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Design and Synthesis of Potent Inhibitor of Apoptosis (IAP) Proteins Antagonists Bearing an Octahydropyrrolo[1,2-*a*]pyrazine Scaffold as a Novel Proline Mimetic

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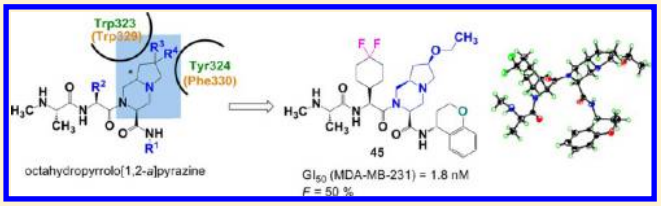
Supporting Information

ABSTRACT: To develop novel inhibitor of apoptosis (IAP) proteins antagonists, we designed a bicyclic octahydropyrrolo-[1,2-*a*]pyrazine scaffold as a novel proline bioisostere. This design was based on the X-ray co-crystal structure of four N-terminal amino acid residues (AVPI) of the second mitochondria-derived activator of caspase (Smac) with the X-chromosome-linked IAP (XIAP) protein. Lead optimization of this scaffold to improve oral absorption yielded compound **45**, which showed potent cellular IAP1 (cIAP1 IC_{50}: 1.3 nM) and XIAP (IC_{50}: 200 nM) inhibitory activity, in addition to potent tumor growth inhibitory activity (GI_{50}: 1.8 nM) in MDA-MB-231 breast cancer cells. X-ray crystallographic analysis of compound **45** bound to XIAP and to cIAP1 was achieved, revealing the various key interactions that contribute to the higher cIAPI affinity of compound **45** over XIAP. Because of its potent IAP inhibitory activities, compound **45** (T-3256336) caused tumor regression in a MDA-MB-231 tumor xenograft model (T/C: −53% at 30 mg/kg).



INTRODUCTION

Apoptosis, or programmed cell death, is a cell suicide mechanism by which multicellular organisms remove damaged or unwanted cells, thus maintaining normal life development and homeostasis.[1] Failure of the apoptosis system is known to play a causative role in triggering carcinogenesis as well as in the chemoresistance of tumor cells.[2−7]

Inhibitor of apoptosis proteins (IAPs) are crucial regulators of apoptosis, and eight mammalian analogues are known at present.[2−5] Among them, the functions and roles of X-chromosome-linked inhibitor of apoptosis protein (XIAP), cellular IAP1 (cIAP1), and cellular IAP2 (cIAP2) have been examined extensively.[4] XIAP inhibits apoptosis by binding directly to initiator caspase-9 through its baculoviral IAP repeat 3 (BIR3) domain, and to effector caspase-3/7 through its BIR2 domain, as well as to linker residues through its XIAP BIR2 domain.[8] Recently, cIAP1 and cIAP2 proteins have been shown to play a critical role in the regulation of tumor necrosis factor (TNF) receptor-mediated apoptosis.[9−12] Degradation of cIAP1/2 is thought to lead to apoptosis of cancer cells. In addition, IAPs have been found to be overexpressed in specific cancer cells and tumor tissues, and such overexpression would generate chemoresistance to cancer cells, suggesting that both XIAP and cIAP proteins could be promising targets for cancer therapy.[13−15]

The second mitochondria-derived activator of caspase (Smac) is an endogenous inhibitor of IAPs, and the crystal and NMR solution structures of the Smac protein in complex with the XIAP BIR3 domain has been reported. These results suggested that Smac has a strong affinity for the XIAP BIR3 domain, with only the four N-terminal amino acid residues (Ala1-Val2-Pro3-Ile4, AVPI) being essential for potent binding.[16−19] Since then, small molecule IAP antagonists that mimic the AVPI binding motif have become attractive targets for drug development. GDC-0152 (Genentech),[20] HGS1029 (Aegera/HGS),[21] TL32711 (TetraLogic),[22] LCL161 (Novartis),[23] and AT-406 (Ascenta)[24] have been tested in clinical trials.[13]

Most of these IAP antagonists have been developed by structure-based design using the co-crystal structure of AVPI with the XIAP-BIR3 domain, as depicted in Figure 1A, and key interactions between AVPI and the XIAP-BIR3 domain are illustrated in Figure 1B.[13−15,18,19] It is well recognized that potential modification of Ala1 is restricted since it interacts with

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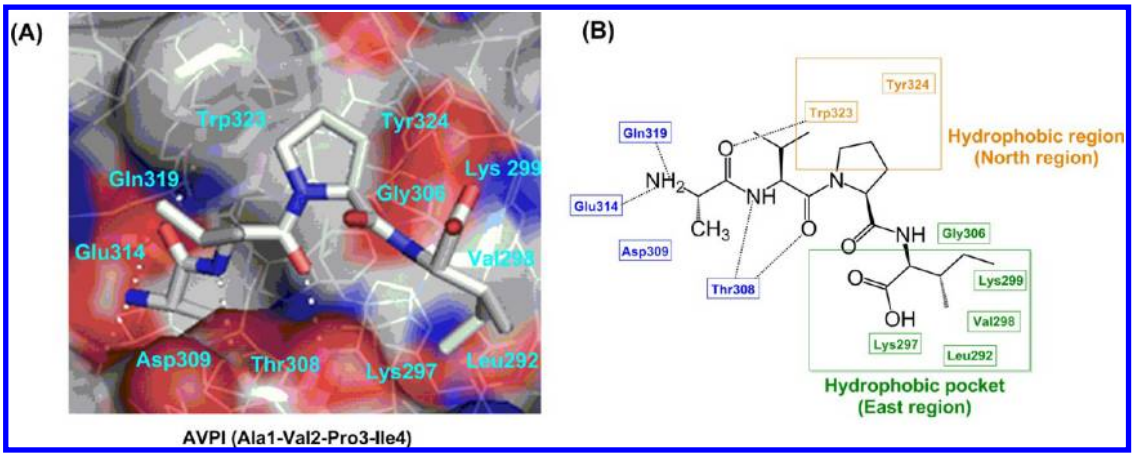
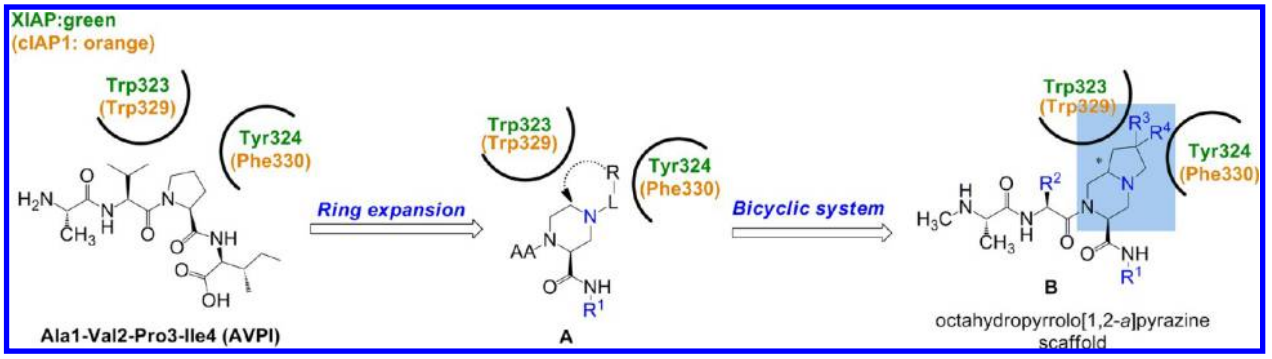


Figure 1. (A) X-ray crystal structure of AVPI in complex with the XIAP BIR3 domain. (B) Key interactions between AVPI and the XIAP BIR3 domain.

Scheme 1. Design of the Octahydropyrrolo[1,2-*a*]pyrazine Scaffold



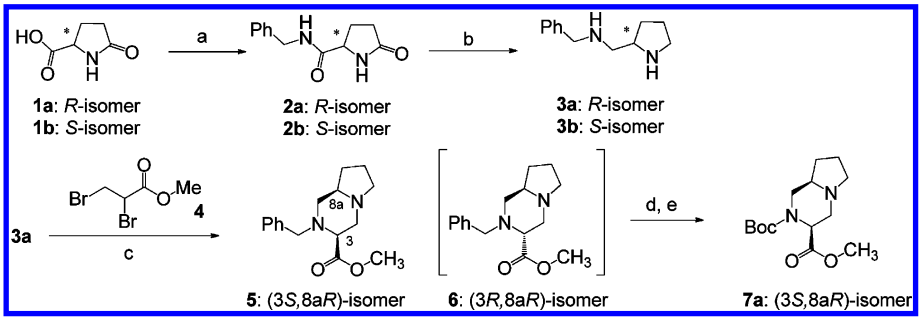
Glu314, Gln319, and Trp323 via strong hydrogen bonds in a tight hydrophilic pocket. The amino and carbonyl groups of Val2 also form strong hydrogen bonds with Thr308, while the side chain is exposed to the solvent region, suggesting that the physicochemical properties of small molecule IAP antagonists could be controlled by replacement of the side chain. The five-membered ring of Pro3 forms van der Waals contacts with the large hydrophobic region (North region) formed by Trp323 and Tyr324 and plays a crucial role in determining the orientation of Ala1 and Ile4. The hydrophobic side chain of Ile4 inserts into another hydrophobic pocket (East region) that consists of the side chains of Leu292 and Val298 and the hydrophobic portion of the side chains of Lys297 and Lys299. On the other hand, we predicted that the carboxylic group of Ile4 that is exposed to the solvent region could be removed without detriment to the potency because of the lack of any specific interaction. Since the hydrophobic pocket in the East region can accommodate a wide variety of hydrophobic substituents, utilizing this region has been well recognized as a promising strategy to enhance IAPs binding affinity.[13−15] However, as far as we know, there have been few reports that describe a design concept to target the hydrophobic groove in the North region. A pioneering study[25] by Oost et al. demonstrated that targeting the hydrophobic groove by introducing a (*S*)-phenoxy group at the 4-position in Pro3 improved the XIAP binding affinity (about three times higher) over that of the corresponding unsubstituted proline derivative. From these results, we envisioned that a conformationally constrained bicyclic scaffold designed to fit in the hydrophobic groove could efficiently interact with the hydrophobic North region.

In this paper, we describe the design, synthesis, structure−activity relationships (SAR), and in vivo efficacy of bicyclic octahydropyrrolo[1,2-*a*]pyrazine derivatives. Crystallographic studies of compound **45** in complex with both XIAP and cIAP1 are also discussed, as well as the origin of the selectivity compound **45** shows for cIAP over XIAP.

Design. The five-membered proline ring in Pro3 was converted to the six-membered nonpeptidic piperazine ring (Scheme 1, **A**).[26] As described above, our key design concept was to construct a conformationally constrained bicyclic scaffold that efficiently interacts with the hydrophobic North region. Thus, we introduced a fused five-membered ring onto the piperazine in the North region to afford a novel bicyclic octahydropyrrolo[1,2-*a*]pyrazine scaffold (Scheme 1, **B**). Because this novel scaffold has a chiral center in the bridge position, we also expected that the conformation of the scaffold could be adjusted precisely by the bridgehead chiral center to fit in the hydrophobic groove of the IAP binding pockets. In addition, the scaffold can be synthesized in a stereoselective manner by means of chiral pool synthesis from various D- and L-proline derivatives. Therefore, further modification would be possible to enhance IAP binding activities as well as improve physicochemical properties.

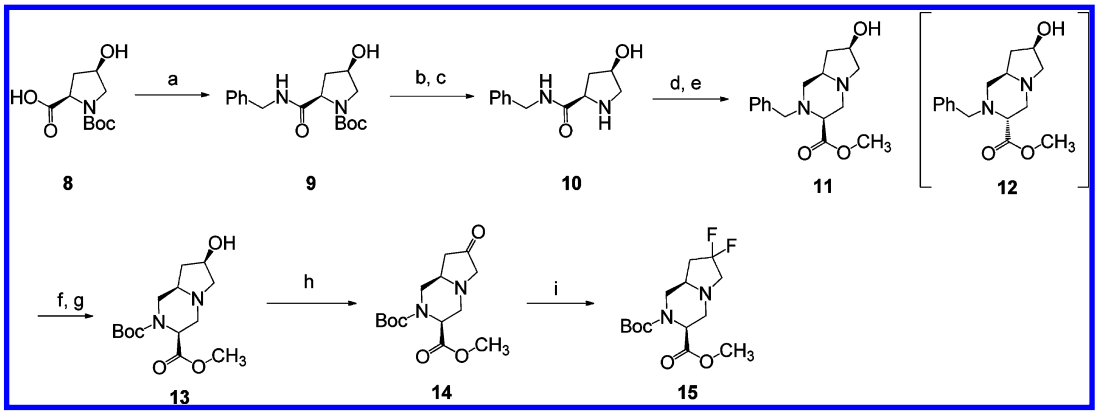
As for the Ala1 residue, *N*-methyl alanine was selected in place of alanine since Wang et al. reported that *N*-methylation improves cell permeability.[13,25,27,28] In addition, we selected a cyclohexyl side chain instead of an isopropyl side chain of Val2

Scheme 2. Synthesis of the (3*S*, 8a*R*)-Octahydropyrrolo[1,2-*a*]pyrazine Scaffold[a]



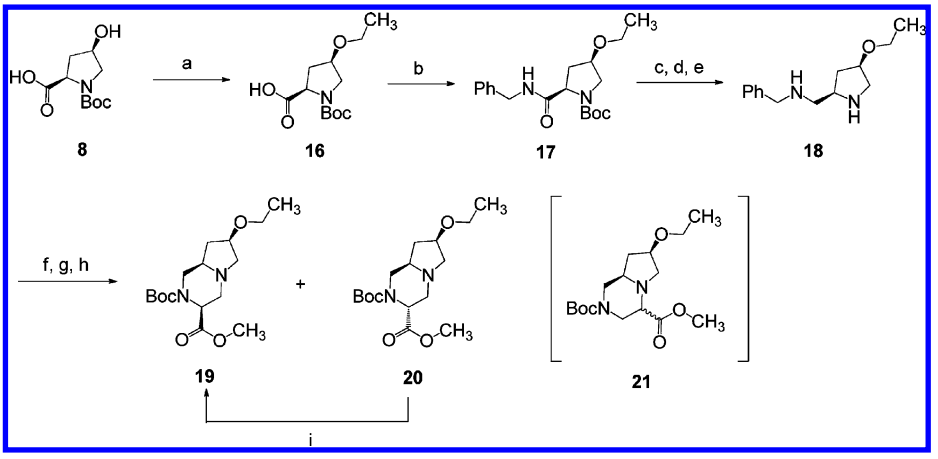
[a]Reagents and Conditions: (a) benzylamine, EDC-HCl, HOBt, CH_3CN, 0 °C to room temperature, 48% (**2a**), 44% (**2b**); (b) $LiAlH_4$, THF, reflux, 93% (**3a**), 90% (**3b**); (c) **4**, Et_3N, toluene, 90 °C, 46% (**5**), 52% (**6**); (d) 10% Pd/C, H_2, HCl-methanol, room temperature; (e) $(Boc)_2O$, sat. $NaHCO_3$, THF, room temperature, 92% (two steps).

Scheme 3. Synthesis of (7*R*)-Hydroxy and 7,7-Difluoro Octahydropyrrolo[1,2-*a*]pyrazine Derivatives[a]



[a]Reagents and conditions: (a) benzylamine, EDC-HCl, HOBt, 4-dimethylaminopyridine, DMF, room temperature, 90%; (b) 4 M HCl, EtOAc, room temperature; (c) Amberlyst A21, methanol, quant (two steps); (d) $LiAlH_4$, THF, reflux; (e) **4**, Et_3N, toluene, 80 °C, 30% (**11**, two steps), 44% (**12**, two steps); (f) 10% Pd/C, H_2, HCl-methanol, room temperature; (g) $(Boc)_2O$, sat. $NaHCO_3$, THF, room temperature, 86% (two steps); (h) SO_3-pyridine, NEt_3, EtOAc/DMSO, 0 °C—room temperature, 91%; (i) Deoxo-Fluor, toluene, 0 °C—room temp, 62%.

Scheme 4. Synthesis of (7*R*)-ethoxy Octahydropyrrolo[1,2-*a*]pyrazine Derivatives[a]



[a]Reagents and conditions: (a) iodoethane, NaH, THF, 0 °C to room temperature, 94%; (b) benzylamine, EDC-HCl, HOBt, CH_3CN, room temperature, 96%; (c) 4 M HCl, EtOAc, room temperature; (d) Amberlyst A21, methanol, quant (two steps); (e) $LiAlH_4$, THF, reflux, 88%; (f) **4**, Et_3N, toluene, 90 °C; (g) 10% Pd/C, H_2, HCl-methanol, room temperature; (h) $(Boc)_2O$, sat. $NaHCO_3$, THF, room temperature, 29% (**19**, three steps), 45% (**20**, three steps); (i) NaOMe, methanol, 60 °C, 90%.

for the optimization study, because conformational rigidity of the Val2 side chain may facilitate high binding affinity.[25]

Chemistry. The synthesis of the (3*S*, 8a*R*)-octahydropyrrolo[1,2-*a*]pyrazine scaffold is outlined in Scheme 2. Condensation of commercially available (*R*)-pyroglutamic acid **1a** with benzylamine in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC-HCl) and 1-hydroxybenzotriazole monohydrate (HOBt) in acetoni-

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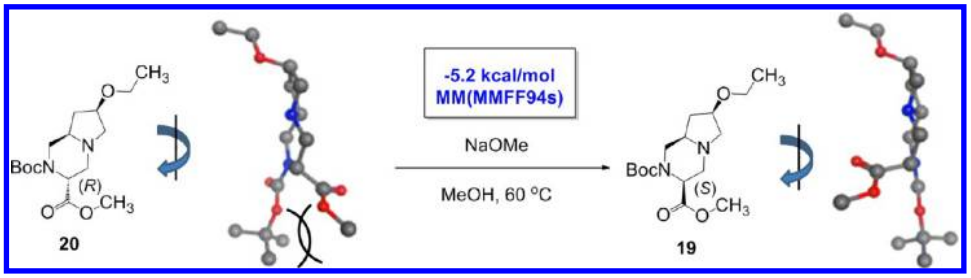
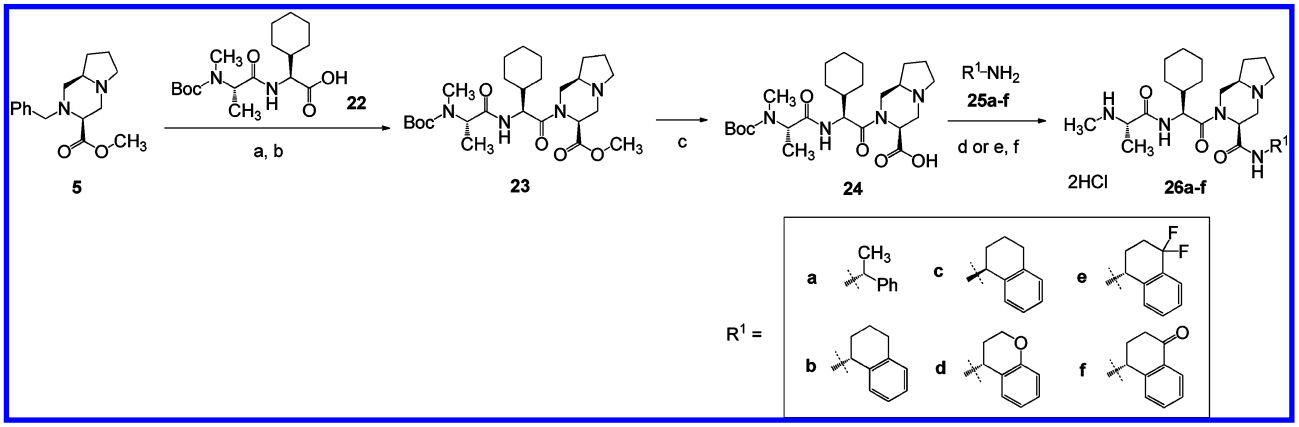
Scheme 5. Calculations for Thermodynamic Stability of Compounds 19 and 20



Scheme 6. Synthesis of Amide Derivatives 26a–f[a]



[a]Reagents and Conditions: (a) 10% Pd/C, H_2, 4 M HCl, EtOH/EtOAc, room temperature; (b) **22**, DMT-MM, *N*-methylmorpholine, THF, 0 °C to room temperature, 43% (two steps); (c) LiOH-H_2O, THF/H_2O, room temperature or 50 °C; (d) amine, DMT-MM, *N*-methylmorpholine, THF, 0 °C to room temperature; (e) **25a–f**, EDC-HCl, HOBt, DMF, 0 °C to room temperature; (f) 4 M HCl, EtOAc or EtOAc/methanol, room temperature, 49% (**26a**, two steps), 44% (**26b**, two steps), 23% (**26c**, two steps), 48% (**26d**, two steps), 29% (**26e**, two steps), 44% (**26f**, two steps).

trile provided amide **2a** in 48% yield. The two amide groups of **2a** were reduced by lithium aluminum hydride in THF to afford diamine **3a** in 93% yield. A similar procedure was applied to the synthesis of *S*-isomer **3b**. Construction of the octahydropyrrolo[1,2-*a*]pyrazine scaffold was carried out according to a reported procedure[29] with a slight modification. Diamine **3a** was reacted with methyl 2,3-dibromopropionate (**4**) in the presence of triethylamine in toluene at 90 °C to afford the stereoisomers **5** (46%) and **6** (52%). Upon silica gel chromatographic separation of the two isomers, the *N*-benzyl group of the desired isomer **5** was converted to the corresponding *N*-*tert*-butoxycarbonyl (Boc) group via standard procedures, giving **7a** in 92% yield (two steps).

With the aim of examining substituent effects at the 7-position of the scaffold, (7*R*)-hydroxy and 7,7- difluoro octahydropyrrolo[1,2-*a*]pyrazine derivatives were synthesized by the route shown in Scheme 3. Condensation of the commercially available (4*R*)-hydroxyproline derivative **8** with benzylamine was conducted according to the same synthetic procedure as that for the preparation of **2a** to give **9** in 90% yield. The Boc group was removed by 4 M HCl in EtOAc and subsequently treated with Amberlyst A21 to furnish salt free **10** in quantitative yield (two steps). Reduction of the amide group of **10** followed by cyclization of the resulting diamine with methyl 2,3-dibromopropionate (**4**) provided (7*R*)-hydroxy derivatives **11** (30%) and **12** (44%), respectively. These isomers were separable by silica gel chromatography, and the *N*-Bn group of **11** was converted to the corresponding *N*-Boc group **13** according to the same synthetic procedure as that for **7a** (86%, 2 steps). The hydroxy group of compound **13** was oxidized to give ketone **14** (91%), which was subjected to difluorination using [bis(2-methoxyethyl)amino]sulfur trifluoride (Deoxo-Fluor) to afford the 7,7-difluoro derivative **15** in 62% yield.

The synthesis of (7*R*)-ethoxy derivative **19** is outlined in Scheme 4. Alkylation of (4*R*)-hydroxyproline derivative **8** with iodoethane and sodium hydride was carried out according to a literature method[30] to provide ethylated compound **16** in 94% yield. Diamine **18** was obtained from **16** through the same condensation and reduction procedures as shown in Scheme 2. Cyclization of **18** with methyl 2,3-dibromopropionate (**4**) proceeded smoothly to provide the corresponding *N*-Bn product mixture, which was subjected to the next two reaction steps without isolation to give the *N*-Boc derivatives **19** (29%, three steps) and **20** (45%, three steps) after purification by silica gel chromatography. *N*-Boc derivatives **19** and **20** were more easily purified by silica gel chromatography than the corresponding *N*-Bn derivatives. We also isolated regioisomer mixture **21** in 3% yield by the scaled up synthesis of **19** (Supporting Information). We believe that the regioselectivity of this cyclization reaction might reflect the higher nucleophilicity of the pyrrolidine amine of **18** than of the benzylamine. Michael addition of nucleophilic pyrrolidine amine to methyl 2-bromoacrylate, an intermediate generated from methyl 2,3-dibromopropionate (**4**) and triethylamine,[31,32] occurred in a selective manner and was followed by intermolecular cyclization to provide bicyclic derivatives **19** and **20** as the major products (Supporting Information).

We were also pleased to find that undesired isomer **20** could be converted into the desired isomer **19**. When isomer **20** was

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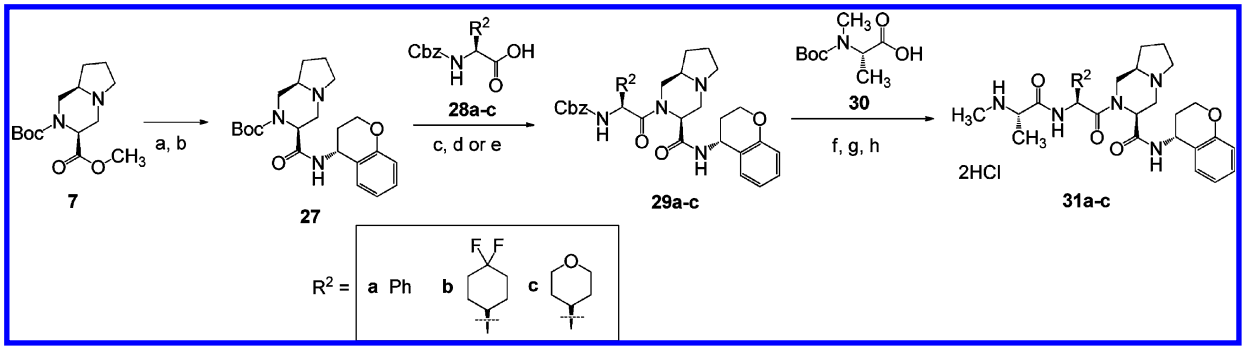
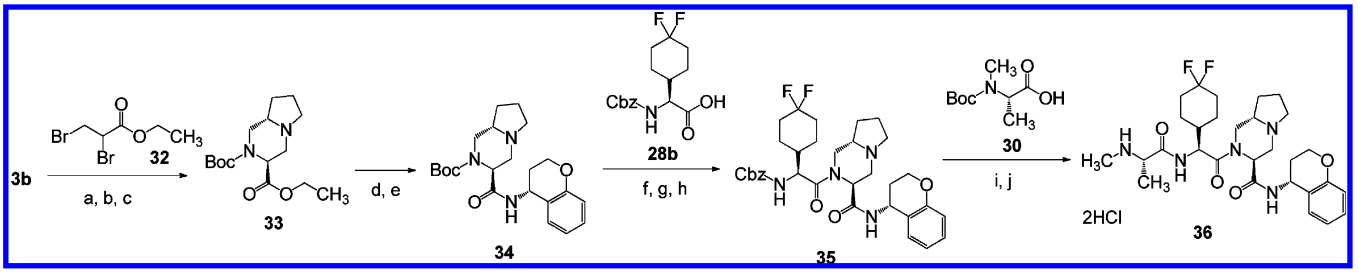
Scheme 7. Synthesis of Side Chain Derivatives 31a−c[a]



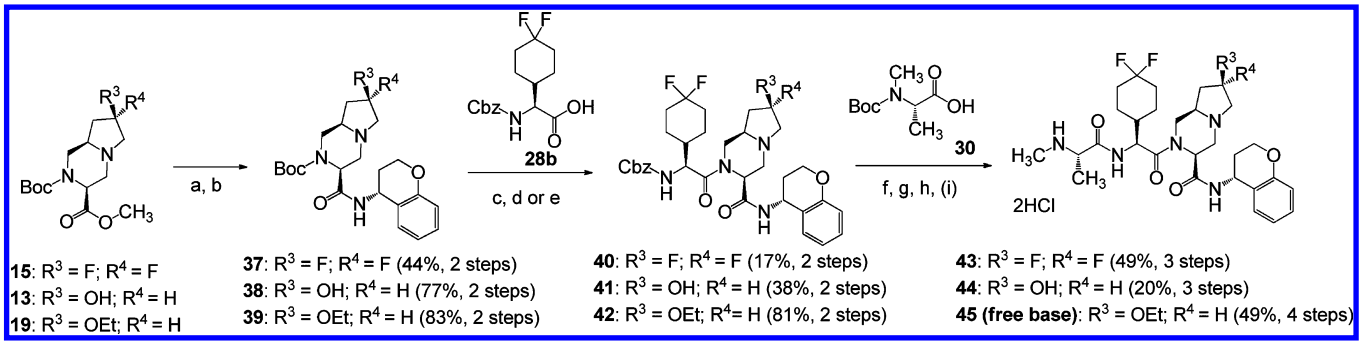
[a]Reagents and conditions: (a) LiOH-H_2O, THF/H_2O, 50 °C; (b) (*R*)-chroman-4-amine hydrochloride, EDC-HCl, HOBt, *i*-Pr_2NEt, DMF, room temperature, 71% (two steps); (c) 4 M HCl, EtOAc, room temperature; (d) **28a** or **28b**, HATU, *i*-Pr_2NEt, DMF, room temperature, 47% (**29a**, two steps), 82% (**29b**, two steps); (e) **28c**, EDC-HCl, HOBt, *i*-Pr_2NEt, DMF, room temperature, 31% (**29c**, two steps); (f) 10% Pd/C, H_2, methanol, room temperature; (g) **30**, HATU, *i*-Pr_2NEt, DMF, room temperature; (h) 4 M HCl, EtOAc, room temperature, 65% (**31a**, three steps), 58% (**31b**, three steps), 72% (**31c**, three steps).

Scheme 8. Synthesis of (3*S*, 8a*S*)-Octahydropyrrolo[1,2-*a*]pyrazine Derivative[a]



[a]Reagents and Conditions: (a) **32**, Et_3N, toluene, 90 °C; (b) 10% Pd/C, H_2, HCl-methanol, room temperature; (c) $(Boc)_2O$, sat. $NaHCO_3$, THF, room temperature, 33% (three steps); (d) LiOH-H_2O, THF/H_2O, 50 °C; (e) (*R*)-chroman-4-amine hydrochloride, EDC-HCl, HOBt, *i*-Pr_2NEt, DMF, room temperature, 42% (two steps); (f) 4 M HCl, EtOAc, room temperature; (g) **28b**, HATU, *i*-Pr_2NEt, DMF, room temperature; (h) 20% $Pd(OH)_2/C$, H_2, methanol, room temperature; (i) **30**, HATU, *i*-Pr_2NEt, DMF, room temperature; (j) 4 M HCl, EtOAc, room temperature, 17% (five steps).

Scheme 9. Synthesis of 7-Substituted (3*S*, 8a*R*)-Octahydropyrrolo[1,2-*a*]pyrazine Derivatives[a]



[a]Reagents and Conditions: (a) LiOH-H_2O, THF/H_2O, 50 °C; (b) (*R*)-chroman-4-amine hydrochloride, EDC-HCl, HOBt, *i*-Pr_2NEt, DMF, room temperature; (c) 4 M HCl, EtOAc, room temperature; (d) **28b**, HATU, *i*-Pr_2NEt, DMF, room temperature; (e) **28b**, EDC, HOBt, *i*-Pr_2NEt, DMF, room temperature; (f) 20% $Pd(OH)_2/C$, H_2, methanol, room temperature; (g) **30**, HATU, *i*-Pr_2NEt, DMF, room temperature; (h) 4 M HCl, EtOAc, room temperature; (i) sat. $NaHCO_3$, room temperature.

treated with NaOMe in methanol at 60 °C, epimerization at the 3-position of **20** proceeded smoothly to give the desired isomer **19** in 90% yield. This result was supported by molecular mechanics (MM) calculations, suggesting that isomer **20** is 5.2 kcal/mol less stable than isomer **19** due to the steric repulsion between the Boc group and methyl ester of **20** (Scheme 5).

Next, we synthesized various amide derivatives **26a−f** as shown in Scheme 6. After removal of the Bn protecting group from compound **5**, the resulting secondary amine was condensed with known carboxylic acid **22**[33] using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) to give tripeptide **23** in 43% yield. It should be noted that use of DMT-MM suppressed epimerization at the carboxylic acid α-position.[34] Hydrolysis of methyl ester **23** followed by condensation with a series of amines **25a−f** provided the desired amide derivatives **26a−f** as HCl salts in 23−49% yield over two steps.

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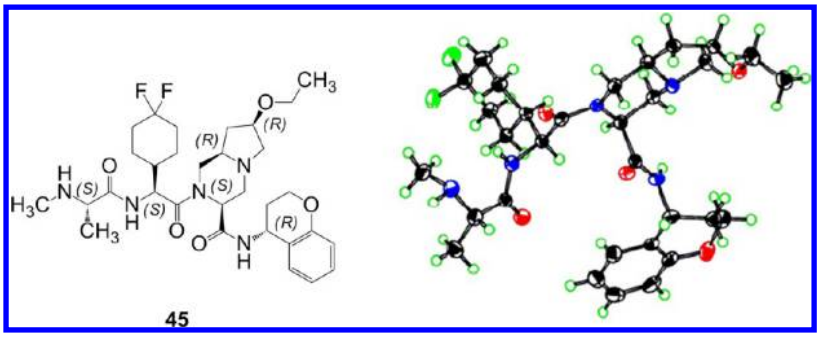
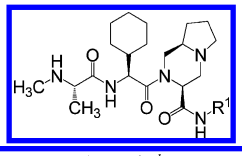
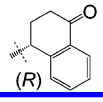


Figure 2. X-ray crystal structure of compound **45**.

Table 1. Biological Data for Octahydropyrrolo[1,2-*a*]pyrazine Derivatives Targeting the East Region



Cmpd	R^1	XIAP BIR3[a,b] IC_{50} (nM)	cIAP1 BIR3[a,b] IC_{50} (nM)	MDA-MB-231 [a,b] GI_{50} (nM)	MS (h/m)[a] (μL·min^{-1}·mg^{-1})	F^c(%)
26a		1400 (1100–1800)	14 (11–17)	20 (17–24)	82/115	63
26b		300 (260–340)	2.7 (2.5–2.9)	6.2 (5.3–7.2)	218/232	6
26c		2200 (1700–2800)	1300 (1000–1600)	NT	236/239	NT
26d		240 (190–300)	2.1 (1.8–2.4)	5.7 (4.9–6.7)	128/116	41
26e		520 (450–610)	4.0 (3.6–4.4)	18 (15–22)	130/208	26
26f		1300 (1000–1700)	7.6 (6.2–9.2)	21 (18–25)	154/161	30

[a]These measurements are described in the Supporting Information. [b]IC_{50} values and 95% confidence intervals (CI) were calculated by nonlinear regression analysis of percent inhibition. [c]Animals used in the study were female BALB/cAJcl mice (seven weeks old; CLEA, Tokyo, Japan). Test compounds were administered orally at a dose of 10 mg/kg and intravenously at a dose of 1 mg/mL/kg. Concentrations of compounds in the plasma were determined by LC/MS/MS.

For the purpose of modifying the cyclohexyl side chain exposed to the solvent contact region, phenyl (**31a**), 4,4-difluorocyclohexyl (**31b**), and 4-tetrahydropyranyl (**31c**) derivatives were synthesized (Scheme 7). Condensation of (*R*)-chroman-4-amine hydrochloride with the carboxylic acid obtained by hydrolysis of compound **7** afforded the corresponding amide **27** in 71% yield (two steps). After removal of the Boc group with 4 M HCl in EtOAc, the resulting amine was coupled with carboxylic acids **28a**−**c**[35] by HOBt/EDC or *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) to provide the amides **29a**−**c** in 31−82% yield (two steps). The benzyloxycarbonyl (Cbz) group of **29a**−**c** was removed with palladium on carbon under hydrogen atmosphere followed by condensation with commercially available *N*-Boc-*N*-methylalanine **30** and final removal of the Boc group to afford the desired compounds **31a**−**c** in 58−72% yield (three steps).

The synthesis of compound **36**, possessing a (3*S*, 8a*S*)-octahydropyrrolo[1,2-*a*]pyrazine scaffold is shown in Scheme 8. Starting from the diamine **3b** prepared in Scheme 1, the scaffold was constructed by a procedure similar to that shown in Schemes 2−4 (33%, three steps). The obtained product **33** was subjected to sequential deprotection and condensation

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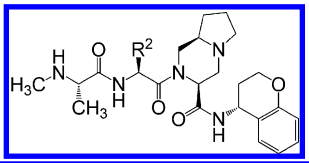
Table 2. Biological Data for Side Chain Octahydropyrrolo[1,2-*a*]pyrazine Derivatives



Cmpd	R^2	XIAP BIR3[a,b] IC_{50} (nM)	cIAP1 BIR3[a,b] IC_{50} (nM)	MDA-MB-231[a,b] GI_{50} (nM)	MS (h/m)[a] ($\mu L \cdot min^{-1} \cdot mg^{-1}$)	$LogD_{7.4}$	F[c] (%)
26d		240 (190–300)	2.1 (1.8–2.4)	5.7 (4.9–6.7)	128/116	2.91	41
31a		190 (150–270)	2.6 (2.4–2.9)	11 (7.9–15)	90/129	2.17	33
31b		480 (340–660)	3.2 (2.8–3.6)	13 (9.1–18)	82/66	2.43	53
31c		470 (390–550)	3.4 (3.2–3.7)	20 (17–23)	15/21	1.75	12

[a]These measurements are described in the Supporting Information. [b]IC_{50} values and 95% confidence intervals (CI) were calculated by nonlinear regression analysis of percent inhibition. [c]Animals used in the study were female BALB/cAJcl mice (seven weeks old; CLEA, Tokyo, Japan). Test compounds were administered orally at a dose of 10 mg/kg and intravenously at a dose of 1 mg/mL/kg. Concentrations of compounds in the plasma were determined by LC/MS/MS.

reactions to afford the desired product **36** in 7% yield in seven steps.

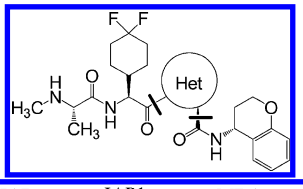
Scheme 9 describes the synthesis of 7-substituted (3*S*, 8a*R*)-octahydropyrrolo[1,2-*a*]pyrazine derivatives **43**–**45** starting from intermediates **15**, **13** and **19**. These products were prepared in a similar manner to the synthesis of **36** shown in Scheme 8. To our delight, (7*R*)-ethoxy derivative **45** could be obtained as salt-free crystals, and its absolute configuration was determined by X-ray single crystal structural analysis (Figure 2).[36]

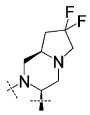
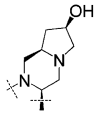
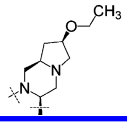
Biological Results and Discussion. Our designed (3*S*, 8a*R*)-octahydropyrrolo[1,2-*a*]pyrazine compounds **26a**–**f**, which possess various hydrophobic substituents targeting the East region of IAPs, were evaluated for biological activity. Their XIAP/cIAP1 binding and tumor growth inhibitory (GI) activities against MDA-MB-231 triple-negative breast cancer cells are shown in Table 1. Human and mouse metabolic stabilities were also examined together with in vivo oral bioavailability in mice. On the basis that the L-configuration of Ile4 regulates the orientation of its hydrophobic side chain, we used chiral control to design (*R*)-*α*-methylbenzyl amide derivative **26a** with an aromatic ring directed to interact effectively with the binding pocket in the East region.[25] As expected, **26a** showed good cIAP1 activity (IC_{50}: 14 nM) together with a promising GI activity (GI_{50}: 20 nM), whereas the binding affinity for XIAP was moderate (IC_{50}: 1400 nM). Furthermore, we applied a fused (*R*)-tetrahydronaphthyl amide to our octahydropyrrolo[1,2-*a*]pyrazine scaffold (**26b**)[13−15] to boost the binding affinities for IAPs. As a result, compound **26b** demonstrated enhanced binding inhibitory activity for cIAP1 (IC_{50}: 2.7 nM) as well as for XIAP (IC_{50}: 300 nM), resulting in potent cellular GI activity (GI_{50}: 6.2 nM). On the other hand, the corresponding (*S*)-tetrahydronaphthyl isomer **26c** showed diminished XIAP/cIAP1 binding inhibitory potency, indicating that the (*R*)-configuration of **26b** is important for suitable orientation of the aromatic ring.

Although compound **26b** showed potent GI activity, its human and mouse metabolic stabilities were decreased compared with those of **26a**, resulting in a poor oral bioavailability (F = 6%) in mice. Judging the benzylic position of the tetrahydronaphthalene moiety to be metabolically labile, we modified this position. (*R*)-Chromanyl amide **26d**, in which a benzylic carbon is replaced by oxygen, exhibited improved metabolic stability and oral bioavailability (F = 41%). In addition, the compound's XIAP/cIAP1 (IC_{50}: 240/2.1 nM) binding inhibitory and cellular GI activities (GI_{50}: 5.7 nM) were maintained. However, when the benzylic position was blocked by difluoro (**26e**) and carbonyl (**26f**) groups, the IAP antagonistic activities decreased. From these results, the (*R*)-chromanyl group of **26d** was selected as a representative hydrophobic replacement for Ile4.

Next, the cyclohexyl side chain of **26d** was replaced with several more hydrophilic cyclic substituents to improve metabolic stability (Table 2). Phenyl derivative **31a** did not show improved metabolic stability and oral bioavailability despite having a lower $LogD_{7.4}$ value of 2.17, indicating that an aromatic group might not be suitable for the side chain. Therefore, we examined polar cyclohexyl analogues such as 4,4-difluorocyclohexyl (**31b**) and 4-tetrahydropyranyl (**31c**). Interestingly, **31b**, having a slightly lower $LogD_{7.4}$ value[37,38] than **26d**, demonstrated improved metabolic stability and oral bioavailability (F = 53%). However, further reduction of lipophilicity (**31c**) turned out to result in poor oral bioavailability, although its metabolic stabilities were much better than that of **26d**. Ultimately, the 4,4-difluorocyclohexyl group in **31b** was selected as the optimal side chain for the Val2 portion. Although the GI_{50} value of **31b** was 5-times lower than

Table 3. Biological Data for 7-Substituted Octahydropyrrolo[1,2-*a*]pyrazine Derivatives



Cmpd	Het	XIAP BIR3[a,b] IC_{50} (nM)	cIAP1 BIR3[a,b] IC_{50} (nM)	MDA-MB-231[a,b] GI_{50} (nM)	MS (h/m)[a] (μL·min^{-1}·mg^{-1})	$LogD_{7.4}$	F[c] (%)
31b		480 (340–660)	3.2 (2.8–3.6)	13 (9.1–18)	82/66	2.43	53
36		140 (110–170)	2.5 (2.3–2.7)	15 (9.8–23)	38/2	1.94	13
43		320 (220–450)	1.7 (1.6–1.8)	8.0 (5.6–11)	170/147	2.56	21
44		410 (360–460)	4.9 (4.3–5.4)	11 (8.8–15)	4/-1	1.60	NT
45		200 (140–280)	1.3 (1.2–1.5)	1.8 (1.6–2.0)	52/40	2.60	50[d]

[a]These measurements are described in the Supporting Information. [b]IC_{50} values and 95% confidence intervals (CI) were calculated by nonlinear regression analysis of percent inhibition. [c]Animals used in the study were female BALB/cAJcl mice (seven weeks old; CLEA, Tokyo, Japan). Test compounds were administered orally at a dose of 10 mg/kg and intravenously at a dose of 1 mg/mL/kg. Concentrations of compounds in the plasma were determined by LC/MS/MS. [d]The compound was dosed discretely.

that of **26d**, we supposed that the introduction of hydrophobic substituents into the scaffold could enhance GI activity.

To further improve the metabolic stability as well as GI activity of **31b**, modification of the (3*S*, 8a*R*)-octahydropyrrolo-[1,2-*a*]pyrazine scaffold was investigated (Table 3). To confirm the role of the bridgehead chiral center for both XIAP/cIAP1 activities and physicochemical properties, (3*S*, 8a*S*)-octahydropyrrolo[1,2-*a*]pyrazine derivative **36** was evaluated. Compound **36** exhibited a unique profile with 3-times higher XIAP binding inhibitory activity (IC_{50}: 140 nM) than **31b**, together with potent cellular GI activity and improved metabolic stability. However, compound **36** showed insufficient oral bioavailability (F = 13%), which we attribute to a low $LogD_{7.4}$ value as observed for **31c**. We, therefore, concluded that the (3*S*, 8a*R*) stereochemistry of the scaffold should be suitable for further modification. Next, X-ray crystallographic analysis of **26b** complexed with cIAP1 suggested that the 7-position would be suitable for modification without any loss of XIAP/cIAP1 binding inhibitory activity since this position is directed toward the solvent contact region (Figure 3).[39] Thus, 7-substituted derivatives **43**–**45** were evaluated. Contrary to our expectations, the metabolic stability of 7,7-difluoro derivative **43** was reduced. (7*R*)-Hydroxy derivative **44** exhibited improved metabolic stability, while its GI activity was not enhanced compared to that of **31b**, presumably because of its low permeability (parallel artificial membrane permeability [PAMPA] $pH_{7.4}$: 3.0 nm/s). Conversion of **44** to

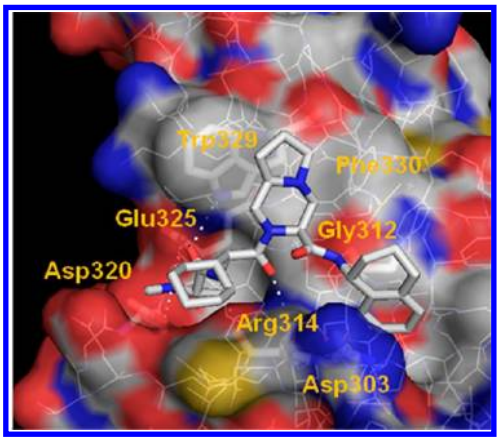


Figure 3. X-ray crystal structure of compound **26b** in complex with the cIAP1 BIR3 domain.

(7*R*)-ethoxy derivative **45** improved membrane permeability (PAMPA $pH_{7.4}$: 44 nm/s) with an appropriate lipophilicity ($LogD_{7.4}$ 2.60), resulting in potent GI activity (GI_{50}: 1.8 nM). Compound **45** also exhibited strong XIAP/cIAP1 binding inhibitory activities (IC_{50}: 200/1.3 nM). Furthermore, compound **45** demonstrated improved metabolic stability to afford good oral bioavailability (F = 50% in a discrete dose pharmacokinetic experiment). Therefore, compound **45** was selected as a candidate for further in vivo pharmacodynamic (PD) and efficacy studies.

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Compound **45** was evaluated in mice using a MDA-MB-231-Luc xenograft model. Administration of compound **45** stimulated caspase-3/7 activity in vivo in a dose-dependent manner (30 and 100 mg/kg), as expected for a pharmacodynamic (PD) effect of IAP inhibition (Figure 4). Reflecting this

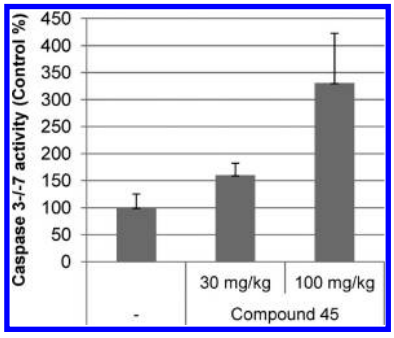

Figure 4. Administration of compound **45** induced caspase activation in MDA-MB-231-Luc xenograft in vivo. Dose levels 30 and 100 mg/kg; $P \leq 0.005$ vs control.

in vivo PD effect, compound **45** induced tumor regression in a dose-dependent manner with a percent tumor growth inhibition (T/C) of −53% and −62% at 30 mg/kg and 100 mg/kg, respectively (Figure 5).[40]

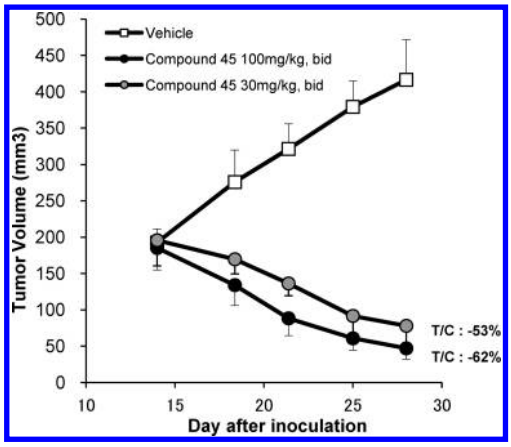

Figure 5. Antitumor efficacy of compound **45** in an MDA-MB-231-Luc xenograft model in mice. Dose levels 30 and 100 mg/kg; $P \leq 0.005$ vs control at day 14.

Structural Analysis and Discussion for Compound 45. To understand the interactions of (3*S*, 8a*R*)-octahydropyrrolo-[1,2-*a*]pyrazine derivative **45** with XIAP/cIAP1 proteins in detail, the crystal structures of **45** in complex with both XIAP[41] and cIAP1[42] have been determined at resolutions of 2.8 Å and 1.8 Å, respectively. The binding modes of key interactions are illustrated in Figures 6 and 7. Key interactions observed in the structure of AVPI in complex with XIAP were conserved in compound **45**, especially in the *N*-methyl alanine moiety (Figure 6A). Similar hydrogen bond interactions were also observed in the complex with cIAP1 (Figure 6B). As described in our design strategy, the octahydropyrrolo[1,2-*a*]pyrazine scaffold was expected to be directed toward the North region to gain hydrophobic interactions. In fact, the scaffold forms van der Waals contacts with Trp323 in XIAP and Trp329 in cIAP1, and its (7*R*)-ethoxy group is directed toward the solvent contact region to avoid steric repulsion with the XIAP/cIAP1

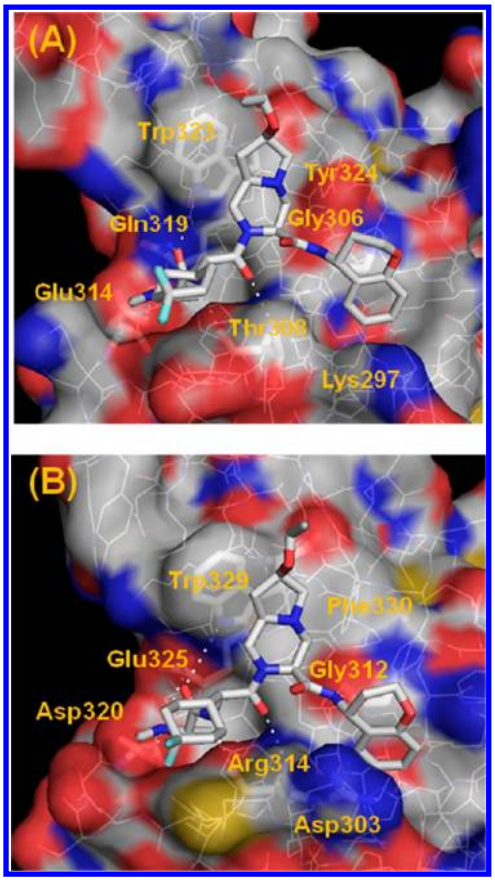

Figure 6. (A) X-ray crystal structure of compound **45** in complex with the XIAP BIR3 domain (front view). (B) X-ray crystal structure of compound **45** in complex with the cIAP1 BIR3 domain (front view).

proteins. However, in XIAP, it appears to cause a steric repulsion between the scaffold and a hydroxy group of Tyr324, which is conformationally restricted due to a hydrogen bond with Gly306 (2.8 Å). In contrast, no steric repulsion was observed in cIAP1, because the corresponding residue in this position is converted to Phe330, which lacks a hydroxy group to cause conformational restriction (Figure 7).[43,44]

Another key difference binding mode was observed in the East region where the (*R*)-chromanyl group inserts into a hydrophobic pocket. In the complex of compound **45** with cIAP1, a cation-π (or π−π) interaction between the guanidine moiety of Arg314 and the (*R*)-chromanyl group may be playing a role in increasing the binding activity (Figure 7B). On the other hand, the corresponding residue in XIAP is Thr308, resulting in only a van der Waals interaction as a major contribution to the binding affinity for XIAP (Figure 7A). On the basis of these observed differences in binding modes, we conclude that compound **45** has a profile of cIAP1-dominant inhibitory activity over XIAP (154-fold).[43−45]

■ CONCLUSION

With the aim of discovering potent IAP antagonists, we designed novel bicyclic octahydropyrrolo[1,2-*a*]pyrazine derivatives based on the co-crystal structures of AVPI in complex with the XIAP BIR3 domain. As expected, our designed lead compound **26b** showed strong binding inhibition against both XIAP (IC_{50}: 300 nM) and cIAP (IC_{50}: 2.7 nM), and effectively induced cell death in triple-negative MDA-MB-231 breast cancer cells (GI_{50}: 6.2 nM). Conversion of the (*R*)-

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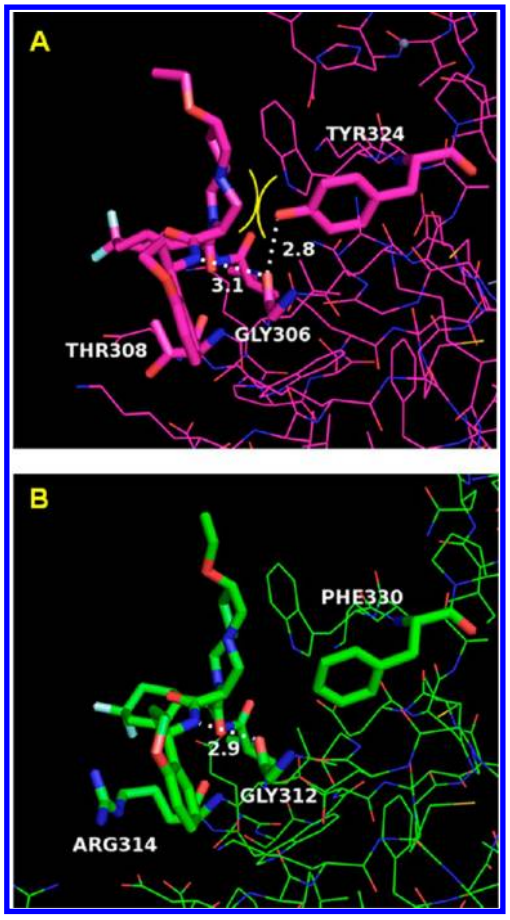

Figure 7. (A) X-ray crystal structure of compound **45** in complex with the XIAP BIR3 domain (side view). (B) X-ray crystal structure of compound **45** in complex with cIAP1 BIR3 domain (side view).

tetrahydronaphthyl group of **26b** to (*R*)-chromanyl as well as difluoro-substitution of the cyclohexylglycine moiety improved the metabolic stability and oral bioavailability. Further improvement of metabolic stability was accomplished by introducing an (*R*)-ethoxy group at the scaffold 7-position to afford the (7*R*)-ethoxy octahydropyrrolo[1,2-*a*]pyrazine derivative **45**. Reflecting its desirable biological, physicochemical, and pharmacokinetic properties, compound **45** showed tumor regression efficacy (T/C=-53% at 30 mg/kg) without severe toxicity in mice. Furthermore, we performed X-ray crystallographic studies of compound **45** and could explain the observed cIAP-dominant profile on the basis of its complex structures with XIAP and cIAP1. Consequently, compound **45** (T-3256336) was demonstrated to be a promising cIAP1-dominant IAP antagonist suitable for further preclinical studies.

EXPERIMENTAL SECTION

The proton nuclear magnetic resonance (^{1}H NMR) spectra were determined on a Bruker AVANCE II (300 MHz) spectrometer. Chemical shifts for ^{1}H NMR were reported in parts per million (ppm) downfield from tetramethylsilane (δ) as the internal standard in deuterated solvent and coupling constants (J) are in Hertz (Hz). The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad. Melting points were determined on a BÜCHI Melting Point M-565 or SRS OptiMelt melting point apparatus, and are uncorrected. Elemental analyses and high-resolution mass spectrometry (HRMS) were carried out by Takeda Analytical Research Laboratories, Ltd. Analytical thin layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (Merck) or NH TLC plate (Fuji Silysia Chemical Ltd.). Column chromatography was carried out on a silica gel column (Chromatorex NH-DM1020, 100−200 mesh, Fuji Silysia chemical) or on Purif-Pack (SI ϕ 60 μM or NH ϕ 60 μM, Fuji Silysia Chemical, Ltd.). Mass spectra (MS) were aquired using an Agilent LC/MS system (Agilent1200SL/Agilent6130MS), Shimadzu LC/MS system (LC-10ADvp high pressure gradient system/LCMS-2010A) or Shimadzu UFLC/MS (Prominence UFLC high pressure gradient system/LCMS-2020) operating in electron spray ionization mode (ESI+) and were used to confirm ≥95% purity of each compound. The column used was an L-column 2 ODS (3.0 × 50 mm I.D., 3 μm, CERI, Japan) with a temperature of 40 °C and a flow rate of 1.2 or 1.5 mL/min. Mobile phase A was 0.05% TFA in ultrapure water. Mobile phase B was 0.05% TFA in acetonitrile which was increased linearly from 5% to 90% over 2 min, 90% over the next 1.5 min, after which the column was equilibrated to 5% for 0.5 min.All commercially available solvents and reagents were used without further purification. Yields were not optimized.

The following abbreviations are used: Boc: (*tert*-butoxy)carbonyl, DIPEA: *N*,*N*-diisopropylethylamine, DMF: *N*,*N*-dimethylformamide, DMT-MM: 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride, DMSO: dimethyl sulfoxide, EDC: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, EDC-HCl: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, Et: ethyl, EtOAc: ethyl acetate; HATU: *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HOBT: 1-hydroxybenzotriazole, *i*: iso, mp: melting point, Me: methyl, Pd/C: palladium on carbon, $Pd(OH)_2$/C: palladium hydroxide on carbon, Pr: propyl, quant.: quantitative yield, sat.: saturated aqueous, *tert*: tertiary, TFA: trifluoroacetic acid, THF: tetrahydrofuran.

***N*-Benzyl-5-oxo-D-prolinamide (2a).** 5-Oxo-D-proline (**1a**, 5.0 g, 38.7 mmol), benzylamine (4.65 mL, 42.5 mmol), EDC (8.5 g, 44.3 mmol), and HOBt (6.3 g, 46.6 mmol) were stirred in acetonitrile (100 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 3 h. The reaction was quenched with 1 M HCl (50 mL), and the mixture was extracted with EtOAc (200 mL). The organic layer was washed with sat. $NaHCO_3$ (50 mL) and brine (50 mL, dried over $MgSO_4$, concentrated under reduced pressure). The resulting precipitate was collected by filtration and washed with diethyl ether (10 mL) to give **2a** (4.02 g, 48%) as a white amorphous powder; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.82−1.96 (1H, m), 2.02−2.36 (3H, m), 3.99−4.09 (1H, m), 4.29 (2H, d, J = 5.9 Hz), 7.18−7.38 (5H, m), 7.85 (1H, s), 8.50 (1H, t, J = 5.9 Hz).

***N*-Benzyl-5-oxo-L-prolinamide (2b).** 5-Oxo-L-proline (**1b**, 5.0 g, 39.0 mmol), benzylamine (5.08 mL, 46.5 mmol) and HATU (14.7 g, 38.7 mmol) were stirred in DMF (100 mL) at room temperature, and the reaction mixture was stirred at room temperature for 18 h. The mixture was diluted with EtOAc (300 mL), and washed with sat. citric acid (50 mL), sat. $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0−10% methanol in EtOAc) to give **2b** (3.75 g, 44%) as a white amorphous powder; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.81−1.97 (1H, m), 2.01−2.36 (3H, m), 4.04 (1H, m), 4.29 (2H, d, J = 5.9 Hz), 7.18−7.37 (5H, m), 7.85 (1H, s), 8.50 (1H, t, J = 5.9 Hz).

1-Phenyl-*N*-[(2*R*)-pyrrolidin-2-ylmethyl]methanamine (3a). To a suspension of lithium aluminum hydride (5.40 g, 142 mmol) in THF (150 mL) was added dropwise a suspension of *N*-benzyl-5-oxo-D-prolinamide (**2a**, 11.0 g, 50.4 mmol) in THF (350 mL) at 0 °C, and the mixture was stirred at 60 °C for 14 h. The mixture was cooled to 0 °C, and then water (10.8 mL), 1 M NaOH (5.4 mL) and water (5.4 mL) were successively added to the mixture. The resulting insoluble material was removed by filtration and the filtrate was concentrated under reduced pressure to give **3a** (8.95 g, 93%) as a pale-yellow oil. The compound was used for the next reaction without further purification; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.18−1.33 (1H, m), 1.48−1.81 (3H, m), 2.09 (2H, br s), 2.30−2.44 (2H, m), 2.63−2.80 (2H, m), 3.00−3.12 (1H, m), 3.68 (2H, s), 7.13−7.37 (5H, m).

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1-Phenyl-*N*-[(2*S*)-pyrrolidin-2-ylmethyl]methanamine (3b). A solution of *N*-benzyl-5-oxo-L-prolinamide (**2b**, 4.90 g, 22.5 mmol) in THF (25 mL) was added dropwise to a suspension of lithium aluminum hydride (2.56 g, 67.5 mmol) in THF (50 mL) at 0 °C, and the reaction mixture was stirred at 60 °C for 18 h. To the mixture were successively added water (2.6 mL), 1 M NaOH (2.6 mL) and water (3.6 mL) at 0 °C. The resulting insoluble material was removed by filtration and the filtrate was concentrated under reduced pressure to give **3b** (3.85 g, 90%) as a pale-yellow oil. The product was used for the next reaction without further purification.

Methyl (3*S*,8a*R*)-2-benzyloctahydropyrrolo[1,2-*a*]pyrazine-3-carboxylate (5). To a suspension of **3a** (13.6 g, 71.5 mmol) in toluene (120 mL) were added triethylamine (22.9 mL, 164 mmol) and methyl 2,3-dibromopropanoate (**4**, 13.4 g, 54.5 mmol) at 0 °C. After being stirred at 90 °C for 5 h, the mixture was allowed to cool at room temperature, and partitioned between diethyl ether (200 mL) and brine (200 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (20−50% EtOAc in *n*-hexane) to give **5** (6.86 g, 46%) and **6** (7.80g, 52%) as pale-yellow oils; **5**: ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.14−1.28 (1H, m), 1.53−1.77 (3H, m), 1.83−2.00 (2 H, m), 2.31 (1 H, dd, J = 10.7, 3.9 Hz), 2.61−2.95 (3H, m), 3.29 (1H, dd, J = 10.7, 2.0 Hz), 3.53 (1H, dd, J = 3.7, 1.8 Hz), 3.62 (3H, s), 3.89 (2H, s), 7.17−7.37 (5H, m); **6**: ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.14−1.25 (1H, m), 1.54−1.72 (3H, m), 1.77 (1H, t, J = 10.2 Hz), 1.88−2.10 (2H, m), 2.15−2.25 (1H, m), 2.75 (1H, dd, J = 10.5, 2.5 Hz), 2.87−2.98 (1H, m), 3.07−3.20 (3H, m), 3.66 (3H, s), 3.74 (1H, d, J = 13.0 Hz), 7.19−7.37 (5H, m).

2-*tert*-Butyl 3-methyl (3*S*,8a*R*)-hexahydropyrrolo[1,2-*a*]-pyrazine-2,3(1*H*)-dicarboxylate (7a). To a solution of **5** (6.80 g, 24.8 mmol) in 5−10% HCl in methanol (50 mL) was added 10% Pd/C (680 mg), and the mixture was stirred at room temperature for 10 h under hydrogen atmosphere. The mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure to give pale-yellow oil, and the oil was dissolved in sat. $NaHCO_3$ (25 mL) and THF (50 mL). To the mixture was added di-*tert*-butyl dicarbonate (5.68 g, 26.0 mmol), and the mixture was stirred at room temperature for 1 h. The mixture was partitioned between EtOAc (300 mL) and water (100 mL). The organic layer was washed with brine (100 mL) and dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10−20% EtOAc in *n*-hexane) to give **7a** (6.50 g, 92%) as a colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.18−1.39 (1H, m), 1.42−1.51 (9H, m), 1.61−1.94 (4H, m), 1.97−2.12 (1H, m), 1.99−2.11 (1H, m), 2.22−2.33 (1H, m), 2.69−2.91 (1H, m), 2.99−3.09 (1H, m), 3.48−3.58 (1H, m), 3.72−3.78 (2H, m), 3.93−4.13 (1H, m), 4.56−4.82 (1H, m).

***tert*-Butyl (2*R*,4*R*)-2-(benzylcarbamoyl)-4-hydroxypyrrolidine-1-carboxylate (9).** To a solution of (4*R*)-1-(*tert*-butoxycarbonyl)-4-hydroxy-D-proline (**8**, 50.0 g, 216 mmol) in acetonitrile (750 mL) were added benzylamine (28.3 mL, 259 mmol), *N*,*N*-dimethylpyridin-4-amine (2.64 g, 216 mmol), HOBt (38.0 g, 281 mmol), and EDC (54.0 g, 281 mmol) at room temperature, and the mixture was stirred at room temperature for 14 h. The mixture was partitioned between EtOAc (1.5 L) and 5% citric acid (750 mL). The organic layer was washed with sat. $NaHCO_3$ (750 mL) and brine (500 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was passed through a pad of NH silica gel and the pad was washed with EtOAc (4.0 L). The eluate was concentrated under reduced pressure to give **9** (62.3 g, 90%) as a white amorphous solid; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.22−1.47 (9H, m), 1.69−1.82 (1H, m), 2.24−2.41 (1H, m), 3.15−3.26 (1H, m), 3.49 (1H, dd, J = 10.7, 5.4 Hz), 4.07−4.42 (4H, m), 5.18−5.34 (1H, m), 7.18−7.37 (5H, m), 8.48 (1H, t, J = 6.0 Hz).

(4*R*)-*N*-Benzyl-4-hydroxy-D-prolinamide (10). A solution of **9** (61.0 g, 190 mmol) in 4 M HCl in EtOAc (500 mL) was stirred at room temperature for 2 h. The solvent was concentrated under reduced pressure, and the residue was passed through a pad of Amberlyst A21, and the pad was washed with methanol (1.0 L). The eluate was concentrated under reduced pressure to give **10** (42.8 g, quant) as a white amorphous solid; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.78 (1H, ddd, J = 13.2, 5.9, 4.8 Hz), 2.30 (1H, ddd, J = 13.2, 9.4, 5.8 Hz), 2.87 (1H, dd, J = 11.2, 3.1 Hz), 3.04 (1H, dd, J = 11.2, 5.1 Hz), 3.17 (1H, s), 3.86 (1H, dd, J = 9.4, 5.9 Hz), 4.17- 4.28 (1H, m), 4.32 (2H, d, J = 5.9 Hz), 5.00 (1H, br s), 7.16−7.40 (5H, m), 8.69 (1H, t, J = 5.9 Hz).

Methyl (3*S*,7*R*,8a*R*)-2-benzyl-7-hydroxyoctahydropyrrolo-[1,2-*a*]pyrazine-3-carboxylate (11). To a suspension of lithium aluminum hydride (12.9 g, 340 mmol) in THF (300 mL) was added dropwise a suspension of **10** (37.4 g, 170 mmol) in THF (300 mL) at 0 °C, and the reaction mixture was heated at reflux for 14 h. The reaction mixture was cooled to 0 °C, and then water (13 mL), 1 M NaOH (13 mL) and water (26 mL) were successively added to the mixture. The resulting insoluble material was removed by filtration and the filtrate was concentrated under reduced pressure. The residue (33.0 g) was dissolved in toluene (330 mL), and then, to the solution were added triethylamine (60 mL, 431 mmol) and methyl 2,3-dibromopropanoate (18.2 mL, 144 mmol). After being stirred at 80 °C for 20 h, the mixture was cooled at room temperature. The mixture was partitioned between EtOAc (500 mL) and water (300 mL), and the aqueous layer was extracted with EtOAc (500 mL). The combined organic layers were dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (5−100% EtOAc in *n*-hexane) to give **11** (12.6 g, 30%) as orange oil and **12** (18.2g, 44%) as a yellowish amorphous solid; **11**: ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.06−1.21 (1H, m), 1.79−1.94 (1H, m), 1.99−2.14 (2H, m), 2.24 (1H, dd, J = 10.6, 4.0 Hz), 2.59 (1H, dd, J = 10.7, 3.0 Hz), 2.74 (1H, d, J = 9.8 Hz), 2.79−2.89 (1H, m), 3.24 (1H, dd, J = 10.7, 1.7 Hz), 3.49−3.56 (1H, m), 3.64 (3H, s), 3.89 (2H, s), 4.14 (1H, dt, J = 6.8, 3.1 Hz), 4.70 (1H, d, J = 4.5 Hz), 7.18−7.38 (5H, m); **12**: ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.03−1.16 (1H, m), 1.81−1.95 (2H, m), 1.98−2.24 (3H, m), 2.65−2.81 (2H, m), 3.06 (1H, dd, J = 10.4, 3.2 Hz), 3.14−3.22 (2H, m), 3.66 (3H, s), 3.69−3.78 (1H, m), 4.08−4.21 (1H, m), 4.71 (1H, d, J = 4.7 Hz), 7.16−7.41 (5H, m).

2-*tert*-Butyl 3-methyl (3*S*,7*R*,8a*R*)-7-hydroxyhexahydropyrrolo[1,2-*a*]pyrazine-2,3(1*H*)-dicarboxylate (13). To a solution of **11** (12.0 g, 41.3 mmol) in 5−10% HCl solution in methanol (120 mL) was added 10% Pd/C (1.2 g), and the reaction mixture was stirred at room temperature for 3 h under hydrogen atmosphere. The mixture was filtered through a pad of Celite and the pad was washed with methanol (300 mL). The filtrate was concentrated under reduced pressure, and then the residue was dissolved in THF (180 mL). To the solution were successively added sat. $NaHCO_3$ (180 mL) and di-*tert*-butyl dicarbonate (9.93 g, 45.5 mmol), and the mixture was stirred at room temperature for 18 h. To the mixture was added EtOAc (300 mL) and the aqueous layer was extracted with EtOAc/THF (3:1, 300 mL). The combined organic layers were dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0−20% methanol in EtOAc) to give **13** (10.7 g, 86%) as a white solid; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.08−1.24 (1H, m), 1.35−1.41 (9H, m), 1.70−1.86 (1H, m), 2.09−2.23 (3H, m), 2.57−2.89 (2H, m), 3.30−3.39 (1H, m), 3.67−3.69 (3H, m), 3.79−3.92 (1H, m), 4.11−4.22 (1H, m), 4.53−4.66 (1H, m), 4.77 (1H, d, J = 4.3 Hz).

2-*tert*-Butyl 3-methyl (3*S*,8a*R*)-7-oxohexahydropyrrolo[1,2-*a*]pyrazine-2,3(1*H*)-dicarboxylate (14). To a solution of **13** (1.62 g, 32.3 mmol) and triethylamine (4.5 mL, 32.3 mmol) in DMSO/EtOAc (1:2.5, 11.3 mL) were added dropwise a solution of sulfur trioxide pyridine complex (2.58 g, 16.2 mmol in DMSO (13.0 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, and then partitioned between EtOAc/THF (2:1, 150 mL) and water (50 mL). The organic layer was washed with sat. $NaHCO_3$ (25 mL) and brine (25 mL) successively, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (30−80% EtOAc in *n*-hexane) to give **14** (1.46 g, 91%) as a colorless oil; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.34−1.45 (9H, m), 2.02−2.14 (1H, m), 2.30−2.40 (1H, m), 2.45−2.62 (2H, m), 2.62−2.71 (1H, m), 2.73−3.00 (1H, m), 3.24−3.35 (1H, m), 3.40−

3.48 (1H, m), 3.66−3.70 (3H, m), 3.90−4.03 (1H, m), 4.62−4.76 (1H, m).

2-*tert*-Butyl 3-methyl (3*S*,8a*R*)-7,7-difluorohexahydropyrrolo[1,2-*a*]pyrazine-2,3(1*H*)-dicarboxylate (15). Deoxo-Fluor (3.63 mL, 20.6 mmol) was added to a solution of **14** (2.80 g, 9.39 mmol) in toluene (28 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, and then allowed to warm at room temperature and stirred for 20 h. The mixture was partitioned between EtOAc (200 mL) and sat. $NaHCO_3$ (50 mL). The organic layer was washed with brine (50 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (20−60% EtOAc in *n*-hexane) to give **15** (1.73 g, 62%) as a colorless oil; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.33−1.44 (9H, m), 1.75−2.00 (1H, m), 2.27−2.60 (4H, m), 2.61−2.89 (1H, m), 3.30−3.43 (2H, m), 3.65−3.70 (3H, m), 3.87−3.98 (1H, m), 4.60−4.73 (1H, m).

(4*R*)-1-(*tert*-Butoxycarbonyl)-4-ethoxy-D-proline (16). Sodium hydride (ca. 60% dispersion in oil, 13.0 g, 325 mmol) was added to a mixture of (4*R*)-1-(*tert*-butoxycarbonyl)-4-hydroxy-D-proline **8** (30.0 g, 130 mmol) in THF (300 mL) at 0 °C, and the reaction mixture was stirred at 0 °C for 30 min. To the mixture was added iodoethane (51.9 mL, 646 mmol), and then the mixture was allowed to warm at room temperature and stirred for 18 h. To the mixture were successively added water (150 mL), 1 M HCl (150 mL), and NaCl (40 g), and the mixture was extracted with EtOAc (500 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc) and crystallization from diethyl ether/*n*-hexane (1:4, 250 mL) to give **16** (31.6 g, 94%) as colorless crystals; mp 58.9−60.4 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.99−1.10 (3H, m), 1.30−1.44 (9H, m), 1.87−2.02 (1H, m), 2.22−2.45 (1H, m), 3.10−3.21 (1H, m), 3.26−3.44 (2H, m), 3.47−3.61 (1H, m), 3.94−4.05 (1H, m), 4.08−4.18 (1H, m), 12.43 (1H, br s).

***tert*-Butyl (2*R*,4*R*)-2-(benzylcarbamoyl)-4-ethoxypyrrolidine-1-carboxylate (17).** To a suspension of **16** (28.0 g, 108 mmol), benzylamine (13.0 mL, 98.7 mmol), and HOBt (16.1 g, 119 mmol) in acetonitrile (300 mL) was added EDC-HCl (31.06 g, 162 mmol) at 0 °C, and the reaction mixture was allowed to warm at room temperature and stirred for 2 h. To the mixture was added water (100 mL), and the mixture was extracted with EtOAc (500 mL). The organic layer was washed with sat. $NaHCO_3$ (150 mL × 2) and brine (100 mL) successively, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was crystallized from diethyl ether/*n*-hexane (1:5, 60 mL) to give **17** (36.0 g, 96%) as colorless crystals; mp 87.5−89.0 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.04 (3H, t, J = 6.9 Hz), 1.23−1.45 (9H, m), 1.79−2.00 (1H, m), 2.22−2.44 (1H, m), 3.18−3.44 (3H, m), 3.53−3.66 (1H, m), 3.85−4.43 (4H, m), 7.15−7.35 (5H, m), 8.01−8.27 (1H, m).

***N*-Benzyl-1-[(2*R*,4*R*)-4-ethoxypyrrolidin-2-yl]methanamine (18).** To a solution of **17** (35.4 g, 102 mmol) in EtOAc (100 mL)/methanol (25 mL) was added 4 M HCl in EtOAc (125 mL). The reaction mixture was stirred at room temperature for 18 h, and then concentrated under reduced pressure. The residue was filtered through a pad of Amberlyst A21 (150 g), the pad was washed with methanol (500 mL), and the filtrate was concentrated. The residue was dissolved in THF (100 mL), and the solution was added to a suspension of lithium aluminum hydride (7.71 g, 203 mmol) in THF (300 mL) at 0 °C. The mixture was refluxed for 15 h. The reaction mixture was cooled to 0 °C, and then sodium sulfate decahydrate (15 g) was slowly added to the mixture. The insoluble material was filtered, and the filtrate was concentrated under reduced pressure. The residue containing a desired product **18** was used for the next reaction without further purification (21.0 g, 88%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (3H, t, J = 7.0 Hz), 1.39−1.53 (1H, m), 2.04−2.24 (4H, m), 2.66−2.82 (2H, m), 2.86−2.98 (1H, m), 2.99−3.10 (1H, m), 3.20−3.35 (1H, m), 3.43 (2H, q), 3.84 (2H, s), 3.94−4.04 (1H, m), 7.14−7.45 (4H, m).

2-*tert*-Butyl 3-methyl (3*S*,7*R*,8a*R*)-7-ethoxyhexahydropyrrolo[1,2-*a*]pyrazine-2,3(1*H*)-dicarboxylate (19) and 2-*tert*-Butyl 3-methyl (3*R*,7*R*,8a*R*)-7-ethoxyhexahydropyrrolo[1,2-*a*]pyrazine-2,3(1*H*)-dicarboxylate (20). To a solution of crude **18** (19.8 g, 84.5 mmol) in toluene (158 mL) were added methyl 2,3-dibromopropanoate (9.1 mL, 71.9 mmol) and triethylamine (28.3 mL, 203 mmol), and the reaction mixture was stirred at 80 °C for 5 h. After being cooled at room temperature, the mixture was partitioned between EtOAc (500 mL) and water (150 mL). The organic layer was dried over $MgSO_4$, and concentrated under reduced pressure. The residue was filtered through a pad of silica gel (100 g), and the pad was washed with EtOAc (500 mL), and the filtrate was concentrated under reduced pressure. The residue was dissolved in 5−10% HCl in methanol (200 mL), and to the solution was added 0% Pd/C (4.9 g). The reaction mixture was stirred at room temperature for 2 h under hydrogen atmosphere (3 atm). The mixture was filtered through a pad of Celite, and the pad was washed with methanol (50 mL). The filtrate was concentrated under reduced pressure, and the residue was dissolved in a mixture of THF (220 mL) and sat. $NaHCO_3$ (220 mL). To the solution was added di-*tert*-butyl dicarbonate (16.8 g, 77.0 mol), and the reaction mixture was stirred at room temperature for 3 h. The mixture was partitioned between EtOAc (350 mL) and water (100 mL), and the organic layer was washed with brine (100 mL), and dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10−100% EtOAc in *n*-hexane, then 0−15% methanol in EtOAc) to give **19** (6.9 g, 29%) and **20** (10.6 g, 45%) as colorless oils. Compound **21**, a mixture of regioisomers, was also obtained as a minor product (3%). **19**: ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.07 (3H, t, J = 7.1 Hz), 1.14−1.27 (1H, m), 1.33−1.43 (9H, m), 1.72−1.88 (1H, m), 2.06−2.27 (3H, m), 2.58−2.85 (1H, m), 2.91−2.97 (1H, m), 3.25−3.41 (3H, m), 3.65−3.70 (3H, m), 3.81−3.96 (2H, m), 4.53−4.67(1H, m); **20**: ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.08 (3H, t, J = 7.1 Hz), 1.38−1.46 (10H, m), 2.08−2.21 (1H, m), 2.64−2.86 (4H, m), 3.03−3.21 (2H, m), 3.26−3.42 (2H, m), 3.57−3.66 (1H, m), 3.64 (3H, s), 3.87−3.98 (1 H, m), 4.25−4.31 (1H, m); **21**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.15−1.23 (3H, m) 1.38−1.50 (9H, m) 1.93−2.32 (2H, m) 2.48−2.76 (1H, m) 2.82−3.13 (2H, m) 3.13−3.34 (2H, m) 3.34−3.54 (2H, m) 3.58−3.80 (4H, m) 3.94−4.44 (3H, m).

2-*tert*-Butyl 3-methyl (3*S*,7*R*,8a*R*)-7-ethoxyhexahydropyrrolo[1,2-*a*]pyrazine-2,3(1*H*)-dicarboxylate (19). A solution of sodium methoxide (1.25g, 23.1 mmol) in methanol (28%, 4.46 g) was added to a solution of **20** (10.6 g, 32.3 mmol) in methanol (42.4 mL), and the mixture was stirred at 60 °C for 5 h. The mixture was allowed to cool at room temperature and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (20−80% EtOAc in *n*-hexane) to give **19** (9.56 g, 90%) as a colorless oil; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.07 (3H, t, J = 7.1 Hz), 1.14−1.27 (1H, m), 1.33−1.43 (9H, m), 1.72−1.88 (1H, m), 2.06−2.27 (3H, m), 2.58−2.85 (1H, m), 2.91−2.97 (1H, m), 3.25−3.41 (3H, m), 3.65−3.70 (3H, m), 3.81−3.96 (2H, m), 4.53−4.67(1H, m).

Methyl (3*S*,8a*R*)-2-[(2*S*)-2-{[*N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-alanyl]amino}-2-cyclohexylacetyl]octahydropyrrolo[1,2-*a*]pyrazine-3-carboxylate (23). To a solution of **5** (400 mg, 1.46 mmol) in ethanol (3 mL) were added 10% Pd/C (60 mg) and 4 M HCl in EtOAc (1 mL), and the reaction mixture was stirred at room temperature for 4 h under hydrogen atmosphere. The mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure to give the residue containing a crude methyl (3*S*, 8a*R*)-octahydropyrrolo[1,2-*a*]pyrazine-3-carboxylate dihydrochloride (370 g) as pale-yellow oil. To the mixture of crude product in THF (5 mL) were added (2*S*)-{[*N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-alanyl]amino}(cyclohexyl)thanoic acid **22** (540 mg, 1.58 mmol), DMT-MM (475 mg, 1.72 mmol), and 4-methylmorpholine (0.314 mL, 2.89 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 4 h. The mixture was partitioned between EtOAc (50 mL) and water (50 mL), and the organic layer was washed with brine (50 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10−100% EtOAc in *n*-hexane) to give **23** (320 mg, 43%) as a pale-yellow oil; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.75−1.33 (10H,

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m), 1.39 (9H, s), 1.49−2.40 (11H, m), 2.62−3.07 (4H, m), 3.37−4.02 (5H, m), 4.04−4.80 (3H, m), 4.93−5.49 (1H, m), 6.95−8.28 (1H, m).

(3*S*,8a*R*)-2-[(2*S*)-2-{[*N*-(*tert*-Butoxycarbonyl)-*N*-methyl-L-alanyl]amino}-2-cyclohexylacetyl]octahydropyrrolo[1,2-a]pyrazine-3-carboxylic acid (24). To a solution of **23** (320 mg, 0.63 mmol) in THF (3 mL) was added lithium hydroxide (34 mg, 0.81 mmol) in water (3 mL) at room temperature, and the mixture was stirred at room temperature for 3 h. The reaction was quenched by the addition of 1 M HCl (0.818 mL), and the mixture was concentrated under reduced pressure to give **24** as a crude amorphous solid. The compound was used for the next reaction without further purification.

(3*S*,8a)-2-{(2*S*)-2-Cyclohexyl-2-[(*N*-methyl-L-alanyl)amino]-acetyl}-*N*-[(1*R*)-1-phenylethyl]octahydropyrrolo[1,2-*a*]-pyrazine-3-carboxamide dihydrochloride (26a). To a solution of **23** (200 mg, 0.49 mmol) in THF (5.7 mL) was added lithium hydroxide (24.8 mg, 0.59 mmol) in water (1.5 mL) at room temperature, and the mixture was stirred at 50 °C for 3 h. To the mixture were added DMT-MM (232 mg, 0.84 mmol) and (1*R*)-1-phenylethanamine (71 mg, 0.59 mmol), and the mixture was stirred at room temperature for 15 h. The mixture was partitioned between EtOAc (30 mL) and water (30 mL), and the organic layer was washed with brine (30 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (30−100% EtOAc in hexane) to give a colorless amorphous solid. The amorphous powder was dissolved in EtOAc (4 mL). To the solution was added 4 M HCl in EtOAc (2 mL), and the mixture was stirred at room temperature for 3 h. The mixture was concentrated under reduced pressure, and the residue was partitioned between EtOAc (30 mL) and sat. $NaHCO_3$ (30 mL), and the organic layer was washed with brine (30 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was dissolved in EtOAc (5 mL). To the solution was added 4 M HCl in EtOAc (10 mL), and the resulting precipitate was collected by filtration to give **26a** (124 mg, 49%) as a white amorphous solid; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.86−1.21 (5H, m), 1.30−1.43 (6H, m), 1.50−1.87 (8H, m), 1.97−2.18 (2H, m), 2.40−2.48 (3H, m), 2.97−3.92 (7H, m), 4.38−4.52 (1H, m), 4.65−4.76 (2H, m), 4.79−4.93 (1H, m), 7.16−7.45 (5H, m), 8.49−9.08 (3H, m), 9.32−10.10 (1H, m), 12.01−12.52 (1H, m); Anal. Calcd for $C_{28}H_{45}Cl_2N_5O_3{\cdot}2H_2O$: C, 55.44; H, 8.14; N, 11.54. Found: C, 55.51; H, 8.42; N, 11.47.

(3*S*,8a*R*)-2-{(2*S*)-2-Cyclohexyl-2-[(*N*-methyl-L-alanyl)amino]-acetyl}-*N*-[(1*R*)-1,2,3,4-tetrahydronaphthalen-1-yl]-octahydropyrrolo[1,2-*a*]pyrazine-3-carboxamide dihydrochloride (26b). To a mixture of **24** (190 mg, 0.48 mmol) in THF (5 mL) were added successively (1*R*)-1,2,3,4-tetrahydronaphthalen-1-amine (68 mg, 0.46 mmol), DMT-MM (232 mg, mmol, 0.84 mmol) and 4-methylmorpholine (0.043 mL, 0.39 mmol) at 0 °C, and the mixture was stirred at room temperature for 3 h. The mixture was partitioned between EtOAc (30 mL) and water (30 mL), and the organic layer was washed with brine (30 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10−100% EtOAc in hexane, then 0−10% methanol in EtOAc) to give a colorless amorphous powder. The amorphous powder was dissolved in EtOAc (1 mL) and methanol (1 mL). To the solution was added 4 M HCl in EtOAc (1 mL), and the mixture was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure, and the residue was washed with ether (10 mL), and dried under a vacuum to give **26b** (120 mg, 44%) as a white amorphous powder; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.86−1.21 (5H, m), 1.30−1.43 (6H, m), 1.50−1.87 (8H, m), 1.97−2.18 (2H, m), 2.40−2.48 (3H, m), 2.97−3.92 (7H, m) 4.38−4.52 (1H, m), 4.65−4.76 (2H, m), 4.79−4.93 (1H, m), 7.16−7.45 (5H, m), 8.49−9.08 (3H, m), 9.32−10.10 (1H, m), 12.01−12.52 (1H, m); Anal. Calcd for $C_{30}H_{47}Cl_2N_5O_3{\cdot}1.0H_2O$: C, 58.62; H, 8.04; N, 11.39. Found: C, 58.69; H, 8.06; N, 11.09.

(3*S*,8a*R*)-2-{(2*S*)-2-Cyclohexyl-2-[(*N*-methyl-L-alanyl)amino]-acetyl}-*N*-[(1*S*)-1,2,3,4-tetrahydronaphthalen-1-yl]-octahydropyrrolo[1,2-*a*]pyrazine-3-carboxamide dihydrochloride (26c). To a solution of **24** (119 mg, 0.24 mmol) in DMF were successively added (1*S*)-1,2,3,4-tetrahydronaphthalen-1-amine (71 mg, 0.48 mmol), HOBt (48.6 mg, 0.36 mmol), DIPEA (0.21 mL, 1.20 mmol) and EDC (138 mg, 0.72 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 18 h. The mixture was partitioned between EtOAc (30 mL) and water (30 mL), and the organic layer was washed with brine (30 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography twice (0−80% EtOAc in *n*-hexane) to give *tert*-butyl [(1*S*)-2-({(1*S*)-1-cyclohexyl-2-oxo-2-[(3*S*,8a*R*)-3-[(1*S*)-1,2,3,4-tetrahydronaphthalen-1-ylcarbamoyl]hexahydropyrrolo-[1,2-*a*]pyrazin-2(1*H*)-yl]ethyl}amino)-1-methyl-2-oxoethyl]-methylcarbamate as colorless oil. This colorless oil was dissolved in EtOAc (1 mL), and to the solution was added 4 M HCl in EtOAc (3 mL), and the mixture was stirred at room temperature for 1 h. The mixture was concentrated under reduced pressure, and the residue was washed with ether (5 mL), and dried under reduced pressure to give **26c** (34 mg, 23%) as a white amorphous powder; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.88−1.27 (5H, m), 1.38 (3H, d, J = 6.8 Hz), 1.43−2.28 (12H, m), 2.34−2.49 (3H, m), 2.60−2.87 (2H, m), 2.96−3.13 (1H, m), 3.46−3.98 (5H, m), 4.09−5.12 (7H, m), 6.83−7.29 (4H, m), 8.38−9.20 (3H, m), 9.21−9.88 (1H, m), 11.61−12.85 (1H, m); Anal. Calcd for $C_{30}H_{47}Cl_2N_5O_3{\cdot}0.5H_2O{\cdot}0.5C_4H_{10}O$: C, 59.80; H, 8.31; N, 10.90. Found: C, 59.60; H, 8.35; N, 11.18.

(3*S*,8a*R*)-2-{(2*S*)-2-Cyclohexyl-2-[(*N*-methyl-L-alanyl)amino]-acetyl}-*N*-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl]-octahydropyrrolo[1,2-*a*]pyrazine-3-carboxamide dihydrochloride (26d). A solution of **23** (1.80 g, 3.54 mmol) in THF (40 mL) was added to aqueous lithium hydroxide monohydrate (193 mg, 4.60 mmol) in water (10 mL), and the reaction mixture was stirred at 50 °C for 3 h. The mixture was allowed to cool at room temperature, and 1 M HCl (4.6 mL) was added to the mixture. The mixture was concentrated under reduced pressure, and the residue was dissolved in DMF (20 mL). To the solution were added (*R*)-chroman-4-amine hydrochloride (984 mg, 5.30 mmol), EDC (4.48 g, 23.4 mmol), HOBt (478 mg, 3.53 mmol) and DIPEA (1.23 mL, 7.05 mmol) successively, and the mixture was stirred at room temperature for 48 h. The mixture was partitioned between EtOAc (30 mL) and water (30 mL), and the organic layer was washed with brine (30 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography twice (first column: 10−100% EtOAc in *n*-hexane, second column: 30−100% EtOAc in *n*-hexane) to give *tert*-butyl [(1*S*)-2-({(1*S*)-1-cyclohexyl-2-[(3*S*,8a*R*)-3-[(4*R*)-3,4-dihydro-2*H*-cromen-4-ylcarbamoyl] hexahydropyrrolo[1,2-*a*]pyrazin-2(1*H*)-yl]-2-oxoethyl}amino)-1-methyl-2-oxoethyl]methylcarbamate as a colorless oil (1.21 g). This colorless oil was dissolved in EtOAc (16 mL), and to the solution was added 4 M HCl in EtOAc (8 mL). The mixture was stirred at room temperature for 4 h. The reaction was quenched by the addition of water (30 mL), the aqueous layer was neutralized by 1 M NaOH (35 mL) and extracted with EtOAc (30 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give a salt free form of **26d** (912 mg, 1.49 mmol) as a colorless oil. A solution of the oil (902 mg, 1.47 mmol) in EtOAc (10 mL) was added to 4 M HCl in EtOAc (1 mL), and the mixture was stirred at room temperature for 3 h. The precipitate was collected by filtration, washed with EtOAc (10 mL), and dried under reduced pressure to give **26d** (1.01 g, 48%) as a white amorphous powder; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.95−1.27 (5H, m), 1.37 (3H, d, J = 6.8 Hz), 1.50−2.18 (12H, m), 2.41−2.48 (3 H, m), 2.95−3.78 (6H, m), 3.81−3.94 (1H, m), 4.20−4.53 (3H, m), 4.58−4.68 (1H, m), 4.71−4.81 (1 H, m), 4.92−5.04 (1 H, m), 6.73−6.95 (2H, m), 7.10−7.62 (2H, m), 8.48−9.20 (3H, m), 9.24−10.44 (1H, m), 11.95−12.60 (1H, m); Anal. Calcd for $C_{29}H_{44}Cl_2N_5O_4{\cdot}3.0H_2O$: C, 53.45; H, 7.73; N, 10.75. Found: C, 53.30; H, 7.78; N, 10.65.

(3*S*,8a*R*)-2-{(2*S*)-2-Cyclohexyl-2-[(*N*-methyl-L-alanyl)amino]-acetyl}-*N*-[(1*R*)-4,4-difluoro-1,2,3,4-tetrahydronaphthalen-1-yl]octahydropyrrolo[1,2-*a*]pyrazine-3-carboxamide dihydrochloride (26e). Compound **26e** was prepared by a method similar to that described for **24** and **26d** using **23** (200 mg, 0.11 mmol), LiOH-H_2O (24.8 mg, 0.59 mmol), (1*R*)-4,4-difluoro-1,2,3,4-tetrahydronaphthalen-1-amine (20 mg, 0.11 mmol), EDC-HCl (226 mg, 1.67 mmol) and HOBt (159 mg, 1.18 mmol). Yield: 29%, white amorphous powder (20.2 mg); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.80−1.31

(6H, m), 1.32−1.43 (3H, m), 1.53−1.92 (8H, m), 1.94−2.17 (3H, m), 2.41−2.48 (3H, m), 2.96−4.55 (9H, m), 4.58−4.68 (1H, m), 4.71−4.84 (1H, m), 4.98−5.17 (1H, m), 7.04−7.79 (5H, m), 8.51−9.18 (3H, m), 9.22−9.54 (1H, m), 11.99−12.59 (1H, m); HRMS-ESI (*m*/*z*): [M + H] calcd for $C_{30}H_{43}F_2N_5O_3$, 560.3407; found. 560.3399.

(3*S*,8a*R*)-2-{(2*S*)-2-Cyclohexyl-2-(*N*-methyl-L-alanyl)amino]-acetyl}-*N*-[(1*R*)-4-oxo-1,2,3,4-tetrahydronaphthalen-1-yl]-octahydropyrrolo[1,2-*a*]pyrazine-3-carboxamide dihydrochloride (26f). Compound **26f** was prepared by a method similar to that described for **24** and **26d** using **23** (200 mg, 0.39 mmol), LiOH-H_2O (24.8 mg, 0.59 mmol), (4*R*)-4-amino-3,4-dihydronaphthalen-1(2*H*)-one (95 mg, 0.59 mmol), EDC-HCl (452 mg, 2.36 mmol), HOBt (106 mg, 0.78 mmol) and DIPEA (0.14 mL, 0.80 mmol). Yield: 44%, white amorphous powder (105 mg); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.63−1.46 (9H, m), 1.52−2.34 (12H, m), 2.54−2.96 (3H, m), 3.08 (1H, br s), 3.43−4.16 (6 H, m), 4.41−4.59 (1H, m), 4.51 (1H, br s), 4.62−4.84 (2H, m), 5.21 (1H, br s), 7.27−7.79 (3H, m), 7.89 (1H, d, J = 7.7 Hz), 8.82 (2H, br s), 9.03 (1H, d, J = 8.5 Hz), 9.33 (1H, br s), 12.09 (1H, br s); HRMS-ESI (*m*/*z*): [M +H] calcd for $C_{30}H_{43}N_5O_4$, 538.3388; found. 538.3366.

***tert*-Butyl (3*S*,8a*R*)-3-[(4*R*)-3,4-dihydro-2*H*-chromen-4-ylcarbamoyl]hexahydropyrrolo[1,2-*a*]pyrazine-2(1*H*)-carboxylate (27).** A solution of 7 (852 mg, 3.00 mmol) in THF (6 mL) was added to lithium hydroxide monohydrate (164 mg, 3.91 mmol) in water (2 mL) at room temperature, and the mixture was stirred at 50 °C for 3 h. To the mixture was added 1 M HCl (3.9 mL), and the mixture was concentrated under reduced pressure to give colorless amorphous powder. This amorphous powder was dissolved in DMF (10 mL), and to the solution were added (*R*)-chroman-4-amine hydrochloride (863 mg, 4.65 mmol), EDC-HCl (1.73 g, 9.02 mmol), HOBt (486 mg, 3.60 mmol) and DIPEA (1.57 mL, 9.01 mmol). The mixture was stirred at room temperature for 15 h, and then partitioned between EtOAc (50 mL) and water (100 mL). The organic layer was washed with brine (100 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (50−100% EtOAc in *n*-hexane) to give **27** (1.12 g, 71%) as a white solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.12−1.30 (1H, m), 1.32−1.47 (9H, m), 1.55−2.09 (7H, m), 2.12−2.27 (1H, m), 2.79−3.06 (2H, m), 3.36−3.51 (1H, m), 3.76−3.98 (1H, m), 4.12−4.28 (2H, m), 4.31−4.53 (1H, m), 5.05 (1H, d, J = 1.3 Hz), 6.63−6.94 (2H, m), 7.06−7.26 (2H, m), 8.11−8.42 (1H, m).

Benzyl {(1*S*)-2-[(3*S*,8a*R*)-3-[(4*R*)-3,4-dihydro-2*H*-chromen-4-ylcarbamoyl]hexahydropyrrolo[1,2-*a*]pyrazin-2(1*H*)-yl]-2-oxo-1-phenylethyl}carbamate (29a). To a solution of **27** (450 mg, 1.12 mmol) in EtOAc (3 mL) was added 4 M HCl in EtOAc (3 mL), and the mixture was stirred at room temperature for 1 h. The mixture was concentrated under reduced pressure, and the residue was washed with ethyl ether (5 mL) and dried under reduced pressure to give (3*S*,8a*R*)-*N*-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl]octahydropyrrolo[1,2-*a*]-pyrazine-3-carboxamide dihydrochloride (410 mg). The obtained product (200 mg, 0.534 mmol), (2*S*)-{[(benzyloxy)carbonyl]amino}-(phenyl)ethanoic acid **28a** (230 mg, 0.806 mmol), HATU (406 mg, 1.07 mmol) and DIPEA (0.558 mL, 3.20 mmol) were mixed in DMF (5 mL) at 0 °C, and the reaction mixture was stirred at room temperature for 18 h. The mixture was partitioned between EtOAc (30 mL) and water (30 mL), and the organic layer was washed with sat. $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10−80% EtOAc in *n*-hexane) to give **29a** (220 mg, 47%) as a pale-yellow amorphous powder; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.93−1.36 (2H, m), 1.42−2.30 (6H, m), 2.69 (2H, s), 2.90 (1H, m), 3.37−3.63 (1H, m), 3.80−4.55 (3H, m), 4.80−5.16 (4H, m), 5.45−5.85 (1H, m), 6.68−6.95 (2H, m), 7.02−7.19 (2H, m), 7.19−7.52 (10 H, m), 7.71−8.36 (2H, m).

(3*S*,8a*R*)-*N*-[(4*R*-3,4-Dihydro-2*H*-chromen-4-yl]-2-{(2*S*)-2-[(*N*-methyl-L-alanyl)amino]-2-phenylacetyl}octahydropyrrolo[1,2-*a*]pyrazine-3-carboxamide dihydrochloride (31a). To a solution of **29a** (220 mg, 0.387 mmol) in methanol (10 mL) was added 10% Pd/C (30 mg), and the reaction mixture was stirred at room temperature for 3 h under hydrogen atmosphere (3 atm). The mixture was filtered through a pad of Celite, and the filtrate was concentrated to give (3*S*,8a*R*)-2-[(2*S*)-2-amino-2-phenylacetyl]-*N*-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl]octahydropyrrolo[1,2-*a*]pyrazine-3-carboxamide as a colorless oil.

The obtained oil, *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-alanine **30** (118 mg, 0.581 mmol), HATU (295 mg, 0.776 mmol) and DIPEA (0.405 mL, 2.33 mmol) were mixed in DMF (5 mL) at 0 °C, and the reaction mixture was stirred at room temperature for 18 h. The mixture was partitioned between EtOAc (30 mL) and water (30 mL), and the organic layer was washed with brine (50 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10−80% EtOAc in *n*-hexane) to give the *tert*-butyl [(1*S*)-2-({(1*S*)-2-[(3*S*,8a*R*)--[(4*R*)-3,4-dihydro-2*H*-chromen-4-ylcarbamoyl]hexahydropyrrolo[1,2-*a*]pyrazin-2(1*H*)-yl]-2-oxo-1-phenylethyl}amino)-1-methyl-2-oxoethyl]-methylcarbamate (200 mg) as a white amorphous powder.

The obtained amorphous powder (200 mg) was dissolved in EtOAc (3 mL), and to the solution was added 4 M HCl in EtOAc (3 mL). The mixture was stirred at room temperature for 2 h, and then concentrated under reduced pressure. The residue was washed with EtOAc (10 mL) and dried under reduced pressure to give **31a** (150 mg, 65%) as a white amorphous powder. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.22−2.14 (10H, m), 2.41 (3H, br s), 2.64−3.29 (3H, m), 3.41−3.97 (4H, m), 4.05−4.76 (4H, m), 4.89−6.26 (2H, m), 6.67−6.99 (2H, m), 7.06−7.64 (7H, m), 8.70−9.61 (3H, m), 12.04 (1H, br s). Anal. Calcd for $C_{29}H_{39}Cl_2N_5O_4 \cdot 1.4H_2O \cdot 0.3Et_2O$: C, 56.67; H, 7.06 N, 10.94. Found: C, 56.85; H, 7.37; N, 11.23.

Benzyl {(1*S*)-1-(4,4-difluorocyclohexyl)-2-[(3*S*,8a*R*)-3-[(4*R*)-3,4-dihydro-2*H*-chromen-4- ylcarbamoyl]hexahydropyrrolo-[1,2-*a*]pyrazin-2(1*H*)-yl]-2-oxoethyl}carbamate (29b). Compound **29b** was prepared by a method similar to that described for **29a** using **27** (1.12g, 2.79 mmol), 4 M HCl in EtOAc (7 mL, 28 mmol), (2*S*)-(4,4-difluorocyclohexyl)[(benzyloxycarbonyl)amino]-ethanoic acid **28b** (985 mg, 3.01 mmol), HATU (1.71 g, 4.50 mmol) and DIPEA (1.57 mL, 9.01 mmol). Yield: 82%, colorless oil (1.51 g); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.12−1.41 (3H, m), 1.51−2.07 (14H, m), 2.09−2.20 (1H, m), 2.87−3.21 (2H, m), 3.46−3.73 (1H, m), 3.94−4.26 (3H, m), 4.32−4.56 (1H, m), 4.86−5.12 (4H, m), 6.71−6.79 (1H, m), 6.80−6.91 (1H, m), 7.07−7.45 (7H, m), 7.63 (1H, d, J = 8.9 Hz), 8.06−8.22 (1 H, m).

(3*S*,8a*R*)-2-{(2*S*)-2-(4,4-Difluorocyclohexyl)-2-[(*N*-methyl-L-alanyl)amino]acetyl}-*N*-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl]-octahydropyrrolo[1,2-*a*]pyrazine-3-carboxamide dihydrochloride (31b). Compound **31b** was prepared by a method similar to that described for **31a** using **29b** (1.50g, 2.46 mmol), 10% Pd/C (300 mg), *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-alanine **30** (716 mg, 3.52 mmol), HATU (1.79 g, 4.71 mmol), DIPEA (1.64 mL, 9.41 mmol) and 4 M HCl in EtOAc (8 mL). Yield: 58%, white amorphous powder (922 mg); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.23−1.48 (5H, m), 1.58−2.22 (14H, m), 2.41−2.48 (3H, m), 2.92−3.18 (1H, m), 3.47−4.29 (7H, m), 4.44−4.75 (2H, m), 4.80−5.06 (2H, m), 6.58−7.54 (4H, m), 8.50−9.28 (3H, m), 9.33−9.73 (1H, m), 11.09−12.69 (1H, m); Anal. Calcd for $C_{29}H_{43}Cl_2F_2N_5O_4$+2.0$H_2O$: C, 51.94; H, 7.06; N, 10.44. Found: C, 51.94; H, 7.20; N, 10.09.

(3*S*,8a*R*)-*N*-[(4*R*)-3,4-Dihydro-2*H*-chromen-4-yl]-2-[(2*S*)-2-[(*N*-methyl-L-alanyl)amino]-2-(tetrahydro-2*H*-pyran-4-yl)acetyl]-octahydropyrrolo[1,2-*a*]pyrazine-3-carboxamide dihydrochloride (31c). To a solution of **27** (450 mg, 1.12 mmol) in EtOAc (3 mL) was added 4 M HCl in EtOAc (3 mL), and the mixture as stirred at room temperature for 1 h. The mixture was concentrated under reduced pressure, and the residue was washed with ethyl ether (10 mL) and dried under reduced pressure to give (3*S*,8a*R*)-*N*-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl]octahydropyrrolo[1,2-*a*]pyrazine-3-carboxamide (410 mg, 1.10 mmol). The obtained product (200 mg, 0.534 mmol), (2*S*)-{[(benzyloxy)carbonyl]amino}(tetrahydro-2*H*-pyran-4-yl)ethanoic acid **28c** (235 mg, 0.801 mmol), HATU (406 mg, 1.068 mmol) and DIPEA (0.558 mL, 3.20 mmol) were mixed in DMF (5 mL) at 0 °C, and the reaction mixture was stirred at room temperature for 18 h. The mixture was partitioned between EtOAc (30 mL) and water (30 mL), and the organic layer was washed with sat. $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by

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silica gel column chromatography (15−100% EtOAc in *n*-hexane) to give benzyl [(1*S*)-2-[(3*S*,8a*R*)-3-[(4*R*)-3,4-dihydro-2*H*-chromen-4-ylcarbamoyl]hexahydropyrrolo[1,2-*a*]pyrazin-2(1*H*)-yl]-2-oxo-1-(tetrahydro-2*H*-pyran-4-yl)ethyl]carbamate **29c** (200 mg) as a white amorphous powder.

The obtained product **29c** (200 mg) was dissolved in 5−10% HCl solution in methanol (10 mL), and then 10% Pd/C (40 mg) was added to the solution. The mixture was stirred at room temperature for 1 h under hydrogen pressure (2 atm). The mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure to give (3*S*,8a*R*)-2[(2*S*)-2-amino-2-(tetrahydro-2*H*-pyran-4-yl)acetyl]-*N*-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl]-octahydropyrrolo[1,2-*a*]pyrazine-3-carboxamide dihydrochloride as colorless oil. The obtained colorless oil, *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-alanine **30** (106 mg, 0.522 mmol), HATU (264 mg, 0.694 mmol) and DIPEA (0.363 mL, 2.084 mmol) were mixed in DMF (5 mL) at 0 °C, and the reaction mixture was stirred at room temperature for 18 h. The mixture was partitioned between EtOAc (30 mL) and water (30 mL), and the organic layer was washed with sat. $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10−100% EtOAc in hexane) to give *tert*-butyl [(1*S*)-2-{[(1*S*)-2-[(3*S*,8a*R*)-3-[(4*R*)-3,4-dihydro-2*H*-chromen-4-ylcarbamoyl]hexahydropyrrolo[1,2-*a*]pyrazin-2(1*H*)-yl]-2-oxo-1-(tetrahydro-2*H*-pyran-4-yl)ethyl]amino}-1-methyl-2-oxoethyl]-methylcarbamate (180 mg) as a white amorphous powder.

The obtained amorphous powder (180 mg) was dissolved in EtOAc (3 mL), and 4 M HCl in EtOAc (3 mL) was added to the solution. The mixture was stirred at room temperature for 2 h and concentrated under reduced pressure. The residue was washed with EtOAc (5 mL) and dried under reduced pressure to give **31c** (150 mg, 72%) as a white amorphous powder; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.38 (6H, d, J = 6.8 Hz), 1.50−2.22 (11H, m), 3.05 (1H, brs), 3.37 (3H, s), 3.41−3.72 (6H, m), 4.19 (3H, t, J = 5.0 Hz), 4.40−4.73 (2H, m), 4.77−5.03 (2H, m), 6.63−6.96 (2H, m), 7.02−7.26 (2H, m), 8.51−9.07 (3H, m), 9.14−9.54 (1H, m), 12.08 (1H, br s). Anal. Calcd for $C_{28}H_{43}Cl_2N_5O_5\cdot0.5HCl\cdot2.0H_2O$: C, 51.36; H, 7.31; N, 10.69. Found: C, 51.36; H, 7.49; N, 10.63.

2-*tert*-Butyl 3-ethyl (3*S*,8a*S*)-hexahydropyrrolo[1,2-*a*]-pyrazine-2,3(1*H*)-dicarboxylate (33). A solution of ethyl 2,3-dibromopropanoate **32** (195 g, 793 mmol) in toluene (500 mL) was added to a solution of **3b** (158.4 g, 832 mmol) and triethylamine (267.3 mL, 1.92 mol) in toluene (1.0 L) at room temperature, and the reaction mixture was stirred at 90 °C for 18 h. After being cooled to room temperature, the mixture was filtered, and the filtrated was partitioned between EtOAc (1.0 L) and water (500 mL). The organic layer was washed with sat. $NaHCO_3$ (500 mL) and brine (500 mL), dried over $MgSO_4$, and concentrated under reduced pressure to give a pale-yellow oil (224 g). A solution of the oil in ethanol (1.0 L) was added to a suspension of 10% Pd/C (22.4 g) in sat.HCl in ethanol (1.0 L), and the mixture was stirred at 50 °C for 18 h under hydrogen pressure (3 atm). The mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure to give a pale-yellow oil. The oil was dissolved in sat. $NaHCO_3$/THF (1:1, 1.0 L), and di-*tert*-butyl dicarbonate (187 g, 857 mmol) was added to the solution. The reaction mixture was stirred at room temperature for 18 h, and then extracted with EtOAc (1.0 L). The organic layer was washed with sat. $NaHCO_3$ (500 mL) and brine (500 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10−100% EtOAc in *n*-hexane) to give **33** (75.1 g, 33%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.27 (3H, t, J = 7.2 Hz), 1.43 (9H, s), 1.44−1.53 (1H, m), 1.69−1.78 (1H, m), 1.80−1.89 (2H, m), 2.70−2.74 (2H, m), 2.97−3.03 (3H, m), 3.22−3.27 (1H, m), 3.73 (1H, dd, J = 13.2, 4.0 Hz), 4.16−4.24 (2H, m), 4.36 (1H, t, J = 5.6 Hz).

***tert*-Butyl (3*S*,8a*S*)-3-[(4*R*)-3,4-dihydro-2*H*-chromen-4-ylcarbamoyl]hexahydropyrrolo[1,2-*a*]pyrazine-2(1*H*)-carboxylate (34).** Lithium hydroxide monohydrate (141 mg, 3.36 mmol) was added to a solution of **33** (670 mg, 2.25 mmol) in THF (4 mL) and water (1 mL) at room temperature, and the reaction mixture was stirred at 50 °C for 3 h. The reaction mixture was allowed to cool at room temperature and neutralized with 1 M HCl (3.1 mL). The mixture was concentrated under reduced pressure, and then the residue was dissolved in DMF (10 mL). To the solution were added (*R*)-chroman-4-amine hydrochloride (500 mg, 2.69 mmol), EDC (1.98 mL, 11.2 mmol), HOBt (363 mg, 2.69 mmol) and DIPEA (0.469 mL, 2.69 mmol) at room temperature, and the reaction mixture was stirred at room temperature for 18 h. The solvent was evaporated under reduced pressure and the residue was partitioned between EtOAc (300 mL) and water (100 mL). The organic layer was dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10−20% methanol in EtOAc) to give **34** (381 mg, 42%) as a white amorphous powder; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.26−1.47 (10H, m), 1.48−2.06 (5H, m), 2.55−2.77 (2H, m), 2.82−3.22 (4H, m), 3.48−3.62 (1H, m), 4.19 (3H, t, J = 5.4 Hz), 5.02 (1H, q, J = 6.9 Hz), 6.70−6.89 (2H, m), 7.06−7.26 (2H, m), 8.31 (1H, d, J = 8.1 Hz).

(3*S*,8a*S*)-2-{(2*S*)-2-(4,4-Difluorocyclohexyl)-2-[(*N*-methyl-L-alanyl)amino]acetyl}-*N*-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl]-octahydropyrrolo[1,2-*a*]pyrazine-3-carboxamide dihydrochloride (36). The amorphous powder **34** (381 mg, 0.949 mmol) was dissolved in 4 M HCl in EtOAc (2 mL), and the mixture was stirred at room temperature for 2 h. The resulting precipitate was collected by filtration, and dried under a vacuum to give (3*S*,8a*S*)-*N*-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl]octahydropyrrolo[1,2-*a*]-pyrazine-3-carboxamide dihydrochloride as colorless amorphous powder (355 mg, 0.984 mmol). To a solution of the obtained amorphous powder (200 mg, 0.534 mmol), (2*S*)-{[(benzyloxy)-carbonyl]amino}(4,4-difluorocyclohexyl)ethanoic acid **28b** (192 mg, 0.587 mmol) and DIPEA (0.186 mL, 1.07 mmol) in DMF (3 mL) was added HATU (406 mg, 1.07 mmol) at room temperature, and the reaction mixture was stirred at room temperature for 2 h. The mixture was partitioned between EtOAc (250 mL) and water (50 mL). The organic layer was washed with brine (50 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was dissolved in methanol (10 mL), and 20% $Pd(OH)_2$/C (35 mg) was added to the solution. The mixture was stirred at room temperature for 5 h under hydrogen pressure (3 atm). The mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure to give (3*S*,8a*S*)-2-[(2*S*)-2-amino-2-(4,4-difluorocyclohexyl)acetyl]-*N*-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl]octahydropyrrolo[1,2-*a*]-pyrazine-3-carboxamide **35** (820 mg) as white amorphous solid.

To a solution of the obtained **35**, *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-alanine **30** (109 mg, 0.536 mmol) and DIPEA (0.186 mL, 1.07 mmol) in DMF (3 mL) was added HATU (305 mg, 0.802 mmol) at room temperature. After being stirred at room temperature for 1 h, the mixture was partitioned between EtOAc (250 mL) and water (100 mL). The organic layer was washed with brine (50 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (30−100% EtOAc in *n*-hexane, then 0−10% methanol in EtOAc) to give colorless oil (90 mg). The oil was dissolved in 4 M HCl in EtOAc (3 mL) and the solution was stirred at room temperature for 1 h. To the solution was added ethyl ether (10 mL), and the resulting precipitate was collected by filtration, and dried under reduced pressure to give **36** (56 mg, 17%) as a white amorphous powder; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.28−1.49 (5H, m), 1.61−2.31 (14H, m), 2.43−2.48 (3H, m), 3.07 (1H, d, J = 8.5 Hz), 3.47−3.73 (3H, m), 3.78−4.30 (5H, m), 4.67 (1H, t, J = 5.3 Hz), 4.80 (1H, t, J = 7.2 Hz), 4.93−5.04 (1H, m), 6.75−6.95 (2H, m), 7.16 (2H, t, J = 6.9 Hz), 8.65−9.04 (3H, m), 9.19−9.47 (1H, m), 10.88−11.28 (1H, m); Anal. Calcd for $C_{29}H_{43}Cl_2F_2N_5O_4\cdot2.5H_2O$: C, 51.25; H, 7.12; N, 10.30. Found: C, 51.10; H, 7.16; N, 10.10.

***tert*-Butyl (3*S*,8a*R*)-3-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl-carbamoyl]-7,7-difluorohexahydropyrrolo[1,2-*a*]pyrazine-2(1*H*)-carboxylate (37).** Lithium hydroxide monohydrate (147 mg, 3.50 mmol) was added to a solution of **15** (700 mg, 2.18 mmol) in THF/water (4.3:1, 25 mL) at room temperature, and the mixture was stirred at 50 °C for 5 h. The reaction mixture was allowed to cool at room temperature and neutralized with 1 M HCl (3.5 mL). The mixture was

concentrated under reduced pressure, and then the residue was dissolved in DMF (20 mL). To the solution were successively added EDC-HCl (2.51 g, 13.1 mmol), HOBt (354 mg, 2.62 mmol), DIPEA (0.761 mL, 4.37 mmol) and (*R*)-chroman-4-amine hydrochloride (609 mg, 3.28 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 18 h. The mixture was partitioned between EtOAc (200 mL) and water (5 mL), and the organic layer was washed with sat. $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (80−100% EtOAc in *n*-hexane) to give **37** (450 mg, 44%) as a white amorphous powder; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.36−1.44 (9H, m), 1.76−2.09 (4H, m), 2.21−2.57 (4H, m), 2.91−3.14 (1H, m), 3.28−3.48 (1H, m), 3.84−3.98 (1H, m), 4.15−4.26 (2H, m), 4.38−4.57 (1H, m), 4.97−5.13 (1H, m), 6.73−6.88 (2H, m), 7.09−7.19 (2H, m), 8.23−8.43 (1H, m).

Benzyl {(1*S*)-1-(4,4-difluorocyclohexyl)-2-[(3*S*,8a*R*)-3-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl-carbamoyl]-7,7-difluorohexahydropyrrolo[1,2-*a*]pyrazin-2(1*H*)-yl]-2-oxoethyl}-carbamate (40). To a solution of **37** (1810 mg, 4.14 mmol) in EtOAc (5 mL) was added 4 M HCl in cyclopentyl methyl ether (5 mL), and the reaction mixture was stirred at room temperature for 1 h. The mixture was diluted with EtOAc (5 mL), and the resulting precipitate was collected by filtration to give (3*S*,8a*R*)-*N*-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl]-7,7-difluorooctahydropyrolo[1,2-*a*]-pyrazine-3-carboxamide dihydrochloride as a colorless amorphous powder (1.46 g).

The obtained colorless amorphous powder (1.53 g), (2*S*)-{[(benzyloxy)carbonyl]amino}(4,4- difluorocyclohexyl)ethanoic acid (1.22 g, 3.72 mmol), HATU (2.83 g, 7.43 mmol) and DIEPA (3.89 mL, 22.3 mmol) were mixed in DMF (15 mL) at 0 °C, and the reaction mixture was stirred at room temperature for 18 h. The mixture was partitioned between EtOAc (200 mL) and water (100 mL). The organic layer was washed with sat. $NaHCO_3$ (100 mL) and brine (50 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (20−60% EtOAc in *n*-hexane) to give **40** (393 mg, 17%) as a white amorphous powder. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.22−1.44 (2H, m), 1.55−2.08 (10H, m), 2.28 (3H, m), 2.63 (1H, s), 3.26 (1H, m), 3.37−3.70 (2H, m), 4.07−4.25 (3H, m), 4.48 (1H, m), 4.80−5.13 (4H, m), 6.68−6.91 (2H, m), 7.07−7.72 (8H, m), 8.09−8.29 (1H, m).

(3*S*,8a*R*)-2-{(2*S*)-2-(4,4-Difluorocyclohexyl)-2[(*N*-methyl-L-alanyl)amino]acetyl}-*N*-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl]-7,7-difluorooctahydropyrrolo[1,2-*a*]pyrazine-3-carboxamide dihydrochloride (43). To a solution of **40** (2.39 g, 3.69 mmol) in 5−10% HCl in methanol (10 mL) was added 10% Pd/C (300 mg), and the reaction mixture was stirred at room temperature for 2 h under hydrogen pressure (3 atm). The mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure to give (3*S*,8a*R*)-2-[(2*S*)-2-amino-2-(4,4-difluorocyclohexyl)acetyl]-*N*-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl]-7,7-difluorooctahydropyrrolo-[1,2-*a*]pyrazine-3-carboxamide dihydrochloride (2.0 g) as a colorless oil.

A solution of the obtained colorless oil in DMF (5 mL) was added a solution of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-alanine (1.04 g, 5.12 mmol), EDC-HCl (1.97 g, 10.3 mmol), HOBt (554 mg, 4.10 mmol) and DIPEA (1.79 mL, 10.3 mmol) in DMF (10 mL), and the reaction mixture was stirred at room temperature for 18 h. The mixture was partitioned between EtOAc (200 mL) and water (100 mL). The organic layer was washed with sat. $NaHCO_3$ (100 mL) and brine (100 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was crystallized from EtOAc (15 mL) and *n*-hexane (5 mL) to give *tert*-butyl [(1*S*)-2-({(1*S*)-1-(4,4-difluorocyclohexyl)-2-[(3*S*,8a*R*)-3-[(4*R*)-3,4-dihyro-2*H*-chromen-4-yl-carbamoyl]-7,7-difluorohexahydropyrrolo[1,2-a]pyrazin-2(1*H*)-yl]-2-oxoethyl}-amino)-1-methyl-2-oxoethyl]methylcarbamate (1.40 g) as colorless crystals. mp 202−204 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.20 (4H, m), 1.39 (9H, s), 1.54−2.07 (12H, m), 2.15−2.43 (3H, m), 2.54 (2H, m), 3.7 (1H, m), 3.35−3.71 (2H, m), 4.03 (2H, m), 4.20 (2H, m), 4.38−5.14 (4H, m), 6.67−6.96 (2H, m), 7.02−7.31 (2H, m), 7.91 (1H, m), 8.12−8.43 (1H, m).

The obtained crystals (250 mg, 0.358 mmol) were dissolved in methanol (2 mL), and to the solution was added 4 M HCl in cyclopentyl methyl ether (5 mL), and the mixture was stirred at room temperature or 1 h. The mixture was diluted with ethyl ether (5 mL), and the resulting precipitate was collected by filtration to give **43** (150 mg, 33%) as colorless amorphous powder. The filtrate was concentrated under reduced pressure to give additional **43** (70 mg, 16%) as a white amorphous powder; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.35 (5H, d, J = 6.8 Hz), 1.42−2.15 (14H, m), 2.41−2.48 (4H, m), 3.15 (2H, s), 3.29−3.97 (5H, m), 4.64−5.12 (3H, m), 6.67−6.96 (2H, m), 7.04−7.34 (2H, m), 8.38 (1H, br s), 8.59−8.97 (2H, m), 9.29 (1H, br s); Anal. Calcd for $C_{29}H_{41}Cl_2F_4N_5O_4\cdot1.0H_2O\cdot0.8C_4H_{10}O$: C, 51.71; H, 6.87; N, 9.36. Found: C, 51.55; H, 6.77; N, 9.39.

***tert*-Butyl (3*S*,7*R*,8a*R*)-3-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl-carbamoyl]-7-hydroxyhexahydropyrrolo[1,2-*a*]pyrazine-2(1*H*)-carboxylate (38).** Compound **38** was prepared by a method similar to that described for **37** using **13** (2.00 g, 6.66 mmol), lithium hydroxide monohydrate (418 mg, 9.96 mmol), (*R*)-chroman-4-amine hydrochloride (1.48 g, 7.97 mmol), DIPEA (2.32 mL, 13.3 mmol), HOBt (1.08 g, 7.99 mmol) and EDC (3.53 mL, 19.9 mmol). Yield: 77%, white amorphous powder (2.15 g); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.08−1.30 (1H, m), 1.31−1.50 (9H, m), 1.60−2.32 (6H, m), 2.62−3.19 (2H, m), 3.21−3.48 (1H, m), 3.72−3.95 (1H, m), 4.03−4.32 (3H, m), 4.32−4.59 (1H, m), 4.69−4.88 (1H, m), 4.90−5.19 (1H, m), 6.59−7.00 (2H, m), 7.04−7.27 (2H, m), 8.06−8.55 (1H, m).

Benzyl {(1*S*)-1-(4,4-difluorocyclohexyl)-2-[(3*S*,7*R*,8a*R*)-3-[(4*R*)-3,4-dihydro-2*H*-chromen-4-ylcarbamoyl]-7-hydroxyhexahydropyrrolo[1,2-*a*]pyrazin-2(1*H*)-yl]-2-oxoethyl}carbamate (41). The amorphous powder **38** (1.00 g, 2.40 mmol) was dissolved in 4 M HCl in EtOAc (10 mL), and the mixture was stirred at room temperature for 15 h. The solution was concentrated under reduced pressure to give (3*S*,7*R*,8a*R*)-*N*-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl]-7-hydroxyoctahydropyrrolo [1,2-*a*]-pyrazine-3-carboxamide dihydrochloride (840 mg) as a colorless amorphous powder.

To a solution of the obtained powder, HOBt (340 mg, 2.52 mmol), DIPEA (1.13 mL, 6.45 mmol) and EDC (1.14 mL, 6.44 mmol) in DMF (10 mL) was added (2*S*)-{[(benzyloxy)carbonyl]amino}(4,4-difluorocyclohexyl)ethanoic acid **28b** (775 mg, 2.37 mmol) at room temperature, and the reaction mixture was stirred at room temperature for 15 h. The mixture was partitioned between EtOAc (300 mL) and water (50 mL). The organic layer was washed with brine (50 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (5−100% EtOAc in *n*-hexane, then 0−10% methanol in EtOAc) to give **41** (575 mg, 38%) as a white amorphous solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.08−1.49 (3H, m), 1.50−2.46 (13H, m), 2.54−3.06 (2H, m), 3.06−3.74 (2H, m), 3.80−4.30 (3H, m), 4.30−4.54 (1H, m), 4.63−5.27 (5H, m), 6.69−6.80 (1H, m), 6.80−6.95 (1H, m), 7.00−7.47 (7H, m), 7.49−8.52 (2H, m).

(3*S*,7*R*,8a*R*)-2-{(2*S*)-2-(4,4-Difluorocyclohexyl)-2-[(*N*-methyl-L-alanyl)amino]acetyl}-*N*-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl]-7-hydroxyoctahydropyrrolo[1,2-*a*]pyrazine-3-carboxamide dihydrochloride (44). To a solution of **41** (500 mg) in 4 M HCl in methanol (10 mL) was added 20% $Pd(OH)_2$/C (100 mg), and the reaction mixture was stirred at room temperature for 18 h under hydrogen atmosphere. The mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure to give (3*S*,7,8a*R*)-2-[(2*S*)-2-amino-2-(4,4-difluorocyclohexyl)acetyl]-*N*-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl]-7-hydroxyoctahydropyrrolo[1,2-*a*]pyrazine-3-carboxamide as a colorless oil (383 mg).

To a solution of the obtained colorless oil (383 mg), *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-alanine **30** (190 mg, 0.935 mmol), HOBt (116 mg, 0.858 mmol) and DIPEA (0.272 mL, 1.562 mmol) in DMF (5 mL) was added EDC (0.413 mL, 0.212 mmol), and the reaction mixture was stirred at room temperature for 3 h, and partitioned between EtOAc (100 mL) and water (25 mL). The organic layer was washed with brine (25 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column

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chromatography (5−100% EtOAc in *n*-hexane) to give *tert*-butyl [(1*S*)-2-({(1*S*)-1-(4,4-difluorocyclohexyl)-2-[(3*S*,7*R*,8a*R*)-3-(4*R*)-3,4-dihydro-2*H*-chromen-4-yl-carbamoyl]-7-hydroxyhexahydropyrrolo-[1,2-*a*]pyrazin-2(1*H*)-yl]-2-oxoethyl}amino)-1-methyl-2-oxoethyl]-methylcarbamate (144 mg) as a white amorphous solid; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.08−1.30 (7H, m), 1.39 (9H, br s), 1.52−.29 (15H, m), 2.57−2.89 (2H, m), 3.08−3.97 (2H, m), 4.09−4.29 (3H, m), 4.30−5.26 (5H, m), 6.62−6.95 (2H, m), 7.04−7.34 (2H, m), 7.54−8.47 (2H, m).

The obtained colorless amorphous solid (130 mg, 0.192 mmol) was dissolved in 4 M HCl in cyclopentyl methyl ether (5 mL), and the solution was stirred at room temperature for 2 h. The solution was concentrated under reduced pressure, and the residue was washed with ethyl ether (20 mL) and dried under reduced pressure to give **44** (93 mg, 20%) as a white amorphous powder; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.23−1.53 (5H, m), 1.54−2.38 (10H, m), 2.41−2.49 (3H, m), 3.02−4.30 (10H, m), 4.32−4.77 (3H, m), 4.78−5.11 (2H, m), 5.78 (1H, br s), 6.65−6.98 (2H, m), 7.04−7.58 (2H, m), 8.28−9.69 (4H, m), 11.71−12.92 (1H, m); Anal. Calcd for $C_{29}H_{43}Cl_2F_2N_5O_5$·2.5$H_2O$: C, 50.07; H, 6.96 N, 10.07. Found: C, 49.95; H, 7.30; N, 9.73.

***tert*-Butyl (3*S*,7*R*,8a*R*)-3-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl-carbamoyl]-7-ethoxyhexahydropyrrolo [1,2-*a*]pyrazine-2(1*H*)-carboxylate (39).** Compound **39** was prepared by the similar method to that described for **37** using **19** (3.60 g, 11.0 mmol), lithium hydroxide monohydrate (736 mg, 13.2 mmol), (*R*)-chroman-4-amine hydrochloride (2.14 g, 11.5 mmol), DIPEA (5.73 mL, 32.9 mmol), HOBt (1.78g, 13.2 mmol) and EDC-HCl (6.30g, 32.9 mmol). Yield: 83%, white amorphous powder (4.04 g); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.09 (3H, t, J = 7.1 Hz), 1.15−1.35 (1H, m), 1.35−1.45 (9H, m), 1.65−2.25 (6H, m), 2.87−3.08 (2H, m), 3.33 (2H, q, J = 7.1 Hz), 3.30−3.45 (1H, m), 3.80−4.00 (2H, m), 4.15−4.35 (2H, m), 4.37−4.53 (1H, m), 5.02−5.14 (1H, m), 6.73−6.89 (2H, m), 7.10−7.19 (2H, m), 8.20−8.40 (1H, m).

Benzyl {(1*S*)-1-(4,4-difluorocyclohexyl)-2-[(3*S*,7*R*,8a*R*)-3-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl- carbamoyl]-7-ethoxyhexahydropyrrolo[1,2-*a*]pyrazin-2(1*H*)-yl]-2-oxoethyl}-carbamate (42). The amorphous powder **39** (2.03 g, 4.56 mmol) was dissolved in 4 M HCl in EtOAc (30.5 mL) and the mixture was stirred at room temperature for 1 h, and then concentrated under reduced pressure. The residue was diluted with EtOAc/diethyl ether (1:1, 20 mL), and the resulting precipitate was collected by filtration to give (3*S*,7*R*,8a*R*)-*N*-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl]-7-ethoxyoctahydropyrrolo [1,2-*a*]pyrazine-3-carboxamide dihydrochloride as a colorless amorphous powder.

To a solution of (2*S*)-{[(benzyloxy)carbonyl]amino}(4,4-difluorocyclohexyl)ethanoic acid (1.64 g, 5.01 mmol), HOBt (0.74 g, 5.48 mmol) and DIPEA (2.50 mL, 14.4 mmol) in DMF (30.5 mL) was added EDC-HCl (2.62 g, 13.7 mmol) at 0 °C, and the mixture was stirred at room temperature for 15 min. To the mixture was added the obtained amorphous powder at 0 °C, and the reaction mixture was stirred at room temperature for 48 h. The mixture was partitioned between EtOAc (300 mL) and water (200 mL), and the organic layer was washed with sat.$NaHCO_3$ (100 mL) and brine (100 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (50−100% EtOAc in *n*-hexane) to give **42** (2.43 g, 81%) as a white amorphous powder; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.10 (3H, t, J = 7.1 Hz), 1.15−1.42 (2H, m), 1.50−2.24 (13H, m), 2.82−3.73 (5H, m), 3.80−4.27 (3H, m), 4.28−5.18 (6H, m), 6.67−6.92 (2H, m), 7.06−7.18 (2H, m), 7.19−7.79 (7H, m), 8.08−8.24 (1H, m).

(3*S*,7*R*,8a*R*)-2-{(2*S*)-2-(4,4-Difluorocyclohexyl)-2-[(*N*-methyl-L-alanyl)amino]acetyl}-*N*-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl]-7-ethoxyoctahydropyrrolo[1,2-*a*]pyrazine-3-carboxamide (45). To a solution of **42** (2.40 g, 3.67 mmol) in 5−10% HCl solution in methanol (48 mL) was added 10% Pd/C (0.72 g), and the reaction mixture was stirred at room temperature for 3 h under hydrogen atmosphere. The mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure to give (3*S*,7*R*,8a*R*)-2-[(2*S*)-2-amino-2-(4,4-diluorocyclohexyl)acetyl]-*N*-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl]-7-ethoxyoctahydropyrrolo[1,2-*a*]pyrazine-3-carboxamide dihydrochloride as a yellow oil.

To a solution of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-alanine **30** (0.82 g, 4.04 mmol), HOBt (0.59 g, 4.37 mmol) and DIPEA (3.19 mL, 18.1 mmol) in DMF (14.4 mL) was added EDC-HCl (2.11 g, 11.0 mmol) at 0 °C, and the mixture was stirred at room temperature for 15 min. To the mixture was added a solution of (3*S*,7*R*,8a*R*)-2-[(2*S*)-2-amino-2-(4,4-difluorocyclohexyl)acetyl]-*N*-[(4*R*)-3,4-dihydro-2*H*-chromen-4-yl]-7-ethoxyoctahydropyrrolo[1,2-*a*]pyrazine-3-carboxamide dihydrochloride and DIPEA (0.64 mL, 0.742 mmol) in DMF (19.2 mL) at room temperature, and the reaction mixture was stirred at room temperature for 16 h. The mixture was partitioned between EtOAc (200 mL) and water (200 mL), and the organic layer was washed with sat. $NaHCO_3$ (100 mL) and brine (100 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (50−100% EtOAc in *n*-hexane) to give *tert*-butyl [(1*S*)-2-({(1*S*)-1-(4,4-difluorocyclohexyl)-2-[(3*S*,7*R*,8a*R*)-3-[(4*R*)-3,4-dihydro-2*H*-chromen-4-ylcarbamoyl]-7-ethoxyhexahydropyrrolo[1,2-*a*]pyrazin-2(1*H*)-yl]-2-oxoethyl}amino)-1-methyl-2-oxoethyl]methylcarbamate (1.69 g, 65%) as a white amorphous solid; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.10 (3H, t, J = 7.1 Hz), 1.10−1.36 (15H, m), 1.55−2.24 (13H, m), 2.57−3.68 (8H, m), 3.86−5.17 (8H, m), 6.70−6.90 (2H, m), 7.07−7.29 (2H, m), 7.65−8.32 (2H, m).

The obtained crystals (1.68 g, 2.38 mmol) were dissolved in 4 M HCl in EtOAc (33.6 mL), and the mixture was stirred at room temperature for 1 h. The mixture was concentrated under reduced pressure. The residue was partitioned between EtOAc/THF (2:1, 120 mL) and sat. $NaHCO_3$ (100 mL), and the organic layer was washed with brine (100 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (5−70% EtOAc in *n*-hexane) and subsequent crystallization from ether/heptane (1:1, 20 mL) to give **45** (1.08 g, 75%) as colorless crystals; mp 171−174 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.01−1.15 (6H, m), 1.15−1.38 (3H, m), 1.54−2.29 (16H, m), 2.63−3.64 (7H, m), 3.88−4.48 (4H, m), 4.59−5.15 (3H, m), 6.70−6.80 (1H, m), 6.80−6.92 (1H, m), 7.07−7.30 (2H, m), 7.88−8.12 (1H, m), 8.12−8.33 (1H, m); Anal. Calcd for $C_{31}H_{45}F_2N_5O_5$: C, 61.47; H, 7.49 N, 11.56. Found: C, 61.54; H, 7.61; N, 11.30.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Includes information methods used in molecular modeling, enzyme assays, cell line and animal models, pharmacokinetics, metabolic stability, and structural analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

PDB ID codes: **26b** with cIAP1: 4HY4, **45** with XIAP: 4HY0, **45** with cIAP1: 4HY5.

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Notes

The authors declare no competing financial interest.

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