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VIRTUAL SCREENING AND MOLECULAR DOCKING OF NDM1 INHIBITOR FOR TREATMENT OF *KLEBSIELLA PNEUMONIA* INFECTION

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ABSTRACT

The outbreak of antimicrobial resistance has become one of the most serious public health problems across in the world. The search for promising antimicrobial inhibitors is still under limelight as bacteria are continuously developing resistance to antimicrobial compounds. The Metallo-β-lactamases (MβLs) is one the New Delhi metallo-beta-lactamase 1 (NDM-1) are principle contributors of the emergence of resistance to their ability to hydrolyze almost all known β-lactam antibiotics including penicillins, cephalosporins, and carbapenems. In this study, we used the computer assisted methodology such as virtual screening and molecular docking studies to apply and identify six potent inhibitors against NDM1.

KEYWORDS: ***NDM1, Antibiotics resistance, Docking, Virtual screening, ADMET.***

INTRODUCTION

Klebsiella pneumoniae is a gram-negative leading opportunistic pathogen, of hospital acquired infections in worldwide. *Klebsiella pneumonia* causes several infections such as usually pneumonia, urinary tract infections, and bacteraemia and wound infections are commonly severe, notoriously affecting incapacitated patients with a suppressed immune status and neonates in intensive care units(Podschun& Ullmann, 1998). The management of infections due to *K. pneumoniae* has been difficult by the emergence of antimicrobial resistance, especially 1980 onwards and are generally acknowledged to be a major source of antimicrobial resistance genes that can spread into other Gram-negative pathogens. The cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole are often used to treat infections due to *K. pneumoniae*, and resistance to these agents generates delays in appropriate empirical therapy with subsequent increased morbidity and mortality in patients (Tumbarello M *et al*, 2007). Hence, clinical therapeutic choices for treating nosocomial infections due to *K. Pneumonia* have become challenging (Tumbarello M et al, 2007; Lautenbach E et al, 2001; Girometti N et al, 2014) β-lactam antibiotics cause an increasing threaten of multi drug-resistant bacteria to public health. One of the main causes of antibiotic resistant is via the expression of β-lactamase. Two different types of β-lactamases have been discovered in clinical bacteria: the serine-β-lactamases and the metallo-β-lactamases (MβLs). MβLs require one or two zinc ions for their hydrolysis activity (Ambler, 1980). According to the known sequences, MβLs have been classified into three subclasses B1, B2, and B3 (Galleni M et al., 2001 and Garau G et al, 2004). New Delhi Metallo-β-lactmase-1 (NDM-1) was originally reported in *Klebsiella pneumoniae* from India, which belongs to the subclass B1 MβLs super family (Yong et al., 2009). To date, the emergence of a large number bacteria containing blaNDM-1 gene has been reported in many other countries (Rolain JM et al 2010; Cornaglia *et al*., 2011). The most troubling aspect is that these bacteria are highly resistant to almost all β-lactam antibiotics (Williamson DA et al., 2012; Jamal W et al., 2012; Kumarasamy KK et al., 2010; Moellering RC, 2010). NDM–1 is a single-chain protein, which N-terminal has a putative signal peptide domain of 18 amino acids, and the core region of the enzyme composed of 270 amino acids. The crystal

structures of NDM-1 (Zhang H & Hao Q, 2011; Kim Y et al., 2011; Guo Y et al., 2011) reveal some characteristics of this enzyme. It contains two zinc ions in the active site, near the bottom of substrate binding pocket (Zhang H & Hao Q, 2011). The expanded volume of the active site and the flexible loops covering the binding pocket may explain the observed extended spectrum β–lactamase (ESBL) activity and catalytic efficiency (Zhang H& Hao Q, 2011; Kim Y et al., 2011; Guo Y et al., 2011). Therefore, NDM1 is a most important drug target for K. Pneumonia antibiotics resistant. Thus the study attempts to identify potential NDM1 inhibitors from binding database based virtual screening and molecular approach. These findings may give valuable insights for rational drug designing and identify novel drugs to fight against the antibiotic resistance of *Klebsiella pneumoniae*NDM-1.

MATERIALS AND METHODS

PROTEIN PREPARATION

Preparation of the target protein structure of the three dimensional structure of *Klebsiella pneumoniae*NDM1 was retrieved from the Protein Data Bank (PDB code: 4Hl2). All water molecules were removed, the hydrogen atoms were added to the protein and all atom force field (OPLS-2005) charges and atom types were assigned. Preparation and refinement were done running Protein Prep job on the structure in a standard procedure. Energy Minimizations were performed until the average root mean square deviation of non-hydrogen atoms reached 0.3 Å (Salam NK et al., 2009)

Ligand preparation

The 3D coordinates for the ligands were generated using Ligprep Module of Schrodinger Software in Maestro 9.0.111 (Schrodinger, NY) using a force field OPLS 2005. Five low energy conformers were generated per ligand which resulted from Chembridge database and Schrodinger utilities were used to remove salts, neutralize and ionise compounds at the physiological pH 7.0 ± 2.0 using Epik state and the large penalties of high energy ionization or tautomer states were removed. The protein was kept as scaling van der Waals radius by 1.0Å and partial atomic charge is less than 0.25 Å at default constraint parameters. The ligand poses that pass the initial screens were subjected to energy minimisation on precompiled Van der Waals and electrostatic grids and pass through filters for the initial geometric and complementary fit between ligands and the receptor. (Kawatkar S et al., 2009, Friesner RA et al., 2006)

Receptor grid generation

The scoring grid was generated using a box size of 30 ˚A ×30 ˚A ×30 ˚A and centered on the centroid within a box of dimension 27 ˚A ×16 ˚A ×46 ˚A that encloses the entire groove near the active site to fit the ligands (Kawatkar S et al., 2009).

Virtual screening

Virtual screening has become a promising tool for identifying active lead/active compounds and has combined with the pipeline of drug discovery in most pharmaceutical companies. Glide module has been used for all the docking protocol (Louise-May S et al., 2007). Among 50,000 small molecules contain chembridge database that compounds have been used for screening and get less toxic compounds from the hits. The ligands were processed with the LigPrep program to assign the suitable protonation states at physiological pH= 7.2±0.2. Conformer generation was carried out with the Conf Gen torsional sampling and Lig and docking used OPLS_2005 force field. The van der Walls radii were scaled using a default scaling factor of 0.80 and default partial cutoff charge of 0.15 to decrease the penalties. There are three modes to screen the compound such as by HTVS, SP and XP in Glide module.

Induce fit docking

The protein structure of NDM1 is applied with the induced-fit docking (IFD) method in the Schrodinger software suite (Friesner RA et al., 2004). The five ligands were prepared using LigPrep and were optimized with the OPLS force field in the Macro Model module in Schrodinger (Stahl M et al., 2006). Ligands were docked to the rigid protein using the soften-potential docking in the Glide program with the vander Waals radii scaling of 0.8 for the proteins. Residues having at least one atom within 5Å of any of the 20 ligand poses were subject to a conformational search and minimization while residues outside the zone were held fixed. In this way, the flexibility of proteins was taken into account (Schrodinger, 2007)

Pharmacokinetic predictions of best fit molecules

The ligands identified in docked mode were subjected to predict the pharmacokinetic properties using Qikprop module of Schrodinger software suite (QikProp, 2011). Structures with unfavorable absorption, distribution, metabolism and elimination have been identified as the major cause

of failure of candidate molecules in drug development. So there is an early prediction of ADME properties, with the objective of increasing the success rate of compounds reaching further stages of the development. Glide score, glide energy, visual inspection and ADME predictions were used as filtering in screening 6 hits for *Klebsiella pneumoniae*NDM1.

RESULTS AND DISCUSSION

Virtual screening, molecular docking, and IFD studies

Binding database and MayBridge databases were first screened against the active site of NDM1 by using virtual screening work flow in the Maestro (Schrodinger, LLC, New York, 2009). The ligands were prepared at pH7.0 ± 2.0 using Epik state and the large penalties of high energy ionization or tautomer states were removed. TheNDM1 protein was kept as scaling van der Waals radius by 1.0 Å and the partial atomic charge is less than 0.25 Å at default constraint parameters. The Glide HTVS was performed with flexible docking algorithm using selected constraints for each grid in OPLS 2005 force field. About 2500 and1500 compounds were screened from May Bridge and Binding database, respectively, using Glide HTVS. Further docking analyses were carried out using Glide SP mode in Schrodinger (Friesner RAet al., 2004) and also screened Glide XP mode in Schrodinger using Top 10 compounds from both databases were selected and the docking studies were carried out. The final Glide XP results are shown in Table 1. The best six docked protein ligand complexes with hydrogen interactions are shown in Fig. 1-6. According to Singh et al 2012, the strength of van der Waals contacts with the receptor is an important feature of each active site; the ratio between H-donor and acceptor character of the grid maps is quite balanced, indicating that both properties are desirable in a well-structured ligand for a tight receptor binding. The compounds screened from the Glide docking studies and further analyzed by a mixed molecular docking/dynamics approach (IFD) (Sherman W et al., 2005 & Nabuurs SB et al., 2007). Flexible receptor docking was carried out for the purpose of comparative study substantiated by Singh et al 2012. In most cases the Glide scores were very close to those generated from IFD (Table 2). Besides, both methods have a similar trend with a few exceptions (Sherman W et al., 2005). A total of 20 pose were generated for each best compound obtained from Glide XP results. The comparison of scores from both Glide XP and IFD shows that the compound I.D 11143 posses less Glide XP score (-11) when compared to IFD Glide score (-12) but the energy remains more or less same for both methods (IFD energy of -69 kcal/mol and Glide energy of -58 kcal/mol). Similarly, the compound I.D 10787 has shown good score in Glide XP (-11) when compared to IFD Glide score (-12) and the energy was more for Glide (-68 kcal/mol) than IFD (-70 kcal/mol).For all the 6 compounds, scores and energies from both the studies were shown in Table 1and 2.

ADME Toxicity Prediction

The best 6 compounds (Table 3), which had the highest docking score, glide energy and no of hydrogen interaction were subjected to predict pharmacokinetic properties using the QikProp module from the Schrodinger, 2009 software. Predicted significant ADME properties suchas permeability through MDCK cells (QPlog MDCK),QikProp predicted log IC50 value for blockage of K^+channels (QPlogHERG), QikProp predicted gut-bloodrrier (QPPCaco), and violations of the Lipinski's rule of five are listed in Table 3.All the Compounds that satisfy ADME properties are considered drug like. Hence our Insilco analysis can conclude that these ligands can be act as *Klebsiella pneumonia* NDM1 inhibitor.

Table 1
Xp docking results of best hits compounds

S. No	Compound Name	Docking score	Glide energy	H-Bond Interaction residues
1	11143	-11	-68	LYS211 and ASN220
2	10787	-11	-68	GLN123,LYS211 and ASN220
3	10938	-11	-72	LYS211 and ASN220
4	180	-11	-70	LYS211 and ASN220
5	11496	-11	-65	GLN123,LYS211 and ASN220
6	11019	-11	-73	GLN123,LYS211 and ASN220

Table 2
IFD docking results of best hits compounds

S. No	Compound Name	Docking score	Glide energy	H-Bond Interaction
1	11143	-12	-69	GLN123,LYS211 and ASN220
2	10787	-12	-70	GLN123,LYS211 and ASN220
3	10938	-12	-73	GLN123,LYS211 and ASN220
4	180	-12	-71	LYS211 and ASN220
5	11496	-12	-67	GLN123,LYS211 and ASN220
6	11019	-12	-74	GLN123,LYS211 and ASN220

Table 3
ADMET properties prediction for IFD docking hits compounds

S. No	Compound Name[a]	MolMW[b]	HB-DR[c]	HB-AC[d]	QPPMDCK[e]	QPlogHERG[f]	QPPCaco[g]	Rule of Five[h]
1	11143	307.28	1	8	25.578	-3.327	26.978	0
2	10787	317.341	2	6	123.441	-2.738	221.615	0
3	10938	399.873	2	5	303.252	-4.521	220.626	1
4	180	294.34	2	4	13.491	-1.492	11.482	0
5	11496	369.297	2	5	212.173	-4.245	87.925	0
6	11019	399.873	2	5	304.143	-4.499	221.224	1

a-Compound I.D's from Maybridge database
b-Molecular weight (acceptable range>600 is good)
c-Hydrogen Bond Donor acceptable range from 1-5
d-Hydrogen bond Acceptor acceptable range from 1-10
e-Predicted apparent MDCK cell permeability in nm/s (acceptable range:\25 is poor,[500 is high)
f-Predicted IC50 value for blockage of HERG K? Channels (concern below -7)
g-Predicted Caco-2 cell permeability in nm/s (acceptable range:\25 is poor and[500 is high)
h-Number of violations of Lipinski's rule of five (maximum is 4)

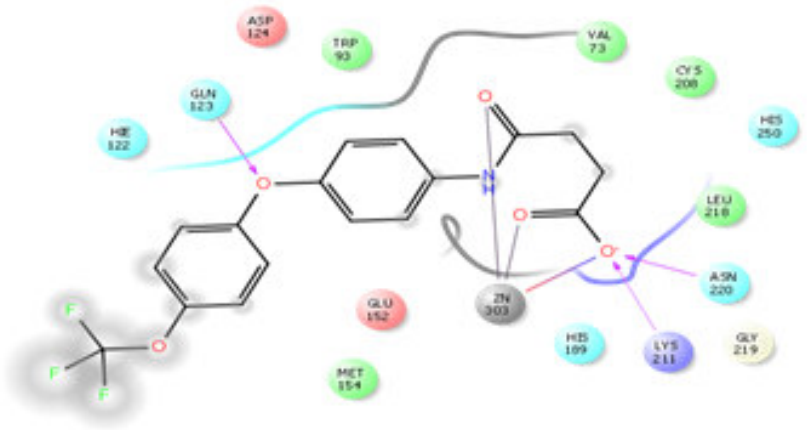

Figure 1
Compound 11143 interaction with NDM1

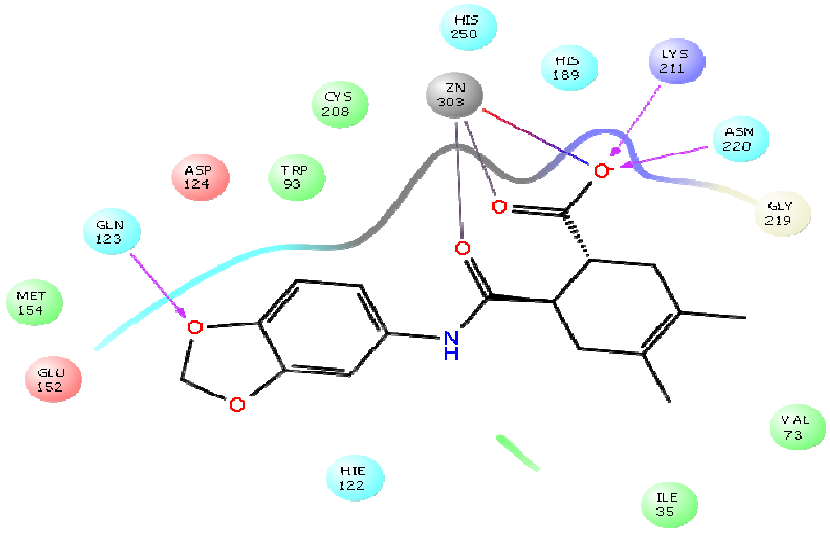

Figure 2
Compound 10787 interaction with NDM1

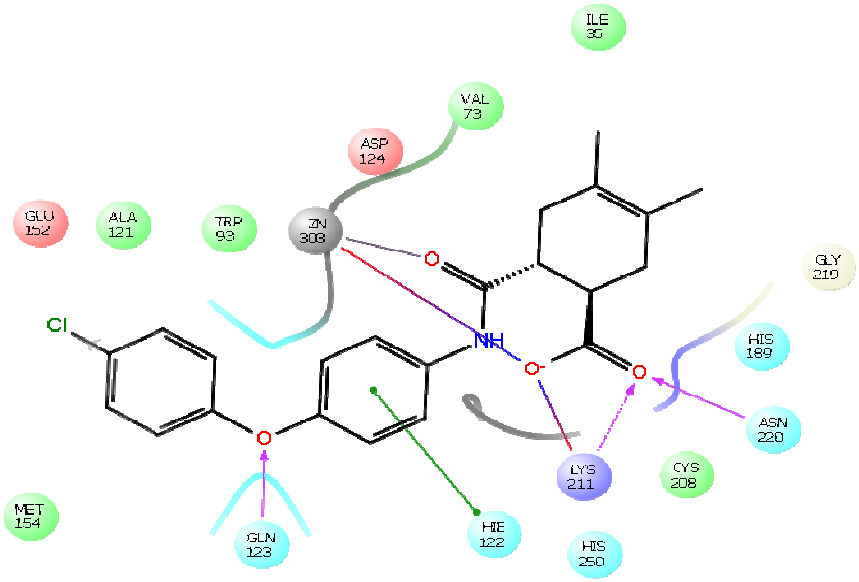

Figure 3
Compound 10938 interaction with NDM1

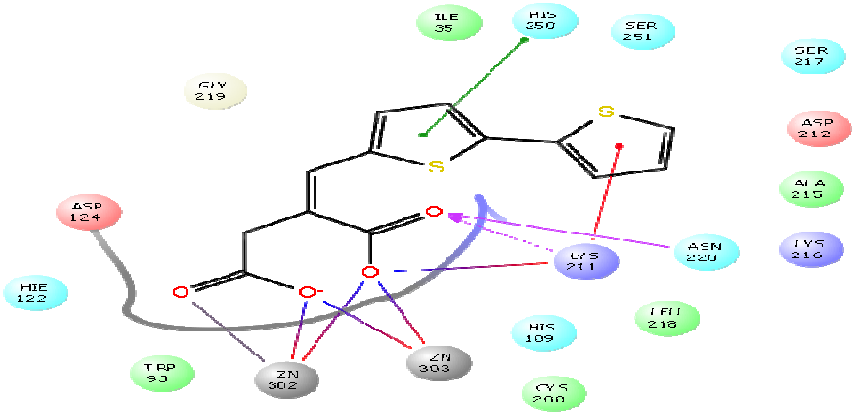

Figure 4
Compound 180 interaction with NDM1

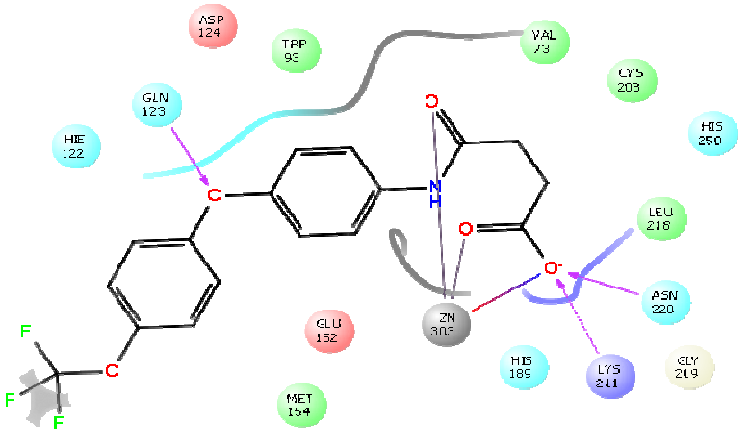

Figure 5
Compound 11496 interaction with NDM1

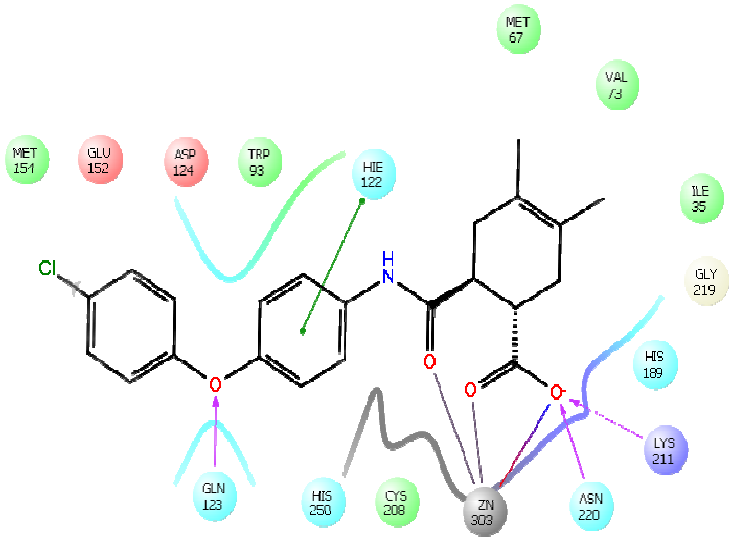

Figure 6
Compound 11019 interaction with NDM1

CONCLUSION

The study lists out the potential compounds against *Klebsiella pneumoniae* NDM1. Also the molecular mechanism of screened compounds gives a lead for drug mechanism against the *Klebsiella pneumoniae* NDM1. Thus the present finding gives a lead for multidrug resistance *Klebsiella pneumoniae* NDM1 against various out breaking disease.

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