Brief Articles

Potent, Orally Bioavailable Diazabicyclic Small-Molecule Mimetics of Second Mitochondria-Derived Activator of Caspases

Yuefeng Peng,[†] Haiying Sun,[†] Zaneta Nikolovska-Coleska,[†] Su Qiu,[†] Chao-Yie Yang,[†] Jianfeng Lu,[†] Qian Cai,[†] Han Yi,[†] Sanmao Kang,[‡] Dajun Yang,[‡] and Shaomeng Wang*[,‡]

Comprehensive Cancer Center and Departments of Internal Medicine, Pharmacology and Medicinal Chemistry, University of Michigan, 1500 E. Medical Center Drive, Ann Arbor, Michigan 48109, Ascenta Therapeutics, Inc., 101 Lindenwood Drive, Suite 405, Malvern, Pennsylvania 19355

Received August 14, 2008

A series of small-molecule Smac mimetics containing a diazabicyclic core structure have been designed, synthesized, and evaluated. The most potent compound (**6**) binds to XIAP, cIAP-1, and cIAP-2 with K_i values of 8.4, 1.5, and 4.2 nM, respectively, directly antagonizes XIAP in a cell-free functional assay and induces cIAP-1 degradation in cancer cells. It inhibits cell growth with an IC_{50} value of 31 nM, effectively induces apoptosis in the MDA-MB-231 cancer cell line, and has a good oral bioavailability.

Introduction

Inhibitor of apoptosis proteins (IAPs)[a] are key apoptosis regulators characterized by the presence of one to three regions known as baculoviral IAP repeat (BIR) domains.[1,2] Among these IAP proteins, cellular IAP-1 (cIAP-1) and cIAP-2 play critical roles in regulation of tumor necrosis factor receptor-mediated apoptosis,[3,4] and X-linked IAP (XIAP) is a central regulator of both death-receptor-mediated and mitochondria-mediated apoptosis pathways.[5] XIAP and cIAP-1 play a role in apoptosis resistance of cancer cells to a variety of anticancer drugs and are considered to be promising cancer therapeutic targets.[5,6]

Second mitochondria-derived activator of caspases (Smac), a potent pro-apoptotic protein, is an endogenous antagonist of IAP proteins.[7,8] Previous studies have established that Smac interacts with XIAP and cIAP-1/2 proteins via its AVPI tetrapeptide motif.[2,9−12] In the past few years, intense research efforts have been devoted to the design and development of Smac mimetics, small molecules which mimic the AVPI binding motif and function as antagonists of IAP proteins.[13−22] Smac mimetics are considered to have the great potential to be developed as a new class of anticancer drugs by promoting apoptosis in cancer cells. Two types of Smac mimetics, monovalent and bivalent, have been reported. The monovalent compounds are designed to mimic the binding of a single AVPI binding motif to IAP proteins,[14−18] and the bivalent compounds contain two AVPI binding motif mimetics tethered together through a linker.[13,19−22] We have shown that the bivalent Smac mimetics can achieve much higher affinities to XIAP and are 1−2 orders of magnitude more potent than the corresponding monovalent Smac mimetic in induction of apoptosis in tumor cells.[19] However, because of their lower molecular weights, properly designed monovalent Smac mimetics can possess major advantages over bivalent Smac mimetics for purposes of drug design.

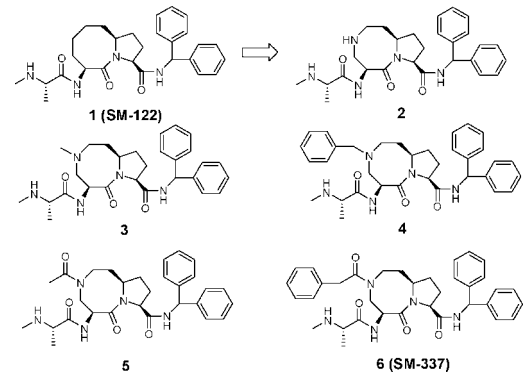


Figure 1. Chemical structures of designed Smac mimetics.

Our laboratory has previously reported the structure-based design, synthesis, and evaluation of a series of conformationally constrained monovalent Smac mimetics.[14,15,17,19] Compound **1** (Figure 1) binds to the XIAP BIR3 protein with a K_i value of 26 nM.[19] Compound **1** directly antagonizes XIAP in a cell-free functional assay and induces apoptosis in cancer cells.[19] It also binds to cIAP-1 and cIAP-2 with K_i values of 1.0 and 1.8 nM (Table 1), respectively, and is thus a potent monovalent Smac mimetic.

To examine if compound **1** can be potentially a drug candidate, we evaluated its pharmacokinetics (PK) in rats. Compound **1** achieved a maximum concentration (C_{max}) of 545 ± 43 nM, an elimination half-life ($T_{1/2}$) of 1.7 ± 0.3 h and $AUC_{(0-\infty)}$ (area-under-the-curve) of 482 ± 92 μg/L h at 25 mg/kg administered orally and an oral bioavailability of 14%. Hence, compound **1** has a modest AUC and oral bioavailability, making it less than an ideal drug candidate for further development. To improve the PK profile, we have decided to modify the core structure of **1**, which led to a new class of Smac mimetics. The most promising new compound (**6**) binds to XIAP and cIAP-1/2 with very high affinities, is more potent than **1** in cell-based

* To whom correspondence should be addressed. Phone: (734)615-0362. Fax: (734)6479647. E-mail: shaomeng@umich.edu.

† Comprehensive Cancer Center and Departments of Internal Medicine, Pharmacology and Medicinal Chemistry, University of Michigan.

‡ Ascenta Therapeutics, Inc.

[a] Abbreviations: IAP, inhibitor of apoptosis protein; XIAP, X-linked IAP; cIAP-1/-2, cellular IAP-1/2; Smac, second mitochondria-derived activator of caspases; BIR, baculoviral IAP repeats (BIR) domain; BIR2, the second BIR domain; BIR3, the third BIR domain; FP, fluorescence polarization; PK, pharmacokinetics; C_{max}, maximum concentration; $T_{1/2}$, elimination half-life; AUC, area-under-the-curve; PARP, poly(ADP-ribose) polymerase.

10.1021/jm801254r CCC: $40.75
Published on Web 12/02/2008

Table 1. Binding Affinities of Smac Mimetics to XIAP, cIAP-1, and cIAP-2, as Determined in Competitive, Fluorescence-Polarization Based Assays (Assays Details Provided in *Supporting Information*)[a]

	$K_i \pm$ SD (nM)		
compd	XIAP	cIAP-1	cIAP2
1	26 ± 4	1.0 ± 0.3	1.8 ± 0.6
2	20.0 ± 14.5	1.2 ± 0.1	4.6 ± 1.5
3	341 ± 65.9	3.3 ± 1.3	17.5 ± 4.3
4	91.8 ± 30.4	3.7 ± 1.4	9.8 ± 4.1
5	5.4 ± 3.0	1.3 ± 0.3	1.9 ± 0.8
6	8.4 ± 1.6	1.5 ± 0.5	4.2 ± 0.6

[a] Data were obtained using 3−5 independent experiments.

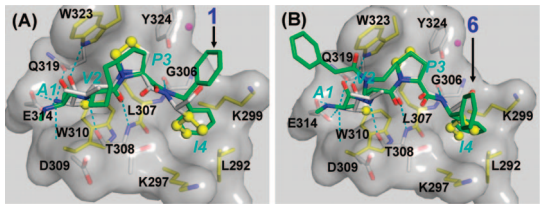
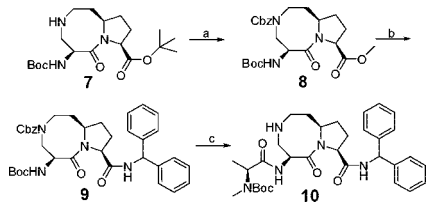


Figure 2. Predicted binding models of compounds **1** and **6** to XIAP BIR3 domain in superposition with Smac AVPI peptide. Compounds **1** and **6** are colored in green and the AVPI peptide in yellow. Binding pockets are shown in transparent surface. Oxygen, nitrogen, and sulfur atoms are colored in red, blue, and yellow, respectively. Hydrogen bonds are depicted in dash lines.

Scheme 1. Synthesis of the Key Intermediate **10**[a]



[a] Reagents and conditions: (a) (i) CbzCl, $NaHCO_3$, 1,4-dioxane, rt, overnight; (ii) $(Boc)_2O$, $NaHCO_3$, 1,4-dioxane, rt, overnight, 65% over three steps. (b) (i) 2 N LiOH, 1,4-dioxane, 3 h, then 1 N HCl; (ii) aminodiphenylmethane, EDC, HOBt, *N*,*N*-diisopropylethylamine, rt, overnight, 82% over two steps. (c) (i) 4 N HCl in 1,4-dioxane, methanol, rt, overnight; (ii) L-*N*-Boc-*N*-methyl-alanine, EDC, HOBt, *N*,*N*-diisopropylethylamine, rt, overnight; (iii) H_2, 10% Pd-C, methanol, overnight, 77% over three steps.

assays, and has improved PK parameters and oral bioavailability as compared to **1**.

Results and Discussion

Our model of **1** complexed with XIAP BIR3 showed that while the 8-membered ring has van der Waals contacts with Trp323 in XIAP BIR3, the middle portion of this 8-membered ring is largely exposed to solvent (Figure 2). On the basis of this model, we predicted that replacement of one of the carbons in the 8-membered ring atoms by a nitrogen may not be detrimental to the binding to XIAP.

To test this idea, we designed and synthesized compound **2** (Figure 1, Schemes 1 and 2), which has a diazabicyclic core structure. This compound binds to XIAP BIR3 with a K_i value of 20 nM in our fluorescence polarization-based (FP-based) XIAP binding assay[23] and is thus as potent as **1**. It also binds to cIAP-1 and cIAP-2 with K_i values of 1.2 and 4.6 nM, respectively, determined in FP-based competitive binding assays for these two proteins. Hence, **2** is a potent Smac mimetic and a promising compound for further optimization.

Modifications of the secondary amino nitrogen in the 8-membered ring of **2** were conducted to explore structure−activity relationships at this site. Introduction of a methyl or benzyl group on the nitrogen resulted in **3** and **4**, and introduction of an acetyl group, or a phenylacetyl group on the nitrogen atom, yielded **5** and **6**, respectively.

Scheme 2. Synthesis of Designed Smac Mimetics[a]

[a] Reagents and conditions: (a) 4 N HCl in 1,4-dioxane, methanol, rt, overnight, 95%. (b) (i) Formaldehyde (37% in water), $NaBH_3CN$, HOAc, methanol, rt, overnight; (ii) 4 N HCl in 1,4-dioxane, methanol, rt, overnight, 71% over two steps. (c) (i) BnBr, $NaHCO_3$, 1,4-dioxane, rt, overnight; (ii) 4 N HCl in 1,4-dioxane, methanol, rt, overnight 77% over two steps. (d) (i) Ac_2O or BnCOCl, *N*,*N*-diisopropylethylamine, CH_2Cl_2, rt, overnight; (ii) 4 N HCl in 1,4-dioxane, methanol, rt, overnight, 85% for **5** and 87% for **6** over two steps.

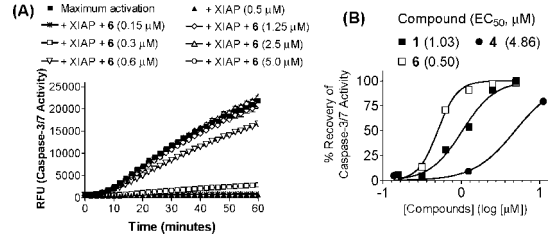


Figure 3. Inhibition of caspase-3/-7 activity by XIAP and antagonism of Smac mimetics to XIAP to recover the activity of caspase-3/-7 in a cell-free functional assay. (A) Caspase-3/-7 was activated by addition of cytochrome c and dATP into cell lysates and recombinant XIAP BIR3 protein at 0.5 μM completely inhibited caspase activation. Compound **6** dose-dependently recovered the caspase-3/-7 activity. (B). Dose-dependent recovery of caspase-3/-7 activity by **1**, **4**, and **6** to the maximum activation. Caspase-3/-7 activity at 30 min time point was used.

In binding to XIAP BIR3, both **5** and **6** have higher binding affinities than **2**, with K_i values of 5.4 and 8.4 nM, respectively. These compounds, with K_i values of 1.3 and 1.5 nM, respectively, are as potent as **2** to cIAP-1. Compound **6** is as potent and compound **5** is twice as potent as **2** in binding to cIAP-2. These binding data for compounds **2**−**6** showed that modifications at this site can significantly impact the binding affinities of these Smac mimetics to XIAP, cIAP-1, and cIAP-2 proteins.

We next evaluated if compounds **2**−**6** behave as antagonists of XIAP in cell-free functional assays. The results for three representative compounds, **1**, **4**, and **6**, are shown in Figure 3. The XIAP BIR3 protein effectively inhibits the activity of caspase-3/-7, and these Smac mimetics dose-dependently antagonize XIAP and restore caspase activity (Figure 3A and Supporting Information). Consistent with the binding affinities, compound **6** has twice the potency of **1** and 10 times that of **4** in antagonizing XIAP in this functional assay.

Previous studies showed that Smac mimetics can effectively inhibit cell growth and induce apoptosis in the MDA-MB-231 human breast cancer cell line.[16,17] We have evaluated these new Smac mimetics for cell growth inhibition in the MDA-MB-231 cancer cell line (Figure 4) and found that, while all these compounds are effective, **5** and **6**, with IC_{50} values of 69 and 31 nM, respectively, are the two most potent; **6** is 8 times more potent than **1** in this cell growth assay. Compounds **3** and **4** are

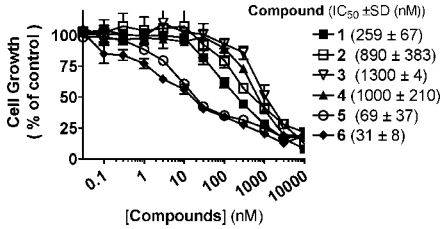


Figure 4. Inhibition of cell growth by Smac mimetics in the MDA-MB-231 cancer cell line. Cells were treated with Smac mimetics for 4 days and cell growth was determined using a WST-based assay.

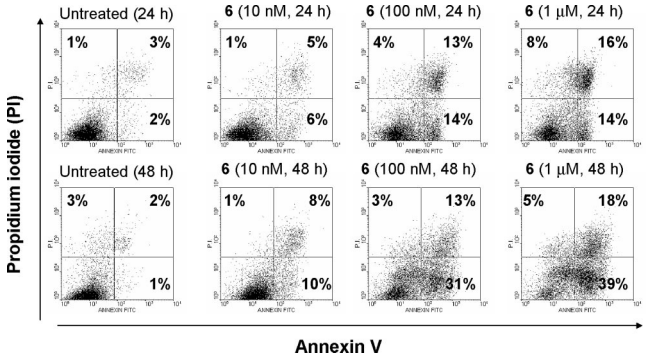


Figure 5. Induction of apoptisis by compound **6** in the MDA-MB-231 cell line. Cells were treated with different concentrations of **6** for 24 or 48 h. Apoptosis was analyzed using Annexin V and propidium iodide double staining by flow cytometry. Percentage of early apoptotic (Annexin V^+/PI^-), late apoptotic (Annexin V^+/PI^+), and dead (Annexin V^-/PI^+) cells are shown, respectively.

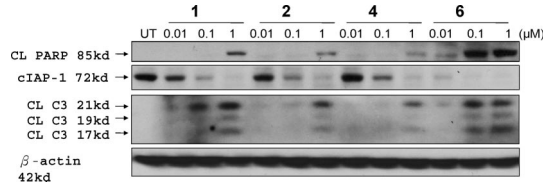


Figure 6. Induction of cIAP-1 degradation, cleavage of PARP, and processing of caspase-3 by compounds **1**, **2**, **4**, and **6** in the MDA-MB-231 cell line. Cells were treated with different concentrations of Smac mimetics for 24 h and levels of cIAP-1, cleaved PAPR (CL PARP), and cleaved caspase-3 (CL C3) were probed by Western blot.

the two least potent, with IC_{50} values of 1300 and 1000 nM, respectively.

We evaluated the most potent compound **6** for its ability to induce apoptosis in the MDA-MB-231 cancer cell line. Compound **6** effectively induces apoptosis in this cancer cell line in a dose- and time-dependent manner (Figure 5). For example, compound **6** at 100 nM for 24- and 48-h treatment induces 31% and 47% of the MDA-MB-231 breast cancer cells to undergo apoptosis, respectively.

Several recent studies have shown that Smac mimetics induce cIAP-1 degradation in cancer cells, mediating apoptosis induction, and cIAP-1 is a direct target of Smac mimetics.[20−22] We have evaluated our Smac mimetics for their ability to induce cIAP-1 degradation and cleavage of caspase-3 and poly(ADP-ribose) polymerase (PARP) in the MDA-MB-231 cell line. We found that **6** at concentrations as low as 10 nM effectively induces the degradation of cIAP-1 and is more potent than **1**, **2**, and **4** (Figure 6). Consistent with the potent activity in the apoptosis assay (Figure 5), at concentrations as low as 100 nM, **6** induces robust cleavage of PARP and processing of caspase-3, two biochemical markers of apoptosis, within 24 h and is more effective than **1**, **2**, or **4**.

We next evaluated the pharmacokinetics in rats of **6** dosed orally and found that it achieves a C_{max} of 532 ± 249 nM, a $T_{1/2}$ of 4.7 ± 2.2 h, and $AUC_{(0-\infty)}$ of 1985 ± 614 μg/L h at 30 mg/kg. The C_{max} value for **6** at 30 mg/kg is 17 times of its IC_{50} value in the cell growth assay in the MDA-MB-231 cancer cell line and it has an oral bioavailability of 24% at doses of 30 mg/kg in rats. In direct comparison, **6** has a longer $T_{1/2}$ and a larger AUC value than **1** and a better oral bioavailability. Hence, compound **6** has a significantly better PK profile than **1**.

The synthesis of compounds **2**−**6** is shown in Schemes 1 and 2. Compound **7** (Scheme 1) was synthesized using a published method.[24] Protection of the amino group in **7** with carbobenzoxy gave a carbamate that was treated with $SOCl_2$ in MeOH to transform the *tert*-butyl ester to a methyl ester. The primary amino group was reprotected with *t*-Boc to afford **8**, hydrolysis of whose methyl ester followed by condensation of the resulting acid with aminodiphenylmethane yielded **9**. Removal of the *t*-Boc protecting group in **9** and subsequent condensation of the resulting amine with L-*N*-*t*-Boc-*N*-methyl alanine furnished an amide. Cleavage by hydrogenation of the Cbz protecting group in this amide afforded the common key intermediate **10**.

The synthesis of compounds **2**−**6** is shown in Scheme 2. Methylation or benzylation of **10** group furnished the alkylated amines and removal of the *t*-Boc protecting group in these compounds provided **3** and **4**, respectively. Condensation of **10** with acetic anhydride or phenylacetyl chloride afforded the respective amides and subsequent removal of the *t*-Boc protecting group gave **5** and **6**, respectively.

Summary

A new class of Smac mimetics has been designed and synthesized through modifications of the [8,5] bicyclic core structure in our initial compound **1**. Several highly potent and cell-permeable Smac mimetics were obtained. The most potent compound, **6**, binds to XIAP, cIAP-1, and cIAP-2 with K_i values of 8.4, 1.5, and 4.2 nM, respectively. Compound **6** potently inhibit cell growth in the MDA-MB-231 cell line with an IC_{50} value of 31 nM and effectively induces apoptosis at 100 nM within 24 h of treatment. Significantly, compound **6** is orally bioavailable and has excellent aqueous solubility. These data reveal that compound **6** is a promising lead compound for further evaluation and optimization. Extensive optimization and in vitro and in vivo testing of **6** and its analogues are under way and will be reported in due course.

Experimental Section

Chemistry. General Methods. NMR spectra were measured at 300 MHz. ^1H chemical shifts are reported relative to DHO (4.79 ppm) as internal standard. Final products were purified by C18 reverse 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.

(5*S*,8*S*,10a*R*)-*N*-Benzhydryl-5-((*S*)-2-(methylamino) propanamido)-6-oxodeca-hydropyrrolo[1,2-*a*][1,5]diazocine-8-carboxamide (2). HCl solution (4 N in 1,4-dioxane, 1 mL) was added to a solution of **10** (35 mg, 0.06 mmol) in methanol (5 mL). The solution was stirred at room temperature overnight and then concentrated to give crude **2**, which was purified by reverse phase semipreparative HPLC to give pure **2** (40 mg, 95%). The gradient ran from 80% of solvent A and 20% of solvent B to 60% of solvent A and 40% of solvent B in 40 min. ^1H NMR (300 MHz, D_2O) δ 7.42−7.29 (m, 10H), 6.03 (d, J = 6.8 Hz, 1H), 5.36 (m, 1H), 4.75 (m, 1H), 4.61 (m, 1H), 3.99 (dd, J = 12.0, 5.4 Hz, 1H), 3.68 (m,

1H), 3.55 (m, 1H), 3.43 (m, 1H), 3.25 (m, 1H), 2.69 (s, 3H), 2.49 (m, 1H), 2.30−1.82 (m, 5H), 1.52 (d, J = 7.1 Hz, 3H). ESI MS: m/z 500.4 $(M + Na)^+$. Anal. ($C_{27}H_{35}N_5O_3 \cdot 2.7CF_3COOH$): C, H, N.

(5*S*,8*S*,10a*R*)-*N*-Benzhydryl-3-methyl-5-((*S*)-2-(methylamino)-propanamido)-6-oxodeca-hydropyrrolo[1,2-*a*][1,5]diazocine-8-carbox-amide (3). Aqueous formaldehyde solution (37%, 0.2 mL) and HOAc (0.2 mL) were added to a solution of **10** (44 mg, 0.076 mmol) in MeOH (5 mL). After addition of $NaBH_3CN$ (50 mg, 0.79 mmol) at 0 °C, the solution was warmed to room temperature and stirred for 3 h. The mixture was concentrated and then partitioned between EtOAc (20 mL) and brine (5 mL). The organic layer was dried over Na_2SO_4 and concentrated, and the residue was purified by chromatography to give the methylated amine. HCl solution (4 N in 1,4-dioxane, 1 mL) was added to a solution of this amine in MeOH (5 mL). The solution was stirred at room temperature overnight and then concentrated to give crude **3**, which was purified by reverse phase semipreparative HPLC to give pure **3** (38 mg, 71% over two steps). The gradient ran from 75% of solvent A and 25% of solvent B to 60% of solvent A and 40% of solvent B in 40 min. ^{1}H NMR (300 MHz, D_2O) δ 7.42−7.25 (m, 10H), 6.07 (d, J = 6.8 Hz, 1H), 5.35 (m, 1H), 4.70 (m, 1H), 4.61 (m, 1H), 3.99 (dd, J = 14.0, 7.0 Hz, 1H), 3.86 (m, 1H), 3.65 (m, 1H), 3.56 (m, 1H), 3.30 (m, 1H), 3.00 (s, 3H), 2.68 (s, 3H), 2.49 (m, 1H), 2.32−1.82 (m, 5H), 1.51 (d, J = 7.1 Hz, 3H). ESI MS: m/z 492.3 $(M + H)^+$. Anal. ($C_{28}H_{37}N_5O_3 \cdot 3.1CF_3COOH$): C, H, N.

(5*S*,8*S*,10a*R*)-*N*-Benzhydryl-3-benzyl-5-((*S*)-2-(methylamino)-propanamido)-6-oxodeca-hydropyrrolo[1,2-*a*][1,5]diazocine-8-carbox-amide (4). Benzyl bromide (0.1 mL) and $NaHCO_3$ (0.3 g) were added a solution of **10** (48 mg, 0.083 mmol) in DMF (5 mL). The mixture was stirred at room temperature overnight and then concentrated before being partitioned between EtOAc (20 mL) and brine (5 mL). The organic layer was dried over Na_2SO_4 then concentrated, and the residue was purified by chromatography to give a benzylated amine. To a solution of this amine in methanol (5 mL) was added HCl solution (4 N in 1,4-dioxane, 1 mL). The solution was stirred at room temperature overnight and then concentrated to give crude product, which was purified by reverse phase semipreparative HPLC to give pure **4** (51 mg, 77% over two steps). The gradient ran from 75% of solvent A and 25% of solvent B to 60% of solvent A and 40% of solvent B in 40 min. ^{1}H NMR (300 MHz, D_2O) δ 7.34 (m, 2H), 7.28−7.02 (m, 13H), 6.05 (d, J = 6.9 Hz, 1H), 5.38 (m, 1H), 4.72 (m, 1H), 4.52 (m, 1H), 4.25 (ABq, J = 8.4 Hz, 2H), 3.97 (dd, J = 13.5, 6.8 Hz, 1H), 3.82−3.56 (m, 2H), 3.49 (m, 1H), 3.18 (m, 1H), 2.67 (s, 3H), 2.35 (m, 1H), 2.08 (m, 1H), 1.75−1.52 (m, 4H), 1.47 (d, J = 7.0 Hz, 3H). ESI MS: m/z 568.3 $(M + H)^+$. Anal. ($C_{34}H_{41}N_5O_3 \cdot 2.9CF_3COOH$): C, H, N.

(5*S*,8*S*,10a*R*)-3-Acetyl-*N*-benzhydryl-5-((*S*)-2-(methylamino)-propanamido)-6-oxodeca-hydro pyrrolo[1,2-*a*][1,5]diazocine-8-carbox-amide (5). Acetic anhydride (0.1 mL) and *N*,*N*-diisopropylethylamine (0.3 mL) were added to a solution of **10** (46 mg, 0.08 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The mixture was stirred at room temperature overnight, concentrated, and then partitioned between EtOAc (20 mL) and brine (5 mL). The organic layer was dried over Na_2SO_4 and then concentrated. The residue was purified by chromatography to give an amide. HCl solution (4N in 1,4-dioxane, 1 mL) was added to a solution of this residue in MeOH (5 mL). The solution was stirred at room temperature overnight and then concentrated to give crude **5**, which was purified by reverse phase semipreparative HPLC to give pure product (38 mg, 85% over two steps). The gradient ran from 70% of solvent A and 30% of solvent B to 50% of solvent A and 50% of solvent B in 40 min. ^{1}H NMR (300 MHz, D_2O) δ 7.38−7.19 (m, 10H), 5.95 (brs, 1H), 4.96 (m, 1H), 4.40 (m, 1H), 4.25 (m, 1H), 3.94 (m, 1H), 3.66 (m, 1H), 3.60−3.35 (m, 3H), 2.63 (s, 3H), 2.25 (m, 1H), 2.15−1.65 (m, 8H), 1.47 (d, J = 7.1 Hz, 3H). ESI MS: m/z 520.3 $(M+H)^+$. Anal. ($C_{29}H_{37}N_5O_4 \cdot 1.0HCl \cdot 1.5CF_3COOH$): C, H, N.

(5*S*,8*S*,10a*R*)-*N*-Benzhydryl-5-((*S*)-2-(methyl-amino) propanamido)-6-*oxo*-3-(2-phenyl-acetyl)decahydro pyrrolo[1,2-*a*][1,5]diazocine-8-carboxamide (6). Phenylacetyl chloride (0.1 mL) and *N*,*N*-diisopropylethylamine (0.3 mL) were added to a solution of **10** (47 mg, 0.08 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred at room temperature overnight, concentrated, and then partitioned between EtOAc (20 mL) and brine (5 mL). The organic layer was dried over Na_2SO_4 then concentrated, and the residue was purified by chromatography to give an amide. HCl (4N in 1,4-dioxane, 1 mL) was added to a solution of this amide in MeOH. The solution was stirred at room temperature overnight and then concentrated to give crude **6**, which was purified by reverse phase semipreparative HPLC to give pure product (44 mg, 87% over two steps). The gradient ran from 70% of solvent A and 30% of solvent B to 50% of solvent A and 50% of solvent B in 40 min. ^{1}H NMR (300 MHz, D_2O) δ 7.27−6.90 (m, 15H), 5.95 (brs, 1H), 4.65 (m, 1H), 4.38 (m, 1H), 4.06 (m, 1H), 3.85 (m, 1H), 3.78−3.30 (m, 6H), 2.55 (brs, 3H), 2.08 (m, 1H), 1.98−1.30 (m, 8H). ESI MS: m/z 596.3 $(M + H)^+$. Anal. ($C_{35}H_{41}N_5O_4 \cdot 1.0HCl \cdot 1.2CF_3COOH$): C, H, N.

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

Supporting Information Available: An experimental section including details of the synthesis and chemical data of intermediates, biochemical and cellular assays, molecular modeling, and pharmacokinetics. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Deveraux, Q. L.; Reed, J. C. IAP family proteins-suppressors of apoptosis. *Genes Dev.* **1999**, *1*, 239–252.
(2) Salvesen, G. S.; Duckett, C. S. IAP proteins: blocking the road to death's door. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 401–410.
(3) Fotin-Mleczek, M.; Henkler, F.; Samel, D.; Reichwein, M.; Hausser, A.; Parmryd, I.; Scheurich, P.; Schmid, J. A.; Wajant, H. Apoptotic crosstalk of TNF receptors: TNF-R2-induces depletion of TRAF2 and IAP proteins and accelerates TNF-R1-dependent activation of caspase-8. *J. Cell Sci* **2002**, *115*, 2757–70.
(4) Deng, Y.; Ren, X.; Yang, L.; Lin, Y.; Wu, X. A JNK-dependent pathway is required for TNFalpha-induced apoptosis. *Cell* **2003**, *115*, 61–70.
(5) Holcik, M.; Gibson, H.; Korneluk, R. G. XIAP: Apoptotic brake and promising therapeutic target. *Apoptosis* **2001**, *6*, 253–261.
(6) Fulda, S. Inhibitor of apoptosis proteins as targets for anticancer therapy. *Expert Rev Anticancer Ther.* **2007**, *7*, 1255–64.
(7) 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.
(8) 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.
(9) 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.
(10) 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.
(11) 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.
(12) 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.
(13) Li, L.; Thomas, R. M.; Suzuki, H.; De Brabander, J. K.; Wang, X.; Harran, P. G. A small molecule Smac mimic potentiates TRAIL- and TNFalpha-mediated cell death. *Science* **2004**, *305*, 1471–4.
(14) 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*, 16686–16687.

(15) 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*, 4147–50.

(16) 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*, 4417–26.

(17) Sun, H.; Nikolovska-Coleska, Z.; Lu, J.; Qiu, S.; Yang, C.-Y.; Gao, W.; Meagher, J.; Stuckey, J.; Wang, S. Design, synthesis, and evaluation of a potent, cell-permeable, conformationally constrained second mitochondria derived activator of caspase (Smac) mimetic. *J. Med. Chem.* **2006**, *49*, 7916–20.

(18) Zobel, K.; Wang, L.; Varfolomeev, E.; Franklin, M. C.; Elliott, L. O.; Wallweber, H. J.; Okawa, D. C.; Flygare, J. A.; Vucic, D.; Fairbrother, W. J.; Deshayes, K. Design, synthesis, and biological activity of a potent Smac mimetic that sensitizes cancer cells to apoptosis by antagonizing IAPs. *ACS Chem. Biol.* **2006**, *1*, 525–33.

(19) Sun, H.; Nikolovska-Coleska, Z.; Lu, J.; Meagher, J. L.; Yang, C.-Y.; Qiu, S.; Tomita, Y.; Ueda, Y.; Jiang, S.; Krajewski, K.; Roller, P. P.; Stuckey, J. A.; Wang, S. Design, synthesis, and characterization of a potent, nonpeptide, cell-permeable, bivalent Smac mimetic that concurrently targets both the BIR2 and BIR3 domains in XIAP. *J. Am. Chem. Soc.* **2007**, *129*, 15279–94.

(20) Varfolomeev, E.; Blankenship, J. W.; Wayson, S. M.; Fedorova, A. V.; Kayagaki, N.; Garg, P.; Zobel, K.; Dynek, J. N.; Elliott, L. O.; Wallweber, H. J.; Flygare, J. A.; Fairbrother, W. J.; Deshayes, K.; Dixit, V. M.; Vucic, D. IAP antagonists induce autoubiquitination of c-IAPs, NF-kappaB activation, and TNFalpha-dependent apoptosis. *Cell* **2007**, *131*, 669–81.

(21) Vince, J. E.; Wong, W. W.; Khan, N.; Feltham, R.; Chau, D.; Ahmed, A. U.; Benetatos, C. A.; Chunduru, S. K.; Condon, S. M.; McKinlay, M.; Brink, R.; Leverkus, M.; Tergaonkar, V.; Schneider, P.; Callus, B. A.; Koentgen, F.; Vaux, D. L.; Silke, J. IAP antagonists target cIAP1 to induce TNFalpha-dependent apoptosis. *Cell* **2007**, *131*, 682–93.

(22) Petersen, S. L.; Wang, L.; Yalcin-Chin, A.; Li, L.; Peyton, M.; Minna, J.; Harran, P.; Wang, X. Autocrine TNFalpha signaling renders human cancer cells susceptible to Smac-mimetic-induced apoptosis. *Cancer Cell* **2007**, *12*, 445–56.

(23) 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*, 261–73.

(24) Peng, Y.; Sun, H.; Wang, S. Design and synthesis of a 1,5-diazabicyclo[6,3,0] dodecane amino acid derivative as a novel dipeptide reverse-turn mimetic. *Tetrahedron Lett.* **2006**, *47*, 4769−4770.

JM801254R