Design and Synthesis of Piperazine-Based Matrix Metalloproteinase Inhibitors

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A new generation of cyclic matrix metalloproteinase (MMP) inhibitors derived from *dl*-piperazinecarboxylic acid has been described. The design involves: incorporation of hydroxamic acid as the bidentate chelating agent for catalytic Zn^{2+}, placement of a sulfonamide group at the 1N-position of the piperazine ring to fill the S1′ pocket of the enzyme, and finally attachment of diverse functional groups at the 4N-position to optimize potency and peroral absorption. A unique combination of all three elements produced inhibitor **20** with high affinity for MMPs 1, 3, 9, and 13 (24, 18, 1.9, and 1.3 nM, respectively). X-ray crystallography data obtained for MMP-3 cocrystallized with **20** gave detailed information on key binding interactions defining an overall scaffold geometry for piperazine-based MMP inhibitors.

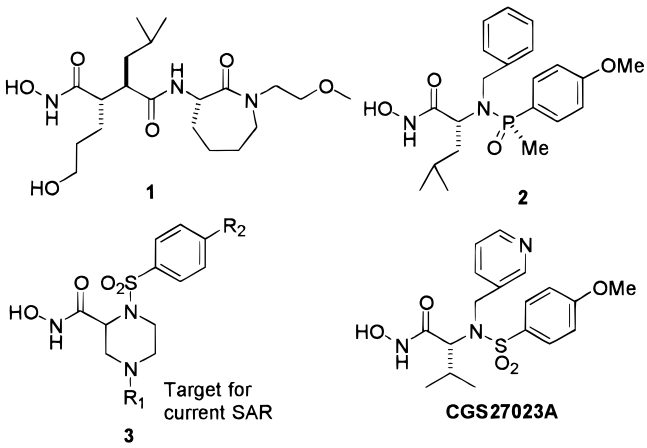
Introduction

Matrix metalloproteinases (MMPs) constitute a family of zinc-dependent endoproteinases that are involved in extracellular matrix remodeling. These enzymes are secreted as zymogens which are subsequently processed by other proteolytic enzymes to generate the active forms. Under normal physiological conditions, the proteolytic activity of the MMPs is controlled at any of the following three known stages: transcription, activation of the zymogens, and inhibition of the active forms by various tissue inhibitors of MMPs (TIMPs).[1] In pathological conditions this equilibrium is shifted toward increased MMP activity leading to tissue degradation.[2] Consequently a considerable amount of effort has been invested in designing orally active MMP inhibitors (MMPIs) with the understanding that such agents will be able to either halt or slow the progression of diseases such as osteoarthritis, tumor metastasis, and corneal ulceration.[3]

Design Strategy for the Cyclic MMPIs

Previous work from our laboratory on both SAR studies and crystallography with partially cyclic and acyclic MMPIs (see **1** and **2**, Chart 1) indicated that the hydroxamic acid unit was critical in terms of preserving high potency.[4] As indicated in Figure 2, with a 4-point attachment to the active surface, this critical unit behaves like a *molecular magnet*. As part of our long-term interests in the design of potent MMPIs containing a central, conformationally constrained, heterocycle ring,[4,5] *dl*-piperazinecarboxylic acid was chosen to provide the central backbone. Convenient features of this starting material include: (a) commercial availability, (b) convenient synthetic access to a variety of analogues,

Chart 1. Hydroxamic Acid-Based MMPIs



and (c) conformational stability of the cyclic backbone. To impart a higher proportion of nonpeptidic character, a decision was made to incorporate a sulfonamide unit at the 1N-position of piperazinecarboxylic acid (see CGS-27023A).[6] The distal nitrogen atom (4N) of the piperazine ring provided the desired site for SAR work reported in this publication.

Chemistry. The piperazine-based MMPIs were synthesized from commercially available *dl*-piperazinecarboxylic acid following the general procedure detailed in Scheme 1. Selective protection of piperazinecarboxylic acid with di-*tert*-butyl dicarbonate under optimized conditions produced the desired monoamine **5**. The crude amine **5** was subsequently treated with *p*-methoxyphenylsulfonyl chloride, and then the resulting sulfonamide was treated with methanolic HCl to provide the desired common intermediate **7** in good overall yield. For SAR studies, intermediate **7** was treated with various reagents to provide the desired substitued piperazine derivatives **8**. The methyl ester **8** was then treated with hydroxylamine in KOH−MeOH to provide the desired hydroxamic acid **9**. For example, treatment of **7** with methanesulfonyl chloride in Et_3N afforded the

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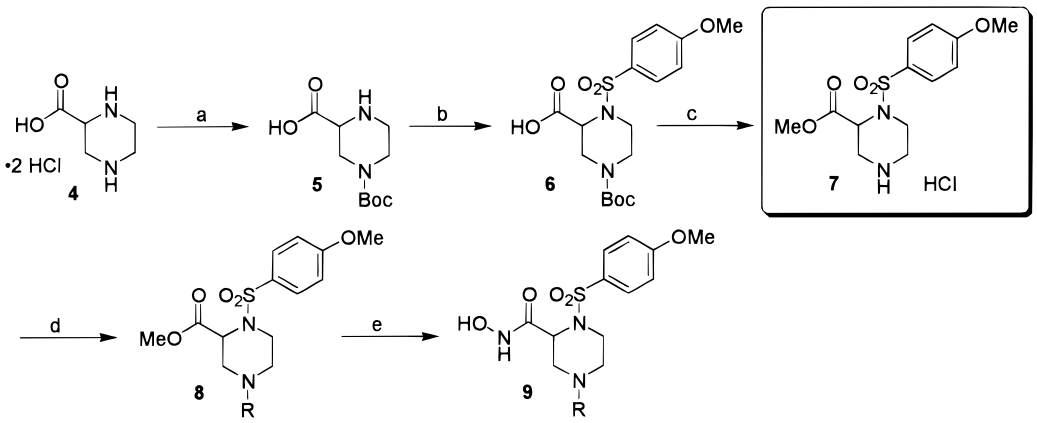
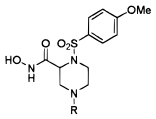
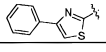
Scheme 1[a]



[a] Reagents: (a) 1.1 equiv (t-BOC)$_2$O, 2 equiv NaOH, dioxane−water; (b) 1 equiv p-MeOPhSO$_2$Cl, Et$_3$N/cat. DMAP; (c) SOCl$_2$, MeOH; (d) sulfonyl chlorides or alkyl halides or isocyanates or acyl halides, etc.; (e) NH$_2$OH in KOH and MeOH.

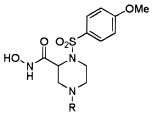
Table 1. In Vitro Profile of Substituted Piperazine-Based MMPIs

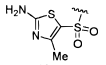
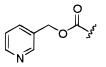
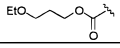


		IC$_{50}$ (nM)[a]				
compd	R	MMP-1	MMP-3	MMP-7	MMP-9	MMP-13
10	H-	175	48	2390	6.9	1.7
11	Me-	1260	269	>10000	25	21
12	n-C$_6$H$_{13}$-	996	71	15740	17	4.2
13	Bn-	324	75	14000	7.6	8.0
14		325	11	3290	28	nd

[a] See Experimental Section for details of the enzyme assays; nd = not determined.

Table 2. In Vitro Profile of Sulfonamide- and Carbamate-Substituted Piperazine-Based MMPIs



		IC$_{50}$ (nM)[a]				
compd	R	MMP-1	MMP-3	MMP-7	MMP-9	MMP-13
15	MeSO$_2$-	8.0	3.5	483	0.6	1.0
16	p-MeOPhSO$_2$-	60	13	1890	1.2	1.3
17		31	2.5	1283	2.1	2.0
18		54	3.2	1018	2.4	1.3
19	Boc-	81	60	1460	2.3	nd
20	CBz-	24	18	232	1.9	1.3
21		18	8.9	502	1.9	1.0
22		22	5.3	483	0.6	0.7

[a] See Experimental Section for details of the enzyme assays; nd = not determined.

bis-sulfonamide which, upon treatment with hydroxylamine in KOH−MeOH, provided the hydroxamic acid **15** (see Table 2). In a similar fashion, treatment of **7** with benzyloxycarbonyl (CBz) chloride in dioxane−water afforded the desired carbamate methyl ester which was then converted to the desired hydroxamic acid **20** (Table 2). Additional details for the remaining analogues are provided in the Experimental Section.

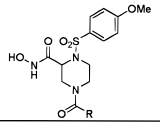
Results and Discussion

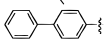
***N*-Alkylpiperazines.** All compounds were tested for the inhibition of MMPs 1, 3, 7, 9, and 13.[7] The SAR work presented herein was performed with the *p*-methoxyphenylsulfonamide moiety appended α with respect to the hydroxamic acid unless otherwise specified. Initial SAR studies were performed with alkyl-substituted piperazine derivatives. The parent compound of the series (compound **10**, Table 1) was a nanomolar inhibitor of MMPs 1, 3, 9, and 13 but exhibited micromolar activity against MMP-7. Substitution of the NH in compound **10** with an alkyl chain did not significantly alter the potency or selectivity of the series. Like the parent compound, analogues **11**−**13** were all potent inhibitors of MMPs 9 and 13, and they were all less potent against MMP-7. All of the compounds in the series demonstrated a preference for MMP-3 vs MMP-1 although the extent of this selectivity varied based upon the *N*-alkyl substituent. Even substitution with a large group such as the heterocycle in **14** did not significantly alter the in vitro profile of the simple *N*-alkylamines.

Sulfonamide- and Carbamate-Substituted Piperazines. The bis-sulfonamide- and carbamate-substituted piperazines all showed enhanced potency for MMPs 3, 9, and 13 relative to the *N*-alkyl derivatives (Tables 1 and 2). Many of these compounds were also quite potent inhibitors of MMP-1. Increasing the steric bulk of the bis-sulfonamide appeared to have very little influence on the in vitro profile of these compounds. The heterocyclic sulfonamides **17** and **18** were only slightly less potent for MMP-1 than the methanesulfonamide **15**. The in vitro profile of the carbamate-based MMPIs was also quite insensitive to the changes in both steric and electronic environments.

Amide-Substituted Piperazines. The amide-substituted piperazines possessed a consistent in vitro profile with potent inhibition of MMPs 9 and 13 and weak inhibition of MMP-7 (Table 3). Some variation was observed with the inhibition of MMPs 1 and 3. The steric

Table 3. In Vitro Profile of Amide-Substituted Piperazine-Based MMPIs



		IC_{50} (nM)[a]				
compd	R	MMP-1	MMP-3	MMP-7	MMP-9	MMP-13
23	CH_3-	73	18	821	1.8	1.0
24	*n*-C_5H_{11}-	115	39	787	1.7	1.7
25	*c*-C_6H_{11}-	219	27	2173	1.8	1.5
26		227	60	1695	4.4	1.0
27	$PhOCH_2$-	92	9.5	908	3.0	2.1
28	Ph-	81	66	1522	3.0	3.0
29		994	184	27680	32	57
30		68	15	710	1.3	1.9
31		25	7.5	642	1.6	2.5
32		62	147	nd	nd	nd
33		1059	173	6086	17	21
34		1320	410	22050	22	27
35		43	23	931	0.9	0.9

[a] See Experimental Section for details of the enzyme assays. nd = not determined.

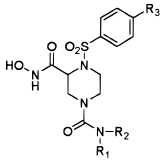
bulk of the amide substituent appears to play little if any role in determining the in vitro potency of the series. The acetamide **23** and the biphenylamide **35** exhibited almost an identical in vitro profile even though these compounds are significantly different in steric requirements. The pyridylamide **29** was significantly less potent than the corresponding benzamide **28**. The presence of a basic nitrogen in this position is clearly not well-tolerated by the MMPs.

Urea-Substituted Piperazines. The urea-substituted piperazines demonstrated a similar in vitro profile to the corresponding amides in Table 3. These compounds were potent inhibitors of MMPs 9 and 13 and weak inhibitors of MMP-7 (Table 4). The parent compound in the series, **36**, was a potent MMPI which only spared MMP-7. This compound possessed moderate selectivity for MMP-3 vs MMP-1. The corresponding *n*-hexylurea **37** possessed little to no selectivity (MMP-1 vs MMP-3), but the corresponding *N*-methyl-*N*-hexylurea **38** was surprisingly selective for MMP-3. Cyclic urea-based inhibitors were also prepared including the morpholino- and *N*-methylpiperazine-based inhibitors **47** and **48**. The inclusion of a ring at this position did not result in a dramatic shift in either potency or selectivity for this series.

Replacement of the *p*-methoxyphenylsulfonamide with a *p*-bromophenylsulfonamide (compound **39**) resulted in a reversal in the in vitro selectivity profile which was generally observed in this series. The compound was significantly less potent for MMP-3 than the corresponding *p*-methoxyphenylsulfonamide **38**. The only other *p*-bromophenylsulfonamide prepared in this series, compound **49**, was also more potent for MMP-1 vs MMP-3.

Absorption Studies. As a first step toward understanding the overall pharmacokinetic profile of the

Table 4. In Vitro Profile of Urea-Substituted Piperazine-Based MMPIs



				IC_{50} (nM)[a]				
compd	R_1	R_2	R_3	MMP-1	MMP-3	MMP-7	MMP-9	MMP-13
36	H-	H-	OMe	115	20	537	2.3	3.6
37	*n*-C_6H_{13}-	H-	OMe	30	28	497	2.6	1.4
38	*n*-C_6H_{13}-	Me-	OMe	71	4.3	3245	6.0	1.9
39	*n*-C_6H_{13}-	Me-	Br	87	175	1200	13	4.5
40	$PhCH_2$-	H-	OMe	99	17	416	3.8	2.4
41[b]	$PhCH_2$-	H-	OMe	68	41	401	6.6	nd
42	$PhCH_2$-	Me-	OMe	322	38	nd	nd	1.8
43	$PhCH_2CH_2$-	H-	OMe	70	27	824	3.6	nd
44	*m*-MeOPh-	H-	OMe	80	13	1100	4.1	2.8
45	*c*-$C_3H_5CH_2$-	*n*-Pr-	OMe	493	65	2093	nd	1.1
46	-$(CH_2)_6$-		OMe	158	54	2694	nd	2.9
47	-$CH_2CH_2OCH_2CH_2$-		OMe	228	124	4350	8.3	54
48	-$CH_2CH_2NMeCH_2CH_2$-		OMe	354	114	2690	5.0	4.1
49	$MeOCH_2CH_2$-	$MeOCH_2CH_2$-	Br	49	385	1134	20	3.2

[a] See Experimental Section for details of the enzyme assays; nd = not determined. [b] Compound **41** is a thiourea.

Table 5. Predicted Peroral Absorption and ex Vivo Efficacy Data

compd	absorption (%)[a]	log *P*[b]	explant IC_{50} (nM)[c]
12	93−98	3.43	>1000
13	98	2.63	>1000
15	51−62	0.24	94
16	5	1.38	<10
17	37	0.57	57
20	59	1.76	43
21	10	1.14	100
23	8−18	0.12	nd
25	13	2.15	nd
27	6	2.17	37
30	14	1.18	35
35	100	3.29	5
37	29−76	2.82	115
38	44−87	3.16	100
39	0	3.85	nd
43	3−4	2.36	108
44	10−23	1.85	22
45	34−37	2.70	44
46	35−37	2.62	370
49	3−6	2.03	nd

[a] Data obtained from both single and multiple determinations; see ref 11 for details. [b] Calculated octanol/water partition coefficient (ClogP, by BioByte Corp., Claremont, CA). [c] Data are expressed as an IC_{50} from multiple concentrations of inhibitor each run in 8 replicate cartilage cultures; nd = not determined.

piperazine-based MMPIs, an attempt was made to determine the permeability of a selected number of analogues. In terms of predicted in vitro peroral absorption values, most of the compounds evaluated showed moderate to good absorption potential.[8] However, it was difficult to identify any definitive trends in the absorption data with physicochemical properties or molecular structure. For example, while the sulfonamides **15** and **17** demonstrated moderate to good absorption, a close relative, **16**, showed a much lower absorption potential. Low predicted absorption of these compounds, in some cases, may have been attributed to low solubility of the molecule. In addition, there was evidence that some compounds in this class were substrates for the various concentration-dependent efflux systems (including P-glycoprotein), encoded by the *MDR1* gene, present in the enterocytes of the intestinal tract (data not shown). Nevertheless, the relatively lipophilic analogues **12** and **13** (*N*-alkyl analogues) and **35** showed the best absorption values. However, attempts to improve the hydrophilic properties of the molecules tended to result in a lower absorption potential in many cases (examples based on calculated log octanol/water partition coefficients: **15**, **16**, **17**, **20**, **21**, **27**, **44**, and **49**). In contrast, compound **39**, which had the highest calculated log octanol/water coefficient of all the analogues (3.85), may have partitioned into the intestinal cell membranes and stayed there, resulting in no transport. Indeed, further experimentation would be necessary to better characterize the intestinal transport properties of these compounds.

Cartilage Degradation. To obtain a preliminary assessment of the efficacy of the piperazine-based MMPIs, a cartilage explant cell culture method was used. The degradation of cartilage matrix was measured ex vivo using cultures of bovine nasal cartilage stimulated with IL-1.[9] An inhibitory effect on matrix degradation in this model may reflect cartilage-protecting potential of compounds. A number of factors can affect the collagen-protecting performance of MMPIs in bovine

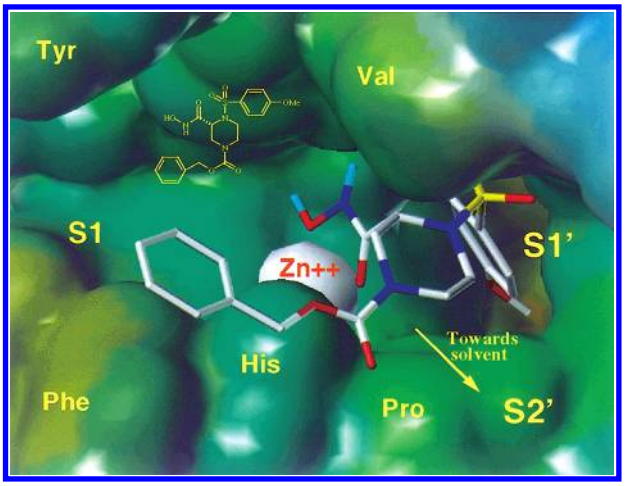


Figure 1. Structure of the stromelysin−**13** complex.

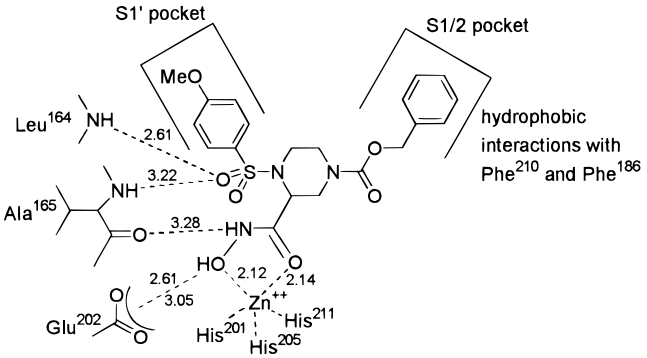


Figure 2. Binding interactions of **13** bound to truncated MMP-3.

explant cultures, including potency for the MMPs, stability of the compounds in the culture medium, and permeability of the MMPIs into cartilage matrix. Preliminary permeability studies done in-house using articular cartilage demonstrate that small MMPIs (MW 400−500) of similar types do penetrate the matrix with ease.[10]

A close examination of the explant data reveals that there is agreement between binding potency and efficacy in this model for a select group of analogues. For example, most of the broad-spectrum MMPIs (**15**, **20**, **21,** etc.) showed efficacy at an IC_{50} level close to 100 nM or less (see Table 5). Some analogues with high IC_{50}'s for the inhibition of MMP-1 (e.g. **12** and **13**) failed to demonstrate any efficacy. Although it has been suggested that MMP-1 plays a prominent role in the BNC explant model,[11] it is difficult to draw any conclusion from these limited examples as to which factor or factors have an overriding effect in terms of determining efficacy in this model.

Structural Analysis. During the progression of this work, crystallography data for a number of enzyme−inhibitor complexes were reported.[12] Most of these structures were for acyclic inhibitors. To understand the binding mode of piperazine-based inhibitors, the crystal structure of **20** bound to truncated MMP-3 was solved. As shown in Figures 1 and 2, the hydroxamic acid unit was tightly anchored through four contact points. Bidentate chelation of Zn^{2+} was achieved using two nearly identical bonds (2.12 and 2.14 Å). Glu-202 was properly poised with two bonding possibilities (2.61 and 3.05 Å). Ala-165 carbonyl formed the expected hydrogen bond

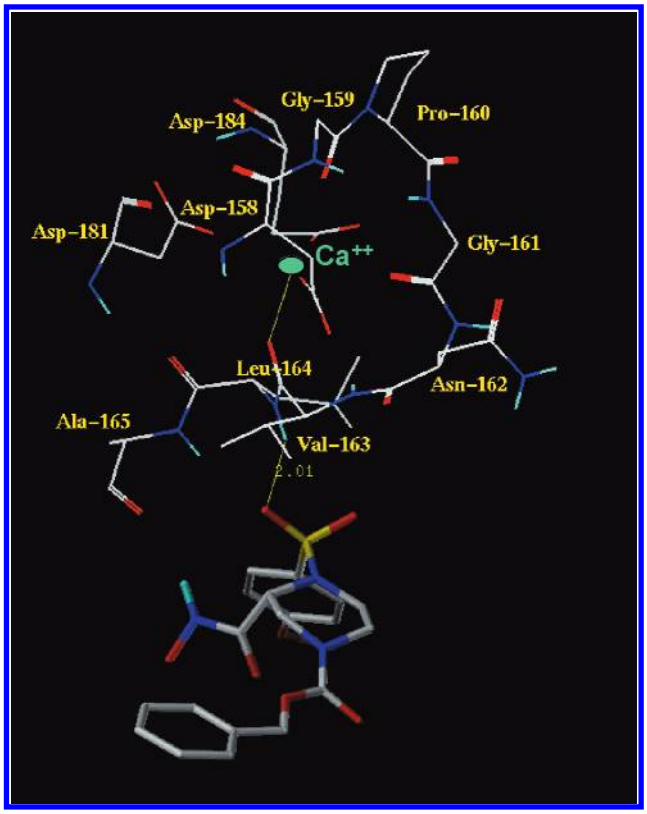


Figure 3. Structure of the stromelysin−**13** complex.

with the hydroxamate nitrogen atom (3.28 Å). One of the oxygen atoms of the sulfonamide unit formed a tight hydrogen bond with Leu-164 (2.89 Å) and was also found in hydrogen bond distance (3.22 Å) with the nitrogen atom of Ala-165. The *p*-methoxyphenyl unit was found to be buried in the deep S1′ pocket. Similar interactions were observed in complexes of **47** with MMPs 1, 3, and 13. These data will be reported in due course.

During the SAR work with the piperazine-based MMPIs, the possible orientation of substituents attached to the distal nitrogen became a major subject of discussions. SAR derived from a diverse series of substituents was quite intriguing. The crystallography data obtained for **20** partly answered this question. The Cbz group was placed in an intermediate position between the S1 and S2′ pockets. Some hydrophobic interactions with Phe-210 and Phe-186 could be observed. Based on the nature of this site, it is possible that some of the polar substituents (as in **17** and **21**) can shift more toward the solvent.The importance of Leu-164 in terms of forming a strong hydrogen bond with the peptidic backbone, or in this case with one of the oxygen atoms of the sulfonamide unit, has been a subject of much discussion.[13] One can hypothesize that the proximity of structural Ca^{2+} to Val-163 carbonyl polarizes the bond considerably, leading to lower p*K* for the Leu-164 amidic proton (see Figures 3 and 4). This, in turn, can help Leu-164 form a strong hydrogen bond with a suitably oriented oxygen atom, supplied by either an amidic carbonyl group or a sulfonamide, as seen for **20**. A careful survey of many MMPIs reveals that a 1,4-relationship between the two heteroatoms, as shown in Figure 2, is generally preserved in order to achieve high binding potency (also see BB-2516 and BY12-9566 in Figure 4).[14]

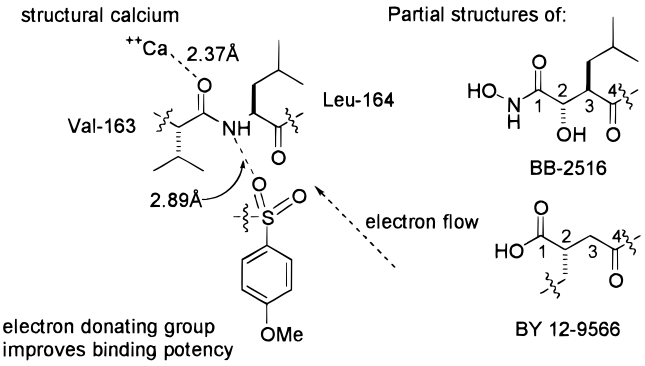


Figure 4. Possible role played by structural Ca^{2+} to induce a critical H-bonding.

Conclusion

The design and synthesis of a new generation of cyclic MMPIs have been described. Variation of substituents attached to the 4N-position of a piperazine scaffold has produced a series of potent inhibitors with varying degree of selectivity for MMPs reported herein. By proper optimization of various substituents, a number of inhibitors with high absorption profiles and desirable efficacies in the bovine nasal cartilage assay were identified. A careful analysis of the X-ray data revealed that certain structural elements (optimized substituents attached to the distal nitrogen, a bidentate ligand for catalytic Zn^{2+}, hydrogen bonding with Leu-164, etc.) are essential for achieving high binding affinity and selectivity. Some explanations, as to why an electron-donating group attached to the phenylsulfonyl unit enhances the binding potency for certain MMPs, have been provided. The required geometry of folding for the piperazine-based MMPIs has also been delineated.

Experimental Section

General Methods and Materials. ^{1}H NMR spectra were measured at 300 MHz on either a Bruker or GE instrument using deuteriochloroform as solvent unless otherwise indicated. Mass spectra were measured at 70 eV in CI (chemical ionization) or ESI (electrospray ionization). Elemental analyses (C, H, N) were performed at either Oneida Research Services or Procter and Gamble Analytical and Stability Laboratories in Norwich. Analytical thin-layer chromatography (TLC) analysis was performed using Merck DC-F_{254} glass-based silica gel plates. Flash chromatography was performed using kieselgel 60 (230−400 mesh) silica gel. All organic extracts were dried over anhydrous $MgSO_4$ or Na_2SO_4 prior to solvent removal on a rotary evaporator under reduced pressure. For the synthesis of a hydroxamic acid from the corresponding methyl ester, a general procedure, as outlined in Fieser & Fieser, Vol. 1, p 478, was followed.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonylpiperazine-2-carboxamidotrifluoroacetic Acid (10).** To a solution of Boc-piperazine **(19)** (95 mg, 0.23 mmol) in 5 mL of CH_2Cl_2 was added trifluoroacetic acid (5 mL) at 0 °C. The reaction was stirred for 4 h at room temperature, followed by removal of solvent under reduced pressure. The crude product was purified by flash silica gel column eluting with CH_3OH/EtOAc (1:9) to give 90 mg (61% yield) of desired product as a foamy solid: ^{1}H NMR (DMSO-d_6) δ 2.63 (td, J = 12.3, 4.0 Hz, 1H), 2.82 (dd, J = 13.6, 2.8 Hz, 1H), 3.01 (d, J = 13.5 Hz, 1H), 3.26−3.34 (m, 1H), 3.48 (td, J = 12.2, 3.3 Hz, 1H), 3.61−3.72 (m, 1H), 3.89 (s, 3H), 4.37 (d, J = 3.5 Hz, 1H), 7.10 (dd, J = 9.0, 1.2 Hz, 2H), 7.80 (dd, J = 8.9, 1.2 Hz, 2H); ion spray MS 338 $[M + Na]^+$, 316 $[M + H]^+$; HRMS (M^+) calcd for $C_{12}H_{18}N_3O_5S$ 316.0967, found (M^+) 316.0971.

1-(4-Methoxyphenyl)sulfonyl-4-(*tert*-butoxycarbonyl)-piperazine-2-carboxylic Acid (6). To a solution of piperazine-2-carboxylic acid dihydrochloride **(4)** (3 g, 14.8 mmol) in 30 mL of *p*-dioxane and 15 mL of water at 0 °C was added slowly aqueous sodium hydroxide (2.3 mL, 50% w/w, 19.4 N, 44.4 mmol), followed by di-*tert*-butyl dicarbonate (3.6 g, 16.3 mmol). After 5 h at room temperature (or overnight at larger scale), triethylamine (4.1 mL, 29.6 mmol) and 4-methoxyphenylsulfonyl chloride (3.0 g, 14.8 mmol) were added, and the reaction was stirred overnight. The reaction mixture was concentrated under reduced pressure and partitioned between EtOAc and 1 N HCl. The EtOAc layer (2 × 100 mL) was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the title compound (4.2 g, 80%) as a solid: ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 9H), 2.78−2.94 (m, 1H), 3.05−3.18 (m, 1H), 3.30−3.44 (m, 1H), 3.54−3.68 (m, 1H), 3.86 (s, 3H), 3.72−3.88 (m, 1H), 4.46−4.62 (m, 2H), 6.95 (dd, J = 9.0, 2.2 Hz, 2H), 7.70 (dd, J = 9.2, 2.2 Hz, 2H); CI$^+$ MS 418 $[M + NH_4]^+$, 401 $[M + H]^+$, 362 $[M + NH_4 - tBu]^+$, 345 $[M + H - tBu]^+$, 301 $[M + H - tBoc]^+$.

Methyl 1-(4-Methoxyphenyl)sulfonylpiperazine-2-carboxylate Hydrochloride (7). To a solution of 1-(4-methoxyphenyl)sulfonyl-4-(*tert*-butoxycarbonyl)piperazine-2-carboxylic acid **(6)** (19.6 g, 48.9 mmol) in 100 mL of methanol was added thionyl chloride (18 mL, 244.5 mmol) dropwise at room temperature. The reaction was stirred overnight. The reaction mixture was concentrated under reduced pressure to a solid residue, which was triturated from 5% methanol/hexane to give the title compound (13.06 g, 76%) as a solid: ^{1}H NMR ($CDCl_3$) δ 2.98 (td, J = 12.8, 3.8 Hz, 1H), 3.15 (dd, J = 12.5, 4.3 Hz, 1H), 3.42 (d, J = 9.8 Hz, 1H), 3.52−3.64 (m, 1H), 3.66 (s, 3H), 3.80−3.91 (m, 2H), 3.88 (s, 3H), 4.86 (d, J = 4.8 Hz, 1H), 6.98 (d, J = 9.2 Hz, 2H), 7.72 (d, J = 9.0 Hz, 2H); CI$^+$ MS 315 $[M + H]^+$.

Methyl 1-(4-Methoxyphenyl)sulfonyl-4-methylpiperazine-2-carboxylate (11a). To a solution of amine hydrochloride **7** (1.2 g, 3.4 mmol) and sodium acetate (0.84 g, 10.26 mmol) in 10 mL of ethanol was added paraformaldehyde (0.21 g, 6.84 mmol), followed by sodium cyanoborohydride (0.45 g, 6.84 mmol) slowly at room temperature. The reaction was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure and stirred in 1 N HCl for 30 min. The mixture was made basic by 1 N NaOH and extracted with EtOAc (3 × 80 mL) in the presence of some solid NaCl. The EtOAc layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel using EtOAc as elute to give the title compound (0.68 g, 62%) as an oil: ^{1}H NMR ($CDCl_3$) δ 2.04 (td, J = 15.4, 3.7 Hz, 1H), 2.24 (s, 3H), 2.21−2.26 (m, 1H), 2.67 (ddd, J = 11.3, 1.4, 1.7 Hz, 1H), 3.21 (ddd, J = 11.4, 1.8, 1.9 Hz, 1H), 3.38 (td, J = 11.0, 3.2 Hz, 1H), 3.61 (s, 3H), 3.54−3.62 (m, 1H), 3.87 (s, 3H), 4.64 (t, J = 1.9 Hz, 1H), 6.94 (dd, J = 8.9, 2.0 Hz, 2H), 7.72 (dd, J = 8.9, 2.0 Hz, 2H); CI$^+$ MS 329 $[M + H]^+$.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-methylpiperazine-2-carboxamide (11).** The methyl ester **11a** (0.45 g, 1.37 mmol) was mixed with NH_2OK (6 mL, 10.2 mmol, 1.7 M in methanol; solution prepared as described in Fieser & Fieser, Vol. 1, p 478) and stirred overnight at room temperature. Silica (1.5 g) was added to the mixture and the solvent was evaporated. The dry silica was poured on top of a flash silica gel column which was subsequently eluted with CH_3OH/EtOAc (1:5) to give 275 mg (61% yield) of desired product as a thick oil: ^{1}H NMR (DMSO-d_6) δ 1.74 (td, J = 15.3, 3.4 Hz, 1H), 1.86 (s, 3H), 1.90 (dd, J = 12.1, 4.2 Hz, 1H), 2.36 (d, J = 10.2 Hz, 1H), 3.12 (d, J = 10.4, 1H), 3.42 (td, J = 11.0, 3.2 Hz, 1H), 3.73 (br d, J = 12.8 Hz, 1H), 3.91 (s, 3H), 4.42 (br s, 1H), 7.08 (dd, J = 8.9, 2.0 Hz, 2H), 7.80 (dd, J = 8.9, 2.0 Hz, 2H); ion spray MS 352 $[M + Na]^+$, 330 $[M^+ + H]^+$; HRMS ($[M + H]^+$) calcd for $C_{13}H_{19}N_3O_5S$ 330.1124, found ($[M + H]^+$) 330.1116.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-(*n*-hexyl)-piperazine-2-carboxamide (12).** The title compound was prepared following the procedure described for compound **11:** yield 38%; ^{1}H NMR (DMSO-d_6) δ 0.92 (t, J = 6.7 Hz, 3H), 1.04−1.36 (m, 8H), 1.58−1.67 (m, 1H), 1.70−1.82 (m, 1H), 1.98−2.12 (m, 2H), 2.52−2.61 (m, 1H), 2.98−3.08 (m, 1H), 3.32−3.59 (m, 2H), 3.82 (s, 3H), 4.22 (s, 1H), 7.08 (d, J = 9.0 Hz, 2H), 7.76 (d, J = 9.0 Hz, 2H), 8.80 (s, 1H), 10.48 (s, 1H); ESI$^+$ MS 400 $[M + H]^+$. Anal. ($C_{18}H_{29}N_3O_5S \cdot 0.25H_2O$) C, H, N.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-phenylmethylpiperazine-2-carboxamide (13).** The title compound was prepared from amine hydrochloride **7** following the procedure described for compound **11**: yield 40%; ^{1}H NMR (5% NaOD) δ 1.45−1.66 (m, 1H), 2.32 (d, J = 11.9 Hz, 1H), 2.96 (d, J = 11.6 Hz, 1H), 3.22−3.51 (m, 4H), 3.64 (d, J = 13.2 Hz, 1H), 3.80 (s, 3H), 4.24 (s, 1H), 6.88 (d, J = 9.0 Hz, 2H), 6.94−7.03 (m, 2H), 7.19−7.30 (m, 3H), 7.59 (d, J = 9.0 Hz, 2H); ion spray MS 428 $[M + Na]^+$, 406 $[M + H]^+$. Anal. ($C_{19}H_{23}N_3O_5S \cdot HCl \cdot 0.5H_2O$) C, H, N.

Methyl 4-[4-(4-Bromophenyl)thiazol-2-yl]-1-(4-methoxyphenyl)sulfonylpiperazine-2-carboxylate (14a). To a solution of amine hydrochloride **7** (300 mg, 0.86 mmol) and acetic acid (50 μL, 0.86 mmol) in ethanol (5 mL) was added 4-bromophenacyl thiocyanate (220 mg, 0.86 mmol). The reaction mixture was stirred at room temperature for 6 h, neutralized with triethylamine, and partitioned between ether and water. The ether layer was washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by flash chromatography (hexanes−ethyl acetate 4/1 v/v) to give 135 mg (28% yield) of the title compound as colorless solid: mp 68−70 °C; ^{1}H NMR ($CDCl_3$) δ 3.18 (dt, J = 11.6, 3.9 Hz, 1H), 3.37 (dd, J = 13.6, 3.0 Hz, 1H), 3.49 (s. 3H), 3.55 (m, 1H), 3.78 (m, 1H), 3.84 (s, 3H), 4.00 (m, 1H), 4.51 (bd, J = 13.2 Hz, 1H), 4.76 (m, 1H), 6.78 (s, 1H), 6.95 (d, J = 8.6 Hz, 2H), 7.47 (d, J = 8.6 Hz, 2H), 7.64 (d, J = 8.6 Hz, 2H), 7.73 (d, J = 8.6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 41.2, 48.0, 50.5, 52.5, 54.7, 55.9, 102.8, 114.2, 121.6, 127.5, 129.4, 131.0, 131.6, 133.6, 150.5, 163.1, 169.3, 170.2.

***N*-Hydroxy-4-[4-(4-bromophenyl)thiazol-2-yl]-1-(4-methoxyphenyl)sulfonylpiperazine-2-carboxamide (14).** To methyl ester **14a** (121 mg, 0.22 mmol) was added NH_2OK (2 mL of 1.7 M solution in methanol; prepared as described in Fieser & Fieser, Vol. 1, p 478) and the reaction mixture was stirred overnight at room temperature. The reaction mixture was neutralized with 1 N aqueous hydrochloric acid and concentrated under reduced pressure. The crude product was purified by flash chromatography (hexanes−ethyl acetate 3/7 v/v) to give 54.6 mg (45% yield) of the title compound as colorless solid: mp 181 °C dec; ^{1}H NMR ($CDCl_3$) δ 3.00 (dt, J = 11.6, 5.7 Hz, 1H), 3.24 (dd, J = 12.6, 5.8 Hz, 1H), 3.68−3.90 (m, 3H), 3.81 (s, 3H), 4.26 (dd, J = 12.5, 1.9 Hz, 1H), 4.59 (m, 1H), 6.98 (s, 1H), 7.06 (d, J = 8.7 Hz, 2H), 7.48 (d, J = 8.7 Hz, 2H), 7.70 (d, J = 8.7 Hz, 2H), 7.82 (d, J = 8.7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 43.1, 47.7, 55.6, 56.2, 104.0, 115.7, 122.3, 128.8, 130.7, 132.0, 132.6, 135.4, 151.5, 165.0, 168.0, 171.9; ion spray MS 553, 555 $[M + H]^+$, 575, 577 $[M + Na]^+$. Anal. ($C_{21}H_{21}BrN_4O_5S_2$) C, H, N.

Methyl 1-(4-Methoxyphenyl)sulfonyl-4-methylsulfonylpiperazine-2-carboxylate (15a). To a solution of amine hydrochloride **7** (1.5 g, 4.3 mmol), triethylamine (1.8 mL, 12.7 mmol), and DMAP (52 mg, 0.43 mmol) in 2 mL of water and 8 mL of *p*-dioxane was added methanesulfonyl chloride (0.43 mL, 5.56 mmol) dropwise at room temperature. The reaction was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure and partitioned between EtOAc and water. The EtOAc layer was washed with 1 N HCl, water, and brine, dried over $MgSO_4$, and concentrated under reduced pressure to give the title compound (0.8 g, 50%): ^{1}H NMR ($CDCl_3$) δ 2.88 (s, 3H), 2.89−2.97 (m, 1H), 3.01 (dd, J = 12.1, 4.0 Hz, 1H), 3.40 (td, J = 12.0, 3.4 Hz, 1H), 3.61 (s, 3H), 3.67−3.80 (m, 2H), 3.85 (s, 3H), 4.21 (ddd, J = 10.3, 1.9, 1.9 Hz, 1H), 4.78−4.81 (m, 1H), 6.97 (d, J = 9.0 Hz, 2H), 7.72 (d, J = 9.0 Hz, 2H); ion spray MS 410 (100, $M + NH_4^+$), 393 (46, $M^+ + H$).

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-methylsulfonylpiperazine-2-carboxamide (15).** The methyl ester **15a** (0.7 g, 1.78 mmol) was mixed with NH_2OH (10 mL, 14.2 mmol,

1.5 M in methanol) and stirred overnight at room temperature. The reaction mixture was acidified with HCl, silica (2 g) was added, and the solvent was evaporated. The dry silica was poured on top of a flash silica gel column which was subsequently eluted with 5% CH_3OH/EtOAc to give 0.31 g (46% yield) of the desired product as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.56−2.64 (m, 1H), 2.79 (s, 3H), 2.78−2.88 (m, 1H), 3.40−3.49 (m, 1H), 3.52−3.70 (m, 2H), 3.73−3.81 (m, 1H), 3.83 (s, 3H), 4.36−4.42 (m, 1H), 7.07 (d, J = 9.0 Hz, 2H), 7.78 (d, J = 9.0 Hz, 2H), 8.92 (s, 1H), 10.65 (s, 1H); ion spray MS 411 [M + NH_4]$^+$, 394 [M + H]$^+$. Anal. ($C_{13}H_{19}N_3O_7S_2$) C, H, N.

***N*-Hydroxy-1,4-bis(4-methoxyphenylsulfonyl)piperazine-2-carboxamide (16).** The title compound was prepared from amine hydrochloride **7** following the sequence of reactions described for compound **15**: yield 54%; ^{1}H NMR (DMSO-d_6) δ 1.63 (td, J = 11.5, 3.1 Hz, 1H), 1.82 (dd, J = 11.8, 4.2 Hz, 1H), 3.22−3.41 (m, 1H), 3.42−3.61 (m, 1H), 3.72−3.88 (m, 2H), 3.81 (s, 3H), 3.89 (s, 3H), 4.47 (br s, 1H), 6.93 (d, J = 9.0 Hz, 2H), 7.10 (d, J = 9.0 Hz, 2H), 7.48 (d, J = 9.0 Hz, 2H), 7.64 (d, J = 9.0 Hz, 2H); ion spray MS 508 [M + Na]$^+$, 503 [M + NH_4]$^+$, 486 [M + H]$^+$. Anal. ($C_{19}H_{23}N_3O_8S_2$) C, H, N.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-[(2-amino-4-methyl-5-thiazolyl)sulfonyl]piperazine-2-carboxamide (17).** The title compound was prepared as a white solid from amine hydrochloride **7** following the procedure described for compound **15**: yield 27%; ^{1}H NMR (DMSO-d_6) δ 2.03 (td, J = 13.6, 3.6 Hz, 1H), 2.22 (s, 3H), 2.19−2.30 (m, 1H), 3.25−3.38 (m, 1H), 3.42−3.60 (m, 1H), 3.74−3.91 (m, 2H), 3.83 (s, 3H), 4.52 (s, 1H), 7.03 (d, J = 9.0 Hz, 2H), 7.72 (d, J = 9.0 Hz, 2H), 7.84 (s, 2H), 8.98 (s, 1H), 10.72 (s, 1H); ion spray MS 514 [M + Na]$^+$, 492 [M + H)$^+$. Anal. ($C_{16}H_{21}N_5O_7S_3$) C, H, N.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-[(3,5-dimethyl-4-isoxazolyl)sulfonyl]piperazine-2-carboxamide (18).** The title compound was prepared as a white solid from amine hydrochloride **7** following the sequence of reactions described for compound **15**: yield 47%; ^{1}H NMR (DMSO-d_6) δ 1.92−2.02 (m, 1H), 2.18 (s, 3H), 2.19−2.26 (m, 1H), 2.44 (s, 3H), 3.24−3.36 (m, 1H), 3.39−3.49 (m, 1H), 3.66−3.82 (m, 2H), 3.80 (s, 3H), 4.42−4.48 (m, 1H), 6.98 (d, J = 9.0 Hz, 2H), 7.64 (d, J = 9.0 Hz, 2H), 8.92 (s, 1H), 10.73 (s, 1H); ion spray MS 492 [M + NH_4]$^+$, 475 [M + H]$^+$. Anal. ($C_{17}H_{22}N_4O_8S_2$) C, H, N.

Methyl 1-(4-Methoxyphenyl)sulfonyl-4-(*tert*-butoxycarbonyl)piperazine-2-carboxylate (19a). To a solution of acid **6** (2 g, 5.61 mmol) in 8 mL of DMF was added potassium *tert*-butoxide (1 g, 8.4 mmol) at room temperature. After 30 min, iodomethane (0.42 mL, 6.73 mmol) was added at 0 °C, and the resulting mixture was stirred at room temperature overnight. The reaction mixture was quenched with water and partitioned between EtOAc and H_2O. The EtOAc layer was washed with 1 N HCl, 1 N NaOH, H_2O, and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. Column chromatography of the residue on silica gel using hexane/EtOAc (4:1) provided the title compound as an oil: 1.25 g (54%); ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 9H), 2.77−2.98 (m, 1H), 3.03−3.19 (m, 1H), 3.31−3.50 (m, 1H), 3.53 (s, 3H), 3.56−3.68 (m, 1H), 3.86 (s, 3H), 3.92−4.21 (m, 1H), 4.39−4.53 (m, 1H), 4.53−4.61 (m, 1H), 6.96 (dd, J = 9.0, 2.2 Hz, 2H), 7.70 (dd, J = 9.1, 2.2 Hz, 2H); CI$^+$ MS 432 [M + NH_4]$^+$, 415 [M + H]$^+$, 376 [M + NH_4 − tBu]$^+$, 359 [M + H − tBu]$^+$, 315 [M + H − tBoc]$^+$.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-(*tert*-butoxycarbonyl)piperazine-2-carboxamide (19).** The methyl ester **19a** (0.33 g, 0.8 mmol) was converted to title compound following the procedure described for compound **15**, providing the desired product as a white foamy solid: yield 61%; ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 9H), 2.72−2.99 (m, 1H), 3.05 (dd, J = 14.6, 3.9 Hz, 1H), 3.54−3.64 (m, 2H), 3.86 (s, 3H), 3.81−3.91 (m, 1H), 4.11−4.30 (m, 2H), 7.04 (dd, J = 8.8, 1.2 Hz, 2H), 7.74 (dd, J = 8.9, 1.2 Hz, 2H); ion spray MS 438 [M + Na]$^+$, 433 [M + NH_4]$^+$, 416 [M + H]$^+$; HRMS (M$^+$) calcd for $C_{17}H_{26}N_3O_7S$ 416.1491, found (M$^+$) 416.1501.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-benzyloxycarbonylpiperazine-2-carboxamide (20).** The title compound was prepared as an amorphous solid from amine hydrochloride **7** following the procedure described for compound **15**: yield 60%; ^{1}H NMR ($CDCl_3$) δ 2.70−2.98 (m, 1H), 3.08 (dd, J = 11.9, 3.2 Hz, 1H), 3.46−3.65 (m, 2H), 3.78−3.90 (m, 1H), 3.86 (s, 3H), 3.96−4.18 (m, 1H), 4.24 (br s, 1H), 4.93−5.06 (m, 2H), 7.06 (dd, J = 8.9, 2.2 Hz, 2H), 7.25−7.40 (m, 5H), 7.72 (dd, J = 8.9, 2.2 Hz, 2H), 8.89 (s, 1H), 10.72 (s, 1H); ion spray MS 472 [M + Na]$^+$, 450 [M + H]$^+$. Anal. ($C_{20}H_{23}N_3O_7S$) C, H, N.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-(3-pyridinylmethoxycarbonyl)piperazine-2-carboxamide (21).** The title compound was prepared as a white solid from amine hydrochloride **7** following the procedure described for compound **15**: yield 47%; ^{1}H NMR (DMSO-d_6) δ 3.01−3.60 (m, 5H), 3.83 (s, 3H), 4.01−4.13 (m, 1H), 4.21−4.28 (m, 1H), 4.98−5.08 (m, 2H), 7.08 (d, J = 9.0 Hz, 2H), 7.34−7.40 (m, 1H), 7.67−7.78 (m, 3H), 8.22 (s, 1H), 8.47−8.56 (m, 2H), 10.77 (s, 1H); ESI$^+$ MS 451 (100, M$^+$ + H). Anal. ($C_{19}H_{22}N_4O_7S\cdot H_2O$) C, H, N.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-(3-ethoxy-1-propoxycarbonyl)piperazine-2-carboxamide (22).** The title compound was prepared from amine hydrochloride **7** following the procedure described for compound **15**: yield 51%; ^{1}H NMR (DMSO-d_6) δ 1.02 (t, J = 6.9 Hz, 3H), 1.61−1.72 (m, 2H), 2.62−2.81 (m, 1H), 2.92−3.01 (m, 1H), 3.27−3.38 (m, 5H), 3.44−3.59 (m, 2H), 3.80 (s, 3H), 3.83−4.01 (m, 3H), 4.13−4.19 (m, 1H), 7.05 (d, J = 9.0 Hz, 2H), 7.66 (d, J = 9.0 Hz, 2H), 8.79 (s, 1H), 10.63 (s, 1H); ion spray MS 463 [M + NH_4]$^+$, 446 M + H]$^+$. Anal. ($C_{18}H_{27}N_3O_8S$) C, H, N.

Methyl 1-(4-Methoxyphenyl)sulfonyl-4-acetylpiperazine-2-carboxylate (23a). To a solution of amine hydrochloride **7** (2 g, 5.7 mmol), triethylamine (3.2 mL, 22.8 mmol), and DMAP (70 mg, 0.57 mmol) in 10 mL of *p*-dioxane was added acetic anhydride (0.81 mL, 8.5 mmol) dropwise at room temperature. The reaction was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure and partitioned between EtOAc and water. The EtOAc layer was washed with 1 N HCl, water, and brine, dried over $MgSO_4$, and concentrated under reduced pressure to give the title compound (1.37 g, 67%). The ^{1}H NMR spectrum shows two distinct rotamers: ^{1}H NMR ($CDCl_3$) δ 2.04 (d, J = 10.0 Hz, 6H), 2.43 (dd, J = 12.4, 4.5 Hz, 1H), 2.94 (dd, J = 12.1, 4.0 Hz, 1H), 3.08 (td, J = 12.6, 3.4 Hz, 1H), 3.21−3.41 (m, 2H), 3.58 (d, J = 10.0 Hz, 6H), 3.56−3.58 (m, 1H), 3.64−3.80 (m, 3H), 3.88 (d, J = 3.4 Hz, 6H), 4.32 (d, J = 14.6 Hz, 1H), 4.48−4.62 (m, 1H), 4.72−4.77 (m, 1H), 4.92 (d, J = 14.1 Hz, 1H), 6.92−7.01 (m, 4H), 7.64−7.78 (m, 4H); ion spray MS 374 [M + NH_4]$^+$, 357 [M + H]$^+$.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-acetylpiperazine-2-carboxamide (23).** The methyl ester (1.3 g, 3.65 mmol) was mixed with NH_2OH (21 mL, 28.8 mmol, 1.5 M in methanol) and stirred overnight at room temperature. After adjusting the pH to 5 with acetic acid, the crude product was mixed with silica gel, and the solvent was evaporated. The dry silica was poured on top of a flash silica gel column which was subsequently eluted with EtOAc followed by a mixture of 20% CH_3OH/EtOAc to give 0.7 g (54% yield) of the desired product. The ^{1}H NMR spectrum shows two distinct rotamers: ^{1}H NMR (DMSO-d_6) δ 1.39 (s, 3H), 2.18−2.26 (m, 1H), 2.28−2.37 (m, 1H), 2.60−2.68 (m, 1H), 2.85−2.91 (m, 1H), 3.03−3.12 (m, 1H), 3.77 (s, 3H), 3.79−4.01 (m, 1H), 6.93 (d, J = 9.0 Hz, 2H), 7.72 (d, J = 9.0 Hz, 2H), 8.92 (s, 1H), 10.65 (s, 1H); ion spray MS 375 [M + NH_4]$^+$, 358 [M + H]$^+$. Anal. ($C_{14}H_{19}N_3O_6S$) C, H, N.

Methyl 1-(4-Methoxyphenyl)sulfonyl-4-cyclohexanecarbonylpiperazine-2-carboxylate (25a). To a solution of amine hydrochloride **7** (2 g, 5.7 mmol), triethylamine (2.4 mL, 17.6 mmol), and DMAP (70 mg, 0.57 mmol) in 4 mL of water and 8 mL of *p*-dioxane was added cyclohexanecarbonyl chloride (1.0 mL, 7.4 mmol) at 0 °C. The reaction was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure and partitioned between EtOAc and water. The EtOAc layer was washed with 1 N HCl, aqueous $NaHCO_3$, water, and brine, dried over $MgSO_4$, and concentrated under reduced pressure to give 1.5 g (62%) of the desired product: ^{1}H NMR ($CDCl_3$) δ 1.12−1.86 (m, 8H), 2.28−2.50 (m, 1H), 2.58−2.73 (m, 1H), 2.88−2.98 (m, 1H), 3.08−3.39 (m, 2H),

3.42−3.58 (m, 3H), 3.60−3.76 (m, 1H), 3.89 (s, 3H), 4.36−4.47 (m, 1H), 4.48−4.77 (m, 2H), 4.92−5.01 (m, 1H), 6.96 (d, J = 9.0 Hz, 2H), 7.62−7.74 (m, 2H); CI^+ MS 425 $[M + H]^+$.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-cyclohexanecarbonylpiperazine-2-carboxamide (25).** The methyl ester **25a** (1.3 g, 3.0 mmol) was mixed with NH_2OH (16.3 mL, 24.5 mmol, 1.7 M in methanol) and stirred overnight at room temperature. After adjusting the pH to 5 with acetic acid, the crude product was mixed with silica gel, and the solvent was evaporated. The dry silica was poured on top of a flash silica gel column which was subsequently eluted with 5% CH_3OH/EtOAc to give 0.58 g (46% yield) of the desired product as a white solid: ^{1}H NMR (DMSO-d_6) δ 0.98−1.24 (m, 6H), 1.34−1.64 (m, 4H), 2.26−2.59 (m, 2H), 2.88−3.14 (m, 2H), 3.21−3.60 (m, 2H), 3.80 (s, 3H), 4.08−4.34 (m, 2H), 7.06 (d, J = 9.0 Hz, 2H), 7.60−7.78 (m, 2H), 8.72−8.96 (m, 1H), 10.58−10.74 (m, 1H); ion spray MS 448 $[M + Na]^+$, 443 $[M + NH_4]^+$, 426 $[M + H]^+$. Anal. ($C_{19}H_{27}N_3O_6S \cdot 0.5H_2O$) C, H, N.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-(1-oxohexyl)-piperazine-2-carboxamide (24).** The title compound was prepared as a solid from amine hydrochloride **7** following the procedure described for compound **25**: yield 54%; ^{1}H NMR (DMSO-d_6) δ 0.80 (t, 3H), 1.07−1.26 (m, 4H), 1.27−1.41 (m, 2H), 2.02−2.21 (m, 2H), 2.86−3.08 (m, 2H), 3.30−3.93 (m, 3H), 3.78 (s, 3H), 3.92−4.28 (m,3H), 7.04 (d, J = 8.9 Hz, 2H), 7.68 (t, J = 10.4 Hz, 2H); ion spray MS 436 $[M + Na]^+$, 414 $[M + H]^+$; HRMS ($M^+ + H$) calcd for $C_{18}H_{27}N_3O_6S$ 414.1699, found ($M^+ + H$) 414.1690.

***N*-Hydroxy-1-(4-bromophenyl)sulfonyl-4(S)-(2-hydroxy-3-methyl-1-oxobutyl)piperazine-2-carboxamide (26).** The title compound was prepared as a white foaming solid from amine hydrochloride **7** following the procedure described for compound **25**: yield 70%; ^{1}H NMR (DMSO-d_6) δ 0.63−0.84 (m, 6H), 2.57−2.72 (m, 1H), 2.83−3.04 (m, 2H), 3.10−3.21 (m, 1H), 3.41−3.62 (m, 2H), 3.83 (s, 3H), 3.88−4.06 (m, 2H), 4.18−4.28 (m, 2H), 4.33−4.64 (m, 2H), 7.08 (d, J = 9.0 Hz, 2H), 7.62−7.82 (m, 2H), 8.82 (s, 1H), 10.62 (s, 1H); ion spray MS 433 $[M + NH_4]^+$, 416 $[M + H]^+$. Anal. ($C_{17}H_{25}N_3O_7S$) C, H, N.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-phenoxyacetylpiperazine-2-carboxamide (27).** The title compound was prepared as a white solid from amine hydrochloride **7** following the procedure described for compound **25**: yield 40%; ^{1}H NMR (DMSO-d_6) δ 2.42−2.60 (m, 1H), 2.90−3.18 (m, 2H), 3.37−3.81 (m, 2H), 3.96 (s, 3H), 3.88−4.18 (m, 1H), 4.23−4.41 (m, 1H), 4.59−4.84 (m, 2H), 6.86 (d, J = 7.9 Hz, 2H), 6.91 (t, J = 8.0 Hz, 2H), 7.12 (d, J = 8.8 Hz, 2H), 7.23 (t, J = 8.4 Hz, 2H), 7.66−7.82 (m, 2H); ion spray MS 467 $[M + NH_4]^+$, 450 $[M + H]^+$. Anal. ($C_{20}H_{23}N_3O_7S$) C, H, N.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-benzoylpiperazine-2-carboxamide (28).** The title compound was prepared as a colorless solid from amine hydrochloride **7** following the procedure described for compound **23**: yield 73%; ^{1}H NMR ($CDCl_3$) δ 3.05−3.80 (m, 6H), 3.90 (s, 3H), 4.60 (m, 1H), 7.01 (d, J = 9.3 Hz, 2H), 7.40−7.46 (m, 5H), 7.79 (d, J = 9.3 Hz, 2H); ion spray MS 420 $[M + H]^+$. Anal. ($C_{19}H_{21}N_3O_6S$) C, H, N.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-nicotinoylpiperazine-2-carboxamide Hydrochloride (29).** The title compound was prepared as a solid from amine hydrochloride **7** following the procedure described for compound **24**: yield 31%; ^{1}H NMR (DMSO-d_6) δ 2.19−3.45 (m, 6H), 3.89 (s, 3H), 3.96−4.04 (m, 1H), 7.04 (d, J = 8.9 Hz, 2H), 7.39−7.46 (m, 1H), 7.64 (d, J = 8.9 Hz, 2H), 8.01−8.22 (m, 1H), 8.56−8.86 (m, 3H); ion spray MS 443 $[M + Na]^+$, 421 $[M + H]^+$. Anal. ($C_{18}H_{20}N_4O_6S$) C, H, N.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-thiophenecarbonylpiperazine-2-carboxamide (30).** The title compound was prepared as a white solid from amine hydrochloride **7** following the procedure described for compound **24**: yield 30%; ^{1}H NMR (DMSO-d_6) δ 2.93−3.12 (m, 1H), 3.50−3.68 (m, 2H), 3.80 (s, 3H), 3.97−4.07 (m, 1H), 4.12−4.18 (m, 3H), 6.98−7.10 (m, 3H), 7.26 (d, J = 1.8 Hz, 1H), 7.64−7.72 (m, 3H), 8.89 (s, 1H), 10.72 (s, 1H); ion spray MS 448 $[M + Na]^+$, 443 $[M + NH_4]^+$, 426 $[M + H]^+$. Anal. ($C_{17}H_{19}N_3O_6S_2$) C, H, N.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-furoylpiperazine-2-carboxamide (31).** The title compound was prepared as a white solid from amine hydrochloride **7** following the procedure described for compound **24**: yield 36%; ^{1}H NMR (DMSO-d_6) δ 2.92−4.15 (m, 6H), 3.83 (s, 3H), 4.24−4.36 (m, 1H), 6.57−6.64 (m, 1H), 6.91 (d, J = 3.4 Hz, 1H), 7.06 (d, J = 9.0 Hz, 2H), 7.82 (s, 1H), 8.91 (s, 1H); ion spray MS 432 $[M + Na]^+$, 427 $[M + NH_4]^+$, 410 $[M + H]^+$; HRMS ($M^+ + H$) calcd for $C_{17}H_{19}N_3O_7S$ 410.1022, found ($M^+ + H$) 410.1020.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-(isoxazole-5-carbonyl)piperazine-2-carboxamide (32).** The title compound was prepared as a white solid from amine hydrochloride **7** following the procedure described for compound **24**: yield 32%; ^{1}H NMR (CD_3OD) δ 3.59−3.76 (m, 2H), 3.82−4.01 (m, 6H), 4.08−4.80 (m, 2H), 4.78−4.98 (m, 2H), 7.01−7.21 (m, 4H), 7.72−7.86 (m, 2H); ion spray MS 428 $[M + NH_4]^+$, 411 $[M + H]^+$. Anal. ($C_{16}H_{18}N_4O_7S$) C, H, N.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-(4-methyl-1,2,3-thiadiazole-5-carbonyl)piperazine-2-carboxamide (33).** The title compound was prepared as a white solid from amine hydrochloride **7** following the procedure described for compound **24**: yield 42%; ^{1}H NMR (DMSO-d_6) δ 2.41−2.62 (m, 2H), 2.71−2.88 (m, 2H), 2.79 (s, 3H), 2.89 (s, 3H), 3.24−3.42 (m, 1H), 3.78−3.87 (m, 2H), 7.02−7.13 (m, 2H), 7.62−7.78 (m, 2H), 9.59 (s, 1H), 10.54 (s, 1H); ion spray MS 459 $[M + NH_4]^+$, 442 $[M + H]^+$; HRMS ($M^+ + H$) calcd for $C_{16}H_{20}N_5O_6S_2$ 442.0855, found ($M^+ + H$) 442.0842.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-(5-methyl-3-phenylisoxazole-4-carbonyl)piperazine-2-carboxamide (34).** The title compound was prepared as a white solid from amine hydrochloride **7** following the procedure described for compound **24**: yield 47%; ^{1}H NMR (DMSO-d_6) δ 2.32−2.43 (m, 3H), 2.92−3.16 (m, 2H), 3.35−3.46 (m, 1H), 3.50−3.68 (m, 2H), 3.83 (s, 3H), 4.24−4.40 (m, 1H), 4.57−4.64 (m, 1H), 7.01−7.13 (m, 2H), 7.40−7.62 (m, 5H), 7.72 (d, J = 9.0 Hz, 2H), 8.80−8.97 (m, 1H), 10.58−10.80 (m, 1H); ion spray MS 518 $[M + NH_4]^+$, 501 $[M + H]^+$; HRMS ($M^+ + H$) calcd for $C_{23}H_{24}N_4O_7S$ 501.1444, found ($M^+ + H$) 501.1455.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-(4-biphenylcarbonyl)piperazine-2-carboxamide (35).** The title compound was prepared as a white solid from amine hydrochloride **7** following the procedure described for compound **24**: yield 45%; ^{1}H NMR (DMSO-d_6) δ 2.78−4.58 (m, 7H), 3.87 (s, 3H), 7.09 (d, J = 9.0 Hz, 2H), 7.29−7.41 (m, 3H), 7.50 (t, J = 7.5 Hz, 2H), 7.64−7.78 (m, 6H), 8.92 (s, 1H); ion spray MS 518 $[M + Na]^+$, 513 $[M + NH_4]^+$, 496 $[M + H]^+$. Anal. ($C_{25}H_{25}N_3O_6S$) C, H, N.

Methyl 1-(4-Methoxyphenyl)sulfonyl-4-amidopiperazine-2-carboxylate (36a). To a solution of amine hydrochloride **7** (1.5 g, 4.3 mmol), triethylamine (1.8 mL, 12.7 mmol), and DMAP (52 mg, 0.43 mmol) in 4 mL of water and 8 mL of *p*-dioxane was added trimethylsilyl isocyanate (0.89 mL, 4.45 mmol) dropwise at room temperature. The reaction was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure and partitioned between EtOAc and water. The EtOAc layer was washed with 1 N HCl, water, and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by column chromatography (EtOAc) to give the title compound (0.33 g, 23%): ^{1}H NMR ($CDCl_3$) δ 2.88 (td, J = 14.2, 4.5 Hz, 1H), 3.14−3.26 (m, 2H), 3.58 (s, 3H), 3.63−3.72 (m, 1H), 3.88 (s, 3H), 3.98−4.06 (m, 1H), 4.24−4.33 (m, 1H), 4.64−4.79 (m, 3H), 6.98 (d, J = 9.0 Hz, 2H), 7.72 (d, J = 9.0 Hz, 2H); CI^+ MS 358 $[M + H]^+$.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-amidopiperazine-2-carboxamide (36).** The methyl ester **36** (0.2 g, 0.56 mmol) was mixed with NH_2OK (3.2 mL, 4.5 mmol, 1.5 M in methanol; solution prepared as described in Fieser & Fieser, Vol. 1, p 478) and stirred overnight at room temperature. The solvent was removed under reduced pressure. The mixture was then dissolved in 1 mL of methanol, acidified with HCl (4 N

in dioxane) to pH ~6. The crude product was subsequently eluted with 20% CH_3OH/EtOAc to give 110 mg (55% yield) of the title compound: ^{1}H NMR (DMSO-d_6) δ 2.66−2.76 (m, 1H), 2.79−2.98 (m, 1H), 3.17−3.21 (m, 1H), 3.44−3.56 (m, 1H), 3.65−3.76 (m, 1H), 3.94 (s, 3H), 4.02−4.08 (m, 1H), 4.19−4.22 (m, 1H), 5.94 (s, 2H), 7.08 (d, J = 9.0 Hz, 2H), 7.74 (d, J = 9.0 Hz, 2H), 8.62 (s, 1H), 10.34 (s, 1H); ion spray MS 397 [M + NH_4]$^+$, 359 [M + H]$^+$; HRMS (M^+ + H) calcd for $C_{13}H_{18}N_4O_6S$ 359.1025, found 359.1035.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-(*n*-hexylaminocarbonyl)piperazine-2-carboxamide (37).** The title compound was prepared as a white solid from amine hydrochloride **7** following the procedure described for compound **36**: yield 26%; ^{1}H NMR ($CDCl_3$) δ 0.80−0.93 (m, 3H), 1.20−1.38 (m, 6H), 1.39−1.49 (m, 2H), 2.50 (td, J = 12.5, 3.0 Hz, 1H), 2.71 (dd, J = 11.8, 3.2 Hz, 1H), 3.02−3.21 (m, 3H), 3.72 (d, J = 11.7 Hz, 1H), 3.86−3.96 (m, 1H), 3.89 (s, 3H), 4.22 (d, J = 13.6 Hz, 1H), 4.53 (d, J = 1.3 Hz, 1H), 5.17−5.23 (m, 1H), 7.00 (d, J = 8.8 Hz, 2H), 7.78 (d, J = 8.9 Hz, 2H), 9.77 (br s, 1H); ion spray MS 465 [M + Na]$^+$, 443 [M + H]$^+$. Anal. ($C_{19}H_{30}N_4O_6S$) C, H, N.

Methyl 1-(4-Methoxyphenyl)sulfonyl-4-(*N*-methyl-*N*-hexylaminocarbonyl)piperazine-2-carboxylate (38a). To a solution of phosgene (20% in toluene, 17 mL, 34 mmol) in dichloroethane (15 mL) at 0 °C was added amine hydrochloride **7** (3 g, 8.5 mmol) slowly. The reaction was stirred for 2 h at 45 °C and overnight at room temperature. The reaction mixture was concentrated under reduced pressure, dissolved in 15 mL of dichloroethane, and added to a solution of *N*-methylhexylamine (2.6 mL, 17.1 mmol) and triethylamine (3.6 mL, 12.6 mmoL) in dichloroethane (15 mL) at 0 °C dropwise. The resulting mixture was stirred overnight at room temperature. The reaction was diluted by 1 N HCl and extracted with EtOAc. The EtOAc layer was washed with 1 N HCl, water, and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by column chromatography (hexane/EtOAc, 1:2) to give the title compound (1.9 g, 49%): ^{1}H NMR ($CDCl_3$) δ 0.84 (t, J = 6.9 Hz, 3H), 1.13−1.28 (m, 6H), 1.39−1.48 (m, 2H), 2.72 (s, 3H), 2.81 (td, J = 12.6, 3.4 Hz, 1H), 2.98−3.18 (m, 3H), 3.40−3.50 (m, 2H), 3.54 (s, 3H), 3.59−3.66 (m, 1H), 3.84 (s, 3H), 3.92−3.98 (m, 1H), 4.59−4.61 (m, 1H), 6.92 (d, J = 9.0 Hz, 2H), 7.67 (d, J = 9.0 Hz, 2H); ion spray MS 473 [M + NH_4]$^+$, 456 [M + H]$^+$.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-(*N*-methyl-*N*-hexylaminocarbonyl)piperazine-2-carboxamide (38).** The methyl ester (0.82 g, 1.8 mmol) was mixed with NH_2OH (10 mL, 14.4 mmol, 1.5 M in methanol) and stirred overnight at room temperature. After adjusting the pH to 5 with acetic acid, the crude product was mixed with silica gel, and the solvent was evaporated. The dry silica was poured on top of a flash silica gel column which was subsequently eluted with EtOAc to 5% CH_3OH/EtOAc to give 0.40 g (49% yield) of the desired product: ^{1}H NMR (DMSO-d_6) δ 0.80 (t, J = 6.9 Hz, 3H), 1.03−1.23 (m, 6H), 1.28−1.41 (m, 2H), 2.40−2.51 (m, 1H), 2.60 (s, 3H), 2.61−2.70 (m, 1H), 2.82−3.02 (m, 3H), 3.21−3.32 (m, 1H), 3.48−3.59 (m, 2H), 3.81 (s, 3H), 4.21−4.26 (m, 1H), 7.04 (d, J = 9.0 Hz, 2H), 7.69 (d, J = 9.0 Hz, 2H), 8.80 (s, 1H), 10.64 (s, 1H); ion spray MS 479 [M + Na]$^+$, 457 [M + H]$^+$. Anal. ($C_{20}H_{32}N_4O_6S$·0.5H_2O) C, H, N.

***N*-Hydroxy-1-(4-bromophenyl)sulfonyl-4-(*N*-methyl-*N*-hexylaminocarbonyl)piperazine-2-carboxamide (39).** The title compound was prepared as a white solid from piperazine-2-carboxylic acid dihydrochloride **4** following the procedure described for compound **38**: yield 51%; ^{1}H NMR (DMSO-d_6) δ 0.80 (t, J = 7.0 Hz, 3H), 1.04−1.23 (m, 7H), 1.27−1.41 (m, 2H), 2.61 (s, 3H), 2.68−2.81 (m, 1H), 2.82−3.02 (m, 2H), 3.25−3.32 (m, 1H), 3.48−3.60 (m, 3H), 4.21−4.27 (m, 1H), 7.64 (d, J = 8.6 Hz, 2H), 7.74 (d, J = 8.6 Hz, 2H), 8.78 (s, 1H), 10.62 (s, 1H); ion spray MS 507, 505 [M + H]$^+$. Anal. ($C_{19}H_{29}N_4O_5SBr$) C, H, N.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-benzylcarbamoylpiperazine-2-carboxamide (40).** To a solution of amine hydrochloride **7** (500 mg, 1.43 mmol) and triethylamine (0.4 mL, 2.86 mmol) in 10 mL of dichloromethane was added benzyl isocyanate (0.19 mL, 1.57 mmol) dropwise at room temperature. The reaction mixture was allowed to stir for 4 h at room temperature, washed with water, dried over $NaSO_4$, and concentrated under reduced pressure. The residue was mixed with NH_2OH (3.4 mL, 5.9 mmol, 1.76 M in methanol) and stirred for 3 h at room temperature. The reaction mixture was neutralized with 1 N aqueous hydrochloric acid and concentrated under reduced pressure. The crude product was purified by flash chromatography (ethyl acetate to ethyl acetate−ethanol 9/1 v/v) to give 220.5 mg (34% yield) of the title compound as a colorless glass: ^{1}H NMR (CD_3OD) δ 2.92 (m, 1H), 3.10 (m, 1H), 3.61−4.22 (m, 4H), 3.88 (s, 3H), 4.28 (m, 2H), 4.36 (m, 1H), 7.12 (d, J = 9.0 Hz, 2H), 7.25 (m, 5H), 7.82 (d, J = 9.0 Hz, 2H); ion spray MS 449 [M + H]$^+$. Anal. ($C_{20}H_{24}N_4O_6S$) C, H, N.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-benzylthiocarbamoylpiperazine-2-carboxamide (41).** The title compound was prepared as a white solid from amine hydrochloride **7** following the procedure described for compound **40**: yield 14%; ^{1}H NMR (CD_3OD) δ 3.17−4.82 (m, 9H), 3.92 (s, 3H), 7.11 (d, J = 9.5 Hz, 2H), 7.24−7.36 (m, 5H), 7.84 (d, J = 9.5 Hz, 2H); ion spray MS 465 [M + H]$^+$; Anal. ($C_{20}H_{24}N_4O_5S_2$·0.25EtOAc) C, H, N.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-(*N*-methyl-*N*-phenylmethylaminocarbonyl)piperazine-2-carboxamide (42).** The title compound was prepared from piperazine-2-carboxylic acid dihydrochloride **(4)** following the procedure described for compound **38**: yield 40%; ^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3H), 2.72−2.81 (m, 1H), 3.21−3.41 (m, 3H), 3.46−3.65 (m, 2H), 3.81 (s, 3H), 4.08−4.32 (m, 3H), 7.06 (d, J = 9.0 Hz, 2H), 7.11−7.18 (m, 2H), 7.20−7.31 (m, 3H), 7.67 (d, J = 9.0 Hz, 2H), 8.80 (s, 1H), 10.66 (s, 1H); ion spray MS 463 [M + H]$^+$. Anal. ($C_{21}H_{26}N_4O_6S$) C, H, N.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-(2-phenylethylaminocarbonyl)piperazine-2-carboxamide (43).** The title compound was prepared from piperazine-2-carboxylic acid dihydrochloride **(4)** following the procedure described for compound **38**: yield 36%; ^{1}H NMR ($CDCl_3$) δ 2.44−2.58 (m, 1H), 2.70 (dd, J = 13.8, 4.0 Hz, 1H), 2.97 (t, J = 5.6 Hz, 2H), 3.01−3.14 (m, 1H), 3.32−3.44 (m, 2H), 3.70 (d, J = 13.0 Hz, 1H), 3.82−3.92 (m, 1H), 3.90 (s, 3H), 4.18 (d, J = 12.8 Hz, 1H), 4.52 (br s, 1H), 5.12−5.23 (m, 1H), 7.01 (dd, J = 9.0, 2.0 Hz, 2H), 7.13−7.32 (m, 5H), 7.78 (dd, J = 9.0, 2.0 Hz, 2H); ion spray MS 485 [M + Na]$^+$, 463 [M + H]$^+$. Anal. ($C_{21}H_{26}N_4O_6S$) C, H, N.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-(3-methoxyphenylaminocarbonyl)piperazine-2-carboxamide (44).** The title compound was prepared as a white solid from piperazine-2-carboxylic acid dihydrochloride **(4)** following the procedure described for compound **38**: yield 57%; ^{1}H NMR (DMSO-d_6) δ 2.84−2.92 (m, 1H), 2.98 (dd, J = 14.2, 3.5 Hz, 1H), 3.51−3.63 (m, 2H), 3.71 (s, 3H), 3.86 (s, 3H), 3.82−3.91 (m, 1H), 4.13 (dd, J = 13.2, 1.8 Hz, 1H), 4.24−4.30 (m, 1H), 6.52 (dd, J = 9.1, 2.5 Hz, 1H), 6.98 (dd, J = 9.0, 2.0 Hz, 2H), 7.02−7.13 (m, 4H), 7.78 (d, J = 9.0 Hz, 2H), 8.43 (s, 1H), 8.89 (s, 1H), 10.77 (s, 1H); ion spray MS 482 [M + Na]$^+$, 465 [M + H]$^+$. Anal. ($C_{20}H_{24}N_4O_7S$) C, H, N.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-(*N*-propyl-*N*-cyclopropylmethyl aminocarbonyl)piperazine-2-carboxamide (45).** The title compound was prepared as a white solid from piperazine-2-carboxylic acid dihydrochloride **(4)** following the procedure described for compound **38**: yield 43%; ^{1}H NMR (DMSO-d_6) δ 0.04−0.13 (m, 2H), 0.38−0. 42 (m, 2H), 0.78 (t, J = 7.8 Hz, 3H), 0.80−0.90 (m, 1H), 1.36−1.44 (m, 2H), 2.48−2.58 (m, 1H), 2.68−2.76 (dd, J = 14.1, 4.1 Hz, 1H), 2.83−2.92 (m, 2H), 2.99−3.12 (m, 2H), 3.22−3.36 (m, 2H), 3.56−3.62 (m, 2H), 3.86 (s, 3H), 4.23−4.30 (m, 1H), 7.08 (d, J = 9.0 Hz, 2H), 7.72 (d, J = 9.0 Hz, 2H), 8.80 (s, 1H), 10.66 (s, 1H); ion spray MS 455 [M + H]$^+$. Anal. ($C_{20}H_{30}N_4O_6S$·0.25H_2O) C, H, N.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-[(hexahydro-1*H*-azepin-1-yl)carbonyl]piperazine-2-carboxamide (46).** The title compound was prepared as a white solid from piperazine-2-carboxylic acid dihydrochloride **(4)** following the

procedure described for compound **38**: yield 62%; ^{1}H NMR (DMSO-d_6) δ 1.31−1.42 (m, 4H), 1.45−1.60 (m, 4H), 2.36−2.48 (m, 1H), 2.58−2.67 (m, 1H), 3.12 (t, J = 5.5 Hz, 4H), 3.28−3.30 (m, 1H), 3.44−3.57 (m, 3H), 3.80 (s, 3H), 4.20−4.23 (m, 1H), 7.05 (d, J = 9.0 Hz, 2H), 7.67 (d, J = 9.0 Hz, 2H), 8.80 (s, 1H), 10.64 (s, 1H); ion spray MS 441 $[M + H]^+$. Anal. ($C_{19}H_{28}N_4O_6S$) C, H, N.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-(4-morpholinylcarbonyl)piperazine-2-carboxamide (47).** The title compound was prepared from piperazine-2-carboxylic acid dihydrochloride **(4)** following the procedure described for compound **38**: yield 87%; ^{1}H NMR (DMSO-d_6) δ 2.50−2.61 (m, 1H), 2.79 (dd, J = 13.5, 4.4 Hz, 1H), 2.90−3.06 (m, 6H), 3.28−3.64 (m, 6H), 3.79 (s, 3H), 4.22 (t, J = 3.5 Hz, 1H), 7.03 (d, J = 8.9 Hz, 2H), 7.68 (d, J = 8.9 Hz, 2H), 8.81 (s, 1H), 10.66 (s, 1H); ion spray MS 451 $[M + Na]^+$, 429 $[M + H]^+$; HRMS (M^+ + H) calcd for $C_{17}H_{24}N_4O_7S$ 429.1444, found (M^+ + H) 429.1446.

***N*-Hydroxy-1-(4-methoxyphenyl)sulfonyl-4-(4*N*-methylpiperazine-1*N*-carbonyl)piperazine-2-carboxamide Hydrochloride (48).** The title compound was prepared from piperazine-2-carboxylic acid dihydrochloride **(4)** following the procedure described for compound **38**. The hydrochloride product was recrystallized from from EtOAc/methanol to give a white solid: yield 40%; ^{1}H NMR (DMSO-d_6) δ 2.03 (s, 3H), 2.08−2.20 (m, 5H), 2.19−2.50 (m, 1H), 2.63 (dd, J = 14.1, 4.2 Hz, 1H), 2.94−3.08 (m, 5H), 3.18−3.42 (m, 2H), 3.50−3.64 (m, 2H), 3.79 (s, 3H), 4.07−4.13 (m, 1H), 7.01 (d, J = 9.0 Hz, 2H), 7.66 (d, J = 9.0 Hz, 2H); ion spray MS 480 $[M + K]^+$, 442 $[M + H]^+$; HRMS (M^+ + H) calcd for $C_{18}H_{28}N_5O_6S$ 442.1760, found (M^+ + H) 442.1752.

***N*-Hydroxy-1-(4-bromophenyl)sulfonyl-4-[*N*-bis(2-methoxyethyl)aminocarbonyl]piperazine-2-carboxamide (49).** The title compound was prepared from piperazine-2-carboxylic acid dihydrochloride **(4)** following the procedure described for compound **38**: yield 45%; ^{1}H NMR ($CDCl_3$) δ 3.01−3.19 (m, 3H), 3.36 (s, 6H), 3.38−3.42 (m, 4H), 3.44−3.57 (m, 5H), 3.59−3.68 (m, 2H), 4.17−4.22 (m, 1H), 4.66−4.71 (m, 1H), 7.60−7.67 (m, 3H), 7.79 (d, J = 9.0 Hz, 2H); ion spray MS 525, 523 $[M + H]^+$. Anal. ($C_{18}H_{27}N_4O_7SBr$) C, H, N.

In Vitro Intestinal Permeability Studies. (Performed as described in: Dowty, M. E.; Dietsch, C. R. *Pharm. Res.* **1997**, *14*, 1792−1797.) Briefly, a portion of rat ileum tissue was freshly excised and mounted in an in vitro diffusion chamber system (NaviCyte, San Diego, CA). The amount of drug transported across the tissue with time at various donor concentrations of drug was determined at 37 °C and pH 7.4. Permeability coefficients, k_p (cm/min) were calculated at steady state with the following equation from Fick's laws of diffusion:

$$k_p = [S]/[AC_o]$$

where S is the slope of the linear (steady state) portion of the cumulative amount transported through the membrane versus time plot in μg/min, A is the cross-sectional area of the tissue surface exposed to solute transport in cm^2 (0.636 cm^2), and C_o is the initial concentration of solute in the donor chamber in μg/mL (20−90 μg/mL). A predicted percent absorption, *Abs*, is obtained from the ratio of the k_p value for the test compound divided by the k_p of the mannitol internal standard (k_p ratio) from the following equation:

$$Abs = [100]/[1 + \exp\{-7.42(k_p \text{ ratio} - 0.812)\}]$$

Cartilage Explant Cultures. Bovine nasal cartilage obtained the same day from a local abattoir was sliced and placed in F-12 media containing ascorbic acid (50 mg/mL) and 2% penicillin−streptomycin−fungizone. Cartilage disks were prepared (7−12 mg wet weight) using a sterile belt punch. Cartilage explants were cultured in Dulbecco's modified Eagle's medium (DMEM) containing glutamine (2 mM), ascorbic acid (50 mg/mL), 2% penicillin−streptomycin−fungizone, and 1% ITS universal culture supplement (Collaborative Biomedical Products, Bedford, MA) for at least 3 days before use. Cartilage degradation assays were performed in 96-well microtiter plates with each well containing 2 cartilage disks in 200 mL of DMEM media supplemented with ascorbic acid and antibiotics as described above but without ITS and 50 ng/mL interleukin-1-α (IL-1-α; Genzyme Inc., Cambridge, MA). Unstimulated control cultures were treated identically except IL-1 was omitted. MMPIs were evaluated in cultures containing DMEM with 1 mM, 500 nM, 250 nM, 125 nM, or 62.5 nM of test compound. Supernatants were removed from the plates at 7-day intervals and stored at −70 °C until analysis for collagen degradation. Fresh media with or without inhibitor was added to the plates and the cultures continued for 21 days. Collagen degradation was measured by the release of hydroxyproline into the culture media as previously described (Ellis, A. J.; et al. *Biochem. Biophys. Res. Commun.* **1994,** *201*, 94−101). Collagen degradation was measured in 8 replicate cartilage cultures, and the data are expressed as the mean ± SD.

X-ray Crystallography with Truncated MMP-3. A truncated form of MMP-3 containing the catalytic domain with residues 83−255 was crystallized as described.[15] 1 mM compound **20** was added to the crystal hanging drop for 3 h. Data were then collected on a Mar-Research 180-mm image plate detector system. These data were processed using the HKL program. The structure was solved and refined to 2.4 Å using XPLOR. The MMP-3−**20** complex structure has been deposited with the Protein Data Bank (PDB code 1D8F).

Expression and Purification of Human Recombinant Truncated MMP-1. Briefly, the DNA sequence coding for Val-82−Pro-249 of proMMP-1 was amplified by polymerase chain reaction from a commercially available plasmid, p35-1 (ATCC, Rockville, MD; Templeton, N. S.; et al. *Cancer Res.* **1990,** *50*, 5431−5437) encoding human interstitial MMP-1. The PCR fragment was ligated into the expression vector, pET-11a (Novagen, Madison, WI), and expressed in *E. coli* BL21(DE3) cells. The protein was solubilized from inclusion bodies in 6 M urea, 0.15 M NaCl (pH 7.5), refolded in 50 mM Tris-HCl (pH 7.5), 10 mM $CaCl_2$, 0.1 mM zinc acetate, then purified to homogeneity over a hydroxamic acid inhibitor affinity column as previously described (Moore, W. A.; Spilburg, C. A. *Biochemistry* **1986,** *25*, 5189−5195). N-Terminal sequence analysis confirmed the presence of three N-terminal sequences of Val-Leu-Thr-Glu-Gly-Asn, Met-Val-Leu-Thr-Glu-Gly-Asn, and Leu-Thr-Glu-Gly-Asn (minor).

Expression and Purification of Human Recombinant proMMP-2. A partial cDNA clone for MMP-2 known as K-121 (Tryggvason, K. *J. Biol. Chem.* **1990,** *265*, 11077−11082) was obtained from ATCC and subcloned into the pBlueScript SK$^-$ (pBS) plasmid. Sanger dideoxy sequencing revealed that the first 134 bp of the coding sequence were missing from the 5′ end of K-121. To restore the missing sequence, two overlapping 90+-mer oligonucleotides were designed and synthesized. These, along with a 3′ antisense oligonucleotide, were used as primers to the K-121 template in a series of polymerase chain reaction (PCR) experiments to synthesize a full-length MMP-2 cDNA. The PCR product was then subcloned into pBS. To express MMP-2 in the mammalian CHO D$^-$ cell system, it was first subcloned into the mammalian expression vector pJT1 (J. Ting, CRD), which contains the DHFR gene. Recombinant MMP-2/pJT1 was Polybrene transfected into CHO D$^-$ cells. Clonal cell populations were isolated and screened for production of MMP-2 mRNA using specific oligonucleotide primers and reverse transcription PCR. Ten clones producing the highest levels of MMP-2 mRNA were selected, and the DHFR/MMP-2 construct was amplified by gradually increasing media methotrexate (MTX, a DHFR inhibitor) concentration. Clonal selection was further narrowed after assessing MMP bioactivity in an MMP fluorescence assay (M. Anastasio, CRD) and on zymogram gels. Those clones showing activity were tested for MMP-2 protein production by sequential Edman degradation protein sequencing (F. Wang, P&GP Cell & Molecular Biology Core) and Western blot. Based on all results, one clone was expanded for growth in roller bottles. Approximately 9 L of serum-free conditioned media with an MMP-2 concentration

of ~35 mg/L were generated. proMMP-2 was purified from conditioned media as previously described (Crabbe, T.; et al. *Eur. J. Biochem.* **1993,** *218*, 431−438) with the following modifications. Conditioned serum-free medium was reduced in volume with an Amicon S1Y30 spiral-wound cartridge prior to chromatography on a gelatin-Sepharose 4B column equilibrated in 25 mM Tris/HCl, 30 mM NaCl, 10 mM $CaCl_2$, pH 7.5 (TNC buffer). The column was washed with equilibration buffer before elution of the bound protein by TNC buffer containing 1 M NaCl followed by 1 M NaCl and 10% (by vol) dimethyl sulfoxide (DMSO) in TNC buffer. proMMP-2 fractions were concentrated in an Amicon ultrafiltration cell with a YM30 membrane and diafiltered against 25 mM MES/NaOH, 30 mM NaCl, 10 mM $CaCl_2$, pH 6.0 (MNC buffer). The concentrate was then chromatographed over a second gelatin-Sepharose 4B column equilibrated in MNC buffer, washed with equilibration buffer to remove nonbinding proteins, and eluted with MNC buffer containing 0.3 M NaCl and 10% (by vol) DMSO. Elution fractions containing progelatinase A were pooled, diluted, and loaded onto an S-Sepharose Fast Flow column equilibrated in MNC buffer and eluted with MNC buffer containing 0.3 M NaCl. The concentrated fractions of purified proMMP-2 were combined and stored in MNC buffer containing 0.3 M NaCl at −70 °C. N-Terminal sequence, amino acid, and mass spectrophotometric analyses confirmed the identity of the purified protein with the expected sequence for progelatinase A.

Expression and Purification of Human Recombinant Truncated MMP-3. Briefly, the DNA sequence coding for Ala-1−Thr-255 of proMMP-3 was amplified by PCR from a recombinant plasmid encoding human synovial preproMMP-3 kindly provided by Dr. Hideaki Nagase (University of Kansas Medical Center, Kansas City, KS) and Dr. Markku Kurkinen (Department of Medicine, UMDNJ−Robert Wood Johnson Medical School). After DNA sequence verification, this DNA fragment was ligated into the expression vector, pET-3d (Novagen, Madison, WI), and expressed in *E. coli* BL21 (DE3) cells as previously described (Marcy, A. I.; et al. *Biochemistry* **1991**, *30*, 6476−6483). proMMP-3 was solubilized form inclusion bodies in 8 M guanidine-HCl, refolded in 100 mM Tris-HCl (pH 7.5), 10 mM $CaCl_2$, 0.5 mM zinc acetate, and activated overnight at 37 °C with 1.5 mM *p*-aminophenylmercuric acetate. Active, truncated MMP-3 was purified to homogeneity over a hydroxamic acid inhibitor affinity column as described (Moore, W. A.; Spilburg, C. A. *Biochemistry* **1986,** *25*, 5189−5195). N-Terminal sequence analysis confirmed the sequence to be consistent with the catalytic domain, Phe-83−Thr-255, of proMMP-3.

Collagenase (MMP-1) Inhibition Assay. proMMP-1 was activated prior to assay by treatment with trypsin. MMP-1 activity was monitored using a fluorescence assay previously described.[7] In a Dynatech MicroFLUOR plate, 10 nM activated collagenase was incubated with 8 μM Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 in 50 mM Tris-HCl, 10 mM $CaCl_2$, 0.15 M NaCl, 0.05% Brij for 20−30 min at 37 °C in the presence of varying concentrations of inhibitor. The reaction was then quenched with 50 mM EDTA and the fluorescence increase monitored on a Perkin-Elmer LS50B spectrofluorimeter (λ_{ex} 328 nm, λ_{em} 393 nm). Activity was measured as a percentage of control activity in the absence of inhibitor. Inhibitor concentrations were run in triplicate, and IC_{50} determinations were calculated from a 4-parameter logistic fit of the data within a single experiment.

Stromelysin (MMP-3) Inhibition Assay. proMMP-3 was activated prior to assay by treatment with *p*-aminophenylmercuric acetate or trypsin. MMP-3 activity was measured by following the degradation of ^{3}H-reduced, carboxymethylated transferrin.[16] In a Multiscreen DP filtration plate (Millipore), 50 ng of activated MMP-3, 30 μg of [^{3}H]transferrin, and varying concentrations of inhibitor were incubated in a buffer of 50 mM Tris-HCl, 10 mM $CaCl_2$, 0.15 M NaCl, 0.05% Brij (pH 7.5) at 37 °C for 3 h. The reaction was quenched by addition of 4.4% TCA, and TCA-soluble fragments were counted for radioactivity. Activity was measured as a percentage of control activity in the absence of inhibitor. Inhibitor concentrations were run in triplicate, and IC_{50} determinations were calculated from a 4-parameter logistic fit of the data within a single experiment.

Gelatinase A (MMP-2), Matrilysin (MMP-7), Neutrophil Collagenase (MMP-8), Gelatinase B (MMP-9), and Collagenase 3 (MMP-13) Inhibition Assays. Inhibition of all enzymes was measured according to the representative procedure described below for MMP-2. proMMP-2 was activated prior to assay by treatment with 1 mM *p*-aminophenylmercuric acetate for 45 min at 37 °C. proMMP-9 was activated with MMP-3 (1:20 MMP-3:MMP-9) and stored at −80 °C until use. MMPs 7, 8, and 13 were all supplied as active enzymes and stored frozen until use. MMP activity was monitored using a fluorescence assay previously described,[7] modified for a microtiter plate format. In a Dynatech MicroFLUOR plate, active enzyme was incubated with 8 μM Mca-Pro-Leu-Dpa-Ala-Arg-NH_2 in 50 mM Tris-HCl (pH 7.5), 10 mM $CaCl_2$, 0.15 M NaCl, 0.05% Brij for 20−30 min at 37 °C in the presence of varying concentrations of inhibitor. The reaction was then quenched with 50 mM EDTA, and the relative fluorescence was monitored on a Perkin-Elmer LS50B spectrofluorimeter (λ_{ex} 328 nm, λ_{em} 393 nm) fitted with a microplate reader attachment. Activity was measured as a percentage of control activity in the absence of inhibitor. Inhibitor concentrations were run in triplicate, and IC_{50} determinations were calculated from a 4-parameter logistic fit of the data within a single experiment.

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