Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 1487–1490

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and SAR of Bicyclic Heteroaryl Hydroxamic Acid MMP and TACE Inhibitors

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Received 6 November 2002; accepted 16 January 2003

Abstract—Potent and selective bicyclic heteroaryl hydroxamic acid MMP and TACE inhibitors were synthesized by a novel convergent route. Selectivity and efficacy versus MMPs and TACE could be controlled by appropriate substitution on the scaffolds and by variation of the P1′ group. Select compounds were found to be effective in in vivo models of arthritis.

Matrix metalloproteinases (MMPs)[1] play a fundamental role in a variety of pathological conditions that involve extracellular matrix destruction (e.g., arthritis, tumor metastasis). TNF-α converting enzyme (TACE)[2] is responsible for the release of soluble TNF-α (tumor necrosis factor-α) whose over expression is characteristic of illnesses such as rheumatoid arthritis and Crohn's disease. Due to the broad therapeutic potential of inhibiting these enzymes this area has been a major focus of pharmaceutical research.[3] The design of inhibitors of these zinc containing enzymes has been facilitated in recent years by the determination of NMR and X-ray inhibitor-enzyme structures elucidating structural requirements that determine potency and selectivity for the different members of this class of enzymes.[4]

The anthranilic acid scaffold as a template for hydroxamic acid based MMP and TACE inhibitors was recently reported.[5,6] Crucial for potent enzyme inhibition in this system are (1) an *ortho* relationship between the hydroxamic acid and sulfonamide P1′ group and (2) a substituent at the other position *ortho* to the P1′ group (Fig. 1).

Incorporation of the required *ortho* substituent into a fused ring was proposed as an entry to a series which could explore interactions of inhibitors with new regions of the enzymes through a rigid scaffold. Heterobicyclic ring systems such as quinolines met these structural requirements and allow for preparation of functionalized inhibitors through ready synthetic access to 1-chloro-2-carboxyquinoline precursors. A novel sulfonamide anion displacement of chloride from these intermediates facilitated the preparation of analogues through a convergent synthesis route (Scheme 1).

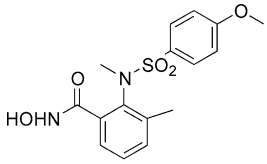

Figure 1. Representative anthranilic acid inhibitor.

The bicyclic heteroaryl ring systems **1** were accessible by condensation of an aniline or amino heterocycle **2** with ethoxymethylenemalonate diethylester **3** followed by thermally induced cyclization.[7] Treatment with phosphorus oxychloride converted the hydroxy substituent to the chloride in **4**. A convergent route to sulfonamide **6** was designed using the anion of the fully elaborated sulfonamide P1′ group **5** to displace chloride on the pyridine ring of **4**. Hydrolysis of the ester **6** with sodium

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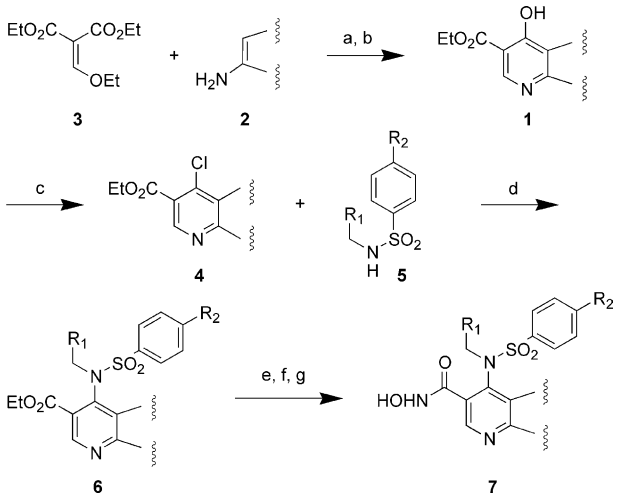
doi:10.1016/S0960-894X(03)00127-6



Scheme 1. (a) 120–150 °C; (b) Dowtherm, reflux; (c) $POCl_3$; (d) NaH, DMF; (e) NaOH; (f) $(COCl)_2$; (g) NH_2OH.

hydroxide followed by activation of the acid with oxalyl chloride and reaction of the intermediate acid chloride with hydroxylamine gave the hydroxamic acid **7**.

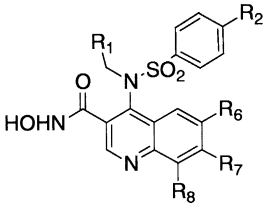
The quinoline scaffold **8** gave potent, low nanomolar inhibitors of MMP-13 and MMP-9, with selectivity versus MMP-1 and TACE (Table 1).[8] Substitution at positions six and seven of the quinoline ring system did not affect activity. In contrast, dramatic effects on TACE and MMP-1 inhibition were seen upon substitution at the eight position. Transposing a bromine substituent from the 6 or 7 position to the 8 position (**8c**,**d**) gave a greater than nine-fold increase in TACE activity.

A systematic investigation of substituents at the eight position (**8h**–**r**) revealed that this effect was general for a variety of groups. In addition, a striking, adverse effect on MMP-1 activity with a sterically bulky *tert*-butyl group at the eight position (**8r**) was seen. To a lesser extent, a C-8 trifluoromethyl group was also deleterious to MMP-1 activity (**8h**). Molecular modeling using the TACE and MMP-1 X-ray structures revealed that the quinoline 8-position interacts with the S2′ pocket of the enzymes.

Replacement of the quinoline nucleus with a heterobicyclic scaffold gave analogues **9** comparable in activity to the quinolines against MMP-13, 9 and 1 but more potent versus TACE (Table 2). In the pyrazolopyridines (**9**: X = N), removal of the R_3 methyl group (**9b**) led to a decrease of activity versus all the enzymes. A methyl or phenyl R_4 substituent gave equivalent activity. The inhibition profiles of the isoxazolopyridine (**9**: X = O) and isothiazolopyridine (**9**: X = S) analogues were not distinct from the pyrazolopyridines, though **9e** appeared to be more selective versus MMP-1.

In these scaffolds (**8** and **9**) modification of the P1′ group by replacement of the methoxy group with fluorine, phenyl or methyl led to a loss of activity versus all

Table 1. Quinoline scaffold inhibitors



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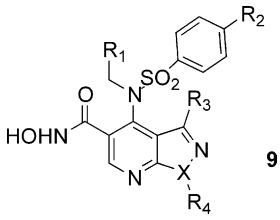
Compd	R_1	R_2	R_6	R_7	R_8	MMP-1[a]	MMP-9[a]	MMP-13[a]	TACE[a]
8a	Ph	OCH_3	Br	H	H	108	8	6	>1000
8b	Ph	OCH_3	H	Br	H	172	11	7	>1000
8c	H	OCH_3	H	H	Br	225	2	2	296
8d	3-Pyr	OCH_3	H	H	Br	65	2	1	124
8e	Ph	OCH_3	CF_3	H	H	172	11	7	>1000
8f	3-Pyr	OCH_3	H	CF_3	H	77	4	1	~1000
8g	Ph	OCH_3	H	CF_3	H	172	11	7	>1000
8h	Ph	OCH_3	H	H	CF_3	933	2	1	190
8i	3-Pyr	OCH_3	H	H	I	75	3	3	124
8j	3-Pyr	OCH_3	H	H	OCH_3	46	2	1	226
8k	3-Pyr	OCH_3	H	H	Ph	151	3	4	120
8l	3-Pyr	OCH_3	H	H	2-Thienyl	136	2	2	161
8m	3-Pyr	OCH_3	H	H	Bn	100	4	3	336
8n	H	OCH_3	H	H	Vinyl	200	5	5	205
8o	Ph	OCH_3	H	H	CH_3	152	—	26	627
8p	Ph	OCH_3	H	H	CH_2CH_3	192	2	4	314
8q	Ph	OCH_3	H	H	*i*-Pr	344	6	9	589
8r	Ph	OCH_3	H	H	*t*-Bu	3100	8	16	401
8s	3-Pyr	Ph	H	CF_3	H	4200	1162	50	124
8t	3-Pyr	CH_3	H	CF_3	H	>1000	>1000	>1000	>1000
8u	H	O-4-Pyr	H	H	H	1012	1	1	124
8v	H	O-CH_2CCCH_3	H	H	Br	956	—	27	82
8w	H	O-CH_2CCCH_3	H	H	OCH_3	875	33	9	17

[a]IC_{50}, nM.

Table 2. Heterobicyclic scaffold inhibitors



9

Compd	R_1	R_2	R_3	R_4	X	MMP-1[a]	MMP-9[a]	MMP-13[a]	TACE[a]
9a	3-Pyr	OCH_3	CH_3	CH_3	N	39	2	2	160
9b	3-Pyr	OCH_3	H	Ph	N	913	6	20	435
9c	3-Pyr	OCH_3	CH_3	Ph	N	115	2	3	138
9d	3-Pyr	OCH_3	CH_3	—	O	111	9	10	147
9e	3-Pyr	OCH_3	CH_3	—	S	550	2	3	535
9f	H	F	CH_3	CH_3	N	177	595	170	>1000
9g	H	OC_4H_9	CH_3	CH_3	N	1922	7	3	86
9h	H	O-4-ClPh	CH_3	CH_3	N	116	1	1	356
9i	H	O-4-Pyr	CH_3	CH_3	N	1805	2	1	>100
9j	H	OCH_2CCCH_3	CH_3	CH_3	N	~1000	~100	~100	30
9k	H	OCH_2CCCH_3	CH_3	—	O	1911	244	150	6
9l	H	OCH_2CCCH_3	CH_3	—	S	2333	95	34	14

[a]IC_{50}, nM.

Table 3. Cartilage explant, dialysis and sponge data for **8u** and **9i**

Compd	Cartilage explant[a,b]	Dialysis[a]	Sponge[a,c]
8u	42% (44%)	90% (88%)[d]	44% (39%)
9i	61% (32%)	54% (55%)[e]	46% (55%)

[a]CGS-27023A values in parenthesis.
[b]% Inhibition at 100 nM.
[c]% Inhibition at 50 mpk po bid.
[d]% Inhibition at 50 mpk po 1 h after dosing.
[e]% Inhibition at 25 mpk po 1 h after dosing.

Table 4. Inhibition of TNF-α release

Compd	TACE[a]	THP[b]	LPS po/ip[c]
8v	82	32	—
8w	17	58	60/95
9j	30	60	48/-
9k	6	57	52/80
9l	14	42	60/81

[a]IC_{50}, nM.
[b]% Inhibition at 3 μM.
[c]% Inhibition at 100 mpk 1 h after dosing.

enzymes, with the exception of TACE activity when R_2 was phenyl (**8s**). In other modifications, MMP activity was maintained so long as the oxygen of R_2 was present. A 4-pyridyloxy substituent gave very potent and selective inhibitors on both the quinoline (**8u**) and pyrazolopyridine (**9i**) scaffolds and these two compounds were chosen for further biological evaluation based on the expected favorable effect of basic substituents on oral bioavailability.

In the secondary in vitro assay, quinoline **8u** demonstrated effective ex vivo inhibition of cartilage degradation (42% @ 100 nM) in the bovine articular cartilage explant assay (Table 3).[9] In the primary in vivo model, it demonstrated oral bioavailability with 90% inhibition of MMP-13 at 50 mpk in the mouse dialysis tubing implant assay.[10] In a secondary in vivo model, the rat sponge-wrapped cartilage implant model of osteoarthritis,[11] **8u** showed 44% inhibition of cartilage degradation at 50 mpk po bid. The pyrazolopyridine **9i** showed comparably favorable activity to **8u** with 61% inhibition @100 nM in the cartilage explant assay, 54% inhibition at 25 mpk po in the dialysis tubing implant model and 46% inhibition at 50 mpk po bid in the sponge wrapped cartilage model. Novartis' sulfonamide-hydroxamate MMP inhibitor CGS-27023A[12] was used as a standard in the above models giving comparable inhibition. These results demonstrate that **8u** and **9i** are orally bioavailable, potent MMP inhibitors active in in vivo models of osteoarthritis.

Based on molecular modeling studies of the TACE X-ray crystal structure[13] showing that an acetylenic P1′ group could fit in the tunnel connecting the S1′ and S3′ pockets,[6] incorporation of a butynyloxy group into the P1′ position of these bicyclic scaffolds gave rise to

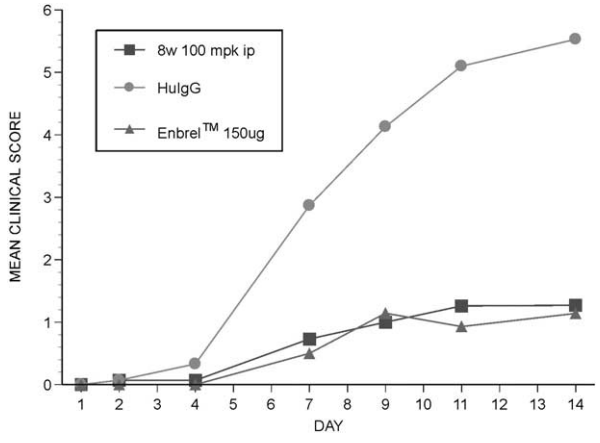


Figure 2. Cartilage induced arthritis assay.

potent and selective TACE inhibitors. The isoxazolopyridine **9k** had an IC_{50} of 6 nM against TACE and was ~300-fold selective versus MMP-1 and ~25-fold selective versus MMP-13. Importantly, in addition to the potent TACE activity, and in contrast to their non-butynyloxy counterparts, these compounds were more potent in vitro in the THP cellular assay of TNF-α release[14] and in the mouse in vivo LPS induced TNF-α release assay[14,15] (Table 4). Compound **8w** was also shown to be effective in the mouse collagen induced arthritis model of arthritis[16] at 100 mpk bid ip with a reduction of the mean clinical score relative to control (HulgG) comparable to Enbrel™ (etanercept) dosed at 150 ug/day (Fig. 2).

In summary, bicyclic heteroaryl scaffolds have utility in the preparation of potent and selective MMP and TACE inhibitors. Variation of substituents on the rings and the P1′ group altered potency and selectivity of the inhibitors through interaction with enzyme subsites. Advanced biological evaluation of inhibitors with appropriate P1′ functionality revealed oral activity in in vivo models of osteo and rheumatoid arthritis.

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