Potent and Selective Mechanism-Based Inhibition of Gelatinases

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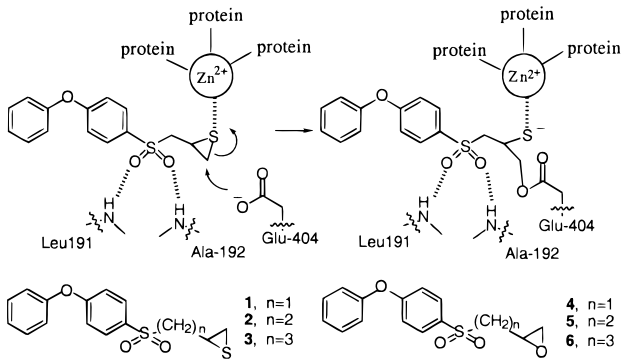
Specific interactions of cells within the extracellular matrix are critical for the normal function of the organism. Alterations of the extracellular matrix are carried out by a family of zinc-dependent endopeptidases called matrix metalloproteinases (MMPs) in various cellular processes such as organ development, ovulation, fetus implantation in the uterus, embriogenesis, wound healing, and angiogenesis.[1,2] Gelatinases, collagenases, stromelysins, membrane-type MMPs, and matrilysin comprise the five major groups of MMPs, of which at least 26 members have been identified in humans to date. The activities of MMPs in physiological conditions are strictly regulated by a series of complicated zymogen activation processes and inhibition by the protein tissue inhibitors of metalloprotainases ("TIMPs").[2,3] Excessive MMP activity has been implicated in cancer growth, tumor metastasis and angiogenesis, arthritis, connective tissue diseases, inflammation, and cardiovascular and autoimmune diseases.[1,2,4] Due to the potential therapeutic value of MMP inhibitors for these conditions, synthetic inhibitors of MMPs are highly sought.[5,6] All the known inhibitors for MMPs take advantage of chelation to the active site zinc ion for inhibition of activity. The known MMP inhibitors usually suffer from toxicity to hosts.[5a,c,7] Besides the issue of undesirable side effects, the design of MMP inhibitors has been complicated by only low levels of specificity among members of the MMP family, which hampers our ability to target specific MMPs in each pathological condition. We describe herein the first mechanism-based inhibitor for MMPs, a novel concept for the selective inhibition of these enzymes. We show that our inhibitor rivals the action of TIMPs in its efficacy in inhibition of MMPs.

Increased level of activity for human gelatinases, MMP-2 and MMP-9, has been implicated in the process of tumor metastasis and angiogenesis.[8] As a result, we have been interested in the selective inhibition of these two key MMPs. For this purpose, we have resorted to the design of mechanism-based inhibitors (also known as "k_{cat} inhibitors" or "suicide substrates"). This type of inhibitor has the potential to impart high selectivity in inhibition of closely related enzymes, such as MMPs.[9] Our strategy for mechanism-based inhibition of MMPs by compound **1** is depicted in Scheme 1. The strategy envisions that coordination of the thiirane with the active-site zinc ion would activate it for modification by a nucleophile in the enzyme active site. The biphenyl moiety in compounds **1**−**6** would fit in the $P_{1'}$ subsite of gelatinases, which is a deep hydrophobic pocket.[10,11] Energy-minimized complexes of MMP-2 and MMP-9[12] with compound **1** indicated that the biphenyl group would fit in the active site analogously to the same group in reversible inhibitors of MMP-2 and MMP-9.[6] This binding mode would bring the sulfur of the thiirane in **1** into the coordination sphere of the zinc ion. The models indicated that the thiirane moiety in compounds **2** and **3**, with longer carbon backbones, would not be able to coordinate with the zinc ion, but would fit in an extended conformation in the active site.

Scheme 1



Scheme 2 shows the synthetic route for compounds **1**−**6**. 4-Phenoxythiophenol **10** was prepared from the commercially available 4-phenoxyphenol **7** via a three-step procedure described by Newman and Karnes for a related system.[13] Subsequent alkylation of **10** with allyl bromide, 4-bromo-1-butene, and 5-bromo-1-pentene, respectively, led to the sulfanyl compounds **11**−**13** in good yields. Epoxidation of **12** and **13** with mCPBA proceeded in 2−3 days, but that for **11** took 7 days and required an excess of mCPBA. Finally, conversion of the epoxides **4**−**6** to their corresponding thiirane derivatives **1**−**3**, respectively, was accomplished by the treatment of each epoxide with ammonium thiocyanate. Although the thiiranes **2** and **3** were isolated in high yields (93 and 85%, respectively), thiirane **1** could only be recovered in a poor 14% yield.

Compounds **1**−**6** were evaluated with MMPs.[14] Whereas inhibitors **2**−**6** showed either no inhibition or relatively poor

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Scheme 2

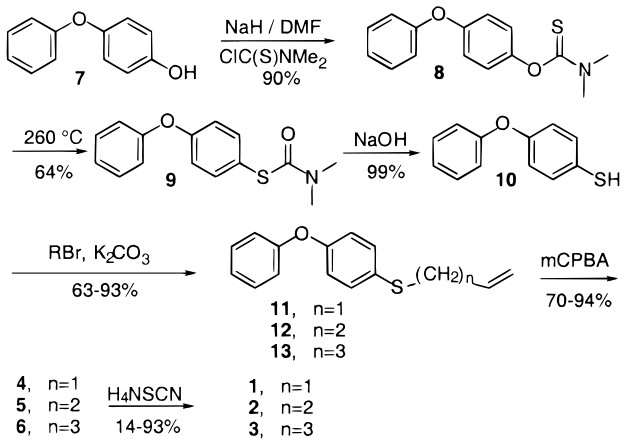

Table 1. Kinetic Parameters for Inhibition of MMPs by the Synthetic Inhibitor

	$10^{-4}k_{on}$ ($M^{-1}s^{-1}$)	$10^{3}k_{off}$ (s^{-1})	K_i (μM)
Inhibitor 1			
MMP-2	11 ± 1	1.8 ± 0.1	0.0139 ± 0.0004
MMP-9	1.4 ± 0.3	7.1 ± 0.5	0.6 ± 0.2
MMP-3	0.018 ± 0.004	5.5 ± 0.4	15 ± 6
MMP-7			96 ± 41
MMP-1			206 ± 60
TIMP-1[18]			
MMP-2	4.4 ± 0.1	1.3 ± 0.2	0.029 ± 0.005
MMP-9	5.2 ± 0.1	1.2 ± 0.2	0.024 ± 0.004
TIMP-2[18]			
MMP-2	3.3 ± 0.1	0.8 ± 0.1	0.023 ± 0.004
MMP-9	2.2 ± 0.1	1.3 ± 0.2	0.058 ± 0.007

inhibition of the MMPs (K_i values of micromolar at best; see Supporting Information), the behavior of inhibitor **1** was different. Inhibitor **1** showed a dual behavior. It served as a mechanism-based inhibitor with a partition ratio of 79 ± 10 (i.e., k_{cat}/k_{inact}) for MMP-2 and of 416 ± 63 for MMP-9.[15] Furthermore, it also behaved as a slow-binding inhibitor, for which the rate constants for the on-set of inhibition (k_{on}) and recovery of activity from inhibition (k_{off}) were evaluated (Table 1). It would appear that coordination of the thiirane with the zinc ion (as seen in the energy-minimized computational models; Scheme 1) would set in motion a conformational change, which is presumed from the slow-binding kinetic behavior. The kinetic data fit the model for slow-binding inhibition.[16] Covalent modification of the enzymes ensued this conformational change. We incubated inhibitor **1** with MMP-2 to the point that less than 5% activity remained. This inhibitor−enzyme complex was dialyzed over 3 days, which resulted in recovery of approximately 50% of the activity. This observation is consistent with modification of the active site Glu-404, via the formation of an ester bond, which is a relatively labile covalent linkage.[17]

We observe selectivity in inhibition of gelatinases by inhibitor **1**. The K_i values are 13.9 ± 0.4 and 600 ± 200 nM for MMP-2 and MMP-9, respectively. In contrast, the corresponding K_i values for the other MMPs tested, including MMP-3, which does show the slow-binding mechanism-based inhibition profile, are in the micromolar range. Interestingly, the values for k_{on} are 611- and 78-fold larger for MMP-2 and MMP-9, respectively, than that for MMP-3. Collectively, these kinetic parameters make inhibitor **1** a potent and selective inhibitor for both MMP-2 and MMP-9, more so for MMP-2. We have determined previously that two molecules of either TIMP-1 or TIMP-2 bind to activated MMP-2 and MMP-9.[18] One binding event is high affinity and would appear physiologically relevant, whereas the second binding event takes place with relatively lower affinity (micromolar).[18] Inhibition of MMP-2 and MMP-9 by TIMP-2 and TIMP-1, respectively, also follows slow-binding kinetics. The kinetic parameters for these interactions at the high affinity site are listed in Table 1. We find it noteworthy that the kinetic parameters for the slow-binding component of inhibition of MMP-2 and MMP-9 by inhibitor **1** (k_{on} and k_{off}) approach closely the same parameters for those of the TIMPs.[18]

We have outlined in this paper a novel example for potent inhibition of human gelatinases by the small-molecule inhibitor **1**, which follows both slow-binding and mechanism-based inhibition in its kinetic profile. This compound appears to behave similarly to TIMP-2 and TIMP-1 in the slow-binding component of inhibition. Furthermore, the inhibitor also exhibits a covalent mechanism-based behavior in inhibition of these enzymes. The selectivity that inhibitor **1** displays (in both affinities and the modes of inhibition) among the other structurally similar MMPs is noteworthy and should serve as a paradigm in the design of inhibitors for other closely related enzymes in the future.

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Supporting Information Available: Detailed procedures for syntheses and kinetic determinations are provided (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) Homogeneous preparations of MMPs were used in our studies. Recombinant human MMP-2 and MMP-9 were prepared as described previously (see Supporting Information). Representative members of the other classes of MMPs, such as stromelysin 1 (MMP-3), matrilysin (MMP-7), and collagenase-1 (MMP-1), were used in our studies.

(15) The partition ratio indicates that there is turnover of the thiirane for each covalent inhibition of the enzyme. The partition ratios were relatively low, such that given the quantities of the enzymes available to us, we were not able to isolate and characterize the product of this turnover.

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(17) The time-dependent loss of activity is not merely due to the slow-binding behavior. For instance, for a k_{off} of 2×10^{-3} s^{-1} (the values are not very different from one another in Table 1) the half-time for recovery of activity ($t_{1/2}$) is calculated at just under 6 min. The fact that 50% of activity still did not recover after dialysis over 3 days strongly argues for the covalency of enzyme modification.

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