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Original Article

DESIGNING A NOVEL β-LACTAMASE INHIBITOR BY USING QSAR AND DOCKING STUDIES

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ABSTRACT

Objective: Betalactamase is an enzyme secreted by gram negative bacteria, which is responsible for their resistance activity against betalactam antibiotics. Classic inhibitors for betalactamase with less efficiency have been reported. Hence it is of interest to identify new inhibitors.

Methods: Using the structure of known inhibitors as query in pubchem structural search 1516 compounds have been identified and subjected for QSAR studies. It is done with hope to find novel inhibitors with better activity and lesser side effects. Along with the known inhibitors, 24 lead compounds obtained by QSAR studies were docked with β-lactamase.

Results: The results clearly showed that the Compound CID- 4964348 showed higher binding affinity in comparison to known inhibitors and other 23 lead compounds. Also the binding pocket of this lead compound is different, while all 3 known inhibitors bind to the same pocket.

Conclusion: Compound CID- 4964348 should be tested for in vitro and in vivo activities in future for consideration as effective β-lactamase inhibitor.

Keywords: Betalactamase, Betalactamase inhibitor, QSAR, Docking studies, Binding pocket

INTRODUCTION

Emergence of resistance to betalactam antibiotics by gram negative organism has become a serious worldwide problem. This resistance activity in gram negative pathogen was mainly contributed by betalactamase. Betalactamase are enzymes synthesized by bacteria which hydrolyze beta-lactam ring of the antibiotics, deactivating the molecule's antibacterial properties and hence rendering them ineffective before they reach the target. It is believed that betalactamase enzymes are evolved from penicillin-binding proteins with which it shares sequence homology [1].

Various classification schemes for betalactamase have been proposed but they are most commonly classified according to two general schemes: molecular classification and functional classification. Molecular classification was proposed by Ambler in the year 1980 that divides betalactamase into four classes A, B, C & D based on their nucleotide and amino acid sequence homology [2]. Functional classification was proposed by Bush et al., in 1995 that divides betalactamase into four groups (group 1-4) based on their substrate and inhibitor profile [3]. In order to overcome the resistance mechanism due to betalactamase, beta-lactam antibiotics are often prescribed with combination of betalactamase inhibitors.

Clavulanic acid was the first clinically useful betalactamase inhibitor discovered in 1970; a natural compound isolated from *Streptomyces clavuligerus*. The combination of amoxicillin and clavulanic acid (2:1), commonly called by the trade name Augmentin was the first betalactamase inhibitor approved worldwide for clinical usage. Sulbactam and tazobactam are the other two inhibitors of betalactamase in clinical usage introduced in the year 1978 and 1980 respectively. These three traditional betalactamase inhibitors are irreversible suicide inhibitors, which show higher binding affinity for class A betalactamase. Studies have shown that all the three molecules share structural similarity with penicillin. Researchers have also proved that in combination with betalactam antibiotics (amoxy-cillin and ticarcillin for clavulanate, ampicillin for sulbactam and piperacillin for tazobactam), these inhibitors provide broad-spectrum anti-microbial activity [4]. Unfortunately *these inhibitors lack its effect against new variants of betalactamase [5, 6].

Apart from these inhibitors in clinical use, numerous inhibitors have been discovered but found to be a failure because of its higher side effects. Therefore drug discovery often involves the use of QSAR to identify chemical structures that could have good inhibitory effects on specific targets and have low toxicity (non-specific activity) [7].

QSARs are the mathematical relationships linking chemical structures with biological activity using physicochemical property as an interface. QSAR method has many advantages such as; reduces side effects, improves success rate, improves bioavailability and bioactivity, improves understanding of drug receptor interaction, improves understanding of molecular recognition process, reduces cost, reduces time to market the drug [7].

Hence the purpose of present study is to design a preferable inhibitor of β-lactamase, alternative to traditional inhibitors (Clavulanic acid, Sulbactam, Tazobactam) with no or less side effect, by using QSAR studies.

MATERIALS AND METHODS

List of β-lactamase inhibitors in clinical use

A record of inhibitors to β-lactamase enzymes, which is in current clinical use has been prepared by using the literature available at Pubmed (http://www.ncbi.nlm.nih.gov/pubmed) [8].

Compilation of structural features of known inhibitors to β-lactamase

The structural features such as IUPAC name, Canonical Smiles and SD file format relevant to known β-Lactamase inhibitors were collected from PubChem at http://pubchem.ncbi.nlm.nih.gov [8] and employed for QSAR analysis.

Chemical library construction

To construct a chemical library similar to that of known inhibitors (clavulanic acid, Sulbactam and Tazobactam), their structures (SMILES) were used as query in PubChem structural search [8]. Compounds with 80% or > 80% similarities were identified and downloaded as database in SDFile format named as NCBI- 1516.

Prediction of lead compounds

Quantitative structure activity relationship (QSAR) studies have been performed with NCBI- 1516 database and SMILES file of known inhibitors of betalactamase to identify the structure related compounds. To perform QSAR study, Jchem-Screenmd tool (http://www.chemaxon.com) [9] has been used which involves various descriptors like Pharmacophore Fingerprint (PF), Chemical Fingerprint (CF), Total polar surface area (TPSA), Mass, BCUT value, LogD, LogP, Hydrogen Donors (HDon), Hydrogen Acceptors (HAcc).

Docking studies

The structurally related compounds attained from QSAR studies, along with the known inhibitors were subjected to docking studies. This study was done to predict compound(s) with best inhibitory or binding property [10]. It comprises of several steps which are as follows;

a) Obtaining PDB structure of β-lactamases

The first step in docking procedure is to get the tertiary structure of β- lactamase enzyme. It was collected from protein data bank at http://www.rcsb.org/pdb/ [11] with PDB ID 3BLM.

b) Converting lead compounds in SMILES format to PDB format

As both the receptor (β-lactamases enzyme) and ligand molecules (lead compounds) has to be in PDB format for docking studies, the lead compounds attained from QSAR studies has been converted to PDB Format by using J Chem- Marvin View tool (http://www.chemaxon.com). The PDB structure of known inhibitors was downloaded from PubChem at http://pubchem.ncbi.nlm.nih.gov

c) Docking and scoring via Patch dock online tool

Predicted lead compounds and known compounds were docked with β-lactamases using Protein-ligand docking tool PatchDock available at http://bioinfo3d.cs.tau.ac.il/PatchDock/ [12]. The scores of docked lead compounds were acquired and saved. The scores of known ligand were also saved for comparison.

Pocket finder

To analyze the binding site of lead compound and known inhibitor with β-lactamases, Q-finder, an online tool was used [13].

RESULTS AND DISCUSSION

Inhibitors to betalactamase in clinical use

The list of betalactamase inhibitors in clinical use (http://www.ncbi.nlm.nih.gov/pubmed) are shown in the following table;

Table1: List of betalactamase inhibitors

S.no	Inhibitor	Target
1	Clavulanic acid	Class-A betalactamase
2	Sulbactam	Class-A betalactamase
3	Tazobactam	Class-A betalactamase

Structural details of known betalactamase inhibitor

Structural features such as IUPAC name, SMILES and SD file format of known inhibitors to

betalactamase mentioned in table 1 were collected from PubChem at http://pubchem.ncbi.nlm.nih.gov.

A) Clavulanic acid

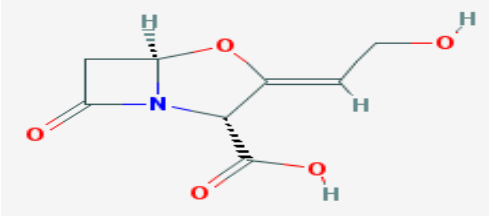
CID : 5280980

IUPACName :(2R,3Z,5R)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane- 2-carboxylic acid.

Canonical SMILES: C1C2N(C1=O)C(C(=CCO)O2)C(=O)O

IsomericSMILES: C1[C@@H]2N(C1=O)[C@H](/C(=C/CO)/O2)C(=O)O

SD file format



B) **Sulbactam**

CID: 130313

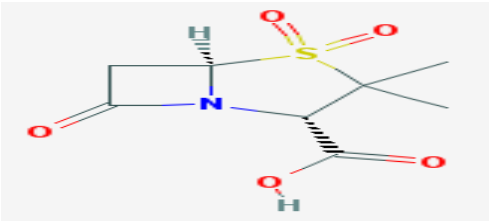
IUPAC Name: (2S,5R)-3,3-dimethyl-4,4, 7-trioxo-4λ6-thia-1-azabicyclo[3.2.0]heptane-2-

carboxylic acid

Canonical SMILES: CC1(C(N2C(S1(=O)=O)CC2=O)C(=O)O)C

IsomericSMILES: CC1([C@@H](N2[C@H](S1(=O)=O)CC2=O)C(=O)O)C

SD file format



C) Tazobactam

CID: 123630

IUPAC Name: (2S,3S,5R)-3-methyl-4,4, 7-trioxo-3-(triazol-1-ylmethyl)-4λ⁶-thia-1-

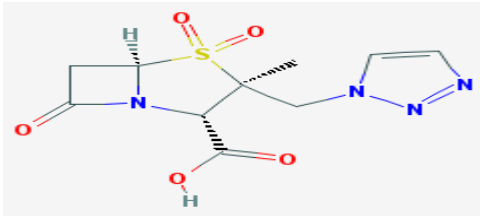
azabicyclo[3.2.0]heptane- 2-carboxylic acid

CanonicalSMILES: CC1(CN2C=CN=N2)C(N2C(CC2=O)S1(=O)=O)C(O)=O

Isomeric SMILES

C[C@]1(CN2C=CN=N2)[C@@H](N2[C@@H](CC2=O)S1(=O)=O)C(O)=O

SD file format



Synthesis of chemical library

The structure of known inhibitors used (clavulanic acid, sulbactam, tazobactam) as a query in PubChem structural search and compounds with 80% or > 80% similarity were downloaded as database.

Table 2: Construction of chemical library

S.no	Known inhibitor (Query)	No. of similar compound in PubChem
1	Clavulanic acid	165
2	Sulbactam	1122
3	Tazobactam	229
TOTAL		**1516**

Totally 1516 compounds were obtained and saved as SD file format with the name of NCBI- 1516.

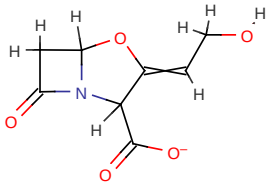
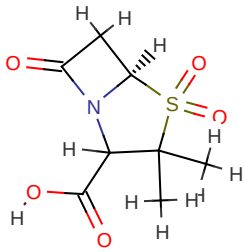
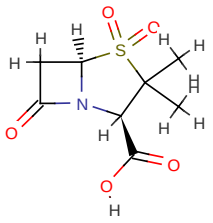
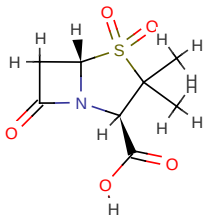
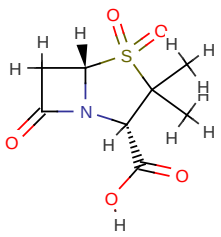
The results have been depicted in table 2.

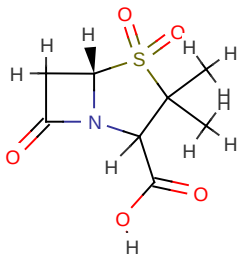
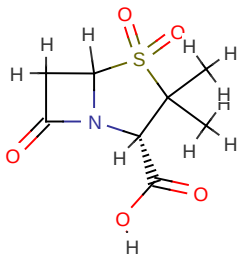
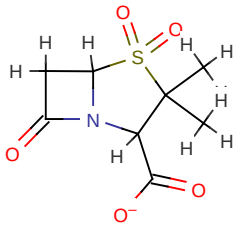
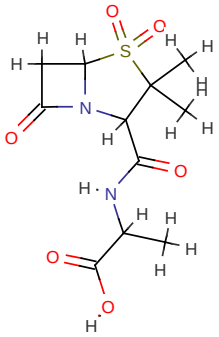
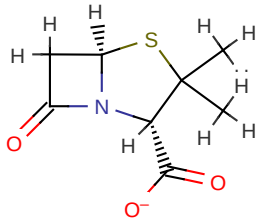
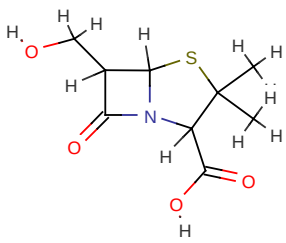
Identification of lead compounds using QSAR study

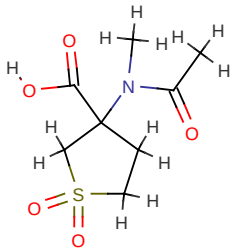
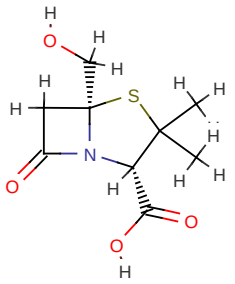
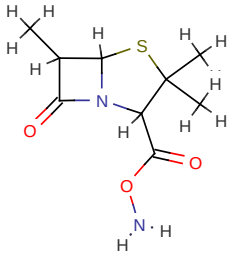
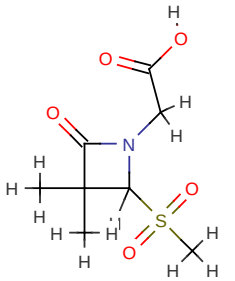
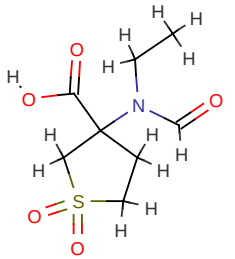
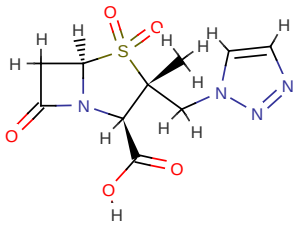
Quantitative structure activity relationship (QSAR) studies were performed with NCBI- 1516 database and SMILES file of known inhibitors of betalactamase using Jchem-Screenmd tool

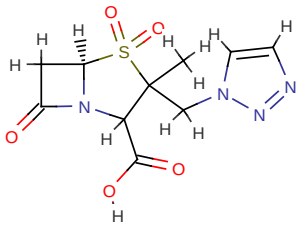
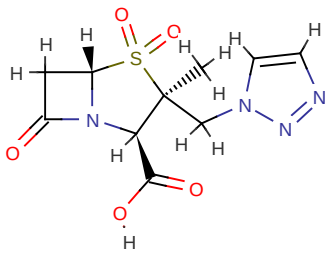
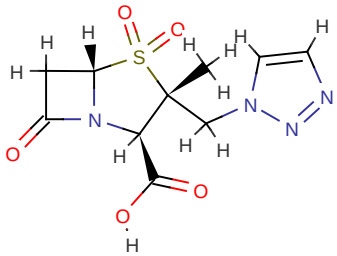
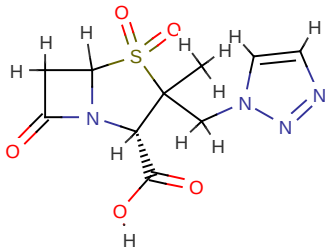
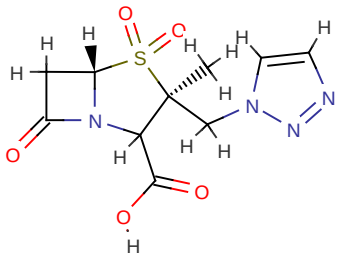
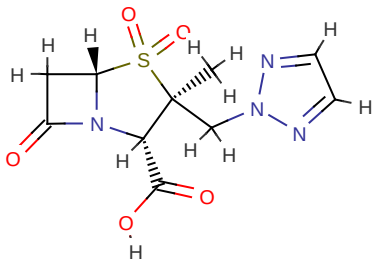
(http://www.chemaxon.com). This resulted in identification of structurally related 24 lead compounds which is shown in the table 3.

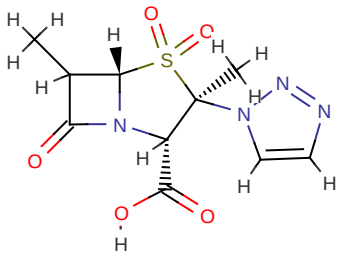
Table 3: Predicted lead compounds in SD file format

S.no	CID	SD file format
1	3762899	
2	50161	
3	667504	
4	820564	
5	7018315	

6	9845669	
7	11841676	
8	3400406	
9	4964348	
10	60087043	
11	20209766	

12	63702440	
13	70486624	
14	71122201	
15	18781969	
16	63687580	
17	1150563	

18	13340877	
19	18637505	
20	28125511	
21	67172806	
22	67803703	
23	67916194	

24	70480661	

Docking studies

Docking score for all the 24 lead compounds was obtained using Protein- ligand docking tool PatchDock available at http://bioinfo3d.cs.tau.ac.il/PatchDock/. The known inhibitors docking score was also obtained for comparison. The result shows that lead compounds CID: 4964348, 1150563, 13340877, 18637505, 28125511, 67172806 and 67803703 scored above all the known inhibitors. Among these seven lead compounds, compound CID- 4964348 scored maximum of 3650. The docking score for known inhibitors and other lead compounds are illustrated in the following table 4 and 5 respectively.

Table 4: Docking score of known inhibitor

S.no	Known inhibitor	Docking score
1	Clavulanic acid	3122
2	Sulbactam	2724
3	Tazobactam	3490

Table 5: Docking score of lead compounds

S.no	CID	Docking score
1	3762899	2826
2	50161	2806
3	667504	2806
4	820564	2806
5	7018315	2806
6	9845669	2806
7	11841676	2806
8	3400406	2802
9	**4964348**	**3650**
10	60087043	2768
11	20209766	3074
12	63702440	2874
13	70486624	2934
14	71122201	3196
15	18781969	2950
16	63687580	2868
17	1150563	3516
18	13340877	3516
19	18637505	3516
20	28125511	3516
21	67172806	3516
22	67803703	3516
23	67916194	3450
24	70480661	3442

Binding site analysis

The binding site of lead compound CID- 4964348 and known inhibitors with β-lactamase was analyzed by Q- Site finder, an online tool. The report shows that all three known inhibitors bind to site 3 where as the lead compound CID- 4964348 bind to site 1(depicted in table 6). This proves that lead compound CID- 4964348 has higher binding affinity towards the target betalactamase enzyme compared to that of known inhibitors (clavulanic acid, sulbactam and tazobactam). The interaction between protein (betalactamase) and drug (lead compound CID- 4964348) has been shown in figure 1(a) & (b).

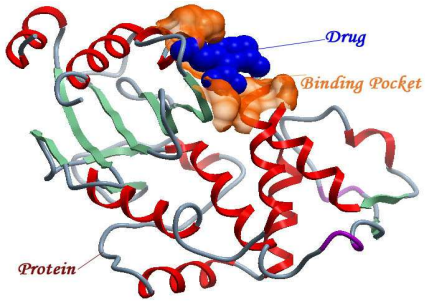


Fig. 1(a)

Fig. 1(a): Binding of the drug (lead compound CID- 4964348) at site 1 of the protein betalactamase.

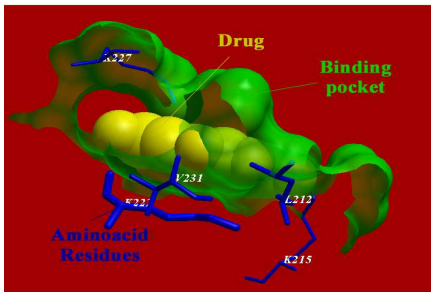


Fig. 1(b)

Fig. 1(b): This figure shows the zoomed image of binding site of the drug to betalactamase.

Table 6: Binding analysis between known and lead compound using Q- Site finder

Compound	Predicted site	Site volume	Protein volume
Clavulanic acid	3	146cubic Angstroms	25481 Cubic Angstroms
Sulbactam	3	146cubic Angstroms	25481 Cubic Angstroms
Tazobactam	3	146cubic Angstroms	25481 Cubic Angstroms
Lead compound CID- 4964348	1	172Cubic Angstroms	25481 Cubic Angstroms

CONCLUSION

Betalactam are the antibiotics which act against bacterial growth by inhibiting cell wall synthesis. But the efficacy of these antibiotics was declined by the synthesis of betalactamase enzyme by bacteria. Since 1970 betalactam antibiotics are prescribed with the combination of betalactamase inhibitor. Unfortunately, the traditional betalactamase inhibitors such as clavulanic acid, sulbactam and tazobactam lose its effect against new variants of betalactamase. This motivated for the search of novel inhibitor with better efficacy than the known inhibitors. The present study is to design a novel betalactamase inhibitor with no or less side effect *by using QSAR studies. To accomplish the aim of the study, list of betalactamase inhibitors namely clavulanic acid, sulbactam and tazobactam (table 1), which is in clinical use was collected from literature available at pubmed. The structural details such as IUPAC name, SMILES and SD file format of these known inhibitors was collected from PubChem and used for the construction of chemical library similar to our study. Using the known inhibitors as query in PubChem structural search with > 80% similarity as criteria, totally 1516 compounds have been identified and saved as SD file database in the name of NCBI1516 (Table2). This database was subjected for further QSAR analysis using Jchem-Screenmd tool, which employs various descriptors like Pharmacophore Fingerprint (PF), Chemical Fingerprint (CF), Total polar surface area (TPSA), Mass, BCUT value, LogD, LogP, Hydrogen Donors (HDon), Hydrogen Acceptors (HAcc). At the end of QSAR study, 24 lead compounds were predicted (table 3). The structurally related 24 lead compounds and known inhibitors were docked with the betalactamase to find the binding property. The lead compound CID- 4964348 obtained maximum docking score than all 3 known inhibitors and other lead compounds (table 4 & 5). The lead compound CID- 4964348 and known inhibitors were analyzed for binding site using pocket finder online tool. This analysis revealed that all the known inhibitors bind to site 3 while the lead compound binds to new site (i.e.) site 1. Thus our insilico study shows that the lead compound CID- 4964348 may have better binding efficacy and greater activity than the traditional inhibitors. In future, however this compound has to be examined extensively in vitro and in vivo activities before possible use in clinical settings as an effective inhibitor.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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