In Silico Evaluation of Some Dihydroxamic Derivatives of Diphenylether, as Hybrid Hydroxamic-Allosteric Inhibitors for MMP13

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Abstract— **Synthetic inhibitors for MMPs may become effective if they include a functional group as hydroxamic acid, carboxylic acid, sulfhydryl, etc. capable of binding the catalytic Zn, while at least one functional group provides a hydrogen bond interaction with the enzyme backbone, and one or more side chains will undergo effective van der Waals interactions with the enzyme subsites.**

Due to the fact that previous clinical trials that have used small inhibitor molecules and especially hydroxamic derivatives (batiamastat, marimastat) have shown poor clinical results and even malignancy rebound, the next generation of MMP inhibitors is directed toward exodomain-substrate interactions.

In the present study we have docked both known and experimentally tested inhibitors and also new proposed inhibitor models. Then we have compared binding affinities of the known compounds with those of three newly proposed ligands.

The purpose of this study was evaluate the affinity degree of these hypothetic hybrid inhibitors for the catalytic domain of MMP13.

The docking study performed with the open source software Autodock Vina, have generated promising results regarding the possibility to propose hypothetical but potent hybrid hydroxamic-allosteric inhibitors for the catalytic domain of MMP. Interactions between the newly proposed ligands and the catalytic site of MMP13 show new interesting alternative options for tunnel-like catalytic site enzymes. It appears that the direct Zn ion coordination is not solely responsible for enzyme inhibition but also allosteric inhibition may play an important role.

Our results show that the proposed inhibitors, nominated as ligands 3, 4 and 5, mainly dihydroxamic derivatives of diphenylether, has both hydroxamic potency but als the ability to perform allosteric inhibition at least for MMP13 catalytic site.

Further studies will consider evaluation of these theoretical inhibitors by docking on other MMPs with different S1' pockets.Regarding the proposed extended docking studies, we suppose that the synthesis of ligand-5 and the experimental data should confirm our molecular docking results.

Keywords— **molecular docking, metalloproteinase, synthetic inhibitor, hydroxamate.**

I. Introduction

Matrix metalloproteinases (MMP) are representative enzymes involved in decomposition of the natural compounds in the extracellular matrix [1,2]. Zn and Ca are essential ions for these enzymes activity. Even if MMP plays important roles in physiological processes, their overexpression plays also crucial roles in pathological processes as multiple sclerosis, arthritis, Alzheimer disease and especially in cancer and metastasis [3].

Interactions between ligands and substrate macromolecules – as enzyme-substrate or enzyme-inhibitor interactions – can be explored by molecular docking. Most of the methods are using molecular modeling algorithms that may involve either the exhaustive exploration of all possible ligand conformations and their relative position according to the enzyme. Some new software as Autodock Vina [4] is faster than the classical ones and are recommended to be used in ligand screening methods. Any MMP catalytic site is characterized by a Zn atom and a conserved zinc binding motif, HExxHxxGxxH. Synthetic inhibitors for MMPs may become effective if they include a functional group as hydroxamic acid, carboxylic acid, sulfhydryl, etc. capable of binding the catalytic Zn, while at least one functional group provides a hydrogen bond interaction with the enzyme backbone, and one or more side chains will undergo effective van der Waals interactions with the enzyme subsites.

II. Purpose

Due to the fact that previous clinical trials that have used small inhibitor molecules and especially hydroxamic derivatives (batiamastat, marimastat) have shown poor clinical results and even malignancy rebound, the next generation of MMP inhibitors is directed toward exodomain-substrate interactions [5]. The third generation of MMP inhibitors show no Zn-biding activity and exploits the presence and depth of the S1' pocket in most metalloproteases. As MMP13 shows a peculiar S1' loop on an additional S1' pocket side, and regarding the previous experimental data obtained by Johnson et al [6], we have imagined three new hybrid compounds that conserve either the hydroxamic group and also develop an exosite-compliant domain.

In the present study we have used the technique of molecular docking to explore the possibility of designing new selective inhibitors for MMPs based on an allosteric

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regulation philosophy. Docking experiments were performed either on experimentally tested inhibitors and also on three new proposed inhibitor models. We have compared binding affinities of the known compounds with those of proposed ligands.

The purpose of this study was evaluate the affinity degree of these hypothetic hybrid inhibitors for the catalytic domain of MMP13.

III. Material and Methods

We considered useful to apply molecular docking method that attempts to predict non-covalent binding between a macromolecule (here a metallo-enzyme, MMP13) and a small molecule (MMP inhibitors) starting from their unbounded structures. Docking methods are reproducing chemical potentials, and thus the bound conformation preference and the free binding energy [7].

In our docking study we have used a new program for molecular docking and virtual screening, Autodock Vina [4]. Vina uses Iterated Global Local Optimizer [8] which, briefly, uses a succession of steps consisting of a mutation, followed by a local optimization. In each step, the method of Broyden-Fletcher-Goldfarb-Shanno (BFGS) [9] is used for the local optimization that uses not only the scoring value but also its gradient.

While the algorithm implemented in Vina is based on a heuristic scoring function, its computes for at least 4 times faster than other docking software. By using multithreading, Vina can further speed up the execution by taking advantage of multiple CPUs or CPU cores. [4, 7].

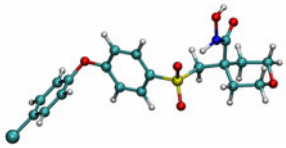
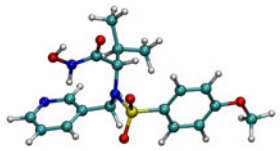
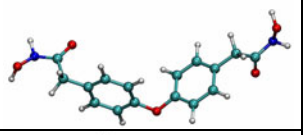
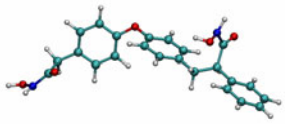
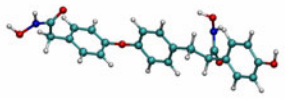
Crystal structures for MMP13 (PDBID:830c) and the two known hydroxamic derivative inhibitors were chosen from ProteinDataBank [10]. In our study, for convenience, the known ligands were nominated as ligand-1(4-[4-(4-chloro-phenoxy)-benzene-sulfonylmethyl]-tetrahydro-pyran-4-carboxylic acid hydroxyamide) [11] and ligand-2 (2-{4-[4-(4-chloro-phenoxy)-benzenesulfonyl]-tetrahydro-pyran-4-yl}-n-hydroxy-acetamide) [12]. New proposed ligands were depicted as ligand-3, 4 and 5 respectively, and they represent ideal constructs, nominated as dihydroxamic derivatives of diphenylether (table 1).

Models visualization and graphic representation was performed by VMD, a molecular visualization program for displaying, animating, and analyzing large biomolecular systems using 3-D graphics and built-in scripting [13].

IV. Results

The docking study performed with the open source software Autodock Vina, have generated promising results regarding the possibility to propose hypothetical but potent hybrid hydroxamic-allosteric inhibitors for the catalytic domain of MMPs. In table 1 we are showing the docking results generated for three newly imagined MMP inhibitors, compared to two already described inhibitors with known crystallographic structure in complex with MMP-13 (collagenase 3). Affinity scores obtained for the described inhibitors are correlated with experimental data described in literature [6].

Table 1 Gibbs free energy of binding for 5 different MMP13 inhibitors

MMP Inhibitor	Gibbs free energy of binding (kcal/mol)	Chemical formula
LIGAND 1 4-[4-(4-chloro-phenoxy)-benzenesulfonyl-methyl]- tetrahydro-pyran-4-carboxylic acid hydroxyamide	-9.6	
LIGAND 2 2-{4-[4-(4-chloro-phenoxy)-benzenesulfonyl]-tetrahydro-pyran-4-yl}-n-hydroxy-acetamide	-7,2	
LIGAND 3 PROPOSED MODEL	-9,8	
LIGAND 4 PROPOSED MODEL	-10,3	
LIGAND 5 PROPOSED MODEL	-11,1	

We observed that ligand-5 experimental has the highest affinity for the MMP-13 catalytic site (free Gibbs energy of -11,1 kcal/mol). Moreover, all three newly imagined inhibitors show higher affinities than previous known inhibitors (ligand 3 = -9,8 kcal/mol and ligand 4 = -10,3 kcal/mol, compared to ligand 1 = -9,6kcal/mol and ligand 2 = -7,2 kcal/mol). The proposed structures for docked ligand-3, -4, and -5 are depicted in figure 1; ligand 5 seems to realize an optimal fit into the catalytic site (Fig. 1).

Due to the fact that we have performed a comparative analysis between experimental data that shows only the Ki and not the free energy values, and while conversion between these constants ranges high output errors, we have

performed also a docking analysis for the compounds used in the mentioned experiments [11]. Thus, on the MMP13 crystallographic structure we have docked ligand-1 and ligand-2 (see table 1) and we have obtained a good agreement with the experimental structure.

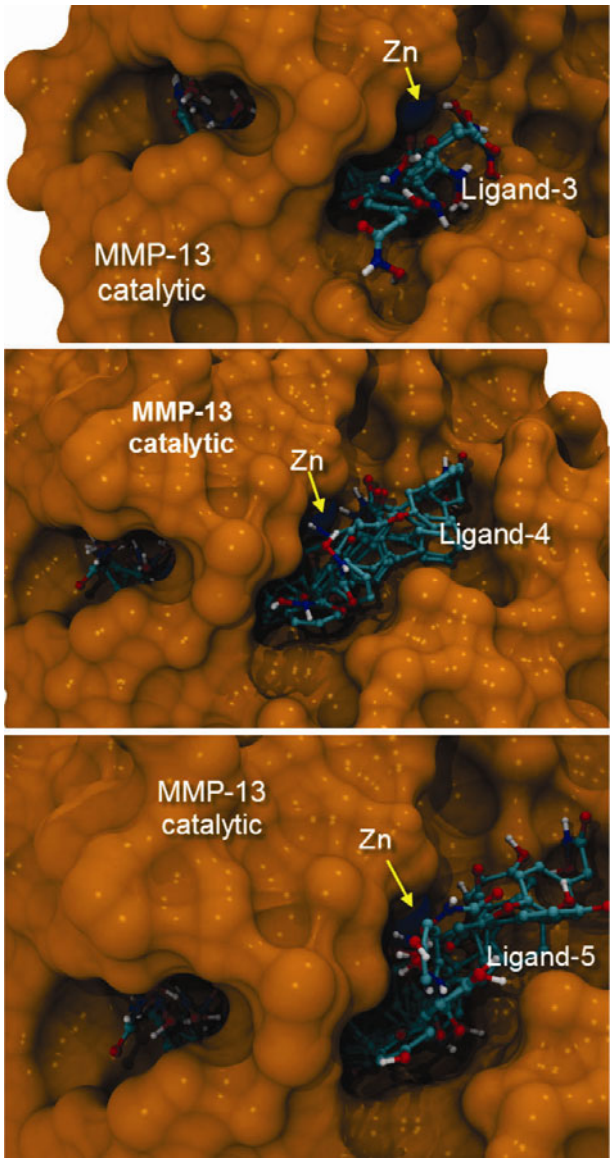


Fig. 1 Docking results for the new proposed dihydroxamic derivatives of diphenylether, nominated as ligand-3, -4 and -5. Posed conformations with the most favorable binding energy are located deep in the catalytic site tunnel

While during docking process, the mobility of the side chains was limited, the position of the hydroxamic group is a little bit displaced from the crystallographic results (Fig. 2). Observing that the inhibitor position in the catalytic site is very close to the crystallographic location, we may consider that the values resulted from docking analysis are reproducible and may be subject for further synthesis process.

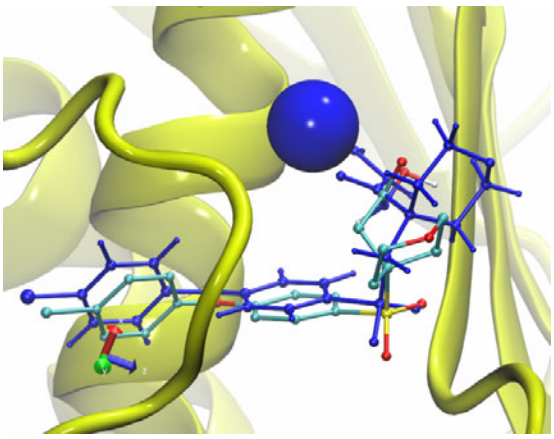


Fig. 2 Ribbon model of MMP13 catalytic site (yellow) with bound ligand 1 (in blue stick-ball model, crystallographic structure; in CPK model, the docked structure by Autodock Vina). Zn ion - large blue sphere.

Then, experimental data [11] shows that ligand-1 (Ki=0.52nM) is a more potent inhibitor than ligand-2 (Ki=1.9nM) [12], fact that is also demonstrated by docking procedure using Autodock Vina.

V. Discussions

Interactions between the newly proposed ligands and the catalytic site of MMP13 show new interesting alternative options for tunnel-like catalytic site enzymes. It appears that the direct Zn ion coordination is not solely responsible for enzyme inhibition but also allosteric inhibition may play an important role. Allosteric control of MMP2 action on gelatin was demonstrated by Ingvarsen et al [14].

As the tested compounds, whose crystallographic structure is described in complex with MMP13 (PDBID:830c), both contain a phenoxy-benzensulfone group, we have imagined a symmetrical molecule (ligand 3) with conserved the aromatic bicyclic structure on which we have grafted two hydroxamic terminal groups, obtaining three dihydroxamic derivatives of diphenylether. Hydrophobic ligand areas are interacting with complementary areas in the MMP13 catalytic tunnel (Fig. 3). Ligand-4 has a supplementary aromatic group, interacting with Pro242 and Ile 243 in the S1' loop. Ligand 5 has a supplementary hydroxyl group that is defining probably hydrogen bridge with Gly 183. The most important aminoacid in this relation between the inhibitor ligand 4 or 5 and the MMP13 catalytic site is

Leu185. The aliphatic non-linear side chain of Leu185 goes parallel with the neighbor aromatic cycle, insuring a large contact surface between the enzyme hydrophobic pocket and the designed inhibitor.

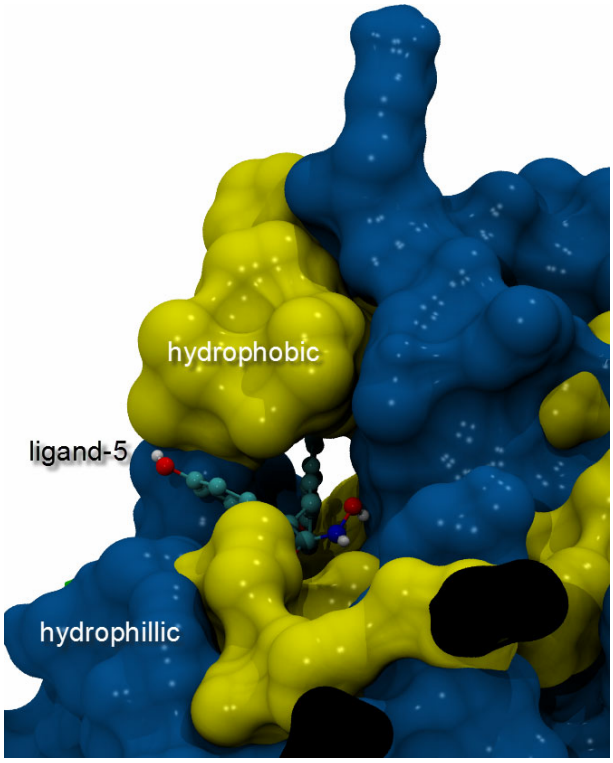


Fig. 3 Surface model of MMP13 catalytic site hydrophobic (yellow) and hydrophillic areas with bound ligand 5 (CPK stick-ball mode).

Ligand-5 is able to form H-bonds with Gly183 and at the same time an extended apolar contact with Leu185, areas that are not classic described as Zn coordination sites. Thus, ligand-5 appears to be the best option prom the proposed inhibitors that will be able to perform allosteric modulation of MMP catalytic site activity.

VI. Conclusions

Our results show that the proposed inhibitors, nominated as ligands 3, 4 and 5, mainly dihydroxamic derivatives of diphenylether, has both hydroxamic potency but also the ability to perform allosteric inhibition at least for MMP13 catalytic site.

Further studies will consider evaluation of these theoretical inhibitors by docking on other MMPs with different S1' pockets.

Regarding the proposed extended docking studies, we suppose that the synthesis of ligand-5 and the experimental data should confirm our molecular docking results.

Acknowledgments

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