be at risk and/or predisposed to the disease but does not yet experience or display any or all of the pathology or symptomatology of the disease.

[0077] "Prodrug" means a compound that is convertible *in vivo* metabolically into an inhibitor according to the present invention. The prodrug itself may or may not also have activity with respect to a given target protein. For example, a compound comprising a hydroxy group may be administered as an ester that is converted by hydrolysis *in vivo* to the hydroxy compound. Suitable esters that may be converted *in vivo* into hydroxy compounds include acetates, citrates, lactates, phosphates, tartrates, malonates, oxalates, salicylates, propionates, succinates, fumarates, maleates, methylene-bis-β-hydroxynaphthoate, gentisates, isethionates, di-*p*-toluoyltartrates, methanesulfonates, ethanesulfonates, benzenesulfonates, *p*-toluenesulfonates, cyclohexylsulfamates, quinates, esters of amino acids, and the like. Similarly, a compound comprising an amine group may be administered as an amide that is converted by hydrolysis *in vivo* to the amine compound.

[0078] The term "saturated" when referring to an atom means that the atom is connected to other atoms only by means of single bonds.

[0079] A "stereoisomer" or "optical isomer" is an isomer of a given compound in which the same atoms are bonded to the same other atoms, but where the configuration of those atoms in three dimensions differs. "Enantiomers" are stereoisomers of a given compound that are mirror images of each other, like left and right hands. "Diastereomers" are stereoisomers of a given compound that are not enantiomers. Chiral molecules contain a chiral center, also referred to as a stereocenter or stereogenic center, which is any point, though not necessarily an atom, in a molecule bearing groups such that an interchanging of any two groups leads to a stereoisomer. In organic compounds, the chiral center is typically a carbon, phosphorus or sulfur atom, though it is also possible for other atoms to be stereocenters in organic and inorganic compounds. A molecule can have multiple stereocenters, giving it many stereoisomers. In compounds whose stereoisomerism is due to tetrahedral stereogenic centers (*e.g.*, tetrahedral carbon), the total number of hypothetically possible stereoisomers will not exceed 2n, where n is the number of tetrahedral stereocenters. Molecules with symmetry frequently have fewer than the maximum possible number of

stereoisomers. A 50:50 mixture of enantiomers is referred to as a racemic mixture. Alternatively, a mixture of enantiomers can be enantiomerically enriched so that one enantiomer is present in an amount greater than 50%. Typically, enantiomers and/or diasteromers can be resolved or separated using techniques known in the art. It is contemplated that that for any stereocenter or axis of chirality for which stereochemistry has not been defined, that stereocenter or axis of chirality can be present in its *R* form, *S* form, or as a mixture of the *R* and *S* forms, including racemic and non-racemic mixtures. As used herein, the phrase "substantially free from other stereoisomers" means that the composition contains $\leq 15\%$, more preferably $\leq 10\%$, even more preferably $\leq 5\%$, or most preferably $\leq 1\%$ of another stereoisomer(s).

[0080] "Effective amount," "Therapeutically effective amount" or "pharmaceutically effective amount" means that amount which, when administered to a subject or patient for treating a disease, is sufficient to effect such treatment for the disease.

[0081] "Treatment" or "treating" includes (1) inhibiting a disease in a subject or patient experiencing or displaying the pathology or symptomatology of the disease (*e.g.*, arresting further development of the pathology and/or symptomatology), (2) ameliorating a disease in a subject or patient that is experiencing or displaying the pathology or symptomatology of the disease (*e.g.*, reversing the pathology and/or symptomatology), and/or (3) effecting any measurable decrease in a disease in a subject or patient that is experiencing or displaying the pathology or symptomatology of the disease. In some embodiments, treatment of a patient afflicted with one of the pathological conditions described herein comprises administering to such a patient an amount of compound described herein which is therapeutically effective in controlling the condition or in prolonging the survivability of the patient beyond that expected in the absence of such treatment. As used herein, the term "inhibition" of the condition also refers to slowing, interrupting, arresting or stopping the condition and does not necessarily indicate a total elimination of the condition. It is believed that prolonging the survivability of a patient, beyond being a significant advantageous effect in and of itself, also indicates that the condition is beneficially controlled to some extent.

B. Pharmaceutical Formulations and Routes of Administration

[0082] For administration to a mammal in need of such treatment, the compounds in a therapeutically effective amount are ordinarily combined with one or more excipients

appropriate to the indicated route of administration. The compounds may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanoic acids, cellulose alkyl esters, talc, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulfuric acids, gelatin, acacia, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and tableted or encapsulated for convenient administration. Alternatively, the compounds may be dissolved in water, polyethylene glycol, propylene glycol, ethanol, corn oil, cottonseed oil, peanut oil, sesame oil, benzyl alcohol, sodium chloride, and/or various buffers. Other excipients and modes of administration are well and widely known in the pharmaceutical art.

[0083] The pharmaceutical compositions may be subjected to conventional pharmaceutical operations such as sterilization and/or may contain conventional pharmaceutical carriers and excipients such as preservatives, stabilizers, wetting agents, emulsifiers, buffers, *etc.*

[0084] The compounds of the present disclosure may be administered by a variety of methods, *e.g.*, orally or by injection (*e.g.* subcutaneous, intravenous, intraperitoneal, *etc.*). Depending on the route of administration, the active compounds may be coated in a material to protect the compound from the action of acids and other natural conditions which may inactivate the compound. They may also be administered by continuous perfusion/infusion of a disease or wound site.

[0085] To administer the therapeutic compound by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. For example, the therapeutic compound may be administered to a patient in an appropriate carrier, for example, liposomes, or a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes.

[0086] The therapeutic compound may also be administered parenterally, intraperitoneally, intraspinally, or intracerebrally. Dispersions can be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

[0087] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases, the composition must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (such as, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

[0088] Sterile injectable solutions can be prepared by incorporating the therapeutic compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the therapeutic compound into a sterile carrier which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient (*i.e.*, the therapeutic compound) plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0089] The therapeutic compound can be orally administered, for example, with an inert diluent or an assimilable edible carrier. The therapeutic compound and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the therapeutic compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The percentage of the therapeutic compound in the compositions and preparations may, of

course, be varied. The amount of the therapeutic compound in such therapeutically useful compositions is such that a suitable dosage will be obtained.

[0090] It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit containing a predetermined quantity of therapeutic compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the therapeutic compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such a therapeutic compound for the treatment of a selected condition in a patient.

[0091] The therapeutic compound may also be administered topically to the skin, eye, or mucosa. Alternatively, if local delivery to the lungs is desired the therapeutic compound may be administered by inhalation in a dry-powder or aerosol formulation.

[0092] Active compounds are administered at a therapeutically effective dosage sufficient to treat a condition associated with a condition in a patient. For example, the efficacy of a compound can be evaluated in an animal model system that may be predictive of efficacy in treating the disease in humans, such as the model systems shown in the examples and drawings.

[0093] The actual dosage amount of a compound of the present disclosure or composition comprising a compound of the present disclosure administered to a subject may be determined by physical and physiological factors such as age, sex, body weight, severity of condition, the type of disease being treated, previous or concurrent therapeutic interventions, idiopathy of the subject and on the route of administration. These factors may be determined by a skilled artisan. The practitioner responsible for administration will typically determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject. The dosage may be adjusted by the individual physician in the event of any complication.

[0094] An effective amount typically will vary from about 0.001 mg/kg to about 1000 mg/kg, from about 0.01 mg/kg to about 750 mg/kg, from about 100 mg/kg to about 500 mg/kg, from about 1.0 mg/kg to about 250 mg/kg, from about 10.0 mg/kg to about 150 mg/kg in one or more dose administrations daily, for one or several days (depending of course of the

mode of administration and the factors discussed above). Other suitable dose ranges include 1 mg to 10000 mg per day, 100 mg to 10000 mg per day, 500 mg to 10000 mg per day, and 500 mg to 1000 mg per day. In some particular embodiments, the amount is less than 10,000 mg per day with a range of 750 mg to 9000 mg per day.

[0095] The effective amount may be less than 1 mg/kg/day, less than 500 mg/kg/day, less than 250 mg/kg/day, less than 100 mg/kg/day, less than 50 mg/kg/day, less than 25 mg/kg/day or less than 10 mg/kg/day. It may alternatively be in the range of 1 mg/kg/day to 200 mg/kg/day. For example, regarding treatment of diabetic patients, the unit dosage may be an amount that reduces blood glucose by at least 40% as compared to an untreated subject. In another embodiment, the unit dosage is an amount that reduces blood glucose to a level that is ± 10% of the blood glucose level of a non-diabetic subject.

[0096] In other non-limiting examples, a dose may also comprise from about 1 microgram/kg/body weight, about 5 microgram/kg/body weight, about 10 microgram/kg/body weight, about 50 microgram/kg/body weight, about 100 microgram/kg/body weight, about 200 microgram/kg/body weight, about 350 microgram/kg/body weight, about 500 microgram/kg/body weight, about 1 milligram/kg/body weight, about 5 milligram/kg/body weight, about 10 milligram/kg/body weight, about 50 milligram/kg/body weight, about 100 milligram/kg/body weight, about 200 milligram/kg/body weight, about 350 milligram/kg/body weight, about 500 milligram/kg/body weight, to about 1000 mg/kg/body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg/body weight to about 100 mg/kg/body weight, about 5 microgram/kg/body weight to about 500 milligram/kg/body weight, *etc.*, can be administered, based on the numbers described above.

[0097] In certain embodiments, a pharmaceutical composition of the present disclosure may comprise, for example, at least about 0.1% of a compound of the present disclosure. In other embodiments, the compound of the present disclosure may comprise between about 2% to about 75% of the weight of the unit, or between about 25% to about 60%, for example, and any range derivable therein.

[0098] Single or multiple doses of the agents are contemplated. Desired time intervals for delivery of multiple doses can be determined by one of ordinary skill in the art employing no more than routine experimentation. As an example, subjects may be administered two

doses daily at approximately 12 hour intervals. In some embodiments, the agent is administered once a day.

[0099] The agent(s) may be administered on a routine schedule. As used herein a routine schedule refers to a predetermined designated period of time. The routine schedule may encompass periods of time which are identical or which differ in length, as long as the schedule is predetermined. For instance, the routine schedule may involve administration twice a day, every day, every two days, every three days, every four days, every five days, every six days, a weekly basis, a monthly basis or any set number of days or weeks there-between. Alternatively, the predetermined routine schedule may involve administration on a twice daily basis for the first week, followed by a daily basis for several months, *etc.* In other embodiments, the invention provides that the agent(s) may taken orally and that the timing of which is or is not dependent upon food intake. Thus, for example, the agent can be taken every morning and/or every evening, regardless of when the subject has eaten or will eat.

C. Examples

[0100] The following examples are included to demonstrate certain non-limiting aspects of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the applicants to function well in the practice of the invention. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1 – MATERIALS AND METHODS

[0101] A novel group of monomeric and dimeric anticancer Smac peptides, including novel lipid-conjugated Smac mimics, was synthesized, characterized and screened for anticancer activity against human metastatic breast cancer cell line, MDA-MB-231. Selected lipidated analogues, monomeric (M11) and dimeric (D7), were displayed favorable pharmacokinetics, plasma stability and *in vivo* efficacy in murine model. The most active lipid-conjugated analogue M11, showed *in vivo* activity while administered both subcutaneously and orally. The modification of Smac mimics with various lipids is a viable approach in the development of novel anticancer leads.

[0102] Generally monomeric (M) analogues have a sequence NMeAla-Cys/Lys-Pro-BHA, which is closely related to various previously-developed analogues. Position 2, which

is occupied by either (L)Lys or (L)Cys, was chosen as a modification/dimerization point. Synthesis of monomers was carried out in solution according to reaction schemes in FIGS. 2 and 3. Reaction conditions for the corresponding steps in FIG. 2 are: **a:** (1) BHA/1,4-dioxane/2h; (2) 4M HCl in 1,4-dioxane/30 min; **b:** (1) Boc-(L)-Cys(SStBu)-OH/TCTU/NMM/DMSO/75°C/10 min/MW; (2) 4M HCl in 1,4-dioxane/30 min **c:** (1) Boc-(L)-Ser(tBu)-OH/TCTU/NMM/DMSO/75°C/10 min/MW; (2) 4M HCl in 1,4-dioxane/30 min; **d:** (1) Boc-(L)-Lys(Fmoc)-OH/TCTU/NMM/DMSO/75°C/10 min/MW; (2) 4M HCl in 1,4-dioxane/30 min; **e:** Boc-N-Me-(L)-Ala-OH/TCTU/NMM/DMSO/75°C/10 min/MW; **f:** 4M HCl in 1,4-dioxane/30 min; **g:** 4M HCl in 1,4-dioxane/30 min; **h:** TFA/DTT/30 min; **i:** C18-Br/CH3OH/TMGU/1h/50°C; **j:** 4M NaOH in CH_3OH (1:9)/r.t./30 min; **k:** 4M HCl in 1,4-dioxane/30 min. Reaction conditions for the corresponding steps in FIG. 3 are: **a:** 4M HCl in 1,4-dioxane/30 min; **b:** (1) DNFB/1,4-dioxane/NMM/2h; (2) 4M HCl in 1,4-dioxane/30 min **c:** (1) N,N-Di-Boc-1H-pyrazole-1-carboxamidine/1,4-Dioxane/NMM//75°C/10 min/MW; (2) 4M HCl in 1,4-dioxane/30 min; **d:** (1) Cholesteryl chloroformate/1,4-dioxane/NMM/2h; (2) 4M HCl in 1,4-dioxane/30 min; **e:** (1) Palmitoyl chloride/1,4-dioxane/NMM/2h; (2) 4M HCl in 1,4-dioxane/30 min; **f:** (1) Lignoceroyl chloride/1,4-dioxane/NMM/2h; (2) 4M HCl in 1,4-dioxane/30 min.

[0103] Dimerization of monomers was carried out utilizing either bis-amine-reactive linkers (D1-D8, **FIGS. 6A-B**) or bis-thiol-reactive linkers (D9-D16, **FIGS. 7A-B**).

[0104] The synthesis of monomers proceeded efficiently and was carried out with minimal purification of the intermediates, due in part to simplicity of final products. Four different lipids were used to modify M compounds in position 2: palmitic acid (Pal, C_{16}), lignoceric acid (Lig, C_{24}), cholesterol (Chol, C_{27}) and stearyl chain (Ste, C_{18}). Palmitic acid and lignoceric acid were appended by reacting the side chain amine group of Lys with palmitoyl chloride or lignoceroyl chloride, respectively. Stearyl ligation was accomplished by reaction with 1-bromooctadecane. Cholesterol was introduced in similar manner using cholesteryl chloroformate giving urethane type connectivity (see **FIG. 3**). Analogue M11 was synthesized using a previously described 1,1,3,3-tetramethylguanidine (TMG) driven alkylation of thiol(s) in organic solvents that we adapted to peptides. Notably, the same S-alkylation protocol was successfully employed in the synthesis of dimers D9-D16. Among all dimers synthesized for this study only three, D7, D8 and D16 underwent lipidation. D7 and D8 were modified with palmityl moiety using either physiologically stable amide bond (D7) or cleavable ester type (D8) connectivity. D16 was modified with stearyl chain afforded

by mBMPB-3,5-bis(bromomethyl)-1-(methyl-S-palmityl)-benzene however efficiency of the reaction was particularly low (<3%).

[0105] An initial evaluation of bioactivity Smac derivatives was carried out *in vitro* using exclusively growth inhibition assay (PrestoBlue™, Invitrogen, Carlsbad, CA) and MDA-MB-231 metastatic human breast cancer cell line. The *in vitro* growth inhibition assay provides more reliable data than pure biophysical method(s) *e.g.* measurement of binding affinity to BIR2/BIR3 XIAP domain, as it takes into account factors including compound cell permeability, binding potency, and stability in the cellular microenvironment. Results are summarized in Table 1 and an example of cell growth curves is presented in **FIG. 4A**.

[0106] Initial screening of monovalent Smac analogues (M1-M11) (**FIG. 1**) suggested that indeed optimal hydrophobicity plays an important role in overall bioactivity of position 2 modified compounds and observed activity gain can be significant in both 2Cys (M2<M3<M11, EC50 (μM): NA<49.9±0.7<4.4±0.1 respectively) and 2Lys series (M4<M6<M7<M8, EC50 (μM): NA<44.7±15.0<19.0±1.6<6.1±0.1 respectively). Exceedingly hydrophobic substituents (Lig, Chol) in position 2 is undesirable, as is the presence of hydrophilic/ionizable amine and guanidine moieties (M4 and M5 respectively). Similar low potency results were observed for dimeric mimics utilizing hydrophilic linkers (D1, D6, D9, D10). Lipidation is clearly beneficial, as introduction of lipid moiety (Pal) into otherwise inactive D6 compound leads to highly active derivatives D7 and D8. Effects of dimerization are also apparent with dimeric analogue D3 being ~13.5× more potent than its monomeric counterpart, M6 (EC50 (μM): 44.7±15.0 versus 3.3±0.2 respectively). The most active dimeric analogues contain relatively hydrophobic linker(s) with ~13-14 atoms (Cα↔C'α). Moreover, spatial geometry of linkers(s) is also important for overall potency, as compounds utilizing relatively similar linkers show diversified bioactivity (D13-D15). For analogous with shorter linkers (D11&D12), a specific linker geometry (*para-* versus *meta-* positions of substituents) seems to have an opposite effect on overall potency.

[0107] To confirm that theSmac mimics promote apoptosis, enzymatic activities of caspases-3/7 and -9 were measured in a metastatic breast cancer cell line, MDA-MB-231. The cells were treated with analogues M11, D3, D7 and D13. Direct comparison of the effects of the treatment at 10 μM concentration is presented in **FIG. 4B**. Caspase-3/7 is selectively affected by the treatment, resulting in ~2.7-6.7 fold increase in enzymatic activity. All tested dimers (D3, D7, D13) are also approximately ~2.5× more potent than monomer,

M11. The lipid-conjugated analogues M11 and D7 displayed dose-dependent effects (**FIG. 4C**).

[0108] To further characterize most potent lipid-conjugated analogues M11 and D7, pharmacokinetic (PK) studies were performed using 2 different delivery routes: subcutaneous (SC, 10 mg/kg dose) and oral (OR, 10 mg/kg dose). For analogue M11, the observed plasma half-life ($t_{1/2}$) is ~2.2 h for SC and ~5.2 h for OR delivery (**FIGS. 4D** and **4E**). Analogue D7 was bioavailable only via SC route, giving value of $t_{1/2}$ ~ 2.8 h.

[0109] M11 and D7 analogues were assessed for plasma stability. The plasma stability results (**FIG. 4F**) indicate that both analogues are remarkably stable in experimental conditions (<31 h). After prolonged exposure (144h), both compounds were largely intact (plasma stability: M11=60.4±0.3% and D7=80.1±0.5%). Under the same experimental conditions, unrelated control peptide mHS172 quickly degraded, falling to less than 1% of initial content within 6 h.

[0110] To test cellular and whole animal utility of lipidated Smac derivatives *in vivo* studies were performed using subcutaneous engraftment mouse model and human metastatic breast cancer line, MDA-MB-231. Based on pharmacokinetic experiment outcomes, monovalent analogue M11 was administered via both SC and OR routes. Bivalent lipidated analogue D7 was administered only SC. The treatment of the experimental, cancer bearing animals with M11 resulted in dose-dependent anticancer effects (**FIG. 5**). Animals treated with 10 doses of the M11 at the escalating dosage from 2.5 to 15 mg/kg showed progressively longer tumor growth delay values (Table 1) reaching ~11.0 days of delay at the 15 mg/kg SC dose. Oral administration of M11 at 30 mg/kg dose resulted in ~7.6 days of tumor growth delay. Based on the anticancer response, the oral bioavailability of M11 is ~23%. Bivalent D7 analogue showed low anticancer *in vivo* activity and was ~2.7× less potent than monovalent M11 (SC route). Compared to a previously-reported analogue, SMAC17-2X47, compound M11 is significantly less active as reported tumor growth delay values for SMAC17-2X were: ~10.2 days at 2.5 mg/kg dose and ~23.4 days at 7.5 mg/kg dose. Similarly, *in vivo* results reported for SM-16435, compound 2739 and SM-120044 showed markedly improved activity profiles with SM-1200 promoting complete and durable tumor regression in mouse models. Modification of Smac mimics with lipids is herein demonstrated to be beneficial to biological activity, as some lipid-modified analogues showed improved bioactivity comparing to "lipid-less" counterparts, *e.g.*, M11 versus M2; M8 versus

M4; D7/D8 versus D6 . Optimal overall hydrophobicity is also important and is related to solubility, which in turn can influence delivery, distribution and pharmacokinetics.

Table 1 Tumor growth delay values obtained for M11 and D7 analogues.

Compound	Dose (mg/kg)	Delivery route	Tumor Growth Delay at 500 mm^3 (days)
M11	2.5	SC	~2.5
M11	7.5	SC	~8.1
M11	15	SC	~11.0
M11	30	OR	~7.6
D7	15	SC	~4.1

[0111] Secondary structures of Smac analogs were probed using Fourier transform infrared (FTIR) spectroscopy. Results are summarized in Table 2. Both tested peptides, M11 and D7, showed dominant turn conformations in hydrated self-films and in POPC multilayers (Figure S2A-D and Table 2). In self-films, M11 had broad absorption from 1682 cm^{-1} to 1662 cm^{-1}, indicating that the peptide assumed a number of turn conformations. A well-defined β-sheet was present, with absorbance centered at 1924 cm^{-1}. This absorbance is representative of self-associattion by the formation of anti-parallel β-sheets under thee experimental conditions. When the M11 was incorporated into a membrane-like environment of POPC multilayers, the turn absorption was better defined with a peak centered around 1675 cm^{-1}, and some loss of β-sheet to disordered conformations (**FIG. 8B**). In both self-films and lipid multilayers, M11 showed very little α-helical structure. The infrared signature of D7 amide I conformational band, although similar to that of M11, had better defined turn band than the latter, which was centered at 1664 cm^{-1}. This signature is representative of typical 310-helix or type III turn conformations (**FIG. 8B**, D7 self-film). The deuterium hydrated self-film of D7 also had a well-defined β-sheet band round 1924 cm^{-1}, similar to that observed for the M11 peptide. D7 also showed a loss of β-sheet conformation to more disordered structures in the POPC environment similar to that of M11. In POPC multilayers environment, D7 showed a dominant absorption around 1664 cm^{-1} (**FIG. 8B**, D7 in POPC). The dimeric lipo-peptide D7 assumed a more stable type III turn structure than M11.

Table 2 Proportions of different components of secondary structure for M11 and D7 peptides in hydrated self-films and lipid multilayers based on infrared spectroscopic analysis.

Peptide*	% Conformation			
	α-helix	β-sheet	turns	disordered
M11 self-film	11.90	26.75	50.98	10.37
M11 in POPC	6.71	18.29	35.27	39.73
D7 self-film	4.43	23.17	55.32	17.08

D7 in POPC	6.40	25.09	36.96	32.55

*Deuterium hydrated peptide self-films and POPC - peptide multilayers were studied with a germanium ATR accessory as described in methods. All IR spectra analyzed for secondary conformation based on secondary structural analysis using GRAMS/AI deconvolution-curve-fitting software. Peak area error is ±5%.

[0112] All monovalent Smac mimics (M1-M11) were synthesized as C-terminal benzhydryl-amides (BHA). Syntheses were carried out in solution according to reaction schemes in FIGS. 2 and 3. For some examples, a CEM Liberty automatic microwave peptide synthesizer (CEM Corporation Inc., Matthews, NC) was employed in manual mode, and applying tert-butoxycarbonyl (Boc) chemistry and standard, commercially-available amino acid derivatives and reagents (Chem-Impex International, Inc., Wood Dale, IL). All compounds were purified by preparative reverse-phase high performance liquid chromatography (RP-HPLC) and their purities were evaluated by matrix-assisted laser desorption ionization spectrometry (MALDI-MS) as well as analytical RP-HPLC.

[0113] **Synthesis of HCl*Pro-BHA (1):** 15 g (48 mMole) of Boc-Pro-OSu was dissolved in anhydrous 1,4-dioxane (40 mL). 8.8 g (8.3 mL, 48 mMole) of benzhydrylamine was added to the solution with vigorous mixing (magnetic stirrer) and reaction was allowed to proceed for additional 2 hours. Solvent was evaporated on rotary evaporator and Boc group cleaved using 4 M HCl in 1,4-dioxane (40 mL/30 min). Solution was concentrated on rotary evaporator and product precipitated using ice cold diethyl ether, giving 15.03 g of dry HCl*Pro-BHA. Yield 98.7%. The precipitated compound was used without further purification.

[0114] **Synthesis of HCl*Cys(StBu)-Pro-BHA (2), HCl*Ser-Pro-BHA (3), and HCl*Lys(Fmoc)-Pro-BHA (4):** Boc-(L)-Cys(SStBu)-OH (5 g, 16.2 mMole), Boc-(L)-Ser(tBu)-OH (5 g, 19.1 mMole), and Boc-(L)-Lys(Fmoc)-OH (5 g, 10.7 mMole), were activated (separately) with TCTU (1:1 ratio), NMM (3 eq) in DMSO (20 mL, 30 min, r.t.) and subsequently reacted with HCl*Pro-BHA (1:1 ratios) in microwave synthesizer (CEM Liberty, CEM Corporation Inc., Matthews, NC) for 10 min at 75 °C. Obtained solutions were diluted with H_2O and extracted with diethyl ether (3×). Ether extracts were combined, washed with brine (3×) and concentrated on rotary evaporator. Obtained oily residues were dissolved in ethyl acetate and precipitated by addition of n-hexane (3×), and dried under the vacuum. Subsequently, each compound was treated with 4 M HCl in 1,4-dioxane (30 mL) for 30 min to remove Boc protecting groups, concentrated on rotary evaporator and crystallized by addition of ice cold diethyl ether giving solid: HCl*Cys(SStBu)-Pro-BHA

(yield 78.5%), HCl*Ser(OH)-Pro-BHA (4.8 g, yield 81.8%), and HCl*Lys(Fmoc)-Pro-BHA (yield 77.2%). The precipitated compounds were used without further purification.

[0115] Synthesis of Boc-NMe-Ala-Cys(StBu)-Pro-BHA (5): Boc-NMe-(L)-Ala-OH was activated with TCTU (1:1 ratio), NMM (3 eq) in DMSO (30 min, r.t.) and subsequently reacted with 2 (1:1 ratio) in microwave synthesizer (CEM Liberty) for 10 min at 75 °C. The reaction mixture was diluted with H_2O and extracted with diethyl ether (3×). Ether extracts were combined, washed with the brine (3×) and concentrated on rotary evaporator. Obtained oily residue was dissolved in minimal amount of ethyl acetate and precipitated by addition of n-hexane (3×) and dried under the vacuum (yield 75.8%). The precipitated compound was used without further purification.

[0116] Synthesis of Boc-NMe-Ala-Lys(Fmoc)-Pro-BHA (6): Boc-NMe-(L)-Ala-OH was activated with TCTU (1:1 ratio), NMM (3 eq) in DMSO (30 min, r.t.) and subsequently reacted with 4 (1:1 ratio) in microwave synthesizer (CEM Liberty) for 10 min at 75 °C. The reaction mixture was diluted with H_2O and extracted with diethyl ether (3×). Ether extracts were combined, washed with the brine (3×) and concentrated on rotary evaporator. Obtained oily residue was dissolved in ethyl acetate and precipitated by addition of n-hexane (3×), and dried under the vacuum (yield 87.0%). The precipitated compound was used without further purification.

[0117] Synthesis of Boc-NMe-Ala-Lys-Pro-BHA (7): Boc-NMe-Ala-Lys-Pro-BHA was obtained by the treatment of 6 with Tesser's base (4M NaOH in CH_3OH (1:9) (vol:vol)) at r.t. for 30 min. The reaction mixture was acidified with diluted HCl and then concentrated on rotary evaporator. Obtained oily residue was purified by preparative reverse-phase high performance liquid chromatography (RP-HPLC) giving 2.1 g of pure product (yield 33.1%).

[0118] Synthesis of H-NMe-Ala-Ser-Pro-BHA (M1): Boc-NMe-(L)-Ala-OH was activated with TCTU (1:1 ratio), NMM (3 eq) in DMSO (30 min, r.t.) and subsequently reacted with 3 (1:1 ratio) in microwave synthesizer (CEM Liberty) for 10 min at 75 °C. The reaction mixture was diluted with H_2O and extracted with diethyl ether (3x). Ether extracts were combined, washed with the brine (3x) and concentrated on rotary evaporator. Obtained oily residue was treated with 4 M HCl in 1,4-dioxane for 30 min to remove Bocprotecting group, concentrated on rotary evaporator, and crystallized by addition of ice cold diethyl ether giving solid M1 (yield 75.6%). Compound M1 was purified by preparative reverse-phase high performance liquid chromatography (RP-HPLC), and its purity evaluated by

matrix-assisted laser desorption ionization spectrometry (MALDI-MS) as well as analytical RP-HPLC (see Table S1).

[0119] Synthesis of H-NMe-Ala-Cys-Pro-BHA (M2): Compound M2 was obtained by the treatment of Boc-NMe-Ala-Cys(StBu)-Pro-BHA with TFA/DTT/H2O (96:4:1) (30 min) and crystallized by addition of ice cold diethyl ether. Compound M2 was purified by preparative reverse-phase high performance liquid chromatography (RP-HPLC), and its purity evaluated by matrix-assisted laser desorption ionization spectrometry (MALDI-MS) as well as analytical RP-HPLC (see Table S1).

[0120] Synthesis of H-NMe-Ala-Cys(StBu)-Pro-BHA (M3): Compound M3 was obtained by the treatment of Boc-NMe-Ala-Cys(StBu)-Pro-BHA with with 4 M HCl in 1,4-dioxane (30 min) and crystallized by addition of ice cold diethyl ether. Compound M3 was purified by preparative reverse-phase high performance liquid chromatography (RP-HPLC) and its purity evaluated by matrix-assisted laser desorption ionization spectrometry (MALDI-MS) as well as analytical RP-HPLC (see Table S1).

[0121] Synthesis of H-NMe-Ala-Lys-Pro-BHA (M4): Compound M4 was obtained by the treatment of Boc-NMe-Ala-Lys-Pro-BHA with 4 M HCl in 1,4-dioxane (30 min) and crystallized by addition of ice cold diethyl ether. Compound M4 was purified by preparative reverse-phase high performance liquid chromatography (RP-HPLC), and its purity evaluated by matrix-assisted laser desorption ionization spectrometry (MALDI-MS) as well as analytical RP-HPLC (see Table S1).

[0122] Synthesis of H-NMe-Ala-Lys(Gu)-Pro-BHA (M5): Compound was obtained in reaction of Boc-NMe-Ala-Lys-Pro-BHA with N,N-Di-Boc-1H-pyrazole-1-carboxamidine (2 eq) in 1,4-dioxane and NMM (10 eq) (75 °C/10min/MW). The resulting solution was concentrated on rotary evaporator and then treated with 4 M HCl in 1,4-dioxane (30 min), and crystallized by addition of ice cold diethyl ether. Compound M5 was purified by preparative reverse-phase high performance liquid chromatography (RP-HPLC), and its purity evaluated by matrix-assisted laser desorption ionization spectrometry (MALDI-MS) as well as analytical RP-HPLC (see Table S1).

[0123] Synthesis of H-NMe-Ala-Lys(DNFB)-Pro-BHA (M6): Compound M6 was obtained in reaction of Boc-NMe-Ala-Lys-Pro-BHA with 2,4-dinitrofluorobenzene (DNFB, 1.2 eq) in 1,4-dioxane and NMM (5 eq/r.t./2 h). The resulting solution was concentrated on rotary evaporator and then treated with 4 M HCl in 1,4-dioxane (30 min), and crystallized by

addition of ice cold diethyl ether. Compound M6 was purified by preparative reverse-phase high performance liquid chromatography (RP-HPLC), and its purity evaluated by matrix-assisted laser desorption ionization spectrometry (MALDI-MS) as well as analytical RP-HPLC (see Table S1).

[0124] Synthesis of H-NMe-Ala-Lys(Fmoc)-Pro-BHA (M7): Compound M7 was obtained by the treatment of Boc-NMe-Ala-Lys(Fmoc)-Pro-BHA with 4 M HCl in 1,4-dioxane (30 min) and crystallized by addition of ice cold diethyl ether. Compound M7 was purified by preparative reverse-phase high performance liquid chromatography (RP-HPLC), and its purity evaluated by matrix-assisted laser desorption ionization spectrometry (MALDI-MS) as well as analytical RP-HPLC (see Table S1).

[0125] Synthesis of H-NMe-Ala-Lys(Pal)-Pro-BHA (M8): Compound was obtained in reaction of Boc-NMe-Ala-Lys-Pro-BHA with palmitoyl chloride (1.1 eq) in 1,4-dioxane and NMM (5 eq/r.t./2 h). The resulting solution was concentrated on rotary evaporator and then treated with 4 M HCl in 1,4-dioxane (30 min), and evaporated again on rotary evaporator. Compound M8 was purified by preparative reverse-phase high performance liquid chromatography (RP-HPLC), and its purity evaluated by matrix-assisted laser desorption ionization spectrometry (MALDI-MS) as well as analytical RP-HPLC (see Table S1).

[0126] Synthesis of H-NMe-Ala-Lys(Lig)-Pro-BHA (M9): Compound was obtained in reaction of Boc-NMe-Ala-Lys-Pro-BHA with lignoceroyl chloride (1.1 eq) in 1,4-dioxane and NMM (5 eq/r.t./2 h). The resulting solution was concentrated on rotary evaporator and then treated with 4 M HCl in 1,4-dioxane (30 min), and evaporated again on rotary evaporator. Compound M9 was purified by preparative reverse-phase high performance liquid chromatography (RP-HPLC), and its purity evaluated by matrix-assisted laser desorption ionization spectrometry (MALDI-MS) as well as analytical RP-HPLC (see Table S1).

[0127] Synthesis of H-NMe-Ala-Lys(Chol)-Pro-BHA (M10): Compound M10 was obtained in reaction of Boc-NMe-Ala-Lys-Pro-BHA cholesteryl chloroformate (1.1 eq) in 1,4-dioxane and NMM (5 eq/r.t./2 h). The resulting solution was concentrated on rotary evaporator and then treated with 4 M HCl in 1,4-dioxane (30 min) and evaporated again on rotary evaporator. Compound M10 was purified by preparative reverse-phase high performance liquid chromatography (RP-HPLC), and its purity evaluated by matrix-assisted

laser desorption ionization spectrometry (MALDI-MS) as well as analytical RP-HPLC (see Table S1).

[0128] **Synthesis of H-NMe-Ala-Cys(S-Ste)-Pro-BHA (M11)**: Compound M11 was obtained by reaction of M2 with stearyl bromide and TMGu (1:1:3) in methanol (50 °C/60 min). The reaction mixture was evaporated on rotary evaporator and compound M11 was purified by preparative reverse-phase high performance liquid chromatography (RP-HPLC), and its purity evaluated by matrix-assisted laser desorption ionization spectrometry (MALDI-MS) as well as analytical RP-HPLC (see Table S1).

[0129] **Analytical RP-HPLC:** Analytical RP-HPLC was performed on a Varian ProStar 210 HPLC system equipped with ProStar 325 Dual Wavelength UV-Vis detector with the wavelengths set at 220 nm and 280 nm (Varian Inc., Palo Alto, CA). Mobile phases consisted of solvent A, 0.1% TFA in water, and solvent B, 0.1% TFA in acetonitrile. Analyses of peptides were performed with an analytical reversed phase C18 Vydac 218TP54 column, 4.6×250 mm, 5µm (Grace, Deerfield, IL) or (*) analytical reversed-phase C18 SymmetryShield™ column, 4.6×250 mm, 5 µm (Waters, Milford, MA) or (#) analytical reversed-phase C4 XBridge™ BEH300 column, 4.6×150 mm, 3.5 µm (Waters, Milford, MA), applying linear gradient of solvent B from 0 to 100% over 100 min (flow rate: 1 ml/min).

[0130] **Synthesis of dimers D1-D8:** Dimers D1-D8 were obtained by dimerization of 7 (1 eq) using various bis-functional-amine-reactive reagents (0.5 eq) and NMM (5 eq) in DMF (overnight). Bis-functional-amine-reactive reagents used were, for:

p-nitrophenyl chloroformate (D1),

suberic acid bis(N-hydroxysuccinimide ester) (D2),

1,5-difluoro-2,4-dinitrobenzene (D3),

1,4-phenylene diisocyanate (D4), and

4,4'-oxybis(phenyl isocyanate) (D5).

[0131] D6- Fmoc-Ida-OH was preactivated with TCTU/NMM (30 min). Fmoc group was deprotected by addition of equal volume of piperidine (final concentration 50% (vol:vol) for 1 h. The reaction mixture was diluted with H_2O and extracted with diethyl ether (3×). Ether extracts were combined, washed with the brine (3x) and concentrated on rotary evaporator.

[0132] D7 was obtained by reaction of D6 (see above) with palmitoyl chloride (1.1 eq) in 1,4-dioxane and NMM (5 eq/r.t./2 h). The resulting solution was concentrated on rotary evaporator.

[0133] D8- Pal-O-Et-N-Ida-OH (N,N-bis(carboxymetyl)-O-palmitoyl-ethanolamine) was preactivated with TCTU/NMM (30 min). The reaction mixture was diluted with H_2O and extracted with diethyl ether (3×). Ether extracts were combined, washed with the brine (3×) and concentrated on rotary evaporator. Pal-O-Et-N-Ida-OH (not commercially available) was obtained by reaction of N-hydroxyethyl-iminodiacetic acid (1 eq) and palmitoyl chloride (10 eq) in TFA (60 min). The product was crystalized by addition of ice cold diethyl ether, and subsequently recrystallized from 4 M HCl/1,4-dioxane/Et_2O system (2×) (TFA->HCl counter ion exchange).

[0134] Each compound was subsequently treated with 4 M HCl in 1,4-dioxane for 30 min to remove Boc protecting groups. Each solution was concentrated on rotary evaporator and D1-D6 were crystallized by addition of ice cold diethyl ether. Obtained crude D1-D8 were purified by preparative reverse-phase high performance liquid chromatography (RP-HPLC), and their purities were evaluated by matrix-assisted laser desorption ionization spectrometry (MALDI-MS) as well as analytical RP-HPLC (see Table S1).

[0135] **Synthesis of dimers D9-D16:** Dimers D9-D16 were obtained by dimerization of M2 (1 eq) using various bis-functional-thiol-reactive reagents (0.5 eq).

[0136] D9 synthesis was carried out in 70% ACN, 10 mM NH_4HCO_3 for 48 h using divinylsulfone (DVS).

[0137] D10 synthesis was carried out in 70% ACN, 50 mM NH_4HCO_3 for 48 h using N,N'-Bis(2-chloroacetylo)ethylenediamine.

[0138] D11- D16, syntheses were carried out in methanol with addition of TMGu (1,1,3,3-tetramethylguanidine) (5 eq) for 60 min at 50 °C, using:

1,4-bis(bromomethyl)benzene (D11),

1,3-bis(bromomethyl)benzene (D12),

4,4'-bis(bromomethyl)biphenyl (D13),

1-(chloromethyl)-4-[4-(chloromethyl)phenoxy]benzene (D14),

1-(bromomethyl)-3-[3-(bromomethyl)benzyl]benzene (D15), and

1-(palmityl-S-methyl)-3,5-bis(bromomethyl)-benzene (D16).

[0139] Subsequently, each reaction mixture was evaporated using rotary evaporator and obtained crude compounds purified by preparative reverse-phase high performance liquid chromatography (RP-HPLC), and their purities were evaluated by matrix-assisted laser desorption ionization spectrometry (MALDI-MS) as well as analytical RP-HPLC (see Table S1).

[0140] **Cell growth inhibition assay:** Experiments were carried out using PrestoBlue™ Cell Viability Reagent (Invitrogen, Carlsbad, CA) according to manufacturer's protocol. Briefly, Smac-susceptible human metastatic breast cancer MDA-MB-231 cells were plated in a 96-well plate at a density of 5×103 cells/well in a total volume of 50 µl of culture media, and treated with various concentrations of tested peptides (50 µl of 0-200 µM peptides in culture media). The cells' viability was assessed after 48 h by fluorescence measurement (Ex/Em:560/590, incubation time 30 min) employing the SpectraMAX M2 microplate reader (Molecular Devices, Sunnyvale, CA). All experiments were carried out in triplicate.

[0141] **Enzymatic activity of caspases-3/7 and -9:** Enzymatic activity of caspases-3/7 and -9 was measured using commercially available Caspase-Glo® 3/7 and Caspase-Glo® 9 assays (Promega Corp., Madison, WI) utilizing manufacturers protocols. Briefly, MDA-MB-231 cells were plated in a white-walled 96-well plate at a density of 5×10^3 cells/well in a total volume of 50 µl of culture media and treated with various concentrations of tested peptides (50 µl of 0-100 µM peptides in culture media) for 24 hours. Subsequently, 100 µl of appropriate Caspase-Glo® reagent was added to each well and cells incubated for additional 60 min. Luminescence values were determined employing the SpectraMAX M2 microplate reader (Molecular Devices, Sunnyvale, CA). All experiments were carried out in triplicate.

[0142] **Pharmacokinetic (PK) studies:** C57BL/6 mice were weighted and individually dosed with M11 or D7 either subcutaneously at 10 mg/kg dose, or orally (gavage) at 10 mg/kg dose. Subsequently small samples of blood were collected at the indicated time-points and centrifuged (3000 rpm/10 min). Obtained plasma samples were transferred into the 0.5 mL centrifuge tubes and immediately diluted with 4 volumes of a DMSO/ACN mixture (1:1) containing 0.1% of TFA. Subsequently samples were centrifuged at 13,000 rpm for 10 min and obtained supernatants were analyzed using the Agilent 6460

Triple Quadrupole LC/MS System (Agilent Technologies, Santa Clara, CA) with N-methylated M11 (M11-Me) as an internal standard.

[0143] **Plasma stability studies:** Tested analog(s) (10 mM stock solutions in DMSO) were added to freshly prepared mouse plasma (1 μL per 400 μL of plasma, c=25 μM) and incubated at 37 °C. Subsequently small samples of plasma (10 μL) were collected at the indicated time-points and immediately diluted with 200 μL of a DMSO/ACN mixture (1:1) containing 0.1% of TFA. Samples were then centrifuged at 13,000 rpm for 10 min and obtained supernatants were analyzed using the Agilent 6460 Triple Quadrupole LC/MS System (Agilent Technologies, Santa Clara, CA) with N-methylated M11 (M11-Me) as an internal standard.

[0144] **Animal experiments:** All animal experiments were approved by the UCLA Animal Care and Use Committee (ARC#1999-173-23) and conformed to local and national guidelines. Each group consisted 8 experimental animals. For subcutaneous engraftment model experiments, BALB/SCID gnotobiotic mice (8 weeks old, females) were obtained from the UCLA AALAC-accredited Department of Radiation Oncology Facility and subcutaneously injected with 2.0x106 cells of human metastatic breast cancer line (MDA-MB-231, leg). After 3 weeks, palpable tumors of approximately 5 mm diameter appeared and treatment was initiated. In general, each animal received either subcutaneously or orally a total of 10 doses of the peptide (2% Cremophor EL, Sigma-Aldrich, St Louis, MO) at indicated doses on days 1-5 and 8-12. Control animals were injected with vehicle. Tumor size was assessed with calliper and its volume calculated using formula: V=L*W2/2 (V=tumor volume, L=length, W=width, L>W) and animals sacrificed as necessary according to the UCLA Animal Care guidelines.

[0145] **FTIR experiments:** Infrared spectra of peptides were recorded at 25 °C using a Bruker Vector 22™ FTIR spectrometer with a DTGS detector and averaged over 256 scans at a gain of 4 with a resolution of 2 cm^{-1}. Peptides were initially freeze-dried several times from 10 mM HCl in D_2O to remove any interfering counter ions and residual H_2O. The peptides were lipid-peptide conjugates and were examined as self-films or in POPC multilayer films that resemble membrane like environments using Attenuated Total Reflectance (ATR) FTIR. Self-films or multilayer lipid-peptide films were prepared by drying the lipo-peptide or mixtures of POPC with peptide (10:1, mole:mole, lipid:peptide ratios) in hexafluoroisopropanol and dried onto a 50×20×2 mm, 45° ATR crystal (Pike Technologies, Madison, WI). Residual solvent was removed from the sample with a stream

of dry nitrogen gas. After evaporation of the solvent, lipid:peptide film was hydrated by passing deuterium-saturated nitrogen gas through the sample chamber for one hour prior to spectroscopy to ensure hydration of the sample. The relative proportions of α-helix, turn, β-sheet, and disordered conformations of solution and multilayer IR spectra were determined by Fourier deconvolution for band narrowing and area calculations of component peaks of the FTIR spectra using curve-fitting software supplied by Galactic Software (GRAMS/AI, version 8.0; Thermo Electron Corp., Waltham, MA). Frequency limits for the different structures were: α-helix (1662-1645 cm-1), β -sheet (1637-1613 and 1710-1682 cm-1), turns (1682-1662 cm-1), and disordered or random (1650-1637 cm-1).

Table 3 Analytical data for synthesized Smac analogues (M-monomers, D-dimers).

Compound	R	Composition	MW Calc / Found	R_T [min]	EC_{50} [μM] MDA-MB-231
M1	Ser^{OH}	$C_{25}H_{32}N_4O_4$	452.55 / 452.08	31.01	NA
M2	Cys^{SH}	$C_{25}H_{32}N_4O_3S$	468.61 / 469.84	35.03	NA
M3	$Cys^{S\text{-}StBu}$	$C_{29}H_{40}N_4O_3S_2$	556.78 / 557.78	45.56	49.9±0.7
M4	Lys	$C_{28}H_{39}N_5O_3$	493.64 / 494.37	29.13	NA
M5	Lys^{Gu}	$C_{29}H_{41}N_7O_3$	535.68 / 536.15	30.48	NA
M6	$Lys^{NH\text{-}DNFB}$	$C_{34}H_{41}N_7O_7$	659.74 / 659.33	47.51	44.7±15.0
M7	$Lys^{NH\text{-}Fmoc}$	$C_{43}H_{49}N_5O_5$	715.89 / 716.30	51.04	19.0±1.6
M8	$Lys^{NH\text{-}Pal}$	$C_{44}H_{69}N_5O_4$	732.06 / 732.87	65.07*	6.1±0.1
M9	$Lys^{NH\text{-}Lig}$	$C_{52}H_{85}N_5O_4$	844.27 / 845.04	67.02#	NA
M10	$Lys^{NH\text{-}Chol}$	$C_{56}H_{83}N_5O_5$	906.30 / 906.42	64.39#	9.7±0.5
M11	$Cys^{S\text{-}Ste}$	$C_{43}H_{68}N_4O_3S$	721.09 / 722.38	62.76#	4.4±0.1
D1	$Lys^{NH\text{-}Urea}$	$C_{57}H_{76}N_{10}O_7$	1013.29 / 1013.40	41.53	NA
D2	$Lys^{NH\text{-}Sub}$	$C_{64}H_{88}N_{10}O_8$	1125.46 / 1125.29	41.85	NA
D3	$Lys^{NH\text{-}DFDNB}$	$C_{62}H_{78}N_{12}O_{10}$	1151.37 / 1152.79	49.52	3.3±0.2
D4	$Lys^{NH\text{-}PDI}$	$C_{64}H_{82}N_{12}O_8$	1147.43 /1147.49	42.62	34.8±2.7
D5	$Lys^{NH\text{-}OPI}$	$C_{70}H_{86}N_{12}O_9$	1239.52 / 1239.88	45.37	14.4±1.4
D6	$Lys^{NH\text{-}Ida}$	$C_{60}H_{81}N_{11}O_8$	1084.37 / 1084.28	35.65*	NA
D7	$Lys^{NH\text{-}Ida\text{-}Pal}$	$C_{76}H_{111}N_{11}O_9$	1322.78 / 1323.54	56.70*	4.2±0.1
D8	$Lys^{NH\text{-}Ida\text{-}N\text{-}EtO\text{-}Pal}$	$C_{78}H_{115}N_{11}O_{10}$	1366.83 / 1366.41	56.77*	4.3±0.1
D9	$Cys^{S\text{-}DVS}$	$C_{54}H_{70}N_8O_8S_3$	1055.37 / 1055.96	43.60	52.4±4.3
D10	$Cys^{S\text{-}CAEDA}$	$C_{56}H_{72}N_{10}O_8S_2$	1077.36 / 1077.49	43.21	NA
D11	$Cys^{S\text{-}pBMB}$	$C_{58}H_{70}N_8O_6S_2$	1039.36 / 1039.21	46.15	9.6±0.6
D12	$Cys^{S\text{-}mBMB}$	$C_{58}H_{70}N_8O_6S_2$	1039.36 / 1039.20	43.15*	7.4±0.4
D13	$Cys^{S\text{-}Bip}$	$C_{64}H_{74}N_8O_6S_2$	1115.46 / 1115.18	46.04*	4.0±0.1
D14	$Cys^{S\text{-}CMPB}$	$C_{64}H_{74}N_8O_7S_2$	1131.46 / 1131.26	48.33	18.4±1.1
D15	$Cys^{S\text{-}BMBB}$	$C_{65}H_{76}N_8O_6S_2$	1129.48 / 1129.63	49.68	NA
D16	$Cys^{S\text{-}mBMPB}$	$C_{75}H_{104}N_8O_6S_3$	1309.88 / 1309.80	62.25#	11.3±0.6

****

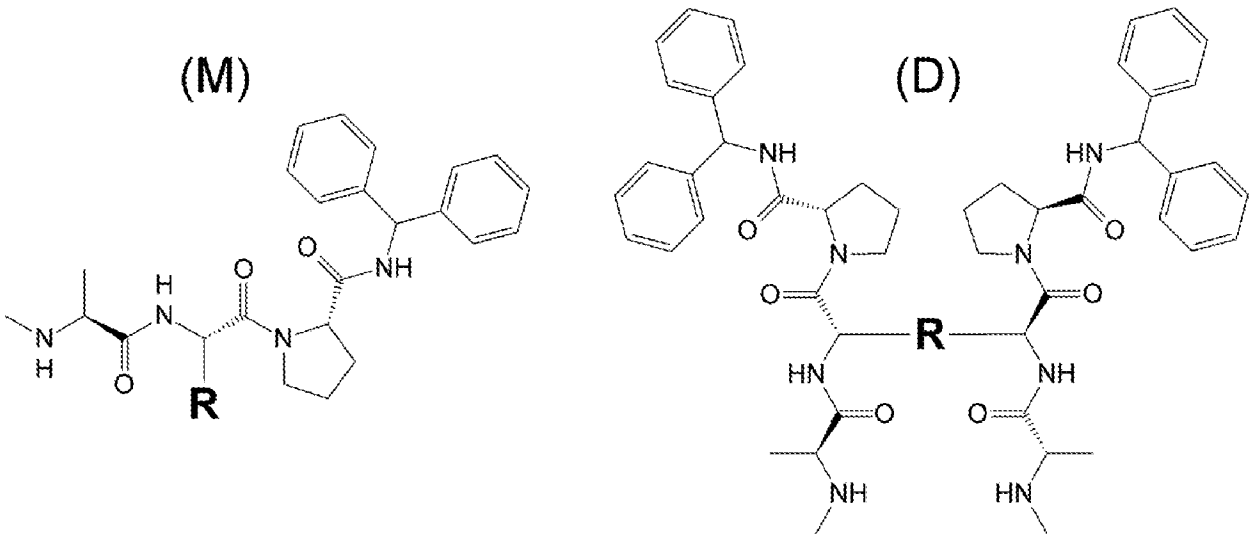
[0146] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be

apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

CLAIMS

1. A method of treating cancer in a subject comprising administering to the subject an effective amount of a 2-lysine- or 2-cysteine-functionalized Smac derivative.

2. The method of claim 1, wherein the 2-lysine- or 2-cysteine-functionalized Smac derivative is a compound of formula M or D:



wherein R is SerOH, CysSH, CysS-StBu, Lys, LysGu, LysNH-DNFB, LysNH-Fmoc, LysNH-Pal, LysNH-Lig, LysNH-Chol, CysS-Ste, LysNH-Urea, LysNH-Sub, LysNH-DFDNB, LysNH-PDI, LysNH-OPI, LysNH-Ida, LysNH-Ida-Pal, LysNH-Ida-N-EtO-Pal, CysS-DVS, CysS-CAEDA, CysS-pBMB, CysS-mBMB, CysS-Bip, CysS-CMPB, CysS-BMBB, or CysS-mBMPB.

3. The method of any of claims 1 or 2, wherein treating cancer is further defined as delaying the growth of a tumor.

4. The method of any of claims 1 to 3, wherein the 2-lysine- or 2-cysteine-functionalized Smac derivative is administered orally, intraadiposally, intraarterially, intraarticularly, intracranially, intradermally, intralesionally, intramuscularly, intranasally, intraocularally, intrapericardially, intraperitoneally, intrapleurally, intraprostaticaly, intrarectally, intrathecally, intratracheally, intratumorally, intraumbilically, intravaginally, intravenously, intravesicularlly, intravitreally, liposomally, locally, mucosally, orally, parenterally, rectally, subconjunctival, subcutaneously, sublingually, topically, transbuccally, transdermally, vaginally, in cremes, in lipid compositions, via a catheter, via a lavage, via continuous infusion, via infusion, via

inhalation, via injection, via local delivery, via localized perfusion, bathing target cells directly, or any combination thereof.

5. The method of any of claims 1 to 4, wherein the 2-lysine- or 2-cysteine-functionalized Smac derivative is a monovalent 2-lysine- or 2-cysteine-functionalized Smac derivative.

6. The method of claim 5, wherein the monovalent 2-lysine- or 2-cysteine-functionalized Smac derivative is functionalized with a lipophilic moiety.

7. The method of claim 6, wherein the lipophilic moiety is a substituted or unsubstituted hydrocarbon comprising at least 6 carbon atoms.

8. The method of claim 6, wherein the lipophilic moiety is selected from the group consisting of palmitoyl, lignoceroyl, stearyl, or cholesteryl.

9. The method of any of claims 1 to 4, wherein the 2-lysine- or 2-cysteine-functionalized Smac derivative is a bivalent 2-lysine- or 2-cysteine-functionalized Smac derivative.

10. The method of claim 9, wherein the bivalent 2-lysine- or 2-cysteine-functionalized Smac derivative comprises two Smac moieties conjugated to each other through the 2-positions.

11. The method of claim 10, wherein the bivalent Smac derivative comprises a lipophilic linker conjugating the two Smac moieties.

12. The method of any of claims 1 to 11, wherein the cancer is melanoma, carcinoma, lymphoma, blastoma, sarcoma, leukemia or lymphoid malignancies, breast cancer, colon cancer, rectal cancer, colorectal cancer, kidney or renal cancer, lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, squamous cell cancer (*e.g.* epithelial squamous cell cancer), cervical cancer, ovarian cancer, prostate cancer, liver cancer, bladder cancer, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, head and neck cancer, glioblastoma, retinoblastoma, astrocytoma, thecomas, arrhenoblastomas, hepatoma, hematologic malignancies including non-Hodgkins lymphoma (NHL), multiple myeloma and acute hematologic malignancies, endometrial or uterine carcinoma, endometriosis, fibrosarcomas, choriocarcinoma, salivary gland carcinoma, vulval cancer, thyroid cancer, esophageal carcinomas, hepatic carcinoma, anal carcinoma,

penile carcinoma, nasopharyngeal carcinoma, laryngeal carcinomas, Kaposi's sarcoma, melanoma, skin carcinomas, Schwannoma, oligodendroglioma, neuroblastomas, rhabdomyosarcoma, osteogenic sarcoma, leiomyosarcomas, urinary tract carcinomas, thyroid carcinomas, Wilm's tumor, as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome

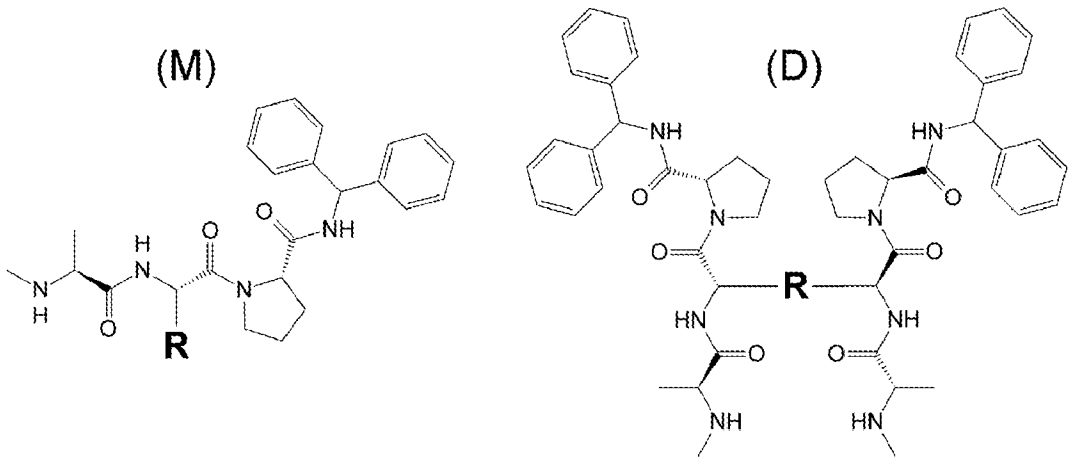
13. The method of claim 12, wherein the cancer is breast cancer.

14. The method of any of claims 1 to 13, wherein said subject is further administered a distinct cancer therapy.

15. The method of any of claims 1 to 14, wherein said distinct cancer therapy comprises surgery, radiotherapy, chemotherapy, toxin therapy, immunotherapy, cryotherapy or gene therapy.

16. The method of any of claims 1 to 15, wherein the cancer is a chemotherapy or radio-resistant cancer.

17. A compound of the formula M or D:



wherein R is SerOH, CysSH, CysS-StBu, Lys, LysGu, LysNH-DNFB, LysNH-Fmoc, LysNH-Pal, LysNH-Lig, LysNH-Chol, CysS-Ste, LysNH-Urea, LysNH-Sub, LysNH-DFDNB, LysNH-PDI, LysNH-OPI, LysNH-Ida, LysNH-Ida-Pal, LysNH-Ida-N-EtO-Pal, CysS-DVS, CysS-CAEDA, CysS-pBMB, CysS-mBMB, CysS-Bip, CysS-CMPB, CysS-BMBB, or CysS-mBMPB, or salt, enantiomer, or diastereomer thereof.

18. The compound of claim 17, wherein the compound is a monovalent 2-lysine- or 2-cysteine-functionalized Smac derivative.

19. The monovalent 2-lysine- or 2-cysteine-functionalized Smac derivative of claim 18, wherein the monovalent 2-lysine- or 2-cysteine-functionalized Smac derivative is functionalized with a lipophilic moiety.

20. The compound of claim 19, wherein the lipophilic moiety is a substituted or unsubstituted hydrocarbon comprising at least 6 carbon atoms.

21. The compound of claim 19, wherein the lipophilic moiety is selected from the group consisting of palmitoyl, lignoceroyl, stearyl, or cholesteryl.

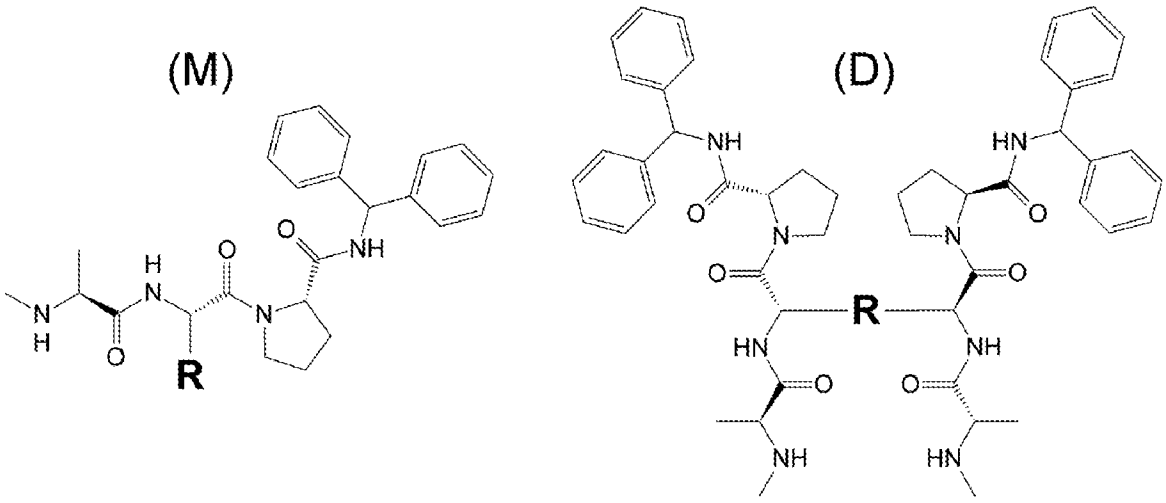
22. The compound of claim 17, wherein the 2-lysine- or 2-cysteine-functionalized Smac derivative is a bivalent 2-lysine- or 2-cysteine-functionalized Smac derivative.

23. The compound of claim 22, wherein the bivalent 2-lysine- or 2-cysteine-functionalized Smac derivative comprises two Smac moieties conjugated to each other through the 2-positions.

24. The compound of claim 23, wherein the bivalent Smac derivative comprises a lipophilic linker conjugating the two Smac moieties.

25. A method of killing or inhibiting the growth of cells comprising contacting the cells with a composition comprising an amount of a 2-lysine- or 2-cysteine-functionalized Smac derivative effective to kill or inhibit the growth of the cells.

26. The method of claim 25, wherein the 2-lysine- or 2-cysteine-functionalized Smac derivative is a compound of formula M or D:



wherein R is SerOH, CysSH, CysS-StBu, Lys, LysGu, LysNH-DNFB, LysNH-Fmoc, LysNH-Pal, LysNH-Lig, LysNH-Chol, CysS-Ste, LysNH-Urea, LysNH-Sub, LysNH-DFDNB, LysNH-PDI, LysNH-OPI, LysNH-Ida, LysNH-Ida-Pal, LysNH-Ida-N-EtO-

Pal, CysS-DVS, CysS-CAEDA, CysS-pBMB, CysS-mBMB, CysS-Bip, CysS-CMPB, CysS-BMBB, or CysS-mBMPB;

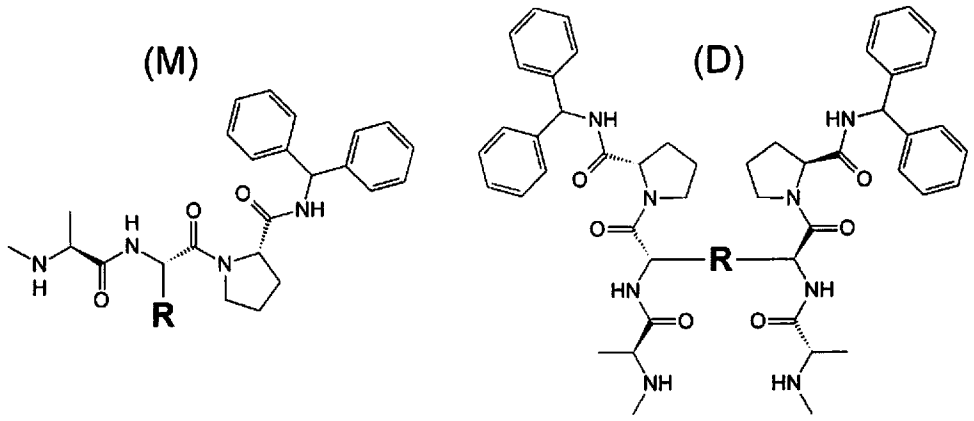
or a salt, prodrug, enantiomer or diastereomer thereof.

27. The method of any of claims 25 to 26, wherein the cells are in a patient's body.

28. The method of any of claims 25 to 27, wherein the cells are cancer cells.

29. The method of any of claims 25 to 28, wherein the cells are in a tumor.

30. The method of any of claims 25 to 29, wherein the tumor is a human breast cancer tumor.



Compound	R	EC_{50} [μM] MDA-MB-231
M1	Ser^{OH}	NA
M2	Cys^{SH}	NA
M3	$Cys^{S\text{-}StBu}$	49.9 ± 0.7
M4	Lys	NA
M5	Lys^{Gu}	NA
M6	$Lys^{NH\text{-}DNFB}$	44.7 ± 15.0
M7	$Lys^{NH\text{-}Fmoc}$	19.0 ± 1.6
M8	$Lys^{NH\text{-}Pal}$	6.1 ± 0.1
M9	$Lys^{NH\text{-}Lig}$	NA
M10	$Lys^{NH\text{-}Chol}$	9.7 ± 0.5
M11	$Cys^{S\text{-}Ste}$	4.4 ± 0.1
D1	$Lys^{NH\text{-}Urea}$	NA
D2	$Lys^{NH\text{-}Sub}$	NA
D3	$Lys^{NH\text{-}DFDNB}$	3.3 ± 0.2
D4	$Lys^{NH\text{-}PDI}$	34.8 ± 2.7
D5	$Lys^{NH\text{-}OPI}$	14.4 ± 1.4
D6	$Lys^{NH\text{-}Ida}$	NA
D7	$Lys^{NH\text{-}Ida\text{-}Pal}$	4.2 ± 0.1
D8	$Lys^{NH\text{-}Ida\text{-}N\text{-}EtO\text{-}Pal}$	4.3 ± 0.1
D9	$Cy^{sS\text{-}DVS}$	52.4 ± 4.3
D10	$Cys^{S\text{-}CAEDA}$	NA
D11	$Cys^{S\text{-}pBMB}$	9.6 ± 0.6
D12	$Cys^{S\text{-}mBMB}$	7.4 ± 0.4
D13	$Cys^{S\text{-}Bip}$	4.0 ± 0.1
D14	$Cys^{S\text{-}CMPB}$	18.4 ± 1.1
D15	$Cys^{S\text{-}BMBB}$	NA
D16	$Cys^{S\text{-}mBMPB}$	11.3 ± 0.6

FIG. 1

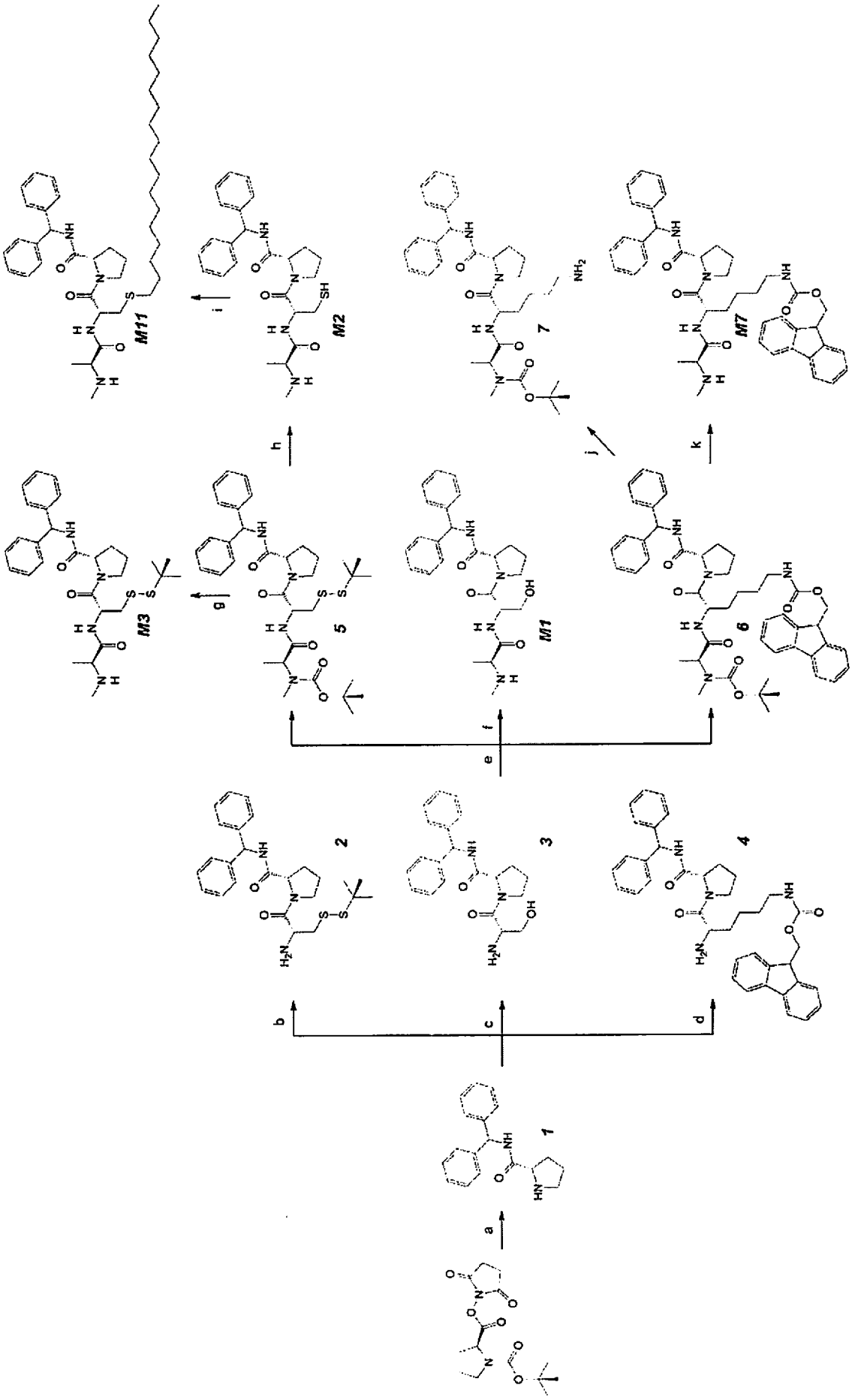


FIG. 2

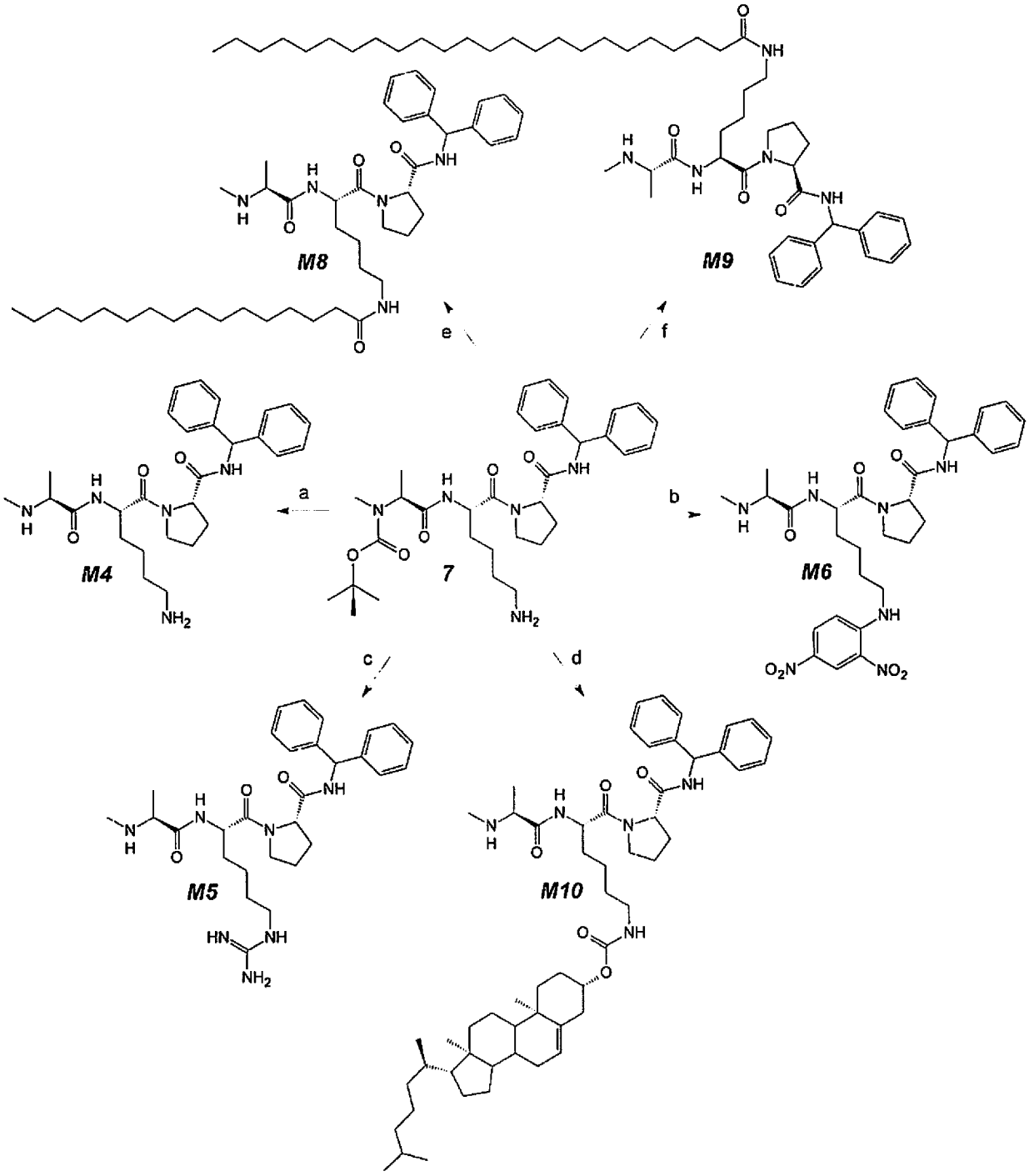


FIG. 3

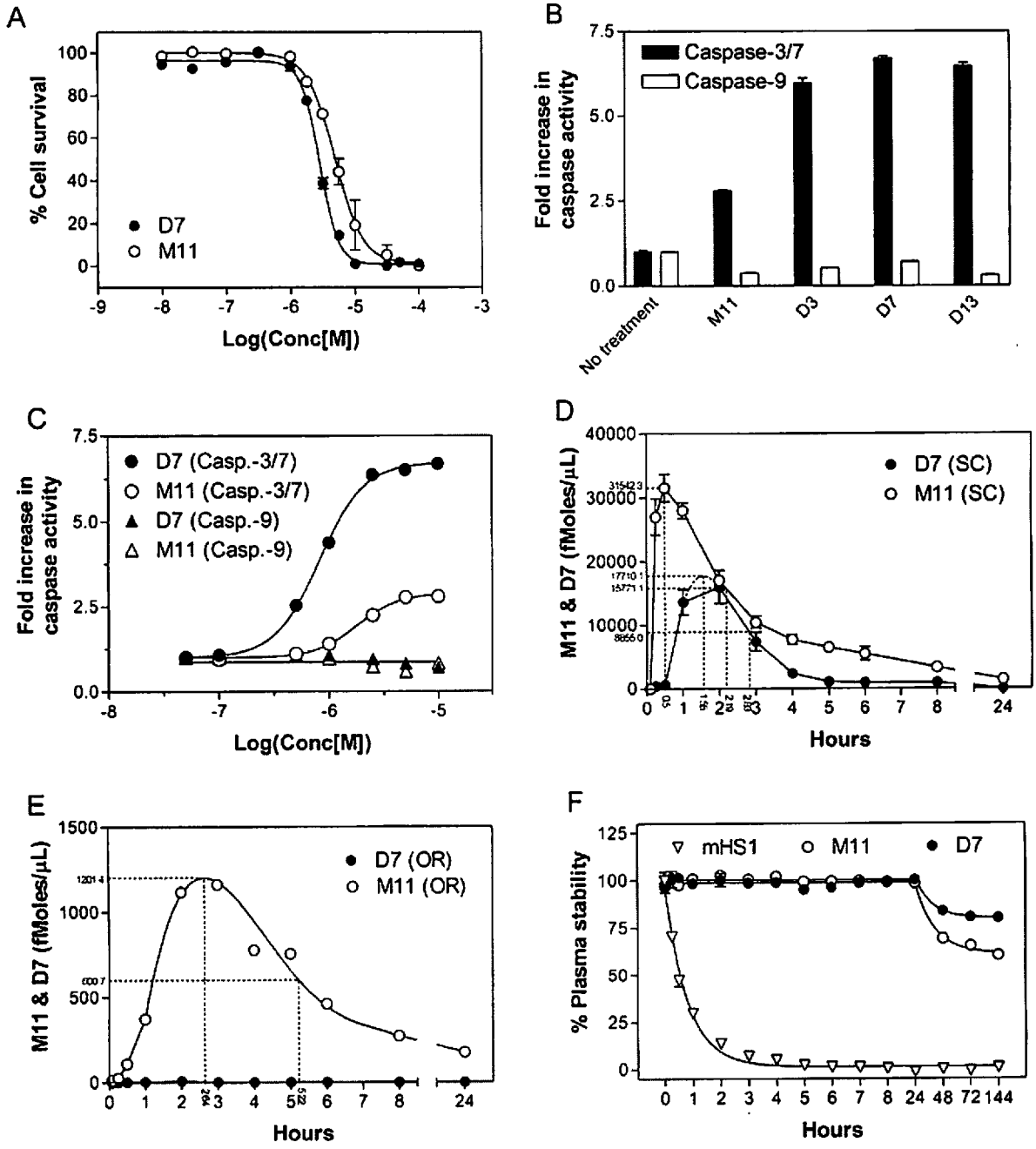

A
% Cell survival
100
80
60
40
20
0
D7
M11
-9 -8 -7 -6 -5 -4 -3
Log(Conc[M])
B
Fold increase in caspase activity
7.5
5.0
2.5
0.0
Caspase-3/7
Caspase-9
No treatment
M11
D3
D7
D13
C
Fold increase in caspase activity
7.5
5.0
2.5
0.0
D7 (Casp.-3/7)
M11 (Casp.-3/7)
D7 (Casp.-9)
M11 (Casp.-9)
-8 -7 -6 -5
Log(Conc[M])
D
M11 & D7 (fMoles/µL)
40000
30000
20000
10000
0
D7 (SC)
M11 (SC)
0 1 2 3 4 5 6 7 8 24
Hours
E
M11 & D7 (fMoles/µL)
1500
1000
500
0
D7 (OR)
M11 (OR)
0 1 2 3 4 5 6 7 8 24
Hours
F
% Plasma stability
125
100
75
50
25
0
mHS1
M11
D7
0 1 2 3 4 5 6 7 8 24 48 72 144
Hours

FIG. 4

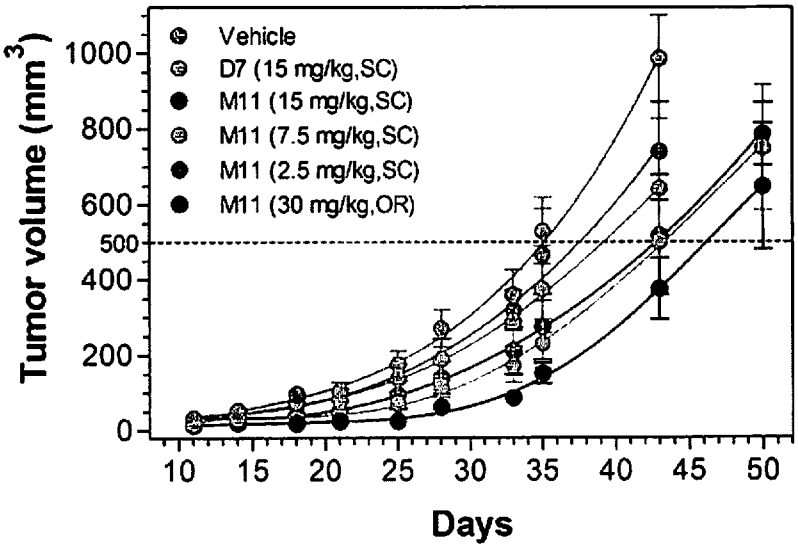
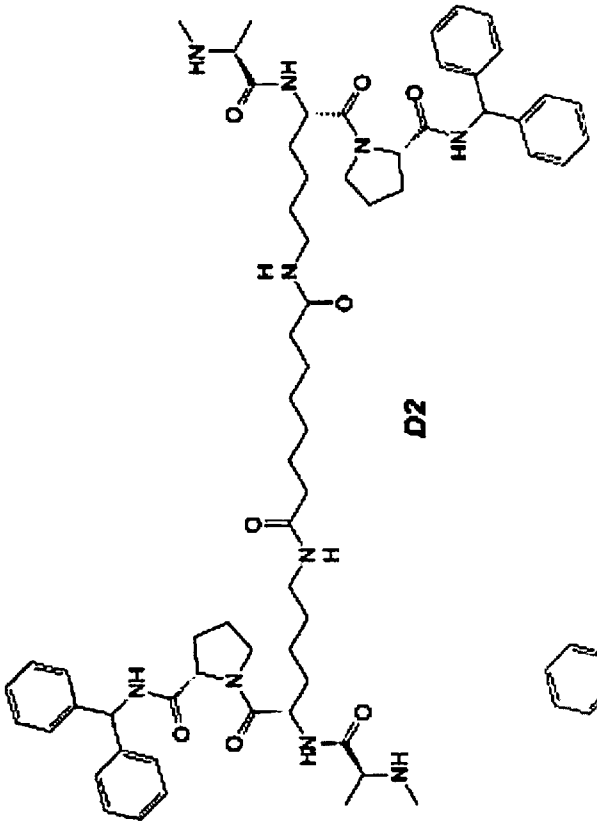
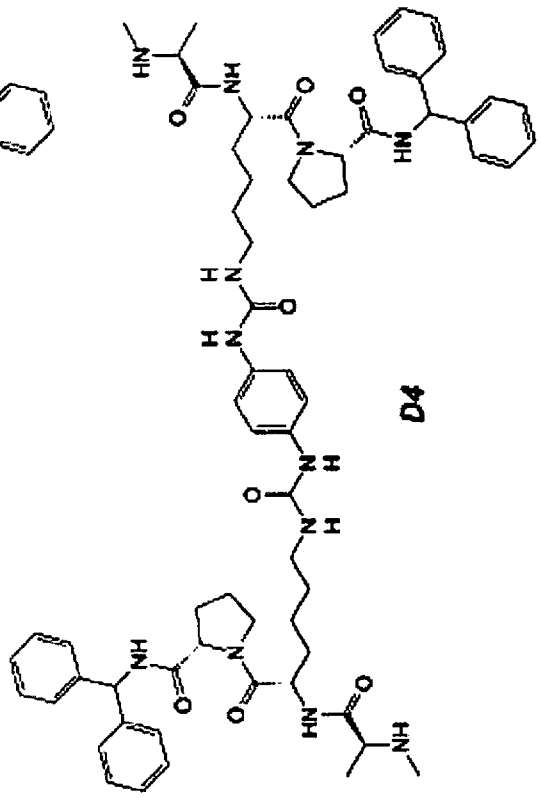
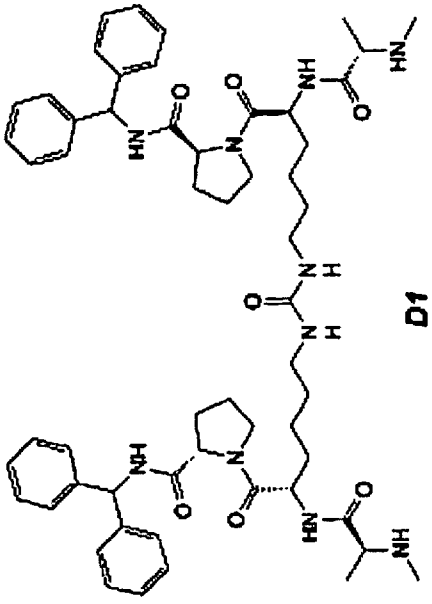

Vehicle
D7 (15 mg/kg,SC)
M11 (15 mg/kg,SC)
M11 (7.5 mg/kg,SC)
M11 (2.5 mg/kg,SC)
M11 (30 mg/kg,OR)
Tumor volume (mm3)
1000
800
600
500
400
200
0
10
15
20
25
30
35
40
45
50
Days

FIG. 5







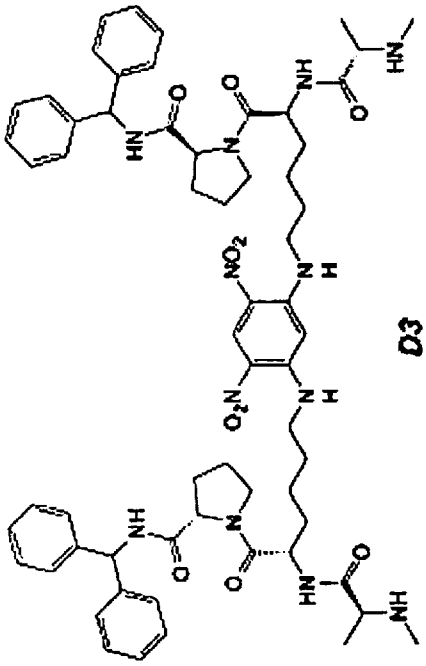


FIG. 6A

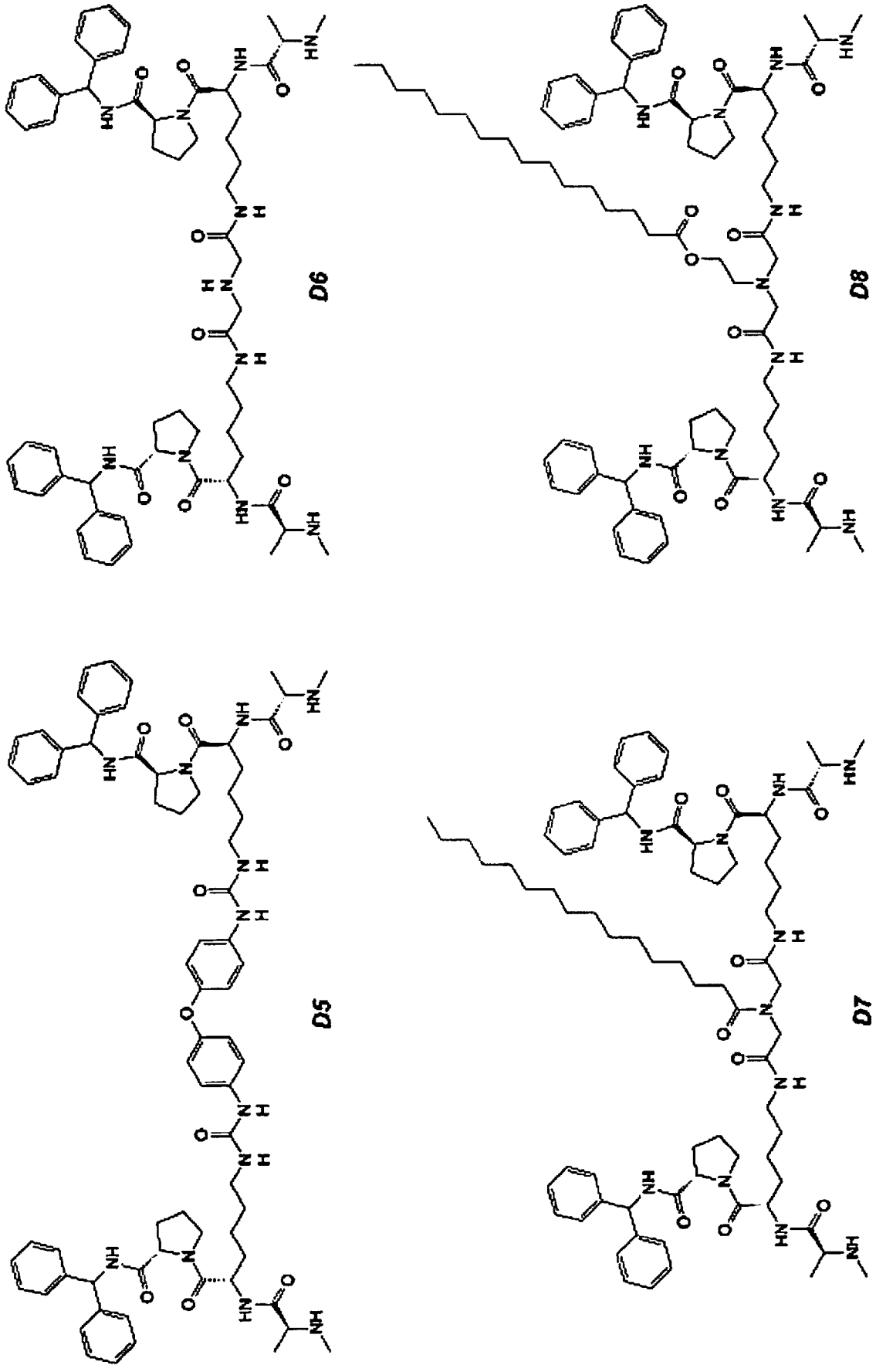
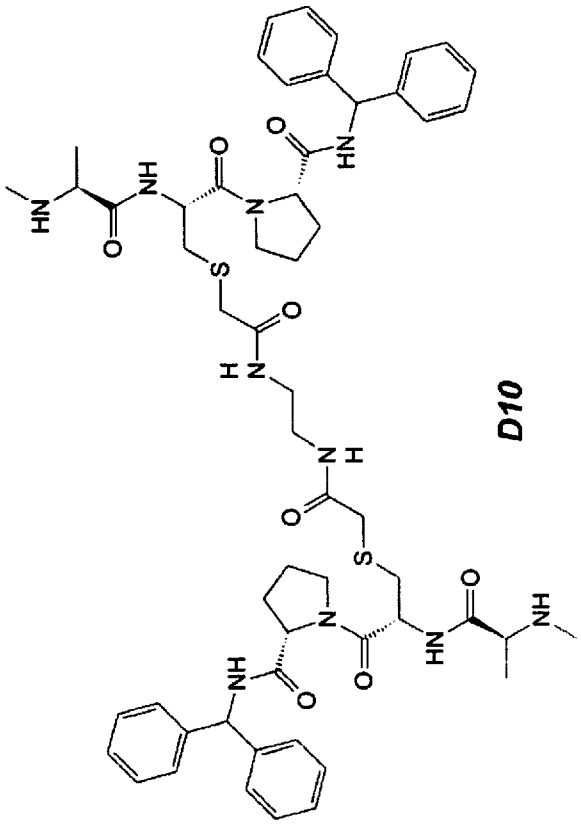
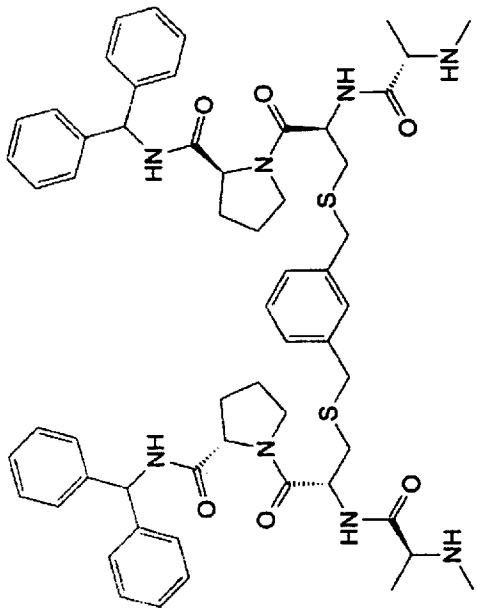


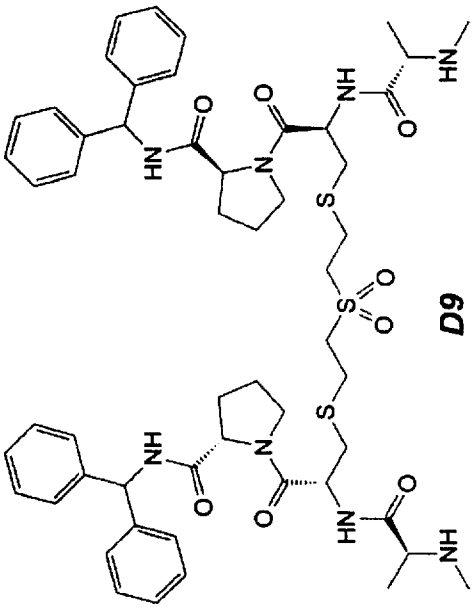
FIG. 6B



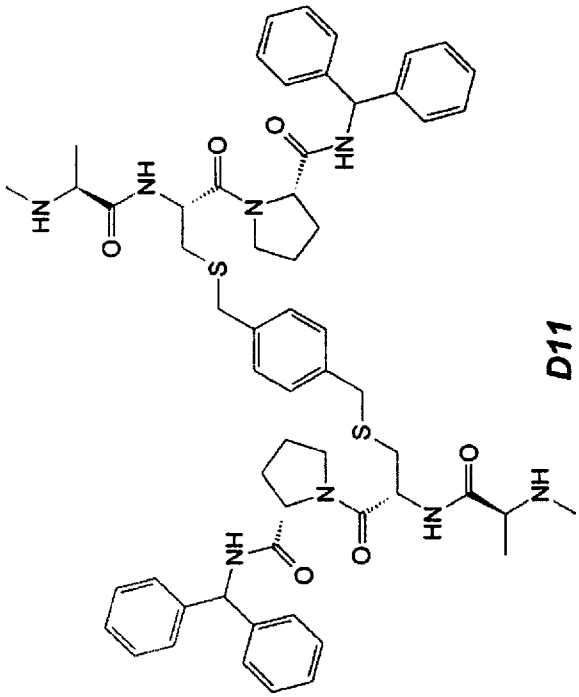
D10



D12



D9



D11

FIG. 7A

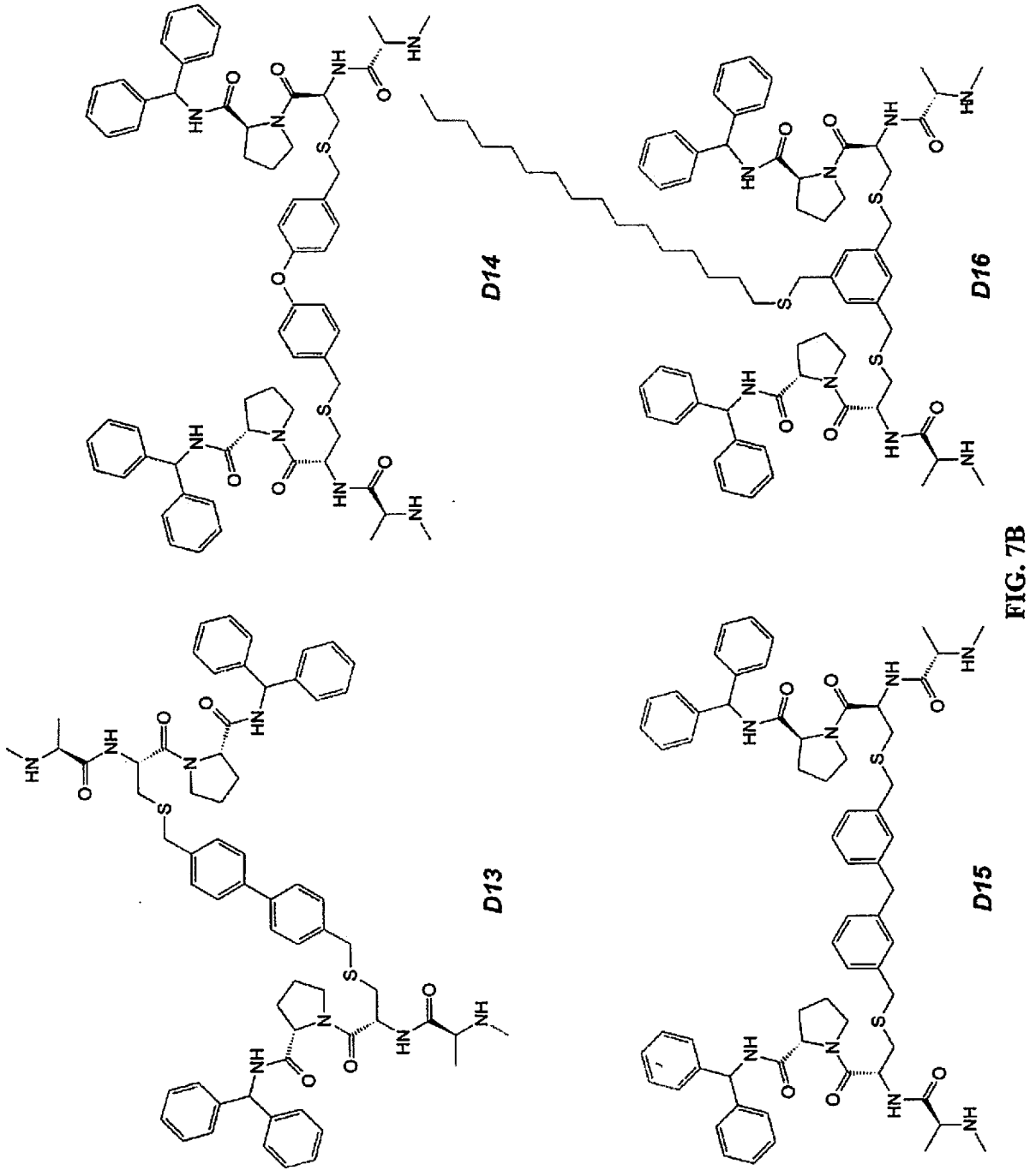


FIG. 7B

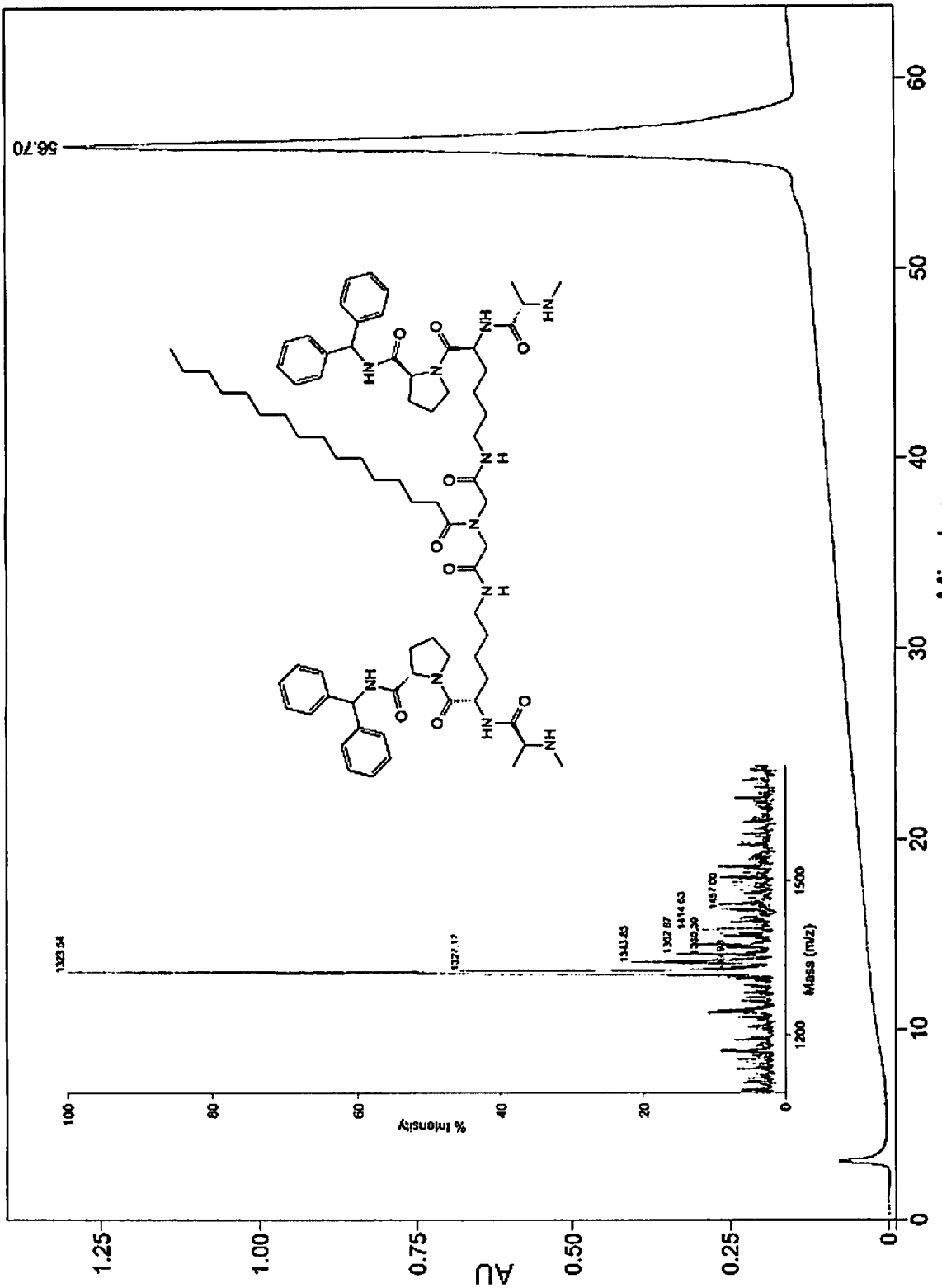

56.70
AU
Minutes
% Intensity
Mass (m/z)
1323.54
1327.17
1343.85
1302.67
1414.63
1330.39
1457.00

FIG. 8A

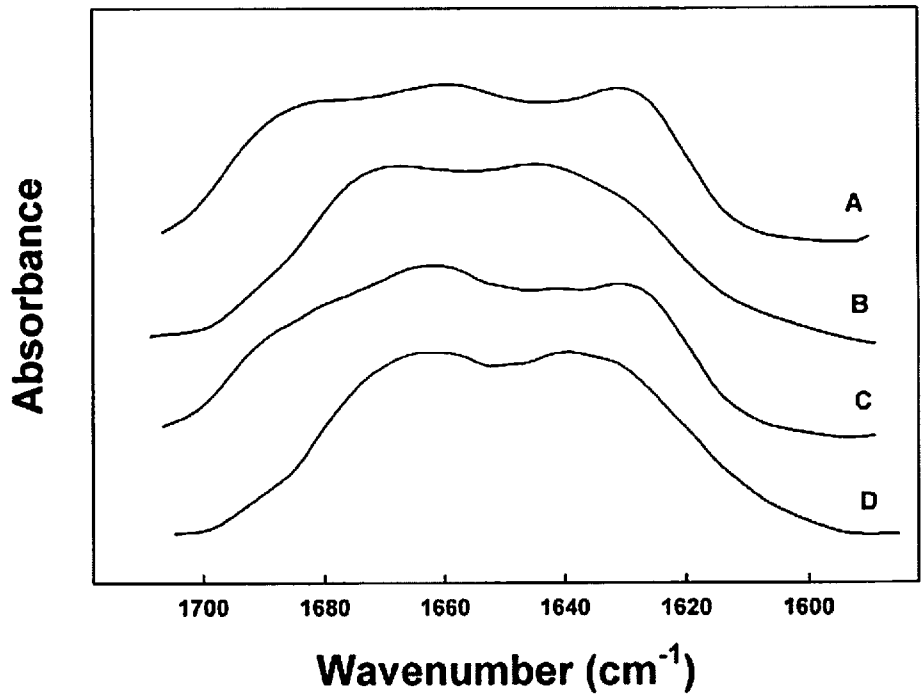

Absorbance
A
B
C
D
1700
1680
1660
1640
1620
1600
Wavenumber (cm-1)

FIG. 8B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/50720

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07D 207/06; C07K 5/06 (2016.01)
CPC - C07D 207/06; C07K 5/06, 5/103
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61P 35/00; C07D 207/06; C07K 5/06 (2016.01)
CPC: C07D 207/06; C07K 5/06, 5/103 USPC: 514/18.9; 548/538

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PATSEER (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, Other Countries (INPADOC), RU, AT, CH, TH, BR, PH); EBSCO; Google Scholar; IP.com; SureChEMBL; KEYWORDS: Smac mimetic*, treat* cancer*, monovalen*, monovalen* Smac, di valen*, lipid* Smac, lysine 2, cysteine 2, IAP*, induc* apopto*, tetrapeptide* derivative*, amino acid*, pyrrolidin*, lysine, lys, cysteine, cys

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2010/0261914 A1 (SHI, Y et al.) 14 October 2010; paragraphs [0017]-[0023], [0034]-[0035], [0071]	1-2, 3/1-2, 17-26, 27/25-26
A	US 2010/0190688 A1 (CHAO, B et al.) 29 July 2010; paragraphs [0001], [0029]-[0034], [0036]-[0037], [0039]-[0041], [0045], [0086], [0090]	1-2, 3/1-2, 17-26, 27/25-26
A	US 2010/0093645 A1 (WANG, S et al.) 15 April 2010; paragraphs [0002], [0021], [0135]	1-2, 3/1-2, 17-26, 27/25-26
A	MICEWICZ, ED et al., Novel dimeric Smac analogs as prospective anticancer agents, Bioorganic & Medicinal Chemistry Letters 24, pages 1452-1457, 2014; abstract; page 1453, figure 1, table 1	1-2, 3/1-2, 17-26, 27/25-26

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
14 October 2016 (14.10.2016)	28 OCT 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/50720

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-16 and 28-30
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.