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INSILICO DESIGN, SYNTHESIS, AND ANTI-DIABETIC EVALUATION OF BENZOTHIAZOLE SUBSTITUTED OXADIAZOLE DERIVATIVES

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ABSTRACT

Diabetes Mellitus is a disease of critical metabolic failure characterized by high blood glucose levels and less insulin. The objective of the study is to carry out the *insilico* design, synthesis and anti-diabetic evaluation of benzothiazole substituted oxadiazole derivatives. For *insilico* designing ACD lab Chemsketch v. 12.0, Molinspiration Online Software, Autodock Vina were used. All the designed derivatives were synthesized and characterized spectrally. The anti-diabetic evaluation was conducted by alpha-glucosidase inhibitory assay. The *in-vitro* anti-diabetic screening revealed that only BZT_4 showed inhibition against alpha-glucosidase.

KEYWORDS: Diabetes mellitus, Alpha-glucosidase, Autodock Vina, Acarbose.

INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder that is characterized by the presence of hyperglycaemia because of impairment of insulin secretion, defective insulin action, or both. The chronic hyperglycaemia of diabetes is associated with long-term microvascular complications affecting the eyes, kidneys, and nerves, as well as an increased threat for cardiovascular disease (CVD).[4] Different types of diabetes mellitus includes: Type-1, Type-2, Gestational diabetes, and other specific types. In clinical treatment, many potential drugs such as Sulfonylureas, Meglitinides, Biguanides, Thiazolidinediones, and alpha-glucosidase inhibitors are used to control high blood sugar problems (hyperglycaemia).[5]

Alpha-glucosidase is a catabolic enzyme located in brush border of the small intestine and is involved in the uptake of a glucose molecule into the blood. It hydrolyzes oligosaccharides, trisaccharides, and disaccharides to glucose and other monosaccharides in the small intestine. Alpha-glucosidase inhibitors competitively inhibit enzymes that convert complex non-absorbable carbohydrates into simple absorbable carbohydrates. Acarbose, Voglibose, and Miglitol are the commercially available α- glucosidase inhibitors. Acarbose is an effective α-glucosidase inhibitor that helps in lowering the blood glucose level as well as minimizing the risk of cardiovascular diseases and organ damage.[6]

Benzothiazole is a privileged bicyclic ring system, finds use in research as a starting material for the synthesis of larger, usually bioactive structures.[7] Oxadiazoles are a class of five-membered heterocyclic compounds having a broad biological activity spectrum including antibacterial, antifungal, analgesic, anti-inflammatory, anticancer, antiviral, anticonvulsant, and anti-diabetic properties.[8]

MATERIALS AND METHODS

ACD Lab Chemsketch v 12.0 software was helpful in drawing chemical structures and calculation of molecular properties. Molinspiration Molecular Viewer allows the calculation of various molecular descriptors as well as the prediction of bioactivity score of important drug targets. Prediction of Activity Spectra for Substances (PASS) estimated the probable profile of biological activity of a drug-like organic compound based on its structural formula.[9]

Protein data bank (PDB)

PDB is a database for the three-dimensional structural data for large biological molecules such as proteins, small molecules, and nucleic acids. Most structures are determined by X-ray diffractions and NMR studies. Each structure published PDB receives a four- character alphanumeric identifier called PDB ID Eg: 2ZQ0 Crystal structure of SusB complexed with acarbose.[10]

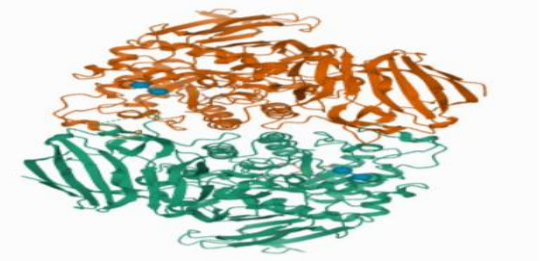


Figure 1: Structure of α-glucosidase (PDB ID-2ZQ0).

Molecular docking

Docking can be used to discriminate between putative binders and non-binders in large databases and to reduce the number of compounds to be subjected to experimental testing. Molecular docking is achieved by Autodock Vina. It is an open-source program offering a complete molecular viewer and graphical support for all the steps inevitable for setup and docking analysis. PyMOL allows to carry out molecular docking, virtual screening, and binding site analysis. PyRx is for docking analysis.[11]

Protein preparation

The 3D structure of alpha-glucosidase was uncovered from the protein data bank (PDB ID-2ZQ0). PyMOL produces a high- quality 3D image of these proteins. The structure should clear up with water molecules (HOH), small molecules, and detergents (DSN). It is achieved by inserting various commands like "remove<>resn<> HOH/DSN" (for water molecules/ detergents). Finally, hydrogen atoms should be added to the protein structure.

Ligand preparation

The 2D chemical structures of ligands are drawn with the help of ACD Lab Chemsketch v 12.0 and generated smiles notation is being converted into 3D PDB format using freely accessible Corina Online Software.

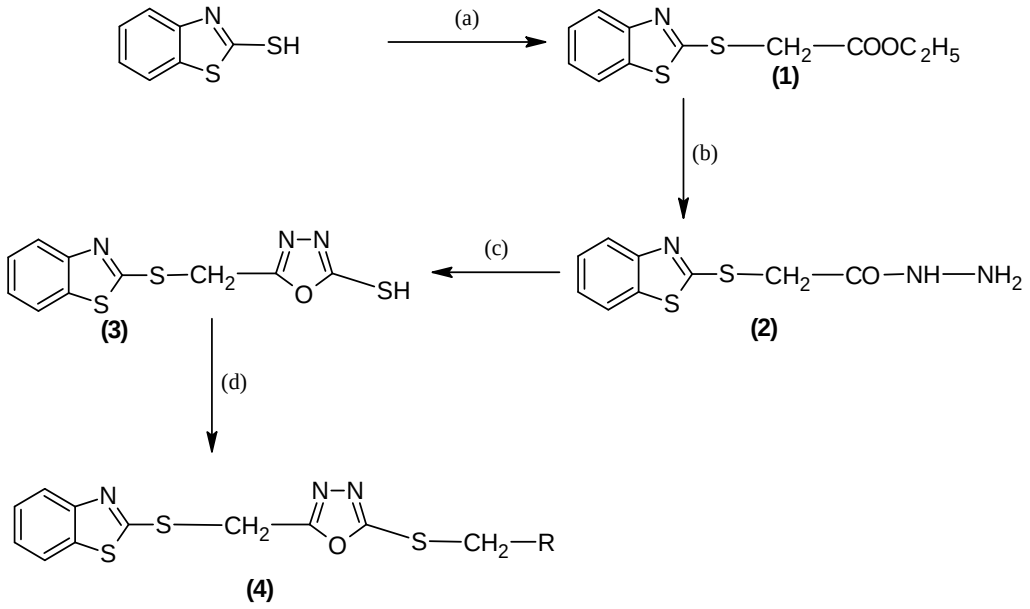
Docking using autodock vina

Docking is performed with PyRx where both the derivative and receptor are loaded in the navigator pane. Then the derivative into ligand molecule and protein is converted into macromolecule. After the preparation, click on the Autodock Vina Wizard start button and adjust the grid size. After processing, docking results were displayed in terms of binding affinity (kcal/mol) with RMSD upper bound and lower bound value. Autodock Vina converts PDB to PDBQT file which is followed by an additional step by adding polar contacts to find out the types of amino acid interactions during ligand-receptor binding.

Visualization with Biovia discovery studio

Binding interactions of proteins and ligands arise from a variety of steric and electrochemical factors. Post docking, visualization using Biovia Discovery Studio 2019 shows binding mode and interactions of hydrogen, hydrophobic and electrostatic bonds.[12]

Synthesis



Scheme 1: Synthesis of benzothiazole substituted derivatives.

R= NH-C_6H_5-Cl, -NHC_4H_9O, -N-C_5H_{11}, - NH-C_6H_5-CH_3, -NH-$C_6H_5NO_2$

(a) $ClCH_2COOC_2H_5$, anhydrous K_2CO_3, Dry Acetone, 70ºC, 5 hrs reflux,

(b) $NH_2NH_2.H_2O$, EtOH, 80ºC, 8hrs reflux,

(c) CS_2, KOH, EtOH, 40ºC, 4hrs reflux,

(d) HCHO, R-NH_2, EtOH, 1,4-dioxane, 30ºC, 6hrs reflux

Alpha-glucosidase inhibitory assay

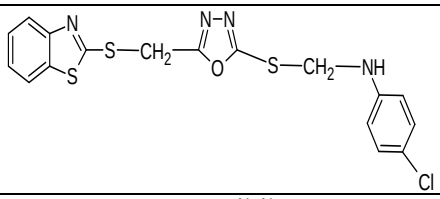
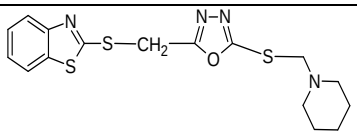
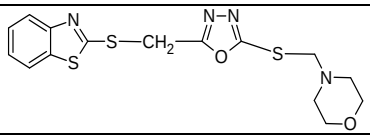
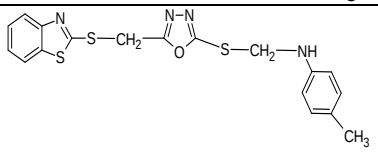
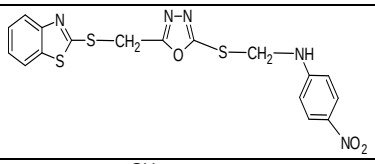
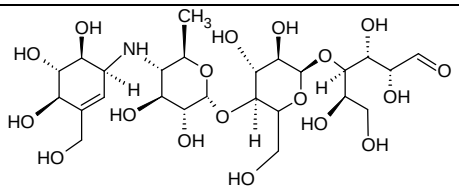
The effect of the sample on α-glucosidase activity was determined according to the method described by Shai *et al*, (2011) with slight modification. 400 µL of α-glucosidase (0.067 U/mL) was preincubated with different concentrations of the sample for 30 min. Then 200 µL of 3.0 mM (*p*NPG) used as substrate dissolved in 0.1M sodium phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37°C for 30 min and stopped by adding 2 mL of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the yellow-colored para-nitro phenol released from pNPG at 400 nm. The results were expressed as a percentage of inhibition. The same procedure was done with Acarbose (1mg/ml stock) which was used as standard.[13]

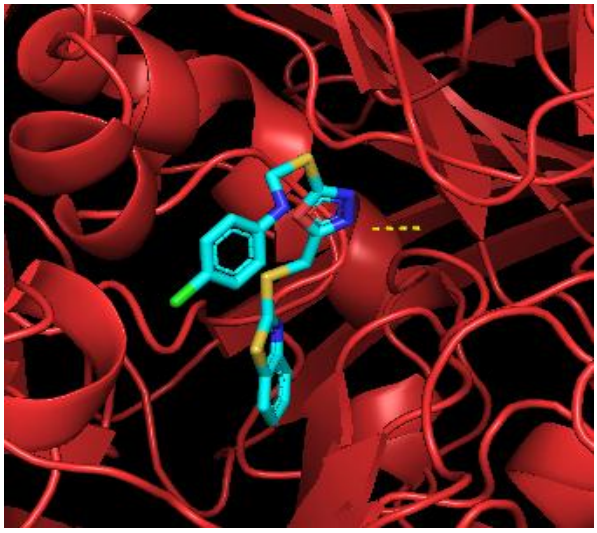
$$\% \text{ inhibition} = \frac{\text{OD of Test - OD of Control}}{\text{OD of Test}} \text{ X } 100$$

RESULTS AND DISCUSSION

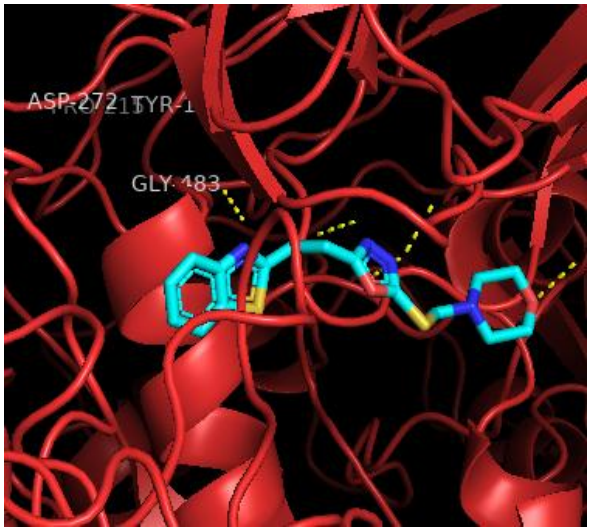
Docking results revealed a high negative docking score (Table 1). It indicates very good interaction and affinity with the binding site of protein 2ZQ0. The designed derivatives and standard Acarbose exhibited various type of interactions towards the receptor. [{5-[(1, 3-benzothiazol-2-ylsulfanyl) methyl]-1, 3, 4-oxadiazol-2-yl} sulfanyl) methyl] derivatives were synthesized through a four-step conventional method (Scheme 1). Five synthesized derivatives were named BZT_1, BZT_2, BZT_3, BZT_4, and BZT_5. Spectral characterization was done by Infrared, ^1H NMR, ^{13}C NMR, and Mass spectroscopy.

Table 1: Docking score of derivatives and standard (Acarbose) with protein 2ZQ0.

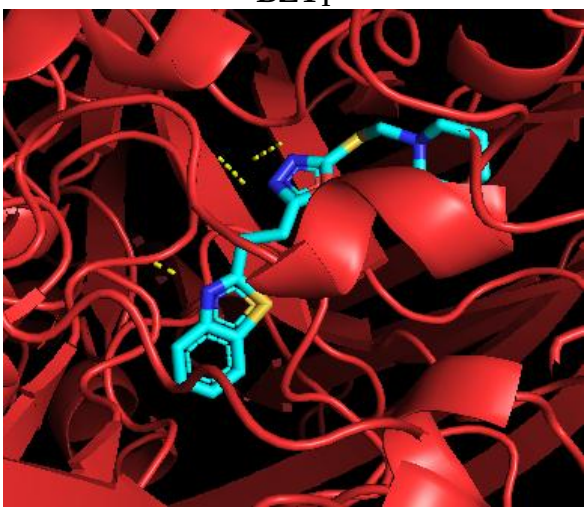
Compound Code	Structure	Docking score (kcal/mol)	Aminoacid interactions
BZT_1		-8.2	GLU-194,SER-218, MET-334, TYR-533
BZT_2		-8.0	ASN-216, GLU-391, TRP-400, LYS-169
BZT_3		-8.3	GLU-525, ILE-292, ARG-154, SER-127
BZT_4		-8.7	GLU-194 ILE-355, SER-218, GLU-526
BZT_5		-8.6	PHE-222, ARG-206, ASP-356, SER-404
Standard (Acarbose)		-7.6	HIS-123, ILE-120, ALA-302, TYR-629



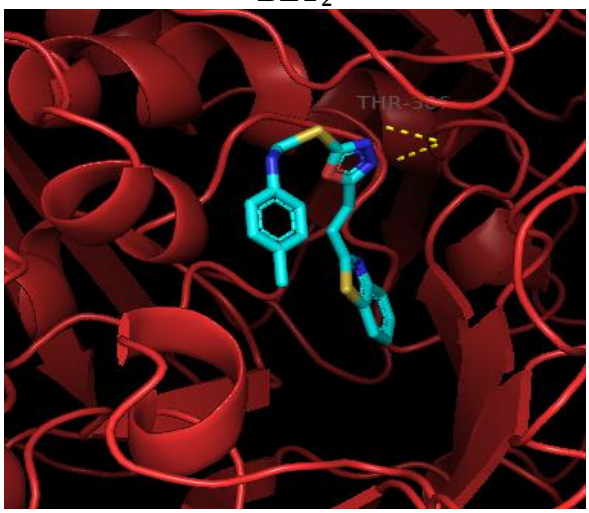
BZT_1



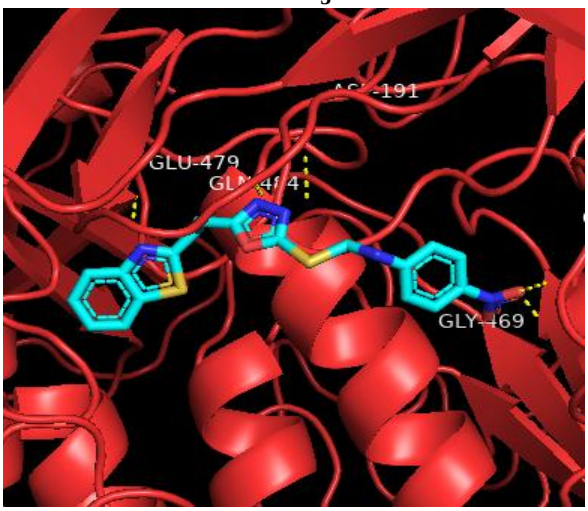
BZT_2



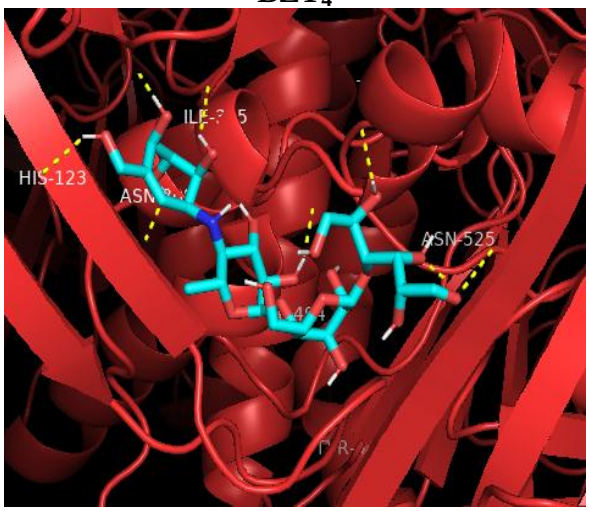
BZT_3



BZT_4



BZT_5



STD

Figure 2: Docking images of derivatives and standard (Acarbose) with 2ZQ0.

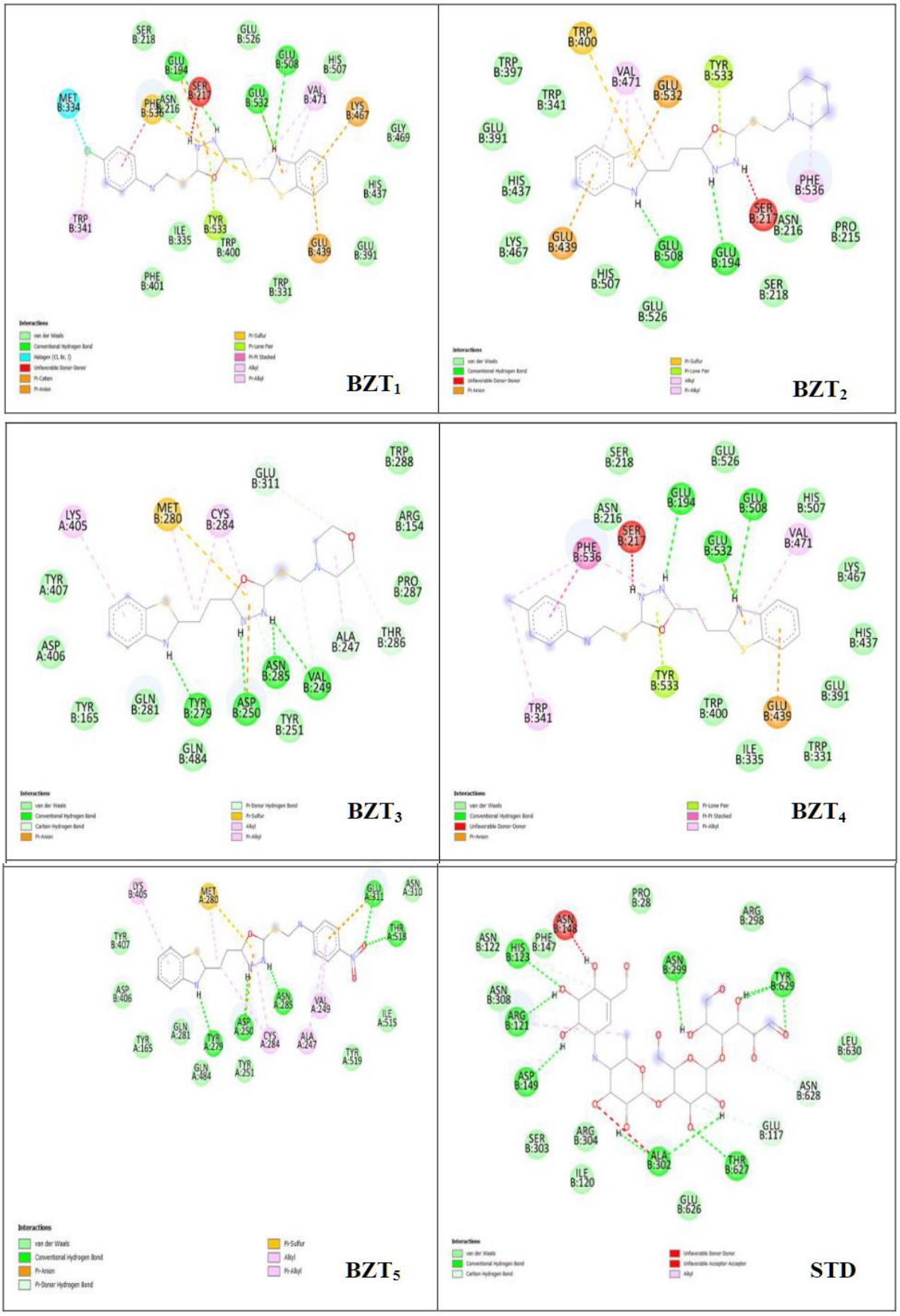


Figure 3: 2D diagram of binding interactions of derivatives and Acarbose on the α-glucosidase receptor.

In the case of alpha-glucosidase enzyme inhibitory assay, BZT_4 showed significant inhibition for alpha-glucosidase enzyme (Fig. 4). The compound exhibited high percentage inhibition (64.06%) and the IC_{50} value of BZT_4 was found to be 48.8µg (Table 2) which is adjacent to the IC_{50} of standard (Acarbose).

Table 2: Alpha- glucosidase inhibitory activity of synthesized Derivatives and Acarbose.

Sample	Concentration (µg)	OD at 400nm	Percentage inhibition (%)	IC_{50} (µg)
BZT_1	25	1.429	20.42	58.11
	50	1.321	26.55	
	100	1.102	48.97	
BZT_2	25	1.543	18.90	72.13
	50	1.492	21.85	
	100	1.300	32.95	
BZT_3	25	1.643	18.54	81.03
	50	1.485	26.86	
	100	1.326	35.23	
BZT_4	25	1.351	28.43	48.80
	50	1.263	41.14	
	100	1.059	64.06	
BZT_5	25	1.644	7.30	92.48
	50	1.614	9.07	
	100	1.375	23.14	
Acarbose	25	0.208	73.13	39.15
	50	0.050	96.46	
	100	0.042	97.64	

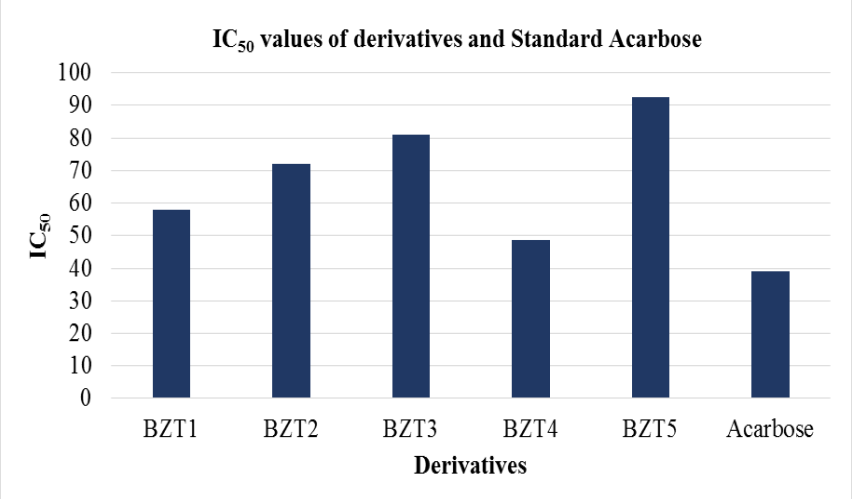


Figure 4: Comparison of IC_{50} of derivatives and Standard Acarbose.

CONCLUSION

A series of benzothiazole substituted oxadiazole derivatives were designed and docked against alpha-glucosidase receptor (PDB ID- 2ZQ0). BZT_4 showed a high docking score and

multiple interactions of residues with the binding site. The reason for good docking value of BZT_4 is due to the presence of an electron-donating methyl group exhibiting positive inductive effect. The selected molecules were synthesized and characterized by Infrared, ^1H NMR, ^{13}C NMR, and Mass spectroscopy. Also, they were evaluated for anti-diabetic activity by alpha-glucosidase inhibitory assay method. The experiment results showed that only BZT_4 has excellent inhibition against alpha-glucosidase (64.06%) with an IC_{50} value of 48.8µg which is adjacent to the IC_{50} of Standard Acarbose. Further structure activity relationship studies may develop more potent and less toxic molecules.

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