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Potent pyrimidinetrione-based inhibitors of MMP-13 with enhanced selectivity over MMP-14

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Abstract—Through the use of computational modeling, a series of pyrimidinetrione-based inhibitors of MMP-13 was designed based on a lead inhibitor identified through file screening. Incorporation of a biaryl ether moiety at the C-5 position of the pyrimidinetrione ring resulted in a dramatic enhancement of MMP-13 potency. Protein crystallography revealed that this moiety binds in the S_1' pocket of the enzyme. Optimization of the C-4 substituent of the terminal aromatic ring led to incorporation of selectivity versus MMP-14 (MT-1 MMP). Structure activity relationships of the biaryl ether substituent are presented as is pharmacokinetic data for a compound that meets our in vitro potency and selectivity goals.

Owing to their likely role in the pathology of various diseases (e.g., osteoarthritis and cancer) therapeutically useful MMP inhibitors have been sought for many years.[1,2] A number of compounds have been advanced into clinical trials. However, these relatively nonselective MMP inhibitors have exhibited musculoskeletal side effects (MSS) characterized by joint stiffness and pain, particularly in the hands and shoulders.[3] In rodents, many of these compounds induce joint fibroplasia and accumulation of type-I collagen.[4] An hypothesis that these effects are caused by inhibition of MMP-1 has recently been discounted through the clinical study of an MMP-13 inhibitor that spares MMP-1.[1] Thus, the pharmacological basis for this side effect remains unknown, but still presumably involves the indiscriminate inhibition of MMPs other than MMP-1. In an effort to determine the role of the various MMPs in disease and to help identify, which MMP(s) is responsible for MSS, a number of murine MMP knock-outs has been produced. Of these, the MMP-14 (MT-1 MMP) knock-out displays a phenotype reminiscent of the histopathology produced in rats by nonselective inhibitors.[5] This observation, combined with the evidence that MMP-13 plays a critical role in the pathology of osteoarthritis (OA),[6] suggests that an MMP-13 inhibitor that spares MMP-14 may reduce articular cartilage degradation while avoiding the MSS side effect, thereby providing an effective therapy for OA.

A component of our program to discover therapeutically useful MMP-13 inhibitors involved identifying compounds that possess a zinc binding group other than the ubiquitous, and generally metabolically labile, hydroxamic acid. File screening revealed pyrimidinetrione **1** as a weak MMP-13 inhibitor (Fig. 1). The utility of pyrimidinetriones for the inhibition of MMPs has also been reported by the Boehringer Mannheim/Hoffman La Roche group,[7] and, very recently, by Bristol Meyers Squibb.[8] Molecular modeling suggested that the pendant aryl ether of **1** would occupy the S_1' pocket, while the methyl group would project into a largely solvent exposed region. If this were so, then replacing the 4-chloro substituent of **1** with an aryloxyaryl ether, to take

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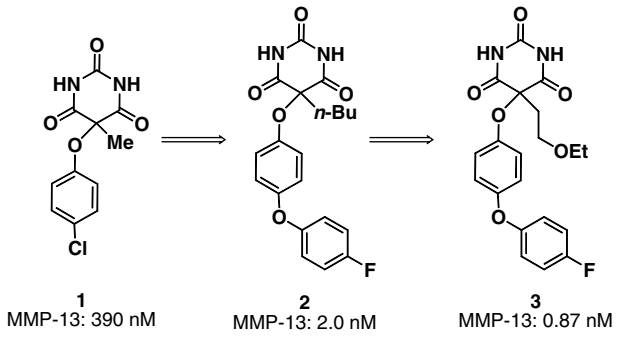


Figure 1. File lead and its initial development.

advantage of the deep S_1' pocket of MMP-13,[9] was expected to improve MMP-13 inhibition potency. This change and concomitant extension of the C-5 substituent to a butyl residue (**2**) did lead to substantial improvement in potency. The overall lipophilicity could be reduced by replacement of the butyl group with an ethoxyethyl group (**3**) (*c*log *P* **2**: 5.05, **3**: 3.11). The X-ray crystal structure of **3** bound to MMP-13 (Fig. 2)[10] confirmed the modeling predictions with the aryloxyaryl ether residing in the S_1' pocket. Other observations include the binding of the pyrimidinetrione to the active site zinc in an enolic form and the apparent displacement of an ordered water by the ether oxygen in the C-5 side chain. The latter could account for the modest increase in potency of **3** over **2**. Unfortunately, none of the above analogs possessed high selectivity (>100-fold) versus MMP-14. We did not anticipate that altering the solvent exposed C-5 side chain or the pyrimidinetrione core would lead to any improvement in selectivity. Thus, our efforts focused on exploring the effect that substitution of the biaryl ether has on MMP-13 potency and selectivity. Such analogs of **3** were prepared using the route outlined in the Scheme 1.

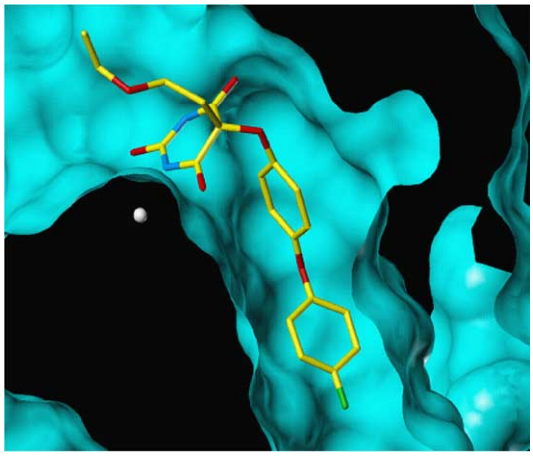
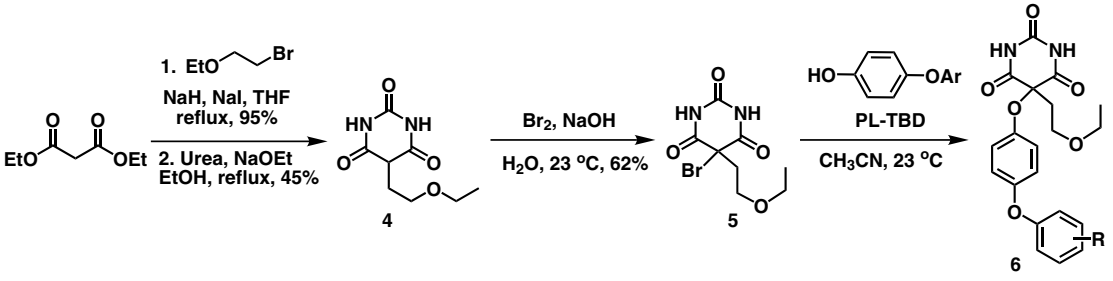


Figure 2. X-ray crystal structure of **3** bound to MMP-13.

Alkylation of diethylmalonate with ethyl 2-bromoethyl ether followed by reaction with urea afforded the monosubstituted pyrimidinetrione **4**. Subsequent bromination of **4** gave bromopyrimidinetrione **5**. Analogs of **3** could be rapidly prepared by treatment of **5** with the appropriate phenol and the polymer bound base PL-TBD[11] in acetonitrile. Aqueous acid workup and silica gel chromatography gave good to excellent yields of the target pyrimidinetriones **6**.

The biological activity of our initial set of substituted biaryl ether derivatives containing C-4, C-3, and C-2 fluoro, chloro, or methyl substituents on the terminal aryl ring (compounds **3** and **7**–**14**, Table 1) demonstrated that C-4 was a promising position for exploration. Thus, the C-4 substituted compounds, **3**, **9**, and **12**, are potent inhibitors and display some selectivity. In contrast, the C-3 analogs, **7**, **10**, and **13**, display only moderate MMP-13 potency and little selectivity. While the C-2 chloro analog **11** is only a weak inhibitor, the C-2 fluoro and methyl analogs, **8** and **14**, display reasonably potent MMP-13 inhibition with the latter also being moderately selective. Nevertheless, the X-ray of **3** co-crystallized with MMP-13 indicated that the opportunity to make significant modifications in this region of the molecule was likely to be limited. We therefore focused our efforts on examining substituents at C-4. The presence of a hydrophobic group at C-4 appears to be beneficial—the halide and methyl analogs (**3**, **9**, **12**, and **15**) are all quite potent MMP-13 inhibitors. There does, however, appear to be a steric limit to the size of the C-4 substituent indicating that the S_1' pocket is deep but narrow. Thus, the trifluoromethyl analog **16** is slightly less potent than the halides and the *tert*-butyl derivative **17** is substantially less so. In contrast, the phenyl derivative **18**, having a large but flat hydrophobic group at C-4, is still very potent. Consistent with this region of the S_1' pocket being hydrophobic, incorporation of hydrophilic groups leads to decreases in MMP-13 potency (compounds **19**–**25**). The compound containing the hydrophilic carboxy (**25**) group loses the most potency.



Scheme 1. Synthesis of pyrimidinetrione analogs **6** from diethylmalonate.

Table 1. MMP-13 and MMP-14 inhibitory activity of analogs **6**[a]

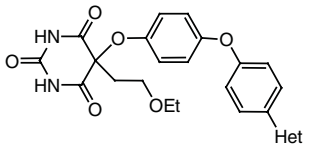
	R (in structure **6**)	MMP-13 IC_{50} (nM)	MMP-14 IC_{50} (nM)	Ratio
3	4-Fluoro	0.87	23	26
7	3-Fluoro	9.2	60	7
8	2-Fluoro	3.1	16	5
9	4-Chloro	1.4	18	13
10	3-Chloro	53	120	2
11	2-Chloro	150	510	3
12	4-Methyl	2.1	23	11
13	3-Methyl	19	50	3
14	2-Methyl	3.1	70	23
15	4-Bromo	0.87	19	22
16	4-Trifluoromethyl	5.0	150	30
17	4-*tert*-Butyl	190	6700	35
18	4-Ph	1.7	36	21
19	4-Methoxy	28	220	8
20	4-Methanesulfonyl	140	8700	62
21	4-Cyano	5.9	200	34
22	4-H_2NCO–	19	620	33
23	4-MeNHCO–	6.0	650	108
24	4-Me_2NCO–	56	9300	166
25	4-HO_2C–	>300	>30,000	—

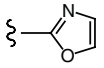
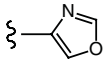
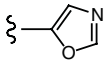
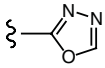
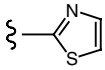
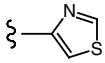
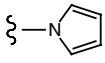
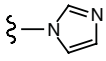
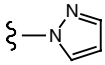
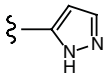
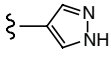
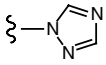
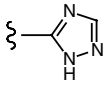
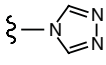
[a] See Ref. 12.

The selectivity of the C-4 substituted analogs for MMP-13 over MMP-14 is generally about 20–30-fold. However, two analogs, the secondary and tertiary amides (**23** and **24**, respectively), stand apart in this regard. The reduction in MMP-13 potency suffered by the former is modest as compared to **3** (~7-fold); however, the comparative MMP-14 potency loss is greater (~28-fold) leading to a selectivity for MMP-13 over MMP-14 of >100-fold. Although the tertiary amide displays an even greater loss in MMP-13 potency (~64-fold relative to **3**), the MMP-14 activity shifts >400-fold, again yielding a compound with >100-fold selectivity. The presence of the heteroatoms in these two analogs appears to induce the selectivity we sought by diminishing the potency against MMP-14 more so than for MMP-13. While these two compounds were not of particular interest in themselves owing to their potency, their selectivity prompted us to consider incorporating what appeared to be their key features—heteroatoms in the appropriate places—into molecules that retain potent inhibition. To this end we prepared a series of five-membered aromatic heterocycles at C-4, some of which can be considered isosteres of an amide (Table 2).

Consistent with the S_1' pocket being very deep but narrow, as was demonstrated with the phenyl derivative **18**, all of the analogs containing five-membered aromatic heterocycles at C-4 display good to excellent MMP-13 inhibition. However, selectivity against MMP-14 varies greatly and appears to be dependent on the presence and positions of the ring heteroatoms. This is best illustrated by the three oxazoles **26**–**28**. The 2-oxazolyl analog, having both an sp^2 nitrogen and an oxygen adjacent (α) to the point of attachment, is potent ($IC_{50} \sim 1$ nM) and very selective (>100-fold). Moving the oxygen atom to the β position, as in **27**, yielded a compound with diminished MMP-13 potency and selectivity, while moving the nitrogen atom to the β position, as in **28**, yielded a potent compound but with little selectivity. The potency and selectivity of **26** was mirrored by the oxadiazole **29**, which has the same arrangement of heteroatoms adjacent to the point of attachment of the heteroaryl ring. This arrangement of heteroatoms—α oxygen and sp^2 nitrogen atoms—appears to be unique in providing both potency and selectivity since other arrangements

Table 2. MMP-13 and MMP-14 inhibitory activity of heteroaryl analogs[a]



	Het	MMP-13 IC_{50} (nM)	MMP-14 IC_{50} (nM)	Ratio
26		1.4	[b]	170[b]
27		18	340	19
28		0.54	3.0	6
29		1.0	220	220
30		3.1	75	24
31		3.6	150	42
32		3.1	22	7
33		0.95	18	19
34		3.8	260	68
35		4.1	27	7
36		0.61	11	18
37		3.9	420	108
38		15	1400	93
39		8.0	230	29

[a] See Ref. 12.
[b] See Ref. 13.

of heteroatoms yielded compounds that were either potent or selective, but not both.

Attempts to rationalize the effects of the heteroatoms in this series by analysis of the SAR or through modeling did not lead to a unified view of how these compounds interact with MMP-13 or MMP-14. Nevertheless, we had identified compounds with the properties that we desired—potent MMP-13 inhibition with a >100-fold selectivity over MMP-14. Broader in vitro examination of oxadiazole **29** revealed it also to be selective against MMP-1 (IC_{50} 13,000 nM), but not particularly selective against MMP-2, 8, or 12 (IC_{50}s 21 nM, 31 nM, 29 nM, respectively). In vivo in rats **29** was found to have a moderate half-life ($t_{1/2} = 4.0$ h), low clearance (0.63 mL/min/kg), and a low volume of distribution (0.20 L/kg). Oral absorption was excellent (~100%).

In conclusion, we have described the discovery of a series of pyrimidinetrione-derived MMP-13 inhibitors that spare MMP-14. These compounds were designed based on structural insights from modeling of a lead compound in the MMP-13 active site. The proposed mode of binding was confirmed by X-ray crystallography of the bound complex of inhibitor **3** with MMP-13. Inhibition of MMP-14 is strongly influenced by the nature of the C-4 substituent on the terminal phenyl group. High selectivity for MMP-13 over MMP-14 can be achieved by appropriate variation of this group. A compound possessing favorable pharmacokinetic properties has been identified thus enabling further investigations into the role that MMP-14 plays in fibroplasia in rats and MSS in humans.

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10. Coordinates have been deposited in the PDB: accession number 1YOU.
11. PL-TBD is polymer supported 1,5,7-triazbicyclo[4.4.0]dec-5-ene resin obtained from Polymer Laboratories Inc. Amherst Fields Research Park, 160 Old Farm Road, Amherst, MA 01002, USA.
12. MMP measurements were made at concentrations of enzyme and substrate such that IC_{50}s approximate the K_is. MMPs 1, 8, 12, 13, and 14 were assayed using the quenched fluorescent substrate Dnp-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys-(Nma)-NH_2 as described by Bickett (Ref. 14): [S] = 10 μM, [E] = 1 nM for MMP-1 and MMP-13; [E] = 3 nM for MMPs-8, 12, and 14. MMP-2 was assayed using the quenched fluorescent substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 as described by Knight (Ref. 15): [S] = 10 μM; [E] for MMP-2 = 0.27 nM.
13. The MMP-14 IC_{50} for **26** of 1400 nM was determined under conditions different from those used for other compounds in Tables 1 or 2. Determination of the MMP-13 potency of **26** under corresponding conditions yielded an IC_{50} of 8.3 nM and therefore a MMP-14 IC_{50} to MMP-13 IC_{50} ratio of 170. We thank Grace Munie and Teresa Sunyer of Pfizer Global Research and Development, St. Louis Laboratories for these determinations.
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