Research Article

Molecular Docking of Benzoxazolone and its Derivatives against *Staphylococcus aureus* Biotin Protein Ligase

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Accepted on: 05-03-2015; **Finalized on:** 30-04-2015.

ABSTRACT

There is an importunate necessity to reinstate the obtainable antibiotics with new drug molecules to contest with the upsurge challenging bacteria. Resistance towards the drug is as a result of certain critical enzymes present in the bacteria. Thus our present study is focused in inhibiting a critical enzyme of *Staphylococcus aureus*, biotin protein ligase (SaBPL) using benzoxazolone along with its derivatives as ligand. Benzoxazolone are major heterocyclic compounds with benzene and a pyrole fused together, mainly used in the field of pharmacological probe designing. A small set of 36 analogues along with benzoxazolone are used as ligand. Benzoxazolone derivatives were docked in the active site region of SaBPL using Schrodinger glide module. All these compounds display good binding interaction with the target protein, *Staphylococcus aureus* biotin protein ligase. Specifically, when there is a functional group attached either to the benzene ring or to the pyrole ring, binding efficacy is more than with simple benzoxazolone structure. For instance, docking result of ligands S2 and S3 ranks top with a glide (dock) score -7.49 and -7.34, glide energy -40.69 and -37.68 Kcal/mol respectively. On the other hand the simple structure benzoxazolone has a glide score -4.37 and glide energy -29.62 Kcal/mol. Hence the analogues of benzoxazolone may perhaps be an improved alternative drug to combat *Staphylococcal* infections.

Keywords: Benzoxazolone, *Staphylococcus aureus*, Biotin protein ligase, molecular docking, glide.

INTRODUCTION

Food-borne diseases and food spoilage are stern issues allied with human health and trading of food products respectively. These harms ensue primarily attributable to the clinically important gram positive bacteria, *Staphylococcus aureus*. These bacteria show opposition to almost all the antibiotics available in the drug market.[1] Hence there is an importunate necessity to reinstate the obtainable antibiotics with new drug molecules to contest with the upsurge challenging bacteria. These bacteria resist the drug in consequence of certain critical enzymes, one such enzyme is the Biotin protein ligase (BPL). BPL is an impending target, causing bacterial infectivity, implicated by the process called Biotinylation. The microbial BPLs can be alienated into two disconnect category I and II. The category I BPLs are simple structures with two-domain with smallest catalytic unit. Some examples of this category are *Aquifex aeolicus*, *Pyrococcus horikoshii*, *Methanococcus jannaschii* and *Mycobacterium tuberculosis*. The category II BPLs is multipart that include N-terminal domain, in additional which is requisite for DNA binding. The BPL from *S. aureus* (SaBPL) fit into category II.[2] Acetyl-CoA carboxylase and pyruvate carboxylase are the two substrates present in SaBPL that are inactive lacking biotinylation. The former is involved in fatty acid biosynthesis and latter in tricarboxylic acid (TCA) cycle. Both mechanisms are essential for *S.aureus* to cause infection. Thus by inhibiting the metabolic pathway of *S.aureus*, its contagion can be controlled. Genetic studies support the observation that *Sa*BPL is an essential gene product in *S. aureus*. Thus *Sa*BPL have been selectively inhibited *in vitro*.[3] Also another enzyme of *S. aureus*, porphobilinogen synthase which is involved in staphylococcal infections has been identified and its action is blocked using potent analog via an *in silico* method.[4]

Benzoxazole are the biologically significant category of heterocyclic compounds, which comprise of a benzene ring complexed with an oxazole structure. Based on the structural features, benzoxazoles could be considered as bioisosters of nucleotides, such as adenine and guanine, which exist in nature, enabling them to relate directly with the biopolymers of a living organism. Amongst this heterocyclic compounds, Benzoxazolone [BOA] are vital derived metabolites, by reason of their fortunate substructure ("privileged scaffold" which means "motif" or pattern), used in the fabrication of probes for pharmacological purpose.Thus the integration of their pattern to the pharmacophore region provide high grade drug likeliness as a result of functional group properties pertinent to ligand binding.[5]

Derivatives of BOA display varied biological actions such as hepatoprotective properties[6], antipsychotic[7], acetylcholinesterase inhibitory activity[8], antimicrobial[9-11], antiHIV[12] and antibacterial[13] activities.

MATERIALS AND METHODS

Protein Preparation

The three dimensional crystal structure of *Staphylococcus aureus* biotin protein ligase complexes with inhibitor (PDB ID: 3v7s)[3] was retrieved from the Protein Data Bank.

The structural features of the protein and its binding pocket residues were analyzed using PDBsum. The ligand or the inhibitor and water molecule present in the protein was removed using Schrodinger Maestro v9.2 Protein Preparation wizard.

This step is essential as it is refining the protein structure by fixing the error in the atomic representation and optimization.

Ligand Sets and Preparation

A series of benzoxazolone derivatives listed in Table 1 are chosen as ligands, as these molecules are reported to have pharmacological activities.[10-13]

The molecules were drawn in chemical structure sketching software ACD/Chemsketch (http://www.acdlabs.com/resources/freeware/chemsketch).

Schrodinger ligprep module was used for preparation of ligands which involves the conversion of 2D structure of the ligand molecules into three dimensional structures with default parameter and force field OPLS 2005.

Table 1: List of compounds used as ligand for molecular docking.[10-13]

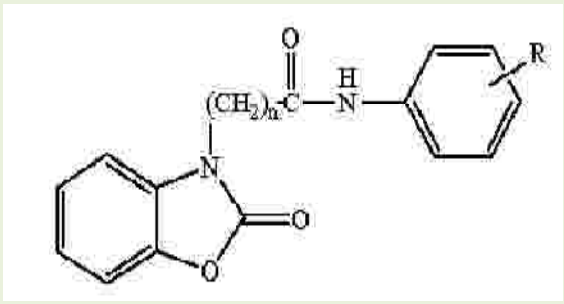
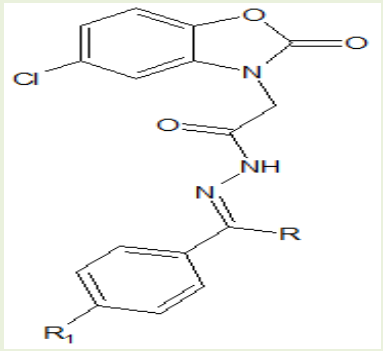
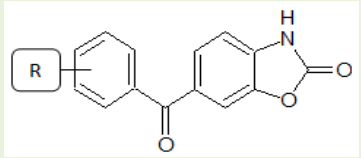
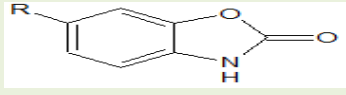
S. No	Compound ID	R	n	R_1	Chemical structure
1	S1	H	1	-	
2	S2	H	2	-	
3	S3	o-CH3	1	-	
4	S4	m-CH3	1	-	
5	S5	p-CH3	1	-	
6	S6	m-CH3	2	-	
7	S7	p-CH3	2	-	
8	S8	p-Cl	1	-	
9	S9	o-Cl	2	-	
10	S10	m-Cl	2	-	
11	S11	p-Cl	2	-	
12	S12	o-OCH3	1	-	
13	H1	H	-	H	
14	H2	H	-	F	
15	H3	H	-	Cl	
16	H4	H	-	Br	
17	H5	H	-	OH	
18	H6	H	-	OCH3	
19	C1	CH3	-	H	
20	C2	CH3	-	F	
21	C3	CH3	-	Cl	
22	C4	CH3	-	Br	
23	C5	CH3	-	OH	
24	C6	CH3	-	OCH3	
25	Kv1	H	-	-	
26	Kv2	2-F	-	-	
27	Kv3	3-F	-	-	
28	Kv4	4-F	-	-	
29	Kv5	3-NO_2	-	-	
30	Kv6	3-$N(CH_3)_2$	-	-	
31	Kv7	3-Cl	-	-	
32	Kv8	3-Br	-	-	
33	Kv9	4-NO_2	-	-	
34	Kv10	4-NH_2	-	-	
35	BOA	H	-	-	
36	MBOA	MeO	-	-	

Table 2: Docking (Glide) score, glide energy and number of hydrogen bonds of the Ligand-Protein (SaBPL) complex.

S. No.	Compound ID	Number of Hydrogen Bonds	Glide score/XP score	Glide energy (Kcal/mol)
1	S2	2	-7.49309	-40.6392
2	S3	2	-7.34502	-37.6814
3	kv4	1	-6.3678	-33.7786
4	kv10	3	-6.03608	-38.2419
5	H2	2	-5.71177	-41.4216
6	C5	2	-5.67125	-46.1151
7	kv3	1	-5.65578	-36.3813
8	S8	3	-5.55756	-44.4529
9	kv2	1	-5.54087	-29.3079
10	kv6	2	-5.5340	-30.1806
11	H3	2	-5.47595	-31.3021
12	kv7	2	-5.45226	-41.2359
13	H5	2	-5.40224	-37.5671
14	kv8	1	-5.3443	-50.3249
15	H4	1	-5.33342	-32.6807
16	C2	1	-5.31116	-32.5073
17	S1	2	-5.18952	-35.6308
18	H1	2	-5.17119	-37.7114
19	S6	2	-5.11707	-28.6182
20	kv5	4	-5.05978	-30.0252
21	MBOA	1	-5.05844	-32.7828
22	S11	2	-5.03583	-37.5718
23	S4	0	-5.03573	-27.5856
24	S5	2	-5.02502	-22.6398
25	S10	1	-5.00268	-34.9168
26	S12	2	-4.89245	-30.8215
27	C4	2	-4.87328	-35.932
28	kv1	1	-4.87156	-36.9001
29	C3	2	-4.84939	-26.6863
30	C6	1	-4.83458	-35.1697
31	S9	2	-4.74694	-33.7582
32	S7	2	-4.65802	-24.4107
33	BOA	1	-4.37807	-29.6244
34	C1	1	-3.49914	-34.051
35	kv9	1	-2.93995	-29.7452
36	H6	2	-2.45376	-30.5487

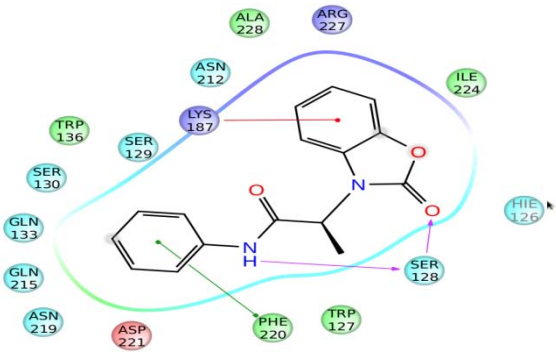


Figure 1: Ligand S2 interacting with residue Ser 128 of the target protein SaBPL

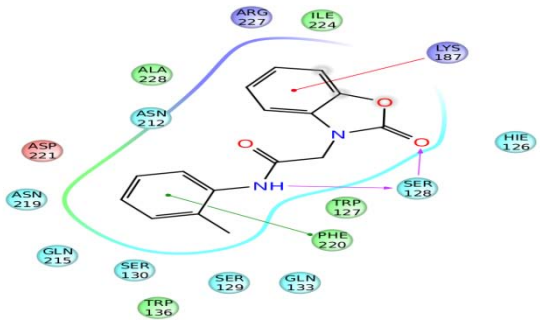


Figure 2: Ligand S3 interacting with residue Ser 128 of the target protein SaBPL

Molecular Docking

Molecular docking is a computational approach that finds best binding orientation between two biomolecules i.e. the ligand and the protein. Docking of Benzoxazolone and its derivatives against the target protein *Staphylococcus aureus* biotin ligase was done using Schrodinger Glide docking module.[14] This docking program uses Monte Carlo based simulation algorithm, and glide SP (Standard Precision) & XP (eXtra Precision) were applied.

RESULTS AND DISCUSSION

Structural Analysis of the docked Protein-Ligand Complex

Crystal structure of receptor protein (SaBPL) is retrieved from ProteinDataBank (PDB), consists of single chain (A) and 329 residues. Ligplot from PDBsum database provides the binding cavity residues information of the target protein. Residues such as Ser128, Ser93, Gln116, Gln95, Asp180, Arg120, Arg122, Arg125, Phe191, Thr94, Gly119, Gly121, Met137, Lys187, Gly190, Leu192, Gly208, Gly210, Ile209, Ile224, Asn212 and Arg227 contribute for the binding interaction of protein with ligand atoms. These binding cavity residues were used as active site region for this docking study.

Docking results given in Table 2 is consolidated based on the glide XP score (or docking score), glide energy and the number of hydrogen bonds interaction between the ligands and the target protein. Table 3 shows the interacting residues of protein and ligands. Ligand S2 ranks top with a glide score -7.49, glide energy -40.69 Kcal/mol and forms two hydrogen bonds.

The hydrogen bond interaction is formed connecting the Oxygen atom (O) of Ser128 with the Hydrogen atom (H) of the ligand S2 shown in Figure 1. The second top place is hold by ligand S3 shown in Figure 2 with a glide score -7.34, glide energy -37.68 Kcal/mol and forms two hydrogen bond interaction same as that of ligand S2. Ligand kv10 takes up the next rank in the docking list with glide score -6.03 and glide energy -38.24, forms three HB interaction as follows: first hydrogen bond interaction is formed amid the Hydrogen atom (HE22) of Gln 116 and Oxygen atom (O) of the ligand.

Second and third HB interaction is formed between Oxygen atom (OD2) of Asp 180 and Hydrogen atom (H34 and H33 respectively). Ligand H2 and C5 both forms two hydrogen bonds, and has glide scores -5.71, -5.67 and glide energy -41.42, -46.11 Kcal/mol respectively. In ligand H2, first HB interaction is formed involving Hydrogen atom (H) of Arg 122 with Oxygen atom (O) of ligand. Second HB interaction is formed flanked by Oxygen atom (O) of Phe191 with Hydrogen atom (H) of ligand. In ligand C5, one HB interaction is formed among Oxygen atom (O) of Arg 227 with Hydrogen atom (H) of ligand and the other HB interaction is formed between Hydrogen atom (HE21) of Gln 215 with Oxygen atom (O) of ligand.

Table 3: Inter molecular hydrogen bond and distance of the docked complex.

LIGAND ID	HYDROGEN BOND Distance (Å)	INTERACTING RESIDUES		
		Protein		Ligand
		Atom	Residue	Atom
S2	1.970	O	Ser 128	H
	2.035	H	Ser 128	O
S3	1.810	O	Ser 128	H
	1.824	H	Ser 128	O
Kv10	1.822	HE22	Gln 116	O
	2.338	OD2	Asp 180	H34
	1.960	OD2	Asp 180	H33
H2	2.087	H	Arg 122	O
	2.438	O	Phe 191	H
C5	2.000	O	Arg 227	H
	2.045	HE21	Gln 215	O
S8	2.134	HH11	Arg 122	O
	2.219	O	Phe 191	H
	1.903	H	Arg 122	O
H5	2.106	HE22	Gln 116	O
	2.166	O	Phe 191	H
Kv5	1.894	HZ2	Lys 187	O
	2.455	HD22	Asn 212	O
	2.428	HD22	Asn 212	O
	2.077	HE21	Gln 215	O
MBOA	2.038	HE22	Gln 116	O
BOA	2.092	HE22	Gln 116	O

Ligand S8 has a glide score -5.55 with glide energy -44.45 Kcal/mol and forms three hydrogen bonds. Firstly the interaction is formed linking the Hydrogen atom (HH11) of Arg 122 with Oxygen atom (O) of ligand. Secondly the interaction is formed relating Oxygen atom (O) of Phe 191 with Hydrogen atom (H) of ligand. Third interaction is formed amidst Hydrogen atom (H) of Arg 122 and Oxygen atom (O) of ligand. Ligand H5 with a glide scores -5.40 and glide energy -37.56 Kcal/mol forms two hydrogen bonds. One of the HB interaction is formed linking the Hydrogen atom (HE22) of Gln 116 and Oxygen atom (O) of ligand, while is between the Oxygen atom (O) of Phe 191 with Hydrogen atom (H) of ligand. Among the 36 compounds used for docking, ligand kv5 has got four hydrogen bonds. This molecule has glide score and glide energy as -5.05 and -30.02 Kcal/mol respectively. Details of the HB interaction of this ligand is as follows: first interaction is flanked by the Hydrogen atom (HZ2) of Lys 187 and Oxygen atom (O) of ligand, and other two interaction is found with the Hydrogen atom (HD22) of Asn 212 and Oxygen atom (O) of ligand. Fourth interaction is formed between the Hydrogen atom (HE21) of Gln 215 and Oxygen atom (O) of ligand. Ligand MBOA has glide score -5.05 and glide energy -32.78 Kcal/mol. The ligand BOA shown in Figure 3 has glide score -4.37 and glide energy -29.62 Kcal/mol. Both MBOA and BOA forms one hydrogen bond and the interaction residue atoms are same. The HB interaction is formed between

Hydrogen atom (HE22) of Gln116 and Oxygen atom (O) of ligand.

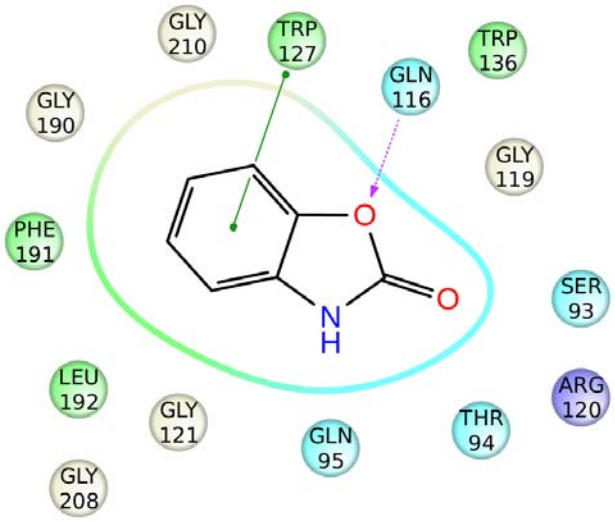


Figure 3: Ligand BOA interacting with residue Gln 116 of the target protein SaBPL

CONCLUSION

Docking study of Benzoxazolone and its derivatives against the disease target *Staphylococcus aureus* biotin ligase (SaBPL) reveals the binding efficiency and interactions between them. *Staphylococcal* infections are severe crisis associated with human wellbeing in regard with causing infection and food spoilage. It's essential to concentrate on inhibiting the source (the bacteria). This could be achieved by blocking the enzymes essential for the bacteria to cause infection. Hence our present study is focused on blocking a key enzyme, Biotin protein ligase of *Staphylococcus aureus*, using a class of heterocycle molecules Benzoxazolone and its derivatives. From the docking analysis it is observed that when the functional group attached either to the benzene ring or to the pyrole ring, better results are obtained. Hence by modifying the functional group and their positions, these derivatives could be used for designing new drug to battle the infections caused by this bacteria.

REFERENCES

1. Amit K, Ajay K, Vandana K, Sandip P, Chandani P and Anil K, Antibacterial potential of some natural food preservatives against *Staphylococcus aureus* isolated from various food samples of Himachal Pradesh (India), World J Sci and Tech, 1, 2011, 48-53.
2. Nicole RP, Steve WP, Grant WB, John CW and Matthew CJW, Purification, crystallization and preliminary crystallographic analysis of biotin protein ligase from *Staphylococcus aureus*, Acta Crystallographica Section F Structural Biology and Crystallization Communications, F64, 2008, 520–523.
3. Tatiana PS, William T, Min YY, Nicole RP, Steven WP, Daniel SP, Renato M, John DT, John CW, Matthew CJW and Andrew DA, Selective inhibition of Biotin Protein Ligase from *Staphylococcus aureus*, J Biological Chemistry, 287, 2012, 17823–17832.
4. Pasupuleti SK, Yellapu NK, Uppu VP, Sthanikam Y, Vimjam S, Gopal S, Katari V, Srikanth L, Valasani KR, Krishna S, *In silico* designing and molecular docking of a potent analog against *Staphylococcus aureus* porphobilinogen synthase, J Pharmacy and Bioallied Sciences, 6(3), 2014, 3-6.
5. Poupaert J, Pascal C and Evelina C, 2(*3H*)-Benzoxazolone and Bioisosters as "Privileged Scaffold" in the Design of Pharmacological Probes, Current Medicinal Chemistry, 12, 2005, 877-885.
6. Lin L, Hui F, Ping Q,Yan M, Lijuan Z, Liping C, Xing L and Jun L, Synthesis and hepatoprotective properties of *Acanthus ilicifolius* alkaloid A and its derivatives, Experimental and Therapeutic Medicine, 6, 2013, 796-802.
7. Brennan JA, Radka G, Steven MG, Rachel LN, Claudine MP, Zoe AH, Qian L, Caitlin W, Sharon L, Farhana P, Margaret L, Deborah S and Goutier W, WS-50030 [7-{4-[3-(1*H*-inden-3-yl)propyl]piperazin-1-yl}-1,3-benzoxazol-2(3*H*)-one]: A Novel Dopamine D2 Receptor Partial Agonist/Serotonin reuptake inhibitor with Preclinical Antipsychotic-like and Antidepressant-like activity, J of Pharmacology and Experimental Therapeutics, 332, 2010, 190-201.
8. Soyer Z, Sulunay P and Vildan A, Synthesis and acetylcholinesterase (AChE) inhibitory activity of some *N*-substituted-5-chloro-2(3*H*)-benzoxazolone derivatives, Marmara Pharmaceutical Journal, 17, 2013, 15-20.
9. Jency S, Sharmila DJ, Patrick G, Emmanuvel L, Viswanathan V and Velmurugan D, Isolation, Purification, Crystal Structure Determination of 1, 3-Benzoxazol-2(3H)-one from *Crossandra Infundibuliformis* flower extract and antimicrobial evaluation, Asian J Phytomedicine and Clinical Research, 2(4), 2014, 210-220.
10. Soyer Z and Erac B, Evaluation of Antimicrobial Activities of Some 2(*3H*)-Benzoxazolone Derivatives, Journal of Pharmaceutical Science, 32, 2007, 167-171.
11. Modiya PR and Patel CN, Synthesis and screening of antibacterial and antifungal activity of 5-chloro-1, 3-Benzoxazol-2(3H)-one derivatives, Organic and Medicinal Chemistry Letters, 2, 2012, 29-39.
12. Kim VD, Huseyin U, Graciela A, Robert S, Jan B, Erik DC and Jacques HP, Synthesis and antiviral activity of 6-benzoyl-benzoxazolin-2-one and 6-benzoyl-benzothiazolin-2-one derivatives, Antiviral Chemistry & Chemotherapy, 10, 1999, 87–97.
13. Bravoa HR, Sylvia VC, Sebastian FD, Madeleine L, and Jose SM, 1, 4-Benzoxazin-3-one, 2-Benzoxazolinone and Gallic Acid from *Calceolaria thyrsiflora* Graham and their Antibacterial Activity, Z Naturforsch, 60c, 2005, 389-393.
14. Maestro 9.0, versuib 70110, Schrodinger, New York (2009).

Source of Support: Nil, **Conflict of Interest:** None.