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Development of XIAP Antagonists Based On De Novo 8,5-Fused Bicyclic Lactams**

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In order to develop original water soluble antagonists of X-linked inhibitor of apoptosis protein (XIAP), a novel bicyclic scaffold was designed based on 8,5-fused bicyclic lactam. During its preparation, a spontaneous rearrangement from 8,5- to 7,5-fused bicyclic lactam was observed and confirmed by MS and NMR analyses, in particular the HMBC spectra. DFT calculations were performed to understand the corresponding mechanism. It was finally prevented through changing the reaction order in the synthesis route and a Smac mimetic with this core structure, **ZJ-1** was successfully obtained. The structure of this new bicyclic scaffold was well confirmed by HRMS and NMR (^{1}H, ^{13}C, NOESY) analyses. **ZJ-1** presented in addition a binding affinity to XIAP-BIR3, nearly 6 times better than that of AVPI, similar to the reported **SM-128** in an in vitro fluorescence polarization (FP) assay. This preliminary result suggests that this new bicyclic scaffold could be very attractive in the development of novel anticancer agents targeting XIAP.

1. Introduction

Inhibitors of apoptosis proteins (IAPs) are a family of functionally and structurally related proteins, which serve as endogenous inhibitors of apoptosis. Several members of the IAP family have been shown to be overexpressed in various cancers.[1] The X-linked inhibitor of apoptosis protein (XIAP) is the best characterized member of all the IAPs and the only member that regulates caspase activity through direct binding to caspases.[2]

Smac/DIABLO (Second mitochondria-derived activator of caspases or Direct IAP binding protein with low pI) has been identified as an endogenous IAP antagonist that is released from mitochondria into the cytosol in response to apoptotic stimuli.[3] It regulates the apoptosis through binding to the BIR3 domain of XIAP, in competition with Caspase 9, using its N-terminal tetrapeptide binding motif, Ala-Val-Pro-Ile (AVPI, Figure 1).[3c,4]

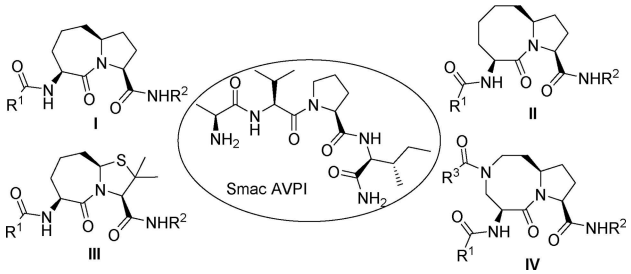

Figure 1. AVPI and representative core structures of bicyclic Smac mimetics.[5]

Starting from AVPI as a leading compound, many laboratories have developed both peptide and non-peptide small molecular Smac mimetics.[5a,e,f,6] Some representative core structures of bicyclic Smac mimetics are shown in Figure 1 and the common feature in these structures is the presence of a bicyclic moiety, based on octahydro-1*H*-pyrrolo[1,2-*a*]azepine (core structure of I) or decahydropyrrolo[1,2-*a*]azocine (core structure of II). Another heteroatom could be well tolerated when it was incorporated into either the 5-membered ring, giving the core structure of III[5e] or the 8-membered ring, giving the core structure of IV.[5d] The binding affinity and the PK profile of the corresponding compounds were even largely improved in the latter case when the nitrogen was extracyclically acylated.[5d] According to the structure data reported by Wang's group,[5c] the 8-membered ring in the core structure of II has extensive contacts with the side chain of Trp323 in XIAP. Moreover, the presence of a hydrophilic group in the 8-

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membered ring, as in the core structure IV, is beneficial to the binding affinity and the PK profile.

In this work, a novel bicyclic scaffold was designed on the basis of 8,5-fused bicyclic lactam, in order to develop original water soluble antagonists of X-linked inhibitor of apoptosis protein (XIAP). A spontaneous rearrangement from 8,5- to 7,5-fused bicyclic lactam was successfully prevented and a Smac mimetic with this core structure, **ZJ-1** was finally obtained. The latter presented a binding affinity to XIAP-BIR3, nearly 6 times better than that of the tetrapeptide AVPI, similar to the reported **SM-128** in an in vitro FP assay. This result is indeed encouraging and more efforts are actually devoted to this stimulating project.

2. Results and Discussion

2.1. Molecular Modeling

To develop original and orally bioavailable Smac mimetics, a new XIAP antagonist based on 8,5-fused bicyclic lactam (Figure 2, **ZJ-1**) was designed by introducing an amide function

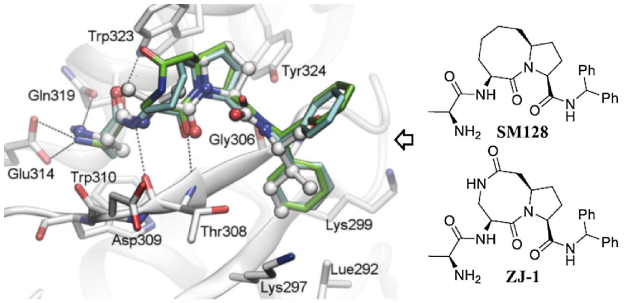


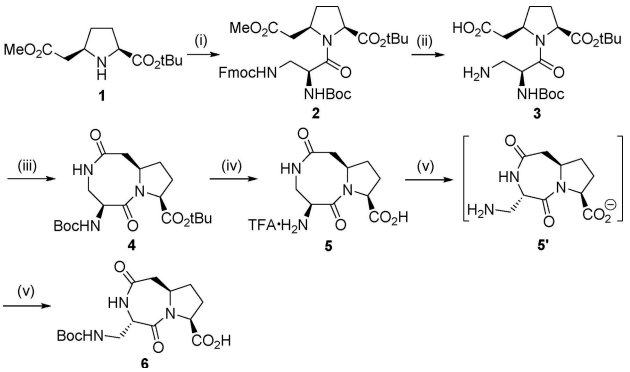
Figure 2. Predicted binding model of **ZJ-1** to XIAP BIR3 in comparison with AVPI and **SM-128**. The binding model was predicted by LeDock, and representation was prepared by VMD. Only the important residues in XIAP BIR3 are shown in white, whereas AVPI, **ZJ-1** and **SM128** in stick style are shown in white, green and cyan colors, respectively. The hydrogen bonds between **ZJ-1** and XIAP BIR3 domain are shown as dashed lines.

into the 8-membered ring of the bicyclic structure II. The binding model of **ZJ-1** to XIAP BIR3 domain was predicted by molecular docking.

As shown in Figure 2, the backbones of AVPI, **ZJ-1** and **SM-128** interact with XIAP BIR3 in the same way, and share almost all kinds of interactions between the ligands and the target. The binding model of **ZJ-1** to XIAP BIR3 is similar to that of **SM-128** (RMSD <0.5 Å for the common atom pairs). The amide function in the 8-membered ring of **ZJ-1** increases the rigidity of the ring, and favors the binding to the TRP323. It was noted that only one benzyl group in **ZJ-1** replaced the Ile4 side chain to interact with the side chains of Lue292, Lys 297, Lys299 through Van der Waals forces and the other one has no specific interaction with BIR3, as already reported.[5c] The latter has been chosen, since it has been suggested that the second phenyl group could control the compound binding orientation and reduce the entropic loss.[5d] Taking together, we predicted that the Smac mimetic containing 8,5-fused bicyclic lactam would have better or similar binding affinity to XIAP BIR3 than or to the reference compounds, AVPI and **SM-128**.

2.2. Chemistry

The first synthesis route adopted to prepare the core structure of **ZJ-1** was outlined in Scheme 1.



Scheme 1. Synthesis of 8,5-fused bicyclic lactam **5** and its spontaneous rearrangement to 7,5-fused bicyclic lactam **6**. Reagents and conditions: (i) Boc-Dap(Fmoc)-OH, BOP, DIEA, DCM (78%); (ii) LiOH, MeCN/H_2O; (iii) BOP, DIEA, DCM (78% for the two steps); (iv) TFA, TIPS, DCM (86%); (v) Boc_2O, Et_3N, MeCN/H_2O (64%). Dap: 2,3-diaminopropionic acid, Fmoc: 9-fluorenylmethyloxycarbonyl, BOP: tert-butyloxycarbonyl, DIEA: diisopropylethylamine, DCM: dichloromethane, TFA: trifluoroacetic acid, TIPS: triisopropylsilane.

The key intermediate, amine **1** was first prepared as reported[7] and then condensed with Boc-Dap(Fmoc)-OH to provide the dipeptide **2** in 78% yield. A simultaneous cleavage was conducted to hydrolyze the methyl ester and to remove the Fmoc group by handling **2** with LiOH in a mixture of solvents MeCN/H_2O, affording the intermediate **3**. Its cyclization through BOP-assisted intramolecular coupling led to the formation of the target bicyclic 8,5-fused bicyclic lactam **4** (78% overall yield for the 2 steps). The protecting groups of the lactam **4**, Boc and *t*Bu were next removed by TFA to furnish the expected free 8,5-fused bicyclic lactam **5** in 86% yield. Its identity was confirmed by HRMS and ^1H, ^{13}C, in particular HMBC NMR analyses (Figure 3). As shown in Figure 3, the correlation spots between H1a, H1b, H4a, H4b and C2 demonstrated without any ambiguity that they were in close proximity (within 3 chemical bonds). On the other hand, H5 correlated with C6, but not at all with C2. This suggested that H5 and C2 were separated from each other by more than 3 chemical bonds. Taken together, the 8-membered ring was clearly evidenced.

In order to pursue the synthesis, it was decided to selectively protect the amine function in **5**. This was carried out using the classical experimental conditions: Boc_2O in the presence of triethylamine in CH_3CN/H_2O. To our surprise, the main product, isolated in 74% yield corresponded to the 7,5-fused bicyclic lactam **6**, instead of the desired product. The identity of **6** has also been confirmed by HRMS and NMR

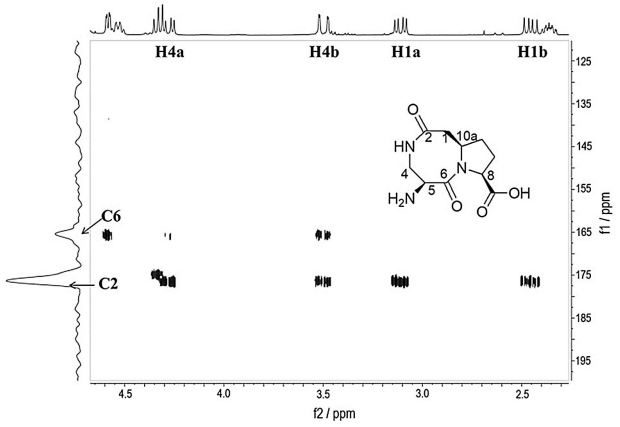


Figure 3. Part of the HMBC spectrum of **5** in D_2O, recorded with a Bruker AVANCE III 400 NMR spectrometer at room temperature.

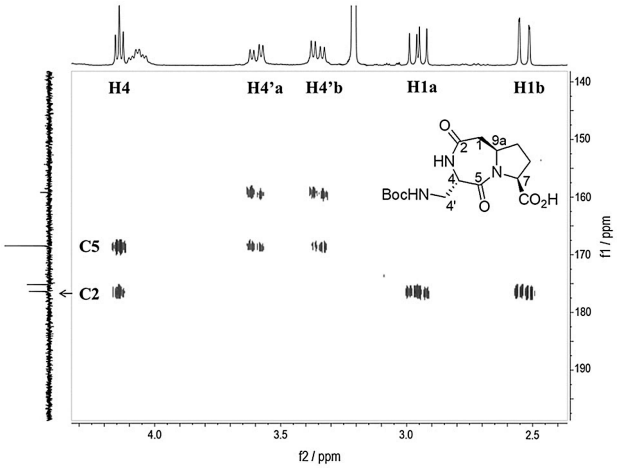


Figure 4. Part of the HMBC spectrum of **6** recorded in $CDCl_3$ at room temperature.

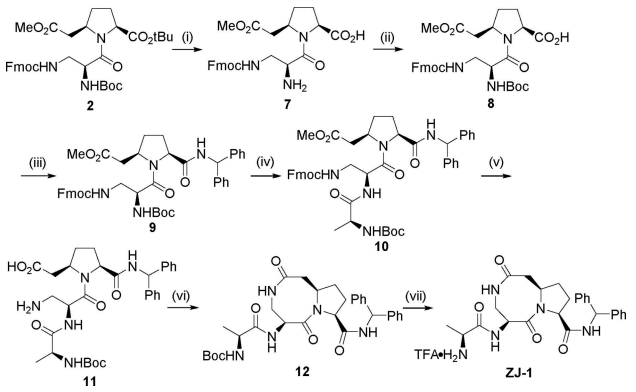
analyses as well (SI and Figure 4). In the HMBC spectrum of **6** (partially shown in Figure 4), only H1a and H1b were still observed to correlate with C2, while no detectable correlation was visible between H4'a, H4'b (corresponding to H4a, H4b in the 8-membered ring) and C2 at all. Instead, a new correlation was observed between H4 (corresponding to H5 in the 8-membered ring) and C2. The correlations were also observed between H4, H4'a, H4'b and C5. This implied that H4'a and H4'b were no more in close proximity to C2 (more than 3 chemical bonds). This could be true only in conditions that a rearrangement converted the free 8,5-fused bicyclic lactam **5** into the free 7,5-fused bicyclic lactam **5'** in the basic medium through transamidation before the protection reaction. The amine function of **5'** was then protected to give finally **6**. Although it is likely that the driving force of this transamidation could be the higher stability of a 7-membered ring compared to an 8-membered one, such a spontaneous rearrangement has not been described in the past to our best knowledge.

Calculations using DFT were undertaken to investigate the reaction mechanism of the rearrangement between **5** and **5'** under basic conditions. The 8,5-fused bicyclic lactam **5** is a relative flexible molecule and a large number of stationary structures corresponding to different conformations of the 8-membered ring can be found on the potential energy surface. An extensive exploration of the conformational space is beyond the scope of this work and we only present three of them in SI. The large changes between dihedral angle in the cycle show that the 5-membered fused ring do not prevent the conformational flexibility. In the most stable structure, the 8-membered cycle adopts a boat like geometry with the two C=O bonds pointing in the same direction of the space. The 7,5-fused bicyclic lactam **5'** is calculated to be more planar than **5** as revealed by the value of the dihedral angle ω_1, ω_4, ω_7 and ω_8 close to 0°. Furthermore, **5'** is found more stable than **5** and DFT calculations predict that the rearrangement from **5** to **5'** is exothermic by 3.1 kcal/mol (Table S1 and Figure S1).

From the most stable conformation of **5** we investigated the reaction pathway leading to the lactam **5'**by DFT calculations in acetonitrile modelized as continuum medium. Despite extensive search to reveal the reaction pathway between **5** and **5'**, only one transition state connecting both structures was found at the DFT level of calculation. Hence, from **5-A** minimum, the reaction proceeds via a transition state lying at 64.3 kcal/mol relative to the energy of the starting structure (Figure S2). This transition state structure is displayed in Figure S2 and results from the intramolecular nucleophilic attack of the NH_2 on the C_2=O to form a new ring. On the same time, a hydrogen atom of the NH_2 group is transferred between both the nitrogen atoms. As a consequence, the geometry of this transition state shows considerably elongated C–N bonds with respect to the equilibrium C–N distance (i = 1.629 Å and h = 1.641 Å, see SI) and a hydrogen is located between both nitrogen atoms. An IRC calculation conducted from the TS confirms that this transition state connects the **5-A** minimum and **5'** structure. Thus from the TS downhill toward product shows hydrogen transfer concerted with the C_2–C_3 bond breaking.

The rearrangement could be also considered as a solvent-assisted reaction with two water molecules. Indeed it is well known that the transfer of hydrogen atom may be facilitated in environment containing nearby H_2O species. In such an environment the transfer of hydrogen atom may occur to a neighboring H_2O molecule, followed by the transfer of another H atom from the solvent to the second site on the solute. Calculations reveal that the rearrangement of **5** to **5'** may proceed via two steps. The first step involves the nucleophilic attack of NH_2 on the C_2=O carbonyl group through TS1 lying 22.1 kcal/mol above the starting reactant. In TS1 the nitrogen-C_2 key distance is 1.953 Å. Downhill from TS1, a tricyclic intermediate INT is formed with the nitrogen-carbon bond of 1.637 Å. From INT the reaction can produce **5'** trough TS2 in which an hydrogen is transferred from NH_2 group to N_3. The geometry of TS2 is found very similar to that found in the non-solvent mediated reaction. Overall the energy barrier for **5** to **5'** reaction is determined by the hydrogen transfer step.

To avoid this rearrangement, the reactions order was changed in the synthesis route, as shown in Scheme 2.



Scheme 2. Synthesis of **ZJ-1**. Reagents and conditions: (i) TFA, TIPS, DCM; (ii) Boc_2O, NEt_3, MeCN (78% for 2 steps); (iii) Ph_2CHNH_2, EDCI, HOBt, DIEA, DCM (61%); (iv) 1) TFA, DCM; 2) Boc-Ala-OH, EDCI, HOBt, DIEA, DCM (74% for 2 steps); (v) LiOH, MeCN/H_2O (quantitative); (vi) BOP, DIEA, DCM; (vii) TFA, DCM (20% for the 2 steps). EDCI: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; HOBt: 1-hydroxybenzotriazole.

The intermediate **2** was treated with 50% TFA to simultaneously remove the Boc and *t*Bu groups and the free amine of **7** was selectively protected with Boc_2O to give the acid **8** in 78% yield for the 2 steps. The latter without purification was coupled with benzhydrylamine in the presence of EDCI, HOBt and DIEA, to afford the compound **9** in 61% yield. Its Boc group was then removed by TFA and the liberated amine condensed with Boc-Ala-OH as above, leading to the tripeptide **10** in 74% yield for the last two steps. A simultaneous cleavage was conducted as earlier to hydrolyze the methyl ester and to remove the Fmoc group quantitatively, and the intermediate **11** was cyclized in the same conditions as above to afford the compound **12**. The Boc group of **12** was finally cleaved and the crude product was purified by HPLC to give pure **ZJ-1** in 20% yield for the 2 steps. It was then characterized by HR MS and NMR (^1H, ^{13}C, COSY and NOESY) analyses. The strong NOE correlation between H5 and H10a shown in Figure 5 clearly demonstrated that H5 and H10a were in *cis* position. Taking into account the absolute configurations of C5 and C10a in **ZJ-1**, this relative position could exist only given that the desired 8,5-fused bicyclic moiety was formed and survived during the TFA treatment and the HPLC purification. The identity of **ZJ-1** was in such a way demonstrated.

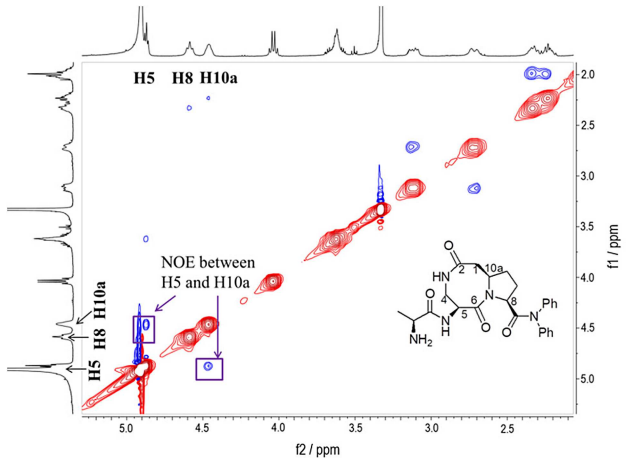


Figure 5. Part of the NOESY spectrum of **ZJ-1**, recorded in $CDCl_3$ at room temperature.

2.3 Biological Activity

The binding affinity of **ZJ-1** to XIAP-BIR3 was evaluated using a fluorescence polarization (FP) assay, developed by Glover[8] with certain modifications, taking in-house prepared AVPI[9] and SM-128 ($k_i = 14$ nM)[5b] as reference compounds. The results are shown in Figure 6.

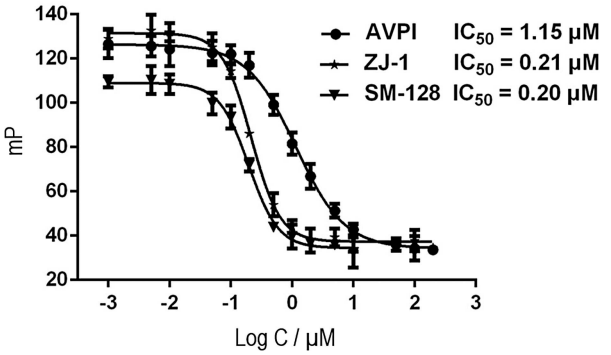


Figure 6. Binding affinity of AVPI, **ZJ-1** and **SM-128** to recombinant XIAP BIR3 protein, determined using the FP assay.

ZJ-1 showed nearly 6 times better binding affinity (0.21 μM) to XIAP-BIR3 than that of AVPI (1.15 μM), similar to **SM-128** (0.20 μM) under the same experimental conditions. These binding data indicate that the bicyclic core structure can tolerate other functional groups such as amide, and suggest that 8,5-fused bicyclic lactam could be used in the design and synthesis of XIAP antagonists. In addition, its better water solubility than the corresponding carbocyclic compounds might be more beneficial for its oral bioavailability.

3. Conclusions

In this work, a new water soluble XIAP antagonist (**ZJ-1**) based on 8,5-fused bicyclic lactam has been designed, synthesized and evaluated. Although the molecular docking study suggested that **ZJ-1** would interact with XIAP BIR3 domain in the same manner as AVPI, the 8,5-fused bicyclic lactam conferred to the new Smac mimetic a certain rigidity which may be helpful for its binding to XIAP. In addition, the introduction of an amide function into the bicyclic structure improved normally the water solubility. During the synthesis, a spontaneous rearrangement from 8,5- to 7,5-fused bicyclic lactam was observed and well characterized by MS and NMR (especially HMBC) analyses. Based on the fact that (3 *S*,6 *S*,10*aS*)-6-amino-5-oxodecahydropyrrolo [1,2-*a*]azocine-3-carboxylic acid, **5** was stable when it was in

TFA salt and the rearrangement observed took place when the 6-amino group was converted to free nucleophile in a basic medium, we anticipated that the rearrangement could be prevented if the 6-amino group would be kept non nucleophile after the 8-membered ring was constructed. The above results clearly verified this hypothesis and the target compound, **ZJ-1** was successfully obtained. In addition, as predicted by molecular modeling, **ZJ-1** indeed showed better binding affinity to XIAP BIR3 than AVPI, similar to **SM-128** with an IC_{50} value of 0.21 μM in the FP assay. The preliminary results revealed that **ZJ-1** is a promising leading compound for further *in vitro* and *in vivo* evaluation. More efforts are devoted to the development of new derivatives and the results will be reported in due course.

Experimental Section

Molecular Docking

The protein structure of XIAP BIR3 domain was extracted from the BIR3/Smac complex[3c] (PDBID: 1G73). Protein structure was prepared by VMD software,[10] in which the atom names were assigned in CHARMM force field format. The protonation state was corrected according to the PDB2PQR's result with pH 7.3.[11] Then the hydrogen bond network was optimized by NAMD program using CHARMM22 force field.[12] The binding position of AVPI peptide of Smac protein was set as the docking site. The structure of **ZJ-1** and **SM-128** were built with Maestro (Schrödinger, LLC), then were minimized using MMFF94 force field. Finally, the docking was performed by ledock program (www.lephar.com), which combines global searching (genetic algorithm) and local optimization (steepest descent). The clustering RMSD was set to 1 angstrom, and 20 conformations was generated. The representation was generated by VMD software.

DFT Calculations

Molecular ground-state structures of 5 and 5′ compounds as well as transition states were obtained by optimizing their geometric parameters with Gaussian 09[13] The calculations have been performed in acetonitrile as in the experiment and solvent have been included in the calculations by means of the polarizable continuum model by the integral equation formalism (IEFPCM).[14] All the geometry optimizations were led without symmetry constraints and followed by a vibrational frequency computation to ensure that a transition state (one imaginary mode) or a minimum (zero imaginary modes) is located on the potential energy surface.

From the transition states, the whole reaction path can be determined by using the Intrinsic Reaction Coordinate (IRC) method.[15] The IRC is determined by a steepest descent based algorithm[16] which, using small steps along the negative gradient in a mass-weighted coordinate system, describes a molecular reaction from the TS towards the reactant and product. Therefore we performed IRC calculations at the same level of theory and in acetonitrile medium to verify that we obtained the correct transition state for the reaction when we examine the structures that are downhill from the saddle point and to facilitate connection between the reactants and the products.

Chemistry

General Methods

All chemicals were purchased as reagent grade and used without further purification unless otherwise noted. Reactions under anhydrous conditions were carried out with freshly distilled DCM or EtOAc from CaH_2 and THF from sodium. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F254 (Merck). Solvents were evaporated under reduced pressure and below 40 °C (bath). Flash column chromatography was performed on silica gel 60 (230–400 mesh; Merck). NMR spectra were recorded with a Bruker AVANCE III 400 NMR spectrometer (400 MHz for ^{1}H NMR and 101 MHz for ^{13}C NMR). The chemical shifts are referenced to the solvent peak, δ = 7.26 ppm (^{1}H) and δ = 77.36 ppm (^{13}C) for $CDCl_3$, at 25 °C, and coupling constants are given in Hz. High-resolution mass spectra (HRMS) were recorded on a ESI/TOF (LCT Premier XE, Waters) spectrometer. Preparative HPLC: Perkin Elmer Series 200 UV/VIS detector; Perkin Elmer Series 200 pump. Analytical HPLC: Perkin Elmer Series 225 Auto sampler; Perkin Elmer Series 220 UV/VIS Detector; Perkin Elmer Series 200 Pump.

tert-butyl (2 *S*,5*R*)-1-((*S*)-3-((9*H*-fluoren-9-yl)methoxycarbonylamino)-2-(*tert*-butoxycarbonyl amino)propanoyl)-5-(2-methoxy-2-oxoethyl) pyrrolidine-2-carboxylate (2). To a solution of 1 (128 mg, 0.46 mmol) in anhydrous DCM (5 mL) at 0 °C were added Boc-Dap (Fmoc)-OH (215 mg, 0.51 mol), BOP (244 mg, 0.55 mmol) and DIEA (0.5 mL, 3.57 mmol). The mixture was stirred for 4 hours at room temperature. The reaction solution was washed with saturated NH_4Cl, $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated to give a crude product, which was purified by chromatography (cyclohexane/EtOAc = 2/1) to give 2 as a colorless oil (234 mg, 78 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76–7.24 (m, 8H,), 5.69 (br, 0.4H), 5.48 (br, 0.9H), 5.23 (br, 0.5H), 4.88–4.16 (m, 6H), 3.70-3.60 (m, 3H), 3.60–3.33 (m, 2H), 3.29 (dd, *J* = 16.0, 3.9 Hz, 0.5H), 2.86 (brd, *J* = 16.1 Hz, 0.4H), 2.61 (dd, *J* = 16.3, 10.4 Hz, 0.4H), 2.44-1.61 (m, 4.7H), 1.51–1.36 (m, 18H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.98, 171.32, 170.96, 170.80, 170.54, 168.80, 156.78, 156.74, 155.37, 144.14, 144.10, 144.05, 141.38, 127.79, 127.77, 127.17, 127.12, 125.37, 125.32, 125.29, 120.06, 120.02, 83.00, 81.88, 80.27, 80.18, 67.27, 67.21, 60.66, 60.49, 56.03, 55.45, 52.80, 51.94, 51.70, 50.96, 47.28, 44.11, 43.75, 38.96, 38.27, 31.08, 29.53, 29.42, 28.40, 28.10, 28.00, 26.66; MS (ESI): m/z 652.3 $(M+H)^+$, 674.3 $(M+Na)^+$; $[\alpha]_D^{21}$: −19.77 (C 1.0, $CHCl_3$).

***tert*-butyl (5*S*,8*S*,10*aR*)-5-((*tert*-butoxycarbonyl)amino)-2,6-dioxo-decahydropyrrolo[1,2-*a*][1,5] diazocine-8-carboxylate (4).** To a solution of **2** (111 mg, 0.17 mmol) in MeCN/H_2O (2/1, 6 mL), was added LiOH (9.0 mg, 0.38 mmol). The mixture was stirred for 1 hour at room temperature and concentrated. The residue was diluted with H_2O. The aqueous solution was washed with ether, and then freeze-dried to give crude **3** (60 mg). To a solution of **3** (55 mg) in anhydrous DCM (26 mL) at 0 °C was added BOP (76 mg, 0.17 mmol), followed by DIEA (0.5 mL, 3.57 mmol). The mixture was stirred at room temperature overnight, washed with saturated NH_4Cl, $NaHCO_3$ and brine, dried over $MgSO_4$, concentrated to give a crude product, which was purified by chromatography (cyclohexane/acetone = 2/1) to provide pure **4** as a colorless oil (48 mg, 78 % for the two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.66 (br, 1H), 5.99 (br, 1H), 4.54-4.46 (m, 1H), 4.42 (t, *J* = 8.0 Hz, 1H), 4.33–4.24 (m, 1H), 3.81-3.68 (m, 1H), 3.36 (dd, *J* = 15.2, 5.2 Hz, 1H), 3.25 (dd, *J* = 14.6, 6.3 Hz, 1H), 2.51 (brd, *J* = 14.5 Hz, 1H), 2.33–2.14 (m, 2H), 2.02-1.86 (m, 2H), 1.47 (s, 9H), 1.41 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.98, 170.98, 169.55, 155.37, 82.09, 80.15, 61.26, 57.06, 55.59, 48.10, 43.91, 35.66, 29.81, 28.43, 28.10; MS (ESI): m/z 420.2 $(M+Na)^+$; HR ESI MS for $C_{19}H_{31}N_3O_6Na$ required 420.2111, found: 420.2104; $[\alpha]_D^{23}$: −16.28 (C 1.0, $CHCl_3$).

(5*S*,8*S*,10*aR*)-5-amino-2,6-dioxodecahydropyrrolo[1,2-*a*][1,5] diazocine-8-carboxylic acid TFA salt (5). To a solution of **4** (48 mg, 0.12 mmol) in DCM (2 mL) at 0 °C was added a solution of TFA (5 mL) and TIPS (50 μL) in DCM (3 mL). The mixture was stirred for 3.5 hours and concentrated. The residue was diluted with H_2O, and washed with ether. The aqueous phase was freeze-dried to give crude **5** as a white solid (37 mg, 86%), used directly in the next step without purification. ^{1}H NMR (400 MHz, D_2O): δ 4.70–4.58 (m, 2H), 4.46–4.33 (m, 2H), 3.59 (d, J = 17.2 Hz, 1H), 3.20 (dd, J = 16.6, 6.8 Hz, 1H), 2.55 (dd, J = 16.4, 9.2 Hz, 1H), 2.50–2.40 (m, 1H), 2.25–2.13 (m, 1H), 2.13–2.00 (m, 1H), 1.97–1.88 (m, 1H); ^{13}C NMR (101 MHz, D_2O): δ 176.33, 174.67, 165.50, 60.81, 54.19, 54.11, 41.03, 41.00, 32.32, 26.18; ESI MS: m/z 242.1 $(M+H)^+$; HR ESI MS for $C_{10}H_{16}N_3O_4$ required 242.1141, found: 242.1131; $[\alpha]_D^{23}$: −8.84 (C 1.0, H_2O).

(4*S*,7*S*,9*aR*)-4-(*tert*-butoxycarbonylaminomethyl)-2,5-dioxooctahydro-1*H*-pyrrolo[1,2-*d*][1,4]diazepine-7-carboxylic acid (6). To a solution of **5** (31 mg, 0.09 mmol) in MeCN/H_2O (2.5 mL/ 0.5 mL) were added Boc_2O (19 mg, 0.09 mmol) and NEt_3 (49 μL, 0.35 mmol). The mixture was stirred for 5 hours at room temperature and concentrated to dryness. The residue was recovered in DCM and washed with H_2O, brine, dried over $MgSO_4$, concentrated to give **6** as a colorless oil (22 mg, 74%). ^{1}H NMR (400 MHz, MeOD): δ 4.51-4.44 (m, 1H), 4.24 (t, J = 6.1 Hz, 1H), 4.21–4.12 (m, 1H), 3.70 (dd, J = 14.4, 5.9 Hz, 1H), 3.45 (dd, J = 14.3, 6.4 Hz, 1H), 3.05 (dd, J = 15.9, 11.6 Hz, 1H), 2.63 (dd, J = 15.8, 1.1 Hz, 1H), 2.28–2.15 (m, 2H), 2.07–1.99 (m, 1H), 1.90–1.78 (m, 1H), 1.44 (s, 9H); ^{13}C NMR (101 MHz, MeOD): δ 176.35, 175.17, 168.46, 159.20, 80.73, 62.65, 59.60, 57.03, 43.04, 40.98, 33.54, 28.66, 28.32; ESI MS: m/z 342.2 $(M+H)^+$; HR ESI MS for $C_{15}H_{24}N_3O_6$ required 342.1665, found: 342.1671.

methyl 2-((2*R*,5 *S*)-1-((*S*)-3-((9*H*-fluoren-9-yl)methoxycarbonylamino)-2-(*tert*-butoxycarbonylamino)propanoyl)-5-(benzhydrylcarbamoyl) pyrrolidin-2-yl)acetate (9). To a solution of **2** (137 mg, 0.21 mmol) in DCM (3 mL) at 0 °C was added a solution of TFA (5 mL) and TIPS (43 μL) in DCM (2 mL). The mixture was stirred for 4 hours and concentrated to dryness to give crude **7**. It was diluted with H_2O and extracted with ether. The aqueous phase was reduced in volume (about 5 mL) and diluted with MeCN (8 mL). To this solution were added Boc_2O (44 mg, 0.2 mmol) and NEt_3 (85 μL, 0.61 mmol, pH > 8). The mixture was stirred for 3 hours at room temperature and then concentrated. The residue was dissolved in EtOAc, washed with 0.5 M HCl and brine, dried over $MgSO_4$, and concentrated to give crude **8**. To a solution of **8** in dry DCM (5 mL) at 0 °C were added benzhydrylamine (47 mg, 0.26 mmol), EDCI (123 mg, 0.64 mmol), HOBt (35 mg, 0.26 mmol) and DIEA (0.5 mL). The mixture was stirred at room temperature overnight and then washed with 0.2 M HCl, saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated to give a crude product, which was purified by chromatography (cyclohexane/acetone = 4/1) to give pure **9** as a colorless oil (70 mg, 44% for 3 steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19-6.99 (m, 19H), 6.27–6.19 (m, 1H), 5.70–5.50 (brm, 1H), 5.41 (br, 1H), 4.97–3.97 (m, 6H), 3.62–3.38 (m, 4.6H), 3.20–3.10 (m, 0.4H), 3.05 (dd, J = 15.8, 4.1 Hz, 0.4H), 2.68 (dd, J = 16.0, 6.2 Hz, 0.6H), 2.58-1.75 (m, 5H), 1.42 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$): δ172.23, 172.14, 171.61, 170.54, 170.18, 169.29, 157.48, 156.96, 155.73, 155.49, 143.97, 143.89, 143.75, 141.81, 141.54, 141.38, 128.74, 128.64, 127.94, 127.86, 127.80, 127.50, 127.45, 127.36, 127.18, 127.15, 125.26, 125.23, 120.06, 80.41, 80.23, 67.41, 67.12, 60.99, 60.24, 57.61, 57.08, 56.35, 56.04, 53.27, 51.95, 51.68, 51.34, 47.21, 47.00, 44.13, 43.27, 38.48, 38.38, 38.26, 31.06, 30.21, 29.82, 28.38, 24.78; $[\alpha]_D^{23}$: −20.35 (C 1.0, $CHCl_3$).

2-((2*R*,5 *S*)-1-((*S*)-3-Amino-2-((*S*)-2-(*tert*-butoxycarbonylamino) propanamido)propanoyl)-5-(benzhydrylcarbamoyl)pyrrolidin-2-yl)acetic acid (11). To a solution of **9** (70 mg, 0.090 mmol) in DCM (3 mL) at 0 °C was added a solution of TFA (1 mL) in DCM (2 mL). The mixture was stirred for 1 hour and concentrated dryness. To this residue in dry DCM (5 mL) were added Boc-Ala-OH (23.5 mg, 0.12 mmol), EDCI (55 mg, 0.29 mmol) and HOBt (17 mg, 0.12 mmol) at 0 °C, followed by DIEA (84 μL, 0.48 mmol). The mixture was stirred at room temperature for 4 hours. The reaction solution was diluted with DCM and washed with 0.2 M HCl, saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuum. The residue obtained was chromatographed on silica gel (cyclohexane/acetone = 3/1) to afford pure compound **10** (61 mg, 74%). To a solution of **10** (61 mg, 0.073 mmol) in MeCN/H_2O (2/1, 6 mL) was added LiOH (5 mg, 0.21 mmol) and the suspension was stirred for 4 hours at room temperature, then concentrated. The residue was diluted with water, washed with ether (3 times) and then freeze-dried to give crude product **11** as a white solid (44 mg, quantitative), which was used in the next step directly without further purification. ^{1}H NMR (400 MHz, MeOD): δ 7.36–7.17 (m, 10H), 6.19 (s, 1H), 4.95-4.82 (m, 1H), 4.72 (t, J = 6.9 Hz, 1H), 4.48 (t, J = 8.6 Hz, 1H), 4.03 (q, J = 6.9 Hz, 1H), 3.00 (dd, J = 13.4, 5.8 Hz, 1H), 2.80 (dd, J = 13.4, 7.5 Hz, 1H), 2.46–2.29 (m, 3H), 2.05–1.83 (m, 3H), 1.41 (s, 9H), 1.24 (d, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, MeOD): δ178.95, 175.92, 173.98, 172.86, 157.56, 143.09, 143.07, 129.43, 129.38, 129.02, 128.73, 128.24, 128.18, 80.54, 62.51, 58.91, 57.98, 54.62, 51.42, 44.34, 44.25, 31.97, 28.70, 28.49, 18.20; ESI MS: m/z 596.3 $(M+H)^+$.

(5 *S*,8 *S*,10*aR*)-5-((*S*)-2-aminopropanamido)-*N*-benzhydryl-2,6-dioxodecahydropyrrolo[1,2-*a*][1,5] diazocine-8-carboxamide (ZJ-1). To a stirred solution of **11** (40 mg, 0.070 mmol) at 0 °C was added BOP (45 mg, 0.10 mmol), then DIEA (59 μL, 0.34 mmol). The mixture was stirred overnight at room temperature. The reaction mixture was washed with 0.2 M HCl, saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated to give a crude product, which was purified by flash chromatography (DCM/MeOH = 20/1) to give product **12**. It was dissolved in DCM (5 mL) and deprotected with TFA (1 mL) at 0 °C for 1 hour. The solution was concentrated to dryness and the residue purified by HPLC to provide pure **ZJ-1** as a white solid (8 mg, 20% for 2 steps). ^{1}H NMR (400 MHz, D_2O): δ 7.49–7.23 (m, 10H), 6.03 (s, 1H), 5.03–4.96 (m, 1H), 4.59–4.51 (m, 1H), 4.45 (t, J = 7.9 Hz, 1H), 4.07 (q, J = 7.1 Hz, 1H), 4.02 (dd, J = 16.3, 5.8 Hz, 1H), 3.38 (dd, J = 16.3, 3.7 Hz, 1H), 3.02 (dd, J = 15.9, 6.0 Hz, 1H), 2.63 (dd, J = 16.0, 7.8 Hz, 1H), 2.38–2.27 (m, 1H), 2.23–2.12 (m, 1H), 2.00–1.87 (m, 2H), 1.53 (d, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, D_2O): δ 176.76, 172.50, 170.50, 168.98, 140.85, 140.58, 128.90, 128.79, 127.81, 127.75, 127.33, 127.19, 62.38, 57.56, 54.71, 54.07, 49.00, 43.94, 40.95, 32.86, 26.88, 16.56; ESI MS: m/z 478.2 $(M+H)^+$; HR ESI MS for $C_{26}H_{32}N_5O_4$ required 478.2454, found: 478.2458.

Fluorescence Polarization (FP) Assay

The buffer solution used in this study was 20 mM Hepes (pH 7.4), 2 mM DTT, 100 mM NaCl and 0.1% BSA in Milli Q water. XIAP-BIR3 (R & D Systems Inc., USA, 200 nM final concentration), AVPFAQK (FITC) (2.0 nM final concentration) and AVPI or **SM-128** or **ZJ-1** (various concentrations from 0.75 nM to 250 μM) were added to the 384-well plates (Corning Incorporate) successively, bringing the final volume in each well to 20 μL. Three controls were also included in the measurement: (1) the buffer alone, (2) 2.0 nM AVPFAQK(FITC) in the buffer (minimal mP), and (3) 2.0 nM AVPFAQK (FITC) and 200 nM XIAP-BIR3 in the buffer (maximal mP). After the samples were incubated at r.t. for 20 min, the FP values were measured by an Envision 2014 multilabel reader (Perkin Elmer). The experiences were performed in triplicate and the IC_{50} of each test compound was calculated using Graphpad Prism 5 software.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: anticancer drugs · lactams · Smac mimetics · spontaneous rearrangement · XIAP antagonists

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