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RESEARCH ARTICLE

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COMPARATIVE ANALYSIS AND IDENTIFICATION OF NOVEL β-LACTAMASE INHIBITORS

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ABSTRACT

β-lactam antibiotics are a broad class of antibiotics, consisting of all antibiotic agents that contain a β-lactam nucleus in its molecular structure. Till now β-lactams like penicillins, cephalosporins, monobactams, and carbapenems have been developed. Resistance to various β-lactam antibiotics among target pathogens were started with its first use.The most widespread and important cause is the destruction of the β-lactam ring, mediated by β-lactamases. Antibiotic resistance can be overcome successfully with the use of β-lactamase inhibitors in combination with β-lactams. Resistance within a few years of the introduction of the antibiotic class seems inevitable that the new antibiotics developed to hit novel targets will also encounter resistance. Hence, it's needed to identify novel therapeutics to be used as β-lactamase inhibitors. Present study was accomplished to develop novel β-lactamase inhibitors and their comparative study with the already present ones. Most promising results were shown by phenolic derivatives of sulbactam.

KEYWORDS

β-lactamases, NDM1, Sulbactam, Molecular docking, ACE Values

INTRODUCTION

The β-lactam antibiotics were discovered in early twentieth century was used against pathogenic bacteria. These antibiotics are used to treat a broad spectrum of gram-positive and gram-negative bacteria. As they are relatively inexpensive and highly efficient semi-synthetic products, they have become an important part of anti-infective chemotherapy for the past many years. The β-lactam antibiotics due to its structural similarities with the binding sites of bacterial substrates, attach to and inactivate the transpeptidases involved in bacterial cell wall synthesis [1]. However, bacteria evolve mechanisms of evading their action resistance against β-lactam antibiotic classes; penicillin resistance emerged soon after it was first introduced in the 1940s [2,3.] Now its a major barrier in the field of medical success [4]. The most common cause of resistance is the production of a β-lactamase by the pathogen, that leads to hydrolysis of the antibiotic. Two types of approaches have been used to prevent the inactivation of β-lactams by the above way they are: (i) structural modification of the antibiotic that prevents the action of the enzyme (ii) inhibition of the enzyme using inhibitors that structurally related to the substrate. The β-lactamases are broadly divided into four major classes, A to D. Classes A, C, and D β-lactamases are serine enzymes, while class B are zinc metalloenzymes. These resistance causing factor may be coded on plasmids and hence mobile within a bacterial community. β-lactamase inhibitors which can mimic the substrate for the enzyme β-lactamase, can be used to overcome this resistance to β-lactam antibiotic classes. The combination of β-lactams with β-lactamase inhibitors restores the activity of the β-lactams, allowing their continued clinical use [5]. β-lactamase inhibitors in clinical use include are clavulanic acid and its potassium salt (usually combined with amoxicillin or ticarcillin), sulbactam and tazobactam.

New Delhi metallo-β-lactamase (NDM-1) is an enzyme which belongs to class B of β-lactamases makes bacteria resistant to a broad range of beta-lactam antibiotics. NDM-1 comes under the member of a large gene family that encodes β-lactamase enzymes called carbapenemases. Bacteria producing carbapenemases are often referred to as "superbugs" because its infections are difficult to treat. Mostly Gram-negative such as *Escherichia coli* and *Klebsiella pneumoniae*, make this enzyme but the gene coding for NDM-1 can spread from one strain of bacteria to another by horizontal gene transfer. NDM-1 is resistant to carbapenes [6]. In this study we try to develop novel β-lactamase inhibitors against NDM-1 through bioinformatics approach. Novel β-lactamase inhibitors were developed by chemically modifying the side chains of already present β-lactamase inhibitors, which were taken as parent compounds. The inhibiting ability of these inhibitors was checked against the β- lactamase proteins obtained from five different strains using molecular docking.

MATERIALS AND METHODS

(i) Selection of NDM-1 β-lactamase producing pathogenic strains

The sequence for NDM-1 gene from *Escherichia coli* was obtained from NCBI with accession no. ADY00041.1. It was then compared with other sequences in the database and the sequences showing highest similarity were selected. Sequences for metallo β-lactamase from four different pathogenic strains with 100% sequence similarity were taken for further studies. The four pathogenic strains with their accession number and gi number are shown in table1.

Table.1
Pathogenic strains with Accession and GI number

S.N.	Source	Accession no./ G.I
1	*Klebsiella pneumoniae*	ADW08084.1/320041713
2	*Acinetobacter junii*	ADU02194.1/315271096
3	*Enterococcus faecium*	ADP37377.1/310892441
4	*Stenotrophomonas maltophilia*	BAJ76899.1/323461480

(ii) Determining the 3D structure of the NDM-1 β-lactamase

The 3D structures of β-lactamases were determined using SWISS-MODEL, which is a fully automated homology modelling server used for protein structures determination. As the number of energetically possible folds in nature are limited hence three dimensional structure of proteins are more conserved than their sequences.It is often possible to identify the homologous protein by comparing target protein with a known structure i.e the template, for the protein sequence of the target whose structure is not known[7].Homology modelling has proven to be the method of choice to generate a reliable three-dimensional model of a protein. This is accessible via EXPASY web server or from the deep view program [8,9].

The energy of the 3D structure obtained by SWISS-MODEL was then minimised using NOMAD-Ref. The NOMAD-Ref is also a web server which provides tools for online calculation of the normal modes(energetically most favourable structure) of large molecules (up to 100,000 atoms) maintaining a full all-atom representation of their structures, as well as access to a number of programs that utilize these collective motions for deformation and refinement of bio molecular structures. NMA has proved useful for structural refinement against experimental data. The addition of a small number of collective degrees of freedom is sufficient to capture most of the intrinsic flexibility of the macromolecule, while retaining local connectivity and stereo chemical properties. In contrast to using classical rigid body's concept, NMA is almost model-free, and the level of detail can be adjusted freely by changing the number of modes used. In some sense, normal modes can be regarded as completely arbitrary collective displacements. The fact that they provide such an efficient refinement space where all the most important biological motions are included, with obvious applications to docking methods and drug design in the presence of induced fit [10.]

(iii) Generation of novel β-lactamase inhibitors

The already used β-lactamase inhibitors were taken as the parent compounds and to these parent compounds side chain modifications were done using chemsketch to generate novel β-lactamase inhibitors. ACD/ChemSketch contains tools for 2D structure generation, 3D optimization and viewing, IUPAC International Chemical Identifier (InChI) generation and conversion, drawing of polymers, organometallics, Markush structures and IUPAC systematic nomenclature capability for molecules with lesser than 50 atoms and 3 rings. The software allowed drawing of novel chemical moieties which were then obtained in terms of canonical smiles (simplified molecular input line entry specification) format. These canonical smiles were then fed to CORINA, online software, which is used to generate 3D coordinates of the structure which were then used for docking. CORINA is a fast

and powerful 3D structure generator for small and medium sized, drug-like molecules. Due to its robustness, comprehensiveness, speed and performance CORINA is preferably used in conversion of large chemical datasets or databases. Regular advancement of CORINA has come up during the past decades and has become the most recognized and preferred world-wide gold standard in industry and academics to generate 3D molecular models of high quality. Currently major user of CORINA is Symyx, NCI/NIH and most major pharmaceutical and chemical companies to convert their 2D structures into 3D. It generates a single, high-quality and low-energy conformation (default), optionally generates multiple conformations for cyclic compounds. It covers a broad range of organic chemistry and organometallic compounds with atoms of up to a coordination number of 6 [11].

(iv) ***Molecular docking***

Molecular docking was carried out using PatchDock software present online. PatchDock is a molecular docking algorithm. The input can be two molecules of any type such as: proteins, DNA, peptides or drugs. The output is a list of promising potential complexes sorted by shape and complementarily criteria. PatchDock algorithm is documented by object recognition and image segmentation techniques used in Computer Vision. Two molecules are given one is the enzyme (· β-lactamase) other is the side chain modified inhibitor, The input for docking was given in the form of pdb files. Their surfaces are divided into concave, convex and flat patches. Once the patches are identified, they can be superimposed using shape matching algorithms. All candidate complexes obtained from the previous step are examined and complexes with unacceptable penetrations of the atoms of the receptor to the atoms of the ligand are discarded. Finally, the remaining candidate complexes are ranked according to a geometric shape complimentarity score that considers both geometric fit and atomic desolvation energy [12,13].

RESULTS

To obtain the optimal docking configuration and scoring function, the structure of enzyme New Delhi Metallo β-Lactamase (NDM-1) was docked with the chemsketch designed set of ligands, whose 3D coordinates were generated using CORINA. PatchDock was used to yield the closest model to the crystallographic structure of Enzyme-ligand complex and to find maximum score and corresponding ACE values.

1. ***3D structure of new delhi metallo β-lactamase (NDM-1) and its energy minimization***

The structure of New Delhi Metallo β-lactamase (NDM-1) was generated using SWISS MODEL. The structure was then subjected for energy minimization using NOMAD-Ref and the cut off value for structure minimization was kept 10 Å. Other parameters were kept default. The structure obtained after energy minimization is as shown in Fig 1.

Energy minimised structure of New Delhi Metallo β-Lactamase (NDM-1)

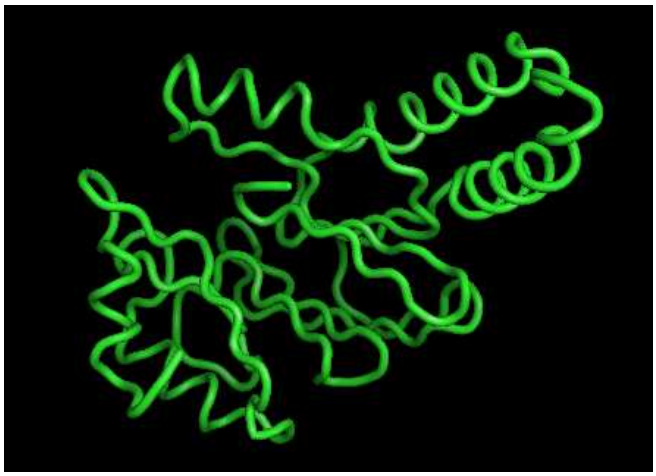

Fig 1

Structure of New Delhi Metallo β-Lactamase (NDM-1) after energy minimization. All possible folds with minimum possible energy is generated.

2. ***Generation of novel β-lactamase inhibitors***

ACD/ChemSketch was used to generate novel β-lactamase inhibitors by side chain modifications of already present β-lactamase inhibitors clavulanic acid, sulbactam and tazobactam taken as parent compounds. The generated inhibitors are shown in fig: 2(a,b,c).Here only highly stable and efficient inhibitors of above 3 inhibitor classes are shown.

Side chain modified inhibitors with better chance of success

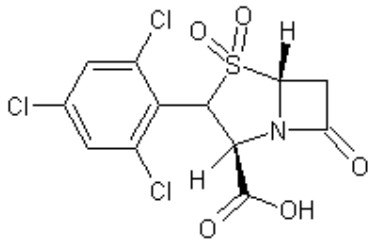

Fig 2a

Sulbactam derivative

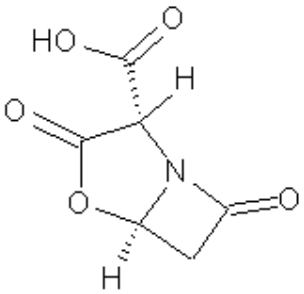

Fig 2b

Clavulanic acid derivative

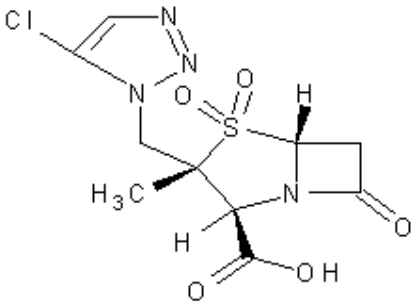

Fig 2c

Tazobactam derivative

Online demo of CORINA software was used for generation of 3D coordinates of these generated organic compounds. The input was submitted in the form of canonical SMILES and 3D structures were obtained which were downloaded as PDB format and used for docking. The generated energetically favourable ligands with their chemical formula and SMILES are presented in Table 2.

Table 2
Synthetic ligands with their chemical formula and SMILES

S.N.	Synthetic ligands with chemical formula	SMILES
1	(2*R*,3*Z*)-6-chloro-3-(hydroxymethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid	OC(=O)[C@H]1C(OC2C(Cl)C(=O)N12)=[C@H]O
2	(2*R*,3*Z*,5*R*)-7-oxo-3-(1*H*-1,2,3-triazol-1-ylmethylidene)-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid	OC(=O)[C@H]1C(\O[C@@H]2CC(=O)N12)=C\n3ccnn3
3	(2*S*,5*R*)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide	O=S2(=O)C(C)(C)[C@@H](N1C(=O)C[C@H]12)C(=O)O
4	(2*S*,5*R*)-3-(4-hydroxyphenyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide	O=S2(=O)C([C@@H](N1C(=O)C[C@H]12)C(=O)O)c3ccc(O)cc3
5	(2*S*,5*R*)-3-(2,4-dihydroxyphenyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide	O=S2(=O)C([C@@H](N1C(=O)C[C@H]12)C(=O)O)c3ccc(O)cc3O
6	(2*S*,5*R*)-7-oxo-3-(2,4,6-trihydroxyphenyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide	O=S2(=O)C([C@@H](N1C(=O)C[C@H]12)C(=O)O)c3c(O)cc(O)cc3O
7	(2*S*,5*R*)-3-(4-chlorophenyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide	O=S2(=O)C([C@@H](N1C(=O)C[C@H]12)C(=O)O)c3ccc(Cl)cc3
8	(2*S*,5*R*)-3-(2,4-dichlorophenyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide	O=S2(=O)C([C@@H](N1C(=O)C[C@H]12)C(=O)O)c3ccc(Cl)cc3Cl
9	(2*S*,5*R*)-7-oxo-3-(2,4,6-trichlorophenyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide	O=S2(=O)C([C@@H](N1C(=O)C[C@H]12)C(=O)O)c3c(Cl)cc(Cl)cc3Cl
10	(2*S*,5*R*)-7-oxo-3-(2,4,6-trichlorophenyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide	O=S2(=O)C([C@@H](N1C(=O)C[C@H]12)C(=O)O)c3c(Cl)cc(Cl)cc3Cl

3D structure of generated ligands is obtained by using CORINA online tool. The result of CORINA is shown in Fig 3, where different colours represent the specific position of atoms and its conjugation with other neighbouring atoms.

3D structure of generated novel β-lactamase inhibitor

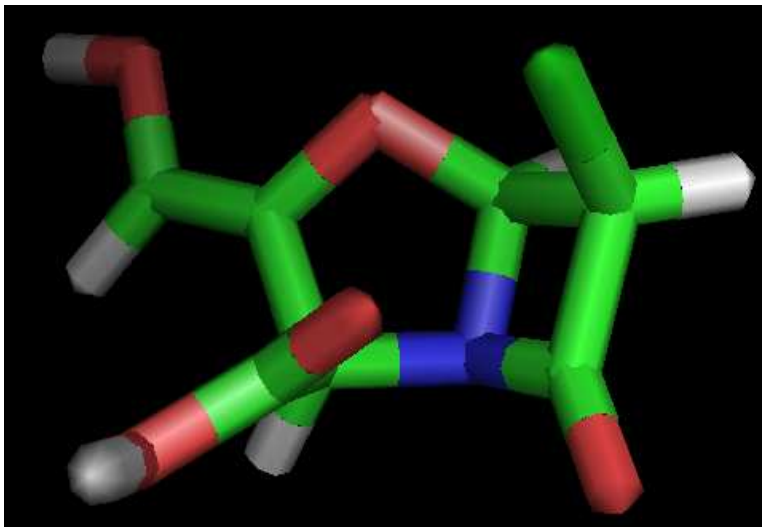

FIG 3

shows 3D structure of one of the ligands as viewed in PyMOL viewer, obtained using CORINA software.

3.Docking of 3D structure of new delhi metallo β-lactamase (NDM-1) with novel β-lactamase inhibitors

The energy minimised PDB file of new delhi metallo β-lactamase (NDM-1) is docked with novel β-lactamase inhibitors with the help of PatchDock online tool. Keeping the target constant (New Delhi metallo β-lactamase (NDM-1) the various side chain modified derivatives of inhibitor classes are tested. The result showing maximum docking score and least atomic contact energy (ACE) signifies the superiority of one inhibitor over other. Docking result for various inhibitors is shown in Table 3. The result of PatchDock which are generated in form of PDB files can be visualized in any PDB file viewer. Here we have used Pymol and YASARA viewer. Result obtained can be seen in Fig 4, Fig 5a(PyMol view) and Fig 5b(YASARA view).

Table 3

Docking result with score and energy using enzyme

S.N.	Ligands	Score	ACE
1	(2*R*,3*Z*)-6-chloro-3-(hydroxymethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid	2882	-98.31
2	(2*R*,3*Z*,5*R*)-7-oxo-3-(1*H*-1,2,3-triazol-1-ylmethylidene)-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid	3400	-77.13
3	(2*S*,5*R*)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide	2554	-96.34

4	(2*S*,5*R*)-3-(4-hydroxyphenyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide	3236	-117.38
5	(2*S*,5*R*)-3-(2,4-dihydroxyphenyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide	3442	-89.01
6	(2*S*,5*R*)-7-oxo-3-(2,4,6-trihydroxyphenyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide	3404	-209.94
7	(2*S*,5*R*)-3-(4-chlorophenyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide	3638	-96.4
8	(2*S*,5*R*)-3-(2,4-dichlorophenyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide	3672	-92.67
9	(2*S*,5*R*)-7-oxo-3-(2,4,6-trichlorophenyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide	3712	-98.59

Docked structure of novel β-lactamase inhibitors and β-lactamase complex

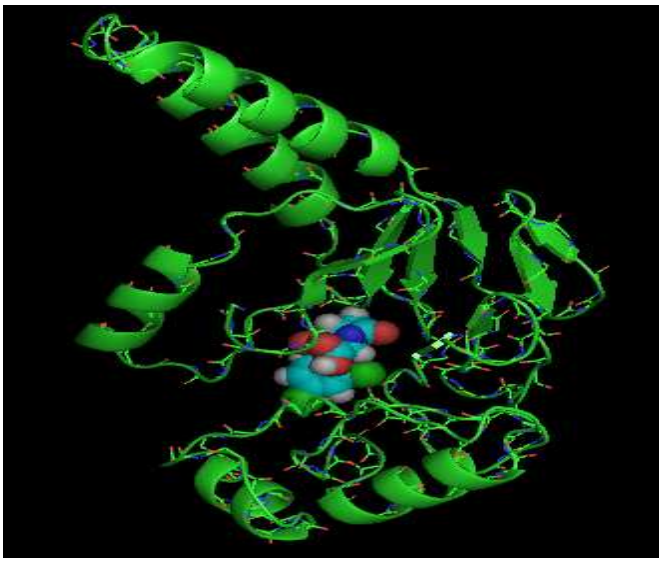

FIG 4

Docked structure of clavulanic acid derivatives complex

Docked structure of Sublactam derivative complex

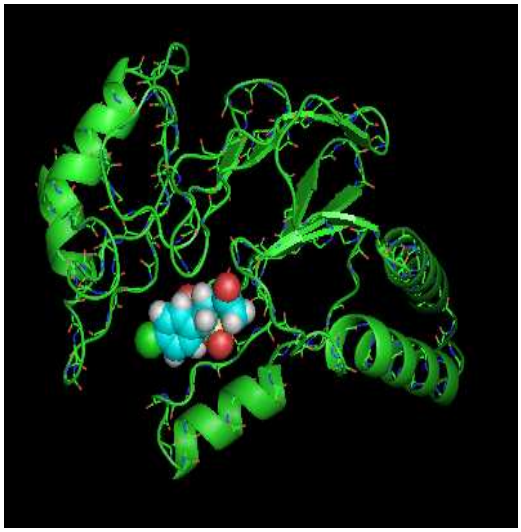

FIG 5a: PyMol View

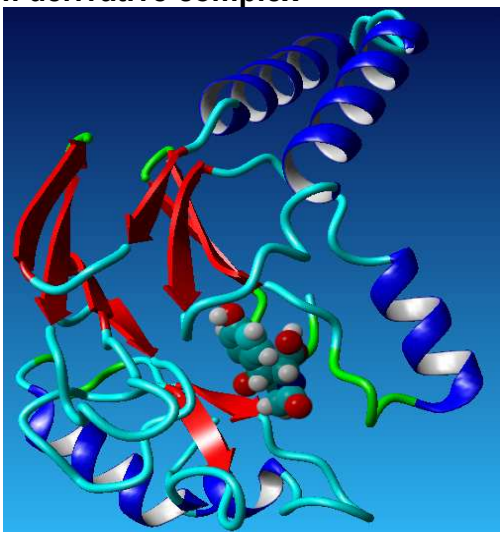

FIG 5b: YASARA View

FIG 5

Docked structure of Sulbactam derivative complex(PyMol and YASARA View)

Counting the number of favourable intermolecular interactions such as hydrogen bonds and hydrophobic contacts makes the basis of scoring. The focus of molecular docking is to computationally simulate the molecular recognition process and aims to achieve an optimized conformation for both the protein and ligand and relative orientation between protein and ligand such that the free energy of the overall system is minimized. In our study the maximum score and minimum energy were found in case of Sulbactam derived ligands. Score shown by Clavulanic and Sulbactam derived ligands was 3156 and 3712 respectively ,which is maximum among the commonly used drugs whose values are found around 2632. Clavulanic acid is mostly recommended against the Antibiotic Intolerance however they have lower score and more energy in comparison to Sulbactam compounds and hence can be deduced to be less effective. So sulbactam derived compounds promise the better potency and effectiveness against the disease. Among the generated ligands most of the compounds do not show good score, however Phenolic derivative shows good score i.e. 3712 and hence could also be used as an option for the treatment of well tolerated, clinically effective β-lactam antibiotics to treat a variety of bacterial infections. In majority of the cases, it can be seen that ACE values are considerably lower in case of docking with enzyme suggesting that the ligands bind more spontaneously and more effectively to NDM-1 β-lactamase.

DISCUSSION

A key to the success of β-lactamase inhibitors has been their close structural resemblance to the substrate antibiotics themselves. Another feature is the mechanism-based irreversible nature of the inactivation [14]. With the completion of the *Klebsiella pneumoniae* genome sequence on 27 Feb 2011, for the first time we have the opportunity to identify novel chemotherapeutic treatments. This requires the exploitation of a variety of technologies. The major challenge is to take the process from discovery of drug candidates. A crucial component will be the forging of partnerships between the pharmaceutical industry and publicly funded scientists to ensure that the promise of the current revolution in biology lives upto our hopes and expectations. A series of structurally different inhibitors were used in the present study to generate and validate a

most efficient docking using PatchDock methodology and considering their score and corresponding energy value. The observation of docking result, mainly of sulbatums gave relevant information that could be easily used to design a more potent inhibitor in comparison to the present inhibitors. This study demonstrated that clavulanic acid and sulbactams derivatives can be an effective and well-tolerated alternative for the treatment of resistance against antibiotics. Previous studies done with chemically synthesised β-lactamase inhibitors indicate them to be better than the ones being usually used in conjugation with β-lactams [15,16].Out of the generated ligands phenolic and chlorine conjugated compounds indicated good inhibition ability. The ability to continue the use of β-lactam antibiotics in combination with the β-lactamase inhibitors is the best possible arsenal in fight against bacterial diseases in this age of increasing antibiotic resistance.

CONCLUSION

Till now among all the inhibitors and their antibiotic conjugates available in the market, clavulanic acid and its derivatives were the most effective one against β-lactamase. Our investigation clearly demonstrates that Sulbactam and its phenolic derivatives possess significant effectiveness against β-lactamase activity. Thus these derivatives and their antibiotic conjugates can be used effectively against β-lactamase enzyme and antibiotic activities can be enhanced.

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