Design, Synthesis, and Evaluation of a Potent, Cell-Permeable, Conformationally Constrained Second Mitochondria Derived Activator of Caspase (Smac) Mimetic

Haiying Sun,[†] Zaneta Nikolovska-Coleska,[†] Jianfeng Lu,[†] Su Qiu,[†] Chao-Yie Yang,[†] Wei Gao,[†] Jennifer Meagher,[‡] Jeanne Stuckey,[‡] and Shaomeng Wang*,[†]

Comprehensive Cancer Center, Departments of Internal Medicine, Pharmacology, and Medicinal Chemistry, and Life Sciences Institute, University of Michigan, 1500 E. Medical Center Drive, Ann Arbor, Michigan 48109

Received September 24, 2006

A potent, cell-permeable, conformationally constrained second mitochondria derived activator of caspase mimetic (SM-131, **2**) has been designed, synthesized, and evaluated. Compound **2** binds to X-linked inhibitors of apoptosis proteins (XIAP) with a K_i of 61 nM in a competitive binding assay and directly antagonizes the XIAP inhibition of caspase-9 activity in a cell-free functional assay. Compound **2** achieves an IC_{50} of 100 nM in inhibition of cell growth and effectively induces cell death in the MDA-MB-231 human breast cancer cell line.

Introduction

Apoptosis, or programmed cell death, is a critical cell process in normal development and homeostasis of multicellular organisms.[1] Apoptosis dysfunction is a hallmark of human cancers and plays a major role in the failure of current anticancer drugs.[2] Design and development of small molecules that target critical apoptosis regulators to induce cancer cells to undergo apoptosis is an attractive therapeutic approach for the development of new classes of anticancer agents.[3,4]

Inhibitors of apoptosis proteins (IAPs) are a class of key apoptosis inhibitors.[5,6] Among all the IAP members, the X-linked IAP (XIAP) has attracted the greatest attention because of its potent biological functions and its role in cancer.[5−9] XIAP has been found to be widely expressed in many human cancer cell lines and tumor tissues,[7] and such overexpression confers apoptosis resistance on cancer cells to various anticancer agents.[8,9] Accordingly, targeting XIAP is a promising therapeutic strategy for the design of a new class of anticancer therapy.[7−9]

Smac/DIABLO (*s*econd *m*itochondria derived *a*ctivator of *c*aspase or *d*irect *IA*P-*b*inding protein with *lo*w pI) is a potent proapoptotic protein.[5,6] It promotes apoptosis, at least in part, by directly interacting with XIAP and other IAP members and inhibiting their antiapoptotic function.[10−15] XIAP contains three baculoviral IAP repeat (BIR) domains.[5,6] Its BIR2 domain, together with the linker before the BIR2 domain, binds to effector caspases-3 and -7 and inhibits their activity, and its BIR3 domain binds to and inhibits an initiator caspase-9.[5,6] Biological and structural studies have demonstrated that Smac binds to the BIR3 domain of XIAP, where caspase-9 binds.[13−17] The binding of Smac protein to XIAP blocks the XIAP−caspase-9 interaction and relieves the inhibition of XIAP to caspase-9.[16,17] This leads to an increased activity of caspase-9 and other caspases in cells and promotes apoptosis.

High-resolution X-ray crystal and NMR solution experiments have shown that the interaction between Smac and the BIR3 domain of XIAP is mediated by the N-terminal four residues (AVPI) in Smac and a well-defined surface groove in XIAP BIR3.[14,15] The binding site in the BIR3 domain of XIAP where Smac and caspase-9 bind is ideally suited for the design of small-molecule Smac mimetics that antagonize XIAP.[8] Because XIAP is widely expressed in human cancers, Smac mimetics may hold great promise for the treatment of many types of human cancers by promoting apoptosis in cancer cells.[18−24]

Design and Synthesis

Our laboratory has previously reported the structure-based design and synthesis of a series of conformationally constrained Smac mimetics.[22,23] Among them, **1** is the most potent Smac mimetic and binds to a recombinant XIAP BIR3 protein with a K_i of 25 nM in our competitive, fluorescence-polarization-based (FP-based) binding assay.[22] However, **1** has very weak activity in cell-based assays,[22] a major shortcoming that must be overcome for the development of our Smac mimetics as new anticancer drugs. Herein, we report the design, synthesis, and biochemical and biological evaluation of a potent Smac mimetic based on **1** but with dramatically improved cellular activity. In addition, we show that our potent Smac mimetics function as direct antagonists of XIAP BIR3 protein in a cell-free functional assay.

Analysis of the chemical structure of **1** showed that it contains a primary amino group that is positively charged at physiological pHs, which may account for its poor cell permeability. Consistent with this observation, peptide-based Smac mimetics in which this free amino group is methylated are found to have potent cellular activity.[24] We have therefore focused our modifications on the primary amino group in **1** to improve its cell permeability.

Our modeled structure of **1** complexed with XIAP BIR3 showed that the primary amino group forms an extensive hydrogen bond network with a number of negatively charged residues in XIAP, including Asp309 and Glu314 residues.[22] Indeed, blocking of this amino group with an acetyl group in Smac-based peptides made the resulting peptides inactive in binding to XIAP BIR3,[21] indicating the critical importance of the hydrogen-bonding network for binding between Smac mimetics and XIAP BIR3. Accordingly, we have designed a series of analogues of **1**, in which the primary amino group has been replaced with a methylated secondary or tertiary amino group or with a hydroxyl group (Figure 1). Under physiological conditions, the methylated secondary or tertiary amino group

* To whom correspondence should be addressed. Phone: 734-615-0362. Fax: 734-647-9647. E-mail: shaomeng@umich.edu.
[†] Comprehensive Cancer Center and Departments of Internal Medicine, Pharmacology, and Medicinal Chemistry.
[‡] Life Sciences Institute.

10.1021/jm061108d CCC: $33.50
Published on Web 11/23/2006

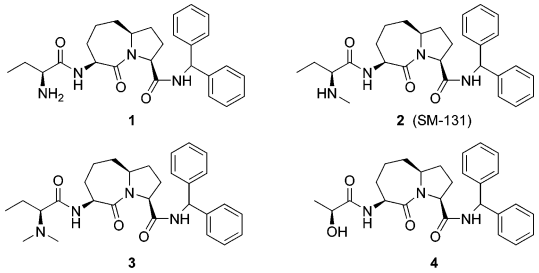
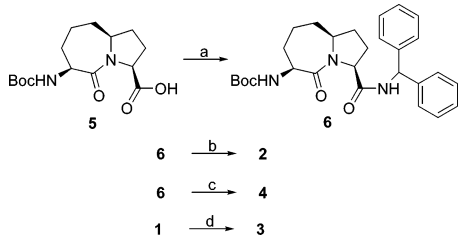


Figure 1. Chemical structures of designed Smac mimetics.

Scheme 1. Synthesis of New Analogues of Compound **1**[a]



[a] Reagents and conditions: (a) aminodiphenylmethane, EDC, HOBt, *N*,*N*-diisopropylethylamine, CH_2Cl_2, 0 °C− to room temp, 92%; (b) (i) 4 N HCl in 1,4-dioxane, MeOH; (ii) (*S*)-*N*-methyl-*N*-Boc-aminobutyric acid, EDC, HOBt, *N*,*N*-diisopropylethylamine, CH_2Cl_2, room temp; (iii) 4 N HCl in 1,4-dioxane, MeOH, 79% over three steps; (c) (i) 4 N HCl in 1,4-dioxane, MeOH; (ii) L-lactic acid, EDC, HOBt, *N*,*N*-diisopropylethylamine, CH_2Cl_2, room temp; 82% over rwo steps; (d) formaldehyde (37.4% solution in water), $NaBH_3CN$, MeOH, 78%.

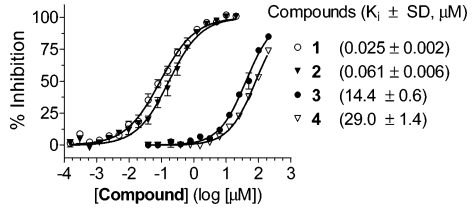


Figure 2. Competitive binding curves of designed Smac mimetics to recombinant XIAP BIR3 protein as determined using a fluorescence-polarization-based assay.

or the hydroxyl group should still be capable of forming one or more hydrogen bonds with XIAP BIR3.

Compounds **2**−**4** were synthesized using the procedures outlined in Scheme 1. Briefly, the key intermediate **5** was synthesized using the same procedure in our previous publication.[22] Condensation of **5** with aminodiphenylmethane gave **6**. Removal of the Boc protecting group in **6** followed by condensation of the resulting ammonium salt with (*S*)-*N*-methyl-*N*-Boc-aminobutyric acid afforded an amide. Removal of the Boc protecting group in this amide by treatment with HCl in methanol yielded **2**. Condensation of the ammonium salt derived from **6** by deprotection of the Boc protecting group with L-lactic acid furnished **4**. Condensation of **1** with formaldehyde followed by reduction of the resulted enamine with $NaBH_3CN$ gave **3**.

Results and Discussion

Compounds **2**−**4**, together with **1**, were first evaluated for their binding affinities to recombinant XIAP BIR3 protein using our FP-based competitive.[25] The results are shown in Figure 2.

Compound **2** binds to XIAP BIR3 with a K_i of 61 nM in this competitive binding assay, while **3** and **4** have K_i of 14.4 and 29.9 μM, respectively. In comparison, **1** has a K_i of 25 nM. Thus, **2** with a secondary amino group is 2 times less potent in our binding assay than **1** with a primary amino group. Compound **3**, with a tertiary amino group, is more than 500 times less potent than **1**. Compound **4** with a hydroxyl group is

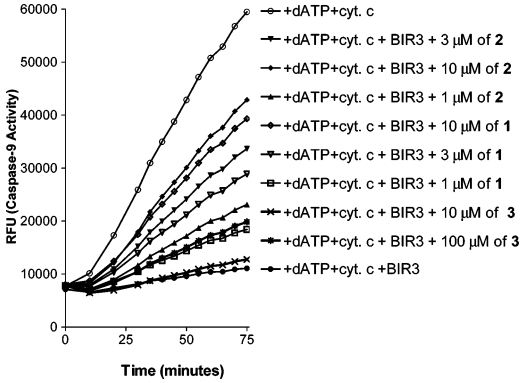


Figure 3. Evaluation of Smac mimetics in cell-free caspase-9 functional assay.

1000 times less potent than **1**. These binding data indicate that primary and secondary amino groups are clearly preferred for Smac mimetics to interact with XIAP BIR3 at this site and to achieve high affinity, whereas a tertiary amino group or a hydroxyl group is highly detrimental to binding.

Our Smac mimetics are designed to target XIAP BIR3, which is known to bind to caspase-9 and effectively inhibits caspase activity. We next sought to evaluate if these Smac mimetics function as direct antagonists of XIAP in a functional assay and if their potencies correlate with their binding affinities to XIAP BIR3. For this purpose, we established a cell-free functional assay using recombinant XIAP BIR3 protein and cell lysates (Supporting Information) and tested our Smac mimetics for their ability to directly overcome the inhibitory effect of XIAP BIR3 protein to caspase-9 activity in this functional assay (Figure 3). In this assay, addition of dATP and cytochrome *c* into cell lysates activates caspase-9 (Figure 3 and Support Information). As expected, recombinant XIAP BIR3 protein effectively inhibits the activity of caspase-9 (Figure 3). Consistent with their high binding affinities, **1** and **2** are potent and effective in relieving the inhibition of XIAP BIR3 to caspase-9 activity. At 3 and 10 μM respectively, **1** and **2** can restore the activity of caspase-9 by 40% and 75%, respectively, in the presence of 0.5 μM XIAP BIR3 protein. In comparison, **3** with a low binding affinity to XIAP BIR3 is much less effective in this functional assay than **1** and **2**. At 10 μM, **3** has a minimal effect, and at 100 μM it restores only 15% of the activity of caspase-9. Thus, these functional data show that our designed Smac mimetics act as direct antagonists of XIAP BIR3 protein and their potencies in this functional assay nicely correlate with their binding affinities to XIAP BIR3.

Our major goal for the design and synthesis of these new Smac mimetics is to improve their cellular activity. For this purpose, we have evaluated them for their activity in a number of cell-based assays.

These compounds were evaluated for their activity in inhibiting cell growth in the MDA-MB-231 human breast cancer cell line using the WST-based assay (Support Information). The results are shown in Figure 4. As can be seen, **1** has an IC_{50} of 50 μM in inhibition of cell growth. In comparison, **2** achieves an IC_{50} of 100 nM and is 500 times more potent than **1**. Although **3** has a much weaker binding affinity than **1**, it is more potent than **1** with an IC_{50} of 2.9 μM, indicating its superior cell permeability. Compound **4** with a hydroxyl group has an IC_{50} of 65 μM, consistent with its very weak binding affinity to XIAP BIR3.

XIAP functions as a potent inhibitor of cell death, and Smac mimetics may effectively induce cell death in certain cancer

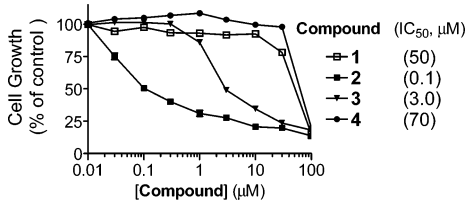


Figure 4. Inhibition of cell growth by Smac mimetics in the MDA-MB-231 human breast cancer cell line. Cells were treated for 4 days, and cell growth inhibition was determined using the WST-based assay.

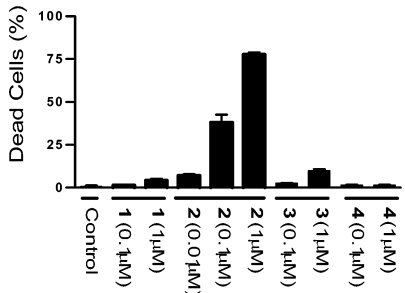


Figure 5. Induction of cell death in the MDA-MB-231 breast cancer cells by Smac mimetics. Cells were treated for 4 days, and cell viability was determined using the Trypan blue exclusion assay.

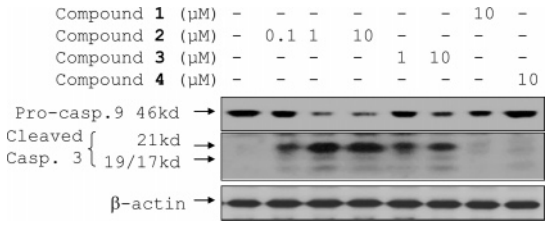


Figure 6. Western blot analysis of activation of caspase-9 and -3 in the MDA-MB-231 breast cancer cells induced by Smac mimetics. Cells were treated for 24 h by Smac mimetics, and procaspase-9 and cleaved caspase-3 were examined using specific monoclonal antibodies.

cells where XIAP acts as the final defensive mechanism to protect cells from undergoing cell death. We have tested these compounds for their ability to induce cell death in the MDA-MB-231 cell line using the Trypan blue exclusion assay (Figure 5 and Support Information). Our data showed that **2** effectively induces cell death in MDA-MB-231 cells in a dose-dependent manner. Significant cell death induction was observed at concentrations as low as 10 nM. When cells were treated with 100 nM and 1 μM **2** for 4 days, 40% and 80% of cells underwent cell death. These data showed that **2** is a highly effective and potent inducer of cell death in the MDA-MB-231 cancer cells. Consistent with their activity in inhibition of cell growth, **1**, **3**, and **4** are much less potent than **2** in induction of cell death in MDA-MB-231 cells, and **3** is clearly more potent than **1** and **4**. Hence, their activities in induction of cell death for **1**−**4** correlate with their potencies in inhibition of cell growth.

Potent and cell-permeable Smac mimetics are predicted to activate caspase-9 and -3 by overcoming the inhibition of XIAP in cancer cells. We have therefore evaluated **1**−**4** for their ability to activate caspase-9 and -3 in the MDA-MB-231 cells using Western blot analysis. The results are shown in Figure 6.

As can be seen, **2**, effectively and in a dose-dependent manner, induces a decrease of procaspase-9 protein, indicative of processing and activation of procaspase-9. Induction of caspase-9 processing is evident using 0.1 μM **2** and becomes more pronounced at 1 and 10 μM. In addition, **2** at concentrations as low as 0.1 μM also causes significant caspase-3 cleavage, indicative of activation of caspase-3. In comparison, **1** and **4** at 10 μM have a minimal effect in induction of caspase-9 processing and cleavage of caspase-3. Compound **3** at 1 and 10 μM clearly induces cleavage of caspase-3, although to lower degree than **2**. These data are highly consistent with the activity of these compounds in inhibition of cell growth and induction of cell death (Figures 4 and 5).

In summary, our chemical modifications on the primary amino group in **1** yielded a series of new analogues. Of these, **2** binds to XIAP BIR3 with a K_i of 61 nM, similar to that of **1**. Consistent with their modes of action, **1** and **2** function as potent and direct antagonists of XIAP BIR3 in promoting the activity of caspase-9 in a cell-free functional assay. However, **2** is 500 times more potent than **1** in inhibition of cell growth in the MDA-MB-231 human breast cell line. Compound **2** is highly effective in induction of cell death and activates caspase-9 and -3 in the MDA-MB-231 cancer cells. Significantly, **2** shows minimal toxicity to normal cells (data not shown). Collectively, these data demonstrate that **2**, which we named as SM-131 (*S*mac *m*imetic 131), is a potent, cell-permeable, and promising Smac mimetic. Compound **2** is an excellent pharmacological tool to elucidate the role of XIAP and other IAP proteins in human cancer and other diseases and warrants extensive evaluation in vitro and in vivo as a potential new anticancer therapy.

Experimental Section

I. Chemistry. General Methods. ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra at 75 MHz on a Bruker ADVANCE300 spectrometer. ^{1}H chemical shifts are reported with TMS (0.00 ppm) or DHO (4.70 ppm) as internal standards. ^{13}C chemical shifts are reported with $CDCl_3$ (77.00 ppm) or 1,4-dioxane (67.16 ppm) as internal standards.

All starting materials, solvents, and silica gel were purchased from Aldrich, Fisher, or Lancaster and were used without further purification. The final products were purified by a C18 reversed-phase semipreparative HPLC column with solvent A (0.1% of TFA in water) and solvent B (0.1% of TFA in CH_3CN) as eluents.

(3*S*,6*S*,10*S*)-[3-(Benzhydrylcarbamoyl)-5-oxooctahydropyrrolo-[1,2-a]azepin-6-yl]carbamic Acid *tert*-Butyl Ester (6). To a mixture of **5** (320 mg, 1 mmol), EDC (230 mg, 1.2 mmol), HOBt (160 mg, 1.2 mmol), and aminodiphenylmethane (185 mg, 1 mmol) in 5 mL of CH_2Cl_2 was added 0.6 mL of *N*,*N*-diisopropylethylamine at 0 °C. The mixture was warmed to room temperature and stirred overnight. The solvent was evaporated and the residue was purified by chromatography on silica gel to give **6** (440 mg, yield 92%). ^{1}H NMR (300 M Hz, $CDCl_3$) δ 7.99 (brd, J = 8.7 Hz, 1H), 7.37−7.19 (m, 10H), 6.22 (d, J = 8.7 Hz, 1H), 5.96 (d, J = 6.1 Hz, 1H), 4.80 (d, J = 7.4 Hz, 1H), 4.28 (m, 1H), 3.75 (m, 1H), 2.43 (m, 1H), 2.24 (m, 1H), 1.91−1.60 (m, 6H), 1.46 (s, 9H), 1.25 (m, 1H), 1.03 (m, 1H); ^{13}C NMR (75 MHz,$CDCl_3$) δ 173.27, 169.66, 155.05, 141.98, 141.20, 128.62, 128.47, 127.34, 127.23, 127.03, 61.00, 60.36, 59.40, 56.69, 54.06, 35.01, 33.41, 31.88, 28.32, 27.41, 25.21.

(3*S*,6*S*,10*S*)-6-((*S*)-2-Methylaminobutyrylamino)-5-oxooctahydropyrrolo[1,2-*a*]azepine-3-carboxylic Acid Benzhydrylamide (2). To a solution of compound **6** (95 mg, 0.2 mmol) in 3 mL of MeOH was added 1 mL of a solution of 4 N HCl in 1,4-dioxane. The solution was stirred at room temperature overnight and then evaporated. The residue was suspended in 5 mL of CH_2Cl_2. To this mixture were added L-*N*-Boc-*N*-methyl-2-aminobutyric acid (50 mg, 0.25 mmol), EDC (58 mg, 0.3 mmol), HOBt (40 mg, 0.3 mmol), and *N*,*N*-diisopropylethylamine (0.2 mL). After the mixture was stirred at room temperature overnight, the solvent was evaporated and the residue was purified by chromatography to give an amide. To a solution of this amide in 3 mL of methanol was added 0.5 mL of 4 N HCl in 1,4-dioxane. The solution was stirred at room temperature overnight and then evaporated to give crude **2**. The crude product was purified by HPLC on a C_{18} reversed-phase column to give the pure compound as a salt with trifluoacetic

acid (95 mg, yield 79%). The gradient ran from 75% of solvent A and 25% of solvent B to 60% of solvent A and 40% of solvent B in 30 min. The purity was confirmed by analytical HPLC to be over 98%. ^{1}H NMR (300 M Hz, D_2O) δ 7.34−7.19 (m, 10H), 5.96 (s, 1H), 4.52−4.46 (m, 2H), 3.91 (m, 1H), 3.73 (m, 1H), 2.56 (s, 3H), 2.18−1.45 (m, 12H), 0.91 (t, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, D_2O) δ 173.73, 172.62, 168.32, 141.54, 141.37, 129.56, 129.45, 128.41, 128.38, 127.92, 127.75, 63.23, 62.67, 60.20, 58.27, 54.45, 33.38, 33.08, 32.05, 29.98, 28.43, 27.58, 24.09, 8.80.

(3*S*,6*S*,10*S*)-6-((*S*)-2-Dimethylaminobutyrylamino)-5-oxooctahydropyrrolo[1,2-*a*]azepine-3-carboxylic Acid Benzhydrylamide (3). To a solution of **1** (salt with HCl, 100 mg, 0.2 mmol) in 5 mL of methanol was added 1 mL of formaldehyde solution (37.4% in water). The solution was cooled to 0 °C and stirred for 10 min. Then $NaBH_3CN$ (80 mg, 1 mmol) was added. After the mixture was stirred for 1 h at 0 °C, 10 mL of water was added to quench the reaction. The mixture was extracted by CH_2Cl_2 three times. The combined organic layers were dried over Na_2SO_4 and then evaporated. The residue was purified by chromatography to give **3**. To a solution of **3** in CH_2Cl_2 was added 3 equiv of trifluoroacetic acid. After removal of solvent, the residue was purified by HPLC to give the pure product (salt with TFA, 94 mg, yield 78%). The gradient ran from 75% of solvent A and 25% of solvent B to 60% of solvent A and 40% of solvent B in 30 min. The purity was confirmed by analytical HPLC to be over 98%. ^{1}H NMR (300 M Hz, D_2O) δ 7.30−7.14 (m, 10H), 5.89 (s, 1H), 4.48−4.36 (m, 2H), 3.79 (m, 1H), 3.66 (m, 1H), 2.76 (s, 3H), 2.71 (s, 3H), 2.10−1.38 (m, 12H), 0.84 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, D_2O) δ 173.46, 172.35, 167.65, 141.61, 141.45, 129.51, 129.43, 128.34, 127.88, 127.70, 70.00, 62.59, 60.13, 58.19, 54.46, 43.06, 41.08, 33.45, 33.07, 28.34, 27.55, 22.26, 8.84.

(3*S*,6*S*,10*S*)-6-((*S*)-2-Hydroxypropionylamino)-5-oxooctahydropyrrolo[1,2-*a*]azepine-3-carboxylic Acid Benzhydrylamide (4). To a solution of **6** (110 mg, 0.23 mmol) in 3 mL of MeOH was added 1 mL of a solution of 4 N HCl in 1,4-dioxane. The solution was stirred at room temperature overnight and then evaporated. The residue was suspended in 5 mL of CH_2Cl_2. To this mixture were added L-lactic acid (22 mg, 0.23 mmol), EDC (58 mg, 0.3 mmol), HOBt (40 mg, 0.3 mmol), and *N*,*N*-diisopropylethylamine aminodiphenylmethane (0.2 mL). After the mixture was stirred at room temperature overnight, the solvent was evaporated and the residue was purified by chromatography to give **4** (84 mg, yield 82%). Compound **4** was purified by HPLC again. The gradient ran from 70% of solvent A and 30% of solvent B to 50% of solvent A and 50% of solvent B. The purity was confirmed by analytical HPLC to be over 98%. ^{1}H NMR (300 M Hz, $CDCl_3$) δ 7.86 (d, J = 8.7 Hz, 1H), 7.65 (d, J = 6.8 Hz, 1H), 7.45−7.15 (m, 10H), 6.22 (d, J = 8.7 Hz, 1H), 4.75 (d, J = 6.4 Hz, 1H), 4.53 (m, 1H), 4.23 (m, 1H), 3.78 (m, 1H), 3.50 (brs, 1H), 2.39 (m, 1H), 2.23 (m, 1H), 2.15−1.58 (m, 6H), 1.43 (d, J = 6.8 Hz, 3H), 1.26 (m, 1H), 1.13 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.77, 172.83, 169.63, 141.90, 141.17, 128.65, 128.51, 127.40, 127.32, 127.28, 127.13, 68.14, 61.10, 59.41, 56.74, 52.59, 34.83, 33.34, 31.15, 27.32, 25.49, 21.15.

Acknowledgment. We are grateful for financial support from the National Cancer Institute, National Institutes of Health (Grant R01CA109025), the Breast Cancer Research Foundation, the Susan G. Komen Foundation, the Prostate Cancer Foundation, the University of Michigan Cancer Center (Core Grant P30CA046592), and Ascenta Therapeutics Inc.

Supporting Information Available: Information on the binding assay, cell-free functional assay, cell growth assay, cell death induction, and Western blot analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Kerr, J. F.; Wyllie, A. H.; Currie, A. R. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* **1972**, *26*, 239−257.
(2) Thompson, C. B. Apoptosis in the pathogenesis and treatment of disease. *Science* **1995**, *267*, 1456−1462.
(3) Nicholson, D. W. From bench to clinic with apoptosis-based therapeutic agents. *Nature* **2000**, *407*, 810−816.
(4) Reed, J. C. Apoptosis-based therapies. *Nat. Rev. Drug Discovery* **2002**, *1*, 111−121.
(5) Deveraux, Q. L.; Reed, J. C. IAP family proteins—suppressors of apoptosis. *Genes Dev.* **1999**, *1*, 239−252.
(6) Salvesen, G. S.; Duckett, C. S. IAP proteins: blocking the road to death's door. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 401−410.
(7) Tamm I.; Kornblau S. M.; Segall, H.; Krajewski, S.; Welsh, K.; Kitada, S.; Scudiero, D. A.; Tudor, G.; Qui, Y. H.; Monks, A.; Andreeff, M. Expression and prognostic significance of IAP-family genes in human cancers and myeloid leukemias. *Clin. Cancer Res.* **2000**, *6*, 1796−1803.
(8) LaCasse, E. C.; Baird, S.; Korneluk, R. G.; MacKenzie, A. E. The inhibitors of apoptosis (IAPs) and their emerging role in cancer. *Oncogene* **1998**, *17*, 3247−3259.
(9) Holcik, M.; Gibson, H.; Korneluk, R. G. XIAP: Apoptotic brake and promising therapeutic target. *Apoptosis* **2001**, *6*, 253−261.
(10) Huang, Y.; Park, Y. C.; Rich, R. L.; Segal, D.; Myszka, D. G.; Wu, H. Structural basis of caspase inhibition by XIAP: differential roles of the linker versus the BIR domain. *Cell* **2001**, *104*, 781−790.
(11) Du, C.; Fang, M.; Li, Y.; Li, L.; Wang, X. Smac, a mitochondrial protein that promotes cytochrome *c*-dependent caspase activation by eliminating IAP inhibition. *Cell* **2000**, *102*, 33−42.
(12) Verhagen, A. M.; Ekert, P. G.; Pakusch, M.; Silke, J.; Connolly, L. M.; Reid, G. E.; Moritz, R. L.; Simpson, R. J.; Vaux, D. L. Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. *Cell* **2000**, *102*, 43−53.
(13) Ekert, P. G.; Silke, J.; Hawkins, C. J.; Verhagen, A. M.; Vaux, D. L. DIABLO promotes apoptosis by removing MIHA/XIAP from processed caspase 9. *J. Cell Biol.* **2001**, *152*, 483−490.
(14) Wu, G.; Chai, J.; Suber, T. L.; Wu, J. W.; Du, C.; Wang, X.; Shi, Y. Structural basis of IAP recognition by Smac/DIABLO. *Nature* **2000**, *408*, 1008−1012.
(15) Liu, Z.; Sun, C.; Olejniczak, E. T.; Meadows, R.; Betz, S. F.; Oost, T.; Herrmann, J.; Wu, J. C.; Fesik, S. W. Structural basis for binding of Smac/DIABLO to the XIAP BIR3 domain. *Nature* **2000**, *408*, 1004−1008.
(16) Srinivasula, S. M.; Hegde, R.; Saleh, A.; Datta, P.; Shiozaki, E.; Chai, J.; Lee, R. A.; Robbins, P. D.; Fernandes-Alnemri, T.; Shi, Y.; Alnemri, E. S. A conserved XIAP-interaction motif in caspase-9 and Smac/DIABLO regulates caspase activity and apoptosis. *Nature* **2001**, *410*, 112−116.
(17) Shiozaki, E. N.; Chai, J.; Rigotti, D. J.; Riedl, S. J.; Li, P.; Srinivasula, S. M.; Alnemri, E. S.; Fairman, R.; Shi, Y. Mechanism of XIAP-mediated inhibition of caspase-9. *Mol. Cell* **2003**, *11*, 519−527.
(18) Fulda, S.; Wick, W.; Weller, M.; Debatin, K. M. Smac agonists sensitize for Apo2L/TRAIL- or anticancer drug-induced apoptosis and induce regression of malignant glioma *in vivo*. *Nat. Med.* **2002**, *8*, 808−815.
(19) Arnt, C. R.; Chiorean, M. V.; Heldebrant, M. P.; Gores, G. J.; Kaufmann, S. H. Synthetic Smac/DIABLO peptides enhance the effects of chemotherapeutic agents by binding XIAP and cIAP1 *in situ*. *J. Biol. Chem.* **2002**, *277*, 44236−44243.
(20) Yang, L.; Mashima, T.; Sato, S.; Mochizuki, M.; Sakamoto, H.; Yamori, T.; Oh-Hara, T.; Tsuruo, T. Predominant suppression of apoptosome by inhibitor of apoptosis protein in non-small cell lung cancer H460 cells: therapeutic effect of a novel polyarginine-conjugated Smac peptide. *Cancer Res.* **2003**, *63*, 831−837.
(21) Kipp, R. A.; Case, M. A.; Wist, A. D.; Cresson, C. M.; Carrell, M.; Griner, E.; Wiita, A.; Albiniak, P. A.; Chai, J.; Shi, Y.; Semmelhack, M. F.; McLendon, G. L. Molecular targeting of inhibitors of apoptosis proteins based on small molecule mimics of natural binding partners. *Biochemistry* **2002**, *41*, 7344−7349.
(22) Sun, H.; Nikolovska-Coleska, Z.; Yang, C.-Y.; Xu, L.; Liu, M.; Tomita, Y.; Pan, H.; Yoshioka, Y.; Krajewski, K.; Roller, P. P.; Wang, S. Structure-based design of potent, conformationally constrained Smac mimetics. *J. Am. Chem. Soc.* **2004**, *126* (51), 16686−16687.
(23) Sun, H.; Nikolovska-Coleska, Z.; Yang, C.-Y.; Xu, L.; Tomita, Y.; Krajewski, K.; Roller, P. P.; Wang, S. Structure-based design, synthesis, and evaluation of conformationally constrained mimetics of the second mitochondria-derived activator of caspase that target the X-linked inhibitor of apoptosis protein/caspase-9 interaction site. *J. Med. Chem.* **2004**, *47*, 4147−4150.

(24) Oost, T. K.; Sun, C.; Armstrong, R. C.; Al-Assaad, A. S.; Betz, S. F.; Deckwerth, T. L.; Ding, H.; Elmore, S. W.; Meadows, R. P.; Olejniczak, E. T.; Oleksijew, A.; Oltersdorf, T.; Rosenberg, S. H.; Shoemaker, A. R.; Tomaselli, K. J.; Zou, H.; Fesik, S. W. Discovery of potent antagonists of the antiapoptotic protein XIAP for the treatment of cancer. *J. Med. Chem.* **2004**, *47*, 4417−4426.

(25) Nikolovska-Coleska, Z.; Wang, R.; Fang, X.; Pan, H.; Tomita, Y.; Li, P.; Roller, P. P.; Krajewski, K.; Saito, N. G.; Stuckey, J. A.; Wang, S. Development and optimization of a binding assay for the XIAP BIR3 domain using fluorescence polarization. *Anal. Biochem.* **2004**, *332*, 261−273.

JM061108D