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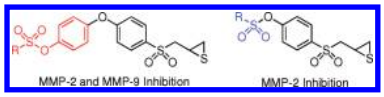
Sulfonate-Containing Thiiranes as Selective Gelatinase Inhibitors

Sebastian A. Testero,† Mijoon Lee,† Rachel T. Staran, Mana Espahbodi, Leticia I. Llarrull, Marta Toth, Shahriar Mobashery,* and Mayland Chang*

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556, United States

ABSTRACT Matrix metalloproteinases (MMPs) are important zinc-dependent endopeptidases. Two members of this family of enzymes called gelatinases (MMP-2 and MMP-9) have been implicated in a number of human diseases, including cancer, neurological and cardiovascular diseases, and inflammation, to name a few. We describe in this report the preparation and evaluation of two structural types of thiirane inhibitors that show selectivity toward gelatinases. The biphenyl series targets both gelatinases, whereas the monophenyl analogues exhibit potent inhibition of only MMP-2. The latter structural type also exhibits improved water solubility and metabolic stability, both traits desirable for progress of these molecules forward in gelatinase-dependent animal models of disease.

KEYWORDS Slow-binding gelatinase inhibitors, thiiranes, matrix metalloproteinases



Matrix metalloproteinases (MMPs), members of a family of 26 closely related zinc-dependent endopeptidases, are involved in important pathological and physiological functions.[1−4] Upregulation of these enzymes has been implicated in cancer progression, neurological disorders, arthritis, and cardiovascular diseases, just to name a few. Because of the important therapeutic potential, MMP inhibitors are highly sought as a means of disease intervention.[5,6] Virtually all known MMP inhibitors were developed to chelate the active-site zinc ions of these enzymes, and as such, they are broad-spectrum inhibitors.[7] This lack of selectivity has been problematic in clinical trials of MMP inhibitors as anticancer agents, as the long-term use of the molecules showed serious side effects.[2,6,8,9]

A general structure for an MMP inhibitor includes a zinc-binding group, typically a zinc chelator. The most explored of such a zinc chelator in inhibitors has been the hydroxamate group.[7] Unfortunately, hydroxamates suffer from many shortcomings, which we will not discuss here in the interest of brevity. Newer generations of inhibitors utilize carboxylates, phosphates and phosphinates, pyrimidines, and thiolates as the entities that interact with the active-site zinc ion.[5,7,10] A few years prior, we disclosed the family of thiirane-based selective gelatinase (MMP-2 and MMP-9) inhibitors, of which compound **1** is the prototypic member (Figure 1).[11] Compound **1** was shown previously to be a potent inhibitor of gelatinases with time-dependent kinetics for the inhibition, a hallmark of either covalent inhibition or slow-binding inhibition. Two mechanisms are possible. The first mechanism is covalent *O*-alkylation of Glu404, and the second is Glu404 catalyzed deprotonation at the methylene adjacent to the sulfone, initiating ring-opening of the thiirane and formation of a stable zinc-thiolate complex. We have demonstrated that the mechanism of inhibition is the second one and that compound **1** is a slow-binding inhibitor.[12] The thiirane moiety in these inhibitors serves as a caged thiolate, which is unleashed in the active site of gelatinases (closely related MMP-2 and MMP-9) to result in potent inhibition of these enzymes.[12] The deprotonation reaction that leads to opening of the thiirane moiety is the rate-limiting step, which leads to slow-binding and, at times, tight-binding inhibition of gelatinases.[13−15]

A major route of metabolism of these compounds is hydroxylation at the *para* position in the terminal phenyl group.[16] A computational exercise in which the terminal phenyl ring was modified led to a focused library of 452 compounds, which were docked and scored for the goodness of their fit in the active site of MMP-2 (also known as gelatinase A).[17] An unexpected finding from this *in silico* docking and scoring exercise was that several of the compounds that scored among the top 50 were sulfonate-containing molecules of the general structure **2**. In the present report, we have synthesized and evaluated a series of sulfonate-containing compounds of the two structural classes **2** and **3** (Figure 1). As will be discussed, both these sets of compounds are exquisite slow-binding inhibitors that exhibit selectivity in targeting gelatinases.

In our continuing efforts to explore the potential for thiirane-based gelatinase inhibitors—both as medicinal compounds and as selective mechanistic tools in elucidation

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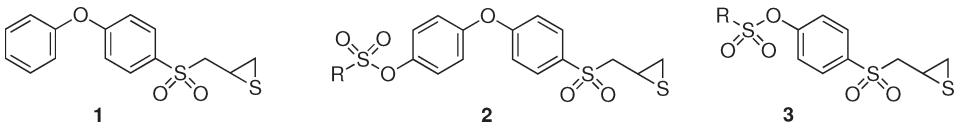
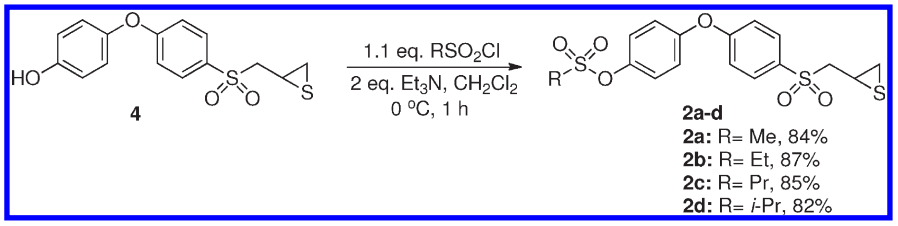
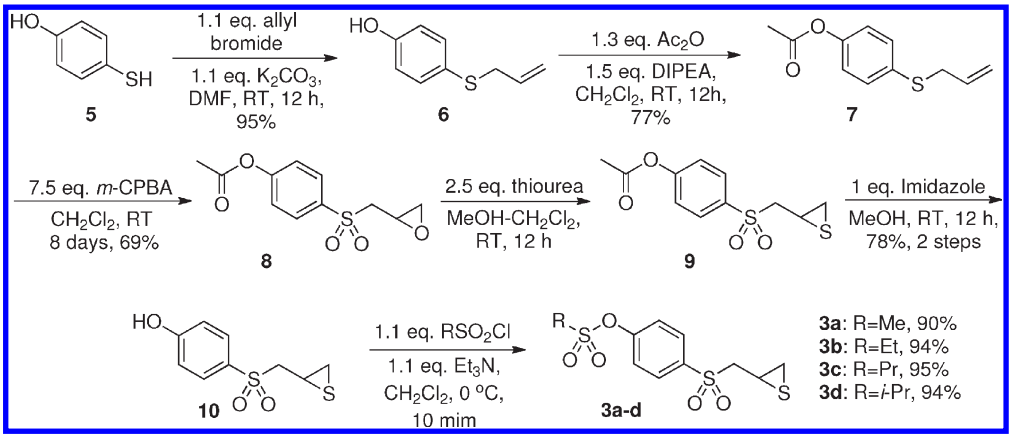


Figure 1

Scheme 1. Syntheses of Sulfonate Derivates of **4**



Scheme 2. Synthetic Route to 4-(Thiiran-2-ylmethylsulfonyl)phenol (**10**) and Syntheses of Sulfonyl Derivatives **3a**−**d**



of the functions of these important enzymes in biological systems—we report herein the syntheses and evaluation of two series of sulfonate-containing thiirane compounds that exert potent and selective inhibition of gelatinases. The compounds in the first series have the structure **2** and were synthesized from the phenolic derivative **4** in one step. The 7-step synthesis of compound **4** was reported earlier in the context of its identification as a major metabolite of compound **1**.[17] The various sulfonates of this type were prepared in 82%−87% yields (Scheme 1). This class of compounds is quite stable, with considerable shelf time. The compounds are easily purified by silica gel chromatography.

In an effort to simplify the syntheses, increase water solubility, and minimize metabolic lability and the structural complexity of these molecules, the next series of compounds eliminated the left-terminal aromatic ring, which gave rise to the structural class **3**. The general synthetic route to these compounds is depicted in Scheme 2. Selective *S*-alkylation of the commercially available 4-hydroxythiophenol **5** with allyl bromide and subsequent acetylation of the phenol provided compound **7** in high yields. Oxidation of the sulfur and the olefin moieties in **7** was achieved by the use of an excess of *m*-CPBA to afford the oxirane **8** in 69% yield. Conversion of the epoxide to the corresponding thiirane was accomplished by treatment with thiourea to obtain the desired product. Partial deacetylation took place at times in this reaction. Regardless, treatment of the resultant mixture of the thiiranes with imidazole in methanol at room temperature gently removed the acetyl group of **9** to afford the phenol **10** in 78% yield for the 2 steps. The final step, conversion of the phenol of **10** to the corresponding sulfonates, proceeds smoothly within 10 min to give derivatives **3a**−**d** in high yields.

The synthetic thiiranes were evaluated by *in vitro* kinetics for their ability to inhibit purified MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, and MMP-14 by the methodology that our laboratories has reported earlier (Table 1).[18] We also investigated the type of inhibition that was observed. As reported earlier in our mechanistic studies of the thiirane-type gelatinase inhibitors, slow-binding behavior is a hallmark of the mechanism of action of this type of inhibitors.[12,19,20] We showed that inhibitor **1** exhibited slow-binding inhibition of gelatinases, which we proposed to be at the root of selectivity.[12] As proposed earlier, gelatinases deprotonate α to the aromatic sulfone moiety, which leads to the opening of the thiirane ring and the release of the thiolate. Hence, the thiirane behaves as a caged thiolate, which becomes available within the active site of gelatinases. Inhibitor **1** either did not inhibit other MMPs or did so very poorly, as merely a

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Table 1. Kinetic Parameters for Inhibition of MMPs by Selected Compounds

inhibitor	enzyme	$k_{on} \times 10^{-3}$ (M^{-1} s^{-1})	$k_{off} \times 10^{3}$ (s^{-1})	K_i (μM)
1[a]	MMP-1			206 ± 60
	MMP-2	1.1 ± 0.1	1.8 ± 0.1	0.0139 ± 0.0004
	MMP-3	0.0018 ± 0.0004	5.5 ± 0.4	15 ± 6
	MMP-7			96 ± 41
	MMP-9	0.14 ± 0.03	7.1 ± 0.5	0.6 ± 0.2
	MMP-14			0.11 ± 0.01
2a[b]	MMP-1			140 ± 7
	MMP-2	45 ± 4	1.0 ± 0.2	0.023 ± 0.005
	MMP-3			0.60 ± 0.03
	MMP-7			18 ± 3
	MMP-9	21 ± 2	0.11 ± 0.10	0.005 ± 0.001
	MMP-14			0.15 ± 0.1
2b	MMP-1			NI (40 μM)
	MMP-2	19 ± 2	1.3 ± 0.2	0.070 ± 0.013
	MMP-3			38% (20 μM)
	MMP-7			8% (40 μM)
	MMP-9	4.6 ± 0.4	1.5 ± 0.2	0.33 ± 0.05
	MMP-14	2.5 ± 0.1	9.0 ± 2.0	0.36 ± 0.81
2c	MMP-1			NI (20 μM)
	MMP-2	38 ± 4	1.3 ± 0.3	0.034 ± 0.008
	MMP-3			22% (20 μM)
	MMP-7			5% (20 μM)
	MMP-9	3.2 ± 0.3	1.6 ± 0.1	0.52 ± 0.06
	MMP-14	7.6 ± 0.6	1.8 ± 0.1	0.24 ± 0.02
2d	MMP-1			NI (30 μM)
	MMP-2	18 ± 2	1.1 ± 0.2	0.061 ± 0.013
	MMP-3			5% (20 μM)
	MMP-7			NI (30 μM)
	MMP-9	5.3 ± 0.5	2.0 ± 0.1	0.38 ± 0.04
	MMP-14	2.5 ± 1.0	2.0 ± 0.8	0.79 ± 0.03
3a	MMP-1	4.2 ± 0.5	1.7 ± 0.2	41 ± 6
	MMP-2	3.3 ± 0.3	1.3 ± 0.2	0.39 ± 0.07
	MMP-3			29% (200 μM)
	MMP-7	0.0045 ± 0.0006	0.50 ± 0.03	11 ± 2
	MMP-9	0.26 ± 0.06	0.25 ± 0.6	3.9 ± 1.7
	MMP-14	0.42 ± 0.05	0.20 ± 0.03	0.48 ± 0.09
3b	MMP-1			7% (200 μM)
	MMP-2	4.3 ± 0.5	0.40 ± 0.01	0.09 ± 0.01
	MMP-3			62% (200 μM)
	MMP-7	0.0023 ± 0.0002	0.6 ± 0.1	26 ± 5
	MMP-9	0.15 ± 0.01	1.8 ± 0.2	12 ± 2
	MMP-14	0.15 ± 0.03	1.6 ± 0.4	11 ± 3
3c	MMP-1			30% (200 μM)
	MMP-2	2.9 ± 0.3	0.8 ± 0.3	0.28 ± 0.11
	MMP-3			58% (200 μM)
	MMP-7	0.0023 ± 0.0003	0.8 ± 0.2	35 ± 10
	MMP-9	0.15 ± 0.01	0.9 ± 0.2	6.2 ± 1.5
	MMP-14	0.047 ± 0.005	1.5 ± 0.1	32 ± 4
3d	MMP-1			NI (200 μM)
	MMP-2	4.5 ± 0.5	1.1 ± 0.2	0.24 ± 0.05
	MMP-3			25% (200 μM)

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Table 1. Continued

inhibitor	enzyme	$k_{on} \times 10^{-3}$ (M^{-1} s^{-1})	$k_{off} \times 10^{3}$ (s^{-1})	K_i (μM)
	MMP-7	0.011 ± 0.001	1.4 ± 0.6	120 ± 11
	MMP-9	0.54 ± 0.08	1.9 ± 0.1	3.5 ± 0.6
	MMP-14	0.086 ± 0.001	1.7 ± 0.4	20 ± 4

[a] Reported in refs 11 and 22. [b] Reported in ref 16.

linear-competitive inhibitor. This behavioral pattern is repeated for the inhibitors reported herein, but the profile of the inhibition for these new compounds appears to be somewhat different, as will be described below.

We looked for the presence or absence of time dependence for inhibition of MMPs. The former is an evidence of slow-binding behavior, and the latter its absence. Slow-binding behavior is defined by a rapid onset of inhibition, described by a second-order rate constant k_{on}, which is large and favorable for the cases listed in Table 1. The rapid onset of inhibition is complemented by a small first-order rate constant for dissociation of the enzyme−inhibitor complex, k_{off}. The ratio of k_{off}/k_{on} describes the dissociation constant K_i for the inhibitor for the given enzyme. We note that all of these compounds were slow-binding inhibitors of gelatinases (MMP-2 and MMP-9), with most exhibiting dissociation constants (K_i) within the nanomolar range. This general statement is true for the biphenyl ether variants of the general structure **2**, as it is for the truncated compounds of structure **3**. Some of these compounds exhibited more expanded profiles for slow-binding inhibition of MMPs. For example, for the first time for the thiirane class of inhibitors, compounds **3a**−**d** served as slow-binding inhibitors of MMP-14 and MMP-7 as well. Yet, this comment has to be placed in context. Notwithstanding the observation of slow-binding behavior, dissociation constants resulting for this pattern of inhibition for **3a** with MMP-7 and MMP-9 and for **3b**, **c**, and **d** with MMP-7, MMP-9, and MMP-14 are high in the micromolar range. Hence, the series of compounds **3** are indeed exhibiting selectivity with interesting profiles. Compound **3a** is a nanomolar inhibitor only for MMP-2 and MMP-14; compounds **3b**, **c**, and **d** are nanomolar inhibitors exclusively for MMP-2. Compounds of series **3** are indeed selective in different ways than those of series **2**. Compounds **3b**, **c**, and **d** can be construed as selective inhibitors of gelatinase A (MMP-2), which with an appropriate level of dosing in animals could limit the inhibition exclusively to MMP-2 *in vivo*.

The thiirane inhibitors were also evaluated for *in vitro* metabolic stability using rat liver microsomes (Table 2). In the biphenyl series, methyl (**2a**) and ethyl (**2b**) had improved metabolic stability over the propyl (**2c**) and isopropyl (**2d**) variants. In general, the monophenyl compounds **3** were more metabolically stable than the corresponding biphenyls **2**. From the *in vitro* half-life, intrinsic clearance (the ability of the liver to metabolize a compound) was calculated.[21] In general, the biphenyl series had higher intrinsic clearance than the monophenyl counterparts. All of the biphenyl inhibitors had intrinsic clearances > 70 % hepatic blood flow (the rate of blood flow through the liver; drugs with high hepatic clearance depend on hepatic blood flow for elimination) of

Table 2. Metabolic Stability, Intrinsic Clearance, and Aqueous Solubility of MMP Inhibitors

inhibitor	half-life (min)	intrinsic clearance [L/(h kg)]	solubility (μg/mL)
1	12.0	7.02	2.3 ± 0.1
2a	24.8	3.39	3.5 ± 0.2
2b	35.9	2.34	4.1 ± 0.3
2c	12.6	6.67	4.0 ± 0.1
2d	16.2	5.19	1.1 ± 0.05
3a	45.3	1.86	167 ± 6
3b	31.8	2.65	114 ± 9
3c	29.8	2.83	96 ± 14
3d	36.1	2.33	230 ± 14

3.96 L/(h kg), indicating that these were high clearance compounds. Inhibitor **3a** had the lowest intrinsic clearance of 1.86 L/(h kg), which was < 50 % of hepatic blood flow.

Aqueous solubility was also measured for the series of thiirane inhibitors (Table 2). Aqueous solubility is a significant contributor to the absorption, distribution, metabolism, and elimination of a drug in the body and, thus, is an important parameter for the selection of compounds with druglike properties. In general, elimination of the terminal aromatic ring increased solubility > 70-fold, except for a 26-fold increase for compound **3b** (ethyl) versus the corresponding biphenyl (**2b**). The increased metabolic stability, moderate intrinsic clearance, and improved water solubility for the monophenyl thiirane series makes these compounds superior drug candidates.

The class of thiirane inhibitors as selective gelatinase inhibitors holds considerable promise as potential therapeutics in treatment of the aforementioned gelatinase-dependent diseases. The prototype compound **1** and the structural variants **2** inhibit both gelatinases effectively. Notwithstanding the broadening of the slow-binding profile of compound **3** to include MMP-7 and MMP-14, the kinetic parameters for these enzyme inhibitions are such that actually only MMP-2 is inhibited potently. This consideration in conjunction with the desirable traits of improved aqueous solubility, better metabolic stability, moderate clearance, and a more simplified chemical structure would make inhibitors of type **3** worthy compounds for investigations of their effects in animal models of disease.

SUPPORTING INFORMATION AVAILABLE General information, experimental procedures, spectroscopic data, and ^{1}H NMR and ^{13}C NMR spectra of new compounds, and general biological assay procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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AUTHOR INFORMATION

Corresponding Author: *M.C.: phone, (574) 631-2965; fax, (574) 631-6652; e-mail, mchang@nd.edu. S.M.: phone, (574) 631-2933; fax, (574)631-6652; e-mail, mobashery@nd.edu.

Author Contributions: † Authors contributed equally to this work.

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