JOC Notes

Design, Synthesis, and Evaluation of a Mechanism-Based Inhibitor for Gelatinase A

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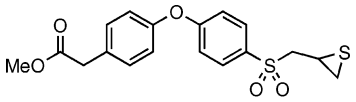

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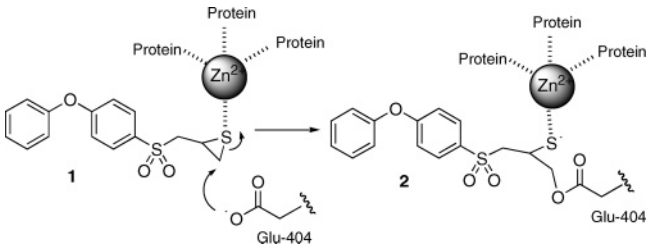
Matrix metalloproteinases (MMPs), of which 26 are known, have been implicated in a number of pathological conditions, including tumor metastasis. We have previously described the first mechanism-based inhibitor for MMPs (*J. Am. Chem. Soc*. **2000**, *122*, 6799−6800), which in chemistry mediated by the active site zinc ion selectively and covalently inhibits MMP-2, -3, and -9. Computational analyses indicated that this selectivity in inhibition of MMPs could be improved by design of new variants of the inhibitor class. We report herein the syntheses of methyl 2-(4-{4-[(2-thiiranylpropyl)-sulfonyl]phenoxy}phenyl)acetate (**3**) and 2-(4-{4-[(2-thiiranyl-propyl)sulfonyl]phenoxy}phenyl)acetic acid (**4**), and show that compound **3** serves as a mechanism-based inhibitor exclusively for MMP-2. This molecule should prove useful in delineating the functions of MMP-2 in biological systems.

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases with important pathological and physiological functions.[1] A total of 26 MMPs are known. Their unregulated and uncontrolled activities have been associated with a number of disease processes, including neurological disorders, arthritis, cardiovascular diseases, and cancer, just to mention a few. Inhibition of MMPs as a means to intervention of disease is highly sought.[2] With very few exceptions, the known inhibitors of MMPs are broad-spectrum molecules, designed to chelate the active-site zinc ions of these enzymes. This broad breadth of activity has been problematic in clinical trials of MMP inhibitors, as the molecules show serious side effects.[3]

We have been interested in selective inhibition of gelatinases, MMP-2 and -9 (also known as gelatinases A and B, respectively). The excessive and unregulated activities of these two enzymes have been indicated in a number of cancer metastases.[4] We disclosed for the first time a novel strategy in mechanism-based inhibition of MMP by a thiirane-containing inhibitor, where the thiirane sulfur first coordinates with the active-site zinc ion.[5] The coordinated thiirane predisposes it to nucleophilic attack by the active site glutamate (Glu-404 in MMP-2) in these enzymes, a process that leads to covalent modification of the enzyme and the attendant loss of activity. This process is depicted below for the prototypic member (compound **1**) of this class of inhibitors (**1** → **2**).



Inhibitor **1** underwent the chemistry shown above with three MMPs: MMP-2, -3, and -9.[5] We have been keen on developing inhibitors of this class that show even a more narrow spectrum of inhibition than that exhibited by **1**. Specific interactions at the bottom of the deep S1′ pocket of MMP-2 and MMP-9 were identified that could be exploited with a structural variant of inhibitor **1** to enhance selectivity toward gelatinases (Figure 1). Two

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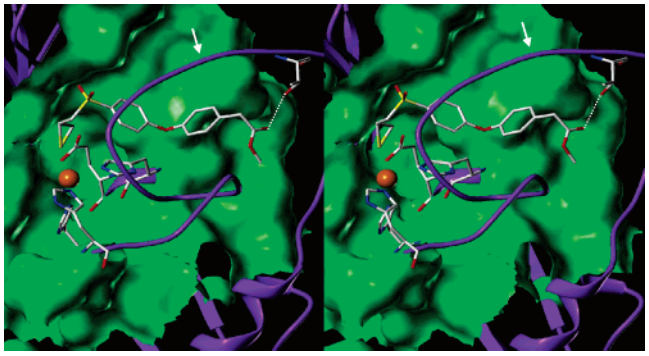
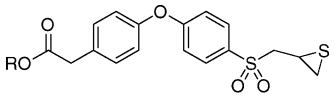


FIGURE 1. Stereoview of compound **3** bound to the active site of MMP-2. A Connolly solvent-accessible surface is constructed in the active site (shown in green), while the protein is rendered in purple. Compound **3** along with the active site Glu-404 and the three histidines that are coordinated to the catalytic zinc are shown in capped-stick representation, colored according to atom types (yellow, red, blue, and white for S, O, N, and C, respectively). The zinc ion is shown as an orange sphere. The white arrow points to the S1′ pocket. The side chain hydroxyl of Thr428 is expected to hydrogen bond (2.8 Å) to the ester carbonyl of compound **3**. The methyl group of compound **4** is located near Leu399, Leu420, and Phe431 resulting in favorable hydrophobic interactions that would likely contribute to the overall binding affinity.

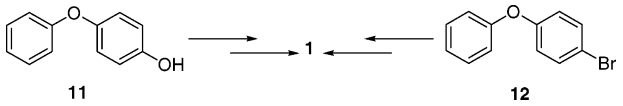
such molecules, **3** and **4**, were designed by computational analyses. We report herein the syntheses of these molecules. Furthermore, we document that compound **3** shows the pattern of slow-binding inhibition that leads to covalent chemistry only with MMP-2.



3 (R = Me)
4 (R = H)

The syntheses of compounds **3** and **4** were accomplished according to Scheme 1. An *N*,*N*-dimethyl glycine-promoted Ullmann coupling reaction[6] between the commercially available aryl bromide **5** and phenol **7**,[7] which was in turn prepared from 4-hydroxythiophenol (**6**) by chemoselective allylation, proceeded smoothly to give **8** in 65% yield. Oxidation of the sulfur and olefin moieties in **8** was achieved by the use of an excess of *m*-CPBA (12 equiv) to afford oxirane **9** in 92% yield, which was treated with thiourea to provide thiirane **3** in 77% yield. Attempts at hydrolysis of the methyl ester of **3** to the corresponding carboxylic acid **4** under various basic conditions were unsuccessful, probably due to the deprotonation of the acidic α-position to the sulfonyl moiety, followed by *β*-elimination of the thiolate. The method of Mascaretti with the use of $(Bu_3Sn)_2O$ in toluene at 80 °C[8] was found to be most effective for this conversion. Under these conditions, the methyl ester **3** was converted to the corresponding tin ester **10**, which was subsequently hydrolyzed on C_{18}-reverse phase silica gel to afford the desired carboxylic acid **4** in 65% yield (with an attendant 12% recovery of **3**).

The synthetic route to compounds **3** and **4** is different than those reported for inhibitor **1**.[5,9] Syntheses of **1** began from the commercially available diphenyl ethers **11** or **12**. The synthetic route of Scheme 1 allows more flexibility in creating structural diversity in the two-ring systems and should find more general applicability for entries into this molecular class of versatile enzyme inhibitors.



11 **12**

Compounds **3** and **4** were evaluated with a representative set of MMPs. As shown in Table 1, compound **4** inhibits MMP-2 with a K_i of 460 nM. Whereas compound **3** exhibits dissociation constants for MMP-2 and -9 in the low nanomolar levels, it behaves as a slow-binding inhibitor that leads to mechanism-based inhibitor only with MMP-2. Therefore, compound **3** is a selective and potent mechanism-based inhibitor of MMP-2 (gelatinase A). Both compounds are merely poor competitive inhibitors (micromolar) of the other MMP that were tested. Hence, high selectivity in inhibition of MMP-2 has been achieved.

Superimposition of the X-ray structures for MMP-2 and MMP-9 reveals some important differences.[10] Midway through the S1′ loop, an arginine residue is present in MMP-9, as opposed to a threonine in MMP-2. The pocket of MMP-9 appears to be more constricted than that of MMP-2, as the backbone of the S1′ loop of MMP-9 is about 2−3 Å inward. This could potentially lead to unfavorable steric interaction for the methyl moiety of compound **3** in MMP-9.

Experimental Section

Methods. Enzyme kinetics were performed as described earlier.[5]

4-(Allylthio)phenol (7). To a stirred solution of 4-hydroxythiophenol (**6**) (4.30 g, 34.1 mmol) in DMF (25 mL) were added K_2CO_3 (4.71 g, 34.1 mmol) and allyl bromide (3.09 mL, 34.1 mmol) at ice-water temperature, and the mixture was stirred for 15 min, prior to stirring overnight at room temperature. After the addition of 1 M aqueous HCl, the mixture was extracted with ether (3×). The combined organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (ethyl acetate/hexane = 1/10 to 1/6) to give **7** (5.74 g, 70%) as a white semisolid. The ^1H and ^{13}C NMR spectra and mass spectrum were identical with the reported values.[7]

Methyl 2-{4-[4-(Allylthio)phenoxy]phenyl}acetate (8). A mixture of **5** (1.51 g, 6.59 mmol), **7** (1.64 g, 9.88 mmol), Cs_2CO_3 (4.30 g, 13.2 mmol), *N*,*N*-dimethylglycine hydrochloride salt (276 mg, 1.98 mmol), CuI (125 mg, 0.659 mmol), and degassed 1,4-dioxane (14 mL) was heated at 90 °C for 22 h under a nitrogen atmosphere. After dilution with water, the mixture was extracted with ethyl acetate. The combined organic layer was

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SCHEME 1

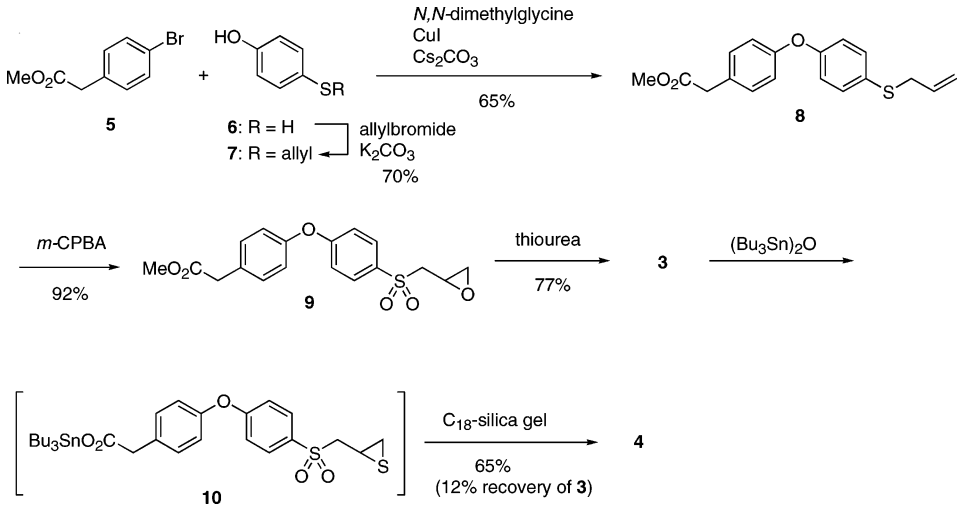

TABLE 1. Kinetic Parameters for Competitive Inhibition of MMPs by the Synthetic Inhibitors

	K_i (nM)	
	3	**4**
MMP-2	50 ± 14^a	460 ± 30
MMP-9	40 ± 2	$(4.1 \pm 0.2) \times 10^3$
MMP-14_{cat}	590 ± 70	$(5.3 \pm 0.3) \times 10^4$
MMP-1	$(1.1 \pm 0.2) \times 10^4$	$(4.5 \pm 0.9) \times 10^3$
MMP-3	$(8.7 \pm 0.5) \times 10^3$	$(5.4 \pm 1.0) \times 10^5$
MMP-7	1.3×10^4	$(2.5 \pm 0.1) \times 10^5$

[a] Parameters for the slow-binding component of inhibition: $k_{on} = (1.2 \pm 0.3) \times 10^4$ M^{-1} s^{-1}, $k_{off} = (6.2 \pm 0.7) \times 10^{-4}$ s^{-1}.

washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (ethyl acetate/hexane = 1/12) to give **8** (1.35 g, 65%) as a pale yellow semisolid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.49 (d, 2H, J = 7.2 Hz), 3.61 (s, 2H), 3.71 (s, 3H), 5.03−5.10 (m, 2H), 5.86 (m, 1H), 6.91−6.97 (m, 4H), 7.23−7.26 (m, 2H), 7.32−7.35 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 38.5, 40.3, 52.1, 117.5, 119.0, 119.1, 129.0, 129.3, 130.6, 132.9, 133.7, 156.0, 156.3, 172.0; HRMS (FAB) calcd for $C_{18}H_{18}O_3S$ (M^+) 314.0977, found 314.0986.

Methyl 2-(4-{4-[(2-Oxiranylpropyl)sulfonyl]phenoxy}-phenyl)acetate (9). To a stirred solution of **8** (500 mg, 1.59 mmol) in CH_2Cl_2 (20 mL) was added *m*-CPBA (ca. 70%, 4.7 g, 19.1 mmol) at ice-water temperature, and the mixture was subsequently stirred at room temperature for 8 days. With ice-cooling, the reaction was quenched with a saturated $Na_2S_2O_3$ solution, followed by saturated $NaHCO_3$ solution, and the mixture was extracted with ethyl acetate (3×). The combined organic layer was washed with saturated $Na_2S_2O_3$ solution, saturated $NaHCO_3$ solution, water, and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (ethyl acetate/hexane = 1/2 to 2/3) to give **9** (528 mg, 92%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.47 (dd, 1H, J = 5.0, 2.0 Hz), 2.82 (m, 1H), 3.26−3.33 (m, 3H), 3.65 (s, 2H), 3.72 (s, 3H), 7.03−7.05 (m, 2H), 7.08−7.10 (m, 2H), 7.32−7.34 (m, 2H), 7.86−7.88 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 40.3, 45.8, 52.1, 59.6, 117.6, 120.6, 130.5, 130.9, 131.1, 132.4, 153.8, 162.8, 171.8; HRMS (FAB) calcd for $C_{18}H_{18}O_6S$ (M^+) 362.0824, found 362.0829.

Methyl 2-(4-{4-[(2-Thiiranylpropyl)sulfonyl]phenoxy}-phenyl)acetate (3). To a stirred solution of **9** (500 mg, 1.38 mmol) in MeOH−CH_2Cl_2 (10:1, 11 mL) was added thiourea (262 mg, 3.45 mmol) at room temperature, and the mixture was stirred overnight. After concentration under reduced pressure, the residue was dissolved in ethyl ether. The ethyl ether solution was washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate/hexane = 2/5) to give **3** (400 mg, 77%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.15 (dd, 1H, J = 5.5, 2.0 Hz), 2.53 (dd, 1H, J = 6.5, 2.0 Hz), 3.05 (m, 1H), 3.17 (dd, 1H, J = 14.5, 7.0 Hz), 3.51 (dd, 1H, J = 14.5, 5.5 Hz), 3.65 (s, 2H), 3.72 (s, 3H), 7.03−7.05 (m, 2H), 7.08−7.10 (m, 2H), 7.33−7.34 (m, 2H), 7.85−7.86 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.2, 26.0, 40.3, 52.1, 62.6, 117.7, 120.5, 130.7, 130.9, 131.1, 131.9, 153.8, 162.8, 171.8; HRMS (FAB) calcd for $C_{18}H_{19}O_5S_2$ ($M + H^+$) 379.0674, found 379.0645.

2-(4-{4-[(2-Thiiranylpropyl)sulfonyl]phenoxy}phenyl)-acetic Acid (4). To a stirred solution of **3** (312 mg, 0.83 mmol) in toluene (11 mL) was added bis(tributyltin)oxide (1.05 mL, 2.06 mmol) at room temperature, and the mixture was stirred at 80 °C for 12 h. The solution was cooled to room temperature and concentrated to dryness under reduced pressure. The residue was dissolved in acetonitrile, and the solution was washed with hexane (3×) and concentrated under reduced pressure to leave the crude tin ester **10** (532 mg) as a pale-yellow oil. Subsequently, **10** was passed through a C_{18}-reverse phase silica gel pad (ODS silica gel 20 g, washed with water, 1:2 water/acetonitrile and acetonitrile) to afford a mixture of **3** and **4**, which was purified by silica gel column chromatography (chloroform/methanol = 30/1 to 10/1) to give **4** (195 mg, 65%) as a white solid with the recovery of some of **3** (38 mg, 12%). Compound **10**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, 9H, J = 7.2 Hz), 1.23−1.38 (m, 12H), 1.54−1.64 (m, 6H), 2.16 (dd, 1H, J = 5.4, 1.8 Hz), 2.54 (dd, 1H, J = 6.0, 1.8 Hz), 3.07 (m, 1H), 3.15 (dd, 1H, J = 13.8, 7.8 Hz), 3.54 (dd, 1H, J = 13.8, 5.1 Hz), 3.64 (s, 2H), 7.03 (m, 2H), 7.08 (m, 2H), 7.35 (m, 2H), 7.86 (m, 2H); mass (FAB) m/z 655 ($M + H^+$); R_f 0.3 (chloroform/methanol = 10/1). Compound **4**: mp 133−134 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.16 (d, 1H, J = 4.0 Hz), 2.54 (d, 1H, J = 5.5 Hz), 3.06 (m, 1H), 3.19 (dd, 1H, J = 14.0, 8.0 Hz), 3.52 (dd, 1H, J = 14.0, 6.0 Hz), 3.68 (s, 2H), 7.05 (br d, 2H, J = 8.5 Hz), 7.10 (br d, 2H, J = 8.5 Hz), 7.35 (br d, 2H, J = 8.5 Hz), 7.86 (br d, 2H, J = 8.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.2, 26.0, 40.2, 62.6, 117.8, 120.5, 130.2, 130.7, 131.3, 132.0, 154.1, 162.7, 177.1; HRMS (FAB) calcd for $C_{17}H_{17}O_5S_2$ ($M + H^+$) 365.0517, found 365.0495; R_f 0.2 (chloroform/methanol = 10/1).

Computational Procedures. The X-ray structure of MMP-2 provided the Cartesian coordinates for the molecular docking study (RCSB code 1QIB). The Sybyl program (Tripos Inc., St. Louis, MO) was used for the manipulation and visualization of all structures and for the protonation of the bound ligand. AM1-BCC charges were computed for the ligand by using the antechamber module from the AMBER 7 suite of programs.[11] The ligand was docked into the active site of MMP-2 with use of a Lamarckian genetic algorithm as implemented in

the AutoDock 3.04 program.[12] Parameters for the docking runs were similar to those used previously,[12] except for the following differences: the quaternion step, the translation step, and the torsion step were set to 0.2, 5, and 5, respectively. The number of evaluations was increased to 2.5×10^7 from 250 000 and the ligand was fully flexible during the docking runs.

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Supporting Information Available: NMR spectra for the synthetic molecules. This material is available free of charge via the Internet at http://pubs.acs.org.

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