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Heteroaryl and Cycloalkyl Sulfonamide Hydroxamic Acid Inhibitors of Matrix Metalloproteinases

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Abstract—Heteroaryl and cycloalkyl sulfonamide-hydroxamic acid MMP inhibitors were investigated. Of these, the pyridyl analogue **2** is the most potent and selective inhibitor of MMP-9 and MMP-13 in vitro.

The normal remodeling of extracellular matrix proteins is controlled by a family of zinc-containing matrix metalloproteinases (MMPs). A variety of pathologies such as rheumatoid arthritis,[1] osteoarthritis,[1] and cancer[2] have now been associated with the abnormal regulation of more than 20 different MMPs. The ability of small molecule inhibitors of various MMPs to effectively treat arthritis and cancer is now being studied in man. Four representative compounds to have reached clinical trials are shown in Figure 1. Marimastat and CG-S27023A are broad spectrum MMP inhibitors while Ro-32-3555 is selective for the collagenases and AG-3340 shows a preference for MMP-1.[3]

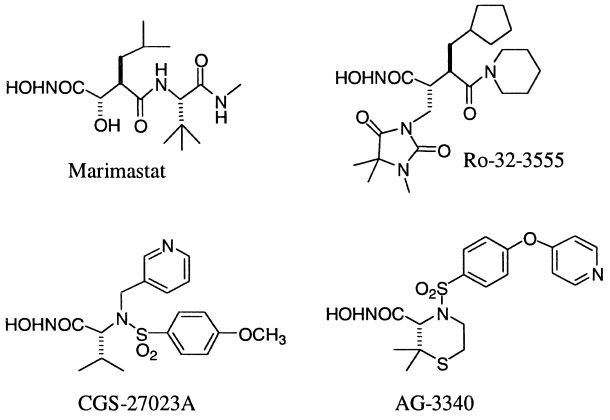

Figure 1. MMP inhibitors in clinical trials.

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We have recently reported a novel series of anthranilic acid-based MMP inhibitors of general structure **1**, which differs from most other inhibitors in having a two carbon linker between the sulfonamide nitrogen and the zinc chelating hydroxamic acid (Fig. 2).[4]

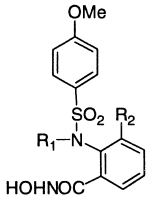

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Figure 2. Anthranilic acid-based MMP inhibitors.

The SAR of the anthranilic acid MMP inhibitors had shown that the sulfonamide, hydroxamic acid, and a third substituent must be on adjacent carbons of the aromatic ring, as shown in Figure 2, to provide compounds with potent activity against MMP-1, MMP-9, and MMP-13.[4] In an effort to assess the effect of heteroatom substitution, ring size, and saturation on the anthranilic acid portion of the molecule we targeted the pyridyl, thienyl, pyrazolyl and cycloalkyl analogues of the anthranilic acids. We now disclose several extensions of this series, demonstrating that the anthranilic acid phenyl ring can be replaced with heteroaryl groups as well as a simple cyclohexyl moiety.

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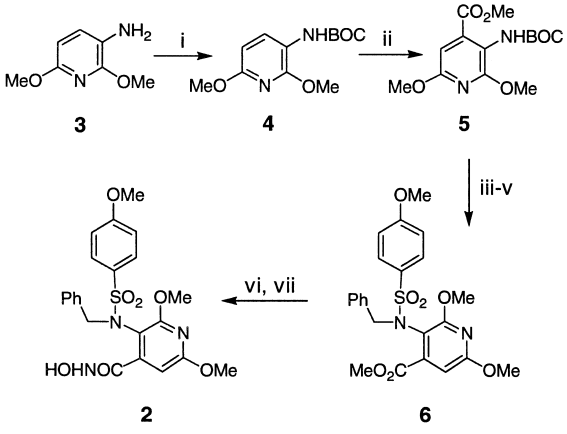
Chemistry

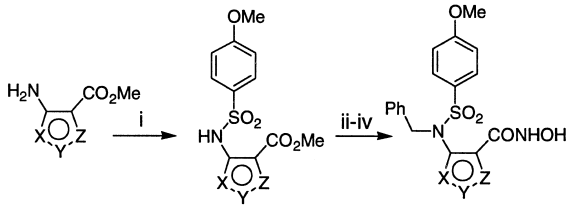
The pyridyl analogue **2**, with the required substitution pattern, was synthesized in eight steps starting from 3-amino-2,6-dimethoxypyridine, as shown in Scheme 1.[5] Thus, reaction of the aminopyridine **3** with BOC-anhydride provided the carbamate **4** in quantitative yield. Metalation of the pyridine ring *ortho* to the carbamate with *n*-butyllithium and TMEDA followed by the addition of methyl chloroformate then gave the methyl ester **5** in 34% yield.[6] Removal of the BOC moiety was accomplished with *p*-toluenesulfonic acid and the resulting amine was converted into the *p*-methoxybenzene sulfonamide in 86% overall yield. The sulfonamide was subsequently alkylated with benzyl bromide and sodium hydride in quantitative yield to give **6**. Hydrolysis of the methyl ester with lithium hydroxide followed by conversion into the corresponding acid chloride and reaction with hydroxylamine then gave the desired pyridine **2**, an analogue of the anthranilic acid series.



Scheme 1. (i) BOC_2O; (ii) (a) *n*-BuLi, TMEDA, (b) $ClCO_2Me$; (iii) *p*-TsOH; (iv) 4-$MeOPhSO_2Cl$, TEA; (v) BnBr, NaH; (vi) LiOH; (vii) (a) $(COCl)_2$, DMF; (b) NH_2OH.

The thiophene and pyrazole heteroaryl analogues of the anthranilic acids were readily available from commercial starting materials as shown in Scheme 2. The appropriate thiophene and pyrazole amino-esters **7a–d** were sulfonylated with *p*-methoxybenzenesulfonyl chloride and pyridine to give sulfonamides **8a–d**. Alkylation of the sulfonamides proceeded with sodium hydride and benzyl bromide. Hydrolysis of the esters and conversion of the resulting carboxylic acids into the hydroxamic acids proceeded as before to give **9a–d**.

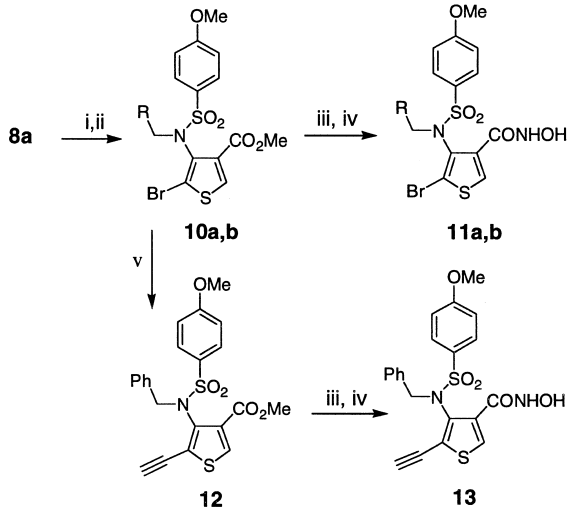
The bromo and alkynyl-substituted thiophenes were synthesized from sulfonamide **8a** as shown in Scheme 3. The alkynes were targeted since they provided a readily accessible substituent roughly equivalent in size to the methyl group that had enhanced the activity of compound **9c**. Bromination of **8a** with *N*-bromosuccinimide in acetic acid provided the corresponding bromothiophene in 81% yield. Benzylation as before, or alkylation with potassium carbonate and 3-picolyl chloride, gave the esters **10a** (R = Ph) and **10b** (R = 3-pyridyl). Esters **10a** and **10b** were then converted into hydroxamates **11a**



	X	Y	Z
a	CH	S	CH
b	CH	CH	S
c	CMe	CH	S
d	NMe	N	CH

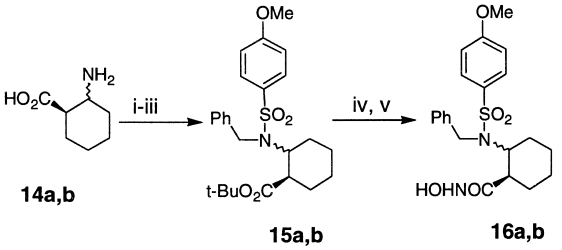
Scheme 2. (i) 4-$MeOPhSO_2Cl$, TEA; (ii) BnBr, NaH; (iii) NaOH; (iv) (a) $(COCl)_2$, DMF, (b) NH_2OH.

(R = Ph) and **11b** (R = 3-pyridyl) as before. Palladium(II) catalyzed coupling of bromothiophene **10a** with TMS-acetylene and subsequent desilylation gave **12**, which was converted into hydroxamic acid **13** as before.



Scheme 3. (i) NBS, AcOH; (ii) BnBr, NaH or 3-$PyCH_2Cl$, K_2CO_3; (iii) NaOH; (iv) (a) $(COCl)_2$, DMF; (b) NH_2OH; (v) (a) TMS-CCH, $(PPh_3)_2PdCl_2$, CuI, (b) Bu_4NF.

The saturated analogues of the anthranilic acid MMP inhibitors were prepared as shown in Scheme 4. Thus, racemic *trans*- and *cis*-2-aminocyclohexane carboxylic acids, **14a** and **14b** respectively, were reacted with *p*-methoxybenzenesulfonyl chloride to give the analogous sulfonamide-carboxylic acids. The acids were then converted into the corresponding *tert*-butyl esters with *N*,*N*-dimethylformamide di-*tert*-butyl acetal in refluxing toluene. The NH-sulfonamides were alkylated with benzyl bromide and sodium hydride to give sulfonamide-esters **15**. The *tert*-butyl esters were next deprotected with trifluoroacetic acid to provide the carboxylic acids. Hydroxamic acid formation was accomplished as before with oxalyl chloride/DMF followed by the addition of hydroxylamine to give **16a** (*trans*) and **b** (*cis*).



Scheme 4. (i) 4-MeOPhSO$_2$Cl, TEA; (ii) (Me)$_2$NCH(O*t*Bu)$_2$; (iii) BnBr, NaH; (iv) TFA; (v) (a) (COCl)$_2$, DMF, (b) NH$_2$OH.

Biology

All of the hydroxamic acid final products were tested in vitro[7,8] for their ability to inhibit MMP-9. Inhibitors of MMP-9 are postulated to have utility as inhibitors of tumor metastasis. Most compounds were also tested against the collagenases MMP-1 and MMP-13, which are presumed to be important in the etiology of osteoarthritis. The in vitro potencies of these compounds are shown in Table 1.

Pyridine analogue **2** is a potent inhibitor of both MMP-9 and MMP-13 with moderate selectivity over MMP-1. Compounds with this selectivity profile may be useful in assessing the basis of musculoskeletal side effects seen in clinical trials of some MMP inhibitors.[9] Pyridine **2** is almost 3-fold more potent against MMP-13 than the corresponding 3-methoxy anthranilic acid analogue (**1b**, Table 1), and much more selective over MMP-1. The reason for this apparent increase in potency is not known. Compound **2** is also a moderately potent inhibitor of TNF-α converting enzyme (TACE), with an IC$_{50}$ of 294 nM.[10] Unfortunately, compound **2** was not orally active against MMP-13 at a dose of 50 mg/kg in the rat dialysis implant bioactivity model.[11]

The thiophene derivatives **9a** and **9b**, lacking a second substituent adjacent to the sulfonamide nitrogen were very weakly active versus MMP-9 in accord with the SAR previously shown for the anthranilic acid derivatives. As expected, incorporation of a methyl substituent next to the sulfonamide nitrogen in thiophene **9c** provides approximately a 100-fold increase in potency against MMP-9. The two bromothiophenes, **11a** and **11b**, were both moderately potent versus MMP-9. As in the anthranilic acid series, the *N*-picolyl sulfonamide is somewhat more active than the *N*-benzyl derivative. The alkynyl substituent of thiophene **13** is less effective at increasing potency, perhaps due to its smaller steric bulk. None of the thiophene hydroxamic acids approached the level of MMP inhibition achieved by the anthranilic acid hydroxamic acids (see **1a**, Table 1). The picolyl derivative **11b** was inactive on oral dosing at 100 mg/kg against MMP-9 in the rat dialysis implant bioactivity model.

Table 1. In vitro potency of sulfonamide-hydroxamic acids

Compound	MMP-1[a]	MMP-9[a]	MMP-13[a]
1a (R_1 = CH_2-3-Py, R_2 = Me)	143	5	8
1b (R_1 = CH_2Ph, R_2 = OMe)	520	23	138
2	1227	15	47
9a	40% (1)[b]	67% (10)[b]	NT[c]
9b	19% (1)[b]	57% (10)[b]	NT
9c	NT	104	NT
9d	22% (1)[b]	900	NT
11a	639	236	427
11b	50% (1)[b]	70	290
13	49% (1)[b]	38% (0.3)[b]	35% (0.3)[b]
16a	174	181	233
16b	361	318	291

[a]IC$_{50}$ (nM).
[b]% Inhibition (μM).
[c]NT = not tested.

The pyrazole analogue **9d** was only weakly active against MMP-9 even with the methyl group on the pyrazole nitrogen acting as the requisite second substituent adjacent to the sulfonamide nitrogen. The electron deficient nature of the pyrazole ring may be responsible for the diminished in vitro activity of **9d** relative to the electron rich thiophenes.

The cyclohexyl analogues **16a** and **16b** are also less active than the anthranilic acid hydroxamic acids **1a** and **1b** versus both MMP-9 and MMP-13. The *trans*-cyclohexyl derivative is more potent than the *cis*-isomer against MMP-1, MMP-9, and MMP-13. The level of activity for the *trans*-cyclohexyl analogue, **16a**, is somewhat high, however, in light of the fact that the corresponding anthranilic acid analogue lacking a 3-substituent (**1**, R_1 = Bn, R_2 = H) is a 555 nM inhibitor of MMP-13.

In conclusion, we have investigated the pyridyl, thiophene, pyrazole and cyclohexyl analogues of the anthranilate hydroxamate class of MMP inhibitors. The pyridyl derivative **2** is more potent and selective in vitro than the related anthranilate **1b**. The thiophene and pyrazole rings proved to be less satisfactory replacements for the phenyl ring of the anthranilate hydroxamic acids. It is unclear whether the decrease in potency observed for these two scaffolds is due to electronic or steric effects. The cyclohexyl ring is also a less effective scaffold for the sulfonamide-hydroxamate pharmacophore, although incorporating additional substituents on the cycloalkyl ring might provide more active compounds. Two of the compounds (**2** and **11b**) were tested in vivo and were inactive at the dose tested.

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