Dual Inhibitors of Matrix Metalloproteinases and Carbonic Anhydrases: Iminodiacetyl-Based Hydroxamate−Benzenesulfonamide Conjugates

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Matrix metalloproteinases (MMPs) and carbonic anhydrases (CAs) are two classes of zinc enzymes with different roles and catalytic targets, such as the degradation of most of the extracellular matrix (ECM) proteins and the regulation of the CO_2/HCO_3^- equilibrium in the cells, respectively. Both families have isoforms which were proved to be involved in several stages of carcinogenic processes, and so the selective inhibition of these enzymes might be of interest in cancer therapy. We report herein the design, synthesis, and *in vitro* evaluation of a series of compounds possessing the iminodiacetic acid as the main backbone and two functional groups attached, namely, the hydroxamic acid and the arylsulfonamide ($ArSO_2NH_2$) moieties, to enable the inhibition of MMPs and CAs, respectively. These compounds were demonstrated to strongly inhibit both MMPs and CAs, some of them from the nanomolar to subnanomolar range. Furthermore, a docking study for MMPs was reported for the most promising compound in order to investigate its binding interactions with the different MMPs.

Introduction

Matrix metalloproteinases (MMPs),[a] also designated as matrixins, are a class of zinc-dependent endopeptidases able to degrade all components of the extracellular matrix (ECM), such as glycoproteins (e.g., collagen), proteoglycans, and fibrins, but also other proteins such as growth-factor-binding proteins, cell-surface receptors, cell-adhesion molecules, or even the precursors of other proteinases. These enzymes are involved in physiological processes related to the degradation of the connective tissue, including tissue remodeling and repair, embryonic development, and angiogenesis, but also other very complex processes such as homeostatic regulation of the extracellular environment and control of the innate immunity.[1−3] However, some pathological situations are associated with imbalance between MMPs and their endogenous regulators (namely, the TIMPs, tissue inhibitors of MMPs), leading to an overexpression of these enzymes, which may contribute to the occurrence of several degenerative and inflammatory diseases.[4,5] It is also well-known that MMPs are involved in several steps of cancer progression, including tumor growth, angiogenesis and apoptosis, as well as tissue invasion and formation of metastasis, and their overexpression was detected in most types of cancers.[2,6] For this reason, MMPs have been considered for long as promising targets in cancer therapy. However, due to their complex roles, namely, with their signaling functions, MMPs may also have protective roles against tumor progression.[7] On the other hand, phase III clinical trials have shown that the use of broad-spectrum inhibitors could cause severe side effects with dose-limiting consequences.[8,9] Altogether, these facts lead to the need of developing a new generation of MMP inhibitors with high selectivity for the well-identified and validated targets in each disease, namely, inhibiting the target MMPs and sparing the antitarget proteins.[10] To date, Overall and Kleifeld[9] identified MMP-1, -2, and -7 as anticancer drug target MMPs and MMP-3, -8, -9, and -12 as antitargets. However, considering the musculoskeletal problems that have been associated with the inhibition of MMP-1,[11] in spite of some controversy,[12] that enzyme is herein considered as an antitarget. Therefore, in a cancer therapy, a candidate drug for targeting the MMPs should be able to inhibit preferably MMP-2 and -7 and to spare some others, more specifically the antitargets MMP-1, -3, -8, -9, and -12, in a ratio higher than 1000-fold IC_{50} or K_I units; nowadays, this is the major challenge in the design of third generation MMP inhibitors.[4,10,12]

The α-carbonic anhydrases (CAs, EC 4.2.1.1) are a class of widespread zinc-containing enzymes which catalyze the CO_2/HCO_3^- interconversion. They play a vital role for most living organisms, participating in several physiological processes related with the respiration and transport of those species, pH homeostasis, several carbonate-dependent biosynthetic reactions, bone resorption, etc. Among the 16 isoforms known in vertebrates, considerable differences are associated with their activity and subcellular localization. They can be cytosolic, membrane-bound, mitochondrial, etc. and can be found in various tissues, such as the gastrointestinal tract, nervous system, kidneys, skin, and eyes.[13] It is also known the involvement of these enzymes in several pathologies (such as glaucoma, epilepsy, hypertension, gastric and duodenal ulcers, tumors), and so CA inhibitors have been largely investigated, and some of them are in clinical use for the treatment of these diseases. They belong mostly to the class of primary sulfonamides (RSO_2NH_2), sulfamates ($ROSO_2NH_2$), and sulfamides ($RNHSO_2NH_2$); one example is acetazolamide (**AAZ**,[14] see Figure 1), in clinical use for more than 50 years as a

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[a] Abbreviations: MMP, matrix metalloproteinase; CA, carbonic anhydrase; ECM, extracellular matrix; TIMP, tissue inhibitor of MMPs; HIFα, hypoxia inducible factor-α; ZBG, zinc-binding group; IDA, iminodiacetic acid; SPE, 4-sulfamoylphenylethyl group; TACE, TNF-α converting enzyme; SAR, structure−activity relationship; SMC, scaffold match constraint.

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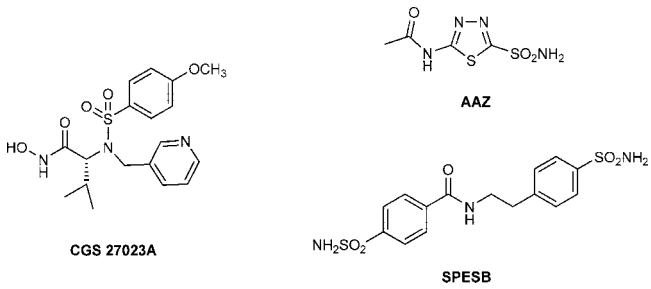


Figure 1. The broad-spectrum MMP inhibitor **CGS 27023A**[20] and the reference CA inhibitors acetazolamide (**AAZ**)[14] and *N*-(4-sulfamoylphenylethyl)-4-sulfamoylbenzamide (**SPESB**).[21]

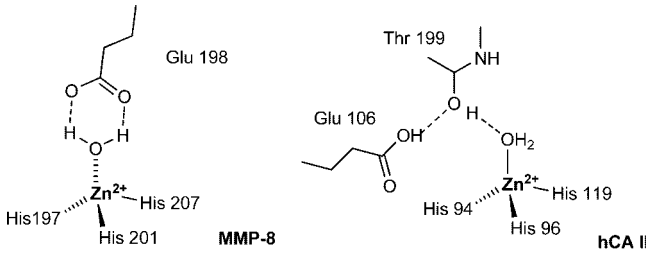


Figure 2. Catalytic coordination site of the Zn(II) in MMP-8 and human carbonic anhydrase II (hCA II). In MMPs, the water molecule is H-bonded to the carboxylate group of the glutamic acid, whereas in CAs it forms a H-bond with the hydroxyl of a threonine residue, which is H-bonded with a glutamic acid.[22]

antiglaucoma and antiepileptic drug.[15] In spite of the long use of CA inhibitors, only recently the roles of some specific isoforms have been more clearly understood, namely, the transmembrane CA IX, which is present only in very few normal tissues but has its expression strongly increased in many types of hypoxic tumors, namely, due to hypoxia inducible factor-α (HIFα).[16] CA IX was proved to be involved in acidification of the extratumoral medium, contributing both to the acquisition of metastatic phenotypes and to chemoresistance to weakly basic anticancer drugs, and probably it also plays a role in providing the bicarbonate necessary for the cell growth.[17,18] These factors point out the high interest of finding selective CA IX inhibitors, which can spare the ubiquitous CA I and II, either to be used in cancer therapy as antitumor drugs against hypoxic tumors or to develop imaging fluorescent agents to target this very specific hypoxic-related CA.[19]

Since both MMP and CA families have members that are related to some carcinogenic and tumor progression processes, we thought that it might be of great interest to exploit possible synergic effects or "cross-reactivity" of dual inhibitors of these enzymes. To date, only a few reports are known about compounds able to inhibit both classes of metalloenzymes, with moderate cross-activity, namely, compounds of hydroxysulfonamide,[22] sulfonylated hydroxamate,[22,23] and hydroxyurea[24,25] types.

Although both MMPs and CAs possess a catalytic zinc ion coordinated to three histidine residues and one water molecule/hydroxide ion, the major differences between their catalytic sites make it difficult for a zinc-binding group (ZBG) to be good for both classes of enzymes (see Figure 2).[22,26] However, following a bifunctional approach, it is possible to conjugate in one molecule two of the preferred ZBGs for MMPs and CAs, namely, a hydroxamic acid and a primary arylsulfonamide, respectively.

Following our previous work reporting a series of iminodiacetylmonohydroxamate derivatives, which proved to act as MMP inhibitors, some of them presenting high activity and selectivity for MMP-2 vs MMP-1 (Chart 1, compounds **1** and **2**),[27] we report herein two series of related compounds, which were designed mainly for dual inhibition of MMPs and CAs (see general formula in Chart 1). A first series of compounds (**3a**−**c**) was developed to evaluate the effect of introducing an (*R*)-isopropyl group in the α-position to the hydroxamic moiety of some of the previously reported inhibitors. Then, a second series of compounds was synthesized, containing a hydroxamic acid (X = NHOH moiety) and a benzenesulfonamide group (R_2 = NH-SPE, SPE = 4-sulfamoylphenylethyl) (compounds **4**, **6a**−**c**) in order to further confer activity against CAs. Finally, compound **7**, conjugating both features of the two series was developed, aiming at the double target inhibition.

Chemistry

All of the compounds reported herein possess the iminodiacetic acid (IDA) as the main scaffold, although they were obtained from either IDA or D-valine as starting materials. Except **4**, all possess an *N*-sulfonylaryl moiety, which provides an aromatic moiety and hydrogen bond acceptor atoms (from the SO_2 group), for hydrophobic interactions with the S_1' pocket of the enzymes and H-bonds with the amino acid residues at the entrance of this cavity, respectively.

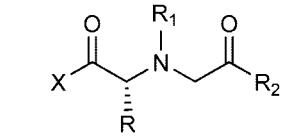
For compounds **3a**−**c**, possessing an alkyl group ((*R*)-isopropyl) α-positioned relative to the hydroxamic acid, the starting material was D-valine (see Scheme 1). This reagent was first N-substituted by reaction with the appropriate arylsulfonyl chloride (aryl = $PhOCH_3$, PhPh, PhOPh), resulting in the corresponding secondary sulfonamide. Considering the subsequent reaction steps, a protection of the carboxylic acid was necessary. The benzyl group was revealed to be the most suitable for this purpose, and the esterification was performed with benzyl bromide in the presence of triethylamine (TEA). Afterward, the coupling of one acetic moiety with the sulfonamide was performed using *tert*-butyl bromoacetate in dry DMF and excess of K_2CO_3, leading to compounds **10a**−**c**. The next step was the catalytic hydrogenolysis of the benzyl ester using Pd/C, affording the corresponding carboxylates **11a**−**c**. The carboxylic groups of these intermediates were condensed with the *O*-benzylhydroxylamine, upon previous activation with ethylchloroformate (ECF) in the presence of *N*-methylmorpholine (NMM), to afford the *O*-benzyl-protected hydroxamic acids, **12a**−**c**. Further *tert*-butyl and benzyl deprotection with TFA and catalytic hydrogenolysis, respectively, afforded the final compounds **3a**−**c**.

Compound **4** (Scheme 2) was prepared from *N*-benzyliminodiacetic acid (BIDA), which was converted to the corresponding anhydride, **14**, upon treatment with oxalyl chloride, and subsequently underwent nucleophilic attack with (4-sulfamoylphenyl)-ethylamine (SPEA) to yield **15**. Further coupling of the carboxylic group with hydroxylamine, using the ECF/NMM method described above, afforded the final hydroxamic analogue, **4**, possessing the 4-sulfamoylphenylethyl (SPE) moiety.

For the synthesis of **6a**−**c** (Scheme 3), the corresponding N-substituted dicarboxylic acids **16a**−**c** were used as starting materials, which were obtained upon derivatization of IDA, as previously reported.[27] In these cases, instead of the anhydride intermediate route used for compound **4**, the first step consisted of peptide coupling of SPEA with one of the carboxylic groups of **16a**−**c**, involving activation with ECF/NMM, to obtain the compounds **5a**−**c**. This step was followed by a second condensation of the remaining carboxylic group with hydroxylamine using the same method, thus affording the final hydroxamic analogues **6a**−**c**.

For the synthesis of compound **7**, the starting material was the intermediate **10c**, prepared as discussed above, whose *tert*-

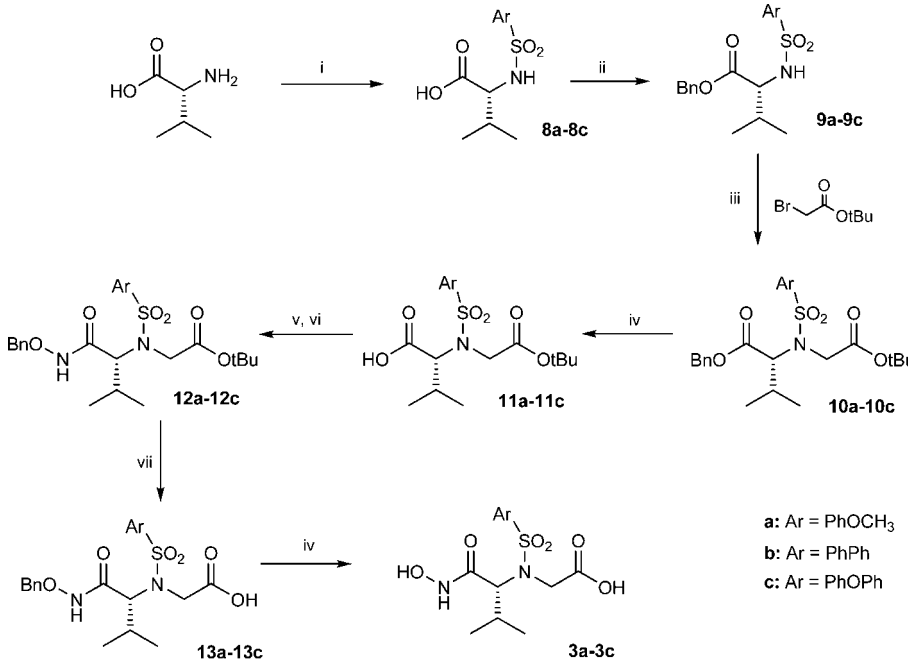
Chart 1



SPE = -CH$_2$CH$_2$-C$_6$H$_4$-SO$_2$NH$_2$

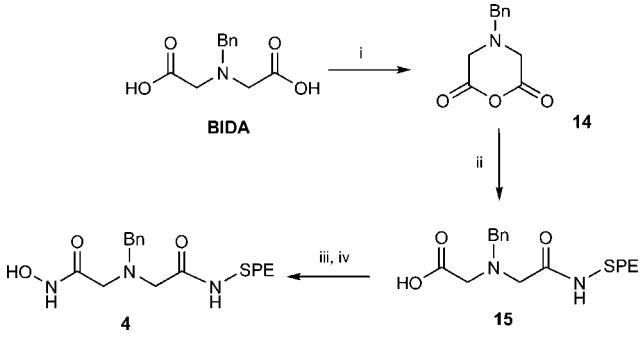
	X	R	R_1	R_2
1	NHOH	H	-SO_2PhOCH_3	OH
2	NHOH	H	-SO_2PhOPh	OH
3a	NHOH	*i*-Pr	-SO_2PhOCH_3	OH
3b	NHOH	*i*-Pr	-SO_2PhPh	OH
3c	NHOH	*i*-Pr	-SO_2PhOPh	OH
4	NHOH	H	CH_2Ph	NH-SPE
5b	OH	H	-SO_2PhPh	NH-SPE
5c	OH	H	-SO_2PhOPh	NH-SPE
6a	NHOH	H	-SO_2PhOCH_3	NH-SPE
6b	NHOH	H	-SO_2PhPh	NH-SPE
6c	NHOH	H	-SO_2PhOPh	NH-SPE
7	NHOH	*i*-Pr	-SO_2PhOPh	NH-SPE

Scheme 1[a]



[a] Reagents and conditions: (i) $ArSO_2Cl$, TEA, 1:1 dioxane/H_2O, room temperature; (ii) BnBr, TEA, MeCN, reflux; (iii) K_2CO_3, KI catalyst, DMF, room temperature; (iv) H_2, Pd/C, MeOH, room temperature; (v) ECF, NMM, THF, 0 °C; (vi) NH_2OBn, MeOH, 0 °C; (vii) TFA, DCM, room temperature.

Scheme 2[a]



[a] Reagents and conditions: (i) $C_2O_2Cl_2$, DCM, reflux; (ii) SPEA, TEA, MeCN, 0 °C; (iii) ECF, NMM, THF, 0 °C; (iv) NH_2OH, MeOH, 0 °C.

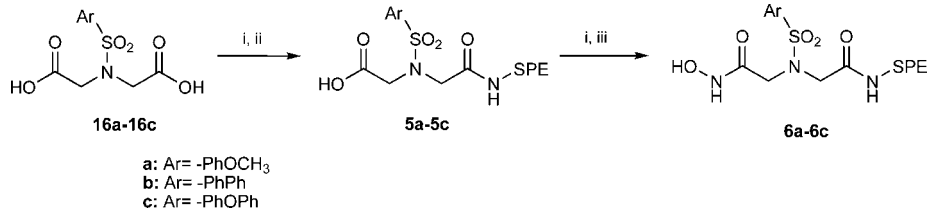
butyl ester hydrolysis with TFA afforded **17** (see Scheme 4). The SPE moiety was then introduced in this compound, by coupling its carboxylic group with SPEA, as described before for **5a**−**c**. After catalytic hydrogenolysis of the benzyl ester, *O*-benzylhydroxylamine was coupled to the carboxylic acid of **19**, forming the corresponding *O*-benzylhydroxamic acid intermediate, **20**. The use of O-protected hydroxylamine instead of the nonprotected one (as in the synthesis of **6a**−**c**) was preferred herein, in order to avoid side reactions with the hydroxyl group and guarantee a better overall yield. Catalytic deprotection of **20** with Pd/C afforded the final compound **7**.

Results and Discussion

MMPs Inhibition. Most of the reported compounds were tested against MMP-1, -2, -8, -9, -14, and -16, and the most active ones were also tested on MMP-13 and TACE (TNF-α converting enzyme), another zinc-dependent metalloproteinase whose active site domain is homologous to that of MMPs. The inhibition results obtained herein are presented in Table 1, which also includes the values for **CGS 27023A**[20] (see Figure 1), a reference broad-spectrum sulfonamide-type inhibitor, as well as two previously reported IDA derivatives (**1** and **2**).[27]

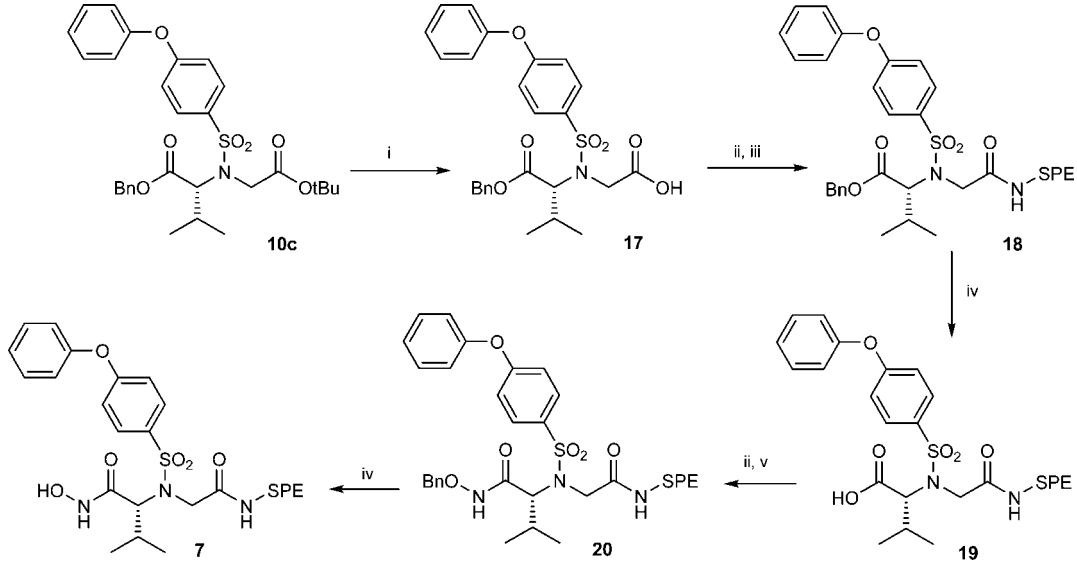
From a brief SAR analysis of the inhibitory profile of these compounds, the type of the N-substituent group (R_1 of Chart

Scheme 3[a]



[a] Reagents and conditions: (i) ECF, NMM, THF, 0 °C; (ii) SPEA, THF, 0 °C; (iii) NH_2OH, MeOH, 0 °C.

Scheme 4[a]



[a] Reagents and conditions: (i) TFA, DCM, room temperature; (ii) ECF, NMM, THF, 0 °C; (iii) SPEA, THF, 0 °C; (iv) H_2, Pd/C, MeOH, room temperature; (v) NH_2OBn, MeOH, 0 °C.

1) was shown to be the most determinant factor for the activity and selectivity of the inhibitors. In fact, compound **4**, possessing a benzyl group as the R_1 substituent, presented lower inhibitory activity (high micromolar range) than its analogous compounds with an arylsulfonyl group, **6a**−**c**, which showed activities from nanomolar to subnanomolar range. This fact is in agreement with our previous work, where it was shown that inhibitors possessing an arylalkyl group attached to the amine of the iminodiacetylhydroxamate derivatives (usually acting as a P_1' substituent, which interacts with the S_1' subsite) presented much weaker binding interaction with the catalytic site of the tested enzymes than their analogues containing an arylsulfonyl group. That difference is attributed to the ability of this group to get a more favorable orientation of the aromatic group for interaction within the S_1' pocket, as well as to provide extra O-donor atoms to establish H-bonding interactions with residues Ala192 and Glu404[27,29] (numeration according to the human MMP-2 structure, entry P08253 of UniProtKB/Swiss-Prot database).[30] Furthermore, among the arylsulfonyl derivatives, those possessing a phenoxybenzenesulfonyl group resulted to be more active than the corresponding biphenylsulfonyl and methoxybenzenesulfonyl analogues, on all tested enzymes.

With regard to the introduction of an isopropyl group in the α-position to the hydroxamate (as in compounds **3a**−**c**), it was mainly aimed at increasing the potency of the inhibitors and, eventually, their bioavailability. In fact, it is known that P_1 substituents often lead to enhancement of the inhibitory activity,[31,32] and on the other hand, an α-substituent may shield the adjacent amide bond and reduce the metabolization of the hydroxamic acid moiety.[33] The (*R*)-isomer was chosen because it is also known that this stereoisomer interacts more favorably with the enzymes[32] and, since most natural amino acids are (*S*) isomers, (*R*) compounds are expected to be less likely to undergo *in vivo* metabolization.

Compounds **3a**−**c** (see Table 1) revealed high inhibitory activities against all of the tested MMPs (nanomolar or subnanomolar), with 1.3−35-fold potency increase when compared to their homologues without the α-(*R*)-isopropyl group (i.e., **1**, **2**). The highest inhibition enhancements were observed on MMP-1 and -8, with a concomitant reduction of the selectivity for MMP-2 relative to these two enzymes. Since for many inhibitors possessing an α-substituent, this group tends to bind into the S_1 cavity of the enzymes, identical behavior was expected for these compounds. The S_1 pocket does not change much between the different MMPs; therefore, modifications in the P_1 substituent of the inhibitors usually do not lead to selectivity enhancement, while lipophilic groups in this position have been reported to give broad-spectrum characteristics to the inhibitors.[34]

The introduction of a 4-sulfamoylphenylethyl (SPE) moiety as the R_2 group (compounds **6a**−**c**) also led to an increase of activity toward all of the tested MMPs, as compared with their homologues containing a carboxylic acid (e.g., **1**, **2**), thus indicating that the SPE group must interact favorably with these enzymes. However, this increase of activity was associated with some decrease of selectivity for the cancer-related enzymes (namely, MMP-2), particularly evident for compound **6c** vs **2**.

The carboxylic analogues (compounds **5b** and **5c**) of hydroxamic inhibitors (**6b** and **6c**) were also tested on some MMPs in order to compare the effect of these functional groups on the activities. The results indicated that these compounds possess moderate activity against the tested enzymes (from 2.2 to 8.2

Table 1. Inhibition Profile of Compounds **3**−**7** and Reference Inhibitors toward the Selected MMPs and CAs

compd	IC_{50} (nM)							K_I (nM)[a]		
	MMP-1	MMP-2	MMP-8	MMP-9	MMP-13	MMP-14	MMP-16	TACE	CA I[b]	CA II[b]	CA IX[c]
3a	235 ± 17	145 ± 16	29.2 ± 3.6	62.0 ± 4.0		353 ± 20					
3b	236 ± 19	24.7 ± 1.3	4.3 ± 0.3	27.7 ± 1.5		306 ± 55					
3c	59 ± 3.5	0.50 ± 0.17	0.40 ± 0.05	0.77 ± 0.03	0.33 ± 0.03	3.9 ± 0.3	1.56 ± 0.19	(2.29 ± 0.36) × 10^3			
4		> 100 × 10^3		> 300 × 10^3		> 100 × 10^3			2339	63	26
5b		(5.12 ± 0.46) × 10^3	(8.20 ± 0.85) × 10^3			(33.9 ± 2.0) × 10^3			116	8.4	3.8
5c		(2.23 ± 0.16) × 10^3	(4.36 ± 0.48) × 10^3			(8.95 ± 0.63) × 10^3			3430	9.3	6.4
6a	532 ± 18	25.3 ± 1.5	59.0 ± 4.0	53.7 ± 2.6		89.8 ± 4.2	44.0 ± 3.2		1765	57	13
6b	542 ± 18	16.2 ± 1.4	35 ± 5	66 ± 6		260 ± 18	186 ± 14		2487	35	13
6c	143 ± 10	1.57 ± 0.27	0.74 ± 0.07	0.51 ± 0.04		2.1 ± 0.2	1.00 ± 0.09		95	65	12
7	7.8 ± 0.7	0.35 ± 0.04	0.29 ± 0.03	0.21 ± 0.04	0.12 ± 0.02	0.32 ± 0.04	0.31 ± 0.04	55.0 ± 5.5	730	4.2	3.3
1	(6.77 ± 0.58) × 10^3	193 ± 20	(1.01 ± 0.04) × 10^3	322 ± 32	213 ± 7	(1.69 ± 0.15) × 10^3	996 ± 24				
2	(1.53 ± 0.06) × 10^3	1.2 ± 0.1	3.7 ± 0.2	3.2 ± 0.2	1.6 ± 0.1	14 ± 1.7	11.4 ± 0.6				
CGS 27023A	56.3 ± 5.8	24.8 ± 4.0	7.7 ± 1.2	5.0 ± 1.3	5.5 ± 1.1	23.2 ± 1.6	6.8 ± 0.8				
AAZ									250	12	25
SPESB									40	5	18

[a] Errors are in the range of 5−10% of the reported value (from three different assays). [b] Human recombinant isozymes, stopped-flow CO_2 hydrase assay method.[28] [c] Catalytic domain of the human, recombinant enzyme, stopped-flow CO_2 hydrase assay method.[28]

μM range). The phenoxybenzenesulfonyl-containing compound (**5c**) was more active than the analogue with a biphenylsulfonyl group, **5b**, as happened with their hydroxamic homologues.

The best results were obtained with compound **7**, which was designed to conjugate the positive effects on MMP inhibition resulting from the insertion of the α-(*R*)-isopropyl group, as in **3c**, with those of the SPE substituent, as in **6c**. Among the series of tested MMPs inhibitors, **7** appears as the most active compound, displaying high inhibitory activity (subnanomolar toward all MMPs, except MMP-1).

The inhibitory activity toward TACE was also evaluated, but only for the most promising compounds, **3c** and **7**. The results showed moderate to high activity (2.3 μM for **3c** and 55 nM for **7**) but still displaying selectivity for the MMPs (e.g., selectivity MMP-2/TACE of 4580 and 157, respectively). This fact may be due to the main differences in the primary and secondary structures of MMPs and TACE, namely, on the S_1' and S_3' sites, where the inhibitors are expected to bind with different affinities.[35,36]

Globally, within these series of new compounds, some potent MMP inhibitors were obtained, with activities in nanomolar to subnanomolar range. Although with reduced selectivity for MMP-2 over some antitargets in cancer therapy (e.g., MMP-8 and -9), in general they presented selectivity for MMP-2 over MMP-1 and TACE.

CA Inhibition. The inhibitory activities of the SPE-containing compounds were evaluated toward a set of physiologically relevant CAs (CA I, II, and IX), and the results are shown in Table 1, together with some others previously reported.[37] As can be seen from this table, all of the tested inhibitors presented high activity against CA II and IX (nanomolar range), and the carboxylic analogues (e.g., **5b**, **c**) were shown to be more active than the corresponding hydroxamates (e.g., **6b**, **c**). In general, all of the compounds demonstrated lower activity toward CA I (high nanomolar to micromolar range) than **AAZ** and the structurally related inhibitor *N*-(4-sulfamoylphenylethyl)-4-sulfamoylbenzamide, **SPESB**[21] (see Figure 1), and they presented similar potency against the CA II isoform.

Concerning the membrane-bound tumor-associated isozyme CA IX, these compounds presented very high inhibitory activity, in most cases higher than **AZZ** and **SPESB** (compound **7** displaying the best result, with a K_I of 3.3 nM). They also demonstrated a selectivity profile that favors the inhibition of CA IX over the ubiquitous isoforms CA I and II (selectivities CA IX/I ranging from 7.9 to 536 and CA IX/II from 1.3 to 5.4), which is a remarkable feature when developing potential antitumor drugs.

Considering the overall inhibition profiles of the compounds reported herein over MMPs and CAs, our studies revealed for some of them high inhibitory potency against both classes of enzymes. In particular, the conjugated introduction of an isopropyl group in the α-position to the hydroxamate and the SPE substituent on the IDA scaffold endowed compound **7** with a subnanomolar activity on most MMPs and a high activity and selectivity toward CA IX and CA II vs CA I. As previously mentioned, based on the multifactorial nature of many disease processes, the capacity of a drug to simultaneously target two pathways associated to the same disease may represent a favorable feature in therapy. Altogether, these results suggest that these bifunctional compounds hold great potential as dual drugs and may provide a more efficient approach to overcome the complex processes of cancer. Further studies will be developed to improve the activity and selectivity of this type

PDB	MMP	GoldScore	GoldScore ZBG	ChemScore	ChemScore ZBG	ASP	ASP ZBG	SMC-GoldScore	SMC-GoldScore ZBG	SMC-ChemScore	SMC-ChemScore ZBG	SMC-ASP	SMC-ASP ZBG
1hfc	1	2.08	0.53	2.38	0.66	2.52	0.60	2.17	0.54	2.31	0.32	2.38	0.18
1bqo	3	4.14	0.94	3.66	0.46	3.17	0.48	4.11	0.93	3.36	0.32	2.01	0.31
1jiz	12	4.31	0.51	1.14	0.41	1.42	0.56	1.37	0.51	1.13	0.25	1.14	0.19
1mnc	8	0.51	0.40	0.47	0.46	0.99	0.42	0.81	0.50	0.53	0.42	0.54	0.16
830c	13	1.81	1.67	0.62	0.56	0.92	0.45	3.16	1.14	0.46	0.24	0.45	0.15
1d5j	3	5.95	0.81	1.31	1.18	1.31	1.18	5.94	0.74	0.77	0.34	0.86	0.31
1d8f	3	9.09	1.62	3.74	1.20	1.66	0.53	5.37	1.08	3.23	0.28	1.56	0.26
1fbl	1	1.14	1.27	1.18	0.59	1.19	0.27	0.92	1.34	1.01	0.45	1.01	0.31
1g05	3	3.23	1.30	0.90	0.64	0.96	0.57	3.16	1.14	0.80	0.26	0.87	0.33
1q3a	10	1.73	1.83	1.44	1.25	1.17	1.61	1.52	1.52	1.09	0.52	1.03	0.49
1rm8	16	2.96	0.44	2.20	0.43	1.84	0.41	2.14	0.36	1.10	0.24	1.83	0.23
1mmq	7	0.62	0.50	0.82	0.92	0.30	0.40	0.64	0.45	0.53	0.49	0.47	0.15
966c	1	1.05	0.26	0.82	0.58	0.99	0.76	1.11	0.27	0.81	0.27	0.72	0.27
2tcl	1	0.88	0.68	1.88	0.49	1.40	0.58	1.48	0.53	1.70	0.26	1.41	0.15
1biw	3	1.71	0.56	1.07	0.57	1.04	0.26	1.66	0.41	1.06	0.26	1.03	0.27
1d8m	3	3.52	1.82	1.41	0.76	3.50	1.89	1.63	0.64	1.43	0.46	1.93	0.27
1d7x	3	2.02	0.64	2.04	0.99	3.14	1.21	1.81	0.53	1.72	0.22	1.51	0.49
2d1o	3	4.49	1.18	3.59	1.01	3.22	0.93	1.38	0.41	1.28	0.24	1.78	0.23
1g49	3	3.58	1.97	1.81	0.84	2.51	1.58	3.50	1.43	1.97	0.65	1.51	0.78
1b3d	3	0.93	0.74	1.58	0.60	1.10	0.35	0.60	0.36	0.94	0.30	1.10	0.25
1a86	8	2.17	0.86	1.96	0.55	1.60	0.60	1.96	0.73	1.71	0.33	1.73	0.31
1mmb	8	1.00	0.70	2.15	0.42	1.08	0.33	1.09	0.51	1.20	0.33	1.02	0.26
1a85	8	1.85	0.72	1.74	0.46	1.94	0.60	1.89	0.64	2.05	0.49	2.17	0.39
1jap	8	0.73	0.50	0.50	0.45	1.30	0.68	0.79	0.46	0.89	0.49	1.21	0.37
1jaq	8	1.56	0.47	4.95	1.63	2.09	0.50	1.53	0.38	0.82	0.15	1.02	0.18
1kbc	8	0.88	0.58	1.28	0.93	1.29	0.48	0.96	0.37	1.40	0.78	1.22	0.21
1y93	12	2.46	2.46	2.51	2.51	2.22	2.22	0.24	0.24	0.36	0.36	0.40	0.40
1rmz	12	2.64	1.37	0.71	0.44	0.86	0.44	2.82	0.76	0.78	0.39	0.77	0.39
1cxv	13	2.56	1.36	0.85	0.74	0.75	0.82	0.97	0.35	0.93	0.54	1.14	0.56
2d1n	13	1.13	1.29	1.31	0.81	1.99	1.75	2.02	1.45	2.72	0.35	2.09	0.51
average		**2.42**	**1.00**	**1.73**	**0.78**	**1.65**	**0.78**	**1.96**	**0.69**	**1.34**	**0.37**	**1.26**	**0.31**

RMSD (Å):
- ≤ 0.7
- ≤1.0
- ≤ 2.0
- ≤ 3.0
- > 3

Figure 3. rmsd evaluation of the binding disposition of the ligands (in Å) docked into the respective MMPs, for the entire molecule and for the ZBG, using different procedures (neutral hydroxamate of the ligand and anionic Glu^- in the protein).

of compound toward those of MMPs and CAs really implicated in cancer, such as MMP-2 and CA IX.

Molecular Modeling. In order to understand the interactions of this type of inhibitor with the MMP active site, modeling studies were carried out by docking compound **7** into all of the assayed MMP structures. The best approach to be used in this modeling study was based on a first evaluation of several inhibitor−enzyme systems reported in the literature.

Docking of Compound 7 with MMPs. In order to test the reliability of our docking calculations, we first tested the ability of GOLD software[38] for that purpose by doing the docking of 30 MMP inhibitors into their crystal structures. These structures represented all of the available crystal structures[39] of MMP complexed with inhibitors characterized by the presence of the hydroxamic acid as ZBG. In the complex model, the ligand and the enzyme were worked up (see Experimental Section), and then the respective ligands were submitted to docking calculations into their corresponding protein using the program Gold 3.0. The three scoring functions available with that software (i.e., GoldScore, ChemScore, and ASP), either without any constraint or with a scaffold match constraint (SMC), were used. In the SMC method, the hydroxamic moiety of the original ligand was used as a scaffold to match the tested ligands. The SMC was applied because it has recently shown good results for the docking of MMP inhibitors.[40] The first-ranked structures obtained from these calculations were then compared with the original complex, and the root-mean-square deviations (rmsd) were determined for the heavy atoms of the ZBG (the molecular segment $C_\alpha CONO$) and for the whole ligand molecules (see Figure 3).

The results show that, from the three scoring functions supplied by Gold, in general ASP gives the best average prediction of the corresponding binding conformation of the inhibitors, in terms of both its coordination to the zinc and the overall accommodation of the ligand in the catalytic site. For the docking with simple methods (without any constraint), ASP predicted the binding with an average rmsd of 0.78 and 1.65 Å for the ZBG and the whole ligand, respectively. ChemScore also gave acceptable results but with slightly worse average rmsd (0.78 Å for the ZBG and 1.73 Å for the ligands).

When the SMC was applied to the hydroxamate scaffold, the prediction ability was improved for all of the scoring functions, both for the ZBG and for the whole molecule. As displayed in Figure 3, the ASP function still demonstrated the best ability on predicting both the position of the ZBG (average rmsd of 0.31 Å) and the entire ligand molecule (rmsd of 1.26 Å).

From this analysis we concluded that the most reliable method to perform docking studies involving MMPs/hydroxamic inhibitors with the Gold program is the ASP fitness function with a hydroxamate SMC.

Compound **7** was then docked into all of the analyzed MMPs by means of Gold software, applying the SMC-ASP procedure discussed above. Figure 4 displays the results for MMP-2. The hydroxamate group coordinates the zinc ion through the two oxygen atoms and also establishes two H-bonds with the enzyme, through the carboxylate of Glu404 and the carbonyl of Ala192 (numeration according to UniProtKB/Swiss-Prot database, entry P08253).[30] The sulfonyl group of the SO_2PhOPh moiety forms H-bonds with the backbone of Leu191 and Ala192, and the phenoxybenzene aromatic rings are accommodated in the S_1' cavity, forming van der Waals contact with its hydrophobic residues, in particular with Leu399, Val400, and Leu420. Regarding the isopropyl group, as expected from the previous SAR analysis,[32] it is placed in the S_1 zone of the enzyme, which is mostly hydrophobic, and allows lipophilic interactions with Leu190. This may account for the higher potency evidenced by this inhibitor (**7**) and others, relative to the compounds without this α-substituent group (compounds **1** and **2**).

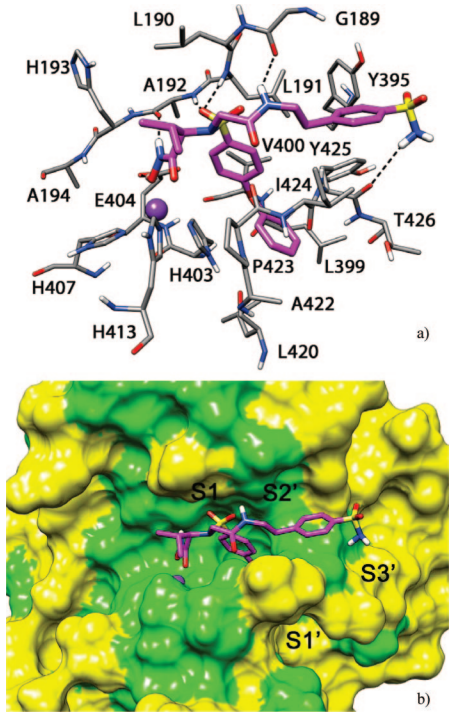


Figure 4. (a) Compound **7** docked into the MMP-2 catalytic site with H-bonds displayed (slashed lines). (b) Surface of the binding site and its subsites, green representing conserved and yellow nonconserved regions, among MMP-2, -8, -9, -13, -14, and -16.

Concerning the SPE moiety, it seems to be well accommodated in the catalytic site and is able to establish several interesting binding interactions with the enzyme. The NH group of the amide connecting the SPE moiety to the IDA backbone is able to form a H-bond with the backbone carbonyl group of Gly189. The benzene ring of the SPE extends toward the $S_2'-S_3'$ region, over the phenol rings of Tyr395 and Tyr425, establishing a close lipophilic interaction with these groups, and finally the nitrogen of the sulfonamide group forms a H-bond with the backbone carbonyl of Tyr425.

Compound **7** shows a high inhibitory activity with almost all of the MMPs tested. The docking studies of this compound into MMP-8, -9, -13,-14, and -16 suggested that the binding interactions shown for the MMP-2 are also maintained in these MMPs. As shown in the Supporting Information (Figures S1−S5), compound **7** presented all of the main interactions reported for MMP-2 also in MMP-8, -9, and -13, in particular the binding of the SPE moiety in the $S_2'-S_3'$ region and displaying the two H-bonds with the backbone of Gly189 and Tyr425 (MMP-2 sequence number). In MMP-14 and -16, the ligand showed the same type of accommodation observed for the other MMPs; however, instead of the H-bond with the backbone of Tyr425, the sulfonamido group forms a H-bond with an asparagine residue that is not conserved and is substituted by a tyrosine in the other analyzed MMPs (Tyr395 in MMP-2).

Analysis of the primary sequence of the binding site of the analyzed MMPs reveals that, as shown in Figure 4b, the region in which inhibitor **7** interacts is highly conserved, thus explaining the very close inhibition activities shown by this compound with all of these MMPs.

The activity of **7** against MMP-1 is almost 30-fold worse than for the other MMPs. As shown in Figure 5, MMP-1 is characterized by a short S_1' pocket, and the ligand is not able to display favorable binding interactions since the arylsulfonamide group is too bulky to fit in the S_1' cavity, thus explaining the decreased activity for this MMP subtype relative to the others studied.

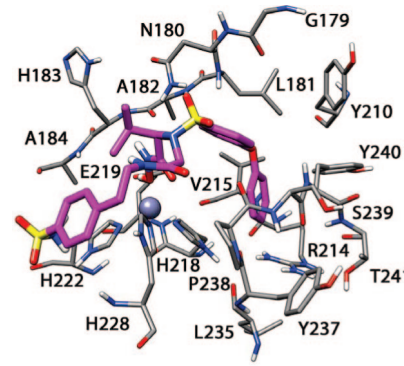


Figure 5. Compound **7** docked into the MMP-1 catalytic site.

Conclusions

A set of new hydroxamic compounds based on the iminodiacetyl scaffold with a variety of substituent groups was developed and evaluated, aimed at obtaining potent and selective inhibitors for some target matrix metalloproteinases (MMPs) involved in cancer, but also providing inhibitory ability against carbonic anhydrase isozymes (CAs), which are also involved in this pathology. Among the different types of P_1' substituents, the *N*-sulfonylaryl groups appeared to be the most relevant ones to achieve the best activity and selectivity results on MMP inhibition. Modifications at the α-position to the hydroxamate by insertion of an isopropyl group, to improve the bioavailability of inhibitors, in most cases led to significant enhancement of inhibitory activity toward MMPs, although with some selectivity reduction. A further substitution was performed at the remaining OH group of the IDA, with the 4-sulfamoylphenylethyl group (SPE), known to display inhibitory activity toward CAs. This feature led to enhancement of the inhibitory activities both on MMPs and on CAs (from nanomolar to subnanomolar range), thus suggesting that these types of bifunctional compounds can be potential double targeting drugs for use in the therapy of some malignant tumors.

A docking study of the most interesting compound allowed the understanding of its interaction with the various MMPs, thus suggesting the basis of its activity profile.

In conclusion, the present paper demonstrates that the introduction of the 4-sulfamoylphenylethyl group in the IDA scaffold maintains the inhibitory potency on MMPs and also provides a good activity against some CAs. This finding encourages further studies toward the identification of new selective dual inhibitors for those enzymes implicated in cancer, such as MMP-2 and CA IX.

Experimental Section

(A) Chemistry. General Methods and Materials. Analytical grade reagents were purchased from Aldrich, Sigma, and Fluka and were used as supplied. Solvents were dried according to standard methods.[41] The chemical reactions were monitored by TLC using alumina plates coated with silica gel 60 F_{254} (Merck). Column flash chromatography separations were performed on silica gel Merck 230−400 mesh ASTM. Melting points were measured with a Leica Galen III hot stage apparatus and are uncorrected. IR spectra were recorded on a Bio-Rad Merlin, FTS 3000 MX. The ^{1}H NMR spectra were recorded on a Varian Unity 300 spectrometer at 25 °C. Chemical shifts (δ) are reported in parts per million (ppm) from standard internal references, namely, tetramethylsilane (TMS) for

organic solvents and sodium 3-(trimethylsilyl)-[2,2,3,3-d_4]propionate (DSS) for D_2O solutions. The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, dd = double doublet, 2d = two overlapping doublets, and bs = broad singlet. ^{13}C NMR spectra were measured for some representative compounds and were recorded with a Bruker Avance II 400 spectrometer at 25 °C, and they are presented in Supporting Information. Mass spectra (FAB) were performed in a VG TRIO-2000 GC/MS instrument and ESI spectra on a Quattro LC mass spectrometer (Micromass, Manchester, U.K.). The high-resolution mass spectra (HRMS) were obtained with a high-resolution Fourier transform ion cyclotron resonance (FTICR) instrument, Finnigan FT/MS 2001-DT, equipped with a 3.0 T superconducting magnet, by electron impact (EI), typically with 15 eV electron beam energies, 5-μm emission currents, and 150 °C sample temperatures. Elemental analyses were performed on a Fisons EA1108 CHNF/O instrument.

General Method for Preparation of *N*-(Arylsulfonyl)-D-valine Compounds (8a−c). To a solution of D-valine (0.50 g, 4.27 mmol) and triethylamine, TEA (0.89 mL, 6.40 mmol), in 1:1 water/dioxane (100 mL) was added the appropriate arylsulfonyl chloride (4.70 mmol), and the mixture was stirred for 12 h. The resulting solution was concentrated, and the residue was dissolved in 5% NaOH (50 mL) and washed with CH_2Cl_2 (4 × 50 mL); the aqueous phase was acidified to pH 1−2 with concentrated HCl, and this solution was extracted with CH_2Cl_2 (3 × 50 mL). After the organic phase was dried over Na_2SO_4 and solvent evaporated under vacuum, the pure products were obtained as white solids.

N*-(4-Phenoxybenzenesulfonyl)-D-valine (8c).** The 4-phenoxybenzenesulfonyl chloride used herein was prepared according to the literature.[27] Final recrystallization with *n*-hexane afforded the product as a white solid: yield 67%; mp 97−99 °C; ^1H NMR ($CDCl_3$) δ 7.78 (d, J = 9.3 Hz, 2H, ArH***), 7.41 (t, J = 8.0 Hz, 2H, Ar***H***), 7.22 (t, J = 7.6 Hz, 1H, Ar***H***), 7.06−6.99 (2d, 4H, Ar***H***), 5.07 (d, J = 9.6 Hz, 1H, N***H***), 3.84−3.79 (2d, 1H, NC***H***(*i*Pr)), 2.16−2.09 (m, 1H, C***H***$(CH_3)_2$), 0.99 (d, J = 6.6 Hz, 3H, CHC***H***$_3$), 0.89 (d, J = 6.6 Hz, 3H, CHC***H***$_3$); *m*/*z* (FAB) 350 $(M + H)^+$, 372 $(M + Na)^+$.

***N*-(4-Methoxybenzenesulfonyl)-D-valine (8a) and *N*-(4-Biphenylsulfonyl)-D-valine (8b).** See characterization in Supporting Information.

General Method for Preparation of *N*-(Arylsulfonyl)-*O*-benzyl-D-valine Compounds (9a−c). To a solution of the respective intermediate **8a**, **8b**, or **8c** (2.60 mmol) and TEA (0.54 mL, 3.90 mmol) in acetonitrile (30 mL) was added benzyl bromide (0.34 mL, 2.86 mmol), and the mixture was stirred overnight under reflux. The solvent was evaporated, and the residue was dissolved in ethyl acetate (30 mL) and then washed with 0.1 M HCl (3 × 30 mL); after addition of *n*-hexane to the organic phase (10 mL), the solution was washed with 5% NaOH (3 × 40 mL), the organic phase was dried over anhydrous Na_2SO_4, and after evaporation of the solvent and drying of the solid residue in vacuum, the pure products were obtained as solids.

N*-(4-Phenoxybenzenesulfonyl)-*O*-benzyl-D-valine (9c).** Yellow solid; 81% yield; mp 57−59 °C; ^1H NMR ($CDCl_3$) δ 7.75 (d, J = 9.0 Hz, 2H, ArH***), 7.40 (t, J = 8.1 Hz, 2H, Ar***H***), 7.38−7.20 (m, 6H, Ar***H***), 7.03 (d, J = 7.8 Hz, 2H, Ar***H***), 6.96 (d, J = 8.7 Hz, 2H, Ar***H***), 5.09 (d, J = 9.6 Hz, 1H, N***H***), 4.92 (s, 2H, C***H***$_2$Ph), 3.81−3.76 (2d, 1H, NC***H***(*i*Pr)), 2.12−2.05 (m, 1H, C***H***$(CH_3)_2$), 0.96 (d, J = 6.9 Hz, 3H, CHC***H***$_3$), 0.84 (d, J = 6.9 Hz, 3H, CHC***H***$_3$); *m*/*z* (FAB) 462 $(M + Na)^+$, 440 $(M + H)^+$.

***N*-(4-Methoxybenzenesulfonyl)-*O*-benzyl-D-valine (9a) and *N*-(4-Biphenylsulfonyl)-*O*-benzyl-D-valine (9b).** See characterization in Supporting Information.

General Method for Preparation of *N*-(Arylsulfonyl)-*N*-*tert*-butoxycarbonylmethyl-*O*-benzyl-D-valine Compounds (10a−c). To a suspension of the derivative **9a**, **9b**, or **9c** (2.07 mmol), K_2CO_3 (2.86 g, 20.7 mmol), and KI (0.069 g, 0.41 mmol) in dry DMF (20 mL) was added *tert*-butyl bromoacetate (0.61 mL, 4.14 mmol), and the mixture was stirred at room temperature for 3 days. The mixture was then taken into 1:1 *n*-hexane/ethyl acetate (40 mL), and that solution was extracted with water (2 × 40 mL), 5% NaOH (40 mL), and brine (40 mL). After drying over anhydrous Na_2SO_4 and evaporation of the solvent, the residue was dissolved in methanol (25 mL) and refluxed with active charcoal (0.5 g) for 3 h; filtration and evaporation of the solvent gave a yellow oil, which had no further purification.

N*-(4-Phenoxybenzenesulfonyl)-*N*-*tert*-butoxycarbonylmethyl-*O*-benzyl-D-valine (10c).** ^1H NMR ($CDCl_3$) δ 7.83 (d, J = 8.4 Hz, 2H, ArH***), 7.40 (t, J = 7.8 Hz, 2H, Ar***H***), 7.35−7.19 (m, 6H, Ar***H***), 7.02 (d, J = 8.1 Hz, 2H, Ar***H***), 6.90 (d, J = 8.7 Hz, 2H, Ar***H***), 5.00, 4.85 (dd, J = 12.3, 12.0 Hz, 2H, C***H***$_2$Ph), 4.24, 3.91 (dd, J = 18.3, 18.6 Hz, 2H, C***H***$_2CO_2t$Bu), 4.02 (d, J = 10.5, 1H, NC***H***(*i*Pr)), 2.08−2.01 (m, 1H, C***H***$(CH_3)_2$), 1.44 (s, 9H, *t*Bu***H***), 1.00 (d, J = 6.6 Hz, 3H, CHC***H***$_3$), 0.87 (d, J = 6.6 Hz, 3H, CHC***H***$_3$); *m*/*z* (FAB) 498 $(M - t\text{Bu} + 2H)^+$, 576 $(M + Na)^+$, 520 $(M - t\text{Bu} + H + Na)^+$.

***N*-(4-Methoxybenzenesulfonyl)-*N*-*tert*-butoxycarbonylmethyl-*O*-benzyl-D-valine (10a) and *N*-(4-Biphenylsulfonyl)-*N*-*tert*-butoxycarbonylmethyl-*O*-benzyl-D-valine (10b).** See characterization in Supporting Information.

Method for Preparation of *N*-(Arylsulfonyl)-*N*-*tert*-butoxycarbonylmethyl-D-valine Compounds (11a−c). The general method for benzyl deprotection by catalytic hydrogenolysis was followed. To a solution of the *O*-benzyl derivative **10a**, **10b**, or **10c** (1.00 g) in methanol (15 mL) was added 10% Pd/C (0.30 g), and the suspension was stirred under H_2 (4 bar) at room temperature for 3 h. After filtration and evaporation of the solvent, the residue was taken into 2 M NaOH (50 mL) and washed with *n*-hexane (2 × 50 mL), and then the aqueous solution was extracted with ethyl acetate (3 × 50 mL). The organic phase was dried over anhydrous Na_2SO_4, and evaporation of the solvent afforded the pure product.

N*-(4-Phenoxybenzenesulfonyl)-*N*-*tert*-butoxycarbonylmethyl-D-valine (11c).** Pale yellow solid; 79% overall yield for **10c** and **11c**; mp 97−99 °C; ^1H NMR ($CDCl_3$) δ 8.01 (d, J = 8.7 Hz, 2H, ArH***), 7.36 (t, J = 7.8 Hz, 2H, Ar***H***), 7.17 (t, J = 7.4 Hz, 1H, Ar***H***), 6.96 (d, J = 7.8 Hz, 2H, Ar***H***), 6.79 (d, J = 8.4 Hz, 2H, Ar***H***), 4.24, 4.10 (dd, J = 18.0, 18.3 Hz, 2H, C***H***$_2CO_2t$Bu), 3.62 (d, J = 10.2, 1H, NC***H***(*i*Pr)), 1.98−1.91 (m, 1H, C***H***$(CH_3)_2$), 1.43 (s, 9H, *t*Bu***H***), 0.86 (d, J = 6.0 Hz, 3H, CHC***H***$_3$), 0.25 (d, J = 5.7 Hz, 3H, CHC***H***$_3$); *m*/*z* (FAB) 452 $(M - t\text{Bu} + 2Na)^+$, 430 $(M - t\text{Bu} + H + Na)^+$, 486 $(M + Na)^+$.

***N*-(4-Methoxybenzenesulfonyl)-*N*-*tert*-butoxycarbonylmethyl-D-valine (11a) and *N*-(4-Biphenylsulfonyl)-*N*-*tert*-butoxycarbonylmethyl-D-valine (11b).** See characterization in Supporting Information.

Method for Preparation of *N*-(Arylsulfonyl)-*N*-*tert*-butoxycarbonylmethyl-*O*-benzyl-D-valinehydroxamic Acids (12a−c). The general method for carboxylic acid/hydroxylamine coupling was followed. To an ice-cooled solution of the intermediate **11a**, **11b**, or **11c** (2.06 mmol) in dry THF (25 mL) was added ethylchloroformate (ECF, 0.23 mL, 2.37 mmol) and *N*-methylmorpholine (NMM, 0.26 mL, 2.37 mmol); the mixture was stirred at 0 °C for 45 min, and the solids were subsequently filtered off. Meanwhile, a solution of *O*-benzylhydroxylamine hydrochloride (0.411 g, 2.58 mmol) in dry methanol was neutralized with KOH (0.144 g, 2.58 mmol), stirring at 0 °C for 30 min, followed with filtration. The first solution was then dropwise added to the hydroxylamine solution, and the mixture was stirred at 0 °C for 4 h under N_2. The reaction mixture was concentrated under vacuum and the residue taken into ethyl ether (30 mL), which was then washed with 1 M HCl (2 × 30 mL); the two layers were separated, and to the organic phase was added petroleum ether (30 mL), and the mixture was further washed with 5% NaOH (3 × 50 mL). After drying the final organic phase over Na_2SO_4 and solvent removal, the pure products were obtained.

N*-(4-Phenoxybenzenesulfonyl)-*N*-*tert*-butoxycarbonylmethyl-*O*-benzyl-D-valinehydroxamic Acid (12c).** Pale yellow solid; 77% yield; mp 45−48 °C; ^1H NMR ($CDCl_3$) δ 8.97 (s, 1H, CONH***OBn), 7.84 (d, J = 9.3 Hz, 2H, Ar***H***), 7.46−7.36 (m, 7H, Ar***H***), 7.22 (t, J = 7.3 Hz, 1H, Ar***H***), 7.05−7.01 (2d, 4H, Ar***H***), 4.92 (s, 2H, C***H***$_2$Ph), 4.14, 3.90 (dd, J = 17.7, 18.3 Hz, 2H, C***H***$_2CO_2t$Bu), 3.30

(d, J = 10.5, 1H, NC***H***(*i*Pr)), 2.15−2.07 (m, 1H, C***H***$(CH_3)_2$), 1.47 (s, 9H, *t*Bu***H***), 0.79 (d, J = 6.9 Hz, 3H, CHC***H***$_3$), 0.49 (d, J = 6.3 Hz, 3H, CHC***H***$_3$); *m*/*z* (FAB) 513 (M − *t*Bu + 2H)$^+$, 591 (M + Na)$^+$, 569 (M + H)$^+$.

***N*-(4-Methoxybenzenesulfonyl)-*N*-*tert*-butoxycarbonylmethyl-*O*-benzyl-D-valinehydroxamic Acid (12a) and *N*-(4-Biphenylsulfonyl)-*N*-*tert*-butoxycarbonylmethyl-*O*-benzyl-D-valinehydroxamic Acid (12b).** See characterization in Supporting Information.

General Method for Preparation of *N*-(Arylsulfonyl)-*N*-hydroxycarbonylmethyl-*O*-benzyl-D-valinehydroxamic Acids (13a−c). To a solution of compound **12a**, **12b**, or **12c** (1.40 mmol) in dichloromethane (5 mL) was added trifluoroacetic acid (TFA, 3.3 mL), and the mixture was stirred at room temperature for 2 h. The solution was concentrated in vacuum, the residue was taken into 5% NaOH (40 mL), and this solution was washed with ethyl ether (2 × 40 mL); the pH of the aqueous phase was lowered to 6 with 2 M HCl, and it was extracted with CH_2Cl_2 (3 × 20 mL). The total organic phase was dried over Na_2SO_4, and the solvent was evaporated in vacuum, affording the pure products as white solids.

N*-(4-Phenoxybenzenesulfonyl)-*N*-hydroxycarbonylmethyl-*O*-benzyl-D-valinehydroxamic Acid (13c).** Herein, the extractions at pH 6 were carried out with ethyl ether instead of CH_2Cl_2, after which the organic phase was washed several times with 1% Na_2HPO_4 solution. Yield 62%; mp 69−70 °C; ^{1}H NMR ($CDCl_3$) δ 8.80 (s, 1H, CONH***OBn), 7.80 (d, J = 8.7 Hz, 2H, Ar***H***), 7.43−7.38 (m, 7H, Ar***H***), 7.23 (t, J = 7.6 Hz, 1H, Ar***H***), 7.05−7.01 (2d, 4H, Ar***H***), 4.89 (s, 2H, C***H***$_2$Ph), 4.29, 4.01 (dd, J = 18.3, Hz, 2H, C***H***$_2$CO$_2$H), 3.36 (d, J = 10.5, 1H, NC***H***(*i*Pr)), 2.17−2.06 (m, 1H, C***H***$(CH_3)_2$), 0.79 (d, J = 6.6 Hz, 3H, CHC***H***$_3$), 0.47 (d, J = 6.0 Hz, 3H, CHC***H***$_3$); *m*/*z* (FAB) 535 (M + Na)$^+$, 513 (M + H)$^+$.

***N*-(4-Methoxybenzenesulfonyl)-*N*-hydroxycarbonylmethyl-*O*-benzyl-D-valinehydroxamic Acid (13a) and *N*-(4-Biphenylsulfonyl)-*N*-hydroxycarbonylmethyl-*O*-benzyl-D-valinehydroxamic Acid (13b).** See characterization in Supporting Information.

General Method for Preparation of *N*-(Arylsulfonyl)-*N*-hydroxycarbonylmethyl-D-valinehydroxamic Acids (3a−c). Catalytic hydrogenolysis of intermediate **13a**, **13b**, or **13c** was performed as described for **11a**−**c**. Final recrystallization afforded the pure products as white solids.

N*-(4-Methoxybenzenesulfonyl)-*N*-hydroxycarbonylmethyl-D-valinehydroxamic Acid (3a).** Recrystallized from CH_2Cl_2/*n*-hexane; 98% yield; mp 80−82 °C; ^{1}H NMR (D_2O, pD ca. 8) δ 7.83 (d, J = 8.4 Hz, 2H, ArH***), 7.11 (d, J = 9.34 Hz, 2H, Ar***H***), 4.28, 3.81 (dd, J = 18.0, 18.3 Hz, 2H, C***H***$_2$CO$_2$*t*Bu), 3.89 (s, 3H, OC***H***$_3$), 3.44 (d, J = 10.5 Hz, 1H, NC***H***(*i*Pr)), 1.97−1.89 (m, 1H, C***H***$(CH_3)_2$), 0.90 (d, J = 6.0 Hz, 3H, CHC***H***$_3$), 0.76 (d, J = 6.6 Hz, 3H, CHC***H***$_3$); *m*/*z* (FAB) 361 (M + H)$^+$, 383 (M + Na)$^+$. Anal. ($C_{14}H_{20}N_2O_7S$) C, N, S. H: calcd, 5.70; found, 5.11.

N*-(4-Biphenylsulfonyl)-*N*-hydroxycarbonylmethyl-D-valinehydroxamic Acid (3b).** Recrystallized from acetonitrile/ethyl ether; 96% yield; mp 100−102 °C; ^{1}H NMR (CD_3OD) δ 8.00 (d, J = 8.4 Hz, 2H, ArH***), 7.81 (d, J = 8.4 Hz, 2H, Ar***H***), 7.69 (d, J = 7.8 Hz, 2H, Ar***H***), 7.51−7.41 (m, 3H, Ar***H***), 4.49, 4.02 (dd, J = 18.3 Hz, 2H, C***H***$_2$CO$_2$H), 3.64 (d, J = 10.5, 1H, NC***H***(*i*Pr)), 2.09−1.98 (m, 1H, C***H***$(CH_3)_2$), 0.88 (d, J = 6.6 Hz, 3H, CHC***H***$_3$), 0.83 (d, J = 6.6 Hz, 3H, CHC***H***$_3$); *m*/*z* (FAB) 429 (M + Na)$^+$, 407 (M + H)$^+$. Anal. ($C_{19}H_{22}N_2O_6S\cdot 2CH_2Cl_2$) C, H, N. S: calcd, 5.57; found, 5.08.

N*-(4-Phenoxybenzenesulfonyl)-*N*-hydroxycarbonylmethyl-D-valinehydroxamic Acid (3c).** Recrystallized from ethyl ether/petroleum ether; 97% yield; mp 80−82 °C; ^{1}H NMR ($CDCl_3$) δ 9.24 (bs, 1H, CONH***OH), 7.78 (d, J = 8.7 Hz, 2H, Ar***H***), 7.42 (t, J = 7.6 Hz, 2H, Ar***H***), 7.23 (t, J = 7.2 Hz, 1H, Ar***H***), 7.07−7.02 (2d, 4H, Ar***H***), 4.37, 3.83 (dd, J = 18.9 Hz, 2H, C***H***$_2$CO$_2$H), 3.55 (d, J = 10.2, 1H, NC***H***(*i*Pr)), 2.25−2.16 (m, 1H, C***H***$(CH_3)_2$), 0.82 (d, J = 6.0 Hz, 3H, CHC***H***$_3$), 0.37 (d, J = 6.9 Hz, 3H, CHC***H***$_3$); *m*/*z* (FAB) 445 (M + Na)$^+$, 423 (M + H)$^+$. Anal. ($C_{19}H_{22}N_2O_7S\cdot 0.3Et_2O$) C, N. H: calcd, 5.65; found, 5.21. S: calcd, 7.23; found, 6.70.

N*-Benzyl-*N*-{[2-(4-sulfamoylphenyl)ethylcarbamoyl]methyl}-aminoacetic Acid (15).** A suspension of *N*-benzyliminodiacetic anhydride hydrochloride, **14** (1 g, 4.14 mmol), prepared as previously reported,[27] in dry acetonitrile (50 mL) was neutralized with dry pyridine (0.34 mL, 4.14 mL) and left stirring for 30 min. The resulting solution was added dropwise to an ice-cooled solution of (4-sulfamoylphenyl)ethylamine (SPEA, 0.691 g, 3.45 mmol) in dry acetonitrile (50 mL); the mixture reacted for 4 h, and it was subsequently filtered and evaporated. The crude material was washed with hot ethanol, affording the pure product as a white solid (0.529 g, 38% yield): mp 181−182 °C; ^{1}H NMR (D_2O, pD ca. 3) δ 7.73 (d, J = 8.7 Hz, 2H, ArH***), 7.42−7.28 (m, 7H, Ar***H***), 4.28 (s, 2H, C***H***$_2$Ph), 4.04 (s, 2H, C***H***$_2$CONHCH$_2$), 3.94 (s, 2H, C***H***$_2$CO$_2$H), 3.36 (t, J = 6.4 Hz, 2H, NHC***H***$_2$CH$_2$PhSO$_2$NH$_2$), 2.75 (t, J = 6.4 Hz, 2H, NHCH$_2$C***H***$_2$PhSO$_2$NH$_2$); *m*/*z* (FAB) 406 (M + H)$^+$.

N*-Benzyl-*N*-{[2-(4-sulfamoylphenyl)ethylcarbamoyl]methyl}-aminoacetohydroxamic Acid (4).** The general method for carboxylic acid/hydroxylamine coupling, as described for compounds **12a**−**c**, was followed with **15**, but using hydroxylamine hydrochloride instead. After filtration and evaporation of the reaction mixture, the residue was dissolved in water (20 mL), and extractions were performed with ethyl acetate (6 × 20 mL); the organic phase was then dried over anhydrous Na_2SO_4 and evaporated. Further recrystallization of the crude with CH_2Cl_2/ethyl ether afforded the pure product as a white solid (0.319 g, 77% yield): mp 70−71 °C; ^{1}H NMR (D_2O, pD ca. 3) δ 7.78 (d, J = 7.2 Hz, 2H, ArH***), 7.44−7.32 (m, 7H, Ar***H***), 4.29 (s, 2H, C***H***$_2$Ph), 3.98 (s, 2H, C***H***$_2$CONHCH$_2$), 3.90 (s, 2H, C***H***$_2$CONHOH), 3.47 (t, J = 6.0 Hz, 2H, NHC***H***$_2$CH$_2$PhSO$_2$NH$_2$), 2.84 (t, J = 6.0 Hz, 2H, NHCH$_2$C***H***$_2$PhSO$_2$NH$_2$); *m*/*z* (FAB) 421 (M + H)$^+$. Anal. ($C_{19}H_{24}N_4O_5S$) C, H, N. S: calcd, 7.46; found, 6.98.

General Method for Preparation of *N*-(Arylsulfonyl)-*N*-{[2-(4-sulfamoylphenyl)ethylcarbamoyl]methyl}aminoacetic Acids (5a−c). To an ice-cooled solution of the appropriate *N*-(arylsulfonyl)iminodiacetic acid, **16a**, **16b**, or **16c**, prepared according to the literature[27] (0.73 mmol), in dry THF (10 mL) were simultaneously and slowly added two THF solutions (10 mL each) of NMM (0.085 mL, 0.77 mmol) and ECF (0.073 mL, 0.77 mmol). This mixture was stirred for 45 min, and then a solution of SPEA (0.146 g, 0.73 mmol) in dry THF (10 mL) was added; the final solution was left stirring at 0 °C for 4 h. The solids were filtered off, the solution was concentrated under vacuum, and the residue was dissolved in a 5% $NaHCO_3$ solution (25 mL), which was then washed with CH_2Cl_2 (3 × 25 mL). The resulting aqueous phase was acidified (pH ca. 1−2) with 2 M HCl and then extracted with ethyl acetate (3 × 25 mL), and the combined organic phases were dried over anhydrous Na_2SO_4 and evaporated. After evaporation of the solvent, recrystallization of the remaining residue with ethanol afforded the pure products as white solids.

N*-(4-Biphenylsulfonyl)-*N*-{[2-(4-sulfamoylphenyl)ethylcarbamoyl]-methyl}aminoacetic Acid (5b).** Yield 34%; mp 127−129 °C; ^{1}H NMR (D_2O, pD ca. 8) δ 7.94−7.87 (2d, 4H, ArH***), 7.76−7.69 (2d, 4H, Ar***H***), 7.61−7.49 (m, 3H, Ar***H***), 7.29 (d, J = 7.8 Hz, 2H, Ar***H***), 3.91 (s, 2H, C***H***$_2$CONHCH$_2$), 3.87 (s, 2H, C***H***$_2$CO$_2$H), 3.39 (t, J = 6.6 Hz, 2H, NHC***H***$_2$CH$_2$PhSO$_2$NH$_2$), 2.79 (s, J = 6.6 Hz, 2H, NHCH$_2$C***H***$_2$PhSO$_2$NH$_2$); *m*/*z* (FAB) 532 (M + H)$^+$, 554 (M + Na)$^+$.

N*-(4-Phenoxybenzenesulfonyl)-*N*-{[2-(4-sulfamoylphenyl)ethylcarbamoyl]methyl}aminoacetic Acid (5c).** Yield 46%; mp 119−121 °C; ^{1}H NMR (D_2O, pD ca. 9) δ 7.78 (d, J = 7.8 Hz, 2H, ArH***), 7.72 (d, J = 7.8 Hz, 2H, Ar***H***), 7.45 (t, 2H, J = 8.0 Hz, Ar***H***), 7.34−7.27 (m, 3H, Ar***H***), 7.12 (d, J = 7.8 Hz, 4H, Ar***H***), 3.83 (s, 2H, C***H***$_2$CONHCH$_2$), 3.80 (s, 2H, C***H***$_2$CO$_2$H), 3.40 (t, J = 6.6 Hz, 2H, NHC***H***$_2$CH$_2$PhSO$_2$NH$_2$), 3.40 (t, J = 6.8 Hz, 2H, NHCH$_2$C***H***$_2$PhSO$_2$NH$_2$); *m*/*z* (FAB) 570 (M + Na)$^+$, 548 (M + H)$^+$.

***N*-(4-Methoxybenzenesulfonyl)-*N*-{[2-(4-sulfamoylphenyl)ethylcarbamoyl]methyl}aminoacetic Acid (5a).** See characterization in Supporting Information.

**General Method for Preparation of *N*-(Arylsulfonyl)-*N*-{[2-(4-sulfamoylphenyl)ethylcarbamoyl]methyl}aminoacetohydroxamic Ac-

ids (6a−c). The general method for carboxylic acid/hydroxylamine coupling, as for the preparation of compounds **12a−c**, was followed with intermediate **5a**, **5b**, or **5c** and using hydroxylamine hydrochloride. The reaction mixture was filtered, and the solvent was evaporated; recrystallization of the residue afforded the pure products as solids.

N*-(4-Methoxybenzenesulfonyl)-*N*-{[2-(4-sulfamoylphenyl)ethylcarbamoyl]methyl}aminoacetohydroxamic Acid (6a).** Recrystallized from acetonitrile; white solid; 82% yield; mp 170−172 °C; ^{1}H NMR (D_2O, pD ca. 9) δ 7.77−7.72 (2d, 4H, ArH***), 7.35 (d, J = 8.4 Hz, 2H, Ar***H***), 7.12 (d, J = 9.0 Hz, 2H Ar***H***), 3.88 (s, 3H, OC***H***$_3$), 3.79 (s, 2H, C***H***$_2$CONHCH$_2$), 3.71 (s, 2H, C***H***$_2$CONHOH), 3.40 (t, J = 6.8 Hz, 2H, NHC***H***$_2$CH$_2$PhSO$_2$NH$_2$), 2.84 (t, J = 6.8 Hz, 2H, NHCH$_2$C***H***$_2$PhSO$_2$NH$_2$); *m*/*z* (FAB) 501 (M + H)$^+$, 535 (M + K)$^+$. Anal. ($C_{19}H_{24}N_4O_8S_2$) C, H, S. N: calcd, 11.19; found, 11.68.

N*-(4-Biphenylsulfonyl)-*N*-{[2-(4-sulfamoylphenyl)ethylcarbamoyl]methyl}aminoacetohydroxamic Acid (6b).** Recrystallized from CH_2Cl_2; white solid; 85% yield; mp 165−166 °C; ^{1}H NMR (D_2O, pD ca. 9) δ 7.98 (d, J = 8.7 Hz, 2H, ArH***), 7.89 (d, J = 7.8 Hz, 2H, Ar***H***), 7.79 (d, J = 8.1 Hz, 2H, Ar***H***), 7.71 (d, J = 7.2 Hz, 2H, Ar***H***), 7.54−7.45 (m, 3H, Ar***H***), 7.34 (d, J = 7.8 Hz, 2H, Ar***H***), 3.83 (s, 2H, C***H***$_2$CONHCH$_2$), 3.78 (s, 2H, C***H***$_2$CONHOH), 3.42 (t, J = 7.1 Hz, 2H, NHC***H***$_2$CH$_2$PhSO$_2$NH$_2$), 2.85 (s, J = 7.4 Hz, 2H, NHCH$_2$C***H***$_2$PhSO$_2$NH$_2$); *m*/*z* (HRMS) for (M + Na)$^+$, calcd 569.11351, found 569.11466; for (M + H)$^+$, calcd 547.13157, found 547.13270. Anal. ($C_{24}H_{26}N_4O_7S_2 \cdot 0.4CH_2Cl_2$) C, N. H: calcd, 4.65; found, 4.24. S: calcd, 11.01; found, 11.44.

N*-(4-Phenoxybenzenesulfonyl)-*N*-{[2-(4-sulfamoylphenyl)ethylcarbamoyl]methyl}aminoacetohydroxamic Acid (6c).** Recrystallized from acetonitrile; pale solid; 31% yield; mp 151−153 °C; ^{1}H NMR (D_2O, pD ca. 9) δ 7.78 (d, J = 9.0 Hz, 2H, ArH***), 7.74 (d, J = 8.1 Hz, 2H, Ar***H***), 7.48 (t, J = 8.1 Hz, 2H, Ar***H***), 7.36 (d, J = 8.4 Hz, 2H, Ar***H***), 7.32 (t, 1H, J = 7.2 Hz, Ar***H***), 7.17−7.12 (2d, 4H, Ar***H***), 3.85 (s, 2H, C***H***$_2$CONHCH$_2$), 3.79 (s, 2H, C***H***$_2$CONHOH), 3.41 (t, J = 6.8 Hz, 2H, NHC***H***$_2$CH$_2$PhSO$_2$NH$_2$), 2.84 (t, J = 6.8 Hz, 2H, NHCH$_2$C***H***$_2$PhSO$_2$NH$_2$); *m*/*z* (HRMS) for (M + Na)$^+$, calcd 585.10843, found 585.10743; for (M + H)$^+$, calcd 563.12648, found 563.12580. Anal. ($C_{24}H_{26}N_4O_8S_2 \cdot 0.4CH_2Cl_2$) C, H, N, S.

N*-(4-Phenoxybenzenesulfonyl)-*N*-hydroxycarbonylmethyl-*O*-benzyl-D-valine (17).** The synthesis of the title compound involved the preparation of **10c**, starting from **9c** as previously described, and subsequent *tert*-butyl deprotection was performed as for compounds **13a−c**. After evaporation of the reaction mixture, the residue was washed with ethyl ether, the solid was dissolved in ethyl acetate, and the resulting solution was washed with 1% NaOH; after the organic phase was dried over anhydrous Na_2SO_4 and the solvent was evaporated, the pure product was obtained as a pale yellow solid: overall yield of 38% for **10c−17**; mp 73−75 °C; ^{1}H NMR ($CDCl_3$) δ 7.76 (d, J = 8.4 Hz, 2H, ArH***), 7.33 (t, J = 7.8 Hz, 2H, Ar***H***), 7.22−7.13 (m, 6H, Ar***H***), 6.94 (d, J = 7.8 Hz, 2H, Ar***H***), 6.79 (d, J = 8.1 Hz, 2H, Ar***H***), 4.97, 4.91 (dd, J = 12.3, 12.0 Hz, 2H, C***H***$_2$Ph), 4.19 (d, J = 18.0, Hz, 1H, C***H***$_2$CO$_2$H), 3.90−3.83 (m, 3H, C***H***$_2$CO$_2$H, NC***H***(*i*Pr)), 2.08−1.97 (m, 1H, C***H***(CH$_3$)$_2$), 0.71−0.65 (2d, 6H, CH(C***H***$_3$)$_2$); *m*/*z* (FAB) 520 (M + Na)$^+$.

N*-(4-Phenoxybenzenesulfonyl)-*N*-{[2-(4-sulfamoylphenyl)ethylcarbamoyl]methyl}-*O*-benzyl-D-valine (18).** A procedure similar to that used for the preparation of **5a−c** was followed, starting from **17**. After the extractions were performed as mentioned in that case, the organic extract was concentrated, and the residue was purified by flash chromatography using 12:1 CH_2Cl_2/MeOH as eluent (R_f 0.60). Evaporation of the solvent under vacuum afforded the pure product as a yellow solid: yield 72%; mp 60−62 °C; ^{1}H NMR ($CDCl_3$) δ 7.88 (d, J = 8.1 Hz, 2H, ArH***), 7.68 (d, J = 8.7 Hz, 2H, Ar***H***), 7.44−7.22 (m, 10H, Ar***H***), 7.04 (d, J = 7.2 Hz, 2H, Ar***H***), 6.93 (d, J = 8.7 Hz, 2H, Ar***H***), 4.92, 4.80 (dd, J = 12.3, 12.0 Hz, 2H C***H***$_2$Ph), 4.73 (s, 2H, SO$_2$N***H***$_2$), 4.12, 3.85 (dd, J = 18.3, 17.7 Hz, 2H, C***H***$_2$CONHCH$_2$), 4.04 (d, J = 9.9 Hz, 1H, NC***H***(*i*Pr)), 3.55 (q, J = 6.8 Hz, 2H, NHC***H***$_2$CH$_2$PhSO$_2$NH$_2$), 2.93 (t, J = 7.2 Hz, 2H, NHCH$_2$C***H***$_2$PhSO$_2$NH$_2$), 2.07−1.95 (m, 1H, C***H***(CH$_3$)$_2$), 0.87−0.83 (2d, 6H, CH(C***H***$_3$)$_2$); *m*/*z* (FAB) 680 (M + H)$^+$, 702 (M + Na)$^+$.

N*-(4-Phenoxybenzenesulfonyl)-*N*-{[2-(4-sulfamoylphenyl)ethylcarbamoyl]methyl}-D-valine (19).** The title compound was prepared following the general method for catalytic hydrogenolysis, starting from **18** and using 1.5 bar of H_2. Recrystallization with ethyl ether afforded the pure product as a pale solid: yield 92%; mp 97−99 °C; ^{1}H NMR ($CDCl_3$) δ 7.87 (d, J = 8.4 Hz, 2H, ArH***), 7.75 (d, J = 9.0 Hz, 2H, Ar***H***), 7.44−7.39 (m, 4H, Ar***H***), 7.22 (t, J = 7.5 Hz, 1H, Ar***H***), 7.06−7.01 (2d, 4H, Ar***H***), 6.92 (bs, 1H, CON***H***CH$_2$), 4.95 (s, 2H, SO$_2$N***H***$_2$), 3.98, 3.80 (dd, J = 17.4, 18.6 Hz, 2H, C***H***$_2$CONHCH$_2$), 3.84 (d, J = 9.6 Hz, 1H, NC***H***(*i*Pr)), 3.68−3.50 (2m, 2H, NHC***H***$_2$CH$_2$PhSO$_2$NH$_2$), 2.93 (q, J = 5.7 Hz, 2H, NHCH$_2$C***H***$_2$PhSO$_2$NH$_2$), 2.03−1.90 (m, 1H, C***H***(CH$_3$)$_2$), 0.94 (d, J = 6.3 Hz, 3H, CHC***H***$_3$), 0.72 (d, J = 6.3 Hz, 3H, CHC***H***$_3$); *m*/*z* (FAB) 612 (M + Na)$^+$, 590 (M + H)$^+$.

N*-(4-Phenoxybenzenesulfonyl)-*N*-{[2-(4-sulfamoylphenyl)ethylcarbamoyl]methyl}-*O*-benzyl-D-valinehydroxamic Acid (20).** Compound **20** was synthesized by a procedure identical to that used for **12a−c**, starting from **19**. After concentration in vacuum of the reaction mixture, the residue was dissolved in ethyl acetate, and this solution was washed with 1 M HCl and water; the organic phase was then dried over anhydrous Na_2SO_4 and the solvent evaporated under vacuum. Flash column chromatography of that residue was then performed with a 12:1 CH_2Cl_2/MeOH eluent (R_f 0.48); evaporation of the solvent under vacuum afforded the pure product as a yellow hygroscopic solid (64% yield): ^{1}H NMR ($CDCl_3$) δ 9.19 (s, 1H, CONH***OBn), 7.83 (d, J = 8.4 Hz, 2H, Ar***H***), 7.77 (d, J = 8.4 Hz, 2H, Ar***H***), 7.39−7.31 (m, 9H, Ar***H***), 7.21 (t, J = 6.9 Hz, 1H, Ar***H***), 7.04−7.00 (m, 4H, Ar***H***), 6.60 (t, J = 6.0 Hz, 1H, CON***H***CH$_2$), 4.90−4.79 (m, 4H, SO$_2$N***H***$_2$, C***H***$_2$Ph), 3.99, 3.76 (dd, J = 17.1 Hz, 2H, C***H***$_2$CONHCH$_2$), 3.59−3.43 (m, 2H, NHC***H***$_2$CH$_2$PhSO$_2$NH$_2$), 3.42 (d, J = 10.8 Hz, 1H, NC***H***(*i*Pr)), 2.91 (t, J = 6.0 Hz, 2H, NHCH$_2$C***H***$_2$PhSO$_2$NH$_2$), 2.04−1.99 (m, 1H, C***H***(CH$_3$)$_2$), 0.81 (d, J = 6.3 Hz, 3H, CHC***H***$_3$), 0.50 (d, J = 6.0 Hz, 3H, CHC***H***$_3$); *m*/*z* (FAB) 695 (M + H)$^+$, 717 (M + Na)$^+$.

N*-(4-Phenoxybenzenesulfonyl)-*N*-{[2-(4-sulfamoylphenyl)ethylcarbamoyl]methyl}-D-valinehydroxamic Acid (7).** Catalytic hydrogenolysis of **20** was performed, using the above-described general method, with 1.5 bar of H_2. Final recrystallization from ethyl acetate/ethyl ether afforded the pure product as a pale yellow solid; yield 95%; mp 85−87 °C;^{1}H NMR (D_2O, pD ca. 9) δ 7.76−7.71 (2d, 4H, ArH***), 7.44 (t, J = 7.0 Hz, 2H, Ar***H***), 7.36 (d, 2H, J = 7.8 Hz, Ar***H***), 7.28 (t, J = 6.3 Hz, 1H, Ar***H***), 7.10 (d, J = 6.9 Hz, 4H, Ar***H***), 4.20, 3.94 (dd, J = 17.1, 18.0 Hz, 2H, C***H***$_2$CONHCH$_2$), 3.63 (d, J = 10.2 Hz, 1H, NC***H***(*i*Pr)), 3.38−3.32 (m, 2H, NHC***H***$_2$CH$_2$PhSO$_2$NH$_2$), 2.80 (t, J = 7.0 Hz, 2H, NHCH$_2$C***H***$_2$PhSO$_2$NH$_2$), 1.84−1.97 (m, 1H, C***H***(CH$_3$)$_2$), 0.80 (d, J = 6.3 Hz, 3H, CHC***H***$_3$), 0.73 (d, J = 5.7 Hz, 3H, CHC***H***$_3$); *m*/*z* (FAB) 605 (M + H)$^+$, 627 (M + Na)$^+$. Anal. ($C_{27}H_{32}N_4O_8S_2 \cdot$ 0.4EtOAc) C, H, N, S.

(B) MMP Inhibition Assays.[42,43] Recombinant human progelatinases A (pro-MMP-2) and B (pro-MMP-9) from transfected mouse myeloma cells and MMP-16 and MMP-14 catalytic domains were supplied by Prof. Gillian Murphy (Department of Oncology, University of Cambridge, U.K.). Pro-MMP-1, pro-MMP-8, pro-MMP-13, and TACE (ADAM-17) were purchased from Calbiochem. Proenzymes were activated immediately prior to use with *p*-aminophenylmercuric acetate (APMA, 2 mM, for 1 h at 37 °C for MMP-2, MMP-1, and MMP-8, 1 mM for 1 h at 37 °C for MMP-9 and MMP-13).

For assay measurements, the inhibitor stock solutions (DMSO, 100 mM) were further diluted, at seven different concentrations (0.01 nM−300 μM) for each MMP in the fluorometric assay buffer (FAB: 50 mM Tris, pH = 7.5, 150 mM NaCl, 10 mM $CaCl_2$, 0.05% Brij 35, and 1% DMSO). Activated enzyme (final concentration 2.9 nM for MMP-2, 2.7 nM for MMP-9, 1.5 nM for MMP-8, 0.3 nM for MMP-13, 1 nM for MMP-14 cd, 15 nM for MMP-16 cd, 2.0 nM for MMP-1, and 7.5 nM for TACE) and inhibitor solutions were incubated in the assay buffer for 4 h at 25 °C. After addition of 200 μM solution of the fluorogenic substrate Mca-Arg-Pro-Lys-

Pro-Val-Glu-Nva-Trp-Arg-Lys(Dnp)-NH_2 (Sigma) for MMP-3 and Mca-Lys-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH_2 (Bachem)[44] for all of the other enzymes in DMSO (final concentration 2 μM), the hydrolysis was monitored every 15 s for 20 min by recording the increase in fluorescence (λ_{ex} = 325 nm, λ_{em} = 395 nm) using a Molecular Device SpectraMax Gemini XS plate reader. The assays were performed in triplicate in a total volume of 200 μL per well in 96-well microtiter plates (Corning, black, NBS). Control wells lack inhibitor. The MMP inhibition activity was expressed in relative fluorescent units (RFU). Percent of inhibition was calculated from control reactions without the inhibitor. IC_{50} was determined using the formula $V_i/V_0 = 1/(1 + [I]/IC_{50})$, where V_i is the initial velocity of substrate cleavage in the presence of the inhibitor at concentration [I] and V_0 is the initial velocity in the absence of the inhibitor. Results were analyzed using SoftMax Pro[45] software and GraFit[46] software.

(C) CA Inhibition Assays. Recombinant human CA isoforms I, II, and IX were prepared as previously reported by our group,[47,48] and their activity was assayed by a stopped-flow CO_2 hydration assay[26] with an Applied Photophysics (Oxford, U.K.) stopped-flow instrument. Phenol red (at a concentration of 0.2 mM) was used as indicator, working at the absorbance maximum of 557 nm, with 10 mM Hepes (pH 7.5) as buffer, 0.1 M Na_2SO_4 (for maintaining constant the ionic strength), following the CA-catalyzed CO_2 hydration reaction for a period of 10−100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor, at least six records of the initial 5−10% of the reaction were used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (1 mM) were prepared in distilled deionized water with 10−20% (v/v) DMSO (which is not inhibitory at these concentrations), and dilutions up to 0.1 nM were done thereafter with distilled deionized water. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay in order to allow for the formation of the E−I complex. The inhibition constants were obtained by nonlinear least-squares methods using PRISM 3, from Lineweaver−Burk plots, as reported earlier, and represent the average from at least three different determinations.[47,48]

(D) Docking of Ligands. MMP−Inhibitor Complex Structures and Docking. The X-ray structures of MMP−inhibitor complexes with different hydroxamate-based inhibitors, available to date, were taken from the Protein Data Bank[39] and were used for this analysis. Solvent molecules and counterions were removed from the original structure whenever they were present, as well as the structural Ca^{2+} ions in the proteins. Hydrogen atoms were added by means of Maestro 7.5.[49] The ligands were extracted from the complexes and were subjected to a conformational search (CS) of 1000 steps, using a water environment model (generalized-Born/surface-area model) by means of Macromodel.[50] The algorithm used was based on the Monte Carlo method with the MMFFs force field and a distance-dependent dielectric constant of 1.0. The ligands were then minimized using the conjugated gradient method until a convergence value of 0.05 kcal/(Å·mol) was reached, using the same force field and parameters used for the CS. The minimized ligands were docked in their respective proteins with the Gold program,[38] version 3.0.1. The region of interest used by Gold was defined in order to contain the residues within 15 Å from the original position of the ligand in the X-ray structures; the zinc ion was set as possessing a trigonal-bipyramidal coordination. The "allow early termination" option was deactivated while the possibility for the ligand to flip ring corners was activated. The remaining Gold default parameters were used, and the ligands were submitted to 100 genetic algorithm runs applying the three fitness functions available with Gold (GoldScore, ChemScore, and ASP), without any other constraints. The best docked conformation for each scoring function was then compared with the experimental conformation of the ligand in the crystal structure and the root-mean-square deviation (rmsd) between the positions of the heavy atoms was calculated, this parameter being considered as a measure of the docking accuracy.

Each scoring function was also tested with a scaffold match constraint, where the hydroxamic moiety of the original ligand in each crystal structure was used as the scaffold to be matched; the scaffold match constraint weight, a parameter defining how closely the ligand atoms should fit onto the scaffold, was set to 10.0.

Docking of Compound 7. The ligand was built using Maestro 7.5 and underwent CS and structure optimization with Macromodel, using the same procedures described above. The structures of the proteins were extracted from the RCSB Protein Data Bank and subsequently treated in the same way as described before: for MMP-2 (entry 1QIB, catalytic domain), MMP-1 (entry 1HFC), MMP-8 (entry 1MNC), MMP-9 (entry 1GKC), MMP-13 (entry 830C), MMP-14 (entry 1BQQ), and MMP-16 (entry 1RM8). Since the MMP-2 structure did not possess any ligand, it was aligned with the MMP-9 complex (using Chimera software),[51] and this ligand was further used to define the zone of interest in MMP-2 for the docking calculations with Gold. To perform the docking calculations on MMPs, we used the same conditions and parameters that were previously used in the validation analysis, which revealed the best prediction of the binding mode of the hydroxamic inhibitors into the MMPs. These conditions involved, namely, the ASP scoring function applying the SMC function.

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Supporting Information Available: Docking results of inhibitor **7** with MMP-8, -9, -13, -14, and -16 and synthesis and characterization of all compounds, as well as chromatographic and purity data for target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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