IN SILICO STUDY OF NEW SCHIFF BASE-AND AMIDE-BORONIC ACID DERIVATIVES AS POTENTIAL INHIBITORS OF β-LACTAMASES

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ABSTRACT

Bacteria are becoming more and more resistant to β-lactam antibiotics. One approach to lower such resistance involves combining inhibitors of β-lactamase with β-lactams antibiotics. As such, the need for innovative inhibitors of β-lactamases is urgent. Therefore, the aim of this research was to design and dock two new series of amides and Schiff bases of the cyclic and noncyclic boronate derivatives into four subtypes from two different classes of the β-lactamase enzymes. *In silico* prediction of the pharmacokinetic profile of the designed compounds was also performed. The results revealed possible enhanced activity of 15 out of the 82 compounds, when matched with 4 existing β-lactamase inhibitors (clavulanic acid, sulbactam, tazobactam and vaborbactam). The 15 compounds showed favorable docking interactions with the residues in the active site of all enzymes. The predicted pharmacokinetic characteristics also showed that the 15 compounds are promising as oral agents. The designed compounds have the potential to act as inhibitors of β-lactamase as shown by their docking results on 4 β-lactamase crystal structures. The pharmacokinetic profile of 15 compounds is also promising, making them suitable candidates for synthesis and *in vitro* testing.

Keywords: β-lactamase, docking, boronic acid, Schiff bases, amides

INTRODUCTION

The β-lactam antibacterial drugs were probably the greatest remarkable and efficient drug-related accomplishment in the previous century. They restrain the synthesis of peptidoglycan, which is the principal element of the microbial cell wall causing bacterial mortality[1]. Expansion of resistance in many bacterial species has originated from the prolonged use of β-lactams[2]. Microbial resistance became an extreme health problem challenging the whole world[2,3]. This resistance has now extended to terrifying stages[4]. Consequently, there is an acute necessity to manage this condition. There are two plans to defeat this resistance: (a) the synthesis of new β-lactamase-stable drugs and (b) the invention of strong β-lactamase inhibitors to be co-administered with a β-lactam antibiotics[4,5].

The β-lactamases are enzymes created by microbes and give resistance to β-lactams drugs[6]. The ambler classification makes use of the amino acid arrangement to categorize β-lactamases into four extensive classes: A, B, C, and D[7]. The A, C, and D groups are serine β-lactamases (SBLs) whereas, the B enzymes are metallo-β-lactamases (MBLs)[8,9]. Both the classes result in a β-lactam ring opening, canceling out drug product and its ultimate effect[10]. All β-lactamase classes share a common characteristic by their catalytic mechanism that includes creation of transition states alongside and tetrahedral intermediates (which mimic the boronic acid and phosphonates framework)[11]. Hence, compounds resembling such intermediates are remarkable candidates to be efficient inhibitors of the β-lactamases.

The β-lactamase inhibitors disturb the ability of the bacteria to deactivate β-lactam antibiotics, and their dispensation with β-lactam antibiotics by co-administration is now the most effective approach to oppose the resistance mechanism[12]. These inhibitors combine the β-lactamase enzyme, therefore the β-lactam antibiotic traps the transpeptidase which has the job of peptidoglycan creation and restrict it, thus initiating cell wall lysis[12,13]. Clavulanic acid, sulbactam and tazobactam were the initial β-lactamase inhibitors[14,15]. Development of several new β-lactamase inhibitors is currently in progress, one pattern is the sulfonamide boronic acid[14,15]. Boronate derivatives are regarded as number 2 skeleton for non-

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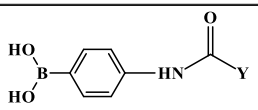
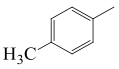
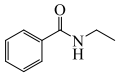
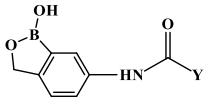
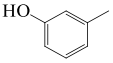
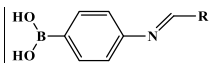
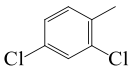
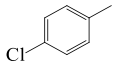
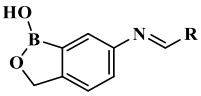
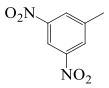
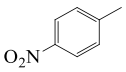
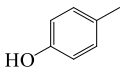
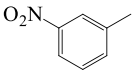
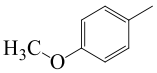
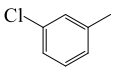
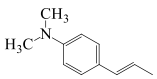
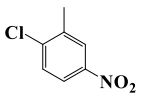
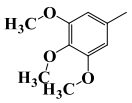
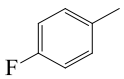
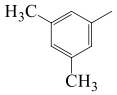
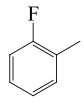
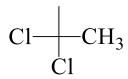
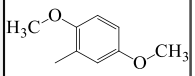
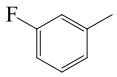
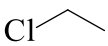
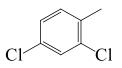
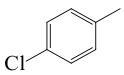
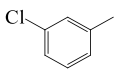
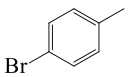
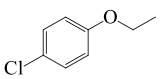
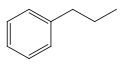
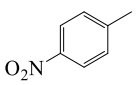
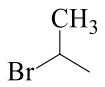
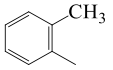
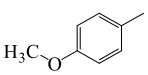
Table I: The structures of the compounds used in this work

A1		**Y12**		**Y27**	
A2		**Y13**		**R 1**	
S1		**Y14**		**R 2**	
S2		**Y15**		**R 3**	
Y1		**Y16**		**R 4**	
Y2		**Y17**		**R 5**	
Y3		**Y18**		**R 6**	
Y4		**Y19**		**R 7**	
Y5		**Y20**		**R 8**	
Y6		**Y21**		**R 9**	
Y7		**Y22**		**R 10**	
Y8		**Y23**		**R 11**	
Y9		**Y24**		**R 12**	
Y10		**Y25**		**R 13**	
Y11		**Y26**		**R 14**	

β-lactam β-lactamase inhibitors. The ability of boron to shape a tetrahedral geometry authorizes it to effectively characterize transient tetrahedral skeletons that are created across hydrolytic reactions[15,16]. Vaborbactam is a strong broad-spectrum inhibitor versus almost all SBLs subclasses (TEM, KPC, CTXM, SHV, CMY), intensifying significantly the efficiency of cefepime carbapenems against class A, C or D β-lactamases[13,17].

Molecular docking is a drug design method, utilized to demonstrate possible molecular interactions and stability in the binding sites[18]. This tool is used in drug design research areas, utilizing computer hardware, software, and algorithms in order to reduce the time and cost of drug production. *In silico* molecular docking is believed to be an encouraging device for drug invention and a practical method for inspection of enormous number of compounds[18,19]. Expectation of potential objectives for a specific receptor utilizing the molecular docking is used to determine the principle or the hit scaffold from the molecular databases using scoring function [20].

Schiff's bases (imines) were originally created by Hugo Schiff in the 19th century. A Schiff's base has a general formula (-N=CH-)[21]. Schiff's bases offer various biological activities including antibacterial, antituberculosis, antifungal, antiviral, antiprotozoal, anticonvulsant, antimalarial, anthelmintic, anti-HIV, anti-inflammatory, antitumor and analgesic activities[22,23].

The amides are important organic functional groups in pharmaceuticals due to their extreme bond polarization and firmness[24]. The most utilized reaction in the synthesis of pharmaceuticals is the amide synthesis. The amides can be distinguished in 25% of drugs in the pharmacies (e.g., valsartan, diltiazem and atorvastatin)[24,25].

The aim of this work is the *in silico* docking for two series of amides and Schiff bases of the cyclic and noncyclic boronate derivatives with four subtypes from different classes of the β-lactamase enzymes. This work is an initial step to choose the best compounds to be synthesized in future work.

MATERIALS AND METHODS

In total, 82 compounds were designed having an amide (A1 and A2 in Table I) or a Schiff base (S1 and S2 in Table I) with noncyclic (A1 and S1 in Table I) or cyclic (A2 and S2 in Table I) boronate. The substitutions on the amide are given by the Y group in Table I, while those on the Schiff base are given by the R group in the same table. All the 82 designed compounds were drawn, and energy minimized using the software Avogadro[26]. The energy minimization was applied using the GAFF force field (General Amber Force Field)[27,28]. The compounds were saved as mol2 files to be used in the docking process. The crystal structures of the β-lactamase enzymes were downloaded from the Protein Data Bank (www.rcsb.org) as the PDB entries 1ERM[29], 2ZD8[30], 6LBL[31] and 6KXI [31]. Water molecules were deleted from the crystal structures before using them, as target proteins for the docking process. The crystal structures had a boronic acid inhibitor in 1ERM, meropenem in 2ZD8 and sulfamoyl heteroarylcarboxylic acids in 6LBL and 6KXI in complex with the protein. These ligands were considered as the original inhibitor and used to define the active site for docking.

GOLD (Genetic optimisation for ligand docking) program version 5.3.0 was the software used for the docking process[32–37]. Default parameters were applied, with 8 Å around the complex ligand to define the active site. After few experiments with the scoring functions offered within GOLD, GoldScore[32] was found to be the best scoring function in terms of its ability to reproduce the crystal pose for the bound ligands. Microsoft Excel was used to analyze the scores of the docked compounds and to rank the best 15 ones using the scores from the four proteins. Accelrys Discovery Studio (DS) Visualizer version 4.0[38] was used to examine the docked poses of the best 15 compounds and to generate the pictures presented in this work. For comparison, 4 standard inhibitors were also docked into the 4 enzymes along with the designed compounds and the bound ligands: clavulanic acid, sulbactam, tazobactam and vaborbactam. It may be important to highlight here that GOLD generates the total score by taking the negative sum of individual energy terms making higher scores indicative of better poses[39], and for the same reason, the scores are unitless[40].

The best achieving compounds in the docking process were further analyzed for their predicted pharmacokinetic characteristics through the online tool SwissADME[41]. The structures of the designed compounds were uploaded to the website and the results were analyzed accordingly.

RESULTS AND DISCUSSION

1ERM and 2ZD8 are type A β-lactamases of TEM-1 and SHV-1 subtypes respectively, while 6LBL and 6KXI are of type B (metallo) β-lactamases belonging to IMP-1 and NDM-1 subtypes, respectively. These enzymes and their subtypes were chosen for the purpose of designing, a universal inhibitor for all the families of β-lactamases. In this work, this was achieved

by selecting the compounds with the best docking scores in all the above enzymes from two series of amides and Schiff bases of the cyclic and noncyclic boronate derivatives, and not just a perfect inhibitor for only a specific enzyme. These universal inhibitors are intended to be able to face and deactivate any type of β-lactamase (mutated or not) as they can modulate and fit the active site of the enzymes from the most important and most common four different subtypes.

The docking protocol used in this work was able to capture the crystal pose for the original inhibitors in the 4 enzymes with a good precision (RMSD values from the original pose of 0.88 Å, 2.36 Å, 0.67 Å and 0.48 Å for 1ERM, 2ZD8, 6LBL and 6KXI, respectively). The docking results of the 82 tested compounds varied for each enzyme, and the same was true for the 4 standard inhibitors (clavulanic acid, sulbactam, tazobactam and vaborbactam). So, for selecting the best 15 compounds

Table II: Docking results and the amino acids interactions within the TEM-1 (1ERM) β-lactamase

Compound	H Bond	Pi-Pi	Pi-Alkyl	Pi-Cation	Halogen	Docking Score
Original inhibitor	SER130, ASN132, GLU166, ALA237, 2ARG244	TYR105	ALA237	LYS234, ARG244		60.38
Clavulanic acid	SER130, ASN132, SER235, 2ARG244					37.34
Sulbactam	TYR105, SER130, SER235, 2ALA237, 2ARG244					43.24
Tazobactam	2SER130, ASN170, SER235	TYR105				45.71
Vaborbactam	SER130, VAL216, SER235, 3ARG244		VAL216	LYS73		54.11
A1+Y3	2SER70, SER235, ALA237		GLY236	ARG244	ASN132	52.76
A1+Y15	ASN170, VAL216, SER235, 2ARG244	2TYR105	VAL216 ALA237			52.21
A1+Y18	VAL216, SER235, 2ARG244	2TYR105	VAL216 ALA237			52.87
A1+Y23	ASN132, SER235, 2ARG244		VAL216 ALA237	ARG244	GLU240	55.09
A1+Y27	SER70, ASN132, ASN170, SER 235, ALA237, ARG244		VAL216			57.28
A2+Y18	SER70, ASN170, ALA237		VAL216			54.49
A2+Y23	ASN132, SER235, ALA237, 2ARG244				GLU240	57.23
A2+Y27	ASN132, SER235, ALA237, ARG244					58.15
S1+R5	ARG244		VAL216, ALA237			55.19
S1+R6	VAL216, SER235, 2ARG244	2TYR105	VAL216, ALA237			52.66
S1+R11	VAL216, ARG244	TYR105	VAL216 2ALA237			54.39
S1+R13	VAL216, SER235, ARG244	2TYR105	VAL216 ALA237			55.16
S2+R5	SER130, SER235, 2ARG244		ALA2327			57.17
S2+R11	SER235, 2ARG244		ALA237			54.34
S2+R13	SER130, ASN132, GLU166		ALA237			55.15

to act as inhibitors, we chose those with the best docking scores and best poses within the four enzymes and not with each enzyme alone. Hence, it is worth mentioning that the scores for the selected 15 compounds in most cases were better than or at least equal to those of the standard inhibitors for each enzyme subtype.

Molecular docking study with the type A TEM-1 (1ERM) β-lactamases

The four standard inhibitors (clavulanic acid, sulbactam, tazobactam and vaborbactam) in addition to the original inhibitor (OI, boronic acid inhibitor)[29] were docked within the active site of this enzyme, and the results are presented in Table II. These results will be as a control for the 15 chosen boronate compounds. Also, the positions of the controls were used to identify the important amino acids residues that define the active site of this enzyme. The interactions of 3D structures of the selected A1+Y27 and clavulanic acid are shown in Fig. 1. A 2-dimensional representation of the interactions is also given in this figure.

Ness and his coworkers identified 10 amino acid residues as the key for the binding to the TERM-1 enzyme[29], and these were SER70, SER130, LYS73, LYS234, SER235, ASN132, ALA237, ARG244, GLU166 as well as and TYR105. They were involved in hydrogen bonding as well as electrostatic and hydrophobic interactions in the original crystal structure. All the 10 amino acids are mentioned in Table II and with the exception of LYS73 and LYS234, all of them are involved in interactions with the 15 chosen designed compounds. None of the designed compounds was able to score better than the

Table III: Docking results and the amino acids interactions within the SHV-1 (2ZD8) β-lactamase

Compound	H Bond	Pi-Pi	Pi-Alkyl	Pi-Cation	Halogen	Docking Score
Original inhibitor	SER70, LYS73, SER130, THR235, ALA237, 4ARG244		TYR105			61.89
Clavulanic acid	ASN132, 3LYS234, 2THR235					47.83
Sulbactam	TYR105, THR235, ALA237, 6ARG244					44.73
Tazobactam	LYS234, 2ALA237, 3ARG244					53.82
Vaborbactam	5ARG244	TYR105	VAL216			67.76
A1+Y3	3SER70, THR235, ALA237, 4ARG244	TYR105	ALA237		ASN132	66.77
A1+Y15	SER70, TYR105, 3LYS234, THR235, ALA237, GLU240 2ARG244	TYR105	ALA237			69.4
A1+Y18	2THR235, ALA237, GLU240, 5ARG244	TYR105	2ALA237			72
A1+Y23	2SER70, ASN132, THR235, 6ARG244		ALA237		GLU240	65.52
A1+Y27	2SER70, ASN132, THR235, ALA237, 2ARG244		VAL216, 2ALA237			69.79
A2+Y18	2ASN132, GLU166, ASN170, THR235, 5ARG244		ALA237			69.88
A2+Y23	SER70, ASN132, THR235, 6ARG244				GLU240	62.9
A2+Y27	SER70, ASN132, 2ALA237, 5ARG244					67.29
S1+R5	THR235, 3ARG244	TYR105	ALA237			71.25
S1+R6	THR235, 8ARG244	2TYR105	VAL216, ALA237			68.61
S1+R11	SER70, THR235, 5ARG244		ALA237			66.62
S1+R13	2LYS73, SER130, THR235, 6ARG244	2TYR105	VAL216 2ALA237	SER70		67.36
S2+R5	5ARG244	TYR105	ALA237			57.98
S2+R11	SER70, THR235, 8ARG244	TYR105				63.33
S2+R13	ASN132, 3ARG244		ALA237			62.3

OI for 1ERM but 11 of the chosen 15 scored better than vaborbactam and all of them scored better than the other 3 standard inhibitors with A2+Y27 scoring the best.

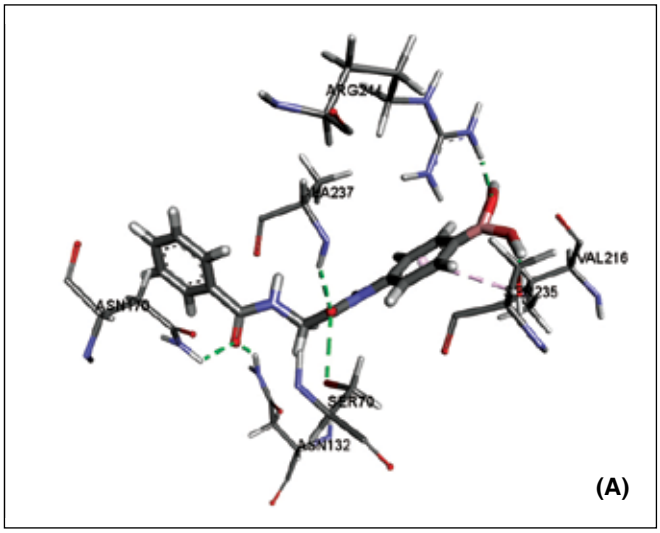
Molecular docking study with the type A SHV-1 (2ZD8) β-lactamases

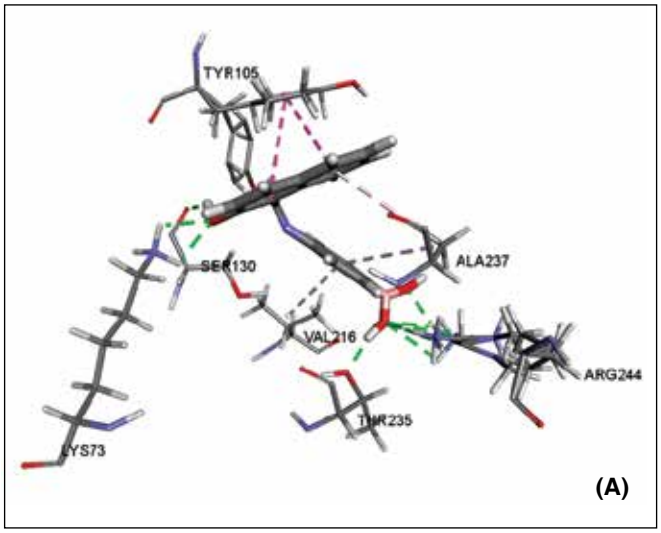
The four standard inhibitors in addition to the OI (meropenem[30]) were docked within the active site of this enzyme, and their interactions are presented in Table III. Nukaga and his coworkers identified 5 amino acid residues as the key for the binding site of the SHV-1 enzyme[30], all of them are included within the key amino acids mentioned in Table III. The interactions of structures of the representatives S1+R13 and vaborbactam are given in Fig. 2.

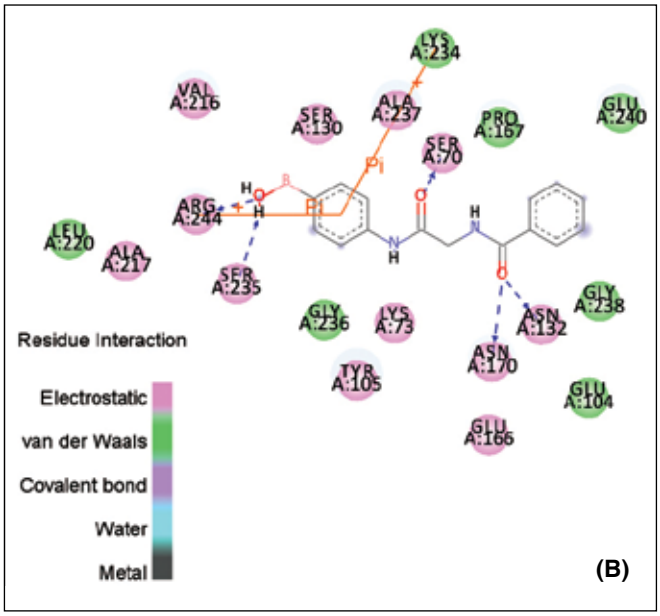
Nine residues were found to be repeating in the interactions of the standard and original inhibitors, and many of them were also found to be interacting with the 15 selected designed compounds. For example, compound S1+R13 bound to 8 of the 9 residues and 3 compounds (S1+R11, S2+R5 and S2+R13) were found to interact with 3 of the 9 residues. This indicates that the chosen designed compounds could bind to the enzyme in a manner that resembles the original and the standard

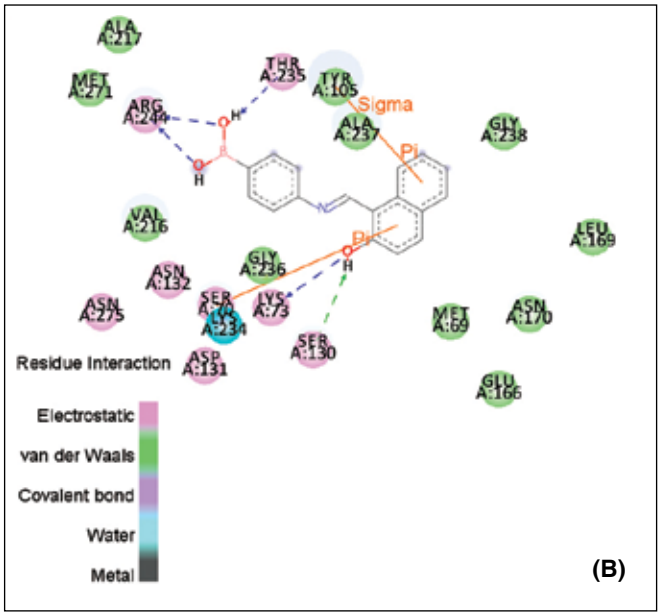
Table IV: Docking results and the amino acids interactions within the IMB-1 (6LBL) β-lactamase

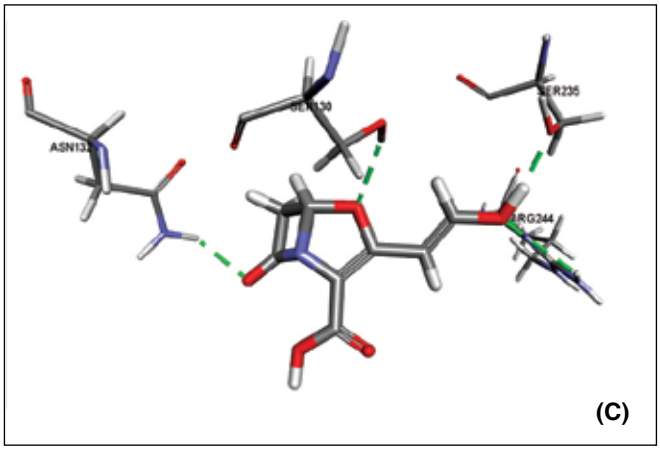
Compound	H bond	Pi-Pi	Pi-Alkyl	Halogen	Pi-Sulfur	Docking Score
Original inhibitor	HIS116, ASP120, HIS196, CYS221, LYS224, 2ASN230	HIS263	VAL61,2TRP64, VAL67		HIS116, HIS196	53.81
Clavulanic acid	HIS118, 2SER119, ASP120, LYS224					40.31
Sulbactam	LYS224, 2ASN230					36.69
Tazobactam	ASP120, LYS224, ASN230, HIS263					56.32
Vaborbactam	ASP120, GLY227, 2ASN230, HIS263	HIS263			PHE87	58.8
A1+Y3	HIS263		VAL61, VAL67, LYS224	TRP64		52.24
A1+Y15	ASP120		CYS221			51.61
A1+Y18	HIS118, ASP120	2TRP64	VAL67, CYS221			57.81
A1+Y23	HIS118, ASP120, ASN230		CYS221, LYS224	LYS224, PRO225		59.5
A1+Y27	2HIS118, ASP120	HIS263	CYS221, LYS224			60.94
A2+Y18	HIS118, ASP120, GLY227		LYS224			57.72
A2+Y23			CYS221	HIS116, HIS118 HIS196, CYS221 HIS263		59.73
A2+Y27	HIS118, HIS196		LYS224			60.8
S1+R5	HIS116, HIS118, ASP120, HIS196	TRP64			CYS221	62.99
S1+R6	ASP120, HIS263		LYS224			54.4
S1+R11	ASP120	TRP64			CYS221	51.19
S1+R13	GLY227, ASN230		VAL67		CYS221	52.75
S2+R5	HIS116, HIS118, HIS196, ASN230	TRP64	HIS263			59.6
S2+R11	HIS118, HIS196, GLY227		LYS224			57.26
S2+R13		HIS263	VAL61		CYS221	53.05











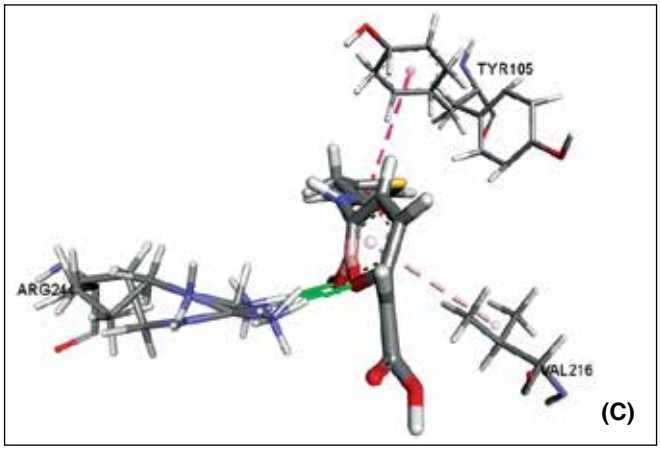
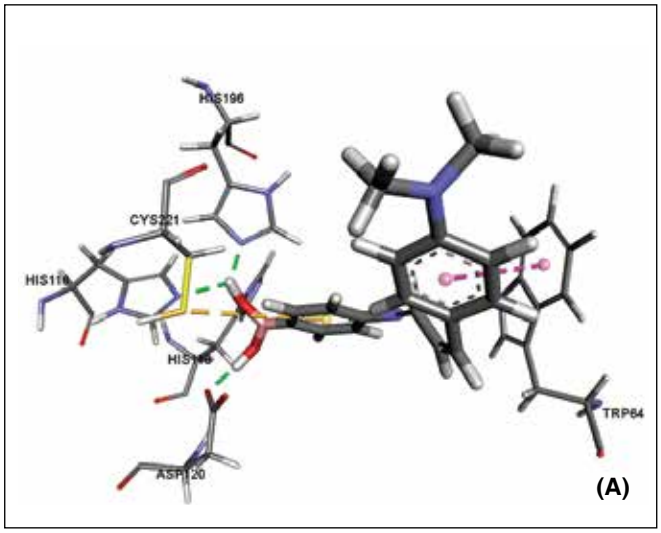
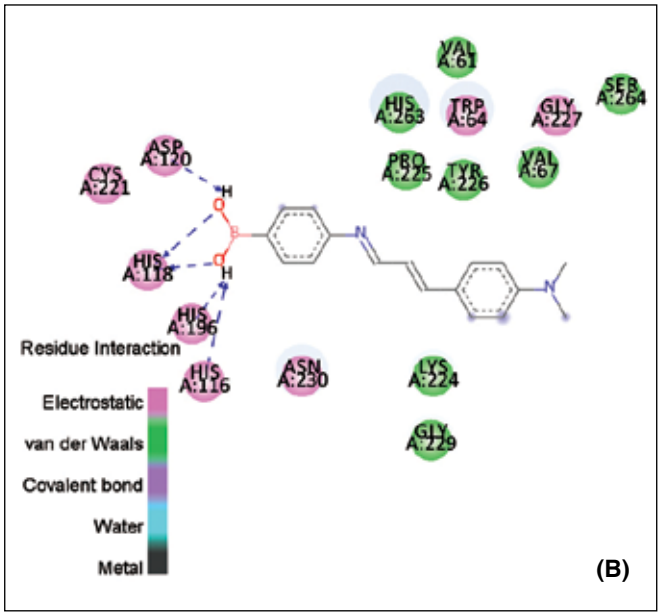


Fig. 1: 3D chemical structure for selected A1+Y27 (A) and clavulanic acid (C) with the TEM-1 (1ERM) enzyme. B: 2D representation of the interactions of A1+Y27 with TEM-1

Fig. 2: 3D chemical structure for selected S1+R13 (A) and vaborbactam (C) with the SHV-1 (2ZD8) enzyme. B: 2D representation of the interactions of S1+R13 with 2ZD8





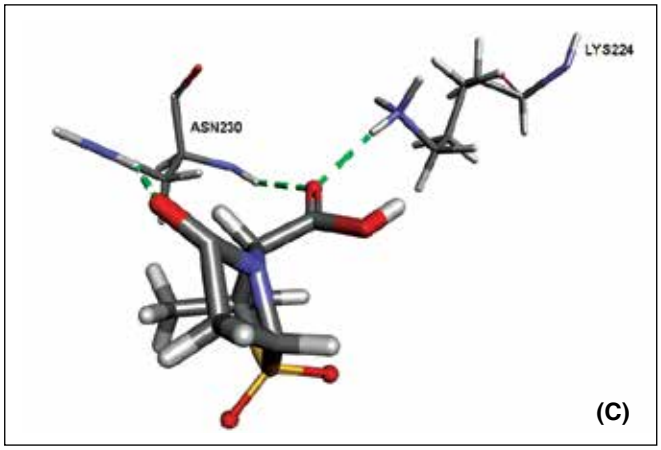
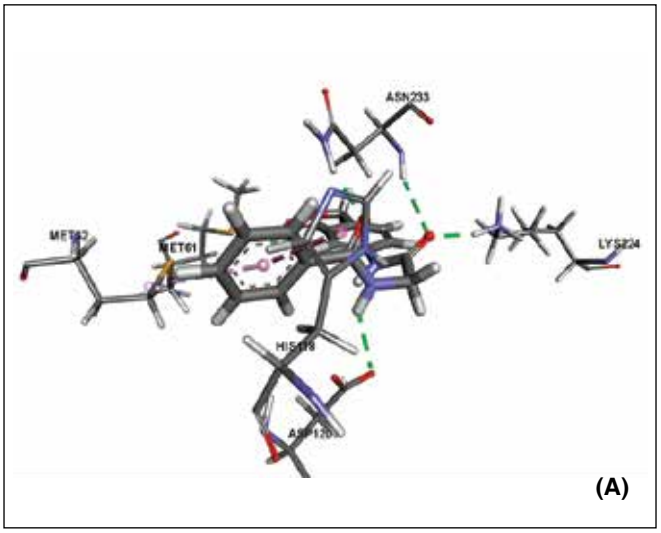
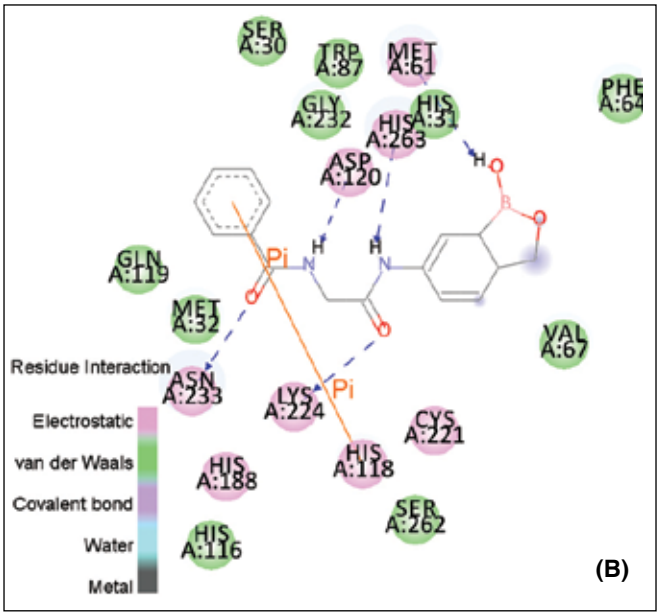


Fig. 3: 3D chemical structure for selected S1+R5 (A) and sulbactam (C) with the IMP-1 (6LBL) enzyme. B: 2D representation of the interactions of S1+R5 with 6LBL





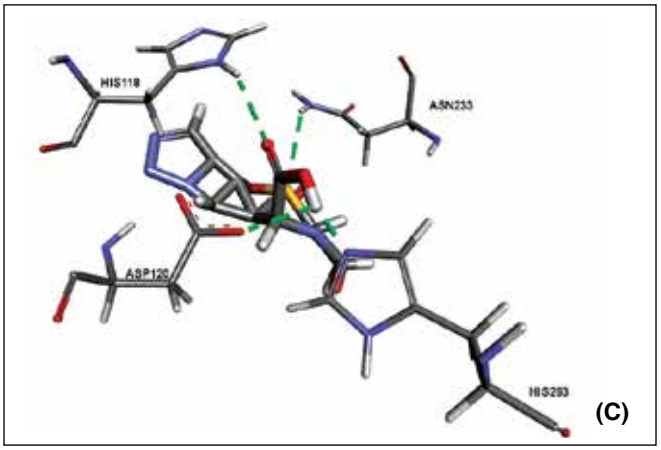


Fig. 4: 3D chemical structure for selected A2+Y27 (A) and tazobactam (C) with the NDM-1 (6KXI) β-lactamase. B: 2D representation of the interactions of A2+Y27 with 6KXI

inhibitors. Six of the selected designed compounds scored better than vaborbactam, 14 scored better than the OI and all of them scored better than tazobactam, clavulanic acid and sulbactam (Table III).

Molecular docking study with the type B IMP-1 (6LBL) β-lactamases

Again, the four standard inhibitors in addition to the original inhibitor (sulfamoyl heteroarylcarboxylic acid[31]) were docked into active site of this enzyme, and their results are given in Table IV. The interactions of the structures of the selected S1+R5 and sulbactam are shown in Fig. 3.

Wachino et al., who published the crystal structure, identified about 11 amino acids (VAL61, VAL67, ASP120, LYS224, ASN230, TRP64, HIS116, HIS118, HIS263, CYS221 and HIS196) which they considered essential for binding to the enzyme[31]. All of them are included in Table IV, especially involving the original inhibitor or the selected designed compounds. The authors of the crystal

Table V: Docking results and the amino acids interactions within the NDM-1 (6KXI) β-lactamase

Compound	H Bond	Pi-Pi	Pi-Alkyl	Halogen	Pi-Sulfur	Docking Score
Original inhibitor	HIS116, ASP120, HIS196, CYS221, LYS224, 2ASN233		TRP87 HIS263		MET32, MET61	56.98
Clavulanic acid	HIS118, ASP120, LYS224					44.29
Sulbactam	HIS116, HIS118, ASN233				TRP87	39.69
Tazobactam	HIS118, ASP120, ASN233, HIS263					53.38
Vaborbactam	ASP120, LYS224, 2ASN233, HIS263	HIS118 HIS263	MET32		TRP87	61.51
A1+Y3	SER30, GLU159, LYS224	HIS118	HIS31, MET32, VAL67	TRP87, MET32, MET61, VAL67		65.61
A1+Y15	SER30, HIS31, GLU159, LYS244	HIS118	VAL67		MET32, MET61	68.39
A1+Y18	SER30, HIS31, GLU159	HIS118 HIS263	MET32		MET61	53.27
A1+Y23	SER30, HIS118, GLU159, ASN233	HIS118	MET32, VAL67	HIS263	MET61	62.2
A1+Y27	SER30, HIS31, HIS118, GLU159, LYS244	HIS118 HIS263	MET32		MET61	63.13
A2+Y18	ASP120	HIS263	VAL67		MET61	57.49
A2+Y23	LYS224	HIS118	MET32	HIS31, HIS118, MET32		59.45
A2+Y27	MET61, ASP120, LYS224, 2ASN233	HIS118	MET32			60.1
S1+R5	SER30, HIS31, GLU159	HIS118	MET32, VAL67			61.76
S1+R6	SER30, HIS118		VAL67		MET32	56.97
S1+R11	SER30, HIS31, GLU159	HIS118	VAL67		MET32, MET61	59.76
S1+R13	HIS31, HIS118, ASP120, 2GLU159	HIS118	MET32, CYS221		MET61	65.94
S2+R5	ASP120	PHE64	VAL67		MET61	55.48
S2+R11	ASP120	HIS263				54.56
S2+R13	LYS224		VAL67		MET61	62.37

Table VI: Pharmacokinetic properties of the tested compounds and the standard inhibitors as predicted by SwissADME

Compound	GI Absorption	BBB* Permeant	Lipinski Violations	Ghose Violations	Veber Violations	Bioavailability Score	Synthetic Accessibility
Sulbactam	High	No	0	0	0	0.56	3.84
Tazobactam	Low	No	0	1	1	0.56	5.59
Clavulanic acid	High	No	0	1	0	0.56	3.75
Vaborbactam	High	No	0	0	0	0.56	3.61
A1+Y3	High	No	0	0	0	0.56	2.34
A1+Y15	Low	No	0	0	1	0.56	2.61
A1+Y18	High	No	0	0	0	0.56	2.37
A1+Y23	High	No	0	0	0	0.56	2.12
A1+Y27	High	No	0	1	0	0.56	1.99
A2+Y18	High	No	0	0	0	0.56	3.16
A2+Y23	High	Yes	0	0	0	0.56	2.88
A2+Y27	High	No	0	0	0	0.56	2.93
S1+R5	High	Yes	0	0	0	0.56	3.12
S1+R6	High	Yes	0	0	0	0.56	2.66
S1+R11	High	Yes	0	0	0	0.56	2.7
S1+R13	High	Yes	0	0	0	0.56	2.71
S2+R5	High	Yes	0	0	0	0.56	3.72
S2+R11	High	Yes	0	0	0	0.56	3.42
S2+R13	High	Yes	0	0	0	0.56	3.51

**BBB: Blood brain barrier*

structure also identified HIS196, HIS118 and HIS116, as also CYS221, HIS263 and ASP120 as important for the catalysis of the enzyme through coordinating the 2 zinc ions, respectively[31]. Therefore, any compound interacting with these specific amino acids will have the potential to inhibit the action of the enzyme. As such, the selected designed compounds through their ability to interact with important active site residues as well as inhibiting the coordination of the zinc ions will be excellent candidates as inhibitors. Regarding how these compounds achieved in terms of their scores, 6 of them scored better than vaborbactam, 9 better than tazobactam, 10 better than the OI and all of them better than clavulanic acid and sulbactam, as can be seen in Table V.

Molecular docking study with the type B NDM-1(6KXI) β-lactamases

The docking results of the four standard inhibitors in addition to the OI (sulfamoyl heteroarylcarboxylic acid[31]) within the active site of this enzyme are shown in Table V. The interactions of structures of the selected A2+Y27 and tazobactam are represented in Fig. 4.

There were 7 amino acid residues (ASP120, MET61, VAL67, TRP87, LYS224, ASN233 and HIS263) identified by the authors of the crystal structure[31] as important for binding to the NDM-1 enzyme. All of them, in addition to others, were among the residues shown in Table V, which interacted with the OI and many of our compounds. In 6KXI, 7 of the designed compounds managed to score better than vaborbactam, and 11 scored better than the OI. They also mostly scored better than the remaining three standard inhibitors.

When analyzing the interactions, of the designed compounds within the 4 enzymes to look for common functional groups, we found that the aliphatic boronates offer better scores and binding interactions than the cyclic boronates, with the same substituents for both the amide and the Schiff bases compounds. However, the amides

appear to give more potent scores and binding interactions than the Schiff bases with the same substituents. This could be due to the hydrogen bonding formed by the carbonyl group, of the amide bond which can potentiate the interaction, while the (-CH=N-) group of the Schiff bases lack such interaction[20,23].

Talking about the functional groups in the chosen 15 compounds, some have nitro group; also, 7 compounds have oxygen in the form of ether, hydroxyl, or carbonyl, and 4 compounds with amino group. All these groups can form hydrogen bonding in different positions and locations within the active site of the enzyme. Another 3 compounds have the chlorine atom that also appears to potentiate the interactions with the enzymes. It is worth mentioning that the chosen 15 compounds have substituents with at least single phenyl ring, in addition to the other aliphatic or aromatic hydrophobic moieties; all are found to potentiate and strengthen the interactions. This is in agreement with previous studies, which identified the binding site of the β-lactamases as hydrophobic in nature that prefers hydrophobic residues[20,23].

ADME study

The 15 compounds that achieved best results in the docking stage, were further analyzed for the prediction of their pharmacokinetic behavior and their drug-likeness. All the compounds, except A1+Y15, were predicted to have high GI absorption and 8 compounds were predicted to cross the blood brain barrier. The latter could be beneficial in the treatment of CNS infections. The bioavailability score of the designed compounds was 0.55, indicating acceptable oral bioavailability that was the same as that predicted for the standard inhibitors used as reference in this work. SwissADME guesses drug-likeness and good oral bioavailability by measuring the number of violations from sets of rules that investigate features such as molecular weight, number of atoms, number of rotatable bonds, polar surface area and others. Such sets include Lipinski[42], Ghose[43] and Veber[44] rules. The tested compounds showed no violations from the Lipinski rules, with only one compound (A1+Y27) exhibiting one violation from the Ghose rules and another compound (A1+Y15) having one violation of the Veber set of rules. The 15 tested compounds, were predicted to be more easily synthesized than 3 of the 4 standard inhibitors, and 14 compounds were more easily synthesizable than the 4 standard inhibitors as predicted by the synthetic accessibility score of SwissADME[41,45]. The results of the pharmacokinetic predictions are presented in Table VI.

CONCLUSION

Molecular docking for 82 cyclic and noncyclic boronate derivatives against four subtypes from two classes of the β–lactamase enzymes indicates possible enhanced activity of 15 compounds compared with the standard inhibitors (clavulanic acid, sulbactam, tazobactam and vaborbactam). These compounds show promising docking interactions with the active site in all enzymes. We can conclude that the active site of both enzymes' classes prefers hydrophobic residues, and the halogenated substituents could potentiate the binding with both enzymes' classes. Also, both the halogenated and the hydrophobic substituents containing oxygen and nitrogen atoms will potentiate the affinity and the binding ability of any compound by forming additional hydrogen bond when they are added to their structures to act as β-lactamase inhibitors. The predicted pharmacokinetic properties of the designed compounds were promising as well in terms of GI absorption, bioavailability, drug-likeness and synthetic accessibility further enhancing the potential of these compounds to be developed into actual drugs.

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