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Design and synthesis of a potent biotinylated Smac mimetic

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Abstract—A biotinylated Smac mimetic (**2**) was designed based upon our previously reported potent, conformationally constrained Smac mimetic (**1**). Smac mimetic (**2**) was synthesized and determined to bind to XIAP protein with a high-affinity (K_i value of 13 nM) and is therefore a useful pharmacological tool for probing the intracellular targets of this class of potent Smac mimetics.

Smac/DIABLO (Second Mitochondria-derived Activator of Caspase or Direct IAP Binding protein with LOw pI) is a protein released from mitochondria in response to apoptotic stimuli.[1,2] Smac/DIABLO promotes apoptosis in cells, at least in part by directly interacting with the inhibitor of apoptosis proteins (IAPs) and functions as a potent endogenous cellular antagonist of IAPs.[1,2] Structural[3,4] and biological[5] studies have demonstrated that Smac binds to a surface groove in the third Birculovirus IAP repeat (BIR3) of X-linked IAP (XIAP) through its exposed four N-terminal residues (alanine–valine–proline–isoleucine or AVPI).[6] Short Smac peptides derived from the N-terminal residues of Smac fused to a carrier peptide for intracellular delivery have been shown to overcome resistance to apoptosis of cancer cells possessing high levels of IAP proteins. Such Smac peptides enhance the activity of anti-cancer drugs in vitro and in vivo, and have little toxicity to normal cells in vitro or to normal tissues in vivo.[7,8] These studies thus strongly suggest that Smac mimetics may have great therapeutic potential to be developed as a new class of anti-cancer drugs.

Smac peptides have several intrinsic limitations such as poor cell-permeability, poor in vivo stability and bioavailability. Consequently, our laboratory[9–11] and others[12,13] are interested in design of Smac peptidomimetics and non-peptidic mimetics with much improved binding affinity, cell-permeability, in vivo stability, and bioavailability, and we have recently reported the design and synthesis of a series of conformationally constrained non-peptidic Smac mimetics.[9,10] Compound **1** (Fig. 1) is the most potent Smac mimetic we have obtained from our previous studies,[9,10] and has a K_i value of 25 nM to XIAP as determined in our fluorescence-polarization-based (FP-based) binding assay.[14] Toward understanding the precise molecular mechanisms of action and probing the intracellular molecular targets of our designed Smac mimetics, we have designed and synthesized a biotinylated Smac mimetic based upon the structure of compound **1**.

In the design of a biotinylated ligand, it is critical to identify a suitable position for biotinylation of the ligand so that the biotin tag will not compromise the binding of the ligand to its target protein(s). Based upon our predicted binding model for compound **1** (Fig. 2), the pro (*S*)-phenyl ring projects from the XIAP binding groove with apparently no specific interaction with the protein.[10] In comparison, the pro (*R*)-phenyl ring buries into a hydrophobic pocket in XIAP, so a biotin tag at this phenyl ring would clash with the protein atoms and decrease ligand binding to XIAP. Based upon this analysis, the biotinylated compound **2** was designed in which the 3-amino-propyl group was attached to the *para*-position of the pro-(*S*)-phenyl ring of compound **1** (Figs. 1 and 2). The biotin tag is then linked to the amino group by the formation of an amide bond (Fig. 1).

The synthesis of compound **2** is detailed in Scheme 1 and 2. First, a previously reported method was used to synthesize the substituted (*S*)-diphenylmethylamine **9**.[15]

Keywords: Smac mimetic; Biotinylated ligand; XIAP.
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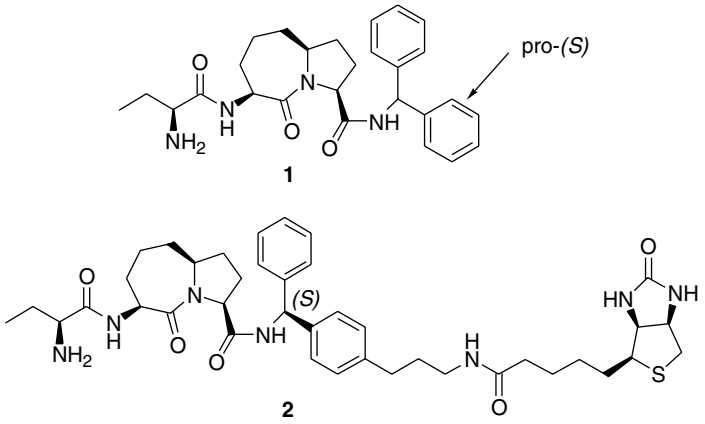


Figure 1. Design of biotinylated Smac mimetic.

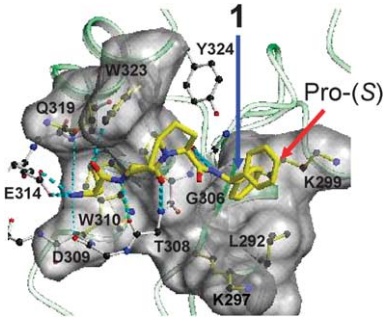
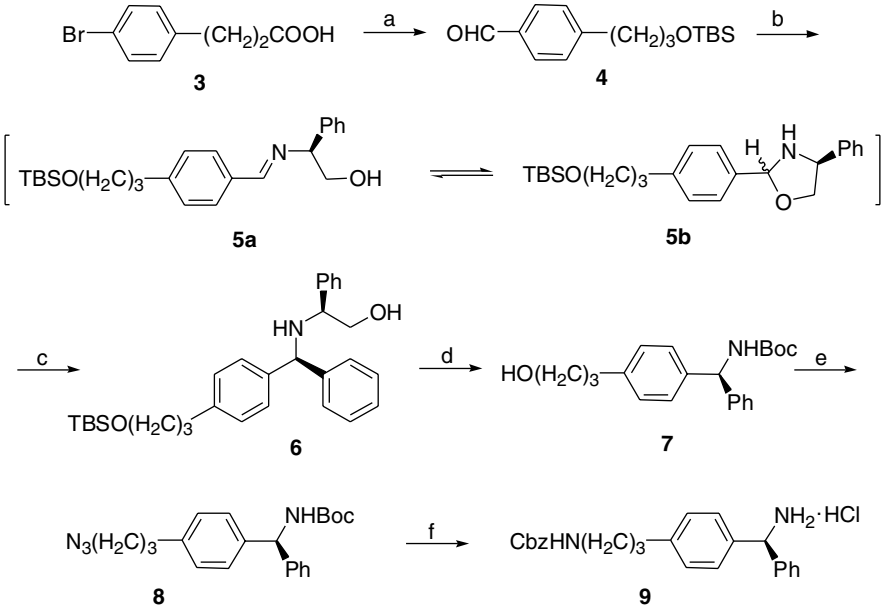
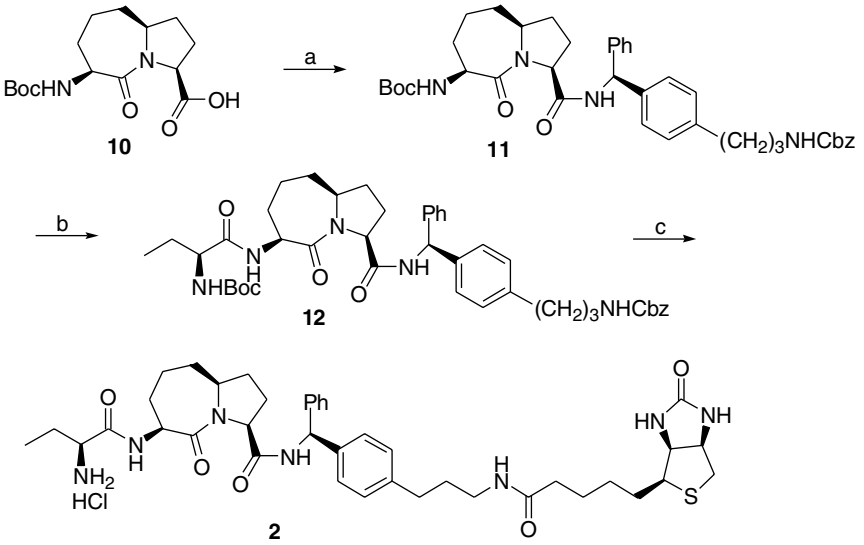


Figure 2. Predicted binding model of compound **1** in complex with XIAP BIR3. The red arrow indicates the labeling position for biotin. Hydrogen bonds formed between **1** and XIAP BIR3 are shown in dashed lines.

Briefly, reduction of the commercially available acid **3** with $NaBH_4$–I_2 gave an alcohol, and protection of the hydroxyl group with TBS yielded a silyl ether. Treatment of this silyl ether with BuLi followed by the addition of the resulted aryl lithium to DMF furnished the aldehyde **4**. Condensation of **4** with (*S*)-phenylglycinol gave aldimine/oxazolidine **5a/5b**. Addition of 4 equiv of phenyl magnesium bromide to **5a/5b** afforded the aminoalcohol **6** as a single isomer. Cleavage of the auxiliary in **6** by $Pb(OAc)_4$ oxidation and removal of the TBS protecting group with 2 N HCl gave an amine. Protection of the amino group with Boc afforded compound **7**. Treatment of **7** with MsCl followed by reaction of the resulted mesylate with NaN_3 yielded azide **8**. Reduction of the azide with PPh_3 in THF–H_2O followed by protection of the resulted amine with carbobenzoxy chloride



Scheme 1. Reagents and conditions: a. (i) $NaBH_4$–I_2, THF; (ii) TBSCl, Et_3N, CH_2Cl_2; (iii) BuLi, −78 °C, then DMF, THF, 85% for three steps; b. (*S*)-phenylglycinol (1.2 equiv), toluene, reflux, 98%; c. PhMgBr (4 equiv), THF, reflux; 56%; d. (i) $Pb(OAc)_4$, MeOH, then 2 N HCl; (ii) $(Boc)_2O$, Et_3N, CH_2Cl_2, 68% for two steps; e. (i) MsCl, Et_3N, CH_2Cl_2; (ii) NaN_3, DMF, 85% for two steps; f. (i) PPh_3, THF–H_2O; (ii) CbzCl, Et_3N, CH_2Cl_2; (iii) 4 M HCl in 1,4-dioxane, MeOH, 82% for three steps.



Scheme 2. Reagents and conditions: a. **9**, EDC, HOBt, *N*,*N*-diisopropylethylamine, CH_2Cl_2, 95%; b. (i) 4 M HCl in 1,4-dioxane, MeOH; (ii) (*S*)-*N*-Boc-2-aminobutyric acid, EDC, HOBt, *N*,*N*-diisopropylethylamine, CH_2Cl_2, 89% for two steps; c. (i) 10% Pd–C, H_2, MeOH; (ii) (+)-biotin *N*-hydroxy-succinimide ester, *N*,*N*-diisopropylethylamine, CH_2Cl_2; (iii) 4 M HCl in 1,4-dioxane, MeOH, 77% over three steps.

furnished a carbomate. Removal of the Boc protective group in this carbomate gave our desired chiral amine **9** (Scheme 1).

Condensation of acid **10**[10] and chiral amine **9** afforded amide **11**. Removal of the Boc protective group in **11** followed by condensation of the resulted ammonium salt with (*S*)-*N*-Boc-2-aminobutyric acid furnished compound **12**. Removal of the Cbz protecting group in **12** by hydrogenation over 10% Pd–C yielded an amine. Condensation of this amine with (+)-biotin *N*-hydroxy-succinimide ester furnished a biotinylated amide. Finally, removal of the Boc protective group from this amide gave the designed biotinylated compound **2**.[16]

Compound **2** was tested for its binding affinity to XIAP BIR3 using our established FP-based assay[14] and was shown to have a K_i value of 13 nM. The binding affinity of **2** is comparable to that of parent compound **1** ($K_i = 25$ nM), hence confirming our design strategy. In our preliminary experiments, we have used compound **2** to probe the intracellular molecular target(s) and shown that compound **2** indeed binds to endogenous XIAP protein in human prostate cancer PC-3 cells (data not shown).

In summary, we have designed and synthesized a biotinylated Smac mimetic, which binds to XIAP with a high-affinity ($K_i = 13$ nM). This potent biotinylated Smac mimetic is being used as a pharmacological tool to prove the cellular targets for this class of potent Smac mimetics in our laboratory.

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