Original Article

IN-SILICO PREDICTION AND DOCKING STUDIES OF NOVEL SYNTHESIZED BENZOXAZOLE DERIVATIVES AS ANTI-TUBERCULAR ACTIVITY

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Abstract

Modification on the benzoxazole moiety has resulted a various types of derivatives all the derivatives have various biological activities. Molecular docking is the study of how two or more molecular structure (Example- drugs, enzymes and proteins) fitted together. Mainly eight types of benzoxazole derivatives (G3-G10) were synthesized from 4-methyl, 2-amino phenol in the prescence of methanol, potassium hydroxide and carbon disulfide for docking studies. Docking is most authentic approaches in drug discovery for the molecular interactions of compound. In molecular docking different types of software has been used such as Chem draw 3D.16.0, molinspiration cheminformatics, swiss target prediction, rcsb PDB, Discovery studio, PyMOL, swissADME, PASS and Auto dock vina 1.5.7. We have estimated the result in the form of binding energy. Novel molecules were favored on the basis of lowest binding energy (-6.9 to -10.4 Calorie/mol) All the novel synthetic derivatives (G3-G10) were shown more negative energy. On the basis of our studies these all compound may be valuable Pharmacophore against antitubercular activity. *In silico* studies predicted biological activity, physiochemical properties, prediction of activity spectra of substances (PASS), pharmacokinetics and druglikeness.

Keywords: Benzoxazole moiety, Discovery studio, Protein, PyMOL software, Auto dock vina.

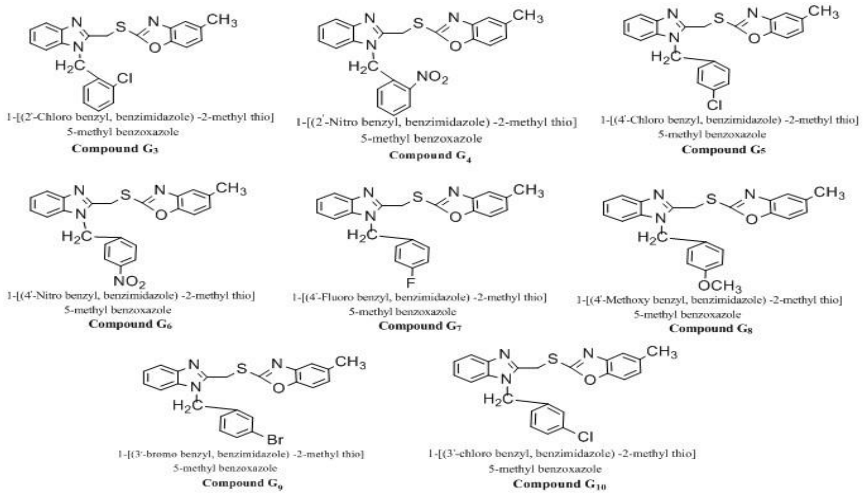
Introduction

Mycobacterium tuberculosis pathogen causes Tuberculosis and take the lengthen time for treatment about six to ninth month. It is the top causes of mortality. In 2017 according the survey report ten million persons were infected by tuberculosis (TB), wherein 3.2 million were adult women and 5.8 million men and one million children. Novel synthesized benzoxazole derivative may inhibit the *mycobacterium tuberculosis* activity [14].

Benzaoxazole is the fused moiety with molecular formula C7H5NO and showed aromaticity [9]. The biological profile of these new generations of benzoxazoles represents more growth with regard to old one derivative. Looking into the therapeutic uses of benzoxazole moiety, it is worthwhile to synthesis definite novel derivative of benzoxazole and screen them for their pharmacological activity [7]. Molecular docking is a technique in which we get to know that how protein interconnected to ligands [8, 10]. Consequently molecular docking is valuable for predict the potency and which kind of indication produced. Docking is one of the most commonly used methods in structure based drugs design [11]. Ability of docking method predicts the binding conformation of accurate target binding and compound ligands. Molecular docking may be three types on the basis of compound ligands i.e. Protein and minute molecules, proteins and nucleic-acid and protein – protein docking [13,1,2]. The aim of ligands

docking is to gives prediction of the compound – receptor complex structure using computation method [12]. This present work beneficially emphasizes the design new drug derivatives (G3-G10). Many different types of software like molinspiration cheminformatics, PyMOL, discovery studio, Chem draw3D 16.0, rcsb PDB, Auto dock vina docking software used in molecular docking of novel synthesize derivatives [3, 4, 6,17].

Figure-1-: Novel synthesized benzoxazole derivatives (G_3-G_{10})



Material and methods:

Ligand Preparation: [5,15,16]

Novel synthesized benzoxazole derivatives (G3-G10) are selected as ligands. Firstly were drawn hypothetically chemical structure (figure-1) of novel Benzaoxazole derivatives by Chem Draw3D.16.0 software and minimized energy and save as lig.pdb file. Further lig.pdb file will open in auto dock vina 1.5.7 and prepared lig.pdbqt file of ligands.

Protein preparation: [5,15,16]

Now predicted the target site (protein) for ligands based on probability by the using Swiss target prediction software after that we were downloaded PDB file of target site by **www.rcsb.org**. Further pdb file open in PyMOL software and remove all extra atoms and extra chain of amino acid and file save as prot.pdb file. Now Prot.pdb file open in Auto dock vina 1.5.7 and prepared prot.pdbqt file of protein.

Molecular Docking:

Preparation of Grid box: [5,15,16]

Auto dock vina 1.5.7 was used for the preparation of grid box. Prot.pdbqt file open by grid macromolecule in auto dock vina1.5.7. Now we were selected amino acid for binding and adjusted gird box (X, Y and Z axis) that all selected amino acid should come under grid box and file save as conf.txt file. Grid box dimensions were showed in **table-1**.

Table no- 1: Dimension of grid box[16]

Compound name		G3	G4	G5	G6	G7	G8	G9	G10
Grid dimension	size_x	40	40	40	40	40	40	40	40
	size_y	40	40	40	40	40	40	40	40
	size_z	40	40	40	40	40	40	40	40
	center_x	55.749	95.414	19.646	35.266	19.646	-1.367	21.017	55.749
	center_y	11.214	107.312	-8.278	-1.144	-8.278	63.921	-2.638	11.214
	center_z	56.132	93.464	23.190	18.615	23.190	16.961	35.074	56.132

The entire prepared file such as lig.pdb, lig.pdbqt, prot.pdb, prot.pdbqt and conf.txt were saved in a same folder and command prompt software was used with some commands and finally The molecular docking outcome were obtained in the form of log file and all ligand.pdbqt. Which ligand site having more negative binding energy was opened in PyMOL software and save as complex file in pdb format. Complex file was opened in Discovery studio for the visualization of ligand and protein interaction in the form of 3D and 2D diagram.

Result and Discussion:

The cheminformatics of the novel synthesized derivatives or ligands as ligands name, IUPAC name and smile file are given in **table-2**.

Table-2: Ligand name, IUPAC name and smile file

S.No	Ligands Name	IUPAC Name	Smile file
1	G_3	1-[(2'-Chloro benzyl, benzimidazole) -2-methyl thio] 5-methyl benzoxazole	Cc5ccc4oc(SCc2nc1ccccc1n2Cc3ccccc3Cl)nc4c5
2	G_4	[(1-(2'- nitro benzyl) benzimidazole--2-yl)]methyl thio,6-methyl benz-oxazole	Cc5ccc4oc(SCc2nc1ccccc1n2Cc3ccccc3N(=O)=O)nc4c5
3	G_5	[(1-(4'-Chloro benzyl) benzimidazole - 2-yl)]methyl thio, 6-methyl benzoxazole	Cc5ccc4oc(SCc2nc1 ccccc1n2Cc3ccc(Cl) cc3)nc4c5
4	G_6	[(1-(4'- nitro benzyl) benzimidazole- 2-yl)]methyl thio, 6-methyl benz-oxazole	Cc5ccc4oc(SCc2nc1ccccc1n2Cc3ccc(N(=O)=O)cc3)nc4c5
5	G_7	1-[(4'-Fluoro benzyl, benzimidazole) -2-methyl thio] 5-methyl benzoxazole	Cc5ccc4oc(SCc2nc1ccccc1n2Cc3ccc(F)cc3)nc4c5

6	G_8	1-[(4'-Methoxy benzyl, benzimidazole) -2-methyl thio] 5-methyl benzoxazole	COc5ccc(Cn4c(CSc2nc1cc(C)ccc1o2)nc3ccccc34)cc5
7	G_9	1-[(3'-bromo benzyl, benzimidazole) -2-methyl thio] 5-methyl benzoxazole	Cc5ccc4oc(SCc2nc1ccccc1n2Cc3cccc(Br)c3)nc4c5
8	G_{10}	1-[(3'-chloro benzyl, benzimidazole) -2-methyl thio] 5-methyl benzoxazole	Cc5ccc4oc(SCc2nc1ccccc1n2Cc3cccc(Cl)c3)nc4c5

Ligands (G3-G10) were drawn by Chem Draw3D 16.0.The lig.pdb file and lig.pdbqt file of the novel derivatives (G3- G10) be not exist in any database because it was a recently drawn molecule. In **table -3** selected target sites, Interacting residues, and PDB Ids by Swiss target prediction and ligands compatibility with PDB.

Table -3: Showing promising PDB Ids and target of the compounds (G3 and G10)[5,16]

S.No	**compound Name**	**Target**	**Interacting residues**	**Common name**	**PDB Id**
1	G_3	Orexin receptor 2	THR-111, PRO 131, VAL 138, PHE227, ILE320, HIS350, VAL 353, TYR354	HCRTR 2	4S0V
2	G_4	Adenosine A1-receptor	PHE 47, LEU 99, ALA 100, VAL 103, ASP 104, LEU 107, VAL 119, ARG 123, VAL126, ALA 127	ADORA 1	6D9H
3	G_5	Cathepsin (B&K)	CYS 29, GLY 73, HIS 111, VAL 176, LEU 181, TRP 221	CTSB	1 CSB
4	G_6	Cathepsin S	LYS 17, TYR 18, TYR 92, ASP87, PRO 91	CTSS	2G7Y
5	G_7	Cathepsin (B&K)	CYS 29, GLY 73, HIS 111, VAL 176, LEU 181, TRP 221	CTSB	1 CSB
6	G_8	Kinase	GLY882, HIS 885, GLY887, VAL 889 GLU 925, ASP 1003, LEU 1010, LEU 1024	JAK 1	4EHZ
7	G_9	Cannabinoids receptor	LEU 165, ILE 169, TYR 172, PHE 191, LYS 192, GLY195, ALA 198, TRP241, ILE 245	CNR 1	6KQI
8	G_{10}	Orexin receptor 2	THR-111, PRO 131, VAL 138, PHE227, ILE320, HIS350, VAL 353, TYR354	HCRTR 2	4S0V

The molecular docking was done by Auto Dock Vina 1.5.7 and the result was predicted in the form of binding energy. Which are given in **table -4.**

Table -4: Showing summary of molecular docking results as binding energies of the novel compounds, hydrogen bond, hydrophobic interaction and pi-pi interaction[5,16]

S.No	**Compound name**	**Binding Energies (Kcalories/mol)**	**No of Hydrogen bonds**	**Hydrophobic Interactions**	**Pi-Pi interaction**
1	G_3	-9.7	-	VAL114,TRP120,ILE-130,GLN-134, THR-135,SER-321,TYR-317,ASN-324 TYR-354,THR111	HIS-350
2	G_4	-6.9	-	ALA-124, ASP104	PHE-47
3	G_5	-7.5	-	MET-196,GLY-198,GLY-197,HIS-199 GLN-23, GLU-122,GLY-73, GLY-27 TRP-27,TRP-30,GLY74	-
4	G_6	-7.4	1	GYS-93,ALA-94,GLY-20,GIN-19, VAL-16, PHE-28, GLY-188,HIS-189	TYR-92
5	G_7	-9.0	1	MET-196,GLY-197,GLY-73, GLU-245 TRP-30,PRO-76,GLY-74,CYS-119, GLU-122,CYS-26,SER-25,GLY-24, HIS-110,GLN23,GLY-27,TRP-221, HIS -199	-
6	G_8	-9.6	1	ASP-921,LEU-922,GLY-887,GLU883 LYS-908,GLY-884,GLY-882,LEU-881,GLY-1020,ASP-1021,ARG-1007, ASP-1003,ASN-1008,ASP-1042,PHE-886	-
7	G_9	-8.1	-	VAL-161, GLY-194,ALA-248, VAL-249,GLY-195, SER-199	TRP-241 PHE-191
8	G_{10}	-10.4	-	GLN-187,MET-191, ILE-130, TRP-120, THR-135,SER-321, GLN-134, HIS-350, THR-111	-

Molecular docking was planned to find out the conformation of interaction of ligands– Target. Some selected Compound showed good docking interaction. **Figure 2-9** are showing 2-D and 3-D interactions between molecules and protein.

Figure 2: 2-D and 3-D interactions between ligand (G_3) and protein (4S0V)

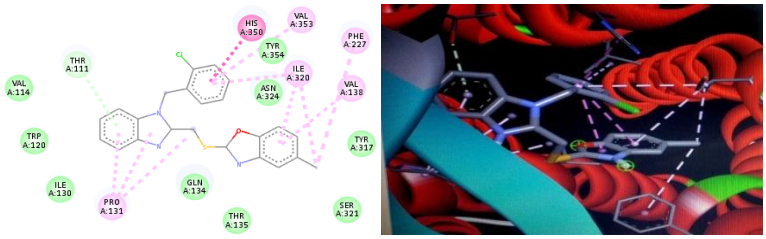


Figure 3: 2-D and 3-D interactions between ligand (G_4) and protein (6D9H)

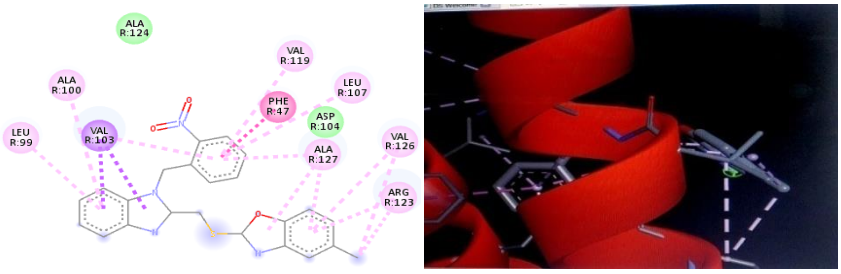


Figure 4: 2-D and 3-D interactions between ligands (G_5) and protein(1CSB)

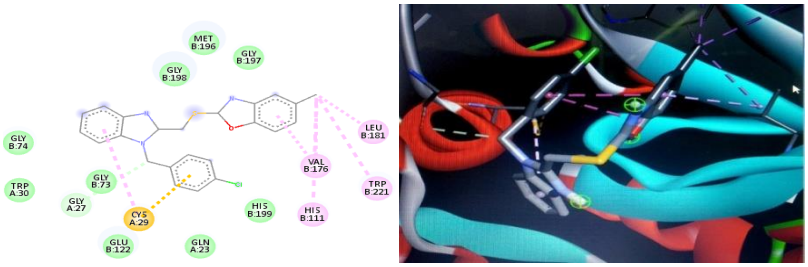


Figure 5: 2-D and 3-D interactions between ligand (G_6) and protein (2G7Y)

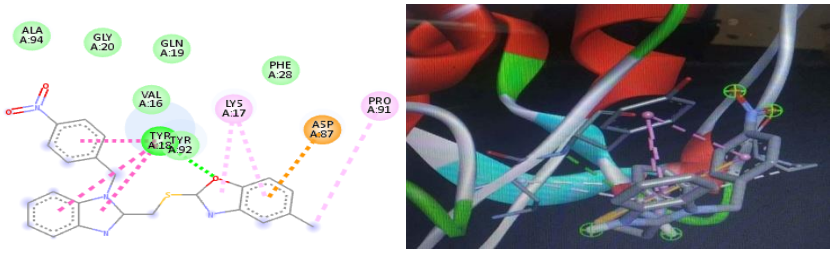


Figure 6: 2-D and 3-D interactions between ligand (G_7) and protein (1CSB)

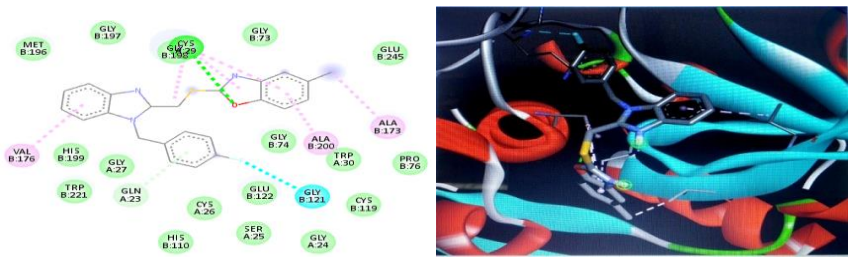


Figure 7: 2-D and 3-D interactions between ligands (G_8) and protein (4EHZ)

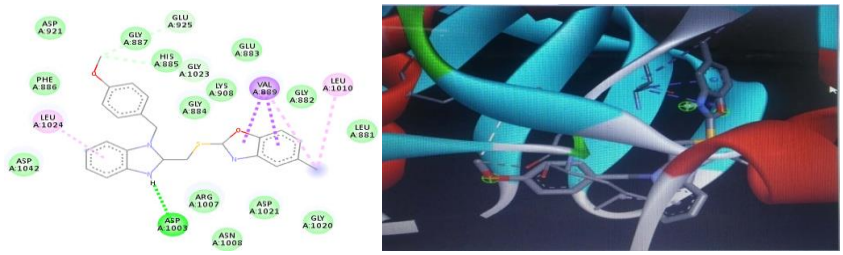


Figure 8: 2-D and 3-D interactions between ligand (G_9) and protein (6KQI)

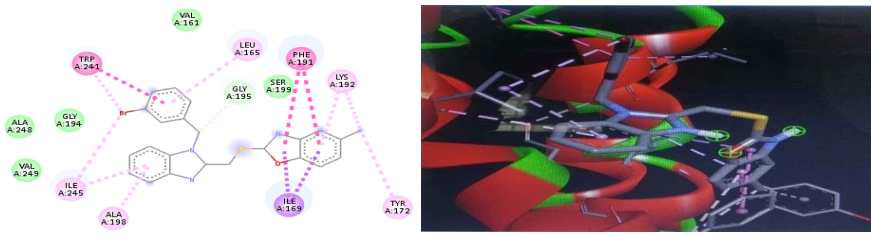
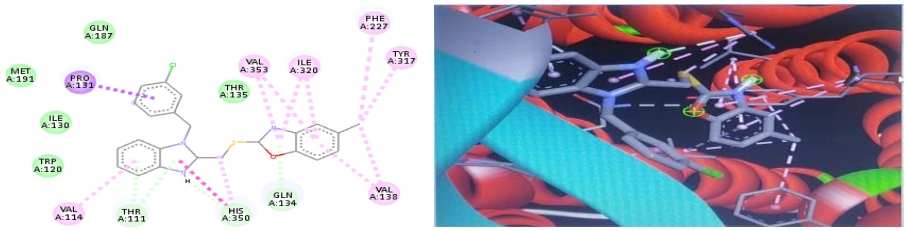


Figure 9: 2-D and 3D interaction between ligands (G_{10}) and protein (4S0V)



Prediction of activity spectra of substance (pass), bioactive score, drugs likeness properties and pharmacokinetics:

SwissADME (www.swiss adme.ch/) was used to predicted the ADME summary and estimate Lipinski rule of 5. Smiles file of ligands enter into swissADME browser to predict drug likeness and ADME Profile. **(Showed in table no-5)**

Table no-5 New designed compound explanatory analysis by SwissADME[16]

Code of compound	**MW≤500 Daltons**	**i-LOG-P**	**MLOG-P ≤ 5**	**TPSA**	**HBA ≤ 5**	**HBD ≤ 10**	**N-rotb**	**nLV ≤ 2**	**GIAB**	**% AB**
G_3	419.93	3.58	4.50	69.15	3	0	5	1	High	81.32
G_4	430.48	2.96	3.0	114.97	5	0	6	0	Low	66.39

G_5	419.93	3.52	4.50	69.15	3	0	5	1	High	81.32
G_6	430.48	2.96	3.0	114.97	5	0	6	0	Low	66.39
G_7	403.47	3.37	4.40	69.15	4	0	5	1	High	81.32
G_8	415.51	3.45	3.67	78.38	4	0	6	0	High	77.34
G_9	464.38	3.63	4.61	69.15	3	0	5	0	High	81.32
G_{10}	419.93	3.48	4.50	69.15	3	0	5	0	High	81.32

M.W-molecular weight in gm/mol, Liphophilicity expressed as LOG-P, iLOG-P- implicit log-P method, WLOG-P –Wildman and Crippen method development, T P S A- Topological polar surface area, H B A- Hydrogen bond acceptor, H B D- Hydrogen bond donor, N - rotb- number of rotatable bond, nLV- Lipinski violation, GIAB- Gastro-intestinal absorption, %-AB – Absorption percentage

Bioactivity score can predict by molinspiration tool by the input of smiles file of novel compound. **(Showed in table no -6)**

Table no-6: New designed molecules bioactivity score by molinspiration tool[16]

Code of compounds	**G P C R ligands**	**Ion-channel modulators**	**Kinase inhibitors**	**Nuclear receptor ligand**	**Protease inhibitors**	**Enzyme inhibitors**
G_3	-0.22	-0.84	-0.42	-0.57	-0.46	-0.31
G_4	-0.28	-0.79	-0.46	-0.64	-0.46	-0.33
G_5	-0.20	-0.82	-0.37	-0.57	-0.40	-0.27
G_6	-0.31	-0.82	-0.46	-0.60	-0.46	-0.32
G_7	-0.19	-0.83	-0.33	-0.53	-0.38	-0.25
G_8	-0.23	-0.86	-0.38	-0.54	-0.40	-0.27

G_9	-0.29	-0.89	-0.42	-0.66	-0.48	-0.31
G_{10}	-0.19	-0.82	-0.38	-0.57	-0.40	-0.26

Bioactive score if ≥0.00 then considerable biological activity, if between -0.50 to 0.0 then sensible activity, if less than -0.50 then in-active

Smiles file of selected derivatives were entered into PASS online web server **(http://www.way2drug.com/)** to predict the antitubercular activity. **(Summary showed in table no-7)**

Table No7- Selected molecule PASS data[16]

Code of compound	**Antitubercular activity**	
	Pa	**Pi**
G_3	0.495	0.020
G_4	0.495	0.019
G_5	0.481	0.022
G_6	0.620	0.009
G_7	0.528	0.004
G_8	0.491	0.020
G_9	0.600	0.010
G_{10}	0.476	0.023

Pa- Probability of activities, Pi- Probability of inactivities

According to PASS prediction preferred molecules have more prospect of acting as anti-tubercular molecules and molecule G_6, G_7 and G_9 have more prospects to anti-tubercular activity.

Conclusion:

Docking have finished of all novel derivative (G3- G10) by using, Chem draw 3D 16.0, Molinspiration cheminformatics, Swiss target prediction, rcsb PDB, Discovery studio, PyMOL and Auto dock vina 1.5.7 software. On the basis of binding energy, we have estimated that more the negative charge of energy on the compound shows the better binding with target site**.** All the synthesized novel derivatives were showed more negative binding energy (-6.9 to-10.4 K.calories/mol). In further research and development, these synthesized derivatives may be helpful as lead molecule against Tuberculosis because according to the PASS prediction studies, molecule $G_{6,}$ G_7 and G_9 have more prospects to anti-tubercular activity.

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Conflict of interest:

NO

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