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Discovery of aminopiperidine-based Smac mimetics as IAP antagonists

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ABSTRACT

A series of structurally unique Smac mimetics that act as antagonists of inhibitor of apoptosis proteins (IAPs) has been discovered. While most previously described Smac mimetics contain the proline ring (or a similar cyclic motif) found in Smac, a key feature of the compounds described herein is that this ring has been removed. Despite this, compounds in this series potently bind to cIAP1 and elicit the expected phenotype of cIAP1 inhibition in cancer cells. Marked selectivity for cIAP1 over XIAP is observed for these compounds, which is attributed to a slight difference in the binding groove between the two proteins and the resulting steric interactions with the inhibitors. XIAP binding can be improved by constraining the inhibitor so that these unfavorable steric interactions are minimized.

In multicellular organisms, apoptosis (programmed cell death) is a crucial mechanism for maintaining homeostasis and for eliminating damaged cells.[1] Under normal circumstances, both extrinsic apoptotic stimuli and intrinsic cellular stresses can lead to a highly coordinated signaling cascade that terminates in the death of the cell. Cancer cells, however, often do not respond to apoptotic cues; this resistance to cell death is widely recognized as one of the hallmarks of cancer.[2] As a result, a number of elements of the apoptotic machinery have received attention as potential targets for cancer therapeutics.

The inhibitor of apoptosis proteins (IAPs) are one of the most widely investigated classes of apoptosis regulators.[3] Certain IAPs (e.g., XIAP, cIAP1, cIAP2), which are frequently overexpressed in cancer cells, can directly bind to and inhibit proteases within the cell that are essential for apoptosis (caspases). The IAP family is characterized by the presence of one to three repeating domains of ~70 amino acids known as BIR (baculovirus IAP repeat) domains, and it is the interaction between these BIR domains and caspases that results in apoptosis inhibition.

It has been established that the interaction between IAP BIR domains and the proapoptotic caspases can be disrupted by an endogenous protein normally localized in the mitochondria, Smac (second mitochondrial activator of caspases).[4,5] Smac is released into the cytoplasm in response to apoptotic stimuli, and binds to the IAP BIR domains in preference to caspases, thus releasing the caspases and allowing apoptosis to occur. The bulk of this binding interaction occurs through the four N-terminal residues of Smac (Ala-Val-Pro-Ile, AVPI),[6,7] and numerous small molecule IAP antagonists that mimic interactions with key hotspots in the Smac binding groove (e.g., the hydrophobic pockets occupied by the methyl side chain of the alanine residue and the *sec*-butyl side chain of the isoleucine residue) have been described in the patent and chemical literature.[8,9] Moreover, a number of these IAP inhibitors are currently under investigation as anticancer agents in clinical trials.[10]

The proline ring of AVPI (**1**) is thought to contribute to the overall binding affinity of Smac towards XIAP-BIR3 by both restricting the conformation of the surrounding peptide chain and by establishing favorable non-bonding interactions between the ring methylene groups with Trp323.[11] Thus, most previously described peptidic Smac mimetics maintain a cyclic structure at that position (Fig. 1). However, several proapoptotic proteins (related to Smac) derived from *Drosophila* do not contain proline at this position (Reaper, Grim),[12,13] and synthetic tetrapeptides based on these have been shown to have modest binding affinity for XIAP-BIR3.[14] Moreover, it is has been previously demonstrated that cIAP1-BIR3 is tolerant of Smac-like peptides containing a valine residue in place of the proline.[15] As part of our medicinal chemistry efforts directed at finding novel IAP antagonists, we devised a strategy to excise the proline ring from examples of previously published Smac mimetics, with the hope that target potency would be maintained. Our intention was that this approach would result in synthetically tractable scaffolds amenable to rapid exploration of structure–activity relationships, and that our learnings could be extrapolated to other series of Smac mimetics. As a result, we

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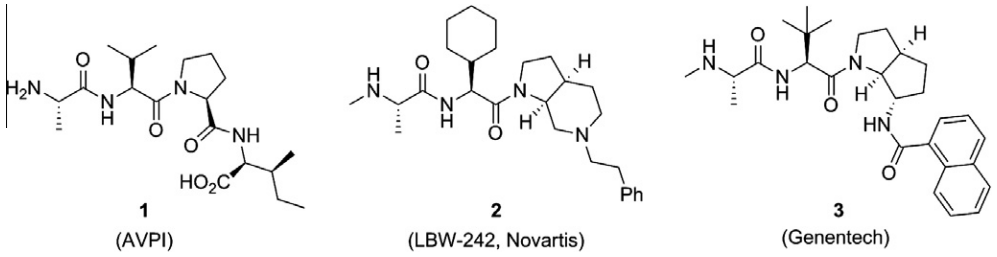


Figure 1. Examples of previously described Smac mimetics.

have discovered a series of aminopiperidine-based inhibitors (structurally related to known compound **2**) that do not contain a proline residue or a similar cyclic structure at that position. Examples of these compounds potently bind to the BIR3 domain of cIAP1, and also produce the phenotype expected from cIAP1 inhibition in cancer cell lines. Interestingly, this series also demonstrates unexpectedly high binding selectivity for cIAP1 over XIAP. Herein, we describe our efforts and the preliminary SAR for the series.

Compound **4** (Fig. 2), in which the absolute stereochemistry of the bridgehead carbon of **2** is maintained, was prepared from commercially available BOC-3-(S)-aminopiperidine and was found to potently bind to the BIR3 domain of cIAP1 (IC_{50} = 40 nM). Moreover, since binding of antagonists to cIAP1 is known to result in autoubiquitination and subsequent protein degradation in certain cancer cell lines,[16,17] we were pleased to observe this activity in MDA-MB231 cells following treatment with this compound (EC_{50} = 113 nM). Encouraged by this promising result, we set about exploring the SAR around this hit.

Initial work was directed at varying the ring size of the cyclic amine, as well as modifying the substituent appended to the ring nitrogen (Table 1). It was found that phenethyl substitution affords the most potent compounds regardless of ring size, although the phenethyl-substituted pyrrolidine (**6**) and homopiperidine (**17**) analogs were found to be less potent than the analogous piperidine (**4**). Interestingly, however, the benzyl-substituted homopiperidine (**16**) is 6 times more potent than the analogous piperidine (**11**) and 12 times more potent than the corresponding pyrrolidine (**5**), suggesting that the larger ring better positions the benzyl group towards the hydrophobic pocket occupied by the isoleucine residue of Smac. Interestingly, acylation of the ring nitrogen results in only modest drops in potency, implying that the basic piperidine nitrogen of **4** may not be optimally interacting with Gly306, a residue involved in hydrogen bonding interactions with Smac[6,7] and many Smac mimetics. This data also suggests that planarization of the ring nitrogen resulting from acylation adversely affects the orientation of the aromatic group such that interactions with the hydrophobic pocket are suboptimal.

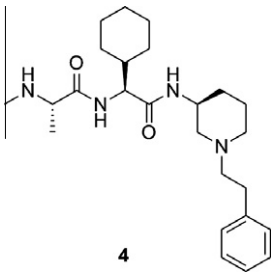
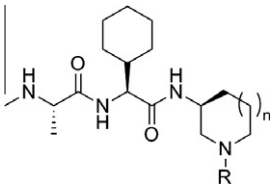
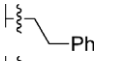
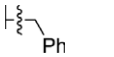
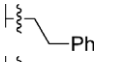
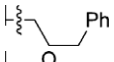
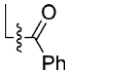
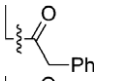
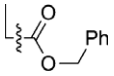
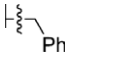
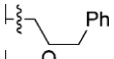
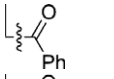
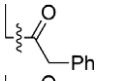
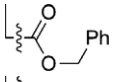
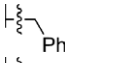
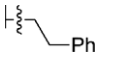


Figure 2. Structure of lead aminopiperidine-based Smac mimetic.

Table 1
SAR around ring size and substituent length



Compound	*n*	R	cIAP1-BIR3 IC_{50} (μM)
4	1		0.04
5	0		3.38
6	0		0.50
7	0		0.74
8	0		5.02
9	0		0.78
10	0		0.74
11	1		1.69
12	1		0.79
13	1		3.36
14	1		0.13
15	1		0.45
16	2		0.27
17	2		0.08

Having established that incorporation of the 3-aminopiperidine motif in these Smac mimetics affords potent inhibitors of cIAP1, we further investigated the effects of varying different regions of the scaffold (Table 2). Replacement of the cyclohexylglycine residue with *tert*-leucine (**18**) maintained binding affinity, as was expected based on work previously conducted in the area.[11,14] We also found that methylation of the amide nitrogen at the aminopiperidine residue (**19**) resulted in a potent inhibitor, even though steric interactions between this methyl group and the aminopiperidine ring are likely to result in a different conformation than would be expected