

Inverse Virtual Screening of Bradyoxetin and a Synthetic Intermediate using OOMD as a Source Database

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INTRODUCTION

Quorum sensing bacteria produce and release chemical signal molecules (like N-acyl homoserine, AHL¹) that increase in concentration as a function of cell density. The responses cover a large spectrum of process such as the virulence in *Staphylococcus aureus*², competence for DNA-uptake in *Bacillus subtilis*³ and *Streptococcus pneumoniae*, sporulation in *Bacillus subtilis*⁴, conjugal plasmid transfer in *Enterococcus faecalis*⁵, and bacteriocin production in lactic acid bacteria. The collapse of (AHL) signaling system in bacteria represents an attractive therapeutic approach towards the development of new antibiotics. Recently, a new extracellular modulator was isolated from a symbiotic bacterium (*Bradyrhizobium japonicum*)⁵ that nodulates soybean. This quorum sensing molecule, containing a novel oxetane ring, was partially characterized and named bradyoxetin (2-{4-[[4-(3-aminooxetan-2-yl)phenyl](imino)methyl]phenyl}oxetan-3-ylamine)(Figure 1). Since there's a lack of information (other than the nodulation of soy bean) it seemed extremely appropriate to investigate this unique organic scaffold using *in silico* techniques such as Inverse Virtual Screening (IVS)⁶ using our own molecular database (OOMD). IVS should provide us initial information on the biological profile of this molecule, as well as other synthetic intermediate, 3-azido-2-phenyl oxetane (Figure 1).

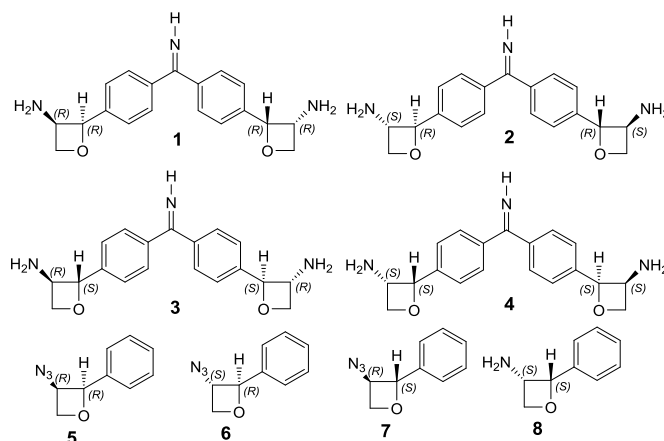


Figure 1

EXPERIMENTAL PROCEDURES

The molecules were entered using Marvin Sketch (ChemAxon, version 14.7.7). During this process the molecules were also protonated accordingly to its structure. The bradyoxetin series were able to accommodate three protons, one in the imine functionality, and other two on the amino groups attached to the oxetanes rings. The pH during protonation was set to be 7.4. The phenyl azido oxetanes were not protonated. During this process Geisteiger charges were also added. The ligand was set to be torsion free and the remaining structures were checked for incongruences in 3D. After this protocol the molecules had their energy minimized by MOPAC using pm7. The molecules were then opened in Auto Dock Tolls and were saved in pdbqt and in smiles for the purpose of expansion of a database of ligands.

The docking studies were performed using Vina1.0.2⁷. The data for the conf.txt files were extracted from the ligand present in the original pdb file (location of the binding site). Exhaustiveness was set to 8 and the grid box set to 20 Å on each side.

With the docking finished, next step was to verify the graphical results. For this, we've used



Discovery Studio v4.1.0.14169 (Accelrys Software Inc.).

RESULTS AND DISCUSSION

The results are expressed in table 1

Mol. Target	1AGW	1DDX	1GKC	1GMY	1LD8	1LF3	1LRH	1QIB	1QJA
Lig. Crist.	-8.1	-9.2	-6.6	-7.7	-13.2	-10.0	-7.7	-9.3	-11.0
5	-5.6	-7.5	-6.9	-5.6	-5.6	-5.9	-6.2	-6.8	-5
6	-5.7	-7.6	-6.9	-5.5	-5.7	-5.5	-6	-6.8	-4.9
7	-5.6	-7.8	-7.2	-5.8	-5.6	-5.4	-6.5	-7.1	-5.5
8	-5.4	-7.4	-6.9	-5.4	-5.6	-5.5	-6.3	-6.5	-4.8
1	-7.8	-9.3	-9.4	-7	-7.8	-7.6	-4.9	-9.3	-6.2
2	-8.1	-9.1	-9.6	-7.2	-8.1	-7.7	-4.7	-9.4	-6.3
3	-8.2	-9.4	-9.5	-7.1	-8.2	-7.7	-4.9	-9.4	-6.4
4	-7.7	-9.0	-9.5	-7.0	-7.7	-7.6	-4.2	-9.7	-6.1
Mol. Target	1W6M	1W22	1Z57	1ZZ1	2ANL	2HY7	2K05	2QHN	2VV9
Lig. Crist.	-4.3	-9.0	-9.5	-7.6	-10.0	-12.6	-33.3	-7.4	-9.1
5	-4.9	-5.3	-8.7	-8.7	-8.1	-7.1	-4.8	-5.6	-6.4
6	-4.6	-5.2	-8.8	-8.6	-7.7	-7.2	-4.7	-5.7	-6.5
7	-4.8	-5.3	-9.3	-8.6	-7.7	-7.4	-5.1	-5.7	-6.8
8	-5.0	-5.3	-8.8	-8.7	-8.2	-7.0	-4.7	-5.6	-6.3
1	-6.2	-7.8	-7.8	-8.7	-8.1	-9.7	-6.9	-7.7	-8.8
2	-6.2	-8.1	-8.1	-8.6	-7.7	-10.2	-7.2	-7.7	-8.8
3	-6.2	-8.2	-8.2	-8.6	-7.7	-10.4	-7.2	-7.7	-8.8
4	-6.2	-7.7	0.0	-8.7	-8.1	-10.0	-6.7	-7.7	-9.1
Mol. Target	2YOE	2ZOQ	3BKY	3BPF	3BZ3	3C4C	3DV3	3EDQ	3ENE
Lig. Crist.	-5.4	-7.8	-9.1	-6.7	-11.1	-10.4	-9.4	-10.7	-9.8
5	-5.7	-6	-5.5	-4.9	-6	-6.2	-5.6	-5.2	-6.1
6	-5.5	-6.1	-5.4	-5.0	-6.1	-6.3	-6.1	-5.5	-6.3
7	-5.8	-6.3	-6	-5.1	-6.1	-6.6	-6.2	-5.8	-6.5
8	-5.5	-6.0	-5.4	-5.1	-6.4	-6.3	-6.0	-5.3	-5.9
1	-8.1	-8.2	-7.1	-6.9	-8.2	-9.3	-9.1	-7.3	-8.2
2	-7.8	-8.6	-6.8	-6.9	-8.6	-9.5	-9.6	-7.4	-8.1
3	-8.0	-8.6	-7.3	-6.8	-8.7	-9.8	-9.6	-7.4	-8.0
4	-8.1	-8.2	-7.1	-6.9	-8.2	-9.3	-9.1	-7.3	-8.5
Mol. Target	3FAP	3FL5	3G0E	3HIG	3JYA	3N8Z	3U1I	4AGN	4EY7
Lig. Crist.	-19.4	-11.5	-10.4	-9.1	-8.4	-9.3	-9.6 ou -7.4	-6.5	-12.7
5	-6.8	-7.7	-6.3	-7.1	-6.1	-6.4	-4.9	-4.8	-7.4
6	-6.4	-7.2	-6.2	-7.1	-6.2	-6.1	-5.6	-4.9	-7.3
7	-6.6	-8.0	-6.5	-6.6	-6.9	-6.4	-5.8	-5.1	-7.8
8	-6.9	-7.5	-6.2	-5.5	-6.2	-5.8	-5.3	-6.5	-7.2
1	-9.1	-6.9	-8.9	-10.2	-8.2	-5.9	-7	-6.6	-10.1
2	-9.4	-6.8	-8.7	-9.9	-8.2	-5.6	-7.3	-6.4	-10.4
3	-9.3	-7.0	-8.6	-9.9	-8.2	-5.9	-7.3	-6.1	-10.6
4	-9.2	-7.5	-9.1	-9.8	-8.2	-5.9	-6.9	-6.5	-10.2
Mol. Target	PFATP	PFHT							
Lig. Crist.	-8.5	-5.4							
5	-6	-5		Legenda:					
6	-6.0	-5.7							
7	-6.1	-5.7		cancer		ativo			
8	-6.0	-5		dengue		inativo			
1	-8.5	-7.4		malária					
2	-8.3	-7.8							
3	-8.3	-7.7							
4	-8.5	-7.4							

Table 1

The most significant results were found in 1GKC⁸, 1W6M⁹, 2YOE¹⁰. In these cases the difference between the natural complex (ligand plus receptor) and the created complex generated through docking was more expressive. In most cases, the targets are involved with cancer. 1GKC is part of the Matrix metalloproteinases (MMPs), the class of enzymes involved in the degradation of extra cellular matrix and surrounding cells are known to be expressed during cancer cell invasion, arthritis and metastasis and MMP-9, in particular seems to be a key protease associated with tumor progression. 1W6M is equally involved with cancer although through a different mode of action. It's presence is associated with cell migration and with tumor malignancy. 2YOE is a GABA receptor modulated by benzodiazepines.

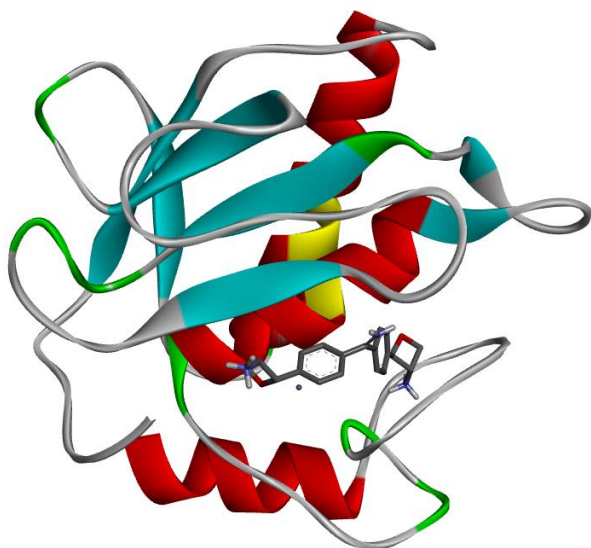


Figure 2: ligand 2 complexed with GKC

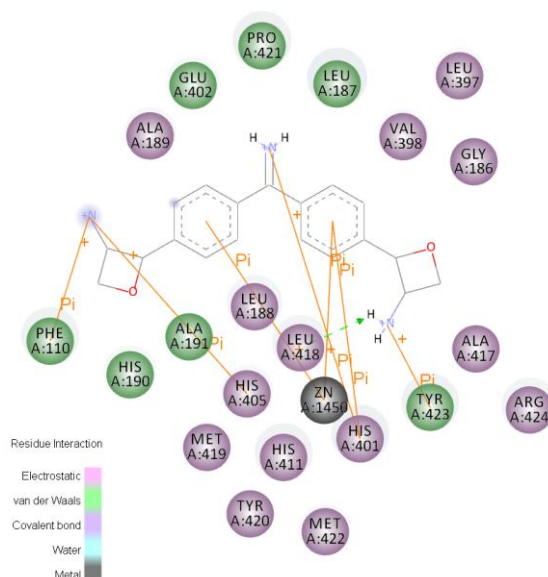


Figure 3: Pharmacophoric map of GKC complexed with ligand 2.

As it can be seen, looking to the pharmacophore map on figure 3, Pi interactions play a major role in binding process of ligand 2. In one of the rings there is a Pi stack created by a Zn atom and His 401. The zinc atom also interacts with the second ring increasing the stability of the complex. The amino groups also play a pivotal role having also Pi interactions with Phe 110, His 405 and Tyr 423. The imine bridge also interacts with His 401 contributing for the low energy of the complex.

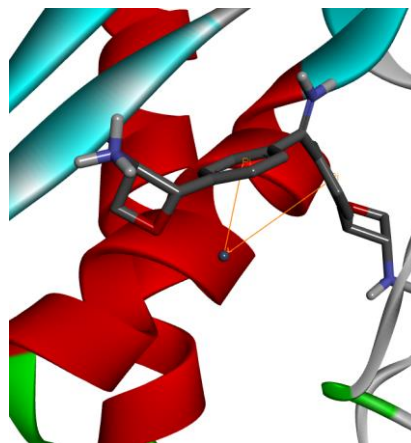


Figure 3: Pi interactions between Zn and aromatic rings of ligand 2

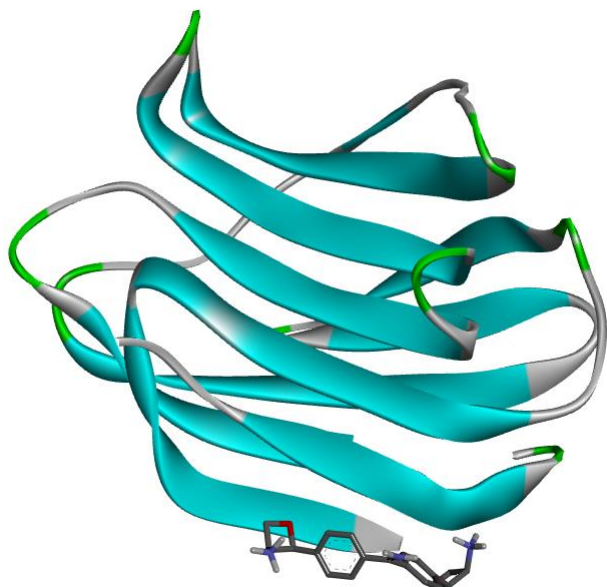


Figure 4: ligand 1 complexed with 1W6M

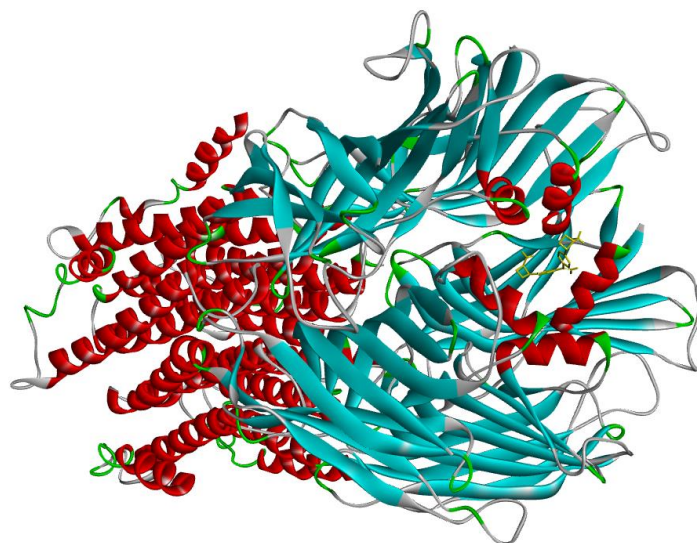


Figure 6: ligand 4 complexed with 2YOE. The ligand is highlighted in yellow.

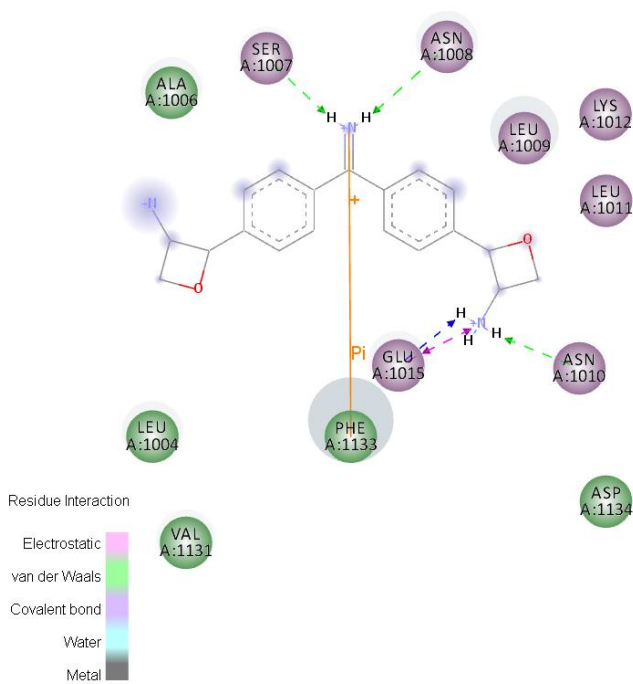


Figure 5: Pharmacophoric map of ligand 2 and 1W6M

As it can be seen in the figure above the number of interactions is much smaller than the previous example. Even so, it is enough to have a better value than the crystallographic complex. The key interactions are a Pi interaction between Phe 1133 and the imine moiety and hydrogen bonds between one of the amine groups and Glu 1015 and Asn 1010.

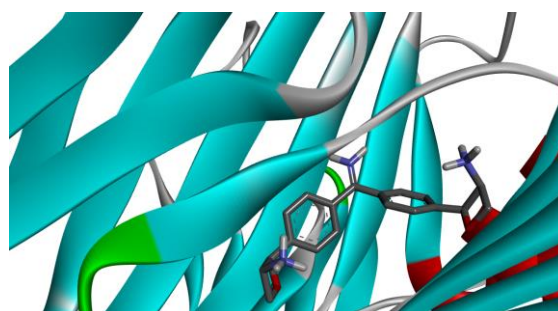


Figure 7: A zoom taken off from Figure 6

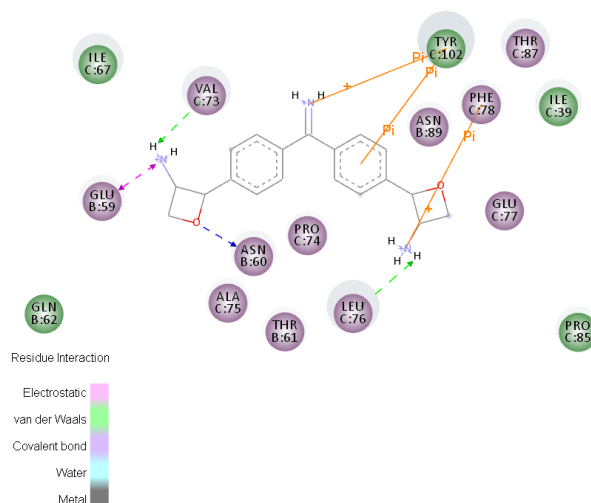


Figure 8: Pharmacophoric map of ligand 8 and 2YOE

Phe 78 and Tyr 102 interact with 2YOE through Pi bonds with the aromatic ring and with the imine. Other interactions seen before are the hydrogen bonds between the protonated amines and the surrounding residues such as Val73, Glu59 and Leu 76.

CONCLUSIONS

The sources for therapeutic molecules seem to be infinite. What were the odds of a symbiotic bacterium that helps nodulation and therefore with nitrogen fixation on soy display good results in an *in silico* screening against cancer mainly. Although the results look promising, a biological study is mandatory to validate the *in silico* results.

The next step should be a refinement docking followed by molecular dynamics.

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