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Original article

Structure-based approach to nanomolar, water soluble matrix metalloproteinases inhibitors (MMPIs)

Emanuele Attolino [a], Vito Calderone [b], Elisa Dragoni [a,c], Marco Fragai [b,c], Barbara Richichi [c], Claudio Luchinat [b,c], Cristina Nativi [b,c,*]

[a] ProtEra s.r.l. viale delle Idee, 22 I-50019 Sesto F.no (FI), Italy
[b] CERM, University of Florence, Sesto F.no (FI), Italy
[c] Department of Chemistry, University of Florence, Sesto F.no (FI), Italy

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ABSTRACT

N-Arylsulfonyl-based MMPs inhibitors (MMPIs) are among the most prominent inhibitors possessing nanomolar affinity. However, their poor bioavailability remains critical for the drug development of this family of molecules. The structural analysis of the complex of NNGH (the most representative member of the family) with MMP-12 provided us with the basis to effectively design simple NNGH analogues with enhanced solubility in water. Following this approach, the sec-butyl residue, not directly involved in the binding with MMP, has been replaced with hydrophilic residues thus yielding new potent inhibitors soluble in water.

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1. Introduction

The decoration of a ligand with hydrophobic groups is often reported as useful strategy to increase its free-energy of binding to a target protein. Due to desolvation, the entropy associated to the binding of a hydrophobic group to the protein surface is usually large and positive with a significant increase of the overall ligand affinity [1]. Ultimately, this increase in affinity parallels the decrease in the solubility of the ligand. However, the increase in affinity only occurs if the hydrophobic group interacts with the protein and water molecules are released upon ligand binding, while if the hydrophobic group sticks-out of the protein surface into the bulk water, it only worsens the solubility without affecting the ligand binding. Therefore, a suitable approach to improve both, affinity and water solubility of a lead compound, lies in the structural analysis of its complex with the target protein to drive the decoration of the scaffold with suitable functional groups. Matrix metalloproteinases (MMPs) are a large class of strictly-related zinc-dependent enzymes which belong to the family of proteolytic enzymes. MMPs are involved in many aspects of physiological cellular processes, as well as in pathologies, such as reumathoid arthritis, pulmonary emphysema, tumor growth and metastasis.

Since many of these pathologies may benefit from the control of MMPs activity [2], several MMPs inhibitors (MMPIs) has been designed and tested in clinical trials [3–8]. However, most of them, are poorly soluble in water and suffer from a modest oral bioavailability [9]. A well-balanced hydrophilic/lipophilic character is crucial for reaching and maintaining high drug levels in plasma.

High lipophilicity negatively affects drug bioavailability, by preventing the drug from reaching the target site and increasing the binding to human serum albumin (HSA) [10]. In addition, a limited drug bioavailability requires the administration of large doses of drug to achieve and maintain an effective therapeutic efficacy. In case of MMPI, it means the increasing of adverse effects due to the modest selectivity which may cause the indiscriminate inhibition of other zinc-peptidases. Side effects are even worse in long-term treatments, required in many MMP-related chronic pathologies [11].

Water soluble inhibitors can be useful in this respect but only few of the huge number of potentially useful MMPIs reported to date [12,13] show a good solubility in water preserving a good bioavailability [14–20]. Water soluble inhibitors are also suitable candidates for topical treatment of pathologies such as pulmonary

* Corresponding author. Department of Chemistry, University of Florence, via della Lastruccia, 3,13 Sesto F.no (FI), Italy. Fax: + 39 0554573540.
E-mail address: cristina.nativi@unifi.it (C. Nativi).

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emphysema, and parodontitis where the solubility in aqueous biological fluids is mandatory.

N-Arylsulfonyl α-aminohydroxamic acid derivatives such as NNGH, **1** [21,22] CGS27023, **2** [21] and AG3340, **3** [23] (Fig. 1) are among the most prominent representatives of a family of nanomolar inhibitors for some MMPs. Extensive NMR and X-ray studies to elucidate the binding interactions between functional groups in the inhibitors and their corresponding subsites in the enzymes [12,24] have provided the basis to effectively design new analogues with potentially more favorable interactions and enhanced solubility in water.

X-Ray structures of the NNGH-MMP-12 complex [25] confirmed that the interaction of inhibitors of this class with the active site of the enzyme involves the binding of the hydroxamic functional group to the catalytic Zn ion and the binding of the aromatic group to the S_1' subsite. On the contrary, the isopropyl group on the sulfonamide nitrogen atom points away from the shallow S_2' pocket and does not directly participate in binding. Capitalizing on such structural insights, we recently reported the synthesis of the water soluble MMPs inhibitor **4** [16] featuring a glucose residue. As expected, the correct functionalisation of the sulfonamidic scaffold allowed to preserve the nanomolar affinity of **4** for several MMPs while increasing its solubility in water.

Relying on this observation, we report here the synthesis and the binding assessment of a family of new arylsulfonamide derivatives **1a** with hydrophilic R'-substituents, replacing *iso*butyl group, and differently para-substituted aryl groups. R and R'-substituents with various degrees of hydrophilicity and R″ substituents with different size and shape were used, featuring new inhibitors with C log*P* [Calculator Plugins, ChemAxon Ltd.] ranging from +0.65 to −2.26 (see Table 1).

Following these leading concepts, eight arylsulfonamide derivatives bearing mono- and poly-hydroxy substituents facing the solvent and different hydrophobic groups targeted to the hydrophobic S_1' pocket have been synthesized. Low nanomolar affinities were achieved for these new inhibitors towards some relevant MMPs such as: MMP-12, implicated in the development of emphysema [22,26], MMP-13 involved in rheumatoid arthritis [27,28], MMP-9 overexpressed in diabetic retinopathy [29–31], MMP-7 involved in colorectal carcinoma [32] and MMP-1, implicated in skin photo-aging damages [33].

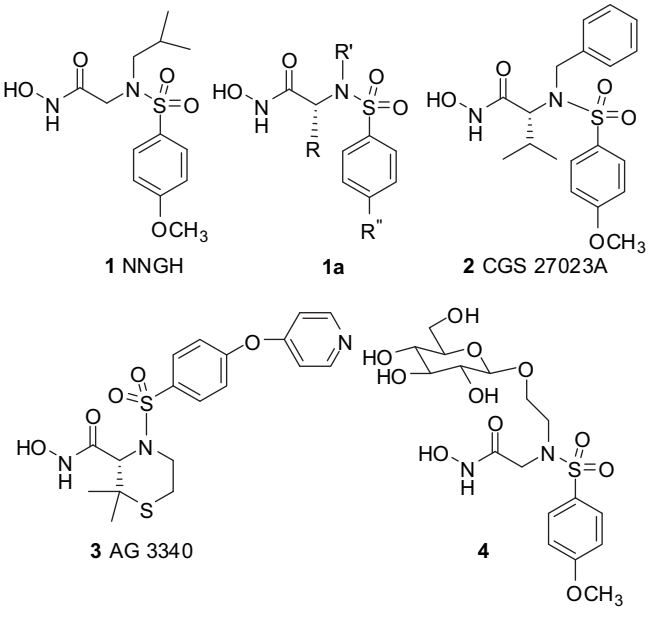
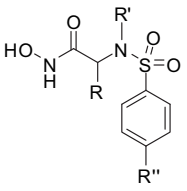


Fig. 1. Structure of inhibitors **1**–**4**.

Table 1
Enzyme inhibition constants of compounds **12**, **17**, **22**, **23**, **24**, **28**, **30** and **32** (data are K_i (nM)) towards several MMPs.[a]



4 R= H, R' = $(CH_2)_2$-Glc, R'' = OMe
12 R = H, R' = $(CH_2)_2$-OH, R" = OMe
17 R = H, R' = $CH_2(CHOH)CH_2OH$, R" = OMe
22 R = CH_2OH, R' = $CH_2(CHOH)CH_2OH$, R" = OMe
23 R = CH_2OH, R' = H, R'' = OMe
24 R = $CH(CH_3)OH$, R' = H, R'' = OMe
28 R = H, R' = $(CH_2)_2$-OH, R" = F
30 R = H, R' = $(CH_2)_2$-OH, R" = biphenyl
32 R = H, R' = $CH_2(CHOH)CH_2OH$, R" = biphenyl

Compound	MMP-1	MMP-7	MMP-8	MMP-12	MMP-13	C log*P*
NNGH [25]	174	13000	9.0	4.3 (1) [16]	3.1 (1) [16]	+1.61
4 [16]	286	2000	9.1 (8.0)	14.3 (7.0)	1.7 (1.1)	−1.76
12	128 (18)	1500 (200)	13 (2)	7.6 (1.1)	1.0 (0.1)	−1.15
17	160 (22)	9000 (170)	24 (3)	8.0 (1.1)	3.6 (0.8)	−1.78
22	32 (5)	344 (65)	8.0 (1.2)	7.0 (0.9)	1.7 (0.3)	−2.26
23	143 (18)	823 (30)	9.0 (1.0)	6.0 (0.7)	2.2 (0.3)	−1.17
24	287 (33)	984 (110)	10.0(1.8)	11.0 (1.7)	2.9 (0.3)	−0.75
28	151 (21)	3000 (400)	173 (23)	40 (7)	46 (6)	−0.85
30	67 (9)	1600 (200)	4.6 (0.6)	31 (4.8)	2.7 (0.5)	+0.65
32	742 (90)	4000 (900)	48 (7)	60 (10)	18 (2)	+0.02

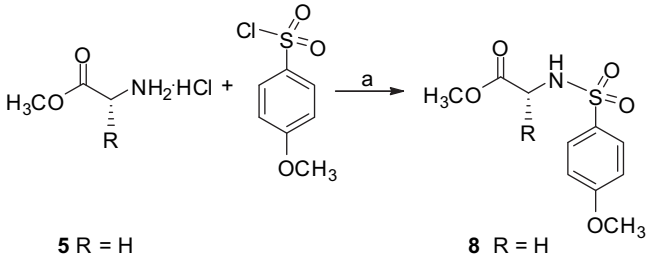
[a] Values are averaged over triplicate experiments with errors reported in parentheses.

2. Results and discussion

2.1. Synthesis

Non-natural, commercially available, GlyOMe **5**, D-SerOMe **6**, and D-ThrOMe **7** were reacted with sulfonyl chloride in the presence of triethylamine and dimethylaminopyridine in dichloromethane as solvent to give sulfonamides **8**–**10** in high yield (90–97%) (Scheme 1).

The sulfonamide of glycine methylester **8** was reacted with ethylene oxide and methyliodide [16] to afford compound **11** which was directly transformed into the corresponding hydroxamic acid **12** by reaction with hydroxylamine hydrochloride and potassium hydroxide, in methanol as solvent (Scheme 2). Compound **8** was also reacted with enantiopure glycerine dimethylacetal **13** under Mitsunobu conditions, to give methylester **14**. Acidic removal (CH_3COOH/H_2O 80:20) of acetal protecting group on **14** afforded a mixture of the desired dihydroxyl derivative **15** and lactone **16**; the latter obtained from **15** by intramolecular trans-esterification.

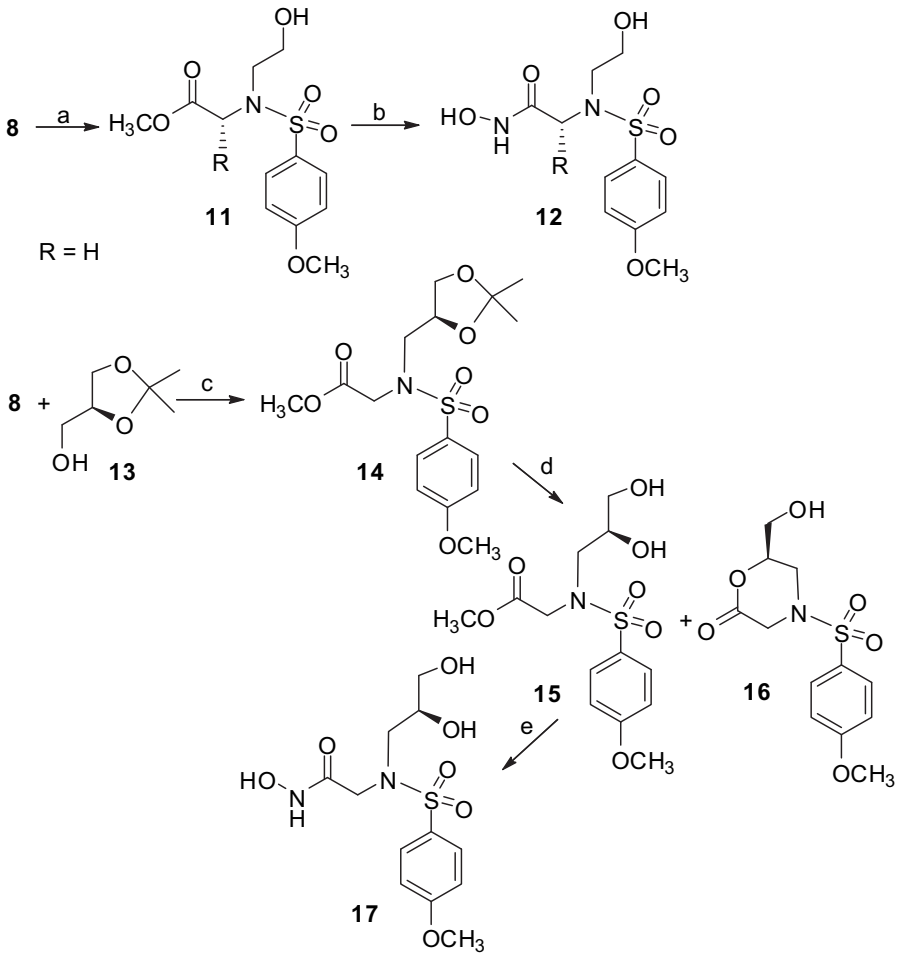


5 R = H
6 R = CH_2OH
7 R = $CH(OH)CH_3$

8 R = H
9 R = CH_2OH
10 R = $CH(OH)CH_3$

[a] a) Et_3N, DMAP, CH_2Cl_2, rt, overnight, 90-97%

Scheme 1.



[a] a) see Ref. 22, 64%; b) $NH_2OH{\cdot}HCl$, KOH, MeOH, rt, 1h + 6h, 38%; c) Ph_3P, DIAD, THF, rt, overnight, 87%; d) CH_3COOH/H_2O 80:20, 60 °C, 4h, 75% (**15** + **16**); e) $NH_2OH{\cdot}HCl$, KOH, MeOH, rt, 1h + 18h, 45% (after HPLC purification)

Scheme 2.

The non-easily separable mixture of **15** and **16** was treated with hydroxylamine hydrochloride and potassium hydroxide in methanol to form hydroxamic acid **17** as white solid in 45% yield (Scheme 2).

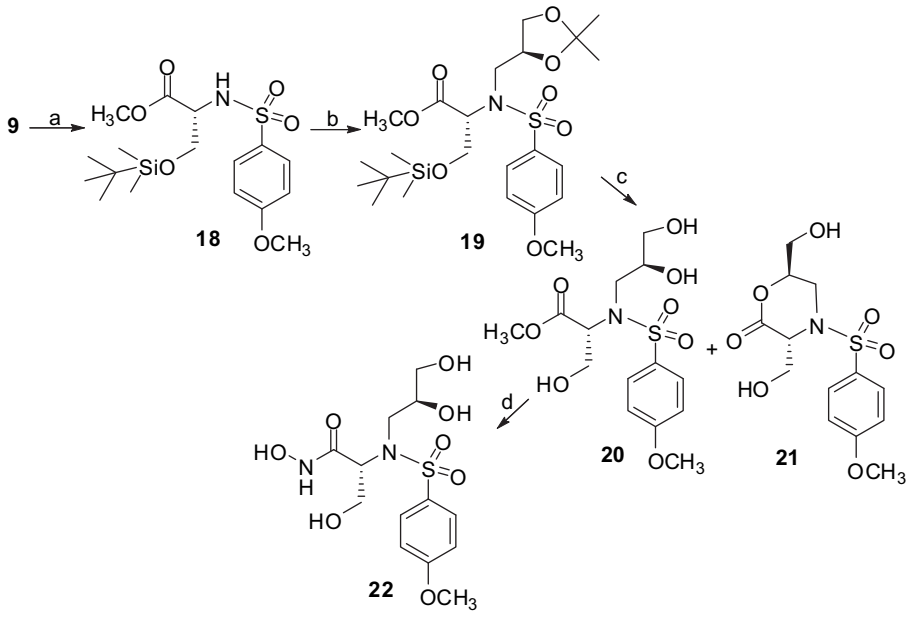
The sulfonamide of serine methyester **9** was firstly transformed, under standard conditions, into the corresponding *tert*butyldimethylsilyl ether **18** then reacted with acetal **13** as reported above, to give compound **19** (Scheme 3).

Acidic removal of silylether and acetal protecting groups afforded a mixture of tri-hydroxyl derivative **20** and lactone **21** which was treated with hydroxylamine hydrochloride and potassium hydroxide to form the expected hydroxamic acid **22** in 50% yield (see Scheme 3). Treatment of sulfonamide of serine methylester **9** and of threonine methylester **10**, with hydroxylamine and potassium hydroxide in methanol as solvent afforded the N-disubstituted hydroxamic acids **23** and **24** respectively (Scheme 4).

Derivatives, **23** and **24**, as well as hydroxamic acids **12**, **17** and **22** are soluble in water and stable as solid or in solution, at room temperature for several months under neutral (pH = 7) or basic conditions (pH = 8.5). The same good stability was observed under slightly acidic conditions (pH = 4.5) with the only exception of derivative **22** which tends to degrade forming the corresponding carboxylic acid.

High-resolution structures of a series of enzymes adducts with homologous ligands we previously reported [34], provided precious information for the designing of new ligands. Taking in consideration these subtle factors, in order to improve inhibitors selectivity, *para*-methoxybenzyl residue on scaffold **8** was replaced by *para*-fluorobenzyl- and biphenyl-moieties. Therefore, the *para*-fluorosulfonamide of glycine methyester **25** and the biphenylsulfonamide of glycine methylester **26** were prepared reacting, as depicted in Scheme 1, ester **5** with *para*-fluorosulfonyl chloride and biphenylsulfonyl chloride respectively (Scheme 5).

Reaction of **25** and **26** with acetylbromoethanol in dimethyformamide as solvent in the presence of sodium hydride afforded sulfonamides **27** and **29** respectively which, after removal of acetyl protecting group under standard conditions, were transformed into the corresponding hydroxamic acids **28** (72%) and **30** (48%) by reaction with hydroxylamine hydrochloride and potassium hydroxide as base, at room temperature (see Scheme 5). Methyl ester **26** was also reacted with **13** to give the sulfonamide **31** which was transformed, as reported for **22**, into the hydroxamic acid **32** in 54% yield.



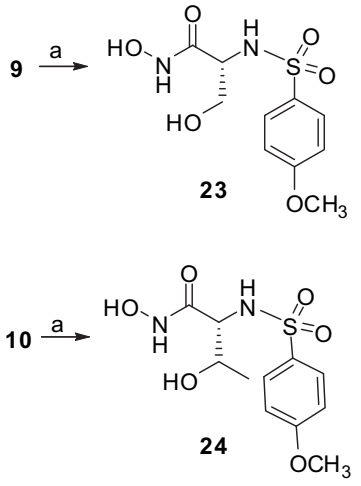
[a] a) $tBuMe_2SiOTf$, Et_3N, rt, 20h, 97%; b) Ph_3P, DIAD, THF, rt, overnight, 85%; c) CH_3COOH/H_2O 80:20, 60 °C, 90%, (**20** + **21**); d) $NH_2OH{\cdot}HCl$, KOH, MeOH, 1h + 15h, 50% (after HPLC purification)

Scheme 3.

3. Biological assays

Compounds **12**, **17**, **22**, **23**, **24**, **28**, **30** and **32** were tested "in vitro" for the inhibition of a panel of six MMPs: MMP-1, -7, -8, -9, -12, -13 (see Experimental Section for details on the protocol used). Assays used the catalytic domain of the proteins. Results are shown in Table 1 in comparison with those of NNGH and compound **4** [16].

Except for MMP-1 and -7, all compounds reported in Table 1 showed low nanomolar K_i values for the MMPs tested. Given the active site topology of MMPs, most inhibitors likely share similar features, i.e., the ability to bind to the metal ion, to the hydrophobic pocket termed S_1', and to the substrate binding groove. Direct evidence was obtained from the X-ray crystal structure of the MMP-12–**17** complex (Fig. 2, PDB code: 1F15). The protein structure clearly resembles those of the previously solved MMP-12 structures [25]. The catalytic and structural zinc atoms as well as the three calcium ions are well visible (Fig. 2A). The inhibitor shows a very well defined electron density for all atoms, which allows to identify the absolute configuration of the non-aminoacidic stereogenic center as *S*. As expected for NNGH-like ligands, the catalytic zinc atom is coordinated by the hydroxamic moiety and the methoxyphenyl ring is nested in the S_1' pocket through hydrophobic and aromatic stacking interactions. The hydroxamic and sulphonate moieties are then establishing favorable interactions with the backbone atoms of the protein through several strong hydrogen bonds. The dihydroxypropyl chain protrudes out of the protein toward the solvent region, placing the two oxygen atoms at hydrogen bond distance from three well ordered water molecules. More importantly, one of the oxygens of the dihydroxypropyl portion is also engaged in a strong hydrogen bond with one of the two sulfonamidic oxygens, locking the inhibitor in the suitable conformation to be buried into the protein cavity. The inhibitor shares the same binding mode of other inhibitors known in the literature and all relevant interactions (with the S_1' pocket, the metal, and the substrate binding groove) are in place.

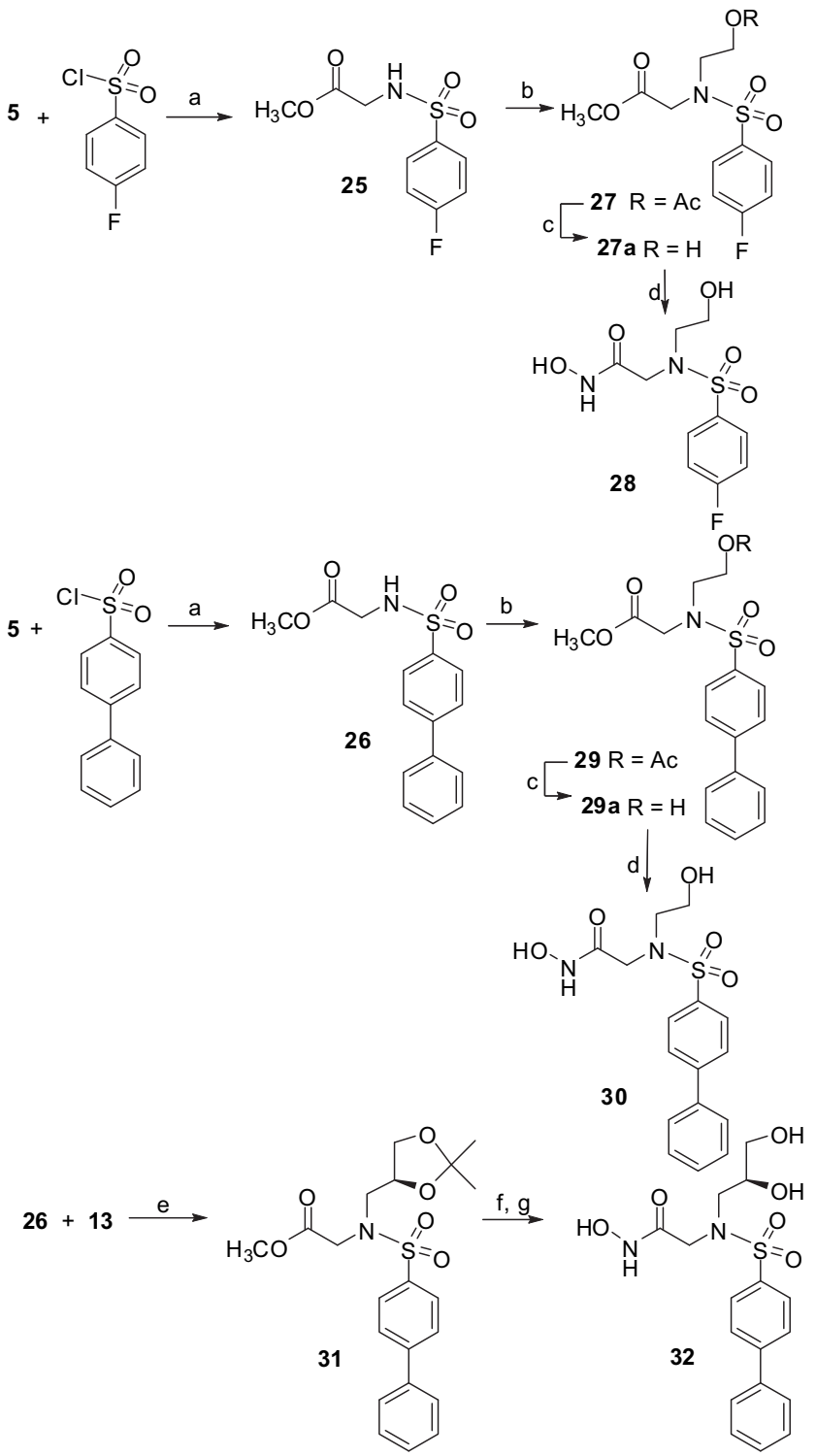


[a] a) $NH_2OH{\cdot}HCl$, KOH, MeOH, rt, 1h + 20h, 47% (**23**); 1h +18h, 36% (**24**).

Scheme 4.

As expected [16], the hydrophilic chain on the sulfonamidic nitrogen protrudes out of the protein toward the solvent region and does not negatively affect the binding properties of the inhibitor, thus confirming the lack of significant participation in binding of such chains.

Relying on the different shape and on the different aminoacidic residues which characterize the S_1' binding pockets of MMPs, we prepared compounds **28** (PDB code: 3F18), **30** and **32** with a fluorine residue (**28**) and a biphenyl residue (**30**, **32**) replacing the methoxy group with the aim of taking advantage of specific additive interactions. However, since besides their intrinsic similarity MMPs exhibit some capability of adapting the binding pocket to the inhibitor shape [35] no real advantages were observed in term of



[a] a) Et_3N, DMAP, Ch_2Cl_2, rt, overnight, 89% (**25**); 81% (**26**); b) $AcOCH_2CH_2Br$, NaH, DMF, 0 °C - rt, 18h, (**27**), 36h (**29**); c) MeONa, MeOH, 1h, 50% (**27a**); 40% (**29a**).; d) $NH_2OH{\cdot}HCl$, KOH, rt, 1h + 18h 72% (**28**); 48% (**30**); e) Ph_3P, DIAD, THF, rt, overnight, 64%; f) CH_3COOH/H_2O 80:20, rt than g) $NH_2OH{\cdot}$ HCl, KOH, 2 h, 54%

Scheme 5.

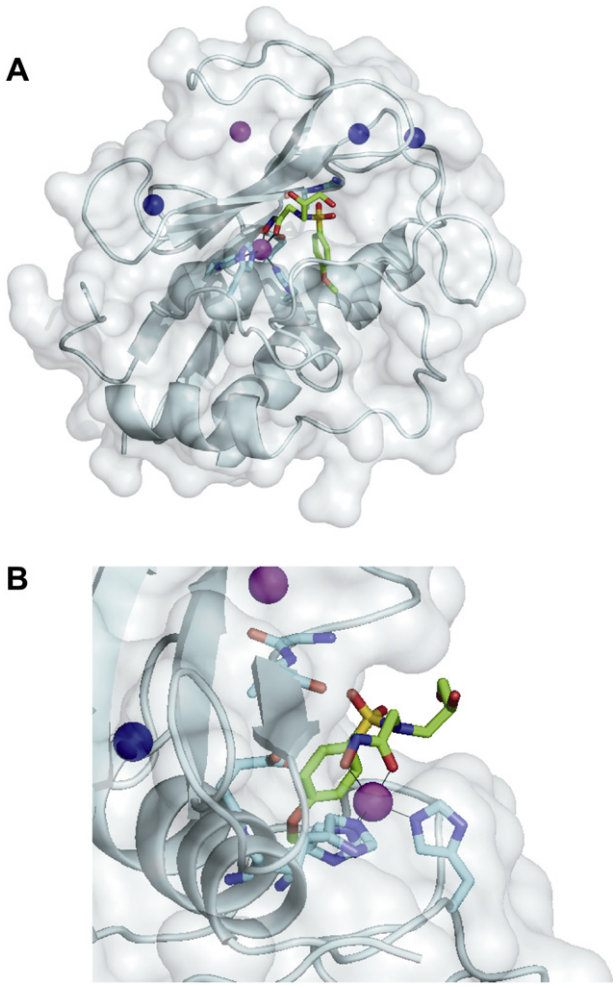


Fig. 2. RX structure of MMP12-**17** complex: A) global view; B) inhibitor's dihydroxyl chain protruding outside the protein.

selectivity. Indeed, the presence of the bulky biphenyl group did not improve substantially the affinity vs the enzyme with a larger S_1' pocket, like MMP-13, with respect to MMP-12 or MMP-8.

The low affinity of all the inhibitors here reported for MMP-7, characterized by a narrow and short S_1' pocket, was not surprising, while the disappointing micromolar K_i value measured in the case of **28** vs MMP-1, featuring at the bottom of the S_1' pocket a positively charged residue, can probably be ascribed to the lack of the expected favorable interactions between the Arg214 and the fluorine atom.

Positive C log*P* values reported in Table 1 for **30** and **32**, featuring one and two hydroxyl residues respectively, clearly indicated a sensible decreasing in hydrophilicity for these inhibitors caused by the replacement of the para-substituted benzene portion with a biphenyl residue. In fact, the presence of at least one hydroxyl group guarantees a global hydrophilic nature for all the others inhibitors, from a minimum of −0.75 for **24** (one hydroxyl group on the aminoacidic skeleton) to a maximum of −2.26 for **22** (three hydroxyls, one on the aminoacidic skeleton and two on the side chain not involved in the binding). Ultimately, the replacement of the methoxy residue with phenyl ring did not increase selectivity vs MMPs, conversely it made inhibitors' worse.

4. Conclusion

In conclusion, the present analysis shows that the structure-based approach to drug design can be easily exploited to modulate the solubility, and consequently the bioavailability, of candidate drugs without affecting their affinity for the target. In particular, the sulfonamidic-based family of MMPIs we reported here represents a rare example of water soluble, structurally simple inhibitors with low nanomolar affinity for MMPs. Noteworthy, the rationale followed to synthesize this class of large spectrum, potent inhibitors clearly demonstrated that the introduction of hydrophilic chains on the nitrogen atom of the NNGH sulfonamidic scaffold does not negatively impact upon the affinity of the inhibitors for MMPs. This is particularly evident in the case of inhibitor **17** which presents the selectivity profile similar to NNGH but a complete solubility in water. This insight could be successfully exploited to develop ditopic devices to efficiently target MMPs and a second biologically relevant target simultaneously.

5. Experimental

All commercially available reagents used for synthesis were employed without further purification; solvents used were of analytical grade and were employed without further purification unless otherwise stated. The following structures: 3F15 (MMP-12-**17**), 3F16 (MMP-12-**23**), 3F18 (MMP-12-**28**), 3B2U (MMP-12-**4**), 3N2 V (MMP-12-**30**) have been deposited to the PDB.

5.1. Synthesis of compound *14*

To a solution of **8** (1.04 g, 4.00 mmol), PPh_3 (1.37 g, 5.20 mmol) and **13** (0.69 g, 5.20 mmol) in dry THF (20 mL) was slowly added DIAD (1.02 mL, 5.20 mmol). The reaction mixture was stirred overnight at room temperature then concentrated to dryness. 25 mL of a mixture cyclohexane/EtOAc 6:1 were added and the suspension was stirred for 30 min at room temperature. The white solid was filtered off and the filtrate was concentrated to dryness. The crude product was purified by flash column chromatography on silica gel ($CHCl_3$/EtOAc 7:1) to afford **14** (1.30 g, 87%) as an oil. $[\alpha]_\Delta^{25}$: +4.39(c 0.41, $CHCl_3$).

HRMS for $C_{16}H_{23}NO_7S$ + H calcd: 374.1273. Found: 374.1268. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.77–7.74 (AA' part of an AA'MM' system, J_{AM} = 9.2 Hz, 2H), 6.97–6.95 (MM' part of an AA'MM' system, J_{AM} = 9.2 Hz, 2H), 4.34–4.22 (m, 3H, H-2a, H-2′, H-2b), 4.09–4.02 (A part of an ABX system, J_{Ax} = 6.4 Hz, J_{AB} = 8.4 Hz, 1H, H-3′a), 3.87 (s, 3H, OCH_3), 3.68–3.64 (B part of an ABX system, J_{Bx} = 6.8 Hz, J_{AB} = 8.4 Hz, 1H, H-3′b), 3.62 (s, 3H, COOCH_3), 3.62–3.52 (A part of an ABX system, J_{Ax} = 4.0 Hz, J_{AB} = 14.8 Hz, 1H, H-1′a), 3.25–3.14 (B part of an ABX system, J_{Bx} = 7.2 Hz, J_{AB} = 14.8 Hz, 1H, H-1′b), 1.35 (s, 3H, C(CH_3)$_2$), 1.30 (s, 3H, C(CH_3)$_2$). ^{13}C NMR (50 MHz, $CDCl_3$): δ 169.7, 162.9, 131.0, 129.4, 114.1, 109.5, 75.4, 67.0, 55.5, 51.9, 50.4, 49.2, 26.6, 25.2.

5.2. Synthesis of compound *15*

A solution of **14** (1.20 g, 3.21 mmol) in 18 mL of a mixture AcOH/H_2O 80:20 was left to stir at 60 °C for 4 h then toluene (20 mL) was added. Concentration of the solvent under vacuum gave a crude product which was purified by a flash column chromatography on silica gel (EtOAc) to afford a mixture of **15** and **16** (total yield 75%) as an oil. An analytical amount of compound **15** has been purified for characterization. $[\alpha]_\Delta^{25}$: −7.00(c 0.50, CH_3OH). Anal. for $C_{13}H_{19}NO_7S$ calcd: C, 46.84; H, 5.74; N, 4.20. Found: C, 47.07; H, 5.01; N, 4.66. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.78–7.73 (AA' part of an AA'MM' system, J_{AM} = 9.2 Hz, 2H), 7.00–6.96 (MM' part of an AA'MM' system, J_{AM} = 9.2 Hz, 2H), 4.12–4.08 (A part of an AB system, J_{AB} = 18.4 Hz, 1H), 4.02–3.97 (B part of an AB system, J_{AB} = 18.4 Hz, 1H), 3.92–3.85 (m, 1H), 3.87 (s, 3H), 3.76–3.56 (m, 1H), 3.72 (s, 3H), 3.39–3.28 (A part of an ABX system, J_{Ax} = 7.6 Hz, J_{AB} = 14.6 Hz, 1H), 3.24–3.31 (B part of an ABX system, J_{Bx} = 4.0 Hz, J_{AB} = 14.6 Hz, 1H),

2.42 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.6, 163.1, 130.1, 129.4, 114.3, 70.1, 63.4, 55.5, 52.4, 52.1, 50.2.

5.3. General procedure for the synthesis of hydroxamic acids. Synthesis of compound **17**

A suspension of KOH (168 mg, 3.00 mmol) and $NH_2OH{\cdot}HCl$ (167 mg, 2.40 mmol) in CH_3OH (2 mL) was stirred at room temperature for 1 h, then a solution of **15** + **16** (0.60 mmol) in CH_3OH (2 mL) was added. The reaction mixture was stirred until no trace of starting material was revealed by TLC analysis (18 h), then concentrated to dryness. The crude product was dissolved in EtOAc, washed with a mixture AcOH/H_2O 1:1 and dried over Na_2SO_4. Concentration of the solvent under vacuum affords a crude product which was purified by HPLC (C_8 column Ultrasphere Beckman Coulter, AcCN/H_2O 10:90, AcCN/H_2O 30:70) to afford **17** (90.0 mg, 45%) as a pale yellow glassy solid. $[\alpha]_D^{25}$: −22.3(c 0.48, CH_3OH). HRMS for $C_{12}H_{19}N_2O_7S$ + H calcd: 335,0913. Found: 335.0909. ESI-MS $[M + H]^+$ calcd: 335.1. Found: 335.1. $[M + Na]^+$ calcd: 357.1. Found: 357.1. ^{1}H NMR (400 MHz, CD_3OD): δ 7.73–7.70 (AA' part of an AA'MM' system, J_{AM} = 8.8 Hz, 2H), 7.05–7.03 (MM' part of a AA'MM' system, J_{AM} = 8.8 Hz, 2H), 3.90–3.78 (m, 3H, CH_2-2, H-2′), 3.88 (s, 3H, OCH_3), 3.52–3.51 (m, 2H, CH-3′), 3.35–3.30 (m, 1H, H-1′a), 3.17–3.11 (B part of an ABX system, J_{Bx} = 8.8 Hz, J_{AB} = 14.4 Hz, 1H, H-1′b). ^{13}C NMR (50 MHz, CD_3OD): δ 167.3, 163.3, 129.7, 129.3, 114.0, 70.5, 63.6, 54.9, 53.4, 50.0.

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Appendix. Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2010.09.057.

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