MOLECULAR DOCKING STUDY OF POTENTIAL DRUG CANDIDATES AGAINST BORRELIOSIS

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Abstract: Lyme borreliosis is one of the most common tick-borne infections, for which there is an extensive need to find a new drug. For this purpose our *in silico* docking study was carried out to identify drug-likeness of chosen small molecules – potential borreliosis drugs. Its results revealed that BesA compound (C2 form) – a membrane fusion protein present in *Borrelia burgdorferi*, can play a significant role as a possible drug target compound and therefore it should be further examined in development of potential drugs for Lyme borreliosis treatment.

Keywords: *Borrelia burgdorferi*, borreliosis, Lyme disease, resistance, molecular docking

1 INTRODUCTION

Lyme borreliosis (Lyme disease) is an infectious disease caused by 12 (out of the 37) species of genus *Borrelia* (*B.*). *B. burgdorferi* is one of the major etiologic agent described in 1984 [1]. This spirochete, gram negative bacterium is transmited to humans through the bite of the *Ixodes* thick [2].

The disease can be treated with various antibiotics (azithromycin, ceftriaxone or doxycycline, erythromycin) [3][4], but it is difficult to treat during the later symptoms and some *B. burgdorferi* strains even express resistance to the antibiotics [5]. Hence, despite the availability of effective antibiotics, there is an extensive need to find a new Lyme borreliosis drug.

Structure based drug design allow us to better understand drug-target interactions. Use of the information provided by structure analysis has proved to be the key approach in current drug discovery. In our study, we applied computer based molecular docking methods. The purpose of molecular docking is to predict the preferred orientation of a small molecule with a protein. We performed *in silico* analysis of potential top fifteen Lyme borreliosis drug candidates suggested in a previous experimental *in vivo* study [6] against membrane associated *B. burgdorferi* protein BesA.

In our docking workflow we prepared protein and ligand structures, performed conformational analysis, set the placement scoring, generated poses of docked molecules, and analyzed the final poses.

2 MATERIALS AND METHODS

2.1 SOFTWARE

Molecular Operating Environment (MOE), developed by Chemical Computing Group Inc [7], is a comprehensive software with number of useful tools including powerful applications designed for molecular docking.

2.2 DATA OF PROTEINS AND SMALL MOLECULES

To be able to perform docking studies we needed to create database of receptor and ligand structures. Currently, there are 84 known *B. burgdorferi* protein structures available in Protein Data Bank (PDB) [8]. It has been known that membrane localized proteins represent largest group (70%) of effective drug targets in any organism [7]. For our study we have used a monomer group of BesA (C2 form) membrane fusion protein, accession code 4KKS (PDB ID). The structure was obtained by X-ray diffraction [9] and its hypothetical biological function is transmembrane transport.

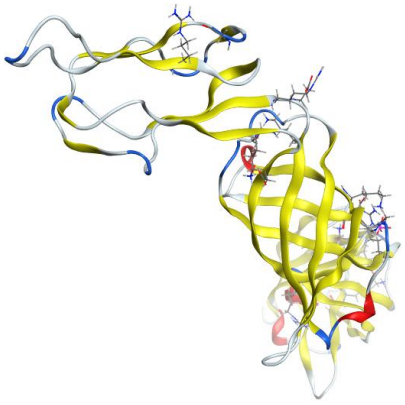

Figure 1: BesA (C2 form) membrane fusion protein (PDB ID 4KKS)

The binding site points of BesA were predicted by MOE's Site Finder application. The table below (Table 1) shows the contact residues.

Size	PLB	Hyd	Side	Contact residues
36	2.55	14	26	ASP67 VAL68 ASP69 LYS73 ASP75 LEU91 LEU127 ASN128 VAL145 LEU155 ILE218 GLY219

Table 1: Analyzed binding site detected in BesA (C2 form) membrane fusion protein (PDB ID 4KKS)

The small molecules desired for docking study were obtained from ChemSpider database [9] in the Mol File format (.mol). Molecules were converted for further analysis to SD format (.sdf) using OpenBabel software [10]. These molecules have been identified as new drug candidates against Borrelia burgdorferi using high-throughput screening in a previous experimental *in vivo* study [6]. The set contained: doxorubicin hydrochloride, josamycin, cefotaxime acid, cefazolin sodium, epirubicin hydrochloride, erythromycin, gramicidin, cephalothin sodium, ceftazidime, ticarcillin disodium, moxifloxacin hydrochloride, linezolid, idarubicin hydrochloride, azlocilin sodium.

2.3 PREPARING PROTEIN DATA

The purpose of this step is to correct the structure and prepare macromolecular data for further computational analysis. The receptors were prepared using following MOE's applications – Protonate 3D to optimize the hydrogen orientations (maximize H-bond networks and minimize the overall self-energy), Energy Minimize for energy refinement and QuickPrep for the correction, protonation, tethering and minimization.

2.4 PREPARING A SMALL MOLECULE DATASET

Important step before any application of the small molecule data is their processing and correction to a suitable form. To perform this step we used these MOE's applications - Wash to correct systemic structure errors, Depict2D to correct bonding patterns, Partial Charges to set atomic partial charges and Energy Minimize to structures' energy minimization.

2.5 DOCKING SIMULATION

For docking study we used MOE's Dock application. For generating poses from ligand conformations we selected Triangle Matcher as Placement method and London dG as a scoring function [7] to estimate the free energy of binding of the ligand from a given pose. Its formulation is as follows:

$$\Delta G = c + E_{flex} + \sum_{h-bonds} c_{HB} f_{HB} + \sum_{m-lig} c_M f_M + \sum_{atoms\ i} \Delta D_i$$

where c represents the average gain/loss of rotational and translational entropy; E_{flex} is the energy due to the loss of flexibility of the ligand (calculated from ligand topology only); f_{HB} measures geometric imperfections of hydrogen bonds and takes a value in [0,1]; c_{HB} is the energy of an ideal hydrogen bond; f_M measures geometric imperfections of metal ligations and takes a value in [0,1]; c_M is the energy of an ideal metal ligation; and D_i is the desolvation energy of atom i. The difference in desolvation energies is calculated according to the formula

$$\Delta D_i = c_i R_i^3 \left\{ \iiint_{u \notin A \cup B} |u|^{-6} du - \iiint_{u \notin B} |u|^{-6} du \right\}$$

where A and B are the protein and/or ligand volumes with atom i belonging to volume B; R_i is the solvation radius of atom i (taken as the OPLS-AA van der Waals sigma parameter plus 0.5 Å); and cici is the desolvation coefficient of atom i. The coefficients {c, c_{HB}, c_M, c_i} were fitted from approximately 400 X-ray crystal structures of protein-ligand complexes with available experimental pK_i data. Atoms are categorized into about a dozen atom types for the assignment of the c_i coefficients. The triple integrals are approximated using Generalized Born integral formulas. In our calculation we set up 5 poses for every docked small molecule (total 75 poses). We compared docked poses and analyzed the output scores.

3 RESULTS

The output database contained the docked poses ranked by the final score S (London dG). The top-scoring poses are found in the table below (Table 2). Other calculated values were the energy of the conformer (E conf) and score from the placement phase (E place). The docking results showed the ligands with the best binding energies - molecule of doxorubicin hydrochloride and idarubicin hydrochloride. These molecules occupied the active sites with the best final score.

	Small Molecule	S score	E conf	E place
1.	doxorubicin hydrochloride	-14.7595	4.2666	-39.5700
2.	idarubicin hydrochloride	-14.1070	1.8779	-43.5461
3.	doxorubicin hydrochloride	-13.7808	0.0000	-46.7254
4.	idarubicin hydrochloride	-13.5485	4.5568	-38.4959
5.	doxorubicin hydrochloride	-13.3276	5.5313	-50.2043
6.	idarubicin hydrochloride	-13.1241	1.8779	-38.6653

Table 2: Binding energies of docked ligands using MOE

Two best docking calculated poses and their ligand interactions are visualized below (Figure 3). Residues involved in the binding site indicate to play an important role in the interaction.

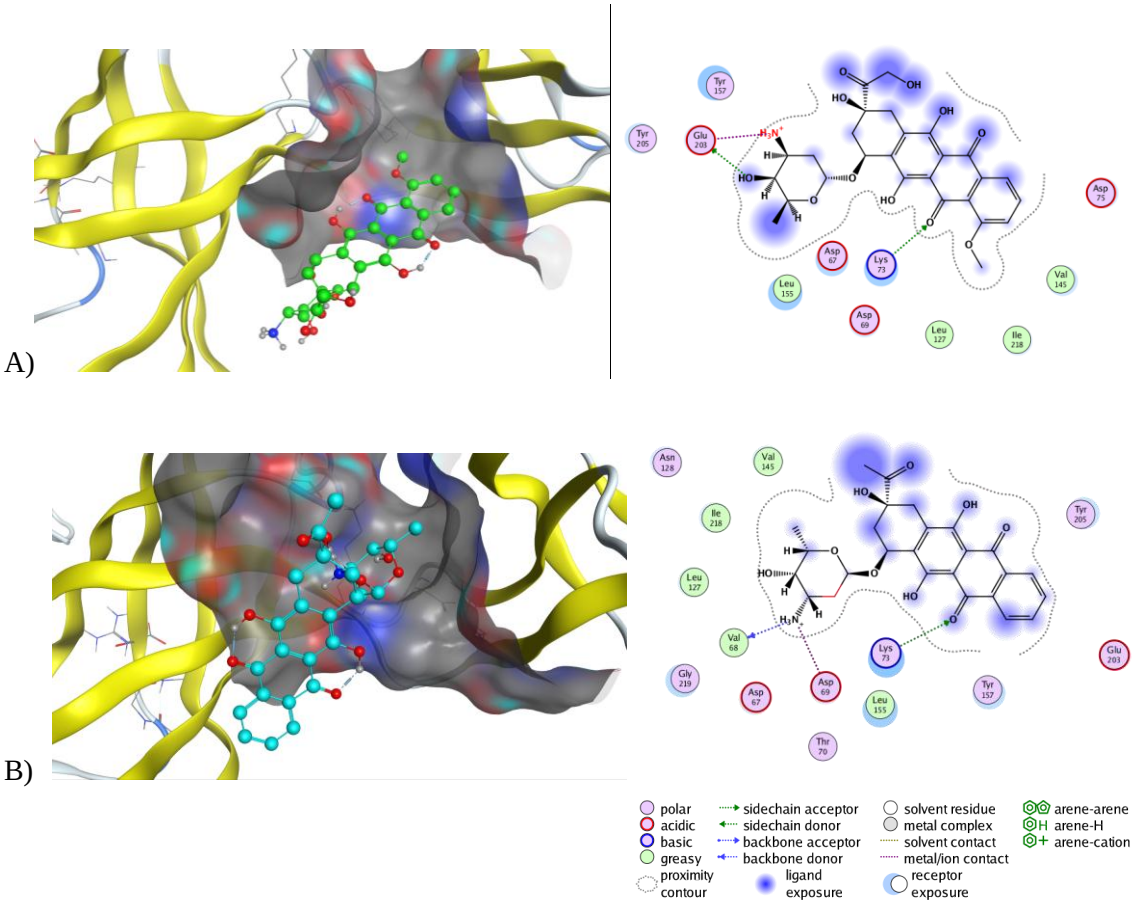

Figure 3: 3D visualization and 2D interaction diagram of docked structures in the binding site of BesA A) doxorubicin hydrochloride B) idarubicin hydrochloride

4 CONCLUSION

The purpose of this study was to predict binding interactions of candidate drugs for borreliosis. Drug resistance development of *B. burgdorferi* strains to available antibiotics is an important point of interest among health practitioners. Molecular docking helps us to examine the binding geometry of interacting molecules with known structures. Our study suggests two drugs - doxorubicin hydrochloride and idarubicin hydrochloride – as potential drug candidates for borreliosis. Their calculated poses provide useful information for a further structural analysis. In further studies we will also compare our results with already available drugs in order to see the differences.

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