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

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Abstract—A series of novel sulfonyl 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. Compounds **6a**–**d** were more potent MMP-2 inhibitors than the positive control LY52. The structure–activity relationships were also briefly discussed.

1. Introduction

The matrix metalloproteinases (MMPs) are a family of structurally related zinc-dependent endoproteinases that degrade and remodel the structural proteins in the extracellular matrix.[1] These include more than 20 subtypes, 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] The 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, which provides a hydrogen bond interaction with the enzyme backbone; and (3) one or more side chains, which undergo effective interactions with the enzyme subsites, such as S1′ pocket and S2′ pocket.[6,7]

Sulfonamide hydroxamates were designed and synthesized as efficient MMP inhibitors since the discovery of CGS 27023A in 1994. CGS 27023A has been used in clinical trials for the treatment of cancer. Cyclic variants, including prinomastat have also been reported (see Fig. 1).[8] The sulfonyl group was incorporated in the inhibitor to improve the enzyme-inhibitor binding, not only by forming hydrogen bonds to the enzyme but also by properly directing the hydrophobic substituent to the S1′ pocket and enabling it to plunge in deeply.

Our group has been developing pyrrolidine scaffold-based MMP-2 inhibitors for a number of years, and numerous compounds have been reported in the literature.[9,10] One of these compounds, LY52 (see Fig. 1) shows low nanomolar activity for MMP-2. 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.[11]

In order to identify more potent MMP-2 inhibitors, based on our previous work and the role of the sulfonyl group in MMP inhibitors, we incorporated the sulfonyl group into pyrrolidine scaffold to form the new inte-

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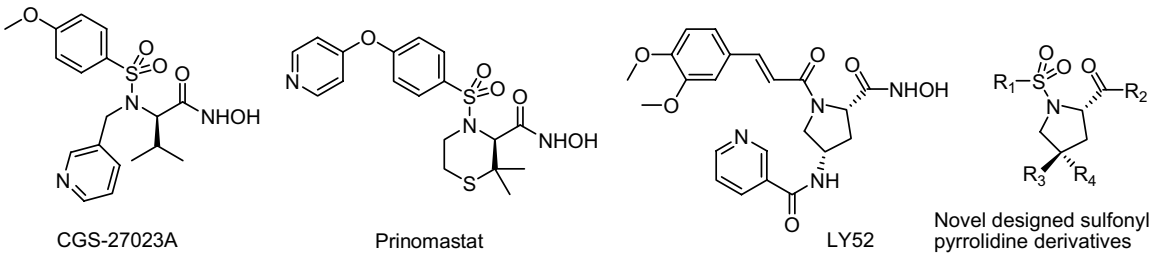


Figure 1. The chemical structures of CGS-27023A, Prinomastat, LY52, and our further designed sulfonyl pyrrolidine derivatives.

grated structural pattern (see Fig. 1). R_1SO_2 group can be any of the various sulfonyl groups, such as toluene-4-sulfonyl, benzenesulfonyl, or methanesulfonyl. COR_2 group can be hydroxamate or carboxylate group. R_3 (R_4) group can be hydroxyl, carbonyl, or ketal group. R_1SO_2 group and R_3 (R_4) group might occupy the S1′ and S2′ pockets, respectively, while the COR_2 group might chelate the active site zinc ion. The pyrrolidine scaffold might bond to the enzyme backbone.

2. Results and discussion

The target compounds were synthesized via the route as shown in Scheme 1. Starting from *trans*-4-hydroxy-L-proline (**1**) as a chiral template, the important intermediate 1-sulfonyl-4-oxo-pyrrolidine-2-carboxylic acid methyl esters (**4a**–**c**) were prepared via the route of esterification,[12] sulfonation, [13] and Jones oxidation.[14] Addition reaction of **4a**–**c** with various diols produced the ketal compounds **5a**–**f**. In this step, the *p*-toluenesulfonic acid acted as a catalyst and the Dean–Stark trap was used to remove the water product.[15] Ammonolysis of **5a**–**f** with NH_2OK provided the final spiro hydroxamate compounds (**6a–f**).[16] The chemical structures of the target compounds were confirmed by IR, ^{1}H NMR and ESI-MS.

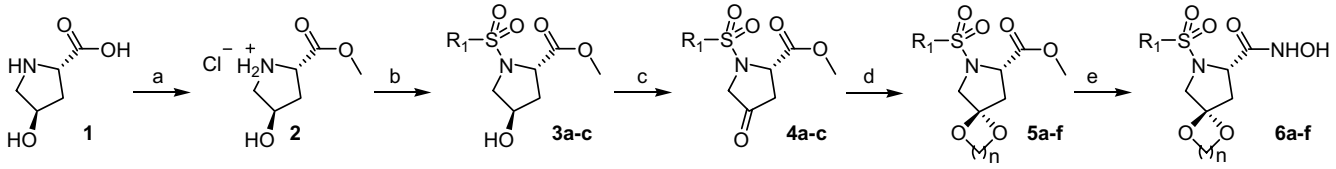
The newly synthesized pyrrolidine derivatives were assayed for the inhibitory activities on MMP-2 and aminopeptidase N (AP-N). [17,18] Similar to MMP-2, AP-N is also a zinc-dependent metalloproteinase involved in the process of tumor invasion and metastasis. Thus the assay was performed on both MMP-2 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). Compounds **6a–d** were more potent MMP-2 inhibitors than the positive control LY52. The FlexX docking of the most potent compounds **6a** and **6d** with MMP-2 was done using Sybyl 7.0 of Tripos Incorporation and the result is shown in Figures 2 and 3. The R_1 group at the N_1 position of the pyrrolidine ring (toluene-4-sulfonyl and benzenesulfonyl, respectively) occupied the deep S1′ pocket of MMP-2, and the hydroxamate group chelated the active site zinc ion 166 with a distance of 1.53 and 2.06 Å, respectively. Compounds **6a** and **6d** interacted well with the MMP-2 active site, especially the deep S1′ pocket and zinc ion 166, consistent with the MMP-2 assay results.

The selective inhibition might be explained by the FlexX docking results of the representative compounds **6a** and **6d** with APN (Figs. 4 and 5). Although compounds **6a** and **6d** chelated well with the active site zinc ion 900, both the ketal group and sulfonyl group at the pyrrolidine ring were solvent exposed and could not occupy the S1′ and S2′ pockets of APN. MMP-2 was a zinc-dependent endopeptidase that could cut the peptide to parts from the specific amino acid residue of the peptide. However, AP-N was a membrane-bound zinc exopeptidase that catalyzed the removal of NH-terminal amino acid from the peptide. 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.

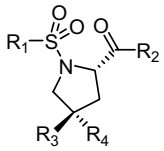
Compounds **6a**–**f** were more potent than their predecessors **5a**–**f**. This activity difference was caused by the ZBG (COR_3), which was the only structural difference between **6a**–**f** and their predecessor. The ZBG is hydroxamate (CONHOH) for **6a**–**f** and carboxylate ($COOCH_3$) for their predecessor, respectively. Both of these groups could chelate zinc ion at the catalytic activity center of the enzyme. However, the hydroxamate group was a more potent ZBG than the carboxylate group as shown in the activity order of **6a**–**f** and their predecessor.

Among compounds **6a**–**c**, R_1SO_2 and COR_2 groups were fixed as toluene-4-sulfonyl and hydroxamate,



Scheme 1. Reagents: (a) CH_3OH, HCl; (b) R_1SO_2Cl; (c) Jones reagent; (d) $HO–(CH_2)_n–OH$; and (e) NH_2OK.

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



No.	R_1	R_2	R_3	R_4	IC_{50}[a] (μM)	
					MMP-2	AP–N
3a	*p*-$CH_3C_6H_4$	OCH_3	OH	H	0.1 ± 0.02	38.4 ± 4.6
3b	C_6H_5	OCH_3	OH	H	0.8 ± 0.1	53.8 ± 3.2
3c	CH_3	OCH_3	OH	H	7.1 ± 0.6	41.6 ± 6.9
4a	*p*-$CH_3C_6H_4$	OCH_3	C=O		0.4 ± 0.04	54.9 ± 4.1
4b	C_6H_5	OCH_3	C=O		2.0 ± 0.1	75.8 ± 10.2
4c	CH_3	OCH_3	C=O		9.5 ± 1.2	92.6 ± 12.4
5a	*p*-$CH_3C_6H_4$	OCH_3	O–$(CH_2)_2$–O		0.1 ± 0.01	155.8 ± 10.3
5b	*p*-$CH_3C_6H_4$	OCH_3	O–$(CH_2)_3$–O		0.2 ± 0.03	173.7 ± 20.7
5c	*p*-$CH_3C_6H_4$	OCH_3	O–$(CH_2)_4$–O		0.3 ± 0.04	124.3 ± 13.5
5d	C_6H_5	OCH_3	O–$(CH_2)_2$–O		0.2 ± 0.04	150.6 ± 18.4
5e	C_6H_5	OCH_3	O–$(CH_2)_3$–O		1.1 ± 0.1	168.1 ± 10.3
5f	C_6H_5	OCH_3	O–$(CH_2)_4$–O		1.5 ± 0.1	134.8 ± 9.5
6a	*p*-$CH_3C_6H_4$	NHOH	O–$(CH_2)_2$–O		0.003 ± 0.0004	48.5 ± 6.7
6b	*p*-$CH_3C_6H_4$	NHOH	O–$(CH_2)_3$–O		0.006 ± 0.0005	61.8 ± 4.9
6c	*p*-$CH_3C_6H_4$	NHOH	O–$(CH_2)_4$–O		0.008 ± 0.001	73.2 ± 11.6
6d	C_6H_5	NHOH	O–$(CH_2)_2$–O		0.008 ± 0.0008	57.8 ± 5.4
6e	C_6H_5	NHOH	O–$(CH_2)_3$–O		0.011 ± 0.001	82.7 ± 12.8
6f	C_6H_5	NHOH	O–$(CH_2)_4$–O		0.012 ± 0.001	106.7 ± 10.4
LY52					0.009 ± 0.0004	141.9 ± 11.7

[a] IC_{50} values are mean of three experiments, standard deviation is given.

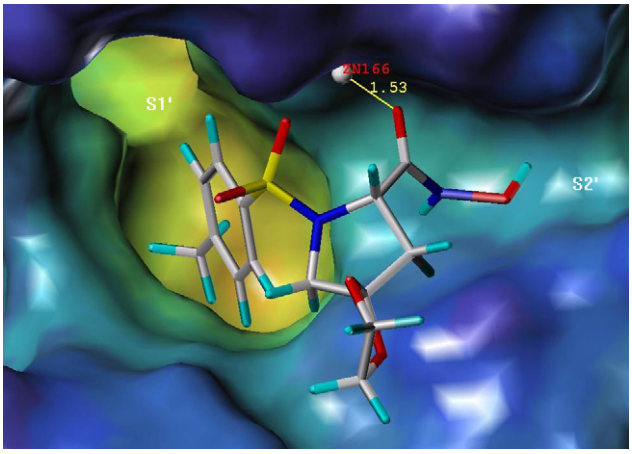


Figure 2. FlexX docking result of compound **6a** with MMP-2.

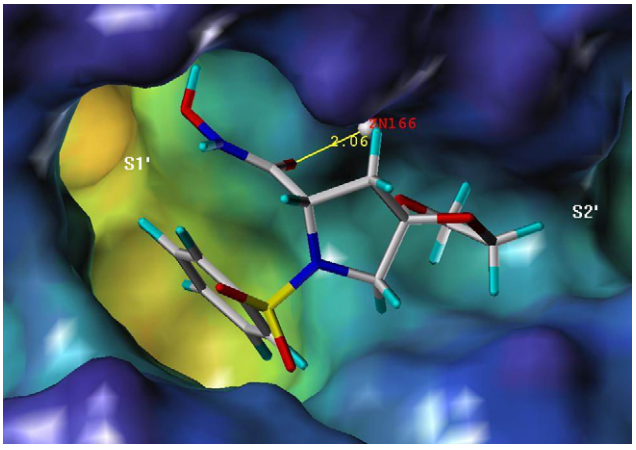


Figure 3. FlexX docking result of compound **6d** with MMP-2.

respectively, and the ketal group at R_3 and R_4 positions was altered at various lengths. So the differences in the inhibitory activities of these compounds were caused by various ketal groups. The introduction of a five-membered ring ketal group (**6a**) displayed the highest activity. As compared with **6a**, the compound bearing a six-membered ring ketal group (**6b**) was in the next place, and the compound containing a seven-membered ring ketal group (**6c**) presented the least activity. The same rule can also be seen among compounds **5a**–**c**, **5d**–**f**, and **6d**–**f**.

Among compounds **5a**–**f** and **6a**–**f**, when the ketal group and ZBG were fixed, and R_1SO_2 group was altered as toluene-4-sulfonyl and benzenesulfonyl, the differences in the inhibitory activities of these compounds were caused by various R_1SO_2 groups. The compound bearing the toluene-4-sulfonyl group was found to be more potent than the compound bearing benzenesulfonyl group. This result might be owing to the toluene-4-sulfonyl group, which could interact with the S1′ pocket and plunge in deeply.

Finally, the binding mode of compound **6d** with MMP-2 was proposed as follows: (1) the hydroxamate chelated the active site zinc ion; (2) the sulfonyl and the hydroxyl groups provided hydrogen bond interactions with the enzyme backbone Ala86 and Glu121, respectively; and (3) two side chains (benzenesulfonyl and ketal) under-

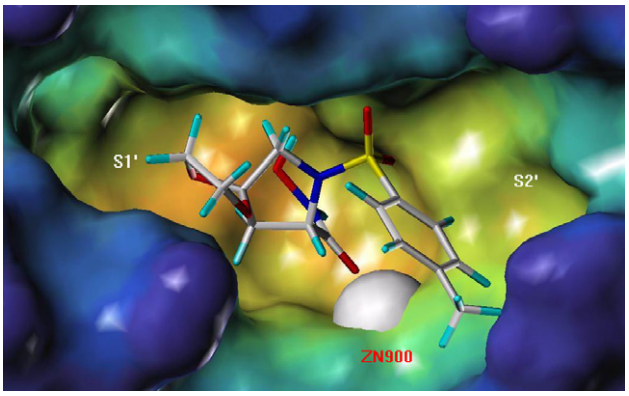


Figure 4. FlexX docking result of compound **6a** with APN.

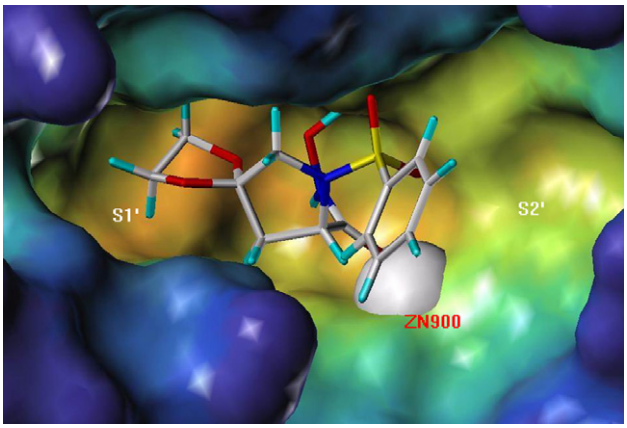


Figure 5. FlexX docking result of compound **6d** with APN.

went effective interactions with the enzyme subsites S1′ pocket and S2′ pocket (Fig. 6). The above binding mode information encouraged us to further design pyrrolidine-scaffold-based MMP-2 inhibitors, which would be reported later.

3. Conclusions

In conclusion, a series of novel sulfonyl pyrrolidine derivatives were designed and synthesized. These pyrrolidine derivatives exhibited highly selective inhibition against MMP-2 as compared with AP-N. Compounds **6a–d** were more potent MMP-2 inhibitors than the positive control LY52. SAR studies indicated that the introduction of toluene-4-sulfonyl group at the N_1 position and a five-membered ring ketal group at the C_4 position at the 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.

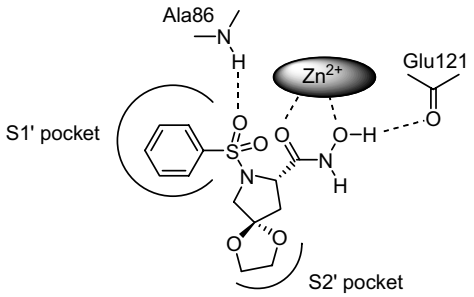


Figure 6. Proposed binding mode of compound **6d** with MMP-2.

4. Experimental

4.1. 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 electro-spray ionization mass spectrometer as the value *m*/*z*. Flash column chromatography was performed using 300 mesh silica gel. The yields were calculated by the last step reaction.

4.1.1. *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 crystallized from the solvent, 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).

4.1.2. 1-Sulfonyl-4-hydroxy-pyrrolidine-2-carboxylic acid methyl ester (3a–c). *Trans*-4-Hydroxy-L-proline methyl ester hydrochloride (**2**) (69 g, 380 mmol) was dissolved in water/dioxane (1:1, 300 ml) with triethylamine (135 ml, 960 mmol). Various sulfonyl chlorides (420 mmol) were added along with 4-(dimethylamino) pyridine (4.6 g, 38 mmol) and the mixture was stirred for 24 h at room temperature. The mixture was then concentrated and extracted with EtOAc (6× 50 ml). Layers were separated and the organic layer was washed with 1 M HCl (2× 100 ml) and brine (1× 100 ml), dried over Na_2SO_4, filtered, and evaporated to give **3a–c** as solid material.

4.1.2.1. 4-Hydroxy-1-(toluene-4-sulfonyl)-pyrrolidine-2-carboxylic acid methyl ester (3a). About 75%; white crystal; mp 78–79 °C; IR (KBr, cm^{-1}): 3512.59 (OH), 2953.46 (CH), 1742.98 (C=O), 1597.79 (C=C), 1343.73 (SO_2), 1156.85 (SO_2); ESI-MS: 300.5 (M+1).

4.1.2.2. 1-Benzenesulfonyl-4-hydroxy-pyrrolidine-2-carboxylic acid methyl ester (3b). About 69%; white crystal; mp 102–103 °C; IR (KBr, cm^{-1}): 3467.77 (OH), 2956.75 (CH), 1725.82 (C=O), 1347.60 (SO_2), 1156.78 (SO_2); ESI-MS: 286.2 (M+1).

4.1.2.3. 4-Hydroxy-1-methanesulfonyl-pyrrolidine-2-carboxylic acid methyl ester (3c). About 35%; white crystal; mp 85–87 °C; IR (KBr, cm^{-1}): 3468.57 (OH), 2959.32 (CH), 1723.68 (C═O), 1344.44 (SO_2), 1147.72 (SO_2); ESI-MS: 224.3 (M+1).

4.1.3. 1-Sulfonyl-4-oxo-pyrrolidine-2-carboxylic acid methyl ester (4a–c). CrO_3 (26.7 g, 267 mmol) was dissolved in H_2SO_4 (conc.) (23 ml). H_2O (77 ml) was added very slowly to the above solution and Jones reagent was prepared. 1-Sulfonyl-4-hydroxy-pyrrolidine-2-carboxylic acid methyl ester (**3a–c**) (100 mmol) was dissolved in acetone (268 ml) and cooled to 0 °C. Jones reagent was added (100 ml) (color changed from orange-red to green), and the mixture was stirred at room temperature for 24 h. The reaction mixture was diluted with water (370 ml) and extracted with EtOAc (1× 250 ml). The organic layers were washed with water (3× 100 ml) and brine (1× 100 ml), dried over Na_2SO_4, and evaporated to give the solid, which was then crystallized from EtOAc to give the desired product as a crystal.

4.1.3.1. 4-Oxo-1-(toluene-4-sulfonyl)-pyrrolidine-2-carboxylic acid methyl ester (4a). About 95%; white crystal; mp 72–73 °C; IR (KBr, cm^{-1}): 2956.12 (CH), 1768.19 (C═O), 1755.58 (C═O), 1598.80 (C═C), 1348.35 (SO_2), 1157.32 (SO_2); ESI-MS: 298.4 (M+1).

4.1.3.2. 1-Benzenesulfonyl-4-oxo-pyrrolidine-2-carboxylic acid methyl ester (4b). About 91%; white crystal; mp 62–64 °C; IR (KBr, cm^{-1}): 2956.07 (CH), 1768.14 (C═O), 1352.74 (SO_2), 1159.45 (SO_2); ESI-MS: 284.2 (M+1).

4.1.3.3. 1-Methanesulfonyl-4-oxo-pyrrolidine-2-carboxylic acid methyl ester (4c). About 82%; white crystal; mp 98–99 °C; IR (KBr, cm^{-1}): 2957.46 (CH), 1754.78 (C═O), 1328.08 (SO_2), 1146.09 (SO_2); ESI-MS: 222.2 (M+1).

4.1.4. General procedure for the preparation of the ketal compounds (5a–f). 1-Sulfonyl-4-oxo-pyrrolidine-2-carboxylic acid methyl ester (**4a** or **4b**) (10 mmol) was dissolved in toluene (100 ml) and various diols (ethylene glycol, 1,3-propanediol, or 1,4-butanediol) (180 mmol). *p*-Toluenesulfonic acid (0.2236 g, 1.3 mmol) was added, and the resulting mixture was heated under reflux for 24 h in the presence of a Dean–Stark trap. The solvent was evaporated in vacuo. The final product was purified by flash column chromatography and **5a–f** were obtained.

4.1.4.1. 7-(Toluene-4-sulfonyl)-1,4-dioxa-7-aza-spiro-[4.4]nonane-8-carboxylic acid methyl ester (5a). Flash column chromatography: dichloromethane/acetone = 100:1–10:1; 31%; colorless oil; IR (KBr, cm^{-1}): 2922.81 (CH), 1743.33 (C═O), 1598.02 (C═C), 1347.99 (SO_2), 1157.66 (SO_2); ^1H NMR ($CDCl_3$, δ ppm): 7.75 (d, 2H, Ar-H, J = 8.20 Hz), 7.32 (d, 2H, Ar-H, J = 8.08 Hz), 4.40 (t, 1H, CH, J = 7.40 Hz), 3.90–3.76 (m, 4H, OCH_2CH_2O), 3.75 (s, 3H, OCH_3), 3.46–3.37 (m, 2H, NCH_2), 2.44 (s, 3H, $ArCH_3$), 2.23 (d, 2H, CH_2, J = 7.40 Hz); ESI-MS: 342.2 (M+1).

4.1.4.2. 2-(Toluene-4-sulfonyl)-6,10-dioxa-2-aza-spiro-[4.5]decane-3-carboxylic acid methyl ester (5b). Flash column chromatography: dichloromethane/acetone = 100:1–10:1; 40%; colorless oil; IR (KBr, cm^{-1}): 2954.88 (CH), 1756.46 (C═O), 1597.96 (C═C), 1347.88 (SO_2), 1158.86 (SO_2); ^1H NMR ($CDCl_3$, δ ppm): 7.77 (d, 2H, Ar-H, J = 8.08 Hz), 7.31 (m, 2H, Ar-H), 4.35 (t, 1H, CH, J = 7.56 Hz), 3.88–3.46 (m, 6H, $2OCH_2$ and NCH_2), 3.75 (s, 3H, OCH_3), 2.43 (s, 3H, $ArCH_3$), 2.32 (m, 2H, CH_2), 1.80–1.57 (m, 2H, CH_2); ESI-MS: 356.3 (M+1).

4.1.4.3. 2-(Toluene-4-sulfonyl)-6,11-dioxa-2-aza-spiro-[4.6]undecane-3-carboxylic acid methyl ester (5c). Flash column chromatography: dichloromethane/acetone = 100:1–10:1; 34%; colorless oil; IR (KBr, cm^{-1}): 2950.83 (CH), 1755.91 (C═O), 1597.99 (C═C), 1348.01 (SO_2), 1158.08 (SO_2); ^1H NMR ($CDCl_3$, δ ppm): 7.75 (d, 2H, Ar-H, J = 8.24 Hz), 7.31 (d, 2H, Ar-H, J = 8.04 Hz), 4.33 (t, 1H, CH, J = 7.72 Hz), 3.65–3.23 (m, 6H, $2OCH_2$ and NCH_2), 3.76 (s, 3H, OCH_3), 2.47 (s, 3H, $ArCH_3$), 2.34–2.11 (m, 2H, CH_2), 1.61–1.51 (m, 4H, $2CH_2$); ESI-MS: 370.3 (M+1).

4.1.4.4. 7-Benzenesulfonyl-1,4-dioxa-7-aza-spiro[4.4]-nonane-8-carboxylic acid methyl ester (5d). Flash column chromatography: dichloromethane/acetone = 100:1–10:1; 36%; white crystal; mp 65–67 °C; IR (KBr, cm^{-1}): 2954.29 (CH), 1749.05 (C═O), 1337.60 (SO_2), 1157.55 (SO_2); ^1H NMR ($CDCl_3$, δ ppm): 7.89 (d, 2H, Ar-H, J = 8.13 Hz), 7.61 (t, 1H, Ar-H, J = 7.43 Hz), 7.54 (t, 2H, Ar-H, J = 8.24 Hz), 4.43 (t, 1H, CH, J = 8.00 Hz), 3.95–3.42 (m, 6H, $2OCH_2$ and NCH_2), 3.74 (s, 3H, OCH_3), 2.25 (m, 2H, CH_2); ESI-MS: 328.3 (M+1).

4.1.4.5. 2-Benzenesulfonyl-6,10-dioxa-2-aza-spiro-[4.5]decane-3-carboxylic acid methyl ester (5e). Flash column chromatography: dichloromethane/acetone = 100:1–10:1; 51%; colorless oil; IR (KBr, cm^{-1}): 2925.72 (CH), 1746.11 (C═O), 1350.36 (SO_2), 1160.47 (SO_2); ^1H NMR ($CDCl_3$, δ ppm): 7.87 (m, 2H, Ar-H), 7.55 (m, 3H, Ar-H), 4.38 (t, 1H, CH, J = 7.60 Hz), 4.30–3.21 (m, 6H, $2OCH_2$ and NCH_2), 3.74 (s, 3H, OCH_3), 2.33 (d, 2H, CH_2, J = 7.56 Hz), 1.76–1.54 (m, 2H, CH_2); ESI-MS: 342.2 (M+1).

4.1.4.6. 2-Benzenesulfonyl-6,11-dioxa-2-aza-spiro[4.6]-undecane-3-carboxylic acid methyl ester (5f). Flash column chromatography: dichloromethane/acetone = 100:1–10:1; 44%; colorless oil; IR (KBr, cm^{-1}): 2951.84 (CH), 1743.41 (C═O), 1348.69 (SO_2), 1159.59 (SO_2); ^1H NMR ($CDCl_3$, δ ppm): 7.87 (d, 2H, Ar-H, J = 7.24 Hz), 7.55 (m, 3H, Ar-H), 4.35 (m, 1H, CH), 3.89–3.19 (m, 6H, $2OCH_2$ and NCH_2), 3.75 (s, 3H, OCH_3), 2.55–2.10 (m, 2H, CH_2), 1.70–1.25 (m, 4H, $2CH_2$); ESI-MS: 356.4 (M+1).

4.1.5. General procedure for the preparation of the final spiro hydroxamate compounds (6a– f). To a solution of compound **5** (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 **6a–f**.

4.1.5.1. 7-(Toluene-4-sulfonyl)-1,4-dioxa-7-aza-spiro[4.4]-nonane-8-carboxylic acid hydroxyamide (6a). Flash column chromatography: dichloromethane/methanol = 10:1; 51%; colorless oil; IR (KBr, cm^{-1}): 3179.95 (OH), 2973.82 (CH), 1673.68 (C=O), 1345.78 (SO_2), 1157.77 (SO_2); ^1H NMR (CD_3OD, δ ppm): 7.77 (d, 2H, Ar-H, $J = 8.16$ Hz), 7.43 (d, 2H, Ar-H, $J = 8.04$ Hz), 4.13 (t, 1H, CH, $J = 7.64$ Hz), 3.91–3.32 (m, 6H, $2OCH_2$ and NCH_2), 2.46 (s, 3H, $ArCH_3$), 2.23–1.97 (m, 2H, CH_2); ESI-MS: 343.3 (M+1).

4.1.5.2. 2-(Toluene-4-sulfonyl)-6,10-dioxa-2-aza-spiro-[4.5]decane-3-carboxylic acid hydroxyamide (6b). Flash column chromatography: dichloromethane/methanol = 10:1; 47%; white crystal; mp 80–81 °C; IR (KBr, cm^{-1}): 3368.44 (OH), 2925.68 (CH), 1674.74 (C=O), 1346.32 (SO_2), 1158.11 (SO_2); ^1H NMR (CD_3OD, δ ppm): 7.79 (d, 2H, Ar-H, $J = 8.20$ Hz), 7.44 (d, 2H, Ar-H, $J = 8.08$ Hz), 4.08 (t, 1H, CH, $J = 7.84$ Hz), 3.84–3.48 (m, 6H, $2OCH_2$ and NCH_2), 2.46 (s, 3H, $ArCH_3$), 2.29–2.10 (m, 2H, CH_2), 1.70–1.55 (m, 2H, CH_2); ESI-MS: 357.3 (M+1).

4.1.5.3. 2-(Toluene-4-sulfonyl)-6,11-dioxa-2-aza-spiro-[4.6]undecane-3-carboxylic acid hydroxyamide (6c). Flash column chromatography: dichloromethane/methanol = 10:1; 47%; colorless oil; IR (KBr, cm^{-1}): 3221.09 (OH), 2922.41 (CH), 1709.27 (C=O), 1346.63 (SO_2), 1159.03 (SO_2); ^1H NMR (CD_3OD, δ ppm): 7.78 (m, 2H, Ar-H), 7.40 (m, 2H, Ar-H), 4.08 (t, 1H, CH, $J = 7.96$ Hz), 3.68–3.04 (m, 6H, $2OCH_2$ and NCH_2), 2.47 (s, 3H, $ArCH_3$), 2.36–2.04 (m, 2H, CH_2), 1.70–1.50 (m, 4H, $2CH_2$); ESI-MS: 371.3 (M+1).

4.1.5.4. 7-Benzenesulfonyl-1,4-dioxa-7-aza-spiro[4.4]-nonane-8-carboxylic acid hydroxyamide (6d). Flash column chromatography: dichloromethane/methanol = 20:1–10:1; 33%; colorless oil; IR (KBr, cm^{-1}): 3165.29 (OH), 2897.67 (CH), 1674.55 (C=O), 1342.93 (SO_2), 1158.80 (SO_2); ^1H NMR (CD_3OD, δ ppm): 7.90 (d, 2H, Ar-H, $J = 7.24$ Hz), 7.72 (m, 1H, Ar-H), 7.63 (t, 2H, Ar-H, $J = 7.88$ Hz), 4.14 (t, 1H, CH, $J = 7.72$ Hz), 3.91–3.47 (m, 6H, $2OCH_2$ and NCH_2), 2.23–2.00 (m, 2H, CH_2); ESI-MS: 329.5 (M+1).

4.1.5.5. 2-Benzenesulfonyl-6,10-dioxa-2-aza-spiro[4.5]-decane-3-carboxylic acid hydroxyamide (6e). Flash column chromatography: dichloromethane/methanol = 10:1; 59%; white crystal; mp 70–72 °C; IR (KBr, cm^{-1}): 3200.36 (OH), 2972.34 (CH), 1672.04 (C=O), 1344.03 (SO_2), 1159.16 (SO_2); ^1H NMR (CD_3OD, δ ppm): 7.92 (d, 2H, Ar-H, $J = 7.36$ Hz), 7.71 (m, 1H, Ar-H), 7.63 (t, 2H, Ar-H, $J = 7.84$ Hz), 4.10 (t, 1H, CH, $J = 7.96$ Hz), 3.85–3.32 (m, 6H, $2OCH_2$ and NCH_2), 2.27–2.14 (m, 2H, CH_2), 1.67–1.52 (m, 2H, CH_2); ESI-MS: 343.4 (M+1).

4.1.5.6. 2-Benzenesulfonyl-6,11-dioxa-2-aza-spiro[4.6]-undecane-3-carboxylic acid hydroxyamide (6f). Flash column chromatography: dichloromethane/methanol = 10:1; 63%; colorless oil; IR (KBr, cm^{-1}): 3306.18 (OH), 2946.47 (CH), 1673.77 (C=O), 1348.15 (SO_2), 1160.01 (SO_2); ^1H NMR (CD_3OD, δ ppm): 7.91 (d, 2H, Ar-H, $J = 7.32$ Hz), 7.71 (m, 1H, Ar-H), 7.63 (t, 2H, Ar-H, $J = 7.76$ Hz), 4.10 (t, 1H, CH, $J = 8.08$ Hz), 3.70–2.96 (m, 6H, $2OCH_2$ and NCH_2), 2.18–2.03 (m, 2H, CH_2), 1.56–1.30 (m, 4H, $2CH_2$); ESI-MS: 357.3 (M+1).

4.2. Biological evaluation

4.2.1. 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 the enzyme were dissolved in sodium borate buffer (pH8.5, 50 mM), and incubated at 37 °C for 30 min. The substrate was added and incubated at 37 °C for another 60 min. Then 0.03% picrylsulfonic acid solution was added and incubated at room temperature for an 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 the OriginPro 7.5 software.

4.2.2. 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 the 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 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 the OriginPro 7.5 software.

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References and notes

1. Murphy, G. J. P.; Murphy, G.; Reynolds, J. J. *FEBS Lett.* **1991**, *289*(1), 4.

2. Bjorklund, M.; Koivunen, E. *Biochim. Biophys. Acta* **2005**, *1755*, 37.
3. Polette, M.; Nawrocki-Raby, B.; Gilles, C.; Clavel, C.; Birembaut, P. *Crit. Rev. Oncol. Hematol.* **2004**, *49*, 179.
4. Verma, R. P.; Hansch, C. *Bioorg. Med. Chem.* **2007**, *15*, 2223.
5. Nagase, H.; Visse, R.; Murphy, G. *Cardiovasc. Res.* **2006**, *69*, 562.
6. Kontogiorgis, C. A.; Papaioannou, P.; Hadjipavlou-Litina, D. J. *Curr. Med. Chem.* **2005**, *12*, 339.
7. Fisher, J. F.; Mobashery, S. *Cancer Metastasis Rev.* **2006**, *25*, 115.
8. Hanessian, S.; Moitessier, N. *Curr. Top. Med. Chem.* **2004**, *4*, 1269.
9. Li, Y. L.; Xu, W. F. *Bioorg. Med. Chem.* **2004**, *12*, 5171.
10. Li, X.; Li, Y.; Xu, W. *Bioorg. Med. Chem.* **2006**, *14*, 1287.
11. Qu, X.; Yuan, Y.; Xu, W.; Chen, M.; Cui, S.; Meng, H.; Li, Y.; Makuuchi, M.; Nakata, M.; Tang, W. *Anticancer Res.* **2006**, *26*(5A), 3573.
12. Jordis, U.; Sauter, F.; Siddiqi, S. M. *Indian J. Chem.* **1989**, *28B*, 294.
13. Natchus, M. G.; Bookland, R. G.; De, B.; Almstead, N. G.; Pikul, S.; Janusz, M. J.; Heitmeyer, S. A.; Hookfin, E. B.; Hsieh, L. C.; Dowty, M. E.; Dietsch, C. R.; Patel, V. S.; Garver, S. M.; Gu, F.; Pokross, M. E.; Mieling, G. E.; Baker, T. R.; Foltz, D. J.; Peng, S. X.; Bornes, D. M.; Strojnowski, M. J.; Taiwo, Y. O. *J. Med. Chem.* **2000**, *43*, 4948.
14. Cheng, M.; De, B.; Almstead, N. G.; Pikul, S.; Dowty, M. E.; Dietsch, C. R.; Dunaway, C. M.; Gu, F.; Hsieh, L. C.; Janusz, M. J.; Taiwo, Y. O.; Natchus, M. G.; Hudlicky, T.; Mandel, M. *J. Med. Chem.* **1999**, *42*, 5426.
15. Smith, E. M.; Swiss, G. F.; Neustadt, B. R.; Gold, E. H.; Sommer, J. A.; Brown, A. D.; Chiu, P. J. S.; Moran, R.; Sybertz, E. J.; Baum, T. *J. Med. Chem.* **1988**, *31*, 875.
16. Pikul, S.; Dunham, K. L. M.; Almstead, N. G.; De, B.; Natchus, M. G.; Anastasio, M. V.; McPhail, S. J.; Snider, C. E.; Taiwo, Y. O.; Chen, L.; Dunaway, C. M.; Gu, F.; Mieling, G. E. *J. Med. Chem.* **1999**, *42*, 87.
17. Baragi, V. M.; Shaw, B. J.; Renkiewicz, R. R.; Kuipers, P. J.; Welgus, H. G.; Mathrubutham, M.; Cohen, J. R.; Rao, S. K. *Matrix Biol.* **2000**, *19*, 267.
18. Lejczak, B.; Kafarski, P.; Zygmunt, J. *Biochemistry* **1989**, *28*(8), 3549.