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1-Hydroxy-2-pyridinone-based MMP inhibitors: Synthesis and biological evaluation for the treatment of ischemic stroke

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Abstract—Matrix metalloproteinase-9 (MMP-9) has been implicated in the breakdown of the blood–brain barrier during cerebral ischemia. As a result, inhibition of MMP-9 may have utility as a therapeutic intervention in stroke. Towards this end, we have synthesized a series of 1-hydroxy-2-pyridinones that have excellent in vitro potency in inhibiting MMP-9 in addition to MMP-2. Representative compounds also demonstrate good efficacy in the mouse transient mid-cerebral artery occlusion (tMCAO) model of cerebral ischemia.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases responsible for the digestion and turnover of extracellular matrix components. Recent studies have shown that MMPs are involved in several neurological diseases, notably in the pathophysiology of cerebral damage after ischemic stroke (IS).[1] The up-regulation of MMP-9 in a rat transient middle cerebral artery occlusion (tMCAO) model correlates with increased blood–brain barrier (BBB) permeability. This breakdown in the BBB then leads to edema, hemorrhage, and infiltration of inflammatory agents, all of which cause significant brain damage. Therefore, MMP-9 is believed to be a potential therapeutic target for acute IS. The goal of our MMP program is to discover and develop novel MMP-2/-9 (gelatinase) inhibitors, which demonstrate in vivo efficacy in animal models of stroke.

To date, the majority of MMP inhibitors (MMPIs) are hydroxamate-based compounds due to their high in vitro potency. However, because of low oral bioavailability, potential metabolic liabilities (hydrolysis and glucuronidation), and side effects of hydroxamates,[2] there is considerable effort to discover non-hydroxamate MMPIs to improve ADME properties.[3] MMP inhibitors typically consist of a Zn-binding group (ZBG) and a recognition motif that binds to subsites within the MMP active site. Recently, hydroxypyridinones (HOPOs) and pyrones have been investigated as novel ZBGs by Cohen's group and a series of full length MMP-3 inhibitors containing these heterocyclic ZBGs were synthesized.[4,5] By far, pyrone-based compounds showed the most promising inhib-

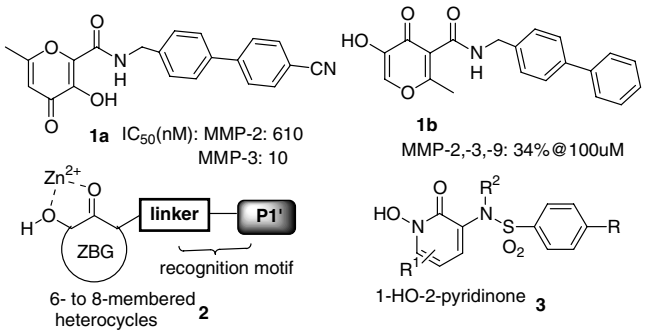

Figure 1. MMPIs with heterocycles as ZBGs.

Keywords: Matrix metalloproteinase inhibitor; MMP-9; 1-Hydroxy-2-pyridone; Stroke.
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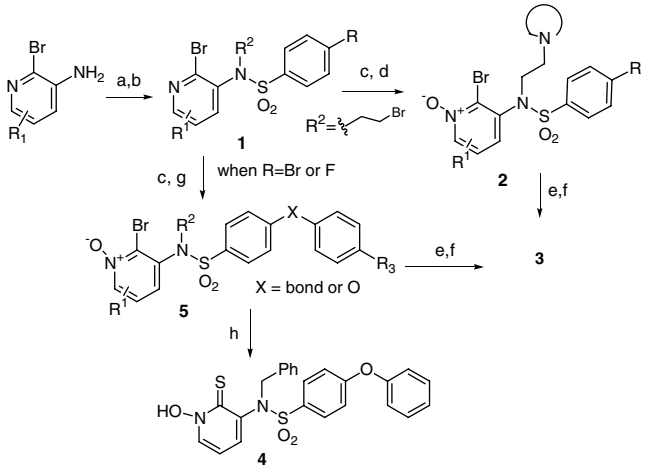
itory activity with IC_{50} values ranging from 10 nM against MMP-3 to 600 nM against MMP-2 (Fig. 1, **1a**).[6] Interestingly, the regio isomer **1b** was basically inactive against MMP-1, -2, -3, and -9,[7] suggesting the position of the P_1' attachment to ZBGs is critical for inhibitory activity against MMPs.

It has been reported that increasing the steric bulk at the α-carbon to the ZBG slows down metabolism of the hydroxamic acid (HA) functionality and improves the PK properties of HA-based MMPIs.[8] We hypothesized that by linking the nitrogen of the HA to the α-carbon to form a ring the steric bulk in the region of the HA as well as the rigidity of the ZBG would be increased, and thus the PK properties of the resulting MMPIs would be improved. Based on this hypothesis and inspired by the reported work from Cohen's research group, we have expanded the scope of the previously reported HOPOs and discovered several classes of potent MMPIs utilizing a variety of 6- to 8-membered heterocycle-derived ZBGs (Fig. 1, **2**). Here we wish to report the first series of 1-hydroxy-2-pyridinone-based gelatinase inhibitors containing a sulfonamide scaffold (Fig. 1, **3**).

Synthesis of 1-hydroxy-2-pyridinone-based sulfonamides **3** is outlined in Scheme 1. The reaction of 3-amino-2-bromopyridine with various commercially available aryl sulfonyl chlorides, followed by alkylation of the sulfonamide nitrogen, gave compound **1**. Oxidation of pyridine **1** in the presence of urea–H_2O_2 complex (UHP) and trifluoroacetic anhydride gave the pyridine N-oxide in high yield.[9] When R^2 is bromoethyl, the substituent can be further derivatized into a nitrogen-containing side chain to yield **2**. Conversion of compound **2** into 2-methoxypyridine N-oxide and subsequent acidic hydrolysis gave **3**. Alternatively, the 4-bromo- or 4-fluoroaryl analog of **1** can be transformed into the desired P_1' groups under Suzuki coupling conditions or by simple displacement with basic phenoxides to give **5**. Intermediate **5** can further be converted into thiopyridinone **4** by treatment with NaSH.

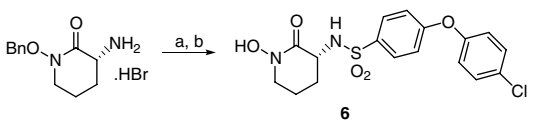


Scheme 1. Reagents and conditions: (a) $ClSO_2Ph(4\text{-}R)$, Pry/CH_2Cl_2, 0 °C—rt, 81%; (b) R^2 halides, Cs_2CO_3, DMF, 70 °C, 70–95%; (c) UHP/TFAA (2.1/2), CH_2Cl_2, 0 °C—rt, 94%; (d) 1.1 equiv Amines, TEA, MeCN, 65 °C, 58–84%; (e) 1.5 equiv NaOMe, MeOH, reflux, 54%; (f) 2 N HCl/MeOH (1/1.5), reflux, 95%; (g) when R = Br, $(OH)_2BPh(4\text{-}R_3)$, 5 mol% $Pd(PPh_3)_4$, Na_2CO_3 (2 M), toluene, 79%; when R = F, $HOPh(4\text{-}R_3)$, Cs_2CO_3, DMF, 90 °C, 60%; (h) NaSH, DMSO/H_2O, 88%.

To better define the scope of HOPOs as zinc chelators, a "fully reduced version" compound **6**, containing 1-hydroxy-2-piperidinone as a ZBG, was also prepared (Scheme 2). Sulfonylation of benzyl-protected (*R*)-3-amino-1-hydroxy-2-piperidinone followed by benzyl cleavage with neat $MeSO_3H$ gave **6**.[10]

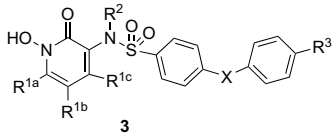
The inhibitory activity of compounds **3a**–**3r**, **4**, and **6** against MMP-2 and MMP-9 was evaluated and the results are shown in Table 1. The biaryl P_1' group (**3a**) does not demonstrate good activity, which is supported by docking experiments to our MMP-9 homology model (Fig. 2).[11] Substitution on the pyridinone ring at any position does not seem favorable. IC_{50} values for compounds **3d**, **3e**, and **3f** increased significantly compared to the unsubstituted **3c**. In contrast, R^2 substitution plays an important role in the inhibitory activity. A simple methyl substitution on **3c** improved the enzyme potency 50- to 100-fold compared to **3b**. The MMP-9 homology model suggests that the binding modes of **3b** and **3c** are considerably different and this may contribute to dramatic changes in affinity for the enzyme. Bulky and hydrophobic groups (**3j** and **3k**) at the R^2 position reduced potency. However, longer side chains (at least a two-carbon extension) containing water-solubilizing groups (**3l**–**3o**) were well tolerated. Figure 2 shows the binding mode of compounds **3c** (orange) and **3l** (white) based on docking of the molecules into the homology model of MMP-9. The modeling indicates that the sulfonamide linker forms a hydrogen-bonding interaction with Leu 188 on the backbone. R^2 substitution extends to the solvent exposed surface of the enzyme. Though SAR indicates that biaryl ether groups fit well into the S_1' subsite, a highly electron withdrawing para-substituent (**3p**) on the terminal phenyl was found to be detrimental to potency. Interestingly, comparison of **3b** and **6** suggests that the 1-hydroxy-2-piperidinone group is still an effective ZBG. This has never been reported in the literature, though 1-hydroxy-2-piperidinone and 1-hydroxy-azepan-2-one have been used in the syntheses of vasopeptidase inhibitors and siderophores.[12] On the other hand, thiopyridinone **4** showed very poor inhibitory activity against MMP-2 and MMP-9. This result is in contrast to the literature report,[4] which found that thio-HOPOs themselves are more potent than their oxygen analogs due to the thiophilicity of zinc. We hypothesized that the larger thiopyridone ZBG hindered the optimal binding to the S_1' site.

The selectivity against several MMPs of representative compounds is summarized in Table 2. As expected, all

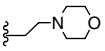
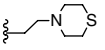
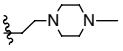
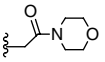


Scheme 2. Reagents and conditions: (a) $ClSO_2Ph(4\text{-}(4'\text{-}Cl)PhO)$, TEA, CH_2Cl_2, rt, 88%; (b) $MeSO_3H$, rt, 80%.

Table 1. IC_{50}[a] (nM) values and SAR of compounds **3**, **4**, and **6**



3

No.	R^{1a}	R^{1b}	R^{1c}	X	R^2	R^3	MMP-2 (nM)	MMP-9 (nM)
3a	–H	–H	–H	bond	–H	–Cl	>1000	>1000
3b	–H	–H	–H	O	–H	–Cl	242	315
3c	–H	–H	–H	O	$-CH_3$	–Cl	5.0	2.4
3d	–H	–H	$-CH_3$	O	$-CH_3$	–Cl	66.2	158.5
3e	–H	–Br	–H	O	$-CH_3$	–Cl	662	1387
3f	$-CH_3$	–H	–H	O	$-CH_3$	–Cl	66.4	34.9
3g	–H	–H	–H	O	$-CH_3$	$-CH_3$	6.1	4.4
3h	–H	–H	–H	O	$-CH_3$	–H	8.3	5.0
3i	–H	–H	–H	O	$-CH_2CH_3$	–H	6.5	3.6
3j	–H	–H	–H	O	Isopropyl	–H	41.0	57.5
3k	–H	–H	–H	O	Benzyl	–H	22.4	33.4
3l	–H	–H	–H	O		–Cl	5.0	4.2
3m	–H	–H	–H	O		–Cl	6.1	4.9
3n	–H	–H	–H	O		–Cl	1.7	1.3
3o	–H	–H	–H	O		–Cl	4.9	4.8
3p	–H	–H	–H	O	$-CH_3$	$-CF_3$	104.5	32.6
3q	–H	–H	–H	O	$-CH_3$	$-OCH_3$	5.6	0.87
3r	–H	–H	–H	O	$-CH_3$	$-OCF_3$	5.7	1.8
4	—	—	—	—	See Scheme 1	—	4591	693
6	—	—	—	—	See Scheme 2	—	108.4	57.9

[a]IC_{50} (nM) values are means of at least two experiments.

compounds showed high selectivity over MMP-1, which has a shallow S_1' subsite, and moderate selectivity for MMP-9 vs MMP-3 (20- to 40-fold). Compounds **3c**, **3l**, and **3q** are also potent MMP-13 inhibitors.

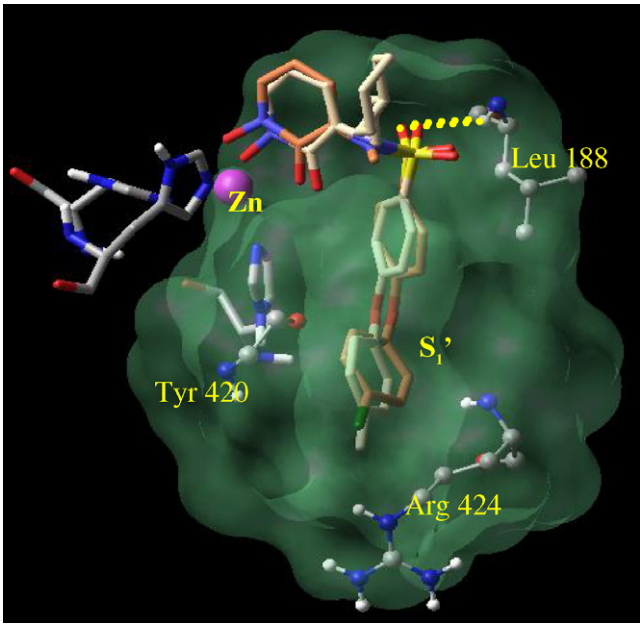


Figure 2. Compounds **3c** (orange) and **3l** (white) docked in homology model of MMP-9.

Table 2. IC_{50}[a] values against selected MMPs of **3c**, **3l**, and **3q**

Compound	MMP-1	MMP-2	MMP-3	MMP-9	MMP-13
3c	>1000	5.0	56.5	2.4	2.5
3l	>1000	5.0	62.9	4.2	4.0
3q	>1000	5.6	79.1	0.87	6.8

[a] IC_{50} (nM) values are means of at least two determinations.

Table 3. Pharmacokinetics parameter[a] of **3c** in rats

Compound	C_0[b] (μM)	$T_{1/2}$[b] (h)	AUC[b] (μg-h/mL)	V_{ss}[b] (L/kg)	CL[b] mL/min/Kg
3c	8.4	47	179	1.75	0.5

[a] Mean value of four animals (rat) at 2 mg/kg iv dose.
[b] C_0, concentration at $T = 0$; $T_{1/2}$, apparent elimination half-life; AUC, area under the concentration–time curve; V_{ss}, volume of distribution at steady state; CL, systemic clearance.

The pharmacokinetics of **3c** was studied in rats following intravenous dosing (Table 3). Compound **3c** demonstrates an excellent PK profile with a long half-life. This compound also exhibited a high plasma concentration in mice at an oral dose of 10 mg/kg dose (34 μM at the 1 h time point).

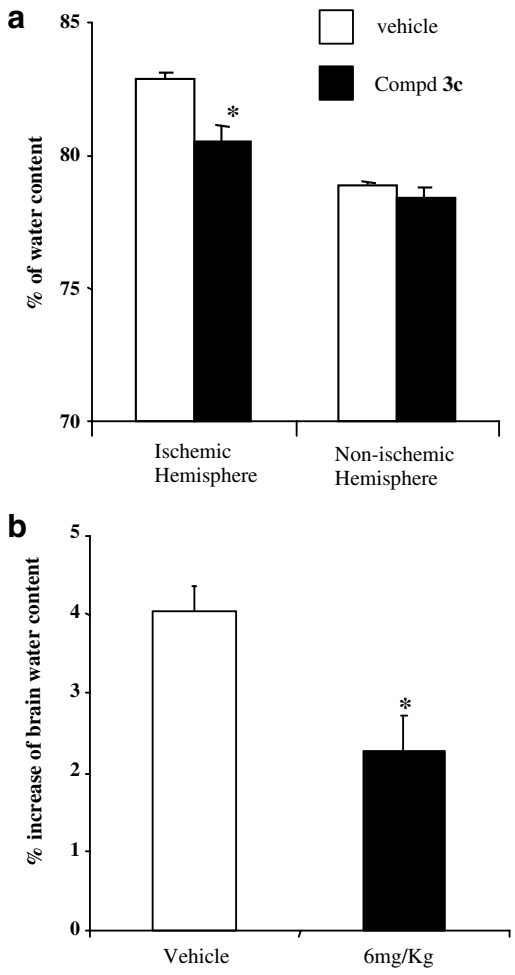


Figure 3. **3c** treatment reduced brain edema after focal ischemia in C57 Black/6 mice (20–25 g, Charles River). Animals were subjected to transient MCA occlusion for 2 h. **3c** ($n = 9$) or vehicle (5 ml/kg, $n = 10$) was given intravenously as a bolus immediately after ischemia and at 2 h following ischemia. Brain edema was evaluated by wet/dry method at 5 h after MCA occlusion. Data are expressed as means ± SEM. $^{*}p < 0.05$ as compared to vehicle-treated group by Student's *t*-test.

The efficacy of compound **3c** on the attenuation of brain injury after IS was evaluated in the mouse tMCAO model.[13] Focal cerebral ischemia of male C57 Black/6 mice was induced by the occlusion of the middle cerebral artery (MCA) and then subjected to reperfusion after 2 h. Compound **3c** (6 mg/kg) or an equal volume of the vehicle (20% PEG400 + 80% of 20% solutol in dH_2O, 5 ml/kg) was administered intravenously as a bolus immediately after ischemia and then again immediately after reperfusion. Five hours after ischemia, animals were euthanized and the brain quickly removed. Brain edema was evaluated by the wet/dry method.[14] The water content in the two hemispheres of the brain tissue was calculated as follows: $100 \times [(\text{wet weight} - \text{dry weight})/\text{wet weight}]$ (%). The percentage increase of the brain water content after ischemia was calculated by the difference in water content between the ipsilateral and contralateral hemispheres. Figure 3a shows, that after focal ischemia, the brain water content increased in the ischemic hemisphere as compared to that of the corresponding non-ischemic hemisphere in the vehicle-treated group. However, this enhancement in brain water content was partially prevented after drug treatment, while no difference was observed in the non-ischemic hemisphere between the drug-treated and vehicle-treated groups. Furthermore, even after normalizing for the water content of the non-ischemic hemisphere, drug treatment was effective in reducing the percentage increase of brain water content, as shown in Figure 3b. These results indicate that **3c** could reduce brain edema induced by focal ischemia in mice.

In summary, a series of novel 1-hydroxy-2-pyridinone-based sulfonamide inhibitors of MMP-2/-9 were designed and synthesized. The optimized compounds possess nanomolar potency in the enzyme assay and demonstrate excellent pharmacokinetic profiles in rats. Compound **3c** reduced early brain edema after transient ischemia in mice and is also orally bioavailable. MMP inhibitors with other heterocyclic 6- to 8-membered ring ZBGs will be reported in due course.

Acknowledgments

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Supplementary data

Protocols for MMP enzyme assays, in vivo tMCAO studies, and the synthetic details. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.10.045.

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