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Design, synthesis, and activity of caffeoyl pyrrolidine derivatives as potential gelatinase inhibitors

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Abstract—The synthesis and biological evaluation of caffonyl pyrrolidine derivatives as MMPs inhibitors are reported in this paper. Inhibiting activities of synthesized compounds on gelatinase (MMP-2 and -9) were tested by using succinylated gelatin as substrate. Structure–activity relationship results from these tested compounds demonstrated that longer and more flexible side chain linked to the pyrrolidine ring at C_4 produced higher activity at gelatinase. Furthermore, aromatic heterocycle and sulfamide in the same position could enhance the activities. Compounds with free phenol hydroxyl group showed higher activity compared to methylated derivatives (or counterparts), which confirms the importance of phenol hydroxyl functionality in the interaction with gelatinase. The anti-metastasis model of mice bearing H_{22} tumor cell was used to evaluate their in vivo inhibiting activities. All tested compounds were orally administered at a dose of 50 or 100 mg/kg, 6 days/week for two weeks. The test results demonstrated that most of these inhibitors showed significant anti-cancer activities (inhibitory rate > 35%) and were devoid of toxic effects. Compound **29** showed the highest inhibitory rate at 69.25%, indicating that it might be a promising lead compound.

1. Introduction

The matrix metalloproteinases (MMPs) are a family of zinc-dependent protienases involved in the degradation of the extracellular matrix. The MMPs have been implicated in the processes of tumor growth, invasion, and metastasis, frequently overexpressed by malignant tumors and have been associated with an aggressive malignant phenotype and poor prognosis in patients with cancer.[1–3] Up to now, the mammal MMP gene family consists of at least 24 structurally related members, among which, gelatinase (MMP-2 and -9) are proved to be high correlation with cancer.

The interaction of MMP inhibitors with MMPs is illustrated in Figure 1 by the complex of CGS27023A with MMP-3.[4] The figure shows the *p*-(methoxyphenyl)sulfonyl extend into S_1' pocket of the enzyme, pyridine is in S_2' pocket, and isopropyl is in S_1 domain. There are two hydrogen bond between the inhibitor with the backbone of the enzyme, which are the O of sulfonylamide with NH of Leu^{164} and NH of hydroxamic acid with carbonyl O of Ala^{165}, respectively. The S_1' pocket of gelatinase is deeper than that of MMP-3 and can accommodate larger group. In our study, we designed compounds with caffonyl group to extend into S_1' pocket.

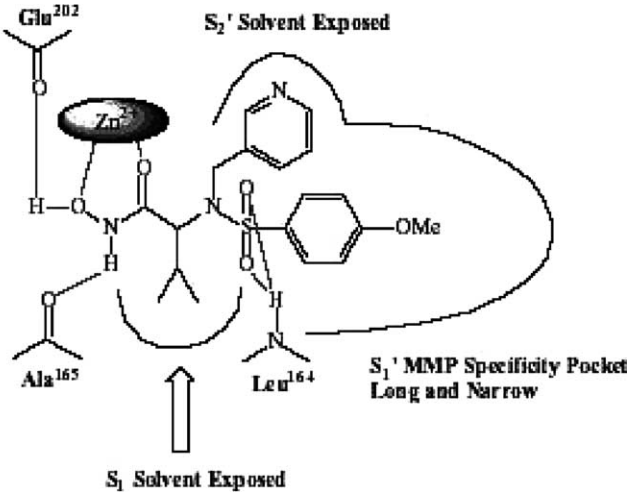

Figure 1. Mode of binding of CGS 27023A with MMP-3.

L-Hydroxyproline is known as one of specific amine acid of collagens, which are the substrate of MMPs. So the

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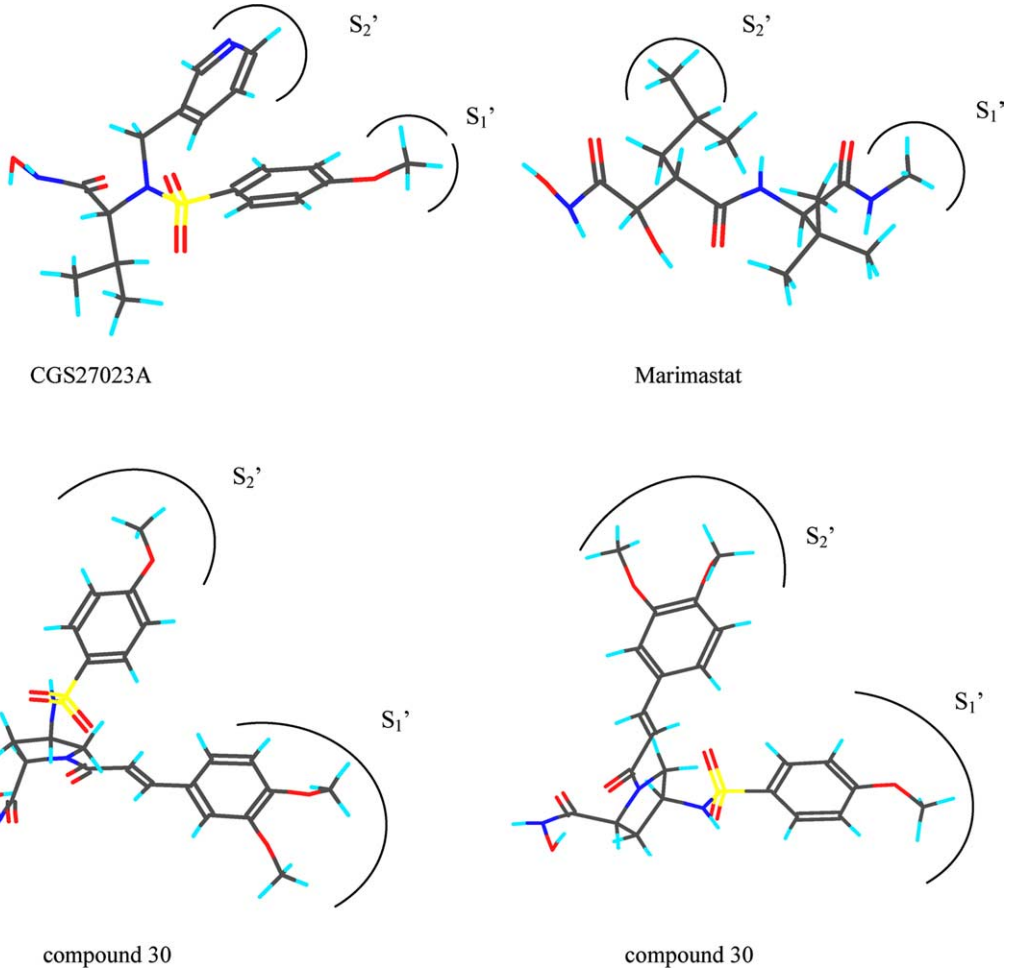


Figure 2. Comparison between designed compound with existing inhibitors. C, H, N, O, S are shown as black, green, blue, red, and yellow, respectively.

derivatives of hydroxyproline may specifically recognize MMPs and then combine with the active site. The caffic acid is proved to inhibit MMP-2 and -9,[5] so we linked caffic acid with hydroxyproline derivatives as to find compounds with activity inhibiting MMPs.

The 3-D structure of one of aimed compounds, compound **30**, was simulated by Chem3D Ultra 7.0 program and showed in Figure 2. From Figure 2, we can deduce that either the caffonyl group or the *p*-(methylphenyl)-sulfonyl group can extend into the S_1' group, which may enhance the flexibility of the compound to combine with the active site.

2. Chemistry

The target compounds were synthesized efficiently following the procedures shown in Schemes 1 and 2.

Briefly, all compounds were derived from 4-L-hydroxyproline, and obtained through a reaction sequence including methylation, esterification, condensation, mesylation, S_N2 reaction upon treatment with sodium azide, hydrogenation over 5% Pd–C/$CaCO_3$, acylation and ester exchange upon treatment with hydroxylamine as illustrated in Scheme 1.

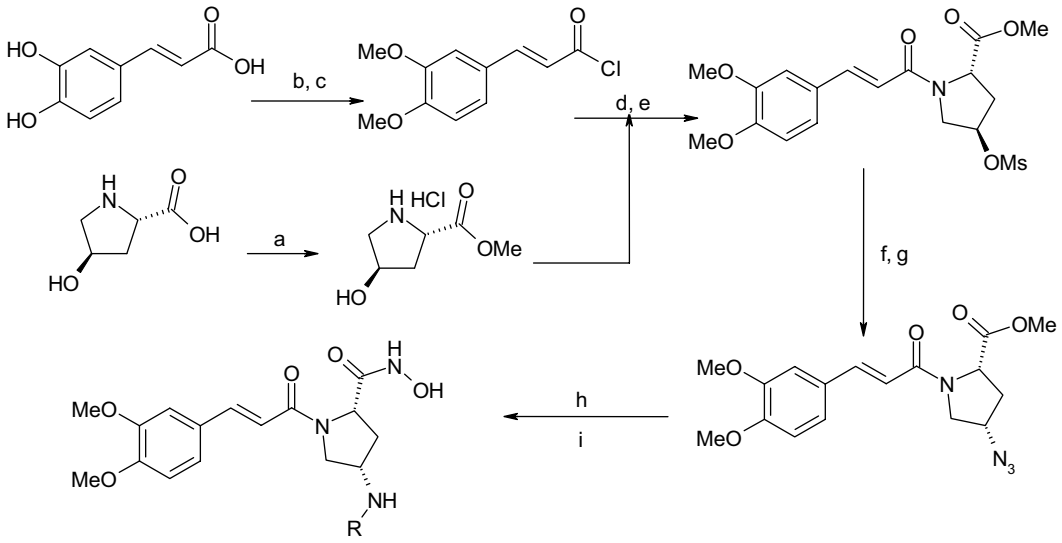
Considered that the caffic acid, which contained free phenol hydroxyl groups could inhibit the activity of MMP-2 and -9, we prepared several compounds with polyphenols as Scheme 2.

3. Results and discussion

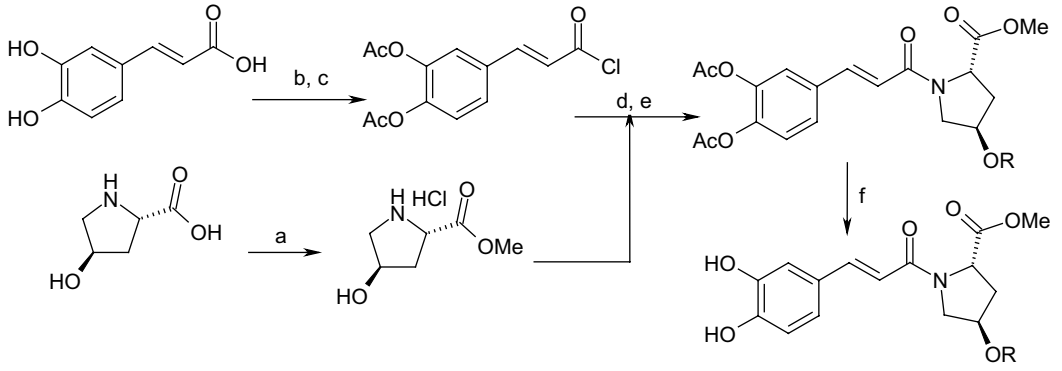
3.1. Structure–activity relationship in vitro

Compounds were evaluated as inhibitors of gelatinases. The results of their inhibitory activities (IC_{50}) were reported in Table 1.

3.1.1. Effect of the size of the side chains linked to the pyrrolidine ring at C_4. It is reported that MMPs have two hydrophobic domains, which are called S_1' pocket and S_2' pocket besides their catalytic activity center as in Figure 3.[5] The side chain R_1 is designed to be located in either of these two pockets. As seen in Table 1, to



Scheme 1. Reagents: (a) MeOH, HCl; (b) Me_2SO_4, NaOH; (c) $SOCl_2$, C_6H_6; (d) Py, Et_3N; (e) MsCl, Et_3N, CH_2Cl_2; (f) NaN_3, DMF; (g) 5% Pd–C, H_2, EtOH; (h) Et_3N, CH_2Cl_2, R–Cl (R = carbonyl or sulfonyl group); (i) NH_2OK, MeOH.



Scheme 2. Reagents: (a) MeOH, HCl; (b) Ac_2O, H_2SO_4; (c) $SOCl_2$, C_6H_6; (d) Py, Et_3N; (e) Et_3N, CH_2Cl_2, R–Cl (R = carbonyl or sulfonyl group); (f) MeOH, THF, HCl.

some extent, the longer the side chain of R_1 was, the more strongly the compounds inhibited the enzyme.

Comparing **23**, **24**, **25**, or **27**, **29**, **31**, **32**, we could confirm that the length of side chains of R_1 was positively relative with the inhibitory activities with an exception of compound **12**. This could be due to its bulky side chain in the interaction with the enzymes' hydrophobic domains.

Effect of the sulfonyl or acryl groups in the side chain linked to the pyrrolidine ring at C_4. The activity of **20** was better than **17**, which confirmed that sulfonyl amide is better than acryl, because sulfonyl groups was easier to form a hydrogen bond with the active domain of enzyme.

3.1.2. Effect of the flexibility of the side chain linked to the pyrrolidine ring at C_4. Compounds **18** and **28** showed higher activities, which might be favored from the flexibility of the hexanyl group, which could modify the configuration to match the 3-D structure (bioactive conformation maybe better) of the enzyme.

3.1.3. Effect of the heteroaryl group of the side chain linked to the pyrrolidine ring at C_4. High activities of **19** and **33** indicated that the heteroaromyl group might be better than aryl group.

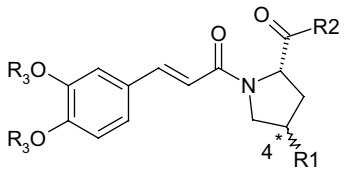
3.1.4. Effect of the free phenol hydroxyl groups. Comparing **9**, **14**, or **10**, **13**, we could draw a conclusion that free phenol hydroxyl group do favor to the inhibitory activity.

3.1.5. Effect of symmetrical structure. The side chain of **32** is *p*-methoxyphenyl-2-propenoyl amide, which was similar to caffonyl group, and so formed a butterfly-like structure, therefore **32** had potent inhibitory abilities.

3.2. Anti-metastasis activity against H_{22} tumor cell in vivo

Compounds were evaluated in the anti-metastasis model of mice bearing H_{22} tumor cell. The results of their

Table 1. SAR of the compounds **6**–**33**



No.	R_1	R_2	R_3	4*	IC_{50} (nmol)	MR
6	OH	MeO	Me	S	657.9 ± 34.6	8.9441
7	OH	MeO	Ac	S	ND*	9.9431
8	OSO_2CH_3	MeO	Me	S	198.9 ± 11.2	10.280
9	OSO_2CH_3	MeO	Ac	S	234.5 ± 21.3	11.279
10	$OSO_2C_6H_4CH_3$-*p*	MeO	Me	S	182.3 ± 26.2	13.102
11	$OSO_2C_6H_4CH_3$-*p*	MeO	Ac	S	160.0 ± 10.7	14.101
12	$OCOC_6H_2(OCOCH_3)_3$-3′,4′,5′	MeO	Ac	S	731.4 ± 64.0	15.650
13	$OSO_2C_6H_4CH_3$-*p*	MeO	H	S	104.9 ± 15.3	11.864
14	OSO_2CH_3	MeO	H	S	168.1 ± 14.9	9.3528
15	N_3	MeO	Me	R	224.9 ± 46.6	9.7197
16	NH_2	MeO	Me	R	ND*	9.2345
17	$NHCOCH_2CH_3$	MeO	Me	R	479.4 ± 30.4	10.586
18	$NHCO(CH_2)_4CH_3$	MeO	Me	R	130.7 ± 15.9	11.978
19	$NHCOC_5H_4N$-3′	MeO	Me	R	91.2 ± 8.1	11.959
20	$NHSO_2CH_3$	MeO	Me	R	155.5 ± 12.7	10.496
21	$NHSO_2C_6H_4CH_3$-*p*	MeO	Me	R	178.0 ± 10.0	13.007
22	$NHCO(CH_2)_2COOH$	MeO	Me	R	103.1 ± 27.4	11.239
23	$NHCOCH_2C_6H_5$	MeO	Me	R	110.6 ± 9.1	12.634
24	$NHCO(CH_2)_2C_6H_5$	MeO	Me	R	93.4 ± 13.3	13.098
25	$NHCOCH{=}CHC_6H_3$(OMe)-4′(*E*)	MeO	Me	R	65.7 ± 7.7	13.993
26	OH	NHOH	Me	R	9.7 ± 1.6	8.849
28	$NHCO(CH_2)_4CH_3$	NHOH	Me	R	7.8 ± 0.9	11.883
27	$NHCOCH_2CH_3$	NHOH	Me	R	15.7 ± 1.5	10.491
29	$NHCOCH_2C_6H_5$	NHOH	Me	R	11.5 ± 0.5	12.539
30	$NHSO_2C_6H_4CH_3$-*p*	NHOH	Me	R	9.7 ± 0.6	12.912
31	$NHCO(CH_2)_2C_6H_5$	NHOH	Me	R	9.4 ± 0.4	13.002
32	$NHCOCH{=}CHC_6H_3$(OMe)-4′(*E*)	NHOH	Me	R	6.7 ± 0.17	13.898
33	$NHCOC_5H_4N$-3′	NHOH	Me	R	9.0 ± 0.6	11.864

ND*: Not determined; MR was determined by Chem3D Ultra 7.0 program.

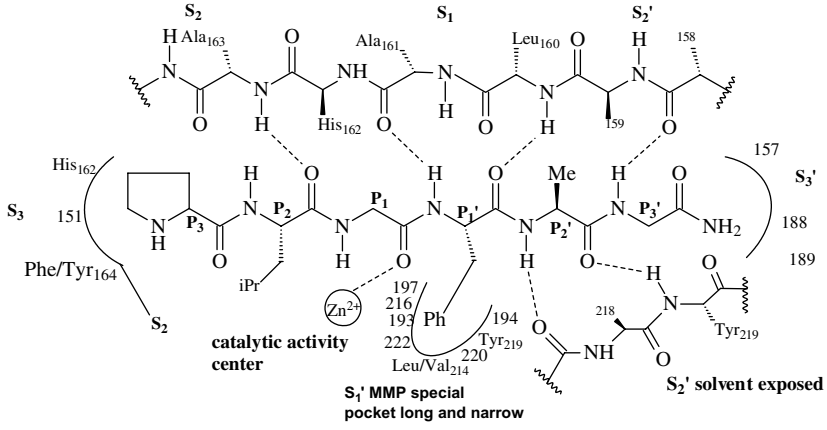


Figure 3. Schematic depiction of the bond between MMPs and substrate.

activities (inhibitory rate) following oral administration in mice were reported in Table 2.

The tested compounds can significantly inhibit the lung metastasis following administration in mice ($P < 0.01$).

Table 2. Anti-tumor activities in vivo

Compound no.	Mice of survived (n)	Body weight (g)	Lung weight (g)	Metastasized nodes on lung surface (n)	Inhibitory rate (%)	lg *P*
Control	10	23.50 ± 3.42	0.168 ± 0.021	46.5 ± 2.12		
12 (100 mg/kg/d)	9(10)*	16.82 ± 3.16	0.148 ± 0.062	22.0 ± 3.78	52.69	1.2687
14 (100 mg/kg/d)	10	17.88 ± 3.27	0.143 ± 0.041	19.5 ± 1.96	58.06	−0.474
19 (100 mg/kg/d)	9(10)*	20.63 ± 2.42	0.153 ± 0.068	17.5 ± 0.93	62.37	0.6211
20 (100 mg/kg/d)	10	23.20 ± 1.16	0.163 ± 0.014	28.8 ± 9.34	38.07	2.939
22 (100 mg/kg/d)	10	21.65 ± 2.31	0.143 ± 0.012	24.5 ± 8.32	47.31	−0.2714
29 (50 mg/kg/d)	10	22.22 ± 3.26	0.166 ± 0.029	14.3 ± 0.57	69.25	1.1552
30 (50 mg/kg/d)	10	22.48 ± 2.50	0.161 ± 0.023	21.7 ± 4.72	53.33	1.236
31 (50 mg/kg/d)	10	23.17 ± 4.25	0.164 ± 0.022	20.0 ± 1.17	56.99	1.5725
32 (50 mg/kg/d)	10	22.68 ± 3.41	0.178 ± 0.073	30.2 ± 12.19	35.05	1.4258
33 (50 mg/kg/d)	9(10)*	21.95 ± 3.42	0.163 ± 0.019	20.6 ± 3.25	55.70	−0.1262

*The animal number in brackets is the original number; lg *P* was determined by Chem3D Ultra 7.0 program.

All animals administrated the tested compounds except **12** did not significantly lose weights ($P < 0.05$), which meant low toxicity of the compounds.

3.2.1. Effect of zinc-binding group. Hydroxamic acid and carbomethoxy are both zinc-binding group and can chelate with zinc at catalytic activity center of the enzyme. Although hydroxamates showed more than 10 times stronger inhibitory activities against gelatinases than carboxylates in vitro, they did not do the same in vivo.[6] Maybe because hydroxamates are not stable under the metabolic system, but carbomethoxy can be metabolized to carboxylic acid, which is also a zinc-binding group.

3.2.2. Effect of the lg *P* of inhibitors. The side chain of **33**, **29**, **31**, and **32** was nicotimide, phenylethanoylamide, phenylpropanoylamide, and *p*-methoxyphenyl-2-propenoylamide, respectively, and we found that phenylethanoylamide was the best side chain in activity. Compound **29** was not too active in vitro, but it showed impressive in vivo activity, which might benefit from its fitting lg *P*. Moreover no toxic effects were observed in compound **29** and this might be a promising lead compound. Compound **29** also suggested that we could modify **31** and enhance its oral bioavailability to gain better inhibitor.

Compound **19** showed higher activity and lower toxic effect, attributing to its well lg *P* and heteroaromyl.

3.2.3. Effect of the flexibility of the side chain linked to the pyrrolidine ring at C_4. Compound **32** was very active in vitro, but was not active in vivo. This result might be due to its high lg *P* value, which makes it difficult to be absorbed. Its molecular rigidity and high polarity also make this compound difficult to transfer across the biomembrane to reach its active site.

3.2.4. Effect of the sulfonyl in the side chains linked to the pyrrolidine ring at C_4. The high activity of **14** might be benefited from sulfonyl group, but it also had intolerable toxic effect, resulting in the rush decrease of body weight of the mice.

4. Conclusion

The pyrrolidine derivatives we designed could conformationally match the active site of MMP-2 and -9, so they showed high anti-MMP-2 and -9 activity in vitro. Furthermore they could be metabolized into active segments, such as derived hydroxyl prolidines and caffic acid, which might also be expected to have anti-neoplastic activities, so they also showed high anti-tumor activity in vivo. Among the inhibitors the best one was compound **29**, which had inhibitory rate as high as 69.25%, showing that it might be a promising lead compound.

5. Experimental section

All reactions except those in aqueous media were carried out by standard techniques for the exclusion of moisture. All reactions were monitored by thin-layer chromatography on 0.25-mm silica gel plates (60GF-254) and visualized with UV light, or iodine vapor. ^{1}H NMR spectra were determined on a Brucker AM300 spectrometer using TMS as an internal standard. ESI MS were determined on an API 4000 spectrometer.

5.1. (2*S*,4*R*) Methyl 4-hydroxy-2-pyrrolidinecarboxylate hydroxychloride (1)

The title compound was prepared as described by Jordis in (1*S*,4*S*)-2-thia-5-azabicyclo[2.2.1]heptane.[7]

5.2. (*E*)-3-(3,4-Dimethoxyphenyl)-2-propenoic acid (2)

Caffic acid (18 g, 0.1 mol) was dissolved in cool 90 mL NaOH solution (4 mol/L), in which process the inner temperature was required under 20 °C. After Me_2SO_4 (20 mL) was added dropwise and stirred for 20 min, 50 mL NaOH solution (4 mol/L) and 20 mL Me_2SO_4 were dropped into the reaction system constantaneously. The resulting mixture was slowly heated to 90 °C in 1 h and maintained at this temperature for 1 h, then the solution was refluxed for 2 h, after 50 mL NaOH solution (4 mol/L) was added, another 2 h of refluxing was applied. The pH of the resulting solution was regulated to 2 by concentrated HCl. Two to three

hours later, mass brown solid were attained by filtrating, and washed to neutrality with water. The gross was recrystallized in EtOH and H_2O to get 16.4 g (yield 78.8%) light yellow crystal, which did not appear blue in $Fe(SCN)_3/FeCl_3$.

5.3. (*E*)-3-(3,4-Diacetyloxyphenyl)-2-propenoic acid (3)

Caffic acid (18 g, 0.1 mol) was dissolved in 30 mL anhydride acetate and 10 d H_2SO_4, and stirred for 10 min at 60 °C. The cold solution was poured into icy water, stirred vigorously, and filtrated to get white solid, washed with water until neutral. The gross was recrystallized in AcOEt and hexane to get 16.4 g (yield 83.0%) white crystal, which did not appear blue in $Fe(SCN)_3/FeCl_3$.

5.4. (*E*)-3-(3,4-Dimethoxyphenyl)-2-propenoic chloride (4)

Compound **2** (20.8 g, 10 mmol) was dissolved in 40 mL $SOCl_2$ and 320 mL benzene, and refluxed for 3 h. The resulting solution was rotary evaporated to get pale yellow crystal.

5.5. (*E*)-3-(3,4-Diacetyloxyphenyl)-2-propenoic chloride (5)

Compound **3** was converted to the title compound as described for compound **4** to get white solid.

5.6. (2*S*,4*R*) Methyl 1-[(*E*)-3-(3,4-dimethoxyphenyl)-2-propenoyl]-4-hydroxy-2-pyrrolidinecarboxylate (6)

Compound **1** (1.85 g, 11 mmol) was suspended in 20 mL Py and 3 mL Et_3N. After stirred for 20 min at room temperature, the resulting mixture was filtrated. The filtrate was cooled to −5 °C, and at this temperature 10 mL CH_2Cl_2 with 2.82 g **4** (10 mmol) was added. After stirred for 3.5 h, the resulting mixture was filtrated to remove white precipitation, and the filtrate was rotary evaporated. The resulting crude oil was chromatographed over flash silica with petro ether–EtOAc (4:1 to 1:4) to provide 2.4 g of pale yellow solid, yield 71.6%, mp 62.5–63.5 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.027–2.134 (m, 1H), 2.305–2.335 (m, 1H), 3.050 (s, 1H), 3.706 (s, 1H), 3.732 (s, 3H), 3.849 (s, 3H), 3.873 (s, 3H), 3.896–3.932 (m, 1H), 4.589 (s, 1H), 4.696 (t, J = 7.8 Hz, 1H), 6.487 (d, J = 15.6 Hz, 1H), 6.783 (d, J = 8.4 Hz, 1H), 7.018 (s, 1H), 7.032 (d, J = 8.4 Hz, 1H), 7.582 (d, J = 15.6 Hz, 1H).

5.7. (2*S*,4*R*) Methyl 1-(*E*)-3-(3,4-diacetyloxyphenyl)-2-propenoyl-4-hydroxy-2-pyrrolidinecarboxylate (7)

The title compound was prepared as described for compound **6** to get 3.0 g white solid, mp 138.7–140.2 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.071–2.080 (m, 1H), 2.129 (dd, J = 8.1, 4.8 Hz, 1H), 2.293–2.299 (6H), 3.689 (s, 1H), 3.904 (dd, J = 4.5, 10.8 Hz, 2H), 4.604 (1H), 4.684 (t, J = 8.1 Hz, 1H), 6.604 (d, J = 15.6 Hz, 1H), 7.181 (d, J = 7.8 Hz, 1H), 7.326 (d, J = 1.5 Hz, 1H), 7.358 (1H), 7.606 (d, J = 15.6 Hz, 1H). ESI MS: m/z (rel intensity) 389.89.

5.8. (2*S*,4*R*) Methyl 1-[(*E*)-3-(3,4-dimethoxyphenyl)-2-propenoyl]-4-[(methylsulfonyl)oxy]-2-pyrrolidinecarboxylate (8)

In nitrogen atmosphere, compound **6** (3.35 g, 10 mmol) was taken in CH_2Cl_2 (5 mL) and Et_3N (4.5 mL) was added at 0 °C. Methanesulfonyl chloride was added dropwise and the resulting mixture was stirred for 4 h at room temperature. The following mixture was partitioned between water and EtOAc. The organic layer was washed with saturated $NaHCO_3$ solution, H_2O, and brine in turn, dried over $MgSO_4$, filtered, and evaporated to give pale yellow oil, which was chromatographed over flash silica with petro ether–acetone (4:1 to 1:2) to provide 3.4 g white crystal, yield 82.3%, mp 139.1–140.0 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.258–2.348 (m, 2H), 2.562–2.645 (m, 1H), 3.067 (s, 3H), 3.770 (s, 3H), 3.899–3.916 (6H), 4.046–4.152 (m, 2H), 4.714 (t, J = 8.1 Hz, 1H), 6.490 (d, J = 15.3 Hz, 1H), 6.850 (d, J = 8.1 Hz, 1H), 7.090 (s, 1H), 7.103 (d, J = 8.1 Hz, 1H), 7.674 (d, J = 15.3 Hz, 1H). ESI MS: m/z (rel intensity) 412.3.

5.9. (2*S*,4*R*) Methyl 1-(*E*)-3-(3,4-diacetyloxyphenyl)-2-propenoyl-4-[(methylsulfonyl)oxy]-2-pyrrolidinecarboxylate (9)

Compound **7** was converted to the title compound as described for compound **8** to get white solid, yield 81.0%, mp 77.8–80.0 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.296–2.370 (m, 8H), 3.072 (s, 3H), 3.781 (s, 3H), 4.024–4.033 (m, 3H), 4.717 (t, J = 8.1 Hz, 1H), 6.496 (d, J = 15.6 Hz, 1H), 7.215 (d, J = 8.4 Hz, 1H), 7.380 (d, J = 1.8 Hz, 1H), 7.397 (d, J = 8.4 Hz, 1H), 7.687 (d, J = 15.6 Hz, 1H), J = 15.6 Hz, 1H). ESI MS: m/z (rel intensity) 426.85 [M−CH_3CO], 384.75 [M−$(CH_3CO)_2$].

5.10. (2*S*,4*R*) Methyl 1-[(*E*)-3-(3,4-dimethoxyphenyl)-2-propenoyl]-4-[(4-methylphenyl)sulfonyl]oxy-2-pyrrolidinecarboxylate (10)

Compound **6** (3.35 g, 10 mmol) was taken in CH_2Cl_2 (5 mL) and Et_3N (4.5 mL) was added at 0 °C. Tolylsulfonyl chloride (3.8 g, 20 mmol) was added dropwise and the resulting mixture was stirred overnight at room temperature. The following mixture was diluted with CH_2Cl_2 and washed with 10% HCl, saturated $NaHCO_3$ solution, and H_2O, dried over $MgSO_4$, filtered, and evaporated to give pale yellow oil, which was chromatographed over flash silica with petro ether–acetone (8:1 to 1:1) to provide 3.9 g white crystal, yield 79.8%, mp 60.2–62.1 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.039–2.208 (m, 1H), 2.412 (s, 3H), 2.444–2.498 (m, 1H), 3.873 (s, 3H), 3.905–3.925 (6H), 3.997–4.014 (m, 3H), 4.651 (t, J = 7.8 Hz, 1H), 6.380 (d, J = 15.3 Hz, 1H), 6.858 (d, J = 8.4 Hz, 1H), 7.004 (d, J = 1.5 Hz, 1H), 7.086 (d, J = 8.4 Hz, 1H), 7.639 (d, J = 15.3 Hz, 1H), 7.348 (d, J = 7.8 Hz, 2H), 7.787 (d, J = 7.8 Hz, 2H). ESI MS: m/z (rel intensity) 488.2.

5.11. (2*S*,4*R*) Methyl 1-(*E*)-3-(3,4-diacetyloxyphenyl)-2-propenoyl-4-[(4-methylphenyl)sulfonyl]oxy-2-pyrrolidinecarboxylate (11)

Compound **7** was converted to the title compound as described for compound **10** to get white solid, yield 78.9%, mp 117.1–120.5 °C (decomposed). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.181 (m, 2H), 2.229 (s, 6H), 2.317 (s, 3H), 3.777 (s, 3H), 3.835–3.999 (m, 3H), 4.647 (t, J = 8.1 Hz, 1H), 6.438 (d, J = 15.6 Hz, 1H), 7.207 (d, J = 4.5 Hz, 1H), 7.333 (d, J = 4.5 Hz, 1H), 7.353 (d, J = 7.8 Hz, 2H), 7.622 (d, J = 15.6 Hz, 1H), 7.784 (d, J = 7.8 Hz, 2H). ESI MS: *m*/*z* (rel intensity) 502.82 [M−CH_3CO], 460.505 [M−$(CH_3CO)_2$].

5.12. (2*S*,4*R*) Methyl 1-[(*E*)-3-(3,4-dimethoxyphenyl)-2-propenoyl]-4-(3,4,5-triacetyloxybenzoyl)oxy-2-pyrrolidinecarboxylate (12)

3,4,5-Trimethoxybenzoic acid was converted to 3,4,5-trimethoxybenzoyl chloride as described for compound **4**, then the title compound was prepared as described for compound **10** to get white solid, yield 80.7%, mp 81.7–84.0 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.070 (s, 3H), 2.246–2.310 (12H), 2.404–2.474 (m, 2H), 3.777 (s, 3H), 3.756–3.925 (m, 1H), 3.963–4.200 (t, J = 7.8 Hz, 1H), 6.590 (d, J = 15.3 Hz, 1H), 7.208 (d, J = 8.1 Hz, 1H), 7.315 (s, 1H), 7.370 (s, 2H), 7.390 (d, J = 8.1 Hz, 1H), 7.665 (d, J = 15.3 Hz, 1H). ESI MS: *m*/*z* (rel intensity) 626.91 [M−CH_3CO].

5.13. (2*S*,4*R*) Methyl 1-[(*E*)-3-(3,4-dihydroxyphenyl)-2-propenoyl]-4-[(4-methylphenyl)sulfonyl]oxy-2-pyrrolidinecarboxylate (13)

Compound **11** (0.9 g, 1.66 mmol) was dissolved in 8 mL MeOH and 8 mL THF, and then 3 mL concentrated HCl was added. The resulting solution was stirred for 20 min at 60 °C and was diluted with H_2O after cooled to room temperature. The following mixture was partitioned between water and EtOAc and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated to give white puff, which was chromatographed over flash silica with petro ether–EtOAc–acetic acid (30:10:1 to 10:30:1) to provide 0.46 g white crystal, which appeared blue in $FeCl_3$ and $Fe(SCN)_3$, yield 60.2%, mp 142.0–144.5 °C. ^{1}H NMR: 2.070 (s, 3H), 2.246–2.310 (12H), 2.404–2.474 (m, 2H), 3.777 (s, 3H), 3.756–3.925 (m, 1H), 3.963–4.200 (t, J = 7.8 Hz, 1H), 6.590 (d, J = 15.3 Hz, 1H), 7.208 (d, J = 8.1 Hz, 1H), 7.315 (s, 1H), 7.370 (s, 2H), 7.390 (d, J = 8.1 Hz, 1H), 7.665 (d, J = 15.3 Hz, 1H). ESI MS: *m*/*z* (rel intensity) 462.29.

5.14. (2*S*,4*R*) Methyl 1-[(*E*)-3-(3,4-dihydroxyphenyl)-2-propenoyl]-4-[(methylsulfonyl)oxy]-2-pyrrolidinecarboxylate (14)

Compound **9** was converted to the title compound as described for compound **11** to get white solid. The mobile phase for VLC employed petro ether–EtOAc–acetic acid (30:10:1–10:30:1), yield 54.8%, mp 179.0–180.5 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.234—2.279 (m, 2H), 2.497 (s, 3H) ESI MS: *m*/*z* (rel intensity) 384.52.

5.15. (2*S*,4*S*) 1-[(*E*)-3-(3,4-Dimethoxyphenyl)-2-propenoyl]-2-carbomethoxy-4-azidopyrrolidine (15)

In nitrogen atmosphere, compound **7** (4.13 g, 10 mmol) was taken in 15 mL dry DMF in the presence of ground NaN_3 (695 mg, 10.7 mmol). The resulting mixture was heated to 55 °C for 10 h and then partitioned between water and EtOAc. The organic layer was then washed with brine, dried over $MgSO_4$, filtered, and evaporated. The resulting crude oil was chromatographed over flash silica with hexane–EtOAc (5:1 to 3:1) to provide 2.87 g of pale yellow oil, which solidified upon standing, yield 79.0%, mp 179.0–180.5 °C. IR: ν_{CH_3} 2953, ν_{N_3} 2104, $\nu_{C=O}$ 1749, 1650, $\nu_{C=C}$ 1597, 1513.

5.16. (2*S*,4*S*) 4-Amine-1-[(*E*)-3-(3,4-dimethoxyphenyl)-2-propenoyl]-2-pyrrolidinecarboxylate (16)

In hydrogen atmosphere, Compound **15** (11 g, 30 mmol) was dissolved in alcohol, and 5% Pd/$CaCO_3$ (3 g) was added. The resulting solution was stirred for 10 h under 760 mmHg pressure, during which the hydrogen was input intermittently. The resulting mixture was filtered and the filtrate was evaporated to give brown oil, which was chromatographed over flash silica with $CHCl_2$–MeOH (4:1 to 1:4) to provide aqua oil, which solidified upon standing, yield 60.8%. ESI MS: *m*/*z* (rel intensity) 333.4. IR: ν_{NH_2} 3345.94, 3257.87, ν_{CH_3} 2936, $\nu_{C=O}$ 1726, 1641, $\nu_{C=C}$ 1590, 1514.

5.17. (2*S*,4*S*) Methyl 1-[(*E*)-3-(3,4-dimethoxyphenyl)-2-propenoyl]-4-propanoylamid-2-pyrrolidinecarboxylate (17)

Compound **16** (334 mg, 1 mmol) was dissolved in 2 mL dry $CHCl_2$ and 0.5 mL Et_3N, and 2 mL $CHCl_2$ with 0.16 mL propanoyl chloride was added dropwise. The resulting solution was stirred for 3 h. The following mixture was partitioned between water and $CHCl_2$ and the organic layer was washed with 1% HCl, 5% $NaCO_3$, and water, in turn dried over $MgSO_4$, filtered, and evaporated to give yellow puff, which was chromatographed over flash silica with petro ether–EtOAc (4:1 to 1:4) to provide 291 mg white crystal, yield 74.6%, mp 132.0–133.7 °C. ^{1}H NMR: 1.144 (t, J = 7.5 Hz, 3H), 1.942 (s, 1H), 1.989 (s, 1H), 2.216 (q, J = 7.5 Hz, 2H), 2.487 (m, 1H), 3.817 (s, 3H), 3.905 (s, 3H), 3.913 (s, 3H), 4.605 (d, J = 9.9 Hz, 1H), 4.806 (s, 1H), 6.518 (d, J = 15.3 Hz, 1H), 6.853 (d, J = 8.1 Hz, 1H), 6.984 (s, 1H), 7.028 (s, 1H), 7.097 (d, J = 8.1 Hz, 1H), 7.668 (d, J = 15.3 Hz, 1H)ESI MS: *m*/*z* (rel intensity) 389.8.

5.18. (2*S*,4*S*) Methyl 1-[(*E*)-3-(3,4-dimethoxyphenyl)-2-propenoyl]-4-hexanoylamid-2-pyrrolidinecarboxylate (18)

Compound **16** was converted to the title compound as described for compound **17**, yield 62.7%. ^{1}H NMR: 0.870 (t, J = 6.6 Hz, 3H), 1.258–1.334 (m, 4H), 1.614 (t, J = 7.1 Hz, 2H), 2.169 (t, J = 7.1 Hz, 2H), 2.487 (m,

2H), 3.818 (s, 3H), 3.905–3.920 (6H), 4.591–4.812 (m, 3H), 6.542 (d, $J = 15.3$ Hz, 1H), 6.840–7.117 (m, 3H), 7.680 (d, $J = 15.3$ Hz, 1H). ESI MS: *m*/*z* (rel intensity) 341.75.

5.19. (2*S*,4*S*) Methyl 1-[(*E*)-3-(3,4-dimethoxyphenyl)-2-propenoyl]-4-[(3-pyridinylcarbonyl)amid]-2-pyrrolidinecarboxylate (19)

Compound **16** was converted to the title compound as described for compound **17** to get pale yellow solid, yield 68.9%. ^{1}H NMR: 2.041–2.155 (m, 2H), 2.535–2.636 (m, 2H), 3.856 (s, 3H), 3.901 (s, 3H), 3.909 (s, 3H), 4.035 (m, 1H), 4.680 (d, $J = 9.6$ Hz, 1H), 5.061 (t, $J = 3.9$ Hz, 1H), 6.526 (d, $J = 15.3$ Hz, 1H), 6.831–7.261 (m, 3H), 7.415 (dd, $J = 4.8$, 7.8 Hz, 1H), 7.679 (d, $J = 15.3$ Hz, 1H), 8.202 (d, $J = 4.8$ Hz, 1H), 8.302 (d, $J = 7.8$ Hz, 1H), 8.737 (d, $J = 4.2$ Hz, 1H), 9.122 (s, 1H). ESI MS: *m*/*z* (rel intensity) 438.5.

5.20. (2*S*,4*S*) Methyl 1-[(*E*)-3-(3,4-dimethoxyphenyl)-2-propenoyl]-4-[(methylsulfonyl)amide]-2-pyrrolidinecarboxylate (20)

Compound **16** was converted to the title compound as described for compound **17** to get white crystal, yield 68.9%, mp 80.3–84.7 °C. ^{1}H NMR: 2.028–2.095 (m, 1H), 2.477–2.575 (m, 1H), 2.979 (s, 3H), 3.776 (s, 3H), 3.884–3.926 (6H), 3.988 (t, $J = 5.4$ Hz, 1H), 4.024–4.238 (m, 2H), 4.584 (dd, $J = 3.3$, 15.6 Hz, 1H), 6.036 (d, $J = 9.6$ Hz), 6.510 (d, $J = 15.6$ Hz, 1H), 6.837 (d, $J = 7.8$ Hz, 1H), 7.081 (d, $J = 1.5$ Hz, 1H), 7.097 (d, $J = 7.8$ Hz, 1H), 7.650 (d, $J = 15.6$ Hz, 1H). ESI MS: *m*/*z* (rel intensity) 411.90.

5.21. (2*S*,4*S*) Methyl 1-[(*E*)-3-(3,4-dimethoxyphenyl)-2-propenoyl]-4-[(4-methylphenyl)sulfonyl]amide-2-pyrrolidinecarboxylate (21)

Compound **16** was converted to the title compound as described for compound **17** to get white crystal, yield 68.9%, mp 103.0–104.0 °C. ^{1}H NMR: 1.760–1.826 (m, 2H), 2.235–2.334 (m, 2H), 2.363 (s, 3H), 3.779 (s, 3H), 3.899–3.907 (6H), 4.098–4.145 (m, 1H), 4.472 (dd, $J = 3.0$, 9.6 Hz, 1H), 6.215 (d, $J = 9.6$ Hz, 1H), 6.348 (d, $J = 15.3$ Hz, 1H), 6.828–7.082 (m, 3H), 7.284 (d, $J = 8.1$ Hz, 1H), 7.616 (d, $J = 15.3$ Hz, 1H), 7.750 (d, $J = 8.1$ Hz, 1H). ESI MS: *m*/*z* (rel intensity) 487.55.

5.22. (2*S*,4*S*) 4-[1-[(*E*)-3(3,4-Dimethoxyphenyl)-2-propenoyl]-5-(carbomethoxy)tetrahydro-1*H*-3-pyrrolidine]amide-4-oxobutanoic acid (22)

Compound **16** (334 mg, 1 mmol) was dissolved in 2 mL dry CH_2Cl_2 and 0.5 mL Et_3N, and 2 mL $CHCl_2$ with 110 mg succinic anhydride was added dropwise. The resulting solution was stirred for 3 h. The following mixture was partitioned between water and CH_2Cl_2 and the organic layer was washed with 1% HCl, 5% $NaCO_3$, and water, in turn dried over $MgSO_4$, filtered, and evaporated to give yellow puff, which was chromatographed over flash silica with petro ether–EtOAc–AcOH (40:10:1 to 10:40:1) to provide 308 mg pale yellow crystal, yield 74.6%, mp 155.3–157.0 °C. ^{1}H NMR: 1.952–2.051 (m, 2H), 2.455 (t, $J = 9.6$ Hz, 2H), 2.677 (t, $J = 9.6$ Hz, 2H), 3.807 (s, 3H), 3.896–3.991 (6H), 4.592 (dd, $J = 1.8$, 9.6 Hz, 1H), 4.762 (d, $J = 3.3$ Hz, 1H), 6.524 (d, $J = 15.6$ Hz, 1H), 6.846 (d, $J = 8.1$ Hz, 1H), 7.039 (d, $J = 1.5$ Hz, 1H), 7.103 (dd, $J = 1.5$, 8.1 Hz), 7.234 (d, $J = 8.7$ Hz, 1H), 7.662 (d, $J = 15.6$ Hz, 1H). ESI MS: *m*/*z* (rel intensity) 433.1.

5.23. (2*S*,4*S*) 1-[(*E*)-3-(3,4-Dimethoxyphenyl)-2-propenoyl]-4-[(2-phenylethanoyl)amide]-2-pyrrolidinecarboxylate (23)

Compound **16** was converted to the title compound as described for compound **17** to get white crystal, yield 68.9%, mp 103.0–104.0 °C. ^{1}H NMR: 2.415–2.465 (m, 2H), 3.481 (m, 2H), 3.700 (s, 3H), 3.900 (s, 3H), 3.914 (s, 3H), 3.853–3.931 (m, 2H), 4.549 (dd, $J = 2.4$, 7.5 Hz, 1H), 4.720 (d, $J = 3$ Hz, 1H), 6.461 (d, $J = 15.6$ Hz, 1H), 6.950 (d, $J = 7.5$ Hz, 1H), 6.811–7.091 (3H), 7.197–7.313 (5H), 7.639 (d, $J = 15.6$ Hz, 1H). ESI MS: *m*/*z* (rel intensity) 452.5.

5.24. (2*S*,4*S*) Methyl 1-[(*E*)-3-(3,4-dimethoxyphenyl)-2-propenoyl]-4-[(2-phenylpropanoyl)amide]-2-pyrrolidinecarboxylate (24)

The starting amine **16** was converted to the title compound as described for compound **17** to get white crystal, yield 59.0%, mp 80.3–81.5 °C. ^{1}H NMR: 1.7852–1.8726 (m, 2H), 2.5115–2.5564 (m, 2H), 2.9756 (t, $J = 7.56$ Hz, 2H), 3.6467 (d, $J = 10.44$ Hz, 1H), 3.7808 (s, 3H), 3.7928–3.8414 (m, 2H), 3.9497 (s, 3H), 3.9816 (s, 3H), 4.5796 (d, $J = 9.84$ Hz, 1H), 4.9794 (m, 1H), 6.4656 (d, $J = 15.36$ Hz, 1H), 6.8892 (d, $J = 8.50$ Hz, 1H), 7.0021 (d, $J = 8.50$ Hz, 1H), 7.0476 (s, 1H), 7.1023–7.1606 (m, 2H), 7.1828–7.1252 (m, 2H), 7.2519–7.2767 (m, 1H), 7.6829 (d, $J = 15.36$ Hz, 1H), 8.2493 (d, $J = 7.86$ Hz, 1H), 8.9080 (1H), 10.7505 (1H). ESI MS: *m*/*z* (rel intensity) 467.05.

5.25. (2*S*,4*S*) Methyl 1-[(*E*)-3-(3,4-dimethoxyphenyl)-2-propenoyl]-4-[(*E*)-3-(4-methoxy)-2-propenoyl]amide-2-pyrrolidinecarboxylate (25)

Compound **16** was converted to the title compound as described for compound **17** to get pale yellow crystal, yield 67.9%, mp 101.9–103.4 °C. ^{1}H NMR: 2.534–2.584 (m, 2H), 3.800 (s, 3H), 3.843 (s, 3H), 3.937 (s, 3H), 3.962 (s, 3H), 3.970–3.988 (2H), 4.656 (d, $J = 9.6$ Hz, 1H), 4.917 (1H), 5.942 (d, $J = 12.6$ Hz, 1H), 6.153 (d, $J = 15.6$ Hz, 1H), 6.832–7.504 (7H), 7.590 (d, $J = 12.6$ Hz, 1H), 7.700 (d, $J = 15.6$ Hz, 1H). ESI MS: *m*/*z* (rel intensity) 495.45.

5.26. (2*S*,4*S*) 1-[(*E*)-3-(3,4-Dimethoxyphenyl)-2-propenoyl]-4-hydroxy-2-N-hydroxyamid-pyrrolidine (26)

Compound **6** (670 mg, 2 mmol) was dissolved in 7 mL dry methanol and 1.5 mL methanol with NH_2OK (prepared by Fieser and Fieser. Vol. 1, p 478). The resulting

solution was stirred at rt for 24 h, then 1.5 g silica gel was added and evaporated to give pale yellow powder, which was chromatographed over flash silica with CH_2Cl_2–MeOH (50:1 to 1:50) to provide 392 mg (58.3%) pale yellow crystal, which appeared red in $FeCl_3$, mp 117.3–118.8 °C. IR: $\nu_{OH,NH}$ 3350–2800 (w), $\nu_{C=O}$ 1634, $\nu_{C=C}$ 1588, 1513, β_{OH} 1141. ESI MS: *m*/*z* (rel intensity) 335.1.

5.27. (2*S*,4*S*) 1-[(*E*)-3-(3,4-Dimethoxyphenyl)-2-propenoyl]-2-N-hydroxyamid-4-propanoylamidepyrrolidine (27)

Compound **17** was converted to the title compound as described for compound **26** to get white crystal, yield 50.7%, mp 123.0–125.4 °C. ^{1}H NMR: 0.996 (t, J = 7.2 Hz, 3H), 1.742–1.763 (m, 1H), 2.073 (q, J = 7.2 Hz, 2H), 2.357–2.383 (m, 1H), 3.776 (s, 3H), 3.807 (s, 3H), 4.240–4.289 (m, 1H), 4.354 (t, J = 6.6 Hz, 1H), 6.850 (d, J = 15.3 Hz, 1H), 6.946 (d, J = 8.4 Hz, 1H), 7.184 (dd, J = 1.5, 8.4 Hz, 1H), 7.333 (d, J = 1.5 Hz, 1H), 7.384 (d, J = 15.3 Hz, 1H). ESI MS: *m*/*z* (rel intensity) 390.1.

5.28. (2*S*,4*S*) 1-[(*E*)-3-(3,4-Dimethoxyphenyl)-2-propenoyl]-4-hexanoylamid-2-N-hydroxyamidepyrrolidine (28)

Compound **18** was converted to the title compound as described for compound **26** to get white crystal, yield 50.8%, mp 105.0–107.5 °C. ^{1}H NMR: 0.841 (t, J = 6.9 Hz, 3H), 1.212–1.298 (m, 4H), 1.444–1.540 (m, 2H), 2.046 (t, J = 7.8 Hz, 2H), 2.484–2.496 (m, 2H), 3.287 (2H), 3.776–3.805 (6H), 4.027–4.061 (m, 1H), 4.233–4.282 (t, J = 6.9 Hz, 1H), 6.847 (d, J = 15.3 Hz, 1H), 6.958 (d, J = 8.4 Hz, 1H), 7.186 (dd, J = 1.2, 8.4 Hz, 1H), 7.329 (d, J = 1.2 Hz, 1H), 7.409 (d, J = 15.3 Hz, 1H). ESI MS: *m*/*z* (rel intensity) 432.3.

5.29. (2*S*,4*S*) 1-[(*E*)-3-(3,4-Dimethoxyphenyl)-2-propenoyl]-2-N-hydroxyamid-4-(2-phenylethanoyl)amidepyrrolidine (29)

Compound **23** was converted to the title compound as described for compound **26** to get white crystal, yield 50.7%, mp 124.9–125.7 °C. ^{1}H NMR: 1.187–1.778 (m, 1H), 2.381–2.409 (m, 1H), 3.401 (s, 2H), 3.704–3.742 (m, 1H), 3.775 (s, 3H), 3.801 (s, 3H), 4.009–4.342 (m, 3H), 6.845 (d, J = 15.3 Hz, 1H), 6.934 (d, J = 8.4 Hz, 1H), 7.172–7.328 (m, 7H), 7.382 (d, J = 15.3 Hz, 1H). ESI MS: *m*/*z* (rel intensity) 528.3.

5.30. (2*S*,4*S*) 1-[(*E*)-3-(3,4-Dimethoxyphenyl)-2-propenoyl]-2-N-hydroxyamid-4-[(4-methylphenyl)sulfonyl]-amidepyrrolidine (30)

Compound **21** was converted to the title compound as described for compound **26** to get white crystal, yield 50.7%, mp 124.9–125.7 °C. ^{1}H NMR: 2.052–2.266 (m, 2H), 3.6122 (m, 1H), 3.784 (s, 3H), 3.813 (s, 6H), 3.875–3.927 (m, 2H), 4.312 (t, J = 5.8 Hz, 1H), 6.553 (d, J = 15.3 Hz, 1H), 6.965 (d, J = 7.8 Hz, 1H), 7.189 (d, J = 7.8 Hz, 1H), 7.301 (s, 1H), 7.351 (d, J = 15.3 Hz, 1H), 7.497 (d, J = 8.1 Hz, 2H), 7.796 (d, J = 8.1 Hz, 2H) ESI MS: *m*/*z* (rel intensity) 488.35.

5.31. (2*S*,4*S*) 1-[(*E*)-3-(3,4-Dimethoxyphenyl)-2-propenoyl]-2-N-hydroxyamid-4-(3-phenylpropanoyl)amidepyrrolidine (31)

Compound **22** was converted to the title compound as described for compound **26** to get white crystal, yield 52.7%, mp 137.9–140.5 °C. ^{1}H NMR: 2.8188 (t, J = 7.56 Hz, 2H), 2.2935–2.3745 (m, 4H), 3.7205–3.7601 (m, 2H), 3.7871 (s, 3H), 3.8221 (s, 3H) 4.1767 (t, J = 7.62 Hz, 1H), 4.3482 (dd, J = 7.14, 19.68 Hz, 1H), 6.8537 (d, J = 15.36 Hz, 1H), 6.9660 (d, J = 8.16 Hz, 1H), 7.1672 (s, 1H), 7.1982–7.2754 (m, 3H), 7.3577–7.4011 (m, 2H), 7.1478 (d, J = 8.16 Hz, 1H), 7.3577 (m, 1H), 7.3887 (d, J = 15.36 Hz, 1H). ESI MS: *m*/*z* (rel intensity) 466.99.

5.32. (2*S*,4*S*) 1-[(*E*)-3-(3,4-Dimethoxyphenyl)-2-propenoyl]-2-N-hydroxyamid-4-[(*E*)-3-(4-methoxy)-2-propenoyl]amide]pyrrolidine (32)

Compound **25** was converted to the title compound as described for compound **26** to get pale yellow crystal, yield 56.6%, mp 154.4–155.7 °C. IR: $\nu_{OH,NH}$ 3203–2836, $\nu_{C=O}$ 1647, $\nu_{C=C}$ 16.2, 1512ESI MS: *m*/*z* (rel intensity) 496.44.

5.33. (2*S*,4*S*) 1-[(*E*)-3-(3,4-Dimethoxyphenyl)-2-propenoyl]-2-N-hydroxyamid-4-(3-pyridinylcarbonyl)amidepyrrolidine (33)

Compound **19** was converted to the title compound as described for compound **26** to get pale yellow crystal, yield 56.6%, mp 154.4–155.7 °C. ^{1}H NMR: 1.9867–2.0840 (m, 2H), 3.8539 (s, 3H), 3.9519 (s, 3H), 6.251 (d, J = 15.36 Hz, 1H), 6.9551 (d, J = 8.28 Hz, 1H), 7.1174 (d, J = 8.28 Hz, 1H), 7.1763 (s, 1H), 7.4110 (d, J = 15.36 Hz, 1H), 7.5421 (1H), 8.7012–8.7295 (m, 1H). ESI MS: *m*/*z* (rel intensity) 439.94.

5.34. Assays of MMP inhibition and anti-tumor activity

5.34.1. MMP inhibition. Gelatinase (MMP-2, -9) and TNBS were purchased from Sigma, and the substance was synthesized as described by Vijaykumar et al.[8] The gelatinase, substance, and inhibitor were dissolved in sodium borate (pH 8.5, 50 mmol/L) and incubated for 30 min at 37 °C, then 0.03%TNBS was added and incubated for another 20 min, the resulting solution was detected under 450 nm wavelength to gain absorption.

5.35. Anti-tumor activities

Male Kunming mice were obtained from Lab Animal Center, Shandong University.

The mice bearing H_{22} tumor cells ascites were obtained from Shandong Medicine Academy of Sciences.

Materials: Aqueous suspensions of inhibitors were prepared by homogenizing the compounds in 0.5%

carboxymethyl cellulose Na, 0.9% benzyl alcohol and 0.4% Tween 80 in saline. A control solution of the vehicle without inhibitors was also prepared.

Bouin solution: Saturated trinitrophenol:saturated formaldehyde:glacial acetic acid = 15:5:1.

Experimental design: Mice bearing H_{22} tumor ascites were injected via the caudal vein and randomly divided into 11 groups. The animals of the control group were treated with the same volume of vehicle, the other groups were given the inhibitors by oral administration, respectively, at a dose of 100 mg/kg/d (carboxylates) or 50 mg/kg/d (hydroxamates) 6 days/week for two weeks. The mice were weighed and sacrificed for autopsy immediately. The lungs with tumor nodes were removed and were weighed, then placed in bouin solution. One day later, the metastasized nodes on the surface of lungs were counted.

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