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Journal
Bioorganic & Medicinal Chemistry Letters, 29(13)

ISSN
0960-894X

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Publication Date
2019-07-01

DOI
10.1016/j.bmcl.2019.04.041

Peer reviewed

HHS Public Access

Author manuscript

Bioorg Med Chem Lett. Author manuscript; available in PMC 2020 July 01.

Published in final edited form as:

Bioorg Med Chem Lett. 2019 July 01; 29(13): 1628–1635. doi:10.1016/j.bmcl.2019.04.041.

Position of lipidation influences anticancer activity of Smac analogs

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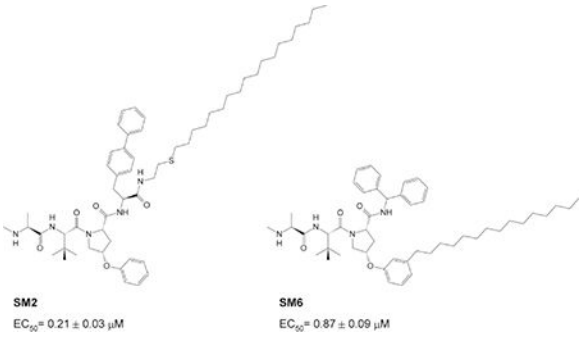
Abstract

A small group of lipid-conjugated Smac mimetics was synthesized to probe the influence of the position of lipidation on overall anti-cancer activity. Specifically, new compounds were modified with lipid(s) in position 3 and C-terminus. Previously described position 2 lipidated analog M11 was also synthesized. The resulting mini library of Smacs lipidated in positions 2, 3 and C-terminus was screened extensively *in vitro* against a total number of 50 diverse cancer cell lines revealing that both the position of lipidation as well as the type of lipid, influence their anti-cancer activity and cancer type specificity. Moreover, when used in combination therapy with inhibitor of menin−MLL1 protein interactions, position 2 modified analog SM2 showed strong synergistic anti-cancer properties. The most promising lipid-conjugated analogs SM2 and SM6, showed favorable pharmacokinetics and *in vivo* activity while administered subcutaneously in the preclinical mouse model. Collectively, our findings suggest that lipid modification of Smacs may be a viable approach in the development of anti-cancer therapeutic leads.

Graphical Abstract



Keywords

Smac mimetics; Anticancer agents; Lipid-conjugated peptides; S-Alkylation of peptides; Apoptosis; Inhibitor of menin—MLL1 protein interactions

Apoptosis, (programmed cell death, PCD) is a physiologically important mechanism controlling homeostasis, host defense, normal development, and the suppression of oncogenesis. Defects in apoptosis are associated with various human pathologies including cancer[1–5], neurodegeneration[6;7], and inflammatory and autoimmune diseases[8;9]. Apoptosis is regulated by Inhibitors of Apoptosis Proteins (IAPs)[10–12] which contain one or more of Baculovirus IAP Repeat (BIR) domains[12;13]. BIRs are capable of binding to and inhibiting various caspases, enzymes belonging to cysteine–aspartyl proteases family, which are crucial for the apoptotic process[14]. Several mammalian IAPs have been identified to date including: 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. XIAP appears to be the most potent caspase inhibitor family member[15;16] which effectively inhibits three caspases: caspase-3, −7, and −9[17–20]. IAPs function is in turn regulated by the second mitochondria derived activator of caspases (Smac), also called direct IAP binding protein with low pI (DIABLO)[21;22] which has been identified as an endogenous pro-apoptotic antagonist of IAP proteins promoting programmed cell death[21–25]. Specifically, N-terminal tetrapeptide AVPI (Ala^1-Val^2-Pro^3-Ile^4) of Smac, so called binding motif[21;22], is responsible for its pro-apoptotic effects, as its binding to BIR2 and/or BIR3 domains abrogates the inhibition of caspases-3, −7, and −9[24;26]. In the case of XIAP, both BIR2 and BIR3 domains are targeted by the homodimeric form of Smac while for cIAP1 and cIAP2 only BIR3 domain is engaged by a single AVPI binding motif[27].

Mimetics of the second mitochondria derived activator of caspases (Smac) are promising therapeutic modalities in anticancer treatment[28–47] with several analogs advancing into clinical trials[32;41;42;46;48–50]. Various monovalent and bivalent Smac analogs have been synthesized to date showing high potency, with bivalent compounds being particularly active[35–37;39;43;44;47], due to their ability to bind both BIR2 and BIR3 XIAP domains.

We recently described potent, lipid-conjugated analogs of Smac[51] with one compound (**M11,** Fig. 1) showing oral availability in murine model. Such encouraging results prompted our further systematic investigation of Smacs' lipidation.

Lipidation is often used in the drug development, including therapeutic peptides[51–55] leading to new analogs with different pharmacological properties. Specifically, lipid-conjugation may result in increased oral availability[56;57] and improved peptide stability, permeability and intestinal absorption[54;58–66].

Mechanistically, attachment of the lipid moiety to therapeutic entity improves its binding to albumin[58;67–69], which in turn increases the drug's presence in circulation. Additional effects such as targeted excretion by the liver[57;58;66] as well as interactions with high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were also described[70].

We previously established[51] that lipidation of Smac analogs in position 2 is a viable method of modification. To ascertain the further utility of this approach we decided to synthesize additional analogs lipidated in position 3 and C-terminus. As changes in position 1 (N-terminus) of Smacs are not well tolerated[40;71] we decided not to probe such modifications.

Generally position 3 modified analogs had a sequence NMeAla-Tle-cis-^{4X}Pro-BHA, (**SM4, SM6**) or NMeAla-Tle-cis-^{4X}Pro-DPEA, (**SM5, SM7**), where X was either hexadecylthio- or 3-pentadecylphenoxy-moiety (NMeAla-(N-methyl)alanine, Tletert-leucine, BHA-benzhydrylamine, DPEA-2,2-diphenylethylamine). The core structure of these peptides is closely related to various potent analogs developed by *Wang group*[35;37–39;43;44]. The *cis*-configuration in position 4 of the substituted proline (*i.e. cis*-^{4X}Pro) was chosen in consensus with the structure of the previously described monovalent potent compound, NMeAla-Tle-(4S)-4-phenoxy-Pro-(R)-tetrahydronaphth-1-yl-amide (K_d = 5 nM) which was developed in Abbott Laboratories[40] (Fig. 1). The sequence of C-terminally lipidated compounds (**SM2, SM3**) was based on the modified analogs we previously described[47]. In this case we decided to use the following sequence NMeAla-Tle-(4S)-4-phenoxy-Pro-Bip-NHCH$_2$CH$_2$-SH, containing both cis-4-phenoxy-proline and biphenylalanine (Bip). The C-terminal cysteamide provided means for further modification/lipidation based on the thiol group reactivity. This report describes the synthesis and biological properties of these novel compounds.

Smac mimetics lipidated in position 3 (**SM4-SM7**) were synthesized as either C-terminal benzhydryl-amides (BHA) or C-terminal 2,2-diphenylethyl-amides (DPEA). Synthesis was carried out in solution according to reaction Scheme 2, using if necessary, the CEM Liberty automatic microwave peptide synthesizer (CEM Corporation Inc., Matthews, NC) which was operated 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). C-terminus-lipidated analogs (**SM2, SM3**) were synthesized sequentially from the non-lipidated parental compound (**SM1**) which was first S-alkylated giving **SM2,** which in turn was oxidized producing the corresponding sulfone (**SM3**) (see reaction Scheme 1). Parental analog **SM1** (containing C-terminal cysteamide) was synthesized by the solid phase method using the CEM Liberty automatic microwave peptide synthesizer (CEM Corporation Inc., Matthews, NC), and 9-fluorenylmethyl-oxycarbonyl (Fmoc) chemistry with cysteamine 4-methoxytrityl resin as a solid support (MilliporeSigma, Burlington, MA). A similar synthetic strategy was also used in the case of **MEV** analogs: **MEV1** was synthesized on the solid support using Fmoc chemistry

(cysteamine 4-methoxytrityl resin) and was subsequently S-alkylated giving **MEV2**. **M11** was synthesized as previously described[51]. All compounds were purified by preparative reverse-phase high performance liquid chromatography (RP-HPLC) to >95% homogeneity and their purity was evaluated by the electrospray ionization mass spectrometry (ESI-MS) as well as an analytical RP-HPLC.

Detailed experimental methods, analytical data for obtained peptides as well as an example of MS-spectra and the corresponding analytical RP-HPLC profile are presented in Supplementary material.

Generally, synthesis of analogs **SM1-SM7** proceeded efficiently and was carried out with minimal purification of the intermediates due to the simplicity of the final products. Overall, three different lipids were used to modify the desired compounds. C-terminal modification of **SM1** was carried out exclusively using the stearyl chain (C_{18}) afforded by 1-bromooctadecane (see Scheme 1). The S-alkylated compound **SM2** was synthesized using a previously described 1,1,3,3-tetramethylguanidine (TMG) driven alkylation of thiol(s) in organic solvents[72] that we adapted to peptides[47;51;73]. Notably, S-alkylation of **MEV1** was carried out in a similar manner, however 2.2 eq. of 1-bromooctadecane and 3 eq. of TMG were used (Scheme 3). Oxidation of thioether-containing compound **SM2** to corresponding sulfone (**SM3**) was carried out using Oxone® ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$, 3 eq.) in methanol/water (9:1) mixture for 5 hours[74].

Preliminary screen of new Smac mimetics was carried out exclusively *in vitro* using growth inhibition assay (PrestoBlue™, Invitrogen, Carlsbad, CA) and various cancer cell lines. In our view this approach 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 not only binding potency but also cell permeability, stability in the cell's microenvironment, the compounds' solubility, etc. Notably, we successfully used the same workflow before in studies that yielded **M11** and potent bivalent analog **SMA17–2X**[47;51]. Obtained results are summarized in Tables 1&2 and an example of cell growth curves is presented in Fig. 2. Initially compounds **SM1-SM7** and **M11**[51] (orally active Smac mimetic lipidated in position 2) were tested against a set of 20 diverse cancer cell lines including: breast cancer, liver cancer, leukemia, lymphoma, melanoma, prostate cancer, colon cancer and head & neck cancer (for full list see Table 2) which were arbitrarily selected. Analysis of obtained results suggested that the C-terminal lipidation strategy produced analogs with greater therapeutic potential which in turn prompted us to test compounds **SM1-SM3** against additional cancer cell lines (in total, 50 cancer cell lines were tested, see Table 1 for complete list). Orally available analog **M11** was also tested in this additional set. Generally, obtained results suggest that the position of lipidation as well as the type of lipid, influence bioactivity. In most cancer cell lines, the highest bioactivity was observed for analogs **SM2/SM3** (C-terminal lipidation), followed by **SM6/SM7** (position 3 lipidation with 3-pentadecylphenoxy-moiety) which were slightly more potent than position 2 modified analog **M11**. In the case of 3-pentadecylphenoxy-lipid-modified compounds, C-terminal benzhydryl-amide (BHA) seems to be preferred moiety (**SM6**) over C-terminal 2,2-diphenylethyl-amide (DPEA) (**SM7**). Position 3 lipidation with hexadecylthio-group does not appear to be a good modification strategy as analogs **SM4** and **SM5** show low

bioactivity. However, in this case the preference for the type of C-terminal amide seems to be reversed as DPEA containing **SM5** appears to be generally more active than BHA containing **SM4**. Oxidation of a thioether group to the corresponding sulfone (**SM2** *versus* **SM3**) also affects bioactivity but observed effects seem to depend on the type of cancer cell line. Specifically, in most tested breast cancer cell lines improvement in bioactivity was observed due to oxidation, and a reversed trend was present in leukemia and the majority of prostate cancer cell lines, with limited influence observed for pancreatic and head & neck cancer cell lines. Lipid-conjugation in position 4 (**SM2** *versus* **SM1**) generally appears to be beneficial although a reverse effect was also observed in the majority of prostate cancer and some lymphoma cell lines. Similar results were also observed before for position 2 lipidation[51]. As *in vitro* bioactivity of our compounds varies, with EC_{50} values from ~140 nM to 63.1 μM depending on the cancer cell line, it is difficult to draw clear conclusions regarding their utility as the same compound may be particularly active against one cancer cell line (e.g. **SM2**: FaDu/EC_{50}=0.19±0.02 μM) and virtually inactive against another (e.g. **SM2**: LNCaP/EC_{50}=63.1±5.7μM, VCaP/NA). Nonetheless, it is important to note that the reported to date *in vitro* results for various Smac mimetics showed even better bioactivity which in some cases was in low nanomolar range[35;39;43–45;47] (i.e. 16: IC_{50}= 0.9±0.2 nM[44], 24: IC_{50}= 1.2±0.3 nM[39], 13: IC_{50}= 3.4±0.6 nM[43], etc.).

Analysis of the data revealed also that analog **SM2** exhibits significant bioactivity against various leukemia cell lines, including mixed lineage leukemia (MLL) cell lines KOPN-8 and Molt-4. Since acute leukemias with translocations of the MLL gene constitute about 5% to 10% of acute leukemias in adults[75] and 70% of acute leukemias in infants[76] and remain mostly incurable diseases[75;77], we decided to test whether **SM2** shows synergistic effects when used in combination therapy with the inhibitor of menin−MLL1 protein interactions, (such interactions are crucial for leukemogenesis in the case of MLL[78]). To this end, we used an analog **MEV2** which is modified/double-lipidated derivative of the previously described compound **MCP-1**[79]. Results are summarized in Table 3. In this case, selected leukemia and lymphoma cell lines were treated with **MEV1** (non-lipidated precursor), **MEV2**, **SM2** and an equimolar mixture of **MEV2** and **SM2**. Findings show that indeed in many cases using the Smac/menin−MLL1 inhibitor combination therapy may be beneficial (KOPN-8, MV-4–11, Nalm-6, SEM, CEM-R, CEM-TL, THP-1, TF-1), however results may vary depending on the type of cancer. To confirm those results we performed an additional flow cytometry apoptosis assay on the KOPN-8 leukemia cell line which has shown promising results in the preliminary cell growth inhibition assay. The KOPN-8 cells were treated with either 1 μM or 10 μM concentrations of **SM2**, **MEV2**, or an equimolar mixture of **SM2** and **MEV2**, cultured for a specific period of time and subsequently stained with the annexin V and propidium iodide (PI). Obtained time course samples were assayed using the BD LSRFortessa™ cell analyzer (BD Biosciences, San Jose, CA). Results (Fig. 3) confirmed that indeed there are strong synergistic effects of lipidated Smac/menin−MLL1 inhibitor combination therapy since an equimolar mixture of **SM2** & **MEV2** promoted markedly higher levels of apoptosis measured as annexinV+/PI+ double positive population in all time points (see Fig. 3B).

To assess whether observed bioactivity of our lipidated Smac mimetics is indeed due to an increase in apoptosis, we measured enzymatic activity of caspases-3/7 and −9 in a metastatic breast cancer cell line, MDA-MB-231 and the MLL rearranged leukemia cell line, KOPN-8, that were treated with various concentrations (0–50 μM) of analogs: **M11**, **MEV2**, **SM2** and **SM6**. Interestingly, in the case of MDA-MB-231 cells, only caspase-3/7 seem to be selectively affected by the treatment with lipidated Smac analogs (Fig. 4), regardless of the position of lipidation resulting in a ~6.4.7÷10.0 fold increase in enzymatic activity. Nonetheless, the most robust response seems to be generated by position 3 lipidated compound **SM6** (~9.3 fold increase). Those results are in line with our previous findings[51] which reported the same caspase-3/7 specificity for both monomeric and dimeric position 2 lipidated Smacs. Moreover, potent effects observed for all lipid modified Smac derivatives are still approximately 30% less effective than our previously described tail-to-tail dimer, **SMAC17–2X**[47]. In addition, menin−MLL1 inhibitor **MEV2** had no effect on the promotion of apoptosis in the MDA-MB-231 cancer cells. However, in the KOPN-8 mixed lineage leukemia cell line only **MEV2** shows any significant increase in enzymatic activity of caspase-3/7 (~3.6×) which perhaps is not surprising since **MEV2** was designed to interfere with menin−MLL1 protein interactions. Similarly to Smacs, **MEV2** does not affect caspase-9 driven apoptosis. In the same system lipidated Smacs exhibit very limited effects (**SM2**: ~1.5 and **SM6**: ~1.4 fold increase, **M11**: inactive).

To characterize further the most promising lipidated analogs **SM2** and **SM6**, we performed preliminary pharmacokinetic (PK) studies in the mouse model. Experimental animals were individually weighed and subsequently received a single subcutaneous (SC) dose (10 mg/kg) of each compound. Blood samples were collected at specified time points *via* retro-orbital bleeding and analyzed using the Agilent 6460 Triple Quadrupole LC/MS System (Agilent Technologies, Santa Clara, CA). For analog **SM2** observed plasma half-life ($t_{1/2}$) is ~28.8±1.0 h (Fig. 5A) and for the compound **SM6** is $t_{1/2}$≈39.9±1.0 h (Fig. 5). Those figures are significantly higher than previously observed values for position 2 lipidated monomeric analog **M11** ($t_{1/2}$≈2.2 h), and also its dimeric counterpart **D7** ($t_{1/2}$≈2.8 h)[51].

The utility of analogs **SM2** and **SM6** was tested further in the preclinical subcutaneous engraftment mouse model *in vivo*. Both analogs were administered subcutaneously at 10 mg/kg or 20 mg/kg doses (2×5 injections) in 2% Cremophor EL (SigmaAldrich, St Louis, MO). The treatment of the experimental, cancer bearing animals with both **SM2** and **SM6** resulted in a dose dependent anticancer response (Fig.5). The treatment of animals with 10 doses of both lipidated Smacs at the escalating dosage from 10 to 20 mg/kg showed progressively longer tumor growth delay values (Table 4) reaching ~8.0 and ~8.9 days of delay for SM2 and SM6 respectively at the 20 mg/kg dose. These results are slightly lower than values previously reported for position 2 lipidated analog **M11** which exhibited ~11.0 days of tumor growth delay at 15 mg/kg dose[51]. Moreover, lipidated Smac derivatives are significantly less active than previously described dimeric derivatives, including **SMAC17–2X**[47] with reported tumor growth delay values: ~10.2 days at 2.5 mg/kg dose and ~23.4 days at 7.5 mg/kg dose. Similarly, more promising *in vivo* activity profiles were also reported for **27**[39], **SM-164**[35], and **SM-1200**[44] with the latter providing complete and durable tumor regression in the preclinical animal model. Despite of lower potency, our data suggest that

lipid modification of Smac mimetics is a viable approach as the specific position of lipidation strongly affects anti-cancer properties/specificity. This provides the means for a “personalized” approach to treatment, especially, as a component(s) in combination therapy. In our view such promising properties warrant further experimentation.

In conclusion, a novel family of monomeric anticancer Smac mimetics lipidated in positions 3 and C-terminus, was synthesized, and characterized *in vitro* and *in vivo*. An extensive screen for anticancer activity against various human cancer cell lines was performed revealing the role of the position of lipidation in overall anti-cancer activity and cancer type specificity. Selected analogs, **SM2** and **SM6**, were characterized further in murine model showing favorable pharmacokinetics, and *in vivo* efficacy. Moreover, **SM2** showed strong synergistic effects when used in combination with inhibitor of menin−MLL1 protein interactions. Collectively, our findings suggest that lipid modification of Smac mimetics is a viable approach in the development of novel anticancer candidates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This project was partially supported by funds from the Adams and Burnham endowments provided by the Dean’s Office of the David Geffen School of Medicine at UCLA (PR) and the NIH/NIAID award 5U19AI067769 (EDM and WHM).

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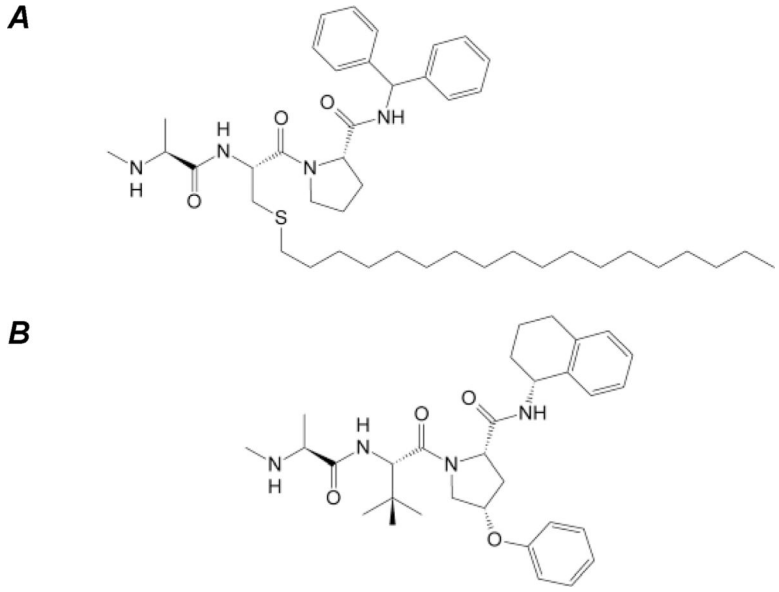


Figure 1.

Structures of (A) orally available Smac analog M1151 and (B) a potent monovalent analog developed by Abbott Laboratories[40].

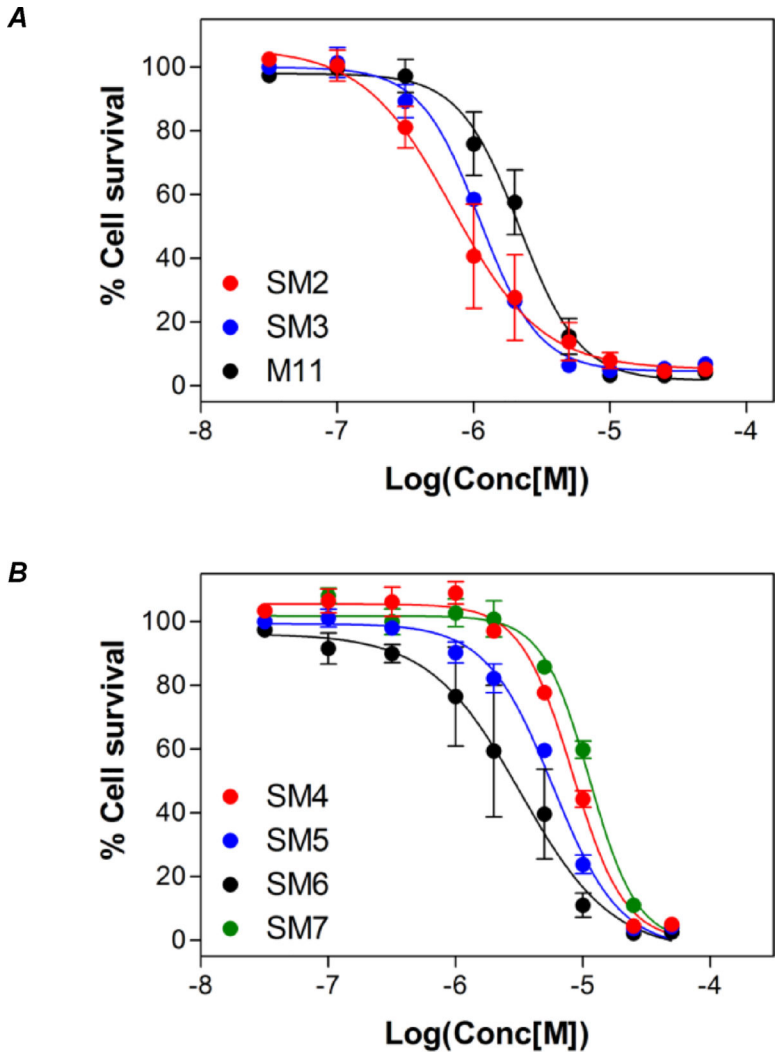
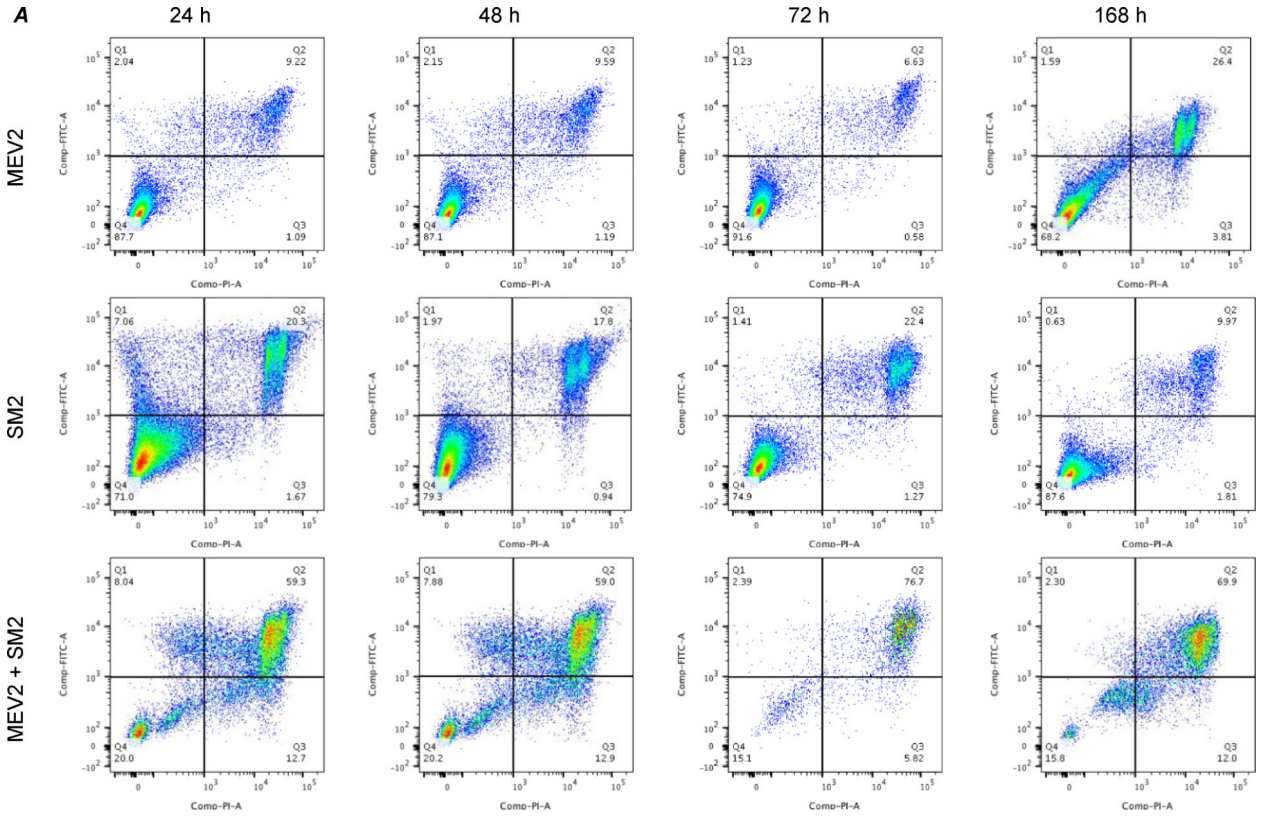


Figure 2.

Examples of cell viability curves obtained for KOPN-8 mixed lineage leukemia cell line treated with (A) compounds lipidated in position 2 (M11) and C-terminus (SM2, SM3), and (B) compounds lipidated in position 3 (SM4-SM7).



B

Compound	Annexin V+/PI+ (%)			
	24 h	48 h	72 h	168 h
MEV2	9.22	9.59	6.63	26.4
SM2	20.3	17.8	22.4	9.97
MEV2+SM2	59.3	59	76.7	69.9

Figure 3.

Apoptotic effects of selected compounds measured by flow cytometry in annexin V/PI assay (A), and corresponding annexin V+/PI+ double positive population values (B). KOPN-8 mixed lineage leukemia cells were treated at 10 μM concentrations with lipidated compounds MEV2, SM2 and equimolar mixture of MEV2 and SM2.

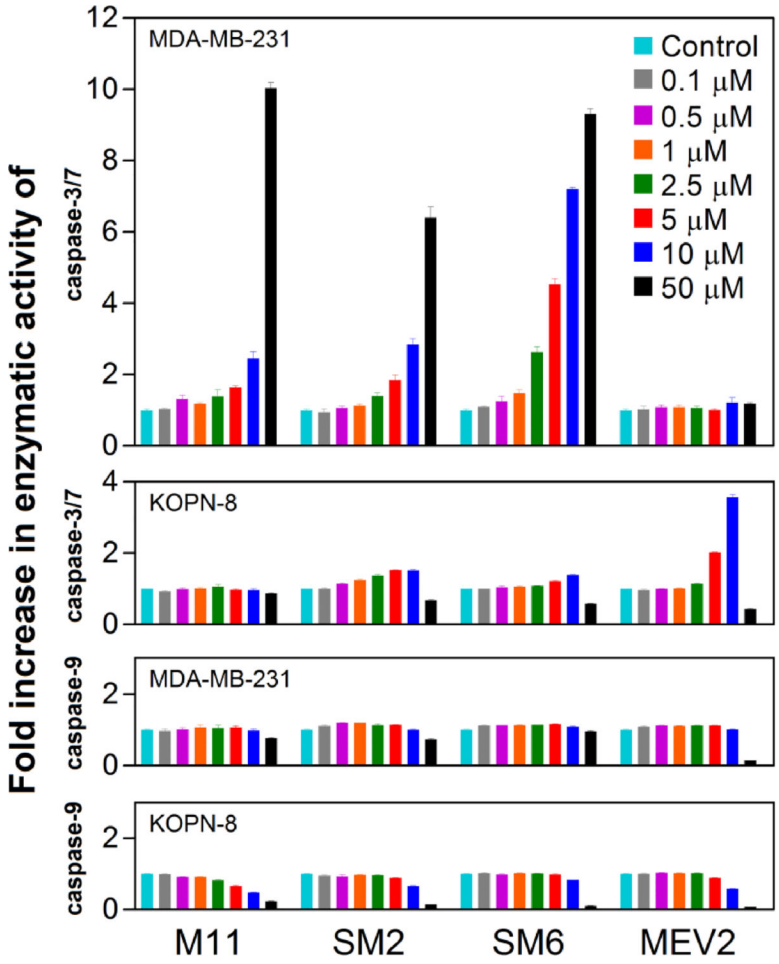


Figure 4.

An increase in enzymatic activity of caspases-3/7 and −9 in MDAMB-231 and KOPN-8 cells treated with peptides M11, SM2, SM6, and MEV2 at various concentrations (dose response).

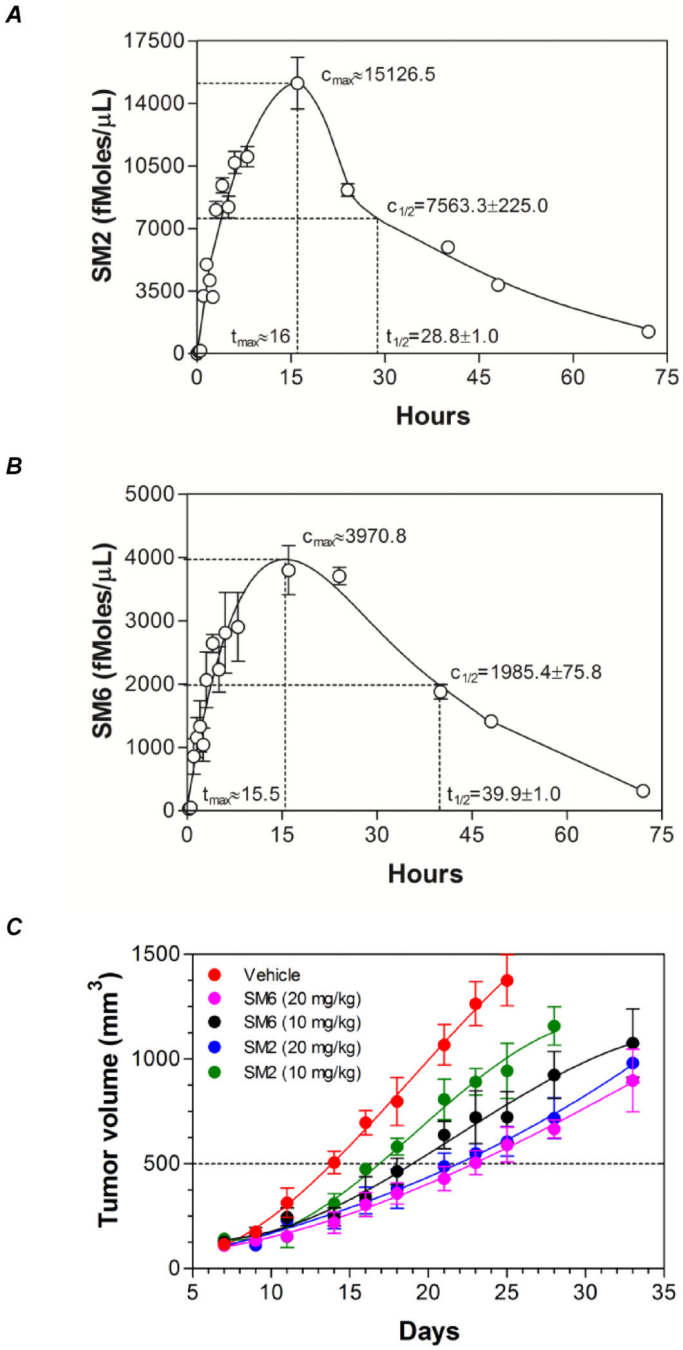
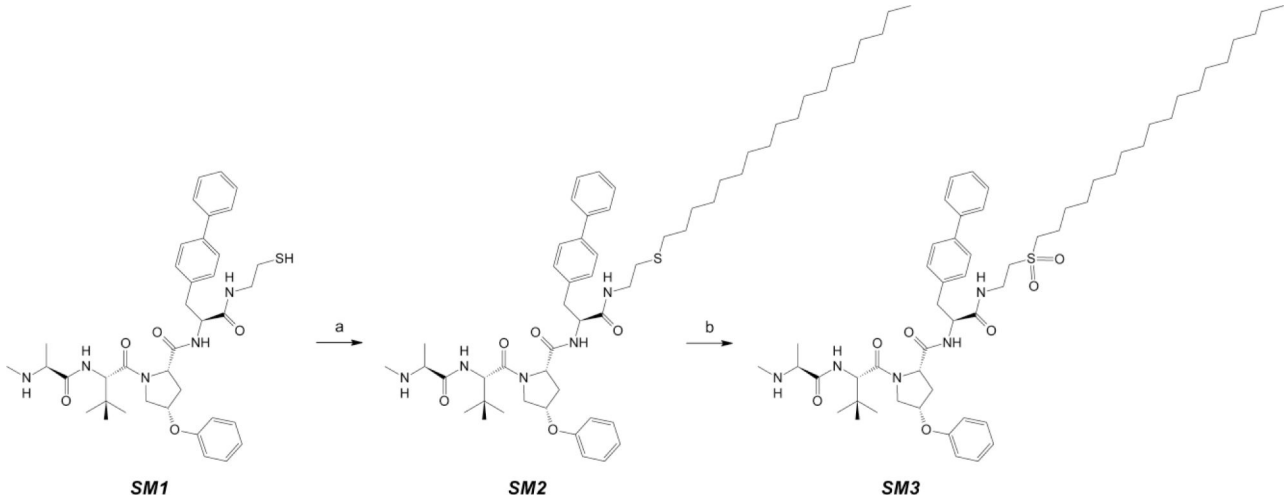


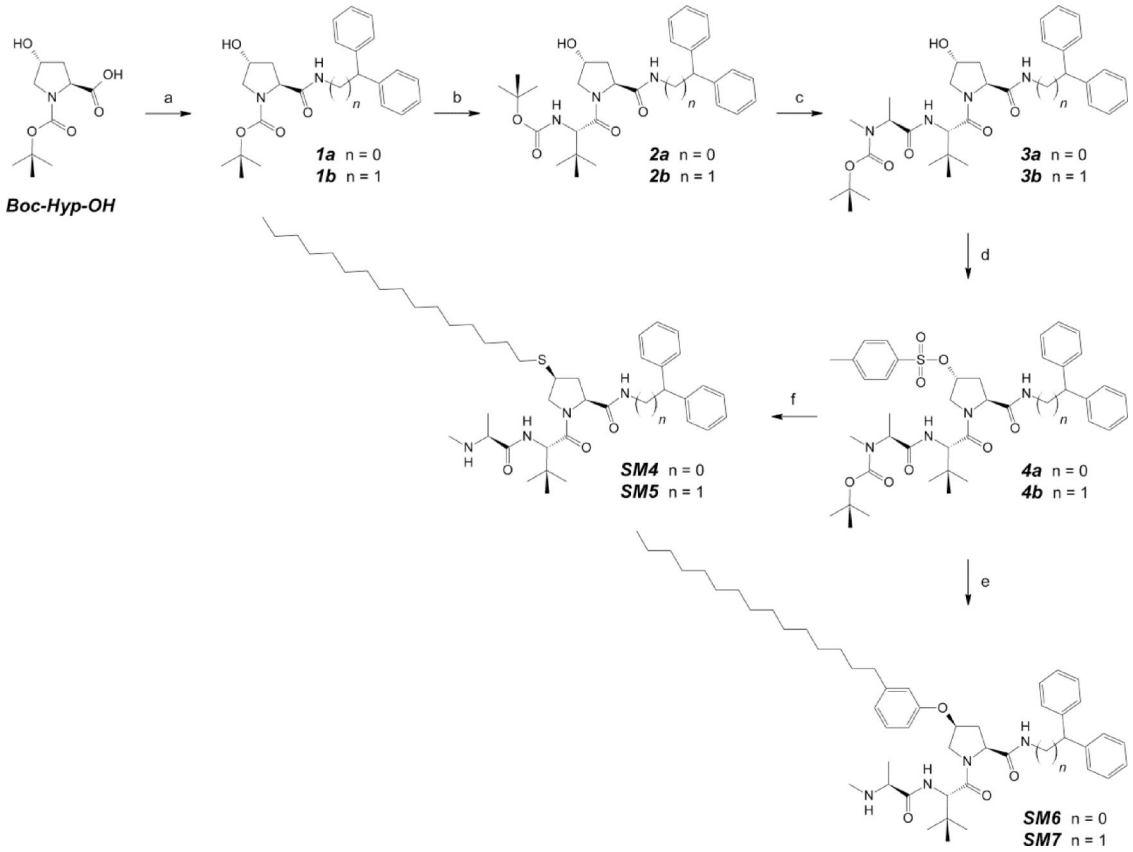
Figure 5.

PK and *in vivo* experiments. Plasma levels after subcutaneous single dose administration of (A) SM2 and (B) SM6 at 10 mg/kg dose. Anticancer effects of SM2 and SM6 in a xenograft mouse model (C).



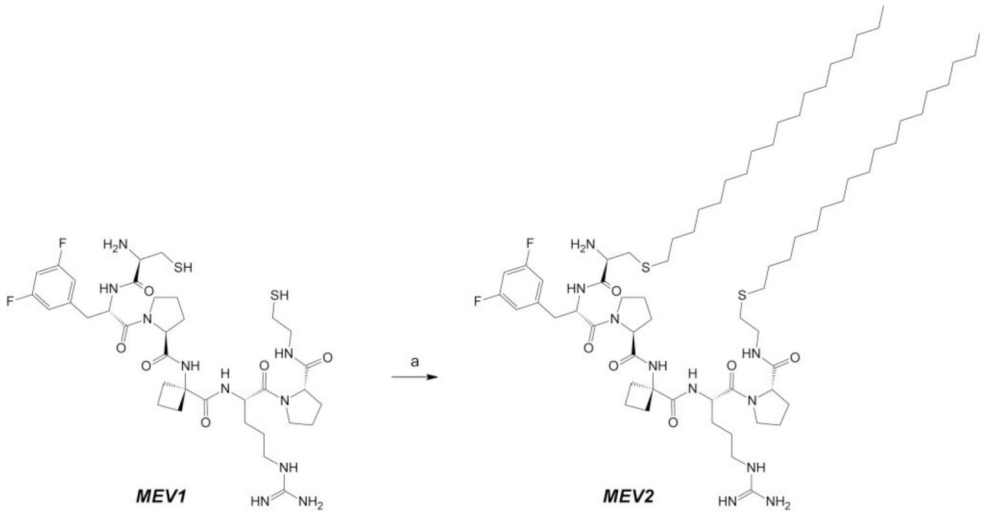
Scheme 1.

Synthesis of C-terminally lipidated Smac derivatives. Conditions: (a) 1-bromooctadecane/BuOH/TMG/48h/90°C; (b) MeOH/H_2O/Oxone®/5h. Abbreviations: BuOH–n-butanol, MeOH-methanol, Oxone®−$2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$, TMG–1,1,3,3-tetramethylguanidine.



Scheme 2.

Synthesis of Smac analogs lipidated in position 3. Conditions: (a) BHA or DPEA/TCTU/NMM/DMSO/75°C/10 min/MW; (b) (1) 4M HCl in 1,4-dioxane/30 min; (2) Boc-(L)-*tert*Leu-OH/TCTU/NMM/DMSO/75°C/10 min/MW; (c) (1) 4M HCl in 1,4-dioxane/30 min (2) Boc-N-Me-(L)-Ala-OH/TCTU/NMM/DMSO/75°C/10 min/MW; (d) Tos-Cl/Py/0°C→r.t./48 h; (e) (1) 3-pentadecylphenol/BuOH/TMG/48h/90°C; (2) TFA/30 min; (f) (1) 1-hexadecanethiol/K_2CO_3/NMP/72 h/90°C; (2) TFA/30 min. Abbreviations: BHA–benzhydrylamine, Boc-Hyp-OH–N-tertbutoxycarbonyl-trans-4-hydroxy-L-proline, Boc-(L)-*tert*Leu-OH–N-tertbutoxycarbonyl-L-*tert*-leucine, Boc-N-Me-(L)-Ala-OH–N-tertbutoxycarbonyl-N-methyl-L-alanine, BuOH–n-butanol, DMSO–dimethylsulfoxide, DPEA–2,2-diphenylethylamine, MW–microwave synthesis, NMM–N-methylmorpholine, NMP–N-methyl-2-pyrrolidone, Py–pyridine, TCTU–O-(6-chloro-1-hydrocibenzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, TFA–trifluoroacetic acid, TMG–1,1,3,3-tetramethylguanidine.



Scheme 3.

Synthesis of MEV2 analog. Conditions: (a) 1-bromooctadecane/BuOH/TMG/48h/90°C. Abbreviations: BuOH–n-butanol,TMG–1,1,3,3-tetramethylguanidine.

Table 1.

Cell growth inhibition of various cancer cell lines induced by analogs SM1-SM3 and M11.

Cell line	SM1	SM2	SM3	M11
Breast Cancer		EC_{50} (μM)		
BTTR	9.0±0.7	2.1±0.2	1.2±0.1	5.5±0.3
BT-474	9.7±1.2	0.32±0.1	0.61±0.1	5.5±0.5
HCC1954	3.9±0.4	0.42±0.1	0.24±0.08	4.4±0.2
MCF-7	10.2±0.4	25.6±3.2	48.2±6.7	24.7±5.5
MDA-MB-231	6.0±0.9	24.7±1.1	69.8±14.4	3.0±0.3
MDA-MB-361	8.9±0.3	1.7±0.5	0.26±0.04	3.1±0.2
T-47D	7.0±0.3	1.3±0.06	1.3±0.03	7.7±0.3
Liver Cancer				
HepG2	8.1±0.5	1.7±0.1	2.1±0.1	6.4±0.3
Hep3B	7.1±0.2	0.21±0.03	0.24±0.04	7.3±0.1
Leukemia				
Jurkat	33.7±3.9	6.2±0.7	11.6±2.3	8.2±0.6
KOPN-8	1.1±0.07	1.0±0.06	1.1±0.04	2.4±0.3
HL-60	2.3±0.5	2.0±0.3	17.1±2.4	18.2±2.9
Molt-4	2.8±0.4	1.0±0.08	24.6±3.1	6.9±1.0
MV-4–11	2.4±0.3	34.9±3.2	29.2±3.7	12.9±1.2
Nalm-6	5.3±0.4	4.1±0.6	50.0±4.3	12.5±2.1
SEM	5.1±0.2	10.3±1.1	15.1±2.2	11.8±1.1
Lymphoma				
CEM-R	2.9±0.1	8.9±0.3	12.6±1.5	7.6±0.6
CEM-TL	1.7±0.2	5.5±0.4	14.2±2.7	3.8±0.5
HH	2.5±0.1	19.9±1.6	18.2±2.1	8.2±1.1
Hut-78	2.8±0.2	3.6±0.2	6.3±0.3	6.9±0.8
THP-1	2.5±0.3	8.7±0.8	12.2±1.5	12.3±1.1
U937	5.7±0.8	NA	NA	16.2±3.5
TF-1	11.7±0.7	3.2±0.4	4.9±0.1	12.2±1.1
Melanoma				
M229	6.3±0.4	2.9±0.1	3.3±0.1	12.4±0.6
M233	6.9±0.3	9.5±0.3	11.3±0.7	21.4±1.7
M249	6.2±0.2	8.0±0.9	5.6±0.2	12.9±0.6
M263	5.6±0.2	2.9±0.1	2.8±0.1	12.8±0.1
SKMEL28	6.1±0.3	9.9±0.6	11.3±1.0	15.9±0.9
Osteosarcoma				

Cell line	SM1	SM2	SM3	M11
MG63	5.4±0.5	2.7±0.1	4.3±0.1	15.1±1.0
Pancreatic Cancer				
AsPC-1	19.7±2.1	1.2±0.08	2.0±0.1	80.3±6.2
BxPC-3	4.1±0.3	1.4±0.04	1.5±0.03	10.8±0.5
COLO 357/FG	8.0±0.2	NA	NA	NA
PANC-1	6.8±0.1	5.1±0.9	4.7±0.6	23.5±2.4
Prostate Cancer				
22Rv1	5.8±0.8	51.0±4.6	10.0±0.7	6.1±0.2
DU145	6.2±0.7	12.8±2.3	12.0±0.3	7.0±0.9
LNCaP	5.6±0.2	63.1±5.7	10.5±0.8	6.6±0.3
PC-3	6.7±0.2	2.2±0.1	2.3±0.2	27.9±3.5
VCap	7.0±0.3	NA	13.9±1.3	6.9±0.3
Colon Cancer				
HCT-116	5.8±0.4	1.5±0.06	1.5±0.04	9.4±0.7
SW480	9.2±1.4	12.9±1.2	25.7±3.3	18.3±0.9
T84	6.2±0.6	2.0±0.1	1.8±0.06	10.9±1.0
Head & Neck Cancer				
FaDu	3.9±0.2	0.19±0.02	0.64±0.1	18.8±4.5
UM-SCC-1	5.8±0.1	1.1±0.05	1.7±0.02	9.0±0.3
UM-SCC-5	5.9±0.7	0.96±0.1	0.73±0.1	8.9±0.6
UM-SCC-6	6.0±0.5	5.4±0.3	4.8±0.3	23.6±3.9
UM-SCC-12	5.5±0.3	1.1±0.1	1.1±0.03	14.9±2.0
UM-SCC-38	4.1±0.3	0.33±0.08	0.14±0.01	9.1±0.5
UM-SCC-74A	7.4±0.5	2.3±0.1	2.2±0.1	16.7±1.4
UM-SCC-178	7.3±0.4	2.7±0.09	3.8±0.1	20.2±1.6

Table 2.

Cell growth inhibition of various cancer cell lines induced by analogs SM4-SM6.

Cell line	SM4	SM5	SM6	SM7
Breast Cancer		EC_{50} (μM)		
BTTR	11.1±0.6	5.1±0.4	1.3±0.3	2.9±0.2
BT-474	NA	10.8±2.1	2.7±0.3	7.0±0.3
HCC1954	NA	17.5±0.6	3.0±0.4	19.8±1.5
MCF-7	NA	16.8±0.7	13.4±1.3	24.4±1.6
MDA-MB-231	32.3±1.3	15.1±0.8	7.2±0.5	10.9±0.2
MDA-MB-361	NA	25.0±1.8	6.8±0.8	9.9±0.4
T-47D	28.2±3.3	19.3±0.9	6.2±0.3	9.0±0.2
Liver Cancer				
HepG2	24.3±2.5	32.0±3.1	2.8±0.2	5.4±0.2
Hep3B	21.4±2.7	12.4±1.7	0.87±0.09	2.2±0.2
Leukemia				
KOPN-8	8.2±0.5	5.9±0.5	3.6±1.1	11.1±0.9
Molt-4	11.2±0.6	12.1±0.8	22.5 ±1.6	NA
Nalm-6	NA	26.9±3.1	0.77±0.05	11.1±1.0
Til-1	3.9±0.2	3.8±0.2	9.0±0.5	12.2±0.7
Lymphoma				
CEM-TL	6.2±0.1	6.5±0.7	3.6±0.9	3.5±0.3
TF-1	12.2±0.5	8.8±0.5	3.2±0.1	5.2±0.2
Melanoma				
M229	22.6±3.3	11.6±2.4	9.8±0.2	14.3±0.4
Prostate Cancer				
DU145	45.4±3.8	12.2±1.6	4.9±0.4	9.3±0.5
VCap	12.9±0.6	8.9±0.2	12.2±0.5	18.1±0.8
Colon Cancer				
T84	NA	11.1±1.7	9.3±0.6	10.6±1.0
Head & Neck Cancer				
FaDu	11.8±0.6	5.4±0.1	0.67±0.04	1.5±0.1

Table 3.

Cell growth inhibition of various cancer cell lines induced by analogs MEV1, MEV2, SM2 and equimolar mixture of SM2 and MEV2.

Cell line	MEV1	MEV2	SM2	MEV2+SM2
Leukemia		EC_{50} (μM)		
Jurkat	NA	21.7±0.7	6.2±0.7	11.8±0.6
KOPN-8	NA	1.0±0.1	1.0±0.06	0.81±0.02
HL-60	NA	12.3±1.2	2.0±0.3	3.6±0.1
Molt-4	NA	6.5±0.5	1.0±0.08	4.2±0.3
MV-4–11	NA	9.2±0.6	34.9±3.2	4.9±0.2
Nalm-6	NA	5.6±0.2	4.1±0.6	2.9±0.2
SEM	NA	6.4±0.2	10.3±1.1	3.2±0.1
Lymphoma				
CEM-R	NA	4.4±0.3	8.9±0.3	2.5±0.07
CEM-TL	NA	5.9±0.3	5.5±0.4	2.9±0.2
THP-1	NA	9.5±0.3	8.7±0.8	2.0±0.1
TF-1	NA	4.2±0.2	3.2±0.4	2.4±0.06

Table 4.

Tumor growth delay values obtained for SM2 and SM6 analogues.

Compound	Dose (mg/kg)	Delivery route	Tumor Growth Delay at 500 mm^3 (days)
SM2	10	SC	~3.0
SM2	20	SC	~8.0
SM6	10	SC	~5.1
SM6	20	SC	~8.9