Discovery of Potent Inhibitors against GTP Pyrophosphokinase of *Neisseria meningitidis* Serogroup B

Nakka Sivakumari, Pasala Chiranjeevi, Dibyabhaba Pradhan and Amineni Umamaheswari

Abstract— *Neisseria meningitidis (N. meningitidis)* is a non-motile, Gram-negative bacterium. *N. meningitidis* is often referred as meningococci and causes cerebrospinal fever and bacterial meningitis. Although antibiotics such as penicillin, ciprofloxacin, ceftriaxone, rifampin and polymixin were used to treat bacterial meningitis, but *N. meningitidis* exhibits multi drug resistance. Therefore, designing novel inhibitors against *N. meningitidis* would be useful for developing therapies directed towards management of cerebrospinal fever and bacterial meningitis. Genome sequence of *N. meningitidis* was explored using sRNAPredict tool to identify 249 sRNA candidates of which 68 were enzymes and rests were non enzymes. The enzyme GTP pyrophosphokinase plays a pivotal role in the growth and metabolisms of the pathogen and non-homologous to Homo sapiens, so it was selected as a potential drug target against *N. meningitidis*. Homology model was built to GTP pyrophosphokinase using Modeller 9v13. Model with lowest DOPE score was selected and validated using PROCHECK, ProSA and ProQ. Published inhibitors such as alpha beta ethylene-ATP, microccin, tetracycline, thiostrepton and viomycin were selected for shape based similarity screening against ASINEX database to generate an in-house library. Docking studies were accomplished using Glide v6.2 for in-house library with GTP pyrophosphokinase which revealed 39 potential leads. Binding free energies (ΔG score) of resulted leads and existing inhibitors were compared to propose five compounds as potent inhibitor of GTP pyrophosphokinase. Lead1 showed lowest ΔG score of -63.65 kcal/mol with strong hydrogen bonding network and with good van der Waals interactions. Five best leads are observed to obey the pharmacological properties at par with 95% of the existing drug molecule. Thus, the lead1 can freezes the functional activity of GTP pyrophosphokinase in the purine metabolism necessary for the replication and DNA repair, to halt proliferation of the of *N. meningitidis*.

Index Terms— Cerebrospinal fever, Glide, ΔG score, GTP pyrophosphokinase, meningitis, *Neisseria meningitidis*, sRNAPredict.

1 INTRODUCTION

Neisseria meningitides, a non-motile Gram negative bacterium [1], has history of large epidemics, which also referred to as meningococci, causes foremost infections including cerebrospinal fever, life threatening sepsis and bacterial meningitis. It can cause severe brain damage and too fatal in 50% of cases if untreated. *Neisseria meningitidis* is a major cause of morbidity and mortality during childhood in industrialized countries and has been responsible for epidemics in Africa and Asia [2]. Pathogen enters the nose through air, attaches and interacts with nasopharyngeal epithelium, passes through the mucosa and enters into the blood vessels. It survives in the blood stream [3], endotoxins and other cell components produced in the host cell cytokines. Cytokines causes inflammation and crosses the blood-brain barrier then enters into cerebrospinal fluid and affects the subarachnoid space which leads to bacterial meningitides. Initially it produces general symptoms like fatigue; it can rapidly progress from fever, headache, neck stiffness, nausea, and partial lesions on skin, coma and death [4].

Neisseria meningitidis serotypes are also showing resistance towards the commonly prescribed antibiotics such as penicillin [5], ciprofloxacin [6], ceftriaxone [7], rifampin [8], and polymyxin. In particular, *N. meningitidis* serogroup B is known for exhibiting multi-drug resistance among other serotypes. Therefore, *N. meningitidis* serogroup B has attained greater interest to researchers for developing effective vaccines and drugs molecules. Herein, *in silico* analysis was carried out to find 249 sRNAs from *Neisseria meningitidis*. GTP pyrophosphokinase is one of the sRNA, non-homologous to humans and plays a crucial role in nucleotide metabolism and energy production, leading to the rise of meningitis symptoms.

The enzyme participates in purine metabolism: it provides components to DNA, RNA and the energy production of the cell. GTP pyrophosphokinase that catalyses reversibly the transfer of a pyrophosphate group from ATP to the 3'-OH group of GDP or GTP with the formation of guanosine 3'-diphosphate 5'-diphosphate or guanosine 3'-diphosphate 5'-triphosphate (ppGpp) and AMP. The synthesis is induced by mRNA and uncharged sRNA which is bound to the amino acyl-t-RNA binding site of the ribosome by a codon-specific association. The enzyme is also termed as stringent factor and is located in the relA gene which plays a pivotal role in bacterial defense mechanism such as stringent response. Hence the development of new lead molecules as inhibitors for selected enzyme as a potential drug target, in turn results in the inhibition of *N. meningitidis* proliferation. Therefore, molecular modeling techniques were accomplished to identify potent lead molecules targeting GTP pyrophosphokinase based on its known inhibitors.

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2 MATERIALS AND METHODS

The complete genome sequence of *N. meningitidis* (strain MC58) was retrieved from the NCBI database to recognize principal enzymes which play vital roles in metabolic pathways of pathogen and non-homologs to human. The genome sequence of pathogen submitted to sRNAPredict tool [9]. The non-human homology of the predicted sRNAs was ensured by performing a BLAST search against humans. sRNAs essential for survival of the pathogen and absent in host were proposed as drug targets of *N. meningitidis*. GTP pyrophosphokinase being a vital enzyme of *N. meningitidis* was selected for lead discovery in the present study.

2.1 Proteomic Analysis of GTP Pyrophosphokinase

The GTP pyrophosphokinase protein sequence was retrieved from the UniProt for further analysis including physicochemical properties analyzed using ProtParam tool.

2.2 Secondary Structure and Functional Elements Prediction

Based on the knowledge of primary protein sequence, the secondary structure analysis gives complete idea of the protein such as information about helices, turns, coils and strands. SOPMA [10] was used to predict the secondary structure of GTP pyrophosphokinase of *N. meningitidis*. The involvement of GTP pyrophosphokinase in pathogen's metabolic pathways was analyzed by the Kyoto Encyclopedia of Genes and Genome [KEGG] [11]. The functional elements of GTP pyrophosphokinase were predicted using ProSite [12], BLOCKS [13] and Pfam [14].

2.3 Homology Modeling and Model Validation

Modeller is a computer program that models three-dimensional structures of proteins and their assemblies by satisfaction of spatial restraints [15]. As an experimentally solved 3D structure of GTP pyrophosphokinase is not available, Modeller 9v13 was used to build 20 models for GTP pyrophosphokinase of *N. meningitidis* along with substrate, GDP. The structure of template (1VJ7) was retrieved from the PDB. Alignment file, PDB coordinate file of template were initialized in the Modeller script file. Of the 20 models generated the best model with the least DOPE (discrete optimized protein energy) score was selected for model validation using PROCHECK, ProSA and ProQ. The validated finest model was visualized with the aid of PyMOL software.

2.4 Prediction of Active Site

The validated 3D model of GTP pyrophosphokinase was analyzed in PyMOL. The residues around 4Å ligand GDP was predicted as active site. CASTp and SiteMap analysis also deciphered the site as largest ligand binding pockets on the protein. The predicted active site residues were also correlated with literature evidences [16], [17], [18]. Therefore, the GDP binding pocket was confirmed as active site of GTP pyrophosphokinase and used for virtual screening. The validated and finest 3D model structure was further taken for the docking studies.

2.5 Shape Screening and Ligand Preparation

ASINEX database was prepared for high throughput virtual screening [22] process by using the LigPrep module from Schrödinger. The number of ligands is more than 600,000 molecules. Each ligand was well structured and defined for a pH range 7.0 ± 2.0 which is around the human physiological pH. Compounds with similar structural geometry have high probability of delivering similar effects. The concept of shape screening emerged with notable success for identifying a new class of inhibitors which encompasses pulling out a set of compound similar to known inhibitors from ASINEX 3D ligand database [19]. Therefore, in virtual screening scenario, it is imperative to implement docking strategies to a set of similar structures of the published inhibitors rather than to a complete ligand database. Shape screening was performed for prepared five published inhibitors from ASINEX 3D database using PHASE v3.2 module.

The existing inhibitors (alpha beta-ethylene-ATP, micrococcin, tetracycline, thiostrepton, and viomycin) obtained from literature [20], [21] and ligands obtained through shape screening were prepared for docking by using Schrodinger LigPrep, Epik and QikProp modules. Epik v2.7 one of the products of Schrödinger suite can automatically be employed by LigPrep to enumerate tautomers and protonation states.

2.6 Docking and Scoring

The molecular docking plays an important role in the rational design of drugs [24]. All the ligands which were shape screened by implementing ASINEX database in PHASE module and then subjected for docking using three stepped Glide v6.0 virtual screening protocol [22], [23], [25]. The modeled structure of the enzyme was imported to Maestro for further protein preparation, minimization and receptor grid generation processes by using protein preparation wizard program in Schrodinger software suite 2014 [26], [27]. The grid was generated centered on the active site residues of the 3D-structure of the GTP pyrophosphokinase; A scaling factor of 1.0 Å was set to van der Waals radii for these residue atoms, with the partial atomic charge less than 0.25. A cubic box of specific dimensions centered on the centroid of the active site residues was generated. The grid was set to 10 x 10 x 10 Å and subjected to systematic three tier Glide docking protocol, which include high throughput virtual screening (HTVS) [28], standard precision (SP), extra precision (XP) docking methods [27]. The docking complexes were then taken for free binding energy (ΔG) calculations using Prime.

MM-GBSA binding free energy can be brought closer to experimental absolute affinities by the inclusion of entropic contributions. Molecular energies (EMM), polar solvation through surface generalized born solvation model (GSGB) and a non-polar solvation term (GNP) composed of non-polar solvent accessible and van der Waals interactions were calculated through MM-GBSA OPLS-2005 [28], [29]. The binding energy was calculated by the following equation.

$$\Delta G_{bind} = \Delta E + \Delta G_{solv} + \Delta G_{SA}$$

Where, ΔE is the minimized energies, ΔG_{solv} solvation free energies, ΔG_{SA} is the difference in surface area energy of the GTP pyrophosphokinase - lead 1 docking complex and sum of the surface energies of GTP pyrophosphokinase and lead 1 respectively. The selected optimized lead molecules were studied for their drug-like properties based on Lipinski's parameters using QikProp [31]. QikProp v3.9 predicts the widest variety of pharmaceutically relevant properties (ADMET) such as octanol/water, water/gas log Ps, log S, log BB, overall CNS activity, Caco-2 and MDCK cell permeability's, log Khsa for human serum albumin binding, and log IC50 for HERG K+-channel blockage, making it an indispensable lead generation and lead optimization.

3 Results and Discussion

The metabolic activity of GTP pyrophosphokinase of *N. meningitidis* is an indispensable process of the pathogen as it is involved in metabolisms of purine synthesis for replication, cell division and proliferation and defense mechanism of pathogen. Thus, designing competitive novel inhibitors against GTP pyrophosphokinase could impede replication, cell division and bacterial proliferation. The genome sequence of *Neisseria meningitidis* (Ref Seq ID: NC_003112.2) codes for 2,114 genes which in turn produces 1,953 proteins. The sRNA prediction led to identify 249 sRNAs from the whole genome sequence of the pathogen of which 68 were enzymes. GTP pyrophosphokinase is one of the 68 enzymes essential for the survival of the pathogen and non-homologous to humans. Hence, it was chosen as a drug target. The GTP pyrophosphokinase sequence of *N. meningitidis* consists of 737 amino acids with 82.5 kDa molecular weight. GTP pyrophosphokinase protein sequence of *Neisseria meningitidis* serogroup B (strain MC58) was accessed through UniProt ID: Q9JY54.

3.1 Proteomic Analysis

The primary sequence analysis was carried out by ProtParam tool gave information about the total number of positively and negatively charged residues are 99 and 93 respectively. The isoelectric point value is 6.36. Aliphatic index is 93.18 and Grand average of hydropathicity (GRAVY) is 0.0331.

3.2 Analysis of Secondary Structure and Functional Elements

The confidence level of secondary structure information (Fig. 1) regarding to helices, sheets, turns, coils of GTP pyrophosphokinase of *N. meningitidis* was predicted using SOPMA [10], [30]. 53.60% of α-helix, 14.25% of extended strands, 4.48% of β-turns and 27.68% of random coils are present in GTP pyrophosphokinase Fig. 1. The KEGG analysis revealed that GTP pyrophosphokinase of *N. meningitidis* is an important enzyme in purine metabolism pathway. The ProSite prediction revealed that no pattern was found in the enzyme. Three possible hits were retrieved from the BLOCKS database. GTP pyrophosphokinase had matched with 6 out of 8 BLOCKS belong to RelA/SpoT with ID as IPB007685 showing an e-value of 5.5e-139. Four domains are present in GTP pyrophosphokinase. HD4 domain with 40-201 amino acids functions as a metal-dependent phosphohydrolase, RelASpoT domain (while SpoT is thought to be a bifunctional enzyme catalysing both ppGpp synthesis and degradation) ppGpp 3'-pyrophosphohydrolase ranges from 260-371 amino acids, TGS domain ranges from 399-473 amino acids which is a possible nucleotide-binding region and the ACT regulatory domain which ranges from 659 -736 amino acids. ACT domain regulates other enzyme domains by binding to amino acids of associated enzyme domains. ACT domain is found at the C-terminus of the RelA protein. These four domains of GTP pyrophosphokinase were predicted with Pfam (Fig. 2).

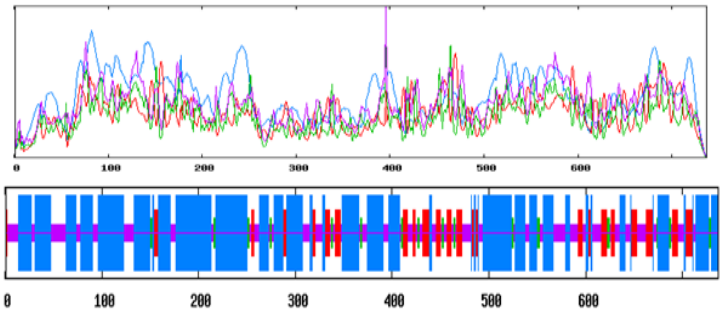

Fig.1. Secondary structure prediction of GTP pyrophosphokinase [Blue color indicates α-helix (53.60%); Magenta color codes for random coils (27.68%); Red color codes for extended strands (14.25%) and Green color codes for β-turns (4.48%)].

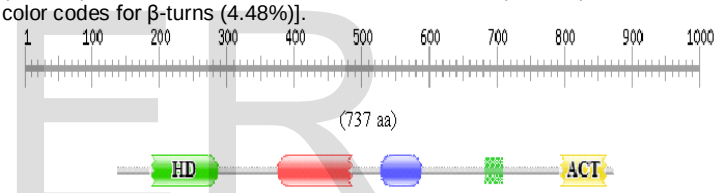

Fig. 2. Pfam matches for GTP pyrophosphokinase, active site is present in RelASpoT domain which is shown in red colour.

3.3 Homology Modeling and Model Validation

GTP pyrophosphokinase of Streptococcus equisimilis (1VJ7) was taken as template showed 39% of identity with GTP pyrophosphokinase of *Neisseria meningitidis*. The alignment file was generated using ClustalX (Fig. 3). 20 models of GTP pyrophosphokinase were generated using Modeller9v13. The 6th model with the lowest DOPE score (-51352.48 kcal/mol) was selected for further validation (Fig. 4a). Stereochemistry assessment of model had shown 92.2% residues in favorable region, 7.3% in additional allowed region and 0.6% in disallowed region and was found to compare favorably with data of crystal structure (1VJ7) (Fig. 4b).

Sequence alignment with template (1VJ7) using ClustalX ProSA-revealed Z-score value of -7.59 (Fig. 5a) and had shown overall residue energies as largely negative. These energies together with pair, combined and surface energies were all negative and had similar surface energy with template (Fig. 5a & 5b). The 3D model of GTP pyrophosphokinase of *N. meningitidis* when submitted to ProQ had shown LGscore of 3.715. The result suggests that the model is of very good quality [31].

3.4 Active Site Analysis

The residues around 4Å of incorporated ligand GDP were pre-

dicted as active site of GTP pyrophosphokinase. CASTp and SiteMap analysis also revealed the same active site residues from the possible largest ligand binding pockets on the protein. The residues such as Asn-196, Trp-201, Lys-204, Trp-205, Arg-261, Lys-263, His-264,Ser-267, Lys-270, Lys-274, Ala-287, Gln-315, Asp-317, Tyr-319, Lys-324, His-332, Thr-333, Val-334, Glu-344, Val-345 and Gln-346 were seemed to be in binding pocket volume of the receptor. These residues are found to be correlating with the literature studies with the active site residues interacting with the co-crystal ligand of 1VJ7.

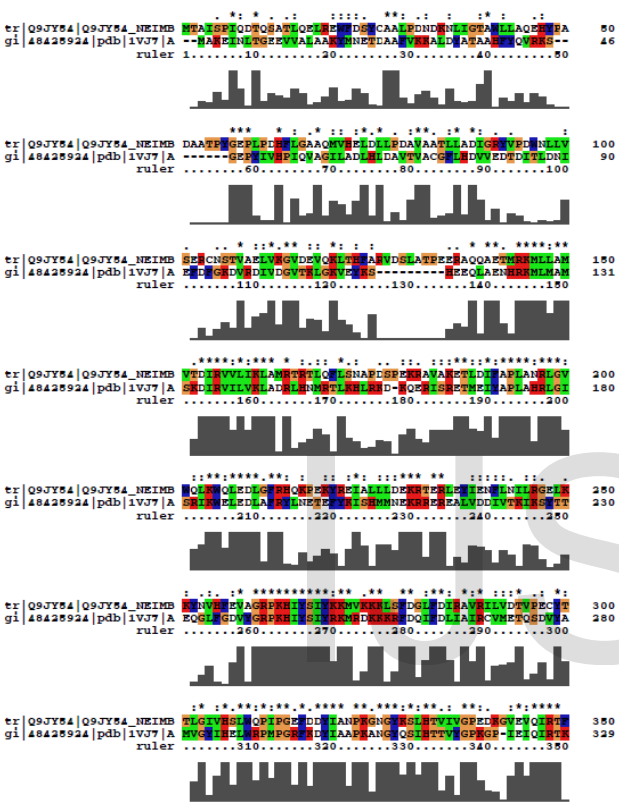

Fig. 3. GTP pyrophosphokinase of *N. meningitidis* (Q9JY54)

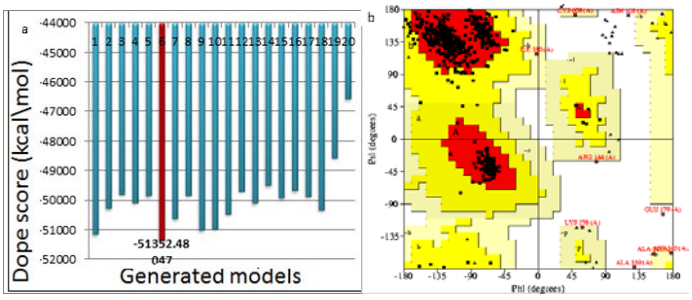

Fig. 4a. DOPE scores for generated 20 models, 4b. Ramachandran plot for predicted GTP pyrophosphokinase model

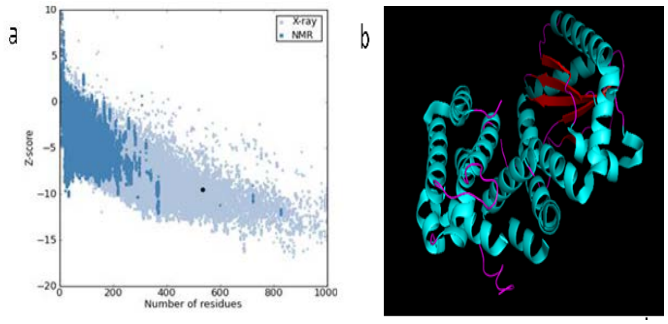

Fig. 5a. Z-score of GTP pyrophosphokinase, 5b. 3D-structure of GTP pyrophosphokinase visualized by PyMOL viewer.

3.5 Virtual Screening and Lead Identification

The preprocessed protein model was subsequently minimized with optimized potentials for liquid simulations (OPLS) 2005 force field and the impact molecular mechanics engine [32] (Schrodinger LLC, 2011); Minimization was performed restraining the heavy atoms with the hydrogen torsion parameters turned off, to allow free rotation of the hydrogens setting the max root mean square deviation (RMSD) of 0.30Å [33] (Schrodinger LLC, 2013). A grid was generated around the active site of the prepared protein and was directed towards virtual screening and docking processes. The most broadly used method for virtual screening is docking of small lead molecules into active site of the target receptor and scoring. In the wide range of different docking programs, semi-rigid docking was preferred, where the ligands are treated as flexible and the receptors as rigid. The Glide module was used for protein-ligand docking. Shape based screening of five published inhibitors from prepared ASINEX 3D database resulted 400 compounds and taken for three tier docking strategy.

3.6 Docking and Scoring

Docking of 400 compounds into the grid of GTP pyrophosphokinase of *Neisseria meningitidis* grid showed 314 compounds docked with significant Glide score. 314 compounds were re-docked using standard precision (SP) docking. Seventy eight compounds were observed to show significant glide score in SP docking hence re-docked using extra precision mode (XP). Obtained compounds were ranked based on XPG score and evaluated through Prime/MM-GBSA free energy calculations (Fig. 6). XP docking resulted in 39 leads and top 5 leads were proposed based on the better Prime MM-GBSA score when compared to existing published inhibitors Prime/MM-GBSA scores (Table 1).

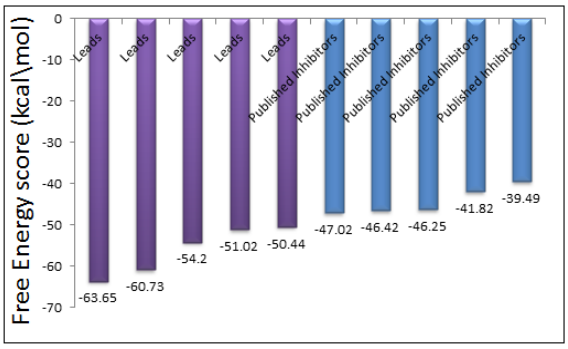

Fig. 6. ΔG of leads and published inhibitors

Docking complex of lead1 – GTP pyrophosphokinase had showed similar binding orientation and more binding affinity when compared to co-crystal ligand. Lead1 formed five hydrogen bonds and one salt bridge with active site residues of GTP pyrophosphokinase. Such as Asn-196, Gln-315, Asp-317, Lys-324, His-332 and Asp-317 also formed one salt bridge with lead 1 (Fig. 7). The good molecular interactions between proposed lead 1 and GTP pyrophosphokinase of *N. meningitidis* would be useful to modulate enzyme into inactive state, which in turn inhibit DNA replication, arrest the cell division and bacteria proliferation due to lack of purine nucleotides synthesized by GTP pyrophosphokinase.

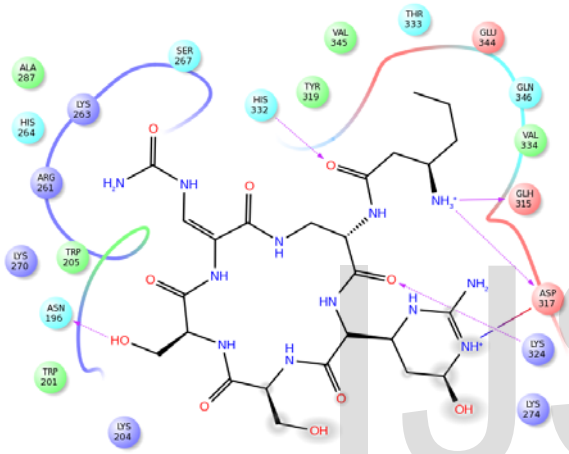

Fig. 7. Molecular interactions of GTP pyrophosphokinase with lead1

TABLE1.
Binding free energy of leads and published inhibitors

S.No	Leads	ΔG scores (kcal/mol)	Published inhibitors	ΔG scores (kcal/mol)
1	3-Amino-hexanoic acid [12-(6-hydroxy-2-imino-hexahydro-pyrimidin-4-yl)-6, 9-bis-h).	-63.65	Alpha beta-ethylene-ATP	-47.02
2	2-(2-Amino-6-hydroxy-9H-purin-8-ylsulfanyl)-1-(4-fluoro-phenyl)-ethanone	-60.73	Microccin	-46.42
3	2-(2-Amino-6-hydroxy-9H-purin-8-ylsulfanyl)-N-benzo[1,3]dioxol-5-yl-acetamide	-54.20	Tetracycline	-46.25
4	3-Amino-1-[16-(3-amino-propionyl)-1,4,10,13-tetraoxa-7,16-diaza-cyclooctadec-7-y	-51.02	Thiostrepton	-41.82
5	5-Hydroxy-2-(4-methoxy-phenyl)-7-[3,4,5-trihydroxy-6-(3,4,5-trihydroxy-6-methyl-	-50.44	Viomycin	-39.49

4 Conclusion

Life threatening diseases explicitly meningitis, septicemia and bacteremia caused predominantly by Gram-negative bacteria *Neisseria meningitidis* strains. These diseases are a growing concern in terms of human mortality worldwide; emphasizing the need to development of new antimicrobial agents against broad-spectrum targets to curb these problems. The present study has focused on GTP pyrophosphokinase of serotype-B strain as a significant target protein since it is non-homologous to humans as well as involved in key unique purine metabolism pathway of all *N. meningitidis* strains as well as other bacterial pathogens. The enzyme catalyses reversibly the transfer of a pyrophosphate group from ATP to the 3'-OH group of GDP or GTP with the formation of guanosine 3'-diphosphate 5'-diphosphate or guanosine 3'-diphosphate 5'-triphosphate ((p)ppGpp) and AMP. Thus, GTP pyrophosphokinase is of significant interest for novel inhibitor design to overcome the challenges of severe meningitis and septicemia etc. A high-quality homology model of GTP pyrophosphokinase as reported through computational validation. Our approach employing Glide for virtual screening along with QikProp ADME/T evaluation provided five novel GTP pyrophosphokinase inhibitors. The five inhibitors identified and proposed through high-throughput virtual screening using GTP pyrophosphokinase homology model would be of interest as inhibitor against *Neisseria meningitidis* serotypes. Among them lead1 showed the best binding affinity, binding orientation and with strong hydrogen bonds network, good van der Waal interactions, pharmacological properties as par with 95% of the existing drug molecule and no reactive functional groups. As GTP pyrophosphokinase important in the purine metabolic pathway of bacteria for replication and further cell division and bacterial proliferation, the identified GTP pyrophosphokinase inhibitors are useful to develop novel inhibitors if synthesized and tested in animal models.

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