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Discovery of a New Class of Potent MMP Inhibitors by Structure-Based Optimization of the Arylsulfonamide Scaffold

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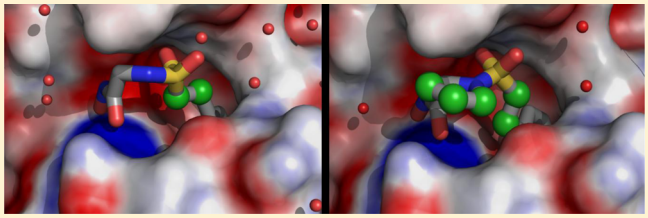
Supporting Information

ABSTRACT: A new class of potent matrix metalloproteinase (MMP) inhibitors designed by structure-based optimization of the well-known arylsulfonamide scaffold is presented. Molecules show an ethylene linker connecting the sulfonamide group with the P1′ aromatic portion and a D-proline residue bearing the zinc-binding group. The affinity improvement provided by these modifications led us to discover a nanomolar MMP inhibitor bearing a carboxylate moiety as zinc-binding group, which might be a promising lead molecule. Notably, a significant selectivity for MMP-8, MMP-12, and MMP-13 was observed with respect to MMP-1 and MMP-7.



KEYWORDS: *MMP, X-ray, synthesis, desolvation, docking, free energy*

Matrix metalloproteinases (MMPs) are a family of extracellular zinc- and calcium-dependent endopeptidases involved in the degradation and remodeling of extracellular matrix components, cell movement, proliferation, and tissues remodeling.[1,2] Aberrant MMPs activities are involved in several pathological states, and the design of synthetic MMP inhibitors represents an opportunity to develop new drug candidates. Recently, we reported on the use of a MMP-9 inhibitor for the potential treatment of Dry Eye Syndrome.[3] Here, we present a new scaffold for potent MMP inhibitors that was discovered by means of an integrated medicinal chemistry study, composed of computer-aided design, synthesis, X-ray analysis, and fluorimetric measurement of the binding affinity toward MMPs. A new strategy to improve the inhibitory activity toward MMPs by increasing the interactions with the S1′ pocket and, especially, by increasing the ligand solvation free energy is further presented. The different contributions to the binding affinity were further investigated with computational methods.[4]

Human macrophagic metalloelastase (MMP-12) was selected as target because of its pathological relevance and of the availability of detailed structural information. Several potent inhibitors for MMP-12, such as phospinic peptides, sulfonamides, and bisphosphonic derivatives, have already been designed and tested.[5−10] For some of them a high selectivity toward MMP-12 has been observed,[5,6,8,10] although the molecular basis of this specificity is still a matter of research. Here, based on the classical arylsulfonamide scaffold, new MMP inhibitors were designed by introducing an ethylene linker between the sulfonamide moiety and the P1′ aromatic portion and by replacing the glycine residue with a D-proline within the zinc-binding group (ZBG).[11,12] The flexible linker was introduced to increase van der Waals interactions with the deep S1′ lipophilic cavity of MMPs,[13] without affecting the overall ligand binding mode. At the same time, the hydroxamate derivative of the rigid D-proline was evaluated as ZBGs for its possible favorable contribution to the solvation free energy of the ligand. It is interesting to note that these chemical substitutions do not sizably affect the ligand logP values, that remain within the tolerance limits described by the rule of three and the Lipinski's rule of five.[12,14] Such ligands **1**−**12** (Chart 1, for chemical structure see Supporting Information) were docked on the crystal structure of MMP-12 (PDB code: 1Y93).[11]

Docking calculations were also carried out on compounds belonging to the hydroxamic acid arylsulfonamide scaffold bearing the same chemical substituents in R1 (**1c**−**12c**). The analysis of the docking-based binding mode of **1**−**12** and **1c**−**12c** within the active site of MMP-12 can be summarized as follows. The hydroxamic ZBG coordinates the catalytic zinc ion by adopting a conformation virtually superimposable to that observed in several crystallographic structures (i.e., PDB codes: 3LK8 and 3F1A)[15] and consistent with the theoretical expectations for complexes between the zinc ion and

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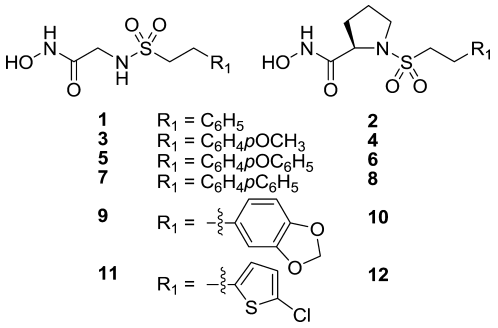
Chart 1. Chemical Structure of Compounds 1−12[a]



[a]Molecules **1c−12c** have the same substituents as **1−12**, but R_1 is directly linked to the sulfonamide moiety. Inhibitors **7***, **7c***, **11***, and **12c*** are endowed with the carboxylic acid zinc-binding group.

hydroxamic acid derivatives.[16] The hydroxamic group establishes two hydrogen bonds with the backbone of A182 and with the side chain carboxylate of the catalytic E219 (Figure 1).

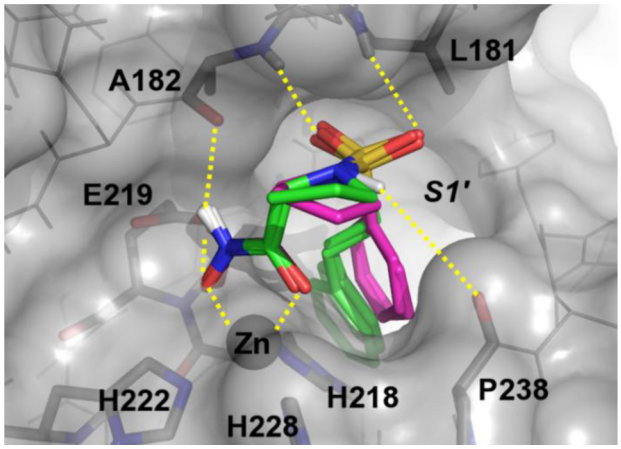
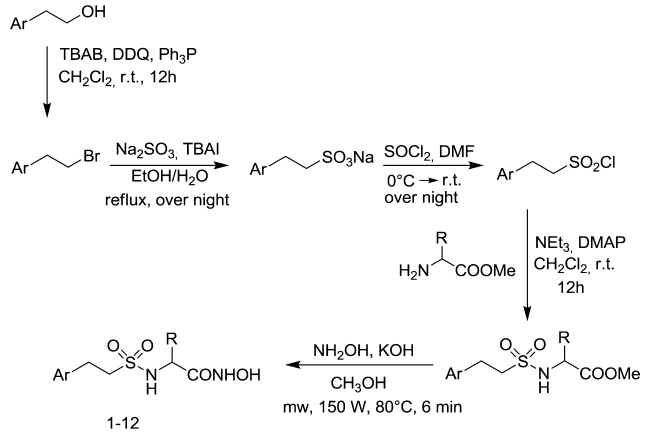


Figure 1. Superposition of the docking poses of **1**, **1c**, **2**, and **2c** toward the catalytic domain of MMP-12. RMSD = 0.173 and 0.208 for glycine- and D-proline-based compounds, respectively. Molecules belonging to the arylsulfonamide scaffold are colored magenta; molecules belonging to the new scaffold are colored green. Polar contacts between MMP-12 and ligands are highlighted as yellow dotted lines.

Ligand interaction is further stabilized by the sulfonamide moiety, which establishes two additional hydrogen bonds with the proton amides of A182 and L181, even though a sulfonamide oxygen is likely to interact also with water solvent. For glycine-based derivatives, as well as for derivatives of all amino acids with the exception of proline, a supplementary hydrogen-bond interaction might occur with the carbonyl oxygen of P238. Extended hydrophobic contacts are established within the S1′ pocket. In this respect, the ethylene linker of **1−12** pushes P1′ groups deeper inside the lipophilic cavity with respect to arylsulfonamides **1c−12c**.

Docking calculations also showed that the D-proline did not alter the overall geometry of zinc coordination or the interactions established by the sulfonamide group with the MMP-12, with respect to what was observed for glycine-based inhibitors.[15] The theoretical free energies of binding calculated by AutoDock[17] (Supporting Information) univocally show that compounds bearing both the ethylene linker and the D-proline moiety exhibit the lowest theoretical K_i values. It is interesting to note that the decrease in theoretical K_i values was found to be more sensitive to the substitution of glycine with D-proline, than to the introduction of the ethylene linker between the sulfonamide moiety and the P1′ group. In fact, for some compounds (**9−9c** and **11−11c**) the binding energy gain provided by the linker is rather small. The analysis of AutoDock energy also revealed that the introduction of the D-proline moiety induces a large decrease of the desolvation energy with minor effects on the enthalpy term. On the contrary, the linker atoms often improve the enthalpy of binding, establishing new van der Waals interactions with the atoms of the protein at the entrance of the S1′ cavity. These theoretical data support the original hypothesis. Then, compounds **1−12** were synthesized according to the general procedure illustrated in Scheme 1.

Scheme 1. Synthetic Procedure for Obtaining Compounds 1−12



The aryl-ethyl alcohol or the corresponding halide was used as starting reagent, depending on availability. The alcohol was treated with tetrabutyl ammonium bromide (TBAB), Ph_3P, and 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) and converted into the corresponding bromide derivative.[18] The bromide was then replaced by the sulfonyl chloride moiety,[19] and the resulting molecule was coupled with the glycine or proline methyl ester. The resulting sulfonamides were treated with hydroxylamine and potassium hydroxide in methanol to obtain the corresponding hydroxamic acids.[16] Activation with microwave was found helpful if not mandatory in most cases.[20] The hydroxamic derivative **7** was not obtained despite several attempts under different experimental conditions. However, the corresponding carboxylic acid derivative of compound **7**, as well as those of compounds **7c**, **11**, and **12c** (here indicated as compounds **7***, **7c***, **11***, and **12c***, respectively), were synthesized and tested. All compounds were further purified by HPLC.

The binding conformation of ligands **1**, **1c**, **2**, **3**, and **3c** toward the catalytic domain of MMP-12 was investigated by X-ray crystallography.[9] The coordinates and the structure factors of new MMP-12 ligand complexes here described were deposited in the Protein Data Bank under the following PDB codes: 3RTS, 3RTT, and 4GUY. Notably, crystallographic data show that the ligand binding mode toward MMP-12 is superimposable to that predicted by docking calculations, with a ligand RMSD lower than 1.5 Å (Figure 2 and Supporting Information).

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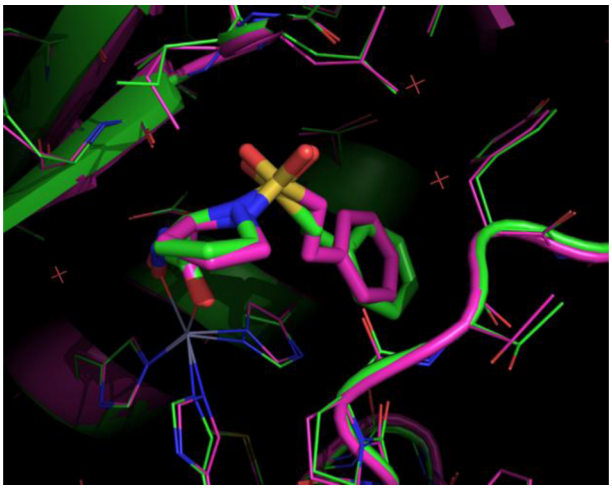

Figure 2. Comparison between the crystallographic- (magenta) and the docking-based (green) binding modes of **2**.

A slight movement of the sulfonamide moiety toward the solvent with respect to the docking pose was observed in the X-ray binding mode of **1**, probably due to the establishment of a H-bond with a crystallographic water molecule that was not accounted for during docking. Overall, ligand interactions with MMP-12 observed by X-ray crystallography are highly consistent with those predicted by docking.

The inhibition constants toward MMP-1, MMP-7, MMP-8, MMP-12, and MMP-13 were further measured by a fluorimetric assay (Table 1). Available K_i values for **1c**, **3c**, **7c**, **7c***, and **12c** belonging to the classic arylsulfonamide scaffold were also reported.[21]

All the investigated compounds are potent inhibitors of the above considered MMPs, notably showing a higher potency in inhibiting MMP-12 and MMP-13 than MMP-1, MMP-7, and, to a lesser extent, MMP-8. Although a significant selectivity was observed with respect to MMP-1 and MMP-7, the lack of a remarkable selectivity toward a specific MMP was attributed to the relative simplicity of the molecular scaffold, which does not bear structural features that are thought to enhance ligand selectivity.[22−25] However, the presented scaffold easily allows for further chemical modifications aimed at improving the selectivity toward a specific MMP. In this respect, the availability of several X-ray structures of MMPs, as well as structural hints provided by selective MMP inhibitors developed so far, such as those presented by Dive,[22] can drive the lead optimization strategy.

Table 1. Experimental K_i Measured by Fluorimetric Assays toward MMP-1, MMP-7, MMP-8, MMP-12, and MMP-13[a]

mol	MMP-8	MMP-12	MMP-13	MMP-1	MMP-7
1c	240	62	42	3400	4000
1	75	23	49	5700	10700
2	43	16	14	1800	7000
3c	59	20	8	77	4000
3	14	10	12	–	–
4	4	1	1	–	–
5	32	10	19	–	–
6	98	5	10	–	–
7c	9	2	2	365	5000
7c*	–	18000	–	29000	–
7*	7	7	5	–	–
8	3	2	1	–	–
9	20	15	35	19700	18500
10	13	5	10	7000	6000
11	37	6	6	–	–
11*	364	272	323	–	–
12	6	9	3	–	–
12c*	275	672	175	–	–

[a]A dash indicates data not available.

In the particular case of MMP-12, the K_i values obtained for the ligands **1−12** span from 1 to 23 nM. Molecules bearing at the same time the D-proline substitution and the ethylene linker exhibit lower K_i toward MMP-12 than the corresponding arylsulfonamide-based inhibitors[21] and, in all cases but one, also lower values than those for molecules bearing only the linker. Indeed **4**, featuring both the D-proline and the ethylene linker, was the most active compound. Even more important are the low K_i values observed for compounds **7***, which has a very low K_i even though being characterized by a carboxylic acid instead of the hydroxamic acid ZBG, which usually exhibits lower affinity for MMP-12 (as observed herein for **7c***).[26] The positive effect of the ethylene linker or D-proline substitution in increasing the affinity of the compounds bearing a carboxylic acid as ZBG is further demonstrated by the nanomolar affinity of compounds **11*** and **12c***. Thereby, **7*** could represent a profitable starting point for the development of a new class of MMP inhibitors (the docking-based binding mode of **7*** is reported in the Supporting Information).[27−29] Finally, the effect of the linker was rather clear by calculating the single atom enthalpy contribution on X-ray adducts (Supporting Information).

To evaluate the contribution of D-proline to ligand affinity, we calculated the free energy of solvation (ΔG_{solv}) for ligands **1−12** and **1c−12c** by means of Gaussian03. Once the molecular geometry of **1−12** and **1c−12c** was optimized in the gas phase at the HF/6-311G* level, ΔG_{solv} was calculated by the Polarizable Continuum Model (PCM) using the 6-311++G(d,p) basis set.[30] The D-proline contribution to the free energy of solvation ($\Delta\Delta G_{solv}$) was then estimated as the difference between the ΔG_{solv} calculated for each D-proline-based inhibitor and the ΔG_{solv} of the corresponding glycine-based derivative (Supporting Information), clearly showing that D-proline introduction into the arylsulfonamide scaffold determines a significant increase of free energy of solvation, which is not related to the chemical substitution in P1′, to the overall size of the ligand, or to the presence of the ethylene linker. Therefore, quantum mechanics (QM) calculations further support the hypothesis that D-proline substitution indirectly improves the binding affinity of MMP-12 inhibitors by increasing their ligand solvation free energy, rather than providing enthalpy gains. The same approach was used to monitor the possible contribution of the linker to the free energy of solvation, but negligible differences were observed, underlining that the linker may improve the affinity of compounds **1−12** by establishing van der Waals interactions with MMP-12, rather than providing favorable solvation effects to the binding.

The analysis of the experimental affinity improvements toward MMP-12 revealed that, on average, the linker provides an enthalpy affinity improvement of 1.5-fold, whereas the D-proline provides a desolvation-based affinity gain of about 3-fold. Therefore, in this series of MMP-12 inhibitors, the solvation free energy provides a contribution twice the enthalpy

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contribution. These findings suggest that the QM $\Delta\Delta G_{solv}$ could be considered as an additional parameter in the evaluation of docking results when structurally related compound are analyzed before the synthetic approach.

In summary, by applying an integrating structure-based approach, a new scaffold for potent MMP inhibitors has been designed. All the related compounds here synthesized and studied by X-ray crystallography and fluorimetric assays show a high affinity toward MMP-12 and a significant selectivity with respect to MMP-1 and MMP-7. Binding modes to MMP-12 predicted by docking were confirmed by X-ray crystallographic data. Computational results indicate that the ethylene linker may contribute to the binding affinity by establishing new van der Waals interactions with the S1′ pocket of the protein. Conversely, *ab initio* PCM calculations show that the introduction of the D-proline ZBG determines a significant increase of theoretical free energy of solvation for these compounds, which might contribute significantly to the observed high affinity. It is worth noting that the predicted water solubility of compounds bearing the D-proline moiety is still highly compatible with the ideal tolerance limits described for hit and lead compounds.[14,31] The contribution of the investigated chemical modifications to the arylsulfonamide scaffold allows the use of ZBGs different from the hydroxamic acid. Notably, **7*** is endowed with a strong inhibitory activity toward MMP-12 and characterized by the carboxylic acid ZBG, which is generally associated with a lower affinity for zinc than hydroxamic acid,[25,27] but with a safer toxicity profile. This compound could be a valuable tool in investigating the biology of MMP-12 catalytic activity and might be a promising lead molecule for the development of medicinally active agents.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Chemistry directions, crystallographic data of PDB IDs 3RST, 3RTT and 4GUY, chemical structures, logP values, fluorimetric assays, QM data, docking analysis, binding mode of **7***, single atom enthalpy contribution, and correlation between experimental and docking data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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