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STRUCTURE BASED DRUG DESIGN FOR NDM1 INHIBITORS IDENTIFICATION OF ANTIBACTERIAL RESISTANCE

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ABSTRACT

The β-lactam antibiotics have long been a cornerstone for the treatment of bacterial infection. Recently, a readily transferable antibiotic resistance factor called the New Delhi metallo-Beta-lactamase-1 (NDM-1) has been found to confer enteric bacteria resistance to nearly all β-lactams antibiotics, including the heralded carbapenems, posing a serious threat to human health. In study to identify suitable NDM-1 inhibitors from Chembridge database using Virtual screening and molecular docking approach. Finally, this study provides a platform for the development of a novel potential inhibitor of NDM-1, which may be considered as a potential drug candidate against bacterial resistance.

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INTRODUCTION

The Last decade of microbial pathogens able to resist antimicrobials treatments is one of the most pressing public health crises (Maynard Smith *et al*., 2000; Palumbi, 2001; Bush *et al*., 2011; Davies *et al*., 2013). Indeed, the European Centre for Disease Prevention and Control (ECDC) estimates that each year, 25 000 people in Europe die directly from drug-resistant bacterial infections (ECDC 2011), while recent estimates provided by the British government suggest that more than half a million people die worldwide from resistant infections. Antibiotic resistance also imposes a significant financial burden on world economies, with the USA alone spending an estimated $35 billion per annum on resistant infections (Davies *et al*., 2013). One of the main causes of antibiotic resistant is via the expression of beta-lactamases. Two different types of beta-lactamases have been discovered in clinical bacteria: the serine- beta-lactamases and the metallo- beta-lactamases (MbLs). MbLs require one or two zinc ions for their hydrolysis activity (Ambler, 1980). According to the known sequences, MbLs have been classified into three subclasses B1, B2 and B3 (Jamal *et al*., 2012; Kumarasamy *et al*., 2010; Moellering, 2010).

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In 2009, New Delhi metallo-beta-lactmase-1 (NDM-1) was originally reported in Klebsiella pneumonia from India, which belongs to the subclass B1 M b Ls superfamily (Moellering, 2010). To date, the emergence of a large number bacteria containing blaNDM-1 gene has been reported in many other countries (Galleni, 2001; Garau, 2004). The most troubling aspect is that these bacteria are highly resistant to almost all beta-lactam antibiotics (Yong *et al.*, 2009; Rolain *et al.*, 2010; Cornaglia *et al.*, 2011; Williamson, 2012). 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 (Jamal *et al.*, 2012) reveal some characteristics of this enzyme. It contains two zinc ions in the active site, near the bottom of substrate binding pocket (Kim *et al.*, 2011) the expanded volume of the active site and the flexible loops covering the binding pocket may explain the observed extended spectrum b–lactamase (ESBL) activity and catalytic efficiency (Guo *et al*., 2011). The lack of efficient drugs against bla NDM-1 -carrying strain requires continuous research effort to solve this trouble. An efficient way is to discover NDM-1 inhibitors, which can protect b -lactam antibiotics from the hydrolysis effect of NDM-1, thus recovering there antibacterial potency. Hitherto the availability of clinically potent inhibitors of NDM-1 is still under limelight.

Therefore, it would be a promising choice to block the NDM-1 with suitable inhibitor. To reveal the resistance Mechanism of bacteria to β-lactam antibiotics, we identified 5 hits compounds from chembridge database based on structure based drug design method database and successively docked with the active site of NDM-1. The selected 5 hits compounds show a good binding affinity at the active site of NDM-1, particularly compounds id 6604 and 6575, produce lower binding energy than the β-lactam antibiotics with NDM-1. These findings may provide useful insights for designing new potent drugs to fight against the antibiotic resistance of NDM-1.

MATERIALS AND METHODS

Protein Preparation

Preparation of target protein structure of the three dimensional structure of 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 ProteinPrep 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 *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 ionize 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 *et al.*, 2009, Friesner *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 *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 *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 ConfGen torsional sampling and Ligand 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 there 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 *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 *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 5 hits for NDM1.

RESULT AND DISCUSSION

Virtual Screening

High-throughput screening is a computational technique to find potent small molecules against protein targets of NDM1. Various parameters such as Glide score, Glide energy and hydrogen bond interactions are used to assess which conformation or binding site orientation is best complement in the protein-binding site. Two main aspects were taken into account to assess the quality of docking methods: (i) Docking accuracy, which identifies the true binding mode of the ligand to the target protein, and (ii) Screening enrichment, which is a measurement of correlation between docking method and true binding ligands rather than random compound selection. As explained in materials and methods we calculated our calculations in HTVS first, SP second and then XP mode. We filtered out 2,500 compounds from the HTVS process against the target NDM1 protein. In the second stage compounds with Glide score > -9.00 were screened for Glide SP docking. In the next stage, only compounds with Glide SP score > -9.00 were screened for Glide XP mode docking. This third run identified 5 hits compounds based on glide score, Glide energy and hydrogen bond interaction.

Table 1. IFD Docking results of NDM1 compounds

S. No	Compound Name	Docking score	Glide energy	H-Bond Interaction	Distance
1	5190595	-11.875	-60	LYS211 N-H…O	2.423
				ASN220 N-H…O	2.231
2	5186496	-11.23	-59	LYS211 N-H…O	2.51
				ASN220 N-H…O	2.71
3	5155037	-10.81	-58	LYS211 N-H…O	2.234
4	5102519	-9.93	-57	LYS211 N-H…O	2.67
				ASN220 N-H…O	2.90
5	4012642	-9.16	-55	LYS211 N-H…O	2.20
				ASN220 N-H…O	2.58

Table 2. Admet properties predicted using Qikprop simulation

S.NO	Compounds	MOL.WT	HB-DR	HB-ACP	QplogHERG	QPlogBB	Rule of 5
1	5190595	236.267	2	4	-0.137	-1.133	0
2	5186496	273.245	4	6	-1.27	-1.828	0
3	5155037	244.219	1	7	-0.394	-1.676	0
4	5102519	291.101	2	5	0.214	-1.102	0
5	4012642	156.141	3	6	-0.908	-1.213	0

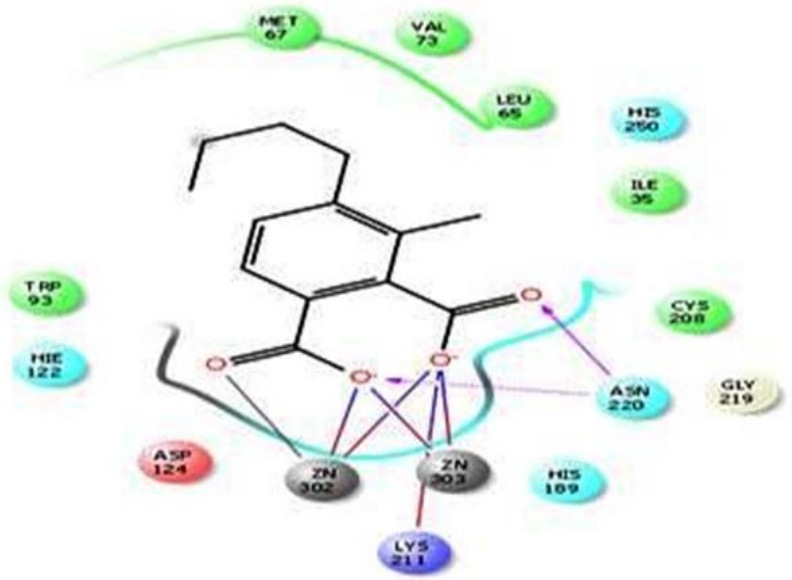

Fig. 1. Compound 5190595 interaction with NDM1

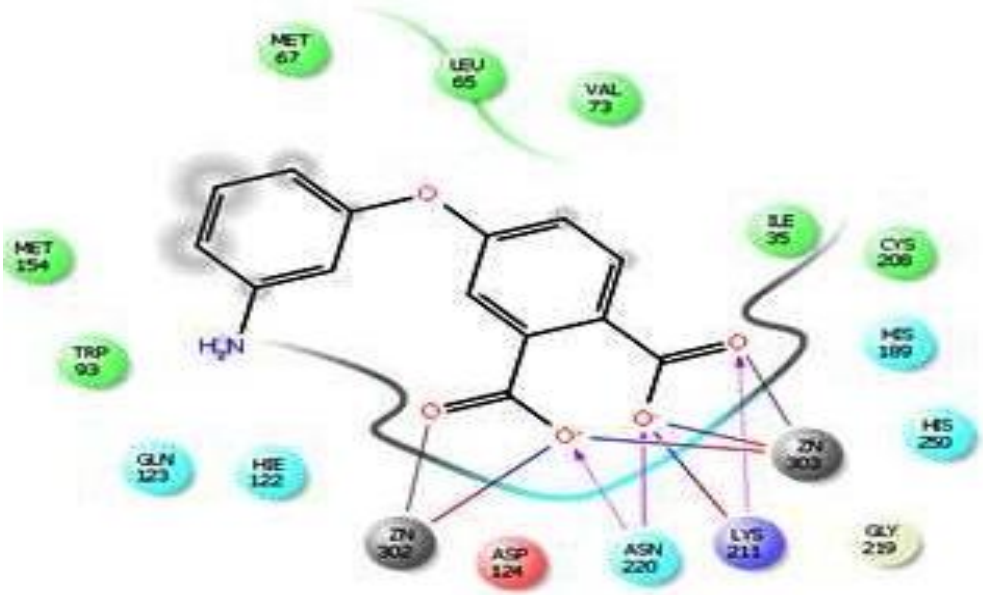

Fig. 2. Compound 5186496 interactions with NDM1

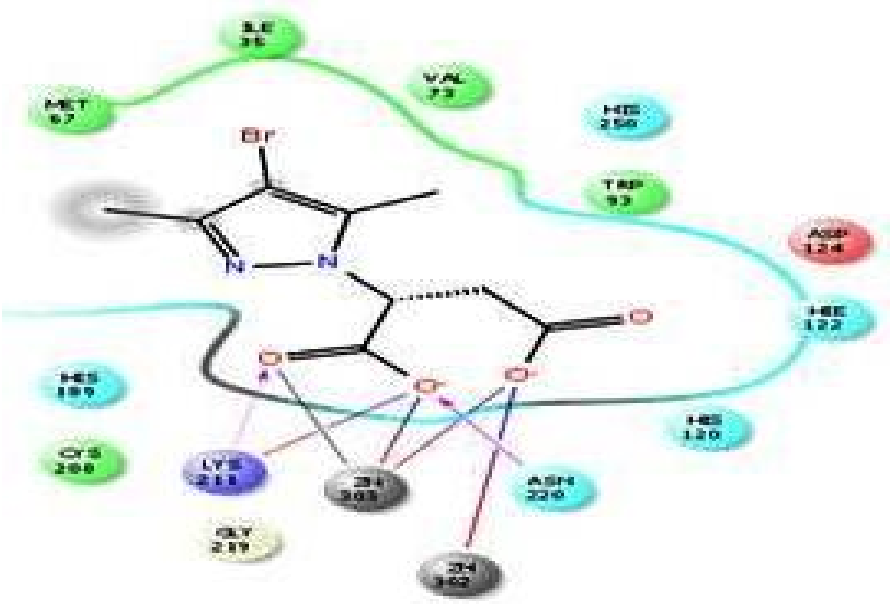

Fig. 3. Compound 5155037 interactions with NDM1

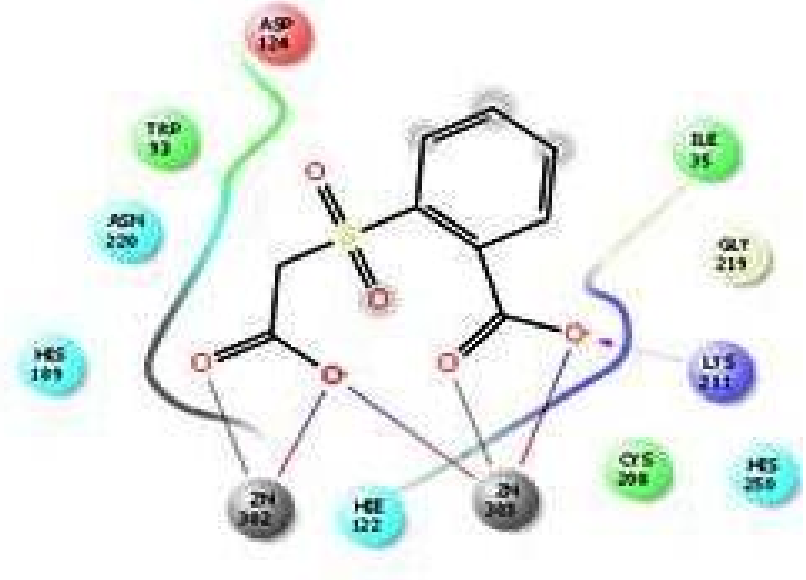

Fig. 4. Compound 5102519 interactions with NDM1

Induce fit docking analysis

With the aim of investigating dynamic behaviour of the active site during the binding of ligand, we performed IFD experiments on best ranked from virtual screening compound using NDM1protein based on glide scores Glide energy and hydrogen bond interaction. Glide scoreis an empirical scoring function that considers the energy contribution, the effects of the hydrophobicity as well as the hydrogen bonding and penalizes the steric clashes. The best 5 compounds out of screened and their corresponding chemical names are: compound 1 (5190595): 4-butyl-3-methylphthalic acid, compound 2 (5186496): 4-(3-aminophenoxy)phthalic acid, compound 3 (5155037) : 2-[(carboxymethyl)sulfonyl]benzoic acid, compound 4 (5102519):2-(4-bromo-3,5-dimethyl-1H-pyrazol-1-yl)succinic acid, compound 5(4012642): 5-methyl-3-oxo-2,3-dihydro-1H-pyrazol-4-yl)acetic acid.

The binding modes of these 5 lead molecules and their interacting residues are shown in Fig. 1-5 and docking results showed in Table 1. The residues HIE 122, LYS211, ASN 220 and two zinc metal ions are involved in ligand interactions and also important for screened compounds binding. From this result it reveals that 5 compounds can bind to the binding pocket of NDM1 and will be able to inhibit this protein.

Binding Mode Analyses of best Compounds

The binding mode of this compound (5190595) at the active site of NDM1 formed hydrogen bond with the key amino acids such as LYS211 and ASN220. The hydrogen atom from two different amino (NH) group interacted with oxygen atom (C=O) from LYS211. The length of hydrogen bond formed is of 2.423 Å and 2.231Å for LYS211 and ASN220 respectively (Fig. 1). The binding modes of this compound at the active site

of NDM1 formed hydrogen bond with the key amino acid were LYS211 and ASN220. The hydrogen atom from two different amino (NH) group interacted with oxygen atom (C=O) from LYS211. The length of hydrogen bond formed is LYS211 of 2.51 Å and 2.71 for ASN220 (Fig. 2). The binding mode of this compound (5155037) at the active site of NDm1 formed hydrogen bond with the key amino acids such as LYS211. The length of hydrogen bond formed is of 2.234 Å LYS211 (Fig. 3). The binding modes of this compound (5102519) at the active site of NDM1 formed hydrogen bond with the key amino acid were LYS211 and ASN220. The hydrogen atom from two different amino (NH) group interacted with oxygen atom (C=O) from LYS211. The length of hydrogen bond formed is LYS211 of 2.67 Å and 2.90 for ASN220 (Fig. 4). The binding modes of this compound (4012642) at the active site of NDM1 formed hydrogen bond with the key amino acid were LYS211 and ASN220. The hydrogen atom from two different amino (NH) group interacted with oxygen atom (C=O) from LYS211. The length of hydrogen bond formed is LYS211 of 2.20 Å and 2.58 for ASN220 (Fig. 5).

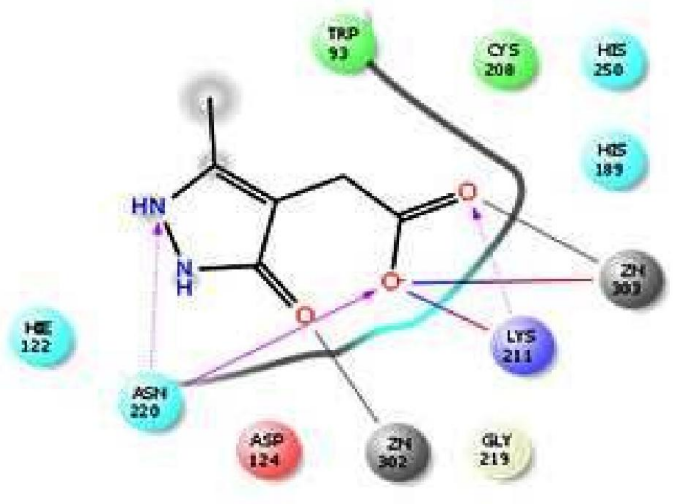

Fig. 5. Compound 4012642 interactions with NDM1

Predicted adme toxicity properties

Qikprop Program predicted pharmacokinetic of the ligands. Predicted ADME properties values (Table 2) were analyzed with the recommended values such as Stars, QPlogHERG which are essential for drug design and The Lipinski's rule of 5, it is a rule of thumb to determine if a chemical compound with a certain biological activity . All the Compounds that satisfy ADME properties are considered drug like. Hence our insilico analysis can conclude that these ligands can be act as NDM1 inhibitor.

Conclusion

Recent studies showed that NDM-1 plays an essential role in the bacterial resistance to antibiotics. Here we identified 5 NDM1 inhibitors using molecular docking and it may be considered as potent and suitable inhibitors for the antibiotic resistance against NDM-1. The Five selected compounds were in the acceptable range of Lipinski's rule of five. All these observations revealed that five selected compounds and their derivative compounds may block NDM-1 activities and provide a significant basis for drug development for therapeutic intervention in bacterial resistance to antibiotics. However, experimental validations are needed to consider these molecules as a suitable drug against the superbug.

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