Supporting Information

Fragment-Based Design of Small Molecule X-Linked Inhibitor of Apoptosis Protein **Inhibitors**

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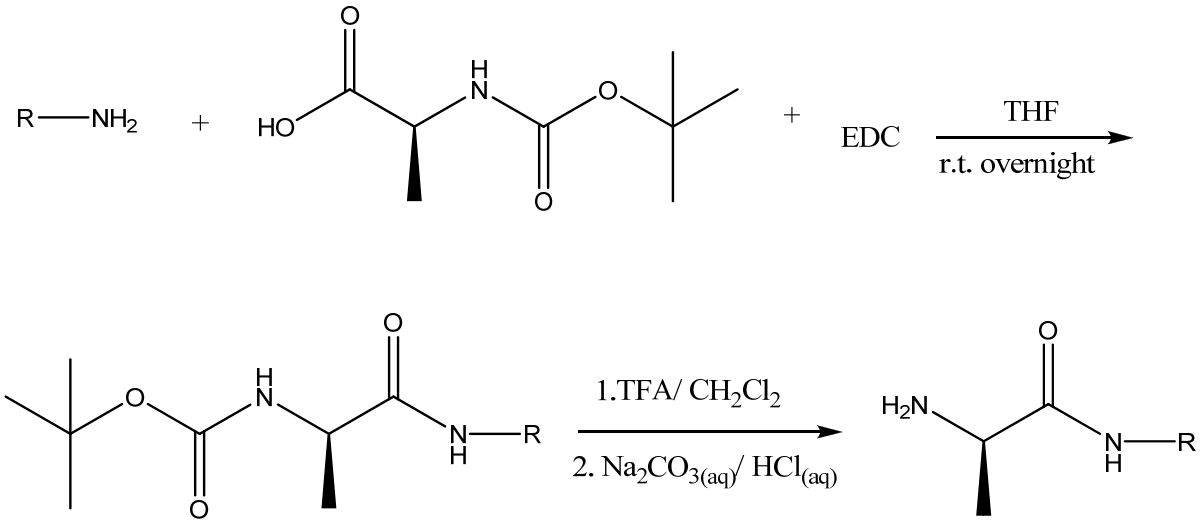
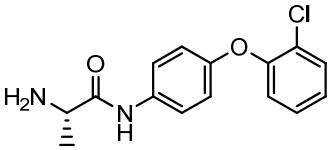
CONTENT :



Supplementary Scheme 1. General scheme for the synthesis of Smac mimetics

(A)



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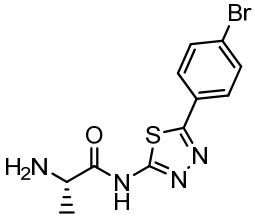
Goldscore: 51.7



BI-75A6

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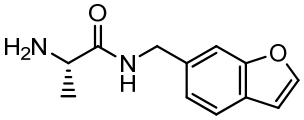

BI-75A11

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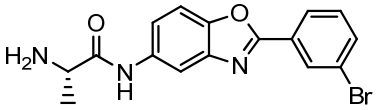

BI-75A2

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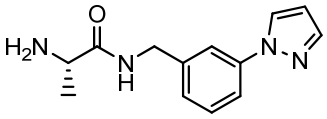

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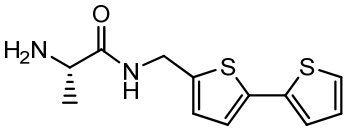

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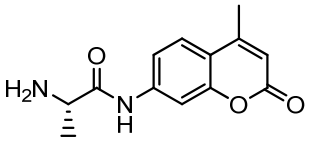

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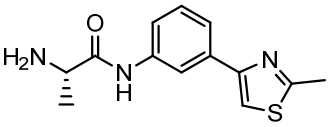

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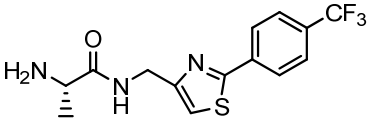
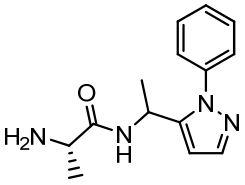


BI-75B1

Goldscore: 55.3



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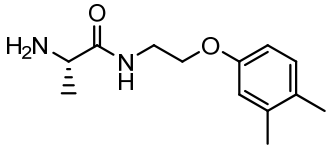

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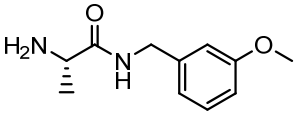
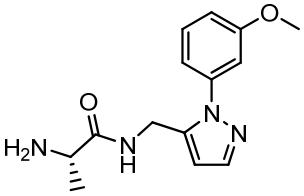
Goldscore:55.6



BI-75B2

Goldscore: 50.0



BI-75A5

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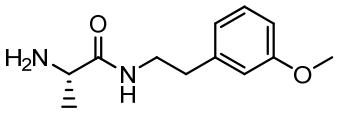

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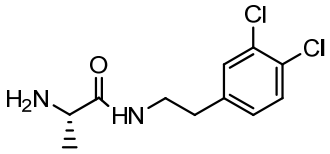
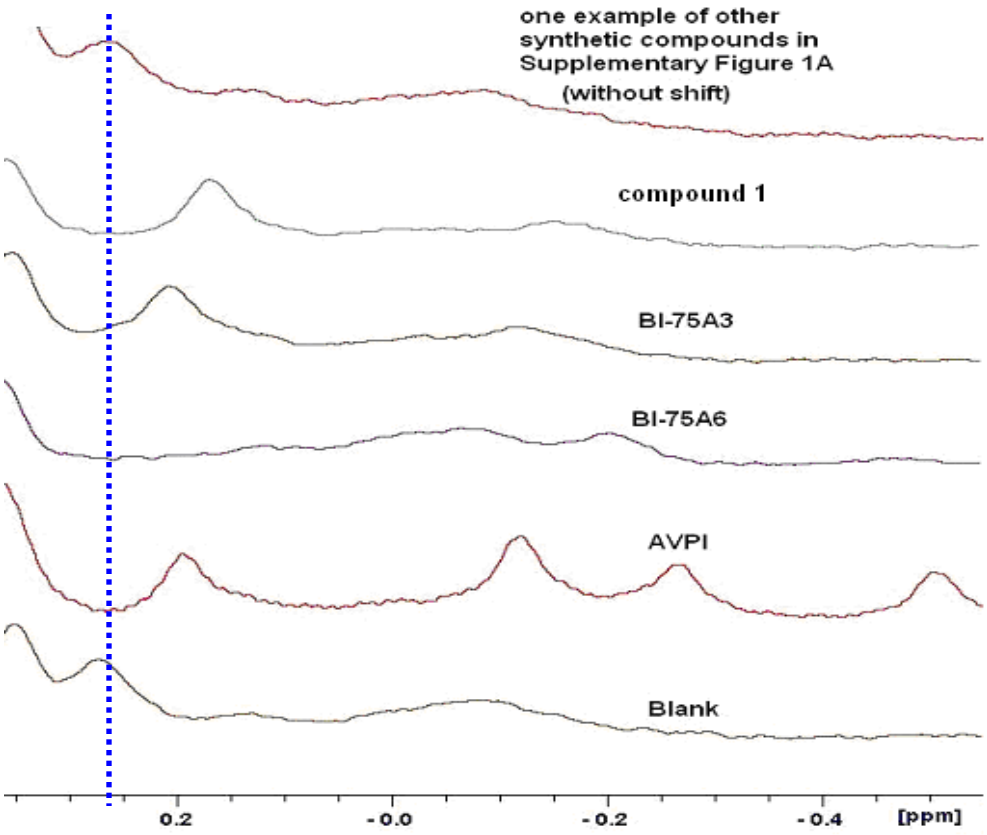
Goldscore: 56.2



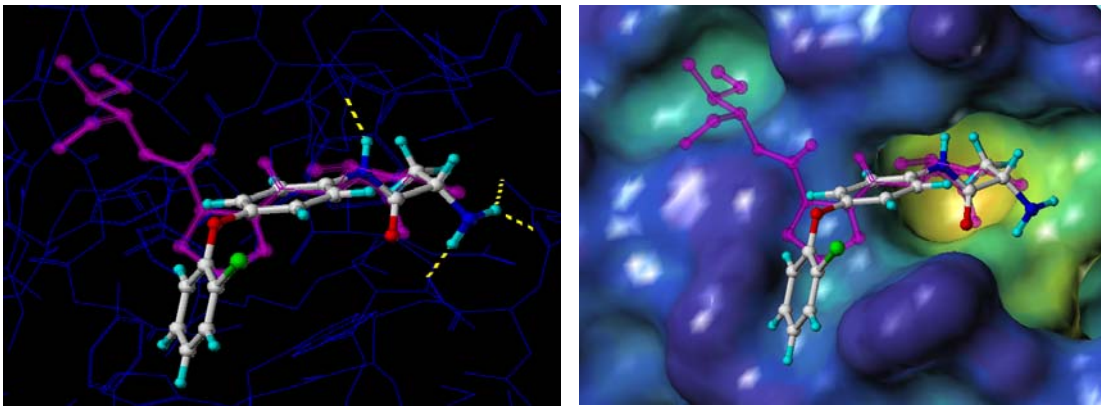
BI-75B3

Goldscore: 56.6

(B)

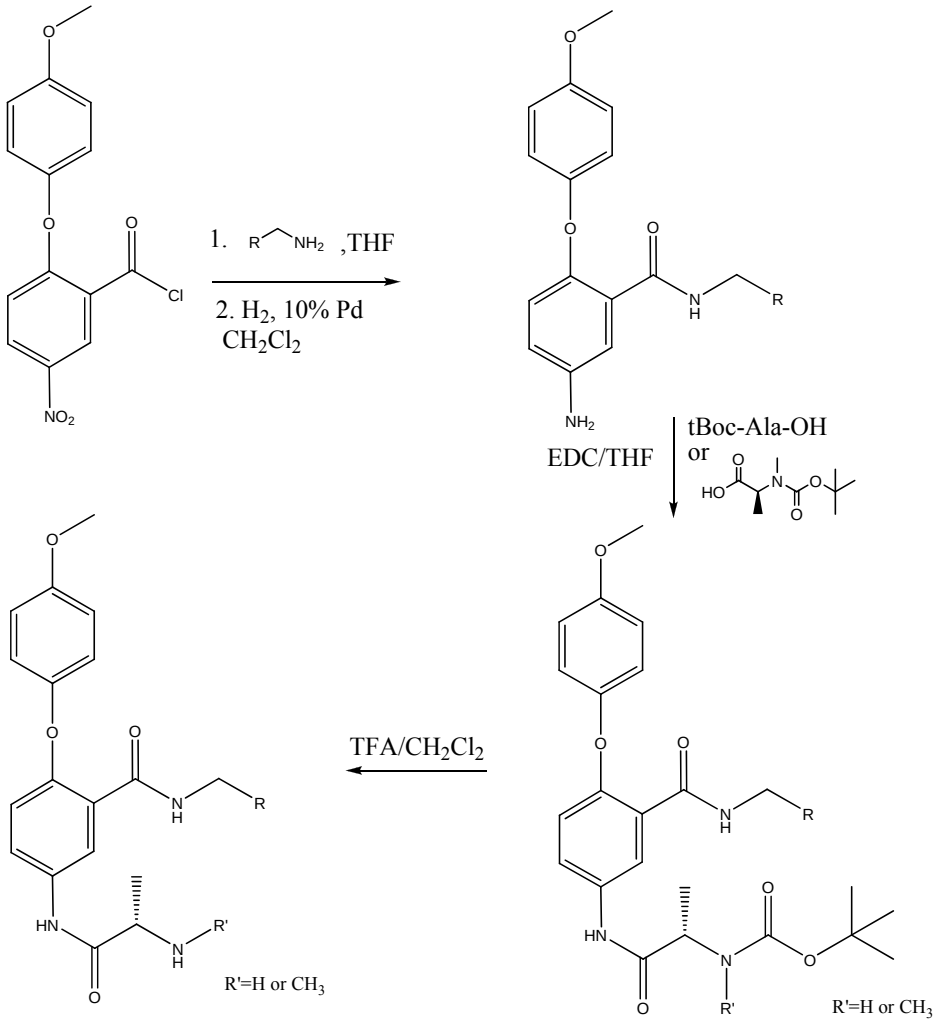


(C)

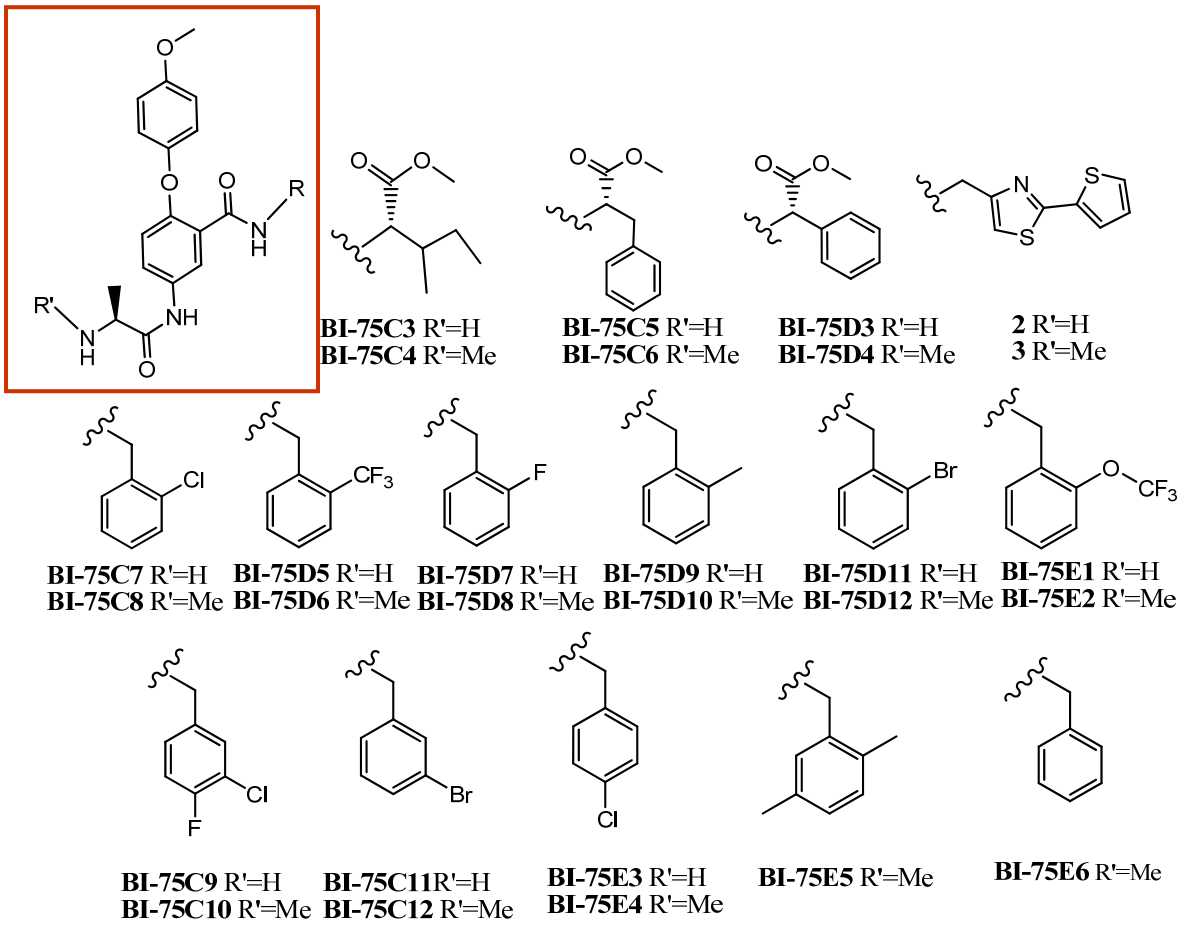


Supplementary Figure 1. (A) The structure and gold scores of synthetic compounds. (B) the 1D NMR spectra for hit compounds. The concentration of Bir3 is 100 μM and the concentrations of compounds are 1 mM. (C) Computational Modeling of AVPI

(magenta) and compound **1** with Bir3 domain of XIAP. The yellow lines in left figure represent the hydrogen bindings between compound **1** and Bir3.

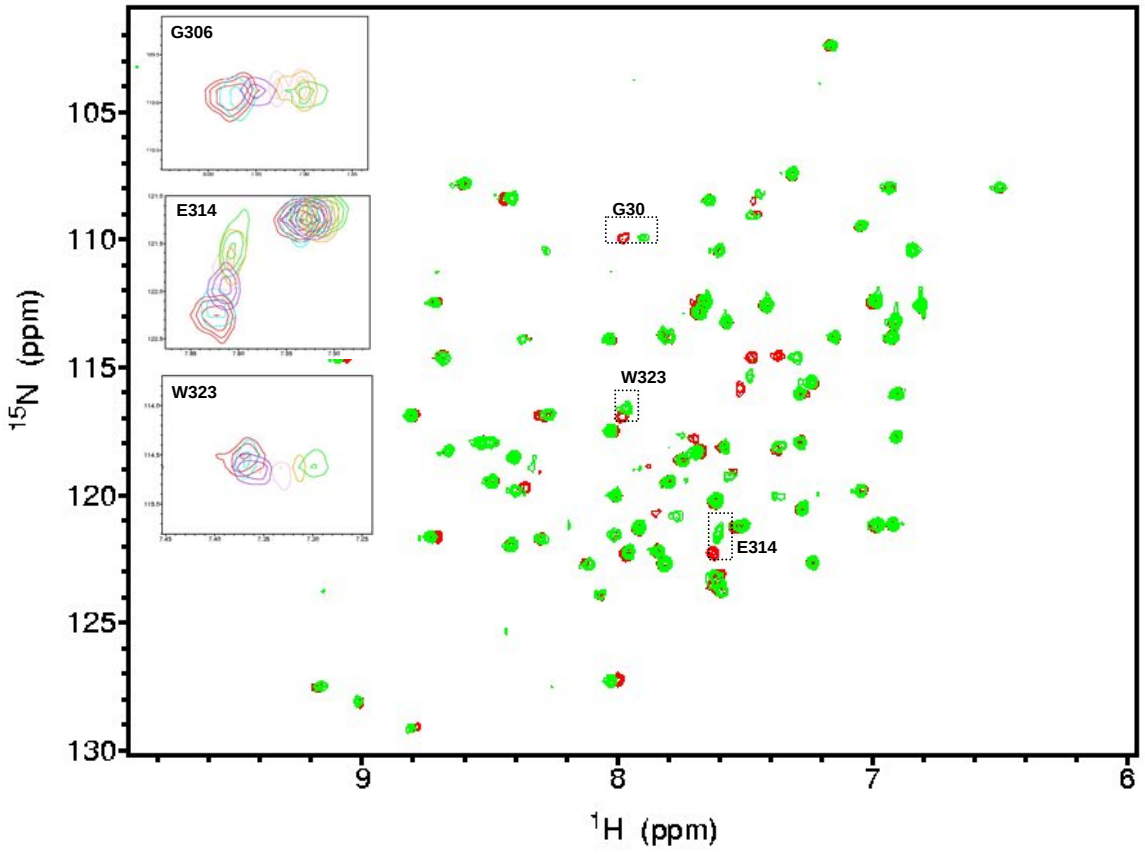


Supplementary Scheme 2. Scheme for the synthesis of compound **1**'s analogues.

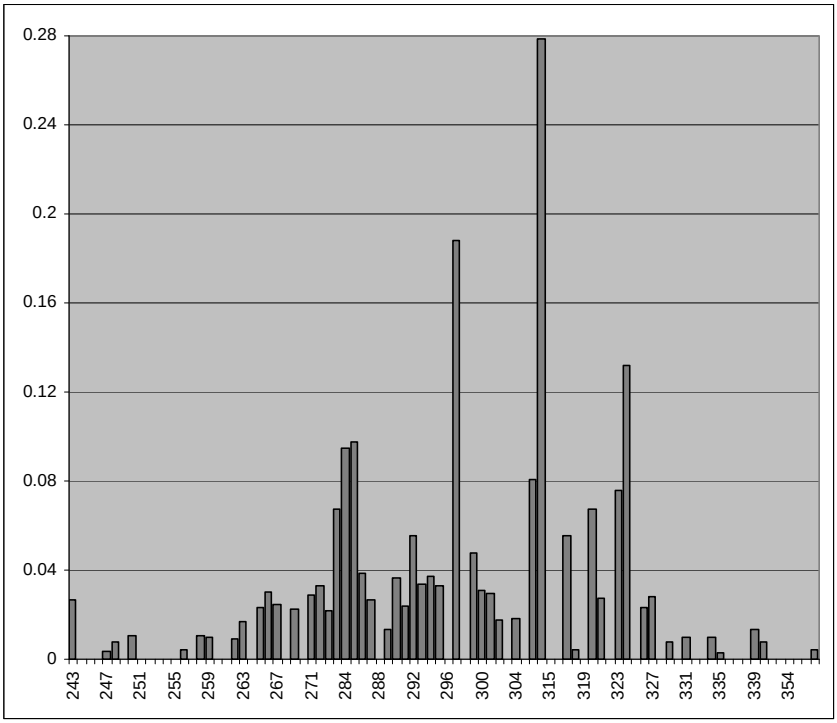


Supplementary Figure 2. Synthetic compound **1**'s analogues.

(A)

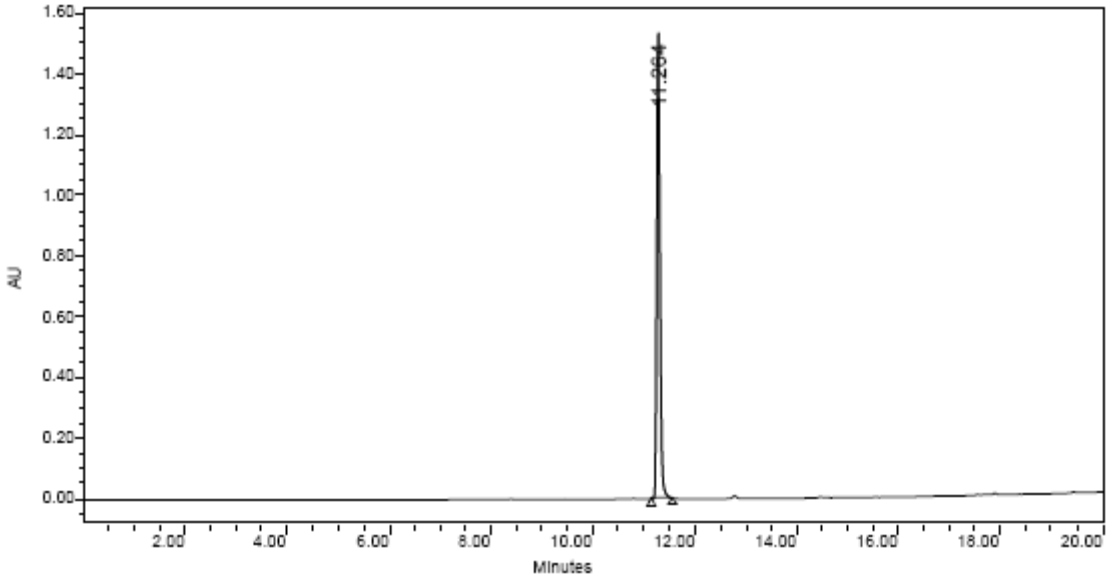

G306
E314
W323
G30
W323
E314
105
110
115
120
125
130
9
8
7
6
15N (ppm)
1H (ppm)

(B)



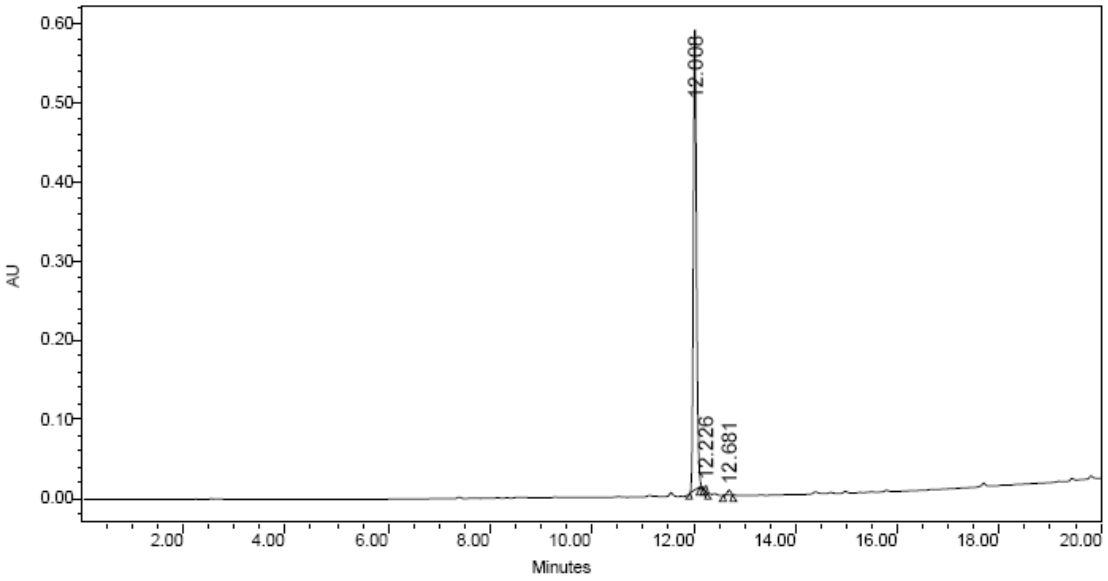
Supplementary Figure 3. Chemical shift mapping studies. (A) Changes in chemical shifts of Bir3 ^{15}N-HSQC spectrum upon addition of compound **3**. (B) Sequential variation of chemical shift changes due to the binding of compound **3**. Chemical shift changes differences ($\Delta\delta$) between ^{15}N-HSQC spectra of free Bir3 and compound **3** bound Bir3 were calculated as $\Delta\delta = [(\Delta H_N)^2 + (0.17\Delta^{15}N)^2]^{1/2}$.

(A)



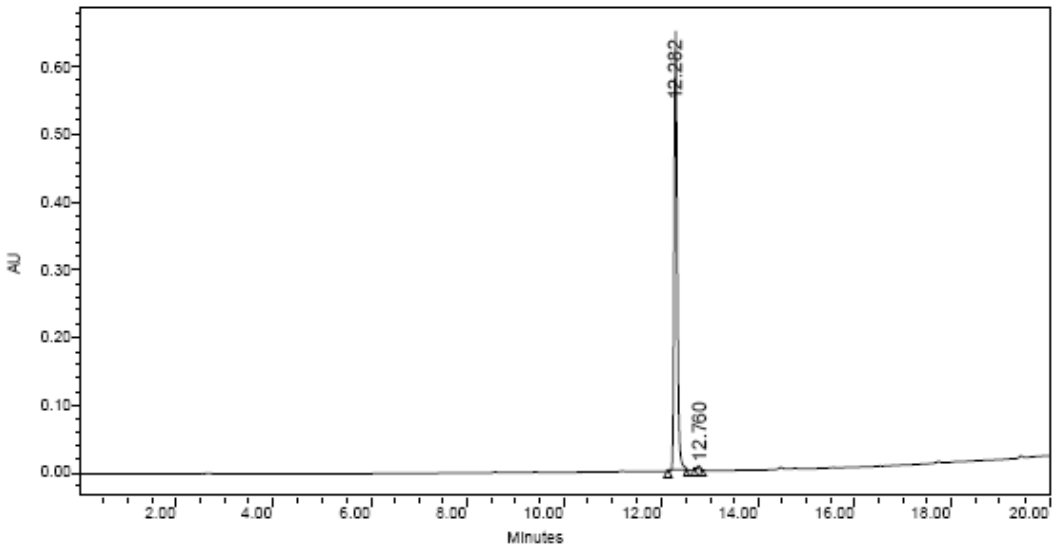
	RT (min)	Area (V*sec)	% Area	Height (V)	% Height
1	11.264	6718167	100.00	1506610	100.00

(B)



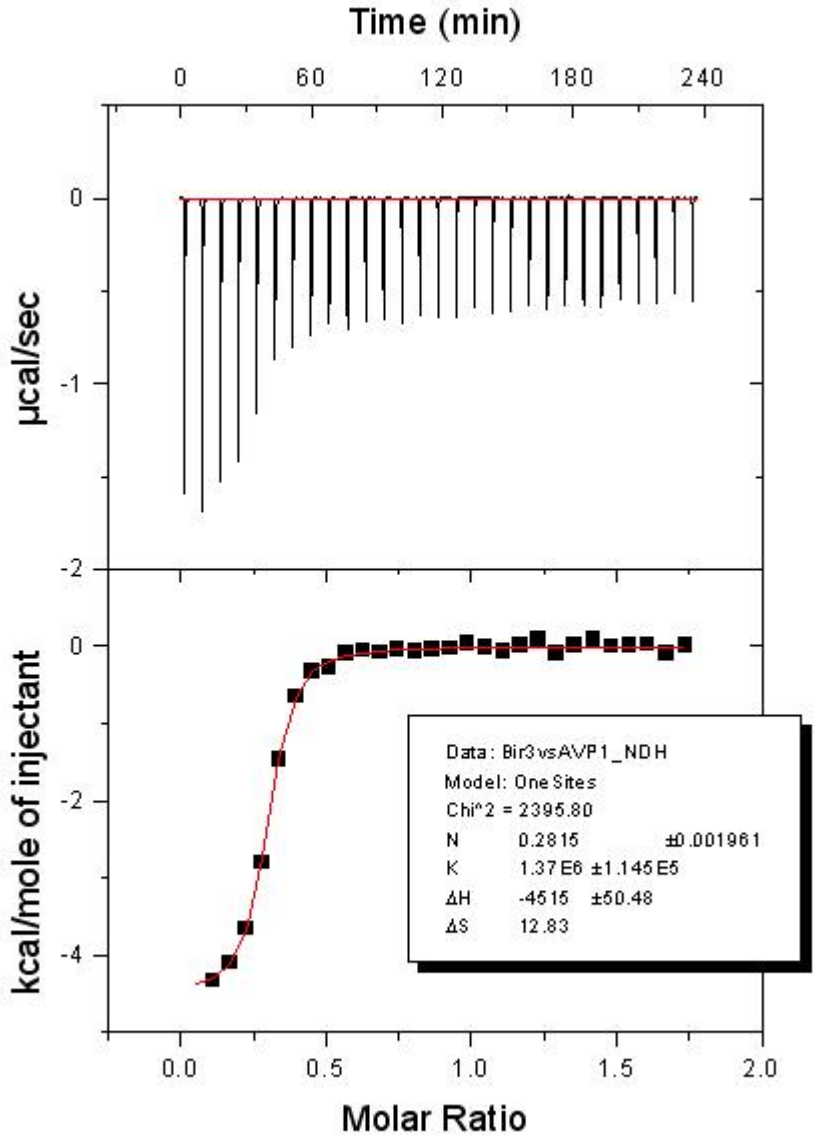
	RT (min)	Area (V*sec)	% Area	Height (V)	% Height
1	12.008	2531800	98.22	582602	98.11
2	12.226	14599	0.57	5049	0.85
3	12.681	31245	1.21	6178	1.04

(C)



	RT (min)	Area (V*sec)	% Area	Height (V)	% Height
1	12.282	2799414	99.12	632585	99.14
2	12.760	24894	0.88	5480	0.86

Supplementary Figure 4. Determination of purity of compound **1**, **2**, and **3** by HPLC. (A) HPLC spectrum for compound **1**. Purity >99.9 %, t_r = 11.26 min. (B) HPLC spectrum for compound **2**. Purity 98.11 %, t_r = 12.08 min. (C) HPLC spectrum for compound **3**. Purity 99.14 %, t_r = 12.28 min.



Supplementary Figure 5. Calorimetric titration of Bir3 with AVPI peptide. The top panel shows raw data in power versus time. The bottom panel shows data after peak integration, subtraction of blank titration data, and concentration normalization. The solid line is the fit to a single binding site model. K_d ~ 0.73 µM.

General Synthetic Procedures of BI-75A2, BI-75A4, BI-75A5, BI-75A6, BI-75A7, BI-75A8, BI-75A9, BI-75A10, BI-75A11, BI-75A12, BI-75B1, BI-75B2, and BI-75B3 (Supplementary Figure 1). N-(tert-Butoxycarbonyl)-L-alanine (1.2 equivalent) and 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC· HCl; 1.5 equivalent) were mixed in THF in round-bottomed flasks, and the respective amines (1 equivalent) in small amount THF were than added drop wise into the flask. The reaction mixture was stirred at room temperature under N_2 overnight. The mixture was extracted with ethyl acetate and saturated $NaHCO_{3(aq)}$, and dried over Na_2SO_4. The crude products were purified by the CombiFlash Companion machine (ISCO, Inc. Lincoln, NE) by 4g RediSep normal-phase flash columns with hexane and ethyl acetate solvent system. Boc-protected L-Alanine contained Smac mimic compounds were obtained after drying the samples in an evaporator and high-pressure vacuum system.

To de-protect Boc groups, the purified compounds from last step were dissolved in dichloromethane (CH_2Cl_2) and than added 10 equivalent trifloroacetic acid. The reaction mixture was stirred at room temperature for 2-3 hours after the starting material was all consumed (checked by TLC). After solvent was removed, the reactions were than quenched by saturated $Na_2CO_{3(aq)}$ solution. The products were extracted with ethyl acetate and the combined organic phase was adjusted to pH 2.0 with concentrated HCl. The final products (salt form) were obtained after drying the samples in an evaporator and high-pressure vacuum system.

(S)-2-amino-N-(2-(3-bromophenyl)benzo[d]oxazol-5-yl)propanamide (BI-75A2). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.38 (s, 1 H), 8.15 (d, *J* = 6.42 Hz, 1 H), 7.64 (d, *J* = 6.30 Hz, 1 H), 7.37 (m, 2 H), 7.05 (s, 1 H), 6.74 (d, *J* = 7.20 Hz, 1 H), 5.00 (m, 1 H), 3.77 (br, 2 H, -NH), 1.28 (d, *J* = 4.92 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 360.03 amu; observed mass of $(M + H)^+$, 359.80 amu

(2S)-2-amino-N-(1-(1-phenyl-1H-pyrazol-5-yl)ethyl)propanamide (BI-75A4) ^{1}H NMR (600 MHz, DMSO-d6) δ 8.64 (s, 1 H, -NH), 8.18 (d, *J* = 11.88 Hz, 1 H), 7.68-7.75 (m, 3 H), 7.49 (t, *J* = 7.5 Hz, 2 H), 7.33 (t, *J* = 4.8, 1 H), 5.14 (m, 1 H), 3.90 (q, *J* = 6.54 Hz, 1 H), 1.53 (d, *J* = 7.08 Hz, 3 H), 1.49 (d, *J* = 7.08 Hz, 3 H). LC-MS expected mass of $(M+)^+$, 259.14 amu; observed mass of $(M + H)^+$, 258.90 amu

(S)-2-amino-N-((1-(3-methoxyphenyl)-1H-pyrazol-5-yl)methyl)propanamide (BI-75A5) ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.15 (s, 1 H), 7.87 (s, 1 H), 7.33 (d, *J* = 15.3 Hz, 1 H), 7.25 (s, 1 H), 7.19 (d, *J* = 6.96 Hz, 1 H), 6.85 (d, *J* = 7.38 Hz, 1 H), 4.42 (d, *J* = 16.2 Hz, 1 H), 4.27 (d, *J* = 16.2 Hz, 1 H), 4.08 (m, 1 H), 3.84 (s, 3 H), 1.50 (d, *J* = 4.92 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 275.15 amu; observed mass of $(M + H)^+$, 274.90 amu

(S)-2-amino-N-(5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl)propanamide (BI-75A6). ^{1}H NMR (600 MHz, MeOD-d4) δ 7.95 (d, *J* = 7.98 Hz, 2 H), 7.73 (d, *J* = 7.98 Hz, 2 H), 4.26-4.29 (m, 1 H), 1.65 (d, *J* = 7.08 Hz, 3 H). ESI mass analysis expected mass of $(M + H)^+$, 326.99 amu; observed mass of $(M + H)^+$, 326.93 amu

(S)-N-(3-(1H-pyrazol-1-yl)benzyl)-2-aminopropanamide (BI-75A7) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.90 (s, 1 H, -NH), 8.24 (s, 1 H), 7.73 (s, 2 H), 7.64 (d, *J* = 6.90 Hz, 1 H), 7.47 (t, *J* = 7.86 Hz, 1 H), 7.27 (d, *J* = 7.08 Hz, 1 H), 6.54 (s, 1 H), 4.55 (dd, *J* =

15.00 Hz, *J*' = 5.28 Hz, 1 H), 4.47 (dd, *J* = 15.00 Hz. *J*' = 5.28 Hz, 1 H), 4.01 (m, 1 H), 2.70 (s, 1 H, -NH), 1.55 (d, *J* = 6.90 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 245.14 amu; observed mass of $(M + H)^+$, 244.90 amu

(S)-2-amino-N-(3-(2-methylthiazol-4-yl)phenyl)propanamide (BI-75A8) ^{1}H NMR (600 MHz, DMSO-d6) δ 8.34 (s, 3 H, -NH, -NH2), 8.20 (s 1 H), 7.88 (s, 1 H), 7.65 (m, 2 H), 7.40 (t, *J* = 7.80 Hz, 1 H), 4.07 (m, 1 H), 2.72 (s, 3 H), 1.48 (d, *J* = 6.60 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 262.10 amu; observed mass of $(M + H)^+$, 261.85 amu

(S)-2-amino-N-(2-(3,4-dimethylphenoxy)ethyl)propanamide (BI-75A9) ^{1}H NMR (600 MHz, MeOD-d4) δ 7.00 (d, *J* = 7.98 Hz, 1 H), 6.72 (s, 1 H), 6.64 (d, *J* = 7.98 Hz, 1 H), 4.02 (t, *J* = 4.50 Hz, 1 H), 3.93 (m, 1 H), 3.61 (t, *J* = 4.50 Hz, 2 H), 2.22 (s, 3 H), 2.18 (s, 3 H), 1.48 (d, *J* = 6.78 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 237.16 amu; observed mass of $(M + H)^+$, 236.95 amu

(S)-2-amino-N-(3-methoxyphenethyl)propanamide (BI-75A10) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.68 (s, 1 H, -NH), 7.81 (s 1 H), 7.70 (t, *J* = 7.68 Hz, 1 H), 7.28 (d, *J* = 6.54 Hz, 2 H), 4.05 (m, 1 H), 3.91 (m, 2 H), 3.72 (s, 3 H), 3.29 (d, *J* = 6.78 Hz, 2 H), 1.93 (d, *J* = 5.34 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 223.14 amu; observed mass of $(M + H)^+$, 222.90 amu

(S)-2-amino-N-(benzofuran-6-ylmethyl)propanamide (BI-75A11) ^{1}H NMR (600 MHz, MeOD-d4) δ 7.75 (s, 1 H), 7.57 (s 1 H), 7.46 (d, *J* = 8.28 Hz 1 H), 7.26 (d, *J* = 8.28 Hz, 1 H), 6.81 (s, 1 H), 4.51 (s, 2 H), 3.93-3.97 (m, 1 H), 1.51 (d, *J* = 6.69 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 219.11 amu; observed mass of $(M + H)^+$, 218.85 amu

(S)-N-(2,2'-bithiophen-5-ylmethyl)-2-aminopropanamide (BI-75A12) ^{1}H NMR (600 MHz, MeOD-d4) δ 7.31 (s, 1 H), 7.16 (s 1 H), 7.04 (s, 1 H), 7.01 (s, 1 H), 6.94 (s, 1 H), 4.57 (q, *J* = 16.76 Hz, 2 H), 3.93 (m, 1 H), 1.51 (d, *J* = 5.64 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 267.05 amu; observed mass of $(M + H)^+$, 267.00 amu

(S)-2-amino-N-((2-(4-(trifluoromethyl)phenyl)thiazol-4-yl)methyl) propanamide (BI-75B1) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.15 (d, *J* = 7.62, 2 H), 7.79 (d, *J* = 7.68 Hz, 2 H), 7.51 (s, 1 H), 4.61 (s, 2 H), 4.01 (m, 1 H), 1.55 (d, *J* = 6.72 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 330.09 amu; observed mass of $(M + H)^+$, 329.85 amu

(S)-2-amino-N-(3-methoxybenzyl)propanamide (BI-75B2) ^{1}H NMR (600 MHz, MeOD-d4) δ 7.24 (t, *J* = 7.62 Hz,1 H), 6.88 (d, *J* = 7.68 Hz, 1 H), 6.87 (s, 1 H), 6.83 (d, *J* = 7.68 Hz, 1 H), 4.39 (s, 2 H), 3.96 (m, 1 H), 3.78 (s, 3 H), 1.52 (d, *J* = 6.84 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 209.13 amu; observed mass of $(M + H)^+$, 208.85 amu

(S)-2-amino-N-(3,4-dichlorophenethyl)propanamide (BI-75B3) ^{1}H NMR (600 MHz, MeOD-d4) δ 7.44 (d, *J* = 8.16 Hz, 1 H), 7.41 (s, 1 H), 7.17 (d, *J* = 8.16 Hz, 1 H), 3.81 (m, 1 H), 3.54-3.59 (m, 1 H), 3.39-3.43 (m, 1 H), 2.71-2.88 (m, 2 H), 1.41 (d, *J* = 6.96 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 261.06 amu; observed mass of $(M + H)^+$, 260.85 amu

(B) General Synthetic Procedures of BI-75A1 Analogues (i) synthesis of BI-75C3, BI-75C5, BI-75C7, BI-75C9, BI-75C11, BI-75D3, BI-75D5, BI-75D7, BI-75D9, BI-75D11, BI-75E1, BI-75E3 Supplementary Figure 2, when R' = H). To a stirring solution of 2-(4-methoxyphenoxy)-5-nitrobenzoyl chloride (1 equivalent) in DMF (5-10 mL) at 0 °C was added 4-Dimethylaminopyridine (DMAP; 1.3 equivalent) in one potion. After 30 minutes, the stirring mixture was added various amine coupling partners (1.2 equivalent) in small amount DMF dropwise. The solution was then stirred overnight at room temperature under nitrogen. The mixture was quenched by saturated $NH_4Cl_{(aq)}$,

extracted with ethyl acetate, and dried over Na_2SO_4. The resulting residue was purified by the CombiFlash Companion machine (ISCO, Inc. Lincoln, NE) with 4g RediSep normal-phase flash columns with hexane and ethyl acetate solvent system to afford nitro products.

To hydrogenate nitro group, the product from last step was dissolved in ethyl acetate (8-20 mL) and the catalytic amount palladium/C (10 wt. %) and a balloon of hydrogen gas were added. The mixture was stirred overnight at room temperature, filtered, and concentrated under reduced pressure. The resulting residue was used for next step without further purification.

N-(tert-Butoxycarbonyl)-L-alanine (1.2 equivalent) and 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC· HCl; 1.5 equivalent) were mixed in 5-10 mL THF in a round-bottomed flask, and the hydrogenated amine products (1 equivalent) in small amount THF was then added dropwise into the flask. The reaction mixture was stirred overnight at room temperature under N_2. The mixture was extracted with ethyl acetate and saturated $NaHCO_{3(aq)}$, and dried over Na_2SO_4. The crude products were purified by the CombiFlash Companion machine (ISCO, Inc. Lincoln, NE) by 4g RediSep normal-phase flash columns with hexane and ethyl acetate solvent system following the recommend procedures. Boc-proteced products were obtained after drying the samples in an evaporator and high-pressure vacuum system.

To de-protect Boc groups, the purified products from last steps were dissolved in dicholormethane (CH_2Cl_2) and than added 10 equivalent trifloroacetic acid. The reaction mixture was stirred at room temperature for 2-3 hours after the starting material was all consumed (checked by TLC). After solvent was removed, the reactions were than quenched by saturated $Na_2CO_{3(aqe)}$ solution. The products were extracted with ethyl acetate and the combined organic phase was adjusted to pH 2.0 with concentrated HCl. The final products (salt form) were obtained after drying the samples in an evaporator and high-pressure vacuum system.

(ii) Synthesis of. BI-75C4, , BI-75C6, , BI-75C8, , BI-75C10, , BI-75C12, , BI-75D4, BI-75D6, BI-75D8, BI-75D10, BI-75D12, BI-75E2, BI-75E4, BI-75E5, and BI-75E6 (Supplementary Figure 2, when R' = CH_3). Follow the synthetic procedures (i), replace N-(tert-Butoxycarbonyl)-L-alanine by Boc-*N*-methyl-L-alanine (1.2 equivalent). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC· HCl; 1.5 equivalent) and the hydrogenated products (1 equivalent) were used in this synthesis to afford corresponding Boc-protected products. The final products (HCl salt form) can be obtained after deprotection of Boc groups, adjusted pH with HCl, and dried in vacuo.

(2S,3R)-methyl 2-(5-((S)-2-aminopropanamido)-2-(4-methoxyphenoxy)benzamido)-3-methylpentanoate (BI-75C3) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.14 (s, 1 H), 7.75 (d, *J* = 8.34 Hz, 1 H), 7.04 (d, *J* = 6.66 Hz, 2 H), 6.98 (d, *J* = 6.66 Hz, 2 H), 6.88 (d, *J* = 8.34 Hz, 1 H), 4.63 (d, *J* = 4.69 Hz, 1 H), 3.89 (m, 1 H), 3.81 (s, 3 H), 3.71 (s, 3 H), 1.96 (m, 1 H), 1.53 (d, *J* = 6.72 Hz, 3 H), 1.45 (m, 1 H), 1.19 (m, 1 H), 0.92 (d, *J* = 7.08 Hz, 3 H), 0.88 (t, *J* = 7.32 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 458.23 amu; observed mass of $(M + H)^+$, 458.05 amu

(2S,3R)-methyl 2-(2-(4-methoxyphenoxy)-5-((S)-2-(methylamino)propanamido)benzamido)-3-methylpentanoate (BI-75C4) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.18 (s, 1 H), 7.74 (d, *J* = 8.34 Hz, 1 H), 7.04 (d, *J* = 6.66 Hz, 2 H), 6.98 (d, *J* = 6.66 Hz, 2 H), 6.88 (d, *J* = 8.34 Hz, 1 H), 4.63 (d, *J* = 4.69 Hz, 1 H), 3.92 (m, 1 H), 3.80 (s, 3 H), 3.71 (s, 3 H), 2.70 (s, 3 H), 1.95 (m, 1 H), 1.59 (d, *J* = 6.84 Hz, 3 H), 1.45 (m, 1 H), 1.18 (m, 1 H), 0.91 (d, *J* = 6.72 Hz, 3 H), 0.88 (t, *J* = 7.38 Hz, 3 H).

LC-MS expected mass of $(M + H)^+$, 472.24 amu; observed mass of $(M + H)^+$, 472.05 amu

(S)-methyl 2-(5-((S)-2-aminopropanamido)-2-(4-methoxyphenoxy)benzamido)-3-phenylpropanoate (BI-75C5) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.63 (d, *J* = 7.38 Hz, 1 H), 8.15 (s, 1 H), 7.70 (d, *J* = 8.76 Hz, 1 H), 7.10 (m, 5 H), 6.99 (d, *J* = 8.64 Hz, 2 H), 6.95 (d, *J* = 7.44 Hz, 2 H), 6.78 (d, *J* = 9.00 Hz, 1 H), 4.92 (m, 1 H), 4.05 (m, 1 H), 3.84 (s, 3 H), 3.72 (s, 3 H), 3.23 (dd, *J* = 15.00 Hz, *J*' = 5.34 Hz, 1 H), 3.14 (dd, *J* = 15.00 Hz, *J*' = 7.32 Hz, 1 H), 1.60 (d, *J* = 6.54 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 492.21 amu; observed mass of $(M + H)^+$, 492.15 amu

(S)-methyl-2-(2-(4-methoxyphenoxy)-5-((S)-2-(methylamino)propanamido)benzamido)-3-phenylpropanoate (BI-75C6). ^{1}H NMR (600 MHz, MeOD-d4) δ 8.22 (s, 1 H), 7.71 (d, *J* = 8.04 Hz, 1 H), 7.12 (m, 5 H), 7.00 (d, *J* = 7.26 Hz, 2 H), 6.95 (d, *J* = 7.26 Hz, 2 H), 6.79 (d, *J* = 8.70 Hz, 1 H), 4.94 (t, *J* = 6.3 Hz, 1 H), 4.04 (m, 1 H), 3.85 (s, 3 H), 3.73 (s, 3 H), 3.24 (dd, *J* = 13.2 Hz, *J*' = 4.8 Hz, 1 H), 3.15 (dd, *J* = 13.2 Hz, *J* = 7.14 Hz, 1 H), 2.76 (s, 3 H), 1.65 (d, *J* = 6.60 Hz, 3 H) LC-MS expected mass of $(M + H)^+$, 506.23 amu; observed mass of $(M + H)^+$, 506.10 amu

(S)-5-(2-aminopropanamido)-N-(2-chlorobenzyl)-2-(4-methoxyphenoxy)benzamide (BI-75C7) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.08 (s, 1 H), 7.68 (d, *J* = 8.52 Hz, 1 H), 7.30-7.18 (m, 4 H), 6.96 (d, *J* = 8.52 Hz, 2 H), 6.92 (d, *J* = 8.76 Hz, 2 H), 6.85 (d, *J* = 8.64 Hz, 1 H), 4.56 (s, 2 H), 4.10 (m, 1 H), 3.79 (s, 3 H), 1.61 (d, *J* = 6.66 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 454.15 amu; observed mass of $(M + H)^+$, 454.00 amu

(S)-N-(2-chlorobenzyl)-2-(4-methoxyphenoxy)-5-(2-(methylamino)propanamido)benzamide (BI-75C8) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.09 (s, 1 H), 7.69 (d, *J* = 7.50 Hz, 1 H), 7.29-7.19 (m, 4 H), 6.97 (d, *J* = 8.10 Hz, 2 H), 6.93 (d, *J* = 7.80 Hz, 2 H), 6.86 (d, *J* = 8.70 Hz, 1 H), 4.57 (s, 2 H), 3.95 (m, 1 H), 3.80 (s, 3 H), 2.74 (s, 3 H), 1.62 (d, *J* = 7.14 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 468.17 amu; observed mass of $(M + H)^+$, 458.00 amu

(S)-5-(2-aminopropanamido)-N-(3-chloro-4-fluorobenzyl)-2-(4-methoxyphenoxy)benzamide (BI-75C9) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.07 (s, 1 H), 7.68 (d, *J* = 7.14 Hz, 1 H), 7.27 (m, 2 H), 6.93 (m, 5 H), 6.86 (m, 1 H), 4.53 (s, 2 H), 4.10 (m, 1 H), 3.79 (s, 3 H), 1.61 (d, *J* = 6.36 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 472.14 amu; observed mass of $(M + H)^+$, 472.00 amu

(S)-N-(3-chloro-4-fluorobenzyl)-2-(4-methoxyphenoxy)-5-(2-(methylamino)propanamido)benzamide (BI-75C10) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.03 (s, 1 H), 7.69 (d, *J* = 6.96 Hz, 1 H), 7.26 (m, 2 H), 6.97-6.83 (m, 6 H), 4.52 (s, 2 H), 4.09 (m, 1 H), 3.78 (s, 3 H), 2.40 (s, 3 H), 1.35 (d, *J* = 6.24 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 486.15 amu; observed mass of $(M + H)^+$, 486.00 amu

(S)-5-(2-aminopropanamido)-N-(3-bromobenzyl)-2-(4-methoxyphenoxy)benzamide (BI-75C11) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.08 (s, 1 H), 7.67 (d, *J* = 8.10 Hz, 1 H), 7.27-7.20 (m, 4 H), 6.96-6.91 (q, *J* = 8.4 Hz, 4 H), 6.86 (d, *J* = 8.88 Hz, 1 H), 4.56(s, 2 H), 4.11 (m, 1 H), 3.79 (s, 3 H), 1.61 (d, *J* = 6.96 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 498.09 amu; observed mass of $(M + H)^+$, 497.95 amu

(S)-N-(3-bromobenzyl)-2-(4-methoxyphenoxy)-5-(2-(methylamino)propanamido)benzamide (BI-75C12) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.08 (s, 1 H), 7.69 (d, *J* = 7.62 Hz, 1 H), 7.28-7.19 (m, 4 H), 6.97-6.92 (q, *J* = 8.52 Hz, 4 H), 6.86 (d, *J* = 8.64 Hz, 1 H), 4.57 (s, 2 H), 3.98 (m, 1 H), 3.79 (s, 3 H), 2.73 (s, 3 H),

1.62 (d, J = 6.96 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 512.11 amu; observed mass of $(M + H)^+$, 511.95 amu

(S)-methyl 2-(5-((S)-2-aminopropanamido)-2-(4-methoxyphenoxy)benzamido)-2-phenylacetate (BI-75D3) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.16 (s, 1 H), 7.74 (d, J = 7.14 Hz, 1 H), 7.27-7.20 (m, 5 H), 7.01 (d, J = 7.98 Hz, 2 H), 6.96 (d, J = 7.98 Hz, 2 H), 6.90 (d, J = 7.98 Hz, 1 H), 5.63 (s, 1 H), 4.08 (m, 1 H), 3.81 (s, 3 H), 3.70 (s, 3 H), 1.60 (d, J = 6.12 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 478.19 amu; observed mass of $(M + H)^+$, 478.05 amu

(S)-methyl 2-(2-(4-methoxyphenoxy)-5-((S)-2-(methylamino)propanamido)benzamido)-2-phenylacetate (BI-75D4) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.14 (s, 1 H), 7.75 (d, J = 6.96 Hz, 1 H), 7.32 (m, 5 H), 7.01 (d, J = 7.26 Hz, 2 H), 6.95 (d, J = 7.50 Hz, 2 H), 6.89 (d, J = 8.46 Hz, 1 H), 5.63 (s, 1 H), 4.10 (m, 1 H), 3.80 (s, 3 H), 3.69 (s, 3 H), 2.49 (s, 3 H), 1.42 (d, J = 5.94 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 492.21 amu; observed mass of $(M + H)^+$, 492.15 amu

(S)-5-(2-aminopropanamido)-2-(4-methoxyphenoxy)-N-(2-(trifluoromethyl)benzyl)benzamide (BI-75D5) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.10 (s, 1 H), 7.69 (d, J = 8.70 Hz, 1 H), 7.66 (d, J = 7.56 Hz, 1 H), 7.53 (d, J = 7.5 Hz, 1 H), 7.46 (t, J = 7.26 Hz, 1 H), 7.41 (t, J = 7.38 Hz, 1 H), 6.98 (d, J = 8.46 Hz, 2 H), 6.94 (d, J = 8.52 Hz, 2 H), 6.87 (d, J = 8.82 Hz, 1 H), 4.78 (s, 2 H), 4.10 (q, J = 6.96 Hz, 1 H), 3.80 (s, 3 H), 1.61 (d, J = 6.90 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 488.17 amu; observed mass of $(M + H)^+$, 488.10 amu

(S)-2-(4-methoxyphenoxy)-5-(2-(methylamino)propanamido)-N-(2-(trifluoromethyl)benzyl)benzamide (BI-75D6) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.13 (s, 1 H), 7.70 (d, J = 8.40 Hz, 1 H), 7.66 (d, J = 7.80 Hz, 1 H), 7.53 (d, J = 7.80 Hz, 1 H), 7.46 (t, J = 6.60 Hz, 1 H), 7.42 (t, J = 7.32 Hz, 1 H), 6.99 (d, J = 8.28 Hz, 2 H), 6.94 (d, J = 8.46 Hz, 2 H), 6.87 (d, J = 8.46 Hz, 1 H), 4.78 (s, 2H), 4.01 (m, 1 H), 3.80 (s, 3 H), 2.74 (s, 3 H), 1.63 (d, J = 6.84 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 502.19 amu; observed mass of $(M + H)^+$, 502.05 amu

(S)-5-(2-aminopropanamido)-N-(2-fluorobenzyl)-2-(4-methoxyphenoxy)benzamide (BI-75D7) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.10 (s, 1 H), 7.67 (d, J = 6.90 Hz, 1 H), 7.30 (t, J = 6.96 Hz, 1 H), 7.24 (q, J = 7.08 Hz, 1 H), 7.04-7.00 (m, 2 H), 6.95 (d, J = 8.52 Hz, 2 H), 6.90 (d, J = 9.30 Hz, 2 H), 6.83 (d, J = 8.76 Hz, 1 H), 4.61 (s, 2 H), 4.14 (m, 1 H), 3.78 (s, 3 H), 1.62 (d, J = 6.96 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 438.18 amu; observed mass of $(M + H)^+$, 438.05 amu

(S)-N-(2-fluorobenzyl)-2-(4-methoxyphenoxy)-5-(2-(methylamino)propanamido)benzamide (BI-75D8) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.11 (s, 1 H), 7.68 (d, J = 8.58 Hz, 1 H), 7.31 (t, J = 7.62 Hz, 1 H), 7.24 (q, J = 6.18 Hz, 1 H),7.05-7.01 (m, 2 H), 6.96 (d, J = 7.26 Hz, 2 H), 6.92 (d, J = 8.40 Hz, 2 H), 6.84 (d, J = 8.88 Hz, 1 H), 4.62 (s, 2 H), 4.03 (m, 1 H), 3.79 (s, 3 H), 2.74 (s, 3 H), 1.63 (d, J = 6.72 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 452.19 amu; observed mass of $(M + H)^+$, 452.10 amu

(S)-5-(2-aminopropanamido)-2-(4-methoxyphenoxy)-N-(2-methylbenzyl)benzamide (BI-75D9) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.09 (s, 1 H), 7.67 (d, J = 8.28 Hz, 1 H), 7.23 (d, J = 7.44 Hz, 1 H), 7.13-7.11 (m, 2 H), 7.06 (m, 1 H), 6.92 (m, 4 H), 6.84 (t, J = 8.04 Hz, 1 H), 4.56 (s, 2 H), 4.10 (m, 1 H), 3.79 (s, 3 H), 2.28 (s, 3 H), 1.61 (d, J = 6.78 Hz, 3 H). LC-MS expected mass of $(M + H)^+$,434.20 amu; observed mass of $(M + H)^+$, 434.10 amu

(S)-2-(4-methoxyphenoxy)-5-(2-(methylamino)propanamido)-N-(2-methylbenzyl)benzamide (BI-75D10) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.05 (s, 1 H), 7.70 (d, *J* = 9.84 Hz, 1 H), 7.23 (d, *J* = 7.26 Hz, 1 H), 7.13 (m, 2 H), 7.07 (m, 1 H), 6.93 (m, 4 H), 6.85 (d, *J* = 8.22 Hz, 1 H), 4.59 (s, 2 H), 4.10 (m, 1 H), 3.79 (s, 3 H), 2.50 (s, 3 H), 2.29 (s, 3 H), 1.43 (d, *J* = 6.54 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 448.22 amu; observed mass of $(M + H)^+$, 448.00 amu

(S)-5-(2-aminopropanamido)-N-(2-bromobenzyl)-2-(4-methoxyphenoxy)benzamide (BI-75D11) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.78 (s, 1 H, -NH), 8.06 (s, 1 H), 7.69 (d, *J* = 8.52 Hz, 1 H), 7.28-7.18 (m, 4 H), 6.95 (dd, *J* = 13.38 Hz, *J*' = 8.52 Hz, 4 H), 6.86 (d, *J* = 8.76 Hz, 1 H), 4.57 (s, 2 H), 4.05 (m, 1 H), 3.80 (s, 3 H), 1.60 (d, *J* = 6.90 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 498.10 amu; observed mass of $(M + H)^+$, 498.00 amu

(S)-N-(2-bromobenzyl)-2-(4-methoxyphenoxy)-5-(2-(methylamino)propanamido)benzamide (BI-75D12) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.78 (s, 1 H, -NH), 8.09 (s, 1 H), 7.69 (d, *J* = 8.82 Hz, 1 H), 7.28-7.21 (m, 4 H), 6.95 (dd, *J* = 11.82 Hz, *J*' = 8.58 Hz, 4 H), 6.86 (d, *J* = 8.64 Hz, 1 H), 4.57 (s, 2 H), 4.11 (m, 1 H), 3.80 (s, 3 H), 2.74 (s, 3 H), 1.62 (d, *J* = 6.84 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 512.11 amu; observed mass of $(M + H)^+$, 512.05 amu

(S)-5-(2-aminopropanamido)-2-(4-methoxyphenoxy)-N-(2-(trifluoromethoxy)benzyl)benzamide (BI-75E1) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.81 (s, 1 H, -NH), 8.09 (s, 1 H), 7.70 (d, *J* = 7.92 Hz, 1 H), 7.41 (d, *J* = 7.08 Hz, 1 H), 7.34 (t, *J* = 7.44 Hz, 1 H), 7.27 (d, *J* = 7.44, 1 H), 7.22 (m, 1 H), 6.98 (d, *J* = 8.04 Hz, 2 H), 6.95 (d, *J* = 8.28 Hz, 2 H), 6.86 (d, *J* = 8.76 Hz, 1 H), 4.68 (s, 2 H), 4.06 (m, 1 H), 3.81 (s, 3 H), 1.60 (d, *J* = 6.72 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 504.17 amu; observed mass of $(M + H)^+$, 504.05 amu

(S)-2-(4-methoxyphenoxy)-5-(2-(methylamino)propanamido)-N-(2-(trifluoromethoxy)benzyl)benzamide (BI-75E2) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.82 (s, 1 H, -NH), 8.11 (s, 1 H), 7.71 (d, *J* = 8.10 Hz, 1 H), 7.41 (d, *J* = 7.32 Hz, 1 H), 7.34 (t, *J* = 7.56 Hz, 1 H), 7.27 (d, *J* = 7.68 Hz, 1 H), 7.22 (t, *J* = 7.26 Hz, 1 H), 6.99 (d, *J* = 8.22 Hz, 2 H), 6.95 (d, *J* = 8.40 Hz, 2 H), 6.86 (d, *J* = 8.70 Hz, 1 H), 4.66 (s, 2 H), 3.96 (m, 1 H), 3.81 (s, 3 H), 2.74 (s, 3 H), 1.62 (d, *J* = 6.72 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 518.18 amu; observed mass of $(M + H)^+$, 518.05 amu

(S)-5-(2-aminopropanamido)-N-(4-chlorobenzyl)-2-(4-methoxyphenoxy)benzamide (BI-75E3) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.07 (s, 1 H), 7.69 (d, *J* = 7.44 Hz, 1 H), 7.26-7.21 (m, 4 H), 6.97 (d, *J* = 8.52 Hz, 2 H), 6.93 (d, *J* = 8.52 Hz, 2 H), 6.86 (d, *J* = 8.58 Hz, 1 H), 4.57 (s, 2 H), 4.07 (m, 1 H), 3.80 (s, 3 H), 1.60 (d, J = 6.42 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 454.15 amu; observed mass of $(M + H)^+$, 454.00 amu

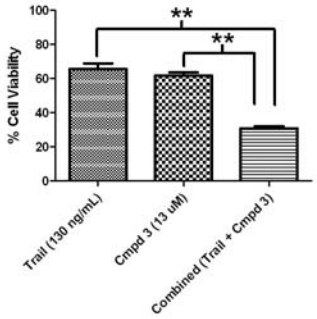
(S)-N-(4-chlorobenzyl)-2-(4-methoxyphenoxy)-5-(2-(methylamino)propanamido)benzamide (BI-75E4) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.09 (s, 1 H), 7.69 (d, *J* = 7.32 Hz, 1 H), 7.28-7.21 (m, 4 H), 6.96 (d, *J* = 7.62 Hz, 2 H), 6.93 (d, *J* = 7.86 Hz, 2 H), 6.86 (d, *J* = 8.52 Hz, 1 H), 4.57 (s, 2 H), 3.99 (m, 1 H), 3.80 (s, 3 H), 2.74 (s, 3 H), 1.62 (d, *J* = 6.36 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 468.16 amu; observed mass of $(M + H)^+$, 467.95 amu

(S)-N-(2,5-dimethylbenzyl)-2-(4-methoxyphenoxy)-5-(2-(methylamino)propanamido)benzamide (BI-75E5) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.23 (s, 1 H), 7.75 (d, *J* = 7.62 Hz, 1 H), 7.03 (dd, *J* = 17.4 Hz, *J*' = 8.10 Hz, 4 H), 6.93 (s, 1 H), 6.87 (m, 2 H), 6.83 (m, 1 H), 4.59 (s, 2 H), 4.06 (m, 1 H), 3.86 (s, 3 H), 3.72 (s, 3 H), 3.69 (s, 3 H), 2.74 (s, 3 H), 1.68 (d, *J* = 5.82 Hz, 3 H).

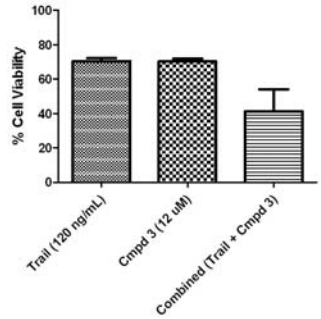
(S)-N-benzyl-2-(4-methoxyphenoxy)-5-(2-(methylamino)propanamido)benzamide (BI-75E6) ^{1}H NMR (600 MHz, MeOD-d4) δ 8.09 (s, 1 H), 7.69 (d, *J* = 7.38 Hz, 1 H), 7.28-7.21 (m, 5 H), 6.95 (dd, *J* = 13.98, *J*' = 7.38 Hz, 4 H), 6.85 (d, *J* = 8.46 Hz, 1 H), 4.57 (s, 2 H), 3.97 (m, 1 H), 3.78 (s, 3 H), 2.74 (s, 3 H), 1.62 (d, *J* = 6.42 Hz, 3 H). LC-MS expected mass of $(M + H)^+$, 434.20 amu; observed mass of $(M + H)^+$, 434.10 amu

A)

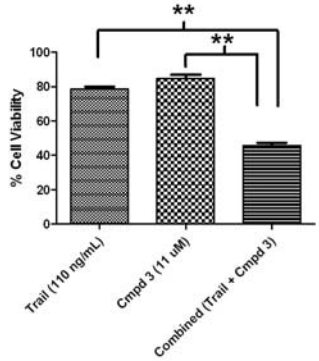
Repeated measures one-way ANOVA data (130 ng/mL and 13 μM)



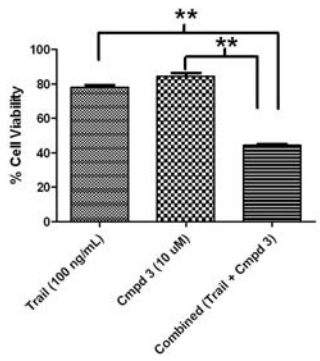
Repeated measures one-way ANOVA data (120 ng/mL and 12 μM)



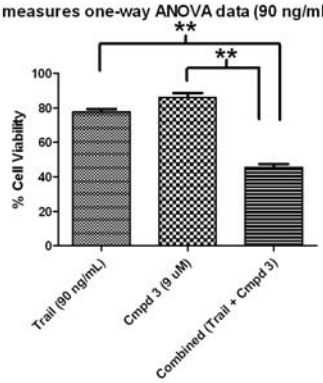
Repeated measures one-way ANOVA data (110 ng/mL and 11 μM)



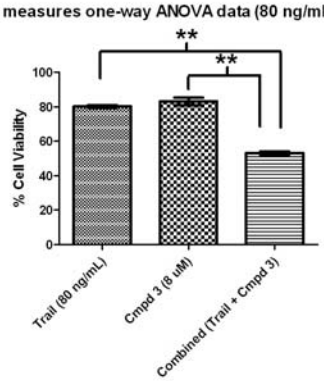
Repeated measures one-way ANOVA data (100ng/mL and 10 μM)



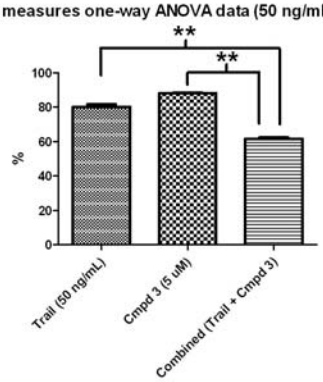
Repeated measures one-way ANOVA data (90 ng/mL and 9 μM)



Repeated measures one-way ANOVA data (80 ng/mL and 8 μM)



Repeated measures one-way ANOVA data (50 ng/mL and 5 μM)



B)

CI For experimental values		
Trail (ng / mL)	Cmpd 3 (uM)	CI
50	5	0.44
80	8	0.49
90	9	0.40
100	10	0.41
110	11	0.50
120	12	0.27
130	13	0.32

Supplementary Figure 6. A) Statistical analysis of MDAMB-231 growth inhibition data by compound **3** alone or in combination with TRAIL. The data are relative to Figure 3 of the manuscript. One-way ANOVA analysis and posttest (Newman-Keuls) comparison were carried out. ** Indicates difference between groups (** P< 0.01). B) Synergy Analysis w/ Calcusyn Software.For two drug combinations the value *CI* for a given effect is derived as in equation (12) where α=1 for a mutually non-exclusive case and α=0 for a mutually exclusive case. Multi-drug combinations greater than 2 follow a similar equation but cannot be performed for the mutually non-exclusive case. D_A refers to the dose of drug A alone required to give the given effect, $D_{A|A+B}$ refers to the dose of drug A in the combination of A+B required to give the given effect. CI < 1 indicates synergistic effect.

$CI_{(A+B)} = (D_{A \mid A+B}) / D_A + (D_{B \mid A+B}) / D_B + \alpha(D_{A \mid A+B})(D_{B \mid A+B}) / (D_A)(D_B)$