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Identification of novel antagonists for DNA processing chain A of *H. influenzae*

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ABSTRACT: Haemophilusinfluenzae is a Gram-negative, coccobacillary, anaerobic bacterium found in upper respiratory tract of human, which majorly causes bacteremia, epiglottitis, pneumonia and acute bacterial meningitis. Moreover H. influenza show multi drug resistance to several antibiotics such as penicillin, rifampicin, chloramphenicol, erythromycin, azithromycin and polymyxin with adverse effects like allergy, dizziness and fever. Therefore, multiple drug resistance nature of H. influenza anticipates the requirement to discover novel antagonists for the treatment of infections mediated by H. influenzae. Three enzymes from sRNA candidates of 5 H. influenzae genomes were predicted using sRNA Predict. Among the 3 enzymes, DNA processing chain A (DprA) of H. influenzaeplays a crucial role in bacterial transformation mechanism and found to be non-homologous to Homo sapiens. Thus, DprA has been targeted for the development of drugs for pneumonia treatment in the present study. Tertiary structure of DprA was modeled using 3UQZ as a template structure with Modeler 9v.15 and validated using PROCHECK, ProSA and ProQ to define the stereo-chemical quality and overall quality of a protein model respectively. RecA binding site residues were defined using Site map and 10x10x10 Å grid was generated around the defined RecA binding sites. The existing seven inhibitors of DprA were subjected to analog search against in-house library containing more than one million compounds using PHASE v.3.8 module. Rigid receptor docking was performed to the obtained 4000 inhibitor analogs towards DprA with Glide v.6.3 in Maestro v.9.6. Virtual screening workflow of rigid receptor docking in Glide v.6.3 with high throughput virtual screening (HTVS) and standard precision (SP) followed by extra precision (XP) docking resulted 38 leads. Comparing the resulted 38 leads to the existing inhibitors resulted three best leads. Lead1 showed docking score of XP Gscore -7.36kcal/mol shows better binding affinity towards DprA. Thus, the proposed three leads in the present study were enough to block the activity of DprA and in turn decrease bacterial transformation and stop the endurance of the bacterial growth of H. influenzae.

Keywords: Haemophilusinfluenzae, Pneumonia, sRNA Predict, DNA processing chain A, Glide, binding free energy, ADMET.

Introduction

Haemophilusinfluenzae is a Gram-negative, coccobacillary, facultative anaerobic bacterium belonging to the Pasteurellaceae family [1]. Aerobic growth requires exogenous heam or protoporphyrin IX and NAD. As expected an upper respiratory parasites, growth is optimal between 35-37°Cwith the little tolerance of higher temperature [2]. *H. influenzae* cause a number of mucosal infections like pneumonia, bacteremia, epiglottitis and acute bacterial meningitidis, including otitis media, conjunctivitis, sinusitis and bronchitis [2],[3]. Pneumonia characterized by early structural lung disease caused by pulmonary infections. The nasopharynx infants are a major ecological reservoir of potential respiratory pathogens. However, statistics also revealed that this pathogen is also a major cause of lower respiratory tract infections in infants and children in developing countries. There are serious side effects like fever, chills and severe allergic reactions are possible but they are rare and also exhibit poor pharmacological properties [4].

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Antibiotic treatment is the cornerstone for management of pneumonia and adequate empiric treatment is associated with improved outcomes [4] also reported it is one of the most predominant pathogen causing bacterial meningitis. Ampicilin, chloramphenicol, cephalosporins, macrolides, azalides, tetracycline and amino glycosides. "Combinational therapy" trimethoprim-sulfamethoxazole, rifampicin-cephalosporin, erythomycine-sulfisoxazole these are the mainstays of treatment. Multidrug resistance nature of *H. influenzae* and adverse effects of existing treatment options, open up new challenges for researchers to discover novel therapeutics for the treatment of pneumonia.In the present study, a novel approach for the identification of drug target was employed. As the sRNAs are the regulators of the bacterial gene expression, identification of sRNAs in the whole genome sequence of the pathogen was performed. sRNAs are small and non-coding RNAs, that have been implicated in regulation of various cellular processes of living system, allowing them to adapt to different environmental conditions. The protein DNA processing chain A (DprA) was selected as a drug target, as it is non-homologous to *Homo sapiens* and also plays a key role in the bacterial transformation. It also promotes genome plasticity in bacteria via RecA driven homologous recombination and cell proliferation of *H. influenzae* leading to cause pneumonia. Double-stranded DNA is internalized as single strands, onto which the transformation dedicated DNA processing protein A (DprA) ensures the loading of RecA to form presynaptic filaments. Rational drug designing approach was adapted by drug target structure modeling, shape screening of

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existinginhibitors and molecular docking studies to discover novel, selective and potent leads against DprA of H. influenzae.

Material and methods

1. SRNAPredict and drug target selection

The genome sequence of H. influenzae was retrieved from the National Center for Biotechnology Information (NCBI) for sRNA prediction [5],[6]. sRNAs are having crucial role in regulating various cellular processes. sRNAPredict tool defines the coordinate based algorithms to incorporate particular or relevant positions of gene predictive features and rapidly identifies the putative intergenicsRNAs present within the coding regions of the genome [7].The sRNA candidates of H. influenzae were selected for non-homologous analysis against proteome of Homosapiens. A potential drug target should not possess homologs particularly with the human proteome in order to avoid unwanted cross reactivity of drug.

2. Homology modeling and validation

Homology modeling is useful in structure-based drug designing applications, especially when a crystallographic structure of the drug target is unavailable. The target protein sequence of DprA of H. influenzae was retrieved from UniProt database (http://www.uniprot.org/). Comparative structure modeling technique was implemented using template structure of Streptococcus pneumoniae to predict tertiary structure of DprA in Modeller 9v15. Target and template sequences were aligned by using ClustalX. Twenty DprA homology models of H. influenzae were constructed and most reliable model with the least DOPE (discrete optimized protein energy) score and the selected model was [8] further validated by PROCHECK [9], ProSA [10], ProQ analysis and target template superposition [12].The validated DprA of H. influenzae was submitted to the protein model database (PMDB). The RecA binding site residues of DprA were visualized using PyMOL.

3. Virtual screening and molecular docking studies

Seven known inhibitors of DprA were retrieved from PubMed. 2D structures of the inhibitors were generated using Marvin sketch and ligands were prepared to expand protonation and tautomeric states at 7.0 ± 2.0 pH units using LigPrep. These seven inhibitors were applied for shape based screening against ASINEX database. Obtained structural analogs were applied for LigPrep and reactive filter in order to avoid false positive hits and ligands not following the Lipinski's filter.

A 10 x 10 x 10 Å grid was generated around the RecA binding site region of DprA. A systematic three tier GLIDE [11],[12] (grid-based ligand docking with energetics) docking protocol, which include high throughput virtual screening (HTVS), standard precision (SP) and extra precision (XP) docking methods were applied. The good scoring ligands were ranked based on XP Gscore and binding orientations were analyzed and it used as criteria to propose potential DprA inhibitors of H. influenzae.

4. ADME/T properties

QikProp v3.6, program was used to calculate ADME/T properties (absorption, distribution, metabolism, excretion and toxicity) of obtained leads from docking [13],[14].

Results and discussion

1. sRNAPredict and drug target selection

The genome of H. influenzae consists of 2.18 Mb size with the GC content of 51.18% contains 1765 coding genes that codes for 1610 proteins. Five sRNAs from the genome of H. influenzae were obtained through sRNAPredict tool. The predicted five sRNAs are non-homologs to human proteome and the drug target DprA was selected for further studies to design novel inhibitors to block its functional activity of transformation, recombination and replication to arrest the growth of the pathogen. Positioning of key interaction residues on the DprA structure revealed an overlap of DprA–DprA and DprA–RecA interaction surfaces.

2. Homology modeling and validation

Homology modeling was performed to generate the three-dimensional models of DprA based on the X-ray structure of Streptococcus pneumonia (3UQZ) which showed 61% identity with the drug target and selected as a template. Twenty homology models of DprA were generated and 18th model showed the least DOPE score of -38984.43kcal/mol and was selected for further validation. Stereochemistry assessment displayed 94.9% residues in the most favourable regions of Ramachandran plot and suggests that the homology model of DprA was deemed to be highly reliable (Fig. 1A). Evaluation of protein structure analysis (ProSA) showed Z score value of -8.16 and protein quality predictor (ProQ) showed LG score of 4.712 (Fig. 1B &1C), represents the model was extremely good quality. The validated structure was submitted to PMDB and it was accepted with less than 3% stereo chemical check failure. The PMDB ID for DprA is PM0000003.

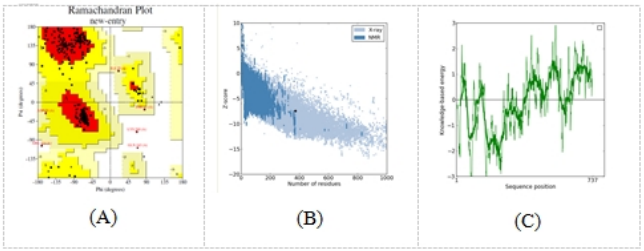

Fig.1: Validation of predicted DprA model (A). PROCHECK evaluation (B). ProSA-Z score plot (C). ProSA-energy plot

3. Virtual screening and docking studies

Seven published inhibitors of DprA were screened against ASINEX database consisting of 4.5 lakhs compounds using Phase v3.2 yielded 2612 structural analogs. Compilation of 2612 using Lipinski's and reactive filters resulted 324 compounds. The 324 analogs were docked with DprA of H. influenzae through virtual screening workflow, HTVS revealed 168 compounds. Top compounds from HTVS were applied to SP reveal 68 compounds and from SP were appliedto XP, 38 ligands were obtained. Among the 38 ligands, 3 leads are having better docking glide score, similar orientations and similar binding interactions, when compared with the seven published inhibitors. Lead 1 formed four hydrogen bonds with RecA binding sites residues of DprA. Backbone atom of Leu-244 formed hydrogen bond with –OH group of ethane-diol moiety of lead 1. Side chain atom of Asn-247 formed hydrogen bond with the –OH group of butane-triol moiety of lead 1. Side chain atom of Tyr-287 formed hydrogen bond with the –OH group of oxanol moiety of lead 1.Arg-248, Glu-249, Val-250, Leu-268, Gln-271, Gly-272, Ala-273 and Met-274 also showed good van der Waal's interactions with RecA binding sites residues of DprA (Fig. 2.). Lead 1 possesses the highest binding affinity towards DprA with the least docking score of -7.35 kcal/mol (TABLE 1). The conformational stability of the DprA–lead1 docking complex was established through hydrogen bond interactions and van der Waals interactions.

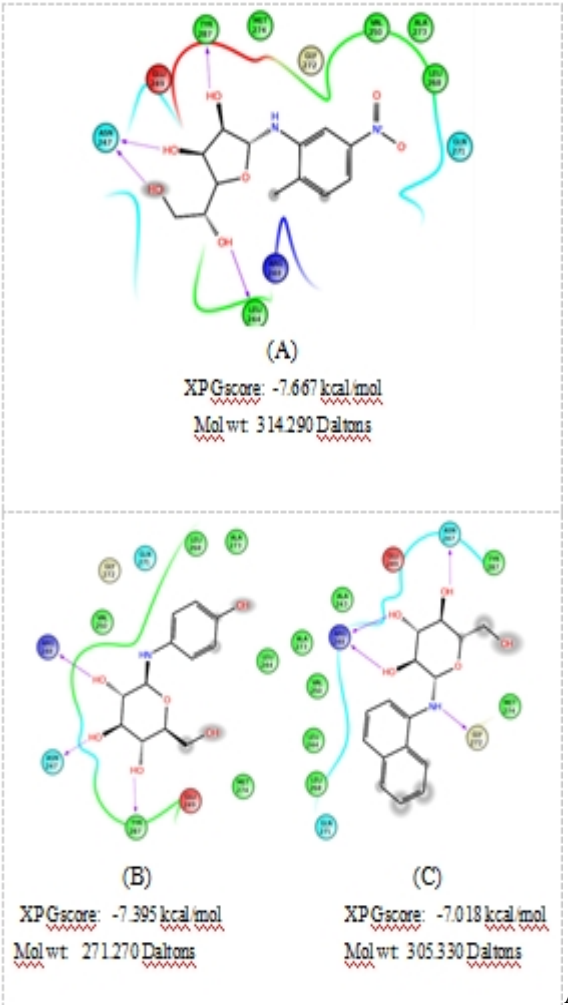

Fig.2: Proposed leads interactions with DprA of H. influenzae (A). Lead 1 (B). Lead 2 (C). Lead 3

Table: 1 Xpgscores of three leads and seven published inhibitors

Compounds	XPG Score (kcal/mol)
Lead1	-7.667
Lead2	-7.395
Lead3	-7.018
PI1	-6.577
PI2	-5.316
PI3	-4.649
PI4	-4.395
PI5	-3.209
PI6	-3.017
PI7	-2.474

4. Pharmacokinetic Properties

The ADMET properties of 3 leads thrived without any violations and biologically active without any toxic functional groups. The pharmacokinetic properties of three leads were within the normal range of FDA approved drugs (TABLE 2).

DprA involves in the bacterial transformation, recombination and replication of H. influenzae by forming active DprA-RecA dimer formation. Hence the crucial DprA residues involved in RecA binding for active dimer formation are targeted with the proposed three leads which are having better binding affinities than the existing inhibitors. The three leads identified in the present study have to be further validated by in vitro bioassays and in vivo studies to support the findings against DprA of H. influenzae will certainly enable better appreciation and aid in the design of better therapeutic strategies against pneumonia.

TABLE: 2ADME Properties of three leads against DprA

S.NO	Parameters	FDA Range	Lead 1	Lead 2	Lead 3
1	Molecular weight	130.0-725.0	344.277	271.270	305.330
2	H bond donors	0.0-6.0	6	1	1
3	H bond acceptors	2.0-20.0	12.5	3	3
4	Polar surface area (PSA)	7.0-200.0	197.65	156.667	127.469
5	Solvent accessible surface area(SASA)	300-1000	549.017	441.0179	503.078
6	Ionization potential (ev)	7.9-10.5	9.13	8.85	8.53
7	Electron affinity (eV)	-0.9-1.7	1.31	1.094	0.66
8	QPpolrz	13-70	27.048	23.757	28.347
9	Log P o/w = log P for octanol/water	-2.0 / 6.5	-0.273	0.098	0.239
10	Log s = log S for aqueous solubility	-6.5 / 0.5	-1.928	-2.958	-4.211
11	Log BB= log BB for brain/blood	-3.0 / 1.2	-3.452	-1.176	-0.337
12	Log KP = log KP for skin permeability	KP in cm/hr	-7.439	-3.953	-2.346
13	Log Khsa= log Khsa Serum Protein Binding	-1.5 / 1.5	1.179	0.098	0.239
14	Lipinski Rule of 5 Violations	maximum is 4	0	0	0
15	Jorgensen Rule of 3 Violations	maximum is 3	0	0	0
16	% Human Oral Absorption in GI (+-20%)	<25% is poor	3	3	3
17	Apparent Caco-2 Permeability (nm/sec)	<25poor,>500 great	4821.428	3319.872	1390.728
18	Apparent MDCK Permeability (nm/sec)	<25poor, >500 great	2708.832	706.607	53.505

Conclusion

Multi drug resistance nature of H. influenzae towards several antibiotics and existing treatments are prone to have adverse effects like allergy, dizziness and fever, which anticipates the requirement to discover novel antagonists for the treating H. influenzae. In order to achieve the aim, H. influenzae genome was submitted to sRNAPredict tool resulted 3 sRNA candidates. These 3 sRNA candidates were further subjected to non-homology analysis, so that pathogen specific and non-homologous to host was defined as drug target. DNA processing chain A (DprA) of H. influenzae was found to be crucial in bacterial transformation mechanism essential for the survival of the pathogen. Tertiary structure of DprA was modeled and validated. Seven published inhibitors were considered for preparing the library of DprA inhibitors by performing shape screening against ASINEX database consisting of 4.5 Lakhs small molecules using Phase v3.2. Rigid receptor docking protocol of Schrodinger was followed with HTVS, SP and XP docking for the library of DprA inhibitors towards DprA grid. The docking analysis revealed 38 ligands and further compared to published inhibitors which revealed three the best leads. These three leads are found to have similar binding patterns with high binding affinities towards DprA of H. influenzae. The proposed three leads were enough to stop the activity of DprA by blocking the RecA binding site residues competitively, which results in the depletion of the bacterial transformation mechanism that isnecessary for the growth and multiplication of the pathogen. Thus the proposed three leads overlay a novel frame work for treating the H. influenzae mediated pneumonia.

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