Communication

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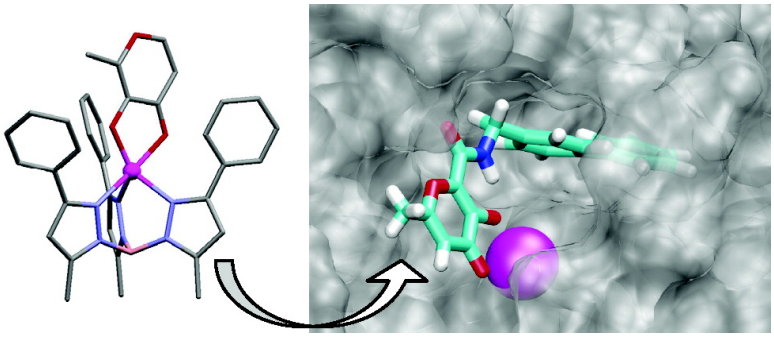

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Potent, Selective Pyrone-Based Inhibitors of Stromelysin-1

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This communication describes the design and synthesis of pyrone-based inhibitors of matrix metalloproteinases (MMPs) that show superior potency over their hydroxamate analogues. The zinc(II)-dependent MMPs have been pursued as chemotherapeutic targets for the treatment of illnesses, such as cancer, arthritis, and heart disease. Consequently, over the past two decades, attempts to interfere with MMP activity have yielded numerous inhibitors.[1] MMP inhibitors (MPIs) are based on a two-part strategy: chelation of the catalytic zinc(II) ion combined with noncovalent interactions within "subsite" pockets in the MMP active site.[1,2]

The majority of MPIs synthesized to date contain a hydroxamic acid as the chelating or zinc-binding group (ZBG).[1,2] Despite recent improvements, hydroxamate-based MPIs have not yet succeeded in clinical trials.[3] This has prompted the investigation of a limited number of non-hydroxamate-based MPIs.[4−6] Herein, we describe inhibitors that utilize a pyrone ZBG, which results in improved potency and novel selectivity relative to similar hydroxamate-based MPIs.[4]

Pyrones were selected for this study due to their synthetic versatility,[7] known biocompatibility,[8] and good aqueous solubility. An earlier study examining the use of maltol (3-hydroxy-2-methyl-4-pyrone) as a ZBG indicated that the 2-methyl substituent was favorably oriented toward the hydrophobic S1′ pocket of stromelysin-1 (MMP-3).[9,10] Several studies show that targeting the S1′ pocket of MMPs yields potent and selective MPIs.[1,2] Therefore, we sought to attach simple aryl groups to the 2-position of maltol in order to exploit this interaction.

To design pyrone-based inhibitors, we used the drug discovery program LUDI (Accelrys) augmented with parameters from a bioinorganic model complex. LUDI uses a constrained docking approach that identifies optimal fragments to link to the pyrone moiety at a specified point of attachment. Structural data of maltol bound to a tris(pyrazolyl)borate model complex[9] were integrated into a known MMP crystal structure to generate the initial receptor complex (Figure S1).[11] The point of attachment to the ZBG was defined as a N−H bond from an amide moiety on the 2-position of the maltol ring (the amide group was built in silico on the ZBG). Fragments were screened and ranked using a LUDI scoring function.[12] The results from an initial screen with MMP-3 using the LUDI link library yielded modest scores for several compounds. Consequently, we created a custom library primarily based on the work of Hadjuk et al.,[13] which generated three high, one moderate, and two low scoring fragments. The result of the LUDI docking for one of the high scoring compounds (**AM-5**, vide infra) is shown in Figure 1. The fragment in Figure 1 was found to reside in the S1′ pocket of MMP-3. The high and low scoring fragments from the custom library were similar in structure; therefore, all six compounds were synthesized to test the accuracy of the LUDI docking and scoring function.

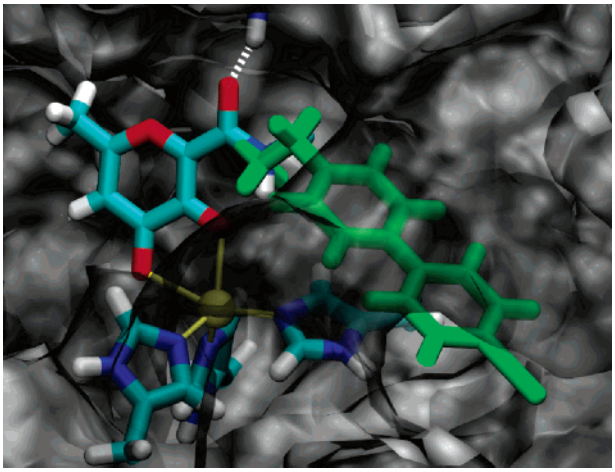
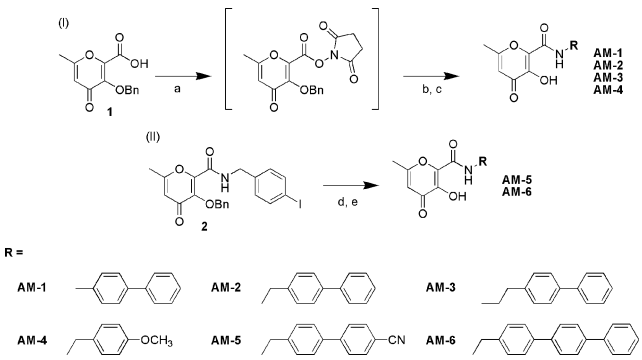


Figure 1. LUDI docking image of backbone fragment (green, in S1′ subsite) with pyrone ZBG (colored by element) in the active site of MMP-3 (gray). This fragment combination leads to the compound designated **AM-5** (see Scheme 1). The zinc(II) ion is shown as a gold sphere; a hydrogen bond between the ZBG and L164 is shown as a dashed line.

Scheme 1 [a]



[a] Key: (I) (a) NHS, DCC, dry THF; (b) "amine", dry THF, 88%; (c) 10% Pd/C, H_2 35 psi, MeOH or 1:1 HCl:CH_3COOH, 60−89%. (II) (d) $ArB(OH)_2$, 2 M K_2CO_3, $Pd(C_2H_3O_2)_2$, PPh_3, toluene, 135 °C, 40−85%; (e) 10% Pd/C, H_2 35 psi, MeOH or 1:1 HCl:CH_3COOH, 60−91%.

Synthesis of the pyrone-based MPIs was performed according to Scheme 1. Two synthetic routes were utilized, based on the commercial availability of the desired amine backbones. 2-Carboxy-3-benzyloxy-6-methylpyran-4(1*H*)-one (**1**) was prepared by a literature method.[7] Compound **1** was then activated with NHS, followed by coupling to the desired amine, and removal of the benzyl protecting group to yield compounds **AM-1**, **AM-2**, **AM-3**, and **AM-4**. The synthesis of **AM-5** and **AM-6** was accomplished similarly, but required the Suzuki coupling of 3-benzyloxy-6-methylpyran-4(1*H*)-one-2-carboxy-*N*-(4-iodobenzylamide) (**2**) with

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Table 1. IC_{50} Values (μM) for MPIs Against MMP-1, MMP-2, and MMP-3: LUDI Scores for MMP-3 (PDB code 1G4K) are Shown

inhibitor	MMP-1	MMP-2	MMP-3	LUDI score
AM-1	>50	36(5)	>50	NS[a]
AM-2	>50	9.3(0.5)	0.24(0.01)	600
AM-3	>50	27(2)	36(1)	NS[a]
AM-4	>50	>50	2.4(0.2)	440
AM-5	>50	0.61(0.01)	0.010(0.002)	640
AM-6	>50	>50	0.019(0.002)	700

[a] NS = no score; no acceptable conformations were found.

4-cyanophenylboronic acid and 4-biphenylboronic acid, respectively, as an intermediate step.

The inhibitory activity of compounds **AM-1** through **AM-6** was evaluated using a fluorescence-based assay;[14] the IC_{50} values are listed in Table 1. **AM-2**, **AM-5**, and **AM-6** were the most potent compounds against MMP-3, with IC_{50} values in the nanomolar range. The IC_{50} values against MMP-3 correlate well with the scores obtained for each fragment using the program LUDI. Although the LUDI scores do not perfectly parallel the relative inhibitory activity, the approach used here does clearly distinguish between poor, moderate, and exceptional MPIs.

Interestingly, the pyrone-based MPIs presented here are more potent than the analogous hydroxamate-based inhibitors,[13] which is contrary to the accepted dogma that hydroxamic acids are the best ZBGs.[15] As expected, the effects of linker length (compare **AM-1**, **AM-2**, and **AM-3**) and backbone substituents (**AM-5** relative to **AM-2**) are consistent with analogous hydroxamate-based MPIs.[13] These results strongly support the concept that ZBGs equal or superior to hydroxamates can be identified and utilized in novel MPI designs.[6,16]

The observed trends in the IC_{50} values of the MPIs described here against MMP-3 suggest that the large aromatic backbone substituents of these compounds occupy the S1′ subsite. This hypothesis was further examined by determining the selectivity of these compounds against different MMPs. Traditionally, the incorporation of bulky groups directed toward the S1′ pocket results in selectivity over MMP-1, which has a shallow S1′ pocket.[1] All six MPIs were found to be poor inhibitors of MMP-1 (Table 1). The poor activity of these compounds against MMP-1 is wholly consistent with the aryl backbone groups occupying the S1′ pocket, which supports the LUDI results (Figure 1) and ZBG orientation predicted by our bioinorganic modeling studies.[9]

The inhibitors were also tested for potency against MMP-2. Like MMP-3, MMP-2 has a deep S1′ pocket, and potency against these two enzymes is expected to be comparable, as found with hydroxamate-based MPIs.[1,2] Interestingly, although **AM-2**, **AM-4**, **AM-5**, and **AM-6** showed a range of potencies against MMP-3, all four compounds were substantially less potent against MMP-2. Indeed, **AM-6** showed >2500-fold selectivity for MMP-3, which, to the best of our knowledge, is the highest selectivity reported for an MPI for MMP-3 over MMP-2.

The observed selectivity of these compounds for MMP-3 over MMP-2 is in contrast to the selectivity observed for most deep S1′ pocket MPIs. Hydroxamate-based MPIs that occupy the S1′ pocket are almost exclusively more potent for MMP-2 than for MMP-3, with few exceptions.[1,2,17] MPIs reported to be selective for MMP-3 over MMP-2 generally target the S3′ subsite;[17] however, on the basis of the LUDI docking, the MPIs presented here have no significant interactions in the S3′ subsite and, indeed, give similar LUDI scores when docked to MMP-2 or MMP-3 (Table S1, Figure S2). Therefore, it is plausible that the observed selectivity originates from the pyrone ZBG. It has been reported that more acidic ZBGs, such as carboxylates (a weaker ZBG than the hydroxamate),[15] are generally more potent for MMP-3 than for MMP-2,[4,17] which is attributed to the difference in the optimal pH for the two enzymes. MMP-3 prefers a more acidic environment (pH ~6.0) compared with other MMPs (including MMP-2), which favor a higher pH (~7.5).[18] By analogy, we propose that the selectivity of the MPIs reported here is due to the greater acidity of the pyrone versus hydroxamate chelator ($\Delta pK_a \sim 1$).[19] These results suggest that the ZBG, and not only the MPI backbone, can provide selectivity between different MMPs without compromising potency. The investigation of additional MPIs, with a range of pK_a's against a wider range of MMPs is ongoing, to interrogate the aforementioned hypothesis more rigorously.

The ability of **AM-5** and **AM-6** to inhibit invasion of neonatal rat cardiac fibroblasts through a collagen membrane was examined, as a gauge of the in vivo potential of these MPIs. At a concentration of 250 nM, the two inhibitors were found to reduce invasion by 67% (**AM-5**) and 55% (**AM-6**) (Figure S3). In summary, we have demonstrated that the use of pyrone ZBGs results in more potent inhibitors than those produced with the widely employed hydroxamate group. Our results also indicate that the use of a non-hydroxamate ZBG reveals a novel route to MMP inhibitor selectivity. Overall, the findings reported here suggest that a chelator-driven approach to metalloprotein drug design can produce potent and selective metalloprotein inhibitors.

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Supporting Information Available: Complete refs 5 and 13, Figures S1−S3, Table S1, and experimental details for syntheses, assays, and computational work. This material is available free of charge via the Internet at http://pubs.acs.org.

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