Articles

N-Aryl Sulfonyl Homocysteine Hydroxamate Inhibitors of Matrix Metalloproteinases: Further Probing of the S_1, S_1', and S_2' Pockets

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A series of *N*-arylsulfonyl *S*-alkyl homocysteine hydroxamic acids were synthesized with variations in three subsites corresponding to P_1, P_1', and P_2'. Enzyme assays with a variety of MMPs revealed activity at the low nanomolar level.

Introduction

The matrix metalloproteinases (MMPs) are zinc-containing proteases including collagenases, stromelysins, and gelatinases. Their key role in the proteolysis of extracellular matrix has fostered intensive efforts worldwide in search of effective inhibitors with a potential for therapeutic applications in a number of diseases such as arthritis and cancer.[1−4] Consequently, several MMP inhibitors were discovered during the past decade, some of which having reached an advanced clinical trial. Derivatives of succinic acid, as exemplified by Marimastat (**1**)[5] or Ro32-3555 (**2**),[6] and more recently *N*-arylsulfonyl α-aminohydroxamic acid derivatives, such as CGS 27023A (**3**)[7] or AG3340 (**4**),[8] have been extensively investigated (Figure 1).

In general, hydroxamic acid analogues have been significantly more effective than the corresponding carboxylic acids, no doubt due to the presence of a zinc atom at the active site of these enzymes.[9] Extensive X-ray and NMR studies of inhibitor/MMP complexes have provided the basis for a better understanding of important binding interactions between functional groups present in the inhibitors and their corresponding subsites in the enzymes.[10−15] These elegant studies have paved the way to effectively design new analogues with potentially more favorable interactions with the enzymes.

As part of our studies on the design and synthesis of inhibitors of MMPs,[16,17] we have reported that N-substituted D,L-homocysteine hydroxamic acids **5** and **6** exhibit potent and broad enzymatic activity (Figure 2).[18] In view of this encouraging result, we thought it necessary to expand the nature of functional groups at the P_1, P_1', and P_2' sites of the inhibitors as functional probes for S_1, S_1', and S_2' pockets of stromelysin and related MMPs. The goal was to see if chemical modifications at a given site would enhance or diminish inhibitory activity in general, but more specifically whether selectivity for a given MMP could be achieved.

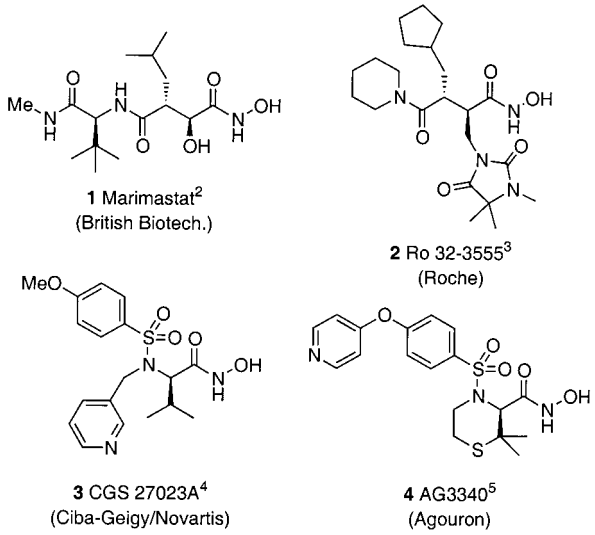

Figure 1. Selected MMP inhibitors.

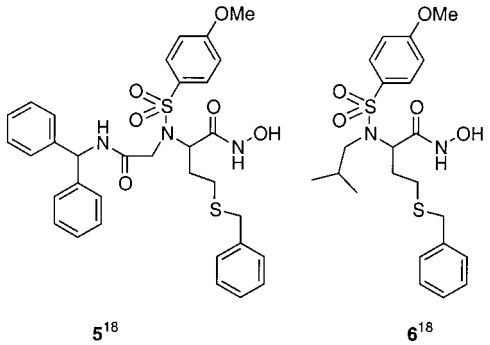

Figure 2. Leads from the previous study.

Results and Discussion

Molecular Modeling and Design. As a prelude to the SAR study, we planned to validate the crucial interactions between **6** and stromelysin-1 (MMP-3) based on available information in the literature.[9] Using the Goodford program GRID,[19−23] we successfully located, among others, the highly hydrophobic S_1 pocket surrounded by Tyr-155, His-166, and Tyr-168 (Figure

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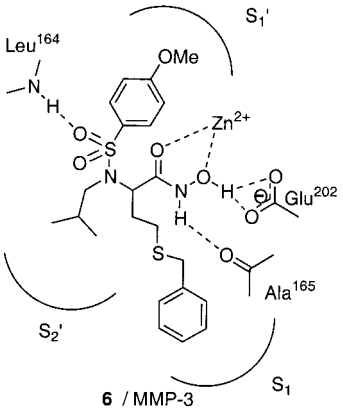


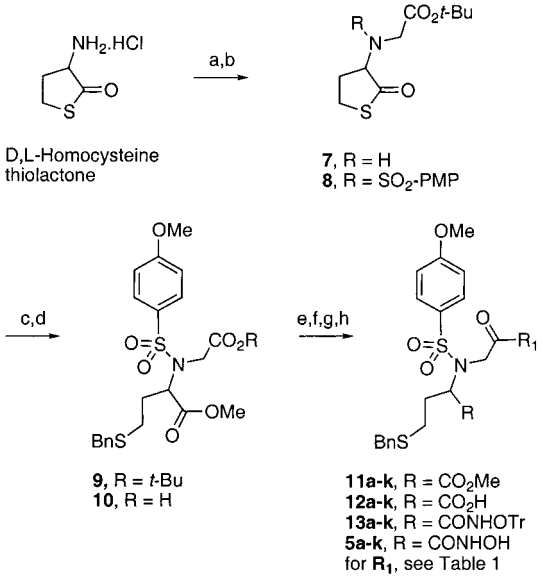
Figure 3. Proposed model of **6**/MMP-3 from docking simulation.

3). This result combined with reported structures of MMP complexes with sulfonamide-based inhibitors[10] brought out insights into the possible binding mode. Docking of compound **6** in the binding site of MMP-3 and further refinement of the complex provide the pictorial model shown in Figure 3: the S_1 pocket is occupied by the *S*-benzyl group. In an effort to check the proposed model, docking with AutoDock,[24] which we proved to be reliable on MMP systems,[25] was performed. The enhanced enzymatic activity of **6** compared to CGS-27023A,[7] possibly due to a more favored P_1/S_1 interaction, instigated the exploration of other hydrophobic substituents at this site. Since compound **5** proved to possess excellent nanomolar to subnanomolar activity against most of the MMPs,[15] we also explored replacements for the diphenylmethyl amide group,[26] presumably occupying the shallow S_2' site in MMP-3.[10] Finally the *p*-methoxyphenyl sulfone moiety occupying the P_1' site was also replaced with other sulfones in an effort to assess its contribution.

Synthesis. We followed a procedure similar to the one previously reported,[18] which allowed the use of a common precursor to access the three groups of compounds as illustrated in Scheme 1. D,L-Homocysteine thiolactone was monoalkylated and the product N-sulfonylated to afford **8**. The thiolactone ring was then opened with sodium methoxide and the resulting thiolate was trapped in situ with benzyl bromide (Scheme 1). The *tert*-butyl ester was converted to the corresponding acid which served as a key intermediate for further functionalization. Amide formation with a variety of amines in a parallel array led to the methyl esters **11a**−**k**. Subsequent saponification of the methyl esters led to the corresponding acids which were individually converted to the hydroxamic acid by coupling with *O*-trityl hydroxylamine followed by TFA-mediated deprotection. In our previous synthesis of **6**, we had alluded to the surprisingly difficult deprotection of an *O*-*tert*-butyl hydroxamate ester with TFA.[18,27] In the present synthesis, we used the *O*-trityl hydroxamate instead, which was smoothly converted to the hydroxamic acid with TFA. A set of *N*,*N*-disubstituted amide derivatives, represented as structures **5a**−**k**, was rapidly assembled.

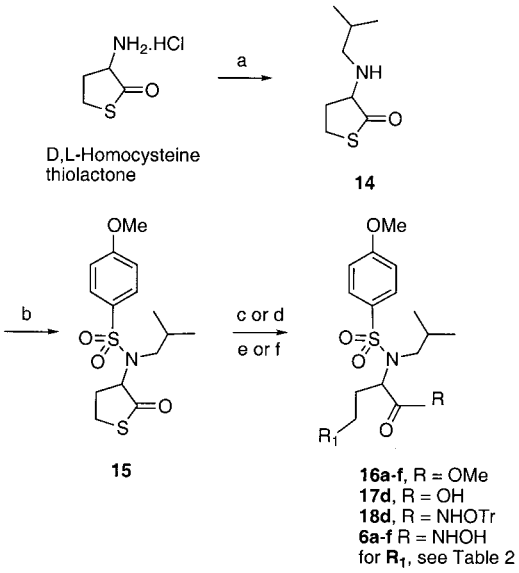
We next turned our attention to the preparation of a set of *N*-aryl sulfonyl hydroxamic acids based on the original structure **6**. The synthesis started with sequential reductive amination of D,L-homocysteine thiolactone and sulfonylation yielding **15** (Scheme 2). Base-catalyzed methanolysis of the thiolactone ring in **15** followed by trapping of the resulting thiolate methyl ester with different electrophiles led to a series of S-alkylated esters. Treatment of the methyl esters with the potassium salt of hydroxylamine afforded the corresponding hydroxamic acids.[28] In one case, we proceeded via a stepwise formation of the *O*-trityl hydroxamate followed by cleavage with TFA. In this manner, we were able to prepare a set of *N*-arylsulfonyl *S*-alkyl homocysteine hydroxamic acids related to **6** in which the P_1 site was

Scheme 1[a]



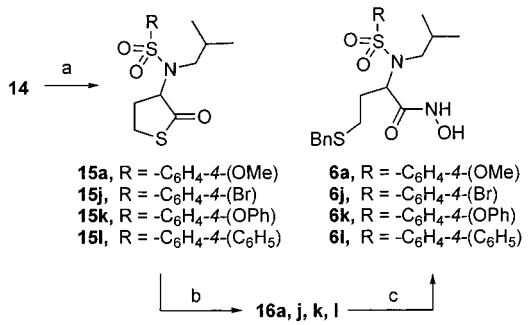
[a] (a) $BrCH_2CO_2t$-Bu, DIPEA, MeCN, 58%; (b) PMP−SO_2Cl, pyridine, 67%; (c) MeONa/MeOH then BnBr, 82%; (d) TFA, CH_2Cl_2, 68%; (e) R_1NH_2, EDC, HOBt, NMM, CH_2Cl_2, 79−98%; (f) (for **12d**, **12e**, **12f**, **12i**, **12j**, **12k**) LiOH, THF/H_2O (26−96%) or (for **12a**, **12b**, **12c**, **12g**, **12h**) LiI, pyridine, reflux (79−83%); (g) $TrONH_2$, EDC, HOBt, NMM, CH_2Cl_2, 59−95%; (h) TFA 5%, CH_2Cl_2.

Scheme 2[a]



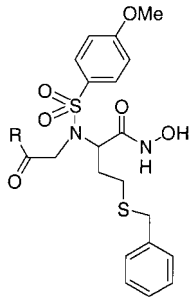
[a] (a) *i*-PrCHO, Et_3N, $NaBH_4$, 73%; (b) PMP−SO_2Cl, pyridine, 80%; (c) MeONa/MeOH then RBr, 20−99%; (d) i: MeONa/MeOH then BnBr, 20−99%; ii: H_2O_2, 75%; (e) NH_2OH, KOH, MeOH, 60−70%; (f) (for d) i: LiOH, H_2O/THF, 81%; ii: $TrONH_2$, EDC, HOBt, THF, 65%; iii: TFA, CH_2Cl_2, 50%.

Scheme 3[a]



[a] (a) R-SO_2Cl, pyridine or Et_3N, CH_2Cl_2, 44−71%; (b) MeONa/MeOH then BnBr, 41−81%; (c) NH_2OH, KOH, MeOH, 31−62%.

Table 1. Variation of P_2' in **5**



R	compd	IC50 (nM)[a] MMP1	MMP2	MMP3	MMP9	MMP13
$NHCH_2C_6H_5$	**5a**	51	0.7	1.6	0.2	0.5
$NHCH_2$(3-pyridyl)	**5b**	20	1.2	2.2	0.2	1.1
$NHCH_2(cC_6H_{11})$	**5c**	nt	0.30	nt	0.01	nt
$NHCH(C_6H_5)_2$	**5d**	nt	2.3	3.7	0.5	4.5
NHCH(2-pyridyl)$_2$	**5e**	39	0.4	1.1	0.2	0.4
$NHCH(cC_6H_{11})_2$	**5f**	nt	8.82	nt	1.88	nt
$NHCH_2C_6H_4$-4-(OMe)	**5g**	19	0.7	0.9	0.2	0.7
$NHCH_2C_6H_3$-3,5-$(OMe)_2$	**5h**	36	1.6	4.7	0.6	2.3
$NHCH_2C_6H_2$-2,4,6-$(OMe)_3$	**5i**	49	1.2	2.5	0.3	1.6
$NH(cC_6H_{11})$	**5j**	16	1.0	1.6	0.2	0.8
$N(CH_2CH_2)_2O$	**5k**	532	16	195	29	23

[a] See Experimental Section for enzyme assay details. nt, not tested.

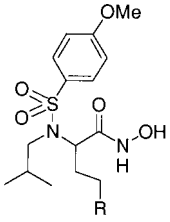
modified with a variety of substituted *S*-benzyl appendages.

Variations in the *N*-arylsulfonyl group occupying the P_1' subsite was the basis of another set of analogues related to **6** as shown in Scheme 3. In this case, we were able to convert the esters into the hydroxamic acids directly by treatment with potassium or sodium salt of hydroxylamine.[29]

Biological Assays. All compounds were tested in vitro as racemic mixtures for the inhibition of stromelysin 1 (MMP-3), gelatinase-A (MMP-2), gelatinase-B (MMP-9), collagenase 1 (MMP-1), and collagenase 3 (MMP-13). The data are summarized in Tables 1, 2, and 3.

The results of introducing diversity at the S_2' pocket with the set of substituted amides **5a**−**k** are shown in Table 1. As expected, the hydroxamic acid derivatives were found to be roughly a 100 times more active than the corresponding carboxylic acids. All compounds exhibited broad spectrum inhibition in the nanomolar range. Although it appeared that the variations of the

Table 2. Variations in the P_1 Site of **6**



R	compd	IC50 (nM)[a] MMP1	MMP2	MMP3	MMP9	MMP13
$-SCH_2C_6H_5$[b]	**6a**	104	0.7	0.7	<0.1	12
$-SO_2CH_2C_6H_5$	**6b**	396	4.22	10.3	0.9	11.8
$-SCH_2C_6H_4$-3-(OMe)	**6c**	297	3.78	5.7	3.2	5.3
$-SCH_2$(3-pyridyl)	**6d**	126	6.3	6.2	3.0	16.2
$-SCH_2$(3-thiophene)	**6e**	188	3.2	5.4	1.0	5.1
$-SCH_2(C_6F_5)$	**6f**	19610	1810	705	149	407
-H[b]	**6g**	384	53	45	7	96
$-SCH_2C_6H_4$-4-(C_6H_9)[b]	**6h**	nt	30	20	0.2	nt
$-CH_2C_6H_4$-4-$(OCH_2C_6H_5)$[b]	**6i**	nt	49	29	8	20

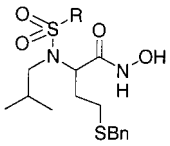
[a] See Experimental Section for enzyme assay details. [b] See ref 18. nt, not tested.

amide side chain caused only moderate changes in the potency, the cyclohexylmethyl analogue **5c** was more potent than the corresponding analogue **5a**, exhibiting picomolar inhibition against MMP-9. Adding an extra cyclohexyl (**5c**−**f**) resulted in loss of activity against MMP-2 and MMP-9, but the dipyridylmethyl amide **5e** was as potent as the monopyridyl analogue **5b**. Introducing a morpholine amide (**5c**−**k**) diminished the binding activity. The fairly bulky cyclohexylmethyl amide group in **5c** seemed to best accommodate the binding site of MMP-2 and MMP-9. These results suggested that the amide substituents in **5** presumed to occupy the shallow S_2' site in MMP-3,[10] allowing considerable diversity for hydrophobic aryl groups in the case of MMP-1 and MMP-13 as well. The high selectivity for analogue **5c** toward MMP-9 compared to other MMPs is noteworthy. Shape complementarity is known to be a crucial feature in enzyme−inhibitor interactions.[30]

The results of the next series (**6a**−**i**) consisting of variations in the P_1 arylthioethyl appendage is shown in Table 2. As previously mentioned, the hydrophobic region of the S_1 pocket is a few ångströms away and required solvent exposed groups as spacers on the inhibitors.[10] The depth of this pocket was probed using *S*-benzyl (**6a**), *p*-phenylbenzyl (**6h**), or benzyloxybenzyl (**6i**) groups that clearly shows the spacial requirements of this pocket. Indeed, the proposed AutoDock model for MMP-3 showed that the S_1 pocket presents roughly a half-sphere shape filled up by the benzyl group of **6a**. Thus, lengthening the side chain as in **6h** and **6i** led to a loss of activity. We then studied the solvent exposed nature of this subsite by replacing the *S*-benzyl groups in **6a** by groups capable of interacting with a polar environment. The sulfone **6b**, the thiophene **6e,** and the pyridyl **6d** analogue were less active than **6a,** while the pentafluorophenyl analogue **6f** was devoid of activity. Variations at the P_1 site were more effective for MMP-9 than for MMP-2, compared to changes at the P_2' site.

Table 3 shows the inhibition data relative to the last series (**6j**−**l**) in which five different aryl groups were chosen for their shape and their electronic properties.

Table 3. Variations in the P_1' Site of **6**



		IC_{50} (nM)[a]				
R	compd	MMP1	MMP2	MMP3	MMP9	MMP13
-C_6H_4-4-(OMe)[b]	**6a**	104	0.7	0.7	<0.1	12
-C_6H_4-4-(Br)	**6j**	164	16	151	4.3	14
-C_6H_4-4-(*O*C_6H_5)	**6k**	1450	1.6	4.2	0.5	3.2
-C_6H_4-4-(C_6H_5)[b]	**6l**	nt	4.1	90	2.5	3.8

[a] See Experimental Section for enzyme assay details. [b] See ref 18. nt, not tested.

The 4-diphenyl analogue **6l**[18] was less active than the more flexible 4-phenoxyphenyl analogue **6k**. Indeed, previous work showed that flexible groups fit better in the presently probed S_1' pocket. [29,31]

In conclusion, we have prepared and tested the in vitro MMP inhibitory activity of a series of acyclic *N*-arylsulfonyl homocysteine hydroxamates while probing the spacial and electronic requirements of different subsites. Low nanomolar and subnanomolar inhibition was found in several derivatives, which are being further evaluated for optimal bioavailablity and related tests. Variations at the P_1, P_1', and P_2' sites revealed that in each case inhibition of MMP-9 was most effective among the MMPs tested.

Experimental Section

Chemistry. Solvents were distilled under positive pressure of dry nitrogen before use and dried by standard methods; THF and ether, from K/benzophenone; CH_2Cl_2 and toluene from $CaCl_2$. All commercially available reagents were used without further purification. All reactions were performed under nitrogen atmosphere. NMR (^1H, ^{13}C) spectra were recorded on AMX-300 and ARX-400 spectrometers in $CDCl_3$ or CD_3OD with tetramethylsilane as the internal standard. Low- and high-resolution mass spectra were recorded on VG Micromass, AEI-MS 902, or Kratos MS-50 spectrometers using fast atom bombardement (FAB) or electrospray techniques. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter in a 1 dm cell at ambient temperature. Analytical thin-layer chromatography was performed on Merck $60F_{254}$ precoated silica gel plates. Visualization was performed by UV or by development using $KMnO_4$ or $FeCl_3$ solutions. Flash column chromatography was performed using (40−60 μm) silica gel at increased pressure. Melting points recorded were uncorrected. For ^{13}C NMR and other analytical data, see Supporting Information.

(2-Oxo-tetrahydro-thiophen-3-ylamino)-acetic Acid *tert*-Butyl Ester (7). To a solution of d,L-homocysteine thiolactone hydrochloride (12.0 g, 78.1 mmol) in MeCN (150 mL) was added DIPEA portionwise (28.6 mL, 2.1 equiv) at 0 °C. After complete dissolution, *tert*-butyl bromoacetate (15.9 g, 1.1 equiv) was added dropwise at 0 °C. The resulting mixture was stirred at room temperature overnight then concentrated. The residue was taken up in EtOAc, and the precipitate was filtered off. The organic solution was concentrated in vacuo then chromatographed (CH_2Cl_2/MeOH 99:1) to afford **7** (18.0 g, 58%): IR (neat/NaCl) 3332.8, 1731.9, 1698.0 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 3.51 (dd, 1H, J = 6.7, 11.5 Hz), 3.40 (m, 2H), 3.30 (m, 2H), 2.57 (m, 1H), 1.95 (m, 1H), 1.46 (s, 9H); ^{13}C NMR (75 MHz, CD_3OD) δ 209.4, 172.3, 82.6, 67.2, 49.9, 33.0, 28.5, 28.3.

[(4-Methoxy-benzenesulfonyl)-(2-oxo-tetrahydro-thiophen-3-yl)-amino]-acetic Acid *tert*-Butyl Ester (8). To a solution of **7** (12.0 g, 51.7 mmol) in pyridine (150 mL) was added *p*-methoxybenzene sulfonyl chloride (16.0 g, 1.5 equiv) at 0 °C. The resulting mixture was stirred overnight at room temperature. The mixture was then concentrated, taken up in CH_2Cl_2, washed successively with water, 0.1 N HCl, 0.1 N $NaHCO_3$, water, and brine, dried over Na_2SO_4, then concentrated in vacuo. The residue afforded, after flash chromatography (CH_2Cl_2/MeOH, 99:1), **8** (13.8 g, 67%): IR (neat/NaCl) 1745.7, 1705.9, 1596.5, 1579.4 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, 2H, J = 8.9 Hz), 6.94 (d, 2H, J = 8.9 Hz), 4.65 (dd, 1H, J = 6.8, 13.2 Hz), 4.05 (d, 1H, J = 18.4 Hz), 3.84 (s, 3H), 3.40 (d, 1H, J = 18.4 Hz), 3.34−3.16 (m, 2H), 2.64 (m, 1H), 2.20 (ddd, 1H, J = 5.1, 7.2, 13.0 Hz), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.7, 169.2, 163.8, 131.8, 130.5, 114.8, 83.0, 67.7, 56.3, 47.9, 30.4, 28.7, 27.5; LRMS (FAB, NBA, *m*/*z*, %) 402 (5) ($M + H^+$), 373 (6), 346 (100), 318 (25), 300 (15), 171 (64), 146 (35), 130 (63), 114 (43); HRMS calcd for $C_{17}H_{24}O_6NS_2$ 402.10449 ($M + H^+$), found 402.10610. ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.7, 169.2, 163.8, 131.8, 130.5, 114.8, 83.0, 67.7, 56.3, 47.9, 30.4, 28.7, 27.5; LRMS (FAB, NBA, *m*/*z*, %) 402 (5) ($M + H^+$), 373 (6), 346 (100), 318 (25), 300 (15), 171 (64), 146 (35), 130 (63), 114 (43); HRMS calcd for $C_{17}H_{24}O_6NS_2$ 402.10449 ($M + H^+$), found 402.10610.

4-Benzylsulfanyl-2-[*tert*-butoxycarbonylmethyl-(4-methoxy-benzenesulfonyl)-amino]-butyric Acid Methyl Ester (9). To a solution of thiolactone **8** (1.67 g, 4.16 mmol) in MeOH (20 mL) was added sodium methoxide freshly prepared from Na° (122 mg, 1.28 equiv) in MeOH (13 mL). After stirring for 30 min, benzyl bromide (0.50 mL, 1.0 equiv) was added. The mixture was stirred for 16 h then concentrated, taken up in EtOAc, filtrated, and finally concentrated in vacuo. The residue was chromatographed (CH_2Cl_2/MeOH, 99:1) to afford **9** (1.78 g, 82%); IR (neat/NaCl) 1746.9, 1596.4 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, 2H, J = 8.9 Hz), 7.30−7.22 (m, 5H), 6.95 (d, 2H, J = 8.9 Hz), 4.52 (dd, 1H, J = 5.5, 8.8 Hz), 4.13 (d, 1H, J = 18.4 Hz), 3.84 (s, 3H), 3.83 (d, 1H, J = 18.4 Hz), 3.66 (s, 2H), 3.51 (m, 3H), 2.58 (m, 1H), 2.46 (m, 1H), 1.96 (m, 1H), 1.82 (m, 1H), 1.46 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 169.3, 163.7, 138.8, 131.4, 130.7, 129.5, 129.1, 127.6, 114.6, 82.6, 58.6, 56.2, 52.9, 47.5, 36.5, 30.7, 28.6, 28.2; LRMS (FAB, NBA, *m*/*z*, %) 523 (9) ($M + H^+$), 468 (14), 408 (8), 296 (14), 227 (14), 206 (14), 171 (29), 91 (100), 57 (45); HRMS calcd for $C_{25}H_{33}O_7NaNS_2$ 546.15961 ($M + Na^+$), found 546.15830.

4-Benzylsulfanyl-2-[carboxymethyl-(4-methoxy-benzenesulfonyl)-amino]-butyric Acid Methyl Ester (10). To a solution of **9** (1.78 g, 3.41 mmol) in CH_2Cl_2 (10 mL) was added TFA (10 mL). After being stirred for 4 h, the mixture was concentrated and chromatographed (CH_2Cl_2/MeOH, 49:1) to afford **10** (1.09 g, 68%); IR (neat/NaCl) 3444.7, 1783.7, 1643.7, 1596.1, 1578.6 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, 2H, J = 8.9 Hz), 7.34−7.24 (m, 5H), 6.97 (d, 2H, J = 8.9 Hz), 4.58 (dd, 1H, J = 5.4, 8.8 Hz), 4.20 (d, 1H, J = 18.8 Hz), 4.00 (d, 1H, J = 18.8 Hz), 3.87 (s, 3H), 3.67 (s, 2H), 3.55 (s, 3H), 2.51 (m, 1H), 2.41 (m, 1H), 1.99 (m, 1H), 1.83 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.1, 171.4, 163.2, 137.9, 129.9, 128.7, 128.4, 126.9, 114.0, 57.9, 55.5, 52.4, 45.8, 35.8, 29.9, 27.2; LRMS (FAB, NBA, *m*/*z*, %) 468 (20) ($M + H^+$), 307 (22), 154 (100), 137 (64), 136 (67); HRMS calcd for $C_{21}H_{25}O_7NaNS_2$ 490.09702 ($M + Na^+$), found 490.09650.

General Procedure for the Preparation of 11a−k. To a solution of **10** (0.90−1.0 mmol in CH_2Cl_2 (10 mL) were added EDC (1.4 equiv), HOBt (1.4 equiv), and NMM (1.8 equiv). After the mixture was stirred for 20 min, the appropriate amine (2.0 equiv) was added, and the resulting mixture was stirred for 15 h. The mixture was dissolved with CH_2Cl_2 and successively washed with water, diluted $NaHCO_3$, diluted HCl water, and brine, dried over Na_2SO_4, then concentrated in vacuo. The residue was purified by flash chromatography to afford **11a−k**. For details, see Supporting Information.

General Procedure for the Preparation of 12a−k. Method A. To a solution of **11** (0.4−0.6 mmol) in refluxing pyridine (6 mL) was added anhydrous lithium iodide (4.0 equiv). The reflux was maintained for 7 h, and then the solution was cooled to room temperature, concentrated in

vacuo, poured into 0.1 N HCl, and extracted with $CHCl_3$. The resulting organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 1:0 to 19:1). For details, see Supporting Information.

Method B. To a solution of **11** (0.4−0.6 mmol) in THF (10 mL) was added lithium hydroxide (2.5 equiv) in water (minimum amount). After being stirred for 16 h, the solution was acidified using diluted HCl and concentrated in vacuo. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 1:0 to 19:1).

General Procedure for the Preparation of 13a−k. To a solution of **12** (0.2−0.4 mmol) in THF (6 mL) was successively added EDC (1.3 equiv), HOBt (1.3 equiv), and NMM (1.5 equiv). After the mixture was stirred for 20 min, *O*-trityl hydroxylamine (1.5 equiv) was added. After stirring for 16 h, the mixture was dissolved with ether, washed with 0.1 N $NaHCO_3$, 0.1 N HCl, water, and brine. The solution was then dried over Na_2SO_4 and concentrated in vacuo. Purification by flash chromatography (hexanes/EtOAc, 1:0 then 3:2) afforded **13**. For details, see Supporting Information.

General Procedure for the Preparation of 5a−k. A solution of **13** (0.1−0.3 mmol) in CH_2Cl_2 (2 mL) saturated with water was added a solution of 10% TFA in CH_2Cl_2 (1 mL) so as to maintain a deep yellow color. The solution was stirred for 1 h, then diluted by CH_2Cl_2, washed with 0.5 N $NaHCO_3$ and then brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash chromatography (CH_2Cl_2/CH_3OH, 1:0 to 19:1) or recrystallization to afford **5**. For ^{13}C NMR and other analytical data see Supporting Information.

2-[(Benzylcarbamoyl-methyl)-(4-methoxy-benzenesulfonyl)-amino]-4-benzylsulfanyl-*N*-hydroxy-butyramide (5a). Yield 65%, colorless oil; R_f = 0.45 (CH_2Cl_2/MeOH, 9:1); IR (neat/NaCl) 3227.4, 1651.8, 1595.6, 1575.9 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.83 (d, 2H, J = 8.9 Hz), 7.35−7.17 (m, 10H), 7.02 (d, 2H, J = 8.9 Hz), 4.41 (s, 2H), 4.25 (dd, 1H, J = 6.7, 7.2 Hz), 4.16 (d, 1H, J = 17.5 Hz), 3.97 (d, 1H, J = 17.5 Hz), 3.85 (s, 3H), 3.60 (d, 1H, J = 13.0 Hz), 3.45 (d, 1H, J = 13.0 Hz), 2.31−2.06 (m, 2H), 2.00 (m, 1H), 1.79 (m, 1H).

4-Benzylsulfanyl-2-((4-methoxy-benzenesulfonyl)-{[(pyridin-3-ylmethyl)-carbamoyl]-methyl}-amino)-*N*-hydroxy-butyramide (5b). Yield 61%, colorless oil; R_f = 0.28 (CH_2Cl_2/MeOH, 9:1); IR (neat/NaCl) 3217.0, 1667.4, 1595.1, 1574.2 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 8.79 (m, 1H), 8.67 (d, 1H, J = 5.2 Hz), 8.44 (d, 1H, J = 8.1 Hz), 7.90 (dd, 1H, J = 5.2, 8.1 Hz), 7.83 (d, 2H, J = 8.8 Hz), 7.30−7.18 (m, 5H), 7.05 (d, 2H, J = 8.8 Hz), 4.61 (s, 2H), 4.25 (dd, 1H, J = 7.4, 7.4 Hz), 4.16 (d, 1H, J = 17.6 Hz), 4.01 (d, 1H, J = 17.6 Hz), 3.85 (s, 3H), 3.60 (d, 1H, J = 13.4 Hz), 3.54 (d, 1H, J = 13.4 Hz), 2.30−2.10 (m, 2H), 1.96 (m, 1H), 1.73 (m, 1H).

4-Benzylsulfanyl-2-[[(cyclohexylmethyl-carbamoyl)-methyl]-(4-methoxy-benzenesulfonyl)-amino]-*N*-hydroxy-butyramide (5c). Yield 69%, colorless oil; R_f = 0.51 (CH_2Cl_2/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 11.61 (s, 1H), 7.82 (d, 2H, J = 9.0 Hz), 7.40−7.20 (m, 5H), 6.95 (d, 2H, J = 9.0 Hz), 6.31 (m, 1H), 4.36 (dd, 1H, J = 5.9, 7.1 Hz), 3.85 (m, 1H), 3.84 (s, 3H), 3.73−3.58 (m, 3H), 3.20−3.04 (m, 2H), 2.55−2.18 (m, 3H), 1.82−1.60 (m, 6H), 1.58−1.40 (m, 1H), 1.35−1.10 (m, 2H), 1.03−0.80 (m, 3H).

2-[[(Benzhydryl-carbamoyl)-methyl]-(4-methoxy-benzenesulfonyl)-amino]-4-benzylsulfanyl-*N*-hydroxy-butyramide (5d). Yield 64%, white solid; R_f = 0.55 (CH_2Cl_2/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 11.15 (s, 1H), 7.82 (d, 2H, J = 9.0 Hz), 7.40−7.15 (m, 15H), 7.01 (d, 1H, J = 8.0 Hz), 6.85 (d, 2H, J = 9.0 Hz), 6.20 (d, 1H, J = 8.0 Hz), 4.34 (dd, 1H, J = 4.0, 8.0 Hz), 3.88 (d, 1H, J = 17.0 Hz), 3.81 (s, 3H), 3.67 (d, 1H, J = 17.0 Hz), 3.62 (d, 1H, J = 13.8 Hz), 3.55 (d, 1H, J = 13.8 Hz), 2.40−2.12 (m, 3H), 1.70 (m, 1H).

4-Benzylsulfanyl-2-[{[(di-pyridin-2-yl-methyl)-carbamoyl]-methyl}-(4-methoxy-benzenesulfonyl)-amino]-*N*-hydroxy-butyramide (5e). Yield 72%, white solid; R_f = 0.31 (CH_2Cl_2/MeOH, 9:1); ^1H NMR (300 MHz, CD_3OD) δ 8.59 (m, 2H), 8.02 (m, 2H), 7.83 (d, 2H, J = 9.0 Hz), 7.81−7.71 (m, 2H), 7.50 (m, 2H), 7.30−7.16 (m, 5H), 7.01 (d, 2H, J = 9.0 Hz), 6.39 (s, 1H), 4.34 (d, 1H, J = 17.7 Hz), 4.28 (dd, 1H, J = 7.2, 7.2 Hz), 4.14 (d, 1H, J = 17.7 Hz), 3.82 (s, 3H), 3.56 (d, 1H, J = 13.0 Hz), 3.51 (d, 1H, J = 13.0 Hz), 2.28−2.09 (m, 2H), 1.99 (m, 1H), 1.75 (m, 1H).

4-Benzylsulfanyl-2-[[(dicyclohexylmethyl-carbamoyl)-methyl]-(4-methoxy-benzenesulfonyl)-amino]-*N*-hydroxy-butyramide (5f). Yield 66%, colorless oil; R_f = 0.60 (CH_2Cl_2/MeOH, 9:1); IR (neat/NaCl) 3299.7, 1660.5, 1596.1, 1577.3 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 11.75 (s, 1H). 7.83 (d, 2H, J = 8.9 Hz), 7.37−7.21 (m, 5H), 6.96 (d, 2H, J = 8.9 Hz), 5.71 (d, 1H, J = 10.1 Hz), 3.97 (dd, 1H, J = 5.7, 8.1 Hz), 3.84 (s, 3H), 3.83 (d, 1H, J = 16.8 Hz), 3.70−3.58 (m, 4H), 2.52−2.20 (m, 3H), 1.88−1.48 (m, 11H), 1.48−1.40 (m, 2H), 1.32−0.86 (m, 10H).

4-Benzylsulfanyl-2-{(4-methoxy-benzenesulfonyl)-[(4-methoxy-benzylcarbamoyl)-methyl]-amino}-*N*-hydroxy-butyramide (5g). Yield 63%, colorless oil; R_f = 0.43 (CH_2Cl_2/MeOH, 9:1); IR (neat/NaCl) 3365.5, 1651.1, 1595.3 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.82 (d, 2H, J = 8.9 Hz), 7.28−7.17 (m, 7H), 7.02 (d, 2H, J = 8.9 Hz), 6.86 (d, 2H, J = 8.9 Hz), 4.32 (s, 2H), 4.25 (dd, 1H, J = 6.6, 7.5 Hz), 4.12 (d, 1H, J = 17.5 Hz), 3.95 (d, 1H, J = 17.5 Hz), 3.84 (s, 3H), 3.75 (s, 3H), 3.60 (d, 1H, J = 13.1 Hz), 3.53 (d, 1H, J = 13.1 Hz), 2.29−2.12 (m, 2H), 1.99 (m, 1H), 1.77 (m, 1H).

4-Benzylsulfanyl-2-[[(3,5-dimethoxy-benzylcarbamoyl)-methyl]-(4-methoxy-benzenesulfonyl)-amino]-*N*-hydroxy-butyramide (5h). Yield 82%, colorless oil; R_f = 0.41 (CH_2Cl_2/MeOH, 9:1); IR (neat/NaCl) 3182.5, 1654.2, 1597.0 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, 2H, J = 8.9 Hz), 7.31−7.18 (m, 5H), 6.92 (d, 2H, J = 8.9 Hz), 6.47 (d, 2H, J = 2.2 Hz), 6.34 (dd, 1H, J = 2.2, 2.2 Hz), 4.45 (dd, 1H, J = 5.5, 15.0 Hz), 4.32 (m, 2H), 3.90 (d, 1H, J = 17.1 Hz), 3.81 (s, 3H), 3.73 (s, 6H), 3.71 (d, 1H, J = 17.1 Hz), 3.60 (d, 1H, J = 13.1 Hz), 3.53 (d, 1H, J = 13.1 Hz), 2.38−2.10 (m, 3H), 1.70 (m, 1H).

4-Benzylsulfanyl-2-{(4-methoxy-benzenesulfonyl)-[(2,4,6-trimethoxy-benzylcarbamoyl)-methyl]-amino}-*N*-hydroxy-butyramide (5i). Yield 49%, colorless oil; R_f = 0.39 (CH_2Cl_2/MeOH, 9:1); IR (neat/NaCl) 3341.5, 3196.2, 1651.8, 1595.6 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.80 (d, 2H, J = 8.9 Hz), 7.29−7.17 (m, 5H), 7.02 (d, 2H, J = 8.9 Hz), 6.20 (s, 2H), 4.49 (d, 1H, J = 13.5 Hz), 4.29 (d, 1H, J = 13.5 Hz), 4.27 (dd, 1H, J = 6.0, 7.1 Hz), 4.12 (d, 1H, J = 16.8 Hz), 3.88 (d, 1H, J = 16.8 Hz), 3.85 (s, 3H), 3.81 (s, 6H), 3.78 (s, 3H), 3.56 (d, 1H, J = 13.0 Hz), 3.50 (d, 1H, J = 13.0 Hz), 2.25−2.11 (m, 2H), 1.95 (m, 1H), 1.72 (m, 1H).

4-Benzylsulfanyl-2-[cyclohexylcarbamoylmethyl-(4-methoxy-benzenesulfonyl)-amino]-*N*-hydroxy-butyramide (5j). Yield 65%, white solid; R_f = 0.50 (CH_2Cl_2/MeOH, 9:1); IR (neat/NaCl) 3252.0, 1651.8, 1595.6 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.85 (d, 2H, J = 9.0 Hz), 7.34−7.17 (m, 5H), 7.05 (d, 2H, J = 9.0 Hz), 4.25 (dd, 1H, J = 5.4, 7.8 Hz), 4.05 (d, 1H, J = 17.5 Hz), 3.88 (d, 1H, J = 17.5 Hz), 3.85 (s, 3H), 3.64 (m, 1H), 3.62 (d, 1H, J = 13.1 Hz), 3.57 (d, 1H, J = 13.1 Hz), 2.30−2.11 (m, 2H), 2.01 (m, 1H), 1.92−1.68 (m, 5H), 1.68−1.56 (m, 1H), 1.42−1.15 (m, 5H).

4-Benzylsulfanyl-2-[(4-methoxy-benzenesulfonyl)−(2-morpholin-4-yl-2-oxo-ethyl)-amino]-*N*-hydroxy-butyramide (5k). Yield 51%, colorless oil; R_f = 0.38 (CH_2Cl_2/MeOH, 9:1); ^1H NMR (300 MHz, CD_2Cl_2) δ 12.14 (s, 1H), 7.80 (d, 2H, J = 9.0 Hz), 7.38−7.20 (m, 5H), 6.98 (d, 2H, J = 9.0 Hz), 4.28 (m, 1H), 4.25 (d, 1H, J = 17.3 Hz), 3.85 (s, 3H), 3.76−3.55 (m, 7H), 3.49−3.31 (m, 2H), 2.40−2.27 (m, 3H), 1.78 (m, 1H).

3-Isobutylamino-dihydro-thiophen-2-one (14). To a solution of D,L-homocysteine hydrochloride (0.201 g, 1.31 mmol) in MeOH (5 mL) was added freshly distilled isobutyraldehyde (0.24 mL, 2.62 mmol) at 0 °C. After complete dissolution, Et_3N (0.2 mL, 1.5 mmol) was added. The resulting mixture was stirred at room temperature for 16 h and then cooled to 0 °C before adding $NaBH_3CN$ (0.172 g, 5.48 mmol) portionwise over 45 min. The mixture was stirred for a further 30 min at 0 °C, quenched with water, and extracted with ether, and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed (CH_2Cl_2/MeOH, 99:1) to afford **14** (0.226 g, 77%), R_f = 0.60

(CH_2Cl_2/MeOH, 1:9); IR (neat/NaCl) 3228.0, 2956.2, 1698.5 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 3.40 (m,1H), 3.25 (m, 2H), 2.60 (m, 1H), 2.56 (dd, 1H, J = 6.6, 10.8 Hz), 2.42 (dd, 1H, J = 6.6, 10.8 Hz), 2.00 (m, 1H), 1.65 (m, 1H), 0.92 (d, 3H, J = 6.6 Hz), 0.88 (d, 3H, J = 6.6 Hz).

***N*-Isobutyl-4-methoxy-*N*-(2-oxo-tetrahydro-thiophen-3-yl)-benzenesulfonamide (15).** To a solution of **14** (0.4 g, 2.31 mmol) in anhydrous pyridine (5 mL) was added *p*-methoxybenzene sulfonyl chloride (0.717 g, 3.47 mmol) at 0 °C. The resulting mixture was stirred for 16 h at room temperature. The mixture was then concentrated, taken up in CH_2Cl_2, washed successively with water, 1 N HCl, saturated $NaHCO_3$, water, and brine, dried over Na_2SO_4, then concentrated in vacuo. The residue afforded, after flash chromatography (CH_2Cl_2/MeOH, 99:1), **15** (0.722 g, 67%), R_f = 0.83 (MeOH/CH_2Cl_2, 1:9); IR (neat/NaCl) 2960.8, 1707.0, 1497.6, 1355.8 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.81 m (d, 2H, J = 9.8 Hz), 6.98 (d, 2H, J = 9.9 Hz), 4.67 (m, 1H), 3.85 (s, 3H), 3.25 (m, 2H), 3.05 (dd, 1H, J = 6.8, 14.5 Hz), 2.74 (dd, 1H, J = 6.8, 14.5 Hz), 2.54 (m, 1H), 2.42 (m,1H), 1.95 (m, 1H), 0.88 (d, 3H, J = 6.5 Hz), 0.86 (d, 3H, J = 6.5 Hz).

General Procedure for the Preparation of 16a−f. To a solution of **15** (0.4 mmol) in MeOH (3 mL) was added at 0 °C sodium methoxide freshly prepared from Na (0.6 mmol) in MeOH (2 mL). After the mixture was stirred for 30 min, the appropriate alkyl bromide (0.8 mmol) was added. The mixture was stirred for 16 h at room temperature then concentrated, taken up in EtOAc, filtered, and concentrated in vacuo. The residue was chromatographed to afford **16**. For details, see Supporting Information.

General Procedure for the Preparation of 6a−f. Method C. To a solution of **16** (0.1−0.2 mmol) in MeOH (1 mL) were added NH_2OH·HCl (2.0 equiv) and NaOMe, 25% in MeOH solution (3.0 equiv). After being stirred for 16 h at room temperature, the solution was acidified with 0.5 N HCl to pH 3, and the organic layer was extracted with EtOAc, dried over Na_2SO_4, concentrated in vacuo, and chromatographed (CH_2Cl_2/MeOH, 9:1) to afford **6**. For ^{13}C NMR and other analytical data, see Supporting Information.

Method D. To a solution of **16** (0.1−0.2 mmol) was added NH_2OK prepared from KOH and NH_2OH (8 equiv, 0.87 M in MeOH). After being stirred for 16 h at room temperature, the solution was acidified with diluted HCl, and the organic layer was extracted with EtOAc and brine, dried over Na_2SO_4, then concentrated in vacuo. Flash chromatography (CH_2Cl_2/MeOH 19:1) afforded **6**.

4-Benzylsulfanyl-*N*-hydroxy-2-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-butyramide (6a). Method C, yield 50%, R_f = 0.20 (CH_2Cl_2/MeOH, 9:1); IR (neat/NaCl) 334.3, 2962.1, 1677.0 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.45 (bs, 1H), 7.82 (d, 2H), 7.23 (m, 5H), 6.95 (d, 2H), 4.35 (t, 1H, J = 7.5 Hz), 3.88 (s, 3H), 3.54 (d, 1H, J = 13.0 Hz), 3.42 (d, 1H, J = 13,0 Hz), 3.12 (dd, 1H, J = 9.8, 13.8 Hz), 2.88 (dd, 1H, J = 4.9, 13.8 Hz), 2.07 (m, 1H), 1.88 (m, 2H), 0.89 (d, 3H, J = 6.6 Hz), 0.85 (d, 3H, J = 6.6 Hz).

***N*-Hydroxy-2-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-4-phenylmethanesulfonyl-butyramide (6b).** Method D, yield 60%, R_f = 0.80 (CH_2Cl_2/MeOH, 9:1); IR (neat/NaCl) 3321.6, 2926.1, 1680.9, 1591.0, 1492.2 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 9.45 (bs, 1H), 7.75 (d, 2H, J = 8.8 Hz), 7.48 (m, 5H), 6.98 (d, 2H, J = 8.8 Hz), 4.51 (t, 1H, J = 7.2 Hz), 4.18 (s, 2H), 3.84 (s, 3H), 3.05 (dd, 1H, J = 5.6, 14.4 Hz), 2.90 (dd, 1H, J = 5.6, 14.4 Hz), 2.75 (m, 1H), 2.64 (m, 1H), 2.35 (m, 1H), 1.82 (m, 1H), 0.85 (d, 3H, J = 6.6 Hz), 0.83 (d, 3H, J = 6.6 Hz).

***N*-Hydroxy-2-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-4-(3-methoxy-benzylsulfanyl)-butyramide (6c).** Method D, yield 65%, R_f = 0.72 (CH_2Cl_2/MeOH, 9:1); IR (neat/NaCl) 3332.2, 2961.5, 1677.1, 1596.8 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 9.54 (br s, 1H), 7.76 (d, 2H, J = 8.8 Hz), 7.27 (m, 1H), 6.99 (d, 2H, J = 8.8 Hz), 6.84 (m, 3H), 4.35 (t, 1H, J = 7.0 Hz), 3.86 (s, 3H), 3.81 (s, 3H), 3.48 (d, 1H, J = 12.9 Hz), 3.38 (d, 1H, J = 12.9 Hz), 3.12 (dd, 1H, J = 7.9, 12.9 Hz), 2.90 (dd, 1H, J = 7.9, 12.9 Hz), 2.10 (m, 2H), 1.98 (m, 1H), 1.43 (m, 2H), 0.90 (d, 3H, J = 6.5 Hz), 0.84 (d, 3H, J = 6.5 Hz).

3-(4-Methoxy-benzenesulfonyl)-5-methyl-2-(2-pentafluorophenylmethylsulfanyl-ethyl)-hexanoic Acid Hydroxyamide (6f). Method D, yield 65%, R_f = 0.45 (CH_2Cl_2/MeOH, 9:1); IR (neat/NaCl) 3334.5, 2964.2, 1678.1, 1596.6, 1505.3 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.77 (d, 2H, J = 7.2 Hz), 7.25 (d, 2H, J = 7.2 Hz), 4.38 (bs, 1H), 3.86 (s, 3H), 3.51 (m, 2H), 3.16 (dd, 1H, J = 7.5, 11.5 Hz), 2.96 (dd, 1H, J = 7.5, 11.5 Hz), 2.24 (m, 2H), 1.96 (m, 1H), 1.51 (m, 1H), 0.91 (d, 3H, J = 4.7 Hz), 0.87 (d, 3H, J = 4.7 Hz).

***N*-Hydroxy-2-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-4-(pyridin-3-ylmethylsulfanyl)-butyramide (6d).** To a solution of the hydroxamate (60 mg, 0.084 mmol) in CH_2Cl_2 (10 mL) was added TFA (1 mL, 10% in CH_2Cl_2) dropwise until a yellow color persists. The mixture was stirred for a further 2 h, then was extracted with CH_2Cl_2, washed with 0.1 N $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. Flash chromatography (CH_2Cl_2/MeOH, 1:0 to 9:1) afforded **6d** (20 mg, 50%), R_f = 0.27 (CH_2Cl_2/MeOH, 9:1); IR (neat/NaCl) 3165.1, 2996.4, 1669.3, 1596.7 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.55 (bs, 1H), 8.48 (m, 3H), 7.68 (d, 2H, J = 8.8 Hz), 7.35 (m, 1H), 6.95 (d, 2H, J = 8.8 Hz), 4.35 (t, 1H, J = 7.1 Hz), 3.86 (s, 3H), 3.55 (s, 2H), 3.12 (dd, 1H, J = 5.3, 13.9 Hz), 2.92 (dd, 1H, J = 5.3, 13.9 Hz), 2.21 (m, 2H), 1.95 (m, 1H), 0.98 (m, 3H, J = 6.6 Hz), 0.85 (d, 3H, J = 6.6 Hz).

***N*-Hydroxy-2-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-4-(thiophen-3-ylmethylsulfanyl)-butyramide (6e).** Method D, yield 60%, R_f = 0.45 (CH_2Cl_2/MeOH 9:1); IR (neat/NaCl) 3335.6, 2961.4, 1674.2, 1595.2 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 9.50 (bs, 1H), 7.78 (d, 2H, J = 7.8 Hz), 7.28 (m, 2H), 7.00 (d, 2H, J = 7.8 Hz), 6.98 (m, 1H), 4.32 (t, 3H, J = 7.3 Hz), 3.88 (s, 3H), 3.51 (d, 1H, J = 5.7 Hz), 3.49 (d, 1H, J = 5.7 Hz), 3.11 (dd, 1H, J = 7.2, 11.2 Hz), 2.90 (dd, 1H, J = 7.2, 11.2 Hz), 2.23 (m, 2H), 1.90 (m, 1H), 0.94 (d, 3H, J = 6.2 Hz), 0.84 (d, 3H, J = 6.2 Hz).

4-Benzylsulfanyl-2-[(4-bromo-benzenesulfonyl)-isobutyl-amino]-*N*-hydroxy-butyramide (6j). Yield 62%, colorless oil; R_f = 0.37 (CH_2Cl_2/MeOH, 9:1); IR (neat/NaCl) 3345.4, 2961.3, 1679.6, 1157.0 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.67 (m, 5H), 7.31 (d, 2H, J = 7.8 Hz), 7.27 (d, 2H, J = 7.8 Hz), 4.35 (t, 1H, J = 6.8 Hz), 3.52 (dd, 1H, J = 5.7, 12.6 Hz), 3.12 (dd, 1H, J = 5.7, 12.6 Hz), 2.22 (m, 2H), 2.11 (m, 1H), 1.91 (m, 2H), 0.89 (d, 3H, J = 6.5 Hz), 0.85 (d, 3H, J = 6.5 Hz).

4-Benzylsulfanyl-*N*-hydroxy-2-[isobutyl-(4-phenoxy-benzenesulfonyl)-amino]-butyramide (6k). Yield 80%, colorless oil; R_f = 0.42 (CH_2Cl_2/MeOH, 9:1); IR (neat/NaCl) 3337.5, 2962.9, 1676.8, 1583.5, 1488.1 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 10.92 (bs,1H), 7.74 (d, 2H, J = 5.8 Hz), 7.39 (t, 2H, J = 5.5 Hz), 7.26 (m, 5H), 7.03 (t, 3H, J = 5.0 Hz), 4.34 (t, 1H, J = 6.6 Hz), 3.49 (dd, 2H, J = 7.5, 13.6 Hz), 3.15 (dd, 1H, J = 10.0, 14.2 Hz), 2.88 (dd, 1H, J = 10.0, 14.2 Hz), 2.22 (m, 1H), 2.1 (m, 2H), 1.8 (m, 1H), 1.4 (m, 1H), 0.90 (d, 3H, J = 6.6 Hz), 0.85 (d, 3H, J = 6.6 Hz).

Molecular Modeling. Molecular modeling studies were performed using software programs from InsightII (Molecular Simulations, 1995, San Diego, CA) using a modified AMBER force field.[32,33] The starting MMP-3 crystallographic structure, retrieved from the Brookhaven Protein Data Bank (code 1HFS in the PDB), was relaxed according to standard procedures, and a GRID binding interaction evaluation was performed. Probes used: Dry, O=, O, N1, 2 planes per angstrom (NPLA = 2), Flexibility of the side chains (MOVE = 1). This brought out the key interaction sites among which the highly hydrophobic S_1 pocket. Compound **6** was manually docked in the binding site of MMP-3 according to the sites shown up above and to the reported structures of similar inhibitors cocrystallized with MMPs.[10,34,35] The resulting complex was then energy minimized. AutoDock study was also performed. Grids surrounding the binding site were computed (61 × 61 points, 0.375 Å spacing) with AutoGrid and used for subsequent docking study with AutoDock using the Lamarckian genetic algorithm as search protocol. The conformations from AutoDock and

manual docking were found to be similar (RMSD < 1 Å). See ref 24 for more details.

Biological Assay. Human purified MMPs were purchased or acquired. MMP-2 gelatinase A and MMP-9 gelatinase B from Boehringer Mannheim (Meylan, France), MMP-3 stromelysin 1 from Valbiotech (Paris, France). All enzymes were activated by APMA (4-aminophenylmercuric acetate). Inhibition of MMP-3 was quantified by using the peptidomimetic substrate (7-methoxycoumarine-4-yl)-Arg-Pro-Lys-Pro-Tyr-Ala-Nva-Trp-Met-Lys(Dnp)-NH_2 (Bachem, Bubbendorf, Switzerland) which is cleaved between Ala and Nva. For inhibition studies of the other enzymes, the substrate Dnp-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(Nma)-NH_2 (Bachem), which is cleaved between amino acids Gly and Cys, was used. The fluorescent cleavage products were measured with a cytofluorometer (Cytofluor 2350, Millipore, PerSeptive Systems, Voisins le Bretonneux, France) equipped with a combination of 340 and 440 nm filters for excitation and emission, respectively. The IC_{50} values were the average of at least two determinations with a standard deviation of less than ±30%.

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Supporting Information Available: Full characterization (^1H,^{13}C NMR, LRMS, and HRMS) of all the compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Leung, D.; Abbenante, G.; Fairlie, D. P. Protease Inhibitors: Current Status and Future Prospects. *J. Med. Chem.* **2000**, *43*, 305−341.

(2) Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. Design and Therapeutic Application of Matrix Metalloproteinase Inhibitors. *Chem. Rev.* **1999**, *99*, 2735−2776.

(3) Michaelides, M. R.; Curtin, M. L. Recent Advances in Matrix Metalloproteinase Inhibitors Research. *Curr. Pharm. Des.* **1999**, *5*, 787−819.

(4) White, A. D.; Bocan, M. A.; Boxer, P. A.; Peterson, J. T.; Schrier, D. Emerging Therapeutic Advances for the Development of Second Generation Matrix Metalloproteinase Inhibitors. *Curr. Pharm. Des.* **1997**, *3*, 45−58.

(5) Beckett, R. P.; Davidson, A. H.; Drummond, A. H.; Whittaker, M. Recent Advances in Matrix Metalloproteinase Inhibitor Research. *Drug Discovery Today* **1996**, *1*, 16−26.

(6) Broadhurst, M. J.; Brown, P. A.; Lawton, G.; Ballantyne, N.; Bottomley, K. M. K.; Cooper, M. I.; Eatherton, A. J.; Kilford, I. R.; Malsher, P. J.; Nixon, J. S.; Lewis, E. J.; Sutton, B. M.; Johnson, W. H. Design and Synthesis of the Cartilage Protective Agent (CPA, Ro32-3555). *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2299−2302.

(7) 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. Discovery of CGS 27023A, a Non-Peptidic, Potent, and Orally Active Stromelysin Inhibitor That Blocks Cartilage Degradation in Rabbits. *J. Med. Chem.* **1997**, *40*, 2525−2532.

(8) (a) Zook, S. E.; Dagnino, R., Jr.; Deason, M. E.; Bender, S. L.; Melnick, M. J. *PCT Int Appl.* WO 97 20,824, 1997; (b) *Chem. Abstr.* **1997**, *127*, 108945s.

(9) (a) Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2000**, *43*, 1858−1865. (b) Barta, T. E.; Becker, D. P.; Bedell, L. J.; De Crescenzo, G. A.; McDonald, J. J.; Munie, G. E.; Rao, S.; Shieh, H.-S.; Stegeman, R.; Stevens, A. M.; Villamil, C. I. **2000**, *10*, 2815−2817. (c) Chollet, A.-M.; Le Diguarther, T.; Murray, L.; Bertrand, M.; Tucker, G. C.; Sabatini, M.; Perré, A.; Atassi, G.; Bonnet, J.; Casara, P. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 295−299. (d) Natchus, M. G.; Bookland, R. G.; Laufersweiler, M. J.; Pikul, S.; Almstead, N. G.; Biswanath, D.; Janusz, M. J.; Hsieh, C. C.; Gu, F.; Pokross, M. E.; Patel, V. S.; Garver, S. M.; Peng, S. X.; Branch, T. M.; King, S. L.; Baker, T. R.; Foltz, D. J.; Mieling, G. E. *J. Med. Chem.* **2001**, *44*, 1060−1071.

(10) Gonnella, N. C.; Li, Y.-C.; Zhang, X.; Paris, C. G. Bioactive Conformation of a Potent Stromelysin Inhibitor Determined by X-nucleus Filtered and Multidimensional NMR Spectroscopy. *Bioorg. Med. Chem.* **1997**, *5*, 2193−2201.

(11) Chen, L.; Rydel, T. J.; Gu, F.; Dunaway, M.; Pikul, S.; McDow Dunham, K.; Barnett, B. L. Crystal Structure of the Stromelysin Catalytic Domain at 2.0 Å Resolution: Inhibitor-induced Conformational Change. *J. Mol. Biol.* **1999**, *293*, 545−557.

(12) Lovejoy, B.; Welch, A. R.; Carr, S.; Luong, C.; Broka, C.; Hendricks, R. T.; Campbell, J. A.; Walker, K. A. M.; Martin, R.; Van Wart, H.; Browner, M. F. Crystal Structures of MMP-1 and MMP-13 Reveal the Structural Basis for Selectivity of Collagenase Inhibitors. *Nat. Struct. Biol.* **1999**, *3*, 217−221.

(13) Pikul, S.; McDow Dunham, K. L.; Almstead, N. G.; De, B.; Natchus, M. G.; Anastasio, M. V.; McPhail, S. J.; Snider C. E.; Taiwo, Y. O.; Rydel, T.; Dunaway, C. M.; Gu, F.; Mieling, G. E. Discovery of Potent, Achiral Matrix Metalloproteinase Inhibitors. *J. Med. Chem.* **1998**, *41*, 3568−3571.

(14) Esser, C. K.; Bugianesi, R. L.; Caldwell, C. G.; Chapman, K. T.; Durette, P. L.; Girotra, N. N.; Kopka, I. E.; Lanza, T. J.; Levorse, D. A.; MacCoss, M.; Owens, K. A.; Ponpipom, M. M.; Simeone, J. P.; Harrison, R. K.; Niedzwiecki, L.; Becker, J. W.; Marcy, A. I.; Axel, M. G.; Christen, A. J.; McDonnell, J.; Moore, V. L.; Olszewski, J. M.; Saphos, C.; Visco, D. M.; Shen, F.; Colleti, A.; Krieter, P. A.; Hagmann, W. K. Inhibition of Stromelysin-1 (MMP-3) by P_1'-Biphenylethyl Carboxyalkyl Dipeptides. *J. Med. Chem.* **1997**, *40*, 1026−1040.

(15) Grams, F.; Crimmin, M.; Hinnes, L.; Huxley, P.; Pieper, M.; Tschesche, H.; Bode, W. Structure Determination and Analysis of Human Neutrophil Collagenase Complexed with a Hydroxamate Inhibitor. *Biochemistry* **1995**, *34*, 14012−14020.

(16) Hanessian, S.; Griffin, A.; Devasthale, P. V. Synthesis of Conformationally Constrained Potential Inhibitors of Mammalian Metalloproteinases. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 3119−3124.

(17) Hanessian, S.; Moitessier, N.; Wilmouth, S. Tetrahydrofuran as a Scaffold for Peptidomimetics. Application to the Design and Synthesis of Conformationally Constrained Metalloproteinase Inhibitors. *Tetrahedron* **2000**, *56*, 8469−8485.

(18) Hanessian, S.; Bouzbouz, S.; Boudon, A.; Tucker, G. C.; Peyroulan, D. Picking the S_1, S_1' and S_2' Pockets of Matrix Metalloproteinases. A Niche for potent Acyclic Sulfonamide Inhibitors. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1691−1696.

(19) GRID version 17, Molecular Discovery Inc., Oxford, U.K. Goodford, P. J. A Computational Procedure for Determining Energetically Favorable Binding Sites on Biologically Important Macromolecules. *J. Med. Chem.* **1985** *28*, 849−857.

(20) Boobbyer, D. N. A.; Goodford, P. J.; McWhinnie, P. M.; Wade, R. C. New Hydrogen-Bond Potentials for Use in Determining Energetically Favorable Binding Sites in Molecules of Known Structure. *J. Med. Chem.* **1989**, *32*, 1083−1094.

(21) Wade, R. C.; Clark, K. J.; Goodford, P. J. Further Development of Hydrogen Bond Functions for Use in Determining Energetically Favorable Binding Sites on Molecules of Known Structure. 1. Ligand Probe Groups with the Ability to Form Two Hydrogen Bonds. *J. Med. Chem.* **1993**, *36*, 140−147.

(22) Wade, R. C.; Clark, K. J.; Goodford, P. J. Further Development of Hydrogen Bond Functions for Use in Determining Energetically Favorable Binding Sites on Molecules of Known Structure. 1. Ligand Probe Groups with the Ability to Form More Than Two Hydrogen Bonds. *J. Med. Chem.* **1993**, *36*, 148−156.

(23) For another successful use of GRID in the inhibitor/MMP complexes study, see: Matter, H.; Schwab, W. Affinity and Selectivity of Matrix Metalloproteinase Inhibitors: A Chemometrical Study from the Perspective of Ligands and Proteins. *J. Med. Chem.* **1999**, *42*, 4506−4523.

(24) Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. Automated Docking Using a Lamarckian Genetic Algorithm and an Empirical Binding Free Energy Function. *J. Comput. Chem.* **1998**, *19*, 1639−1662.

(25) Hanessian, S.; Moitessier, N.; Therrien, E. Design and synthesis of MMP inhibitors guided by molecular modeling. Overview of the available structural data, comparative docking study and design of potentially selective inhibitors. *J. Comput.-Aided Mol. Des.* In press.

(26) Rockwell, A.; Melden, M.; Copeland, E. A.; Hardman, K.; Decicco, C. P.; DeGrado, W. F. Complementarity of Combinatorial Chemistry and Structure-Based Ligand Design − Application to the Discovery of Novel Inhibitors of matrix Metalloproteinases. *J. Am. Chem. Soc.* **1996,** *118*, 10337−10338.

(27) Hanessian, S.; Johnstone, S. Synthesis of Hydroxamic Esters via Alkoxyaminocarbonylation of β-Dicarbonyl Compounds. *J. Org. Chem.* **1999**, *64*, 5896−5903.

(28) (a) Fieser, L. F.; Fieser, M. Reagents for Organic Synthesis; J. Wiley and Sons, Inc.: New York, 1967; Vol. 1, p 478. (b) Hauser, C. R.; Renfrow, W. B., Jr. *Org. Synth. Coll. Vol.* **1943**, *2*, 67−68.

(29) Tamura, Y.; Watanabe, F.; Nakatani, T.; Yasui, K.; Fuji, M.; Komurasaki, T.; Tsuzuki, H.; Maekawa, R.; Yoshioka, T.; Kawada, K.; Sugita, K.; Ohtani, M. Highly Selective and Orally Active Inhibitors of Type IV Collagenase (MMP-9 and MMP-2): N-Sulfonylamino Acid Derivatives. *J. Med. Chem*. **1998**, *41*, 640−649.

(30) Böhm, H.-J.; Klebe, G. What Can We Learn from Molecular Recognition in Protein−Ligand Complexes for the Design of New Drugs? *Angew. Chem., Int. Ed. Engl*. **1996**, *35*, 2588−2614.

(31) Kiyama, R.; Tamura, Y.; Watanabe, F.; Tsuzuki, H.; Ohtani, M.; Yodo, M. Homology Modeling of Gelatinase Catalytic Domains and Docking Simulations of Novel Sulfonamide Inhibitors. *J. Med. Chem*. **1999**, *42*, 1723−1738.

(32) Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S., Jr.; Weiner P. K. A New Force Field for Molecular Mechanical Simulation of Nucleic Acids and Proteins. *J. Am. Chem. Soc*. **1984**, *106*, 765−784.

(33) Weiner, S. J.; Kollman, P. A. A Combined Ab Initio Quantum Mechanical and Molecular Mechanical Method for Carrying out Simulations on Complex Molecular Systems: Applications to the $CH_3Cl + Cl^-$ Exchange Reaction and Gas Phase Protonation of Polyethers. *J. Comput. Chem*. **1986**, *6*, 718−730.

(34) Lovejoy, B.; Welch, A. R.; Carr, S.; Luong, C.; Broka, C.; Hendricks, T.; Campbell, J. A.; Walker, K. A. M.; Martin, R.; Van Wart, H.; Browner, M. F. Crystal Structure of MMP-1 and -13 Reveal the Structural Basis for Selectivity of Collagenase Inhibitors. *Nat. Struct. Biol*. **1999**, *6*, 217−221.

(35) Pikul, S.; McDow Dunham, K. L.; Almstead, N. G.; De, B.; Natchus, M. G.; Anastasio, M. V.; McPhail, S. J.; Snider, C. E.; Taiwo, Y. O.; Rydel, T.; Dunaway, C. M.; Gu, F.; Mieling, G. E. Discovery of Potent, Achiral Matrix Metalloproteinase Inhibitors. *J. Med. Chem*. **1998**, *41*, 3568−3571.

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