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Structure based drug design for identification of novel drugs against UDP-3-O-acyl N-acetyl glucosamine deacetylase enzyme of the Human Pathogen *N.gonorrhoeae FA1090.*

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ABSTRACT

Gonorrhoeae is a most common sexually transmitted disease caused by the bacteria *Neisseria gonorrhoeae*. The majority of the drugs in current clinical use have been designed without knowledge of the targets. Analysis on the whole genome of the bacteria *Neisseria gonorrhoeae FA1090* revealed many drug targets. The UDP-3-O-acyl N-acetyl glucosamine deacetylase (lpxC/envA) was least homologous to humans and so was considered to be a potential drug target. *Insilico* modeling and structure based drug designing using techniques like docking and QSAR (Quantitative Structural Activity Relationship) studies was initiated to evaluate the present day drugs such as Ceftriaxone and also to design new inhibitors like Oxyazoline Hydroxamic acid and use of natural compounds such as Epicatechin and Beta-phenethylamine. It was observed that though these commercial drugs have a better interaction with the protein, they have a high toxicity risk like mutagenecity and tumorigenicity. Thus we recommend the use of ligands that are medicinal plant extracts having a better docking energy with the protein and that have least toxicity.

Keywords: LPXC, Sexually Transmitted Diseases, *Neisseria gonorrhoeae*, QSAR, Docking, Toxicity.

INTRODUCTION

Neisseria gonorrhoeae, also known as Gonococci (plural), or Gonococcus (singular), is a species of Gram-negative kidney bean-shaped diplococci bacteria responsible for the sexually transmitted disease gonorrhoea. *Neisseria gonorrhoeae* infections are acquired by sexual contact and usually affect the mucous membranes of the urethra in males and the endocervix and urethra in females, although the infection may disseminate to a variety of tissues. The pathogenic mechanism involves the attachment of the bacterium to nonciliated epithelial cells via pili (fimbriae) and the production of lipopolysaccharide endotoxin.With the availability completely sequence bacterial genomes the efforts are focused primarily on pathogens which encompass the majority of all genome projects, and have generated a large amount of sequence data for *insilico* analysis. These data pose a major challenge in the post-genomic era, i. e. to fully exploit this treasure trove for the identification and characterization of virulent factors in these pathogens, and to identify novel putative targets for therapeutic intervention. Similar studies in the virulent strain FA1090of *Nisseria gonorrhoeae* revealed several new and putative drug targets. All these drug targets were essential for the survival and were either non human homologs or least homologous and are considered to be novel drug targets. The Genome analysis of the virulent strain FA1090 of *Nisseria gonorrhoeae* revealed that the UDP-3-O-acyl N-acetyl glucosamine deacetylase (lpxC/envA) and lipopolysaccharide heptosyltransferase-I (rfaC) play a key role in lipopolysaccharide biosynthesis are also found to be involved in amino acid metabolism (arginine, proline, histidine) and glycan structures - biosynthesis-2 pathways(Debmalaya Barh *et al.* ,2009). Molecular docking and virtual screening based on molecular docking have become an integral part of many modern structure-based drug discovery efforts. The binding of small molecule ligands to large protein targets is central to numerous biological processes. The accurate prediction of the binding modes between the ligand and protein, (the docking problem) is of fundamental importance in modern structure-based drug design (TaylorRD *et al*., 2002). Molecular docking for the screening of Anti-SARS drugs (D.Q.Wei *et al*.,2006) and the docking studies of anti

tyrosinase activity of the Thai mango seed kernel extract (Saruth Nithitanakool *et al.*, 2009) are just a few good examples of docking studies that have shown a better results for further analysis. Studies revealed that docking results of Argus lab are biologically meaning full (Joy.s *et al*., 2006). In the present investigation the protein UDP-3-O-acyl N-acetyl glucosamine deacetylase (lpxC/envA) was further studied for its interactions with different ligands using Argus Lab4.0.1.

METHODOLOGY:

Insilico subtractive genomic approach based on the strategy that an essential survival gene non-homologous or less homologous to human host gene is a candidate for a given pathogen has been used for the analysis. The protein sequence of one of protein UDP-3-O-acyl N-acetyl glucosamine deacetylase (lpxC/envA) that was considered a putative drug target was retrieved from National Center for Biotechnology Information (NCBI) and was further analysed.

Template Search:

The protein sequence of UDP-3-O-acyl N-acetyl glucosamine deacetylase (lpxC/envA) was searched for suitable templates in BLASTp against PDB as this target protein did not have any structural information with the Protein Data Bank(PDB).The protein UDP-3-O-acyl N-acetyl glucosamine deacetylase (lpxC/envA) showed identities of 51% with chain A crystal of lpxc from *P.aeruginosa* (2VSE|A). The homology modeling of the UDP-3-O-acyl N-acetyl glucosamine deacetylase protein was performed using swissPDB modeler (Schwede *et al.*, 2003) with the template structure 2VSE|A from PDB. After modeling, the protein was validated using tools like procheck.

Active Site Identification:

CASTp server was analysed further for the identification of the most potential active site where the ligand can bind and interact with modeled protein and also providing information about the volume and area of the site (Dundas *et al*., 2006).

Ligand screening and QSAR Studies:

The protein was further studied for suitable ligands and four different ligands were taken for further analysis. One was an antibiotic cefatroxine that is being used in recent past for the disease two naturally occurring compounds used in treatment Beta-phenethylamine from the plants of Sida species and epicatechin found in the plant Acacia arabica that are used for treatment of gonorrhoeae and the fourth is an lpxc inhibitor in *E.coli*(H.Russell Onishi *et al.,*1996). All the structures of the ligands are obtained from the databases software Chem Draw and Chem Sketch shown in (Table.1). All the four ligand molecules (Ceftriaxone, Epicatechin, Oxyazoline Hydroxamic acid and Beta-phenethylamine) were screened for their drug likeness by considering the Lipinski rule. QSAR studied using mol inspiration server and the toxicity were checked using Osiris tool.

Docking

Before going in to the docking studies the water molecules if any were removed and hydrogen were added on to modeled protein. The modeled protein was docked against the obtained four ligands using software Argus Lab 4.0.1. to find the reasonable binding geometries and explore the protein-ligand interactions. A commercially validated and most predominantly used drug in the disease treatment was compared against two other most possible and valued rich medicinal plant extracts and last one being an inhibitor of the protein UDP-3-O-acyl N-acetyl glucosamine deacetylase (lpxC/envA) in *E.coli*. Docking of the protein ligand complex was mainly targeted only on to the predicted active site.

RESULTS:

The target protein UDP-3-O-acyl N-acetyl glucosamine deacetylase was modeled using Swiss PDB server a homology modeling server against a template structure 2VSE|A of PDB which was validated using procheck and was also refined. After the refinement the protein was found to contain 11 Helices, 21 strands and 28 turns (Figure.1).

Potential Binding site in modeled (lpxc):

CASTp server was analysed for the identification of binding site in the modeled protein and 38 active sites(pocket) were identified out of which the pocket with the maximum area and volume was considered the potential binding site .The 38^{th} pocket had a maximum area and volume and was considered to be the best active site. The active site contained 55 amino acids (highlighted in yellow colour (Figure :2).

Validation of the drugs:

QSAR studies of the ligands/drugs reveal that all the ligands except the natural compound Beta phenethylamine have a high Molecular Polar Surface Area (TPSA) which is not according to the Lipinski rule of five (Lipinski C.A *et al*., 1997) (Table.2).Toxicity studies were carried out using the server Osiris in which the Mutagenic, Tumorigenic irritation and reproductive effects of the ligands were analysed and it was found that the drug Ceftriaxone was highly mutagenic and tumourigenic and the Oxyazoline Hydroxamic acid showed low mutagenic risk while the other ligands has no such risks (Table.3).

Interaction studies:

Ceftriaxone which is the commonly being used drug on docking shows energy of -8.37kcal/mol and six hydrogen interactions (Fig.3 (a)). Although Ceftriaxone is an important antibiotic used in the treatment it has a high risk of toxicity(mutagenic and tumorigenic) and also has the highest TPSA value of 214.978 which would affect the absorption of the drug and also has a high molecular weight that are against Lipinski rule of five. Oxyazoline Hydroxamic acid an inhibitor of lpxc on docking shows a energy of -5.80kcal/mol with three hydrogen interactions and also has low toxic effect

but a TPSA of 97.983 makes the ligand difficult for absorption as a drug. Epicatechin a polyphenolic compound in the plant *Acacia arabica* was also analysed and showed a docking energy of -7.23 with four hydrogen bond interactions. Epicatechin though had no toxicity risk but was found to have a TPSA of 110.374 that affects the absorption of the drug. Beta –phenethylamine which is an alkaloid found in the Sida species plants (like *Sida acuta, Sida rhombifolia, Sida spinosa*) when analysed showed a docking energy of -9.67 with two (Fig.3(b)) hydrogen bond interactions and a very low TPSA value of 26.023 and no toxicity risk. Thus with the least binding energy, least TPSA, with a reasonable hydrogen bond interaction and with no toxicity risk (Fig.4) at all ensures this ligand to be a good drug that can act in an effective way against the UDP-3-O-acyl N-acetyl glucosamine deacetylase lpxc protein.

DISCUSSION:

The comparative docking studies against the UDP-3-O-acyl N-acetyl glucosamine deacetylase (lpxc) protein that is essential for the survival of the pathogen *Neisseria gonorrhoeae* revealed that the drug Cefetriaxone that is being used, is a mutagenic and tumourigenic. Beta-phenethylamine, an extract from the medicinal plant of Sida Species showed a better docking energy and also non toxic though it had just two hydrogen bond interactions thus making it a potential drug candidate against the protein. These potential drug candidates can further be validated in wet lab studies like site- directed mutagenesis for its proper function in-vivo with the target protein(Storici *et al* .,2006)

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Fig.1: Modeled lpxc protein of *Neisseria gonorrhoeae*

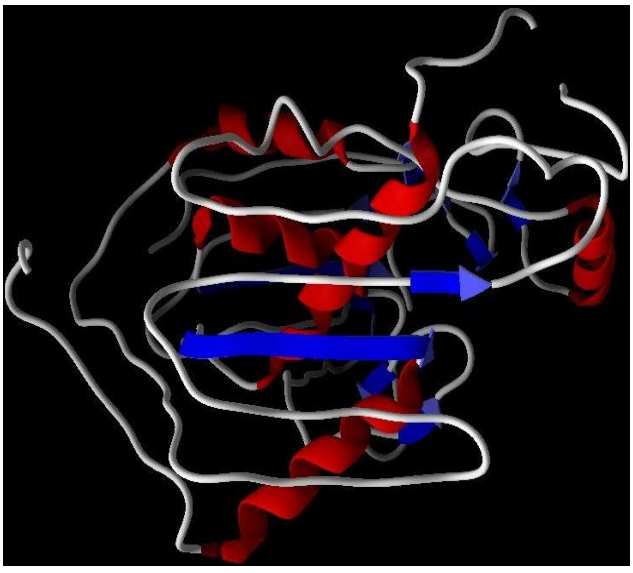

Table.1: Linear structures of the Ligands

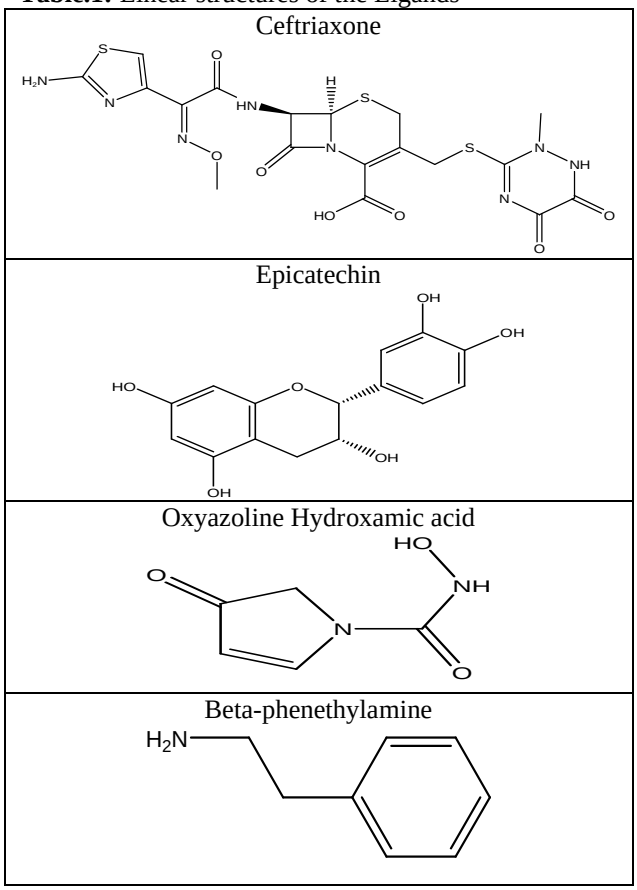

Fig.1: Modeled lpxc protein of *Neisseria gonorrhoeae*

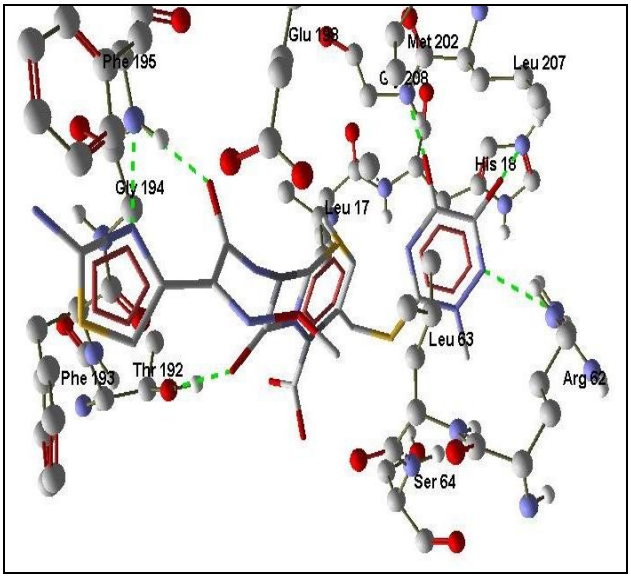

Figure .3A: Hydrogen bond interactions with ligands (a) Ceftriaxone

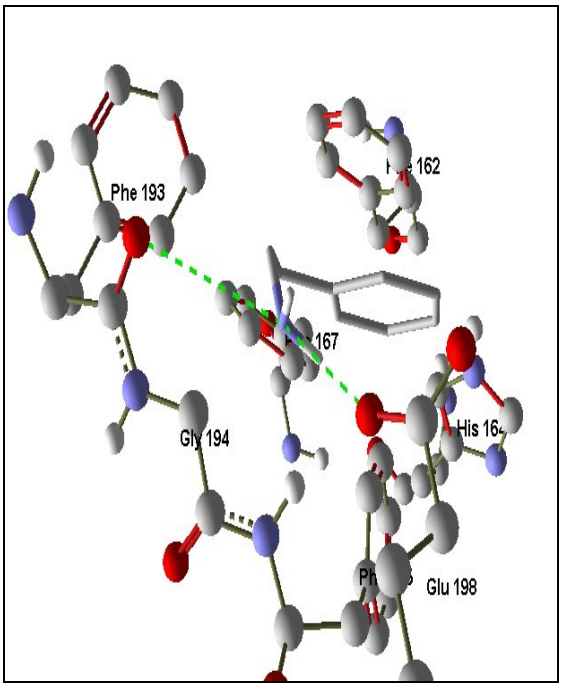

Figure .3B: Hydrogen bond interactions with ligands (b) Beta-phenethylamine

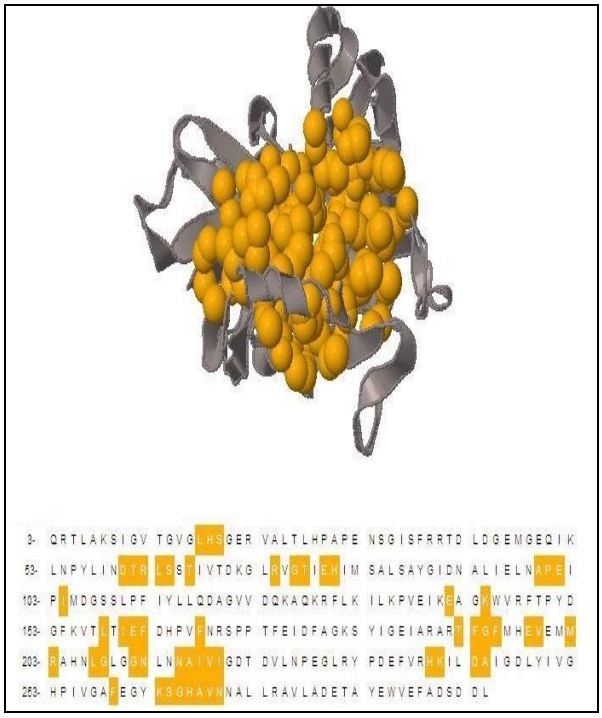

Fig.2: Active site of the modeled protein lpxc highlighted in yellow

Table. 2: Validation of drugs by Molinspiration

Drugs	miLogP	TPSA	natoms	Mol Wt	nON	nOHNH	nviolations	Nrotb	Volume
Ceftriaxone	-1.679	214.978	36	554.592	15	5	2	8	422.23
Epicatechin	1.369	110.374	21	290.271	6	5	0	1	244.141
Oxyazoline Hydroxamic acid	-1.116	97.983	12	170.12	5	4	0	1	135.098
Beta-phenethylamine	0.921	26.023	9	121.183	1	2	0	2	128.936

Note : miLogP: LogP (partition coefficient) TPSA: Molecular Polar Surface Area n atoms: number of atoms MW: molecular weight nON: hydrogen bond acceptor nOHNH: hydrogen bond donor nviolations: number of violations nrotb: number of rotatable bon..

Table.3: Toxicity risk as predicted by Osiris

Drugs	Mutagenic	Tumorigenic	Irritant	Reproductive effect
Ceftriaxone	High risk	High risk	No risk	No risk
Epicatechin	No risk	No risk	No risk	No risk
Oxyazoline Hydroxamic acid	Low risk	No risk	No risk	No risk
Beta-phenethylamine	No risk	No risk	No risk	No risk