In Silico Structure Based Drug Designing of A Potent Inhibitor for Purine Nucleoside Phosphorylase A Therapeutic Target for Schistosomiasis

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ABSTRACT

Schistosomiasis (also known as bilharzia, bilharziasis, bilharziosis or snail fever) is a human disease syndrome caused by infection from one of several species of parasitic trematodes of the genus *Schistosoma.* Schistosomiasis is a major source of morbidity and mortality in many tropical and sub-tropical countries as well as for travelers from developed countries. Control of the disease depends mainly on chemotherapy, with praziquantel becoming the exclusive drug. Extensive use of praziquantel with concerns about the possibility of drug resistance development, unavailability of an applicable vaccine, and the absence of a reasonable alternative to praziquantel all represent a real challenge. One of the suggested solutions is to exploit the advantages of compounds that proved efficacious at the experimental level with a good safety profile. Purine nucleoside phosphorylase is known to be essential for the recovery of purine bases and nucleosides in schistosomes, due to an absence of the enzymes for de novo synthesis, making it a sensitive point in the parasite's metabolism. The present paper discusses PNP as an attractive target for drug design for Schistosomiasis. This potential drug candidate developed on Chemsketch, modeller9v7 and Ligbuilder followed by their rigid docking on Hex and flexible docking using AutoDock and Quantum with IC_{50} 2.511e-0.004 and -7.9 kcal/mol (affinity) might be effective source in curing Schistosomiasis in near future.

Keywords: Schistosomiasis ,metabolism ,praziquantel

INTRODUCTION

In terms of socioeconomic and public health impact, schistosomiasis, also known as snail fever (caused by infection from one of several species of parasite treamatodes of the genus *Schistosoma),* is second only to malaria as the most devastating parasitic disease in tropical countries.

Patients with acute schistosomiasis (Katayama fever) present several weeks after contact with infested water. Symptoms are likely secondary to immune complex formation following egg deposition in tissues; the illness resembles serum sickness. Patients with symptomatic chronic schistosomiasis may present months to years after primary exposure. Those who present to the ED setting are most likely to complain of the following, Bloody diarrhea, Abdominal pain, RUQ pain, cramping, Hematemesis , Ascites , Hematuria, dysuria, Vulvar or perianal lesions, Dyspnea on exertion (with pulmonary hypertension) and Seizures and/or mental status changes , Paralysis.[1]

The disease is found in tropical countries in Africa, the Caribbean, eastern South America, Southeast Asia and in the Middle East. In these areas as of 2010 it affects approximately 238 million people 85% of whom live in Africa. An estimated 600 million people worldwide are at risk from the disease. Worldwide an estimated 12,000 to 200,000 people die related to schistosomiasis yearly. Although schistosomiasis is not eradicable, the disease can be prevented and transmission controlled with a single, annual dose of the drug praziquantel. However extensive use of praziquantel with concerns about the possibility of drug resistance development, unavailability of an applicable vaccine, and the absence of a reasonable alternative to praziquantel all represent a real challenge.

In the past, new synthetic organic molecules were tested in animals or in whole organ preparations. This has been replaced with a molecular target approach in which in-vitro screening of compounds against purified, recombinant proteins or genetically modified cell lines is carried out with a /high throughput. This change has come about as a consequence of better and ever improving knowledge of the molecular basis of disease. Following on from the genomics explosion and the huge increase in the number of potential drug targets, there has been a move from the classical linear approach of drug discovery to a non linear and high throughput approach. The field of bioinformatics has become a major part of the drug discovery pipeline playing a key role for validating drug targets. By integrating data from many inter-related yet heterogeneous resources, bioinformatics can help in our understanding of complex biological processes and help improve drug discovery.

Purine Nucleoside Phosphorylase

Purine nucleoside phosphorylase (also known as PNPase) is an enzyme involved in purine metabolism. PNP metabolizes adenosine into adenine, inosine into hypoxanthine, and guanosine into guanine, in each case creating ribose phosphate. NP encodes the enzyme purine nucleoside phosphorylase that together with adenosine deaminase (ADA) serves a key role in purine catabolism, referred to as the salvage pathway. Mutations in either enzyme result in a severe combined immunodeficiency (SCID). [2]

According to researches three crystal structures for the enzyme purine nucleoside phosphorylase (PNP) from Schistosoma mansoni, a component of the purine salvage pathway were described. PNP is known to be essential for the recovery of purine bases and nucleosides in schistosomes, due to an absence of the enzymes for de novo synthesis, making it a sensitive point in the parasite's metabolism. [3] In all three

structures reported, acetate occupies part of the base-binding site and is directly bound to the conserved glutamic acid at position 203. One of the structures presents the crystallization additive sulfobetaine 195 (NDSB195) occupying simultaneously the ribose and phosphate binding sites, whilst a second presents only phosphate in the latter. Considerable flexibility is observed in the active site, principally due to variable structural disorder in the regions centered on residues 64 and 260.

MATERIALS AND METHODOLOGY

Sequence Retrieval and Alignment

The FASTA sequence of purine nucleoside phosophorylase was retrieved from NCBI under the gi id of 49258474. [4] The retrieved sequence was henceforth aligned using NCBI-BLASTp program for homologous sequences, which could be further used as template for homology modeling.

Homology Modeling

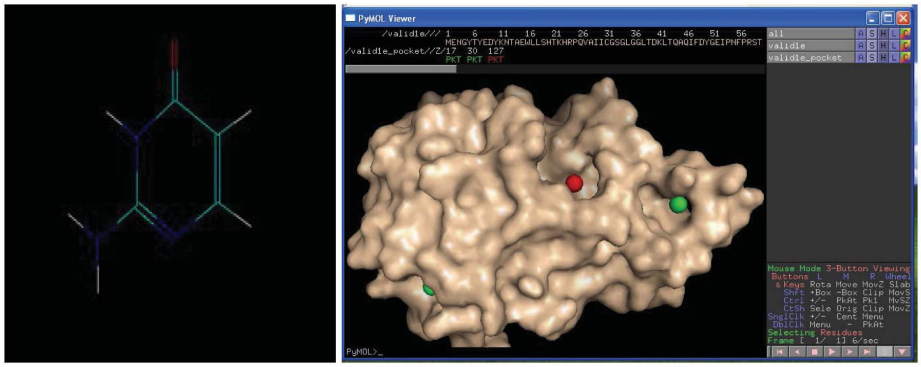
Subsequent to using BLASTp for ascertaining the best template for PNP, homology modeling was carried out using Modeller 9v7. The available structure present in PDB with ID 2Q7O was used as respective template for modeling.

Validation of the Generated Models

Different online and offline software have been developed for energy minimization. During the current analysis, SAVS PROCHECK and Verify3D programs were utilized. While PROCHECK, a structure verification program relies on Ramachandran plot [5], determines the quality of the predicted structure by assessing various parameters such as lengths, angles and planarity of the peptide bonds, geometry of the hydrogen bonds, and side chain conformations of protein structures as a function of atomic resolution. The Verify3D determines the compatibility of an atomic model (3D) with its own amino acid sequence (1D) by assigning a structural class based on its location and environment (alpha, beta, loop, polar, nonpolar etc.) and comparing the results to valid structures [6].

Pocket Identification and Building Inhibitor

After running ligsite we got five pockets and we selected the pocket no 2 because this is the largest pocket. Finding pocket is very essential in drug discovery. The pocket is large enough so that the inhibitor can easily grow inside the pocket during running the Ligbuilder. The inhibitor was drawn after studying functional group common to all inhibitors and drawn on Chemsketch and saved with .mol2 extension.



(A) **(B)**

Fig(A): represents the ligand designed in chemsketch before docking, **Fig(B)** represents the different pockets in the receptor for docking (Pymol view)

Active Site Prediction

The hex software fits the ligand in the free space near the active site. For performing rigid docking using hex, the closest residue to the pocket is determined with the help of moltracer, which is software used to find the closest residues ie SER-33 atom OG-348 by calculating distance in angstroms.

Virtual Screening

Out of several novel ligands generated, 10 ligands were selected on the basis of maximum binding affinity measured in kcal/mol. The selected 10 were then analyzed for drug relevant properties of valid structures based on Lipinski's rule of five [7] and ADMET properties using OSIRIS property explorer and Molinspiration.

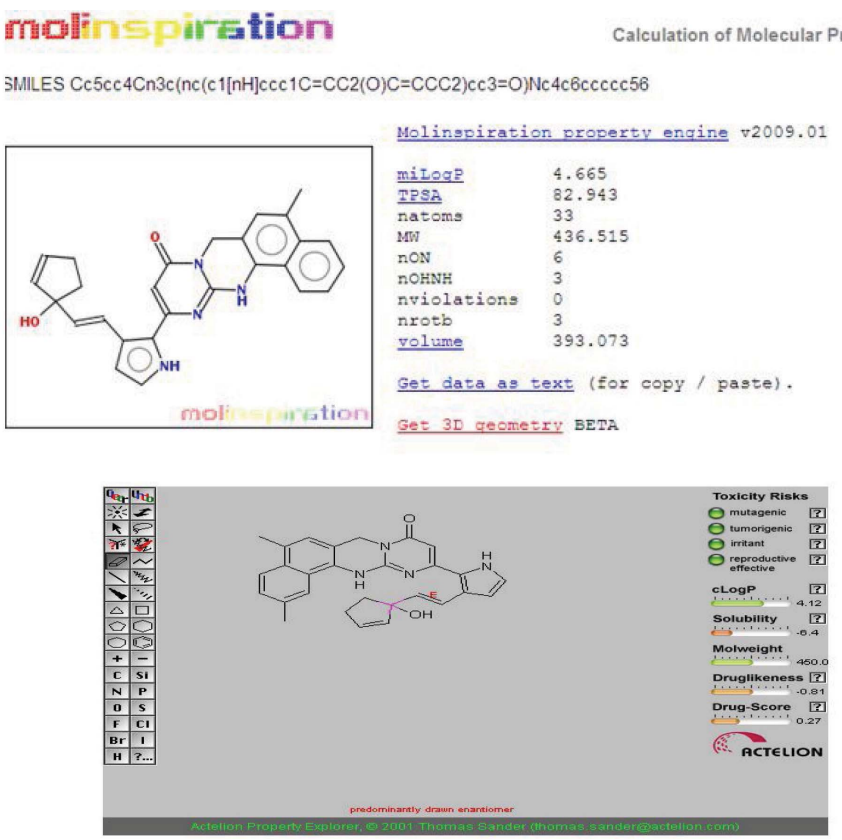
Flexible Docking Using Autodock and Quantum

AutoDock performs the docking of the ligand to a set of grids describing the target protein; AutoGrid pre-calculates this grid. In addition to using them for docking, the atomic affinity grids can be visualized. This can help, for example, to guide organic synthetic chemists design better binders. The grid dimensions used for flexible docking of purine nucleoside phosophorylase with ligand are 26x18x28.

The ligands after quantum docking [8] were then analyzed using their IC_{50} and binding affinity values.

RESULTS AND DISCUSSIONS

Alignment of PNP sequence for its homologous sequences using BLASTp server gave results of 99% identity with its homologous which was hence considered as template for further homology modeling. The PDB structure of the template sequence was retrieved with PDB id 2Q70. Homology modeling generated several models which were then considered for energy minimization using Ramchandran plot. Out of five generated models, the first model was selected which yielded results of 90.1% core, 9.9% allowed, 0.0% general, 0.0% disallowed, bad contacts 0. Moltracer was then



used to calculate residues which were closest to the pocket predicted.

RESULTS OF MOLTRACER

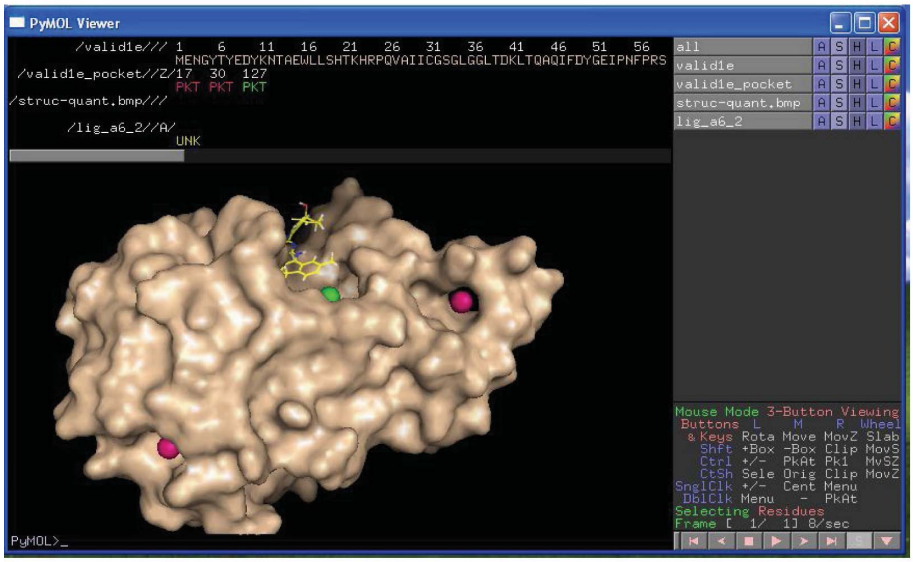
No.	Residue	Atom	Distance(Angstrom)
1.	SER-33	HG-350	3.40452683937137
2	SER-33	OG-348	3.82167947373926
3.	TYR-88	HH-903	3.9550423512271
4.	SER-33	CB-347	4.37315218120751
5.	HIS-86	NE2-874	4.43607416529525

The second residue SER33 Atom OG-348 with distance of 3.82167947373926 was finalized for further work.

For virtual screening different chemical properties were calculated based on Lipinski's rule of five and ADMET properties, leading to selection of one best ligand with miLogP 4.665, TPSA 82.943, Natoms 33, MW 436.515, nON 6, nOHNH 3, nViolation

0, nrotb 3 and volume 393.075. The selected ligand was seen to be in allowed mutagenic, tumorigenic, irritant and reproductive effect range using OSIRIS and MOLINSPIRATION. The calculated SMILES was Cc5cc4Cn3c (nc(c1[nH] ccc1 C=CC2(O)C=CCC2)cc3=O)Nc4c6ccccc56.

The ligand after quantum rigid docking was found to have IC-50 values as 2.115e-0.004(Quantum) and -7.9 kcal/mol (affinity), 1.312 and 2.172(distance from r.m.s.d



Ligand bound to the largest active pocket after docking

l.b and u.b)-AUTODOCK was decided as the lead compound for the drug.

CONCLUSION

During the present course of research, we have focused on structure based drug designing of a potent inhibitor to work against purine nucleoside phosophorylase, which is essential for the recovery of purine bases and nucleosides in schistosomes. The designed drug candidate besides being energy minimized is in allowed ranges of mutagenic and carcinogenic categories. It also fulfills all criteria of Lipinski's rule of five, and falls in considerable ranges of IC_{50}, affinity ranges and distances from r.m.s.d values. Thus, the ligand can be considered as a novel inhibitor to work against PNP as a therapeutic drug target for Schistosomiasis. We hope thus designed novel drug would be able to pass through clinical trial phases and used as a drug candidate in near future for treatment of Schistosomiasis.

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