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Original article

Design, synthesis and preliminary evaluation of novel pyrrolidine derivatives as matrix metalloproteinase inhibitors

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Abstract

A series of novel pyrrolidine derivatives were designed, synthesized and assayed for their inhibitory activities on matrix metalloproteinase 2 (MMP-2) and aminopeptidase N (AP-N). The results showed that these pyrrolidine derivatives exhibited highly selective inhibition against MMP-2 as compared with AP-N. The hydroxamates **8a–c** were equally or more potent MMP-2 inhibitors than the positive control LY52. The binding mode of the most potent compound **8a** with MMP-2 was proposed. Structure–activity relationships were also briefly discussed.

Keywords: Pyrrolidine derivatives; Synthesis; MMP-2 inhibitors; IC_{50}

1. Introduction

The matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that are involved in the degradation of all components of the extracellular matrix [1]. Currently, more than 20 mammalian MMPs have been discovered, among which MMP-2 is highly involved in the process of tumor invasion and metastasis and has been considered as a promising target for cancer therapy [2,3].

It has been reported that besides the catalytic activity center zinc(II) ion of MMP-2, there are two hydrophobic domains, which are called S1′ pocket and S2′ pocket, respectively. S1′ pocket, the key domain of MMP-2, is deeper and narrower than that of most other MMP subtypes, and S2′ pocket is solvent exposed [4,5]. Currently identified MMP-2 inhibitors shared the following structural character and binding mode: (1) a zinc binding group (ZBG, such as hydroxamate and carboxylate) capable of chelating the active site zinc ion; (2) at least one functional group provides a hydrogen bond interaction with the enzyme backbone; (3) one or more side chains which undergo effective interactions with the enzyme subsites, such as S1′ and S2′ pockets [6,7].

LY52 (see Fig. 1) is a caffeinoyl pyrrolidine derivative designed in our lab to fit and extend into the active pocket of MMP-2 [8]. This compound could significantly block the proteolytic activity of MMP-2. LY52 also suppressed human ovarian carcinoma cell line SKOV3 invasion *in vitro*. Furthermore, a significant inhibition of pulmonary metastasis of Lewis lung carcinoma cells was observed in LY52-administrated mice [9]. We might conclude from these results that LY52 might suppress invasion and metastasis of carcinoma cells via inhibition of MMP-2 proteolytic activities.

In order to identify more potent MMP-2 inhibitors, based on our previous work, we introduced some functional groups (R_1CO, R_2 and COR_3) into *trans*-4-hydroxy-L-proline to form the new integrated structural pattern (see Fig. 1). R_1CO group can be various acyl groups, such as acetyl, (*E*)-3-(3,4-dimethoxyphenyl)acryloyl or 3,4-dimethoxyphenylpropanoyl. R_2 group can be hydroxyl, amino, sulfonyl, or imide group, with *R* or *S* configuration. COR_3 group can be hydroxamate, carboxyl or carboxylate group. R_1CO and R_2 groups might occupy the S1′ and S2′ pockets, respectively, while the COR_3 group might chelate the active site zinc ion. The pyrrolidine scaffold might bond to the enzyme backbone.

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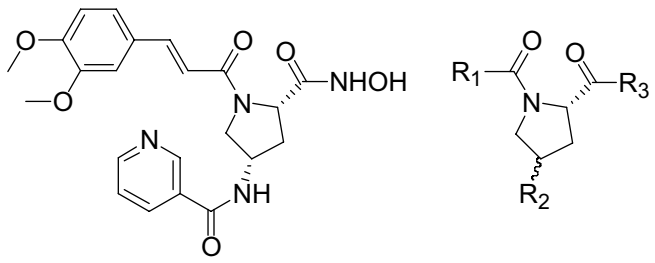


Fig. 1. Chemical structures of LY52 (left) and our further designed pyrrolidine derivatives (right).

2. Chemistry

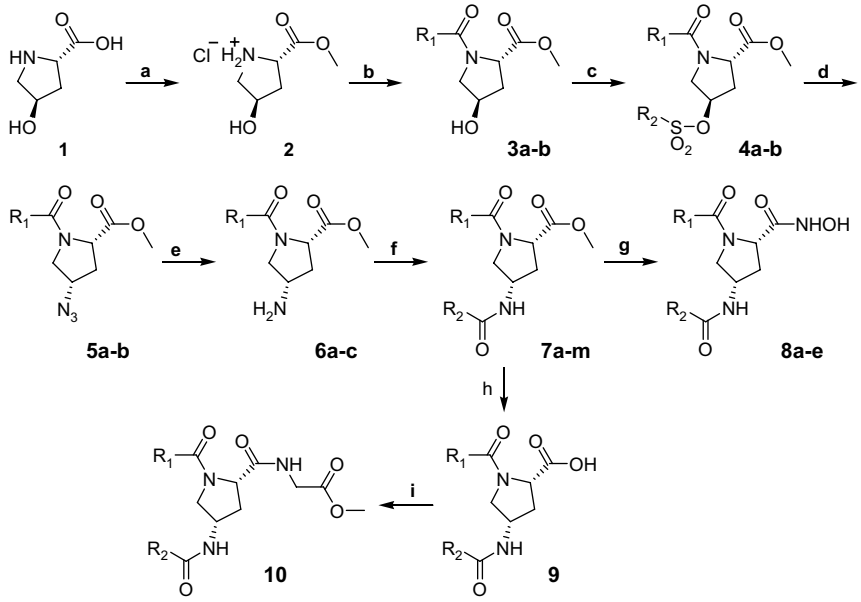
The target compounds were synthesized via the route shown in Scheme 1. Starting from *trans*-4-hydroxy-L-proline (**1**) as a chiral template, the important intermediate (2*S*,4*S*)-methyl 1-acyl-4-aminopyrrolidine-2-carboxylates (**6a–c**) were prepared via the route of esterification [10], acylation, sulfonation [11], SN_2 nucleophilic substitution [12] and catalytic hydrogenation [13]. In the nucleophilic substitution step, (3*R*,5*S*)-5-(methoxycarbonyl)-1-acylpyrrolidin-3-yl sulfonates (**4a**,**b**) reacted with sodium azide to produce configurationally reversed azides (**5a**,**b**), which were then catalytically hydrogenated using 5% Pd–$CaCO_3$ to afford the intermediates **6a–c**. Acylation of **6a–c** with various acyl chlorides provided compounds, (2*S*,4*S*)-methyl 4-acylamido-1-acylpyrrolidine-2-carboxylates (**7a–m**) [14], some of which were further ammonolyzed or hydrolyzed to obtain the target compounds (2*S*,4*S*)-1-acyl-4-(acylamido)-*N*-hydroxypyrrolidine-2-carboxamides (**8a–e**) and (2*S*,4*S*)-4-(3,4,5-trimethoxybenzamido)-1-acetylpyrrolidine-2-carboxylic acid (**9**) [15]. Further coupling of compound **9** with glycine methyl ester gave methyl 2-((2*S*,4*S*)-4-(3,4,5-trimethoxybenzamido)-1-acetylpyrrolidine-2-carboxamido)acetate (**10**). The chemical structures of the target compounds were confirmed by IR, ^1H NMR and ESI-MS.

3. Biological evaluation and discussion

The newly synthesized pyrrolidine derivatives were assayed for the inhibitory activities on MMP-2 and AP-N [16,17]. Similar to MMP-2, AP-N is also a zinc-dependent metalloproteinase involved in the process of tumor invasion and metastasis. Thus the assay was performed on both of MMP-2 and AP-N so as to identify the compound selectivity. LY52 was used as the positive control.

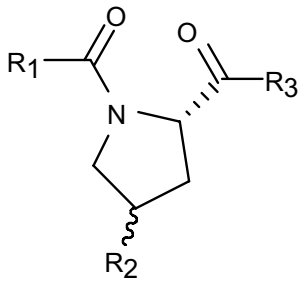
The results showed that these pyrrolidine derivatives exhibited highly selective inhibition against MMP-2 as compared with AP-N, thus confirming our strategy for designing MMP-2 inhibitors (Table 1). The hydroxamates **8a–e** were equally or more potent MMP-2 inhibitors than the positive control LY52. The FlexX docking of the most potent compounds **8a–c** with MMP-2 was done using Sybyl 7.0 of Tripos Incorporation and the result is shown in Figs. 2–4. The R_2 group at C_4 position of pyrrolidine ring (3,4,5-trimethoxybenzamido, nicotinamido, cinnamamido groups, respectively) occupied the deep S1′ pocket of MMP-2, and the hydroxamate group chelated the active site zinc ion 166 with a distance of 0.95, 2.13 and 2.05 Å, respectively. Compounds **8a–c** interacted well with MMP-2 active site, especially the deep S1′ pocket and zinc ion 166, consistent with MMP-2 assay results.

The selective inhibition might be explained by the FlexX docking results of representative compounds **8a–c** with AP-N (Figs. 5–7). Although compounds **8a–c** chelated well with the active site zinc ion 900, the R_2 and R_1CO groups at pyrroliding ring were both solvent exposed and could not occupy the S1′ and S2′ pockets of AP-N. MMP-2 was a zinc-dependent endopeptidase that could cut the peptide to



Scheme 1. Reagents: (a) CH_3OH, HCl; (b) R_1COCl; (c) R_2SO_2Cl; (d) NaN_3; (e) 5% Pd–$CaCO_3/H_2$; (f) R_2COCl; (g) NH_2OK; (h) NaOH/HCl; (i) $H_2NCH_2COOCH_3$.

Table 1
The structures and IC_{50} values of pyrrolidine derivatives



Compound no.	R_1	R_2	R_3	**4***	IC_{50}[a] (μM)	
					MMP-2	AP-N
3a	CH_3	OH	OCH_3	*R*	2.0 ± 0.2	38.6 ± 4.1
3b	CH═$CHC_6H_3(OCH_3)_2$-3′,4′(*E*)	OH	OCH_3	*R*	2.9 ± 0.2	81.8 ± 10.4
4a	CH_3	$OSO_2C_6H_4CH_3$-*p*	OCH_3	*R*	0.3 ± 0.1	30.4 ± 4.9
4b	CH═$CHC_6H_3(OCH_3)_2$-3′,4′(*E*)	OSO_2CH_3	OCH_3	*R*	1.1 ± 0.04	46.6 ± 4.9
5a	CH_3	N_3	OCH_3	*S*	2.6 ± 0.3	73.7 ± 9.4
5b	CH═$CHC_6H_3(OCH_3)_2$-3′,4′(*E*)	N_3	OCH_3	*S*	3.4 ± 0.4	71.3 ± 5.5
6a	CH_3	NH_2	OCH_3	*S*	1.5 ± 0.1	24.1 ± 4.5
6b	CH═$CHC_6H_3(OCH_3)_2$-3′,4′(*E*)	NH_2	OCH_3	*S*	ND	70.6 ± 8.2
6c	$CH_2CH_2C_6H_3(OCH_3)_2$-3′,4′	NH_2	OCH_3	*S*	2.1 ± 0.3	68.4 ± 6.4
7a	CH_3	$NHCOC_6H_5$	OCH_3	*S*	2.7 ± 0.3	122.5 ± 9.6
7b	CH_3	$NHCOC_6H_4Cl$-*p*	OCH_3	*S*	4.2 ± 0.4	121.7 ± 23.4
7c	CH_3	$NHCOC_6H_4Br$-*p*	OCH_3	*S*	6.1 ± 1.0	149.3 ± 15.2
7d	CH_3	$NHCOC_6H_4NO_2$-*p*	OCH_3	*S*	5.2 ± 0.6	72.3 ± 5.5
7e	CH_3	$NHCOC_6H_2(OCH_3)_3$-3′,4′,5′	OCH_3	*S*	0.1 ± 0.01	98.4 ± 12.3
7f	CH_3	$NHCOC_5H_4N$-3′	OCH_3	*S*	1.0 ± 0.1	78.4 ± 8.4
7g	CH_3	$NHCOC_5H_4N$-4′	OCH_3	*S*	2.2 ± 0.2	74.8 ± 8.7
7h	CH_3	$NHCOC_4H_3N_2$-2′,5′	OCH_3	*S*	2.1 ± 0.3	82.6 ± 4.9
7i	CH_3	NHCOCH═CHC_6H_5(*E*)	OCH_3	*S*	0.1 ± 0.01	135.3 ± 26.9
7j	CH_3	NHCOCH═$CHC_6H_3(OCH_3)_2$-3′,4′(*E*)	OCH_3	*S*	0.8 ± 0.1	161.8 ± 18.3
7k	CH═$CHC_6H_3(OCH_3)_2$-3′,4′(*E*)	$NHCOC_5H_4N$-3′	OCH_3	*S*	3.0 ± 0.04	137.3 ± 13.0
7l	$CH_2CH_2C_6H_3(OCH_3)_2$-3′,4′	$NHCOC_5H_4N$-3′	OCH_3	*S*	2.2 ± 0.2	196.4 ± 23.5
7m	$CH_2CH_2C_6H_3(OCH_3)_2$-3′,4′	NHCOCH═CHC_6H_5(*E*)	OCH_3	*S*	1.6 ± 0.3	232.7 ± 42.8
8a	CH_3	$NHCOC_6H_2(OCH_3)_3$-3′,4′,5′	NHOH	*S*	0.001 ± 0.0001	33.5 ± 4.8
8b	CH_3	$NHCOC_5H_4N$-3′	NHOH	*S*	0.005 ± 0.0004	29.8 ± 1.4
8c	CH_3	NHCOCH═CHC_6H_5(*E*)	NHOH	*S*	0.004 ± 0.0005	56.1 ± 5.9
8d	$CH_2CH_2C_6H_3(OCH_3)_2$-3′,4′	$NHCOC_5H_4N$-3′	NHOH	*S*	0.012 ± 0.002	62.2 ± 7.3
8e	$CH_2CH_2C_6H_3(OCH_3)_2$-3′,4′	NHCOCH═CHC_6H_5(*E*)	NHOH	*S*	0.011 ± 0.001	96.6 ± 8.3
9	CH_3	$NHCOC_6H_2(OCH_3)_3$-3′,4′,5′	OH	*S*	0.016 ± 0.002	51.4 ± 6.4
10	CH_3	$NHCOC_6H_2(OCH_3)_3$-3′,4′,5′	$NHCH_2COOCH_3$	*S*	0.109 ± 0.012	121.5 ± 18.4
LY52					0.009 ± 0.0004	141.9 ± 11.7

ND: not determined.
[a] Mean value of three experiments, and standard deviation is given.

parts from the specific amino acid residue of peptide. The motif HEFGHAMGLEH in the catalytic domain where the zinc was coordinated by three histidine residues, and the 'cysteine switch' motif PRCGNPD in the propeptide were conserved structural signatures [2]. However, AP-N was a membrane-bound zinc exopeptidase that catalyzed the removal of NH-terminal amino acid from peptide. AP-N contained the HELAH conserved motif where the zinc was coordinated by two histidine residues and one glutamate residue. The glutamate of the conserved motif acted as a base in catalysis [18]. Due to the structural differences between MMP-2 and AP-N, there were different structural requirements for their respective inhibitors. As these pyrrolidine derivatives exhibited highly selective inhibition against MMP-2, the following structure–activity relationships (SARs) were mainly discussed about MMP-2 inhibition.

Compounds **8a–e** were more potent than their predecessors **7e**, **7f**, **7i**, **7l** and **7m**. This activity difference was caused by the ZBG (COR_3), which was the only structural difference between **8a–e** and their predecessors. The ZBG is hydroxamate (CONHOH) for **8a–e** and carboxylate ($COOCH_3$) for their predecessors. Both these groups could chelate zinc ion at catalytic activity center of the enzyme. However, the hydroxamate group was a more potent ZBG than carboxylate group as shown in the activity order of **8a–e** and their predecessors.

Compound **9** was the only compound containing carboxyl (COOH) ZBG, which exhibited almost equal potency to the hydroxamates. The FlexX docking of **9** with MMP-2 was

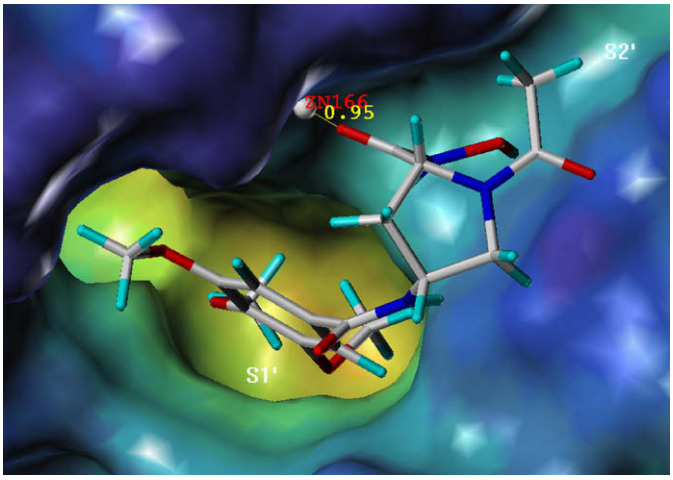


Fig. 2. FlexX docking result of compound **8a** with MMP-2.

also done to elucidate its potency (Fig. 8). The 3,4,5-trimethoxybenzamido group at C_4 position of pyrrolidine ring interacted well with the deep S1′ pocket of MMP-2, and the carboxyl group chelated the active site zinc ion 166 with a distance of 1.96 Å. In comparison with the docking result of counterpart **8a**, some structure–activity relationship information concluded that the hydroxamate group was a better ZBG than carboxyl group.

Among compounds **3a**, **4a**, **5a**, **6a** and **7a–j**, R_1CO and COR_3 groups were fixed as acetyl and carboxylate, respectively, and R_2 group was altered as various length, configurations and substitutions. So the differences in the inhibitory activities of these compounds were caused by various R_2 groups. Introduction of longer R_2 group (**7i**) displayed higher activity. Compound **3a** contained a hydroxyl group at C_4 position of pyrrolidine ring and it was too small to occupy the S1′ pocket entirely. The inhibitory activity of **3a** might be caused by the formation of hydrogen bond with the enzyme backbone, thus stabilizing the binding between the compound and the enzyme. Sulfonation of **3a** produced **4a** with increased activity, due to the sulfonyl group easier to form a hydrogen bond

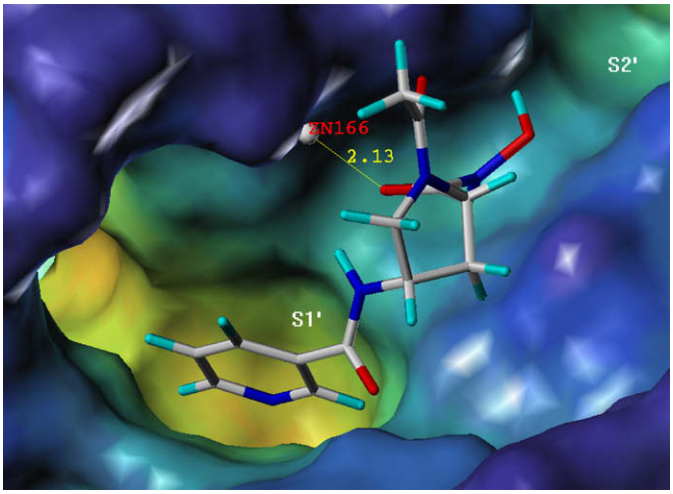


Fig. 3. FlexX docking result of compound **8b** with MMP-2.

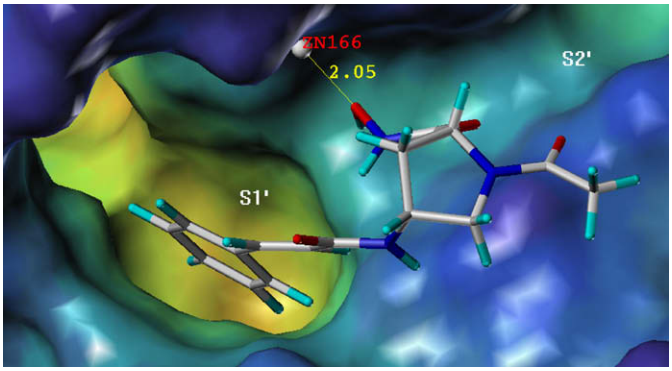


Fig. 4. FlexX docking result of compound **8c** with MMP-2.

with the active domain of enzyme. As compared with **7a**, chloro, bromo and nitro substitutions at the phenyl moiety (**7b–d**) moderately impaired the inhibitory activity, while replacement of the phenyl moiety by heterocyclic moiety (**7f–h**) favored the inhibitory activity. Moreover, the *R* or *S* configuration of these compounds did not seem important to inhibitory activities.

Among compounds **7f**, **7k** and **7l**, R_2 and COR_3 groups were fixed as nicotinamide and carboxylate, respectively, and R_1CO group was altered as acetyl, (*E*)-3-(3,4-dimethoxyphenyl)acryloyl and 3,4-dimethoxyphenylpropanoyl. So the differences in the inhibitory activities of these compounds were caused by various R_1CO groups. The compound bearing the shorter R_1CO group (acetyl, **7f**) was found to be the most potent one, thus we might conclude that compounds with small R_1CO group would exhibit high activities. The compound bearing 3,4-dimethoxyphenylpropanoyl group (**7l**) was in the next place, and the compound containing (*E*)-3-(3,4-dimethoxyphenyl)acryloyl group (**7k**) presented the least activity. This result might be owing to the flexibility of the 3,4-dimethoxyphenylpropanoyl group, which could adjust the conformation to match the catalytic activity center of the enzyme.

Finally, the binding mode of the most potent compound **8a** with MMP-2 was proposed as follows: (1) the hydroxamate chelated the active site zinc ion; (2) the carbonyl, amido and hydroxyl groups provided hydrogen bond interactions with

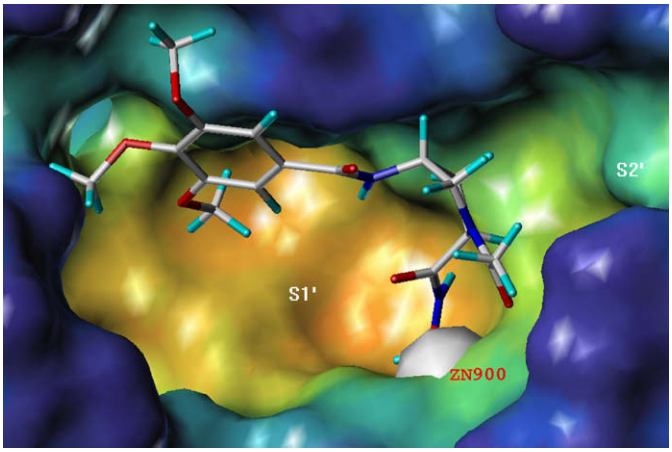


Fig. 5. FlexX docking result of compound **8a** with AP-N.

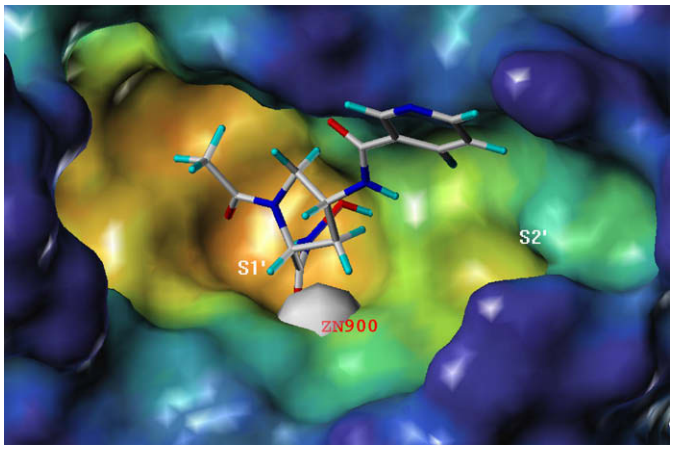


Fig. 6. FlexX docking result of compound **8b** with AP-N.

the enzyme backbone Ala86, Ala84 and Glu121; (3) two side chains (3,4,5-trimethoxybenzamido and acetyl) underwent effective interactions with the enzyme subsites, S1′ and S2′ pockets (Fig. 9). The above binding mode information encouraged us to further design pyrrolidine-scaffold-based MMP-2 inhibitors, which would be reported later.

4. Conclusions

A series of novel pyrrolidine derivatives were designed and synthesized. These pyrrolidine derivatives exhibited highly selective inhibition against MMP-2 as compared with AP-N. The hydroxamates **8a–c** were equally or more potent MMP-2 inhibitors than the positive control LY52. SAR studies indicated that moderately long R_2 group and short R_1 group at pyrrolidine ring favored the inhibitory activity against MMP-2. The FlexX docking was consistent with the above SAR results. Further assays of these compounds on cell culture and animal models are underway.

5. Experimental

5.1. Synthetic methods and spectroscopic details

Melting points were determined using X-6 digital display binocular microscope (uncorrected). Infrared spectra were measured on a Nicolet Nexus 470 FT-IR spectrometer using smear KBr crystal or KBr plate. ^{1}H NMR spectra were recorded on a Bruker Avance (400 MHz) spectrometer; *J* values are in Hz. Mass spectra were recorded on an electrospray ionization mass spectrometer as *m*/*z* value. Flash column chromatography was performed using 300 mesh silica gel. The yields were calculated in the last step reaction.

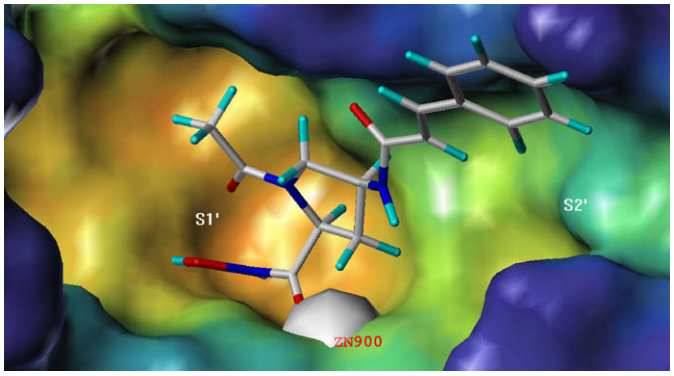


Fig. 7. FlexX docking result of compound **8c** with AP-N.

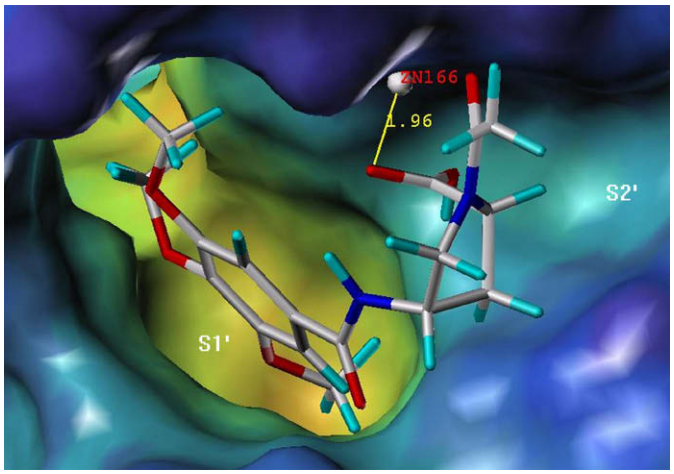


Fig. 8. FlexX docking result of compound **9** with MMP-2.

5.1.1. *trans*-4-Hydroxy-L-proline methyl ester hydrochloride (**2**)

A slurry of *trans*-4-hydroxy-L-proline (**1**) (100 g, 763.5 mmol) in methanol (650 ml) was treated with dry hydrogen chloride until homogeneous. The solution was heated to the reflux temperature for 3 h and concentrated *in vacuo*. Upon cooling, the product was collected by filtration, washed with acetone and ether, and dried under reduced pressure to yield *trans*-4-hydroxy-L-proline methyl ester hydrochloride (**2**) as white crystal (120 g, 87%); mp 157–160 °C (lit. [10] mp 156–160 °C). ^{1}H NMR (CD_3OD, δ ppm): 4.86 (s, 2H, NH_2^+), 4.63 (m, 1H, CH), 3.88 (s, 3H, CH_3), 3.50 (m, 1H, CH), 3.35 (m, 2H, CH_2), 2.47–2.20 (m, 2H, CH_2), 1.31 (s, 1H, OH).

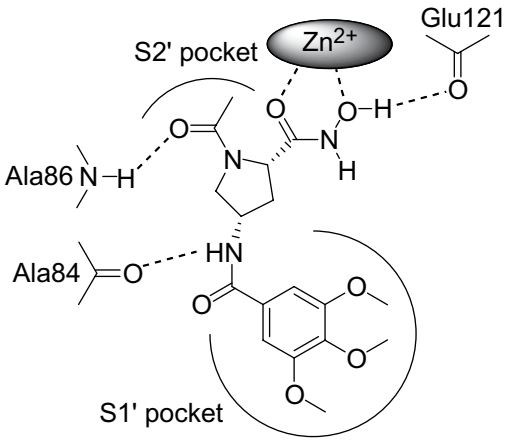


Fig. 9. Proposed binding mode of compound **8a** with MMP-2.

*5.1.2. (2S,4R)-Methyl 1-acyl-4-hydroxypyrrolidine-2-carboxylates (**3a**,**b**)*

To a stirred solution of *trans*-4-hydroxy-L-proline methyl ester hydrochloride (**2**) (64.6 g, 360 mmol) in dioxane–H_2O (1:1, 200 ml) at 0 °C was added $NaHCO_3$ (62.8 g, 750 mmol) in small portions over 10 min, together with acetic anhydride (37 ml, 390 mmol), dropwise over 30 min. After 2 h at 23 °C, the resulting solution was concentrated, diluted with H_2O (100 ml) and saturated with NaCl, and extracted with chloroform (10 × 100 ml). The pooled extracts were dried, filtered, and concentrated to a solid that was recrystallized from ethyl acetate–ether to yield (2*S*,4*R*)-methyl 1-acetyl-4-hydroxypyrrolidine-2-carboxylate (**3a**) as white crystal (51.9 g, 78%); mp 81–82 °C (lit. [11] mp 80–81 °C). IR (KBr, cm^{-1}): 3367.34 (OH), 2938.12 (CH), 1746.51 (C=O), 1613.95 (C=O), 1196.52 (C–O); ^1H NMR ($CDCl_3$, δ ppm): 4.56 (t, 1H, CH, $J = 7.8$ Hz), 3.78 (m, 2H, CH_2), 3.73 (s, 3H, OCH_3), 3.51 (m, 1H, CH), 2.32 (m, 2H, CH_2), 2.08 (s, 3H, CH_3CO), 1.97 (s, 1H, OH); ESI-MS: 188.5 (M + 1).

trans-4-Hydroxy-L-proline methyl ester hydrochloride (**2**) (18.5 g, 100 mmol) was suspended in pyridine (200 ml), and Et_3N (30 ml) was added. After stirring for 20 min at room temperature, the resulting mixture was cooled to −5 °C, and a solution of (*E*)-3-(3,4-dimethoxyphenyl)acryloyl chloride (22.7 g, 100 mmol) in dichloromethane (1000 ml) was added. After stirring overnight at room temperature, the resulting mixture was filtered and the filtrate was evaporated to give crude oil. The crude oil was dissolved in dichloromethane (2000 ml), washed with 3 M HCl and H_2O, and dried to give yellow solid, which was then recrystallized from dichloromethane to provide (2*S*,4*R*)-methyl 4-hydroxy-1-((*E*)-3-(3,4-dimethoxyphenyl)acryloyl)pyrrolidine-2-carboxylate (**3b**) as yellow crystal (27.7 g, 81%); mp 60–62 °C. IR (KBr, cm^{-1}): 3416.25 (OH), 2951.09 (CH), 1744.64 (C=O), 1645.18 (C=O), 1513.81 (C=C), 1267.46 (C–O), 1023.50 (=CH); ^1H NMR ($CDCl_3$, δ ppm): 7.61 (d, 1H, =CH, $J = 15.36$ Hz), 7.05 (d, 1H, Ar–H, $J = 7.60$ Hz), 6.99 (s, 1H, Ar–H), 6.80 (d, 1H, Ar–H, $J = 8.28$ Hz), 6.51 (d, 1H, =CH, $J = 15.40$ Hz), 4.72 (t, 1H, CH, $J = 7.92$ Hz), 4.62 (s, 1H, CH), 3.94 (m, 2H, CH_2), 3.91 (s, 6H, $ArOCH_3$), 3.75 (s, 3H, $COOCH_3$), 2.35 (m, 1H, OH), 2.12 (m, 2H, CH_2); ESI-MS: 336.6 (M + 1).

*5.1.3. (3R,5S)-5-(Methoxycarbonyl)-1-acylpyrrolidin-3-yl sulfonates (**4a**,**b**)*

To a solution of *p*-toluene sulfonyl chloride (12.5 g, 65.6 mmol) in pyridine (25 ml) at 0 °C was added (2*S*,4*R*)-methyl 1-acetyl-4-hydroxypyrrolidine-2-carboxylate (**3a**) (11.2 g, 59.9 mmol). After 15 h at 0 °C, the mixture was poured into 1 M HCl (180 ml), and the solution was extracted with ethyl acetate (3 × 60 ml). The pooled extracts were washed with 1 M HCl (3 × 30 ml), saturated $NaHCO_3$ (2 × 30 ml) and brine (2 × 30 ml), and then dried and concentrated to a tan oil that was crystallized from ether to yield (3*R*,5*S*)-5-(methoxycarbonyl)-1-acetylpyrrolidin-3-yl 4-methylbenzenesulfonate (**4a**) as white crystal (11.3 g, 55%); mp 70–71 °C (lit. [11] mp 70–72 °C). IR (KBr, cm^{-1}): 2923.81 (CH), 1751.25 (C=O), 1647.97 (C=O), 1596.11 (C=C), 1336.45 (SO_2), 1199.34 (C–O), 1172.48 (SO_2); ^1H NMR ($CDCl_3$, δ ppm): 7.79 (d, 2H, Ar–H, $J = 8.28$ Hz), 7.37 (d, 2H, Ar–H, $J = 8.00$ Hz), 5.14 (m, 1H, CH), 4.52 (m, 1H, CH), 3.80 (m, 2H, CH_2), 3.72 (s, 3H, OCH_3), 2.47 (s, 3H, $ArCH_3$), 2.16 (m, 2H, CH_2), 2.07 (s, 3H, CH_3CO); ESI-MS: 342.5 (M + 1).

To a mixture of (2*S*,4*R*)-methyl 4-hydroxy-1-((*E*)-3-(3,4-dimethoxyphenyl)acryloyl)pyrrolidine-2-carboxylate (**3b**) (6.72 g, 20 mmol) and Et_3N (2.02 g, 20 mmol) in dichloromethane (100 ml) at 0 °C was added dropwise a solution of methanesulfonyl chloride (2.3 g, 20 mmol) in dichloromethane (100 ml). The mixture was stirred for 4 h at room temperature, then washed with saturated $NaHCO_3$ solution (2 × 70 ml) and H_2O (2 × 70 ml). The organic phase was dried to give (3*R*,5*S*)-5-(methoxycarbonyl)-1-((*E*)-3-(3,4-dimethoxyphenyl)acryloyl)pyrrolidin-3-yl methanesulfonate (**4b**) as pale yellow solid (6.6 g, 80%); mp 139–140 °C. ^1H NMR ($CDCl_3$, δ ppm): 7.69 (d, 1H, =CH, $J = 15.36$ Hz), 7.11 (d, 1H, Ar–H, $J = 8.32$ Hz), 7.03 (s, 1H, Ar–H), 6.86 (d, 1H, Ar–H, $J = 8.32$ Hz), 6.49 (d, 1H, =CH, $J = 15.40$ Hz), 5.38 (s, 1H, CH), 4.73 (t, 1H, CH, $J = 7.88$ Hz), 4.12 (m, 2H, CH_2), 3.93 (s, 6H, $ArOCH_3$), 3.79 (s, 3H, $COOCH_3$), 3.07 (s, 3H, CH_3SO_2), 2.33 (m, 2H, CH_2); ESI-MS: 414.5 (M + 1).

*5.1.4. (2S,4S)-Methyl 1-acyl-4-azidopyrrolidine-2-carboxylates (**5a**,**b**)*

(3*R*,5*S*)-5-(Methoxycarbonyl)-1-acylpyrrolidin-3-yl sulfonate (**4a** or **4b**) (9.04 mmol) and NaN_3 (46.2 mmol) were stirred in dry DMF (10 ml) at 45–55 °C overnight. The solvent was evaporated, and the residue was mixed with ethyl acetate (25 ml) and H_2O (15 ml). After separation of the layers, the organic phase was washed with H_2O until neutral and then with 0.1 M HCl and dried. Evaporation of ethyl acetate gave (2*S*,4*S*)-methyl 1-acetyl-4-azidopyrrolidine-2-carboxylate (**5a**) or (2*S*,4*S*)-methyl 4-azido-1-((*E*)-3-(3,4-dimethoxyphenyl)acryloyl)pyrrolidine-2-carboxylate (**5b**), which was suitable for the next step. Azide **5a**: colorless oil; 95%; IR (KBr, cm^{-1}): 2954.70 (CH), 2108.36 (N_3), 1747.83 (C=O), 1654.99 (C=O), 1205.16 (C–O); ^1H NMR ($CDCl_3$, δ ppm): 5.03 (m, 1H, CH), 3.84 (m, 2H, CH_2), 3.81 (s, 3H, OCH_3), 2.52 (m, 1H, CH), 2.24 (m, 2H, CH_2), 2.09 (s, 3H, CH_3CO); ESI-MS: 213.4 (M + 1). Azide **5b**: yellow oil; 93%; IR (KBr, cm^{-1}): 2952.70 (CH), 2105.74 (N_3), 1748.66 (C=O), 1649.85 (C=O), 1513.90 (C=C), 1266.96 (C–O), 1023.57 (=CH); ^1H NMR ($CDCl_3$, δ ppm): 7.61 (d, 1H, =CH, $J = 15.47$ Hz), 7.25 (d, 1H, Ar–H, $J = 8.21$ Hz), 7.07 (s, 1H, Ar–H), 6.86 (d, 1H, Ar–H, $J = 8.36$ Hz), 6.44 (d, 1H, =CH, $J = 15.38$ Hz), 5.04 (s, 1H, CH), 4.23 (m, 2H, CH_2), 3.85 (s, 6H, $ArOCH_3$), 3.71 (s, 3H, $COOCH_3$), 2.71 (m, 1H, CH), 2.21 (m, 2H, CH_2); ESI-MS: 361.4 (M + 1).

*5.1.5. (2S,4S)-Methyl 1-acyl-4-aminopyrrolidine-2-carboxylates (**6a–c**)*

(2*S*,4*S*)-Methyl 1-acyl-4-azidopyrrolidine-2-carboxylate (**5a** or **5b**) (7.8 mmol) in methanol (20 ml) was hydrogenated in the presence of catalytic amount of 5% Pd–$CaCO_3$ at room temperature and 1 atm pressure. After 16 h, the catalyst was

filtered off and the solvent was removed under vacuum to give (2*S*,4*S*)-methyl 1-acetyl-4-aminopyrrolidine-2-carboxylate (**6a**) or (2*S*,4*S*)-methyl 4-amino-1-((*E*)-3-(3,4-dimethoxyphenyl)acryloyl)pyrrolidine-2-carboxylate (**6b**). When **5b** was hydrogenated in the presence of excessive catalyst, both the acryloyl and azido groups were hydrogenated to give (2*S*,4*S*)-methyl 1-(3-(3,4-dimethoxyphenyl)propanoyl)-4-aminopyrrolidine-2-carboxylate (**6c**). Compound **6a**: white solid; 97%; mp 55–57 °C; IR (KBr, cm^{-1}): 3268.58 (NH_2), 2954.78 (CH), 1744.78 (C=O), 1652.40 (C=O), 1205.29 (C–O); ^1H NMR ($CDCl_3$, δ ppm): 4.97 (m, 1H, CH), 4.41 (m, 1H, CH), 3.75 (m, 2H, CH_2), 3.65 (s, 3H, OCH_3), 2.26 (m, 2H, CH_2), 2.09 (s, 3H, CH_3CO), 1.83 (m, 2H, NH_2); ESI-MS: 187.3 (M + 1). Compound **6b**: yellow solid; 80%; mp 60–62 °C; IR (KBr, cm^{-1}): 3205.41 (NH_2), 2952.71 (CH), 1744.66 (C=O), 1651.18 (C=O), 1513.74 (C=C), 1264.35 (C–O), 1023.18 (=CH); ^1H NMR ($CDCl_3$, δ ppm): 7.74 (d, 1H, =CH, $J = 15.31$ Hz), 7.23 (d, 1H, Ar–H, $J = 8.27$ Hz), 7.08 (s, 1H, Ar–H), 6.79 (d, 1H, Ar–H, $J = 8.35$ Hz), 6.43 (d, 1H, =CH, $J = 15.43$ Hz), 5.17 (s, 1H, CH), 4.77 (t, 1H, CH, $J = 7.75$ Hz), 4.03 (m, 2H, CH_2), 3.97 (s, 6H, $ArOCH_3$), 3.74 (s, 3H, $COOCH_3$), 2.38 (m, 2H, CH_2), 1.87 (m, 2H, NH_2); ESI-MS: 335.7 (M + 1). Compound **6c**: yellow oil; 82%; IR (KBr, cm^{-1}): 3346.24 (NH_2), 2953.17 (CH), 1744.65 (C=O), 1656.07 (C=O), 1515.45 (C=C), 1261.62 (C–O); ^1H NMR ($CDCl_3$, δ ppm): 7.19 (d, 1H, Ar–H, $J = 8.25$ Hz), 7.00 (s, 1H, Ar–H), 6.72 (d, 1H, Ar–H, $J = 8.39$ Hz), 5.15 (m, 1H, CH), 4.65 (m, 1H, CH), 4.05 (m, 2H, CH_2), 3.89 (s, 6H, $ArOCH_3$), 3.71 (s, 3H, $COOCH_3$), 2.95–2.42 (m, 6H, $3CH_2$), 1.87 (m, 2H, NH_2); ESI-MS: 337.7 (M + 1).

5.1.6. General procedure for the preparation of (2S,4S)-methyl 4-acylamido-1-acylpyrrolidine-2-carboxylates (7a–m)

To a mixture of (2*S*,4*S*)-methyl 1-acyl-4-aminopyrrolidine-2-carboxylate (**6a**, **6b**, or **6c**) (5 mmol) and *N*-methyl morpholine (5 mmol) in dichloromethane (50 ml) at room temperature was added dropwise a solution of acyl chloride (5 mmol) in dichloromethane (50 ml). The mixture was stirred at room temperature for 10 h and the solvent was evaporated *in vacuo*. The final product was purified by flash column chromatography and **7a–m** was obtained.

*5.1.6.1. (2S,4S)-Methyl 1-acetyl-4-(benzamido)pyrrolidine-2-carboxylate (**7a**).* Flash column chromatography: chloroform–acetone = 10:1–4:1; 56%; colorless oil; IR (KBr, cm^{-1}): 3314.68 (NH), 2952.91 (CH), 1747.36 (C=O), 1652.10 (C=O), 1537.68 (C=C), 1203.62 (C–O); ^1H NMR ($CDCl_3$, δ ppm): 7.83 (d, 1H, NH, $J = 9.49$ Hz), 7.81 (t, 2H, Ar–H, $J = 1.75$ Hz), 7.46 (m, 3H, Ar–H), 4.98 (m, 1H, CH), 4.53 (m, 1H, CH), 3.77 (s, 3H, OCH_3), 3.84–3.65 (m, 2H, CH_2), 2.55–2.47 (m, 2H, CH_2), 2.07 (s, 3H, CH_3CO); ESI-MS: 291.3 (M + 1).

*5.1.6.2. (2S,4S)-Methyl 4-(4-chlorobenzamido)-1-acetylpyrrolidine-2-carboxylate (**7b**).* Flash column chromatography: chloroform–acetone = 10:1–4:1; 46%; colorless oil; IR (KBr, cm^{-1}): 3312.24 (NH), 2953.12 (CH), 1747.23 (C=O), 1640.35 (C=O), 1540.70 (C=C), 1204.25 (C–O); ^1H NMR ($CDCl_3$, δ ppm): 8.01 (d, 1H, NH, $J = 8.64$ Hz), 7.79 (d, 2H, Ar–H, $J = 8.45$ Hz), 7.41 (d, 2H, Ar–H, $J = 8.40$ Hz), 4.96 (m, 1H, CH), 4.52 (m, 1H, CH), 3.79 (s, 3H, OCH_3), 3.86–3.67 (m, 2H, CH_2), 2.58–2.02 (m, 2H, CH_2), 2.10 (s, 3H, CH_3CO); ESI-MS: 325.5 (M + 1).

*5.1.6.3. (2S,4S)-Methyl 4-(4-bromobenzamido)-1-acetylpyrrolidine-2-carboxylate (**7c**).* Flash column chromatography: chloroform–acetone = 10:1–4:1; 57%; colorless oil; IR (KBr, cm^{-1}): 3308.78 (NH), 2953.23 (CH), 1747.17 (C=O), 1640.45 (C=O), 1540.62 (C=C), 1204.02 (C–O); ^1H NMR ($CDCl_3$, δ ppm): 7.96 (d, 1H, NH, $J = 8.41$ Hz), 7.71 (d, 2H, Ar–H, $J = 8.52$ Hz), 7.57 (d, 2H, Ar–H, $J = 8.56$ Hz), 4.94 (m, 1H, CH), 4.52 (m, 1H, CH), 3.79 (s, 3H, OCH_3), 3.86–3.67 (m, 2H, CH_2), 2.58–2.02 (m, 2H, CH_2), 2.09 (s, 3H, CH_3CO); ESI-MS: 371.4 (M + 2).

*5.1.6.4. (2S,4S)-Methyl 4-(4-nitrobenzamido)-1-acetylpyrrolidine-2-carboxylate (**7d**).* Flash column chromatography: chloroform–acetone = 10:1–4:1; 48%; yellow oil; IR (KBr, cm^{-1}): 3307.94 (NH), 2954.37 (CH), 1746.17 (C=O), 1652.65 (C=O), 1526.40 and 1348.65 (NO_2), 1204.88 (C–O); ^1H NMR ($CDCl_3$, δ ppm): 8.28 (d, 1H, NH, $J = 6.70$ Hz), 8.04 (d, 2H, Ar–H, $J = 7.46$ Hz), 7.98 (d, 2H, Ar–H, $J = 8.24$ Hz), 4.99 (m, 1H, CH), 4.56 (m, 1H, CH), 3.81 (s, 3H, OCH_3), 3.91–3.73 (m, 2H, CH_2), 2.62–2.03 (m, 2H, CH_2), 2.12 (s, 3H, CH_3CO); ESI-MS: 336.5 (M + 1).

*5.1.6.5. (2S,4S)-Methyl 4-(3,4,5-trimethoxybenzamido)-1-acetylpyrrolidine-2-carboxylate (**7e**).* Flash column chromatography: chloroform–acetone = 4:1; 56%; white crystal; mp 150–152 °C; IR (KBr, cm^{-1}): 3439.94 (NH), 2948.89 (CH), 1743.28 (C=O), 1655.76 (C=O), 1586.03 (C=C), 1127.10 (C–O); ^1H NMR ($CDCl_3$, δ ppm): 7.92 (d, 1H, NH, $J = 8.67$ Hz), 7.13 (s, 2H, Ar–H), 4.99 (d, 1H, CH, $J = 3.98$ Hz), 4.56 (d, 1H, CH, $J = 9.79$ Hz), 3.94 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.71 (m, 2H, CH_2), 2.53 (m, 2H, CH_2), 2.10 (s, 3H, CH_3CO); ESI-MS: 381.4 (M + 1).

*5.1.6.6. (2S,4S)-Methyl 1-acetyl-4-(nicotinamido)pyrrolidine-2-carboxylate (**7f**).* Flash column chromatography: chloroform–acetone = 1:1; 57%; colorless oil; IR (KBr, cm^{-1}): 3303.87 (NH), 2953.60 (CH), 1746.25 (C=O), 1652.53 (C=O), 1541.27 (C=N), 1202.86 (C–O); ^1H NMR ($CDCl_3$, δ ppm): 9.06 (s, 1H, Ar–H), 8.71 (s, 1H, Ar–H), 8.22 (d, 1H, Ar–H, $J = 8.43$ Hz), 8.15 (d, 1H, NH, $J = 7.68$ Hz), 7.40 (t, 1H, Ar–H, $J = 6.18$ Hz), 4.95 (s, 1H, CH), 4.52 (d, 1H, CH, $J = 7.92$ Hz), 3.78 (s, 3H, OCH_3), 3.89–3.70 (m, 2H, CH_2), 2.58–2.02 (m, 2H, CH_2), 2.09 (s, 3H, CH_3CO); ESI-MS: 292.4 (M + 1).

*5.1.6.7. (2S,4S)-Methyl 1-acetyl-4-(isonicotinamido)pyrrolidine-2-carboxylate (**7g**).* Flash column chromatography: chloroform–acetone = 1:1; 68%; yellow oil; IR (KBr, cm^{-1}): 3295.63 (NH), 2954.80 (CH), 1745.57 (C=O),

1652.24 (C=O), 1541.28 (C=N), 1203.96 (C–O); ^{1}H NMR ($CDCl_3$, δ ppm): 8.71 (m, 2H, Ar–H), 8.33 (d, 1H, NH, $J = 8.30$ Hz), 7.70 (m, 2H, Ar–H), 4.92 (m, 1H, CH), 4.54 (m, 1H, CH), 3.79 (s, 3H, OCH_3), 3.91–3.70 (m, 2H, CH_2), 2.60–2.02 (m, 2H, CH_2), 2.09 (s, 3H, CH_3CO); ESI-MS: 292.5 (M + 1).

*5.1.6.8. (2S,4S)-Methyl 1-acetyl-4-(pyrazine-2-carboxamido)-pyrrolidine-2-carboxylate (**7h**).* Flash column chromatography: chloroform–acetone = 10:1–1:1; 37%; white crystal; mp 51–52 °C; IR (KBr, cm^{-1}): 3347.47 (NH), 2954.33 (CH), 1741.40 (C=O), 1652.27 (C=O), 1529.54 (C=N), 1204.19 (C–O); ^{1}H NMR ($CDCl_3$, δ ppm): 9.39 (d, 1H, Ar–H, $J = 5.28$ Hz), 8.77 (d, 1H, Ar–H, $J = 2.40$ Hz), 8.73 (d, 1H, Ar–H, $J = 8.79$ Hz), 8.59 (d, 1H, NH, $J = 3.92$ Hz), 4.98 (m, 1H, CH), 4.59 (m, 1H, CH), 3.80 (s, 3H, OCH_3), 3.97–3.76 (m, 2H, CH_2), 2.64–2.58 (m, 2H, CH_2), 2.11 (s, 3H, CH_3CO); ESI-MS: 293.3 (M + 1).

*5.1.6.9. (2S,4S)-Methyl 1-acetyl-4-(cinnamamido)pyrrolidine-2-carboxylate (**7i**).* Flash column chromatography: chloroform–acetone = 10:1–4:1; 64%; colorless oil; IR (KBr, cm^{-1}): 3285.26 (NH), 2952.54 (CH), 1747.59 (C=O), 1656.66 (C=O), 1544.07 (C=C), 1206.76 (C–O), 981.86 (=CH); ^{1}H NMR ($CDCl_3$, δ ppm): 7.63 (d, 1H, =CH, $J = 15.70$ Hz), 7.49 (m, 2H, Ar–H), 7.43 (d, 1H, NH, $J = 8.51$ Hz), 7.35 (m, 3H, Ar–H), 6.45 (d, 1H, =CH, $J = 15.69$ Hz), 4.84 (m, 1H, CH), 4.49 (m, 1H, CH), 3.76 (s, 3H, OCH_3), 3.88–3.58 (m, 2H, CH_2), 2.57–2.01 (m, 2H, CH_2), 2.09 (s, 3H, CH_3CO); ESI-MS: 317.5 (M + 1).

*5.1.6.10. (2S,4S)-Methyl 4-((E)-3-(3,4-dimethoxyphenyl)acrylamido)-1-acetylpyrrolidine-2-carboxylate (**7j**).* Flash column chromatography: chloroform–acetone = 10:1–4:1; 43%; white crystal; mp 61–63 °C; IR (KBr, cm^{-1}): 3288.56 (NH), 2953.27 (CH), 1746.92 (C=O), 1651.71 (C=O), 1514.28 (C=C), 1261.91 (C–O), 1023.44 (=CH); ^{1}H NMR ($CDCl_3$, δ ppm): 7.51 (d, 1H, =CH, $J = 15.61$ Hz), 7.10 (d, 1H, Ar–H, $J = 8.82$ Hz), 7.05 (m, 1H, NH), 6.99 (m, 1H, Ar–H), 6.81 (d, 1H, Ar–H, $J = 8.32$ Hz), 6.21 (d, 1H, =CH, $J = 15.57$ Hz), 4.84 (m, 1H, CH), 4.46 (m, 1H, CH), 3.87 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.81–3.56 (m, 2H, CH_2), 2.51–1.95 (m, 2H, CH_2), 2.06 (s, 3H, CH_3CO); ESI-MS: 377.5 (M + 1).

*5.1.6.11. (2S,4S)-Methyl 1-((E)-3-(3,4-dimethoxyphenyl)acryloyl)-4-(nicotinamido)pyrrolidine-2-carboxylate (**7k**).* Flash column chromatography: dichloromethane–acetone = 10:1–1:1; 55%; yellow crystal; mp 70–72 °C; IR (KBr, cm^{-1}): 3377.87 (NH), 2952.67 (CH), 1744.19 (C=O), 1648.06 (C=O), 1513.59 (C=N), 1269.94 (C–O), 1024.37 (=CH); ^{1}H NMR ($CDCl_3$, δ ppm): 9.10 (s, 1H, Ar–H), 8.72 (d, 1H, Ar–H, $J = 5.99$ Hz), 8.30 (d, 1H, Ar–H, $J = 8.71$ Hz), 8.15 (d, 1H, Ar–H, $J = 7.97$ Hz), 7.66 (d, 1H, =CH, $J = 15.34$ Hz), 7.38 (q, 1H, NH, $J = 4.22$ Hz), 7.08 (d, 1H, Ar–H, $J = 8.34$ Hz), 7.03 (s, 1H, Ar–H), 6.83 (d, 1H, Ar–H, $J = 8.31$ Hz), 6.54 (d, 1H, =CH, $J = 15.35$ Hz), 5.05 (m, 1H, CH), 4.67 (m, 1H, CH), 3.90 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.99–3.78 (m, 2H, CH_2), 2.63–2.12 (m, 2H, CH_2); ESI-MS: 440.7 (M + 1).

*5.1.6.12. (2S,4S)-Methyl 1-(3-(3,4-dimethoxyphenyl)propanoyl)-4-(nicotinamido)pyrrolidine-2-carboxylate (**7l**).* Flash column chromatography: dichloromethane–acetone = 10:1–1:1; 39%; white crystal; mp 40–42 °C; IR (KBr, cm^{-1}): 3326.38 (NH), 2952.93 (CH), 1744.85 (C=O), 1651.39 (C=O), 1516.08 (C=N), 1262.30 (C–O); ^{1}H NMR ($CDCl_3$, δ ppm): 8.98 (s, 1H, Ar–H), 8.65 (m, 1H, Ar–H), 8.05 (m, 2H, Ar–H and NH), 7.31 (m, 1H, Ar–H), 6.68 (m, 3H, Ar–H), 4.86 (m, 1H, CH), 4.46 (m, 1H, CH), 3.78 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 3.87–3.60 (m, 2H, CH_2), 2.85–2.81 (m, 2H, CH_2), 2.54–2.44 (m, 4H, CH_2CH_2); ESI-MS: 442.6 (M + 1).

*5.1.6.13. (2S,4S)-Methyl 1-(3-(3,4-dimethoxyphenyl)propanoyl)-4-(cinnamamido)pyrrolidine-2-carboxylate (**7m**).* Flash column chromatography: dichloromethane–acetone = 10:1–1:1; 53%; yellow crystal; mp 49–51 °C; IR (KBr, cm^{-1}): 3293.07 (NH), 2951.62 (CH), 1746.11 (C=O), 1656.55 (C=O), 1516.24 (C=C), 1205.72 (C–O), 1028.00 (=CH); ^{1}H NMR ($CDCl_3$, δ ppm): 8.00 (s, 1H, NH), 7.60 (d, 1H, =CH, $J = 15.68$ Hz), 7.51 (m, 2H, Ar–H), 7.36 (m, 3H, Ar–H), 7.24 (d, 1H, Ar–H, $J = 8.61$ Hz), 6.76 (m, 2H, Ar–H), 6.41 (d, 1H, =CH, $J = 15.68$ Hz), 4.77 (m, 1H, CH), 4.47 (m, 1H, CH), 3.87 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.72–3.68 (m, 2H, CH_2), 2.94–2.86 (m, 2H, CH_2), 2.63–2.43 (m, 4H, CH_2CH_2); ESI-MS: 467.6 (M + 1).

*5.1.7. General procedure for the preparation of (2S,4S)-1-acyl-4-(acylamido)-N-hydroxypyrrolidine-2-carboxamides (**8a–e**)*

To a solution of compound **7** (2 mmol) in methanol (7 ml) at room temperature was added dropwise a solution of NH_2OK (6 mmol) in methanol (3.4 ml). The mixture was stirred at room temperature for 24 h and the solvent was evaporated *in vacuo*. The residue was purified by flash column chromatography to give **8a–e**.

*5.1.7.1. (2S,4S)-4-(3,4,5-Trimethoxybenzamido)-1-acetyl-N-hydroxypyrrolidine-2-carboxamide (**8a**).* Flash column chromatography: chloroform–methanol = 10:1–1:1; 42%; white crystal; mp 98–100 °C; IR (KBr, cm^{-1}): 3238.49 (OH and NH), 2940.34 (CH), 1633.03 (C=O), 1584.66 (C=C), 1126.40 (C–O); ^{1}H NMR (CD_3OD, δ ppm): 10.49 (s, 1H, NH), 8.63 (d, 1H, NH, $J = 6.52$ Hz), 7.15 (s, 2H, Ar–H), 4.78–4.72 (m, 2H, CH), 3.93 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.79–3.69 (m, 2H, CH_2), 2.57–2.31 (m, 2H, CH_2), 2.11 (s, 1H, OH), 2.08 (s, 3H, CH_3CO); ESI-MS: 382.5 (M + 1).

*5.1.7.2. N-((3S,5S)-5-(Hydroxycarbamoyl)-1-acetylpyrrolidin-3-yl)nicotinamide (**8b**).* Flash column chromatography: dichloromethane–methanol = 10:1–1:1; 59%; white crystal;

mp 160–162 °C; IR (KBr, cm^{-1}): 3381.51 (OH and NH), 2935.07 (CH), 1629.01 (C=O), 1545.37 (C=N); ^1H NMR (CD_3OD, δ ppm): 8.98 (t, 1H, Ar–H, $J = 9.52$ Hz), 8.68 (s, 1H, Ar–H), 8.26 (m, 1H, Ar–H), 7.98 (s, 1H, NH), 7.95 (s, 1H, NH), 7.54 (m, 1H, Ar–H), 4.72 (m, 1H, CH), 4.39 (m, 1H, CH), 3.95–3.65 (m, 2H, CH_2), 3.30 (s, 1H, OH), 2.76–2.35 (m, 2H, CH_2), 2.09 (s, 1H, CH_3CO); ESI-MS: 293.4 (M + 1).

*5.1.7.3. (2S,4S)-1-Acetyl-4-(cinnamamido)-N-hydroxypyrrolidine-2-carboxamide (**8c**).* Flash column chromatography: dichloromethane–methanol = 10:1–1:1; 61%; white crystal; mp 185–187 °C; IR (KBr, cm^{-1}): 3281.59 (OH), 3223.28 (NH), 2928.65 (CH), 1679.29 (C=O), 1653.98 (C=O), 1624.68 (C=O), 1534.83 (C=C), 1209.10 (C–O), 974.10 (=CH); ^1H NMR (CD_3OD, δ ppm): 8.09 (d, 1H, NH, $J = 7.64$ Hz), 7.54–7.51 (m, 3H, Ar–H, =CH and NH), 7.41–7.26 (m, 4H, Ar–H), 6.45 (m, 1H, =CH), 4.74 (m, 2H, CH), 3.85–3.49 (m, 4H, CH_2), 2.17 (s, 1H, OH), 2.09 (s, 3H, CH_3CO); ESI-MS: 318.5 (M + 1).

*5.1.7.4. N-((3S,5S)-5-(Hydroxycarbamoyl)-1-(3-(3,4-dimethoxyphenyl)propanoyl)pyrrolidin-3-yl)nicotinamide (**8d**).* Flash column chromatography: dichloromethane–methanol = 40:1–10:1; 64%; white crystal; mp 90–92 °C; IR (KBr, cm^{-1}): 3253.73 (OH and NH), 2935.30 (CH), 1647.80 (C=O), 1515.91 (C=N), 1263.02 (C–O); ^1H NMR (CD_3OD, δ ppm): 10.82 (s, 1H, NH), 9.18 (d, 1H, NH, $J = 7.38$ Hz), 8.98 (s, 1H, Ar–H), 8.62 (d, 1H, Ar–H, $J = 3.48$ Hz), 8.11 (d, 1H, Ar–H, $J = 8.02$ Hz), 7.32 (m, 1H, Ar–H), 6.67 (m, 3H, Ar–H), 4.75 (m, 1H, CH), 4.64 (m, 1H, CH), 3.80 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.91–3.61 (m, 2H, CH_2), 3.44 (s, 1H, OH), 2.85–2.81 (m, 2H, CH_2), 2.69–2.32 (m, 4H, CH_2CH_2); ESI-MS: 443.7 (M + 1).

*5.1.7.5. (2S,4S)-1-(3-(3,4-Dimethoxyphenyl)propanoyl)-4-(cinnamamido)-N-hydroxypyrrolidine-2-carboxamide (**8e**).* Flash column chromatography: dichloromethane–methanol = 40:1–10:1; 43%; white crystal; mp 85–87 °C; IR (KBr, cm^{-1}): 3257.07 (OH and NH), 2934.53 (CH), 1655.92 (C=O), 1624.51 (C=O), 1515.95 (C=C), 1262.66 (C–O), 1027.60 (=CH); ^1H NMR (CD_3OD, δ ppm): 10.72 (s, 1H, NH), 8.22 (d, 1H, NH, $J = 7.68$ Hz), 7.58 (d, 1H, =CH, $J = 15.68$ Hz), 7.48 (t, 2H, Ar–H, $J = 3.74$ Hz), 7.30 (m, 3H, Ar–H), 6.72 (m, 3H, Ar–H), 6.44 (d, 1H, =CH, $J = 15.70$ Hz), 4.72 (m, 1H, CH), 4.53 (m, 1H, CH), 3.82 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.64–3.52 (m, 2H, CH_2), 3.46 (s, 1H, OH), 2.83 (m, 2H, CH_2), 2.59–2.16 (m, 4H, CH_2CH_2); ESI-MS: 468.5 (M + 1).

*5.1.8. Procedure for the preparation of (2S,4S)-4-(3,4,5-trimethoxybenzamido)-1-acetylpyrrolidine-2-carboxylic acid (**9**)*

To a stirred solution of ester **7e** (2 mmol) in methanol (5 ml) at 0 °C was added 1 M NaOH (2.1 ml, 2.1 mmol). The mixture was slowly warmed to 23 °C and treated with 1 M HCl (2.1 ml, 2.1 mmol). After 15 min, the solvent was evaporated *in vacuo*. The residue was purified by flash column chromatography (chloroform–methanol = 5:1) to give **9** as white crystal; 87%; mp 246–248 °C; IR (KBr, cm^{-1}): 3388.34 (OH and NH), 2941.41 (CH), 1732.34 (C=O), 1628.52 (C=O), 1585.08 (C=C), 1126.63 (C–O); ^1H NMR ($CDCl_3$, δ ppm): 8.01 (s, 1H, NH), 7.59 (s, 1H, OH), 7.06 (s, 2H, Ar–H), 4.84 (d, 1H, CH, $J = 5.82$ Hz), 4.68 (d, 1H, CH, $J = 4.41$ Hz), 3.92 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.83–3.67 (m, 2H, CH_2), 2.95–2.33 (m, 2H, CH_2), 2.16 (s, 3H, CH_3CO); ESI-MS: 367.5 (M + 1).

*5.1.9. Procedure for the preparation of methyl 2-((2S,4S)-4-(3,4,5-trimethoxybenzamido)-1-acetylpyrrolidine-2-carboxamido)acetate (**10**)*

Compound **9** (5 mmol) was dissolved in tetrahydrofuran (10 ml), then *N*-hydroxysuccinimide (5 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (5 mmol) were added. The mixture was stirred at 0 °C for 8 h, then glycine methyl ester hydrochloride (5 mmol) and *N*-methyl morpholine (5 mmol) were added. The mixture was stirred at 0 °C for another 8 h. The solvent was evaporated *in vacuo* and the residue was purified by flash column chromatography (chloroform–methanol = 10:1) to give **10** as white crystal; 59%; mp 50–52 °C; IR (KBr, cm^{-1}): 3303.73 (NH), 2944.39 (CH), 1751.98 (C=O), 1651.83 (C=O), 1585.09 (C=C), 1126.51 (C–O); ^1H NMR ($CDCl_3$, δ ppm): 8.77 (d, 1H, NH, $J = 6.63$ Hz), 8.26 (t, 1H, NH, $J = 5.33$ Hz), 7.14 (s, 2H, Ar–H), 4.86 (d, 1H, CH, $J = 8.78$ Hz), 4.72 (m, 1H, CH), 3.90 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 4.04–3.65 (m, 4H, CH_2), 2.51–1.99 (m, 2H, CH_2), 2.07 (s, 3H, CH_3CO); ESI-MS: 438.5 (M + 1).

5.2. Biological evaluation

5.2.1. MMP-2 inhibition assay

The pyrrolidine derivatives were assayed for the inhibitory activities against MMP-2 in 96-well microtiter plates using succinylated gelatin as the substrate. The compound and enzyme were dissolved in sodium borate buffer (pH 8.5, 50 mM) and incubated at 37 °C for 30 min. The substrate was added and incubated at 37 °C for another 60 min. The 100% and blank groups were also carried out, in which the 100% group contained not compound and the blank group contained only the enzyme. Then 0.03% picrylsulfonic acid solution was added and incubated at room temperature for additional 20 min. The resulting solutions were measured under 450 nm to gain OD_{450} values, which were then used to calculate the inhibitory rates by $[OD_{450}(100\%) - OD_{450}(\text{compound})]/[OD_{450}(100\%) - OD_{450}(\text{blank})] \times 100\%$. The IC_{50} values were obtained from the above inhibitory rates using OriginPro 7.5 software.

5.2.2. AP-N inhibition assay

The pyrrolidine derivatives were further assayed for the inhibitory activities against AP-N using L-leucine *p*-nitroanilide as the substrate. The compound and enzyme were dissolved in phosphate sodium buffer (pH 7.2, 50 mM) and incubated at 37 °C for 30 min. The substrate was added and incubated at

37 °C for another 60 min. The 100% and blank groups were also carried out, in which the 100% group contained not compound and the blank group contained only the enzyme. The resulting solutions were measured under 405 nm to gain OD_{405} values, which were then used to calculate the inhibitory rates by $[OD_{405}(100\%) - OD_{405}(\text{compound})]/[OD_{405}(100\%) - OD_{405}(\text{blank})] \times 100\%$. The IC_{50} values were obtained from the above inhibitory rates using OriginPro 7.5 software.

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