Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 793–797
Bioorganic & Medicinal Chemistry Letters

Structure-based design, synthesis and biochemical testing of novel and potent Smac peptido-mimetics

Haiying Sun,[a] Zaneta Nikolovska-Coleska,[a] Jianyong Chen,[a] Chao-Yie Yang,[a] York Tomita,[b] Hongguang Pan,[b] Yoshiko Yoshioka,[b] Krzysztof Krajewski,[c] Peter P. Roller[c] and Shaomeng Wang[a,*]

[a]*University of Michigan Comprehensive Cancer Center, Departments of Internal Medicine and Medicinal Chemistry, University of Michigan, 1500 E. Medical Center Drive, Ann Arbor, MI 48109-0934, USA*
[b]*Lombardi Cancer Center, Georgetown University Medical Center, W213 Research Building, 3970 Reservoir Road NW. Washington, DC 20057-1469, USA*
[c]*Laboratory of Medicinal Chemistry, National Cancer Institute, FCRDC, Bldg 376, Frederick, MD 21702-1201, USA*

Received 23 June 2004; revised 29 October 2004; accepted 1 November 2004
Available online 25 November 2004

Abstract—Structure-based design, chemical synthesis and biochemical testing of a series of novel Smac peptido-mimetics as inhibitors of XIAP protein are described. The most potent compound, **6j**, has a binding affinity (K_i value) of 24 nM to XIAP BIR3 protein and is 24 times more potent than the native Smac AVPI peptide. Further optimization of these potent Smac mimetics may ultimately lead to the development of a novel class of anticancer drugs for the treatment of human cancer by overcoming apoptosis-resistance of cancer cells through targeting the inhibitor of apoptosis proteins.

Apoptosis, or programmed cell death, is an essential cell suicide process[1] that is important for normal development, host defense and suppression of oncogenesis.[2] Inappropriate control of apoptosis plays a role in many human diseases, including cancer, autoimmune diseases and neurodegenerative disorders.[2–4] In recent years, key apoptosis regulators have become attractive molecular targets for designing new therapies to treat a variety of human diseases and conditions.[3,4]

Inhibitor of apoptosis proteins (IAPs) were recently identified and characterized as an important class of intrinsic cellular inhibitors of apoptosis, although their functions may not be limited to the regulation of apoptosis.[5,6] IAP proteins potently inhibit both intrinsic and extrinsic apoptosis pathways.[5,6] XIAP (X-linked IAP) is the most potent inhibitor of apoptosis among all known IAP proteins.[5,6] XIAP contains three BIR (Baculovirus IAP repeat) domains as well as a C-terminal RING finger domain.[6] The third BIR domain (BIR3) selectively targets caspase-9, an initiator of apoptosis, while the linker region between BIR1 and BIR2 inhibits effectors caspase-3 and -7.[6–9]

Smac/DIABLO (second mitochondria-derived activator of caspase or direct IAP binding protein with low pI), a protein released from mitochondria in response to apoptotic stimuli, directly interacts with the BIR3 domain of XIAP, cIAP-1 and cIAP-2 and a single BIR domain in ML-IAP, and functions as a direct endogenous inhibitor of IAP proteins.[11,12] Biological study[9] and high-resolution experimental structures[13,14] have convincingly demonstrated that Smac binds to the same surface groove in the XIAP BIR3 domain where caspase-9 binds via its N-terminally exposed four residues (AVPI), in a manner similar to that in caspase-9/XIAP interaction.[10] Consistent with the structural data, Smac peptides as short as 4-residues, derived from Smac protein bind to the recombinant XIAP BIR3 domain protein with the same affinities as the mature Smac protein.[14,15] Several recent studies have shown that short Smac peptides fused to a carrier peptide for intracellular delivery (cell-permeable Smac peptides) overcome resistance of cancer cells with high levels of XIAP protein to apoptosis and enhance the activity of anticancer drugs in vitro and in vivo.[16–18]

Keywords: Smac; Peptido-mimetics; XIAP.
* Corresponding author. Tel.: +1 734 615 0362; fax: +1 734 647 9647; e-mail: shaomeng@umich.edu

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.11.008

Of great significance, these cell-permeable Smac peptides have little toxicity to normal cells in vitro and to normal tissues in vivo.[16–18] Collectively, these studies suggest that potent and cell-permeable Smac mimetics may have great therapeutic potential, as a new class of anticancer drugs, for overcoming apoptosis-resistance of cancer cells with high levels of IAP proteins.

Natural Smac peptides have several intrinsic limitations (e.g., poor in vivo stability and poor bioavailability) as pharmacological tools and as potentially useful therapeutic agents for the treatment of cancer. Furthermore, the natural Smac AVPI peptide has only a moderate binding affinity to XIAP BIR domain protein (K_i = 0.58 μM as determined in our binding assay).[19] Towards developing potent Smac mimetics with improved binding affinities, cellular permeability, in vivo stability and pharmacokinetics, a number of laboratories, including ours, are actively pursuing the design of Smac peptido-mimetics[15,21] and non-peptidic Smac mimetics.[22] In this paper, we wish to report our structure-based design, synthesis and biochemical testing of a series of novel and potent Smac peptido-mimetics.

High-resolution, experimental three-dimensional (3D) structures of the BIR3 domain of XIAP in complex with Smac protein and peptide have recently been determined (Fig. 1). The N-terminal tetrapeptide of Smac (AVPI) recognizes a surface groove on the BIR3 domain of XIAP through several hydrogen-bonding interactions and van der Waals contacts. The experimental structures (Fig. 1) show that the backbone carbonyl group of I4′ of Smac does not have specific interactions with the protein, and the hydrophobic side chain of I4′ of Smac inserts into a hydrophobic pocket. Hence, a simple benzyl amine was used to replace the I4′ residue (**1** in Table 1). Our computational modeling indicated that this simple benzyl amino group mimics the I4′ for hydrophobic and hydrogen-bonding interactions with the protein (Fig. 1). Compound **1**, Smac AVPI peptide and all other Smac peptido-mimetics reported in this study (Table 1) were evaluated for their binding affinities to XIAP BIR3 protein in a fluorescence-polarization-based (FP-based) assay we have recently developed.[19] In this competitive binding assay, these designed Smac mimetics were measured for their affinities to displace a fluorescently labelled, high-affinity, mutated Smac

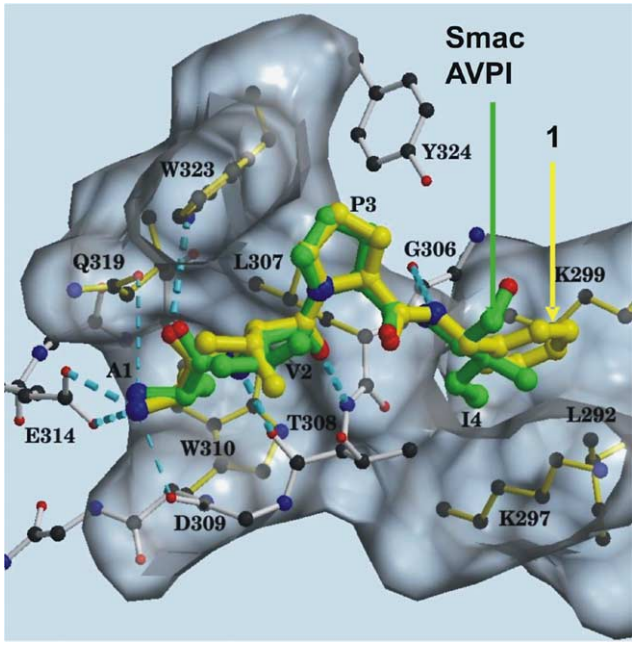
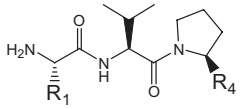
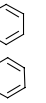
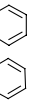


Figure 1. Superposition of modeled structure of compound **1** (yellow) complexed with XIAP BIR3 and the X-ray structure of Smac protein (green) complexed with XIAP BIR3. Compound **1** in complex with XIAP BIR3 was modeled based upon the X-ray structure of XIAP BIR3 in complex with Smac and minimized using the Sybyl program.[23] For clarity, only the AVPI residues of Smac are shown. Carbon atoms are colored in yellow, green and black in compound **1**, Smac AVPI peptide, and in the protein, respectively. Nitrogen and oxygen atoms in these molecules are colored in blue and red, respectively. Hydrogen bonds are depicted in blue dashed lines.

Table 1. Chemical structures of Smac peptido-mimetics and their binding affinities to XIAP BIR3 protein as determined using a fluorescence-polarization-based binding assay[19]



Compounds	R_1	R_4	$K_i \pm$ SD (μM)
1	CH_3	-CO-NH-CH_2- 	0.29 ± 0.07
6a	CH_3	-CO-NH-CH_2- 	13.40 ± 1.6
6b	CH_3	-CO-NH-CH_2- 	2.45 ± 0.7
6c	CH_3	-CO-NH-CH_2- 	4.41 ± 1.5
6d	CH_3	-CO-NH-CH_2- 	1.27 ± 0.2
6e	CH_3	-CO-NH-CH_2- 	0.22 ± 0.07
6f	CH_3	-CO-NH-CH_2- 	0.18 ± 0.07
6g	CH_3	-CONH-	4.9 ± 2.1
6h	CH_3	-CO-NH-CH_2-CH_2- 	0.15 ± 0.09
6i	CH_3	-CO-NH- 	0.028 ± 0.020
6j	C_2H_5	-CO-NH- 	0.024 ± 0.020
10	CH_3	-CH_2-CH_2-CH_2- 	1.2 ± 0.4
12a	H	-CO-NH-$(CH_2)_2$- 	68 ± 7
12b	C_2H_5	-CO-NH-CH_2- 	0.081 ± 0.06
12c	*i*-C_3H_7	-CO-NH-CH_2- 	4.15 ± 1.2
12d	*n*-C_3H_7	-CO-NH-CH_2- 	54 ± 7

peptide from recombinant XIAP BIR3 domain protein.[19]

Compound **1** was determined to have a K_i value of 0.29 μM in our FP-based assay, and is thus 2 times more potent than Smac AVPI peptide (K_i = 0.58 μM) under the same assay conditions. Compound **1** was subsequently used as the template for further design. Based upon **1**, a series of new compounds with different hydrophobic groups (compounds **6a**–**h** in Table 1) were designed, synthesized and tested to further explore the importance of the hydrophobic interactions between the phenyl ring in **1** and XIAP.

Replacement of the benzyl group in **1** with an isobutyl group (**6a**) reduced the binding affinity by about 45 times, indicating that isopropyl is not large enough for achieving optimal hydrophobic interactions at this site. Consistent with this result, replacement of the isobutyl group with a larger 2′-ethylbutyl group (**6b**) improved the binding affinity by 5 times over **6a**, but **6b** is still 8 times less potent than **1**. Replacement with a cyclopropylmethyl group (**6c**) resulted in a reduction of 15 times over **1** but **6c** is 3 times more potent than **6a**, suggesting that not only the size but also the shape of the hydrophobic groups are important for hydrophobic interactions at this site. Replacement of the phenyl ring in **1** with a saturated cyclohexyl ring (**6d**) caused a moderate reduction of 4 times, indicating that an aromatic ring appears to be more effective for hydrophobic interactions. Accordingly, we synthesized two compounds with a five-membered aromatic ring (**6e** and **6f** in Table 1). Compounds **6e** and **6f** have K_i values of 0.22 and 0.18 μM, respectively, and both compounds are as potent as **1**.

For effective hydrophobic interactions, the length of the linker between the proline residue and the phenyl ring in **1** is important. Compounds **6g** and **6h** were designed to investigate the optimal length for the linker. While compound **6g** with one carbon-atom shorter linker than **1** is 16 times less potent than **1**, compound **6h** with a carbon-atom longer linker than **1** is 2 times more potent than **1** with a K_i value of 0.15 μM (Table 1), confirming the importance of the length of the linker.

The amino group of I4′ in AVPI and of the benzylamine in compound **1** forms a hydrogen bond with the backbone carbonyl group of G306 of XIAP (Fig. 1). To probe the importance of this hydrogen bond for binding, we synthesized **10** in which the amide group in **1** is replaced by two carbon atoms (–CH_2–CH_2–). Compound **10** has a K_i value of 1.2 μM, which is 4 times less potent than **1**, indicating that the amide group plays a modest role in the binding between **1** and XIAP.

Although the backbone carbonyl group of I4′ of Smac points toward the solvent (Fig. 1), we hypothesized that it may play a role in restricting the conformation of the hydrophobic side chain of I4′ for effective hydrophobic interactions with XIAP. To test this idea, we designed **6i** in which an additional phenyl ring was introduced. We reasoned that this additional phenyl may enhance the binding affinity of the resulting compound (**6i**) through: (a) controlling the second phenyl ring in an optimal orientation for effective hydrophobic interactions; (b) reducing the conformational flexibility of the second phenyl ring as compared to compound **1**. Compound **6i** was determined to have a K_i value of 0.028 μM (28 nM) and is 10 times more potent than **1**, representing a potent Smac peptido-mimetic.

Previous X-ray structure showed that the methyl group in alanine in position 1 (A1′) interacts with a small but well-defined hydrophobic pocket in XIAP BIR3 (Fig. 1) and this hydrophobic pocket appears to be large enough to accommodate a slightly larger hydrophobic group than a methyl group. To probe the interactions at this site, we have designed and synthesized several simple Smac mimetics (compounds **12a**–**d** in Table 1).

Replacement of the methyl group in compound **1** by a hydrogen atom resulted in compound **12a**. Compound **12a** has a K_i value of 68 μM and is more than 200 times less potent than **1**, highlighting the importance of the hydrophobic interactions. Consistent with a previous study[14] on Smac peptides, replacement of the methyl group in compound **1** by an ethyl group (**12b**) improves the binding affinity by 3 times. Consistent with our modeling analysis that this hydrophobic pocket is quite small, compound **12c**, in which an isopropyl was used to replace the methyl group in compound **1**, is 14 times less potent than **1**. Moreover, compound **12d**, in which an *n*-propyl group was used to replace the methyl group in compound **1**, is more than 180 times less potent than **1**. These data indicate that this small hydrophobic pocket in XIAP is not large enough to accommodate an isopropyl or *n*-propyl group, but can accommodate a methyl or an ethyl group. Indeed, replacement of the methyl group with an ethyl group in **6i** resulted in **6j**, which has a K_i value of 24 nM. Hence, compound **6j** is 12 times more potent than **1** and 24 times more potent than the natural Smac AVPI peptide, representing a highly potent new Smac mimetic.

To conclusively confirm that compound **6j** binds to the binding groove in XIAP BIR3 where Smac binds, we performed an analysis using nuclear magnetic resonance (NMR) methods. The human XIAP BIR3 domain (residues 241–356) fused to His-tag was expressed from BL21(DE3) cells in M9 medium containing ^{15}N ammonium chloride to uniformly label protein with ^{15}N, and was purified. ^{15}N heteronuclear single quantum coherence spectroscopy (HSQC) NMR spectra were recorded with samples containing 100 μM of the ^{15}N protein in 50 mM Tris (pH 7.2), 50 μM $Zn(Cl)_2$, 1 mM DTT with 100 μM of **6j** or without it at 30 °C. Overlaying of the two ^{15}N HSQC spectra of the BIR3 domain of human XIAP with **6j** (red) and without (black) showed that **6j** bound to the protein and caused induced chemical shifts in several residues in XIAP BIR3 (Fig. 2). To identify, which residues in XIAP BIR3 were affected by compound **6j**, ^{13}C and ^{15}N double labeled XIAP BIR3 was prepared and 3D NMR triple resonance experiments were performed to make backbone atom resonance assignments. HNCA, HNCACB,

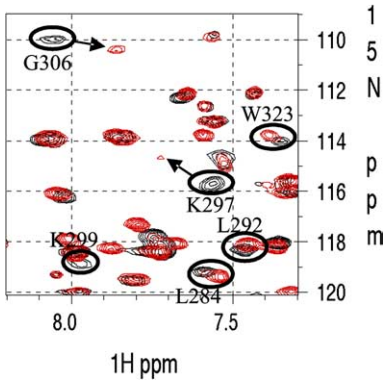


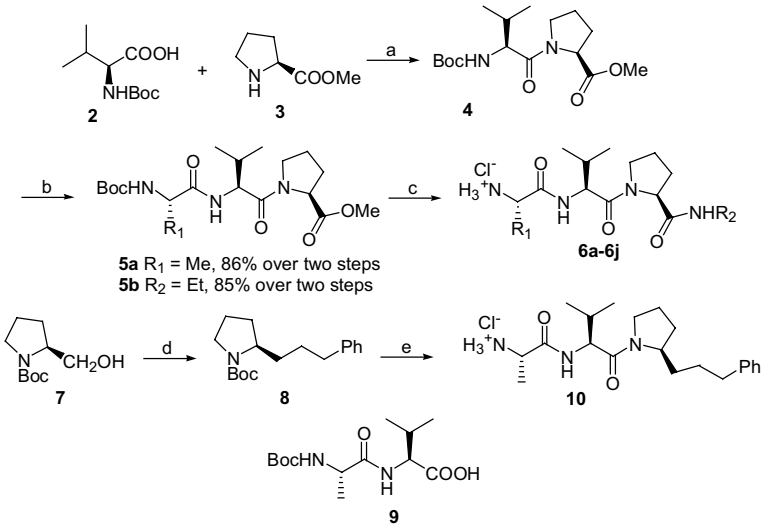
Figure 2. Overlaid ^{15}N heteronuclear single quantum coherence spectroscopy (HSQC) NMR spectra of XIAP BIR3 with (red) or without (black) compound **6j**.

HN(CO)CBCA, HNCO, TOCSY-HSQC, C(CO)NH and the published results[14,20] were used to nearly complete the backbone assignments, except for the two flexible loops (residues 276–280 and 308–314).[20] Based upon the nearly complete backbone assignments of XIAP BIR3, we found that the residues G306, K297, L292, K299 are affected by compound **6j** (Fig. 2). Moreover, these residues were also found to be affected by the Smac AVPI peptide in our NMR analysis (data not shown). Based upon the experimental complex structures of Smac/XIAP BIR3 (Fig. 1), these residues in XIAP BIR3 that are affected by compound **6j** and the Smac AVPI peptide are in direct contact with Smac protein. Taken together, our NMR experimental results conclusively confirm that compound **6j** binds to the binding groove in XIAP BIR3 where Smac protein binds. Our experiments also showed that our potent mimetic **6j** does not unfold XIAP BIR3 protein.

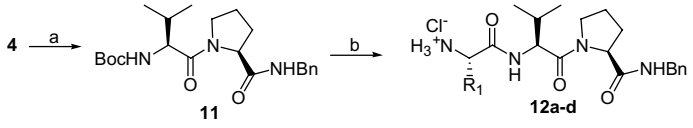
The synthesis of peptido-mimetics with modification at the I4 residue is shown in Scheme 1. Briefly, condensation of commercially available amino acid derivatives **2** and **3** in the presence of EDC and HOBt produced compound **4**. Removal of the Boc protective group in compound **4** followed by condensation with L-*N*-Boc-alanine or L-*N*-Boc-2-aminobutyric acid resulted in amides **5a** and **5b**, respectively. Hydrolysis of the ester group in **5a** and **5b** afforded two acids. Condensation of the two acids with different amines furnished a series of amides. Removal of the Boc protective groups in these amides afforded the desired compounds **6a**–**j**.

The synthesis of compound **10** is provided in Scheme 2. Briefly, oxidation of the hydroxyl group in commercially available compound **7** generated an aldehyde. Olefination of this aldehyde with the ylide derived from phenyltriphenylphosphonium bromide by treatment with BuLi furnished an alkene. Hydrogenation of this alkene catalyzed by 10% Pd–C afforded compound **8**. Removal of the Boc protective group in **8** followed by condensation with dipeptide derivative **9** yielded an amide. Removal of the Boc protective group in this amide gave compound **10**.

The synthesis of compounds (**12a**–**d**) with modifications at A1 of Smac is shown in Scheme 2. Hydrolysis of the ester group in **4** followed by condensation with benzyl amine afforded compound **11**. Removal of the Boc protective group in **11** by treatment with HCl in 1,4-dioxane–methanol, followed by condensation of the resulting salt with different L-*N*-Boc-amino acids, gave a series of amides. Removal of the N-terminal Boc protective group and the C-terminal benzyl group provided a series of the amide derivatives, **12a**–**d**.



Scheme 1. Synthesis of peptido-mimetics with modifications at Ile residue. Reagents and conditions: (a) EDC, HOBt, *N*,*N*-diisopropylethylamine, CH_2Cl_2, 92%; (b) i. 4 N HCl in 1,4-dioxane, MeOH, ii. L-*N*-Boc-alanine or L-*N*-Boc-aminobutyric acid, EDC, HOBt, *N*,*N*-diisopropylethylamine, CH_2Cl_2; (c) i. 2 N LiOH, 1,4-dioxane, then 1 N HCl, ii. amine, EDC, HOBt, *N*,*N*-diisopropylethylamine, CH_2Cl_2, iii. 4 N HCl in 1,4-dioxane, MeOH, structures were shown in Table 1, the yield over three steps is 48–72%; (d) i. Dess–Martin periodinane, CH_2Cl_2, ii. phenylethyltriphenylphosphonium bromide, n-BuLi, −78 °C, THF, iii. 10% Pd–C, H_2, 51% over three steps; (e) i. 4 N HCl in 1,4-dioxane, MeOH, ii. **9**, EDC, HOBt, *N*,*N*-diisopropylethylamine, CH_2Cl_2, 4 N HCl in 1,4-dioxane, MeOH, 72% over three steps.



Scheme 2. Synthesis of peptido-mimetics with modifications at Ala residue. Reagents and conditions: (a) i. 2 N LiOH, 1,4-dioxane, then 1 N HCl, ii. benzylamine, EDC, HOBt, *N*,*N*-diisopropylethylamine, CH_2Cl_2, 88% over two steps; (b) i. 4 N HCl in 1,4-dioxane, MeOH, ii. L-*N*-Boc-amino acid, EDC, HOBt, *N*,*N*-diisopropylethylamine, CH_2Cl_2, iii. 4 N HCI in 1,4-dioxane, MeOH, structures are shown in Table 1, the yield over three steps is 68–74%.

In summary, our structure-based design, synthesis and biochemical testing have yielded highly potent Smac peptido-mimetics. Compounds **6i** and **6j** have K_i values of 28 and 24 nM, respectively, for their binding affinities to the XIAP BIR3 protein, and are at least 20 times more potent than the natural Smac AVPI peptide. Furthermore, these compounds have reduced peptidic properties as compared to the natural Smac peptide. Preliminary evaluations of compound **6j** in human breast cancer MDA-MB-231 cells and prostate PC-3 cancer cells with high levels of XIAP proteins show that **6j** is effective in enhancing apoptosis induced by chemotherapeutic agents. Additional structural optimization of these Smac peptido-mimetics and extensive biological testing are underway and will be reported in due course. It is predicted that highly potent and cell-permeable Smac mimetics with in vivo stability may have the therapeutic potential to be developed as an entirely new class of anticancer drugs for the treatment of human cancers by overcoming apoptosis-resistance of cancer cells to current therapeutic agents.

Acknowledgements

We are grateful for the financial support from the Prostate Cancer Research Foundation and from the National Institutes of Health (1R01CA109025 to S.W.).

References and notes

1. Kerr, J. F.; Wyllie, A. H.; Currie, A. R. *Br. J. Cancer* **1972**, *26*, 239–257.
2. Thompson, C. B. *Science* **1995**, *267*, 1456–1462.
3. Nicholson, D. W. *Nature* **2000**, *407*, 810–816.
4. Reed, J. C. *Nat. Rev. Drug Discovery* **2002**, *1*, 111–121.
5. Deveraux, Q. L.; Reed, J. C. *Genes Dev.* **1999**, *1*, 239–252.
6. Salvesen, G. S.; Duckett, C. S. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 401–410.
7. Huang, Y.; Park, Y. C.; Rich, R. L.; Segal, D.; Myszka, D. G.; Wu, H. *Cell* **2001**, *104*, 781–790.
8. Ekert, P. G.; Silke, J.; Hawkins, C. J.; Verhagen, A. M.; Vaux, D. L. *J. Cell Biol.* **2001**, *152*, 483–490.
9. 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. *Nature* **2001**, *410*, 112–116.
10. Shiozaki, E. N.; Chai, J.; Rigotti, D. J.; Riedl, S. J.; Li, P.; Srinivasula, S. M.; Alnemri, E. S.; Fairman, R.; Shi, Y. *Mol. Cell* **2003**, *11*, 519–527.
11. Du, C.; Fang, M.; Li, Y.; Li, L.; Wang, X. *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. *Cell* **2000**, *102*, 43–53.
13. Wu, G.; Chai, J.; Suber, T. L.; Wu, J. W.; Du, C.; Wang, X.; Shi, Y. *Nature* **2000**, *408*, 1008–1012.
14. Liu, Z.; Sun, C.; Olejniczak, E. T.; Meadows, R.; Betz, S. F.; Oost, T.; Herrmann, J.; Wu, J. C.; Fesik, S. W. *Nature* **2000**, *408*, 1004–1008.
15. 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. *Biochemistry* **2002**, *41*, 7344–7349.
16. Fulda, S.; Wick, W.; Weller, M.; Debatin, K.-M. *Nature Med.* **2002**, *8*, 808–815.
17. Arnt, C. R.; Chiorean, M. V.; Heldebrant, M. P.; Gores, G. J.; Kaufmann, S. H. *J. Biol. Chem.* **2002**, *277*, 44236–44243.
18. Yang, L.; Mashima, T.; Sato, S.; Mochizuki, M.; Sakamoto, H.; Yamori, T.; Oh-Hara, T.; Tsuruo, T. *Cancer Res.* **2003**, *63*, 831–837.
19. 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. *Anal. Biochem.* **2004**, *332*, 261–273.
20. Sun, C.; Cai, M.; Meadows, R. P.; Xu, N.; Gunasekera, A. H.; Herrmann, J.; Wu, J. C.; Fesik, S. W. *J. Biol. Chem.* **2000**, *275*, 33777–33781.
21. 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. *J. Med. Chem.* **2004**, *47*, 4417–4426.
22. Sun, H.; Nikolovska-Coleska, Z.; Yang, C. Y.; Xu, L.; Tomita, Y.; Krajewski, K.; Roller, P. P.; Wang, S. *J. Med. Chem.* **2004**, *47*, 4147–4150.
23. Sybyl, a molecular modeling system, is supplied by Tripos, Inc., St. Louis, MO 63144.