Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1553–1556

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Biaryl Ether Retrohydroxamates as Potent, Long-Lived, Orally Bioavailable MMP Inhibitors

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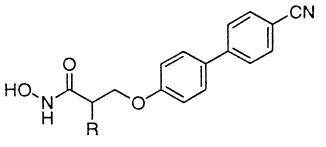
Received 18 October 2000; accepted 5 January 2001

Abstract—A novel series of biaryl ether reverse hydroxamate MMP inhibitors has been developed. These compounds are potent MMP-2 inhibitors with limited activity against MMP-1. Select members of this series exhibit excellent pharmacokinetic properties with long elimination half-lives (7 h) and high oral bioavailability (100%). © 2001 Elsevier Science Ltd. All rights reserved.

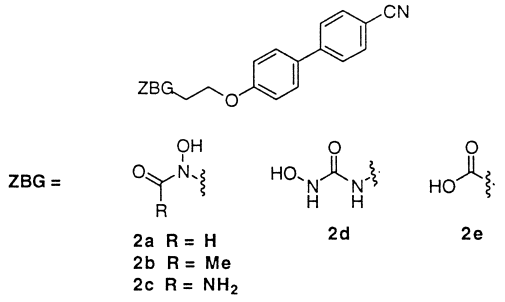
The matrix metalloproteinases (MMPs) are a family of zinc endopeptidases that collectively have the ability to degrade all the components of the extracellular matrix. Under physiological conditions MMPs play an important role in normal tissue turnover and development. However, increased MMP activity has been associated with a variety of pathological conditions such as arthritis, cancer, multiple sclerosis, and arteriosclerosis.[1] Therefore, inhibition of MMP activity represents an attractive therapeutic target and has been the subject of intense research in both industrial and academic laboratories, resulting in several structurally diverse compounds advancing to clinical trials.[2]

Our program has focused on the discovery of selective MMP inhibitors as antitumor agents. Although the role of each of the MMPs in the various pathologies is not known with certainty, an increasing body of evidence suggests a critical role for the gelatinases in tumor progression and metastasis.[3] Furthermore, selective agents may avoid the musculoskeletal side effects observed in patients treated chronically with broad spectrum inhibitors.[4]



1a R = H
1b R = Me

We have previously disclosed a series of biaryl ether hydroxamates (**1**) as potent stromelysin and gelatinase inhibitors, discovered through the use of SARs by NMR.[5] Compounds such as **1** are selective MMP inhibitors since they possess limited activity against MMP-1 and MMP-7. These two enzymes differ from other MMP family members in that they have a relatively small S1′ pocket, which presumably cannot accommodate the large biaryl substituent. However, poor pharmacokinetic properties due to rapid hydroxamate hydrolysis precluded further development of this series. We now wish to report that the replacement of the hydroxamate of **1** with an *N*-formylhydroxylamine (retrohydroxamate) **2a** leads to potent, long-lived and orally bioavailable MMP inhibitors.



2a R = H
2b R = Me
2c R = NH_2

The retrohydroxamate **2a** was prepared as shown in Scheme 1. Reaction of 1,2-dibromoethane with the biaryl phenol **3** gave the bromide **4** (25%), which was reacted with *N*,*O*-bis-Boc protected hydroxylamine to give **5** in 85% yield. Acid mediated deprotection (75%)

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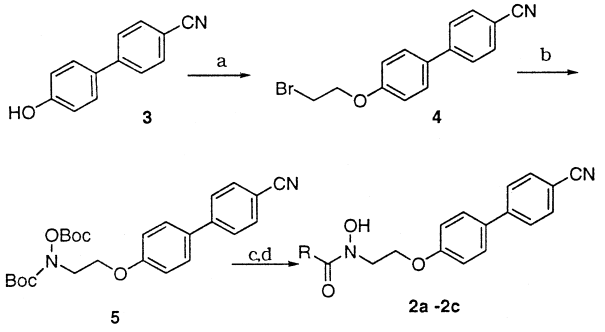
0960-894X/01/$ - see front matter
PII: S0960-894X(01)00031-2

followed by formylation with formic acetic anhydride gave **2a** in 25% recrystallized yield. Alternatively acetylation or reaction with TMSNCO gave the *N*-acetyl hydroxylamine **2b** and the *N*-hydroxyurea **2c**, respectively.

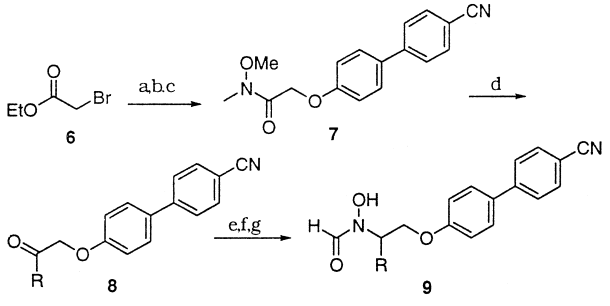
The α-alkyl and aryl substituted compounds were prepared according to Scheme 2. The key step was the addition of an alkyl or aryl Grignard to the Weinreb amide intermediate **7**. Alkylation of ethyl bromoacetate with phenol **3**, followed by ester hydrolysis and amide coupling afforded the intermediate **7** in 60% overall yield. Reaction of **7** with Grignard reagents gave the ketone **8**, which was then converted to the retro-hydroxamate through a three-step procedure involving oxime formation, reduction and formylation (40–50%).

The α-heteroatom methylene substituted compounds were synthesized as in Scheme 3, through the alkylation of the key iodoketone intermediate **12**. Thus, reaction of phenol **3** with epibromohydrin gave the epoxide **11**. Epoxide opening of **11** with iodine and triphenyl phosphine (91%) followed by Dess–Martin alcohol oxidation (75%) gave **12**. Iodide displacement with a variety of heteroatom nucleophiles gave the ketone **13**, which was converted to **14** following the same three-step sequence of reactions described in Scheme 2.

The α-ethyl linked hydantoin compounds were prepared following the same general procedures used for the preparation of **14**, except reversing the order of introduction of the α-substituent and the biaryl phenol (Scheme 4).



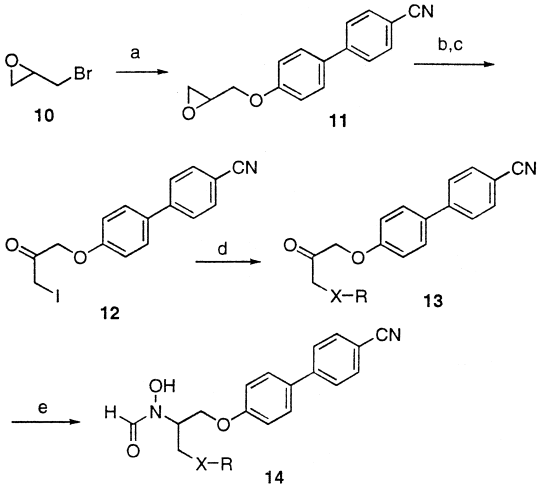
Scheme 1. (a) $Br(CH_2)_2Br$, K_2CO_3, DMF; (b) BocNHOBoc, NaH, DMF; (c) 4 N HCl, dioxane; (d) HCOOAc, THF (for **2a**); Ac_2O (for **2b**): TMSNCO, Et_3N, THF (for **2c**).



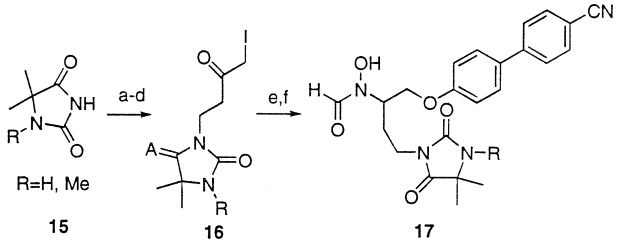
Scheme 2. (a) **3**, K_2CO_3, THF; (b) LiOH, dioxane, H_2O; (c) HNMe(OMe), Et_3N, BOPCl; (d) RMgBr, THF, −78 °C; (e) $NH_2OH{\cdot}HCl$, pyridine, THF; (f) $BH_3{\cdot}Pyr.$, 6 N HCl, EtOH; (g) HCOOAc, THF.

Thus, alkylation of hydantoin **15** followed by mCPBA epoxidation, epoxide opening with iodine and Dess–Martin oxidation gave the key iodo ketone intermediate **16** (45% overall). Alkylation of **16** with phenol **3**, and further elaboration as in the previous schemes provided **17**.

The biaryl ether hydroxamates, although potent MMP inhibitors in vitro, had very poor pharmacokinetic properties with elimination half-lives of less than 30 min due to rapid hydrolysis to the corresponding carboxylic acid. The acids are generally two orders of magnitude weaker inhibitors (cf. **2a** vs **2e**). This led to an investigation of alternate zinc binding groups that would

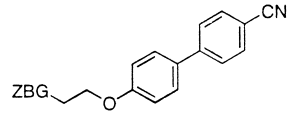


Scheme 3. (a) **3**, K_2CO_3, THF; (b) I_2, Ph_3P, CH_2Cl_2; (c) Dess–Martin, CH_2Cl_2; (d) HX-R, K_2CO_3, DMF; (e) same as e, f, and g in Scheme 2.



Scheme 4. (a) 3-Buten-1-ol, Ph_3P, DEAD; (b) mCPBA; (c) I_2, Ph_3P; (d) Dess–Martin; (e) **2**, K_2CO_3; (f) same as e, f, and g in Scheme 2.

Table 1. Alternate chelators: MMP enzyme inhibition potency[7]

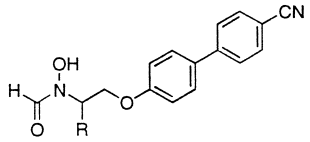


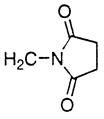
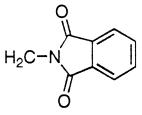
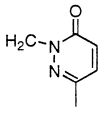
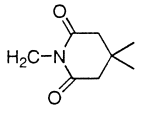
Compd	ZBG	IC_{50} (μM)		
		MMP-2	MMP-3	MMP-1
1a	CONHOH	0.032	0.057	3.3
2a	N(OH)CHO	0.4	0.62	>50
2b	N(OH)COMe	5.9	4.0	nd[a]
2c	$N(OH)CONH_2$	35	>100	>50
2d	NHCONHOH	>100	80	>50
2e	COOH	8.0	>10	nd[a]

[a]nd = Not determined.

potentially be more stable in vivo (Table 1). Replacement of the hydroxamate with either an N-terminal (**2d**) or internal *N*-hydroxyurea (**2c**) led to a dramatic loss of activity. Reversing the regiochemistry of the hydroxamate moiety led to the *N*-formyl and *N*-acetyl hydroxylamines (retrohydroxamates) **2a** and **2b**, respectively.[6] The *N*-formyl hydroxylamine **2a** was only 10-fold weaker than the 'normal' hydroxamate **1a** whereas the *N*-acetyl derivative **2b** was significantly weaker. Consequently, attention was focused on the *N*-formyl analogues. The α-methyl derivative **9a** was more potent than **2a** and only 3-fold less potent than the corresponding hydroxamate **1b** (Table 2). More importantly **9a** had significantly better pharmacokinetic properties than **1b** when dosed intravenously and orally in rat. This compound exhibited a half-life of 1.6 h and oral bioavailability of 33% as opposed to 0.3 h and 7%, respectively, for **1b**. Thus, a systematic investigation of the effect of α-substitution on potency and pharmacokinetics was undertaken. Introduction of larger alkyl and aryl groups (**9b**–**9d**) or aryl ether and thioether (**14a** and **14b**) had a deleterious effect on MMP-2 potency. Heteroalkyl groups such as succinimide, phthalimide and pyridazinone gave a boost in MMP-2 inhibitory potency, however they all exhibited poor pharmacokinetic properties as evidenced by the short (< 1 h) half-lives after intravenous administration in cynomolgous monkeys. Interestingly, introduction of a glutarimide led to a 10-fold increase in MMP-1 potency.

Table 2. MMP enzyme inhibition potency and elimination half-lives: effect of α-substitution

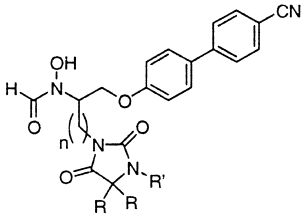


Compd	R	IC_{50} (nM)			Half-life, iv
		MMP-2	MMP-3	MMP-1	
1b	—	19	45	2500	0.3 h[a]
9a	Me	76	620	>10,000	1.6 h[a]
9b	*i*-Bu	950	120	>10,000	nd
9c	pF-Ph	480	300	>10,000	nd
9d	CH_2-(*p*-F-Ph)	650	180	14,000	nd
14a	CH_2OPh	260	130	>10,000	nd
14b	CH_2SPh	160	36	4100	0.3 h[b]
14c		5.2	11	>10,000	0.6 h[b]
14d		37	21	>10,000	0.3 h[b]
14e		3.4	37	4200	0.5 h[b]
14f		25	37	360	nd

[a]Rat.
[b]Cynomolgous monkey.

One of the heterocycle substituents that has been extensively investigated in several series of MMP inhibitors is the hydantoin moiety. In particular two MMP inhibitors that advanced into clinical studies, RO 32-3555 and D-2163, contain a 4,4-dimethyl-*N*-methyl hydantoin.[8] The analogous 4,4-dimethyl-*N*-methyl hydantoin substituted retrohydroxamate **14g** was prepared and found to exhibit approximately the same potency against MMP-2 ($IC_{50} = 58$ nM) as the α-methyl compound **9a** ($IC_{50} = 76$ nM), although significantly more potent against MMP-3 ($IC_{50} = 9.1$ nM vs 620 nM). The *N*-ethyl analogue **14h** showed improved potency against MMP-2 ($IC_{50} = 12$ nM) and over 100-fold selectivity over MMP-1 (Table 3). Pharmacokinetic evaluation of these two compounds in cynomolgous monkey showed that

Table 3. Hydantoin substituted retrohydroxamates: MMP enzyme inhibition potency and pharmacokinetics



Compd	*n*	R	R′	IC_{50} (nM)			$t_{1/2}$ iv[a] (h)	AUC, iv[a] μmol·h/L	AUC po (dose)[b] μmol·h/L (mg/kg)	F (%)
				MMP-2	MMP-3	MMP-1				
14g	1	Me	Me	58	9.1	2300	2.5	15.2	11.8 (10)	23%
14h	1	Me	Et	12	27	2400	1.8	12.3	nd[c]	nd[c]
14i	1	Me	H	7.8	24	2100	7.7	24	27.2 (3)	110%
14j	1	H	H	8.2	14	2400	7.3	59.8	68 (3)	100%
17a	2	Me	Me	45	17	>10,000	0.5	8	1.8 (10)	6%
17b	2	Me	H	6.9	35	14,000	12.7	47.5	2.4 (3)	5%

[a]Dosed at 3 mg/kg, cynomolgous monkey.
[b]Dosed as a solution in 10% ethanol:0.2% hydroxypropyl-methylcellulose, $n = 2$.
[c]nd = Not determined.

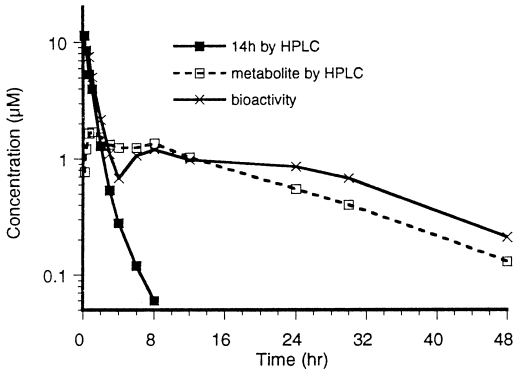


Figure 1. Blood levels after iv administration of **14h**.

these compounds have half-lives of 1.8 and 2.5 h, respectively. Compound **14g** was also administered orally and found to possess 23% oral bioavailability. However, HPLC analysis of blood samples revealed the presence of a long-lived metabolite that is common to both **14g** and **14h**. Analysis of the same samples via a stromelysin bioassay indicated that the bioactivity correlated with the concentrations of metabolite as measured by HPLC at the time points when no detectable parent was present (Fig. 1).

HPLC–mass spectrometry analysis indicated a mass of 422 for the metabolite corresponding to the loss of methyl and ethyl for **14g** and **14h**, respectively. Thus, the putative bioactive metabolite **14i** was prepared and shown to co-elute with the metabolite seen in vivo. Gratifyingly this compound is a potent MMP-2 inhibitor ($IC_{50} = 7.8$ nM) with only weak potency against MMP-1. As expected this compound is long-lived with a half-life after intravenous administration of 7.7 and 6 h in cynomolgous monkey and marmoset, respectively. Remarkably **14i** also possesses excellent oral bioavailabilty in both cynomolgous monkey and marmoset ($F = 110$ and 85%, respectively).[9] The unsubstituted hydantoin analogue **14j** has a similar in vitro profile and is also long-lived and orally bioavailable indicating that the *gem*-dimethyl moiety does not effect the pharmacokinetic profile. The homologated hydantoin analogues **17a** and **17b** have similar MMP-2 potency, but are significantly more selective against MMP-1. However, despite the close structural similarity to **14i** these compounds suffer from low oral biovailability ($F = 5$–6%).

In summary, a series of biaryl ether retrohydroxamates has been identified that are potent MMP-2 inhibitors. Select members of this series possess long elimination half-lives and show excellent oral bioavailability. Further work to optimize the in vitro profile of these compounds and in vivo efficacy is presented in the following paper.[10]

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