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β-*N*-Biaryl ether sulfonamide hydroxamates as potent gelatinase inhibitors: Part 1. Design, synthesis, and lead identification

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Abstract—A new series of β-*N*-biaryl ether sulfonamide hydroxamates as novel gelatinase inhibitors is described. These compounds exhibit good potency for MMP-2 and MMP-9 without inhibiting MMP-1. The structure–activity relationships (SAR) reveal the biaryl ether type P1′ moiety together with methanesulfonamide is the optimal combination that provides inhibitory activity of MMP-9 in the single-digit nanomolar range.

Matrix metalloproteinases (MMPs) are a family of structurally related zinc-dependent proteolytic enzymes that digest extracellular matrix proteins such as collagen, elastin, laminin, and fibronectin. The uncontrolled activity of the gelatinase sub-family of MMPs, gelatinase A (MMP-2) and gelatinase B (MMP-9), has been implicated in a number of pathological events leading to cancer, inflammatory diseases, cardiovascular diseases, and neurological disorders.[1] Therefore, inhibiting MMP-2 and/or MMP-9 activity as a potential treatment in these therapeutic areas is highly desirable.[2]

During the past two decades, the sulfonamide-based hydroxamates, particularly the α-sulfonamide hydroxamates (Fig. 1, **A**), have attracted a significant level of attention in the design of MMP inhibitors.[3] These synthetic inhibitors contain two key features which include: (1) a hydroxamate moiety as the Zn-binding group (ZBG) and (2) a sulfonyl group that provides a vital H-bonding interaction with the enzyme backbone. This critical interaction directs the P1′ group into the S1′ pocket. In addition, having the P1 group at the α-position in a favorable configuration may contribute to the potency and/or slow down the metabolism of the hydroxamate moiety.[4] A representative example of a drug candidate based on this design, CGS-27023A (Fig. 1), has advanced in clinical trials for the potential treatment of cancer.[5]

In our program directed toward novel gelatinase inhibitors, we envisioned the β-*N*-biaryl ether sulfonamide hydroxamates (Fig. 1, **B**) by simply switching the P1′

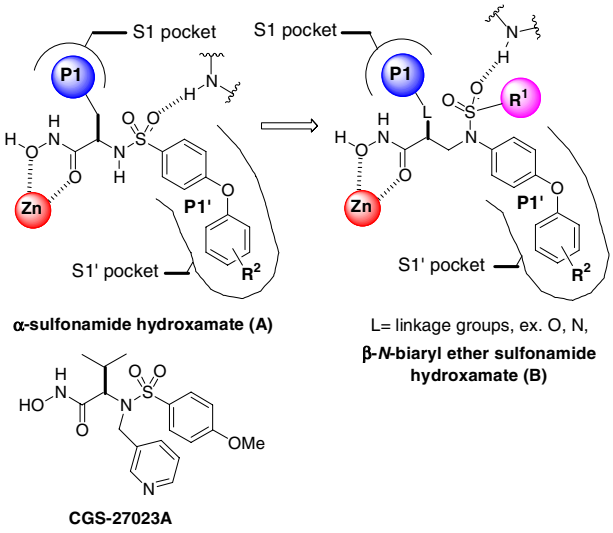

Figure 1. α-Sulfonamide hydroxamates (**A**) versus β-*N*-biaryl ether sulfonamide hydroxamates (**B**), and structure of CGS-27023A.

Keywords: Gelatinase; Matrix metalloproteinase; MMP inhibitor; Hydroxamate.
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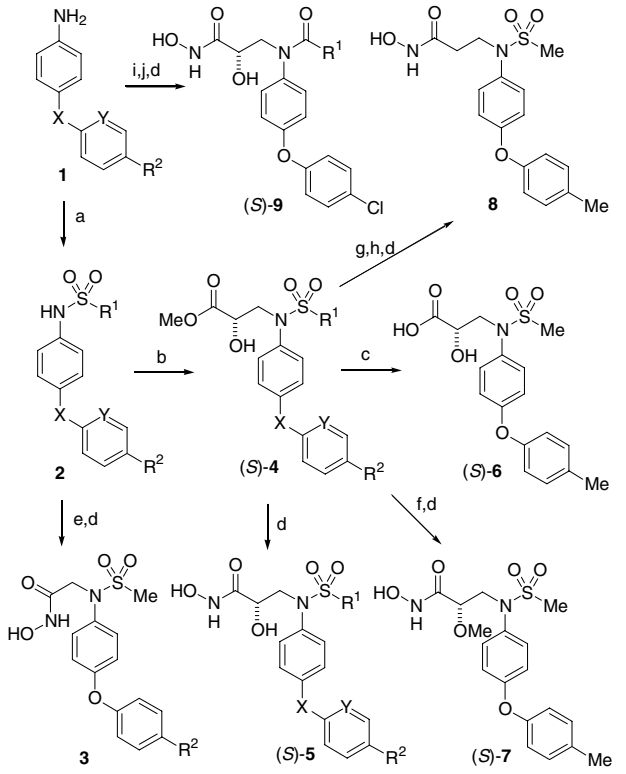
moiety from the sulfonyl group to the nitrogen atom based on the traditional approach illustrated by structure **A**.[6] The sulfonamide unit was transferred from the α- to β-position to further maintain a favorable distance between the hydroxamate moiety and P1′ group. Various substituents could be further introduced at the α-position by using a heteroatom (e.g., O, N) linker (L) to provide a possible interaction with the S1 subsite. Additionally, the substituent on the sulfonamide (R^1) may also offer potential interactions with the S2′ and/or the S3′ subsites. Herein, we disclose our preliminary results in the discovery of β-*N*-biaryl ether, sulfonamide-based hydroxamates as novel MMP-2/MMP-9 inhibitors. Evaluating the enzyme structure–activity relationships (SAR) by modifying the R^1 substituent, the P1′ group, the chirality of the α-carbon center, and the position of *N*-biaryl ether sulfonamide moiety facilitated in identifying new chemical leads.

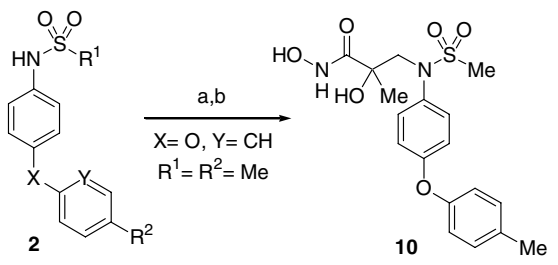
To test the feasibility of this design and rapidly explore the SAR of the R^1 substituent and P1′ group, a two-step synthetic sequence was investigated to efficiently assemble the key intermediates (*S*)-**4** (Scheme 1). The synthesis of (*S*)-**4** began with the formation of sulfonamides **2** from commercially available biaryl ether anilines **1** and diverse sulfonyl chlorides. Under basic conditions, sulfonamides **2** could promote facile ring-opening reactions with epoxides, such as methyl (*S*)-glycidate, to afford (*S*)-**4** with the desired skeleton constructed.[7] This ring-opening process could be performed either thermally in the presence of a catalytic amount of the phase transfer reagent $BnEt_3NCl$, or by using microwave irradiation, with similar efficiency. The esters (*S*)-**4** were further converted to the corresponding hydroxamates (*S*)-**5** using standard hydroxamate formation conditions (hydroxylamine hydrochloride salt with sodium methoxide in methanol).[8] The carboxylic acid (*S*)-**6** and hydroxamate (*S*)-**7** were also prepared from (*S*)-**4** by hydrolysis or by alkylation followed by hydroxamate formation, respectively. These sequences were applicable in the synthesis of both enantiomers, depending on the chirality of the epoxide employed. Removal of the hydroxyl group of (*S*)-**4** gave sulfonamides **8**. This extended analog could be directly compared to the α-*N*-biaryl ether sulfonamides **3**. Alternatively, direct epoxide opening of methyl (*S*)-glycidate with **1** followed by acylation and hydroxamate formation gave β-*N*-biaryl ether amide hydroxamates (*S*)-**9**. Compound **10** was prepared in a similar fashion (Scheme 2).



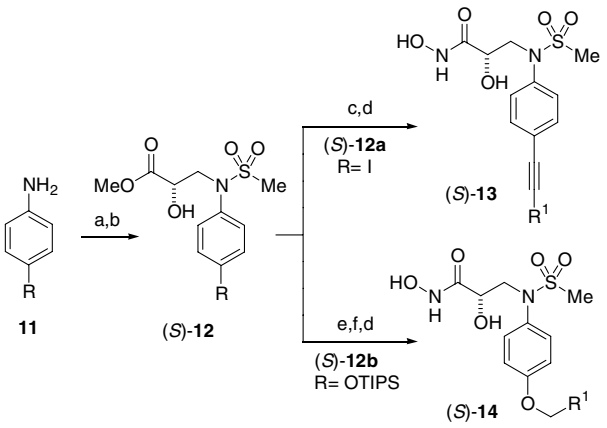
Scheme 1. Reagents and conditions: (a) R^1SO_2Cl, pyridine, 0 °C to rt, 1 h, 48–96%; (b) method A: methyl (*S*)-glycidate, K_2CO_3, $BnEt_3NCl$, dioxane, 60–90 °C, 55–91%. Method B: methyl (*S*)-glycidate, K_2CO_3, DMF, 80–100 °C, 71–86%; (c) $LiOH_{(aq)}$, THF, 90%; (d) $HONH_2{\cdot}HCl$, NaOMe, MeOH, 35–93%; (e) ethyl bromoacetate, K_2CO_3, DMF, microwave, 120 °C, 30 min, 86–93%; (f) MeI, NaH, DMF, rt, 30 min, 55%; (g) Tf_2O, 2,6-lutidine, −20 °C to 0 °C, 91%; (h) i—LiI, THF, 30 min; ii—Zn, cat. $NiCl_2{\cdot}6H_2O$, cat. H_2O, THF, 20 h, 55%; (i) methyl (*S*)-glycidate, DMSO, 60 °C, 24 h, 70%; (j) Ac_2O or *iso*-butyryl chloride, pyridine, CH_2Cl_2, rt, 92–93%.

Additionally, other potential P1′ moieties containing an extended linker, such as an acetylene ((*S*)-**13**) or a more flexible OCH_2 ((*S*)-**14**), were also synthesized (Scheme 3). For preparation of (*S*)-**13**, the Sonogashira coupling was applied to introduce the linear biaryl acetylene from (*S*)-**12a** (R = I). On the other hand, when R is a triisopropylsiloxy group (OTIPS, (*S*)-**12b**), the deprotection followed by an alkylation with substituted aryl halides and then hydroxamate formation gave the desired compound (*S*)-**14**.

Several compounds were prepared and assayed in vitro against MMP-9 and MMP-2 (Table 1)[9] to rapidly identify suitable leads and prove the proposed concept. Broad spectrum MMP inhibitors have been implicated in musculoskeletal syndrome (MSS), a phenomenon observed in clinical trials.[10] It has been speculated that the MSS results from long-term inhibition of MMP-1.[11] Therefore, the inhibitory activities of MMP-1 were also tested to determine the selectivity for MMP-2/-9 versus MMP-1. In general, these compounds showed moderate activities against MMP-2 and slightly better potency toward MMP-9, without inhibiting MMP-1 ($IC_{50} > 10$ μM). This is consistent with literature exam-



Scheme 2. Reagents and conditions: (a) methyl 2-methylglycidate, K_2CO_3, DMF, 120 °C, 20 min, 88%; (b) $HONH_2{\cdot}HCl$, NaOMe, MeOH, 67%.



Scheme 3. Reagents and conditions: (a) $MeSO_2Cl$, pyridine, 0 °C, 93–98%; (b) methyl (*S*)-glycidate, K_2CO_3, $BnEt_3NCl$, dioxane, 60–90 °C, 71–73%; (c) $R^1C{\equiv}CH$, $PdCl_2(PPh_3)_2$ (10 mol%), CuI (10 mol%), Et_3N, THF, rt, 2 h, 81–93%; (d) $HONH_2{\cdot}HCl$, NaOMe, MeOH, 89–96%; (e) TBAF, THF, 0 °C to rt, 1 h, 72%; (f) R^1CH_2Br, K_2CO_3, acetone, 50–55 °C, 72–74%.

ples showing evidence that the shallow S1′ pocket of MMP-1 typically favors a shorter P1′ group. The β-*N*-biaryl ether sulfonamide **8** has an IC_{50} value of 6.6 nM against MMP-9 that is about 5-fold more potent than **3b** (31 nM, an α-*N*-biaryl ether sulfonamide). Notably, this β-*N*-biaryl ether skeleton exhibited some selectivity (ca. 4- to 12-fold) for MMP-9 over MMP-2 as observed in compounds **5a**, **7**–**8**, and **10**. The introduction of small α-substituents has a minor effect on IC_{50} (e.g., (*S*)-**5a**, (*S*)-**7**, and **10** vs **8**). Surprisingly, the chirality of the α-position ((*R*)-**5a** vs (*S*)-**5a** and (*R*)-**7** versus (*S*)-**7**) marginally influenced potency. These results strongly suggested that small substituents, such as OH, Me, and OMe, were well tolerated at the α-position in the present skeleton. On the other hand, using the carboxylic acid as the ZBG (e.g., (*S*)-**6)** eliminated activity for both MMP-2 and MMP-9. Meanwhile, changing the sulfonamide to an amide also caused a 120-fold loss of potency ((*S*)-**5a** versus (*S*)-**9a**). These preliminary results clearly demonstrated the advantage and potential of a β-*N*-biaryl ether sulfonamide moiety in the design of MMP inhibitors.

With this novel skeleton identified, the attention was then focused on investigating the SAR around the sulfonyl group (R^1) and the P1′ moiety based on the lead compound (*S*)-**5a**. Various substituents on the sulfonamide were initially evaluated with a fixed P1′ group, 4-methyl-biphenyl ether. The significant shift of the activity to micromolar range, observed from the results compiled in Table 2, revealed the increased steric influence close to the SO_2. For example, changing the size from methyl ((*S*)-**5a**) to ethyl ((*S*)-**5b**), *n*-propyl ((*S*)-**5c**), *iso*-propyl ((*S*)-**5d**), NMe_2 ((*S*)-**5i**), and phenyl ((*S*)-**5e**) resulted in ca. 3- to 44-fold loss of potency against both MMP-2 and MMP-9. However, moving the phenyl group slightly away by inserting a methylene, for example by replacing the phenyl with a Bn ((*S*)-**5f**), brought the potency back to double-digit nanomolar range against MMP-9. A similar trend for examples (*S*)-**5g**–**h** was also observed. These results suggested that altering the interaction between S2′/S3′ subsites is critical and that the methyl substituent seems to be a superior functionality based on the in vitro results.

The optimization of the P1′ group was next performed. Several directions were explored, including the linker group between the biaryl, the shape of the P1′ unit, and the substitution effect on the aryl ring. Among the compounds tested, the oxygen linker (biaryl ether) was found to be optimal. As shown in Table 2, replacing the oxygen linker with a sulfur atom decreased the IC_{50} around 5-fold for both MMP-2 and MMP-9 ((*S*)-**5n** versus (*S*)-**5a**). Moreover, complete loss of activity was found by using SO_2 ((*S*)-**5p**) as the linker. The activities also dropped significantly when the more flexible and longer OCH_2 ((*S*)-**14a**–**c**) was employed as the linker group. The rigid, linear P1′ moiety, elongated by an acetylene unit ((*S*)-**13a**–**c**), provided double-digit nanomolar activities. These results were better than (*S*)-**5q** which contained a shorter biphenyl P1′ group. Some of the analogs represented by (*S*)-**13** and (*S*)-**14** showed a slight preference for the MMP-2 over the MMP-9 enzyme. This preference may be attributed to the tunnel-like S1′ subsite of MMP-2, which may tolerate the longer P1′ moiety better than the MMP-9 S1′

Table 1. Lead structure/skeleton identification: SAR of **3**, **5a**, and **6**–**10**

Compound	R^1	R^2	MMP-1 IC_{50}[a] (μM)	MMP-2 IC_{50}[a] (nM)	MMP-9 IC_{50}[a] (nM)
3a	—	H	>10	147[b]	383[b]
3b	—	Me	—	73	31
8	—	—	>10	61[b]	6.6[b]
(*R*)-**5a**	Me	Me	>10	67[b]	12[b]
(*S*)-**5a**	Me	Me	>10	61[b]	7.8[b]
10[c]	—	—	>10	20[b]	5.5[b]
(*S*)-**6**	—	—	—	<10%[d]	<10%[d]
(*R*)-**7**	—	—	>10	102	13
(*S*)-**7**	—	—	>10	36[b]	3.8[b]
(*S*)-**9a**	Me	—	—	1262	933
(*S*)-**9b**	*i*-Pr	—	—	286	242

[a] Single experiment.
[b] Values are means of at least two experiments.
[c] Racemic mixture.
[d] % inhibition at 10 μM.

Table 2. SAR of (*S*)-**5**, (*S*)-**13**–**14** on substituents of sulfonamide and P1′ moiety

Compound	X	Y	R^1	R^2	MMP-1 IC_{50}[a] (μM)	MMP-2 IC_{50}[a] (nM)	MMP-9 IC_{50}[a] (nM)	Fold selectivity MMP-2/MMP-9
(*S*)-**5a**	O	CH	Me	Me	>10	61	7.8	7.8
(*S*)-**5b**	O	CH	Et	Me	>10	142	28	5.1
(*S*)-**5c**	O	CH	*n*-Pr	Me	>10[b]	509[b]	124[b]	4.1
(*S*)-**5d**	O	CH	*i*-Pr	Me	>10[b]	1233[b]	286[b]	4.3
(*S*)-**5e**	O	CH	Ph	Me	>10[b]	2382[b]	344[b]	6.9
(*S*)-**5f**	O	CH	Bn	Me	>10[b]	582[b]	93[b]	6.3
(*S*)-**5g**	O	CH	(pyridin-3-yl)CH_2	Me	>10[b]	401[b]	69[b]	5.8
(*S*)-**5h**	O	CH	(pyridin-4-yl)CH_2	Me	>10[b]	344[b]	54[b]	6.4
(*S*)-**5i**	O	CH	NMe_2	Me	>10[b]	1057[b]	243[b]	4.3
(*S*)-**5j**	O	CH	Me	H	>10	247	39	6.3
(*S*)-**5k**	O	CH	Me	Cl	>10	35	4.7	7.4
(*S*)-**5l**	O	CH	Me	CF_3	—	139	14	9.9
(*S*)-**5m**	O	N	Me	CF_3	—	2535	472	5.4
(*S*)-**5n**	S	CH	Me	Me	—	297	54	5.5
(*S*)-**5o**	S	CH	Me	F	—	283	104	2.7
(*S*)-**5p**	SO_2	CH	Me	H	—	>10 μM[b]	>10 μM[b]	—
(*S*)-**5q**	—	CH	Me	H	—	87%[b,c]	79%[b,c]	—
(*S*)-**13a**	—	—	4-MePh	—	>10	47	44	1.1
(*S*)-**13b**	—	—	4-ClPh	—	>10[b]	56	47	1.2
(*S*)-**13c**	—	—	4-MeOPh	—	>10[b]	32	91	0.4
(*S*)-**14a**	—	—	4-FPh	—	>10[b]	2317[b]	4819[b]	0.5
(*S*)-**14b**	—	—	4-ClPh	—	>10[b]	893[b]	984[b]	0.9
(*S*)-**14c**	—	—	4-CF_3Ph	—	>10[b]	2917[b]	1489[b]	1.9

[a] Values are means of at least two experiments.
[b] Single experiment.
[c] % inhibition at 10 μM.

Table 3. PK[a], protein binding, and in vitro microsomal stability of (*S*)-**5a** and **10**

Compound	C_{max}[b] (μM)	$t_{1/2}$[b] (h)	AUC[b] (μg h/mL)	Vss[b] (L/kg)	CL[b] (mL/min/kg)	HLM/RLM[c] ($t_{1/2}$, min)	PB[c] (%)
(*S*)-**5a**	1.3	0.43	0.18	1.4	47.5	>2 h/14	95
10	1.5	0.72	0.22	1.3	41.5	109/10	83

[a] Mean value of 4 animals (rats) at 0.5 mg/kg iv dose.
[b] C_{max}, plasma concentration at t = 5 min; $t_{1/2}$, apparent elimination half-life; AUC, area under curve; Vss, volume of distribution at steady state; CL, clearance level.
[c] HLM, human liver microsome; RLM, rat liver microsome; PB, protein binding.

pocket. The nature of the substituent (R^2) on the aryl ring is also important. For instance, without substitution at the *para*-position ((*S*)-**5j**, R^2 = H) the compounds were ca. 4-fold less potent. The chlorine substituent ((*S*)-**5k**) exhibited similar activities as the methyl ((*S*)-**5a**). Electron-withdrawing groups, such as CF_3 or F, only slightly influenced the activity, (*S*)-**5a** versus (*S*)-**5l** and (*S*)-**5n** versus (*S*)-**5o**. Finally, replacement of the phenyl ring with a heteroaryl ((*S*)-**5m**) caused the potency to drop significantly. This is consistent with the fact that the hydrophobic residues that surround the S1′ pocket typically favor a more lipophilic P1′ moiety.

Compounds (*S*)-**5a** and **10** were initially selected and their pharmacokinetics (PK, rats) and other ADME properties evaluated (Table 3). In general, a relatively poor PK profile was obtained for both analogs. The low plasma exposure (low C_{max} and AUC values) may be attributed to the high clearance rate (CL) and poor rat liver microsomal (RLM) stability. Notably, several analogs exhibited low protein binding (PB), particularly **10** (PB = 83%). In addition, these compounds had moderate water solubility (28–70 μg/mL) at pH 7.0, but were relatively insoluble (<5 μg/mL) at pH 2.0.

In summary, a promising series of gelatinase (MMP-2 and MMP-9) inhibitors bearing a novel β-*N*-biaryl ether sulfonamide moiety has been identified. The preliminary SAR suggested that the methanesulfonyl group together with biaryl ether type P1′ moiety is the optimal combination to afford single-digit nanomolar activities against MMP-9. This skeleton also exhibited great selectivity for MMP-9/MMP-2 over MMP-1. Notably, some analogs showed low PB. The introduction and optimization of an α-amino substituent to further increase potency and improve the ADME properties has been investigated. These results will be disclosed in the following communication.

Acknowledgments

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