# PHARMACOPHORE IDENTIFICATION FOR MATRIX METALLOPROTEINASES BY IN SILICO INVESTIGATIONS

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**Keywords**: Matrix metalloproteinases (MMP), pharmacophores, in silico modeling.
**Abstract**: Pharmacophores are a collection of universal chemical properties that characterizes the specific action of a ligand in the active site of a three-dimensional conformational model of a molecule. Aim. To define the pharmacophores, from some MMP-inhibitor complexes, defined experimentally in protein databank. Material and methods. We have used LiganScout software that supports the pharmacophore alignment and of important ligand molecules, based on their properties, in arbitrary combinations. The alignment of the two elements is realized by pairing only regardless the number of aligned elements. We have chosen 9 files crystalographically defined as MMP-inhibitor complexes: 1eub, 1fls, 1xuc, 1xud, 1xur, 1you, 1ztq, 456c and 830c, respectively. Results and discussions. Different type of inhibitors shows different pharmacophores, with respect to Zn coordination and for the hydrophobic tunnel in the enzyme binding pocket. Conclusions: it is important to understand substrate selectivity in order to develop new synthetic MMP inhibitors. Not only the Zn ion coordination in the P site is important but also the hydrophobicity of S1 tunnel can be a step in further computer design for potent inhibitor or enzyme modulation factors.

## INTRODUCTION

Matrix metalloproteinases (MMPs) are endopeptidases that use a $Zn^{2+}$-dependent mechanism for catalytic activity. The MMPs belong to a larger family of proteases known as the metzincin superfamily. Collectively they are capable of degrading large classes of extracellular matrix proteins, but also can process a number of other bioactive molecules. They are known to be involved in the cleavage of cell surface receptors, the release of apoptotic ligands (such as the FAS ligand), and chemokine/cytokine in/activation (van Lint, 2007). MMPs are also thought to play a major role on cell behaviors such as cell proliferation, migration (adhesion/dispersion), differentiation, angiogenesis, apoptosis and host defense.

In the collagenase family we can find MMP1 (fibroblast collagenase), MMP13 (collagenase-3) MMP8 (neutrophil collagenase) (Borkakoti N. 2000, Verma RP 2007). Even if all three mentioned members play an important role in collagen cleavage, the affinity for different collagen types is not similar. Thus, MM8 cleaves mainly type I collagen while MMP1 shows an affinity 15 times higher for type II collagen. MMP8 can be found stored in the neutrophil granules while MMP1 and 13 are synthesized following cytokine and inflammatory mediators action (Malemud CJ 2006).

MMP-like enzymes show a common functional structure with a central catalytic domain on which we can observe many supplementary domains and short peptide insertions. Matrilysin is the minimal enzyme in their category, being composed by a signal peptide, a propeptide and th catalytic domain. None of the enzymes in the family have all the additions. (Parks WC 1997, Bode W 1994, Flannery CR 2006).

## MATERIAL AND METHODS

Pharmacophores represent a collection of universal chemical properties that characterizes the specific action of a ligand in the active site of a three-dimensional conformational model of a molecule. The mentioned properties are figured by the presence of hydrogen bridges, electrostatic interactions or hydrophobic areas. This method is efficient regarding the computational processing and makes the pharmacophore modeling a universal, comprehensive and editable process. The selectivity can be adjusted by adding or omitting some characteristics. In our paper we have used the LigandScout 2.0 software (Wolber G. 2005, Wolber G 2007). This software supports the pharmacophore alignment and of important ligand molecule, based on their properties, in arbitrary combinations. The alignment of the two elements is realized by pairing only regardless the number of aligned elements. Thus, it is required to define a structure as marker element.

In order to define the pharmacophores, from the 21 MMP-inhibitor complexes we have chosen 9 files that are crystalographically defined in PDB databank: 1eub, 1fls, 1xuc, 1xud, 1xur, 1you, 1ztq, 456c and 830c, respectively. We have chosen these complexes with experimentally defined affinity constants for a further usage of the pharmacophores, based on these complexes. The pharmacophores represents important filters regarding in silico evaluation of new MMP inhibitors.

For automatic generation of MMP pharmacophore models we have imported the selected PDB files in LigandScout application. Due to the fact that PDB files do not include information regarding atom hybridization status and bond type,

LigandScout application uses a complex algorithm to analyze the ligand structure in order to assign the bond type according to molecular geometry. Thus, it is recommended the manual check for the automatic deduction for the ligand structure. The model must be then simplified regarding a more complex analysis with more sophisticated applications as Discover Studio Catalyst.

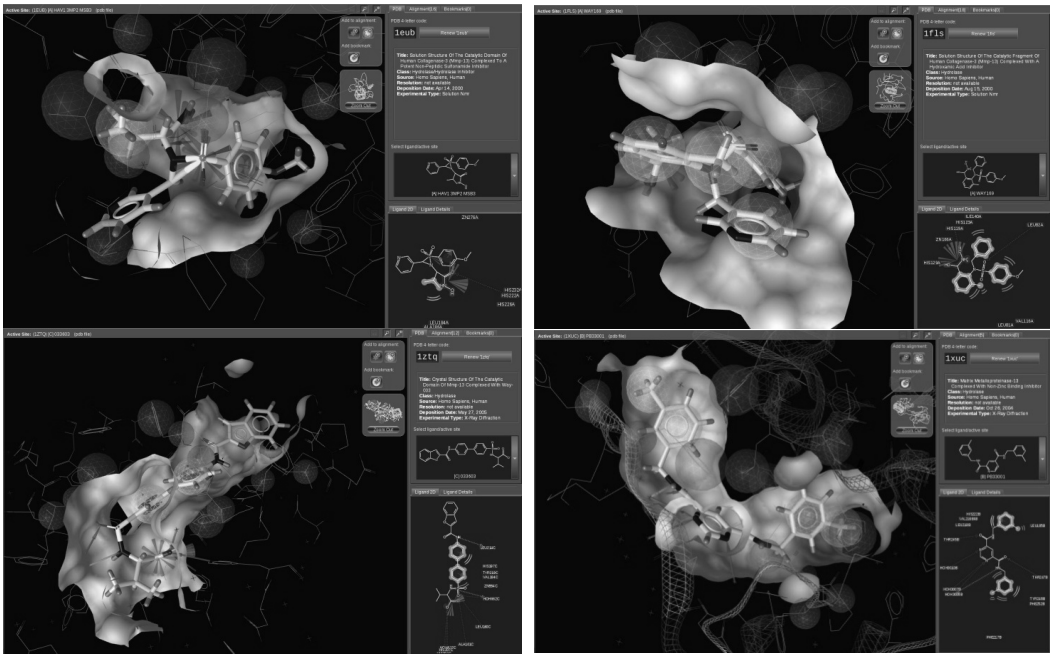
## RESULTS AND DISCUSSIONS

The MMP active site is composed of two separate regions: a groove at the protein surface surrounding the protruded catalytic Zn ion and a specificity site, nominated as S1'; the latter shows a higher degree of variability among the MMP in the family. Inside the groove, all tested inhibitors adopt extended conformations, express come hydrogen bonds with the enzyme and provide support for Zn ion coordination (figure 1).

The key structure for the inhibitors CGS 27023 and WAY-151693 in the complex structure 1eub and 1fls respectively in PDB database is the isopropyl substituent and the basic 3-pyridyl substituent. Zn coordination is insured by the hydroxamic group while this inhibitor is part of the sulfonamide hydroxamates inhibitor family.

The non hydroxamic inhibitor WAY-170523 in the complex 1ztq shows the replacement of the hydroxamic acid group with a carboxylate group; the latter is not Zn chelating but the inhibitory action is due to correct positioning of the benzofuran moiety of the P1' group by a biphenyl P1' linker that fill the hydrophobic S1' tunnel.

Another inhibitor, found in the 1xuc, 1xud and 1xur complex is represented by pyrimidine dicarboxamide. This inhibitor binds deeply in the S1' pocket and the pyridyl substitution do not approach the catalytic zinc more than 5,5A. This inhibitor shows a bent conformation with the pyridyl substitutions close to Leu218.



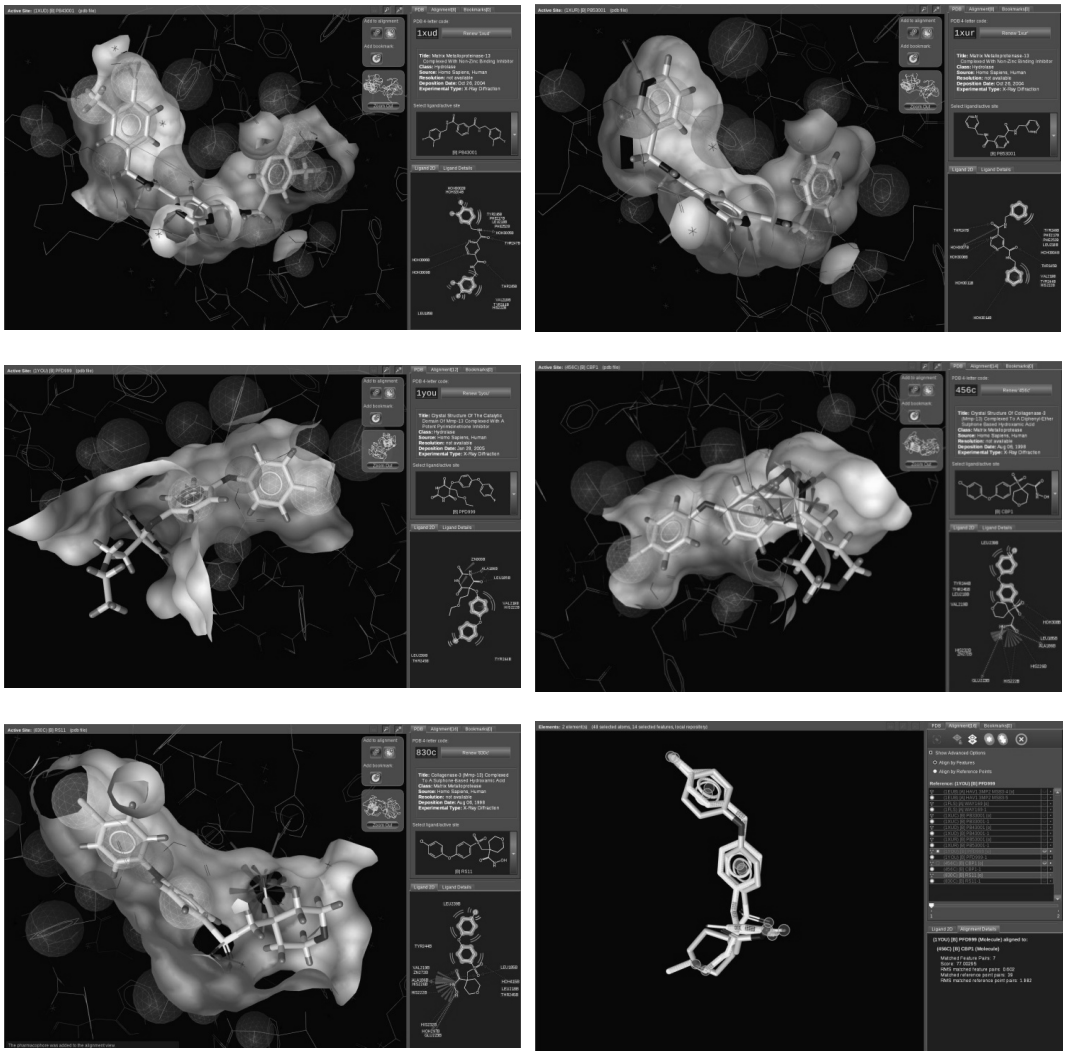



**Figure 1.** Pharmacophores defined by selected complexes MMP-synthetic inhibitors

For the pyrimidinetrione-based inhibitors of MMP13 in 1you complex in PDB, the aryloxyaryl ether residing in the S1 pocket while the pyrimidinetrione binds to the active zinc in the catalytic site. The three dimensional alignment of two inhibitors shown a score of around 77 from 100, that is remarkable for two different inhibitors in two different crystallographic determined structures.

## CONCLUSIONS

We have seen that various MMPs exhibit different selectivity for various matrix proteins. Thus, it is important to understand such substrate selectivity to develop new synthetic MMP inhibitors

We have also shown that not only the Zn ion coordination in the P site is important but also the hydrophobicity of S1 tunnel can be a step in further computer design for potent inhibitor or enzyme modulation factors.

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