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Non-peptidic small molecule inhibitors of XIAP

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Abstract—Non-peptidic small molecule SMAC mimetics were designed and synthesized that bind to the BIR3 domain of XIAP using structure-based design. Substituted five-membered heterocycles such as thiazoles and imidazoles were identified that serve as replacements for peptide fragments of the lead.

Apoptosis is a tightly regulated process that is critical for normal development and homeostasis.[1,2] The deregulation of this process can lead to a variety of diseases such as neurodegenerative disorders and cancers. Members of the inhibitor of apoptosis (IAP) family of proteins are upregulated in cancers and inhibit programmed cell death through their ability to directly inhibit members of the caspase family of apoptotic enzymes.[3–5] Human X-linked IAP (XIAP) is believed to inhibit specific caspases via its ~70 amino acid baculovirus IAP repeat (BIR) domains.[6–8] The BIR3 domain of XIAP binds to the amino terminus of the linker peptide on the small subunit of caspase-9, which becomes exposed after proteolytic processing of the procaspase-9. This prevents the formation of the catalytically active caspase-9 homodimer, the initiator caspase in the mitochondrial pathway of apoptosis.[9–11] Recently, a mammalian protein called SMAC was identified that triggers apoptosis by abrogating the inhibitory effects of IAPs on caspases.[12] SMAC promotes caspase-9 activation by competing with the caspase for the same binding groove on the BIR3 domain. The basis for SMAC binding to the BIR3 domain of XIAP has been studied by both NMR and X-ray structural analysis.[13,14] From these studies it was found that SMAC binds to XIAP BIR3 from interactions involving its first four amino acid residues (AVPI).

SMAC mimetics may be useful for treating cancers that are resistant to proapoptotic drugs due to the overexpression of IAPs. Indeed, several groups have reported that SMAC-derived peptides that bind to XIAP can sensitize cancer cell lines to undergo programmed cell death.[15–22] Based on the NMR structure of a SMAC peptide complexed with the BIR3 domain of XIAP, we reported on a novel series of peptide-based XIAP antagonists.[18] Compounds in this series bind to the BIR3 domain of XIAP with single-digit nanomolar affinity and promote cell death in several human cancer cell lines. Furthermore these compounds inhibit the growth of tumors in a MDA-MB-231 breast cancer mouse xenograft model. This earlier study clearly validates the use of small molecule XIAP antagonists against cancers that overexpress XIAP. For therapeutic applications, however, it is highly desirable to reduce the peptidic character of this initial series to improve their proteolytic stability, cell permeability, and pharmacokinetics.[23–26] Here we describe the discovery of non-peptidic small molecules that bind to the BIR3 domain of XIAP.

Figure 1A shows the structure of the AVPI peptide when bound to Bir3[13,14] and highlights the structural elements that earlier SAR identified as essential for binding.[18] The SAR of peptide analogs showed the first alanine residue with a charged N-terminus was critical for binding to XIAP BIR3 domain. Thus, this element was retained in our analogs. The second important element was the peptide amide bond of the valine residue, which forms a pair of hydrogen bonds to both the backbone amide and carbonyl of Thr 308 of XIAP. Thus, our strategy involved the synthesis of a library containing

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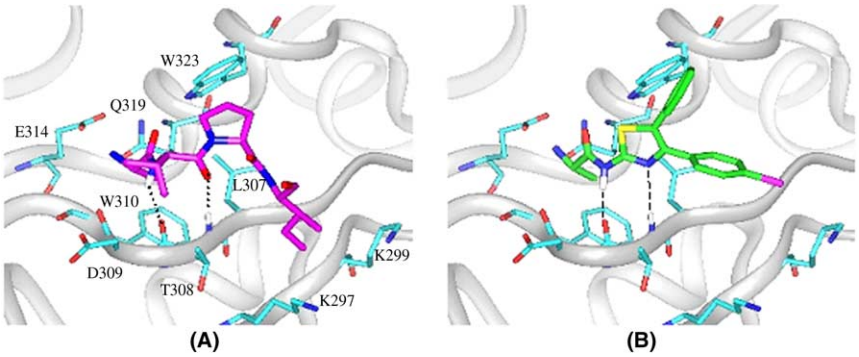
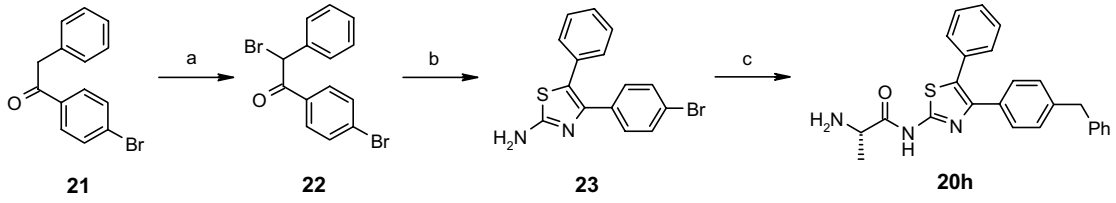
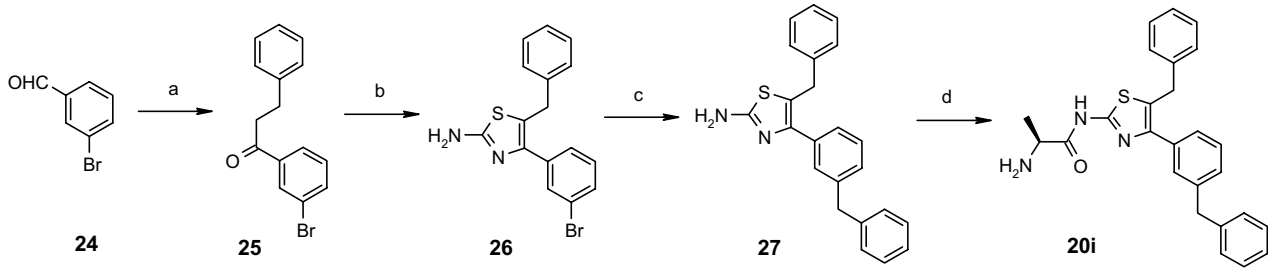


Figure 1. (A) A ribbon depiction of the AVPI terminus of the SMAC peptide bound to the BIR3 domain of XIAP (PDB ID: 1G3F). The peptide is colored magenta. Residues that make important contacts are rendered. Earlier SAR indicates that the N-terminal amino, the Ala-Val peptide bond, the carbonyl of the Val-Pro peptide bind and the hydrophobic interactions of the Ile and Pro are important for tight binding. (B) Model of **20g** docked into the Bir3/SMAC peptide structure. Fifteen NOEs between **20g** and the protein were used to define its orientation.

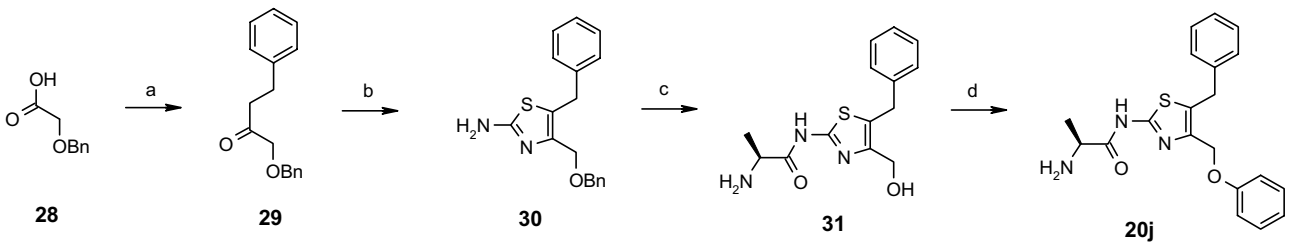
compounds with an alanine linked to fragments that could form hydrogen bonds with the XIAP BIR3 domain. Once a motif of interest was identified, it was further optimized, guided by structural information on the compound bound to BIR3 domain. Compounds were prepared by coupling alanine with amines that were either obtained from Abbott compound library or synthesized as shown in Schemes 1–3 utilizing parallel synthesis. The resulting compounds were initially assayed by NMR, which is able to confirm binding to the SMAC



Scheme 1. Reagents and conditions: (a) Br_2, AcOH, $CHCl_3$, rt; (b) thiourea, EtOH, reflux; (c) (i) benzylzinc bromide, $Cl_2Pd(dppf)$, THF, reflux; (ii) Boc–Ala–OH, EDC, HOAt, DCM; (iii) 50% TFA, 5% TES–H, DCM.



Scheme 2. Reagents and conditions: (a) (i) phenethylmagnesium chloride, THF; (ii) $(COCl)_2$, DMSO, TEA, DCM, −78 °C; (b) (i) Br_2, AcOH, $CHCl_3$, rt; (ii) thiourea, EtOH, reflux; (c) benzylzinc bromide, $Cl_2Pd(dppf)$, THF, reflux; (d) (i) BOC–Ala–OH, EDC, HOAt, DCM; (ii) 50% TFA, 5% TES–H, DCM.



Scheme 3. Reagents and conditions: (a) (i) $(COCl)_2$, DMSO, TEA, DCM, −78 °C; (ii) phenethylzinc bromide, $Pd(PPh_3)_4$, THF, reflux; (b) (i) TESOTf, TEA, DCM, −10 °C to rt; NBS, THF; (ii) thiourea, EtOH, reflux; (c) (i) Boc–Ala–OH, EDC, HOAt, DCM; (ii) BCl_3–DMS, DCM, −78 °C; (d) (i) $(BOC)_2O$, $NaHCO_3$, dioxane, rt; (ii) phenol, diisopropyl azodicarboxylate, PPh_3, THF, 0–55 °C; (iii) 50% TFA, 5% TES–H, DCM.

binding site by the observation of chemical shift perturbations and to quantitate weak binding in the millimolar to micromolar range.[31,32] Compounds estimated to have K_d less than 10 μM based on NMR were evaluated using a fluorescence polarization assay in which an actual K_d could be determined.[34] A number of compounds shown in Table 1 have low micromolar binding affinities, which are much better than the parent dipeptide (AV-NH_2). The valine sidechain in the SMAC peptide is solvent exposed (Fig. 1A) and can be replaced with various substituents such as those in compounds **2**, **3**, and **4** without affecting binding. Compound **5** lacking the corresponding carbonyl group of the valine of the SMAC peptide was inactive, reinforcing the importance of the hydrogen bond between this carbonyl group and the amide of Thr 308. Substitution of this amide carbonyl by a homologated ketone (compound **6)** or ester (compound **7)** was well tolerated. Esters, ketones, and amides are all good hydrogen bond acceptors and gave similar potencies. Interestingly, nitrogen-containing heterocyclic amines were found to be substantially more active (Table 1, compound **8**–**13**). Presumably, the sp^2-hybridized ring nitrogen is a good mimic for the amide carbonyl of the SMAC peptide.[27] Compounds in Table 2 were then prepared to evaluate a variety of heterocyclic moieties as potential amide replacements. Compound **14** and **15** showed similar binding affinity as compounds **16**, **17**, and **18** indicating that different types of heterocycles could be tolerated.

The Pro residue of the SMAC peptide occupies a hydrophobic pocket formed by both Leu 307 and Trp 323 (Fig. 1A). Based on the molecular modeling using the structure of the XIAP-SMAC peptide complex as a guide, substitution at the heterocycle 5-position should provide a vector to access this hydrophobic site. As seen in Table 2 (**19a**, **19b**, and **19c)**, a large hydrophobic substituent at the thiadiazole 5-position results in a clear increase in the binding affinity.

From the peptide SAR, substitution of the terminal Ile residue of the SMAC tetrapeptide by Phe or a large hydrophobic cap elicits a large increase in affinity.[18]

Table 1.

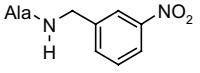
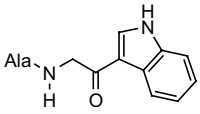
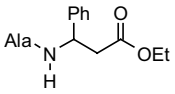
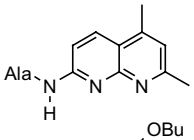
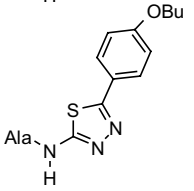
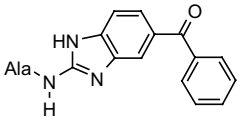
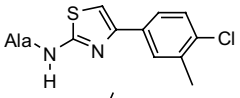
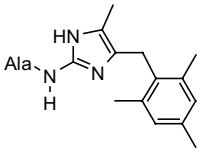
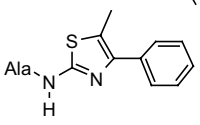
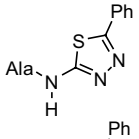
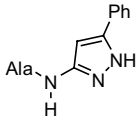
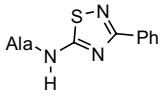
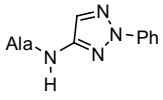
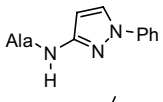
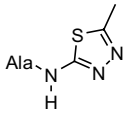
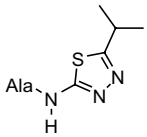
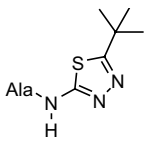
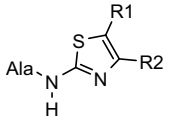
		NMR K_d, μM	FPA K_d, μM
1	AV-NH_2	812	N/T
2	AM-OEt	600	N/T
3	AY-OMe	742	N/T
4	AE-OEt	600	N/T
5		>2000	N/T
6		660	N/T
7		560	N/T
8		<10	20
9		<10	25
10		<10	30
11		<10	30
12		<10	2
13		<10	15

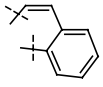
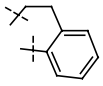
Table 2.

		NMR K_d, μM
14		161
15		170
16		84
17		40
18		84
19a		>2000
19b		63
19c		21

Thus, a series of thiazole compounds was prepared to access this site via substitution at the 4-position while maintaining substitution at the 5-position. The preparation of a few representative compounds is shown in Schemes 1–3. The diphenyl thiazole derivatives were prepared as shown in Scheme 1. Bromination of the ketone **21** followed by condensation with the thiourea gave rise to the thiazole **23**.[28] Negishi coupling with benzylzinc bromide[29] followed by amide formation with Boc-Ala and subsequent deprotection of the Boc group completed compound **20h**. The synthesis of the benzyl phenyl thiazole derivatives was accomplished by the addition of phenethyl Grignard to benzaldehyde **24**. After Swern oxidation of the benzylic alcohol, the ketone was carried through the same transformations as described in Scheme 1 to give rise to compound **20i**. The benzyl aryloxy thiazole derivatives were synthesized by reacting the acid chloride of the benzyl glycolic acid **28** with phenethylzinc bromide under palladium catalysis.[30] The ketone **29** was converted to compound **30** by bromination and condensation with thiourea. Coupling of compound **30** with Boc-alanine and subsequent deprotection of the benzyl group using BCl_3 gave rise to compound **31** with concomitant deprotection of the Boc group. After reprotection of the amino group as Boc carbamate, ether formation under the Mitsunobu conditions followed by deprotection of the Boc group gave compound **20j**. This series of compounds elucidated the SAR for positions R_1 and R_2 (Table 3). With regard to R_1, several substituents were well tolerated, although there was a preference for aromatic hydrophobic groups such as phenyl (**20a**, **20d** vs **20f**). For R_2 substituents, hydrophobic groups of moderate size increased binding (**20e** vs **20f**). The vector of the substituent branching out of R_2 phenyl group appeared critical to maintain the affinity (**20h** vs **20i**). Tethering between the thiazole and hydrophobic group did not increase binding (**20j**, **20k**). Larger groups such as naphthyl or indanyl group disrupted binding (**20l**, **20m**, **20n**). Compound **20g** had submicromolar binding affinity (0.74 μM in FPA) to BIR3 and is equipotent to the AVPIA peptide (0.64 μM in FPA).[13] Hence, **20g** represents a fairly potent small molecule XIAP BIR3 antagonist.

Table 3.



	R_1	R_2	NMR K_d, μM	FPA K_d, μM
20a	H	4-Me–Ph	47	N/T
20b	H	3-Me–4-MeO–Ph	36	N/T
20c	H	1-Naphthyl	357	N/T
20d	^{t}Bu	Ph	<10	4.8
20e	Ph	Me	10	15
20f	Ph	Ph	<10	1.1
20g	Ph	4-Br–Ph	<10	0.74
20h	Ph	4-Bn–Ph	<10	1.2
20i	Bn	3-Bn–Ph	>100	>100
20j	Bn	CH_2O–Ph	<10	4.1
20k	Bn	CH_2O–Bn	<10	4.1
20l	Bn	CH_2O–1-Naphthyl	66	21
20m	Bn	CH_2O–2-Naphthyl	36	14
20n	Bn	CH_2O–2-Indanyl	32	22
20o			<10	7.1
20p			<10	5.6

To validate our binding hypothesis, we carried out structural studies by NMR on compound **20g** bound to the XIAP BIR3 domain. A 1:1 complex of protein and **20g** was formed and 3D ^{13}C-edited ^{13}C, ^{15}N-filtered NOESY experiments were used to identify contacts between **20g** and the protein. Using these constraints, we docked **20g** into the BIR3 structure (Fig. 1B).[33] The NOE contacts observed between the alanine of **20g** and Trp 310, Trp 323, Asp 309, Gln 319, and Leu 307 indicate that it adopts the same position as the alanine in the AVPI peptide structure (Fig. 1). Similar to the SMAC peptide, the positive charge of the N-terminal amino group can interact with the negative charge of Glu 314. This is an important interaction since mutation of Glu 314 to alanine greatly reduces binding of the SMAC peptide.[13] The NOE constraints also indicate that the carbonyl group of the alanine of **20g** is on the same side as the indole of Trp 323 in a position to form the same hydrogen bond as the peptide. The amide in **20g** that connects the alanine to the thiazole is also positioned by these constraints to interact with the carbonyl of Thr 308 of the BIR3 domain. The thiazole C-5 phenyl substituent (R_1) of **20g** has NOEs to one Leu 307 methyl while the alanine methyl of **20g** shows NOEs to the other Leu 307 methyl. These two groups flank Leu 307 while the R1 phenyl sits next to Trp 323 filling the pocket occupied by the proline of the SMAC peptide. Orientation of **20g** using the above structural constraints places the sp^2 nitrogen of the thiazole in position to hydrogen bond with the amide of Thr 308. As expected, the R_2 4-Br–phenyl group is pointing toward the hydrophobic groove filled by the Ile of the SMAC peptide. NOEs from the R_2 4-Br–phenyl and Lys 297 indicate that this group sits on top of the hydrophobic pocket formed by the aliphatic sidechains of Lys 299 and 297.

In summary, we have discovered submicromolar small molecule non-peptidic antagonists that mimic the SMAC peptide binding to XIAP. By using properly substituted heterocycles, we were able to replace several amino acid residues while still preserving the key interactions. Our compounds bind to the BIR3 SMAC peptide binding site in the desired orientation with similar affinity to the natural AVPI peptide. Further optimization of these leads could lead to the discovery of more potent non-peptidic SMAC mimetics that could serve as a useful treatment for cancer.

References and notes

1. Green, D. R.; Reed, J. C. *Science* **1998**, *281*, 1309.
2. (a) Dragovich, T.; Rudin, C. M.; Thompson, C. B. *Oncogene* **1998**, *17*, 3207; (b) Nunez, G.; Benedict, M.

A.; Hu, Y.; Inohara, N. *Oncogene* **1998**, *17*, 3237; (c) LaCasse, E. C.; Baird, S.; Korneluk, R. G.; MacKenzie, A. E. *Oncogene* **1998**, *17*, 3247.
3. Deveraux, Q. L.; Reed, J. C. *Gene Dev.* **1999**, *13*, 239.
4. 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.; Reed, J. C. *Clin. Cancer Res.* **2000**, *6*, 1796.
5. Krajewska, W.; Krajewska, S.; Banares, S.; Huang, X.; Turner, B.; Bubendorf, L.; Kallioniemi, O. P.; Shabaik, A.; Vitiello, A.; Peehl, D.; Gao, G. J.; Reed, J. C. *Clin. Cancer Res.* **2003**, *9*, 4914.
6. Deveraux, Q. L.; Takahashi, R.; Salvesen, G. S.; Reed, J. C. *Nature* **1997**, *388*, 300.
7. Takahashi, R.; Deveraux, Q.; Tamm, I.; Welsh, K.; Assa-Munt, N.; Salvesen, G. S.; Reed, J. C. *J. Biol. Chem.* **1998**, *273*, 7787.
8. Suzuki, Y.; Nakabayahi, Y.; Nakata, K.; Reed, J. C.; Takahashi, R. *J. Biol. Chem.* **2001**, *276*, 27058.
9. 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.
10. 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.
11. 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.
12. Chai, J.; Du, C.; Wu, J.-W.; Kyin, S.; Wang, X.; Shi, Y. *Nature* **2000**, *406*, 855.
13. Liu, Z.; Sun, C.; Olejniczak, E. T.; Meadows, R. P.; Betz, S. F.; Oost, T.; Herrmann, J.; Wu, J. C.; Fesik, S. W. *Nature* **2000**, *408*, 1004.
14. Wu, G.; Chai, J.; Suber, T. L.; Wu, J.-W.; Du, C.; Wang, X.; Shi, Y. *Nature* **2000**, *408*, 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.
16. Tamm, I.; Trepel, M.; Cardo-Vila, M.; Sun, Y.; Welsh, K.; Cabezas, E.; Swatterthwait, A.; Arap, W.; Reed, J. C.; Pasqualini, R. *J. Biol. Chem.* **2003**, *278*, 14401.
17. Franklin, M. C.; Kadkhodayan, S.; Ackerly, H.; Alexandru, D.; Distefano, M. D.; Elliott, L. O.; Flygare, J. A.; Mausisa, G.; Okawa, D. C.; Ong, D.; Vucic, D.; Deshayes, K.; Fairbrother, W. J. *Biochemistry* **2003**, *42*, 8223.
18. 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**, ASAP.
19. Arnt, C. R.; Chiorean, M. V.; Heldebrant, M. P.; Gores, G. J.; Kaufmann, S. H. *J. Biol. Chem.* **2002**, *277*, 44236.
20. Guo, F.; Nimmanapalli, R.; Paranawithana, S.; Wittman, S.; Griffin, D.; Bali, P.; O'Bryan, E.; Fumero, C.; Wang, H. G.; Bhalla, K. *Blood* **2002**, *99*, 3419.
21. Yang, L.; Mashima, T.; Sato, S.; Mochizuki, M.; Sakamoto, H.; Yamori, T.; Oh-Hara, T.; Tsuruo, T. *Cancer Res.* **2003**, *15*, 831.
22. Fulda, S.; Wick, W.; Weller, M.; Debatin, K.-M. *Nat. Med.* **2002**, *8*, 808.
23. Wu, T. Y. H.; Wagner, K. W.; Bursulaya, B.; Schultz, P. G.; Deveraux, Q. L. *Chem. Biol.* **2003**, *10*, 759.
24. Nikolovska-Coleska, Z.; Xu, L.; Hu, Z.; Tomita, Y.; Li, P.; Roller, P. P.; Wang, R.; Fang, X.; Guo, R.; Zhang, M.; Lippman, M. E.; Yang, D.; Wang, S. *J. Med. Chem.* **2004**, *47*, 2430.
25. Schimmer, A. D.; Welsh, K.; Pinilla, C.; Wang, Z.; Krajewska, M.; Bonneau, M.-J.; Pedersen, I. M. Kitada, S.; Scott, F. L.; Bailly-Maitre, B.; Glinsky, G.; Scudiero, D.; Sausville, E.; Salvesen, G.; Nefzi, A.; Ostresh, J. M.; Houghten, R. A.; Reed, J. C. *Cancer Cell* **2004**, *5*, 25.
26. Sun, H.; Nikolovska-Coleska, Z.; Yang, C.; Xu, L.; Tomita, Y.; Krajewski, K.; Roller, P. P.; Wang, S. *J. Med. Chem.* **2004**, *47*, 4147.
27. Fincham, C. I.; Higginbottom, M.; Hill, D. R.; Horwell, D. C.; O'Toole, J. C.; Ratcliffe, G. S.; Rees, D. C.; Roberts, E. *J. Med. Chem.* **1992**, *35*, 1472.
28. Huff, J. R.; Baldwin, J. J.; Solms, S. J.; Guare, J. P.; Hunt, C. A. *J. Med. Chem.* **1988**, *31*, 641.
29. Chambers, R. R.; Collins, C. J.; Maxwell, B. E. *J. Org. Chem.* **1985**, *50*, 4960.
30. Maggiotti, V.; Wong, J.-B.; Razet, R.; Cowley, A. R.; Gouverneur, V. *Tetrahedron: Asymmetry* **2002**, *13*, 1789.
31. Expression and purification of BIR3 protein: The BIR3 protein was prepared as described.[13,18] NMR samples contained 0.8 mM protein in either 90% H_2O/10% ^2H_2O, or 100% ^2H_2O, 10 mM phosphate at (pH 7.2), 150 mM NaCl, and 1 mM ^{2}H-dithiothreitol.
32. NMR spectroscopy: All NMR experiments were acquired at 303 K on a Bruker DRX600 NMR spectrometer. Resonances were assigned using both NOESY and HCCH TOCSY experiments. NOE distance restraints were obtained from three-dimensional ^{13}C-edited NOESY and ^{13}C-edited filtered NOESY spectra acquired with a mixing time of 80 ms.
33. Structure calculations: BIR3/Ligand structures were calculated using a docking protocol implemented with the program Xplor-NIH. Ligands were docked into the previously determined BIR3/SMAC average structure followed by energy minimization to optimize the conformation of the complex. Fifteen inter-molecular NOEs were obtained for docking ligand **20g**.
34. Ligand binding: A fluorescence polarization anisotropy (FPA) competition assay was used to determine the affinities of compounds to BIR3 protein. Fluorescence polarization measurements were carried out on an Analyst 96-well plate reader (LJL, Molecular Dynamic, Sunnyvale, CA). 6-Carboxyfluorescein (FAM) labeled peptides with the sequences AVPIAQK(FAM)-NH_2 was used as probes. Dissociation constants were determined from titration curves with in-house written software using the analytical expressions of Wang.[35]
35. Wang, Z.-X. *FEBS Lett.* **1995**, *360*, 111.