Molecular Modeling and Docking Analysis of Novel Drug like Compounds for NDM-1

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ABSTRACT

NDM-1 (New Delhi metallo-β-lactamase 1) is an enzyme that is carried in certain gram negative bacteria like E. coli and Klebsiella, and makes the bacterium resistant to the beta lactam antibiotics, including carbapenems, with the exception of the monobactam agent, aztreonam. Beta-lactamase is also known as carbapenemases, due to its resistance towards carbapenem antibiotics. Resistance to β –lactam antibiotics mediated by metallo-β-lactamases is an increasingly worrying clinical problem. NDM-1 has been found in several clinically important carbapenem-resistant pathogens, there is a need for inhibitors of this enzyme that could protect broad spectrum antibiotics from hydrolysis and thus extend their utility. In the presented research, the 3D structure of NDM-1 protein was modeled using homology modeling by Modeller9v8. Evaluation of the constructed model is done by PROCHECK, PROSA, ERRAT and Verify3d servers. DEPTH server is used to predict active sites of Protein. Pubchem and ChEMBL, Drug Bank, and SuperNatural databases is used to find novel compounds. These compounds were dokced with the modeled structure, and those showing least Binding Energy were selected. These final compounds were then tested among Online Validation Servers (Molinspiration, and OSIRIS) for Drug Likeness. Also they were validated using ADMET descriptors protocol of Accelrys Discovery Studio.

Keywords

NDM-1 metallo-β-lactamase, homology modeling, docking, DEPTH, ADMET

1. INTRODUCTION

The most common cause of urinary infections is bacteria from the Enterobacteriaceae family. They can also cause sepsis, pneumonia, or wound infections. Symptoms and signs reflect the site of the infection. Most common symptoms are fever and fatigue. If bacteria enter the bloodstream, patients may go into shock. There is no difference in symptoms whether infection is caused by bacteria that express NDM-1 and those that do not. However, patients infected with bacteria producing NDM-1 will not respond to most conventional antibiotics and are at high risk for complications.

During the past decade the increase of antibiotic resistance in Enterobacteriaceae has become a major concern worldwide. These bacteria developes resistance against beta-lactams antibiotics as well as antibiotics of carbapenems family which often representing last-resource drugs for infection caused by these bacteria.Carbapenem resistance appeared in early 2000s, troublesome for public health because these bacteria are a common source of nosocomoial infections. According to their sequence similarities, β-lactamases can be generally divided into four classes, named as A, B, C, and D. Classes A, C, and D of β-lactamases contain serine groups in their active sites, while the enzymes in class B are metalloproteins, or called "metallo-β-lactamases", that require one or two zinc ions for their activity. Among all the β-lactamases, metallo- β-lactamases are the major culprit causing bacteria to resist antibiotics, due to the reason that they can degrade all β-lactams except monobactams and that they are special for their constant and efficient carbapenemase activity [1,2]. Bacteria that produce carbapenemases are often referred to in the news media as "superbugs" because infections caused by them are difficult to treat. Such bacteria are usually only susceptible to polymyxins and tigecycline. The most prevalent carbapenemase so far in Enterobacteriaceae is the KPC-type class-A carbapenemase, which has been found in Klebsiella pneumonia, especially in the United States, Asia, the United Kingdom, Israel and southern Europe[2]. Interestingly, acquired carbapenemases have been mainly restricted to geographical areas and to specific bacterial species, and outbreaks as well as spread in other countries have been often associated with imported cases from countries where the bacteria are endemic. Population mobility is known to be a main factor in globalization and spreading of antimicrobial drug-resistant organisms [3].

The New Delhi metallo-beta-lactamase (NDM-1) is a novel type of MBL named after the city of origin, which has been recently criticized, following a common practice with transferable MBLs since VIM-1 was named after Verona, Italy [4]. The enzyme is active against other compounds that contain a chemical structure known as a beta-lactam ring. Unfortunately, many antibiotics contain this ring, including the penicillins, cephalosporins, and the carbapenems. NDM-1 was first reported in 2009 in a Swedish patient of Indian origin, who travelled to New Delhi and acquired a urinary tract infection due to a carbapenem-resistant K. pneumoniae strain resistant to all antibiotics tested except colistin [5]. Faecal samples collected from this patient during his stay at the nursing home yielded an NDM-1 positive E. coli as well [5]. The most common bacteria that make this enzyme are Gram-negative such as Escherichia coli and Klebsiella pneumoniae, but the gene for NDM-1 can spread from one strain of bacteria to another by horizontal gene transfer.The NDM-1 encoding gene is located on different large plasmids that are easily transferable to susceptible E. coli J53 at a high frequency [5]. These plasmids also harbour genes conferring resistance to almost all antibiotics, thus making their rapid

propagation in clinically relevant bacteria a serious threat for therapy. Following this first case, sporadic cases of infection due to NDM-1 positive bacteria have been detected, including United Kingdom [6], in the United States from patients who received care in India [7], New Delhi [8], in Pakistan and in Canada. In the August issue of the journal The Lancet: Infectious Diseases, a multinational team reported the emergence and spread of 180 cases of patients infected by bacteria carrying the NDM-1, including 37 cases in the United Kingdom and 143 cases in various sites in Pakistan and India, thus suggesting a widespread dissemination [9]. Most plasmids detected in these bacteria were easily transferable and capable of wide rearrangement, suggesting a widespread transmission and plasticity among bacterial populations. NDM-1 is a newly identified problem, only recognized since about December 2009 in the medical literature. To date, there are more than 100 cases identified outside the Indian subcontinent. This is not a pandemic like bird flu or swine flu, but the number of cases is increasing and the concern is that these highly resistant bacteria could supplant more antibiotic-sensitive strains. If this happens, the antibiotic arsenal that has been built up over the last 80 years will be seriously compromised. Currently There are no clinically useful inhibitors of NDM-1 metallo-β-lactamase; however, for metallo-β- lactamases several studies have been undertaken with a variety of experimental inhibitors.

Biomedical Advanced Research and Development Authority (BARDA Department of Health and Human Services, U.S.) given the contract to GlaxoSmithKline of Philadelphia for advanced research and development of an antibiotic called GSK2251052 for ventilator-associated pneumonia, complicated urinary tract infections, and complicated intra-abdominal infections, in which an operation would not remove all of the infected tissue. Phase II clinical trials using the drug to treat ventilator-associated pneumonia, and Phase III clinical trials using the drug to treat complicated intra-abdominal infections. In addition, GlaxoSmithKline also doing initial laboratory testing to determine whether this drug also provides protection against multi-drug resistant pathogens, including those containing the New Delhi Metallo-beta-lactamase-1 (NDM-1) resistance gene. [20]. PMX-30063 from PolyMedix , has shown activity in an in vitro laboratory test against the New Delhi metallo-beta-lactamase-1 (NDM-1) drug resistant strain of Klebsiella pneumonia. The drug candidate is currently undergoing Phase II trial in patients with acute bacterial skin and skin structure infections that are caused by Staphylococcus bacteria. [21]

Till date, crystal structure of NDM-1 metallo-β- lactamase of Escherechia coli is not present in public repository databases, so determining the 3D structure provides a new opportunity for the discovery of more potent inhibitors, particularly in the application of structure based virtual screening to identify lead compounds.In this study, an approach has been taken that combines modelling of 3D structure of NDM-1 protein of Escherechia coli and computational docking process to identify a series of potent inhibitors from different database and there ADMET and Toxicity Prediction.

2. METHODOLOGY

2.1 Protein homology modeling

The entire sequence of Escherechia coli NDM-1, which contains 236 amino acids, was taken from NCBI Protein database with an accession of ADZ45496.1. According to the score of BLAST search, crystal structure of New Delhi Metallo-Beta-Lactamase-1 from Klebsiella pneumoniae [40], was selected as a structural template to perform homology modeling to develop the 3D structure of NDM-1. The PDB code of the crystal structure is 3RKK, which was released in 2011 with a resolution of 2.35 Å. The entire sequence of 3RKK contains 237 amino acids. Modeller9v8 [11] was used to construct the 3D models of NDM-1 protein. The model having least RMSD value by aligning it with the template is selected for further analysis. The selected model is then validated by using PROCHECK[12], ERRAT [13] and VERIFY 3D [14] [15programs of Structural Analysis and Verification Server (SAVES) (http://nihserver.mbi.ucla.edu/SAVES]. The PROCHECK assessed the "stereo chemical quality" of the protein structure. The Verify3D program assessed the 3D protein structure using three-dimensional profiles by analyzing the compatibility of an atomic model (3D) with its own amino acid sequence (1D). The quality of model was also validated by ProSA (Wiederstein et al., 2007) [16]server (https://prosa.services.came.sbg.ac.at/prosa.php), a web server for Protein Structure Analysis. DEPTH server is used to predict active sites of Protein.

2.2 Screening of compounds from different databases

Ligands from other modeled structures of NDM-1 were retrieved from PDB. Similarity search of these ligands and drug tigecycline were performed in different databases, to find novel compounds. During our work Pubchem, ChEMBL, Drug Bank, and SuperNatural database was used for screening of structurally similar inhibitors of or metallo--β--lactamases. These compounds included i)Tigecycline ii)GOL iii) M5P iv) 113 and v) ZZ7. After screening, a total of 50 compounds were obtained that were structurally similar to available inhibitors of metallo-β-lactamases. The screening of database was performed by providing molecular constraints (property based search) and the physicochemical properties such as log P value, H-bond donors, H-bond acceptors, molecular weight and rotational bonds (Lipinski's rule) were also kept into consideration.

2.3 Screening of compound using molecular docking

Virtual screening was performed by docking of inhibitors obtained from different databases to the active site of NDM-1 protein by using AutoDock Vina software[17]. Docking was performed to obtain a population of possible conformations and orientations for the ligand at the binding site. Using the Autodock Tools [18] (http://autodock.scripps.edu/resources/adt) software, polar hydrogen atoms were added to the NDM- 1 protein and its nonpolar hydrogen atoms were merged. The protein receptor (NDM-1) and the inhibitors were converted from PDB format to PDBQT format. All bonds of ligands were set to be rotatable except N-C bonds. In the configuration file of Autodock Vina software, the grid box with a dimension of 20 x 20 x 20 points was used around the active site to cover the entire enzyme binding site and accommodate ligands to move freely. The best conformation was chosen with the lowest docked energy, after the docking search was completed. The interactions of complex NDM-1 protein-ligand conformations, including hydrogen bonds and the bond lengths were analyzed using Swiss-Pdb Viewer [19] v4.0, Pymol software (http://www.pymol.org.) ADMET prediction of the selected ligand or inhibitors was performed using Accelrys Discovery

Studio.(http://accelrys.com/product/discovery-studio/) The toxicity predictions of the ligand molecules were carried out using the 'Toxicity Prediction (Extensible)' protocol of Discovery Studio.

3. RESULTS AND ANALYSIS

3.1 Result of Protein homology modeling

Homology search for NDM-1 metallo-β-lactamase of Escherechia coli resulted into a large number of sequences by running BLASTP against PDB database. The target sequence showed an identity of 38% with metallo-β- lactamase from K.pneumoniae (PDB ID: 3RKK). Chain A of the template was used as a template for making 3D models of NDM-1 protein in Modeller. The sequence similarity of Escherichia coli NDM-1 metallo-β- lactamase with K. Pneumoniae From a total of 50 3D-models of NDM-1 metallo-β-lactamase, the best 3D-model (Figure 1 &2) had lowest RMSD value. The quality of the 3D model was evaluated using the PROCHECK program and assessed using the Ramachandran plot (Figure 3). It is evident from the Ramachandran plot that the predicted model has most favorable regions, the allowed regions, the generic regions and the disallowed regions. Such a percentage distribution of the protein residues determined by Ramachandran plot shows that the predicted model is of good quality. The model show all the main chain and side chain parameters to be in the 'better' region. The overall quality factor of 3D model predicted by ERRAT server was 85.650. Verify 3D server predicted that 95.36% of the residues in NDM-1 metallo- β-lactamase had an averaged 3D-1D, so the model was also verified by the Verify 3D. The quality of the 3D model of NDM-1 metallo- Beta- lactamase as evaluated by ProSA web server (https://prosa.services.came.sbg.ac.at/prosa.php) provided a z-score of -7.77 which falls within the range of values observed for the experimentally determined structures of similar lengths .(Figure 4)

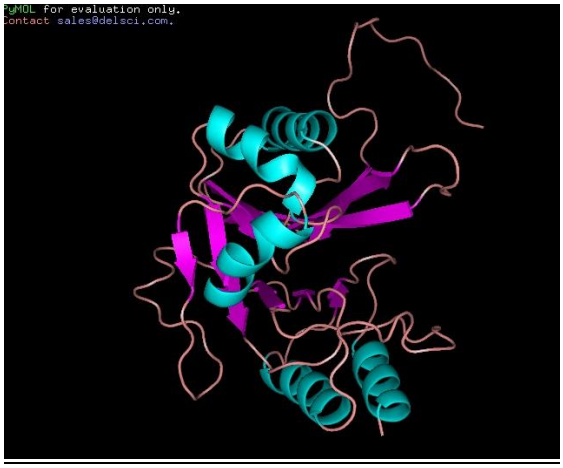

Figure 1: Modeled Structure

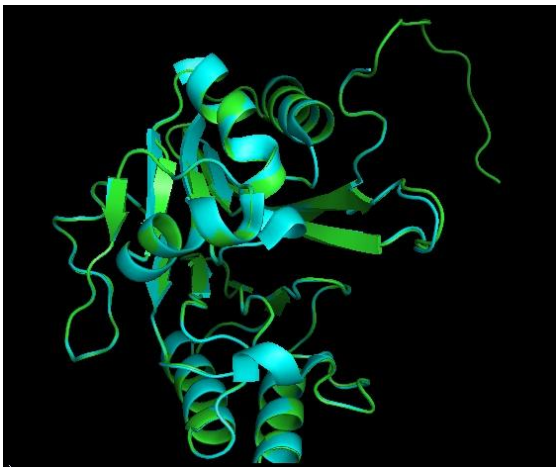

Figure 2: Modeled Structure aligned with Template

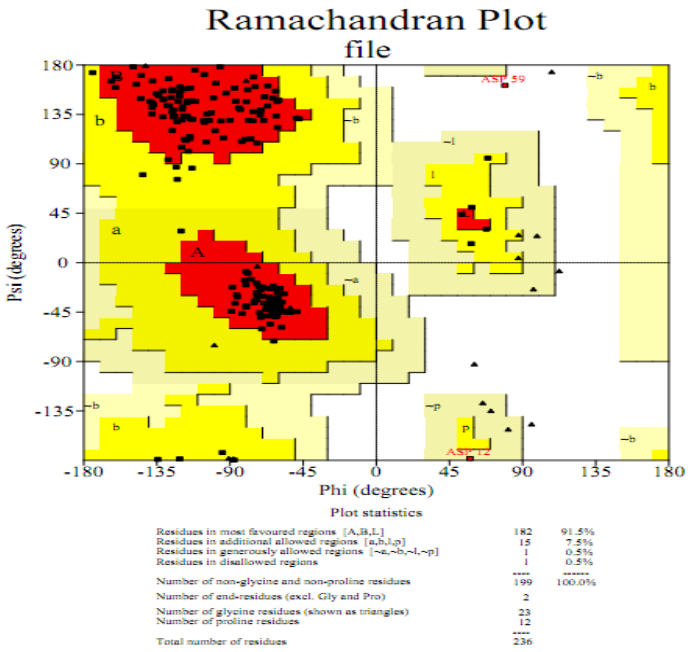

Figure 3: Ramachandran Plot Result

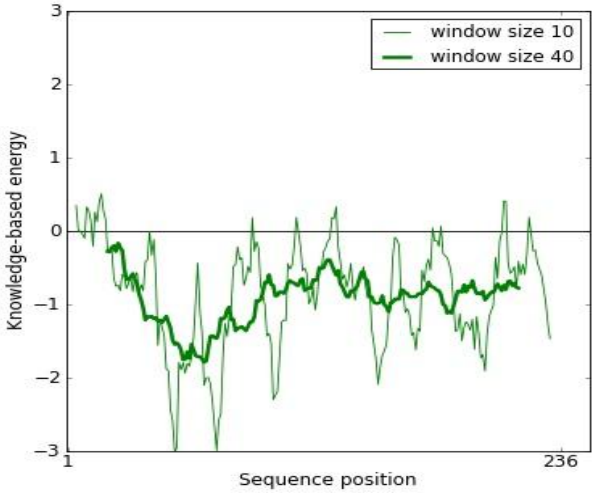

Figure 4: PROSA Result

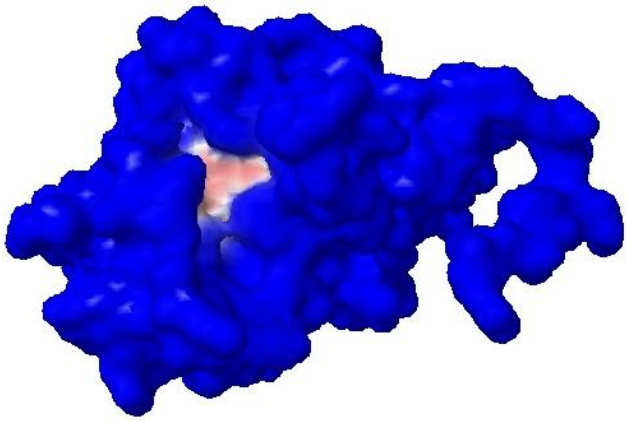

Figure 5: PROSA Result for Active site analysis

3.2 Docking Result Analysis

The most important requirement for interaction of NDM-1 protein and inhibitors was the proper orientation and conformation of inhibitor into the NDM-1 enzyme active site. A total of 50 compounds were obtained from different databases in mol2 format. The best 15 inhibitors were selected on the basis of binding affinity and the extent of binding towards NDM-1. The optimal interactions and the best affinity score were used as criteria to interpret the best conformation among the 10 generated conformations for each inhibitor from different databases. Overall, the best confirmation showed that the free energy of binding (ΔGbind kacl/mol) for the best 15 inhibitors were good and represented in Table 1 and Figure 6A to 6E shows the docking result with minimum binding energy.

Table 1: Docking results

No.	ID (Pubchem/chEMBL)	Binding Energy [KJ/mol]	No. of Hydrogen Bonds formed
1	**CID21680727**	**-7.43**	**2**
2	**Ampicillin (CID 6249)**	**-6.3**	**1**
3	Sigma_c3416	-6.23	3
4	Stock1n-13321	-5.07	2
5	Stock1n-31653	-5.53	2
6	**CID 50909816**	**-7.98**	**4**
7	**CID 2174**	**-7.28**	**3**
8	**CID 4084**	**-6.33**	**1**
9	CHEMBL1232898	-5.29	1
10	CHEMBL1235251	-6.11	2
11	CHEMBL55731	-5.51	1
12	CHEMBL1231663	-6.05	3
13	CID 31161	-5.1	4
14	CID 4478475	-5.12	6
15	CID 44399514	-5.61	4

3.3 ADMET prediction analysis of Compounds

ADMET stands for Absorption, Distribution, Metabolism, Excretion and Toxicity. These describe the disposition of a pharmaceutical compound within an organism. The ADMET criteria influence the performance and pharmacological activity of the compound as a drug. ADMET prediction was performed using Accelrys Discovery Studio. For the predictions, two protocols were used; 'ADMET descriptors' protocol (Table 4) and 'Toxicity Predictions (Extensible)' protocol. (Table 5) Following are the levels of prediction (Table 3) defined for ADMET descriptors protocol:

1. Human Intestinal Absorption
2. Aqueous Solubility
3. Blood Brain Barrier penetration
4. Hepatotoxicity
5. Plasma Protein Binding

Table 2: Lipinski's Rule of five analysis results

No.	ID (Pubchem/chEMBL)	Molecular weight	No. of H Bond acceptors	No. of H Bond Donors	XLogP	No. of violations
1	CID 21680727	350.389	6	2	0.2	0
2	Ampicillin (CID 6249)	349.404	5	3	-1.1	0
3	Sigma_c3416	376.383	9	3	0.679	0
4	Stock1n-13321	333.382	7	2	1.098	0
5	Stock1n-31653	458.467	10	4	0.503	0
6	CID 50909816	351.397	5	2	0.2	0
7	CID 2174	349.404	5	3	-1.1	0
8	CID 4084	361.415	5	2	3	0
9	CHEMBL1232898	441.543	5	4	0.1	0
10	CHEMBL1235251	437.466	9	3	-3.1	0
11	CHEMBL55731	403.493	7	4	-1.7	0
12	CHEMBL1231663	380.415	7	4	-1.4	0

13	CID 31161	316.26	7	4	2.3	0
14	CID 4478475	320.25	8	6	1.1	0
15	CID 44399514	238.193	6	2	0.8	0

Table 3: Levels of ADMET predictions

Human Intestinal Absorption		Aqueous Solubility		Blood Brain Barrier penetration		Hepatotoxicity		Plasma Protein Binding	
1	Good	**0**	Extremely low	**0**	Very high penetrant	**0**	**Non Toxic** (Unlikely to cause dose-dependent liver injuries.)	**0**	Binding is < 90%
2	Moderate	**1**	No, very low, but possible	**1**	High			**1**	Binding is > 90%
3	Poor	**2**	Yes, low	**2**	Medium				Binding is > 95%
4	Very Poor	**3**	Yes, good	**3**	Low	**1**	**Toxic** (Likely to cause dose-dependent liver injuries.)	**2**	
		4	Yes, optimal	**4**	Undefined				
		5	No, too soluble						
		6	Warning: molecules with one or more unknown AlogP98 types						

Table 4: ADMET descriptors prediction

No.	**ID (Pubchem/chEMBL)**	**Blood Brain Barrier penetration**	**Intestinal absorption**	**Aqueous solubility**	**Hepato-toxicity**	**CYP2D6 binding**	**Plasma protein Binding**
1	CID 21680727	4	1	4	0	0	0
2	Ampicillin (CID 6249)	4	1	4	1	0	0
3	Sigma_c3416	4	3	4	1	0	0
4	Stock1n-13321	3	0	4	0	0	0
5	Stock1n-31653	4	2	4	1	0	0
6	CID 50909816	4	1	4	0	0	0
7	CID 2174	4	1	4	1	0	0
8	CID 4084	3	0	4	0	0	0
9	CHEMBL1232898	3	0	3	1	0	0
10	CHEMBL1235251	4	3	4	0	0	0
11	CHEMBL55731	4	3	5	0	0	0
12	CHEMBL1231663	4	3	4	1	0	0
13	CID 31161	4	0	4	1	0	1
14	CID 4478475	4	3	5	1	0	0
15	CID 44399514	4	1	4	1	0	2

Table 5: Toxicity predictions

S No.	ID (Pubchem/ chEMBL)	TOPKAT Aerobic Biodegradability	TOPKAT Ames Mutagenicity	TOPKAT Developmental Toxicity Potential	TOPKAT Skin Irritancy None vs. Irritant	TOPKAT Weight of Evidence Rodent Carcinogenicity
1	CID_21680727	Degradable	Non-Mutagen	Toxic	Non-Irritant	Non-Carcinogen
2	**Ampicillin (CID 6249)**	**Degradable**	**Non-Mutagen**	**Non-Toxic**	**Non-Irritant**	**Non-Carcinogen**
3	Sigma_c3416	Non-Degradable	Non-Mutagen	Non-Toxic	Mild Irritant	Non-Carcinogen
4	**Stock1n-13321**	**Degradable**	**Non-Mutagen**	**Non-Toxic**	**Mild Irritant**	**Non-Carcinogen**
5	Stock1n-31653	Non-Degradable	Non-Mutagen	Non-Toxic	Non-Irritant	Non-Carcinogen
6	**CID_50909816**	**Degradable**	**Non-Mutagen**	**Toxic**	**Non-Irritant**	**Non-Carcinogen**
7	**CID_2174**	**Degradable**	**Non-Mutagen**	**Non-Toxic**	**Non-Irritant**	**Non-Carcinogen**
8	**CID_4084**	**Degradable**	**Non-Mutagen**	**Non-Toxic**	**Non-Irritant**	**Non-Carcinogen**
9	CHEMBL2132898	Degradable	Non-Mutagen	Toxic	Mild Irritant	Non-Carcinogen
10	CHEMBL1235251	Degradable	Non-Mutagen	Toxic	Mild Irritant	Non-Carcinogen
11	CHEMBL55731	Degradable	Non-Mutagen	Non-Toxic	Mild Irritant	Non-Carcinogen
12	CHEMBL1231663	Non-Degradable	Non-Mutagen	Non-Toxic	Mild Irritant	Non-Carcinogen
13	CID_31161	Non-Degradable	Non-Mutagen	Toxic	Mild Irritant	Non-Carcinogen
14	CID_4478475	Degradable	Non-Mutagen	Toxic	Mild Irritant	Non-Carcinogen
15	CID_44399514	Degradable	Non-Mutagen	Toxic	Mild Irritant	Non-Carcinogen

4. COMPARATIVE ANALYSIS OF RESULTS

On the basis of above tables, and results, 5 compounds were identified which can be analysed in invitro conditions and then based on that results can be developed as a Drug to cure infections caused by NDM-1. Those 5 compounds are:

1) CID 6249

2) Stock1n-13321

3) CID 2174

4) CID 4084

5) CID 50909816

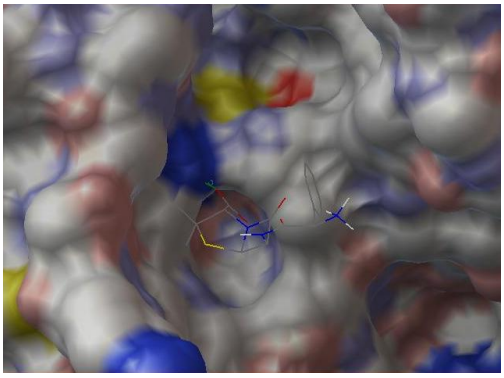

Figure 6 (A): Docking of CID 6249 into NDM-1

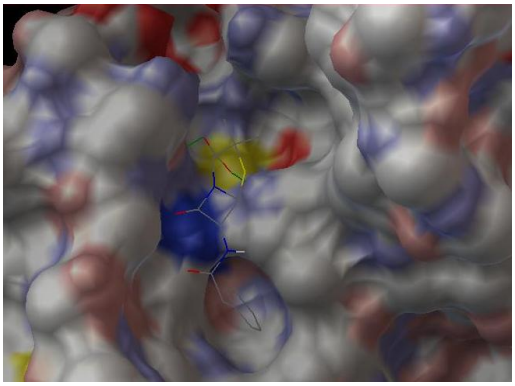

Figure 6 (B): Docking of Stock1n-13321 into NDM-1

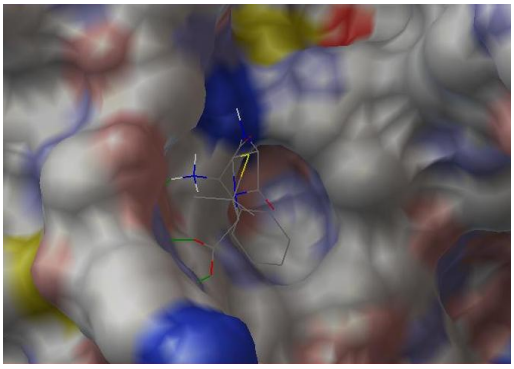

Figure 6 (C): Docking of CID 2174 into NDM-1

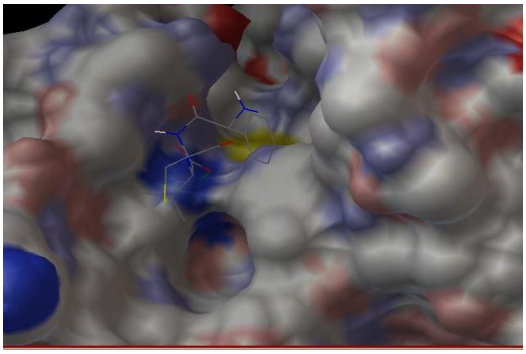

Figure 6 (D): Docking of CID 4084 into NDM-1

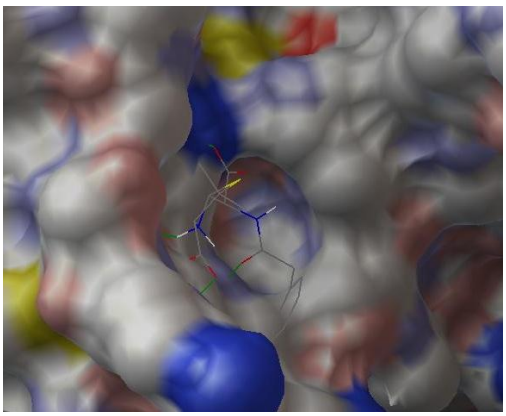

Figure 6 (E): Docking of CID 50909816 into NDM-1

All 15 best inhibitor have been analyzed on the basis of Lipinski rule of five (Table 2).

5. CONCLUSION

New Delhi Metallo beta lactmse (NDM-1) is emerging as a major threat. According to a recent study in USA, NDM-1 and other metallo beta lactamases are killing thousands of people every year. It is also known as "Superbug" because the infections caused are very difficult to treat. It is only susceptible to Tigecycline and polymyxins, which are also not quite efficient sometimes. In order to find novel drug like compounds to inhibit NDM-1. Virtual screening methods are routinely and extensively used to reduced cost and time of drug discovery process. It has been clearly demonstrated that the approach utilized in this study is successful in finding 15 potent inhibitors from the different database. In this work, 3D model of NDM-1 metallo-β–lactamase was predicted and it was used for screening potent compounds from different database.Docking results indicate that out of 50 compounds, there were 15 inhibitory compounds for NDM-1 metallo-β–lactamase that showed interaction with the protein to a great extent and these inhibitors could be used against NDM-1 metallo-β-lactamase. Hydrogen bonding plays an important role for the structure and function of biological molecules, especially for inhibition in a complex. The inhibitors were docked deeply within the binding pocket region and forming interactions. On the basis of minimum energy concept, compound CID 50909816 can be considered as better in comparison to other compounds. But on the basis of Overall analysis of Lipinski's rules and ADMET predictions; CID 6249, Stock1n-13321 and CID 2174 can be considered as better options for being inhibitors of NDM-1.

- These molecules have a low logP value indicating their easy absorption.
- Molecular weight around 348 indicate high solubility of these molecules.
- They are neither carcinogenic nor mutagenic.

On the basis of overall analysis, it can be concluded that these compounds can prove to be good inhibitors of NDM-1. They should be further tested for stability in invitro conditions. It can be hoped that these compounds could be effective for designing a novel drug for curing NDM-1

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