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β-*N*-Biaryl ether sulfonamide hydroxamates as potent gelatinase inhibitors: Part 2. Optimization of α-amino substituents

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Abstract—The introduction and the optimization of an α-amino substituent based on a series of α-hydroxy-β-*N*-biaryl ether sulfonamide hydroxamates is described. The modification leads to a new series of MMP-2/MMP-9 inhibitors with enhanced inhibitory activities and improved ADME properties. An efficacy study on reducing the ischemia-induced brain edema in the rat transient middle cerebral artery occlusion (tMCAo) model is also demonstrated.

Gelatinases, a sub-family of matrix metalloproteinases (MMPs), include gelatinase A (MMP-2) and gelatinase B (MMP-9). These enzymes are responsible for the degradation of extracellular matrix proteins such as denatured collagen, elastin, laminin, and fibronectin. The up-regulation of MMP-2 and/or MMP-9 activity has been implicated in a number of pathological events leading to cancer, inflammatory diseases, cardiovascular diseases, and neurological disorders.[1] Recent studies have demonstrated that the gelatinase expression level and activities increased markedly following an ischemic insult.[2] This results in vascular breakdown and increased permeability of the blood–brain barrier (BBB) that ultimately leads to edema, hemorrhaging, and significant brain damage.[3] Therefore, inhibiting MMP-2 and/or MMP-9 activity as a potential treatment of acute ischemic stroke (IS) is highly desirable.[4]

Several cyclic amides (**1**)[5] or sulfonamides (**2**)[6] bearing a biaryl or biaryl ether P1′ moiety on the nitrogen atom have been recently identified (Fig. 1). Inhibitors containing these substitution patterns have demonstrated excellent inhibitory activities against MMPs and several have been considered as potential drug candidates. We have previously identified a novel series of α-hydroxy-β-sulfonamide hydroxamates (Fig. 1, **3**) during a program directed toward the discovery of gelatinase inhibitors for the potential treatment of acute IS. Compounds **3**, which also contain a *N*-biaryl ether unit, exhibited significant activity for MMP-2/MMP-9, but lacked suitable solubility and sufficient pharmacokinetic (PK) properties preventing further development.[7] Herein we disclose the introduction and the optimization of an α-amino group that led to a new series of gelatinase inhibitors with enhanced inhibitory activities and improved ADME properties.[8] An efficacy study on reducing the ischemia-induced brain edema in the rat transient mid-

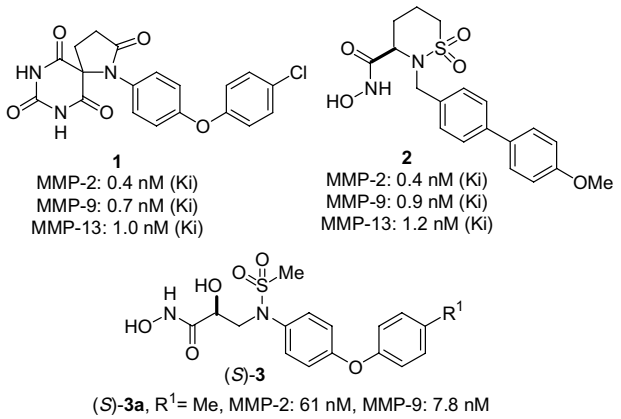

Figure 1. Potent MMP inhibitors bearing a P1′ moiety on the nitrogen atom.

Keywords: Gelatinase; Matrix metalloproteinase; MMP inhibitor; Stroke; Hydroxamate.
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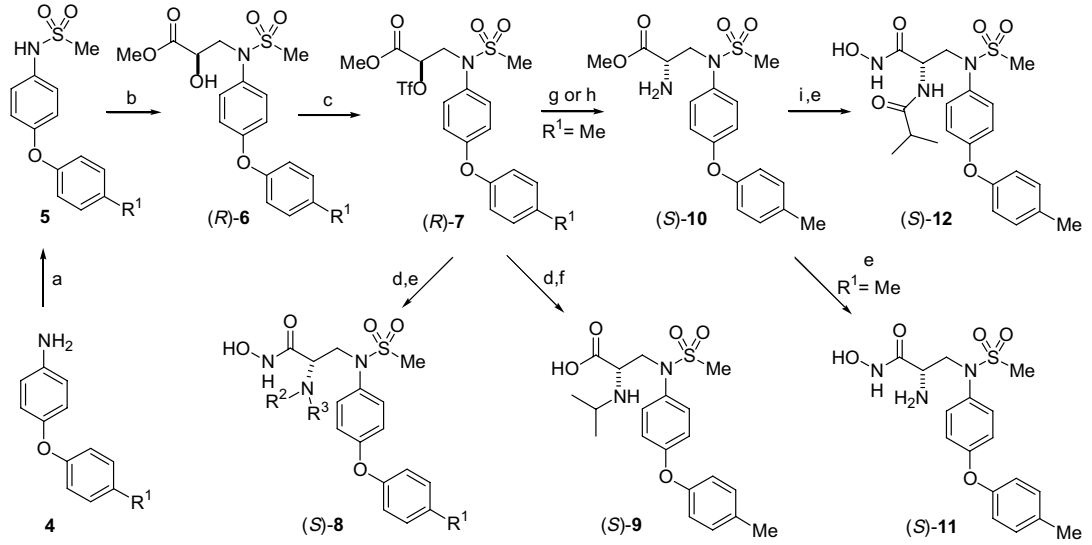
dle cerebral artery occlusion (tMCAo) model is also described.[9]

In an initial attempt to increase potency and improve the ADME properties, a diverse set of substituents was introduced to the α-position of the lead **3** by an S_N2 displacement of the activated hydroxyl group. The synthesis of key intermediate (*R*)-**6** with opposite configuration of the OH group began from the biaryl ether **4** in two steps, formation of the sulfonamide followed by ring opening of the epoxide (Scheme 1).[10] The activation of the OH group with triflic anhydride gave (*R*)-**7**, which could undergo a facile S_N2 displacement reaction with various amines.[11] For example, compound (*S*)-**8** bearing a secondary or tertiary (cyclic or acyclic) amino group at the α-position could be easily obtained from (*R*)-**7** by an S_N2 displacement followed by a hydroxamate formation sequence. The displacement of (*R*)-**7** with isopropyl amine followed by hydrolysis under basic conditions gave the acid (*S*)-**9**. Treatment of (*S*)-**7** with sodium azide followed by Pd-catalyzed hydrogenation or direct S_N2 displacement of (*S*)-**7** with ammonia in methanol provided (*S*)-**10**, which was converted to (*S*)-**11** and amide (*S*)-**12** using similar transformations as described above.

The inhibitory activities (IC_{50}) against MMP-2 and MMP-9 in comparison with lead compound **3** are compiled in Table 1. Initially, efforts were primarily focused on analogs with a secondary amino group attached at the α-position. In general, as observed with the original lead 3, these compounds retained good activity for MMP-2 and MMP-9 without inhibiting MMP-1 ($IC_{50} > 10$ μM). By simply changing the OH to a NH_2 ((*S*)-**3a** vs (*S*)-**11**) led to an 11- and 16-fold loss in potency against MMP-2 and MMP-9, respectively. However, switching the OH to an alkylamino, such as isopropylamino ((*S*)-**8b**), increased the potency dramatically to single-digit nanomolar range for MMP-2 and sub-nanomolar for MMP-9. A similar improvement was observed for (*S*)-**8a** (vs (*S*)-**3c**). Meanwhile, the chirality of the α-position became critical in terms of potency when the bulkier group was attached.[12] For example, the (*S*)-**8b** was 45- and 60-fold more potent than its enantiomer ((*R*)-**8b**) against MMP-2 and MMP-9, respectively. As expected the corresponding carboxylic acid (*S*)-**9** was inactive. In addition, the α-amide substituent ((*S*)-**12**) exhibited similar activities as the lead compound (*S*)-**3a**.

On the other hand, increasing the size of the alkyl substituent to isobutyl ((*S*)-**8c**) or cyclopentyl ((*S*)-**8d**) had only minimal influence on potency. Analogs containing the pyridinylmethyl substituent (e.g., (*S*)-**8e**–**f**), that adopted the same form as CGS-27023A,[13] had comparable activities with the exception of (*S*)-**8g** which was 10-fold less potent. Interestingly, the analogs containing a benzyl group (e.g., (*S*)-**8h**–**i**) typically gave good potency with approximately 16- to 28-fold selectivity for MMP-9 over MMP-2. Moreover, the analogs bearing an α-aniline type substituent, for example, (*S*)-**8j**–**n**, also provided excellent inhibitory activities while retaining the MMP-9 selectivity, particularly for those having H-bonding acceptor capability at the meta-position (e.g., F and OMe in (*S*)-**8l** and (*S*)-**8n**, respectively). Compound (*S*)-**8o** showed decreased potency possibly due to the steric influence of the naphthalene ring.

Since bulky substituents surrounding the hydroxamate moiety may slow down its metabolism,[14] the introduction of a tertiary amino group at the α-position was next examined (Table 2). Based on several acyclic, tertiary amino substituents tested, the combination of isopropyl with several alkyl groups (e.g., (*S*)-**8p**–**r**) did not have a beneficial effect and caused about 4- to 30-fold loss of potency for MMP-2 and MMP-9. To our delight, cyclic



Scheme 1. Reagents and conditions: (a) $MeSO_2Cl$, pyridine, 0 °C to rt, 1 h, 48–96%; (b) Method A: methyl (*R*)-glycidate, K_2CO_3, $BnEt_3NCl$, dioxane, 60–90 °C, 55–91%; Method B: methyl (*R*)-glycidate, K_2CO_3, DMF, 80–100 °C, 71–86%; (c) Tf_2O, 2,6-lutidine, −20 to 0 °C, 85–94%; (d) R^2NHR^3, CH_2Cl_2, 0 °C to rt, 40–96%; (e) $HONH_2$·HCl, NaOMe, MeOH, 35–93%; (f) $LiOH_{(aq)}$, THF, rt, %; (g) i—NaN_3, acetone, H_2O, rt, 95%; ii—H_2, Pd/C, EtOAc, rt, 90%; (h) NH_3 in MeOH (2 M), CH_2Cl_2, 0 °C to rt, 2 h, 82%; (i) *i*-PrCOCl, 2,6-lutidine, CH_2Cl_2, rt, 1 h, 95%.

Table 1. IC_{50} of **3**, **8a–o**, **9**, and **11–12** against MMP-2 and MMP-9

Compound	R^1	R^2NR^3	MMP-2 IC_{50}[a] (nM)	MMP-9 IC_{50}[a] (nM)	Fold selectivity (MMP-2/MMP-9)
(*S*)-**3a**	Me	—	61	7.8	7.8
(*S*)-**3b**	Cl	—	35	4.7	7.4
(*S*)-**3c**	CF_3	—	139	14	9.9
(*S*)-**8a**	CF_3	*i*-PrNH	9.2	2.4	3.8
(*R*)-**8b**	Me	*i*-PrNH	178	31	5.7
(*S*)-**8b**	Me	*i*-PrNH	4.0	0.52	7.7
(*S*)-**8c**	Me	*i*-BuNH	11	1.3	8.8
(*S*)-**8d**	Me	(Cyclopentyl)NH	10	1.7	5.9
(*S*)-**8e**	Me	(Pyridin-4-yl)CH_2NH	9.5	1.8	5.3
(*S*)-**8f**	Me	(Pyridin-3-yl)CH_2NH	17	2.8	6.1
(*S*)-**8g**	Me	(Pyridin-2-yl)CH_2NH	83[b]	23[b]	3.6
(*S*)-**8h**	Me	$PhCH_2NH$	202[b]	7.1	28.4
(*S,R*)-**8i**	Me		24	1.5	16.0
(*S,S*)-**8i**	Me		12	0.54	22.2
(*S*)-**8j**	Me	PhNH	13	0.52	25.0
(*S*)-**8k**	Me	(4-F)PhNH	64	1.3	49.2
(*S*)-**8l**	Me	(3-F)PhNH	53	0.89	59.6
(*S*)-**8m**	Me	(3-Cl)PhNH	333[b]	20[b]	16.6
(*S*)-**8n**	Me	(3-MeO)PhNH	27	0.62	43.5
(*S*)-**8o**	Me	(Naphthylen-2-yl)NH	1.4 μM[b]	86[b]	16.6
(*S*)-**9**	—	—	>10 μM[b]	>10 μM[b]	—
(*S*)-**11**	—	—	670	121	5.5
(*S*)-**12**	—	—	70	5.4	13.0

[a] Values are means of at least two experiments.
[b] Single experiment.

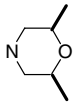
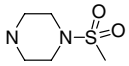
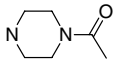
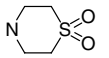
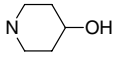
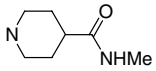
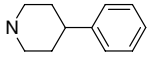
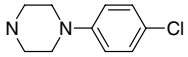
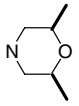
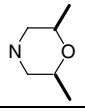
amino substituents such as morpholine ((*S*)-**8s**) provided similar potency to the isopropyl analog (*S*)-**8a**. Several cyclic amines were examined, including substituted morpholine, piperazine, piperidine, etc. ((*S*)-**8t–z**), and all showed excellent activity in inhibiting both MMP-2 and MMP-9 in single-digit nanomolar range. However, the cyclic amine bearing an extra phenyl ring at the 4-position was unfavorable and caused the potency to drop significantly as exemplified by (*S*)-**8aa** and (*S*)-**8bb**. Further investigation by docking studies of these analogs with a MMP-9 homology model revealed that the cyclic amino substituents seem to be pointing to the solvent area, whereas the benzyl ((*S*)-**8h**) and phenyl ((*S*)-**8j**) groups aligned closer to the S1 subsite. The hydrophobic interaction of the phenyl or benzyl with the S1 subsite may have contributed somewhat to the selectivity for MMP-9 versus MMP-2. These observations may also explain the minor influence on potency as well as the decreased MMP-9/-2 selectivity when a cyclic amino group was attached.

Additionally, it has been previously observed in compounds **3** that analogs with Me and Cl substituents (R^1) provided slightly better potency than those with CF_3.[7] Indeed, a similar trend was also obtained when the cyclic amines, such as morpholine and *cis*-dimethylmorpholine, were incorporated at the α-position. In general, these analogs (R^1 = Me or Cl) exhibited excellent potency and were ca. 2- to 4-fold more potent than those with $R^1 = CF_3$ ((*S*)-**8cc**, (*S*)-**8ee** and (*S,cis*)-**8dd**, (*S,cis*)-**8ff** vs (*S*)-**8s** and (*S,cis*)-**8t**, respectively). In addition, the *S*-enantiomer of the compounds bearing a cyclic amino group at the α-center, such as ((*S*)-**8ee**), was consistently greater than 250-fold more potent than their corresponding *R*-enantiomer in both the MMP-2 and MMP-9 enzymatic assays.

Attempts to increase the stability of the hydroxamate moiety by adding a substituent at the nitrogen atom were briefly examined. The coupling of acid (*S*)-**13** with *O*-trityl protected hydroxylamine provided intermediates (*S*)-**14**, which could undergo alkylation followed by trifluoroacetic acid (TFA) deprotection to furnish the analogs (*S*)-**16** (Scheme 2). *N*-Methylation [compound (*S*)-**16a** (R = Me)] significantly decreased the potency to 447 nM and 160 nM for MMP-2 and MMP-9, respectively. However, (*S*)-**16b** (R = *i*-Bu) gained some potency back (261 nM and 88 nM for MMP-2 and MMP-9, respectively). It is likely that the isobutyl group provided some hydrophobic interaction with the S1 subsite.

Several potent compounds were selected to evaluate their PK (rats) and other ADME properties (Table 3). The compounds bearing a secondary amino group at the α-position, such as (*S*)-**8b** and (*S*)-**8l**, showed relatively poor microsomal stabilities (RLM < 9 min), which was reflected in the PK profile (low plasma exposure (AUC) and high clearance level (CL)). Among the compounds tested, (*S*)-**8ee** provided a better PK profile, though the half-life in RLM was only 10 min. The analogs containing an α-cyclic amino group, for example, (*S*)-**8s** and (*S*)-**8ee**, exhibited great water solubility (>70 μg/mL) at both pH 2.0 and pH 7.0. Additionally, these compounds also showed low protein binding

Table 2. IC_{50} of **8** with tertiary α-alkylamino substituents

Compound	R^1	R^2NR^3	MMP-2 IC_{50}[a] (nM)	MMP-9 IC_{50}[a] (nM)	Fold selectivity (MMP-2/MMP-9)
(*S*)-**8a**	CF_3	*i*-PrNH	9.2	2.4	3.8
(*S*)-**8p**	CF_3	*i*-PrNMe	24	8.8	2.7
(*S*)-**8q**	CF_3	*i*-PrNCH$_2$Ph	281[b]	68[b]	4.1
(*S*)-**8r**	CF_3	*i*-PrNCH$_2$CH$_2$NHCbz	262[b]	29[b]	9.0
(*S*)-**8s**	CF_3		9.3	2.9	3.2
(*S*,*cis*)-**8t**	CF_3		5.6	1.1	5.1
(*S*)-**8u**	CF_3		9.9	3.2	3.1
(*S*)-**8v**	CF_3		8.6	4.8	1.8
(*S*)-**8w**	CF_3		4.8	1.7	2.8
(*S*)-**8x**	CF_3		7.6	2.3	3.3
(*S*)-**8y**	CF_3		5.7	1.9	3.0
(*S*)-**8z**	CF_3		5.5	2.5	2.2
(*S*)-**8aa**	CF_3		47[b]	17[b]	2.7
(*S*)-**8bb**	CF_3		119[b]	256[b]	2.7
(*S*)-**8cc**	Cl		5.5	2.0	2.8
(*S*,*cis*)-**8dd**	Cl		2.5	0.46	5.4
(*R*)-**8ee**	Me		1318[b]	295[b]	4.5
(*S*)-**8ee**	Me		5.2	1.9	2.7
(*S*,*cis*)-**8ff**	Me		2.8	0.68	4.1

[a] Values are means of at least two experiments.
[b] Single experiment.

(PB), 58% and 74% for (*S*)-**8ee** and (*S*)-**8s**, respectively.[15] Finally, the enzyme selectivity of (*S*)-**8ee** with selected MMPs was briefly evaluated, which provided the IC_{50} values of >10 μM, 137 nM, and 3.5 nM for MMP-1, MMP-3, and MMP-13, respectively.

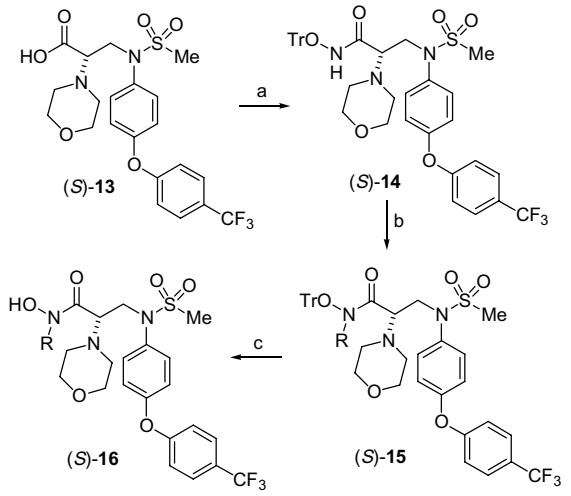
The preliminary in vivo efficacy of (*S*)-**8s** in reducing the ischemia-induced brain edema was evaluated in the rat tMCAo model.[16] Vehicle-treated animals ($n = 21$) typically increased brain water content (i.e., edema) in the ischemic hemisphere 24 h after tMCAo by 4.61% (± 0.39%), on the average (Fig. 2). However, 3 h delayed treatment with (*S*)-**8s** ($n = 19$; dose: 2 mg/kg) was able to reduce tMCAo-induced brain edema to 3.3% (±0.38%), a statistically significant difference ($P < 0.05$). These data suggest that compounds similar to (*S*)-**8s** may have therapeutic utility in reducing brain edema after a transient ischemic insult such as an embolic stroke.

Table 3. PK,[a] protein binding, and in vitro microsomal stability of selected compounds

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*)-**8b**	2.68	0.16	0.25	0.49	35.0	49/<9	84
(*S*)-**8l**	0.62	0.6	0.13	2.89	66.9	<9/<9	99.7
(*S*)-**8s**	0.86	0.2	0.13	0.99	65.4	>2 h/>2 h	74
(*S*)-**8ee**	3.7	6.0	0.67	0.66	12.8	> 2 h/10	58

[a] Mean value of four 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.

In conclusion, a new series of α-amino-β-*N*-biaryl ether sulfonamide hydroxamates as potent gelatinase inhibitors has been described. These compounds exhibited low single-digit nanomolar activities against both MMP-2 and MMP-9 and spared MMP-1. Particularly, the analogs bearing an α-cyclic amino group showed good water solubility, low protein binding, and an improved PK profile (ex. (*S*)-**8ee**). (*S*)-**8s** reduced ischemia-induced brain edema in the rat tMCAo model, also demonstrating a potential utility of these analogs for the treatment of brain injury such as an embolic stroke.



Scheme 2. Reagents and conditions: (a) $TrONH_2$, EDC, HOBt, DMAP, rt, 24 h, 73%; (b) MeOTs or *i*-BuI, Cs_2CO_3, MeCN/DMF, rt, 75–79%; (c) TFA, Et_2O, rt, 65% ((*S*)-**16a**, R = Me), 75% ((*S*)-**16b**, R = *i*-Bu).

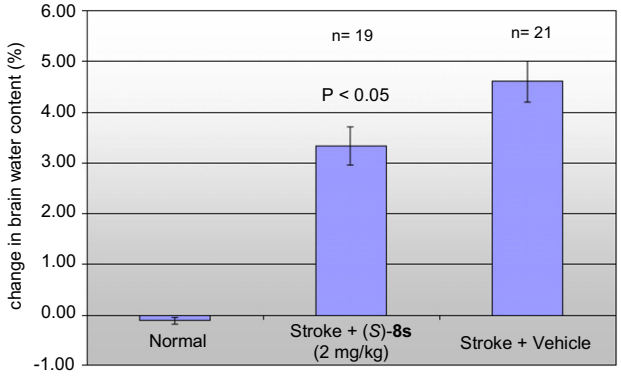


Figure 2. Brain edema in (*S*)-**8s**-treated animals (n = 19; dose: 2 mg/kg).

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References and notes

1. Recent reviews, see: (a) Fingleton, B. *Curr. Pharm. Des.* **2007**, *13*, 333; (b) Fingleton, B. *Exp. Opin. Ther. Targets* **2003**, *7*, 385; (c) Vihinen, P.; Ala-aho, R.; Kähäri, V.-M. *Curr. Cancer Drug Targets* **2005**, *5*, 203; (d) George, S. J. *Exp. Opin. Invest. Drugs* **2000**, *9*, 993; (e) Yong, V. W. *Exp. Opin. Invest. Drugs* **1999**, *8*, 255; (f) Yong, V. W.; Power, C.; Forsyth, P.; Edwards, D. R. *Nat. Rev. Neurosci.* **2001**, *2*, 502.
2. (a) Clarke, A. W.; Krekoski, C. A.; Bou, S.-S.; Chapman, K. R.; Edwards, D. R. *Neurosci. Lett.* **1997**, *238*, 53; (b) Joan Montaner, J.; Alvarez-Sabín, J.; Molina, C.; Anglés, A.; Abilleira, S.; Arenillas, J.; González, M. A.; Monasterio, J. *Stroke* **2001**, *32*, 1759; (c) Romanic, A. M.; White, R. F.; Arleth, A. J.; Ohlstein, E. H.; Barone, F. C. *Stroke* **1998**, *29*, 1020.
3. Asahi, M.; Wang, X.; Mori, T.; Sumii, T.; Jung, J. C.; Moskowitz, M. A.; Fini, M. E.; Lo, E. H. *J. Neurosci.* **2001**, *21*, 7724.
4. (a) Jiang, X.-F.; Namura, S.; Nagata, I. *Neurosci. Lett.* **2001**, *35*, 41; (b) Lapchak, P. A.; Chapman, D. F.; Zivin, J. A. *Stroke* **2000**, *31*, 3034.
5. Kim, S.-H.; Pudzianowski, A. T.; Leavitt, K. J.; Barbosa, J.; McDonnell, P. A.; Metzler, W. J.; Rankin, B. M.; Liu, R.; Vaccaro, W.; Pitts, W. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1101.
6. Cherney, R. J.; Mo, R.; Meyer, D. T.; Hardman, K. D.; Liu, R.-Q.; Covington, M. B.; Qian, M.; Wasserman, Z. R.; Christ, D. D.; Trzaskos, J. M.; Newton, R. C.; Decicco, C. P. *J. Med. Chem.* **2004**, *47*, 2981.
7. Yang, S.-M.; Scannevin, R. H.; Wang, B.; Burke, S. L.; Wilson, L. J.; Karnachi, P.; Rhodes, K. J.; Lagu, B.; Murray, W. V. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1135.
8. α-Amino-β-sulfone hydroxamates as potent MMP-13 inhibitors have been reported recently, see: (a) Becker, D. P.; Barta, T. E.; Bedell, L.; DeCrescenzo, G.; Freskos, J.; Getman, D. P.; Hockerman, S. L.; Li, M.; Mehta, P.; Mischke, B.; Munie, G. E.; Swearingen, C.; Villamil, C. I. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2719; (b) Becker, D. P.; DeCrescenzo, G.; Freskos, J.; Getman, D. P.; Hockerman,

S. L.; Li, M.; Mehta, P.; Munie, G. E.; Swearingen, C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2723.
9. (a) Huang, Z.; Huang, P. L.; Panahian, N.; Dalkara, T.; Fishman, M. C.; Moskowitz, M. A. *Science* **1994**, *265*, 1883; (b) Longa, E. Z.; Weinstein, P. R.; Carlson, S.; Cummins, R. *Stroke* **1989**, *20*, 84; (c) Hara, H.; Huang, P. L.; Panahian, N.; Fishman, M. C.; Moskowitz, M. A. *J. Cereb. Blood Flow Metab.* **1996**, *16*, 605.
10. (a) Yang, S.-M.; Murray, W. V. *Tetrahedron Lett.* **2008**, doi:10.1016/j.tetlet.2007.11.184; (b) Albanese, D.; Landini, D.; Lupi, V.; Penso, M. *Ind. Eng. Chem. Res.* **2003**, *42*, 680, and the references cited therein.
11. (a) Kogan, T. P.; Somers, T. C.; Venuti, M. C. *Tetrahedron* **1990**, *46*, 6623; (b) Uenishi, J.; Hamada, M.; Aburatani, S.; Matsui, K.; Yonemitsu, O.; Tsukube, H. *J. Org. Chem.* **2004**, *69*, 6781.
12. The present skeleton, indeed, tolerates well small α-substituents such as OH, Me, and OMe. The influence on potency by the α-chirality is minimal, see Ref. 7.
13. MacPherson, L. J.; Bayburt, E. K.; Capparelli, M. P.; Carroll, B. J.; Goldstein, R.; Justice, M. R.; Zhu, L.; Hu, S.; Melton, R. A.; Fryer, L.; Goldberg, R. L.; Doughty, J. R.; Spirito, S.; Blancuzzi, V.; Wilson, D.; O'Byrne, E. M.; Ganu, V.; Parker, D. T. *J. Med. Chem.* **1997**, *40*, 2525.
14. (a) Reiter, L. A.; Robinson, R. P.; McClure, K. F.; Jones, C. S.; Reese, M. R.; Mitchell, P. G.; Otterness, I. G.; Bliven, M. L.; Liras, J.; Cortina, S. R.; Donahue, K. M.; Eskra, J. D.; Griffiths, R. J.; Lame, M. E.; Lopez-Anaya, A.; Martinelli, G. J.; McGahee, S. M.; Yocum, S. A.; Lopresti-Morrow, L. L.; Tobiassen, L. M.; Vaughn-Bowser, M. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3389; (b) Noe, M. C.; Natarajan, V.; Snow, S. L.; Mitchell, P. G.; Lopresti-Morrow, L.; Reeves, L. M.; Yocum, S. A.; Carty, T. J.; Barberia, J. A.; Sweeney, F. J.; Liras, J. L.; Vaughn, M.; Hardink, J. R.; Hawkins, J. M.; Tokar, C. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2808.
15. In the early ADME profiling, (*S*)-**8s** and (*S*)-**8ee** both showed low hERG binding (<10% at 10 μM), negative AMES II testing, clean Cerep selectivity panel, and were inactive to P450 isoenzymes (IC_{50} > 10 μM).
16. Focal cerebral ischemia of male Sprague–Dawley rats was induced in the right hemisphere by occlusion of the middle cerebral artery for 2 h, at which point the filament was removed to allow for brain reperfusion. (*S*)-**8s** was administered to the test animals 3 h post-filament insertion via an intravenous bolus delivering 2.0 mg/kg drug solution combined with minipump implantation, which delivered a continuous intravenous infusion of a 30 mg/mL drug solution for the duration of the study. Control animals also receiving tMCAo were administered an equivalent amount of the vehicle solution (20% PEG400 + 80% of 20% solutol in distilled H_2O) both as a bolus and through minipump infusion. Twenty-four hours after onset of ischemia animals were euthanized and the brain was quickly removed to evaluate water content by the wet/dry method. The water content in two hemispheres of the brain tissue was calculated as follows: 100 × [(wet weight − dry weight)/wet weight] (%). The percentage increase of the brain water content after ischemia was calculated by the difference in water content between the ischemic ipsilateral and non-ischemic contralateral hemispheres. See: Yanamoto, H.; Nagata, I.; Niitsu, Y.; Xue, J. H.; Zhang, Z.; Kikuchi, H. *Exp. Neurol.* **2003**, *182*, 261.