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The Discovery of Anthranilic Acid-Based MMP Inhibitors. Part 1: SAR of the 3-Position

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Abstract—A novel series of anthranilic acid-based inhibitors of MMP-1, MMP-9, and MMP-13 was prepared and evaluated both in vitro and in vivo. The most potent compound, **6e**, has in vivo activity in a rat sponge-wrapped cartilage model.

The matrix metalloproteinases (MMPs), comprised of collagenases, stromelysins, gelatinases and membrane-type MMPs, are a family of over 20 zinc-containing enzymes that play a role in the normal remodeling and degradation of extracellular matrix proteins. The aberrant control of MMP levels has been implicated in the etiology of a variety of disease states including atherosclerosis,[1] rheumatoid arthritis and osteoarthritis,[2] and cancer.[3] The potential exists for potent, orally bioavailable small molecule inhibitors of MMPs to treat a broad spectrum of pathologies, and has been investigated in the clinical trials of agents such as marimastat,[4] Ro 32-3555,[5] CGS-27023A,[6] and AG3340[7] (Fig. 1).

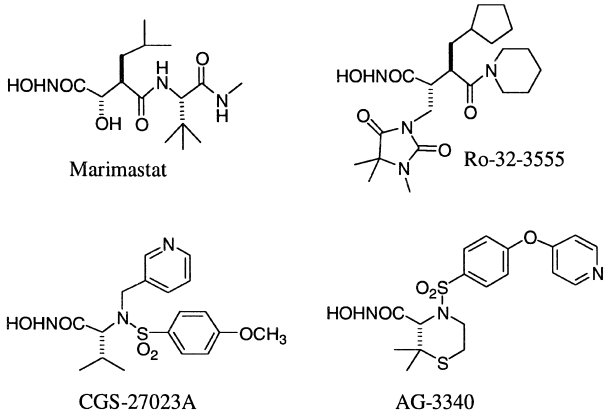

Figure 1. MMP inhibitors in clinical trials.

In that regard the discovery of sulfonamide-based hydroxamic acid inhibitors of stromelysin (MMP-3), exemplified by CGS-27023A, is the seminal work in the rapidly expanding area of nonpeptide MMP inhibitors.[8] The disclosure of numerous sulfonamide analogues related to CGS-27023A, including the biaryl sulfonamides,[9] piperazine,[10] thiazine,[11] thiazepine,[11] and diazepine[12] ring systems, now prompts us to report on the synthesis and biological evaluation of a novel series of anthranilic acid-based MMP inhibitors.

Although a substantial number of nonpeptide sulfonamide hydroxamate MMP inhibitors have been studied, almost all of these compounds have been derived from α-amino acids, with a single carbon linking the sulfonamide nitrogen and the zinc chelating hydroxamic acid moiety.[13] We were interested in ascertaining whether novel, potent MMP inhibitors could be made by using an aromatic ring as the linker between the sulfonamide nitrogen and the hydroxamate (Fig. 2).

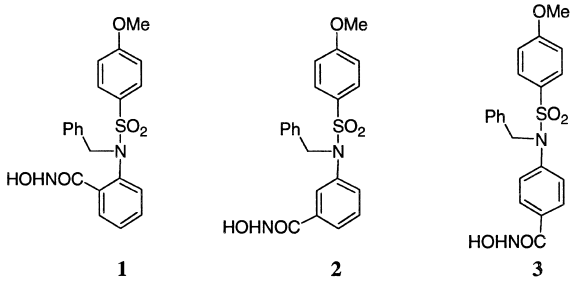

Figure 2. Sulfonylated anthranilate hydroxamic acids.

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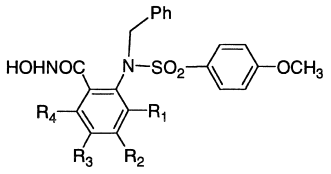
In the event, the anthranilic acid derivative **1** had IC_{50}s below 1 μM versus MMP-1, MMP-9, and MMP-13 (Table 1). In contrast, the 3- and 4-aminobenzoic acid derivatives, **2** and **3**, had no appreciable activity versus MMP-1 at 1 μM. With this result in hand we set out to synthesize a series of sulfonylated anthranilic acid analogues of **1**.

Table 1. In vitro potencies of compounds **1**, **6a**–**6d**

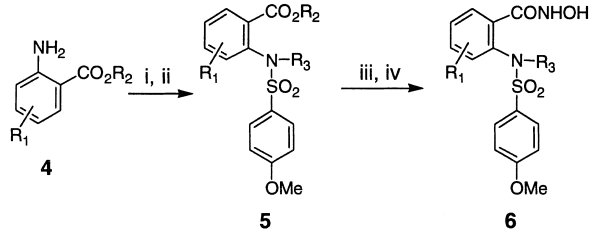


Compound	R_1	R_2	R_3	R_4	MMP-1[a]	MMP-9[a]	MMP-13[a]
1	H	H	H	H	639	650	555
6a	Me	H	H	H	115	23	50
6b	H	Me	H	H	884	346	982
6c	H	H	Me	H	553	353	728
6d	H	H	H	Me	1573	440	717

[a]IC_{50}, nM.

Chemistry

The desired sulfonamide hydroxamic acids were prepared as shown in Scheme 1.[14] Sulfonylation of the appropriate anthranilic acid with 4-methoxybenzenesulfonyl chloride provided the sulfonamides **5a** (R_2, R_3 = H). The sulfonamides were then concomitantly *N*- and *O*-alkylated to provide compounds **5b** (R_2, R_3 = Bn). Hydrolysis of the ester followed by acid chloride formation and conversion into the hydroxamic acid gave compounds **6**.



Scheme 1. (i) 4-MeOPhSO_2Cl, TEA; (ii) R_3X, NaH; (iii) NaOH; (iv) (a) $(COCl)_2$, DMF; (b) NH_2OH.

In order to examine the effect of substitution on the anthranilic acid ring on inhibitory potency, analogues of **6** derived from 3-, 4-, 5-, and 6-methyl 2-amino-benzoic acid were synthesized. The IC_{50} values of methyl analogues **6a**–**6d** (Table 1) clearly demonstrate that while the 4-, 5-, and 6-methyl compounds (**6b**–**6d**) are no more active than the parent compound, the 3-methyl derivative **6a** is an order of magnitude more potent than the unsubstituted anthranilate. Furthermore, compound **6e**, the *N*-3-picolyl analogue of **6a**, is as potent as the CGS-27023A standard (Table 2).

The carboxylic acid analogue of **6a**, and the NH-sulfonamide analogue are approximately 100-fold less active than **6a**. A series of analogues of **6a** in which the 3-substituent was varied was therefore targeted next.

Compounds **6f** and **6g** (Table 2) were synthesized from the commercially available 3-substituted anthranilic acids in the same general manner as shown in Scheme 1. The 3-nitro derivative, **6h**, was synthesized via nitration of **5** (R_1 = H, R_2 = Me, R_3 = Bn). The *N*,*N*-dimethyl aniline **6i** was prepared via tin chloride reduction of the nitro aryl intermediate **5** (R_1 = 3-NO_2, R_2 = Me, R_3 = Bn), followed by methylation of the resulting aniline with iodomethane/potassium carbonate in DMF and subsequent ester hydrolysis and hydroxamate formation. The synthesis of the 3-trifluoromethyl analogue **6j** commenced with the sulfonylation of benzylamine to give **7**. Reaction of **7** with 2-fluoro-3-trifluoromethylbenzonitrile in the presence of sodium hydride then provided **8**. The nitrile was then converted into the corresponding hydroxamic acid via carboxamide **8** as shown in Scheme 2.

Additional 3-substituted anthranilic acid analogues were readily prepared according to Scheme 3. Thus, sulfonylation of 3-hydroxy methyl anthranilate gave **9**, which was elaborated as shown to give compounds **6k** and **6l**.

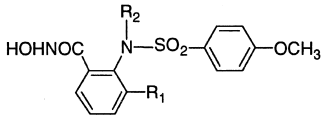
The 3-carbomethoxy derivative was synthesized from aldehyde **11** as shown in Scheme 4. Bis-bromination of the 3-methyl derivative **5c**, followed by hydrolysis gave aldehyde **11**. Oxidation to the carboxylic acid was accomplished with sulfamic acid and sodium chlorite.

Biology

All of the anthranilate hydroxamic acids were tested in vitro[15] for their ability to inhibit MMP-1, MMP-9, and MMP-13 (Table 1). Inhibitors of MMP-9 are potentially valuable as inhibitors of tumor metastasis,[3] while MMP-13 inhibitors may offer protection from the cartilage degradation associated with osteoarthritis.[2] Selectivity for MMP-9 and MMP-13 over MMP-1 was sought in order to examine whether the inhibition of MMP-1 is the source of musculoskeletal side effects seen in clinical trials of broad spectrum MMP inhibitors.[16]

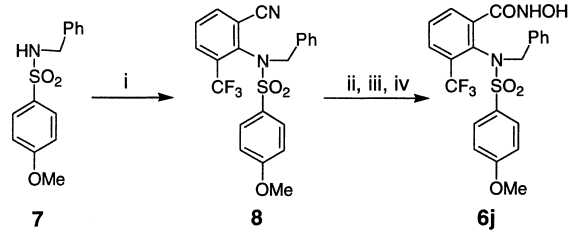
The 3-substituted anthranilate hydroxamic acids shown in Table 2 are potent in vitro inhibitors of both MMP-9 and MMP-13. In particular, the 3-methyl derivative **6e**, methyl ester **6m**, and the bis-hydroxamic acids, **6k** and **6l**, are less than 10 nM against both MMP-9 and MMP-13. Compounds **6k** and **6m** are also potent inhibitors of TNF-α converting enzyme (TACE) with IC_{50} values of 81 and 38 nM, respectively.[17] Compounds **6l** and **6m** are the only inhibitors of the series that are at least 50-fold selective for MMP-13 over MMP-1, with the geminal dimethyl group of **6l** providing a 25-fold reduction in potency versus MMP-1, relative to **6k**. The 3-methoxy analogue **6f** and the 3-trifluoromethyl derivative, **6j**, are potent gelatinase inhibitors with greater than 20-fold and 5-fold selectivity over MMP-1 and MMP-13, respectively. Dimethylaniline **6i** is a less potent MMP-9 inhibitor that displays a similar gelatinase-selective profile.

Table 2. In vitro potency of 3-substituted anthranilate hydroxamic acids

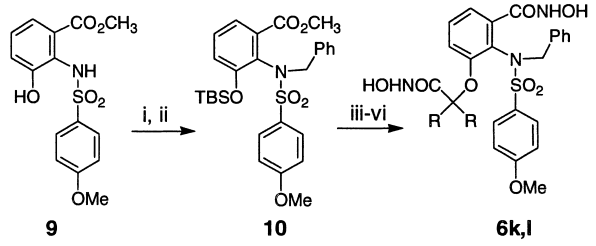


Compound	R_1	R_2	MMP-1[a]	MMP-9[a]	MMP-13[a]
1	H	CH_2Ph	639	650	555
6a	CH_3	CH_2Ph	115	23	50
6e	CH_3	CH_2-3-Py	143	5	8
6f	OCH_3	CH_2Ph	520	23	138
6g	Cl	CH_2Ph	398	31	NT
6h	NO_2	CH_2Ph	202	13	42
6i	$N(CH_3)_2$	CH_2Ph	19%[b]	64	531
6j	CF_3	CH_2Ph	41%[b]	27	136
6k	$OCH_2CONHOH$	CH_2Ph	24	2	1
6l	$OC(CH_3)_2CONHOH$	CH_2Ph	597	4	6
6m	CO_2CH_3	CH_2-3-Py	207	6	4
CGS-27023A	—	—	15	9	8

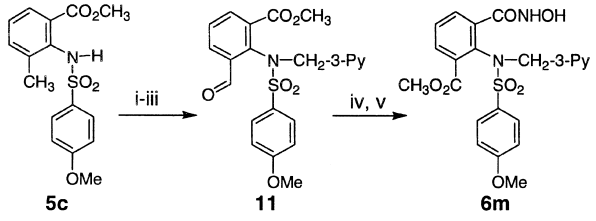
[a]IC_{50}, nM. Inhibitor concentrations were run in triplicate. IC_{50} determinations were calculated from a 4-parameter logistic fit of the data within a single experiment.
[b]% Inhibition at 1 μM.



Scheme 2. (i) NaH, 2-F-3-CF_3PhCN; (ii) NaOH; (iii) $NOBF_4$; (iv) (a) $(COCl)_2$, DMF; (b) NH_2OH.



Scheme 3. (i) TBDMSCl, imidazole; (ii) BnBr, NaH; (iii) Bu_4NF; (iv) $BrC(R)_2CO_2Et$, NaH; (v) NaOH; (vi) (a) $(COCl)_2$, DMF; (b) NH_2OH.



Scheme 4. (i) NBS; (ii) NaOH; (iii) 3-Picolyl chloride-HCl, K_2CO_3; (iv) $NaClO_2$, H_2NSO_3H; (v) (a) $(COCl)_2$, DMF; (b) NH_2OH.

Structural analysis by NMR of the solution structure of compound **6e** bound in the active site of MMP-13 indicates that the *para*-methoxyphenyl group lies in the $S1^1$ pocket of the enzyme and that the pyridine ring is solvent exposed.[18] The sulfonyl oxygens are within hydrogen bonding distance to the peptide backbone. The anthranilate aromatic ring occupies the $S2^1$ pocket of the active site. This binding scheme is quite similar to that of CGS-27023A, with the anthranilate aryl ring taking the place of the isopropyl group of CGS 27023A.[19]

The in vivo bioactivity of several of the anthranilate-hydroxamates (**6a**, **e**, **g**, **k**, and **m**) was assessed through the use of a dialysis tubing implant assay.[20] All of the compounds tested were compared to CGS-27023A in the same experiment. None of the *N*-benzyl substituted sulfonamides had significant in vivo activity. The most active of the compounds tested in this assay is the *N*-picolyl derivative **6e**, which is essentially equipotent to CGS-27023A at 50 mg/kg, po against MMP-9. It has an ED_{50} of 33 mg/kg, po in this assay. However, against MMP-13 in the dialysis implant model, compound **6e** has only 60% of the activity of CGS-27023A at the same oral dose. Ester **6m**, with 30% of the activity of CGS 27023A versus MMP-13 at 50 mg/kg ip, was significantly less potent than **6e**.

The 3-methyl anthranilate-hydroxamate **6e** was also tested in a bovine articular cartilage explant assay.[5b] At a dose of 1 μM, **6e** provides a level of inhibition of collagen degradation comparable to CGS 27023A (49%). Evaluation of **6e** in an in vivo rat sponge-wrapped cartilage model[21] demonstrated that the administration of 50 mg/kg/day ip of **6e** via osmotic pump gave a 51% inhibition of collagen degradation. Oral dosing of **6e** at 50 mg/kg/bid provided a 27% inhibition of collagen degradation, compared to a 52% inhibition of CGS-27023A at the same dose.

In conclusion, we have synthesized a series of anthranilate-hydroxamic acid MMP inhibitors. These compounds are potent inhibitors of MMP-9 and MMP-13 in vitro. Compound **6e** is also active in an in vitro cartilage degradation assay and displays oral activity in an in vivo mouse bioactivity model as well as ip activity in a rat sponge-wrapped cartilage model. The further exploration of the SAR of these novel MMP inhibitors will be reported in due course.

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