

Identification and structural investigation of potential novel drug candidates against lethal human pathogen

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Abstract: *Neisseria meningitidis* is responsible for causing meningococcal meningitis along with acute septicaemia in human beings. Functional genomics strategies proved cruciality of certain genes/proteins in *Neisseria meningitidis* pathogenesis. During the present studies, three important *Neisseria meningitidis* proteins i.e., Dead box RNA-Helicase, Polyribonucleotide nucleotidyl-transferase PNPase and Ribonuclease-III were targeted for homology modeling and protein-ligand docking studies not only to determine their three dimensional architectures but also to identify their potential novel inhibitors. The Biscoumarin, malonitrile and indole derivatives showed the best inhibitory mode against all of the three enzymes. Since, these enzymes are assembled in Gram-negative bacteria to form RNA degradosome assembly therefore their inhibition will definitely shut off the degradosome assembly and ultimately the decay of RNA, which is an essential life process. This is the first ever structural investigation of these drug targets along with identification of potential novel drug candidates. We believe that these small chemical compounds will be proved as better drugs and will provide an excellent barrier towards *Neisseria meningitidis* pathogenesis.

Keywords: *Neisseria meningitidis*, homology modelling, transcription. RNA degradosome, molecular docking

INTRODUCTION

Neisseria meningitidis is a virulent human pathogen causing meningitis as well as life-threatening septicaemia throughout the world (Hammerschmidt *et al.*, 1996). *N. meningitidis* is a gram-negative encapsulated diplococcus having 2.1-2.2 MB genome with almost 2000 genes (Racloz and Luiz, 2010; Tettelin *et al.*, 2000). It has been classified into 13 serogroups based on the differences between capsular polysaccharide structures, but among all these serogroups only 6 serogroups (A, B, C, W-135, X and Y) have been reported to be pathogenic (Rosenstein *et al.*, 2001).

Being a fatal and rapidly-developing disease, meningococcal meningitis with associated sepsis is a global health issue that mostly affects children and young adults (WHO, 2001). Effective conjugated capsular polysaccharide protein vaccines have been developed against strains of all pathogenic serogroups of *N.meningitidis* except serogroup B, which is still the predominant cause of meningitis in Europe, Australia and New Zealand. It is responsible for 64% of the cases in these regions (Racloz and Luiz, 2010; Snape *et al.*, 2008). The capsular polysaccharide vaccines are found to be disputed and challenging against *N. meningitidis* serogroup B as it is identical to human carbohydrate [α (238)N-acetyl neuraminic acid or polysialic acid], so being a self-antigen, it can elicit autoimmune response

(Häyrinen *et al.*, 1995).

Due to the unavailability of *N. meningitidis* serogroup B vaccine or anti-meningococcal drug, it is essential to identify drug candidates which would potentially treat the meningococcal disease. Structure-based drug designing can be a significant strategy if potential drug targets are identified. Understanding the 3D structures of proteins provides valuable insights into the molecular basis of their functions. Functional genomics studies of *N. meningitidis* have been carried out and many genes which belong to different functional categories were identified to be involved in meningococcal pathogenesis (Sun *et al.*, 2000). During the present study, we have selected the proteins or gene products involved in transcription including Dead box RNA-Helicase (NMB1422), Polyribonucleotide nucleotidyl transferase PNPase (NMB0758) and Ribonuclease III (NMB0686), for their detailed structural analyses, as transcription is one of the key processes occurring in a living organism. Here, we predict and validate the 3D structures of these proteins using different bioinformatics strategies. Moreover, we have also performed molecular docking studies on these drug targets and have identified different small molecules as their novel inhibitors.

MATERIALS AND METHODS

Retrieval of target sequences

The protein sequences of all the three pathogenic factors involved in transcription i.e. RNase III (NMB0686), PNPase (NMB0758) and Dead box RNA Helicase

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(NMB1142) were retrieved in FASTA format from UniProt Knowledgebase (Apweiler *et al.*, 2004).

Template search

Homology modeling is a theoretical method established on amino acid sequence and structure relationship in which similar sequences tend to adopt practically identical structures (Sander and Schneider 1991). In order to select a suitable template, all the three target protein sequences were subjected to Psi-BLAST (Altschul *et al.*, 1997) against Protein Data Bank (PDB) (Kouranov *et al.*, 2006) using default parameters. The optimal templates for NMB1142, NMB0758 and NMB0686 proteins were selected based on highest similarities, minimum gaps and bound ligands. The PDB files of selected templates were obtained from Protein Data Bank (Kouranov *et al.*, 2006).

Optimization of structure-based pairwise sequence alignments

Knowledge of the secondary structure of a protein having unknown structure is needed to perceive the structural details. During this study, the secondary structures of NMB1422, NMB0758, NMB0686 were predicted using the online server PsiPred (Jones 1999). Multiple sequence alignment of all the three target proteins with their respective sequence homologues were carried out using CLUSTALX (Larkin *et al.*, 2007). The information from secondary structure predictions and multiple sequence alignments were used to make optimal structure-based pair-wise sequence alignments between targets and their respective template proteins.

Homology modeling and validation of the predicted three-dimensional models

Homology modeling studies of *N. meningitidis* RNA Helicase (NMB1422) complexed with AMP, PNPase (NMB0758) complexed with RNase-E, RNA and Mn^{+2} and RNase III (NMB0686) complexed with double stranded RNA and Mg^{+2} were performed by protein structure-modeling program MODELLER v9.15 (Eswar *et al.*, 2006). Validation was performed by inspecting the overall stereochemistry of the models in Ramachandran plots obtained from PROCHECK standalone software (Laskowski *et al.*, 1993). PROSA (Sippl. 1993) studies were carried out to check the energetic architecture of the models as a function of amino acid sequence position. The root mean square deviation (RMSD) between the main chain atom of the models and the templates was calculated by superimposing the structures of the templates over the predicted models to assess the reliability of the models using PyMol (DeLano 2002).

Molecular Modeling studies of the three dimensional Models

DS-Visualizer (Visualizer DS., 2005) and PyMol (DeLano 2002) were used for the structural investigation of overall fold as well as the active/binding site residues of three dimensional homology models.

Molecular docking studies

Molecular docking studies were performed for the pathogenic proteins DEAD-Box RNA helicase, Polynucleotide phosphorylase (PNPase) and Ribonuclease III using Autodock Vina (Trott and Olson 2010). During these studies, the homology models of these proteins were used as receptors while certain small chemical molecules were used as ligands. For molecular docking studies, structures of 64 small synthetic compounds belonging to different chemical scaffolds that had been synthesized in different laboratories of International Centre for Chemical and Biological Sciences (ICCBS) were used as ligands. The three-dimensional structures of ligands were constructed using MarvinSketch software (Csizmadia 1999), ChemDraw (Spessard 1998) and DSVisualizer (Visualizer 2005). The PDBQT files of protein receptors and ligands were prepared by AutoDockTools v 1.5.6 (Morris *et al.*, 2009).

Analysis of docking results

Interatomic distances between the ligand and receptor proteins, hydrogen bonding and the surface view of docked complexes were analyzed using DSVisualizer (Visualizer DS, 2005), Visual Molecular Dynamics (Humphrey *et al.*, 1996) and Ligplot (Wallace *et al.*, 1995) softwares.

RESULTS

DEAD-Box RNA Helicase

Sequence Alignment and Homology Modeling

Psi-BLAST search tool of NCBI database identified *Homo sapiens* AMP bound DEAD-box RNA helicase crystal structure (PDB id: 2I4I) as an optimal template for *N. meningitidis* DEAD-Box RNA Helicase (fig.1). Psi-BLAST derived alignment having 58% sequence similarity was further edited especially in terms of gap adjustment based on secondary structure prediction and multiple sequence alignment results. The finalized alignment was utilized for homology model building of *N. meningitidis* DEAD-box RNA helicase and was validated using PROCHECK (Laskowski *et al.*, 1993) and PROSA (Sippl. 1993) standalone softwares to select an optimal model conformation. The PROCHECK (Laskowski *et al.*, 1993) analysis of the selected model conformation showed 91.4% residues in most favored regions with no residue in disallowed region. The PROSA tool (Sippl. 1993) showed the model to have an overall good energy profile. So, the homology model of *N. meningitidis* RNA Dead-Box helicase was considered as valid and was utilized for understanding the active site mechanics.

The homology model of *N. meningitidis* DEAD-box helicase consists of distinct Rec-A like fold (fig. 2a) containing domains consisting of central β sheets that are flanked by α helices. The overall three dimensional architecture of *N. meningitidis* DEAD-box helicase model was found to be quite similar with that of *Homo sapiens* DEAD-box helicase.

Table 1: Showing the docking score of representative compounds from different chemical scaffolds in Kcal/mol. The molecular docking was performed using Auto dock Vina for the proteins *Nesseria meningitidis* DEAD-box RNA Helicase, PNPase and RNase III

Dead Box Helicase NMB1142		Polynucleotide phosphorylase NMB0758		Ribonuclease III NMB0686	
Compound code	Docking Score (Kcal/mol)	Compound code	Docking Score (Kcal/mol)	Compound code	Docking Score (Kcal/mol)
AH-22	-10.0	MK-05	-10.5	MK-14	-7.9
MK-04	-10.0	MK-19	-9.9	MK-30	-7.8
MK-19	-10.0	MK-23-i	-9.9	MK-05	-7.7
AH-44	-9.7	AH-43	-9.6	MK-07	-7.6
MK-07	-9.5	MK-07	-9.0	MK-08	-7.2
MK-23-i	-9.0	AH-28	-8.9	MK-23-g	-7.2
MK-08	-9.3	MK-06	-8.8	AH-43	-7.1
MK-24-g	-8.8	MK-24-c	-8.7	MK-24-g	-7.0
MK-06	-8.4	MK-09	-8.7	AH-30	-6.9
MK-28	-8.5	MK-02	-8.4	MK-20-c	-6.7
MK-02	-7.9	MK-30	-8.1	MK-06	-6.6
MK-20-b	-7.6	MK-20-a	-7.7	MK-02	-6.3
MK-32	-7.4	MK-32	-7.3	MK-34	-6.1
AH-40	-6.1	AH-40	-7.0	AH-34	-5.2

All the motifs of *N. meningitidis* DEAD-box helicase are conserved with that of human DEAD-box helicase, except motif IV. In humans the motif IV is VFCKTK whereas, in *N. meningitidis* DEAD-box helicase it is VFVETK. The key residue K249 is substituted by E249 in *N. meningitidis*. Interestingly, a unique insertion between motifs I and Ia is present in human DEAD box helicase (DDX3) performing a vital role in nucleic acid binding and is absent in *N. meningitidis* DEAD-box helicase (Garbelli *et al.*, 2011). This distinguishing feature makes the DEAD box of *N. meningitidis* an important drug target.

Structural Investigation of adenylate binding pocket

The interactions of AMP with amino acid residues in the adenylate binding pocket of *N. meningitidis* DEAD-box helicase including Phe182, Tyr200, Glu201, Asn202, Pro203, Thr204, Gln207, Gln225, Thr226, Gly227, Thr228, Gly229, Lys230, Thr231, Ala232 and Gln285 were found to be almost similar with that of human DEAD-box helicase (fig. 2b).

Analysis of Docking Results

Molecular docking was performed for the *N. meningitidis* DEAD-Box RNA Helicase using a library of sixty-four compounds taken from different laboratories of ICCBS, University of Karachi, Pakistan, using the program AutoDock Vina. Out of sixty-two compounds from different chemical scaffolds, the compounds having the highest docking score [table 1] and showing the best interaction including were selected (fig. 3). The compound from acridine family i.e. 9-(4-hydroxyphenyl)-3,3,6,6-tetramethyl-3,4,6,7,9,10-hexahydroacridine-1,8(2H,5H)-dione (code: AH-22) showed the highest docking score of -10.0 Kcal/mol. AH-22 was observed to make two H-bonds with E20 and E18

residues while it formed Pi-Pi T-shaped interaction with Y19. The interactions between AH-22 and the helicase were further strengthened by van der Waals forces between the ligand and N21, T23, Q26, G46, G48 and E323 residues of the helicase (fig. 3). The analysis revealed that AH-22 compound mimic adenylate moiety in interacting with *N. meningitidis* RNA DEAD-Box Helicase. Here, we believe that this compound can compete with adenylate in binding with enzyme and ultimately can act as a potential inhibitor.

The biscoumarin derivative i.e., 3-[(2,3-dimethoxyphenyl)(6-fluoro-4-hydroxy-2-oxo-2H-chromen-3-yl)methyl]-6-fluoro-4-hydroxy-2H-chromen-2-one (code: MK-04) showed second highest affinity with a docking score of -10.0 Kcal/mol. The ligand was found to be involved in H-bonding with two adenylate binding residues i.e. G46, T47 and an additional H327 residue which is also involved in Pi-Pi stacking with the ligand (fig. 3). The fluorine atom of the ligand formed halogen bond with E20 residue. P24 and V326 residues were involved in making Pi-alkyl bonds whereas important catalytic residues including Y19, N21, T23 along with I25, V210 and E323 residues were engaged in van der Waals interactions with the ligand.

The compound 3-[(1H-indol-3-yl)(naphthalen-2-yl)methyl]-1H-indole (code: MK-19) an indole derivative gave -10.0 Kcal/mol docking score. It was found to be involved in H-bonding with the key residues i.e., E20 and Q26 (fig. 3). The ligand formed Pi-Pi stacked interaction and Pi-Pi donor hydrogen bond with Y19 and Pi-alkyl interaction with A51 and V326 residues. N21, T23, G46, T47, G48, E323 and H327 residues were found to further strengthen the contact with the ligand through van der Waals interactions.



Fig. 1: Structure-based pair-wise sequence alignment of DEAD-box RNA Helicase from *N. meningitidis* (NMB1422) and from *H. sapiens* (PDB id: 2I4I). The amino acid residues which are involved in adenylate binding are highlighted as magenta and the secondary structures are also mentioned.

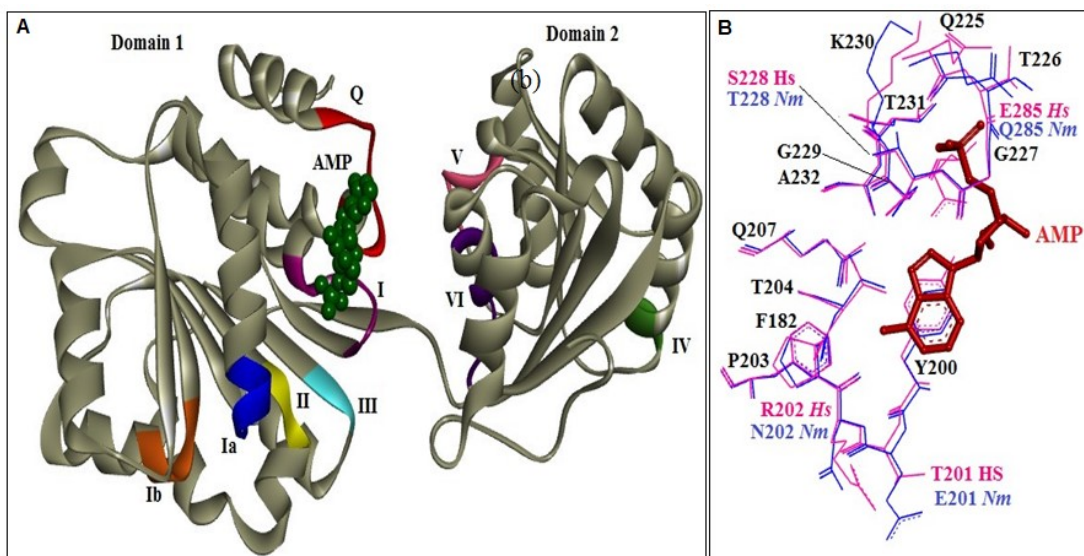


Fig. 2: The homology model of *Nesseria meningitidis* DEAD-box Helicase bound with AMP. (a) The model showing two clear domains and the motifs are highlighted by different colors. AMP showed by green color is bound at adenylylation site. (b) Superimposed image of Interaction of AMP with DEAD-box Helicase from *Nesseria meningitidis* (blue) and *Homo sapiens* (magenta) in Adenylylation binding pocket. The conserved residues are shown in black.

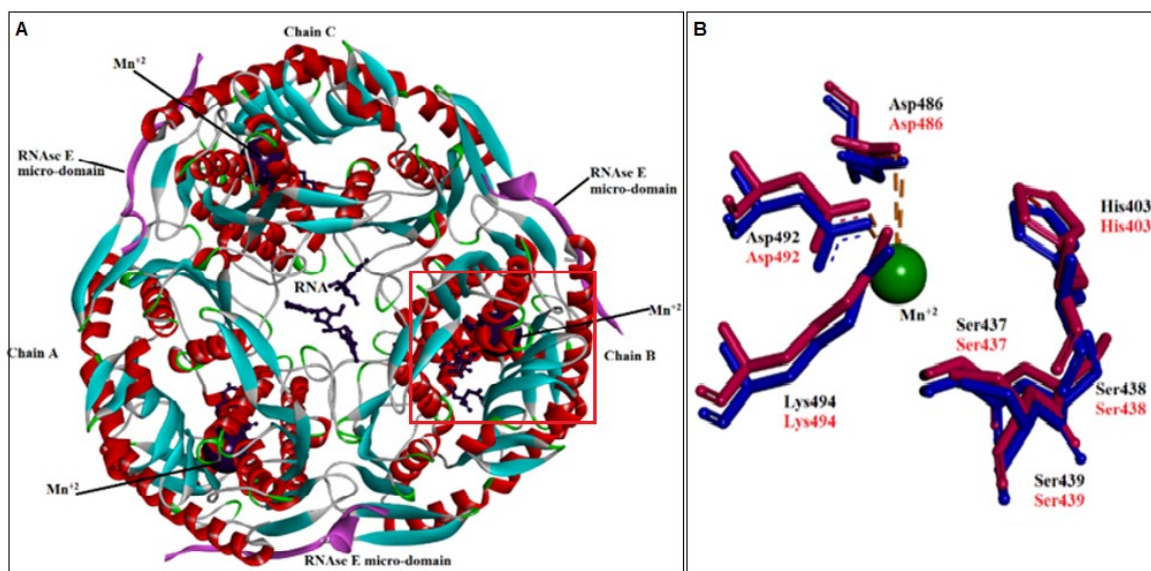


Fig. 5: Homology model of *N. meningitidis* Polynucleotide Phosphorylase (PNPase). (a) The trimeric central channel is formed by trimerization of chain A, B and C. RNase E micro-domain is shown by light purple color. Mn⁺² ions are represented by blue. RNA is bound at the central catalytic channel of the enzyme. (b) Superimposed image of interaction of Mn⁺² (green) with PNPase from *N. meningitidis* (pink) and *Escherichia coli* (blue) bound at active site of the enzyme. The amino acid residues at the active site are mentioned respectively.

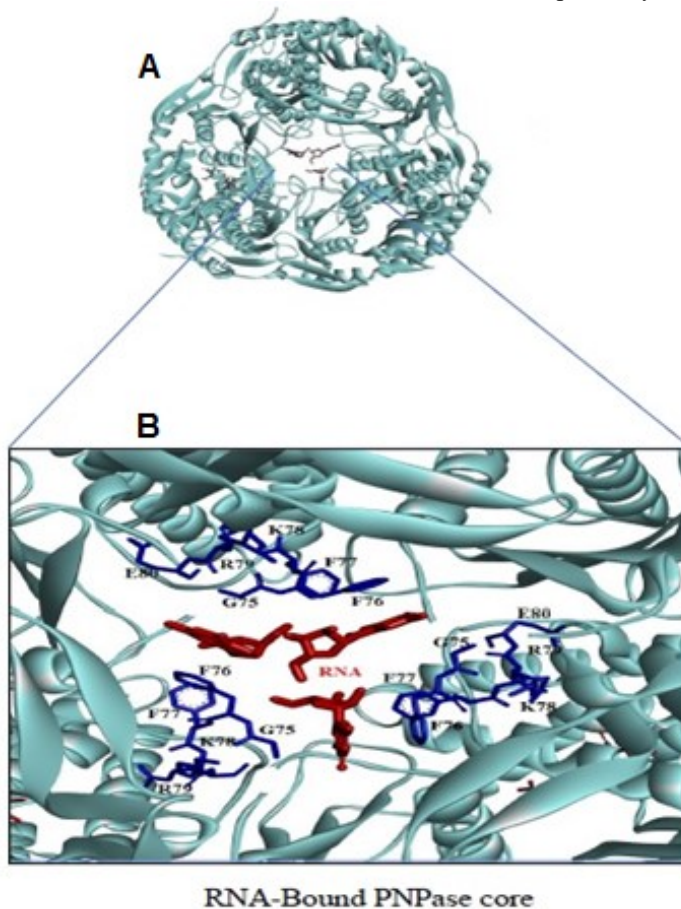


Fig. 6: RNA-bound PNPase core. (a)The interaction of RNA with *N. meningitidis* PNPase bound at the central catalytic pore of the trimeric assembly. (b) The conserved FFRR loop of PNPase assembly involved in the interaction with RNA is shown.

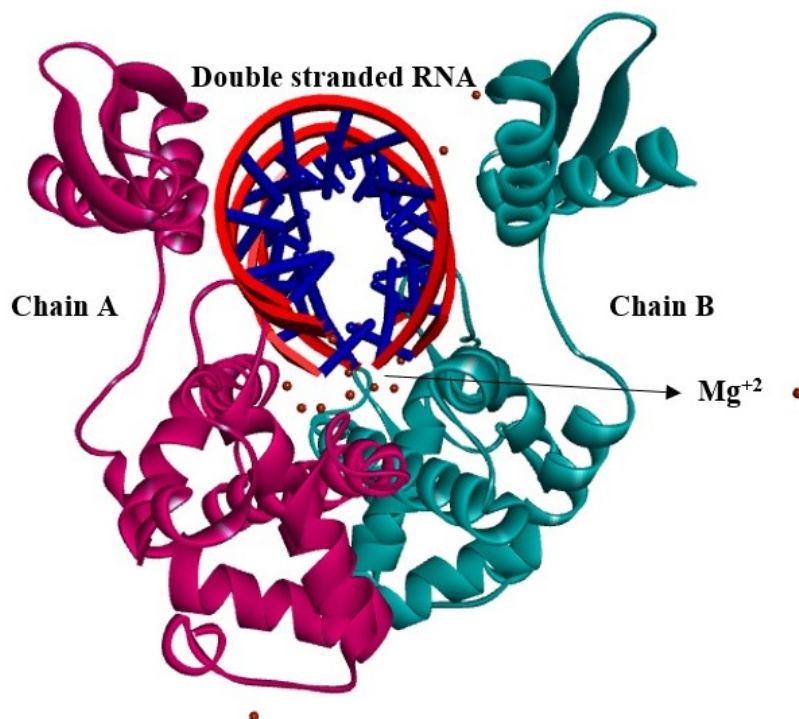


Fig. 9: Homology model of *Nesseria meningitidis* Ribonuclease III homodimer bound with double-stranded RNA and Mg⁺² ions. The two chains are clearly visible. Chain A and chain B shown by magenta and cyan colors respectively. The double-stranded RNA is bound at the catalytic valley of the enzyme. The divalent metal ions of Mg⁺² are represented by red colored dots.

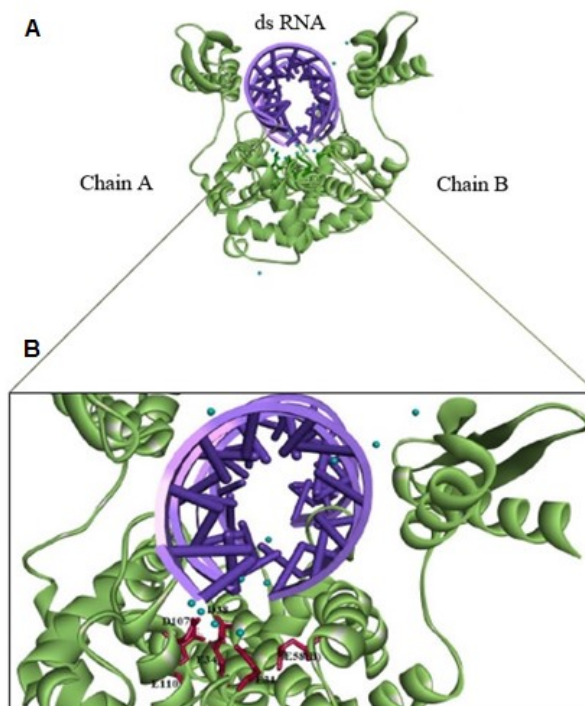


Fig. 10: The catalytic valley of *Neisseria meningitidis* RNase III. (a) The interaction of RNA with RNaseIII bound in the catalytic valley which is formed by the dimerization of the Chain A and B (b) The active site residues involved in the interaction with RNA are shown in sticks representation by pink color. The bivalent Mg⁺² ions shown by cyan colored dots are also present inside the catalytic valley.

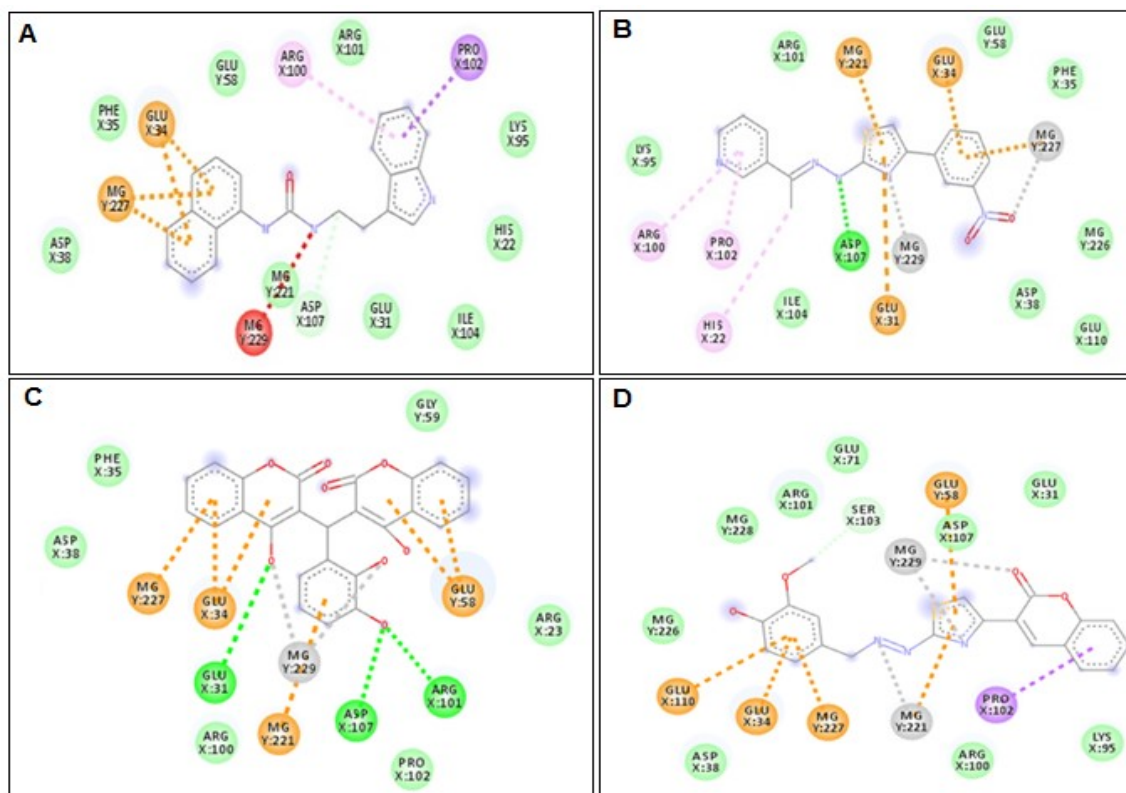


Fig. 11: Enzyme-ligand interactions of the four topmost compounds docked in the catalytic valley of *Neisseria meningitidis* Ribonuclease III showing the optimum interaction. Compound codes are (A) MK-14 (B) MK-30 (C) MK-05 (D) MK-07

The compound 2-(3-cyano-4,6-bis(4-fluorophenyl)-6-methyl-5,6-dihydropyridin-2(1H)-ylidene) malononitrile (code: AH-44) belonging to the family malononitrile gave docking score -9.7 Kcal/mol. It formed conventional H-bonding with Q26, T45, K49 and T50 residues. It also formed Pi-alkyl bond with A51 (fig. 3). Moreover, Pi-Pi stacking and halogen bonds are formed with Q93. The van der Waals interactions are formed with T23, T47 and G48 residues. This ligand showed a very good interaction with most of the active residues of *N. meningitidis* RNA DEAD-Box Helicase.

Polynucleotide Nucleotidyl Phosphorylase Sequence Alignment and Homology Modeling

N. meningitidis Polynucleotide phosphorylase (NMB0758) having 707 amino acids was subjected to Psi-BLAST search against PDB to find out the optimal template. Crystal structure of Polynucleotide phosphorylase from *Escherichia coli* bound with RNase-E micro-domain, RNA and Mn^{+2} (PDB id: 3GCM) was selected as a template as it belongs to the same enzyme family having 77% similarity (fig. 4). Since *E. coli* functional PNPase exists in a trimeric state, *N. meningitidis* PNPase was also modeled as a trimer with bound RNase-E micro-domain, RNA and Mn^{+2} using Modeller v.9.15 (Eswar et al., 2006).

For model validation, all the ten predicted conformers of *N. meningitidis* PNPase model were evaluated using PROCHECK (Laskowski et al., 1993) and PROSA (Sippl, 1993) standalone software to choose a model conformation of optimal quality. The PROCHECK (Laskowski et al., 1993) analysis of the selected model showed 90.7% residues in most favored regions with no residue in the disallowed region suggesting a good model quality. The energy profile and Z-score analysis also showed the model to have an overall good internal energy. Hence, *N. meningitidis* PNPase model was concluded as a valid model which was later used for extracting information regarding the three dimensional fold of the enzyme and its active site.

Structural Investigation of Active site and RNA binding Cavity

During the present studies, the overall fold of *N. meningitidis* PNPase model was found to be quite similar with that of template i.e. *E. coli* PNPase. The main catalytic residues i.e. S437, S438 and S439 were quite conserved in *N. meningitidis* PNPase model (fig. 5) suggesting similar catalytic operations within the two enzymes. Mn^{+2} binding in the catalytic channel activates the enzyme. Analysis of *N. meningitidis* PNPase interactions with Mn^{+2} revealed identical metal

coordination facilitated by H403, D486, D492 and K494 residues.

Each monomer of *E. coli* PNPase homotrimer is known to have a conserved FFRR loop based on F77, F78, R79 and R80 residues which are believed to be major RNA binding residues. The corresponding residues of this loop in the *N. meningitidis* PNPase model were found to be F77, F78, K79 and R80 i.e. having three identical and one conservatively substituted residue (fig. 6). The present studies suggested that the *N. meningitidis* PNPase trimeric structure is almost identical in terms of overall three dimensional architecture including active site and RNA binding cavity. Here, we believe that the PNPase from *E. coli* and *N. meningitidis* are involved in RNA degradation in the similar manner.

Analysis of Docking Results

In molecular docking studies the compounds found to be binding in the active-site of PNPase were selected. Out of sixty-two compounds from different chemical scaffolds these top four compounds were selected i.e., MK-05, MK-19, MK-23-I and AH-43. These compounds have shown higher docking scores as well as good interaction with the target residues. We predict that these compounds would efficiently inhibit *N. meningitidis* PNPase, which is a potent drug target.

The compound 3-[(2,3-dihydroxyphenyl)(4-hydroxy-2-oxo-2H-chromen-3-yl)methyl]-4-hydroxy-2H-chromen-2-one (code: MK-05) gave the highest docking score i.e., -10.5 Kcal/mol. Y380, S434, S437 and S438 residues were bound to the ligand through H-bonding (fig. 7). D486 and F382 residues were found to be involved in Pi-anion interaction and Pi-donor H-bond with the ligand. Pi-Pi-stackings were found to be form between the ligand and K397 and R399 residues. The S439, D492 and K494 residues were bound to the ligand by carbon-hydrogen bonds. The van der Waals interactions were observed with K262, T387, E400, H403, G436, G483 and G487 residues. The compound MK-05 has been found to interact with all the key residues including active site residues of *N. meningitidis* PNPase and is considered as a potential inhibitor for *N. meningitidis* PNPase.

The compound 3-[(1H-indol-3-yl)(naphthalen-2-yl)methyl]-1H-indole (code: MK-19), an indole derivative, having -9.9 Kcal/mol docking score was observed to form H-bonding with one of the active-site residues, i.e., S438 (fig. 7). The Pi-Pi stacking was observed with the residues F382 and H403. The residue D486 was found to form Pi-anion interaction while K397 and R399 residues were involved in Pi-alkyl bonds with the ligand. Additionally, Y380, T387, E400, S434, G436, S437, H487, D492 and K494 residues were involved in van der Waals interactions with the ligand.

The compound 4-phenyl-3-(pyridin-4-yl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (code: MK-23-i), a triazole

derivative, when docked against *N. meningitidis* PNPase produced considerable results with a docking score of -9.9 Kcal/mol. Conventional H-bonding was formed with the active site residue S438 (fig. 7). R399, D486 and D492 residues were found to form Pi-cation and Pi-anion bonds. S437 another active site residue established Sulfur-X interaction with the ligand. The Pi-Pi stacking was found with F382 residue. The van der Waals interactions were found to be formed with Y380, K397, E400, H403, S434, G436, S437, S439, G483, H487, K494, D508 and K510 residues.

The compound 2-(3-cyano-6-methyl-4,6-di-p-tolyl-5,6-dihydropyridin-2(1H)-ylidene) malononitrile (code:AH-43) from malononitrile family showed a docking score of -9.6 kcal/mol and was observed to form H-bonding with G436, S438 and S439 residues (fig. 7). Pi-Pi stacked interactions were observed between ligand and F382 and H487 residues PNPase. K397 and R399 residues were involved in alkyl and Pi-alkyl interactions. The van der Waals interactions were observed between K262, Y380, T387, E400, G483 and N435 residues and the ligand. H403, S434 and S437 residues formed Carbon-Hydrogen bonds. Briefly, AH-43 was found to interact with all the active site residues and is considered as a good drug candidate for *N. meningitidis* PNPase inhibition.

Ribonuclease III

Sequence Analysis and Homology Modeling

The crystal structure of RNase III from *Aquifex aeolicus* complexed with double-stranded RNA (PDB id: 2NUG) at 1.70 Å resolution was taken as template for homology modeling of *N. meningitidis* Ribonuclease III (NMB0686). The sequence similarity between the two proteins was observed to be 54% (fig. 8). Based on the fact that *A. aeolicus* functional RNase III exists in a dimeric state, *N. meningitidis* RNase III was modeled as a dimer, complexed with double-stranded RNA and Mg⁺² using MODELLER 9.15 software (Eswar *et al.*, 2006).

The PROCHECK (Laskowski *et al.*, 1993) analysis of predicted model of NMB0686 showed 91.2% residues were in the most favored regions suggesting the good model quality. The PROSA (Sippl. 1993) analysis showed that most of the residues have negative interaction energy. Overall analysis of homology model of *N. meningitidis* RNase III by PROCHECK (Laskowski *et al.*, 1993) and PROSA (Sippl. 1993) proves that the predicted model was valid.

Structural Investigation of RNase III Catalytic Valley

The predicted homology model of *N. meningitidis* ribonuclease III was observed to be a homodimer consisting of one endoND and a dsRNA binding domain (fig. 9). RNase III proteins are characterized by a conserved nine-residue signature motif in their endoND. In *A. aeolicus* this conserved motif was ₃₇ETLEFLGD₄₅ whereas in *N. meningitidis* this conserved motif was

found to be ${}_{37}\text{ERFEFVGD}_{45}$. On performing multiple sequence alignment of bacterial RNase III proteins using CLUSTALX (Larkin *et al.*, 2007) fifteen amino acid residues were found to be identical that were F15, A24, H27, L39, E40, G43, D44, G65, E110, K153, L156, Q157, Y170, F184 and A209. In *N. meningitidis* RNase III, L39 was conservatively replaced by F39.

The overall fold of *N. meningitidis* RNase III was found quite similar with that of the template. The enzyme forms a large catalytic valley on dimerization. The catalytic valley was observed to be composed of amino acid residues E37, E40, D44, D107, E110 from chain A and E64 from chain B and Mg^{+2} ions (fig. 10). The active site residues are highly acidic therefore the dibasic Mg^{+2} ions stabilize the binding of dsRNA in the catalytic valley of the enzyme. The active site residues, the domains and the motifs were found to be conserved in both the enzymes. Therefore, we believe that ribonuclease III from *N. meningitidis* and *A. aeolicus* will bind to and cleave dsRNA in an almost similar manner.

Analysis of Docking Results

Molecular docking was performed for the *N. meningitidis* Ribonuclease III in the catalytic valley of the enzyme and in the presence of Mg^{+2} ions because all the amino acid residues of active site are acidic in nature therefore in order to stabilize the interaction presence of Mg^{+2} ions is essential. Out of seventy-two compounds from different chemical scaffolds, the compounds having the highest docking score and showing the maximal interaction with active site residues were selected including MK-14, MK-30, MK-05 and MK-07.

The compound 3-[2-(1H-indol-3-yl)ethyl]-1-(naphthalen-1-yl)urea (code: MK-14), an indole derivative, showed the highest docking score of -7.9 kcal/mol. MK-14 was found to form two Pi-anion interactions with active site residue, E34, and Pi-cation interactions with Mg^{+2} metal to stabilize the interaction (fig. 11). The other active site residues E31, D38, E58 and D107 were observed to be involved in van der Waals interaction with the compound MK-14. The analysis of docking revealed that the compound MK-14 can competitively bind in the catalytic valley of RNase III and can act as its potent inhibitor.

The compound 3-{2-[(Z)-2-[1-(pyridin-4-yl)ethylidene]hydrazin-1-yl]-1,3-thiazol-4-yl}phenol (code: MK-30), a derivative of hydrazinyl thiazole substituted pyridine showed high binding affinity with -7.8 Kcal/mol. docking score. The ligand was found to form Pi-anion interactions with active site residues, E31 and E34 (fig. 11). Moreover, it was observed that compound formed hydrogen bonding with the catalytic residue D107. The key active site residue E110 and other catalytic residues D38 and E58 were observed to be involved in van der Waals interaction with the compound MK-30. Mg^{+2} ions were also involved in the stabilization

of this interaction. The present study suggested that the compound MK-30 can act as a good inhibitor of RNase III on the basis of its good interaction with the enzyme and a high docking score.

The compound 3-[(2,3-dihydroxyphenyl)(4-hydroxy-2-oxo-2H-chromen-3-yl)methyl]-4-hydroxy-2H-chromen-2-one (code: MK-05) from biscoumarin family showed a docking score of -7.7 Kcal/mol with RNase III enzyme. The ligand was found to be involved in hydrogen bonding with the active site residues E31 and D107; in Pi-anionic interactions with E34 and E58 residues; and in van der Waals interaction with D38 residue (fig. 11). Since the compound MK-05 showed a strong interaction with the enzyme along with a high docking score, it can act as a competitive inhibitor of *N. meningitidis* RNase III.

The compound 3-{2-[(Z)-2-[(4-hydroxy-3-methoxyphenyl) methylidene] hydrazin-1-yl]-1,3-thiazol-4-yl}-2H-chromen-2-one (code: MK-07) a thiazolyl coumarin showed a high docking score -7.6 Kcal/mol. The compound was found to form Pi-anion interactions with the main active site residues E34, E58 and E110 (fig. 11). The remaining active site residues E31, D38 and D107 were found to be involved in van der Waals interactions. The compound also formed Pi-cation and metal-acceptor bonds with Mg^{+2} ions to fortify the interaction between the enzyme and the ligand. Considering the high docking score and strong binding mode of the compound MK-07 it is suggested that it would be a good inhibitor of the enzyme.

DISCUSSION

DEAD box Helicases, entitled because of the strictly conserved amino acid sequence Aspartate-Glutamic acid-Alanine-Aspartate (D-E-A-D) and found nearly in all forms of life are ATPases that unwind RNA and guarantee the correct RNA folding (Linder 1989). RNA binding DEAD-box Helicases belong to superfamily SF2 (Gorbalenya and Koonin 1993) and perform variety of functions during RNA metabolism such as transcription, pre-mRNA splicing, transport and decay of RNA, initiation of translation, ribosome biogenesis and adaptation to cold shock (Redder *et al.*, 2015; Rocak and Linder 2004). DEAD box proteins are found to have eight conserved sequence motifs Motif I, Ia, Ib, II, and III in domain-1 and Motifs IV, V, and VI in domain-2. Based on genetic, biochemical, and structural data, specific functions have been assigned to these motifs such as, motifs I (Walker A) and II (Walker B) are known to be involved in the binding of adenylate (Gorbalenya and Koonin 1993).

The overall three dimensional structure of *N. meningitidis* DEAD box helicase homology model bound with AMP was found to be quite similar with that of the template i.e.,

Homo sapiens DEAD box helicase. It contains the Rec-A like fold and all the signature motifs of DEAD-box helicase were also conserved except the motif IV. The absence of nucleic acid binding insertion in *N. meningitidis* DEAD box helicase, that is found in human makes it an important drug target.

The adenylate binding pocket was also similar with human DEAD box helicase. The adenylate binding site is important because the binding of adenylate with the DEAD box helicase triggers a conformational change in the protein which leads to binding and unwinding of RNA. Adenylate-binding site occupancy by any other ligand will make the site unavailable for adenylate and ultimately will inhibit enzyme's function. Therefore, we have screened a number of chemical compounds with respect to their interactions in the adenylate binding site of RNA DEAD box helicase in order to find out potential chemical molecules which can mimic adenylate in interacting with AMP binding site.

Molecular docking was performed to identify the small molecules which could inhibit the enzyme activity. Sixty-two compounds were screened. The derivatives of acridine, biscoumarin, indole and malononitrile gave the highest docking scores as well as good interactions and hence these compounds are considered as potential inhibitors of *N.meningitidis* DEAD box RNA helicase.

Polyribonucleotide Nucleotidyl Phosphorylase (PNPase) is a phosphorolytic exoribonuclease found in a wide variety of species. It is the most essential ribonuclease that is required for controlling gene expression in prokaryotic domain and is considered as a main chopping tool in RNA metabolism (Belasco 2017). In *N. meningitidis* serogroup C, the enzyme PNPase is found to affect aggregation and adhesion to human cells and is required for full virulence (Engman *et al.*, 2016).

PNPase is a 3' to 5' exonuclease that performs the processive cleavage of single stranded RNA on the expense of inorganic phosphate and produces nucleoside diphosphates (Sarkar and Fisher 2006; Regnier *et al.*, 1987). PNPase forms a ring like trimeric central channel in which the RNA binding domains and active site resides. The trimeric channel provides a route and traps RNA and promotes its degradation and hence the activity of the enzyme is regulated (Shi *et al.*, 2008; Symmons *et al.*, 2000). In Gram-negative bacteria Ribonuclease E (RNase E) is a requisite endoribonuclease and is critically involved in mRNA decay (Carpousis *et al.*, 1994). RNase E binds with PNPase and DEAD box Helicase along with glycolytic enzyme enolase to form a multiprotein complex for mRNA degradation that is RNA degradosome assembly (Carpousis *et al.*, 1994; Py *et al.*, 1994). Considering the importance of interaction between PNPase and RNase E we have performed the modeling

of *N. meningitidis* PNPase bound with RNase E micro-domain, RNA and Mn^{+2} ions.

Since the trimeric assembly of PNPase was observed in several species, the protein was modelled as a homotrimer. The overall fold of *N.meningitidis* PNPase homology model was found to be quite similar with that of *E.coli* PNPase. The amino acid residues involved in catalytic process S437, S438 and S439, in metal ion binding and the RNA binding loop were also conserved. Basic amino acid residues at the central catalytic core of PNPase stabilize RNA binding.

Being an important drug target, molecular docking was performed to identify novel drug candidates. The chromene, indole, triazole and malononitrile derivative compounds gave the highest score and the best mode of interaction in molecular docking studies. Therefore, it is suggested that these compounds would act as good inhibitors of *N.meningitidis* PNPase.

Ribonuclease III (RNase III) a member of super family ribonuclease III is a double-stranded RNA specific endoribonuclease and plays a significant role in multiple RNA processing, posttranscriptional gene expression control and in defence against viral infections. RNase III is a Mg^{+2} dependent phosphodiesterase and cleaves two phosphodiester bonds of double-stranded RNA and generates two nucleotides, 5' phosphoryl, 3' overhangs and 3' hydroxyl ends in the products (Filippov *et al.*, 2000; Nicholson 1999; Robertson 1982). RNase III from bacterial cells perform their function in two ways either as double-stranded RNA processing enzymes and cleave dsRNA and RNA hairpins into small duplexes or as double-stranded RNA binding proteins and bind with dsRNA without their cleavage (Mohanty and Kushner 2018; Nicholson 1999; Dasgupta *et al.*, 1998). The bacterial RNase III proteins are composed of a single endonuclease domain (endoND) followed by a dsRNA-binding domain (Robertson 1982).

The homology model of *N.meningitidis* RNase III complexed with Mn^{+2} ions and double stranded RNA was found to be quite similar with its template i.e., RNase III from *A.aeolicus*. Active site residues, motifs and domains were also similar between the two enzymes. The catalytic valley is formed by amino acid residues from the both chains of the homodimer.

Considering the importance of RNase III in pathogenesis molecular docking was performed to find its inhibitors as the potential drug candidates. The compounds from indole, thiazole, chormone, biscoumarin and thiazolyl coumarin showed the highest docking scores and the best interaction among a library of sixty-two compounds and hence considered as good inhibitors.

CONCLUSION

Transcription is one of the major processes occurring in living organisms. By regulating the process of transcription, we can overcome the pathogenesis of pathogenic bacteria and other microorganisms. Homology modeling and molecular docking are the important techniques of bioinformatics which are extensively applied for drug designing. Present work demonstrated homology modeling and molecular docking studies of important enzymes of transcription involved in *N. meningitidis* serogroup B pathogenesis including DEAD-box Helicase (NMB1422), Polynucleotide Phosphorylase PNPase (NMB0758) and Ribonuclease III (NMB0686). It has provided insights into the enzymes' active sites, their interaction with RNA and mechanism of RNA decay in *N. meningitidis*. The identification of possible potent inhibitors of these enzymes via molecular docking is the first step towards drug discovery against lethal meningococcal diseases. It is the first ever structural investigation of potential drug candidates against these drug targets. The Biscoumarin, malonitrile derivatives and indole derivatives have shown the best inhibitory mode in all of the three enzymes. Since, these enzymes are assembled in Gram-negative bacteria to form RNA degradosome assembly for RNA decay therefore their inhibition will shut off the degradosome assembly resulting in the decay of RNA, which is an essential life process and ultimately leading towards the death of the pathogenic bacteria. In our opinion these small chemical moieties will provide an excellent barrier towards the pathogenesis of *N. meningitidis*.

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