BIOINFORMATION
Discovery at the interface of physical and biological sciences

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Volume 8(24)

Screening of inhibitors for S130G inhibitor resistant mutants of TEM type beta-lactamase

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Received October 21, 2012; Accepted October 26, 2012; Published December 08, 2012

Abstract:
Bacteria are remarkably adaptable organisms that acquire an almost limitless competence to survive under unpleasant conditions. The drastic emergence of antibiotic resistance among β-Lactamases is the most serious threat to hospitals and nosocomial settings. β-lactam inhibitors came into existence in order to overcome the problem of antibibiotic resistance in bacteria. The emergence of inhibitor resistant mutants has raised the alarms. In this study we have used structured based virtual screening approach and have screened out some inhibitors against S130G TEM mutant. All the compounds were tested in presence and absence of conserved active site water molecules. These compounds were found be showing much higher efficacy than known β-lactamase inhibitors. Amino acids G130, S70, N132, G130, Y105 and V216 were found crucial for the interaction of inhibitors within the active site.

Background:
Bacteria are remarkably adaptable organisms that acquire an almost limitless competence to survive under unpleasant conditions. β-lactam antibiotics are the most common treatment for bacterial infections **[1].** Production of β-Lactamases is the most important mechanism of resistance against β-lactam drug in Gram-negative bacteria **[2, 3].** These enzymes hydrolysed the amide bond of the β-lactam ring inorder to inactivate the antibiotics **[4, 5].** The drastic emergence of antibiotic resistance among β-Lactamases is the most serious threat to hospitals and nosocomial settings **[6].** Class A β-lactamases, that are considered to be responsible for numerous failures in the treatment of infectious diseases, are most widespread enzymes **[7].** TEM and SHV are class A type β-lactamases commonly found in *Escherichia coli* and *Klebsiella pneumoniae*, pathogens and are considered to be mainly responsible for urinary tract, respiratory tract, and bloodstream infections **[8].** TEM-1 was the first plasmid-mediated β-lactamase in Gram - negatives, reported in 1965 from an *Escherichia coli* and was named after the patient from which it was isolated (Temoniera) **[9].** Since then this Class-A β-lactamase has spread worldwide among different bacterial species **[10].** β-lactam inhibitors came into existence in order to overcome the problem of antibibiotic resistance in bacteria **[11].** The emergence of inhibitor resistance strains fuelled the already persisting problem and has seriously challenged the future of the β-lactam antibiotics **[12].** Mutations in several positions of the enzyme are responsible for increased catalytic activity against these antimicrobials and for resistance to β-lactamase inhibitors, turning the enzyme into an extended-spectrum or inhibitor resistant β-lactamase **[13].** One of the important position where the mutation leads to inhibitor resistance is S130G **[14].** Ser130 is an important active site residue that is considered to be playing very important roles that ranges from protonating the lactam nitrogen leaving group to facilitating proton transfer to the β-lactam nitrogen during acylation leading to β-lactam ring opening promoting during substrate hydrolysis **[15, 16].** It has been reported earlier that mutation at this residue position compensates for the loss of activity of enzyme and makes the enzyme to less susceptible to β-lactamase inhibitors **[14].** That in turn increases the acquired dosage to many folds. Today, it seems to be very important for developing an inhibitor against such resistant mutants. Virtual screening by molecular docking is increasingly important in drug discovery **[17, 18].** Thus, in the view of present background we have performed structure based virtual screening of inhibitors against S130 mutation carrying TEM-76 type Class-A β-lactamase.

Methodology:

Protein preparation

The crystal structure of TEM-1 and TEM-76 were extracted from protein data bank (pdb id: 1AXB and 1YT4). Each structure was refined by removing the heteroatoms and water molecules. The minimization was performed by using a CHARMm force field **[19]** with Dependent Dielectric implicit solvent model along and conjugates gradient method.

Library Design

Known inhibitors of beta-lactamases were retrieved from pubchem. A library of 1442 compounds extracted from zinc database **[20]** was prepared on the basis of physicochemical properties of known inhibitors. Further the compounds were refined for correct protonation.

Virtual Screening

GOLD (Genetic Optimization for Ligand Docking) 5.0 **[21]** was used for virtual screening of the compounds dataset against selected targets in present study. Docking annealing parameters for van der Walls and hydrogen bonding were set to 5.0 and 2.5 respectively. The parameters used for genetic algorithm were population size 100, selection pressure 1.2, number of operations 1,00,000, number of islands 5, niche size 2, migrate 10, mutate 100 and cross -over 100. The procedure was repeated twice to confirm the accuracy of our results. Top 100 compounds on the basis with GOLD fitness score were selected after the first phase of screening. These top 100 selected compounds were further screened, and this time these compounds were docked with the active site of TEM-76 in presence of water molecules. The water molecules within 5Å around the active site of TEM-76 were retained. Finally, best five compounds with highest Fitness score against TEM-76 in both in presence and absence of water molecules. These finally selected compounds were subjected to dock into the active site of wild type TEM (TEM-1, pdb id: 1AXB) using the same protocol as mentioned above to confirm their effectivenss.

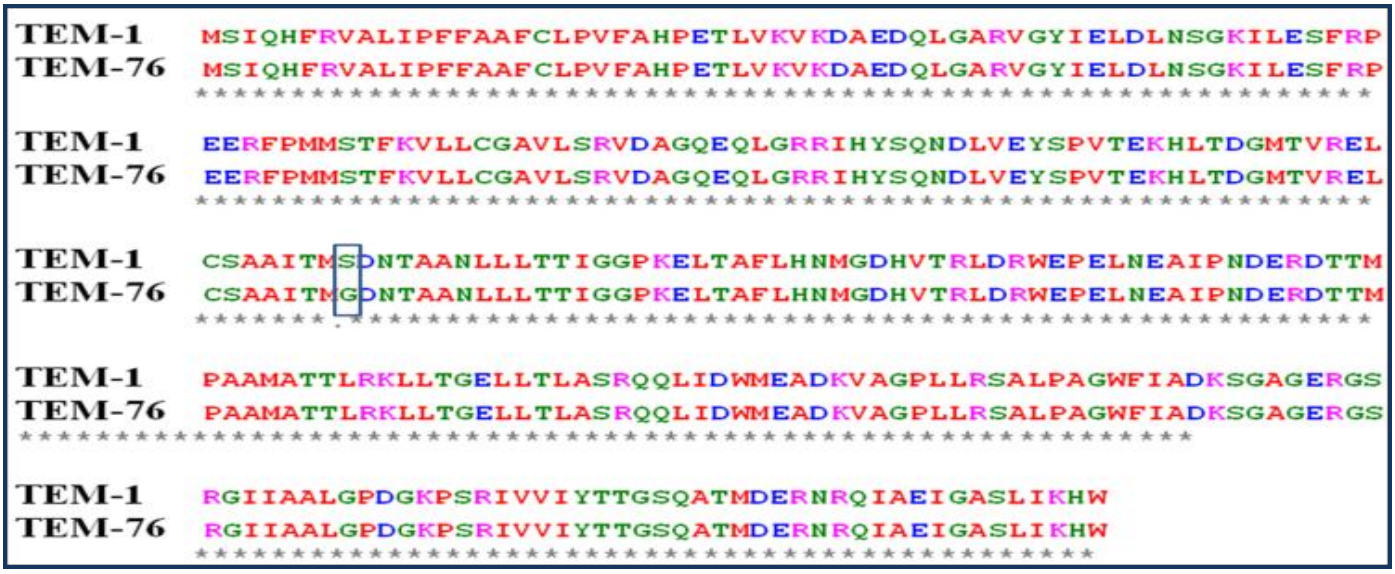

Figure 1: Alignment of TEM-1 with TEM-76

Alignment and Visualisation

The alignment of wild type and S130G mutant of TEM was done using clustalX. The interaction of the above selected compounds within the active site of their respective targets was done using ligplot **[22]** and pymol.

Discussion:

Virtual screening of chemical databases has been an efficient method for discovery and development of new compounds **[23].** In this study we have used virtual screening approach in order to find out compounds with better affinity against the S130G mutant of TEM betalactamase. The alignment of TEM-76 with its wild type is shown in **(Figure 1).** We have also analysed the affinity of these compounds against TEM-1 (wild type) and the results were found to be quite impressive. Out of the total of 1,442 compounds selected for screening, five compounds were found to be affective against both wild type and S130G mutant of TEM type β-lactamase. Presence of water molecules within the active site of β-lactamases is considered to be important in the hydrolysis of drugs, these water molecules appears to serves as the proton donor that is necessary for drug resistance in class A enzymes **[24].**

The compounds that were reported here were checked both in presence and absence of active site water molecules. Out of the five compounds selected, ZINC02775438 was found to be most affective followed by ZINC01738195 and ZINC06143162 **Table 1 (see supplementary material).** These compounds were able to make stable complex within the active site of TEM-76 with goldfitness score of 64.05, 61.88 and 61.13 respectively. It has been reported earlier that TEM-76 shows little susceptibility against three clinically used inhibitors viz., clavulanate, tazobactam, and sulbactam. These traditional inhibitors bind within the active site of TEM-76 with gold fitness score ranging between 32-42 **Table 2 (see supplementary material).** LN-1-255, a 6-alkylidene-2'-substituted penicillin sulfone inhibitor, as an effective β-lactamase inhibitor **[25]** docked with a gold fitness score of 42.28 against TEM-. Penem-1and Penem-2 **[26]** able to make complex with gold fitness score of 42.44 and 43.84 respectively against TEM-76 and was comparable with LN-

1255. The Gold fitness score of all these inhibitors that were set as a reference for comparing the efficacy of screened inhibitors. Hydrophobic interactions and hydrogen bonds play an important role in stabilizing a ligand energetically within the active site of a protein **[27-29].** It was observed that the mutated Glycine residue at 130th position was mostly involved in making hydrophobic contacts with the selected inhibitors. Along with G130 other residues were also found to be playing important role in the binding of inhibitor within the active site of TEM-76. K-73 was found to be a key residue that was actively involved in hydrogen bond formation at several instances. Apart from K-73 there were some other residues that were actively involved in the positioning of inhibitor within the active site they include S70, N132, G130, Y105 and V216. G130 that was the mutant residue was found to be making only hydrophobic contacts with the inhibitors **Table 3 (see supplementary material), (Figure 2 & 3).** Finally we recommend these compounds as potential inhibitors that can be used in future against the S130G and wild type class A TEM betalactamase. However, the outcome of these need to be validated through the experiment analyses.

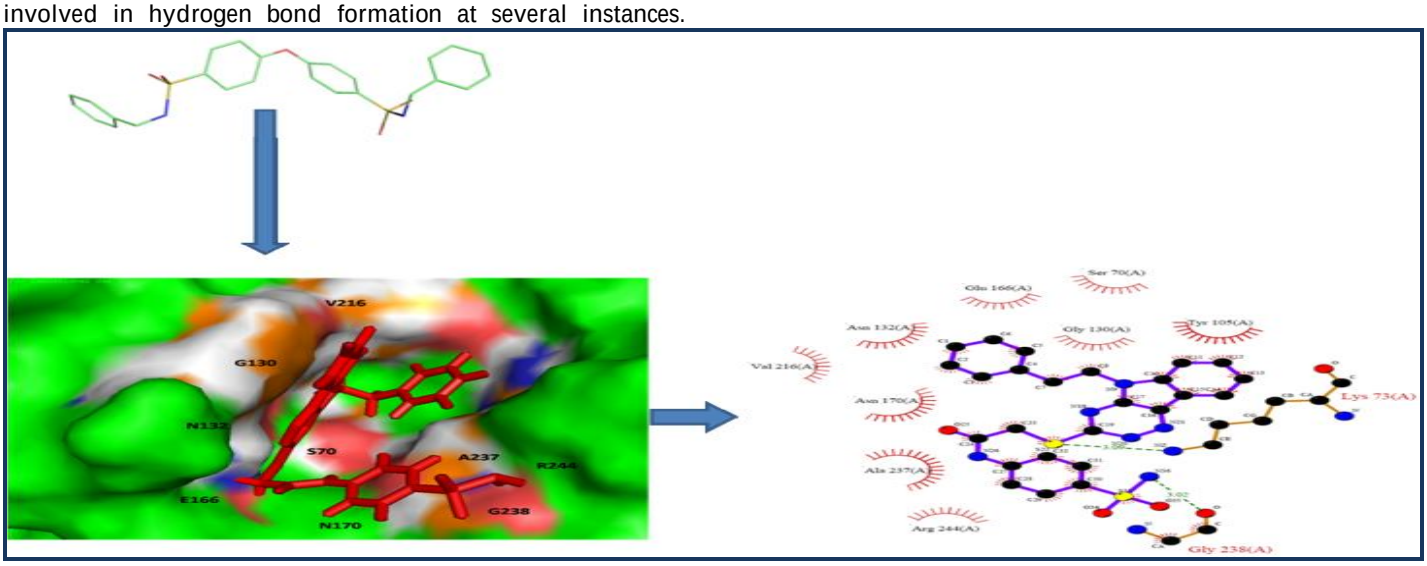

Figure 2: Binding of ZINC02775438 within the active site of TEM-76

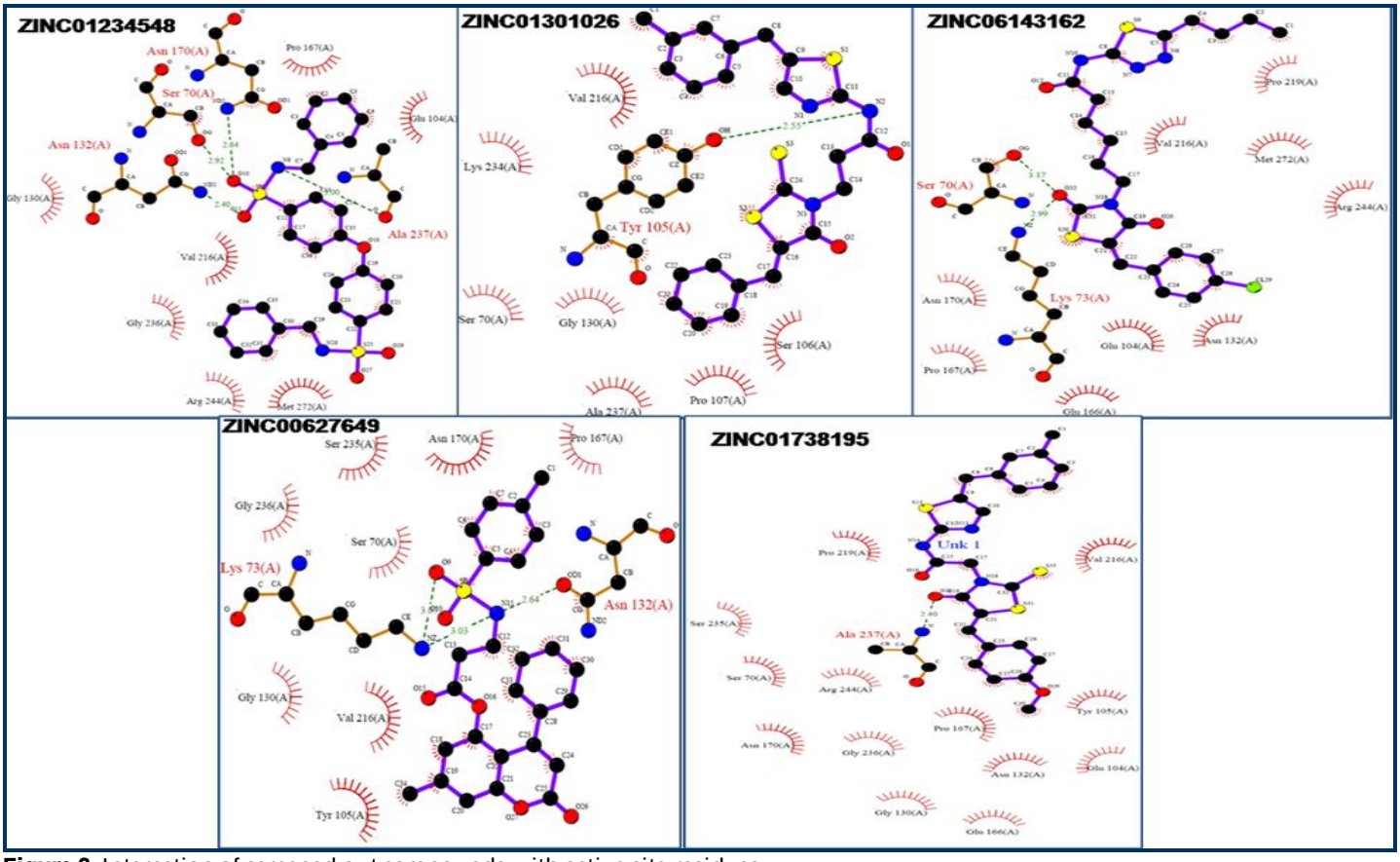

Figure 3: Interaction of screened out compounds with active site residues

Conclusion:
In this study, we have screened out six inhibitors on the basis of their *insilico* binding affinity agsinst S130G inhibitor resistant mutant of TEM using the GOLD 5.0 program. The compounds reported in this study were having better binding affinity both against TEM-76 and its wild type as compare to the known β-Lactamase inhibitors in current use. All the compounds reported in this study were reataining the potential to bind within the active site of TEM-76 also in the presence of water molecule that is considered to be important agent in the hydrolysis of drug. The binding modes exihibited by various docked compounds illustrated the importance of specific residues within the active site region of the targets. Apart from G130, role of some other important aminoacids have also been revealed, that were found to be playing important role in the positioning of inhibitor within the active site. Thus based on above outcomes we conclude that these inhibitors can behave as a lead to drugs against the targets selected for our study. However, some experimentally work need for validating these outcomes.

Acknowledgement:
Authors acknowledge the facilities of Distributed Information Sub-centre, Interdisciplinary Biotechnology Unit, A.M.U., Aligarh. This work was supported by DBT grants, BT/PR11610/BRB/10/669/2008 and BT/PR11453/BID/07/296/2009 to AUK

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Edited by P Kangueane
Citation: Baig *et al.* Bioinformation 8(24): 1225-1229 (2012)

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Supplementary material:

Table 1: Gold Fitness Score of Finally selected compounds against TEM-1 (Wild Type) and TEM

Compounds	Goldfitness score		
	TEM-76		TEM-1
	Without solvent	With solvent	
ZINC02775438	64.05	55.86	61.37
ZINC01738195	61.88	56.58	63.02
ZINC06143162	61.13	57.09	63.39
ZINC01301026	61.16	55.13	65.82
ZINC00627649	61.08	52.50	63.09
ZINC01234548	60.93	53.05	65.43

Table 2: Gold Fitness Score of Known β-lactamase inhibitors against TEM-76

Compounds	Gold Fitness Score
Clavulanic acid	41.37
Sulbactam	32.72
Tazobactam	41.56
LN1255	42.28
Penem-1	42.44
Penem-2	43.84

Table 3: Detailed description of the residues involved in the binding of inhibitors within the active site of TEM-76

	Residues	
	Hydrogen Bonding	Hydrophobic Interaction
ZINC02775438	K73, G238	S70, Y105, G130, N132, E166, N170, V216, A237, G238, R244
ZINC01738195	A237	S70, E104, Y105, N170, V216, P219, S235, R244
ZINC06143162	S70, K73	S70, Y105, G130, N132, P167, N170, V216, S235, G236
ZINC01301026	Y105	S70, Y105, S106, P107, G130, V216, K234, A237
ZINC00627649	K73, N132	S70, Y105, G130, N132, P167, N170, S235, G236