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Structure-based design and synthesis of novel non-zinc chelating MMP-12 inhibitors

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Abstract—A new class of MMP-12 inhibitors was discovered and optimized using structure-based drug design methods. Modeling studies using a known MMP-12 crystal structure identified a new interaction mode for these new MMP-12 inhibitors. Further optimization resulted in the discovery of a compound displaying nanomolar activity against MMP-12 and which was co-crystallized with MMP-12.

Matrix metalloproteinases (MMPs) are a family of zinc dependent endoproteinases, involved in the turnover and remodeling of an extracellular matrix and therefore are therapeutic targets for the treatment of many inflammatory, malignant, and degenerative diseases.[1] For example, inhibitors of MMP-12 are sought for the prevention of chronic obstructive pulmonary disease (COPD) development.[2] The vast majority of reported MMP inhibitors can be viewed as zinc chelating compounds either via a hydroxamate moiety or, less effectively, via other groups, including reverse hydroxamate, thiol, carboxylate, phosphonic acid, and phosphinate functions. Indeed, X-ray crystal structures of MMPs with bound inhibitors reveal that inhibitor binding interactions typically include coordination of the active site zinc by a ligand (e.g., hydroxamic acid) and occupancy of the S1′ pocket with a hydrophobic group. However, in the development of new therapeutic agents targeting MMPs, there remains considerable room for new structural classes not coordinating the active site zinc atom.[3] Moreover, exploiting the structural differences between the MMP subtypes[4] (Table 1), currently an important issue in this area, should lead to more specific inhibitors, postulated to avoid the toxic side effects supposedly linked to broad spectrum inhibitors. We now describe the discovery of a non-zinc chelating compound class optimized for its MMP-12 activity using a structure-based design process.

Table 1. Comparison between various MMP amino acid sequences close to the inhibitor putative binding site

MMP-12	I180	L214	T215	V235	T239	K241	V243
MMP-1	N	R	V	L	S	T	S
MMP-2	L	L	V	L	I	T	T
MMP-3	V	L	V	L	L	H	L
MMP-7	T	Y	A	V	T	G	G
MMP-8	I	L	V	L	N	A	R
MMP-9	L	L	V	L	M	R	T
MMP-13	L	L	V	L	I	T	T
MMP-14	F	L	V	I	F	Q	M

To identify non-competitive or uncompetitive inhibitors from those that are competitive, the MMP-12 high-throughput screen was conducted in the presence of 5 mM acetohydroxamic acid (AH).[5] Compound **1** (Table 2) was thereby identified as an initial lead. Compound **1** alone exhibited weak inhibition of MMP-12 (IC_{50} = 13 μM) but proved to be much more potent (IC_{50} = 0.23 μM) in the presence of AH and showed a complete lack of activity against MMP-1, -3, and -9. The data measured in the presence of AH

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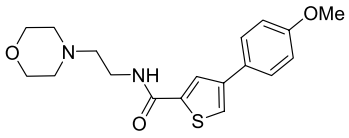
Table 2. Compound **1**, initial hit identified from a mass screen of MMP-12 catalytic domain in the presence of 5 mM acetohydroxamate (AH)

Enzyme	IC_{50} (μM)	Enzyme	IC_{50} (μM)
MMP-12	13	MMP-2	26
MMP-12 + 5 mM AH	0.23	MMP-3	>101
MMP-13	24	MMP-8	25
MMP-1	>101	MMP-9	>101

suggest that compound **1** achieves a synergistic inhibition of MMP-12 with AH and thus binds to the protein in a different way compared to AH. Moreover, compound **1** does not contain an obvious substituent that would chelate the zinc atom. As part of a structure-based design program, modeling studies were initiated using the crystal structure of the inactive E219A mutant MMP-12 determined by Lang et al.[6] We replaced Ala219 by a Glu having the side-chain conformation observed for Glu202 in MMP-13 (pdb code 830c). Docking experiments were conducted with AGdock (proprietary software).[7a] For each of the suggested binding modes, compound **1** was involved in hydrophobic interactions with the S1′ pocket through the phenyl moiety, more precisely with Tyr240 methylene groups. Additionally, the amide moiety forms two hydrogen bonds with the backbone NH of Leu181 and the backbone carbonyl of Pro238. Several binding modes were observed from the docking experiments for the ethyl-morpholine moiety. The preferred orientation is stabilized by hydrophobic contacts between the morpholino methylene groups and the π-electron of the backbone Gly179-Ile180 peptidic bond (Fig. 1). This type of interaction, that is, interaction between an aliphatic group and π-electrons, has already been observed in crystal structure data.[7b]



The overall orientation of the compound clearly matches the preferred region for hydrophobic contacts, as calculated by MOE[7c] contact statistics (green region on Fig. 1). Noteworthy is that the His218 side chain, which constitutes the zinc finger structure, is kept neutral for the docking experiments.[7d] The neutral state of the thiophene moiety creates a hydrophobic field in its vicinity. As a result, the thiophene group makes no interaction other than hydrophobic contacts with His218 (lower side) and Leu181 (upper side), and does not chelate the zinc atom, being at a distance of more than 4 Å. In summary, the thiophene ring constitutes a good linker in optimizing both length and orientation of the compound. This newly discovered interaction mode of MMP-12 inhibitors has motivated us to choose compound **1** as an initial lead candidate for optimization. The docked complex suggests that potency could be improved by optimizing interactions with specific residues lying in the S1′ pocket, that is, through hydrogen bonding either to Lys241 backbone NH or carbonyl oxygen, as well as hydrogen bonding to Thr239 carbonyl oxygen or side- chain hydroxyl group. Furthermore, targeting Lys241 and Thr239 should improve selectivity versus MMP-2,3,9,13, and 14. Moreover, previous studies[4] have shown that MMP selectivity for zinc binding inhibitors can be achieved by optimizing the size of the moiety located in the S1′ pocket, which is differently shaped for each MMP subtype.

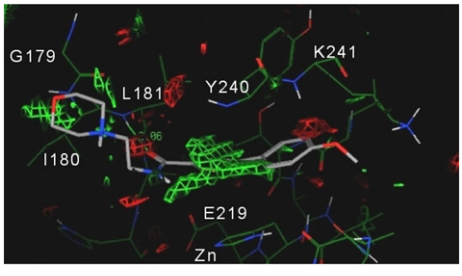


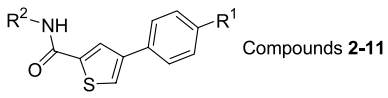
Figure 1. Overlap of compound **1** docked into the active site of MMP-12 cat domain and hydrophobic (green) and polar (red) preferred regions—MOE contact statistics.

On the basis of these results, our main strategy was to optimize the occupancy of the S1′ pocket and hence maximize hydrophobic interactions. On the basis of docking studies of compounds extended on the phenyl ring side with hydrophobic substituents, analogues represented by structures **2**–**5** have been designed and synthesized. As illustrated in Table 3, compounds **2**–**5** were found to be much more potent than the initial hit.

In parallel with the affinity measurements, we also examined the physico-chemical properties of our inhibitors. Not surprisingly, compounds **2**–**5** of Table 3 exhibit poor solubility and high variability of the microsomal metabolic stability, probably related to the solubility issues. A reason accounting for the poor solubility observed in a majority of compounds synthesized to explore the SAR lies in their high lipophilicity, due to their design to fit in the hydrophobic S1′ pocket. More generally, we observed a poor microsomal metabolic stability on a large number of compounds bearing an aminoethylmorpholino group, probably related to the hydrolysis of the amide bond by liver enzymes. To address this issue, a variety of substituents have been investigated for the replacement of this amide substituent, which is open to the solvent according to our docking studies. Table 3 provides a couple of examples of the groups selected with compounds **6** and **7**. A common feature is the presence of a carboxylate group, which probably helps in maintaining some solubility via solvent interactions for these molecules, which are mainly hydrophobic.

Since the majority of compounds displaying the best affinity were biphenyl thienyl derivatives, with a linear, almost planar, shape, presumably favoring efficient crystal packing, compounds were designed to disrupt the planarity of the molecule. Compound **9** is one typical example of this design where we tried to break the planarity. The affinity and solubility data show that compound **9** displays a better solubility than the

Table 3. In vitro profile of compounds **2**–**11**



Compound	R^1	R^2	IC_{50}, (μM) MMP-12	IC_{50}, (μM) MMP-13	Sol (ug/ml)[a]	$t_{1/2}$ (min)[b]
2	Phenyl	2-Morpholin-4-yl-ethyl	0.4		<1	304
3	4-Pyridyl	2-Morpholin-4-yl-ethyl	0.1		3	112
4	Cyclohexyl	2-Morpholin-4-yl-ethyl	0.46		<1	37
5	4-Acetylphenyl	2-Morpholin-4-yl-ethyl	0.057		<1	54
6	Trifluoromethoxy	*trans*-4-Methylcyclohexane-carboxylic acid	0.4		20	248
7	Trifluoromethoxy	4-Methylphenylacetic acid	0.4		100	NM[c]
8	Phenyl	4-Methylphenylacetic acid	0.07	>1.2	<1	NC[d]
9	2-Hydroxyphenyl	4-Methylphenylacetic acid	0.09	2.5	134	NM[c]
10a	Trifluoromethoxy	2-Carboxy-1-phenyl-ethyl, *R* enantiomer	0.18	2.2	>500	NM[c]
10b	Trifluoromethoxy	2-Carboxy-1-phenyl-ethyl, *S* enantiomer	8.3		>500	
11	4-Pyridyl	2-Carboxy-1-phenyl-ethyl, *R* enantiomer	0.014	0.27	>500	99

[a] Thermodynamic solubility measured at pH 7.4.
[b] Metabolic stability in human hepatic microsomes.
[c] Non-metabolized.
[d] Not characterized because of solubility issues.

non-substituted biphenyl compound **8** and remains as potent as compound **8**.

Motivated by these results, we decided to explore the amide side chain, supposedly exposed to the solvent. We planned to carryout interactions with the protein residues in the vicinity of this moiety, although interactions with this region of the protein were extremely difficult to predict. Targeted residues were Ile180 for hydrophobic interactions and Thr239 to form an acceptor or donor hydrogen bond. Several molecules were designed in that manner and compound **10** is an example where we have been able to predict which one of the enantiomers was the most active one.

Docking studies have shown that the *R* isomer **10a** would be stabilized by hydrophobic contacts between its phenyl ring and Ile180, which placed the carboxylate group in the most hydrophilic region (Fig. 2), whereas the *S* enantiomer would have its phenyl ring in the hydrophilic region and its carboxylate anion in the vinicity of the hydrophobic Ile180. As expected, on synthesis and testing of both enantiomers, the R compound proved to be the most active one.

Lastly, this (2*R*)-amino(phenyl)propanoic acid was combined with one of the active hydrophobic phenyl-pyridyl substituents to generate compound **11**, which is soluble and displays good affinity for MMP-12 (IC_{50} = 14 nM) and, to a less extent, for MMP-13 (IC_{50} = 270 nM). Eventually, the binding mode of compound **11** was confirmed by an X-ray crystal structure of MMP-12 catalytic domain complexed with compound **11** with a resolution[8] of 2.5 Å (Fig. 3).

The data clearly show that the biaryl moiety fits into the S1′ pocket of the enzyme, where binding interactions are mainly hydrophobic. Indeed, the core structures of thiophene and phenyl aromatic rings make hydrophobic contacts with Thr215, Tyr240 methylene, and His218. Similarly, lipophilic interactions take place observed between the 4-pyridyl group and Lys241 γ- and ε-methylene groups on the upper side, as well as Val235 on the lower side. Additionally, key hydrogen bonds exist between the carbonyl oxygen and the backbone NH of Leu181 and NH of Ala182, as well as between one carboxylate oxygen atom and the backbone NH of Tyr240, whereas the other carboxylate oxygen atom binds to Gly179 carbonyl oxygen via a water molecule.

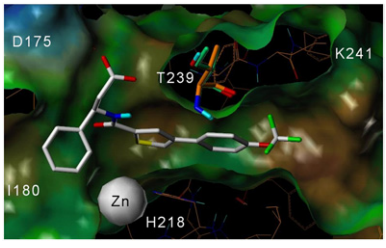


Figure 2. Compound **10a** docked into the active site of MMP-12 cat domain. Hydrophobic regions are colored in brown, while polar surface areas are colored in blue.

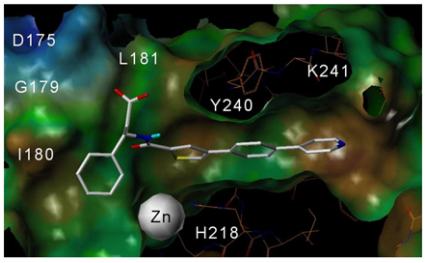
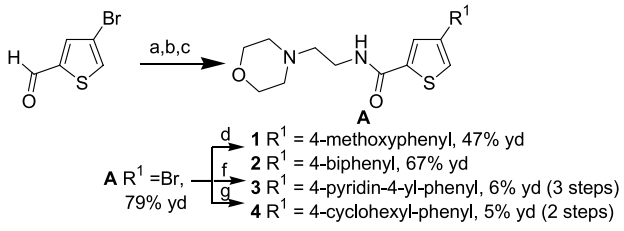


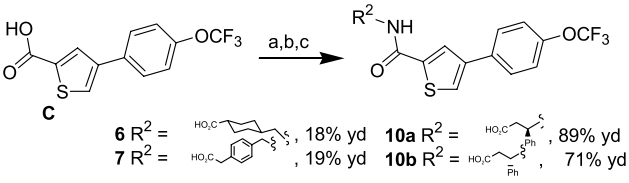
Figure 3. X-ray structure of a MMP-12 catalytic domain complexed with compound **11**.

Synthesis of inhibitor **1** and related analogues is based on the versatile palladium-catalyzed coupling chemistry. Compounds **1**–**4** were synthesized, as shown in Scheme 1. Briefly, an oxidation and amide formation sequence yielded the common intermediate **A**, which was either directly coupled under Suzuki conditions with boronic acids to give products **1** and **2** or transformed into a triflate to yield final product **3** or eventually derivatized into a stannane to undergo a Stille coupling to yield **4**. Compound **5** was prepared in the same manner using 4-(4-bromophenyl)-*N*-(2-morpholin-4-ylethyl)thiophene-2-carboxamide intermediate that was obtained through a Sandmeyer sequence. Compounds **6**, **7**, and **10** were prepared from a common intermediate **C**, derived from the starting aldehyde following a Suzuki sequence, through amide bond formation with readily available amines as shown in Scheme 2. Eventually, preparation of compound **11** is achieved following the chemical route shown in Scheme 3.

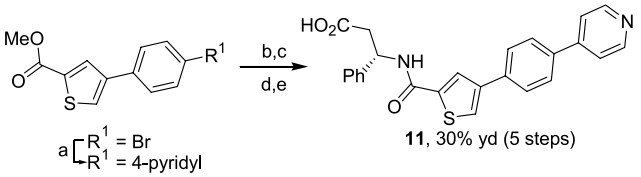
In summary, we have discovered a series of potent MMP-12 inhibitors incorporating a thiophene template, an unusual central linker compared to established MMP binding groups. Structure-based drug design methods led to compounds with an improved potency for MMP-12, achieving a 1000-fold enhancement compared to the initial hit. In contrast to the well-established MMP inhibitors, the compounds described do not interact with the zinc active-site atom and nevertheless display affinities for MMP-12 in the nanomolar range. X-ray crystallography data have confirmed the unique binding mode predicted from docking experiments. Issued from an activity-based and property-based optimization program, compounds such as compound **11** may be regarded as novel, non-peptidic, low molecular weight, non-zinc binding leads for continued development of MMP inhibitors as drugs. On the basis of our crystal structure and molecular modeling model, this compound could be viewed as the starting point for selectivity optimization over MMP-13 and physicochemical profile enhancement.



Scheme 1. Preparation of compounds **1**–**4**. Reagents and conditions: (a) $AgNO_3$, EtOH/NaOH aq, 40 °C; (b) $(COCl)_2$, CH_2Cl_2, DMF cat., 0 °C then rt; (c) 4-(2-aminoethyl)morpholine, Et_3N, CH_2Cl_2, rt; (d) R_1boronic acid, $Pd(PPh_3)_4$, K_3PO_4 aq 2 M, DME, 80 °C; (f) i—4-hydroxyphenylboronic acid, Suzuki cond.; ii—trifluoromethanesulfonic anhydride, CH_2Cl_2, pyridine, rt; iii—pyridine-4-boronic acid, Suzuki cond.; (g) i—hexamethylditin, $Pd(PPh_3)_4$, DME, 80 °C; ii—1-bromo-4-cyclohexylbenzene, LiCl, $CuBr_2$, $Pd(PPh_3)_4$, dioxane, 80 °C.



Scheme 2. Preparation of compounds **6**, **7**, and **10**. Reagents and conditions: (a) $(COCl)_2$, CH_2Cl_2, DMF cat., 0 °C then rt; (b) R_2NH_2, Et_3N, CH_2Cl_2, rt; (c) lithium hydroxide, EtOH/H_2O.



Scheme 3. Preparation of compound **11**. Reagents and conditions: (a) pyridylboronic acid, $Pd(PPh_3)_4$, K_3PO_4 aq 2 M, DME, 80 °C; (b) MeOH, HCl, 65 °C; (c) lithium hydroxide, EtOH/H_2O; (d) (2*R*)-amino(phenyl) propanoate, HATU, DIEA, DMF, rt; (e) lithium hydroxide, EtOH/H_2O and then HCl 2 M.

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