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Synthesis of Chiral 2-(4-Phenoxyphenylsulfonylmethyl)thiiranes as Selective Gelatinase Inhibitors

Mijoon Lee,[†] M. Margarida Bernardo,[‡] Samy O. Meroueh,[†] Stephen Brown,[†] Rafael Fridman,[‡] and Shahriar Mobashery*,[†]

Department of Chemistry and Biochemistry and the Walther Cancer Research Center, University of Notre Dame, Notre Dame, Indiana 46556, and Department of Pathology, School of of Medicine and Proteases and Cancer Program, Karmanos Cancer Institute, Wayne State University, Detroit, Michigan 48201

mobashery@nd.edu

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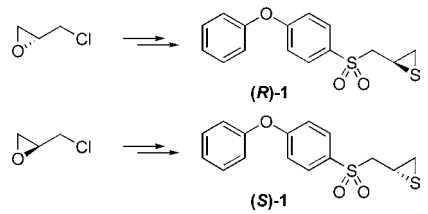
ABSTRACT



Compound 1, 2-(4-phenoxyphenylsulfonylmethyl)thiirane, is a selective inhibitor of gelatinases, which is showing high promise in studies of animal models for cancer metastasis and stroke. The (*R*)-1 and (*S*)-1 enantiomers of compound 1 were each synthesized in this study and were shown to be equally active in inhibition of gelatinases.

Restructuring of the extracellular matrix is an important biological event for both physiological and pathologial processes. These events are highly regulated in physiological settings. However, when the regulation of these events goes awry, a number of pathological events ensue, including cancer growth, tumor metastasis and angiogenesis, arthritis, connective tissue diseases, inflammation, and cardiovascular, neurological, and autoimmune diseases.[1−8]

Matrix metalloproteinases (MMPs) constitute a family of 26 closely related zinc-dependent endopeptidases involved in these processes. Of these enzymes, gelatinases (MMP-2 and -9) have been implicated in a number of diseases, and inhibitors for these are highly sought. Unfortunately, virtually all of the known inhibitors for gelatinases are broad-spectrum.[9−14] There are a mere handful known to exhibit selectivity toward subsets of MMP members.[15]

We recently reported the design and in vitro evaluation of racemic 2-(4-phenoxyphenylsulfonylmethyl)thiirane (compound **1**).[16] This compound is a potent and selective inhibitor for human gelatinases. The gelatinase inhibitor is highly active in in vivo models for cancer metastasis and for brain damage after stroke in mice, both of which are processes known to be mediated by the activity of gelatinases.[17,18]

[†] University of Notre Dame.
[‡] Wayne State University.

(1) Bergers, G.; Benjamin, L. E. *Nat. Rev. Cancer* **2003**, *3*, 401−410.
(2) Van't Veer, L.; Weigelt, B. *Nat. Med.* **2003**, *9*, 999−1000.
(3) Steeg, P. S. *Nat. Med.* **2003**, *9*, 822−823.
(4) Nelson, A. R.; Fingleton, B.; Rothenberg, M. L.; Matrisian, L. M. *J. Clin. Oncol.* **2000**, *18*, 1135−1149.
(5) Kerbel, R.; Folkman, J. *Nat. Rev. Cancer* **2002**, *2*, 727−739.
(6) Hanahan, D.; Weinberg, R. A. *Cell* **2000**, *100*, 57−70.
(7) Forget, M.-A.; Desrosier, R. R.; Beliveau, R. *Can. J. Physiol. Pharmacol.* **1999**, *77*, 465−480.
(8) Chambers, A. F.; Groom, A. C.; MacDonald, I. C. *Nat. Rev. Cancer* **2002**, *2*, 563−572.
(9) Lee, M.; Fridman, R.; Mobashery, S. *Chem. Soc. Rev.* **2004**, *33*, 401−409.
(10) Skiles, J. W.; Gonnella, N. C.; Jeng, A. Y. *Curr. Med. Chem.* **2001**, *8*, 425−474.
(11) Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. *Chem. Rev.* **1999**, *99*, 2735−2776.
(12) Overall, C. M.; Lopez-Otin, C. *Nat. Rev. Cancer* **2002**, *2*, 657−672.
(13) Egeblad, M.; Werb, Z. *Nat. Rev. Cancer* **2002**, *2*, 161−174.
(14) Coussens, L. M.; Fingleton, B.; Matrisian, L. M. *Science* **2002**, *295*, 2387−2392.
(15) Brown, S.; Meroueh, S. O.; Fridman, R.; Mobashery, S. *Curr. Top. Med. Chem.* **2004**, *4*, 1227−1238.

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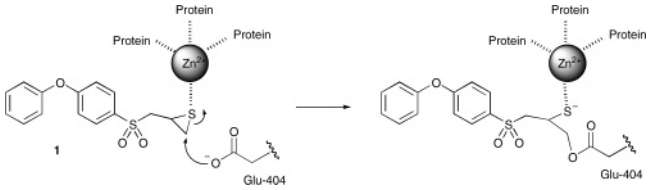
The biphenyl moiety fits in the deep hydrophobic pocket in the active sites of gelatinases, and the inhibitor interacts with the active-site zinc ion through the thiirane moiety. This interaction activates the thiirane for nucleophilic attack by the active site glutamate in these enzymes, resulting in irreversible inhibition (Scheme 1). The mechanism of action of this inhibitor is unique among those reported for MMPs.

Scheme 1



Whereas we have used a racemic mixture of inhibitor **1** in all previous investigations (including animal studies), we have been curious if one or both of the enantiomers would exhibit the biological activity. In the field of enzyme inhibition, it is widely assumed that one enantiomer is active while the other is not. To provide the answer to this question, we report herein the facile syntheses of optically active (*R*)- and (*S*)-enantiomers of compound **1**, starting from commercially available (*R*)- and (*S*)-epichlorohydrin, respectively (Figure 1). We also report on the enzyme inhibitory

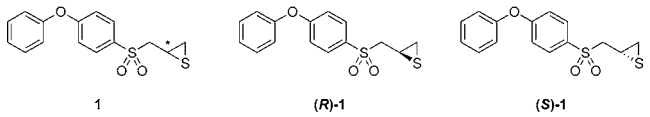
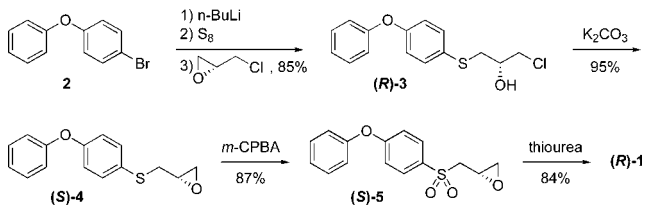


Figure 1. Selective gelatinase inhibitor.

properties of the enantiomers and provide answers on interactions of these molecules within the active site of MMP-2.

Synthesis commences with lithiation of 4-phenoxyphenylbromide (**2**), which was then treated with sulfur to

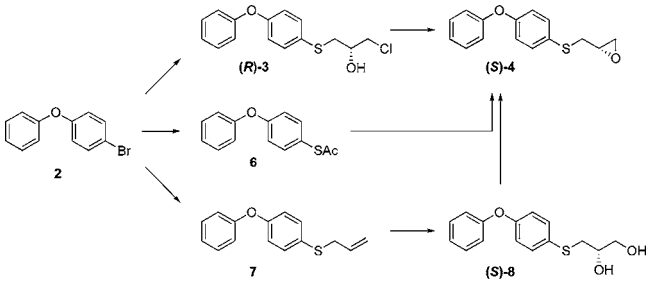
Scheme 2



generate the corresponding thiolate. The thiolate was allowed to react with (*R*)-epichlorohydrin (97% ee) (Scheme 2). Optical purity of the resultant compound ((*R*)-**3**) was 97%, which indicates that the nucleophilic attack of the thiolate occurred exclusively at the C-3 of epichlorohydrin and the stereocenter was not scrambeld during this step. We cannot rule out the possibility of nucleophilic attack at C-1, and the released Cl^- subsequently attacked at C-3 epoxide opening, though C-3 attack is the most likely route. The enantiomeric excess was determined by chiral HPLC (Supporting Information).

We also explored alternative routes to the chiral thiiranes, starting with the same starting material **2** (Scheme 3). After

Scheme 3



the thiolate was generated by the reaction of **2** with *n*-BuLi and sulfur, we allowed it to react with acetyl chloride, yielding the thioacetate **6**. Compound **6** directly gives (*S*)-**4** by treatment with epichlorohydrin. Another approach is by the reaction of the thiolate with allyl bromide to give the allyl sulfide **7**, which could further be elaborated to the corresponding chiral diol, (*S*)-**8**, by the Sharpless dihydroxylation reaction.[19] The resulting diol could be converted to the epoxide under Mitsunobu condition.[20] After careful consideration of the yields, the need for purification, and the issue of enantioselectivity, we opted for the original route outlined in Scheme 2.

The remainder of the synthetic route was similar to that of a published method,[21] which involves the formation of the epoxide ring, sulfide oxidation, and thiirane ring formation. Throughout the synthesis, the stereocenter was intact. The last step, conversion of the oxirane to the thiirane ring, involves inversion of stereochemistry.[22,23] This was performed in the presence of thiourea to give the desired product, which was recrystallized from 1-butanol.

The optical purity was determined by NMR in the presence of the chiral shift reagent europium tris[3-(heptafluoropro-

(16) Brown, S.; Bernardo, M. M.; Li, Z. H.; Kotra, L. P.; Tanaka, Y.; Fridman, R.; Mobashery, S. *J. Am. Chem. Soc.* **2000**, *122*, 6799−6800.
(17) Gu, Z.; Cui, J.; Brown, S.; Fridman, R.; Mobashery, S.; Strongin Alex, Y.; Lipton Stuart, A. *J. Neurosci.* **2005**, *25*, 6401−6408.
(18) Kruger, A.; Arlt, M. J. E.; Gerg, M.; Kopitz, C.; Bernardo, M. M.; Chang, M.; Mobashery, S.; Fridman, R. *Cancer Res.* **2005**, *65*, 3523−3526.
(19) Corey, E. J.; Guzman-Perez, A.; Noe, M. C. *J. Am. Chem. Soc.* **1995**, *117*, 10805−10816.
(20) Takano, S.; Seya, K.; Goto, E.; Hirama, M.; Ogasawara, K. *Synthesis* **1983**, 116−117.
(21) Lim, I. T.; Brown, S.; Mobashery, S. *J. Org. Chem.* **2004**, *69*, 3572−3573.
(22) Sanders, M. *Chem. Rev.* **1966**, *66*, 297−339.
(23) Hauptman, E.; Fagan, P. J.; Marshall, W. *Organometallics* **1999**, *18*, 2061−2073.

pylhydroxymethylene)-(+)-camphorate], $Eu(hfc)_3$. The NMR sample was prepared by adding 5 mg of (*R*)-**1** and 1 mg of $Eu(hfc)_3$ in 0.2 mL of $CDCl_3$. In this condition the other enantiomer ((*S*)-**1**) was not detected in NMR. Thus, we can say that the enantiomeric excess of (*R*)-**1** is >90%.

Synthesis of (*S*)-**1** was performed according to the same procedure, with the sole substitution of (*S*)-epichlorohydrin as the reagent of choice. The optical purity was comparable to the case of the other enantiomer.

The racemic form of inhibitor **1** binds the active site of gelatinases with high selectivity.[16] The compound has a complicated profile for its inhibition of these two enzymes. There is an onset of noncovalent slow-binding inhibition (rapid k_{on}), resulting in a complex that cannot readily reverse itself (slow k_{off}). The ratio of k_{on}/k_{off} furnishes the dissociation constant (K_i) for the inhibitor, a measure of the propensity for the formation of the complex between the inhibitor and the enzyme. The K_i values are in the nanomolar range for gelatinases. The noncovalent complex leads to covalent inhibition of the enzyme. The side-by-side analyses were performed for the racemate, and for the (*R*)-**1** and (*S*)-**1** enantiomers (Table 1). For approach to the kinetic methodologies, consult Brown et al.[16]

Table 1. Kinetic Parameters for Inhibition of Gelatinases by the Synthetic Inhibitors

	$10^{-4}k_{on}$ (M^{-1} s^{-1})	$10^{4}k_{off}$ (s^{-1})	K_i (nM)
MMP-2			
(*R*)-**1**	2.2 ± 0.5	5.3 ± 0.5	24 ± 6
(*S*)-**1**	1.7 ± 0.4	4.0 ± 0.3	23 ± 6
(±)-**1**	2.0 ± 0.5	5.7 ± 0.3	28 ± 7
MMP-9			
(*R*)-**1**	0.22 ± 0.05	11.0 ± 0.2	500 ± 10
(*S*)-**1**	0.28 ± 0.05	8.8 ± 1.4	310 ± 70
(±)-**1**	0.28 ± 0.09	12.4 ± 0.2	400 ± 150

In an unexpected outcome to these analyses, both the (*R*)-**1** and (*S*)-**1** enantiomers were equally active.

While it is unusual for the enantiomers of a racemic mixture to function equivalently as inhibitors, it is not without precedence.[24,25] For example, Ryu et al. reported that both enantiomers of 2-benzyl-3-epoxybutanoic acid having configurations of (2*S*,3*R*) and (2*R*,3*S*) are equally efficient inhibitors of carboxypeptidase A as evidenced by their kinetics and by the X-ray crystal structures of the inhibited enzymes.[24]

The computational study is supportive of the experimental findings that both enantiomers of inhibitor **1** are able to bind to the active site of MMP-2 (Figure 2). In both cases, the requisite thiirane carbon for the nucleophilic attack by Glu-404 is presented properly for the reaction with coordination of the thiirane sulfur to the zinc ion.

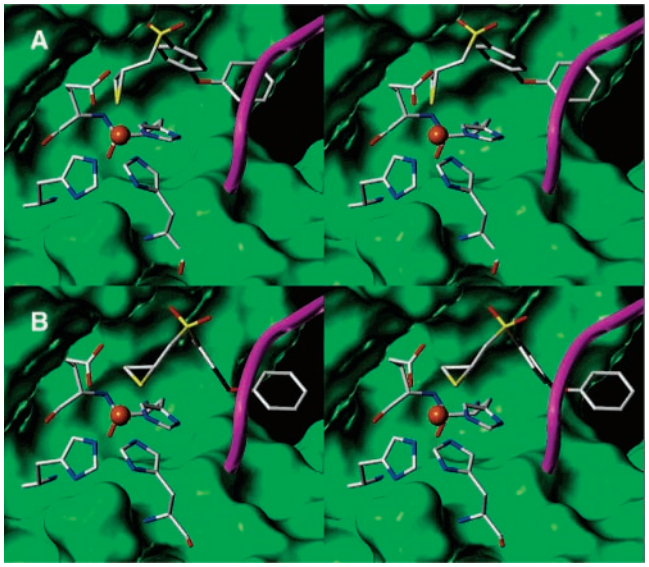


Figure 2. Stereoview of the energy-minimized complexes of (A) (*R*)-**1** and of (B) (*S*)-**1** bound to the active site of MMP-2. A Connolly solvent-accessible surface is constructed in the active site (shown in green), while the loop that constitutes the S1′ subsite of the enzyme is drawn as a purple tube. The compounds, along with the active-site Glu-404 (at 11 o'clock) and the three histidines shown in capped-sticks representation, are 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.

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Supporting Information Available: Experimental procedures and chromatographic and spectroscopic data for compounds **1**, **3**, **4**, and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(24) Ryu, S. E.; Choi, H. J.; Kim, D. H. *J. Am. Chem. Soc.* **1997**, *119*, 38−41.

(25) Lai, M. T.; Liu, H. W. *J. Am. Chem. Soc.* **1990**, *112*, 4034−4035.