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The Discovery of Anthranilic Acid-based MMP Inhibitors. Part 3: Incorporation of Basic Amines

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Abstract—Anthranilic acid derivatives bearing basic amines were prepared and evaluated in vitro and in vivo as inhibitors of MMP-1, MMP-9, MMP-13, and TACE. Piperazine **4u** has been identified as a potent, selective, orally active inhibitor of MMP-9 and MMP-13.

In an effort to find novel therapies for the treatment of diseases such as atherosclerosis,[1] rheumatoid arthritis, osteoarthritis,[2] and cancer[3] we have recently disclosed a series of sulfonamide hydroxamic acid inhibitors of MMP-1, MMP-9, MMP-13, and TACE, based on an anthranilic acid scaffold.[4] It has been postulated that the aberrant control of specific MMPs and TACE is a causative factor in the etiology of these pathologies. The SAR of the anthranilic acid 3- and 5-positions (**4**, R^3 and R^5) as well as the P1′ moiety (**4**, R^1), leading to compounds exemplified by **1** (Fig. 1), with nanomolar level in vitro activity, and oral bioavailability, has been discussed.[5]

In the course of our work on the anthranilate hydroxamates, it became clear that oral activity was greatly enhanced by the incorporation of a basic amine moiety into the inhibitor molecule. In fact, few compounds in this series lacking a basic amine displayed any activity in an in vivo bioactivity model. A similar circumstance has been reported for the sulfonamide-hydroxamates, exemplified by the oncology clinical candidate CGS-27023A.[6]

We now report on the synthesis and evaluation of a series of anthranilate hydroxamates, of general structure **4**, containing basic amine groups at R^2 or R^3. Analysis of NMR and molecular modeling studies of anthranilate hydroxamates bound to the active site of MMP-13 suggested that polar functionality at these two positions would be solvent exposed.[7]

Chemistry

In general, the desired sulfonamide hydroxamic acids were prepared as previously described (Scheme 1).[4,8] Sulfonamide formation was followed by attachment of the desired basic moieties and the fully functionalized anthranilate sulfonamides were then converted into the hydroxamic acids. Analogues **4a**–**4e** were obtained after alkylation of **3a** (R^1 = OMe; R^2 = H; R^3, R^4 = Me; R^5 = H) or **3b** (R^1 = OMe; R^2 = H; R^3, R^4 = Me; R^5 = Br) with the appropriate picolyl or benzylic chloride using potassium carbonate in DMF. The synthesis of benzylic amine **4f** began with the alkylation of **3a** with 4-carbomethoxybenzyl bromide, followed by selective ester hydrolysis (NaOH) and reduction of the

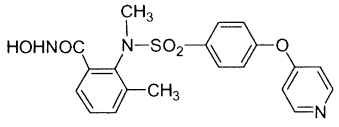

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Figure 1. Sulfonylated anthranilate hydroxamic acids.

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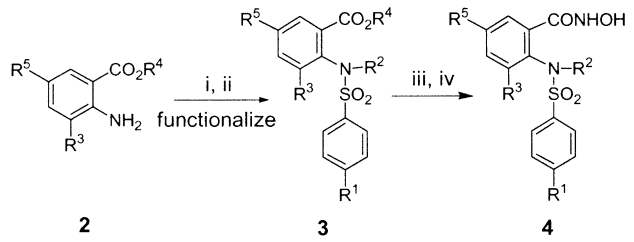


Scheme 1. (i) 4-R^1PhSO_2Cl, TEA; (ii) R^2X, NaH or K_2CO_3; (iii) NaOH; (iv) $(COCl)_2$, DMF, NH_2OH.

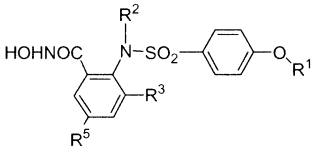
resulting acid to the alcohol with borane–THF. The alcohol was next converted into the corresponding bromide (PPh_3/CBr_4) and then displaced with dimethylamine. Aryl piperazine derivative **4g** resulted from alkylation of **3a** with 4-bromobenzyl bromide followed by Buchwald amination of the aryl bromide with 1-methylpiperazine. Propargylic amines **4h–4j** were obtained by alkylating **3a** or **3b** with propargyl bromide and subsequent Mannich alkylation. Variants **4k–4r** bearing amines at the anthranilate 3-position were prepared by amine displacement of the benzylic bromides resulting from NBS bromination of *N*-methyl sulfonamides **3c** (R^1 = OMe; R^2 = Me; R^3, R^4 = Me; R^5 = H) or **3d** (R^1 = OMe; R^2 = Me; R^3, R^4 = Me; R^5 = Br). The 5-aryl derivatives **4s–4t** were obtained by Suzuki couplings of the aryl boronic acids with **3e** (R^1 = OMe; R^2 = Me; $R^3 = CH_2N[(CH_2)_2]_2NMe$; R^4 = Me; R^5 = Br). The biaryl ether analogues **4u** and **4v** arose from **3f** (R^1 = OPh-4-Cl; R^2 = Me; R^3, R^4 = Me; R^5 = H or Br).

Biology

All of the anthranilate hydroxamic acids were tested in vitro[9] for their ability to inhibit MMP-1, MMP-9, MMP-13, and TACE[10] (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] Inhibitors of TACE are potentially valuable for the treatment of rheumatoid arthritis, Crohn's disease and other inflammatory diseases.[11] Selectivity over MMP-1 was sought in order to examine whether the inhibition of MMP-1 is a possible source of the musculoskeletal side effects seen in clinical trials of broad spectrum MMP inhibitors.[12]

The in vitro potencies against the MMPs and TACE for the series of anthranilate hydroxamic acid analogues in which a variety of basic amines have been linked to the sulfonamide nitrogen (**4a–4j**) are shown in Table 1. All of these compounds are potent inhibitors of MMP-9 and, with the exception of **4d**, MMP-13. They are comparable in vitro to CGS-27023A. The picolyl analogues, **4a–4c**, and the benzyl derivatives **4f–4g**, are essentially equipotent (3–20 nM) with similar selectivity profiles. Incorporation of a 5-bromo substituent into the gelatinase selective compound **4d** provides **4e**, a 3 nM MMP-13 inhibitor with enhanced potency against all four enzymes screened and no selectivity for MMP-9. The propargylic amines **4h–4j** are also excellent (6–19 nM) inhibitors of MMP-9 and MMP-13 with weak selectivity

Table 1. In vitro potency of substituted anthranilate hydroxamic acids



Compd	R^1	R^2	R^3	R^5	IC_{50}, (nM)			
					MMP-1	MMP-9	MMP-13	TACE
4a	Me	CH_2-3-Py	Me	H	143	5	8	231
4b	Me	CH_2-3-Py	Me	H	329	7	18	356
4c	Me	CH_2-3-Py	Me	H	391	8	18	645
4d	Me	CH_2Ph-4-$O(CH_2)_2NC_5H_{10}$	Me	H	176	7	56	277
4e	Me	CH_2Ph-4-$O(CH_2)_2NC_5H_{10}$	Me	Br	35	2	3	108
4f	Me	CH_2Ph-4-CH_2NMe_2	Me	H	213	3	11	243
4g	Me	CH_2Ph-4-$N[(CH_2)_2]_2NMe$	Me	H	142	7	20	146
4h	Me	$CH_2CCCH_2NEt_2$	Me	H	470	11	19	218
4i	Me	$CH_2CCCH_2NEt_2$	Me	Br	150	14	7	36
4j	Me	$CH_2CCCH_2N[(CH_2)_2]_2NMe$	Me	Br	129	10	6	26
4k	Me	Me	CH_2NEt_2	H	744	8	84	210
4l	Me	Me	$CH_2N[(CH_2)_2]_2O$	H	608	5	14	174
4m	Me	Me	CH_2ProMe	H	517	5	6	200
4n	Me	Me	CH_2Im	H	781	10	43	157
4o	Me	Me	$CH_2N[(CH_2)_2]_2NMe$	H	306	1	12	154
4p	Me	Me	$CH_2N[(CH_2)_2]_2NMe$	Br	194	2	5	26
4q	Me	Me	$CH_2N[(CH_2)_2]_2NPh$	Br	444	3	3	87
4r	Me	Me	$CH_2N[(CH_2)_2]_2NBoc$	Br	674	7	6	63
4s	Me	Me	$CH_2N[(CH_2)_2]_2NMe$	Ph-4-OCF_3	745	2	4	210
4t	Me	Me	$CH_2N[(CH_2)_2]_2NMe$	2-Naphthyl	343	2	3	49
4u	Ph-4-Cl	Me	$CH_2N[(CH_2)_2]_2NMe$	H	155	1	0.8	122
4v	Ph-4-Cl	Me	$CH_2N[(CH_2)_2]_2NMe$	Br	82	0.5	0.7	80
CGS-27023A	—	—	—	—	15	9	8	231

over MMP-1. Thus, compound **4h**, unsubstituted at the anthranilate 5-position, is a 19 nM MMP-13 inhibitor. Its 5-bromo analogue, **4i**, is a slightly more potent (7 nM) MMP-13 inhibitor and a substantially more potent TACE inhibitor (36 nM). Excellent TACE activity is also displayed by propargylic piperazine **4j** (26 nM), which retains activity against MMP-9 and MMP-13. However, as we had seen before,[5] despite their potency against isolated enzyme, compounds **4i** and **4j** did not display significant TNF-α inhibitory activity in a THP-1 cellular assay at 3 μM.[13]

The feasibility of incorporating amines at the 3-position of the anthranilic acid ring was explored with benzylic amines **4k**–**4v** (Table 1). The diethylamine **4k** and imidazole **4n** were the least active members of this series against MMP-1 and MMP-13, but remained potent inhibitors of MMP-9 (8–10 nM). The proline methyl ester **4m** displayed selectivity approaching 100-fold for MMP-13 over MMP-1.

The piperazines **4o**–**4v** all proved to be potent inhibitors of MMP-13. The initial analogue, **4o**, is an excellent MMP-9 (1 nM) and MMP-13 (12 nM) inhibitor. Addition of a 5-bromo substituent gives **4p** with good activity versus MMP-13 (5 nM) and TACE (26 nM), but reduced MMP-1 selectivity. Larger substituents on the piperazine, as in the *N*-phenyl and *N*-Boc piperazines, **4q** and **4r**, retained MMP-13 activity and provided the desired selectivity over MMP-1. Similarly, substitution of 5-aryl groups (**4s**–**4t**) for the 5-bromo moiety of **4p** also provided potent MMP-13 inhibitors (3–4 nM) with more than 100-fold selectivity over MMP-1. Extension of the P1′ group with the biaryl ethers **4u** and **4v** boosted potency against all four enzymes relative to their shorter P1′ analogues **4o** and **4p**, but the greater increase for MMP-13 made these compounds more than 100-fold selective over MMP-1.

It should be noted that at room temperature biaryl ether **4u** exists as a 1:1 mixture of atropisomers due to restricted rotation about the sulfonamide N–C bond. The racemate can be separated by chiral HPLC on a Chiralpak AD column eluting with ethanol. A 9:1 mixture of atropisomers is converted into a 3:2 mixture on heating for 0.5 h in refluxing ethanol. We have been unable to resolve these atropisomers in large quantity by either resolution or chemical synthesis. Compound **4u** was the only anthranilate hydroxamic acid that we investigated by chiral HPLC.

A single crystal X-ray structure of **4v** bound to the active site of MMP-13 was determined and is shown in Figure 2. As expected from our previous NMR structure determinations,[6] the anthranilate phenyl ring lies near the S2′ subsite, the biaryl ether is buried in the S1′ pocket and the piperazine ring is solvent exposed. The potency of **4v** versus MMP-1, despite its lengthy P1′ group, is consistent with previous data from this series[5] and indicates that the arginine residue that forms the bottom of the MMP-1 S1′ pocket is pushed aside to some degree by the P1′ substituent, extending the depth of the pocket.

The in vivo bioactivity after oral dosing of some of the anthranilate-hydroxamates (**4a**, **4d, 4g**–**4j**, **4l**, **4n**–**4q**, and **4s**–**4v**) against MMP-13 was initially assessed through the use of a dialysis tubing implant assay.[14] All of the compounds tested were compared to Novartis' sulfonamide-hydroxamate clinical lead, CGS-27023A, in the same experiment. Of these, **4g** (50 mpk), **4p** (50 mpk), **4u** (25 mpk), and **4v** (25 mpk) are 10–30% more potent than an equal dose of CGS-27023A, with **4u** and **4v** being the best.

Anthranilate-hydroxamates **4g**, **4p**, **4u**, and **4v** were also tested side-by-side with CGS-27023A in a bovine articular cartilage explant assay.[15] At a concentration of 1 μM, compounds **4p** and **4v** provided a level of inhibition of cartilage degradation 60 and 20% higher than CGS-27023A, respectively. Compounds **4g** and **4u** were roughly equivalent to CGS-27023A at 1 μM in this assay.

Hydroxamates **4p**, **4u**, and **4v** were next evaluated on oral dosing in an in vivo rat sponge-wrapped cartilage model.[16] All three compounds provided significant inhibition of collagen degradation in this model. However, while **4p** and **4v** at 50 mg/kg/bid were equipotent with a 50 mg/kg/bid dose of CGS-27023A, **4u** surpassed CGS-27023A. Oral dosing of **4u** at 50 mg/kg/bid provided a 73% inhibition of collagen degradation compared to a 55% inhibition by CGS-27023A at the same dose. Even a 25 mg/kg/bid dose of **4u** provided better protection from cartilage degradation than a 50 mg/kg/bid dose of CGS-27023A (38 vs 30%).

In summary, we have synthesized a series of anthranilate-hydroxamic acid MMP and TACE inhibitors bearing basic amine moieties. Many of these compounds are potent inhibitors of MMP-9 and MMP-13 in vitro. Four of these compounds were evaluated in vivo to assess their potential for treating osteoarthritis. Compounds **4g**, **4p**, **4u**, and **4v** are active in an in vitro cartilage degradation assay, display oral activity in an in

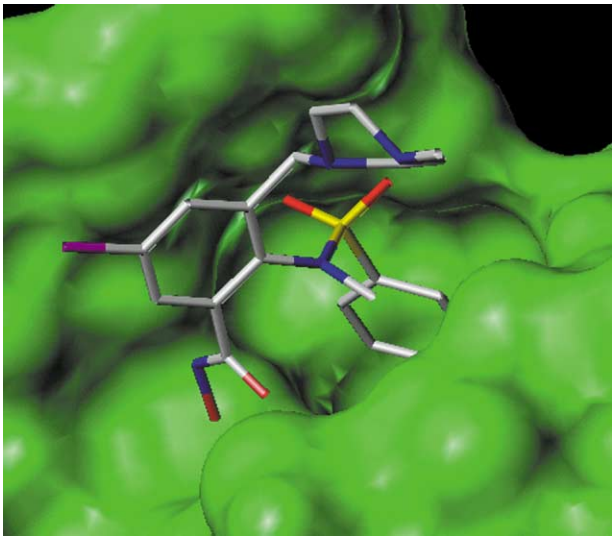


Figure 2. X-ray structure of compound **4v** in the active site of MMP-13.

vivo mouse bioactivity model and also demonstrated oral activity in a rat sponge-wrapped cartilage model. In particular, we have identified piperazine **4u** as a 0.8 nM MMP-13 inhibitor, with almost 200-fold selectivity over MMP-1, that is twice as potent as CGS-27023A in this efficacy model.

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