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Strategic Targeting of Multiple Water-Mediated Interactions: A Concise and Rational Structure-Based Design Approach to Potent and Selective MMP-13 Inhibitors

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In memoriam Claudia Nothnagel

Water is an essential molecule in biological systems based on its role as the solvent for life.[1] Understanding the dynamics of the interaction between water and proteins represents a highly active field of research in molecular recognition, chemical biology, and drug discovery.[2,3] In chemical biology, water-mediated interactions offer tremendous opportunities for the development of novel chemical structures with biological activity, but due to its small size on the one hand and to its overwhelming abundance as a solvent on the other hand, water is often neglected when it comes to the detailed study of biological processes on a molecular or atomic level. Here, we report the very efficient use of X-ray crystallographic data containing structural water molecules for the design and synthesis of potent and selective matrix metalloproteinase-13 (MMP-13) inhibitors by targeting multiple water-mediated interactions between the protein target and the inhibitor.

MMP-13 is a highly relevant and validated target for a multitude of severe diseases, such as cancer, osteoarthritis and rheumatoid arthritis.[4] MMP-13 is a member of the zinc-dependent endopeptidase family. It is the dominant MMP involved in type II collagen cleavage in the degradation process of extracellular matrix during growth and tissue remodeling.[4b,5,6] Early attempts at finding inhibitors against MMPs resulted in peptidomimetics derived from natural substrates with modified moieties close to the scissile amide bond.[4g] The potency of these inhibitors was further enhanced by introducing zinc-chelating groups in order to bind to the zinc ion in the active site of the enzyme. Hydroxamates turned out to be the most efficient zinc binders.[7,8] Because of unsatisfying bioavailability and severe side effects due to a lack of selectivity, all clinical candidates containing strong zinc binding groups failed in clinical trials.[9] While doxycycline, an antibiotic tetracycline that exhibits off-target MMP inhibition, has been the only inhibitor to reach the market so far, this does indicate that the target protein family is indeed druggable.[4g] In order to overcome the deleterious side effects of strong zinc binding inhibitors, a new class of MMP inhibitors has been developed recently that does not bind to the catalytic zinc but rather binds deep within the S1′ pocket.[10] This finding leads to new opportunities for the discovery of selective MMP-13 inhibitors based on the structural differences in the S1′ binding site among different MMPs.

In chemical biology and medicinal chemistry, there is a constant need for novel small molecules modulating biological activity in order to achieve insights into the underlying biological processes on a molecular level. In particular, pharmaceutical companies spend a considerable amount of their budget in the development of potent and selective scaffolds of biologically active molecules. Those small-molecule modulators can either be discovered by extensive and resource-intensive screening campaigns or by rational design approaches. Rather than performing screening activities, we approached this problem by analyzing co-crystal structures of the target protein including structural water molecules in order to define the pharmacophore and substitution pattern for inhibitor scaffolds. Here, our focus was on using structural water molecules as binding partners for novel small-molecule modulators.

Analysis of the co-crystal structure PDB 2OW9[10h] (Figure 1) allowed us to design a novel scaffold of MMP-13 inhibitors

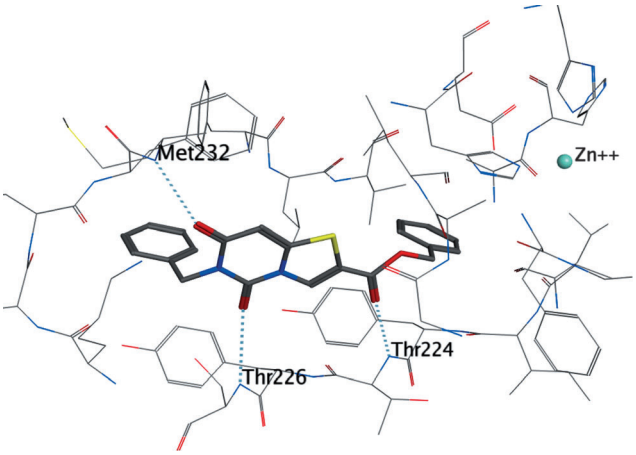

Figure 1. Analyzing the pharmacophore of the co-crystallized inhibitor in PDB 2OW9[10h] allowed for the generation of novel phthalimide scaffold **4**.

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that was subsequently optimized with regard to its binding affinity by targeting water-mediated interactions.

Conserving the hydrogen-bonding capabilities to the backbone NH of Thr224, Thr226 and Met232 as well as the π–π interaction to His201 yielded phthalimide scaffold **4**. Molecular modeling of **4** in the S1′ binding site using force field MMFF94x[11] supported the expected binding orientation of **4** (Figure 2).

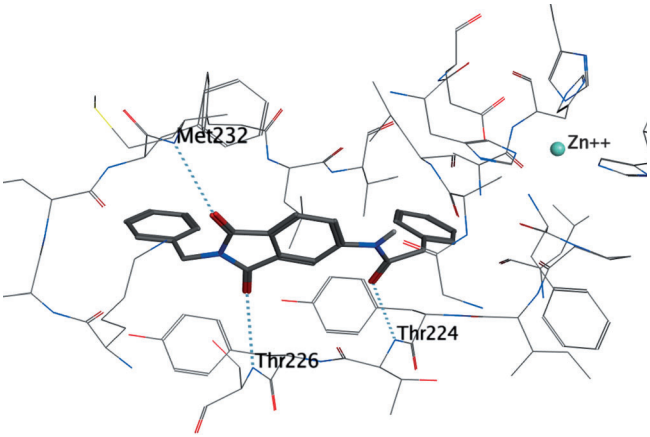
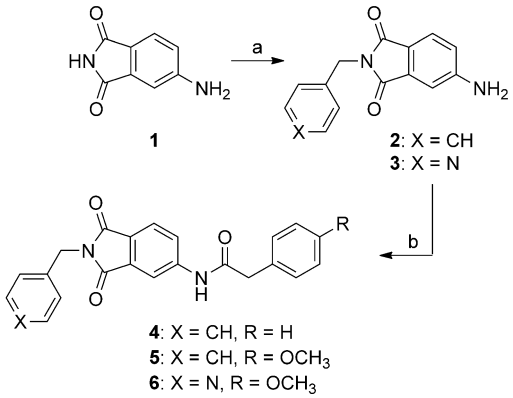


Figure 2. Rational design of phthalimide inhibitor **4** in the MMP-13 S1′ binding site: direct hydrogen bonding to the protein.

Furthermore, molecular modeling of **4** within the S1′ binding sites of MMP-2 (PDB 3AYU),[12] MMP-12 (PDB 1Y93),[13] and MMP-14 (PDB 3MA2)[14] indicated that **4** does not fit into the MMP-2, MMP-12 and MMP-14 binding sites due to a clash in the selectivity loop deep within the S1′ pocket. This finding suggested good selectivity of phthalimide scaffold **4** for MMP-13 over MMP-2, MMP-12 and MMP-14, which is in contrast to classical zinc binding inhibitors.

Based on our molecular modeling results and supported by the positive evaluation in medicinal chemistry filtering processes such as the Lipinski concept,[15] the parent compound of phthalimide scaffold **4** was synthesized in a two-step synthesis starting from 4-aminophthalimide **1** via 2-substituted 5-amino-isoindoline-1,3-diones **2**[16] and **3** (Scheme 1). The ease of its synthesis makes the phthalimide scaffold an ideal candidate for subsequent library synthesis for the optimization of binding properties.

Compound **4** was tested for its biological activity against MMP-2, MMP-12, MMP-13 and MMP-14 at an inhibitor concentration of 6.5 μM in single-point determinations, which were averaged over three measurements. Compound **4** showed inhibitory activity of 25% at 6.5 μM against MMP-13 and moderate selectivity over the antitargets MMP-2, MMP-12 and MMP-14, which supported our molecular modeling results (Table 1). The promising potency and selectivity data of the designed phthalimide scaffold allowed us to perform the second step of our design concept, which was targeting water-mediated interactions in order to improve the potency of the inhibitor structure.



Scheme 1. Synthesis of phthalimides **4**–**6**. *Reagents and conditions*: a) BnBr (**2**)/4-(bromomethyl)pyridine (**3**) (1.0 equiv), KOH (1.0 equiv), DMF, RT, 18 h, 59% (**2**); 77% (**3**); b) phenylacetyl chlorides (1.2 equiv), DIPEA (1.5 equiv), THF, RT, 2 h, 81% (**4**); 86% (**5**); 50% (**6**).

Table 1. MMP inhibitory data for compounds **4**–**6**.

Compd	MMP-13	MMP-2	MMP-12	MMP-14
4	9.80,[a] 25[b]	4[b]	11[b]	8[b]
5	1.57[a]	>100[a]	>100[a]	>100[a]
6	0.49[a]	>100[a]	>100[a]	>100[a]

[a] IC_{50} values [μM]; confidence intervals are given in the Supporting Information. [b] % Inhibition at 6.5 μM; for further details, see the Supporting Information.

Analyzing the close surroundings within 5 Å of the terminal phenyl groups of the designed phthalimide scaffold in the S1′ binding site of PDB 2OW9 with a resolution of 1.74 Å revealed several water molecules as attractive binding partners based on their distance from the inhibitor scaffold: water molecules HOH747, HOH813, HOH836, HOH915 on the right-hand side and HOH794, HOH822, HOH890 on the left-hand side (numbering based on protein chain A of PDB 2OW9; Figure 3).

In order to get a better understanding of the water molecules that are in the proximity of phthalimide scaffold **4**, over-

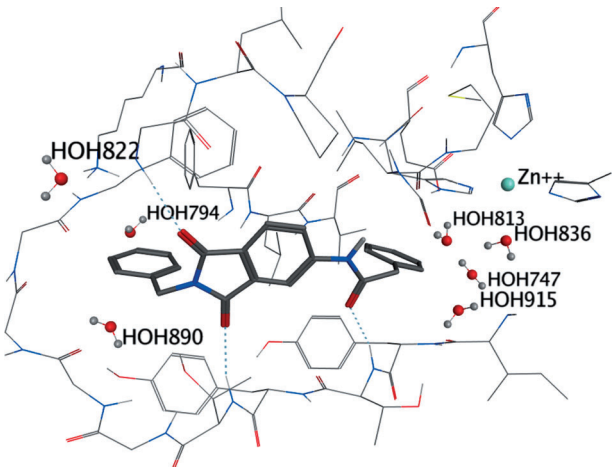


Figure 3. Analysis of water molecules in proximity to phthalimide scaffold **4** within the S1′ binding site of PDB 2OW9.

lays of different MMP-13 co-crystal structures were analyzed. The overlay of PDB 2OW9 and PDB 2OZR[10h] indicated that on the right-hand side, HOH747, HOH813 and HOH836 can be considered as structural water molecules that can be targeted as valuable binding partners in order to improve the binding affinity of phthalimide scaffold **4**. Compared with their counterparts in PDB 2OZR, those water molecules are located at very similar positions and share the same binding motifs to the target protein: HOH747 binds to the backbone NH of Leu 164, HOH813 binds to the carboxylate side chain of Glu 202, and HOH836 binds to the catalytic zinc ion as well as to the backbone carbonyl of Pro 221. HOH915 on the other hand, which is also located in close proximity to the phthalimide scaffold, binds differently compared with its closest counterpart in PDB 2OZR (Figure 4).

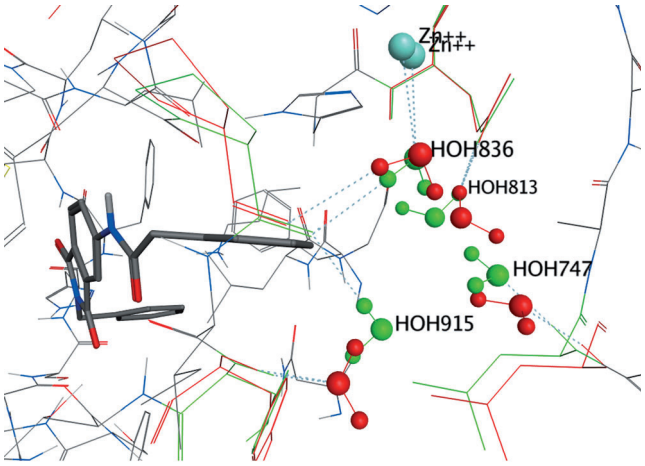


Figure 4. Detailed analysis of water molecules in proximity to the right-hand side of phthalimide scaffold **4**. Overlay of PDB 2OW9 (water molecules and binding amino acid residues in green) and PDB 2OZR (water molecules and binding amino acid residues in red).

On the left-hand side, HOH794 and HOH822 in PDB 2OW9 bind in very similar positions compared with their counterparts in PDB 1XUD.[10a] Again, those water molecules share the same binding motifs: HOH794 binds to the side chain carboxamide NH of Asn 194, and HOH822 binds to the backbone NH of Phe 231 as well as to the side chain amino functionality of Lys 119. HOH890 and its counterpart from PDB 1XUD do not show hydrogen bonding to the MMP-13 target protein (Figure 5).

First, the right-hand portion of inhibitor scaffold **4**, the phenylacetic acid fragment, was modified with a *para*-methoxy substituent in order to introduce an interaction with the structural water molecule that is bound to Glu 202. Energy-minimized poses of the *para*-methoxy-substituted inhibitor structure in the S1′ binding site indeed predict hydrogen bonding to the water molecule HOH813 interacting with Glu 202 (Figure 6).

Molecular modeling of *para*-methoxy-substituted phthalimide scaffold **5** into the S1′ binding site showed a hydrogen-bonding network ranging from the methoxy group to the catalytic zinc ion involving structural water molecules HOH813 and HOH836, as well as the carboxylate from the bridging Glu 202 (Figure 6). Therefore, phthalimide inhibitor compound **5** can be considered a supramolecular inhibitor, which is predicted to bind in the non-zinc-binding S1′ binding site while interacting via structural water molecules with the catalytic zinc ion.

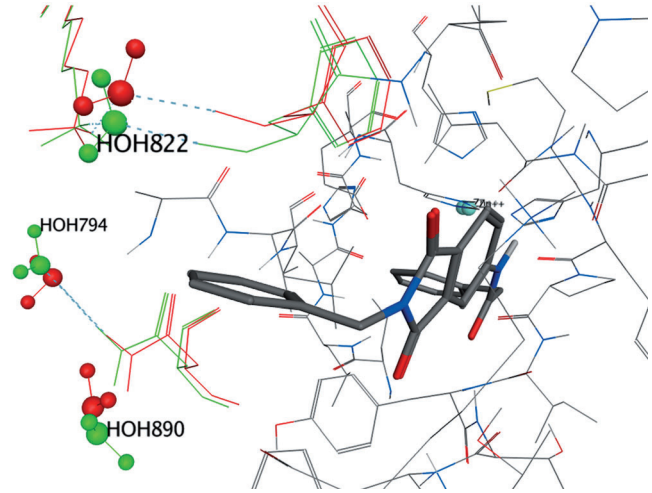


Figure 5. Detailed analysis of water molecules in proximity to the left-hand side of phthalimide scaffold **4**. Overlay of PDB 2OW9 (water molecules and binding amino acid residues in green) and PDB 1XUD (water molecules and binding amino acid residues in red).

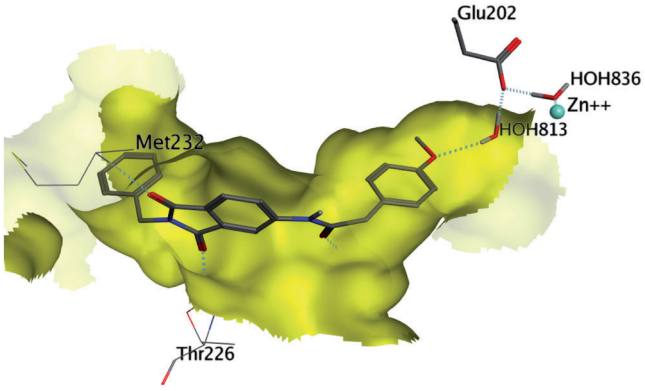


Figure 6. Targeting structural water molecules by introducing a *para*-methoxy group to the phthalimide inhibitor scaffold; compound **5** shows water-mediated binding to Glu 202 by HOH813.

para-Methoxy-substituted compound **5** was synthesized (Scheme 1) and evaluated in vitro. Compound **5** exhibited a drastic improvement in potency against MMP-13 over parent compound **4**. Not only the potency but also the selectivity improved over the antitargets MMP-2, MMP-12 and MMP-14 (Table 1). The sixfold increase in affinity for MMP-13 equals a change in Gibbs free energy at 298 K of about $-4.5\ kJ\,mol^{-1}$. This value is within the estimated range from crystalline salt hydrates for the maximal change in Gibbs free energy when transferring a water molecule from bulk solvent and including

it in an interaction between a protein and an inhibitor molecule (max. −7.0 kJ mol^{-1}).[3i,k]

Encouraged by the successful implementation of a water-mediated interaction on the right-hand side of the inhibitor structure and based on our analysis of the water molecules in proximity to the left-hand side of phthalimide scaffold **4** (Figure 5), we identified structural water molecules HOH794 and HOH822 as possible binding partners to further optimize our inhibitor scaffold.

Replacing the benzylic fragment by a 4-pyridylmethylene fragment in order to form a hydrogen bond to water HOH822 yielded compound **6** (Figure 7). Compound **6** was subsequently synthesized (Scheme 1), and the determination of its inhibitory activity confirmed an additional improvement in potency against MMP-13 while retaining the very attractive selectivity profile with respect to the antitargets (Table 1).

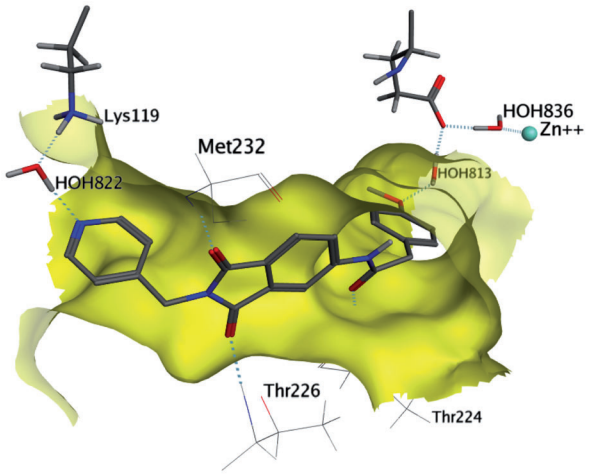


Figure 7. Targeting structural water molecules by introducing a 4-pyridylmethylene fragment to the phthalimide inhibitor scaffold; compound **6** shows additional water-mediated binding to Lys 119 by HOH822.

Compound **6** represents a novel chemical scaffold with nanomolar inhibitory activity against MMP-13, which was designed as a chemical scaffold that binds to the biological target by direct hydrogen bonds, as well as through a network of water-mediated interactions (Figure 8).

In conclusion, we have demonstrated how to use co-crystal structures of therapeutically relevant proteins very efficiently for the design of inhibitors by targeting multiple water-mediated interactions with the target protein. By following this rational design concept of targeting multiple structural water molecules as binding partners for small organic molecules, we could enhance the binding affinity of our rationally designed phthalimide scaffold by a factor of 20. This led to a potent and selective nanomolar MMP-13 inhibitor without any screening activities by a highly decreased number of synthesized compounds compared with classical medicinal chemistry or screening approaches and emphasizes the importance of structural water molecules for the design and discovery of novel small-molecule inhibitors. We are currently expanding this structural

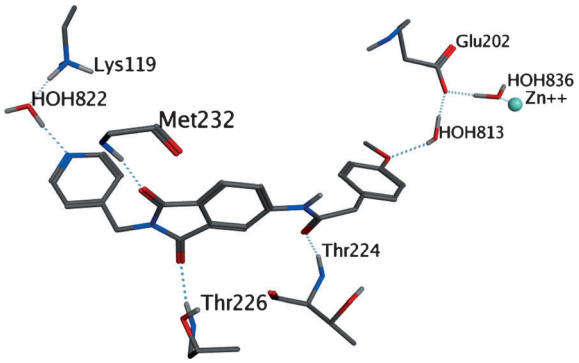


Figure 8. Interaction map of inhibitor **6**: hydrogen-bonding network to structural water molecules in addition to direct hydrogen bonding to Thr 224, Thr 226 and Met 232.

design concept of targeting structural water molecules to other target proteins to show the broad applicability of this approach in the efficient design of novel molecules with tailored biological activity.

Experimental Section

Details of the synthetic protocols and characterization data for novel compounds can be found in the Supporting Information along with details of the biological methods used to evaluate these compounds.

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Keywords: chemical biology · matrix metalloproteinase inhibitors · structure-based drug design · structure–activity relationships · water-mediated interactions

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