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. © 2001 Elsevier Science Ltd. All rights reserved.

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 AG33407 (Fig. 1).

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 diazepine12 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 &alpha;-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 1. MMP inhibitors in clinical trials.

Figure 2. Sulfonylated anthranilate hydroxamic acids.
In the event, the anthranilic acid derivative 1 had IC_{50} values 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.

### Chemistry

The desired sulfonamide hydroxamic acids were prepared as shown in Scheme 1.\textsuperscript{14} Sulfonylation of the appropriate anthranilic acid with 4-methoxybenzenesulfonyl chloride provided the sulfonamides 5a (R₂, R₃ = H). The sulfonamides were then concomitantly N- and O-alkylated to provide compounds 5b (R₂, R₃ = Bn). Hydrolysis of the ester followed by acid chloride formation and conversion into the hydroxamic acid gave compounds 6.

![Scheme 1](image)

**Scheme 1.** (i) 4-MeOPhSO_2Cl, TEA; (ii) R₃X, 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.

### Table 1. In vitro potencies of compounds 1, 6a-6d

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>MMP-1*</th>
<th>MMP-9*</th>
<th>MMP-13*</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>639</td>
<td>650</td>
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<tr>
<td>6a</td>
<td>Me</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>115</td>
<td>23</td>
<td>50</td>
</tr>
<tr>
<td>6b</td>
<td>Me</td>
<td>Me</td>
<td>H</td>
<td>H</td>
<td>884</td>
<td>346</td>
<td>982</td>
</tr>
<tr>
<td>6c</td>
<td>H</td>
<td>H</td>
<td>Me</td>
<td>H</td>
<td>553</td>
<td>353</td>
<td>728</td>
</tr>
<tr>
<td>6d</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>Me</td>
<td>1573</td>
<td>440</td>
<td>717</td>
</tr>
</tbody>
</table>

*IC_{50} nM.

### Biology

All of the anthranilate hydroxamic acids were tested in vitro\textsuperscript{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,\textsuperscript{3} while MMP-13 inhibitors may offer protection from the cartilage degradation associated with osteoarthritis.\textsuperscript{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.\textsuperscript{16}

The 3-carboxyethoxy 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 chloride.

### 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 (R₁ = H, R₃ = Me, R₄ = Bn). The N,N-dimethyl aniline 6i was prepared via tin chloride reduction of the nitro aryl intermediate 5 (R₁ = 3-NO₂, R₃ = Me, R₄ = 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.
Structural analysis by NMR of the solution structure of compound 6e bound in the active site of MMP-13 indicates that the \textit{para}-methoxyphenyl group lies in the S1\textsubscript{1} pocket of the enzyme and that the pyridine ring is solvent exposed.\textsuperscript{18} The sulfonyl oxygens are within hydrogen bonding distance to the peptide backbone. The anthranilate aromatic ring occupies the S2\textsuperscript{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.\textsuperscript{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.\textsuperscript{20} All of the compounds tested were compared to CGS-27023A in the same experiment. None of the \textit{N}-benzyl substituted sulfonamides had significant in vivo activity. The most active of the compounds tested in this assay is the \textit{N}-picolyl derivative 6e, which is essentially equipotent to CGS-27023A at 50 mg/kg, po against MMP-9. It has an ED\textsubscript{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.\textsuperscript{5b} At a dose of 1 \textmu M, 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\textsuperscript{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.

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>R\textsubscript{1}</th>
<th>R\textsubscript{2}</th>
<th>MMP-1\textsuperscript{a}</th>
<th>MMP-9\textsuperscript{a}</th>
<th>MMP-13\textsuperscript{a}</th>
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<tr>
<td>1</td>
<td>H</td>
<td>CH\textsubscript{2}Ph</td>
<td>639</td>
<td>650</td>
<td>555</td>
</tr>
<tr>
<td>6a</td>
<td>CH\textsubscript{3}</td>
<td>CH\textsubscript{3}Ph</td>
<td>115</td>
<td>23</td>
<td>50</td>
</tr>
<tr>
<td>6e</td>
<td>CH\textsubscript{3}</td>
<td>CH\textsubscript{2}-3-Py</td>
<td>143</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>6f</td>
<td>OCH\textsubscript{3}</td>
<td>CH\textsubscript{3}Ph</td>
<td>520</td>
<td>23</td>
<td>138</td>
</tr>
<tr>
<td>6g</td>
<td>Cl</td>
<td>CH\textsubscript{3}Ph</td>
<td>398</td>
<td>31</td>
<td>NT</td>
</tr>
<tr>
<td>6h</td>
<td>NO\textsubscript{2}</td>
<td>CH\textsubscript{3}Ph</td>
<td>202</td>
<td>13</td>
<td>42</td>
</tr>
<tr>
<td>6i</td>
<td>N(CH\textsubscript{2})\textsubscript{2}</td>
<td>CH\textsubscript{3}Ph</td>
<td>19%\textsuperscript{b}</td>
<td>64</td>
<td>531</td>
</tr>
<tr>
<td>6j</td>
<td>CF\textsubscript{3}</td>
<td>CH\textsubscript{3}Ph</td>
<td>41%\textsuperscript{b}</td>
<td>27</td>
<td>136</td>
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<tr>
<td>6k</td>
<td>OCH\textsubscript{2}CONHO\textsubscript{H}</td>
<td>CH\textsubscript{3}Ph</td>
<td>24</td>
<td>2</td>
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<td>6l</td>
<td>OCH\textsubscript{2}CONHO\textsubscript{H}</td>
<td>CH\textsubscript{3}Ph</td>
<td>597</td>
<td>4</td>
<td>6</td>
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<tr>
<td>6m</td>
<td>CO\textsubscript{2}CH\textsubscript{3}</td>
<td>CH\textsubscript{2}-3-Py</td>
<td>207</td>
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<td>4</td>
</tr>
<tr>
<td>CGS-27023A</td>
<td>—</td>
<td>—</td>
<td>15</td>
<td>9</td>
<td>8</td>
</tr>
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</table>

\textsuperscript{a}IC\textsubscript{50}, nM. Inhibitor concentrations were run in triplicate. IC\textsubscript{50} determinations were calculated from a 4-parameter logistic fit of the data within a single experiment.

\textsuperscript{b}% Inhibition at 1 \textmu M.

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![Scheme 2](attachment:image1.png)

**Scheme 2.** (i) NaH, 2-F-3-CF\textsubscript{3}PhCN; (ii) NaOH; (iii) NOBF\textsubscript{4}; (iv) (a) (COCl)\textsubscript{2}, DMF; (b) NH\textsubscript{2}OH.

![Scheme 3](attachment:image2.png)

**Scheme 3.** (i) TBDMSCl, imidazole; (ii) BnBr, NaH; (iii) Bu\textsubscript{4}NF; (iv) BrC(R)\textsubscript{2}CO\textsubscript{2}Et, NaH; (v) NaOH; (vi) (a) (COCl)\textsubscript{2}, DMF; (b) NH\textsubscript{2}OH.

![Scheme 4](attachment:image3.png)

**Scheme 4.** (i) NBS; (ii) NaOH; (iii) 3-Picolyl chloride-HCl, K\textsubscript{2}CO\textsubscript{3}; (iv) NaClO\textsubscript{2}, H\textsubscript{2}NSO\textsubscript{3}H; (v) (a) (COCl)\textsubscript{2}, DMF; (b) NH\textsubscript{2}OH.

Structural analysis by NMR of the solution structure of compound 6e bound in the active site of MMP-13 indicates that the \textit{para}-methoxyphenyl group lies in the S1\textsuperscript{1} pocket of the enzyme and that the pyridine ring is solvent exposed.\textsuperscript{18} The sulfonyl oxygens are within hydrogen bonding distance to the peptide backbone. The anthranilate aromatic ring occupies the S2\textsuperscript{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.\textsuperscript{19}
References and Notes

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14. All new compounds gave satisfactory 1H NMR, IR, and MS data in accord with the assigned structure.
(b) Moy, F. J.; Chanda, P. K.; Cosmi, S.; Edris, W.; Levin, J. I.; Powers, R. J. Biomol. NMR, in press.