Original Article

AN IN SILICO APPROACH TO DISCOVER POTENTIAL INHIBITORS AGAINST MULTI_DRUG RESISTANT BACTERIA PRODUCING NEW-DELHI METALLO-B-LACTAMASE 1 (NDM-1) ENZYME

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ABSTRACT

New Delhi Metallo-β-lactamase 1 (NDM-1) is a monomeric, plasmid-encoded enzyme that can hydrolyze β-lactam antibiotics over a wide range. Presence of this enzyme renders the host bacteria immune to carbapenems, the so-called last resort drugs, as reported in *Klebsiella pneumonia, Escherichia coli* and *Acenetobacter baumanii*. Multi-drug resistance of nosocomial pathogens as above possess a serious threat towards the successful treatment of any patient in a clinical facility; making it a scenario of prime concern in the field of healthcare. Objective of the present study was to establish a potential inhibitor of the NDM-1 enzyme among naturally available antibacterial substances through molecular docking studies. Extensive data mining, Molecular docking and Energy minimization was performed to select the best inhibitor compound amongst the prepared 550 bioactive compound library. Piperine exhibited the lowest binding energy and maximum number of non-covalent interactions with the drug target. The study concludes that Piperine and its derivatives may be effective as an inhibitor of the NDM-1 enzyme and hence, can be regarded as a potential drug candidate for treating MDR bacteria containing NDM-1 enzyme.

Key words: Carbapenemase; Molecular Docking; Multi-drug resistance; New Delhi Metallo-β-lactamase 1; Piperine.

INTRODUCTION

Micro-organisms capable of resisting antimicrobial drugs of a wide variety of structure and function that are targeted at eradicating it are said to be multiple- or multi-drug resistant micro-organisms. [1] Multi-drug resistance is usually conferred by enzymes encoded from chromosomal or extra-chromosomal gene. It may be acquired by natural selection, different modes of horizontal gene transfer or mutations etc. These forms of microbes possess the greatest threat towards public health.[2]

Bacteria usually acquire multi-drug resistance through horizontal gene transfer; usually conjugation, where plasmids carrying the genes for the respective enzymes are transferred from one bacteria to another. β-lactamases constitute a class of such enzymes and these are sub-divided into Serine β lactamases and Metallo β lactamases on the basis of their mode of actions. Metallo β lactamase tightly binds to antibiotics using a highly-conserved fold called the MBL-fold found in their tertiary structures and hydrolyze the β lactam rings, rendering the antibiotics dysfunctional.[3,4] Carbapenems are usually considered as the last resort drugs against MBL-producing bacteria.[5]

In 2009, *Klebsiella pneumonia* producing carbapenemase was reported for the first time in a Swedish patient who acquired a urinary tract infection from the pathogen while travelling from New Delhi, India. [6]The resistance-rendering enzyme was later named after its place of initial discovery, as New Delhi metallo β-lactamase 1 or NDM-1. Later, the same enzyme was also found to be present in *Escherichia coli* and *Acenetobacter baumanii*. [7,8] All three of them are common nosocomial pathogens known to cause urinary and respiratory tract infections in immune-compressed and normal individuals. While the treatment option of infected individuals with carbapenems has been ruled out; emergence of three different forms carbapenemase producing bacteria within such a short span of time is a clear indication that the plasmid-coded enzyme would soon be transferred to the susceptible forms of bacteria creating a pandemic. [9]NDM-1 possessing bacteria have been found to be susceptible towards synthetic drugs- Tigecycline and Colistin. Tigecycline inhibits protein synthesis at the ribosome while Colistin acts as a detergent at the cytoplasmic membrane.[10,11] However, problems associated with Tigecycline (low-plasma concentrations) and colistin (toxicity) makes them less-appealing treatment options[12,13,14]. The risks associated with the usage of these synthetic drugs and paucity of compounds competent enough to treat Carbapenemase-resistant bacteria directs us towards the medicinal plants that have always been used as household remedies. Leads discovered from plants continues to provide us radically us radically drugs against various pharmacological targets involving cancer, malaria, AIDS, etc.[15]

NDM-1 is a metallo-β-lactamase consisting of two bound Zn+ions-Zn1 (tightly-bound) and Zn2 (loosely-bound); both contributes to its catalytic activity, specifically cleaving the amide bond in the β-lactam ring.[16]It shares a common fold structure (MBL-fold) with the other proteins of the class-B MBL superfamily; the structure is highly conserved and can be described as a four-layered β-sandwich where two α-helices flank two mixed β-sheet forms.[17] Tight-binding of the ligand to the enzyme as well as the binding energy plays a crucial role and it affects the catalytic capability of the enzyme.[18] So, an inhibitor can be designed that can bind more effectively to NDM-1 than any commonly available competitive inhibitor at lower binding energy, thus acting as a competitive inhibitor.

In this study, 550 natural compounds with antimicrobial activity were initially selected out of which 17 were considered for the molecular docking studies on the basis of drug-like properties based on Lipinski rule of 5. L-Captopril was docked as a control[19]. Piperine and Norwedelic acid exhibited lower binding energy values and greater non-covalent interactions with NDM-1 than L-Captopril; thus portraying their potential as an inhibitor. Thus, these two bioactive compounds maybe further synthesized for wet lab procedures.

MATERIALS AND METHODS

Data Mining, Structure retrieval and Drug-likeness prediction

A list of medicinal plants available in Assam was downloaded from the homepage of "Assam Industrial Development Corporation" (AIDC) (http://aidcltd.com (out of which 20 species were selected

on the basis of availability. Available literature was searched for the presence of bioactive compounds that exhibited antibacterial properties. Based on it, 550 compounds were selected and their respective 2-Dimensional structures were derived from PubChem Substance Database (http://www.ncbi.nlm.nih.gov/pcsubstance). Similarly, the 3-Dimensional co-ordinates as well as structure were obtained from PDB (ID-4EXS) (http://www.rcsb.org/pdb/home). The selected bioactive compounds were drawn in Molinspiration (http://www.Molinspiration.com/cgi-bin/properties) to predict bioactivities and molecular properties. Out of 550, only 17 compounds seemed to exhibit appreciable drug-like properties such as logP≤5, molecular weight≤500, number of H-bond acceptors≤10, number of H-bond donors≤5 etc. The 3D structures of the selected compounds were drawn and the corresponding.pdb files were downloaded using CORINA (http://www.molecular-networks.com/online_demos/corina_demo_interactive).

All of the structures were viewed using Rasmol (http://rasmol. org/OpenRasMol.html).

Table 1: List of the 17 compounds selected for docking studies

Name of the compound	Source
Echitaminic acid	*Alstonia scholaris*
Isoboonein	*Alstonia scholaris*
Scholaracine	*Alstonia scholaris*
Kaempferol	*Alstonia scholaris*
M-coumaric acid	*Solanum indicum*
N-feruloyltyramine	*Solanum indicum*
Norwedelic acid	*Wedelia chinensis*
Alpha-pinene	*Wedelia chinensis*
Rosemarinic acid	*Ocimum sanctum*
Carvacrol	*Ocimum sanctum*
Beta ocimene	*Ocimum sanctum*
Andrographolide	*Andrographis paniculata*
Piperine	*Piper longum*
Linalyl acetate	*Mentha arvensis*
Luvangetin	*Aegle marmelos*
Psoralen	*Aegle marmelos*
Gamma fagarine	*Aegle marmelos*

Table 2: Showing Ki and Free binding energy of the respective ligands with the enzyme

Ligand	Inhibition constant (µM)	Binding Energy (kcal/mol)
L-Captopril	457.37	-4.56
Echitaminic acid	126.22	-5.32
Isoboonein	148.12	-5.22
Scholaracine	9.99	-6.82
Kaempferol	10.15	-6.81
M-coumaric acid	266.55	-4.88
N-feruloyltyramine	56.88	-5.79
Norwedelic acid	9.90	-6.83
Alpha-pinene	196.63	-5.06
Rosemarinic acid	18.72	-6.45
Carvacrol	231.94	-4.96
Beta ocimene	773.16	-4.25
Andrographolide	17.54	-6.49
Piperine	6.03	-7.12
Linalyl acetate	512.30	-4.49
Luvangetin	48.59	-5.88
Psoralen	348.17	-4.72
Gamma fagarine	168.38	-5.15

The control L-Captopril docked with NDM-1 with a binding energy of -4.56Kcal/mol and Ki value of 457.37 µM [Figure 1-A]. It can be also be deduced from the LigPLot [Figure 1-B] that the control has non-covalent interactions with the amino acid residues Asn103 and Val 113 of the enzyme.

1. Molecular Docking using AutoDock 4.2:

In silico docking techniques are commonly used to investigate the complementarily of a ligand and a protein target at the molecular level. Docking was done using AutoDock.[20] AutoDock 4 is a suite of automated docking tools that helps us to study the protein-ligand interactions and appoints a binding energy to each conformation. AutoDock actually consists of two main programs:

A. AutoGrid4- It pre-calculates these grids.

B. AutoDock4- It performs the docking of the ligand to a set of grids describing the target protein.

Each ligand enlisted in the Table-1 was docked with the enzyme using Lamarckian Genetic Algorithm. The enzyme was prepared by adding polar hydrogens and Kollman charges. 3D affinity grid field was generated using a grid map of 60X60X60 points. The other parameters were used in default settings except that number of GA runs was reset to 50 instead of 10. In order to mimic the solvation effect expressed by inhibitors in protein environment, the distance-dependent dielectric constant was set to 5. The lowest binding energy among the rmsd cluster generated by docking all-ligand root mean square deviation within 1 Å was recorded for each protein-ligand interaction and the complexes that exhibited lowest binding energy were selected, as the best docking is always the one with the lowest energy.[21]

C. Energy Minimization

The docked complexes of NDM-1 with Scholaracine, Piperine, Norwedelic acid and Kaempferol underwent energy minimization performed by using sp4 force-field Ammp molecular dynamics

software of the VEGA ZZ 3.0.1.[22] After assigning correct bond types, force fields as well as charges were applied according to SP4 and gasteiger algorithms. The enzyme-ligand complexes were solvated in a 50X50X50 dimensioned box with 2129 water molecules and then, neutralized with 50 Cl⁻ions. The energy minimization step was done using 300 steps of Conjugate gradient minimization method.

D. Visualisation of the interactions in the Docked complex:

The docked complexes were loaded onto UCSF Chimera package 1.7 (http://www.cgl. ucsf.edu/chimera) to visualise and locate the H-bonds formed between the amino acid residues of the enzyme and ligand molecule.[23] Also, LigPlots of the docked complexes were derived using LigPlot+ version V.1.4.5 to derive explicit images of the hydrophobic interactions respective to amino acid residues in the enzyme under study.[24]

RESULTS AND DISCUSSION

The parameters used to screen the best inhibitor compound among the 17-compounds library was "free energy of binding" and "inhibition constant or Ki". Also, the binding energy and Ki of the compounds were expected to be lesser than that of the L-Captopril. The lowest binding energy signifies the least energy cost to the cell for the enzymatic reaction to occur while in case of the inhibition constant, the smaller the value of Ki, the stronger the interaction between the enzyme and inhibitor; thus the greater inhibitory effect.
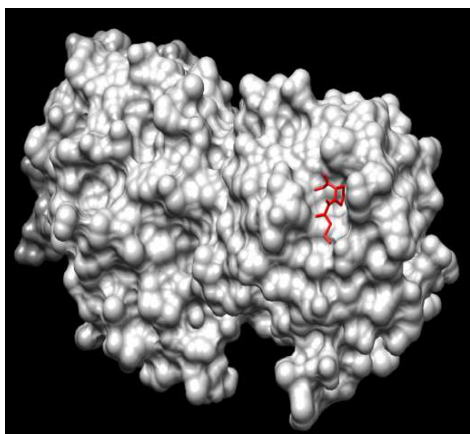

Fig. 1[A]: Docked complex of NDM-1 with L-Captopril as viewed in Chimera 1.7;
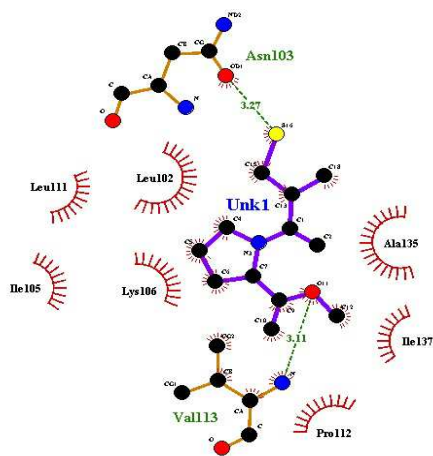

Fig. 1[B]: LigPlots of the NDM-1 complexed with L-Captopril (H-bonds in green)

It is clearly evident from Table-2 that in comparison to L-Captopril, the compounds Scholaracine, Piperine, Kaempferol and Norwedelic

acid exhibited lower binding energy and Ki value. The energy-minimized models of NDM-1 with Scholaracine, Piperine, Kaempferol and Norwedelic acid exhibited an rmsd of 12.56, 8.80, 11.77 and 9.98 Å² respectively. The energy-minimized structures exhibited significant rise in the number of interactions, namely, hydrogen bonds and hydrophobic interactions [Figure-2].
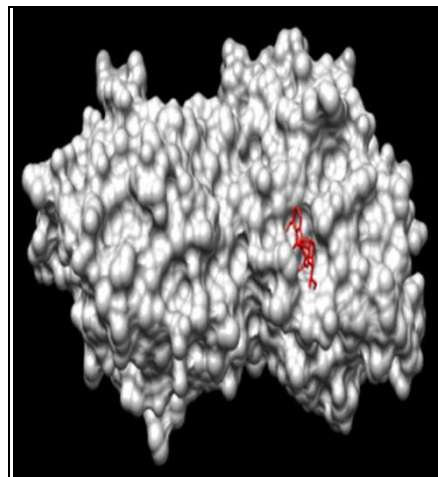

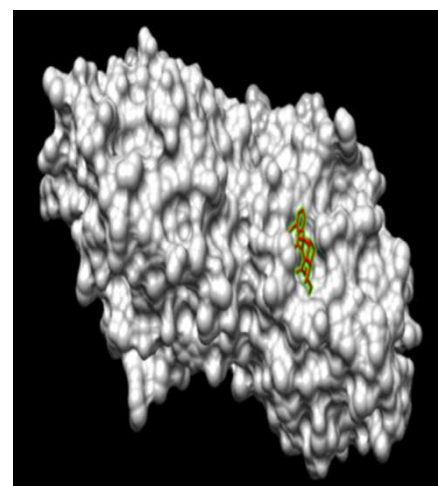**Fig. 2[A]: Docked complexes of NDM-1 with Scholaracine as viewed in Chimera 1.7;**

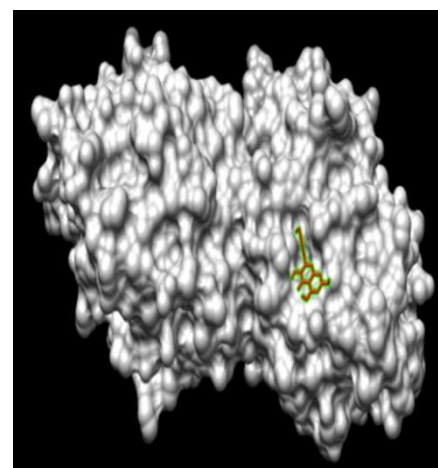**Fig. 2[B]: Docked complexes of NDM-1 with Piperine as viewed in Chimera 1.7;**

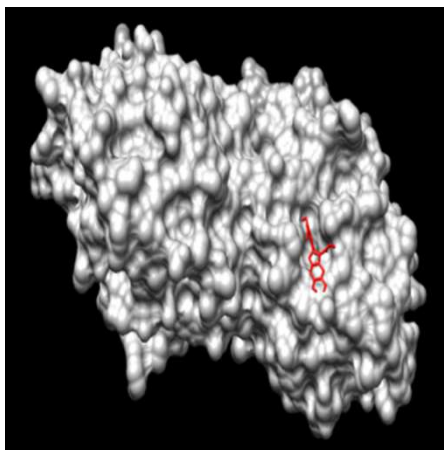**Fig. 2[C]: Docked complexes of NDM-1 with Kaempferol as viewed in Chimera 1.7**

Fig. 2[D]: Docked complexes of NDM-1 with Norwedelic acid as viewed in Chimera 1.7.

Fig. 3 [C]: LigPlot of the NDM-1 complexed with Kaempferol;

We also studied the mode of interaction of the four selected compounds with NDM-1 enzyme using LigPlot+. As we can see in Figure-3, the maximum number of interactions between enzyme and ligand was observed in NDM-1complexed with Piperine and Norwedelic acid; and also, both compounds seemed to bind in close proximity of the enzyme active site.
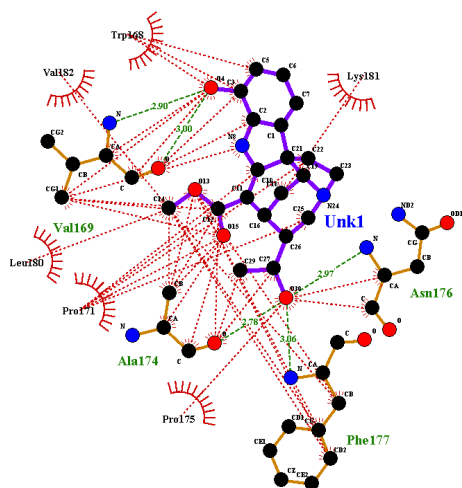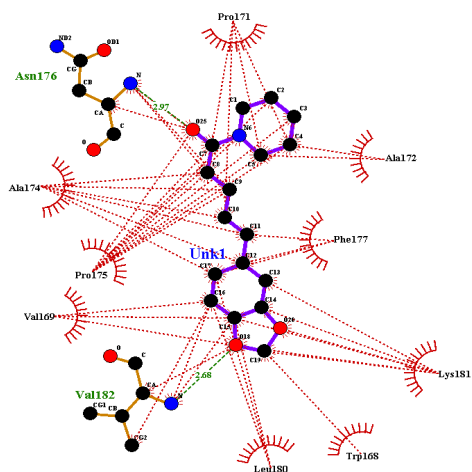

Fig. 3 [D]: LigPlot of the NDM-1 complexed with Norwedelic acid.
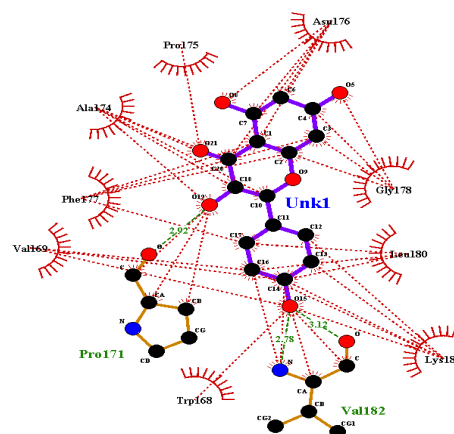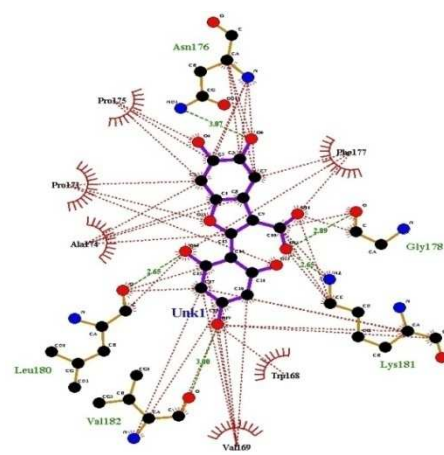

Fig. 3[A]: LigPlot of the NDM-1 complexed with Scholaracine.

Moreover these two particular complexes had more non-covalent interactions with the enzyme than the control, L-Captopril as seen in Figure1[B]. The maximum number of hydrogen bonds and hydrophobic interactions can be observed between Piperine and the amino acid residues Asn176, Ala174, Pro175, Val169, Val182, Leu180, Trp168, Lys181, Phe177, Ala172 and Pro171 in Figure 3[B]. Similarly, the second best interactions could be seen between the amino acid residues Asn176, Pro175, Pro173, Ala174, Leu180, Val182, Val169, Trp168, Lys181, Gly178 and Phe177 of NDM-1 and Norwedelic acid in Figure 3[D] stabilizing the complex. From the data presented, it can be implicated that the complexes of Piperine and Norwedelic acid with NDM-1 were very much stable in comparison to the known competitive inhibitor, L-Captopril and we can be hopeful that these two compounds will exhibit the same results in wet lab procedures.

CONCLUSION

Studying the protein-ligand interactions is very essential for identifying novel inhibitors of NDM-1 in structure-based drug designing. In such a virtual screening approach to analyse a putative protein-ligand complex, docking is favourably used to obtain a reliable energy evaluation that can implicate the quality of a complex as well as provide further insights into drug development process.[25]Our aim was to identify a competitive inhibitor compound against New Delhi Metallo-β Lactamase from natural sources, using the two parameters- binding affinity and inhibition constant for screening the compounds from a 550-compound library. Since the NDM-1 enzyme has a tendency to bind tightly to β-

Fig. 3 [B]: LigPlot of the NDM-1 complexed with Piperine;

lactam rings, the compounds selected for the screening purposes were devoid of it so that any of them could easily resist the hydrolase activity of the enzyme. From the study, we were able to learn that with Piperine and Norwedelic acid exhibited the maximum likeliness to possibly inhibit the NDM-1 enzyme that confers multi-drug resistance to some species of bacteria. However, further study under in vitro conditions is necessary to validate the potential of this compound as a drug against MDR bacteria producing NDM-1.

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