Current Opinion
CO 3 (3), 318– 328 (2023)
Received 25 February 2023 | **Revised** 24 March 2023 | **Accepted** 26 **April** 2023 | **Online Available 25 May** 2023
https://doi.org/10.52845/currentopinion.v3i3.217

OPEN ACCESS JOURNAL
ISSN(O) 2795- 935X

Original Article

Molecular Docking Studies of Novel Benzotriazole Derivative as Potent Antimicrobial Agent

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Abstract:
The Benzo fused heterocyclic nucleus Benzotriazole founds an important class for new drug development. Molecular Docking study is a key tool in Computer Aided Drug Designing. The main objective of this work is to perform preliminary docking screening using SAR studies, Physicochemical Properties SWISS ADME property Explorer, PASS prediction Activity spectra, and the Lipinski Rule of Five. This study is also an attempt to explore the antimicrobial activity of Benzotriazole derivative by Molecular docking with *Staphylococcus aureus* tyrosyl t-RNA synthetase (PDB: 1JIJ) via Autodock 4.2. Among all the Benzotriazole derivatives, 2-(1H-1,2,3-Benzotriazol-1-yl)-N-(4-methoxyphenyl) acetamide is the most effective and showed maximum binding energy (-8.9 K/Cal) showed interaction with TYR36, GLY38, ALA39, ASP40, THR42, LEU70, and GLN196.
Keywords: Benzofused, Heterocyclic compounds, Anthelmintic, Molecular Docking

Introduction

A heterocyclic compound is any class of organic compounds in which at least one noncarbon element is present, most often oxygen, nitrogen, or Sulphur [1]. The most significant benefit of medicinal chemistry has been discovering the link between molecular structure and bioactivity. The fact that heterocyclic compounds constitute the majority of therapeutic medicines is widely recognized [2].

Benzo fused rings have been demonstrated in numerous studies to be promising components for creating novel antibacterial structures [3]. Heterocyclic compounds are of particular interest to medicinal chemists due to their unique chemical and biological properties [4]. A five-membered ring with 3N-atoms is triazoles. It has one N-atoms being pyrrole-like and the other 2 being pyridine-like. In 1,2,3-triazoles (II), all 3N-atoms are nearby connected with 2C-atoms and two double bonds. When the 4,5-position or the "d" site of the II are attached to the benzene ring, it forms Benzotriazoles (III) [5].

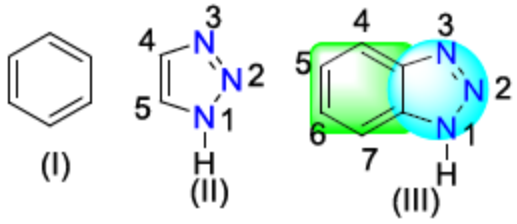


Figure 1 Structure of Benzotriazole

The BT structure has been shown to have an extensive range of uses. It is currently employed as a synthetic auxiliary or after reactions with various carbonyl groups as a good leaving group.[6-7]. Apart from medicinal applications, BT is used in industry as a photographic emulsion fixing agent, [8] anti-tarnish agents for copper and its alloys, and a corrosion inhibitor in anti-freeze and water cooling systems. [9]

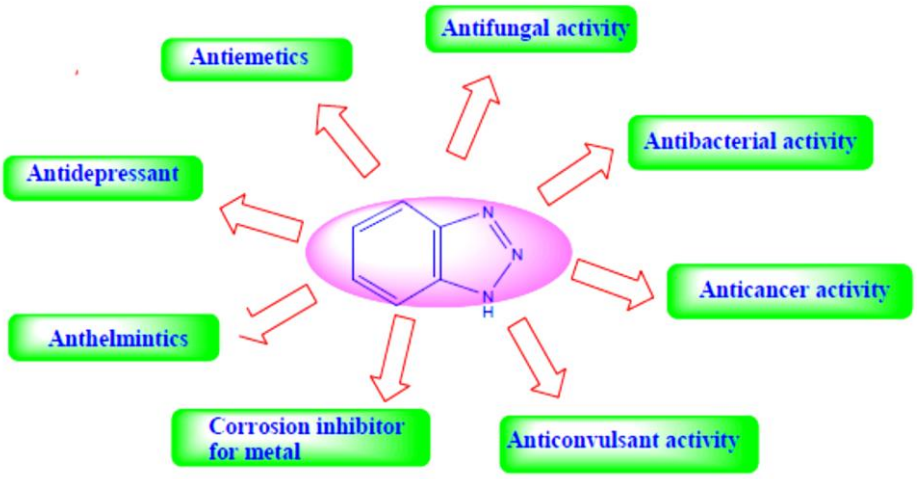


Figure 2 Pharmacological Activities of Benzotriazole [10-15]

The main objective of this work is to perform preliminary docking screening using SAR studies, OSIRIS molecular property explorer, PASS prediction Activity spectra, and Rule of Five. This study is also an attempt to explore the antimicrobial activity of Benzotriazole derivatives by Molecular docking studies.

Methodology

In silico screening - In silico screening are now widely used to observe key parameters that might help in the evaluation of a compound's chemical and physical qualities.

Evaluation of Physicochemical parameter:

Structure-Activity Relationship [SAR] Studies

From the Literature survey, [11-17] it was analyzed that Benzotriazole possesses a Hydrophobic pocket, electron-donating site, Electron Withdrawing atom, and Hydrogen Bond Donor/Acceptor Site as shown in **Figure 3**.

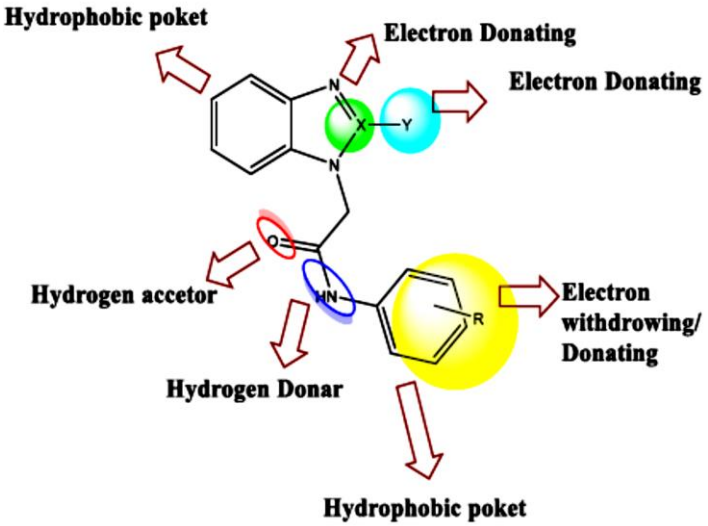


Figure 3 Structural feature of targeted benzotriazole Nucleus

Prediction of PASS and Lipinski Rule of Five

PASS online (http://way2drug.com/PassOnline/) was used to undertake studies on the prediction of activity spectra for drugs (PASS) [18]. All data are summarized in **Table 1**.

Molecular properties such as oral bioavailability are usually associated with some basic molecular descriptors, such as log P (partition coefficient), molecular weight (MW), and the acceptors and donors for hydrogen bonding in a molecule. Using these molecular properties, Lipinski [19] established a divisive rule for in-silico studies. The rule is important to keep in mind during in-silico studies when a pharmacologically active lead structure is optimized step-wise to increase the activity and selectivity of the compound as well as to ensure drug-like physicochemical properties are maintained as described by Lipinski's rule [19]. Limits of Lipinski's 'Rule of Five states that the compounds are more likely to be orally bioavailable if they obey the following criteria: -

S.No.	Parameters	Limits
1.	Molecular weight	180-500 dalton
2.	ClogP	0.4-5.6
3.	Hydrogen Bond Donor [HBD]	<5
4.	Hydrogen Bond Acceptor [HBA]	<10
5.	Polar Surface Area [PSA]	≤140 A^2
6.	Molar refractivity [MR]	40-130m^3/mol

These parameters were calculated by using Marvin sketch 5.0 and Chem draw software. Proposed Benzotriazole compounds show limits under the standard values of the Lipinski rule of five which are shown in **Table 1**.

Physicochemical Parameter:

Molinspiration (http://www.molinspiration.com), an online calculator for physicochemical characteristics was used to calculate properties. [20] The structures were created using the Molinspiration platform, the SMILES (simple molecular-input line-entry system) was created, and pharmacokinetic characteristics, bioactivity scores, and other metrics were computed. Additionally, produced SMILES were put into the PreADMET server (http://preadmet.bmdrc.org/) [21] and Swiss ADME (http://www.swissadme.ch)189 to predict pharmacologically relevant physicochemical characteristics such as ADME and Toxicity**.**[22]

Molecular Docking:

Molecular docking is a frequently used tool in computer-aided structure-based rational drug design. It evaluates how small molecules called ligands (proposed Benzotriazole derivatives) and the target macromolecule (*Staphylococcus aureus* tyrosyl t-RNA synthetase (PDB: 1JIJ) fit together [23].

Auto Dock Tools (ADT) is a program package of automated docking tools and designed to predict how small molecules bind to a target protein of a known 3D- structure. Besides generating binding energies in these docking studies, the position of the ligand in the enzyme binding site can be visualized [24]. It can be useful for developing potential ligands and also for understanding the binding nature [25-26]. Docking Studies were performed in order to check the binding affinity of Benzotriazole derivatives with *Staphylococcus aureus* tyrosyl t-RNA synthetase (PDB: 1JIJ) [27].

Crystal structure of *Staphylococcus aureus* tyrosyl t-RNA synthetase (PDB: 1JIJ) (**Figure 4)** was downloaded from Protein Data Bank (http://www.pdb.org/pdb/home/home.do), which was resolved at 2.92 Å, and 2.70 Å respectively. The optimized ligand molecules were docked into refined *Staphylococcus aureus* tyrosyl t-RNA synthetase enzyme model using "Ligand Fit" in Autodock 4.2 [27].

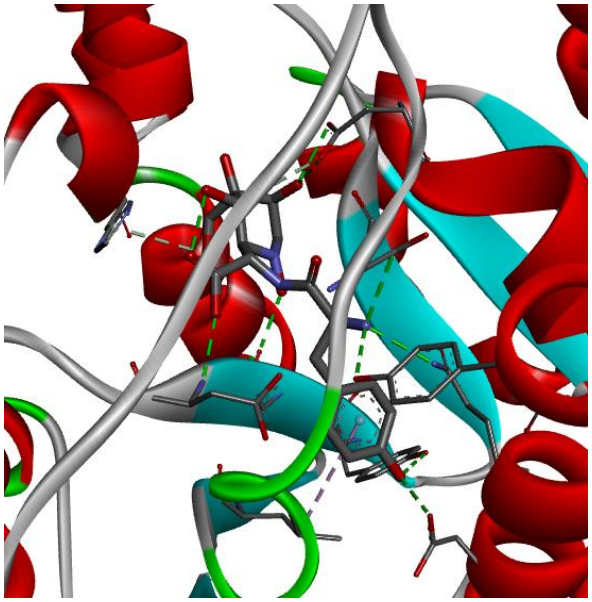


Figure 4 Crystal structure of *Staphylococcus aureus* tyrosyl t-RNA synthetase (PDB: 1JIJ) [Downloaded from https://www.rcsb.org/structure/1JIJ retrieved on 12/02/2023]

PDB sum's Ligplot/2D Interaction of standard ligand with enzyme (1JIJ) showing all amino acid residues of active pocket shown in **Figure 5**

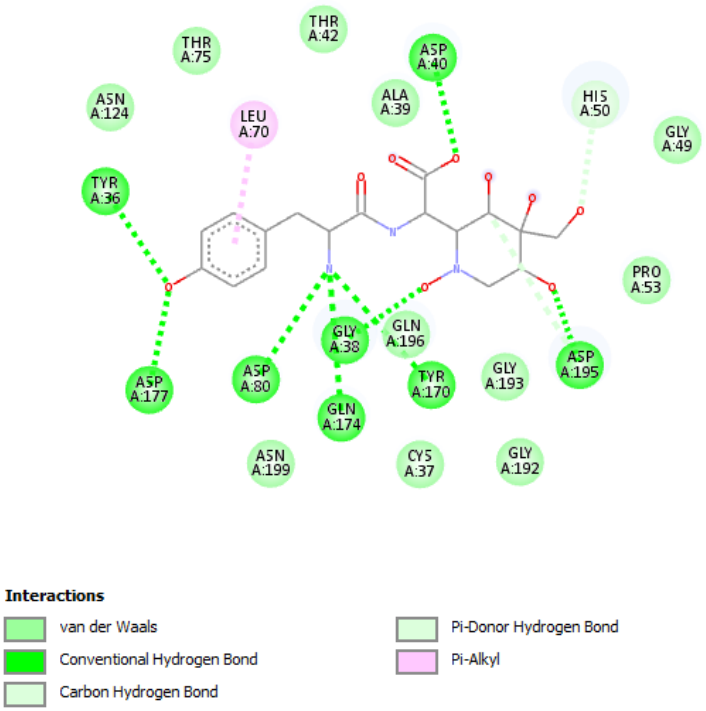


Figure 5- 2D Interaction of standard Ligand results for 1JIJ showing all amino acid residues

All proposed Benzotriazole derivatives were drawn in Chem Draw 3D. All compounds are subjected to minimize the energy and the 3D structure of ligands was converted into PDBQT using Autodock 4.2. The Grid Box for the *Staphylococcus aureus* tyrosyl t-RNA synthetase in complex with SB-239629 (Standard Ligand) was generated with the help of Autodock 4.2 with dimensions X= 70, Y = 74, Z = 70 Å, covering all amino acid residues (Ser 165, Val236, Thr237, Met233, Glu 108 etc). The center grid box was set at 8.671 x - 8.036 x 0.67 dimension as shown in

Figure 6 [28]. Autodock Studies were performed using Lamarakian Genetic algorithm via Autodock 4.2.

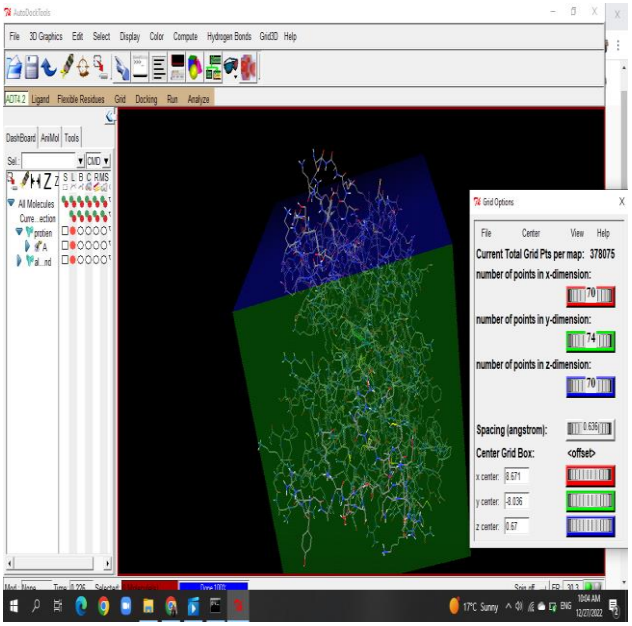


Figure 6 Grid box for the *Staphylococcus aureus* tyrosyl t-RNA synthetase (PDB ID: 1JIJ) with the standard ligand

Result and Discussion

Computational techniques provide the advantage of making novel medications faster and for less cost. Virtual screening, and in silico ADME/T prediction, are innovative techniques for detecting protein-ligand interaction are three major functions of computing in drug development. [29]. PreADMET is then utilized to double-check the drug likeness. The concept of drug-likeness was developed as a way to give valuable guidance throughout the early phases of drug development to increase the likelihood of a chemical entering and passing clinical trials. It may be described as the total of the molecular physicochemical characteristics that distinguish medicines from other substances [30].

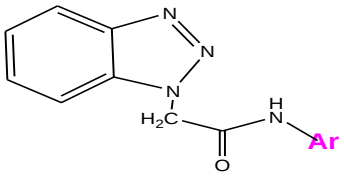
Prediction using PASS *(Prediction of Activity Spectra for Substances) [18]*

It is a software product designed as a tool for evaluating the general biological potential of an organic drug-like molecule. *PASS* provides simultaneous predictions of many types of biological activity based on the structure of organic compounds. Calculated values of all compounds were interpreted in **Table 1** in terms of *Pa (probability "to be active") more than Pi (probability "to be inactive") as potent antimicrobial agents. Compounds **BT 4** possess more scores as shown in **Table 1**.*

Physiochemical properties and Lipinski Rule of Five:

Physiochemical properties must be defined to predict the molecule's potential as a drug. Physicochemical characteristics are the quantifiable qualities of molecules that are connected to interactions with various substrates and surroundings in drug development [31]. For more than a century, physicochemical characteristics of substances have been utilized to predict or anticipate pharmacokinetic processes. Lipophilicity is the most well-known characteristic which is related to diffusion through cell membranes, solubility, interactions with receptors, metabolism, and toxicity. The chemical must attach to a binding pocket in order to activate proteins, such as receptors and enzymes [19 - 21]. Aside from lipophilicity, other important physicochemical characteristics for binding are molecular size, hydrogen bond acceptors/donors, and charge [22] Table 1 describes the physicochemical parameters of all proposed Benzotriazole derivatives among which 2-(1H-1,2,3-Benzotriazol-1-yl)- N-(4-methoxyphenyl) acetamide (BT4) compound showed more potential.

Table 1 Physicochemical Parameter and Prediction of Activity by PASS & Lipinski Rule



S. No.	Compound Name (ID)	Ar	PASS Prediction		Physicochemical Properties							Follow Lipinski Rule
			Antimicrobial Activity		Mol. Weight	No. of rotatable bonds	HBA	HBD	TPSA(A^2)	log P	MR (m^3 / mol)	
			Pa	Pi								
1.	Benzotriazole	-	0,311	0,098	201.09	3	3	1	56.88	2.13	71.62	Yes
2.	2-(1H-1,2,3-Benzotriazol-1-yl)-N-phenylacetamide (BT1)	Phenyl	0,165	0,130	252.27	4	3	1	59.81	2.16	72.62	Yes
3.	2-(1H-1,2,3-Benzotriazol-1-yl)-N-(p-tolyl)acetamide (BT2)	4-Methyl phenyl	0,266	0,144	266.30	4	3	1	59.81	2.48	77.63	Yes
4.	2-(1H-1,2,3-Benzotriazol-1-yl)-N-(4-chloro-phenyl)-acetamide (BT3)	4-Chloro phenyl	0,155	0,143	286.72	4	3	1	59.81	2.61	77.59	Yes
5.	2-(1H-1,2,3-Benzotriazol-1-yl)-N-(4-methoxyphenyl)acetamide (BT4)	4-methoxy Phenyl	0,343	0,072	282.30	5	4	1	69.04	2.22	79.12	Yes
6.	Standard Ligand		0,247	0,002	265.01	2	4	2	72.31	3.20	73.97	Yes

HBA: *Hydrogen Bond Donor,* ***HBA:*** *Hydrogen Bond Acceptor,* ***MW:*** *Molecular Weight,* ***TPSA:*** *total Polarizable Surface area,* ***MR:*** *Molar Refractivity.*

ADMET Prediction:

ADMET prediction is very crucial for any compound which is used to be applied as a medication in the future [30]. ADME prediction using various parameters was also calculated for these compounds by the PreADMET web server (www.preadmet.bmdrc.org), which was summarized in **Table** 2.

Table 2: ADME predicted data of Benzotriazole derivatives from PreADMET

Compound ID	BBB	CaCO2+ Cell Permeability	CYP inhibition	HIA	MDCK	PGP inhibition	Plasma Protein Binding	Skin Permeability
Benzotriazole	1.114	19.214	Non	92.141	21.0005	Non	91.164	-3.8828
BT1	1.143	20.504	Non	95.714	22.4985	Non	100	-3.4828
BT2	0.741	20.837	Non	95.750	24.7306	Non	100	-3.4130

BT3	0.434	21.672	Non	95.988	14.8835	Non	99.550	-3.5288
BT4	0.542	23.898	Non	96.113	14.1237	Non	90.164	-3.6901

Throughout the drug research and development process, toxicology plays a critical role. The objective of toxicity screening was to identify derivatives with safety profiles as soon as possible [32]. **Table 3** summarizes the toxicity screening result of Benzotriazole derivatives calculated using online PreADMET software.

Table 3.: Toxicity parameter of Benzotriazole derivatives predicted by PreADMET

Compound ID	Algae at	Ames test	Carcino Mouse	Carcino Rat	Daphni a at	hERG inhibition	Medak a at	Minno w at
Benzotriazole	0.04263	Mutagen	Positive	Negative	0.033253	Medium Risk	0.004126	0.01604
BT1	0.08506	Mutagen	Negative	Negative	0.097544	Medium Risk	0.016303	0.023663
BT2	0.05264	Mutagen	Positive	Negative	0.073256	Medium Risk	0.009786	0.019644
BT3	0.03781	Mutagen	Positive	Negative	0.050814	Medium Risk	0.005195	0.011414
BT4	0.06747	Mutagen	Negative	Negative	0.113123	Medium Risk	0.023006	0.039922

Molecular docking:

The binding affinities of newly synthesized derivatives and target proteins (1JIJ) were studied using molecular docking. **Table 4** showed the proposed benzotriazole derivative's binding affinity to the target protein with amino acid interaction.

Table 4: Docking Score and interaction of synthesized derivatives with 1JIJ

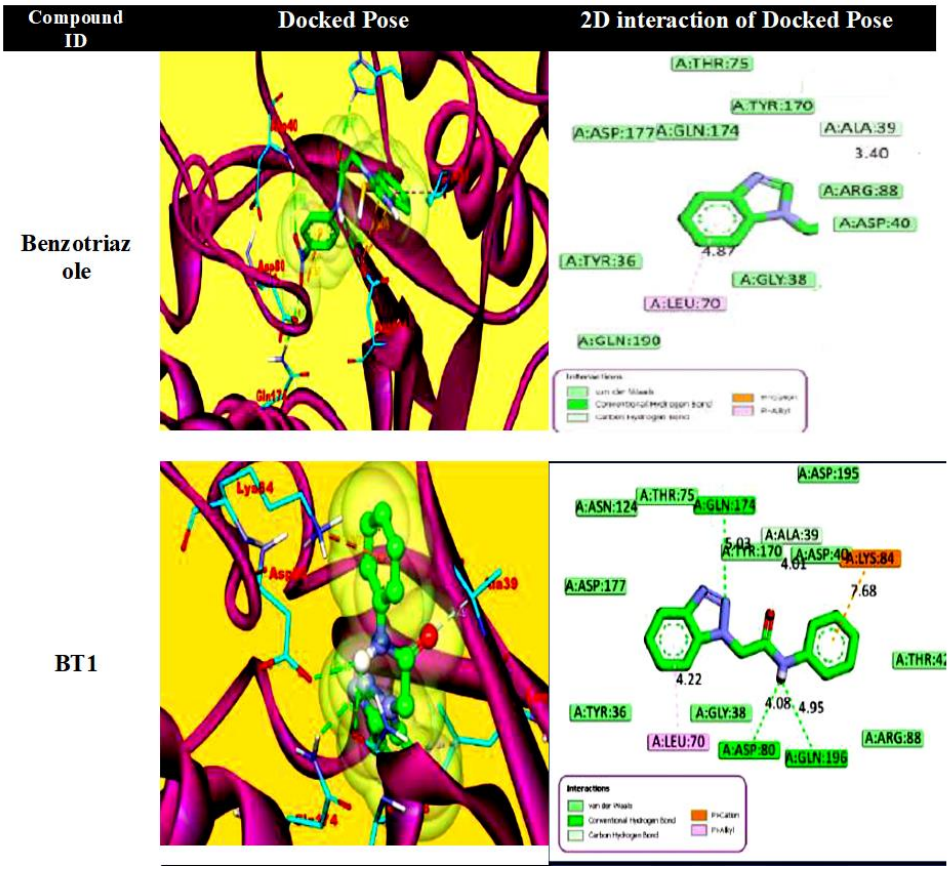
Compound ID	Binding affinity (kcal/mol)	Amino acid in Hydrogen bond	Interacting Amino Acid
Benzotriazole	-7.2	ASP80, GLN174, GLN196	TYR36, GLY38, ALA39, ASP40, THR42, GLY58, ASP80, LEU70, THR75
BT1	-8.0	ASP80, GLN174, GLN196	ASP80, LEU70, THR75, LYS84, ARG88, SN124, TYR170, GLN174, ASP177, ASP195,
BT2	-8.1	ASP80, GLN174, GLN196	THR42, HIS50, GLY58, ASP80, LEU70, THR75, LYS84, ARG88, ASN124, TYR170
BT3	-7.6	ASP40, ASP80, GLN174, GLN196	TYR36, GLY38, ALA39, ASP40, THR42, HIS50, LEU70, THR75, ASP80, LYS84, ARG88, ASN124,
BT4	-8.9	ASP80, ARG88, GLN174, GLN196	TYR36, GLY38, ALA39, ASP40, THR42, LEU70, THR75, ASP80, LYS84, ARG88, ASN124,
Standard Ligand	-7.8	TYR36, ASP40, GLY58, ASP80, TYR170, ASP177, GLN174, ASP195	TYR36, CYS37, ALA39, ASP40, THR42, PRO53, GLY49, HIS50, GLY58 ASP80, LEU70, THR75, GLN174

Molecular docking predicts the binding relationship between macromolecules and a small ligand. It is a theoretical method of examining a compound for potential pharmacological action before its actual synthesis. Understanding binding affinity is critical to comprehending intermolecular interaction [33].

The magnitude of the binding interaction is referred to as binding affinity. Hence binding affinity (kcal/mol) is used to the categorized result obtained after virtual screening [34]. PDB: 1JIJ, were chosen as studies Tyrosyl-tRNA synthetase catalyzes the covalent binding of amino acids to their tRNA and plays an important role in protein synthesi [27]. The ligand present in the protein molecule was used as a reference ligand for comparing binding affinity shown in **Figures 4 & 5** The binding domain was prepared as a grid box in AutoDock after visualization of the binding interaction of reference ligand in discovery studio (**Figure 5**). The docking findings revealed that all of the compounds in the binding pocket had major bonding interactions showed in Figure 6 and The binding affinity of the benzotriazole derivatives to the target protein with amino acid interaction was shown in **Table 4.**

All Benzotriazole derivatives have more binding affinity as compare to Standard ligand SB-219383 with the Staphylococcus aureus tyrosyl-tRNA synthetase (PDB: 1jij). **Figure 6** shows the 2D and 3D interaction of N-substituted Benzotriazole derivatives with the amino acid in the active domain. The binding affinity against Staphylococcus aureus tyrosyl-tRNA synthetase was -7.2 to - 8.9 kcal /mol as compare to -7.8 kcal/mol for reference ligand SB-219383. This docking result reveals that Benzotriazole derivatives showed stronger affinity

The compound BT4 (2-(1H-1,2,3-Benzotriazol-1-yl)-N-(4-methoxyphenyl) acetamide) also shows significant binding affinity towards the active pocket of tyrosyl-tRNA synthetase. The amino acid involved in binding with ligands and present in the binding domain were Ala39, Asp40, Leu70, Asp80, Lys84, Asp195, and Gln196. This amino acid interacts with the ligand through carbon-hydrogen bonding, Pi-cation, Pi-anion, alkyl Pi-alkyl, Van der Waals, and traditional hydrogen bonding. All these interactions and binding affinity studies suggest that the derivatives are stronger inhibitors of t-RNA synthetase protein even stronger than the reference ligand.



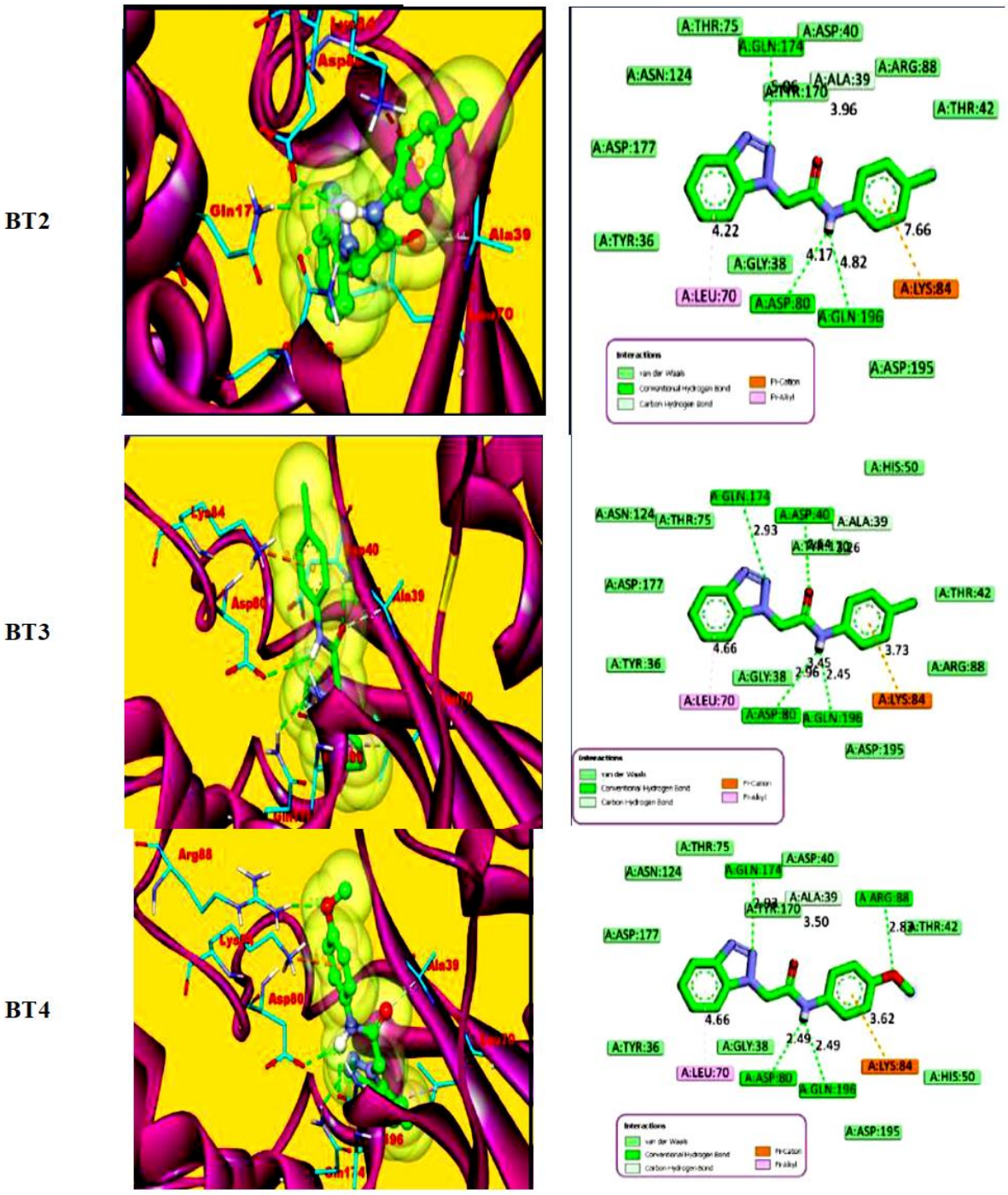


Figure 6 2D and 3D interaction of N-substituted Benzotriazole derivatives with the amino acid in the active domain

Conclusion

Bio-molecules, natural products, and marketed medications all contain benzo-fused nitrogen with five-membered heterocycles as a basic nucleus. Benzotriazole was chosen for study because of the importance of these compounds as pharmacophores, biopotential the literature, and their structural closeness to nucleic acid bases. According to the in-silico study, all proposed benzotriazole nitrogen-containing five-membered heterocyclic compounds follow all drug-likeness rules which suggest that all derivatives have drug-like properties. Further, they have considerable pharmacokinetic features required for drug action like logP value, BBB, HIA, and protein binding. In concern to toxicity, study derivatives possess moderate toxicity. This compound exhibits a higher affinity to bind with tyrosyl t-RNA synthetase enzyme, than the reference ligand under research.

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