BIOINFORMATION
Discovery at the interface of physical and biological sciences

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Volume 10(8)

Virtual screening of phytochemicals to novel targets in *Haemophilus ducreyi* towards the treatment of Chancroid

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Received July 10, 2014; Accepted July 14, 2014; Published August 30, 2014

Abstract:
Conventionally, drugs are discovered by testing chemically synthesized compounds against a battery of *in vivo* biological screens. Information technology and Omic science enabled us for high throughput screening of compound libraries against biological targets and hits are then tested for efficacy in cells or animals. Chancroid, caused by *Haemophilus ducreyi* is a public health problem and has been recognized as a cofactor for Human Immunodeficiency Virus (HIV) transmission. It facilitates HIV transmission by providing an accessible portal entry, promoting viral shedding, and recruiting macrophages as well as CD4 cells to the skin. So, there is a requirement to develop an efficient drug to combat Chancroid that can also diminish HIV infection. *In-silico* screening of potential inhibitors against the target may facilitate in detection of the novel lead compounds for developing an effective chemo preventive strategy against *Haemophilus ducreyi*. The present study has investigated the effects of approximately 1100 natural compounds that inhibit three vital enzymes viz. Phosphoenolpyruvate phosphotransferase, Acetyl-coenzyme A carboxylase and Fructose 1, 6-bisphosphatase of *Haemophilus ducreyi* in reference to a commercial drug Rifabutin. Results reveal that the lead compound uses less energy to bind to target. The lead compound parillin has also been predicted as less immunogenic in comparison to Rifabutin. Further, better molecular dynamics, pharmacokinetics, pharmacodynamics and ADME-T properties establish it as an efficient chancroid preventer.

Keywords: *Haemophilus ducreyi*, Chancroid, Rifabutin, Molecular docking, Molecular dynamics, RMSD

Background:
Chancroid is a sexually transmitted infection caused by the Gram negative bacterium *Haemophilus ducreyi*. The disease manifests as genital ulceration which may be accompanied by regional lymphadenitis and bubo formation. Chancroid remains a major cause of the genital ulceration syndrome. This has been shown to be a major co-factor in the transmission of HIV-1 infection both through cross sectional cohort and prospective longitudinal studies **[1, 2].** Infection of *Haemophilus ducreyi* occurs in genital and non-genital skin, mucosal surfaces, and regional lymph nodes **[3].** Generally, one or a few painful, infected sores at the site of the infection characterize chancroid. The lesions occur most often on the penis, with good visibility that is easily distinguishable in males. The genital ulcers of chancroid dole out as a portico of admission for HIV infection in both males and females. The occurrence of genital ulcers has been reported in many individuals diagnosed with HIV. In addition, if the individual gets infected with HIV, it increases the severity of ulcers when they get infected with chancroid. Chancroid and HIV together augment each other's infectivity. The lymphadenitis is excruciating and may form an abscess. It is sometimes indispensable to aspirate the infected inguinal nodes to prevent rupture and to afford symptomatic relief. In its infection to foreskin keratinocytes, fibroblasts co-cultures have stimulated a profound secretion of pro-inflammatory cytokines IL-6 and IL-8, but not IL-1α and TNF-α. The persuasive activity of polymorphonuclear

leucocytes is held liable for localized accumulation activity of inflammatory neutrophils **[4, 5].**

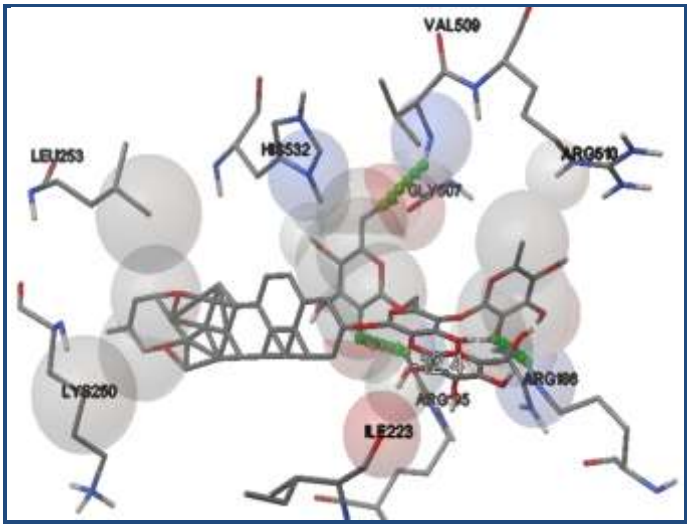

Figure 1: Detailed positions of amino acids present in the vicinity of ligand along with formation of 3 hydrogen bonds as generated by Autodock Vina. Near residues are VAL509, HIS532, ARG510, ARG 186, ILE223, LYS250, LEU253 and GLY507 while the H bond forming amino acids are ARG186, ARG195 and VAL 509 (green dotted structures).

Therefore, effective diagnosis and treatment of chancroid may play an important part in slowing down the HIV-1 epidemic in those parts of the world where both diseases are prevalent. The connotation between chancroid and HIV transmission stimulated several laboratories to investigate *Haemophilus ducreyi* pathogenesis during the past 15 years **[6].** In this investigation, the screening of natural antimicrobial compounds against putative novel drug targets of *H. ducreyi* using subtractive proteomics and *in-silico* drug designing approach has been carried out.

Methodology:

Retrieval and selection of target

Proteome of *Haemophilus ducreyi* was retrieved from Uniprot knowledgebase **[7]**. Total 121 proteins of *Haemophilus ducreyi* were retrieved. The target enzymes were selected by using subtractive proteomic approach against proteome of *Homo sapiens*. All the proteins were analyzed using BLASTP **[8]**. The enzymes whose similarity was lowest while aligning with proteome of *Homo sapiens* were selected. Finally, Acetyl Co-A carboxylase, Fructose 1, 6, bisphosphatase and Phosphoenolpyruvate phosphotransferase were selected and searched for coordinate files in protein data bank.

Homology modeling and validation

Three dimensional coordinate files were not found, therefore, complex was done with IgG using Autodock Vina at default parameters and similarly with Rifabutin and enzyme complex. Simulation of molecular dynamics was completed using NAMD graphical interface module incorporated in VMD.

Prediction of toxicity

Finally, the prediction of toxicity was carried out by the tox-predict application of the Open Tox server (http:// www. opentox.org/toxicity-prediction) **[21]** and Osiris property explorer (http://www.organic-chemistry.org/prog /peo/) **[22]**, which uses an algorithm of similarity search of structure for prediction of various toxicity values.

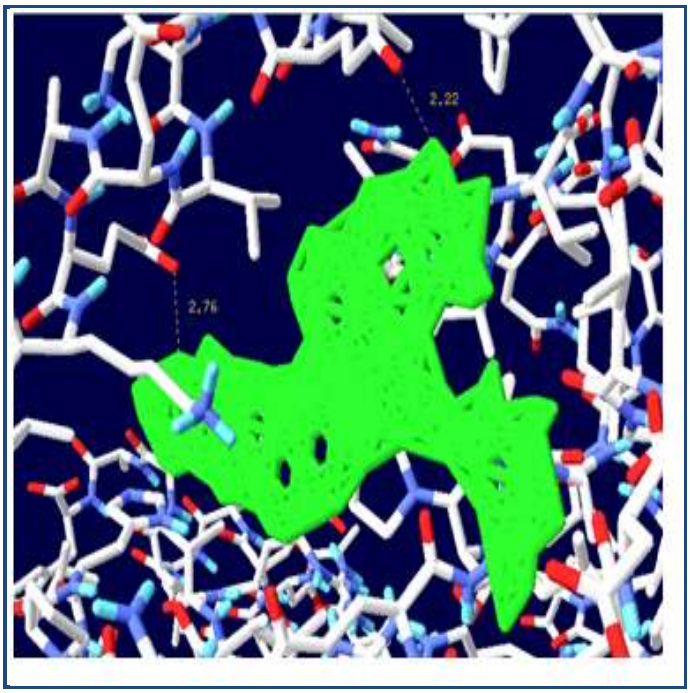

Figure 2: Prediction of H-bonds by SPDBV where 2 H-bonds was detected. The bond lengths of H bonds predicted are of 2.22Å and 2.76Å, this low value of bond length predicts high bond energy and ensuring better binding.

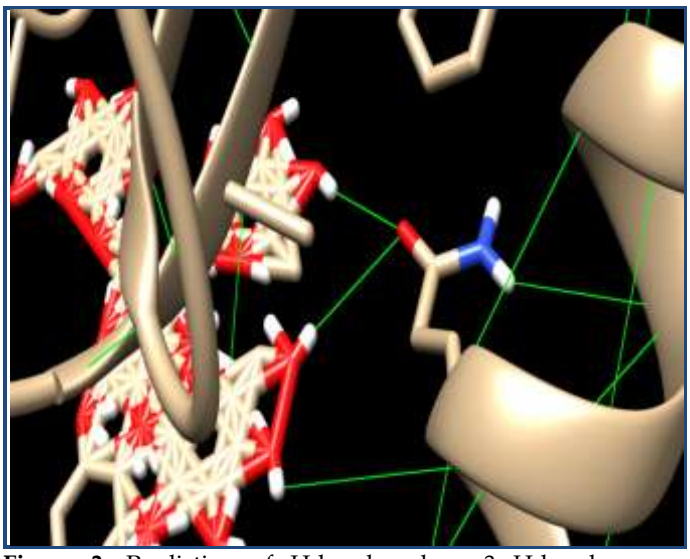

Figure 3: Prediction of H-bonds where 3 H-bonds were detected, O of GLN243, OE1 of GLN243 and O of ALA11 O in Phosphoenolpyruvate phosphotransferase. It ensures the role of other residues like GLN243 and ALA11 that participate in formation of H bonds.

Results & discussion:

Natural compounds always play profound roles in their existence in day to day life and generate curiosity towards their mode of action to combat diseases. The correlation between HIV and Chancroid has projected the challenge to develop new class of drugs other than traditional bactericidal

drugs like Rifabutin due to their colossal side effects like neutropenia, liver enzyme elevation, uveitis and malaise with myalgia **[23]**. Addressing these challenges, novel strategies are required to combat the issues of efficacy, ADME properties, toxicity and immunogenicity. Phosphoenolpyruvate phosphotransferase was selected as the potent target due to the large conserved sequences which refrains it from being mutated.

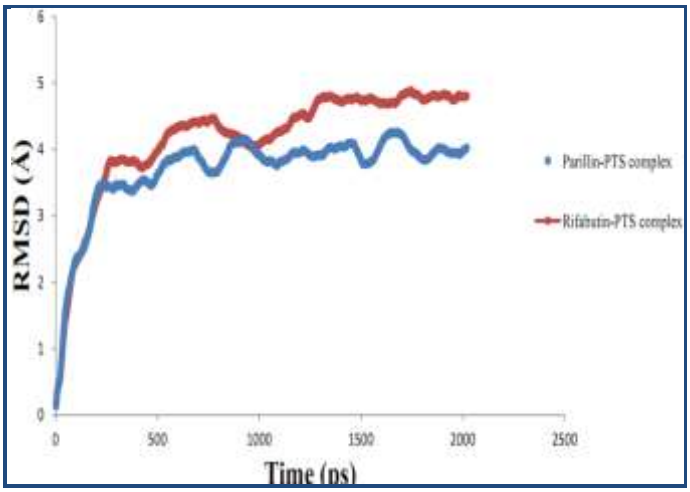

Figure 4: RMSD curve of Phosphoenolpyruvate phosphotransferase in complex with Parillin and Rifabutin. The stability of complex is inversely proportional to RMSD and calculated over a time window of 2190 picoseconds. The high values of Rifabutin-PTS-complex (red curve) states a highly deviated state thus ensuring low stability of the complex while the low value of Parillin-PTS-complex (blue curve) ensures high stability of complex**.**

Modeling and validation of target enzyme

During the model validation progression, Phosphoenolpyruvate phosphotransferase was best validated by all the servers. Errat provided an overall quality factor of 90.340 to the enzyme. ProQ predicts the model as an 'extremely good model' with predicted LG score of 5.132. Z-score of -11.11 and local model quality calculated by ProSA also validates the model beyond gratification. Ramachandran plot analysis through RAMPAGE states that total 534 amino acids i.e. 94.5% lie in the favored region thus imparting a solid base to the model.

Molecular docking and calculation of RMSD

Using Q site finder ASN (346), LEU (347), PRO (348), LYS (349), GLU (350), PRO (353), TRP (357) of Phosphoenolpyuvate phosphotranferase were foreseen to participate actively as binding pocket of the enzyme. Binding analysis commenced in a rigid fashion using Hex 8.0 on a correlation type of shape along with electrostatics with 5D FFT mode. Parameters included grid dimension of 0.6, receptor and ligand range of 180 with step size of 7.5 and twist rage of 360 with a step size of 5.5. The Etotal value of lead molecule parillin was -472.13. Rigid docking of Rifabutin with same target at same parameters resulted in an Etotal value of -342.49 thus supporting that fact that parillin binds in a better manner than Rifabutin. In a semi flexible fashion using Autodock Vina. Parillin was found to have very low binding affinity of -12.4 kcal/mol with Phosphoenolpyruvate phosphotransferase while Rifabutin showed -9.7 kcal/mol as shown in **Table 1 (see supplementary material).** The binding pocket comprised of VAL509, LEU253, HIS532, GLY507, ARG510, ARG186, ARG195, LYS250, ILE223 amino acids. Three hydrogen bonds were also detected as ARG186: HE 1, ARG195:HH22 1 and VAL509:HN 1 as shown in **Figure 1**. Prediction of hydrogen bonds using SPDBV resulted into 2 H bonds. First H-bond was formed between GLN 243 OE1 (42.992, 35.708, 3.555, 50.00) and LIG H (43.535, 33.599, 3.117, 99.99) with a bond length of 2.76 Å. Second H-bond was formed between GLU249 OE1 (39.688, 34.358, 16.037, 50.00) and LIG H (41.354, 32.156, 16.111, 99.99) with a bond length of 2.22 Å as depicted in **Figure 2**. Further UCSF Chimera was also used to predict hydrogen bonds and 3 hydrogen bonds were predicted from the complex generated from Hex 8.0 by relaxing the constraints by 2 Å. First bond formed between Lig1 het H and GLN243 O with a bond length of 3.797 Å, second bond was formed between Lig1 het H and GLN243 OE1with a bond length of 2.217 Å, third bond was formed between Lig1 het H and ALA11 O with the bond length of 3.515 Å as shown in **Figure 3**. This simplifies the fact that there is enormous possibility of proper binding as there is an immense possibility of formation of hydrogen bonds. The highest peak reached by the RMSD curve of parillin complex was around 4.5Å while that of Rifabutin complex was around 5Å. Both the complexes were simulated at equal time window of 2000 picoseconds as depicted in **Figure 4**. The complex of parillin with Phosphoenolpyruvate phosphotransferase was more stable by being less deviated in comparison to the complex formed by Rifabutin with Phosphoenolpyruvate phosphotransferase.

In-silico prediction of immunogenicity

This study also focuses on the immunogenicity caused by stabilization of the complexes formed by interaction of target and lead molecule. The binding simulation of complexes of Rifabutin and parillin were done with IgG (4HDI). Binding analysis was initiated in a rigid fashion using Hex 8.0 on default parameters except the correlation type of shape and electrostatics with 5D FFT mode. The Etotal value of parillin complex was -531.69. Rigid docking of Rifabutin with same target at same parameters resulted in an Etotal value of -581.58 which is smaller than that of parillin thus supporting the fact that Rifabutin complex binds in a better way than parillin complex.

Prediction of toxicity

The toxicity analysis renders positive results towards low toxicity. **Table 2 (see supplementary material)** describes various parameters of toxicity calculated via open tox server and Osiris property explorer. Parillin was found to be prominent in the field of cLogP, pKa= -SMARTS, biodegradability, acute toxicity to fish, carcinogenicity, skin irritation, eye irritation, mutagenicity, reproductive effect and drug score.

Conclusion:

In the current study, various discrepancies have been permeated by incorporating computational binding simulations of lead compound along with their molecular dynamics simulation. During the model validation progression, Phosphoenolpyruvate phosphotransferase was best validated by all the servers. The above mentioned enzyme

has been superiorly inhibited in rigid and semi flexible manner by parillin in comparison to Rifabutin. Molecular dynamics simulation also enhances the authenticity of inhibition. Regarding immunogenicity, the interaction of IgG with Rifabutin and parillin complexes reveals that Rifabutin activates immune response more strenuously than parillin. The interactions of IgG were also simulated for molecular dynamics and had furnished positive results towards the fact that parillin is less immunogenic. The ADME-T properties and prediction as non-carcinogenic and non-irritant further establishes it firmly as possible drug candidate. The current study presents a novel target and a novel system of medication towards the inhibition of *Haemophilus ducreyi*.

Acknowledgement:
We gratefully acknowledge TEQIP–II and G. B. Pant Engineering College, Pauri Garhwal for financial support and providing instrumentation facilities. Pranav Tripathi is thankful to AICTE (All India Council for Technical Education) for fellowship.

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Edited by P Kangueane
Citation: **Tripathi *et al.*** Bioinformation 10(8): 502-506 (2014)

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Supplementary material:

Table 1: *In-silico* docking of phytochemicals and rifabutin with phoshoenolpyruvate phosphotransferase accomplished by Hex 8.0 and Autodock Vina rendering Parillin as the lead molecule.

Ligand name	Hex 8.0	Autodock vina
Amorfrutin	-199.23	-9.6
nene	-324.76	-5.3
arillin	-472.13	-12.4
gitoxin	-20.66	-9.2
Gallotanin	-239.31	-8.4
6-gingerol	-148.65	-6.1
Rutin	-202.72	-8.8
Soya cerebroside	-92.25	-6.9
Rifabutin	-342.49	-9.7

Table 2: This table summarizes the ADME-T properties of the lead compound parillin thus establishing it as a potent contender for a new drug candidate against *Haemophilus ducreyi*. The establishment as non irritant, non mutagenic, non carcinogenic ensure that the lead molecule has potency to work as a new drug candidate after *in-vitro* and *in-vivo* testings.

ADME-T Properties	Description
cLogP	-1.44
Molar refractivity	247.525Cm^3
pKa= -SMARTS	9.80
Biodegradability	Class 2
Acute toxicity to fish	-0.19mmol/L
Carcinogenicity	Non carcinogenic
Skin irritation	No irritation
Eye irritation	No irritation
Mutagenic	No
Reproductive Effect	No
Drug score	0.19