Journal of Medicinal Chemistry Article

J. Med. Chem. **2009,** *52,* 6347–6361 **6347**
DOI: 10.1021/jm900335a

Design, Synthesis, Biological Evaluation, and NMR Studies of a New Series of Arylsulfones As Selective and Potent Matrix Metalloproteinase-12 Inhibitors

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Received March 17, 2009

Overexpression of macrophage elastase (MMP-12), a member of the matrix metalloproteinases family, can be linked to tissue remodeling and degradation in some inflammatory processes, such as chronic obstructive pulmonary disease (COPD), emphysema, rheumatoid arthritis (RA), and atherosclerosis. On this basis, MMP-12 can be considered an attractive target for studying selective inhibitors that are useful in the development of new therapies for COPD and other inflammatory diseases. We report herein the design, synthesis, and in vitro evaluation of a new series of compounds, possessing an arylsulfonyl scaffold, for their potential as selective inhibitors of MMP-12. The best compound in the series showed an IC_{50} value of 0.2 nM, with good selectivity over MMP-1 and MMP-14. A docking study was carried out on this compound in order to investigate its binding interactions with MMP-12, and NMR studies on the complex with the MMP-12 catalytic domain were able to validate the proposed binding mode.

1. Introduction

MMP-12 (macrophage elastase) is a member of the matrix metalloproteinases (MMPs),[a] a family of endopeptidases able to degrade all the components of the extracellular matrix (ECM). In adult tissue, macrophages are the major source of MMP-12. Several studies have demonstrated that MMP-12 is involved in inflammatory processes and contributes to tissue remodeling and tissue degradation.[1] Patients suffering from chronic obstructive pulmonary disease (COPD) showed higher levels of MMP-12 in lung tissue and bronchoalveolar lavage,[2] and MMP-12 knockout mice were resistant to emphysema after chronic cigarette smoke exposure.[3]

These observations suggest that MMP-12 plays an important role in lung tissue destruction in mice, and MMP-12 inhibitors can be potentially useful for the treatment of lung pathologies. In fact, it was recently reported that a selective MMP-12 inhibitor, AS111793[4] (Figure 1), was able to prevent inflammation induced by exposure to cigarette smoke in mice. Unfortunately, only a few MMP inhibitors have entered clinical trials for cancer or arthritis and none have been successful thus far. In fact, many broad spectrum MMP inhibitors have been found to have dose-limiting toxicity in the form of musculoskeletal side effects, including joint stiffness and inflammation.[5] Although the human joint side effects are reversible upon withdrawal of the drug, musculoskeletal syndrome (MSS) has halted the clinical trials of many nonselective MMP inhibitors. While the inhibition of specific MMPs, such as MMP-1 or MMP-14, has been postulated as the cause of MSS, the exact basis for this pathology is not yet clear. For this reason, we started a project aimed at finding selective MMP-12 inhibitors (sparing MMP-1 and MMP-14) that may be useful in the development of new therapies against COPD, emphysema, and other relevant inflammatory diseases, such as RA[6] and atherosclerosis,[7] as has been more recently observed.

MMP-12 is a zinc-dependent protease secreted as a 54 kDa pro-form protein that undergoes self-activation to produce several active forms of the enzyme. It shares common structural domains with other MMPs. The most closely related human matrix metalloproteinases are stromelysin-1 (MMP-3) and interstitial collagenase (MMP-1), each with a 49% similarity to MMP-12.

Recently, the crystal structure of the catalytic domain of MMP-12 complexed to a hydroxamic acid inhibitor was reported.[1] The S1′-specificity pocket is large and extends into a channel through the protein, which puts MMP-12 into a class with MMPs -8 and -13, containing large and open specificity pockets.

In another study, Markus et al.[8] published the solution structure of the catalytic domain of MMP-12 complexed with a carboxylate inhibitor, using the wild-type protein sequence. The inhibitor binds in the S1′ pocket, and the carboxylate

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[a] Abbreviations: MMP, matrix metalloproteinase; ECM, extracellular matrix; COPD, chronic obstructive pulmonary disease; MSS, musculoskeletal syndrome; RA, rheumatoid arthritis; ZBG, zinc-binding group; SAR, structure−activity relationship; EDC, *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; MSS, musculoskeletal syndrome; HSQC, heteronuclear single quantum correlation; NNGH, *N*-isobutyl-*N*-[4-methoxyphenylsulfonyl]glycyl hydroxamic acid; AHA, acetohydroxamic acid; NOE, nuclear Overhauser effect; APMA, *p*-aminophenylmercuric acetate.

Published on Web 09/23/2009

functionality coordinates the catalytic zinc ion. The NMR structure of MMP-12 in the absence of an inhibitor was solved by Bhaskaran et al.[9] using a mutant form of MMP-12 able to prevent autolysis.

Finally, the crystal structures of the MMP-12 catalytic domain complexed with nonzinc chelating inhibitors have been determined.[10] This study pointed out the importance of hydrophobic interactions between the inhibitors and residues in the S1′ pocket in preserving activity and achieving selectivity toward this enzyme.

In spite of the increasing interest in MMP-12,[11] only a modest number of papers regarding the development of inhibitors have been reported to date. Among them, the most promising are the phosphinic peptides discovered by Dive et al.[12] and the nonzinc chelating, nonpeptidic inhibitors published by Dublanchet et al.[13]

In our previous papers,[14,15] we described the synthesis and biological evaluation of *N*-*i*-propoxy-*N*-biphenylsulfonylaminobutyl hydroxamic acids of type **A** (Figure 1) as selective inhibitors of MMP-2 for cancer therapy. In particular, compound **1a** (Figure 1) was found to be a potent antiangiogenic agent, able to block the chemoinvasion of HUVEC cells in the micromolar range.

In the present paper, we report the optimization of template **A** geometry between the hydroxamic zinc-binding group (ZBG) and the P1′ sulfonyl group in order to obtain potent and selective inhibitors of MMP-12. To improve selectivity, we decided to replace substituents R and N-O-R_1 of **A** with an aromatic ring that should confer a major degree of rigidity to the system. Therefore, in a preliminary study of SAR, some sulfone-based benzoic hydroxamates (compounds of type **B**) were synthesized to evaluate the effects of conformational restriction and the presence of different P1′ substituents on enzyme inhibition (Figure 1).

On the basis of docking studies, a second series of sulfone-based inhibitors was then obtained by inserting a methylene between the ZBG and the aromatic ring in order to modify the geometry of chelation (compounds of type **C**). Finally, with the aim of verifying the influence on activity of a small alkyl group (R_2) α to the ZBG, new α-methyl-substituted phenylacetic derivatives were developed (Chart 1). In these studies, biphenylsulfonyl or phenoxybenzenesulfonyl moieties were chosen as P1′ substituents because they were considered suitable for fitting well into a channel-like S1′ pocket as is seen in MMP-12. Both hydroxamic and carboxylic acids were selected as ZBGs, given their different binding modes,[16] good chelating properties, and presence in the vast majority of reported MMP inhibitors. In fact, despite the debate over the feasibility of using hydroxamates as ZBGs due to in vivo stability problems, they are still frequently used because of studies showing the importance of inhibitor structure in determining the bioavailability and toxicity of compounds.[17] As a matter of fact, many hydroxamate-based MMP inhibitors were active in in vivo models and pharmacologically effective.[4,18]

Additional support for the use of hydroxamates can be found in the field of histone deacetylase (HDAC) inhibitors, where the relative safety of some hydroxamates for anticancer

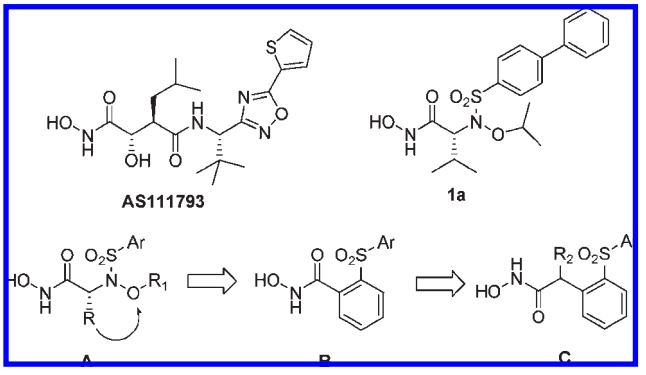
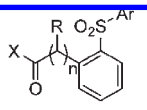


Figure 1. Progression of the SAR.

Chart 1



2-19

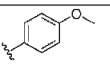
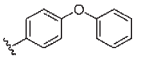
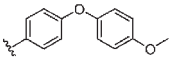
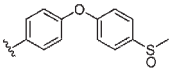
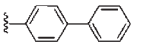
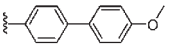
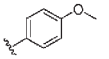
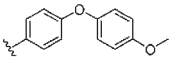
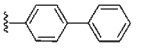
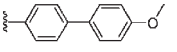
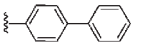
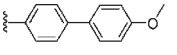
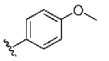
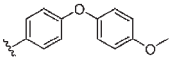
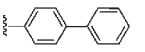
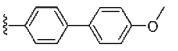
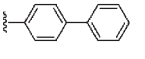
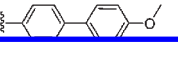
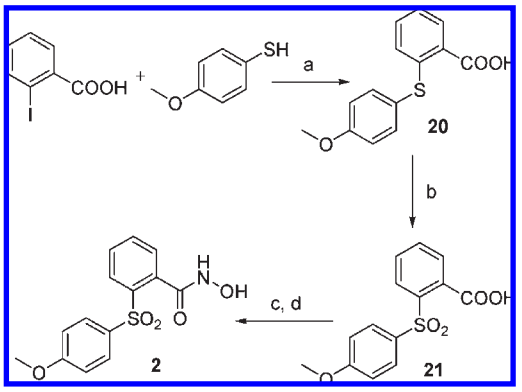
Compd	n	Ar	R	X
2	0		H	NHOH
3	0		H	NHOH
4	0		H	NHOH
5	0		H	NHOH
6	0		H	NHOH
7	0		H	NHOH
8	1		H	NHOH
9	1		H	NHOH
10	1		H	NHOH
11	1		H	NHOH
12	1		CH_3	NHOH
13	1		CH_3	NHOH
14	1		H	OH
15	1		H	OH
16	1		H	OH
17	1		H	OH
18	1		CH_3	OH
19	1		CH_3	OH

Table 1. In Vitro[a] Activity (IC_{50} nM Values) of Benzoic Hydroxamic Acids **2**−**7**

compd	MMP-1	MMP-2	MMP-3	MMP-8	MMP-9	MMP-12	MMP-13	MMP-14	MMP-16
2	> 100000	35000 ± 2500	> 50000	72000 ± 8500	24000 ± 2200	14000 ± 1000	28000 ± 1800	25000 ± 1000	31000 ± 2000
3	> 10000	1200 ± 110	27000 ± 1500	620 ± 38	690 ± 72	1200 ± 160	2700 ± 230	1300 ± 200	1300 ± 100
4	> 10000	1300 ± 160	22000 ± 2500	1900 ± 100	410 ± 45	920 ± 80	2700 ± 100	4000 ± 310	6000 ± 290
5	> 200000	9900 ± 1100	60000 ± 4600	9200 ± 370	51000 ± 1000	5800 ± 370	20000 ± 2100	120000 ± 10000	110000 ± 10000
6	73000 ± 7100	1300 ± 100	45000 ± 2600	1000 ± 110	7700 ± 600	590 ± 28	1400 ± 150	8300 ± 360	5100 ± 320
7	28000 ± 2600	100 ± 10	4000 ± 410	87 ± 5	930 ± 89	70 ± 2	260 ± 26	1300 ± 100	1200 ± 100
1a	490 ± 76	0.80 ± 0.20	50 ± 2	1.6 ± 0.1	6.7 ± 1.6	0.20 ± 0.05	4.1 ± 0.2	9.8 ± 0.2	51 ± 3

[a] Assays were run in triplicate. The final values given here are the mean ± SD of three independent experiments.

Scheme 1. Synthesis of Benzoic Hydroxamate **2**[a]



[a] Reagents and conditions: (a) Cu, KOH, H_2O, microwave, 180 W, 140 °C, 12 min; (b) oxone, MeOH, THF, H_2O; (c) $TBDMSiONH_2$, EDC, CH_2Cl_2; (d) TFA, CH_2Cl_2, 0 °C.

and antimalaria therapy has been established. Furthermore, a member of this family of hydroxamate zinc-chelating agents, Zolinza (Vorinostat), was launched on the market in the USA last year.[19] These results, obtained from different fields of drug therapy where hydroxamic acid derivatives have been used, seem to suggest that the nature of the scaffold around the ZBG can be very important not only for determining the potency and selectivity among the different metalloproteins but also for conferring hydrolytic and/or metabolic stability to the compounds, as previously pointed out.

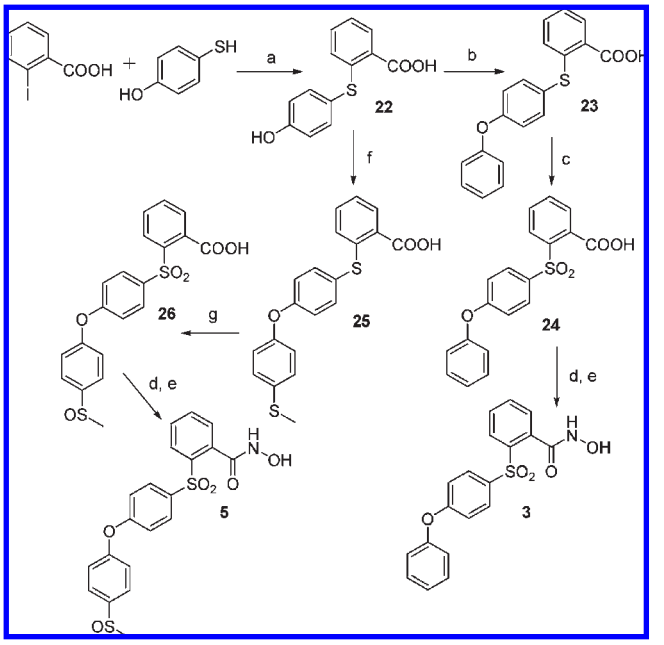
With regards to compounds presented herein, other scientists in the past years have disclosed some aryl sulfonyl hydroxamates as selective MMP-2/MMP-9/MMP-13 inhibitors.[20] Among these sulfonylated compounds, the vast majority of which were arylsulfonamides, a sulfone hydroxamate, somewhat similar to our compounds, was also described as an inhibitor of the above cited MMPs. More recently, we independently developed and patented some thioaryl substituted derivatives as zinc protease inhibitors having similar structures to compounds **B** and **C**.[21]

2. Chemistry

The benzoic hydroxamate **2** (Table 1) was synthesized as described in Scheme 1. Through a microwave-assisted, Cu-catalyzed Ullmann-type reaction[22] between 2-iodobenzoic acid and 4-methoxythiophenol, it was possible to obtain the carboxylic acid **20**, which was oxidized using oxone in methanol/THF/H_2O to the corresponding sulfone **21**. This was converted into the hydroxamic acid **2** by condensation with *O*-(*tert*-butyldimethylsilyl)hydroxylamine in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and subsequent deprotection with trifluoracetic acid in dichloromethane.

The phenoxybenzenesulfonyl benzoic derivatives **3** and **5** (Scheme 2) were prepared starting from the reaction of

Scheme 2. Preparation of Phenoxyphenyl Benzoic Hydroxamates **3** and **5**[a]



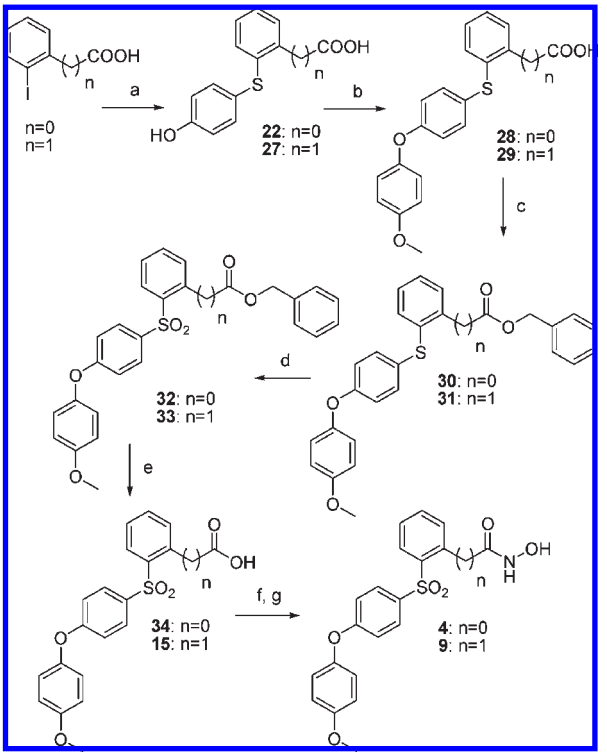
[a] Reagents and conditions: (a) Cu, KOH, H_2O, microwave, 180 W, 140 °C, 12 min; (b) $Cu(OAc)_2$, TEA, CH_2Cl_2, 4 Å molecular sieves, phenylboronic acid; (c) oxone, MeOH, THF, H_2O; (d) $TBDMSiONH_2$, EDC, CH_2Cl_2; (e) TFA, CH_2Cl_2, 0 °C; (f) $Cu(OAc)_2$, TEA, CH_2Cl_2, 4 Å molecular sieves, 4-(methylthio)phenylboronic acid; (g) oxone, MeOH, THF, H_2O.

4-mercaptophenol with 2-iodobenzoic acid to give **22** under the same conditions used before for compound **20**. The use of the microwave for this type of reaction allowed for a reduction in the reaction time with respect to the procedure reported in the literature[22] (from 12 h to 12 min), always maintaining high yields (> 80%).

The phenolic intermediate **22** was subsequently converted to diaryl ethers **23** and **25** by copper(II)-promoted coupling[23] with the appropriate arylboronic acids. The reaction was carried out using $Cu(OAc)_2$ as the source of Cu(II), in the presence of triethylamine (TEA) and powdered 4 Å molecular sieves at room temperature. To prepare the corresponding sulfonyl derivatives, **23** and **25** were oxidized with oxone and finally converted to hydroxamic acids **3** and **5** by the route previously described. Oxidation of **25** with 6 equiv of oxone afforded compound **26**, with a sulfone and a sulfoxide group, which was separated from its bis-sulfone analogue by silica gel column chromatography.

Synthesis of 4-methoxyphenoxy benzen sulfone derivatives **4**, **9**, and **15** was carried out as reported in Scheme 3. Phenylacetic compounds ($n = 1$) **9** and **15** were obtained in a similar way to that reported in Scheme 2 for benzoic derivatives,

Scheme 3. Preparation of compounds **4**, **9**, and **15**[a]



[a] Reagents and conditions: (a) 4-mercaptophenol, Cu, KOH, H_2O, microwave, 180 W, 140 °C, 12 min; (b) $Cu(OAc)_2$, TEA, CH_2Cl_2, 4 Å molecular sieves, 4-methoxyphenylboronic acid; (c) BnBr, Cs_2CO_3, DMF, 0 °C−rt; (d) oxone, MeOH, THF, H_2O; (e) KOH, H_2O, 100 °C; (f) $TBDMSiONH_2$, EDC, CH_2Cl_2; (g) TFA, CH_2Cl_2, 0 °C.

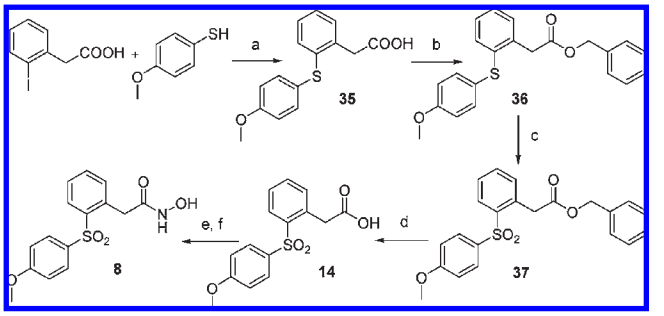
starting from commercially available 2-iodophenylacetic acid. Before oxidation with oxone, carboxylic acids **28** and **29** were protected as benzylic ester by reaction with benzyl bromide in the presence of Cs_2CO_3 in DMF. This further step was necessary to improve the following oxidation reaction, which exhibited poor yields if conducted on carboxylic acids. Hence, benzylic esters **30** and **31** were oxidized to sulfonyl derivatives and subsequently hydrolyzed to the corresponding carboxylic acids **34** and **15** by saponification with KOH in good yields. Benzoic (**4**) and phenylacetic (**9**) hydroxamates were finally obtained from the corresponding carboxylates as described for the previous compounds.

A similar sequence of reactions was carried out to synthesize 4-methoxybenzen hydroxamate **8** and its carboxylic analogue **14**, as detailed in Scheme 4.

Biphenylsulfonyl derivatives **6**, **7**, **10**, **11**, **16**, and **17** were prepared from 4-bromobenzenethiol via a Cu-catalyzed Ullmann-type reaction (Scheme 5), giving carboxylic acids **38** and **39,** which were then protected as benzylic esters. Palladium-catalyzed cross-coupling (Suzuki conditions[24]) of protected arylbromides with phenylboronic or 4-methoxyphenylboronic acid afforded biphenyl derivatives **42**−**45**. Treatment with oxone, followed by basic hydrolysis of the esters, yielded carboxylates **50**, **51**, **16**, and **17**, which were finally converted to their corresponding hydroxamates by condensation with *O*-(*tert*-butyldimethylsilyl)hydroxylamine and acid cleavage with TFA.

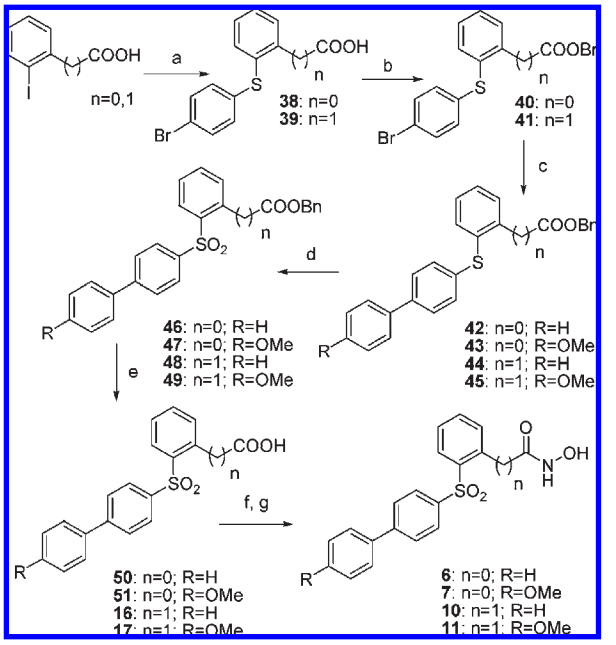
To prepare the α-methyl substituted biphenyl derivatives **12** and **13**, benzyl esters **48** and **49** were alkylated[25] by treatment with methyl iodide in the presence of sodium hexamethyldisilylamide (NaHMDS) in THF at −78 °C (Scheme 6). After alkylation, racemic esters **52** and **53** were hydrolyzed to the corresponding carboxylates and then to the hydroxamates **12** and **13**, as reported in the previous schemes.

Scheme 4. Synthesis of Compounds **8** and **14**[a]



[a] Reagents and conditions: (a) Cu, KOH, H_2O, microwave, 180 W, 140 °C, 12 min; (b) BnBr, Cs_2CO_3, DMF, 0 °C−rt; (c) Oxone, MeOH, THF, H_2O; (d) KOH, H_2O, 100 °C; (e) $TBDMSiONH_2$, EDC, CH_2Cl_2; (f) TFA, CH_2Cl_2, 0 °C.

Scheme 5. Synthesis of Biphenylic Derivatives **6**, **7**, **10**, **11**, **16**, and **17**[a]



[a] Reagents and conditions: (a) 4-bromobenzenethiol, Cu, KOH, H_2O, microwave, 180 W, 140 °C, 12 min; (b) BnBr, Cs_2CO_3, DMF, 0 °C−rt; (c) phenylboronic or 4-methoxyphenylboronic acid, $Pd(PPh_3)_4$, K_3PO_4, dioxane/H_2O, 85 °C; (d) oxone, MeOH, THF, H_2O; (e) KOH, H_2O, 100 °C; (f) $TBDMSiONH_2$, EDC, CH_2Cl_2; (g) TFA, CH_2Cl_2, 0 °C.

3. Results and Discussion

MMPs Inhibition. All final hydroxamic acids were tested in vitro, with a fluorometric assay on purified enzymes, for their ability to inhibit MMPs. Data obtained for benzoic hydroxamates **2**−**7** (type **B**) are shown in Table 1. Inhibitor **1a** was used as a reference compound.

From a brief SAR analysis of the inhibition profile for these compounds, it becomes apparent that the introduction of an aromatic ring between the ZBG and the sulfonyl group causes a drastic decrease in activity for all tested enzymes with respect to the reference compound **1a**. This was a moderately selective inhibitor, with nanomolar activity on

MMP-3, -8, -9, -13, -14, and -16 and subnanomolar activity on MMP-2 and MMP-12. Noteworthy was the good selectivity for MMP-2 and -12 over MMP-1, one of the two enzymes, together with MMP-14, whose inhibition was postulated to be responsible for musculoskeletal syndrome. Interestingly, the newly synthesized compounds of type **B** show different selectivity profiles based on the kind of arylsulfonyl group (P1′) they possess. In fact, the more rigid biphenylsulfonyl derivatives, **6** and **7**, have the best activity on MMP-12 and good selectivity over MMP-1, -3, and -14, with **7** being the most active of the two (IC_{50} = 70 nM on MMP-12) because of its longer P1′ group. In contrast, the more flexible phenoxybenzenesulfonyl derivatives, **3** and **4**, show the best activity on MMP-9 and good selectivity over MMP-1 and MMP-3.

The presence of a methoxybenzenesulfonyl group in P1′, as in compound **2**, caused a reduction in activity for all MMPs tested, probably due to the small dimensions of this substituent. A similar drop in activity is observed when a sulfoxide group is introduced on the terminal ring of the phenoxybenzenesulfonyl substituent, as in compound **5**.

Figure 2A shows the docking results for compound **4** in MMP-12. The *p*-methoxyphenoxybenzene group was inserted into the S1′ pocket and the sulfonyl group was able to interact with L181 and A182. The ZBG forms an H-bond with A182 without interacting with E219.

As already reported,[26] the alignment of all the available experimental structures of MMPs complexed with ligands containing the hydroxamate function as a ZBG suggested that the hydroxamate interacts with the zinc ion in the same binding position for all the MMPs. Figure 2B shows the superimposition of compound **4** and one of the X-ray structures of the MMP-12/hydroxamate ligand complexes (PDB code: 1RMZ[11]). From this picture, it is evident that the ZBG of compound **4** does not possess the common binding geometry of the hydroxamate-based ligands because it lacks interactions with E219. This analysis led to the hypothesis that the hydroxamic acid could not interact with the typical binding geometry of hydroxamic acid ligands due to the direct bond of the ZBG with the benzenesulfonyl group. Therefore, the distorted interaction of the ZBG with the zinc could be the cause of the decreased activity.

To improve the activity of these ligands by allowing for a good interaction of the ZBG, the benzoic hydroxamate function was modified with different scaffolds and submitted to docking calculations. From the docking analysis it was apparent that substitution of the benzoic hydroxamate function with phenylacetic hydroxamate looked very promising.

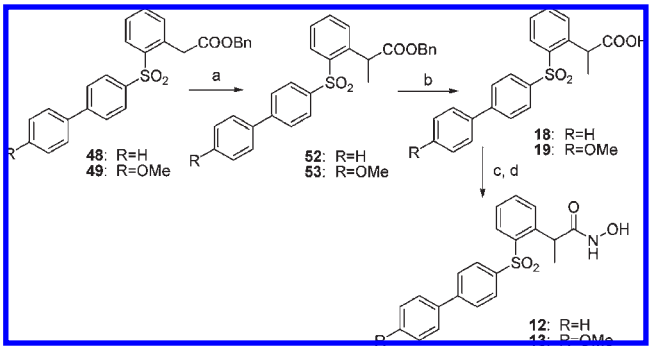
As shown in Figure 3B, the introduction of a methylene between the ZBG and the aromatic ring allowed for good coordination of the ZBG with the zinc ion and the interaction with A182 and E219. The docking of compound **9** also showed the formation of H-bonds between the sulfonyl group and L181 and A182, whereas the *p*-methoxyphenoxybenzene group was inserted into the S1′ pocket (Figure 3A).

On the basis of these docking studies, new phenylacetic hydroxamates were synthesized and tested for their MMP inhibitory activities. As shown in Table 2, in agreement with docking, the introduction of a methylene between the ZBG and the aromatic ring led to a marked increase in inhibitory activity for all tested MMPs.

This increase is more evident in phenoxybenzenesulfonyl than in biphenylsulfonyl derivatives. In fact, comparing compound **9** with its benzoic analogue **4** (Table 1), the first shows a strong increase in activity ranging from 400 (on MMP-2) to 4600 (on MMP-12) times that of **4**. However, with biphenylsulfonyl compound **11** and its benzoic analogue **7**, activity is increased only 2−15 times due to the introduction of the methylene spacer.

A strong improvement of inhibitory activity, particularly marked on MMP-12, was also obtained for compound **8**, bearing a *p*-methoxybenzenesulfonyl group in P1′, relative to its benzoic analogue **2**. In fact, **8** shows an IC_{50} of 32 nM on

Scheme 6. Preparation of α-Methyl Alkylated Compounds **12** and **13**[a]



[a] Reagents and conditions: (a) NaHMDS, MeI, THF, −78 °C; (b) KOH, H_2O, 100 °C; (c) $TBDMSiONH_2$, EDC, CH_2Cl_2; (d) TFA, CH_2Cl_2, 0 °C.

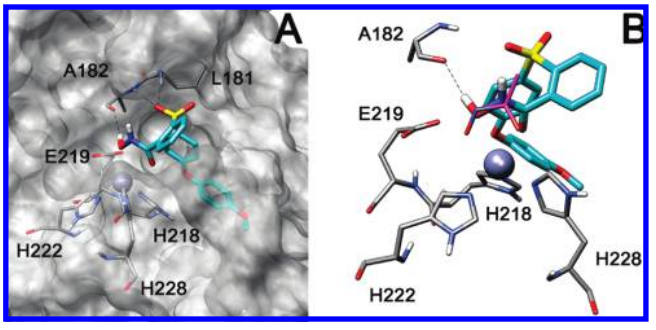


Figure 2. (A) Docking analysis of compound **4** in the MMP-12 binding site. (B) Superimposition of compound **4** (cyan) and the X-ray structure of one of the MMP-12 hydroxamate-based ligands (magenta, PDB code: 1RMZ). The H-bond interactions of the hydroxamic group of compound **4** are also reported.

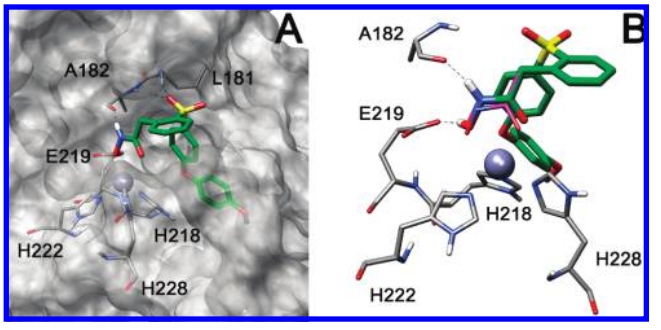


Figure 3. (A) Docking analysis of compound **9** in the MMP-12 binding site. (B) Superimposition of compound **9** (cyan) and the X-ray structure of one of the MMP-12 hydroxamate-based ligands (magenta, PDB code: 1RMZ). The H-bond interactions of the hydroxamic group of compound **9** are also reported.

Table 2. In Vitro[a] Activity (IC_{50} nM Values) of Phenylacetic Hydroxamic Acids **8**−**13**

compd	MMP-1	MMP-2	MMP-3	MMP-8	MMP-9	MMP-12	MMP-13	MMP-14	MMP-16
8	4100 ± 170	230 ± 10	730 ± 32	280 ± 18	98 ± 7	32 ± 1	84 ± 3	200 ± 8	130 ± 13
9	3500 ± 300	3.5 ± 0.2	21 ± 2	5.1 ± 0.3	0.80 ± 0.10	0.20 ± 0.02	4.1 ± 0.3	33 ± 1	32 ± 1
10	29000 ± 3500	38 ± 3	23000 ± 1600	370 ± 19	310 ± 28	6.0 ± 0.4	62 ± 7	940 ± 47	220 ± 19
11	8300 ± 360	9.7 ± 0.3	1600 ± 110	120 ± 10	94 ± 3	4.8 ± 0.4	25 ± 2	710 ± 60	130 ± 10
12[b]	12000 ± 1300	73 ± 4	5600 ± 500	390 ± 25	340 ± 37	6.0 ± 0.9	74 ± 9	860 ± 120	470 ± 24
13[b]	> 10000	7.0 ± 0.3	1400 ± 100	54 ± 3	69 ± 2	2.7 ± 0.2	12 ± 1	360 ± 29	150 ± 10
1a	490 ± 76	0.80 ± 0.20	50 ± 2	1.6 ± 0.1	6.7 ± 1.6	0.20 ± 0.05	4.1 ± 0.2	9.8 ± 0.2	51 ± 3

[a] Assays were run in triplicate. The final values given here are the mean ± SD of three independent experiments. [b] Racemates.

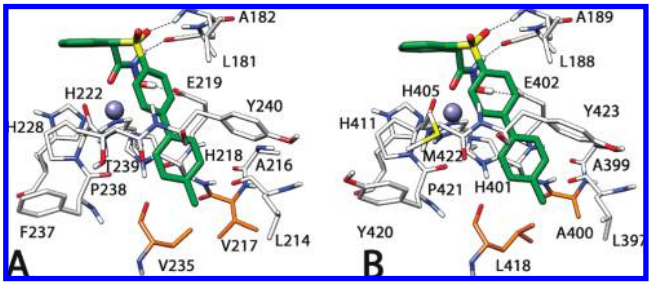


Figure 4. Docking analysis of compound **11** in the MMP-12 (A) and MMP-9 binding site (B). The two nonconserved residues, V217(A400) and V235(L418), are colored orange.

MMP-12, being about 400 times more active than **2** on this enzyme.

Moreover, as seen for the previous series of compounds, derivative **9** bearing a phenoxybenzenesulfonyl group displays its best activity on MMP-9 (0.8 nM) (besides MMP-12), while biphenylsulfonyl compounds **10** and **11** have the best activity on MMP-2 (besides MMP-12). Thus, the presence of a phenoxybenzenesulfonyl group in P1′ causes a marked increase in potency on MMP-9 in both series of compounds (except for compound **5**). This element can be important in COPD treatment, considering that expression of MMP-9 is increased in bronchoalveolar lavage of patients with COPD.[27] The lack of activity of compounds **10** and **11** against MMP-9 was also investigated by means of a docking analysis. However, comparison of the binding interactions of these two compounds with MMP-12 and MMP-9 highlighted that the compounds interacted with both MMPs with a very similar disposition. This observation did not explain the different activity showed against MMP-12 and MMP-9. As shown in Figure 4, the sulfonyl group of compound **11** formed a H-bond with L181 (L188 in MMP-9) and the *p*-methylbiphenyl group was inserted into the S1′ pocket. Analysis of the two S1′ pockets revealed that they were very similar and, regarding the interaction of the *p*-methylbiphenyl group, there were two nonconserved residues, V217 and V235 (A400 and L418 in MMP-9). However, the different interaction of these two residues with the *p*-methylbiphenyl group was not sufficient to explain its 20-fold decrease in activity.

Because it was not possible to explain the different binding activities from the binding interaction analysis of compounds **10** and **11** with MMP-12 and MMP-9, we suggest that the different activities could be due to a differential ability of the two compounds to enter the S1′ pocket of the two MMPs.

With regards to the selectivity profile, type **C** compounds exhibit nanomolar activity on MMP-12 and maintain good selectivity over MMP-1, -3, and -14. The introduction of a methyl group α to the hydroxamate (as in compounds **12** and **13**) has only a small effect on the inhibitory potency, as can be seen by comparing compound **10** with **12** and compound **11** with **13**.

Hence, the best compound of this series is **9**, with an IC_{50} of 0.2 nM on MMP-12 and a 17500-fold selectivity for MMP-12 over MMP-1 and 165-fold selectivity over MMP-14. This compound showed the same activity as reference compound **1a** on MMP-12 but an increased selectivity for MMP-12 over MMP-1. Moreover, it proved to be a subnanomolar inhibitor of MMP-9 (another MMP implicated in the pathogenesis of COPD), as well.

Finally, the carboxylic analogues of type **C** inhibitors (compounds **14**−**19**) were tested on the principal MMPs in comparison with **1b**, the carboxylate analogue of **1a** (Table 3).

As expected, the carboxylates showed a decrease in activity (> 100-fold) on all the tested MMPs with respect to the corresponding hydroxamates but they also showed a different inhibitory profile. In fact, differently from the hydroxamic homologues, the most potent compound of this series on MMP-12 was the *p*-methoxybiphenyl derivative **17** (IC_{50} = 139 nM), which also showed good activity on MMP-2, -13, and -8. This was particularly striking when compared with **1b**, which was poorly active on these enzymes. However, the carboxylic analogue of **9**, compound **15**, was the most selective on MMP-12 over MMP-1, -3, and -14.

The results obtained with carboxylates indicate that the nature of the ZBG, in addition to the structure of the inhibitors, can strongly influence the inhibitory profile.

NMR Studies. To verify the modeled binding modes of the inhibitors synthesized, NMR experiments were conducted in solution. Synergic use of docking and NMR data was applied successfully in the case of MMPs.[28] We selected compound **9**, the most active on MMP-12 (IC_{50} 0.2 nM), for the study.

The proton and carbon chemical shifts of **9** (15 mM in DMSO) are reported in the Experimental Section. The compound is rather insoluble in water. The 1D spectrum is quite complex, showing the presence of multiple species for the methylene moiety. *Z* and *E* forms of the hydroxamic group are easily identified[29] and quantified by HN and OH signals. The *E* form largely predominates (86.9% compared to 13.1% of the *Z* form). The complication regarding the methylene is probably due to hindered rotation around the bonds bearing the substituents in the aromatic moiety near the hydroxamic group.

The $^{1}H,^{15}N$-HSQC spectrum of the ^{15}N-labeled wt-MMP-12 catalytic domain, complexed to the weak inhibitor acetohydroxamic acid (AHA) for stability reasons, is shown in Figure 5. The dispersion of the peaks clearly indicates that the protein is well folded. Chemical shift data are in good agreement with the assignments published elsewhere for its complex with *N*-isobutyl-*N*-[4-methoxyphenylsulphonyl]glycyl

Table 3. In Vitro[a] Activity (IC_{50} nM Values) of Phenylacetic Carboxylic Acids **14**−**19**

compd	MMP-1	MMP-2	MMP-3	MMP-8	MMP-9	MMP-12	MMP-13	MMP-14	MMP-16
14	> 100000	58000 ± 2300	> 100000	74000 ± 3300	> 100000	89000 ± 7100	55000 ± 3700	77000 ± 5900	93000 ± 8800
15	> 10000	710 ± 45	> 10000	1200 ± 140	420 ± 44	380 ± 25	940 ± 93	8100 ± 550	6500 ± 320
16	23000 ± 1000	510 ± 27	74000 ± 1300	290 ± 12	7300 ± 830	1500 ± 140	260 ± 16	5400 ± 600	4300 ± 380
17	4400 ± 330	47 ± 3	7500 ± 750	99 ± 6	730 ± 58	140 ± 22	78 ± 10	740 ± 58	730 ± 84
18[b]	51000 ± 1500	2400 ± 100	> 100000	1900 ± 110	20000 ± 1200	1900 ± 140	1800 ± 190	18000 ± 1700	6300 ± 580
19[b]	> 10000	190 ± 10	8000 ± 570	290 ± 12	3000 ± 230	310 ± 29	190 ± 17	2100 ± 100	1100 ± 160
1b	> 100000	1500 ± 100	54000 ± 4100	2000 ± 100	24000 ± 1800	410 ± 26	7900 ± 390	10000 ± 1600	18000 ± 1000

[a] Assays were run in triplicate. The final values given here are the mean ± SD of three independent experiments. [b] Racemates.

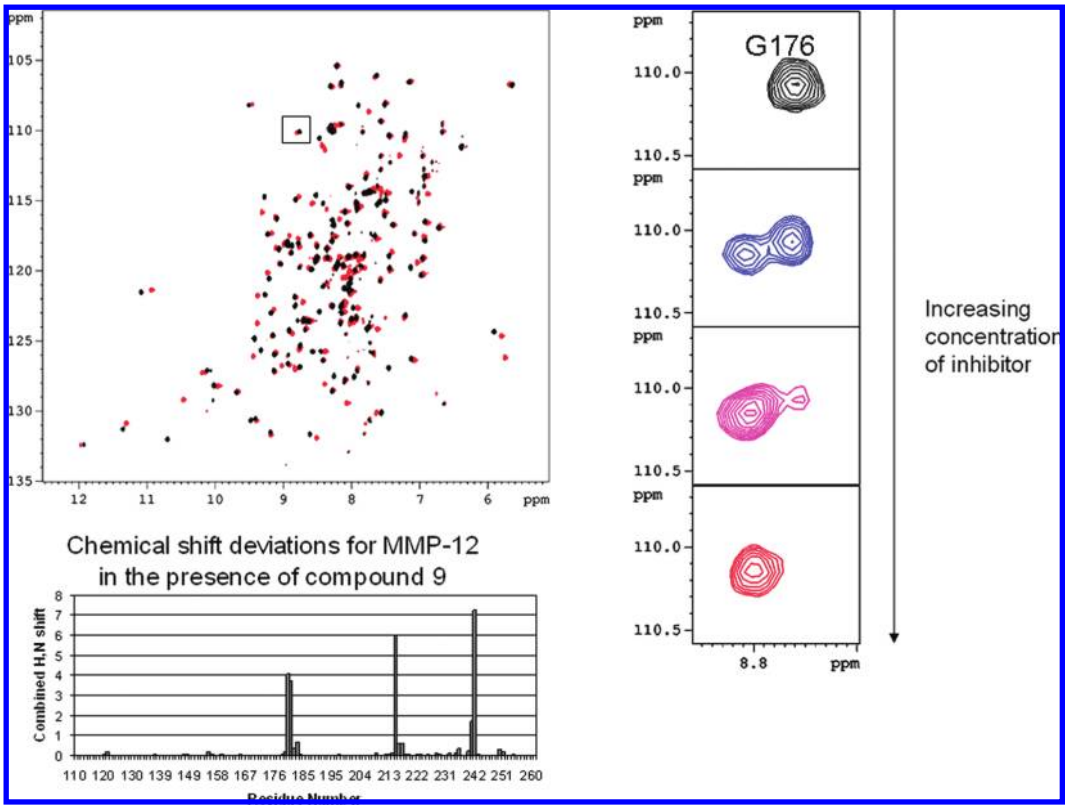


Figure 5. (top) $^{1}H,^{15}N$-HSQC spectrum of the wt-MMP-12 catalytic domain complexed with acetohydroxamic acid (black) and after addition of 1 equiv of **9** (red). Magnification of the region around peak G176 at different steps in the inhibitor addition demonstrates the slow exchange regime on the NMR time scale. (bottom) Combined ^{15}N and ^{1}H chemical shift deviation (calculated as $((\Delta\delta_N{}^2+\Delta\delta_H{}^2)/2)^{1/2}$, where $\Delta\delta$ is the observed chemical shift change) observed after addition of 1 equiv of **9**.

hydroxamic acid (NNGH).[11] However, as expected, some peaks display slightly different chemical shifts in the regions close to the inhibitor.

Addition of **9** caused a substantial change in a large part of the spectrum. Figure 5 shows how the complex forms in a slow exchange regime on the NMR time scale, as expected by the low value of the dissociation constant. Peaks of the "free form" (complexed to the weaker inhibitor AHA) tend to reduce in intensity and a new set of peaks, originating from the form of the enzyme bound to **9**, arises. The titration was performed in steps until the peaks corresponding to the free form of the protein disappeared.

On the basis of the pattern observed in the 3D-$^{1}H,^{15}N$-NOESYHSQC spectrum, the new set of peaks was assigned and the difference in chemical shift was measured (Figure 5).

Although changes may arise from both structural rearrangement of the protein as it harbors the ligand or allosteric effects, it is reasonable to think that the largest shifts correspond to regions in close contact with **9**.

Differences were graphically mapped onto the structure of MMP-12 (PDB code: 1RMZ) (Figure 6A). Dramatic changes are revealed in the region close to the catalytic zinc atom (179−183 region), where binding takes place, and in the S1′ pocket (214−217, 236−242 region). It is important to notice that these changes are not relative to the free form of the protein (which is not stable) but to its complex with the weaker acetohydroxamic inhibitor. Accordingly, what we observe reflects the structural differences of the two inhibitors.

As **9** contains three aromatic rings, it is expected to cause sizable changes in the shifts of the protein due to the very well-known anisotropic properties of this chemical moiety. In addition, the induced shift is highly orientation dependent, causing shielding in the region above and under the plane of the ring and deshielding in the region along the plane. Figure 6B shows that the sign of the largest shifts reflects the orientation of each **9** ring. The figure also shows how the best results obtained from docking calculations indeed agree with the observed chemical shift deviations. For example, residues L181 and K241 display the most sizable proton deviations (negative, indicating shielding): −1.09 ppm and −1.70 ppm, respectively. Figure 6B clearly

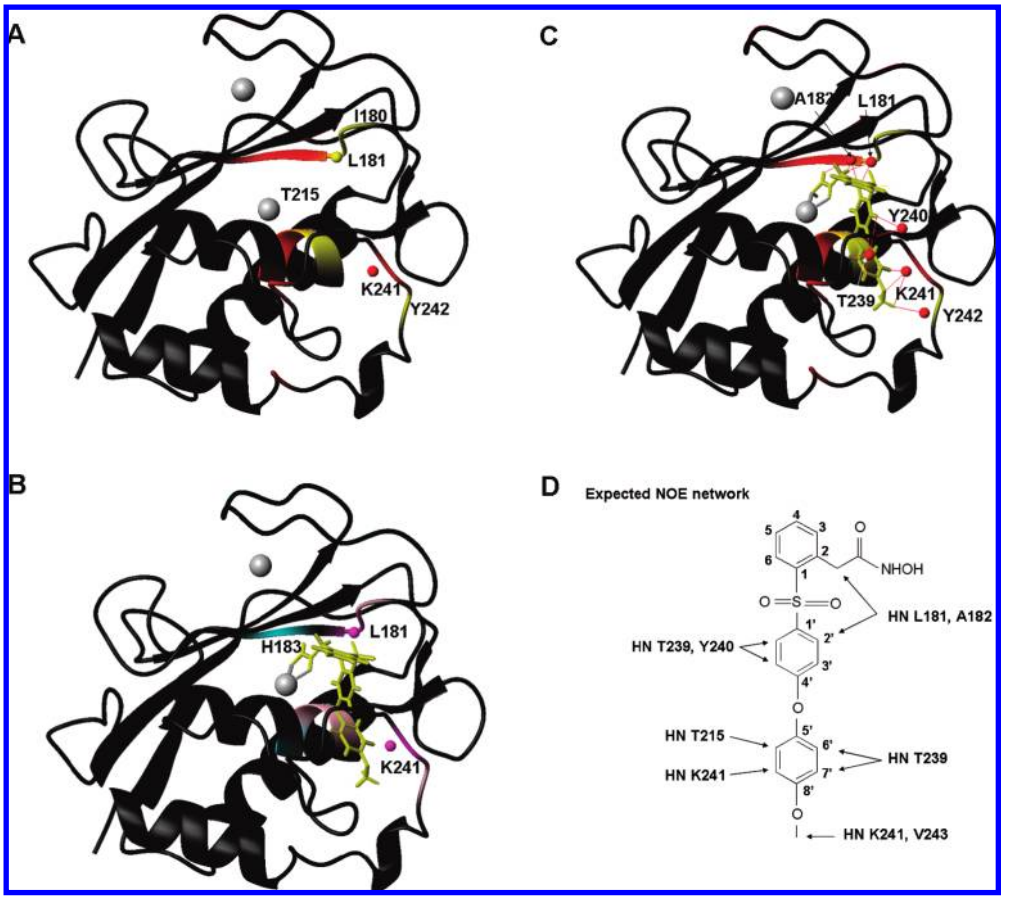


Figure 6. (A) Combined chemical shift deviation mapped onto the MMP-12 structure (PDB code: 1RMZ). Combined deviation larger than 2 and 0.3 are displayed in yellow and red, respectively. (B) Positive nitrogen and proton chemical shift deviations larger than 1 and 0.5, respectively, are displayed in magenta; nitrogen chemical shift variations more negative than −1 (no hydrogen display shifts more negative than −0.5) are displayed in cyan. (C) Distances smaller than 0.4 nm among MMP-12 amide protons and **9** hydrogen atoms are displayed as red lines. Amide nitrogens expected to give rise to intermolecular NOE effects are shown as red spheres. Color coding for the protein ribbons are according to panel A. (D) Expected NOE effect based on distances found in the best docked structure of **9** with the MMP-12 catalytic domain. In panels A, B, and C, amide protons are displayed as spheres.

shows that these are indeed the only two amide protons residing on top of two aromatic rings of the inhibitor, where the shielding effect is the largest. Accordingly, the largest positive deviation (1.1 ppm, indicating deshielding) is found for the amide nitrogen of residue H183, which lies in the plane of the aromatic ring directly linked to the hydroxamic moiety.

The docked structure suggests hydrogen bonding of one sulfonyl oxygen of **9** to either the amide of residue L181 or A182 (or both). To ascertain its presence, we studied the temperature dependence of amide proton chemical shifts. We found temperature coefficients of −9.2 ppb/K and −2.4 ppb/K, respectively, in the absence of **9**. In the bound form these values become −12.3 ppb/K and −2.2 ppb/K. Because temperature coefficients more positive than −4.5 ppb/K suggest the presence of hydrogen bonds,[30] we conclude that amide A182 is hydrogen bonded both in the complex with AHA (our reference) and in that with **9**, while amide L181 appears to be uninvolved in both cases. Interestingly, in the solid state, the X-ray structure of AHA indicates a hydrogen bond between amide L181 (rather than A182) and a water molecule (PDB code 1Y93[11]). Apparently, because the two amides are very close in space, this H-bond can be established with residue A182 in solution. However, what matters for the present discussion is that amide A182 appears to be hydrogen bonded in the complex with **9**, in agreement with docking calculations.

To further confirm the found structure, we looked for intermolecular NOE cross peaks. Figure 6C highlights residues whose amide proton is at least 0.4 nm apart from the nonexchangeable hydrogen atoms of the inhibitor. The expected NOEs are reported in panel D.

Figure 7 shows the superimposition of 3D-^{1}H,^{15}N-NOESYHSQC strips for MMP-12 in the absence and in the presence of **9** in the regions of interest. Indeed, new cross peaks appear in the presence of the inhibitor. Because slow exchange is occurring, the signals arising from bound **9** are likely to be significantly shifted from those found in its free form. On the other hand, we can still classify the new cross peaks as aromatic or aliphatic and use this information to confirm our structure.

Figure 7 shows how the expected NOE pattern in Figure 6D is in very good agreement with the experimental observations. The amide protons L181 and A182 "see" an extra peak at 8.4 (labeled a, b in panel A of Figure 7), and HN Y240 and K241 "see" aromatic peaks at around 7 ppm (labeled b, d in panel B of Figure 7). Amide F237 shows a weak extra aromatic peak (labeled a in panel B of Figure 7), which was not expected but possible because its distance from the 7′ proton is 0.58 nm in the structure. The extra peak labeled e in panel B of Figure 7 is probably due to amide Y242 seeing its own aromatic side chain.

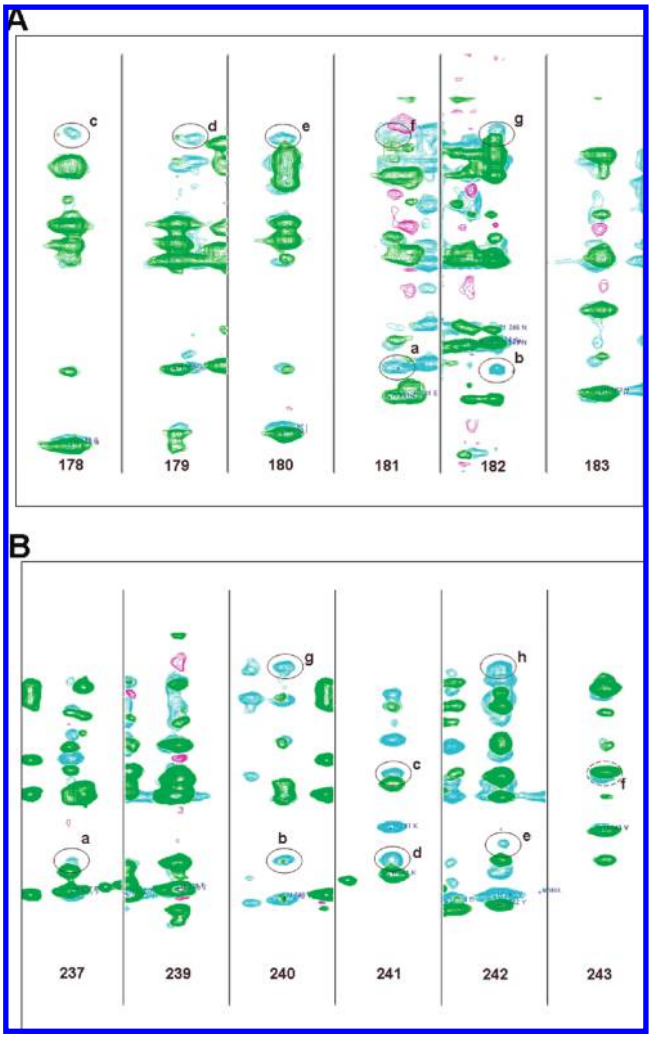


Figure 7. Superimposition of 3D-^{1}H,^{15}N-NOESYHSQC strips for MMP-12 in the absence (green) and presence (cyan) of **9** relative to the regions of interests (negative contours in magenta). (A) Residues 178−183. (B) Residues 237−243. New cross peaks arising in the presence of the inhibitor are highlighted with circles. Residue number is displayed for each amide nitrogen of the strips.

Finally, the methyl protons of the methoxy group are seen by residues Y240 and V243 (labeled c, f in panel B of Figure 7). As for the cross peaks connecting the methylene of **9** and amides L181, A182, and H6′ with amide T215, strong overlap does not allow for verification (data not shown for residue T215).

NOE data also reveal that the presence of the inhibitor causes some extra modifications in the structure: amide protons Y240 and Y242 see extra peaks at 0.2 ppm (g, h in panel B of Figure 7), which are most likely ascribable to the methyls of L214, indicating that the loop is slightly deformed to accommodate the inhibitor. In addition, the region from G178 to A182 comes closer to the methyls of I180 (c, d, e, f, g in panel A of Figure 7). This indicates that the side chain of I180, which in the free form of the protein fills a cavity near the catalytic zinc, moves toward the loop in the region 178−182 in order to make room for **9**.

4. Conclusions

A new series of arylsulfones was designed and synthesized as potential MMP-12 selective inhibitors. The first benzoic derivatives showed poor activity on MMPs. This activity was greatly increased by the introduction of a methylene between the ZBG and the aromatic ring, allowing for a better interaction of the ZBG with the catalytic zinc ion. Five phenylacetic hydroxamates were identified to have nanomolar IC_{50} values on MMP-12. Among them, the most promising inhibitor was compound **9**, with an IC_{50} value of 0.2 nM, a 17500-fold selectivity for MMP-12 over MMP-1, and a 165-fold selectivity over MMP-14. Moreover, this phenoxybenzenesulfonyl compound was a subnanomolar inhibitor of MMP-9, another MMP implicated in the pathogenesis of COPD. The introduction of a small alkyl group, such as a methyl, α to the hydroxamate (as in compounds **12** and **13**), had only a small effect on inhibitory potency. The carboxylic analogues of phenylacetic hydroxamates were also tested on the principal MMPs, showing a decrease in activity but also a different inhibitory profile with respect to the corresponding hydroxamates. In fact, in the carboxylic series, the most potent compound on MMP-12 was the *p*-methoxybiphenyl derivative **17** (IC_{50} = 139 nM). Docking of **9** into the MMP-12 catalytic domain revealed the formation of H-bonds between the sulfonyl group and L181 and A182, whereas the *p*-methoxyphenoxybenzene group was inserted into the S1′ pocket. NMR experiments on the ^{15}N-labeled wt-MMP-12 catalytic domain complexed with **9** provided experimental support for the docking calculations, both through induced shifts and NOE effects. NOE data also revealed that the presence of the inhibitor causes some small extra modifications in the enzyme structure. Further investigations could lead to optimization of the inhibitory activity of this new class of arylsulfones toward MMP-12 to be used in treatment of some inflammatory diseases such as COPD, RA and atherosclerosis.

5. Experimental Section

Chemistry. Melting points were determined on a Kofler hotstage apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were determined with a Varian Gemini 200 MHz spectrometer. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane and referenced from solvent references. Coupling constants *J* are reported in hertz; ^{13}C NMR spectra were fully decoupled. The following abbreviations are used: singlet (s), doublet (d), triplet (t), double−doublet (dd), broad (br), and multiplet (m).Where indicated, the elemental compositions of the compounds agreed to within ±0.4% of the calculated value. Chromatographic separations were performed on silica gel columns by flash column chromatography (Kieselgel 40, 0.040−0.063 mm; Merck). Reverse phase chromatography were performed using ISOLUTE SPE columns Flash C18 (STEPBIO). Reactions were followed by thin-layer chromatography (TLC) on Merck aluminum silica gel (60 F254) sheets that were visualized under a UV lamp, and hydroxamic acids were visualized with $FeCl_3$ acqueous solution. Evaporation was performed in vacuo (rotating evaporator). Sodium sulfate was always used as the drying agent. Microwave-assisted reactions were run in a CEM Discover LabMate microwave synthesizer. The purity of the final compounds was determined by reverse-phase HPLC on a Merck Hitachi D-7000 liquid chromatograph. HPLC purity was determined to be >95% for all final products using a Discovery C18 column (250 mm × 4.6 mm, dp = 5 μm, Supelco), with a gradient of 40% water/60% methanol at a flow rate of 1.4 mL/min, with UV monitoring at the fixed wavelength of 250 nm. See the Supporting Information for compound purity analysis data for final compounds. Commercially available chemicals were purchased from Sigma-Aldrich.

2-(4-Methoxyphenylthio)benzoic Acid (20). A suspension of 2-iodobenzoic acid (1.0 g, 4.03 mmol), 4-methoxythiophenol (565 mg, 4.03 mmol), KOH (451 mg, 8.1 mmol), and copper powder (25.6 mg, 0.403 mmol) in deionized water (2 mL) was submitted to microwave irradiation (two cycles of 6 min each at the power of 180 W, $T_{max} = 140$ °C, 100 psi), cooling the sample during irradiation with a flow of compressed air. The obtained suspension was dissolved in a solution of KOH 2N and then filtered. The filtrate was acidified with HCl 1N, and the formed precipitate was filtrated and dried under reduced pressure ($T =$ 50 °C). The crude solid was purified by trituration with acetone to yield **20** (850 mg, 81% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 3.82 (s, 3H), 6.63 (d, $J = 8.0$ Hz, 1H), 7.06−7.20 (m, 3H), 7.30−7.40 (m, 1H), 7.45−7.49 (m, 2H), 7.91 (dd, $J_1 =$ 7.8 Hz, $J_2 = 1.0$ Hz, 1H).

2-(4-Methoxy-benzenesulfonyl)benzoic Acid (21). A solution of oxone (2.2 g, 3.6 mmol) in H_2O (15 mL) was added slowly to a solution of **20** (300 mg, 1.15 mmol) in THF/MeOH (1:1, 12 mL). The reaction was stirred at room temperature for 24 h, and the organic solvents were evaporated under reduced pressure. The obtained suspension was diluted with H_2O, and the product was extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure to afford **21** as a white solid (202 mg, 60% yield). ^{1}H NMR (DMSO-d_6) δ: 3.83 (s, 3H), 7.11−7.15 (m, 2H), 7.56−7.73 (m, 3H), 7.90−7.94 (m, 2H), 8.07−8.11 (m, 1H).

***N*-Hydroxy-2-(4-methoxy-benzenesulfonyl)benzamide (2).** 1-[3-(Dimethylamino)propyl]-3-ethyl carbodiimide hydrochloride (EDC) was added portionwise (65 mg, 0.34 mmol) to a stirred and cooled solution (0 °C) of the carboxylic acid **21** (100 mg, 0.34 mmol) and *O*-(*tert*-butyldimethylsilyl)hydroxylamine (50 mg, 0.34 mmol) in freshly distilled CH_2Cl_2 (3 mL). After stirring at room temperature overnight, the mixture was washed with water and the organic phase was dried and evaporated in vacuo to afford the *O*-silylate (82 mg, 57% yield) as an oil. ^{1}H NMR ($CHCl_3$) δ: 0.25 (s, 6H), 0.97 (s, 9H), 3.82 (s, 3H), 7.10−7.15 (m, 2H), 7.37−7.42 (m, 1H), 7.65−7.70 (m, 2H), 7.95−8.08 (m, 3H), 11.29 (s, 1H).

TFA (0.8 mL, 10.8 mmol) was added dropwise to a stirred and ice-chilled solution of *O*-silylate (81 mg, 0.19 mmol) in CH_2Cl_2 (4 mL). The solution was stirred under these reaction conditions for 5 h, and the solvent was removed in vacuo to give a solid. The crude product was triturated with *n*-hexane/$CHCl_3$ to give **2** (30 mg, 28% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 3.82 (s, 3H), 7.08−7.13 (m, 2H), 7.37−7.41 (m, 1H), 7.64−7.69 (m, 2H), 7.96−8.08 (m, 3H), 9.17 (s, 1H), 10.91 (br s, 1H). Anal. ($C_{14}H_{13}NO_5S$) C, H, N.

2-(4-Hydroxy-phenylsulfanyl)benzoic Acid (22). Compound **22** was synthesized following an analogous procedure to that described above for the preparation of **20**, starting from 2-iodobenzoic acid (1.5 g, 6.05 mmol) and 4-mercaptophenol (763 mg, 6.05 mmol). The crude solid was purified by trituration with acetone to yield **22** (1.11 g, 75% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 6.65 (d, $J = 7.0$ Hz, 1H), 6.86−6.91 (m, 2H), 7.09−7.20 (m, 1H), 7.29−7.40 (m, 3H), 7.90 (d, $J = 7.8$ Hz, 1H), 9.98 (br s, 1H).

2-(4-Phenoxy-phenylsulfanyl)benzoic Acid (23). To a suspension of **22** (600 mg, 2.43 mmol), phenylboronic acid (592 mg, 4.86 mmol), $Cu(OAc)_2$ (485 mg, 2.43 mmol), and 4 Å molecular sieves in powder (1.0 g) in anhydrous CH_2Cl_2 (25 mL) was added triethylamine (1.7 mL, 12.1 mmol). The reaction mixture was stirred overnight at room temperature and filtered. The filtrated was treated with HCl 1N and extracted with CH_2Cl_2. The organic phases were dried with Na_2SO_4 and evaporated under reduced pressure. The obtained crude was purified by trituration with *n*-hexane to give **23** (236 mg, 30% yield) as a solid. ^{1}H NMR (DMSO-d_6) δ: 6.73 (d, $J = 8.0$ Hz, 1H), 7.06−7.25 (m, 6H), 7.35−7.56 (m, 5H), 7.91 (d, $J = 7.8$ Hz, 1H).

2-(4-Phenoxy-benzenesulfonyl)benzoic Acid (24). A solution of oxone (455 mg, 0.74 mmol) in H_2O (3 mL) was added slowly to a solution of **23** (200 mg, 0.62 mmol) in THF/MeOH (1:1, 8 mL). The reaction was stirred at room temperature for 24 h, and the organic solvents were evaporated under reduced pressure. The obtained suspension was diluted with H_2O, and the product was extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure to afford **24** as a white solid (190 mg, 86% yield). ^{1}H NMR (DMSO-d_6) δ: 6.98−7.07 (m, 3H), 7.13−7.23 (m, 2H), 7.37−7.45 (m, 2H), 7.58−7.70 (m, 3H), 7.94−8.02 (m, 2H), 8.30 (d, $J = 6.8$ Hz, 1H).

***N*-Hydroxy-2-(4-phenoxy-benzenesulfonyl)benzamide (3).** Compound **3** was synthesized according to the procedure described above for the preparation of **2**, starting from carboxylic acid **24** (180 mg, 0.51 mmol). The *O*-silylate intermediate was purified by trituration with *n*-hexane/CH_2Cl_2 to afford the protected hydroxamate (40 mg) as an oil. ^{1}H NMR (DMSO-d_6) δ: 0.24 (s, 6H), 0.97 (s, 9H), 6.98−7.06 (m, 3H), 7.11−7.23 (m, 2H), 7.37−7.45 (m, 2H), 7.57−7.79 (m, 5H), 7.97−8.25 (m, 1H).

After treatment with TFA, the crude product was triturated with *n*-hexane to give **3** (15 mg, 12.5% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 7.02−7.12 (m, 3H), 7.15−7.23 (m, 2H), 7.40−7.51 (m, 2H), 7.57−7.79 (m, 5H), 8.01−8.25 (m, 1H), 10.2 (br. s, 1H). Anal. ($C_{19}H_{15}NO_5S$) C, H, N.

2-[4-(4-Methylsulfanyl-phenoxy)-phenylsulfanyl]benzoic Acid (25). Compound **25** was synthesized according to the procedure described above for the preparation of **23**, starting from phenolic intermediate **22** (400 mg, 1.6 mmol) and 4-(methylthio)phenylboronic acid (539 mg, 3.2 mmol). The obtained crude was purified by C18 reverse phase chromatography ($MeOH/H_2O$ 3:7, $MeOH/H_2O$ 1:1, $MeOH/H_2O$ 9:1, MeOH 100%) to yield **25** as solid (225 mg, 38%). ^{1}H NMR ($CDCl_3$) δ: 2.50 (s, 3H), 6.82 (d, $J = 8.0$ Hz, 1H), 7.02−7.06 (m, 3H), 7.12−7.36 (m, 5H), 7.50−7.54 (m, 2H), 8.11 (d, $J = 7.8$ Hz, 1H).

2-[4-(4-Methanesulfinyl-phenoxy)-benzenesulfonyl]benzoic Acid (26). A solution of oxone (2 g, 3.2 mmol) in H_2O (20 mL) was added slowly to a solution of **25** (200 mg, 0.54 mmol) in THF/MeOH (1:1, 6 mL). The reaction was stirred at room temperature for 24 h, and the organic solvents were evaporated under reduced pressure. The obtained suspension was diluted with H_2O, and the product was extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure to afford **26** as a mixture of SO and SO_2 derivatives of which the first is the prevalent one (114 mg, 70% yield). ^{1}H NMR (DMSO-d_6) δ: 3.20 (s, 3H), 7.17−7.23 (m, 4H), 7.64−7.71 (m, 3H), 7.89−8.04 (m, 4H), 8.30 (d, $J = 7.6$ Hz, 1H), 13.80 (s, 1H).

***N*-Hydroxy-2-[4-(4-methanesulfinyl-phenoxy)-benzenesulfonyl]-benzamide (5).** Compound **5** was synthesized according to the procedure described above for the preparation of **2**, starting from carboxylic acid **26** (114 mg, 0.26 mmol). The *O*-silylate intermediate was purified by flash chromatography on silica gel (CH_2Cl_2: EtOH 95:5, CH_2Cl_2:EtOH 9:1, CH_2Cl_2:EtOH 8:2) to afford the protected SO hydroxamate as an oil. ^{1}H NMR ($CDCl_3$) δ: 0.25 (s, 3H), 0.26 (s, 3H), 1.01 (s, 9H), 3.04 (s, 3H), 7.03−7.21 (m, 4H), 7.50−8.18 (m, 8H), 9.55 (br. s, 1H). After treatment with TFA, the crude product was triturated with Et_2O to give **5** (8.4 mg, 7% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 3.20 (s, 3H), 7.17−7.34 (m, 5H), 7.48−8.00 (m, 6H), 8.13 (d, $J = 7.3$ Hz, 1H), 9.4 (br s, 1H), 11.46 (s, 1H). Anal. ($C_{20}H_{17}NO_6S_2$) C, H, N.

[2-(4-Hydroxy-phenylsulfanyl)-phenyl]acetic Acid (27). Compound **27** was synthesized following an analogous procedure to that described above for the preparation of **20**, starting from 2-iodophenylacetic acid (1.0 g, 3.8 mmol) and 4-mercaptophenol (482 mg, 3.82 mmol). The crude solid was purified by trituration with acetone to yield **27** (2.5 g, 84% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 3.73 (s, 2H), 6.77−6.81 (m, 2H), 6.92−7.00 (m, 1H), 7.14−7.32 (m, 5H), 9.80 (br s, 1H), 12.38 (br s, 1H).

2-[4-(4-Methoxy-phenoxy)-phenylsulfanyl]benzoic Acid (28). Compound **28** was synthesized according to the procedure described above for the preparation of **23**, starting from

phenolic intermediate **22** (400 mg, 1.6 mmol) and 4-(methoxy)-phenylboronic acid (494 mg, 3.2 mmol). The obtained crude was purified by C18 reverse phase chromatography (MeOH/H_2O 3:7) to yield **28** as solid (190 mg, 33%). ^{1}H NMR ($CDCl_3$) δ: 3.82 (s, 3H), 6.80 (d, J = 8.0 Hz, 1H), 6.90−7.18 (m, 7H), 7.26−7.35 (m, 1H), 7.45−7.52 (m, 2H), 8.12 (dd, J_1 = 7.7 Hz, J_2 = 1.6 Hz, 1H).

{2-[4-(4-Methoxy-phenoxy)phenylsulfanyl]phenyl}acetic Acid (29). Compound **29** was synthesized according to the procedure described above for the preparation of **23**, starting from phenolic intermediate **27** (1.9 g, 7.3 mmol) and 4-(methoxy)phenylboronic acid (2.2 g, 14.6 mmol). The obtained crude was purified by flash chromatography on silica gel (CH_2Cl_2/Et_2O 9:1, CH_2Cl_2/Et_2O 8:2, CH_2Cl_2/MeOH 9:1) to yield **29** as solid (840 mg, 31%). ^{1}H NMR (DMSO-d_6) δ: 3.75 (s, 5H), 6.88−7.06 (m, 6H), 7.12−7.36 (m, 6H), 12.34 (s, 1H).

2-[4-(4-Methoxy-phenoxy)phenylsulfanyl]benzoic Acid Benzyl Ester (30). A solution of carboxylic acid **28** (162 mg, 0.46 mmol) in anhydrous DMF (1.0 mL) was cooled to 0 °C. Cesium carbonate (150 mg, 0.46 mmol) was added, and the mixture was stirred for 40 min at 0 °C. Benzyl bromide (0.05 mL, 0.46 mmol) was added, and the reaction mixture was stirred at 0 °C for 30 min and then at room temperature overnight. The mixture was poured into H_2O and extracted with Et_2O. The combined extracts were washed with H_2O and saturated NaCl, dried over Na_2SO_4, filtered, and evaporated under reduced pressure to afford **30** (183 mg, 91% yield) as a yellow solid that was utilized in the next step without further purification. ^{1}H NMR ($CDCl_3$) δ: 3.82 (s, 3H), 5.40 (s, 2H), 6.80 (d, J = 7.1 Hz, 1H), 6.90−7.13 (m, 7H), 7.21−7.30 (m, 1H), 7.34−7.51 (m, 7H), 8.02 (dd, J_1 = 7.7 Hz, J_2 = 1.6 Hz, 1H).

{2-[4-(4-Methoxy-phenoxy)phenylsulfanyl]phenyl}acetic Acid Benzyl Ester (31). Compound **29** (1.56 g, 4.27 mmol) was submitted to the same procedure described above for the preparation of **30**, to afford benzylic ester **31** as a yellow solid (1.7 g, 97% yield). ^{1}H NMR ($CDCl_3$) δ: 3.80 (s, 3H), 3.90 (s, 2H), 5.12 (s, 2H), 6.78−7.00 (m, 6H), 7.17−7.32 (m, 11H).

2-[4-(4-Methoxy-phenoxy)benzenesulfonyl]benzoic Acid Bbenzyl Ester (32). Compound **32** was synthesized according to the procedure described above for the preparation of **24**, starting from benzylic ester **30** (180 mg, 0.41 mmol) and using 15 equiv of oxone. After stirring at room temperature for 3 days, the reaction was treated as described above to give **32** as a white solid (148 mg, 77% yield). ^{1}H NMR ($CDCl_3$) δ: 3.82 (s, 3H), 5.40 (s, 2H), 6.83−6.99 (m, 6H), 7.32−7.46 (m, 5H), 7.53−7.61 (m, 3H), 7.84−7.89 (m, 2H), 8.05−8.09 (m, 1H).

{2-[4-(4-Methoxy-phenoxy)benzenesulfonyl]phenyl}acetic Acid Benzyl Ester (33). Compound **33** was synthesized according to the procedure described above for the preparation of **32**, starting from benzylic ester **31** (200 mg, 0.44 mmol). The crude product was triturated with Et_2O to give sulfone **33** (206 mg, 96% yield) as white solid. ^{1}H NMR ($CDCl_3$) δ: 3.81 (s, 3H), 4.08 (s, 2H), 5.03 (s, 2H), 6.87−6.99 (m, 6H), 7.30−7.35 (m, 5H), 7.43−7.59 (m, 3H), 7.72−7.78 (m, 2H), 8.13 (dd, J_1 = 7.7 Hz, J_2 = 1.6 Hz, 1H).

2-[4-(4-Methoxy-phenoxy)benzenesulfonyl]benzoic Acid (34). A suspension of ester **32** (140 mg, 0.3 mmol) and KOH (50 mg, 0.9 mmol) in H_2O (1.0 mL) was refluxed for 18 h. After being cooled to RT, the reaction was treated with HCl 1N and then extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and concentrated to give a crude product, which was purified by trituration with Et_2O/*n*-hexane to yield **34** (106 mg, 94% yield) as yellow oil. ^{1}H NMR ($CDCl_3$) δ: 3.80 (s, 3H), 6.87−7.00 (m, 5H), 7.31−7.42 (m, 1H), 7.63−7.75 (m, 3H), 7.90−7.95 (m, 2H), 8.16−8.20 (m, 1H).

{2-[4-(4-Methoxy-phenoxy)benzenesulfonyl]phenyl}acetic Acid (15). Compound **15** was synthesized according to the procedure described above for the preparation of **34**, starting from benzylic ester **33** (200 mg, 0.41 mmol). The crude product was purified by trituration with Et_2O/*n*-hexane to yield **15** (133 mg, 81% yield) as white solid; mp: 159 °C. ^{1}H NMR (DMSO-d_6) δ: 3.76 (s, 3H), 3.91 (s, 2H), 6.98−7.13 (m, 6H), 7.41−7.59 (m, 3H), 7.79−7.83 (m, 2H), 8.02 (d, J = 7.4 Hz, 1H), 12.33 (s, 1H). ^{13}C NMR (DMSO-d_6) δ: 37.88, 55.45, 115.28, 116.83, 121.73, 127.93, 128.99, 129.74, 133.47, 134.02, 134.12, 139.17, 147.33, 156.47, 162.24, 171.29.

***N*-Hydroxy-2-[4-(4-methoxy-phenoxy)benzenesulfonyl]benzamide (4).** Compound **4** was synthesized according to the procedure described above for the preparation of **2**, starting from carboxylic acid **34** (100 mg, 0.26 mmol). The *O*-silylate intermediate was treated with TFA, and the crude product was triturated with diisopropyl ether to give **4** (12 mg, 12% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 3.82 (s, 3H), 6.93−7.13 (m, 5H), 7.33−7.51 (m, 5H), 7.85−7.89 (m, 1H), 8.01−8.20 (m, 1H), 12.33 (s, 1H). Anal. ($C_{20}H_{17}NO_6S$) C, H, N.

***N*-Hydroxy-2-{2-[4-(4-methoxy-phenoxy)benzenesulfonyl]-phenyl}acetamide (9).** Compound **9** was synthesized according to the procedure described above for the preparation of **4**, starting from carboxylic acid **15** (500 mg, 1.06 mmol). The *O*-silylate intermediate was treated with TFA, and the crude product was purified by trituration with diisopropyl ether to give **9** (78 mg, 15% yield) as a white solid; mp: 75 °C; NMR assignment of a 15 mM solution in DMSO-d_6 (atom numbering as in Figure 5D. The assignment reports the shifts of the main form): ^{1}H: DMSO-d_6 (600 MHz) δ 10.55 (1H, HN), 8.83 (1H, OH), 4.05, 3.69 (2H, CH_2), 7.38 (1H, H-3), 7.64 (1H, H-4), 7.53 (1H, H-5), 8.03 (1H, H-6), 7.88 (2H, H-2′), 7.03 (2H, H-3′), 7.11 (2H, H-6′), 7.00 (2H, H-7′), 3.77 (3H, Me), 9.95 (1H, HN Z form), 9.18 (1H, OH Z form); ^{13}C: DMSO-d_6 (600 MHz) δ 165.8 (1C, carbonyl), 35.3 (1C, CH_2), 139.4 (1C, C-1), 133.2 (1C, C-2), 132.4 (1C, C-3), 133.0 (1C, C-4), 127.3 (1C, C-5), 128.7 (1C, C-6), 134.0 (1C, C-1′), 129.5 (2C, C-2′), 116.4 (2C, C-3′), 162.2 (1C, C-4′), 146.8 (1C, C-5′), 121.4 (2C, C-6′), 114.9 (2C, C-7′), 156.8 (1C, C-8′), 55.0 (1C, Me). Anal. ($C_{21}H_{19}NO_6S$) C, H, N.

2-(2-(4-Methoxyphenylthio)phenyl)acetic Acid (35). Compound **35** was synthesized following an analogous procedure to that described above for the preparation of **20**, starting from 2-iodophenylacetic acid (1.0 g, 3.8 mmol) and 4-methoxythiophenol (469 μL, 3.8 mmol). The crude solid product so obtained, consisting of **35** (870 mg, 83% yield), was utilized in the next step without further purification; mp: 86 °C. ^{1}H NMR ($CDCl_3$) δ: 3.78 (s, 3H), 3.87 (s, 2H), 6.82−6.86 (m, 2H), 7.16−7.30 (m, 6H).

Benzyl 2-(2-(4-Methoxyphenylthio)phenyl)acetate (36). Ester **36** was synthesized following an analogous procedure to that described above for the preparation of **30**, starting from carboxylic acid **35** (700 mg, 2.5 mmol). The crude oil product so obtained, consisting of **36** (691 mg, 74% yield), was utilized in the next step without further purification. ^{1}H NMR ($CDCl_3$) δ: 3.79 (s, 3H), 3.89 (s, 2H), 5.13 (s, 2 H), 6.81−6.85 (m, 2H), 7.15−7.28 (m, 7H), 7.33 (m, 4H).

Benzyl 2-(2-(4-Methoxyphenylsulfonyl)phenyl)acetate (37). A solution of oxone (9.3 g, 15.2 mmol) in H_2O (37 mL) was added slowly to a solution of **36** (691 mg, 1.9 mmol) in THF/MeOH (3:1, 36 mL). The reaction was stirred at room temperature for 3 days and the organic solvents were evaporated under reduced pressure. The obtained suspension was diluted with H_2O, and the product was extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure to afford **37** as a yellow oil (682 mg, 91% yield). ^{1}H NMR ($CDCl_3$) δ: 3.80 (s, 3H), 4.07 (s, 2H), 5.01 (s, 2H), 6.86−6.92 (m, 2H), 7.28−7.37 (m, 6H), 7.42−7.57 (m, 2H), 7.74−7.81 (m, 2H), 8.12 (dd, J_1 = 7.5 Hz, J_2 = 1.8 Hz, 1H).

2-(2-(4-Methoxyphenylsulfonyl)phenyl)acetic Acid (14). Compound **14** was synthesized according to the procedure described above for the preparation of **34**, starting from benzylic ester **37** (681 mg, 1.7 mmol). The crude product was purified by trituration with Et_2O to yield **14** (355 mg, 89% yield) as white solid; mp 146 °C. ^{1}H NMR ($CDCl_3$) δ: 3.84 (s, 3H), 4.03 (s, 2H), 6.93−6.99 (m, 2H), 7.35 (d, J = 7.5 Hz, 1H), 7.43−7.60 (m, 2H), 7.76−7.83 (m, 2H), 8.10 (dd, J_1 = 7.7 Hz, J_2 = 1.5 Hz, 1H).

^{13}C NMR (DMSO-d_6) δ: 37.84, 55.76, 114.75, 127.88, 128.88, 129.45, 132.36, 133.32, 133.98, 139.46, 162.95, 171.32.

***N*-Hydroxy-2-(2-(4-methoxyphenylsulfonyl)phenyl)acetamide (8).** Compound **8** was synthesized according to the procedure described above for the preparation of **2**, starting from carboxylic acid **14** (150 mg, 0.5 mmol). The *O*-silylate intermediate was utilized in the next step without further purification. ^{1}H NMR ($CDCl_3$) δ: 0.12 (s, 6H), 0.91 (s, 9H), 3.72 (s, 2H), 3.86 (s, 3H), 6.97−7.02 (m, 2H), 7.36−7.44 (m, 1H), 7.50−7.56 (m, 1H), 7.61−7.65 (m, 1H), 7.77−7.81 (m, 2H), 7.94 (d, J = 7.7 Hz, 1H), 9.13 (s, 1H). After treatment with TFA, the crude product was triturated with Et_2O/*n*-hexane to give **8** (96 mg, 91% yield) as a white solid; mp 109 °C. ^{1}H NMR (DMSO-d_6) δ: 3.67 (s, 2H), 3.83 (s, 3H), 7.09−7.13 (m, 2H), 7.35 (d, J = 7.7 Hz, 1H), 7.47−7.66 (m, 2H), 7.82−7.87 (m, 2H), 8.02 (d, J = 7.7 Hz, 1H), 10.58 (s, 1H). ^{13}C NMR (DMSO-d_6) δ: 35.66, 55.74, 114.70, 127.52, 128.83, 129.63, 132.36, 132.69, 133.18, 134.65, 139.55, 162.93, 165.77. Anal. ($C_{15}H_{15}NO_5S$) C, H, N.

2-(4-Bromo-phenylsulfanyl)benzoic Acid (38). Compound **38** was synthesized following an analogous procedure to that described above for the preparation of **20**, starting from 2-iodobenzoic acid (3.0 g, 12.1 mmol) and 4-bromobenzenethiol (2.2 g, 12.1 mmol). The crude solid was purified by trituration with acetone to yield **38** (3.1 g, 82% yield) as a white solid; mp 219 °C. ^{1}H NMR (DMSO-d_6) δ: 6.77 (d, J = 7.3 Hz, 1H), 7.20−7.28 (m, 1H), 7.36−7.49 (m, 3H), 7.65−7.71 (m, 2H), 7.91 (dd, J_1 = 7.7 Hz, J_2 = 1.5 Hz, 1H), 13.26 (br s, 1H).

[2-(4-Bromo-phenylsulfanyl)phenyl]acetic Acid (39). Compound **39** was synthesized following an analogous procedure to that described above for the preparation of **20**, starting from 2-iodophenylacetic acid (5.0 g, 19.1 mmol) and 4-bromobenzenthiol (3.6 g, 19.1 mmol). The crude solid product so obtained, consisting of **39** (4.3 g, 88% yield), was utilized in the next step without further purification. ^{1}H NMR (DMSO-d_6) δ: 3.74 (s, 2H), 7.07 (d, J = 8.1 Hz, 2H), 7.30−7.42 (m, 4H), 7.49 (d, J = 8.1 Hz, 2H).

2-(4-Bromo-phenylsulfanyl)benzoic Acid Benzyl Ester (40). Compound **38** (3.0 g, 9.7 mmol) was submitted to the same procedure described above for the preparation of **30** to afford benzylic ester **40** as a yellow solid (3.7 g, 94% yield); mp 69 °C. ^{1}H NMR ($CDCl_3$) δ: 5.39 (s, 2H), 6.83 (d, J = 8.0 Hz, 1H), 7.10−7.18 (m, 1H), 7.23−7.58 (m, 10H), 8.01 (dd, J_1 = 7.7 Hz, J_2 = 1.6 Hz, 1H).

[2-(4-Bromo-phenylsulfanyl)phenyl]acetic Acid Benzyl Ester (41). Compound **39** (3.3 g, 10.2 mmol) was submitted to the same procedure described above for the preparation of **30**, to afford benzylic ester **41** as a yellow oil (4.1 g, 97% yield). ^{1}H NMR ($CDCl_3$) δ: 3.87 (s, 2H), 5.01 (s, 2H), 6.95−7.00 (m, 2H), 7.27−7.44 (m, 11H).

General Procedure to Synthesize Compounds 42−45 by Suzuki Coupling. A solution of aryl bromide **40** or **41** (1 equiv) in anhydrous dioxane (16 mL/mmol)/H_2O (2.5 mL/mmol) was sequentially treated under nitrogen with K_3PO_4 (2.3 equiv), the appropriate arylboronic acid (1.1 equiv), and $Pd(PPh_3)_4$ (5% equiv). The resulting mixture was stirred for 4 h at 85 °C. After being cooled to room temperature, the mixture was treated with $NaHCO_3$ and extracted with EtOAc The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure.

Benzyl 2-(Biphenyl-4-ylthio)benzoate (42). The title compound was prepared from aryl bromide **40** and phenylboronic acid following the general procedure. The crude oil was purified by flash chromatography on silica gel (CH_2Cl_2/*n*-hexane 3:7) to yield **42** (90% yield) as a yellow oil, which solidified on standing . ^{1}H NMR ($CDCl_3$) δ: 5.41 (s, 2H), 6.91 (d, J = 8.0 Hz, 1H), 7.09−7.17 (m, 1H), 7.23−7.51 (m, 9H), 7.59−7.69 (m, 6H), 8.03 (dd, J_1 = 7.7 Hz, J_2 = 1.6 Hz, 1H).

2-(4′-Methoxy-biphenyl-4-ylsulfanyl)benzoic Acid Benzyl Ester (43). The title compound was prepared from aryl bromide **40** and 4-methoxyphenylboronic acid following the general procedure. The crude oil was purified by flash chromatography on silica gel (CH_2Cl_2/*n*-hexane 6:4) to yield **43** (72% yield) as a solid. ^{1}H NMR ($CDCl_3$) δ: 3.86 (s, 3H), 5.41 (s, 2H), 6.90 (d, J = 8.0 Hz, 1H), 6.98−7.02 (m, 2H), 7.08−7.15 (m, 1H), 7.22−7.60 (m, 12H), 8.02 (dd, J_1 = 7.7 Hz, J_2 = 1.6 Hz, 1H).

[2-(Biphenyl-4-ylsulfanyl)phenyl]acetic Acid Benzyl Ester (44). The title compound was prepared from aryl bromide **41** and phenylboronic acid following the general procedure. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/*n*-hexane 4:6) to yield **44** (72% yield) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 3.93 (s, 2H), 5.01 (s, 2H), 7.20−7.56 (m, 18H).

[2-(4′-Methoxy-biphenyl-4-ylsulfanyl)phenyl]acetic Acid Benzyl Ester (45). The title compound was prepared from aryl bromide **41** and 4-methoxyphenylboronic acid following the general procedure. The crude product was purified by flash chromatography on silica gel (*n*-hexane/EtOAc 9:1) to yield **45** (68% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 3.85 (s, 3H), 3.93 (s, 2H), 5.10 (s, 2H), 6.93−6.98 (m, 2H), 7.19−7.50 (m, 15H).

General Procedure to Synthesize Sulfones 46−49. A solution of oxone (18 equiv) in H_2O (20 mL) was added slowly to a solution of sulfinyl derivatives **42−45** (1 equiv) in THF/MeOH (3:1, 20 mL). The reaction was stirred at room temperature for 3 days, and the organic solvents were evaporated under reduced pressure. The obtained suspension was diluted with H_2O, and the product was extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure.

Benzyl 2-(Biphenyl-4-ylsulfonyl)benzoate (46). The title compound was prepared from sulfinyl derivative **42** following the general procedure. The crude product was purified by trituration with *n*-hexane/Et_2O to yield **46** as a white solid (80% yield); mp 124 °C. ^{1}H NMR ($CDCl_3$) δ: 5.42 (s, 2H), 7.33−7.65 (m, 15H), 7.98−8.02 (m, 2H), 8.13−8.18 (m, 1H).

Benzyl 2-(4′-Methoxybiphenyl-4-ylsulfonyl)benzoate (47). The title compound was prepared from sulfinyl derivative **43** following the general procedure. The crude product was purified by trituration with *n*-hexane/Et_2O to yield **47** as a white solid (82% yield); mp 118 °C. ^{1}H NMR ($CDCl_3$) δ: 3.85 (s, 3H), 5.42 (s, 2H), 6.96−7.00 (m, 2H), 7.33−7.64 (m, 12H), 7.94−7.99 (m, 2H), 8.12−8.16 (m, 1H).

[2-(Biphenyl-4-sulfonyl)phenyl]acetic Acid Benzyl Ester (48). The title compound was prepared from sulfinyl derivative **44** following the general procedure. The oil so obtained, consisting of **48** (90% yield), was utilized in the next step without further purification. ^{1}H NMR ($CDCl_3$) δ: 4.10 (s, 2H), 4.98 (s, 2H), 7.20−7.64 (m, 15H), 7.88−7.92 (m, 2H), 8.20 (dd, J_1 = 7.5 Hz, J_2 = 1.8 Hz, 1H).

[2-(4′-Methoxy-biphenyl-4-sulfonyl)phenyl]acetic Acid Benzyl Ester (49). The title compound was prepared from sulfinyl derivative **45** following the general procedure. The solid product so obtained, consisting of **49** (85% yield), was utilized in the next step without further purification; mp 86 °C. ^{1}H NMR ($CDCl_3$) δ: 3.85 (s, 3H), 4.09 (s, 2H), 4.97 (s, 2H), 6.95−7.01 (m, 2H), 7.20−7.35 (m, 6H), 7.45−7.70 (m, 6H), 7.84−7.89 (m; 2H), 8.21 (dd, J_1 = 7.5 Hz, J_2 = 1.8 Hz, 1H).

General Procedure to Synthesize Carboxylic Acids 50, 51 and 16, 17. A suspension of ester **46−49** (1 equiv) and KOH (3 equiv) in H_2O (3.3 mL/mmol) was refluxed for 18 h. After being cooled to rt, the reaction was treated with HCl 1N and then extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and concentrated .

2-(Biphenyl-4-ylsulfonyl)benzoic Acid (50). The title compound was prepared from benzylic ester **46** following the general procedure. The solid product so obtained, consisting of **50** (86% yield), was utilized in the next step without further purification; mp 195 °C. ^{1}H NMR (DMSO-d_6) δ: 7.43−7.54 (m, 3H), 7.62−7.79 (m, 5H), 7.87−7.91 (m, 2H), 8.02−8.06 (m, 2H), 8.17−8.21 (m, 1H), 13.62 (s, 1H).

2-(4′-Methoxybiphenyl-4-ylsulfonyl)benzoic Acid (51). The title compound was prepared from benzylic ester **47** following the general procedure. The solid product so obtained, consisting of **51** (80% yield), was utilized in the next step without further purification; mp 203 °C. ^{1}H NMR (DMSO-d_6) δ: 3.80 (s, 3H), 7.03−7.07 (m, 2H), 7.61−7.78 (m, 5H), 7.83−7.87 (m, 2H), 7.98−8.02 (m, 2H), 8.15−8.20 (m, 1H).

[2-(Biphenyl-4-sulfonyl)phenyl]acetic Acid (16). The title compound was prepared from benzylic ester **48** following the general procedure. The crude product was purified by trituration with Et_2O to yield **16** as a white solid (88% yield); mp: 182−183 °C. ^{1}H NMR (DMSO-d_6) δ: 3.95 (s, 2H), 7.42−7.77 (m, 8H), 7.86−7.96 (m, 4H), 8.13 (d, J = 7.7 Hz, 1H), 12.38 (br s, 1H). ^{13}C NMR (DMSO-d_6) δ: 38.02, 127.10, 127.72, 127.99, 128.64, 129.04, 129.26, 133.69, 134.09, 134.45, 138.08, 138.70, 139.64, 144.96, 171.34.

[2-(4′-Methoxy-biphenyl-4-sulfonyl)phenyl]acetic Acid (17). The title compound was prepared from benzylic ester **49** following the general procedure. The crude product was purified by trituration with *n*-hexane/Et_2O to yield **17** as a white solid (94% yield); mp 189 °C. ^{1}H NMR (DMSO-d_6) δ: 3.80 (s, 3H), 3.94 (s, 2H), 7.03−7.07 (m, 2H), 7.44 (d, J = 7.3 Hz, 1H), 7.57−7.71 (m, 4H), 7.86 (m, 4H), 8.10 (d, J = 7.5 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ: 37.95, 55.27, 114.51, 126.97, 127.73, 128.03, 128.35, 129.21, 130.25, 133.63, 134.09, 134.31, 138.77, 138.86, 144.59, 159.82, 171.32.

General Procedure to Synthesize Hydroxamic Acids 6, 7 and 10, 11. 1-[3-(Dimethylamino)propyl]-3-ethyl carbodiimide hydrochloride (EDC) was added portionwise (1.5 equiv) to a stirred solution of the carboxylic acid **50**, **51** or **16**, **17** (1 equiv), and *O*-(*tert*-butyldimethylsilyl)hydroxylamine (1.5 equiv) in freshly distilled CH_2Cl_2 (25 mL/mmol). After stirring at room temperature overnight, the mixture was washed with water and the organic phase was dried and evaporated in vacuo to afford the *O*-silylate intermediate. TFA (57 equiv) was added dropwise to a stirred and ice-chilled solution of *O*-silylate (1 equiv) in CH_2Cl_2 (8 mL/mmol). The solution was stirred under these reaction conditions for 5 h, and the solvent was removed in vacuo.

2-(Biphenyl-4-ylsulfonyl)*N*-hydroxybenzamide (6). The title compound was prepared from carboxylic acid **50** following the general procedure. The crude product was purified by trituration with *n*-hexane/Et_2O to yield **6** as a white solid (90% yield); mp 132 °C. ^{1}H NMR (DMSO-d_6) δ: 7.43−7.53 (m, 4H), 7.71−7.74 (m, 4H), 7.86−7.93 (m, 2H), 8.03−8.18 (m, 3H), 10.96 (s, 1H). ^{13}C NMR (DMSO-d_6) δ: 127.11, 127.24, 128.66, 129.04, 129.68, 129.96, 130.48, 133.69, 134.98, 138.29, 138.46, 140.01, 144.92, 164.00. Anal. ($C_{19}H_{15}NO_4S$) C, H, N.

***N*-Hydroxy-2-(4′-methoxybiphenyl-4-ylsulfonyl)benzamide (7).** The title compound was prepared from carboxylic acid **51** following the general procedure. The crude product was purified by trituration with *n*-hexane/Et_2O to yield **7** as a white solid (97% yield); mp 144 °C. ^{1}H NMR (DMSO-d_6) δ: 3.80 (s, 3H), 7.03−7.07 (m, 2H), 7.40−7.45 (m, 1H), 7.67−7.71 (m, 4H), 7.81−7.88 (m, 2H), 8.05−8.16 (m, 3H), 10.95 (s, 1H). ^{13}C NMR (DMSO-d_6) δ: 55.25, 114.50, 126.50, 128.33, 128.66, 129.65, 129.86, 130.45, 133.60, 134.93, 138.60, 139.13, 144.54, 159.74, 164.02. Anal. ($C_{20}H_{16}O_5S$) C, H, N.

2-[2-(Biphenyl-4-sulfonyl)phenyl]*N*-hydroxy-acetamide (10). The title compound was prepared from carboxylic acid **16** following the general procedure. The crude product was purified by trituration with CH_2Cl_2/Et_2O to yield **10** as a white solid (55% yield); mp: 174 °C. ^{1}H NMR (DMSO-d_6) δ: 3.72 (s, 2H), 7.34−7.75 (m, 8H), 7.87−8.02 (m, 4H), 8.12 (d, J = 7.5 Hz, 1H), 8.87 (s, 1H), 10.62 (s, 1H). ^{13}C NMR (DMSO-d_6) δ: 35.73, 127.11, 127.68, 127.93, 128.64, 129.04, 129.26, 132.94, 133.58, 135.00, 138.13, 138.53, 139.64, 144.96, 165.72. Anal. ($C_{20}H_{17}NO_4S$) C, H, N.

***N*-Hydroxy-2-[2-(4′-methoxy-biphenyl-4-sulfonyl)phenyl]acetamide (11).** The title compound was prepared from carboxylic acid **17** following the general procedure. The crude product was purified by trituration with CH_2Cl_2/Et_2O to yield **11** as a white solid (57% yield); mp 158 °C. ^{1}H NMR (DMSO-d_6) δ: 3.71 (s, 2H), 3.80 (s, 3H), 7.04−7.07 (m, 2H), 7.37−8.13 (m, 10H), 10.61 (s, 1H). ^{13}C NMR (DMSO-d_6) δ: 35.71, 55.23, 114.50, 126.91, 127.66, 127.90, 128.33, 129.17, 130.26, 132.85, 133.47, 134.94, 138.77, 138.93, 144.58, 159.78, 165.73. Anal. ($C_{21}H_{19}NO_5S$) C, H, N.

General Procedure to Synthesize Compounds 52, 53. A solution of benzylic ester **48**, **49** (1 equiv) in dry THF (6 mL/mmol) was cooled to −78 °C under argon and treated dropwise with a 1 M solution of NaHMDS in THF (1.1 equiv). After stirring at −78 °C for 1 h, MeI (5 equiv) was added. The mixture was stirred in these conditions for 1 h and then allowed to warm up to −50 °C for 2 h. The reaction mixture was quenched with AcOH (3 equiv) in Et_2O (4 mL/mmol) and filtered over celite. The filtrate was concentrated and the crude product was purified by flash chromatography.

2-[2-(Biphenyl-4-sulfonyl)phenyl]propionic Acid Benzyl Ester (52). The title compound was prepared from benzylic ester **48** following the general procedure. The crude product was purified by flash chromatography on silica gel (*n*-hexane/EtOAc 15:85) to yield **52** (68% yield) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.41 (d, J = 7.14 Hz, 3H), 4.81 (q, J = 7.14 Hz, 1H), 4. 87 (s, 2H), 6.94−7.01 (m, 2H), 7.11−7.19 (m, 3H), 7.39−7.62 (m, 10H), 7.86−7.91 (m, 2H), 8.26 (dd, J_1 = 7.8 Hz, J_2 = 1.5 Hz, 1H).

2-[2-(4′-Methoxy-biphenyl-4-sulfonyl)phenyl]propionic Acid Benzyl Ester (53). The title compound was prepared from benzylic ester **49** following the general procedure. Yellow solid (70% yield). ^{1}H NMR ($CDCl_3$) δ: 1.40 (d, J = 7.14 Hz, 3H), 3.86 (s, 3H), 4.82 (q, J = 7.14 Hz, 1H), 4.87 (s, 2H), 6.95−7.00 (m, 4H), 7.15−7.20 (m, 3H), 7.40−7.61 (m, 7H), 7.83−7.87 (m, 2H), 8.25 (d, J = 7.7 Hz, 1H).

2-[2-(Biphenyl-4-sulfonyl)phenyl]propionic Acid (18). Compound **18** was synthesized according to the procedure described above for the preparation of **34**, starting from benzylic ester **52** (330 mg, 0.72 mmol). The crude product was purified by trituration with toluene to afford **18** (214 mg, 81% yield) as white solid; mp: 209 °C. ^{1}H NMR (DMSO-d_6) δ: 1.18 (d, J = 7.08 Hz, 3H), 4.55 (q, J = 7.08 Hz, 1H), 7.40−7.62 (m, 5H), 7.69−7.78 (m, 3H), 7.90−7.96 (m, 4H), 8.17 (d, J = 7.8 Hz, 1H), 12.34 (br s, 1H). ^{13}C NMR (DMSO-d_6) δ: 18.98, 127.10, 127.68, 127.79, 128.70, 129.08, 129.88, 134.25, 138.02, 139.73, 140.95, 144.90, 174.07.

2-[2-(4′-Methoxy-biphenyl-4-sulfonyl)phenyl]propionic Acid (19). Compound **19** was synthesized according to the procedure described above for the preparation of **34**, starting from benzylic ester **53** (360 mg, 0.74 mmol). The crude product was purified by trituration with Et_2O to afford **19** (210 mg, 72% yield) as white solid; mp: 153 °C. ^{1}H NMR (DMSO-d_6) δ: 1.17 (d, J = 7.08 Hz, 3H), 3.80 (s, 3H), 4.56 (q, J = 7.08 Hz, 1H), 7.03−7.08 (m, 2H), 7.46−7.75 (m, 5H), 7.82−7.87 (m, 4H), 8.15 (d, J = 7.8 Hz, 1H), 12.32 (br s, 1H). ^{13}C NMR (DMSO-d_6) δ: 18.98, 55.25, 114.53, 126.90, 127.77, 128.32, 128.95, 129.85, 130.17, 134.16, 138.22, 138.84, 140.90, 144.52, 159.82, 174.09.

2-[2-(Biphenyl-4-sulfonyl)phenyl]*N*-hydroxy-propionamide (12). Hydroxamic acid **12** was synthesized according to the procedure described above for the preparation of **2**, starting from carboxylic acid **18** (140 mg, 0.44 mmol). The *O*-silylate intermediate was utilized in the next step without further purification. After treatment with TFA, the crude product was triturated with toluene to afford **12** (58 mg, 41%) as white solid; mp: 177 °C. ^{1}H NMR (DMSO-d_6) δ: 1.06 (d, J = 7.14 Hz, 3H), 4.39 (q, J = 7.14 Hz, 1H), 7.44−7.59 (m, 4H), 7.66−8.13 (m, 9H), 8.82 (s, 1H), 10.35 (s, 1H). ^{13}C NMR (DMSO-d_6) δ: 20.01, 37.31, 127.10, 127.46, 127.66, 127.95, 128.66, 129.03, 130.70, 133.69, 138.04, 139.75, 140.66, 144.96, 169.19. Anal. ($C_{21}H_{19}NO_4S$) C, H, N.

***N*-Hydroxy-2-[2-(4′-methoxy-biphenyl-4-sulfonyl)phenyl]propionamide (13).** Hydroxamic acid **13** was synthesized according to the procedure described above for the preparation of **2**,

starting from carboxylic acid **19** (80 mg, 0.20 mmol). The *O*-silylate intermediate was purified by flash chromatography on silica gel (*n*-hexane/EtOAc 6:4) to afford the protected hydroxamate (101 mg) as an oil. ^{1}H NMR ($CDCl_3$) δ: 0.06 (s, 3H), 0.08 (s, 3H), 0.89 (s, 9H), 1.16 (d, J = 6.95 Hz, 3H), 3.86 (s, 3H), 4.35 (q, J = 6.95 Hz, 1H), 6.96−7.02 (m, 2H), 7.38−7.47 (m, 1H), 7.51−7.63 (m, 3H), 7.69−7.74 (m, 3H), 7.86−7.90 (m, 2H), 8.03−8.07 (m, 1H), 8.84 (br s, 1H). After treatment with TFA, the crude product was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 96:4) to give **13** (24 mg, 32% yield) as a white solid; mp: 153 °C. ^{1}H NMR (DMSO-d_6) δ: 1.06 (d, J = 6.78 Hz, 3H), 3.81 (s, 3H), 4.40 (q, J = 6.78 Hz, 1H), 7.04−7.08 (m, 2H), 7.50−8.11 (m, 10H), 8.81 (br s, 1H), 10.33 (br s, 1H). ^{13}C NMR (DMSO-d_6) δ: 20.01, 37.29, 55.25, 114.50, 126.90, 127.42, 127.95, 128.35, 128.55, 130.19, 130.65, 133.62, 138.20, 138.88, 140.63, 144.58, 159.82, 169.21. Anal. ($C_{22}H_{21}NO_5S$) C, H, N.

MMP Inhibition Assays.[31] Recombinant human progelatinase A (pro-MMP-2) and B (pro-MMP-9), MMP-16 and MMP-14 catalytic domains were a kind gift of Prof. Gillian Murphy (Department of Oncology, University of Cambridge, UK). Pro-MMP-1, pro-MMP-8, pro-MMP-3, and pro-MMP-13 were purchased from Calbiochem. Pro-MMP-12 was purchased by R&D Systems.

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). Pro-MMP-3 was activated with trypsin (5 μg/mL) for 30 min at 37 °C followed by soybean trypsin inhibitor (SBTI) (62 μg/mL). Pro-MMP-12 was autoactivated by incubating in FAB for 30 h at 37 °C.

For assay measurements, the inhibitor stock solutions (DMSO, 10 mM) were further diluted, at 7 different concentrations (0.01 nM-300 μM) for each MMP in the fluorometric assay buffer (FAB: Tris 50 mM, pH = 7.5, NaCl 150 mM, $CaCl_2$ 10 mM, Brij 35 0.05% and DMSO 1%). 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, 5 nM for MMP-3, 1 nM for MMP-14 cd, 15 nM for MMP-16 cd, 2.0 nM for MMP-1, and 1 nM for MMP-12) and inhibitor solutions were incubated in the assay buffer for 4 h at 25 °C.[32] After the addition of a 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) for all the other enzymes in DMSO (final concentration 2 μM), the hydrolysis was monitored every 15 s for 20 min, recording the increase in fluorescence (λ_{ex} = 325 nm, λ_{em} = 395 nm) using a Molecular Devices 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 inhibition was calculated from control reactions without the inhibitor. IC_{50} was determined using the formula: $V_i/V_o = 1/(1 + [I]/IC_{50})$, where V_i is the initial velocity of substrate cleavage in the presence of the inhibitor at concentration [I] and V_o is the initial velocity in the absence of the inhibitor. Results were analyzed using SoftMax Pro software[33] and GraFit software.[34]

Docking Calculations. The X-ray structure of MMP-12 complexed with *N*-isobutyl-*N*-[4-methoxyphenylsulfonyl]glycyl hydroxamic acid[11] (PDB code 1RMZ) was taken from the Protein Data Bank [http://www.pdb.org/] and was used for the docking calculations.

The ligands were built using Maestro 7.5[35] 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 8.5.[36] 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. We subjected the ligands to this protocol in order to achieve reasonable starting structures.

Automated docking was carried out by means of the Gold program,[37] version 3.0.1. The region of interest used by Gold was defined in order to contain the residues within 10 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 by applying the ChemScore fitness function. The best docked conformation was taken into account.

NMR Studies. All NMR measurements were made at 300 K on a Bruker Avance III Ultra Shield Plus 600 MHz spectrometer provided with a two channel BBI probe. Hydrogen and carbon assignment of **9** 15 mM in DMSO was accomplished by a series of 2D spectra, namely 2D-NOESY, 2D-COSY, 2D-TOCSY, ^{1}H,^{13}C-HSQC, 2D-^{1}H,^{1}H-TOCSY-^{1}H,^{13}C HSQC, and 2D-^{1}H,^{13}C-HMBC.

The ^{15}N-labeled catalytic domain of wt-MMP-12 was purchased by PROTERA srl (cloned from human cDNA, expressed in *Escherichia coli*) and provided at a concentration of 0.3 mM in a buffer containing 20 mM Tris (pH 7.2), 10 mM $CaCl_2$, 0.1 mM $ZnCl_2$, 0.3 M NaCl, 0.2 M AHA. The enzyme consists of the catalytic domain of human MMP-12 (residues 106−263 Swissprot accession P39900).

The NMR backbone assignment of MMP-12 (BioMagResBank entry 6444) was further adjusted in our experimental condition by 3D-^{1}H,^{15}N-NOESYHSQC using a mixing time of 100 ms to avoid spin diffusion. Compound **9** was added stepwise using few microliters of a concentrated solution in DMSO. The experiments were repeated after concentrating the protein to 1 mM in order to improve the signal/noise ratio and better ascertain the presence of intermolecular NOEs.

Acknowledgment. Molecular graphics images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH grant P41 RR-01081). We thank Bruker Biospin srl for providing a 600 MHz Bruker Avance III Ultra Shield Plus spectrometer, and the CBM (Consorzio per il Centro di Biomedicina Molecolare S.c.r.l., AREA Science Park, Basovizza, Trieste, Italy) for hosting the instrumentation.

Supporting Information Available: Table reporting the HPLC purity analysis and the combustion analysis data of the final products. Additional experiments and dose−response curves for proving the dependence of inhibitory activity on preincubation time. This material is available free of charge via the Internet at http://pubs.acs.org.

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