# Design In Silico, Synthesis and Binding Evaluation of a Carbohydrate-Based Scaffold for Structurally Novel Inhibitors of Matrix Metalloproteinases

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The matrix metalloproteinases (MMPs) are members of a continuously growing family of Zn-dependent endopeptidases that function extracellularly. This clan of enzymes, specialized in endopeptidase activity, is involved in both normal and pathological tissue remodeling. Activation and over-expression of MMPs seem to be connected with pathological conditions such as arthritis, cardiovascular diseases, multiple sclerosis, and cancer-cell metastasis.[1–3] Since preclinical studies clearly showed that the inhibition of MMPs would be therapeutic for such diseases, the generation of effective and selective inhibitors has become an extremely attractive goal.

Early approaches to the identification of potential MMP inhibitors (i.e. substrate-based design of peptidomimetics and random screening of natural product or compound libraries) have recently been replaced by structure–activity relationship (SAR) studies. Deeper insights into enzyme–ligand interactions have been possible from SAR studies, and structure elucidation through X-ray and NMR spectroscopy of MMP catalytic domain/inhibitor complexes showed that the interaction of the inhibitor with zinc in the active site is very important in determining biological potency.[4–6]

Beside the active-site zinc(II) ion, all MMPs are characterized by a hydrophobic cavity, conventionally designated the $S_1'$ pocket, which offers the greatest opportunity for selective-inhibitor design because there is considerable variation between the MMPs in its dimensions and the residues that line the pocket. Thus peptidomimetics that incorporate a zinc ligand and $S_1'$ side chain represent one of the most common classes of MMP inhibitors (i.e. Marimastat,[7] Batimastat[8]), while another class of zinc ligand compounds are sulfonamide-based inhibitors such as NNGH[9,10] or AG3340.[11]

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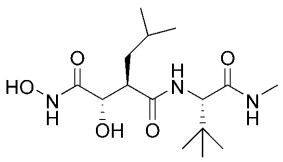
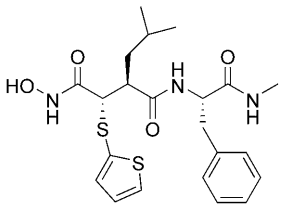


Marimastat (British Biotech)



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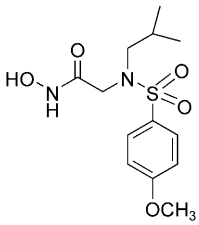
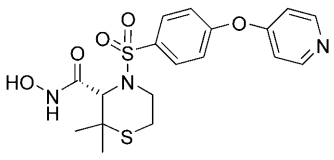


NNGH (Biomol)



AG-3340 (Agouron)

Generally, the structures of MMP inhibitors are related to very few molecular scaffolds, the vast majority of which contain hydroxamic acids as the zinc-binding group (ZBG), followed by carboxylic acid inhibitors.

As a matter of fact, the most common strategy for designing new potential inhibitors is based on the superimposition of new structures onto those of known inhibitors.[12] Although successful in terms of inhibitory activity, this strategy generates molecules that tend to exhibit the same shortcomings of the model compounds. For example, the low selectivity exhibited by known MMP inhibitors (MMPIs), which makes them poorly discriminating towards the active sites of the enzymes and is one of the main reasons for their failure in clinical trials,[13,14] is related to their structural similarity.

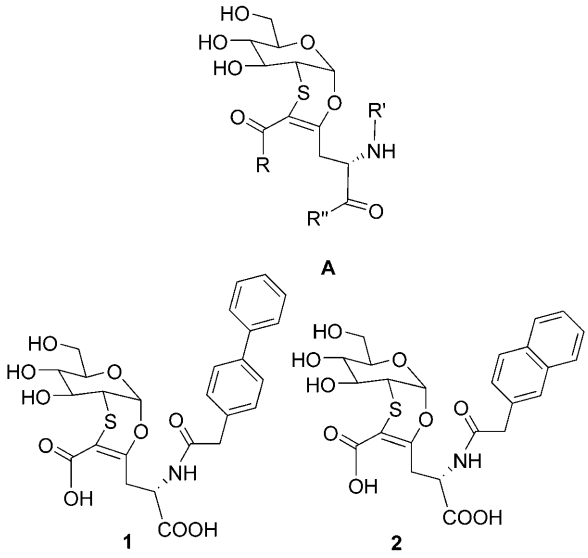
The design of new scaffolds that are structurally diverse from known compounds is therefore necessary, and in this respect the availability of the 3D structure of MMP with its active site represents a great advantage. A virtual screening of new molecules, performed before undertaking their synthesis, can markedly reduce time and costs, thus giving a better chance of success. In addition, docking analyses can provide information on the conformation of the ligand within the binding pocket, directing suitable modifications of the inhibitor structure.

The results provided by in silico studies need to be confirmed and integrated by experimental data on ligand–protein interaction. NMR is a powerful tool for monitoring ligand–protein interaction, especially in the presence of relatively weak binding ligands.

The integrated analysis of ligand–protein interactions and in silico studies provides a convenient strategy for choosing starting molecular leads and checking the lead-optimization process in order to solve the two main problems of the search for a "new generation" of efficient inhibitors namely 1) good selectivity and 2) oral bioavailability. Approaches to the design of inhibitors in which the available space in the MMPs' $S_1'$-specific pocket is filled with large hydrophobic groups are currently generating compounds that are insoluble in physiological media. Therefore a balanced contribution from the hydrophilic and lipophilic portions should be pursued to assure a good bioavailability.

The development of novel carbohydrate-based bioactive molecules was recently reported and suggested that carbohydrate-containing compounds that can overcome bioavailability problems could provide a source of new drug candidates.[15]

In an effort to improve our comprehension of the bioactive conformation of new potential inhibitors, we focused our attention on sulfur-containing constrained structures.[16,17] Bicyclic *O*-glycoamino acidic scaffolds such as **A** (Scheme 1) are



**Scheme 1.**

constrained versatile structures that can be prepared as diastereomerically pure $\alpha$-*O*-glyco derivatives through a totally chemo-, regio-, and stereoselective Diels–Alder reaction under very mild conditions. Despite the naphthyl- and biphenyl- residues, scaffolds **A** are soluble in water due to their carbohydrate portion. A further advantage offered by bicyclic scaffolds **A** is a reduced degree of conformational freedom, which can improve the reliability of predictions in virtual screening. Moreover, the orthogonally protected functional groups of **1** and **2**, will allow further manipulation of the architecture to generate a family of functionally related inhibitors.

For many MMPs, the shape and structure of the $S_1'$ pocket have been extensively investigated in order to design selective inhibitors.[18–20] In the case of macrophage metalloelastase (MMP-12), the wide $S_1'$ pocket is able to accommodate relatively large hydrophobic groups and to provide an appreciable interaction with the protein.

In order to identify new selective and water-soluble MMP-12 ligands, we designed 240 $\alpha$-*O*-glycodipeptide mimetic derivatives with different lipophilic groups. The bicyclic *O*-glycopeptide scaffold **A** has three different positions that can be easily functionalized, and 80 different lipophilic moieties were considered. These lipophilic molecules had been previously selected from among the moieties already known to be accommodated into the $S_1'$ pocket.

A virtual screening of all molecules has been performed by using the Lamarckian Genetic Algorithm (LGA) of the AutoDock 3.0.5 program.[21] The PDB file of the MMP12 catalytic domain (PDB code 1RMZ) corresponding to Gly106–Gly263, solved by X-ray crystallography at 1.3 Å resolution, was used, and the protein charges were assigned by using the AMBER force field.[22] A box of $19.75 \times 19.75 \times 19.75$ Å$^3$ with a grid spacing of 0.275 Å and centered near to the catalytic zinc was defined as docking space. The structures of the glycopeptide derivatives were generated by using Chem3D pro, and their geometry was minimized by semiempirical methods. The correct charges were then assigned according to the Gasteiger–Marsili method. A total of 50 docking runs were performed for each ligand, and the results were ranked according to docking energies. One of the main problems related to virtual screening is the assessment of the binding constant. It can be directly derived from the AutoDock binding energy, but this should be considered as a scoring function useful for selecting results[23] that need to be experimentally validated. With this in mind, a set of ten commercial molecules that exhibit in silico, weak, medium, and high affinity for the $S_1'$ cavity of MMP-12 were selected, and the binding constant was determined by NMR spectroscopy. Among the molecules analyzed, compounds **1** and **2** (Scheme 1), which are characterized by a side chain featuring a biphenyl group (**1**) or a naphthyl group (**2**) linked to the homoglutamic nitrogen and by a carboxylic residue as binding group for zinc, provided the best results in terms of docking energy and cluster population. For both compounds, the value of the docking energy was of the same order of magnitude as that of ligands with micromolar affinity.

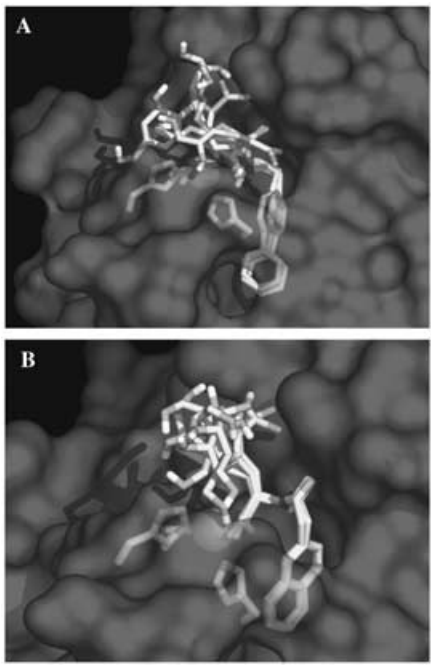
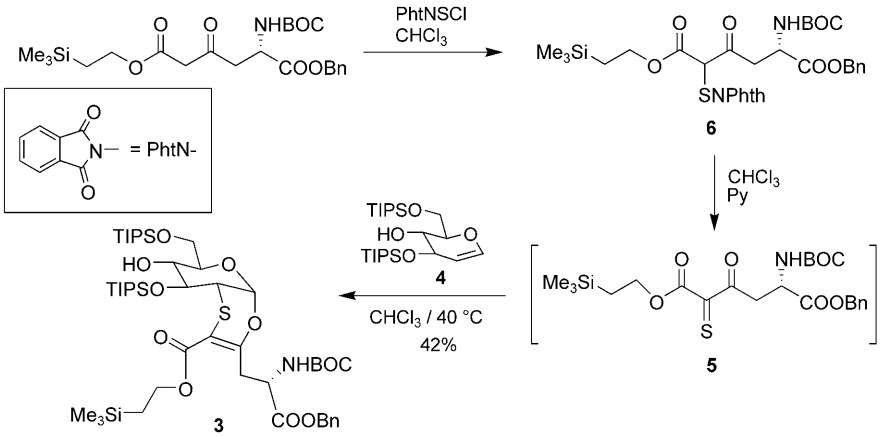



**Figure 1.** Docking of compounds A) **1** and B) **2** into the 3D structure of the MMP-12 catalytic domain (PDB code 1RMZ).

Inspection of the models (Figure 1) indicated that, as expected, binding is due to hydrophobic interactions of **1** and **2** with the $S_1'$ pocket through the aromatic moieties. The monosaccharidic residue located outside the protein does not appear to contribute to binding. Interestingly, AutoDock also revealed that the non-amino acidic carboxylic residue of compound **2** (Figure 1 B) is oriented toward the catalytic zinc ion, whereas it sticks out from the protein surface in the case of the biphenyl derivative **1** (Figure 1 A).



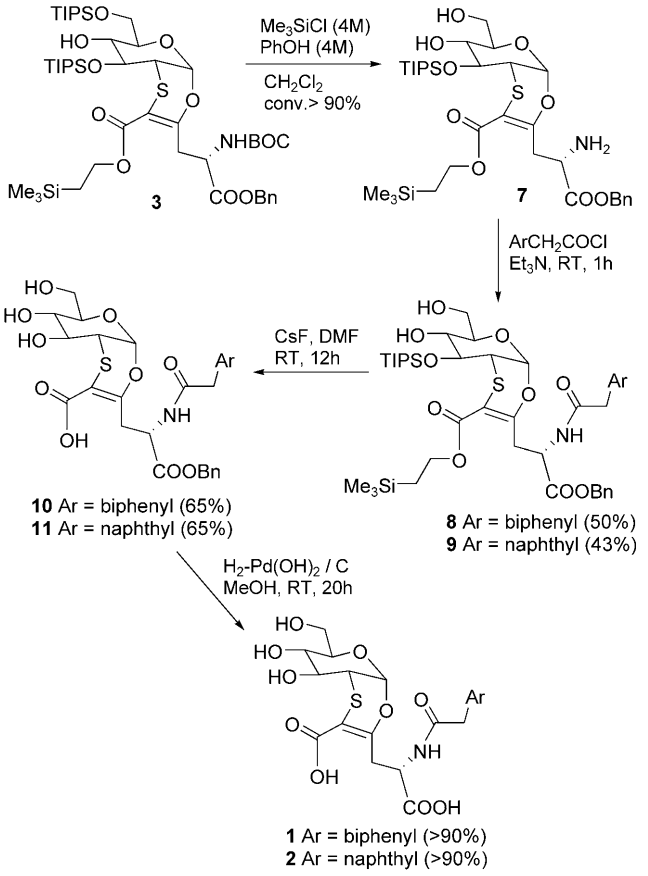

**Scheme 2.**

Carboxylic acid-based inhibitors **1** and **2** (Scheme 1) were prepared as enantiomerically pure compounds from *O*-glyco derivative **3**, obtained by cycloaddition of the electron-rich dienophile **4** with the electron-poor diene **5**, which, in turn, was synthesized according to a literature procedure (Scheme 2).[24] Diene **5** is a highly reactive intermediate that is generated "in situ" from the phthalimidesulfenyl derivative **6** and trapped by the glucal **4**.

Selective removal of the *tert*-butoxycarbonyl group (Boc) with trimethylsilyl chloride and phenol afforded the amino derivative **7**, which was subsequently transformed into the corresponding amides **8** and **9** by treatment of the crude with biphenyl-4-yl- and naphthalene-2-yl-acetyl chlorides, respectively (Scheme 3). Treatment of **8** and **9** with CsF in DMF at room temperature allowed the simultaneous removal of the triisopropylsilyl (TIPS) group at C-3 and deprotection of the trimethylsilylethyl ether to afford the monoesters **10** and **11**. Hydrogenation of the last two compounds gave the water-soluble dicarboxylic acids **1** and **2** in quantitative yield.

Binding of **1** and **2** to the $^{15}$N-enriched catalytic domain of macrophage metalloelastase (MMP-12)[25] was monitored by $^1$H,$^{15}$N HSQC NMR spectroscopy. The sample of MMP-12 catalyt-




**Scheme 3.**

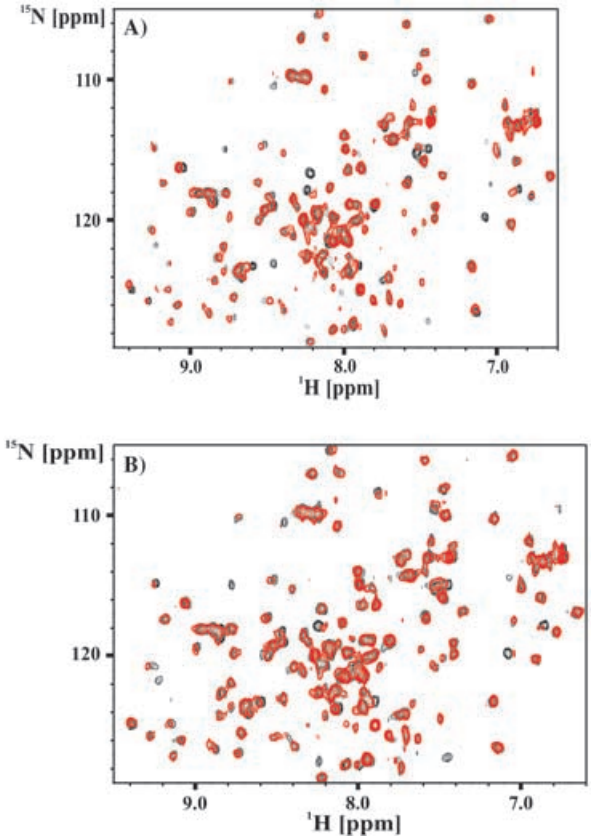



**Figure 2.** A) $^{1}H,^{15}N$ HSQC spectrum of the MMP-12 catalytic domain without (black) and in the presence of (red) 1 mM of compound **1**. B) $^{1}H,^{15}N$ HSQC spectrum of MMP-12 catalytic domain without (black) and in the presence of (red) 1 mM of compound **2**.

ic domain consisted of 0.1 mM protein in 10 mM Tris-HCl buffer, 10 mM $CaCl_2$, 0.1 mM $ZnCl_2$, 0.3 M NaCl, 200 mM acetohydroxamic acid at pH 7.2, and 10% $D_2O$. 2D NMR spectra were recorded at 298 K on a Bruker DRX 700 spectrometer equipped with a TXI probe. Amide NH resonances were detected through $^{1}H,^{15}N$ HSQC experiments[26,27] implemented with the sensitivity enhancement scheme.[28,29] $^{1}H,^{15}N$ HSQC spectra of the MMP-12 catalytic domain were acquired before and after the addition of a solution of compounds **1** and **2** in $[D_6]$DMSO to protein samples. The final concentration of compounds **1** and **2** was 1 mM with 1% of $[D_6]$DMSO.

The presence of compound **1** or **2** induced relevant changes in the HSQC spectra, thus unequivocally revealing the interaction with the protein (Figure 2). The variation induced in the spectra of the free MMP-12 domain by **1** and **2** are similar but not identical, according to their different structures and to the models provided by docking studies. Indeed, in the presence of glyco derivative **1** or **2**, many correlation peaks of the MMP-12 catalytic domain were shifted or broadened beyond detection.

The residues involved in the interaction were identified by using the 3D structure of the protein and the 2D $^{1}H,^{15}N$ HSQC correlation peaks. Binding of compounds **1** and **2** mainly affects amino acids that form the active site of the enzyme (Phe213, Ala216, His218, Glu219, His222, Ser229); this corroborates the docking analysis.

To assess the binding properties of compounds **1** and **2**, their abilities to inhibit the hydrolysis of fluorescence-quenched peptide substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-$NH_2$ (Biomol, Inc.)[30] were tested. The assay provided an $IC_{50}$ value of 490 μM for compound **1** and of 720 μM for compound **2**.

Compounds **1** and **2** represent the first two examples of carbohydrate-based inhibitors of MMP12.[31] The presence of free hydroxyl and carboxylic groups makes these two molecules soluble in water even in the presence of large lipophilic groups. An appreciable interaction of **1** and **2** with the protein proved that the monosaccharidic fragment did not affect the affinity of biphenyl- or naphthyl-containing derivatives.[32] Binding is mainly due to hydrophobic interactions of the aromatic rings with the $S_1'$ pocket, whereas monosaccharidic portions do not appear to contribute. Docking studies and $^{1}H,^{15}N$ HSQC experiments matched in pointing to a lack of real interaction of the carboxylic residues of **1** and **2** with the active site of the protein and in confirming the correctness of the lipophilic portions selected. In conclusion, the presence of an inhibitory activity of **1** and **2** probably suggests that the carbohydrate-containing skeleton we propose represents a veritable scaffold suitable for designing more powerful inhibitors.

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