Bioorganic & Medicinal Chemistry 16 (2008) 7932–7938

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis of new sulfonyl pyrrolidine derivatives as matrix metalloproteinase inhibitors

Xian-Chao Cheng [a], Qiang Wang [a], Hao Fang [a], Wei Tang [b], Wen-Fang Xu [a,*]

[a] Institute of Medicinal Chemistry, School of Pharmaceutical Sciences, Shandong University, No.44 Wenhuaxi Road, Jinan 250012, China
[b] Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, The University of Tokyo 113-8655, Japan

ARTICLE INFO

Article history:
Received 26 May 2008
Revised 23 July 2008
Accepted 24 July 2008
Available online 30 July 2008

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

ABSTRACT

A series of new sulfonyl pyrrolidine derivatives was 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 compounds **4c**, **4j**, **5a**, and **5b** were equally or more potent MMP-2 inhibitors than the positive control LY52. The FlexX docking was done to explain the reason for the different potency between MMP-2 and AP-N. Structure–activity relationships were also briefly discussed.

1. Introduction

Matrix metalloproteinases (MMPs) are a family of structurally related zinc-containing endopeptidases that are involved in the degradation of the macromolecular components in the extracellular matrix (ECM) of connective tissue.[1] This family of enzymes is composed of more than 20 known members, among which MMP-2 is highly involved in the process of tumor invasion and metastasis and has attracted tremendous attention 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 majority of MMP inhibitors contain an effective zinc binding group (ZBG, such as hydroxamate and carboxylate), which interact with the active site zinc ion. To improve selectivity, MMP inhibitors are substituted with side chains that interact with the enzyme subsites, such as S1′ pocket and S2′ pocket.[6,7]

Sulfonamide hydroxamate CGS-27023A was designed and synthesized as efficient MMP inhibitors in 1994. This compound has been used in clinical trials for the treatment of cancer. Cyclic variants, including prinomastat have also been reported (see Fig. 1).[8] The extensive structure–activity relationship (SAR) studies and X-ray crystallography data disclosed the important role of sulfonyl group of these inhibitors in the MMP inhibition potency. The present of the sulfonyl group is crucial, enabling the establishment of hydrogen bonds with the enzyme backbone, and also orienting the hydrophobic substituent in the S1′ pocket.

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) showed high inhibitory activity against MMP-2 with IC_{50} values in the nanomolar range. 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-administered mice.[11]

Further design and synthesis of LY52 analogues as MMP-2 inhibitors were conducted in our lab. Based on the role of the sulfonyl group in MMP inhibitors, we incorporated the sulfonyl group into pyrrolidine scaffold to form the new integrated structural pattern (see Fig. 1). R_1SO_2 group can be various sulfonyl groups, such as toluene-4-sulfonyl, benzenesulfonyl or methanesulfonyl. R_2 group can be hydrogen or various acyl groups. COR_3 group can be hydroxamate or carboxylate group. R_1CO group and R_2 group 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.

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

* Corresponding author. Tel./fax: +86 531 88382264.
E-mail addresses: chengxianchao2000@yahoo.com.cn (X.-C. Cheng), xuwenf@sdu.edu.cn (W.-F. Xu).

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.07.073

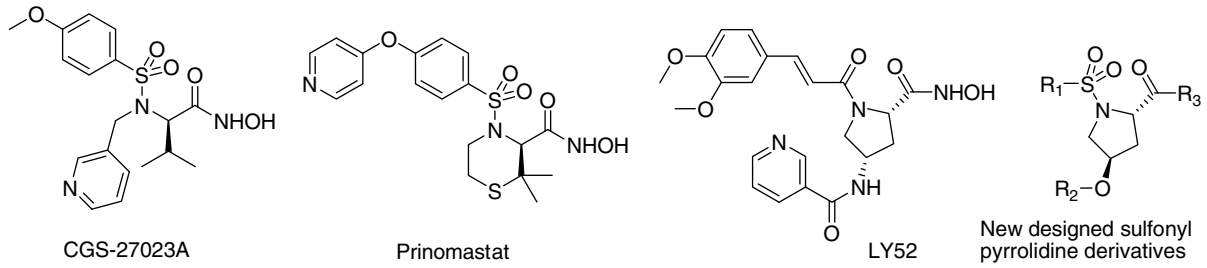


Figure 1. Chemical structures of CGS-27023A, Prinomastat, LY52 and our further designed sulfonyl pyrrolidine derivatives.

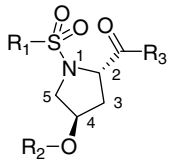
template, the intermediate trans-4-hydroxy-L-proline methyl ester hydrochloride (**2**) was prepared according to the literature.[12] 1-Sulfonyl-4-hydroxy-pyrrolidine-2-carboxylic acid methyl ester (**3a–c**) was prepared by sulfonation of compound **2** with various sulfonyl chlorides in the presence of 4-(dimethylamino) pyridine as catalyst.[13] Esterification of **3a–c** afforded the target compounds 4a–**r** and ammonolysis of **3a–c** with NH_2OK provided the target compounds **5a–c**.[13,14] The chemical structures of the target compounds were confirmed by IR, ^1H NMR, and ESI-MS.

The newly synthesized pyrrolidine derivatives were assayed for the inhibitory activities on MMP-2 and AP-N.[15,16] Similarly as 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 compounds **4c**, **4j**, **5a,** and **5b** were equally or more potent MMP-2 inhibitors than the positive control LY52. The FlexX docking of these compounds with MMP-2 was done using Sybyl 7.0 of Tripos Incorporation and the result was shown in Figures 2–4. The R_1 group at N_1 position of pyrrolidine ring (toluene-4-sulfonyl, benzenesulfonyl, respectively) occupied the deep S1′ pocket of MMP-2, and the ZBG chelated the active site zinc ion 166 with a distance of 2.16, 2.19, and 1.47 Å, respectively. These compounds interacted well with MMP-2 active site, especially the deep S1′ pocket and zinc ion 166, consistent with MMP-2 assay results.

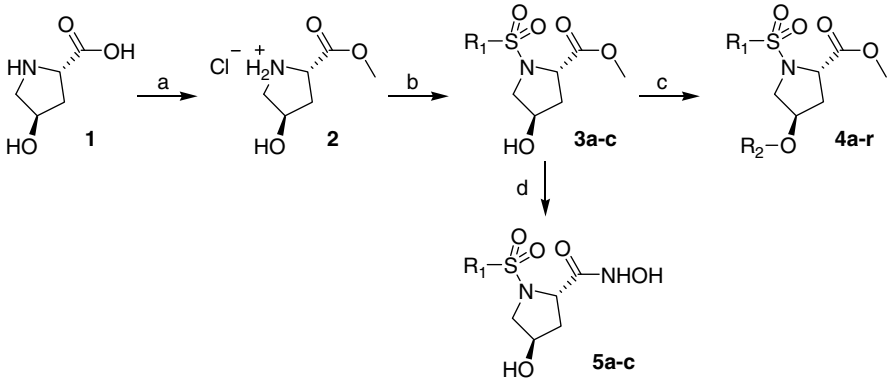
The selective inhibition might be explained by the FlexX docking results of representative compound **5b** with AP-N (Fig. 5). Although compound **5b** chelated well with the active site zinc ion 900, the R_2 and R_1SO_2 groups at pyrrolidine ring were both solvent exposed and could not occupy the S1′ and S2′ pockets of AP-N. MMP-2 was a zinc-dependent endopeptidase that could cut the peptide to parts from the specific amino acid residue of peptide.

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



No.	R_1	R_2	R_3	IC_{50}[a] (μM)	
				MMP-2	AP-N
3a	p-$CH_3C_6H_4$	H	OCH_3	0.1 ± 0.02	38.4 ± 4.6
3b	C_6H_5	H	OCH_3	0.8 ± 0.1	53.8 ± 3.2
3c	CH_3	H	OCH_3	7.1 ± 0.6	41.6 ± 6.9
4a	p-$CH_3C_6H_4$	C_6H_5CO	OCH_3	3.2 ± 0.3	24.8 ± 2.6
4b	p-$CH_3C_6H_4$	p-ClC_6H_4CO	OCH_3	2.2 ± 0.2	51.2 ± 6.1
4c	p-$CH_3C_6H_4$	p-$CH_3C_6H_4SO_2$	OCH_3	0.007 ± 0.001	101.4 ± 10.7
4d	p-$CH_3C_6H_4$	$C_6H_5SO_2$	OCH_3	0.05 ± 0.006	74.6 ± 9.4
4e	p-$CH_3C_6H_4$	CH_3SO_2	OCH_3	0.2 ± 0.01	41.4 ± 3.5
4f	p-$CH_3C_6H_4$	(E)C_6H_5CH=$CHCO$	OCH_3	0.04 ± 0.008	168.6 ± 14.2
4g	C_6H_5	C_6H_5CO	OCH_3	3.5 ± 0.4	43.8 ± 2.6
4h	C_6H_5	p-ClC_6H_4CO	OCH_3	2.7 ± 0.2	36.4 ± 4.7
4i	C_6H_5	p-$CH_3C_6H_4SO_2$	OCH_3	0.04 ± 0.003	61.3 ± 8.2
4j	C_6H_5	$C_6H_5SO_2$	OCH_3	0.01 ± 0.001	47.3 ± 1.5
4k	C_6H_5	CH_3SO_2	OCH_3	1.3 ± 0.1	39.5 ± 7.3
4l	C_6H_5	(E)C_6H_5CH=$CHCO$	OCH_3	0.09 ± 0.01	134.7 ± 11.2
4m	CH_3	C_6H_5CO	OCH_3	5.2 ± 0.4	53.6 ± 5.2
4n	CH_3	p-ClC_6H_4CO	OCH_3	1.5 ± 0.2	47.5 ± 3.9
4o	CH_3	p-$CH_3C_6H_4SO_2$	OCH_3	0.2 ± 0.01	68.9 ± 7.1
4p	CH_3	$C_6H_5SO_2$	OCH_3	0.7 ± 0.06	28.4 ± 4.5
4q	CH_3	CH_3SO_2	OCH_3	8.9 ± 1.0	31.6 ± 2.9
4r	CH_3	(E)C_6H_5CH=$CHCO$	OCH_3	0.5 ± 0.07	114.3 ± 12.6
5a	p-$CH_3C_6H_4$	H	NHOH	0.002 ± 0.0002	21.7 ± 1.9
5b	C_6H_5	H	NHOH	0.004 ± 0.0003	33.5 ± 4.1
5c	CH_3	H	NHOH	0.02 ± 0.001	26.3 ± 3.2
LY52				0.009 ± 0.0004	141.9 ± 11.7

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



Scheme 1. Reagents: (a) CH_3OH, HCl; (b) R_1SO_2Cl; (c) R_2COCl or R_2SO_2Cl; (d) NH_2OK.

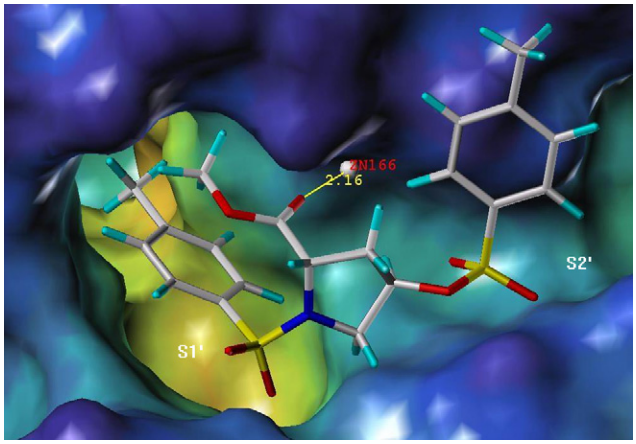


Figure 2. FlexX docking result of compound **4c** with MMP-2.

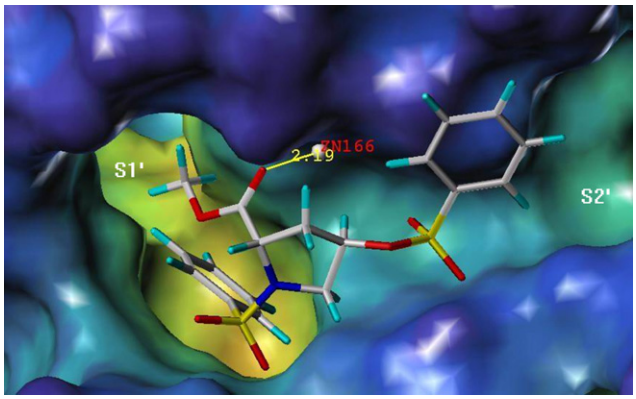


Figure 3. FlexX docking result of compound **4j** with MMP-2.

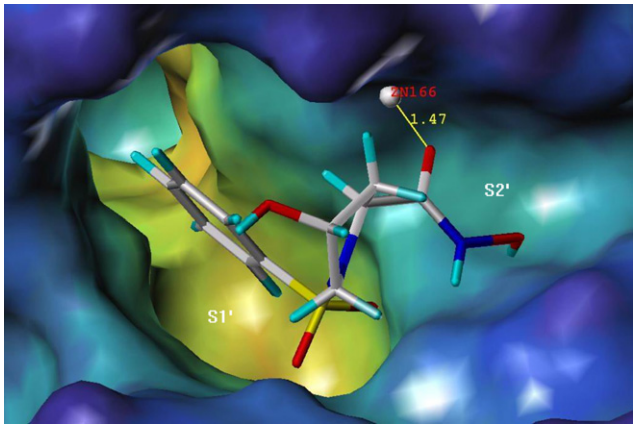


Figure 4. FlexX docking result of compound **5b** with MMP-2.

However, AP-N was a membrane-bound zinc exopeptidase that catalyzed the removal of NH-terminal amino acid from 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 **5a–c** was more potent than their predecessor **3a–c**. This activity difference was caused by the ZBG (COR_3), which was the only structural difference between **5a–c** and their predecessor.

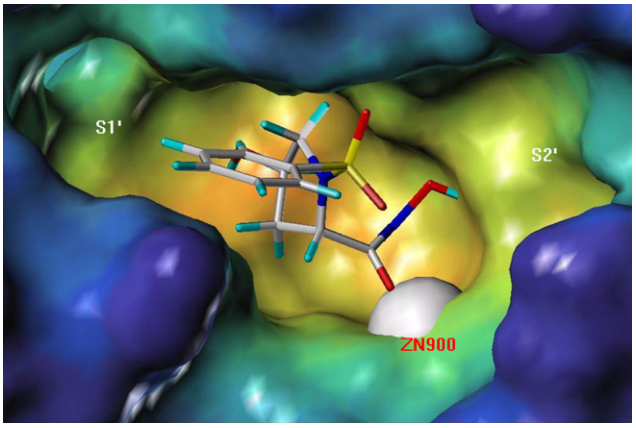


Figure 5. FlexX docking result of compound **5b** with AP-N.

The ZBG is hydroxamate (CONHOH) for **5a–c** and carboxylate ($COOCH_3$) for their predecessor, respectively. Both of these two 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 **5a–c** and their predecessor.

Among compounds **4a–f**, R_1SO_2 and COR_3 groups were fixed as toluene-4-sulfonyl and carboxylate, respectively, and R_2 group was altered as various length and substitutions. So the differences in the inhibitory activities of these compounds were caused by various R_2 groups. For compound **4c**, R_1SO_2 and R_2 groups were both toluene-4-sulfonyl, thus forming a butterfly like structure. The high potency of **4c** might be due to the symmetrical structure, which could adjust the conformation to extend into the catalytic activity center of the enzyme, respectively. Substitution on aromatic ring also has impact on bioactivity. For example, compound **4b** which was substituted with chlorine in para position showed a slightly better inhibitory activity compared with **4a**. Increasing conjugate system between ester and aromatic ring (**4a**) produced **4f** with improved potency.

Among compounds **4g–l**, R_1SO_2 and COR_3 groups were fixed as benzenesulfonyl and carboxylate, respectively, and R_2 group was altered as various length and substitutions. So the differences in the inhibitory activities of these compounds were caused by various R_2 groups. The SARs were similar to compounds **4a–f**. For compound **4j**, R_1SO_2 and R_2 groups were both benzenesulfonyl, thus forming a butterfly like structure. The high potency of **4j** might be due to the symmetrical structure, which could adjust the conformation to extend into the catalytic activity center of the enzyme, respectively. Substitution on aromatic ring also has impact on bioactivity. For example, compound **4h** which was substituted with chlorine in para position showed a slightly better inhibitory activity compared with **4g**. Increasing conjugate system between ester and aromatic ring (**4g**) produced **4l** with improved potency. However, compounds **4g–l** was less potent than its counterpart **4a–f**, which indicated that benzenesulfonyl substitution at N_1 position of pyrrolidine ring was less favorable than the toluene-4-sulfonyl substitution.

Among compounds **4m–r**, R_1SO_2 and COR_3 groups were fixed as methanesulfonyl and carboxylate, respectively, and R_2 group was altered as various length and substitutions. So the differences in the inhibitory activities of these compounds were caused by various R_2 groups. However, the SARs were not similar to compounds 4a–**f** and **4g–l**. For compound **4q**, R_1SO_2 and R_2 groups were both methanesulfonyl, thus forming a butterfly like structure. However, this compound did not present high potency. This might be due to the too small methanesulfonyl group, which could not match the

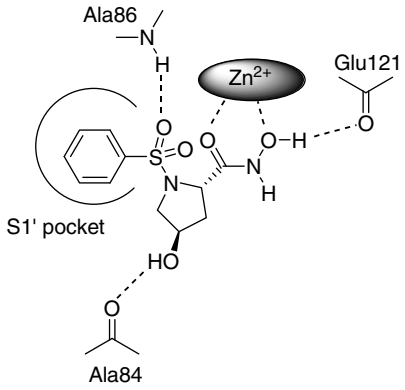


Figure 6. Proposed binding mode of compound **5b** with MMP-2.

catalytic activity center of the enzyme. Compound **4o** displayed high potency, and this could be explained by the toluene-4-sulfonyl at C_4 position of pyrrolidine ring, which was large enough to plunge in the S1′ pocket.

Finally, the binding mode of the compound **5b** with MMP-2 was proposed as follow: (1) the hydroxamate chelated the active site zinc ion; (2) the sulfonyl, and hydroxyl groups provided hydrogen bond interactions with the enzyme backbone Ala86, Ala84, and Glu121; (3) the side chain (benzenesulfonyl) underwent effective interactions with the enzyme S1' 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 new sulfonyl pyrrolidine derivatives was designed and synthesized. These pyrrolidine derivatives exhibited highly selective inhibition against MMP-2 as compared with AP-N. The compounds **4c**, **4j**, **5a**, and **5b** were equally or more potent MMP-2 inhibitors than the positive control LY52. SAR studies indicated that introduction of butterfly like symmetrical structure (toluene-4-sulfonyl 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.

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 Hertz. 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 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, 2-CH), 3.88 (s, 3H, CH_3), 3.50 (m, 1H, 4-CH), 3.35 (m, 2H, 5-CH_2), 2.47–2.20 (m, 2H, 3-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 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 with 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). Yield: 75%; white crystal; mp 78–79 °C; IR (KBr, cm^{-1}): 3513 (OH), 2953 (CH), 1743 (C=O), 1598 (C=C), 1344 (SO_2), 1157 (SO_2); ESI-MS: 300.5 $[M+1]^+$.

4.1.2.2. 1-Benzenesulfonyl-4-hydroxy-pyrrolidine-2-carboxylic acid methyl ester (3b). Yield: 69%; white crystal; mp 102–103 °C; IR (KBr, cm^{-1}): 3468 (OH), 2957 (CH), 1726 (C=O), 1348 (SO_2), 1157 (SO_2); ESI-MS: 286.2 $[M+1]^+$.

4.1.2.3. 4-Hydroxy-1-methanesulfonyl-pyrrolidine-2-carboxylic acid methyl ester (3c). Yield: 35%; white crystal; mp 85–87 °C; IR (KBr, cm^{-1}): 3469 (OH), 2959 (CH), 1724 (C=O), 1344 (SO_2), 1148 (SO_2); ESI-MS: 224.3 $[M+1]^+$.

4.1.3. General procedure for the preparation of 1-(toluene-4-sulfonyl)-4-(acyloxy or sulfonyloxy)-pyrrolidine-2-carboxylic acid methyl ester (4a–f)

4-Hydroxy-1-(toluene-4-sulfonyl)-pyrrolidine-2-carboxylic acid methyl ester (**3a**) (3 g, 10 mmol) was dissolved in dichloromethane (50 ml) and triethylamine (1.01 g, 10 mmol) was added. A solution of acyl chloride or sulfonyl chloride (10 mmol) in dichloromethane (50 ml) was added dropwise at 0 °C. The resulting mixture was stirred overnight at room temperature. The solvent was evaporated in vacuo. The residue was purified by flash column chromatography to give **4a–f**.

4.1.3.1. 4-Benzoyloxy-1-(toluene-4-sulfonyl)-pyrrolidine-2-carboxylic acid methyl ester (4a). Flash column chromatography: dichloromethane/acetone = 100:1; 45%; white crystal; mp 95–97 °C; IR (KBr, cm^{-1}): 2953 (CH), 1720 (C=O), 1599 (C=C), 1350 (SO_2), 1160 (SO_2); ^{1}H NMR ($CDCl_3$, δ ppm): 7.72 (d, 2H, 1-SO_2Ar-H, J = 8.28 Hz), 7.63 (d, 2H, 1-SO_2Ar-H, J = 8.36 Hz), 7.55 (m, 1H, 4-OCOAr-H), 7.36 (t, 2H, 4-OCOAr-H, J = 7.76 Hz), 7.14 (d, 2H, 4-OCOAr-H, J = 8.09 Hz), 5.29 (s, 1H, 4-CH), 4.40 (m, 1H, 2-CH), 3.80 (s, 3H, OCH_3), 3.89–3.73 (m, 2H, 5-CH_2), 2.51–2.30 (m, 2H, 3-CH_2), 2.20 (s, 3H, $ArCH_3$); ESI-MS: 404.4 $[M+1]^+$.

4.1.3.2. 4-(4-Chloro-benzoyloxy)-1-(toluene-4-sulfonyl)-pyrrolidine-2-carboxylic acid methyl ester (4b). Flash column chromatography: dichloromethane/acetone = 100:1; 57%; white crystal; mp 122–124 °C; IR (KBr, cm^{-1}): 2924 (CH), 1724 (C=O), 1593 (C=C), 1350 (SO_2), 1159 (SO_2); ^{1}H NMR ($CDCl_3$, δ ppm): 7.71 (d, 2H, 4-OCOAr-H, J = 8.25 Hz), 7.57 (d, 2H, 1-SO_2Ar-H, J = 9.03 Hz), 7.33 (d, 2H, 4-OCOAr-H, J = 8.60 Hz), 7.14 (d, 2H, 1-SO_2Ar-H, J = 8.05 Hz), 5.36 (s, 1H, 4-CH), 4.39 (m, 1H, 2-CH), 3.82 (s, 3H, OCH_3), 3.88–3.73 (m, 2H, 5-CH_2), 2.52–2.30 (m, 2H, 3-CH_2), 2.23 (s, 3H, $ArCH_3$); ESI-MS: 438.3 $[M+1]^+$.

4.1.3.3. 1-(Toluene-4-sulfonyl)-4-(toluene-4-sulfonyloxy)-pyrrolidine-2-carboxylic acid methyl ester (4c). Flash column chromatography: dichloromethane/acetone = 100:1; 42%; white crystal; mp 85–86 °C; IR (KBr, cm^{-1}): 2945 (CH), 1748 (C=O), 1599 (C=C), 1354 (SO_2), 1171 (SO_2); ^{1}H NMR ($CDCl_3$, δ ppm): 7.70 (d, 2H, 4-OSO_2Ar-H, J = 8.24 Hz), 7.61 (d, 2H, 1-SO_2Ar-H, J = 8.32 Hz), 7.32 (t, 4H, 4-OSO_2Ar-H and 1-SO_2Ar-H, J = 8.68 Hz), 4.97 (m, 1H, 4-CH), 4.27 (t, 1H, 2-CH, J = 7.92 Hz), 3.75 (s, 3H, OCH_3), 3.77–3.55 (m, 2H, 5-CH_2), 2.46 (s, 3H, $ArCH_3$), 2.44 (s, 3H, $ArCH_3$), 2.38–2.16 (m, 2H, 3-CH_2); ESI-MS: 454.2 $[M+1]^+$.

4.1.3.4. 4-Benzenesulfonyloxy-1-(toluene-4-sulfonyl)-pyrrolidine-2-carboxylic acid methyl ester (4d). Flash column chromatography: dichloromethane/acetone = 100:1; 61%; white crystal; mp 95–97 °C; IR (KBr, cm^{-1}): 2941 (CH), 1745 (C=O), 1597 (C=N), 1351 (SO_2), 1187 (SO_2); ^{1}H NMR ($CDCl_3$, δ ppm): 7.75 (d, 2H, 4-OSO_2Ar-H, J = 8.08 Hz), 7.70 (d, 2H, 1-SO_2Ar-H, J = 8.27 Hz), 7.67 (m, 1H, 4-OSO_2Ar-H), 7.55 (t, 2H, 4-OSO_2Ar-H, J = 7.95 Hz), 7.29 (d, 2H, 1-SO_2Ar-H, J = 8.10 Hz), 5.02 (m, 1H, 4-CH), 4.28 (t, 1H, 2-CH, J = 7.78 Hz), 3.73 (s, 3H, OCH_3), 3.72–3.56 (m, 2H, 5-CH_2), 2.43 (s, 3H, $ArCH_3$), 2.40–2.17 (m, 2H, 3-CH_2); ESI-MS: 440.3 $[M+1]^+$.

4.1.3.5. 4-Methanesulfonyloxy-1-(toluene-4-sulfonyl)-pyrrolidine-2-carboxylic acid methyl ester (4e). Flash column chromatography: dichloromethane/acetone = 100:1; 35%; colorless oil; IR (KBr, cm^{-1}): 2955 (CH), 1754 (C=O), 1598 (C=C), 1348 (SO_2), 1160 (SO_2); ^{1}H NMR ($CDCl_3$, δ ppm): 7.77 (d, 2H, Ar-H, J = 8.28 Hz), 7.35 (d, 2H, Ar-H, J = 8.09 Hz), 5.20 (m, 1H, 4-CH), 4.38 (t, 1H, 2-CH, J = 8.07 Hz), 3.76 (s, 3H, OCH_3), 3.74 (m, 2H, 5-CH_2), 2.82 (s, 3H, CH_3SO_2), 2.56–2.50 and 2.32–2.25 (m, 2H, 3-CH_2), 2.44 (s, 3H, $ArCH_3$); ESI-MS: 378.3 $[M+1]^+$.

4.1.3.6. 4-(3-Phenyl-acryloyloxy)-1-(toluene-4-sulfonyl)-pyrrolidine-2-carboxylic acid methyl ester (4f). Flash column chromatography: dichloromethane/acetone = 100:1; 74%; white crystal; mp 110–112 °C; IR (KBr, cm^{-1}): 2953 (CH), 1745 (C=O), 1716 (C=O), 1636 (C=O), 1345 (SO_2), 1159 (SO_2), 1022 (=CH); ^{1}H NMR ($CDCl_3$, δ ppm): 7.75 (d, 2H, 1-SO_2Ar-H, J = 8.20 Hz), 7.56 (m, 1H, 4-Ar-H), 7.47–7.39 (m, 4H, 4-Ar-H), 7.25 (d, 2H, 1-SO_2Ar-H, J = 8.16 Hz), 6.46 (d, 1H, 4-OCOC=CH, J = 16.00 Hz), 5.91 (d, 1H, 4-OCOCH=C, J = 16.00 Hz), 5.26 (s, 1H, 4-CH), 4.37 (m, 1H, 2-CH), 3.80 (s, 3H, OCH_3), 3.83–3.65 (m, 2H, 5-CH_2), 2.47–2.39 (m, 2H, 3-CH_2), 2.16 (s, 3H, $ArCH_3$); ESI-MS: 430.3 $[M+1]^+$.

4.1.4. General procedure for the preparation of 1-benzenesulfonyl-4-(acyloxy or sulfonyloxy)-pyrrolidine-2-carboxylic acid methyl ester (4g–l)

1-Benzenesulfonyl-4-hydroxy-pyrrolidine-2-carboxylic acid methyl ester (**3b**) (2.86 g, 10 mmol) was dissolved in dichloromethane (50 ml) and triethylamine (1.01 g, 10 mmol) was added. A solution of acyl chloride or sulfonyl chloride (10 mmol) in dichloromethane (50 ml) was added dropwise at 0 °C. The resulting mixture was stirred overnight at room temperature. The solvent was evaporated in vacuo. The residue was purified by flash column chromatography to give **4g–l**.

4.1.4.1. 1-Benzenesulfonyl-4-benzoyloxy-pyrrolidine-2-carboxylic acid methyl ester (4g). Flash column chromatography: dichloromethane/acetone = 100:1; 54%; white crystal; mp 110-111 °C; IR (KBr, cm^{-1}): 2954 (CH), 1751 (C=O), 1710 (C=O), 1599 (C=C), 1335 (SO_2), 1158 (SO_2); ^{1}H NMR ($CDCl_3$, δ ppm): 7.86 (m, 2H, 4-OCOAr-H), 7.56 (m, 3H, 4-OCOAr-H), 7.39 (m, 5H, 1-SO_2Ar-H), 5.40 (s, 1H, 4-CH), 4.40 (m, 1H, 2-CH), 3.80 (s, 3H, OCH_3), 3.91–3.73 (m, 2H, 5-CH_2), 2.53–2.31 (m, 2H, 3-CH_2); ESI-MS: 390.2 $[M+1]^+$.

4.1.4.2. 1-Benzenesulfonyl-4-(4-chloro-benzoyloxy)-pyrrolidine-2-carboxylic acid methyl ester (4h). Flash column chromatography: dichloromethane/acetone = 100:1; 64%; white crystal; mp 120-122 °C; IR (KBr, cm^{-1}): 2923 (CH), 1755 (C=O), 1725 (C=O), 1593 (C=C), 1348 (SO_2), 1163 (SO_2); ^{1}H NMR ($CDCl_3$, δ ppm): 7.87 (m, 2H, 1-SO_2Ar-H), 7.52 (d, 2H, 4-OCOAr-H, J = 6.80 Hz), 7.43 (m, 3H, 1-SO_2Ar-H), 7.32 (d, 2H, 4-OCOAr-H, J = 6.74 Hz), 5.40 (s, 1H, 4-CH), 4.41 (m, 1H, 2-CH), 3.80 (s, 3H, OCH_3), 3.89–3.73 (m, 2H, 5-CH_2), 2.53–2.31 (m, 2H, 3-CH_2); ESI-MS: 424.2 $[M+1]^+$.

4.1.4.3. 1-Benzenesulfonyl-4-(toluene-4-sulfonyloxy)-pyrrolidine-2-carboxylic acid methyl ester (4i). Flash column chromatography: dichloromethane/acetone = 100:1–10:1; 46%; white crystal; mp 62-63 °C; IR (KBr, cm^{-1}): 2956 (CH), 1747 (C=O), 1599 (C=C), 1353 (SO_2), 1173 (SO_2); ^{1}H NMR ($CDCl_3$, δ ppm): 7.81 (d, 2H, 1-SO_2Ar-H, J = 7.24 Hz), 7.59 (m, 3H, 1-SO_2Ar-H), 7.51 (t, 2H, 4-OSO_2Ar-H, J = 7.84 Hz), 7.32 (d, 2H, 4-OSO_2Ar-H, J = 8.16 Hz), 4.99 (m, 1H, 4-CH), 4.28 (t, 1H, 2-CH, J = 7.92 Hz), 3.73 (s, 3H, OCH_3), 3.71–3.58 (m, 2H, 5-CH_2), 2.45 (s, 3H, $ArCH_3$), 2.41–2.17 (m, 2H, 3-CH_2); ESI-MS: 440.3 $[M+1]^+$.

4.1.4.4. 1-Benzenesulfonyl-4-benzenesulfonyloxy-pyrrolidine-2-carboxylic acid methyl ester (4j). Flash column chromatography: dichloromethane/acetone = 100:1–10:1; 68%; white crystal; mp 75–76 °C; IR (KBr, cm^{-1}): 2925 (CH), 1741 (C=O), 1360 (SO_2), 1168 (SO_2); ^{1}H NMR ($CDCl_3$, δ ppm): 7.81 (d, 2H, 4-OSO_2Ar-H, J = 7.32 Hz), 7.71 (m, 2H, 4-OSO_2Ar-H), 7.66 (d, 1H, 4-OSO_2Ar-H, J = 7.48 Hz), 7.54 (m, 5H, 1-SO_2Ar-H), 5.03 (s, 1H, 4-CH), 4.29 (t, 1H, 2-CH, J = 7.96 Hz), 3.73 (s, 3H, OCH_3), 3.70–3.60 (m, 2H, 5-CH_2), 2.43-2.16 (m, 2H, 3-CH_2); ESI-MS: 426.1 $[M+1]^+$.

4.1.4.5. 1-Benzenesulfonyl-4-methanesulfonyloxy-pyrrolidine-2-carboxylic acid methyl ester (4k). Flash column chromatography: dichloromethane/acetone = 100:1; 74%; colorless oil; IR (KBr, cm^{-1}): 2955 (CH), 1751 (C=O), 1351 (SO_2), 1173 (SO_2); ^{1}H NMR ($CDCl_3$, δ ppm): 7.89 (d, 2H, 1-SO_2Ar-H, J = 7.25 Hz), 7.63 (m, 1H, 1-SO_2Ar-H), 7.57 (t, 2H, 1-SO_2Ar-H, J = 7.80 Hz), 5.20 (s, 1H, 4-CH), 4.40 (t, 1H, 2-CH, J = 8.18 Hz), 3.76 (s, 3H, OCH_3), 3.79–3.74 (m, 2H, 5-CH_2), 2.80 (s, 3H, CH_3SO_2), 2.55–2.29 (m, 2H, 3-CH_2); ESI-MS: 364.2 $[M+1]^+$.

4.1.4.6. 1-Benzenesulfonyl-4-(3-phenyl-acryloyloxy)-pyrrolidine-2-carboxylic acid methyl ester (4l). Flash column chromatography: dichloromethane/acetone = 50:1; 43%; white crystal; mp 85–86 °C; IR (KBr, cm^{-1}): 2953 (CH), 1744 (C=O), 1714 (C=O), 1640 (C=O), 1347 (SO_2), 1162 (SO_2), 1021 (=CH); ^{1}H NMR ($CDCl_3$, δ ppm): 7.87 (m, 2H, Ar-H), 7.42 (m, 8H, Ar-H), 6.45 (d, 1H, 4-OCOC=CH, J = 16.00 Hz), 5.81 (d, 1H, 4-OCOCH=C, J = 16.08 Hz), 5.26 (s, 1H, 4-CH), 4.37 (m, 1H, 2-CH), 3.78 (s, 3H, OCH_3), 3.84-3.65 (m, 2H, 5-CH_2), 2.45–2.24 (m, 2H, 3-CH_2); ESI-MS: 416.3 $[M+1]^+$.

4.1.5. General procedure for the preparation of 1-methanesulfonyl-4-(acyloxy or sulfonyloxy)-pyrrolidine-2-carboxylic acid methyl ester (4m–r)

4-Hydroxy-1-methanesulfonyl-pyrrolidine-2-carboxylic acid methyl ester (**3c**) (2.23 g, 10 mmol) was dissolved in dichloromethane (50 ml) and triethylamine (1.01 g, 10 mmol) was added. A solution of acyl chloride or sulfonyl chloride (10 mmol) in dichloromethane (50 ml) was added dropwise at 0 °C. The resulting mixture was stirred overnight at room temperature. The solvent was evaporated in vacuo. The residue was purified by flash column chromatography to give **4m–r**.

4.1.5.1. 4-Benzoyloxy-1-methanesulfonyl-pyrrolidine-2-carboxylic acid methyl ester (4m). Flash column chromatogra-

phy: dichloromethane/acetone = 100:1; 52%; white crystal; mp 65-66 °C; IR (KBr, cm^{-1}): 2957 (CH), 1745 (C=O), 1714 (C=O), 1326 (SO_2), 1147 (SO_2); ^1H NMR ($CDCl_3$, δ ppm): 8.00 (d, 2H, Ar-H, J = 7.20 Hz), 7.58 (t, 1H, Ar-H, J = 7.44 Hz), 7.45 (m, 2H, Ar-H), 5.54 (s, 1H, 4-CH), 4.71 (t, 1H, 2-CH, J = 8.12 Hz), 3.79 (s, 3H, OCH_3), 3.93–3.75 (m, 2H, 5-CH_2), 3.03 (s, 3H, CH_3SO_2), 2.70–2.39 (m, 2H, 3-CH_2); ESI-MS: 328.3 $[M+1]^+$.

4.1.5.2. 4-(4-Chloro-benzoyloxy)-1-methanesulfonyl-pyrrolidine-2-carboxylic acid methyl ester (4n). Flash column chromatography: dichloromethane/acetone = 100:1; 51%; white crystal; mp 105–107 °C; IR (KBr, cm^{-1}): 2959 (CH), 1746 (C=O), 1716 (C=O), 1593 (C=C), 1329 (SO_2), 1146 (SO_2); ^1H NMR ($CDCl_3$, δ ppm): 7.95 (d, 2H, Ar-H, J = 6.72 Hz), 7.43 (d, 2H, Ar-H, J = 6.74 Hz), 5.53 (s, 1H, 4-CH), 4.74 (t, 1H, 2-CH, J = 8.07 Hz), 3.80 (s, 3H, OCH_3), 3.97–3.71 (m, 2H, 5-CH_2), 3.05 (s, 3H, CH_3SO_2), 2.70–2.34 (m, 2H, 3-CH_2); ESI-MS: 362.2 $[M+1]^+$.

4.1.5.3. 1-Methanesulfonyl-4-(toluene-4-sulfonyloxy)-pyrrolidine-2-carboxylic acid methyl ester (4o). Flash column chromatography: dichloromethane/acetone = 100:1-10:1; 73%; white crystal; mp 95-96 °C; IR (KBr, cm^{-1}): 2959 (CH), 1747 (C=O), 1596 (C=C), 1338 (SO_2), 1155 (SO_2); ^1H NMR ($CDCl_3$, δ ppm): 7.79 (d, 2H, Ar-H, J = 8.28 Hz), 7.38 (d, 2H, Ar-H, J = 8.12 Hz), 5.08 (s, 1H, 4-CH), 4.48 (t, 1H, 2-CH, J = 8.08 Hz), 3.75 (s, 3H, OCH_3), 3.80–3.60 (m, 2H, 5-CH_2), 2.95 (s, 3H, CH_3SO_2), 2.47 (s, 3H, $ArCH_3$), 2.62–2.55 and 2.27–2.16 (m, 2H, 3-CH_2); ESI-MS: 378.3 $[M+1]^+$ and 395.2 $[M+NH_4]^+$.

4.1.5.4. 4-Benzenesulfonyloxy-1-methanesulfonyl-pyrrolidine-2-carboxylic acid methyl ester (4p). Flash column chromatography: dichloromethane/acetone = 100:1-10:1; 58%; white crystal; mp 78-80 °C; IR (KBr, cm^{-1}): 2956 (CH), 1751 (C=O), 1340 (SO_2), 1151 (SO_2); ^1H NMR ($CDCl_3$, δ ppm): 7.91 (d, 2H, Ar-H, J = 8.68 Hz), 7.72 (t, 1H, Ar-H, J = 7.48 Hz), 7.60 (t, 2H, Ar-H, J = 7.96 Hz), 5.12 (s, 1H, 4-CH), 4.48 (t, 1H, 2-CH, J = 8.12 Hz), 3.74 (s, 3H, OCH_3), 3.80-3.61 (m, 2H, 5-CH_2), 2.95 (s, 3H, CH_3SO_2), 2.62–2.05 (m, 2H, 3-CH_2); ESI-MS: 364.2 $[M+1]^+$ and 381.3 $[M+NH_4]^+$.

4.1.5.5. 1-Methanesulfonyl-4-methanesulfonyloxy-pyrrolidine-2-carboxylic acid methyl ester (4q). Flash column chromatography: dichloromethane/acetone = 100:1; 32%; yellow oil; IR (KBr, cm^{-1}): 2940 (CH), 1748 (C=O), 1336 (SO_2), 1173 (SO_2); ^1H NMR ($CDCl_3$, δ ppm): 5.28 (s, 1H, 4-CH), 4.64 (t, 1H, 2-CH, J = 8.16 Hz), 3.79 (s, 3H, OCH_3), 4.02–3.66 (m, 2H, 5-CH_2), 3.11 (s, 3H, 4-OSO_2CH_3), 3.04 (s, 3H, 1-SO_2CH_3), 2.76–2.17 (m, 2H, 3-CH_2); ESI-MS: 302.3 $[M+1]^+$ and 319.2 $[M+NH_4]^+$.

4.1.5.6. 1-Methanesulfonyl-4-(3-phenyl-acryloyloxy)-pyrrolidine-2-carboxylic acid methyl ester (4r). Flash column chromatography: dichloromethane/acetone = 50:1; 43%; white crystal; mp 70–72 °C; IR (KBr, cm^{-1}): 2954 (CH), 1747 (C=O), 1713 (C=O), 1634 (C=O), 1336 (SO_2), 1151 (SO_2), 1026 (=CH); ^1H NMR ($CDCl_3$, δ ppm): 7.71 (d, 1H, 4-OCOC=CH, J = 16.00 Hz), 7.54 (m, 2H, Ar-H), 7.41 (m, 3H, Ar-H), 6.41 (d, 1H, 4-OCOCH=C, J = 16.00 Hz), 5.43 (s, 1H, 4-CH), 4.67 (t, 1H, 2-CH, J = 8.04 Hz), 3.80 (s, 3H, OCH_3), 3.87–3.70 (m, 2H, 5-CH_2), 3.04 (s, 3H, CH_3SO_2), 2.64–2.29 (m, 2H, 3-CH_2); ESI-MS: 354.2 $[M+1]^+$ and 371.3 $[M+NH_4]^+$.

4.1.6. General procedure for the preparation of 4-hydroxy-1-sulfonyl-pyrrolidine-2-carboxylic acid hydroxyamide (5a–c)

To a solution of compound **3** (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 5a–**c**.

4.1.6.1. 4-Hydroxy-1-(toluene-4-sulfonyl)-pyrrolidine-2-carboxylic acid hydroxyamide (5a). Flash column chromatography: dichloromethane/methanol = 10:1; 36%; white crystal; mp 130–132 °C; IR (KBr, cm^{-1}): 3331 (OH), 2924 (CH), 1682 (C=O), 1599 (C=O), 1332 (SO_2), 1157 (SO_2); ^1H NMR (CD_3OD, δ ppm): 7.98 (d, 1H, 2-CONH, J = 8.48 Hz), 7.75 (d, 2H, Ar-H, J = 8.27 Hz), 7.39 (m, 2H, Ar-H), 4.32 (m, 1H, 2-CH), 4.09 (t, 1H, 4-CH, J = 7.68 Hz), 3.73 (s, 1H, 4-OH), 3.30 (s, 1H, 2-CONOH), 3.62–3.21 (m, 2H, 5-CH_2), 2.41 (s, 3H, $ArCH_3$), 2.11–1.93 (m, 2H, 3-CH_2); ESI-MS: 301.4 $[M+1]^+$.

4.1.6.2. 1-Benzenesulfonyl-4-hydroxy-pyrrolidine-2-carboxylic acid hydroxyamide (5b). Flash column chromatography: dichloromethane/methanol = 10:1; 41%; white crystal; mp 155–157 °C; IR (KBr, cm^{-1}): 3216 (OH), 2948 (CH), 1668 (C=O), 1332 (SO_2), 1158 (SO_2); ^1H NMR (CD_3OD, δ ppm): 7.89 (m, 2H, Ar-H), 7.68 (m, 1H, Ar-H), 7.60 (t, 2H, Ar-H, J = 7.84 Hz), 4.33 (m, 1H, 2-CH), 4.13 (t, 1H, 4-CH, J = 7.76 Hz), 3.66–3.28 (m, 2H, 5-CH_2), 2.13–1.94 (m, 2H, 3-CH_2); ESI-MS: 287.3 $[M+1]^+$.

4.1.6.3. 4-Hydroxy-1-methanesulfonyl-pyrrolidine-2-carboxylic acid hydroxyamide (5c). Flash column chromatography: dichloromethane/methanol = 10:1; 46%; white crystal; mp 150–151 °C; IR (KBr, cm^{-1}): 3416 and 3285 (OH), 2926 (CH), 1678 (C=O), 1336 (SO_2), 1145 (SO_2); ^1H NMR (CD_3OD, δ ppm): 4.43 (s, 1H, 2-CH), 4.24 (t, 1H, 4-CH, J = 8.12 Hz), 3.62-3.46 (m, 2H, 5-CH_2), 3.33 (m, 2H, 4-OH and 2-CONOH), 2.96 (s, 3H, CH_3), 2.33–2.15 (m, 2H, 3-CH_2); ESI-MS: 225.4 $[M+1]^+$.

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 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.

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 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.

Acknowledgments

This work was supported by the National High Technology Research and Development Program of China (863 project; Grant No. 2007AA02Z314), the National Natural Foundation Great Research Program (No. 90713041), the National Natural Foundation Research Grant (Grant No. 30772654), the Doctoral Foundation of Ministry of Education of the People's Republic of China (Grant No. 20060422029) and the Shandong Province Natural Science Foundation (Grant No. Y2004C02). This work was also supported in part by Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan.

References and notes

1. Murphy, G. J. P.; Murphy, G.; Reynolds, J. J. *FEBS Lett.* **1991**, *289*, 4–7.
2. Bjorklund, M.; Koivunen, E. *Biochim. Biophys. Acta* **2005**, *1755*, 37–69.
3. Polette, M.; Nawrocki-Raby, B.; Gilles, C.; Clavel, C.; Birembaut, P. *Crit. Rev. Oncol. Hematol.* **2004**, *49*, 179–186.
4. Verma, R. P.; Hansch, C. *Bioorg. Med. Chem.* **2007**, *15*, 2223–2268.
5. Nagase, H.; Visse, R.; Murphy, G. *Cardiovasc. Res.* **2006**, *69*, 562–573.
6. Kontogiorgis, C. A.; Papaioannou, P.; Hadjipavlou-Litina, D. J. *Curr. Med. Chem.* **2005**, *12*, 339–355.
7. Fisher, J. F.; Mobashery, S. *Cancer Metastasis Rev.* **2006**, *25*, 115–136.
8. Hanessian, S.; Moitessier, N. *Curr. Top. Med. Chem.* **2004**, *4*, 1269–1287.
9. Li, Y. L.; Xu, W. F. *Bioorg. Med. Chem.* **2004**, *12*, 5171–5180.
10. Li, X.; Li, Y.; Xu, W. *Bioorg. Med. Chem.* **2006**, *14*, 1287–1293.
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*, 3573–3578.
12. Jordis, U.; Sauter, F.; Siddiqi, S. M. *Indian J. Chem.* **1989**, *28B*, 294–296.
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*, 4948–4963.
14. 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*, 87–94.
15. 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*, 267–273.
16. Lejczak, B.; Kafarski, P.; Zygmunt, J. *Biochemistry* **1989**, *28*, 3549–3555.