Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 2189–2192

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

The Discovery of Anthranilic Acid-Based MMP Inhibitors. Part 2: SAR of the 5-Position and P1[1] Groups

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Received 10 April 2001; accepted 6 June 2001

Abstract—A novel series of anthranilic acid-based inhibitors of MMP-1, MMP-9, MMP-13, and TACE was prepared and evaluated. Selective inhibitors of MMP-9, MMP-13, and TACE were identified, including the potent, orally active MMP-13 inhibitor **4p**.

The matrix metalloproteinases (MMPs), including collagenases, stromelysins, gelatinases, and membrane-type MMPs, comprise a group of over 20 zinc-containing enzymes that play a role in the normal remodeling and degradation of extracellular matrix proteins. The potential exists for potent, orally bioavailable small molecule inhibitors of MMPs to treat a broad spectrum of pathologies, including atherosclerosis,[1] rheumatoid arthritis, osteoarthritis,[2] and cancer,[3] in which the aberrant control of MMP levels has been implicated as a causative factor. For example, the nonpeptide sulfonamide hydroxamate CGS-27023A has been in oncology clinical trials (Fig. 1).

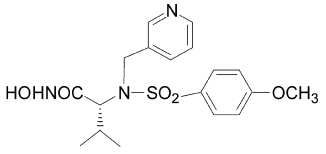
We have recently disclosed a novel series of sulfonamide hydroxamic acid inhibitors of MMP-1, MMP-9, MMP-13, and TACE (TNF-α converting enzyme), based on an anthranilic acid scaffold.[4] The SAR of the anthranilic acid 3-position leading to compounds exemplified by **1** (Fig. 1), with nanomolar level in vitro activity, and oral bioavailability, has been discussed.

We now wish to report the MMP/TACE SARs for variations at the phenylsulfonyl P1[1] moiety (**4**, R^1), as well as the anthranilic acid 5-position (**4**, R^5). Compounds selective for MMP-9, MMP-13, or TACE have been obtained by the judicious choice of substituents at these two key locations.

Chemistry

In general, the desired sulfonamide hydroxamic acids were prepared as previously described (Scheme 1).[4,5]

Variants at R^5 of hydroxamic acid **4** were prepared by starting with the appropriately substituted anthranilic acid, **2**, or via derivatization of the 5-bromo-sulfona-



CGS-27023A

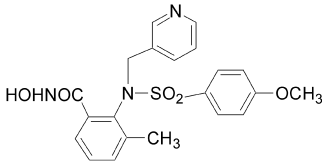


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Figure 1. Sulfonamide hydroxamic acid MMP inhibitors.

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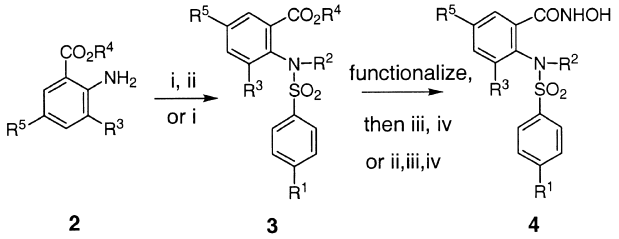
PII: S0960-894X(01)00419-X



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

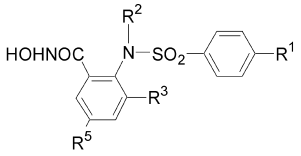
mide-ester **3a** (R^1 = OMe; R^2 = Bn or CH_2-3-Py; R^3 = Me; R^4 = Me; R^5 = Br). Thus, Suzuki couplings of **3a** with aryl boronic acids provided compounds **4c–e** after conversion of the ester into the requisite hydroxamate. The 5-diethylaminomethyl analogue, **4f**, was prepared via Stille coupling of **3b** (R^1 = OMe; R^2 = H; R^3 = Me; R^4 = Me; R^5 = Br) with tributyl(vinyl)tin to give olefin **3c**, followed by benzylation of the NH–sulfonamide, $OsO_4/NaIO_4$ oxidation of the olefin, reductive amination of the resulting aldehyde and hydroxamic acid formation. The *N*,*N*-dimethylaniline **4g** was prepared via Buchwald coupling of **3a** with tris(dimethylamino)borane.[6] Similarly, coupling of **3d** (R^1 = OMe; R^2 = H; R^3 = Br; R^4 = Me; R^5 = Me) with phenylboronic acid and 2-furanboronic acid led to **4h** and **4i**, analogues at the anthranilate 3-position.

Alkoxy P1[1] derivatives **4j–4m**, **4v–4y**, and thioether **4r** were available via fluoride displacement from a 4-fluorophenyl sulfonamide, **3e** (R^1 = F; R^2 = Me, Bn or CH_2-3-Py; R^3 = Me; R^4 = H, Me or Bn; R^5 = Me or Br), with the appropriate alcohol or thiol and NaH/DMF, or from the phenol **3f** (R^1 = OH; R^2 = Bn; R^3 = Me; R^4 = Me; R^5 = Br) via a Mitsunobu alkylation (**4u**). Biaryl P1[1] groups were introduced from the 4-bromophenyl sulfonamide **3g** (R^1 = Br; R^2 = CH_2-3-Py; R^3 = Me; R^4 = Me; R^5 = H) using Suzuki couplings (**4s–4t**). Biaryl ethers were prepared from the requisite biaryl sulfonyl chloride (**4p–4q**), or via aryl ether formation (**4n–4o**) according to the method of Chan from phenol **3f**.[7]

Biology

All of the anthranilate hydroxamic acids were tested in vitro[8] for their ability to inhibit MMP-1, MMP-9, MMP-13, and TACE[9] (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.[10] Selectivity for MMP-9 or MMP-13 or TACE over MMP-1 was sought in order to examine whether the inhibition of MMP-1 is

Table 1. In vitro potency of substituted anthranilate hydroxamic acids



Compound	R^1	R^2	R^3	R^5	MMP-1[a]	MMP-9[a]	MMP-13[a]	TACE[a]
1	OMe	CH_2-3-Py	Me	H	143	5	8	231
4a	OMe	CH_2-3-Py	Me	Br	124	24	20	43
4b	OMe	CH_2-3-Py	Me	Me	132	15	11	70
4c	OMe	CH_2-3-Py	Me	Ph	195	3	4	64
4d	OMe	CH_2-3-Py	Me	Ph-3-CF_3	542	1	2	294
4e	OMe	CH_2-3-Py	Me	2-Naphthyl	745	2	1	194
4f	OMe	CH_2Ph	Me	CH_2NEt_2	1050	5	113	44%(1)[b]
4g	OMe	CH_2-3-Py	Me	NMe_2	74	2	39	633
4h	OMe	CH_2-3-Py	Ph	Me	103	7	3	133
4i	OMe	CH_2-3-Py	2-Furyl	Me	18	8	1	61
4j	OEt	CH_2Ph	Me	Me	291	NT	24	173
4k	O-*n*-Bu	CH_2Ph	Me	Me	33%(1)[b]	144	137	377
4l	OCH_2Ph	CH_2Ph	Me	Me	25%(1)[b]	554	959	429
4m	$O(CH_2)_2Ph$	CH_2Ph	Me	Me	747	46	75	467
4n	OPh	CH_2Ph	Me	Me	376	4	6	37%(1)[b]
4o	OPh-4-*t*Bu	Me	Me	Br	25%(10)[b]	125	44	19%(1)[b]
4p	O-4-Py	Me	Me	H	3245	7	4	32%(1)[b]
4q	O-4-Py	Me	H	H	48%(10)[b]	153	38	22%(1)[b]
4r	SPh	Me	Me	Me	1314	8	3	727
4s	Ph-4-OMe	CH_2-3-Py	Me	H	2268	152	18	4%(1)[b]
4t	Ph-3,4-(-OCH_2O-)	CH_2-3-Py	Me	H	37%(10)[b]	43%(1)[b]	26	9%(1)[b]
4u	OCH_2Ph	CH_2Ph	Me	Br	18%(10)[b]	3448	1752	285
4v	OCH_2Ph	Me	Me	Br	57%(10)[b]	189	163	57
4w	OCH_2-3-Thienyl	Me	Me	Br	4640	232	142	56
4x	OCH_2-2-Thiazolyl	Me	Me	Br	45%(10)[b]	952	661	23
4y	OCH_2-3-Py	Me	Me	Br	42%(10)[b]	28%(1)[b]	48%(1)[b]	28
CGS-27023A	—	—	—	—	15	9	8	231

[a]IC_{50}, nM.
[b]% Inhibition (concentration, μM).

a possible source of the musculoskeletal side effects seen in clinical trials of broad spectrum MMP inhibitors.[11]

The in vitro potencies against the MMPs and TACE for a series of anthranilate hydroxamic acid analogues, **4a**–**4i**, in which substitution at the 5-position of the anthranilate phenyl ring was explored are shown in Table 1. All of these compounds, with the exception of analogue **4f**, are potent inhibitors of both MMP-9 and MMP-13, comparable to the 5-unsubstituted derivative **1**. Aryl or heteroaryl groups at the anthranilate 3- (**4h** and **4i**) or 5-positions (**4c**–**4e**) provide excellent activity versus MMP-13. Furthermore, the 5-aryl compounds, **4c**–**4e**, display high levels of selectivity over MMP-1, from almost 50- to over 700-fold. Interestingly, incorporating basic alkylamino substituents at the 5-position, as in diethylaminomethyl derivative **4f** and dimethylaniline **4g**, results in compounds that are potent against MMP-9 and selective over MMP-1 and MMP-13.

TACE activity is also affected by the choice of the anthranilate 5-substituent. Thus, the 5-bromo derivative **4a** is 5 times more potent against TACE than the 5-unsubstituted parent, **1**. Although a 5-bromo substituent (**4a**) provided the greatest TACE potency in the series **4a**–**4i**, all of the 5-substituents in this series which were not excessively bulky (**4d** and **4e**) or basic (**4f** and **4g**) had enhanced TACE activity relative to compound **1**. Unfortunately, despite their potency against isolated enzyme, compounds **4a**–**4i** did not display significant TNF-α inhibitory activity in a THP-1 cellular assay at 3 μM.[12]

Variations of the $P1^1$ substituent of the anthranilate hydroxamic acids are shown for compounds **4j**–**4y** in Table 1. NMR studies have shown that the R^1 group of compound **4** occupies the $S1^1$ pocket of MMP-13.[13] Lengthening of $P1^1$ alkoxy moieties (**4j**–**4l**) results in a loss of MMP-1 activity, as expected from the shallow nature of the $S1^1$ pocket of this enzyme. However, MMP-9 and MMP-13 activity for **4k** and benzyl ether **4l** also diminish beyond useful levels. Inserting an additional methylene spacer in **4l** leads to the phenethyl analogue **4m** and restores some MMP-9 and MMP-13 activity. On the other hand, the more rigid $P1^1$ biaryl ethers, **4n**–**4q**, and thioether **4r** retain or improve their potency against MMP-9 and MMP-13 relative to methyl ether **1**. Surprisingly, comparison of the neutral phenyl ethers, **4n** and **4o**, shows that a bulky *para*-substituent is required for these to realize greater than 100-fold selectivity over MMP-1. It is possible that the arginine residue that normally forms the bottom of the MMP-1 $S1^1$ pocket is pushed aside to some degree by the $P1^1$ biphenyl ether substituent of **4n**, extending the depth of the pocket. Thioether **4r** and the more polar 4-pyridyl ethers, **4p** and **4q**, do not need additional substitution and are among the most selective members of the series (>250-fold). It is important to note that compound **4q**, with its lengthy $P1^1$ moiety, no longer requires the 3-substituent on the anthranilate ring (R^3 = H) that our initial series needed to attain acceptable potency.[4] The biaryl derivatives **4s** and **4t** are also potent and selective MMP-13 inhibitors, with **4t** possessing over 300- and 35-fold selectivity over MMP-1 and MMP-9, respectively.

Although benzyl ether **4l** was only a weakly active TACE inhibitor, it was the first member of this series that was more potent against TACE than against MMP-1, MMP-9, and MMP-13. The optimization of this lead into a potent and selective TACE inhibitor is represented with compounds **4u**–**4y**. The 5-bromo analogue **4u** provides a slight increase in TACE potency over **4l** and substantially improves selectivity for TACE. Next, a 5-fold increase in potency was realized in going from *N*-benzyl analogue **4u** to the corresponding *N*-methyl sulfonamide **4v**. Replacement of the terminal phenyl ring of **4v** with a 3-thienyl group (**4w**) offered no improvement.

Thiazole analogue **4x**, however, is over twice as potent as **4v** and is approximately 400-, 40-, and 30-fold selective over MMP-1, MMP-9, and MMP-13, respectively. Still greater TACE selectivity is obtained with 3-picolyl ether **4y**, while the analogous 2- and 4-picolyl derivatives (not shown) displayed a 6- to 8-fold diminution of TACE activity.

Examination of the X-ray structure of TACE suggests that the terminal phenyl or heteroaryl ring of **4u**–**4y** is positioned in the channel connecting the $S1^1$ and $S3^1$ pockets.[14] The TNF-α inhibitory activity of compounds **4u**–**4y** in a THP-1 cellular assay was disappointingly poor, however. Thiophene **4w** was the most potent derivative in cells, affording only 22% inhibition of TNF-α at 3 μM.

The in vivo bioactivity against MMP-13 for some of the anthranilate-hydroxamates (**4c**–**4e**, **4h**–**4i**, and **4p**–**4t**) after oral dosing was assessed through the use of a dialysis tubing implant assay.[15] All of the compounds tested were compared to Novartis' sulfonamide-hydroxamate clinical lead, CGS-27023A,[16] in the same experiment. Despite the fact that the compounds tested had in vitro potencies comparable to CGS-27023A against MMP-13, only **4h** was as potent as CGS-27023A in vivo. Compounds **4c**, **4e**, **4p**, and **4r** were approximately 80% as potent CGS-27023A.

Anthranilate-hydroxamates **4c**, **4e**, and **4p** were also tested side by side with CGS-27023A in a bovine articular cartilage explant assay.[17] At a concentration of 1 μM, compound **4e** provided a level of inhibition of cartilage degradation slightly superior to CGS-27023A (**4e**: 83%/CGS-27023A: 70%, $n = 2$). Compounds **4c** and **4p** were roughly equivalent to CGS-27023A at 1 μM in this assay.

Hydroxamates **4c**, **4e**, and **4p** were then evaluated in an in vivo rat sponge-wrapped cartilage model.[18] Only pyridyl ether **4p** demonstrated significant inhibition of collagen degradation in this model. Oral dosing at 50 mg/kg/bid provided a 35% inhibition ($n = 2$) of collagen degradation compared to a 51% inhibition by CGS-27023A at the same dose.

In conclusion, we have expanded upon our initial series of anthranilate-hydroxamic acid MMP inhibitors. We have found that through the proper choice of

substituents, these compounds can be manipulated to provide potent and selective inhibitors of MMP-9 or MMP-13 or TACE. The best MMP-13 inhibitors of the series were evaluated in vitro and in vivo to assess their potential for treating osteoarthritis. Compounds **4c**, **4e**, and **4p** are active in an in vitro cartilage degradation assay and display oral activity in an in vivo mouse bioactivity model. Pyridyl ether **4p**, a potent MMP-9 and MMP-13 inhibitor with greater than 800-fold selectivity over MMP-1, has also demonstrated oral 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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