Synthesis of a Cyclic Analogue of Galardin

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A cyclic analogue 4 of galardin, a known MMP inhibitor, is designed to improve its selectivity. The synthesis of 4 starts from dimethyl (*S*)-malate using diaselective alkylation and subsequent cyclization and amide formation as key steps. The compound 4 showed MMP inhibitory activity on all MMPs tested with IC_{50} ranging from 20.1 μM to 104 μM.

Keywords Matrix metalloproteinases inhibitor, alkylation, cyclization, amide

Matrix metalloproteinases (MMPs) are a family of zinc-containing endopeptidases involved in the degradation and remodeling of the extracellular matrix.[1] They are promising therapeutic targets for treatment of cancer, arthritis, joint destruction, and cardiovascular disease. Therefore, MMP inhibitors are expected to provide therapeutic agents for a number of disease states.[2] However, since MMPs play a significant role in human physiology, the use of nonselective inhibitors might produce a variety of side effects. In fact, clinical studies using nonselective inhibitor marimastat for treatment of cancer have shown that this drug has a dose limiting side effect. The side effect is believed to be mechanism based and hypothesized to result from inhibition of MMP-1.[2] Based on these results, many efforts have been directed to the development of selective MMP inhibitors.

The use of conformationally restricted analogues of biologically relevant molecules is a well-established approach for enhancing receptor selectivity. By using this strategy and the known MMP inhibitors with succinyl hydroxamate structure such as marimastat and galardin as lead compounds,[2] several cyclic analogues such as **2**[3] and **3**[4] have been designed and synthesized (Scheme 1). Some of these cyclic analogues have displayed marked selectivity. Stimulated by these studies, we designed the compound **4** in which an additional ring between C2 and the nitrogen of hydroxamate in **1** was introduced to see if this structure change could also improve the selectivity.

As outlined in Scheme 2, the target molecule **4** was synthesized from dimethyl (*S*)-malate. Treatment of dimethyl (*S*)-malate with LiHMDS followed by trapping the generated anion with benzyl bromide provided (2*S*, 3*R*)-3-benzyl-2-hydroxysuccinic acid dimethyl ester (**5**).[5] Alkylation of **5** with *tert*-butyl bromoacetate mediated by Ag_2O gave triester **6**. The *tert*-butyl group of **6** was removed assisted by gaseous hydrogen chloride and the generated acid was reduced with borane-methyl sulfide complex to afford alcohol **7**. Mesylation of **7** followed by an S_N2 reaction with *O*-benzyl hydroxyamine produced **8**. Lactam **9** was obtained by treatment of **8** with potassium hydroxide in methanol. Hydrolysis of the ester moiety in **9** provided the corresponding acid, which was coupled with *L*-tryptophan *N*-methyl amide (**11**) gave amide **10**. Finally, hydrogenation of **10** to remove the benzyl protecting group afforded **4**.

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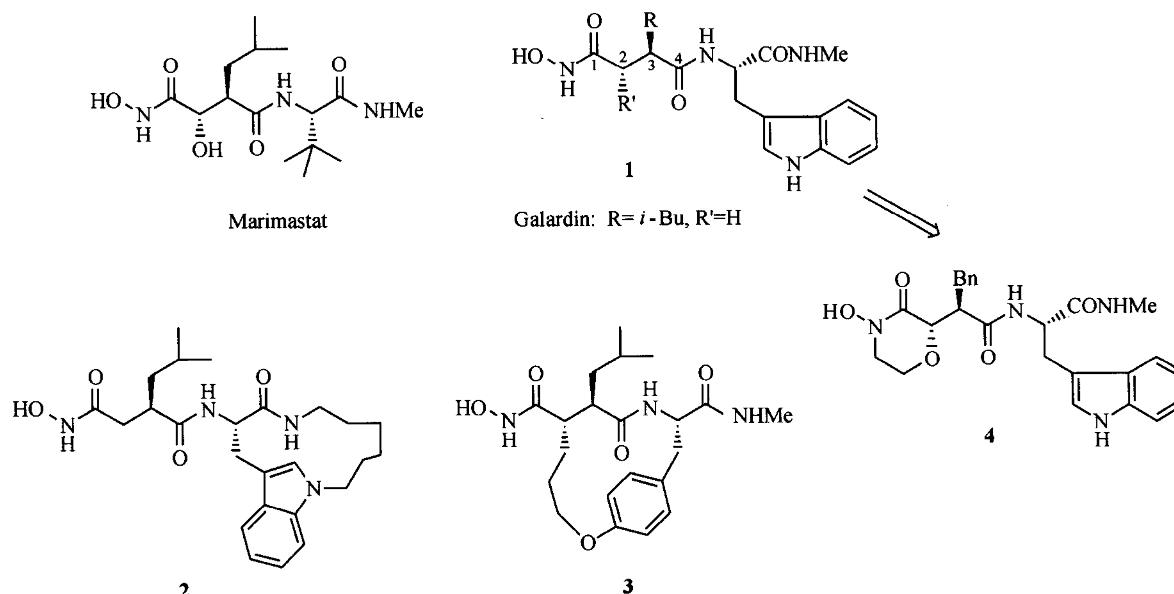
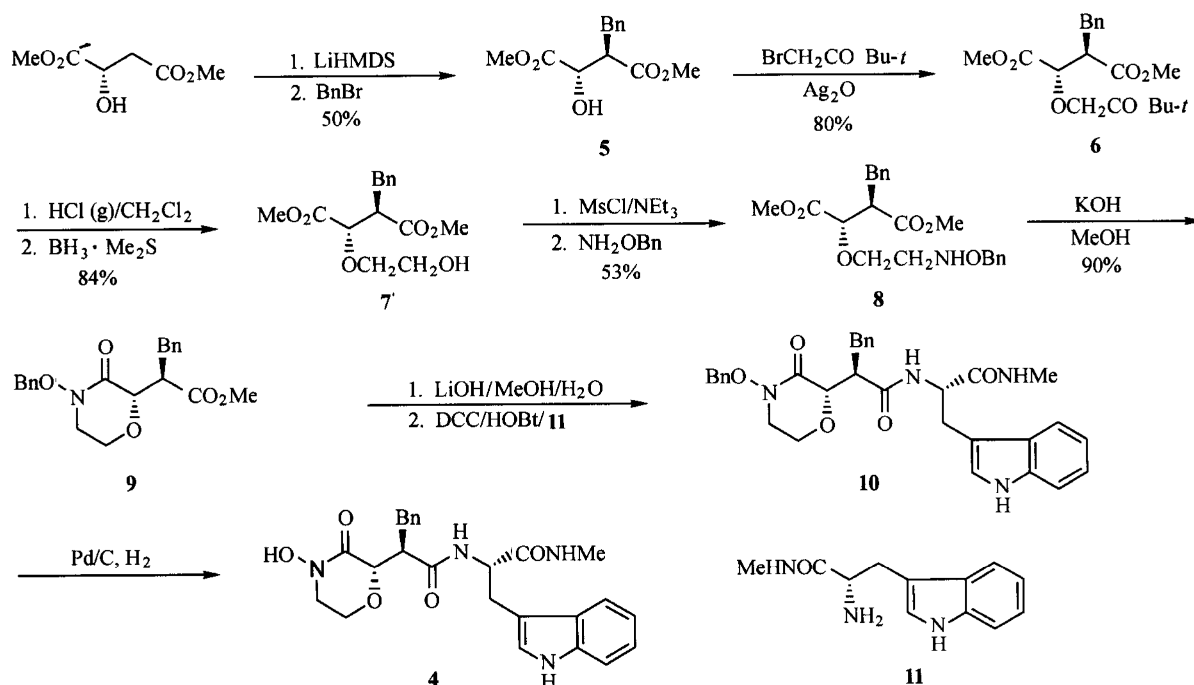
Scheme 1



Scheme 2



We tested the inhibitory activity for compound **4** on four MMP catalytic domains. The assays were carried out using a chromogenic thiopeptolide substrate Ac-Pro-Leu-Gly-thioester-Leu-Leu-Gly-Oet[6] by measuring UV absorption at 412 nm kinetically for 3 minutes. Ye *et al*. reported[7] that Boc-*L*-Trp-OH inhibited tromelysin catalytic domain, a similar construct as the S1CD, with an IC_{50} of 10 μM. Compound **4** showed MMP inhibition on all MMPs tested with IC_{50} ranging from 20.1 μM to 104 μM and had a slight better inhibitory activity on S1CD than Boc-*L*-Trp-OH (Table 1, Fig. 1). Since MMPs are zinc-containing enzymes, and peptide hydroxamides usually bind to MMPs with their metal chelating group hydroxamide. The peptide hydroxamide we tested also showed inhibition on the MMPs.

Table 1 Inhibition of C1CD, C3CD, S1CD, and MT2CDs by compound **4** and other MMP inhibitors

Inhibitor	IC_{50} (μM)			
	C1CD	C3CD	S1CD	CDMMP-15
Peptide hydroxamide	0.73 ± 0.09	2.8 ± 0.5	5.39 ± 1.27	134 ± 10
4	64.8 ± 4.8	69 ± 9	20.1 ± 5.0	104 ± 9
Boc-*L*-Trp-OH			27.8 ± 4.7	

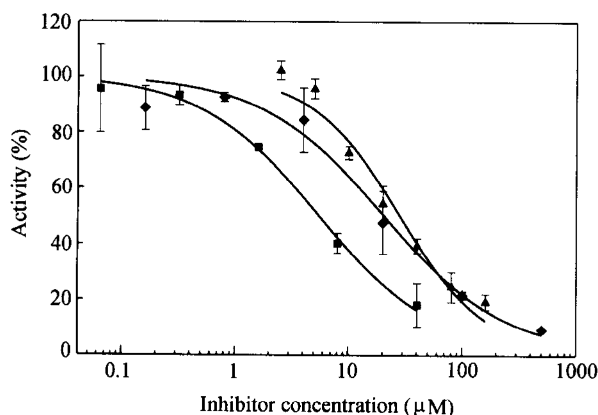


Fig. 1 Inhibition of S1CD by compound **4** (diamond), the peptide hydroxamide (square), and Boc-*L*-Trp-OH (triangle).

As it is apparent, the cyclic analogue **4** has MMP inhibition on all MMPs tested with IC_{50} in higher micromolar range. While compounds **1**, **2** and **3** displayed inhibitory activity on some MMPs tested with IC_{50} in lower nonomolar range.[2-4] The present result indicated that introduction of an additional ring between C2 and the nitrogen of hydroxamate in **1** decreased the inhibition potency to some MMPs.

Experimental

(2*S*,3*R*)-3-*Benzyl*-2-(*tert-butoxycarbonylmethaneoxy*) *succinic acid dimethyl ester* (**6**) To a solution of **5** (4.4 g, 15.5 mmol) in 100 mL of DMF was added Ag_2O (8.1 g, 35 mmol) under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 48 h before it was filtered through celite. The filtrate was concentrated to dryness and the residue was purified on silica gel column eluted with 7/1 hexane/ethyl acetate to afford 5.2 g (80%) of **6**. $[\alpha]_D^{20}$ −40.4(*c* 0.88, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ: 7.29—7.16(m, 5H), 4.23(d, *J* = 16.8 Hz, 1H), 4.15(d, *J* = 5.0 Hz, 1H), 3.90(d, *J* = 16.8 Hz, 1H), 3.59 (s, 3H), 3.31—3.24 (m, 1H), 3.15—2.95 (m, 2H), 1.48 (s, 9H); MS *m/z*: 310 (M^+ − *t*-Bu). HRMS (EI) found *m/z*: 310.1087 (M^+ − *t*-Bu); $C_{15}H_{18}O_7$ requires 310.1052.

(2*S*,3*R*)-3-*Benzyl*-2-(2-*hydroxyethyloxyoxy*) *succinic acid dimethyl ester* (**7**) A solution of **6** (5.4 g, 14.8 mmol) in 200 mL of dry methylene chloride was saturated with gaseous hydrogen chloride at 0℃ for 1 h. After the reaction was completed as monitored by TLC, the excess of hydrogen chloride was removed by passing a stream of nitrogen through the mixture. The solvent was evaporated and the residual oil was purified by column chromatography to afford the corresponding acid. This acid was dissolved in 100 mL of dry THF and then borane-methyl sulfide complex (1.8 mL, 18 mmol) was added dropwise at 0℃ under argon. After the reaction mixture was stirred at room temperature for 16 h, the reaction was quenched by adding 8 mL of methanol. The solution was concentrated and the residual oil was purified by chromatography eluted with 5/4 hexane/ethyl acetate to provide 3.7 g (84%) of **7**. $[\alpha]_D^{20}$ −24.3 (*c* 0.72, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ: 7.31—7.16(m, 5H), 4.10(d, *J* = 7.2 Hz, 1H), 3.70(s, 3H), 3.67—3.61(m, 4H), 3.62(s, 3H), 3.29—3.25(m, 1H), 3.12—2.85(m, 2H); MS *m/z*: 296(M^+). HRMS (EI) found *m/z*: 264.0952 (M^+ − HOMe); $C_{14}H_{16}O_5$ requires 264.0997.

(2*S*, 3*R*)-3-*Benzyl*-2-[2-(*N-benzyloxyamino*) *ethyloxyoxy*) *succinic acid dimethyl ester* (**8**) To a solution of **7** (3.3 g, 11.1 mmol) in 40 mL of dry methylene chloride were added triethylamine (4.6 mL, 33.3 mmol) and mesyl chloride (1.7 mL, 22.2 mmol) with vigorous stirring. The reaction mixture was stirred for 4 h before it was diluted with methylene chloride and washed with brine. After the organic layer was dried over Na_2SO_4 and concentrated, the residual oil was dissolved in 40 mL of dry dioxane. To this stirred solution triethylamine (3.1 mL, 22.2 mmol) and *O*-benzylhydroxyamine (4.1 g, 33.3 mmol) were added at room temperature. The reaction mixture was heated at 100℃

for 3 days and then the solvent was evaporated via rotavapor. The residue was diluted with 100 mL of chloroform before it was washed with saturated aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$ and concentrated. Purification of the residue on silica gel column eluted with 6/1 hexane/ethyl acetate gave 2.4 g (53%) of **8**. $[\alpha]_D^{20}$ − 22.9 (*c* 1.1, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ: 7.45—7.15(m, 10H), 6.10 (br s, 1H), 4.74(s, 2H), 3.95(d, *J* = 7.2 Hz, 1H), 3.68—3.55(m, 4H), 3.28—3.24(m, 1H), 3.18—3.05(m, 3H), 2.92—2.84(m, 1H); MS*m/z*: 402 (M^+ + H^+). HRMS (EI) found *m/z*: 401.1823 (M+); $C_{22}H_{27}NO_6$ requires 401.1838.

2-(R)-[2-(S)-4-Benzyloxy-3-oxo-morpholine-2-yl]-phenylpropionic acid methyl ester (**9**) To a solution of **8** (1.8 g, 4.5 mmol) in 13 mL of dry methanol was added a solution of KOH (0.29 g, 5.2 mmol) in 10 mL of dry methanol at room temperature. After the starting material disappeared monitored by TLC, the reaction mixture was neutralized with 2 N HCl. The solution was partitioned between 100 mL of methylene chloride and 20 mL of brine. The organic layer was dried over $MgSO_4$ and concentrated, the residue was chromatographed to provide 1.5 g (90%) of **9**. $[\alpha]_D^{20}$ − 96.7 (*c* 1.8, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ: 7.45—7.15 (m, 10H), 5.08—5.03(m, 2H), 4.09—4.01(m, 2H), 3.88—3.81(m, 1H), 3.71(s, 3H), 3.69—3.54(m, 2H), 3.33—3.17(m, 2H), 2.87—2.83 (m, 1H); MS *m/z*: 370(M^+ + H^+). HRMS (EI) found *m/z*: 338.1400 (M^+ − OMe); $C_{20}H_{20}NO_4$ requires 338.1392.

2-(R)-[2-(S)-4-Benzyloxy-3-oxo-morpholine-2-yl]-phenylpropionyl-(S)-tryptophan N-methylamide (**10**) A mixture of **9** (280 mg, 0.76 mmol) in 1.5 mL of 1 N aqueous LiOH and 5 mL of methanol was stirred at room temperature for 20 h. After the mixture was acidified with 2 N HCl, it was diluted with 50 mL of chloroform. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified on a short silica gel column to afford the corresponding acid, which was dissolved in 4 mL of methylene chloride. To this stirred solution were added HOBt (70 mg, 0.46 mmol), (*S*)-tryptophan methylamide (150 mg, 0.69 mmol) and DCC (95 mg, 0.46 mmol). After the resultant solution was stirred for 20 h at room temperature, it was filtered and the filtrate was diluted with methylene chloride. The solution was washed with 1 N HCl, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$ and concentrated and the residue was chromatographed to provide 140 mg (33%) of **10**. $[\alpha]_D^{20}$ − 113.0 (*c* 1.4, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ: 8.30(s, 1H), 7.65(d, *J* = 7.2 Hz, 1H), 7.33—7.11(m, 13H), 7.01(s, 1H), 6.79(d, *J* = 7.8 Hz, 1H), 5.90(d, *J* = 4.2 Hz, 1H), 4.84—4.78(m, 2H), 4.75—4.71(m, 1H), 3.94(d, *J* = 3.0 Hz, 1H), 3.56—3.38 (m, 3H), 3.31—3.25 (m, 1H), 3.143.05(m, 3H), 2.99—2.95(m, 1H), 2.89—2.86(m, 1H), 2.57(d, *J* = 4.2 Hz, 1H); MS *m/z*: 401 (M^+). HRMS (EI) found *mz* 401.1824 (M^+); $C_{22}H_{27}NO_6$ requires 401.1838.

2-(R)-[2-(S)-4-Hydroxy-3-oxo-morpholine-2-yl]phenylpropionyl-(S)-tryptophan N-methylamide (**4**)

A solution of **10** (60 mg, 0.11 mmol) in 2 mL of methanol was hydrogenated at 1 atm over 10 mg of 10% Pd/C and 40 mg of $NaHCO_3$ for 8 h. The mixture was filtered and the filtrate was concentrated. The residue was chromatographed eluting with 4/1 chloroform/methanol to give 46 mg (90%) of **4**. $[\alpha]_D^{20}$ − 78.6 (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ: 8.20 (s, 1H), 7.66(d, *J* = 7.2 Hz, 1H), 7.55—6.95 (m, 10H), 6.35—6.31(m, 1H), 4.90—4.76(m, 1H), 3.96—3.94(m, 1H), 3.87—3.85(m, 1H), 3.57—3.45(m, 2H), 3.33—3.29(m, 2H), 3.18—3.05 (m, 2H), 3.05—3.01(m, 1H), 2.90—2.86 (m, 1H), 2.65(s, 3H); MS *m/z*: 465(M^+ + H^+). HRMS (EI) found *m/z*: 417.1729 (M^+ − $NHCH_3$ − OH); $C_{24}H_{23}N_3O_4$ requires 417.1709.

MMP *assays for inhibitors* The MMPs used in the assays include the recombinant catalytic domains of collagenase 1 (C1CD), collagenase 3 (C3CD), stromelysin 1 (S1CD), and MT2-MMP (MT2CD), and their expression and purification will be reported elsewhere. A thiopeptolide Ac-Pro-Leu-Gly-thioester-Leu-Leu-Gly-OEt was purchased from Bachem (King of Prussia, PA, USA), and used as the chromogenic substrate. A peptide hydroxamide 4-Abz-Gly-Pro-*D*-Leu-*D*-Ala-NHOH was also purchased from Bachem and used for comparison, along with the known inhibitor Boc-*L*-Trp-OH. Inhibitors were dissolved in DMSO, and their inhibitory activity was assayed in a total volume of 100 μL, containing 50 mM Tris (pH 7.0) for C1CD, C3CD and MT2CD or 50 mM MES (pH 6.0) for S1CD, 10 mM

$CaCl_2$, 100 μM the thiopeptolide, 1 mM 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), and the individual enzyme (12.5 nM C1CD, 70 nM C3CD, 200 nM S1CD or 100 nM MT2CD).

Inhibitors were evaluated at various concentrations from 1 to 500 μM (compound **4**), 1 to 200 μM (the hydroxamide peptide), and 1 to 500 μM (Boc-*L*-Trp-OH). IC_{50} values were calculated using a least squares algorithm by fitting the percent of activity versus inhibitor concentration to the following equation for a sigmoid inhibition pattern.

$$\% \text{ activity} = 100/[1 + ([I]/IC_{50})^{slope}]$$

where [I] is the inhibitor concentration, IC_{50} is the concentration of inhibitor where 50% enzyme activity was inhibited.

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