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ARTICLE TYPE

Characterization of a selective inhibitor for matrix metalloproteinase-8 (MMP-8)

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Experimental Procedures

Chemicals and reagents. Human recombinant active MMP-2 and MMP-7, and the catalytic domains of human recombinant MMP-3 and MMP-14 were purchased from EMD Biosciences (La Jolla, CA, USA). The catalytic domains of human recombinant MMP-1, MMP-8, and MMP-9 were obtained from Enzo Life Sciences (Farmingdale, NY, USA). Human recombinant MMP-13 was purchased from R&D Systems (Minneapolis, MN, USA) in its pro form and was activated at a concentration of 100 μg/mL with 1 mM *p*-aminophenylmercuric acetate in reaction buffer (50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% Brij-35 (w/v), pH 7.5). Fluorogenic substrate MOCAcPLGLA$_2$pr(Dnp)AR-NH_2 (MMP-2, MMP-7, MMP-9, and MMP-14) was obtained from Peptides International (Louisville, KY, USA). Fluorogenic substrates MOCAcRPKPVE(Nva)WRK(Dnp)-NH_2 (MMP-3) and McaKPLGL(Dpa)AR-NH_2 (MMP-1, MMP-8, and MMP-13) were purchased from R&D Systems (Minneapolis, MN, USA).

Ethyl 2-amino-4-ethoxycarbonylmethyl-4,5,6,7-tetrahydrobenz[b]thiophene-3-carboxylate (4). Diethylamine (4.1 mL, 40 mmol) was added dropwise to a solution of compound **3** (7.36 g, 40 mmol), ethyl cyanoacetate (4.97 g, 44 mmol), sulfur (1.34 g, 42 mmol) in 45 mL of pyridine. The mixture was stirred at room temperature for 27 h, and then the reaction mixture was concentrated under reduced pressure. The title compound was purified as a viscous oil from the residue by silica-gel column chromatography (hexane/EtOAc – 15:1) (7.09 g, 57%). Its spectroscopic data were identical to that reported in the literature.[1]

(R)-1,2,3,4-Tetrahydro-3-isoquinolinecarboxylic acid (6). A total of 77 mL concentrated HCl was added to the mixture of (*R*)-phenylalanine **5** (10.0 g, 60.6 mmol) and formalin (37%, 23 mL). The reaction mixture was heated to 95 °C for 1 h. More formalin (37%, 77 mL) and concentrated HCl (20 mL) were added, and the mixture was kept at the same temperature for another 3 h. At this point, the mixture was cooled in an ice bath and the solid precipitate was collected by filtration. The crude product was suspended in MeOH/H_2O (3:1) and the suspension was brought to reflux. Concentrated NH_4OH was added to this solution until the pH reached to 5-6. The resultant precipitate was collected by filtration and was allowed to dry *in vacuo* at room temperature to afford the title compound (6.97 g, 65%). Its NMR and MS spectra were identical to the reported data.[2]

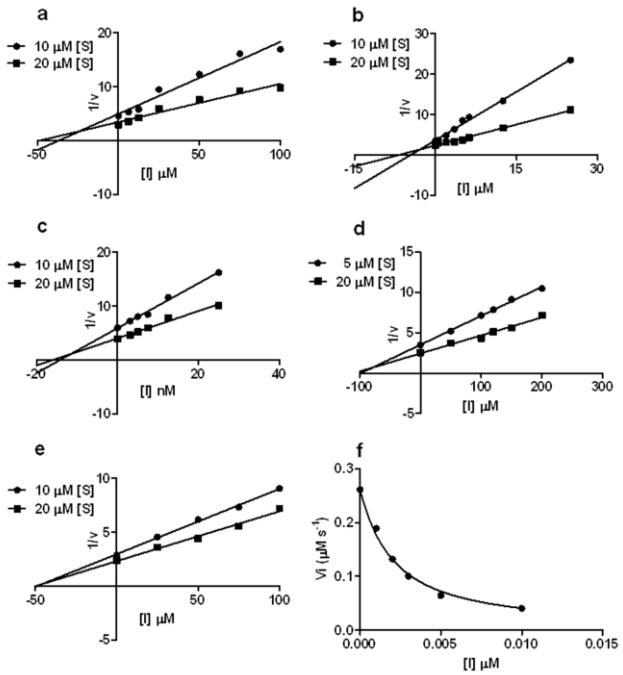
(3R)-1,2,3,4-Tetrahydro-2-[(4-methoxyphenyl)sulfonyl]-3-isoquinolinecarboxylic acid (8). Compound **6** (3.54 g, 20 mmol) and 2 equiv. of potassium carbonate (5.52 g, 40 mmol) were dissolved in 260 mL of THF/H_2O (v/v, 1:1). 4-Methoxylbenzenesulfonyl chloride **7** (5.17 g, 25 mmol) was added dropwise to the above solution. This reaction mixture was stirred for 2 h at room temperature. Then, the reaction solution was acidified by the addition of concentrated HCl until the pH was adjusted to ~4-5, and the solution was extracted with ethyl acetate three times. The combined organic layer was dried over anhydrous Na_2SO_4, the suspension was filtered, and the filtrate was concentrated under reduced pressure. The crude material was purified by column chromatography on silica gel (DCM/MeOH – 30:1) to afford the desired product (5.62 g, 81%). ^{1}H NMR (400 MHz , $CDCl_3$) δ 10.32 (br s, 1H),7.74-7.78 (m, 2H), 7.11-7.18 (m, 2H), 7.03-7.07 (m, 2H), 6.91-6.94 (m, 2H), 4.98 (t, *J* = 4.8, 1H) , 4.63 (d, *J* = 15.6 , 1H), 4.48 (d, *J* = 15.6, 1H , 3.84 (s, 3H), 3.16 (d, *J* = 4.4, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.0, 163.2, 131.6, 130.7, 130.5, 129.6, 128.9, 127.1, 127.0, 126.3, 114.3, 55.8, 53.8, 44.5, 31.7. HRMS-ESI (*m*/*z*): $[M + H]^+$, calcd for $C_{17}H_{18}NO_5S$, 348.0906; found 348.0900.

(3R)-1,2,3,4-Tetrahydro-2-(4-methoxybenzenesulfonyl)-isoquinoline-3-hydroxamate (2). To a solution of compound **8** (1.01 g, 2.92 mmol) and *N*-methylmorpholine (0.42 mL , 3.79 mmol) in 8 mL THF at ice-water temperature was added ethyl chloroformate (0.41 g, 3.791 mmol) dropwise and the mixture was stirred for 30 min at room temperature**.** The solid was filtered off and the filtrate was added to the solution of hydroxylamine hydrochloride (0.41 g, 5.83 mmol) and TEA (0.59 g, 5.83 mmol) in 5 mL DMF for 10 min. The reaction mixture was stirred for 1 h at room temperature. The solvent was evaporated *in vacuo*. The residue was dissolved in ethyl acetate and was washed with water . The organic layer was dried over anhydrous Na_2SO_4, the suspension was filtered, and the filtrate was concentrated to provide a crude brown oil. Subsequent column chromatography on silica gel (DCM/MeOH – 100:1) yielded the desired product (0.54 g, 51%).^{1}H NMR (400 MHz, $CDCl_3$) δ 9.30 (br s, 1H), 7.70-7.72 (m, 2H), 7.13-7.16 (m, 2H), 7.04-7.09 (m, 2H), 6.89–6.91(m, 2H), 4.61-4.58 (m, 1H), 4.50 (d, *J* =14.8, 1H), 4.34 (d, *J* = 14.8, 1H), 3.84 (s, 3H), 3.20 (dd, *J* = 15.6, 4.0, 1H), 2.66 (dd, *J* = 15.6, 6.4, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 163.2, 133.3, 132.6, 130.6, 129.9, 128.6, 127.4, 126.9,

126.6, 115.0, 56.3, 53.3, 45.7, 31.9 . HRMS-ESI (*m/z*): $[M + H]^+$, calcd for $C_{17}H_{19}N_2O_5S$, 363.1015 ; found 363.0996.

Enzyme inhibition. The K_m values for the reaction of enzymes with the fluorogenic substrates were: 12.0 ± 1.0 μM (MMP-1), 18.9 ± 1.0 μM (MMP-2), 5.3 ± 0.3 μM (MMP-3), 6.6 ± 0.6 μM (MMP-7), 9.0 ± 1.9 μM (MMP-8), 5.0 ± 0.1 μM (MMP-9), 7.3 ± 0.77 μM (MMP-13), and 5.6 ± 0.4 μM (MMP-14).[3] Inhibitor stock solutions were prepared in DMSO and at least five different concentrations were used for each assay. The substrate hydrolysis was monitored with a Cary Eclipse fluorescence spectrophotometer (Varian, Walnut Creek, CA, USA). The methodology for the assays was reported previously.[4]

For linear competitive inhibition profiles, the data were plotted in SigmaPlot (Systat Software, Inc., San Jose, CA, USA), and the slope of the fluorescence units plotted versus time gave the reaction velocity, *v*. The reciprocals of these values (1/*v)* were then plotted versus inhibitor concentration, [I], in Microsoft Excel and were fit to a least-squares regression line. Two different substrate concentrations were used for each enzyme to obtain two intersecting lines according to the method of Dixon,[5] from the intersect for which the K_i values were evaluated. The standard deviation was calculated using the LINEST function.



Supplementary Figure 1. Representative K_i curves of compound **1**, a competitive inhibitor of (a) MMP-1, (b) MMP-2, (c) MMP-8, (d) MMP-9, (e) MMP-14, and (f) a tight-binding inhibitor of MMP-13.

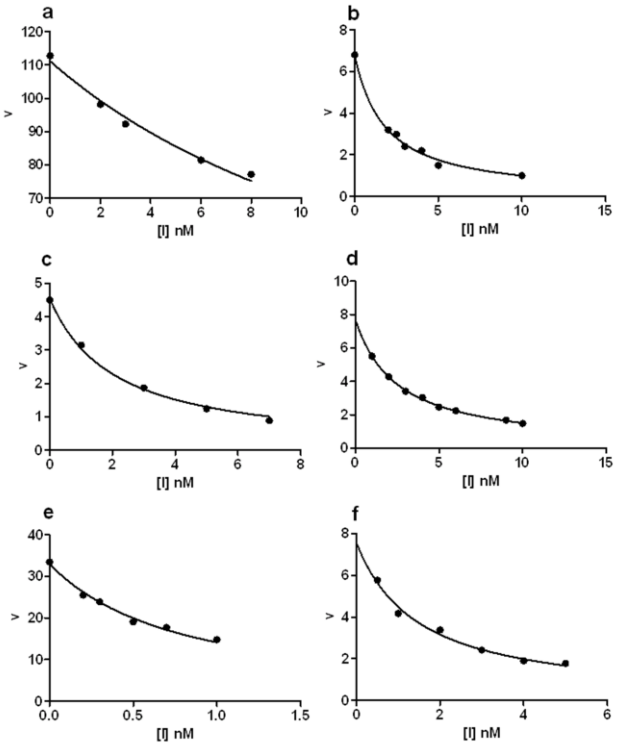
In the cases of inhibition of MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-13, and MMP-14 by compound **2**, where the K_i values were in the low nanomolar or picomolar range, the Morrison equation for competitive tight-binding inhibitors was used:[6, 7]

$$\frac{v_i}{v_0} = 1 - \frac{([E] + [I] + K_i^{app}) - \sqrt{([E] + [I] + K_i^{app})^2 - 4[E][I]}}{2[E]} \qquad \text{(Eq. 1)}$$

where v_i is the inhibited velocity, v_0 is the uninhibited velocity, [E] is the total enzyme concentration, [I] is the inhibitor concentration, and K_i^{app} is the apparent dissociation constant. To calculate K_i from the latter, the following equation was used:[7]

$$K_i^{app} = K_i\left(1 + \frac{[S]}{K_m}\right) \qquad \text{(Eq. 2)}$$

in which [S] is the substrate concentration. The K_m values that we used for these calculations were given earlier.[3]



Supplementary Figure 2. Representative K_i curves for compound **2**, a tight-binding inhibitor of (a) MMP-1, (b) MMP-2, (c) MMP-8, (d) MMP-9, (e) MMP-13 and (f) MMP-14.

Notes and references

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