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Novel 5,5-Disubstitutedpyrimidine-2,4,6-triones as Selective MMP Inhibitors

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Abstract—The 5,5-disubstitutedpyrimidine-2,4,6-triones represent a new class of MMP inhibitors showing selectivity for the gelatinases A and B, collagenase-3, and human neutrophil collagenase. The SAR presented here is in good agreement with an X-ray structure of compound **5** bound to the catalytic domain of stromelysin-1. While of the barbiturate structural class, compound **5** did not show any toxic or sedative effects.

Matrix metalloproteinases (MMPs) are a family of zinc dependent endopeptidases involved in the breakdown of components of the extracellular matrix which facilitates connective tissue remodeling. This process is important in embryonic development, pregnancy, growth, and wound healing. Normally the activity of MMPs is controlled by a tight balance between synthesis of active MMPs and the presence of endogenous inhibitors, such as the TIMPs (tissue inhibitor of metalloproteinases). In a number of disease states, including cancer, this balance is lost. Overexpressed MMPs are important in at least three events in malignancy progression: (1) primary tumor growth, which requires the breakdown of the surrounding connective tissue; (2) metastasis, involving the movement of tumor cells across the vascular basement membrane; and (3) angiogenesis, the growth and entry of new blood vessels into the growing tumor.

The gelatinases A and B (MMPs-2 and -9) appear to be the MMPs most highly expressed in and around the widest variety of tumors and have been implicated in tumor aggressiveness and increased metastatic potential.[1] To date most MMP inhibitors have been strong zinc binding agents and thus broad spectrum MMP inhibitors.[2] The lack of selectivity many of these compounds exhibit may contribute to the unacceptable toxicities shown in human trials.

During a program to identify new, non-hydroxamate inhibitors of gelatinases A and B for use as antitumor agents, we reinvestigated a compound that had been shown to be a weak inhibitor of stromelysin in a high throughput screen. Against stromelysin, compound **1** showed an IC_{50} of 30 μM and thus was of little interest in an earlier arthritis project. Interestingly, when compound **1** was assayed against the oncology targets of gelatinases A and B it was found to be at least 30-fold more active against these enzymes than against stromelysin-1 (IC_{50} of 1.3 μM and 0.33 μM, respectively, against gelatinases A and B). This trend of selectivity for gelatinases over stromelysin-1 was observed with all tested compounds of the pyrimidine-2,4,6-trione class.

Chemistry

Commercial methyl biphenylacetate and methyl 4-phenoxyphenylacetate were converted into the corresponding malonate derivatives by reaction with NaH and dimethylcarbonate in THF at reflux. The malonate anion, prepared with NaH in THF, was then alkylated with the required alkyl halide. Conversion to the 5,5-disubstituted-pyrimidine-2,4,6-triones was accomplished by reaction of the malonate derivatives with $Mg(OCH_3)_2$ and urea (ratio 1:2.8:2) in a minimum amount of methanol at reflux. The thiocarbonyl derivative **10** was prepared as above by substituting thiourea for urea. The *N*-alkylated derivative **11** was prepared by reaction of **5** with NaH and methyl iodide in THF and chromatographic separation of the mono- and dialkylated products. Reaction of the appropriate monosubstituted malonate with urea and $NaOCH_3$ (ratio 1:3:2) in methanol at reflux, gave the 5-monosubstituted pyrimidine trione **12**.

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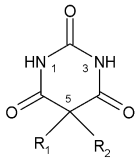
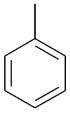
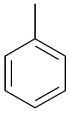
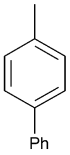
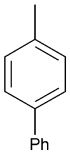
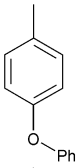
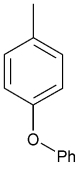
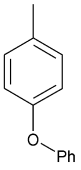
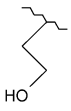
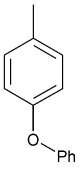
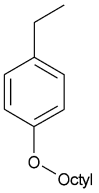
Table 1.



Compd	R_1	R_2	IC_{50} (μM)		
			Stromelysin	Gelatinase A	Gelatinase B
1	Hexyl		30	1.3	0.33
2	Methyl		ND[a]	50	11
3	Hexyl		ND	0.868	0.863
4	Ethyl		9	0.898	0.696
5	Methyl		2	0.081	0.052
6	Hexyl		2	0.021	0.018
7			0.93	0.019	0.017
8			ND	0.059	0.118
9	Methyl		1	0.53	0.164

[a]ND = Not determined.

Assay

Enzyme assays were carried out in a microtiter-format using the fluorogenic substrate described by Bickett et al.[3] Full length forms of gelatinases A[4] and B[5] and stromelysin-1[6] were prepared as described. Assays with gelatinases were run at pH 7.5, 50 mM TrisHCl, 200 mM NaCl, 5 mM $CaCl_2$, 20 μM $ZnCl_2$, and 0.05% (wt/v) Brij-35 with a final substrate concentration of 10 μM and concentration for gelatinase A 0.08 nM; gelatinase B 0.05 nM. For stromelysin-1 the assay buffer was pH 6.5, 50 mM Mes, 2.5 mM EDTA, 5 mM $CaCl_2$, and 0.05% (wt/v) Brij-35. Substrate concentration was 10 μM, and enzyme concentration was 10 nM.

Early modeling work with the pyrimidine-2,4,6-trione **1** suggested that the phenyl group at C-5 would fit into the S1′ pocket and that since this pocket is a very deep cleft, replacing the phenyl with a longer aromatic unit should improve potency. However, compound **3**, with the longer biphenyl moiety showed only modest improvement in gelatinase A inhibitory activity and had a decreased potency against gelatinase B (compare compounds **1** and **3** in Table 1). Modeling of **3** suggested that the biphenyl was too rigid and that the second phenyl group would be forced into the S1′ pocket wall.

To improve the flexibility of the C-5 aromatic unit the 4-phenoxyphenyl derivative **5** was prepared and, in support of modeling, showed a >10-fold improvement in potency. An X-ray structure of **5** complexed with the catalytic domain of stromelysin-1[7] was obtained and the interactions with the protein are shown in Figures 1 and 2.

The effect of the size of the alkyl group at C-5 was explored in the phenyl, biphenyl, and 4-phenoxyphenyl

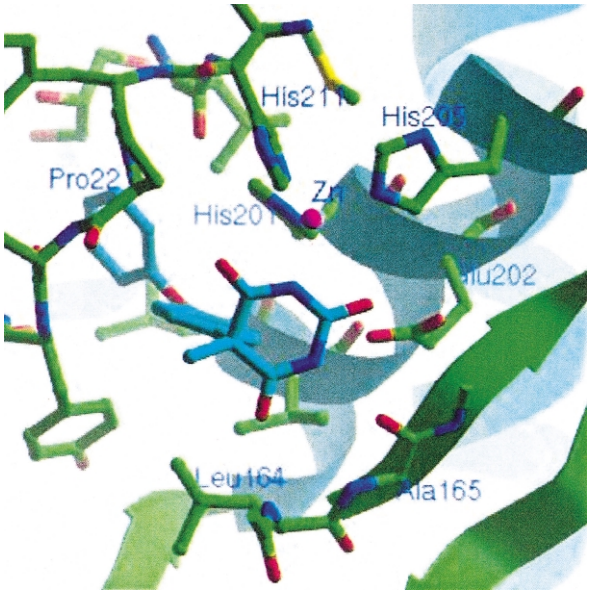


Figure 1. X-ray structure of compound **5** (blue) in the active site of stromelysin-1.

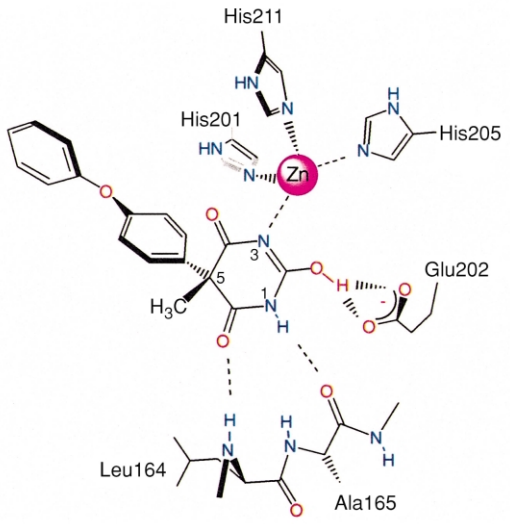
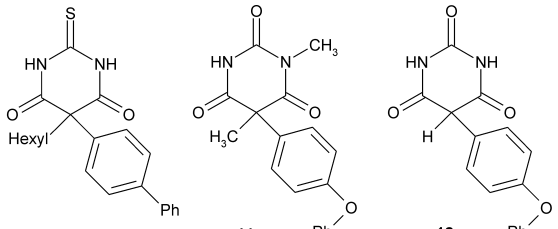


Figure 2. Hydrogen bonds and zinc coordination between protein and compound **5**.

classes. In contrast to the phenyl series, where potency was lost with the shorter alkyl substituent at C-5 (compound **1** vs **2**, Table 1), in the biphenyl series shortening of the C-5 hexyl to an ethyl group did not change potency (compare compounds **3** and **4** in Table 1). However in the phenoxyphenyl series a moderate improvement in activity was observed when the C-5 methyl in **5** was replaced by hexyl (compound **6**, Table 1). These observations suggest that there is some difference in how the alkyl group at C-5 interacts with the protein in the three different compound classes and further suggests that in the phenyl series it is the hexyl group of **1** which is occupying the S1′ pocket.

Replacing the alkyl at C-5 with the methyl benzyl ether group, as shown in compound **7**, resulted in similar potency to the hexyl derivative **6**. However, replacing the alkyl with the more hydrophilic ethanol moiety in compound **8** resulted in some loss in activity toward gelatinase A and a 7-fold loss against gelatinase B. The effect of additional flexibility and the requirement for the second aryl group at C-5 was explored by replacement of the phenoxyphenyl group in **5** with the benzyl alkoxy unit shown in **9**. This modification resulted in some loss in inhibitory activity against both gelatinase A and gelatinase B (>6-fold loss against gelatinase A and about a 3-fold loss against gelatinase B).

Confirmation of the interactions between **5** and the zinc atom and Glu202 shown in the X-ray structure (Figs. 1 and 2), was obtained by replacing the C-2 carbonyl with a thiocarbonyl, compound **10**. This modification caused a >13-fold loss in potency against both gelatinases. This result supports structural information suggesting that enolization of the N-3, C-2 amide unit of the pyrimidine trione provides N-3 as the fourth zinc ligand and that the resultant hydroxyl at C-2 provides the hydrogen for a bidentate hydrogen bond to Glu202. In thioamides it is reported that the thione is favored over the thiol tautomer[8] and further, should Glu202 be protonated, it is also reported that the thiocarbonyl of thioamides is a poor hydrogen-bond acceptor.[9] The importance of the amide hydrogens at N-1 and N-3 was shown by the loss of activity exhibited by *N*-methyl derivative **11**. The dialkylated derivative was also prepared and was similarly inactive.



The requirement for the tetrahedral carbon at C-5 was shown by preparation of compound **12**. Replacing the alkyl group at C-5 with a hydrogen, as shown in **12**, allowed enolization[10] and places C-4, C-5, and C-6 of the pyrimidine trione ring and the phenyl moiety in the same plane, thus the phenoxyphenyl unit is unable to enter the S1′ pocket and results in a considerable loss of activity. Each of these results also agrees with the X-ray structure of a different class of pyrimidine triones with human neutrophil collagenase (MMP-8).[11]

None of the pyrimidine triones showed activity against matrilysin (MMP-7) (IC_{50} >50 μM) and were only weakly active against collagenase-1 (MMP-1) (IC_{50} >2 μM) and stomelysin-1 (MMP-3) (see Table 1). In addition to inhibiting gelatinases A and B (MMP-2 and -9), compounds **5** and **6** were assayed against human neutrophil collagenase (MMP-8) (IC_{50} for compounds **5** and **6**, 0.081 and 0.13 μM, respectively), and collagenase-3 (MMP-13) (IC_{50} for **5** and **6**, 0.065 and 0.078 μM, respectively).

The pyrimidine triones belong to the barbiturate compound class and therefore might be expected to exhibit barbiturate-like activities. This was explored with a tolerability study using compound **5** at an ascending single oral dose (10, 30, 100, and 1000 mg/kg) and an ascending 5 day oral dose (100, 300, and 1000 mg/kg/day) in CD-1 mice. In neither study was there any indication of toxicity or sedative effects, however some increase in the hours the mice were awake and feeding was observed in the treated animals (C. Slater and L. Hall, Hoffmann-La Roche, personal communication).

In summary, the 5,5-disubstitutedpyrimidine-2,4,6-triones have been shown to be a novel and nontoxic class of MMP inhibitors. The selectivity shown for MMPs-2, -9, -8, and -13 make them very attractive as antitumor agents. The SAR presented here is in excellent agreement with the information available from the X-ray structures of pyrimidine-2,4,6-triones with stromelysin-1[7] and human neutrophil collagenase.[11]

However, analysis of these X-ray structures and a published X-ray structure of gelatinase A[12] does not provide a rationale for the selectivity shown by the pyrimidine triones toward the gelatinases over stromelysin-1. The explanation for this will require a co-crystal structure of gelatinase A or B with a pyrimidine trione.

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