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MOLECULAR DOCKING STUDIES ON PENICILLIN-BINDING PROTEIN 3 OF *STREPTOCOCCUS PNEUMONIA- HUNGARY 19A-6* STRAIN

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ABSTRACT

Streptococcus pneumoniae in human upper respiratory tract causes mucosal diseases. HUNGARY19A-6 strain of *Streptococcus pneumoniae* is extremely virulent in pneumococcal pathogenesis which contains Protein coding gene which synthesis Penicillin-binding protein 3. In this article we performed the homology modeling studies of the virulent protein which is synthesized by the protein coding gene and validated the nature of the receptor as a future drug target for HUNGARY19A-6 strain of *Streptococcus pneumonia*. We have also identified specific ligands for the above mentioned protein by using the virtual structure based ligand screening approach. Protein-ligand complexes have been analyzed by docking studies using Discovery Studio and interactions have also been visualized along with the validation of pharmacokinetic descriptors.

KEYWORDS: **pneumonia, *Streptococcus pneumonia,* HUNGARY19A-6, homology, Docking, Penicillin-binding**

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1 INTRODUCTION

Pneumonia is a bacterial lung infection caused by *Streptococcus pneumonia. Streptococcus pneumonia* is one of the most significant microbe which causes bacterial disease in humans (1-2). *S. pneumoniae* is the most common cause of bacterial meningitis in adults, children, and dogs. The transition from commensal bacterium to an opportunistic pathogen is often occurred after another infection in respiratory tract, eg., pneumococcal pneumonia has been a leading secondary infection for causing death during the period of influenza based pandemics. Invading and colonization of virulence factors into host are exhibited by bacterial pathogens. Most of the virulence factors are displayed on the cell surface includes adhesins that mediate the attachment of toxic substance in host cells. Almost all strains of *S. pneumoniae* produce Pneumolysin, a vital toxin for causing damage in human tissue (3). Polysaccharide capsule of *S. pneumoniae* acts as a virulence factor for the organism; at present there are more than 90 serotypes of *S. pneumoniae* with difference in their prevalence, virulence and extent of drug resistance. It has been estimated that worldwide pneumococcal infections are responsible for the death of more than 1 million people (4-5). General vaccination with the 7-valent pneumococcal conjugate vaccine was recommended in Germany during July 2006 for children greater than 2 years (6-7). In the United States and elsewhere, resistance to a range of antibiotics is increasing among clinical isolates of *S. Pneumoniae* (8-11) The genome of *S. pneumoniae* HUNGARY19A-6 virulent strain is of single chromosome with 2245615 base pairs having 39.6% of GC content. In this present study the Penicillin -binding protein 3 (B1IB40) is been studied.

2 MATERIALS AND METHODS

The sequence of the Penicillin-binding protein 3 (B1IB40) was obtained from UniProtKB. Since this protein do not have a structure, homology model building was performed using Modeler9v7(13). The template structure was obtained from protein data bank (PDB Id: 2IZ1: A). The modeled structures were validated using SAVS, an online server. (12). The CASTp server was used to analyze binding sites of the protein molecules(14). Further, on the basis of high throughput method lead molecules having more affinity with the target proteins were obtained from DrugPort database(15). Then the structurally similar compounds were obtained using PubChem database(16). Finally a datset was created for potential ligands inhibiting the target proteins from the *Streptococcus pneumoniae* HUNGARY19A-6 strain using vegaZZ software. Accelrys Discovery Studio 2.0 was used to analyze specific protein-ligand docked complexes and finally toxicity of the ligand molecules were analysed using ADMET descriptors(17).

3 RESULTS AND DISCUSSION

3.1 Homology modeling

Homology modeling was performed for Penicillin-binding protein 3 (Sequence ID: B1IB40) and was modeled using the template structure (PDB Ids: 2IZ1: A). The modeled protein was validated through SAVS and the validation results are shown in Table I. From the result it is found that 88.4% residues in target proteins are present in the allowed region of Ramachandran Plot. The final modeled protein structures and their corresponding Ramachandran plots are shown in (Figure 1).

Table 1

The percentage of residues of modeled structure present in the allowed region of Ramachandran plot as predicted by SAVS with its similarity and template description.

Target Protein	Sequence length	Template	Description of template	Length	Similarity(%)	Ramachandran Plot (%)
Penicillin Binding protein (B1IB40)	413	2IZ1(A)	Lactococcus lactis	470	30.7	88.4

3.2 Ligand Search

Ligands for proteins Penicillin-binding protein 3 (Sequence ID :B1IB40) were retrieved from DrugPort sharing more identity with related protein sequence for which already a drug exists. The best 3 analogs for each ligands were obtained from PubChem were chosen from the hit. The docking was performed with those analogs using Discovery Studio 2.0 software. Dock score was calculated for all the analogs based on non bonding interactions.

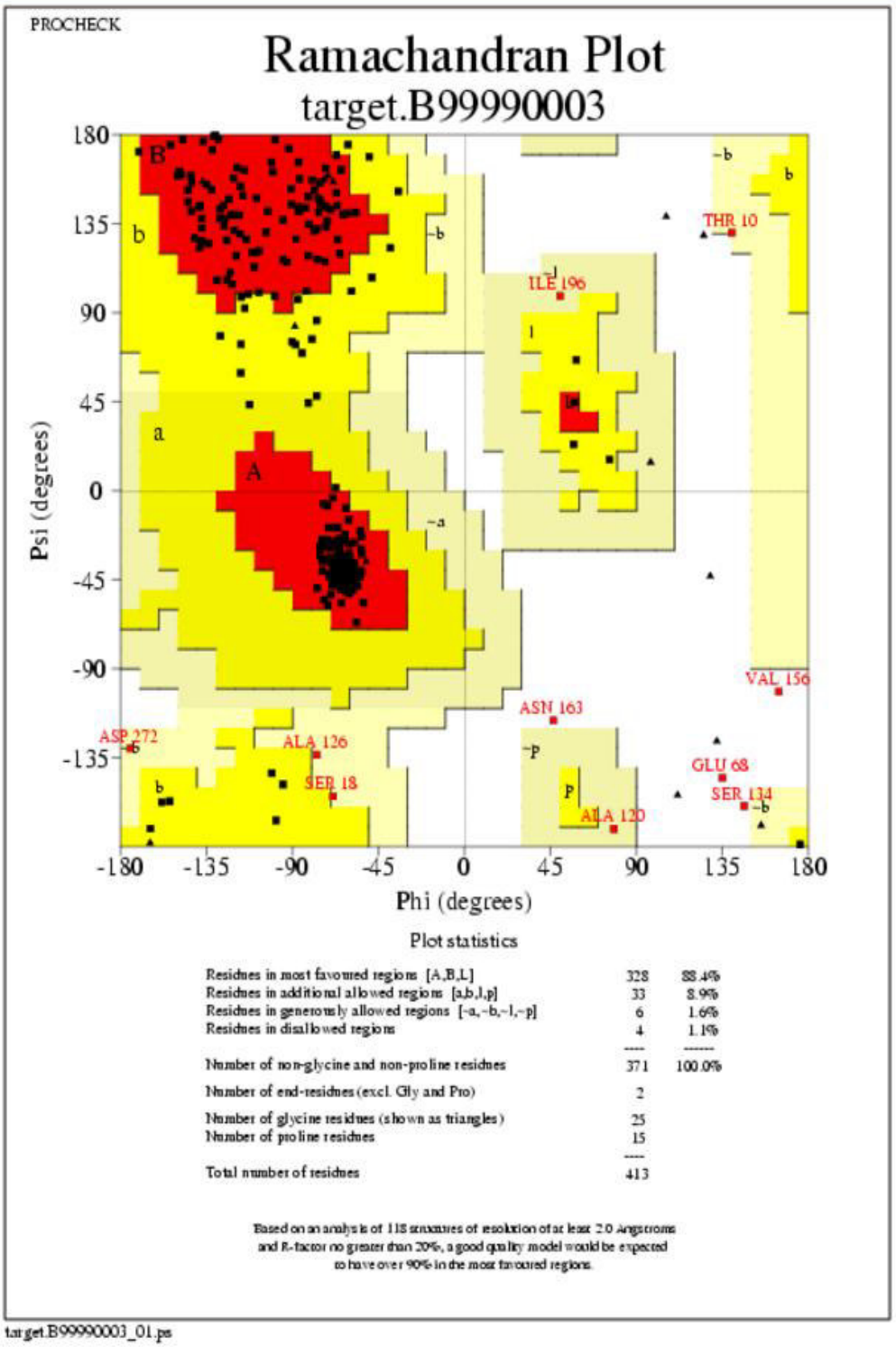


Figure 1
Structure of Penicillin Binding protein (B1IB40) and its Ramachandran plot.

3.3 Docking

The Clindamycin ligand molecule had the best analog compounds (2S,4R)-N-[2-chloro-1-[(2R,3R,4S,5R,6R)-3,4,5-trihydroxy-6-methylsulfanyloxan-2-yl]propyl]-1-methyl-4-propylpyrrolidine-2-carboxamide.,(2S,4R)-N-[(1S,2S)-2-chloro-1-[(2R,3R,4S,5R,6R)-3,4,5-trihydroxy-6-methylsul fanyloxan-2-yl]propyl]-1-methyl-4-propylpyrrolidine-2-carboxamide.,[(2R,3R,4S,5R,6R)-6-[(1S,2S)-2-chloro-1-[[(2S,4R)-1-methyl-4-propylpyrrolidine-2carbonyl]amino]propyl]-4,5-dihydroxy-2-methylsulfanyloxan-3-yl] dihydrogen phosphate with dock score of 40.0,31.368,42.102 respectively. The Cefazolin ligand molecule had a best analog compounds (6R,7R)-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfanylmethyl]-8-oxo-7-[[2-(tetrazol-1-yl)acetyl]amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylicacid.,6R,7R)-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfanylmethyl]-8-oxo-7-[[2-(tetrazol-1-yl)acetyl]amino]-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate., (7R)-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfanylmethyl]-8-oxo-7-[[2-(tetrazol-1-yl)acetyl]amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid with dock score of 53.52,54.84,62.71respectively.ThePentamidine ligand molecule had a best analog compounds 4-[5-(4-carbamimidoyl phenoxy)pentoxy]benzenecarboximidamide.,2-[4-[(E)-4-[4-(4,5-dihydro-1H-imidazol-2-yl)-2-methoxyphenoxy]but-2-enoxy]-3-methoxyphenyl]-4,5-dihydro-1H-imidazole.,2-[4-[(Z)-4-[4-(4,5-dihydro-1H-imidazol-2-yl)-2-methoxyphenoxy]but-2-enoxy]-3-methoxyphenyl]-4,5-dihydro-1H-imidazole with dock score of 44.19,63.94,60.18 respectively. Glyburide, Pioglitazone, Glimepiride were the other ligands having the analog compounds 5-chloro-N-[2-[4-[[cyclohexyl(methyl) carbamoyl]-methylsulfamoyl]phenyl]ethyl]-2 -methoxy-N-methylbenzamide., 5-[[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione.,4-ethyl-3-methyl-N-[2-[4-[(4-methylcyclohexyl)carbamoylsulfamoyl]phenyl]ethyl]-5-oxo-2H-pyrrole-1-carboxamide with dockscore of 45.399,48.542,47.00. The analog compounds with docking score more than 30.0 were considered to be the best for which ADMET studies were performed. The top scoring analogs, PLP(Piecewise Linear Potential) ,PMF(Potential of Mean Force) and Dock score are tabulated in Table II. The protein-ligand interactions at the binding site are shown in (figure 2). Atomic interactions between receptor Penicillin-binding protein 3 (B1IB40) and its ligands are given in Table III.

Table 2

The dock score for ligands obtained from drug port for Penicillin-binding protein 3 (B1IB40)

Ligands	Analogues	PLP1	PLP2	Jain	-PMF	Dock score
Clindomycin	(2S,4R)-N-[2-chloro-1-[(2R,3R,4S,5R,6R)-3,4,5-trihydroxy-6-methylsulfanyloxan-2-yl]propyl]-1-methyl-4-propylpyrrolidine-2-carboxamide.	51.07	46.28	0.34	53.06	40.0
	(2S,4R)-N-[(1S,2S)-2-chloro-1-[(2R,3R,4S,5R,6R)-3,4,5-trihydroxy-6-methylsulfanyloxan-2-yl]propyl]-1-methyl-4-propylpyrrolidine-2-carboxamide.	5.5	48.93	-1.97	49.85	31.368
	[(2R,3R,4S,5R,6R)-6-[(1S,2S)-2-chloro-1-[[(2S,4R)-1-methyl-4-propylpyrrolidine-2carbonyl]amino]propyl]-4,5-dihydroxy-2-methylsulfanyloxan-3-yl] dihydrogen phosphate.	42.68	51.39	-0.57	75.91	42.102
Cefazolin	(6R,7R)-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfanylmethyl]-8-oxo-7-[[2-(tetrazol-1-yl)acetyl]amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.	63.87	54.43	-0.21	115.44	53.525
	(6R,7R)-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfanylmethyl]-8-oxo-7-[[2-(tetrazol-1-yl)acetyl]amino]-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate	63.34	55	-0.14	73.61	54.849
	(7R)-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfanylmethyl]-8-oxo-7-[[2-(tetrazol-1-yl)acetyl]amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	48.45	41.02	-0.85	73.63	62.712
Pentamidine	4-[5-(4-carbamimidoylphenoxy)pentoxy] benzenecarboximidamide	83.37	73.1	1.06	38.35	44.19
	2-[4-[(E)-4-[4-(4,5-dihydro-1H-imidazol-2-yl)-2-methoxyphenoxy]but-2-enoxy]-3-methoxyphenyl]-4,5-dihydro-1H-imidazole	66.69	64.02	0.33	110.06	63.949

	2-[4-[(Z)-4-[4-(4,5-dihydro-1H-imidazol-2-yl)-2-methoxyphenoxy]but-2-enoxy]-3-methoxyphenyl]-4,5-dihydro-1H-imidazole	94.9	86.51	2.35	95.88	60.189
Other ligands	**Glyburide** 5-chloro-N-[2-[4-[[cyclohexyl(methyl) carbamoyl]-methylsulfamoyl]phenyl] ethyl]-2-methoxy-N-methylbenzamide	73.14	73.6	1.71	101.94	45.399
	Pioglitazone: 5-[[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione.	84.16	84.8	1.81	126.84	48.542
	Glimepiride:4-ethyl-3-methyl-N-[2-[4-[(4-methylcyclohexyl)carbamoylsulfamoyl]phenyl]ethyl]-5-oxo-2H-pyrrole-1-carboxamide.	59.46	65.99	0.66	135.45	47.005

3.4 ADMET

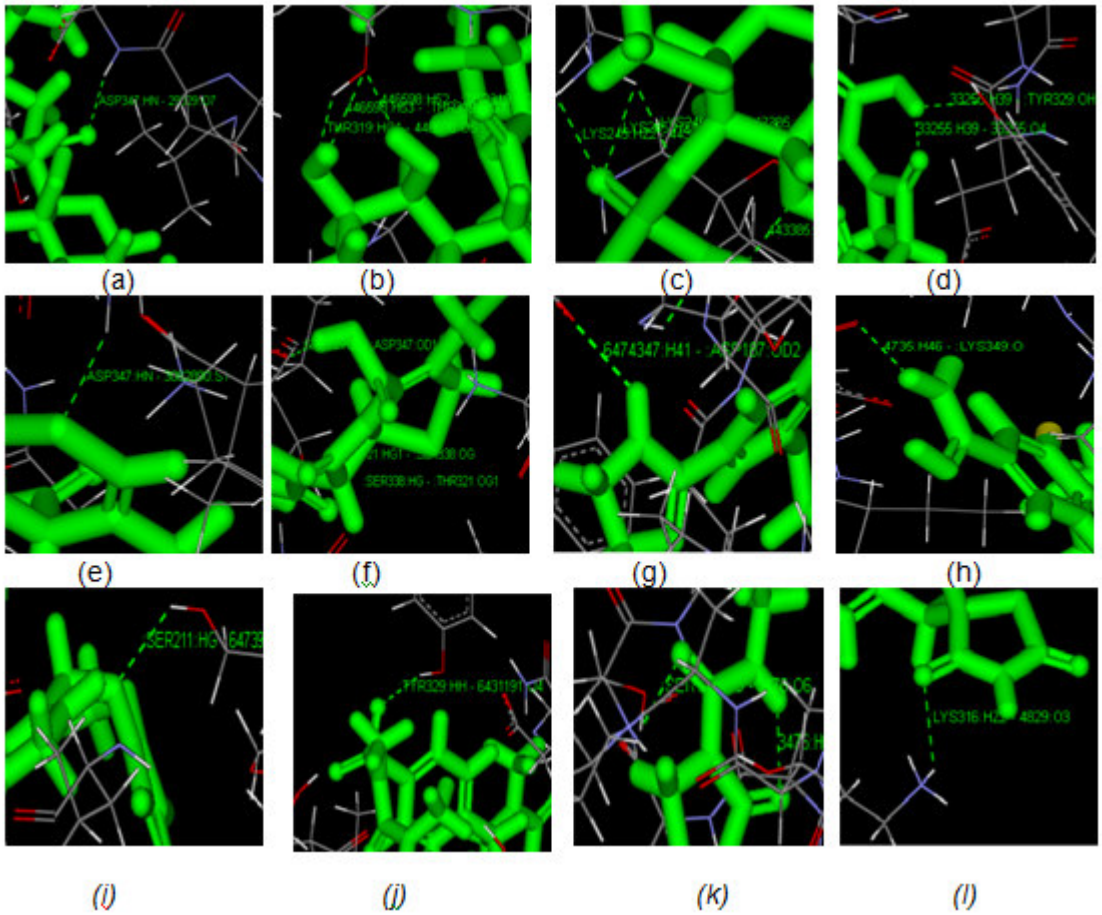
properties for the analogs of ligands having better dock score and maximum interaction with the active site residues were analyzed. The plot of polar surface area (PSA) vs logP is shown in (Figure 3). Based on our analysis, it has been found that the analogs which had maximum dock score have proper lopP, Absorption and Blood Brain Barrier values.

Table 3

Atomic interactions between receptor Penicillin-binding protein 3 (B1IB40) and its ligands

Drugs	ligand	Receptor (B1IB40)		Ligand	Distance
		Amino acid	Atom	Atom	
Clindamycin	Analog 1	ASP347	HN	O7	2.47389
	Analog 2	THR119	OG1	H52	1.80197
	Analog 3	LYS245	HZ2	O11	2.03746
Cefazolin	Analog 1	TYR329	OH	H39	2.16536
	Analog 2	ASP347	HN	S1	1.17689
	Analog 3	ASP347	OD1	H39	1.00817
Pentamidine	Analog 1	LYS349	O	H46	1.90674
	Analog 2	ASP187	OD2	H41	2.12432
	Analog 3	SER211	HG	O1	2.45535
Glyburide	Analog 1	TYR329	HH	O4	1.81182
Pioglitazone	Analog 1	LYS316	HZ2	O3	2.41357
Glimepiride	Analog 1	SER211	HG	O2	2.02786

Figure 2
Docking result of protein B1IB40 with its respective ligands.



(a) (2S,4R)-N-[2-chloro-1-[(2R,3R,4S,methylsulfan2yl]propyl]-1-methyl-4- propyl pyrrolidine-2- carboxamide.
(b)(2S,4R)-N-[(1S,2S)-2-chloro-1-[(2R,3R,4S,5R,6R)-3,4,5-trihydroxy-6-methylsulfanyloxan-2-yl]propyl]-1-methyl-4-propylpyrrolidine-2-carboxamide.
(c) [(2R,3R,4S,5R,6R)-6-[(1S,2S)-2-chloro-1-[[(2S,4R)-1-methyl- 4propylpyrrolidine-2carbonyl]amino]propyl]-4,5 -dihydroxy-2-methyl sulfanyloxan-3-yl]dihydrogenphosphate.
(d)(6R,7R)-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfanylmethyl]-8-oxo-7-[[2-(tetrazol-1-yl)acetyl]amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
(e)(6R,7R)-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfanylmethyl]-8-oxo-7-[[2-(tetrazol-1-yl)acetyl]amino]-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate.
(f) (7R)-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfanylmethyl]-8-oxo-7-[[2-(tetrazol-1-yl)acetyl]amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
(g) 4-[5-(4-carbamimidoylphenoxy)pentoxy]benzenecarboximidamide.
(h) 2-[4-[(E)-4-[4-(4,5-dihydro-1H-imidazol-2-yl)-2-methoxyphenoxy]but-2-enoxy]-3-methoxyphenyl]-4, 5-dihydro-1H-imidazole
(i) 2-[4-[(Z)-4-[4-(4,5-dihydro-1H-imidazol-2-yl)-2-methoxyphenoxy]but-2-enoxy]-3-methoxyphenyl]-4, 5-dihydro-1H-imidazole.
(j) 5-chloro-N-[2-[4-[[cyclohexyl(methyl)carbamoyl]-methylsulfamoyl]phenyl]ethyl]-2-methoxy- N-methyl benzamide.
(k) 5-[[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione.
(l) 4-ethyl-3-methyl-N-[2-[4-[(4-methylcyclohexyl)carbamoylsulfamoyl]phenyl]ethyl]-5-oxo-2H-pyrrole-1-carboxamide.

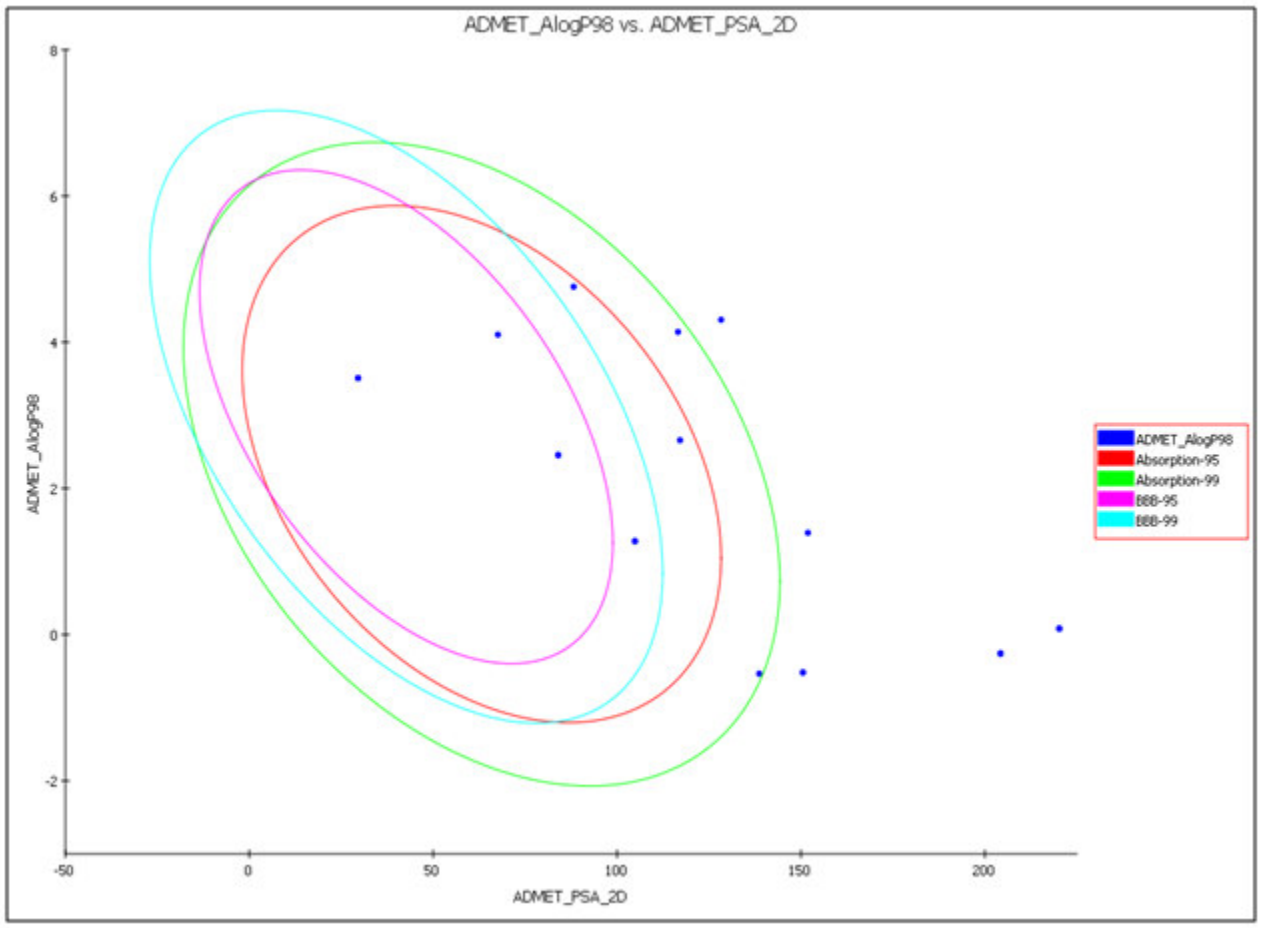


Figure 3
ADMET plot for ligands.

4 CONCLUSION

The results conclude based on docking studies that 2-[4-[(Z)-4-[4-(4,5-dihydro-1H-imidazol-2-yl)-2-methoxyphenoxy]but-2-enoxy]-3-methoxyphenyl]-4,5-dihydro-1H-imidazole is the best ligand for Penicillin-binding protein 3 (B1IB40) with the Dock score of 63.949 with 1 Hydrogen bond interaction with Asp 187 in receptor OD2 and ligand H41 with a bond distance of 2.12. ADMET descriptors were also analyzed for the drug candidates. Hence, this protein can be considered as the drug targets and the above mentioned ligand having highest dock score may be considered as the drug candidate.

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