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IN SILICO DESIGN AND DOCKING STUDIES OF NOVEL BENZOTHIAZOLE DERIVED TRIAZOLE AS A POTENTIAL ANTIDIABETIC AGENTS

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ABSTRACT
Diabetes is one of the pre-dominant metabolic disorders all over the world. It is the prime reason of mortality and morbidity due to hyperglycaemia which is link with numerous obstacles. In the present scenario, we have attempted to design and evaluated a small library of 25 derivatives of benzothiazole in search of potent antidiabetic agents through in silico studies using Glide 5.5 extra precision (XP) maestro 12.3 Schrodinger software. Compounds F18, F25, F3, F5, F16 and F20 showed good activities on α - glucosidase receptor. Molecular docking studies were performed to predict the binding interaction of the compounds with the active site of enzyme. Results of in-silico studies showed that most of the compound have excellent drug likeness properties, pharmacokinetic profile and are preferable as orally available drug. Hence this study provides several insights that can be adopted towards the development of novel glucosidase inhibitors and potential antidiabetic agents.

KEYWORDS: Benzothiazole, Antidiabetic potential, in silico studies, Schrodinger.

INTRODUCTION
Diabetes has reached an epidemic level by becoming a major health issue round the globe. Diabetes or diabetes mellitus is a multifactorial metabolic disease/polygenic disorder characterized by increased glucose levels/insulin resistance over prolonged periods in liver and peripheral tissues. It is categorized as Type I, Type II, and gestational diabetes. Type II diabetes (non-insulin-dependent diabetes mellitus) occurs in 80–90% of the patients, often characterized by hyperglycaemia and insulin resistance in the initial stages leading finally to serious complications such as stroke, cardiovascular diseases, atherosclerosis, chronic eye damage, kidney failure, obesity, etc. collectively known as insulin resistance associated disorders (IRAD).[1] The International Diabetes Federation (IDF) estimated that the global prevalence of diabetes is predicted to grow from 463 million at present to 700 million by 2045.[2] Current treatment includes insulin secretagogues, sulfonylureas and meglitinides (stimulate insulin secretion), biguanides (suppress hepatic glucose production), insulin sensitizers, thiazolidinediones–glitazones (improve insulin sensitivity and peripheral glucose uptake), αglucosidase inhibitors (delay digestion and absorption of intestinal carbohydrate), glucagonlike peptide 1 agonists, dipeptidyl peptidase-IV inhibitors.[3]

Alpha-glucosidase inhibitors (AGIs) such as acarbose, miglitol and voglibose are oral drugs used in the management of diabetes, primarily to reduce post-prandial glucose concentrations. Their use in individuals with impaired glucose tolerance (IGT) has been shown to delay progression to diabetes.[4] Acarbose, the first approved drug in α-glucosidase inhibitor category, was used to delay the release of glucose from polysaccharides by binding with α-glucosidase. Voglibose was used to discontinue the uptake and hydrolysis of saccharides by selectively inhibiting α-glucosidase vs. pancreatic α-amylase and lactase. Miglitol, the first pseudomonosaccharide α-glucosidase inhibitor, was approved to reduce postprandial glucose.[5] These drugs lead to various side effects such as diarrhoea, vomiting, flatulence, severe stomach pain, allergic reactions, etc.,[6] Based on this background, numerous efforts have been carried out to discover new α -glucosidase inhibitors from diverse sources, such as chemical synthetic compounds.

Heterocycles are important pharmacophores and have significance to create privileged chemical structures possessing pharmacological activities. Five membered heterocyclic which incorporate oxygen, nitrogen and sulphur are found in broad spectrum therapeutic agents which have an enormous significance in drug discovery and drug development processes.

Benzothiazole (BTA) is a fused benzoheterocycle formed by the fusion of the thiazole ring with a benzene ring. 2-

substitued benzothiazole has emerged in its usage as a core structure in the diversified therapeutical applications. The studies of structure–activity relationship interestingly reveal that change of the structure of substituent group at C-2 position commonly results the change of its bioactivity. The benzothiazole scaffold has been extensively investigated for anti-cancer[7], anti-bacterial[8], anti-tuberculosis[9], anti-diabetic[10], anthelmintic[11], anti-tumour[12], anti-viral[13], anti-oxidant[14], anti-inflammatory[15], anti-glutamate and antiparkinsonism[16], anticonvulsant[17], muscle relaxant activities[18], neuroprotective[19], Enzyme inhibitors.[20]

This study aims to discover potentially active Benzothiazole analogues through the structure based virtual screening to identify the potent compounds. The present research work involved the preliminary *insilico* screening of 25 novel analogues for quantifying their drug likeness using Molinspiration software. ADMET properties of these compounds are evaluated through the Swiss ADME and preADMET software. The docking studies are performed on glide docking software of Schrödinger. The ligands are docked with the protein (PDB ID:3TOP).

METERIALS AND METHODS

ACD/ChemSketch

ACD/ChemSketch is a molecular modelling program used to create and modify images of chemical structures. Also, a software that allows molecules and molecular models displayed in two and three dimensions, to understand the structure of chemical bonds and the nature of the functional groups. ChemSketch is a comprehensive structure editor with a variety of tools and functionality that ease the communication of scientific and chemical information. Draw molecular structures, generate structures from InChI or SMILES strings, ACD/Labs Version 2021.1.1 software is the latest version. Chemical structures and SMILES notations of the title com-pounds were obtained by using ACD labs Chemsketchversion12.0.
https://www.acdlabs.com/resources/freeware/chemsketch/

MOLINSPIRATION

Molinspiration offers broad range of cheminformatics software tools supporting molecule manipulation and processing, including SMILES and SDfile conversion, normalization of molecules, generation of tautomer's, molecule fragmentation, calculation of various molecular properties needed in QSAR, molecular modelling and drug design, high quality molecule depiction, molecular database tools supporting substructure and similarity searches. It also supports fragment-based virtual screening, bioactivity prediction and data visualization. Molinspiration tools are written in Java, therefore can be used practically on any computer platform. Molinspiration supports internet chemistry community by offering free on-line services for calculation of important molecular properties (log P, polar surface area, number of hydrogen bond donors and acceptors and others), as well as prediction of bioactivity score for the most important drug targets (GPCR ligands, kinase inhibitors, ion channel modulators, nuclear receptors).

PHARMACOKINETIC PARAMETERS AND TOXICITY POTENTIAL

Swiss ADME

In SwissADME, for specialized models, whose predictions are compiled in the Pharmacokinetics section, evaluate individual ADME behaviours of the molecule under investigation.

PreADMET

On the basis of 2D structural models, drawn in ACD Chemsketch, Toxicity properties of proposed compounds were calculated using online preADMET program and observed toxicity properties.

DOCKING

SCHRODINGER MAESTRO 12.5 VERSION

Molecular docking is a computational technique which is widely used to predict the nonbonding interaction of the ligand with the 3D structure of protein that play a crucial role in the hit identification and lead optimisation. In this study, the molecular docking was performed by using Schrodinger Maestro 12.5 Version.

Schrodinger ligand preparation product, Ligprep was used to prepare high quality, all atom 3D structures. The ligand preparation included 2D–3D conversions, generating variations, correction, verification and optimization of the structures. The PDB ID used for ∝-glucosidase receptor is 3TOP. The structures of protein used in this work were downloaded from the Protein Data Bank RSCD PDB. Protein preparation is done by using the Protein Preparation Wizard module of Glide. Initially, the protein structure was imported and pre-processed to be used as a receptor for docking. The typical operations in pre-processing include addition of hydrogen atoms, assignment of atomic charges, assignment of bond orders, creating zero order bond to metals, creating disulphide bonds, filling missing side chains using prime, and elimination of water molecules beyond 5A° from het groups generating het states using epic at pH 7.4 that are not involved in ligand binding. Missing chains and loops can also be added if necessary. Preprocessed protein was optimized with PROPKA and then minimized with OPSL3 force field function, which is followed by a convergence of heavy atoms of RMSD 0.3 Å. Receptor grid were generated using Receptor grid generation in the Glide application of Maestro. The receptor grid was generated by specifying the binding (active) site residues, which was identified by SiteMap tool. Site map analysis is used for finding, visualizing, and evaluating binding sites in protein. Visualization helps in finding potential hydrophobic and hydrophilic regions. The Glide's receptor grid generation wizard was used to generate a three-dimensional (3D) grid with a maximal size of 20 × 20 × 20 Å with 0.5 Å spacing.

There is enough option to apply any constraints such as precision constraints, H-bond constraint, etc., in the receptor grid generation wizard. Once the receptor grid is generated, the ligands are docked to the protein using Glide (Grid based Ligand Docking with Energetics) docking protocol. The ligands were docked using "Extra precision mode" (XP) in glide docking module. The docked conformers were evaluated using Docking Score.

RESULTS AND DISCUSSION

Structure of the Proposed novel Benzothiazole analogue

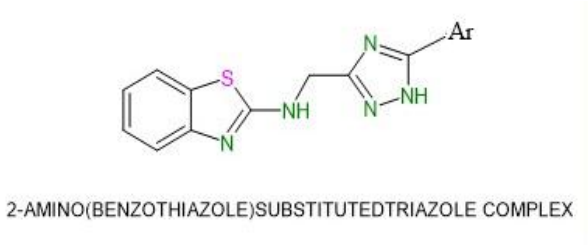

Fig 01: Proposed Benzothiazole analogue.

Proposed analogues were subjected to *in silico* screening studies with aid of software like ACD Chemsketch, Molinspiration, Swiss ADME, preADMET and Glide programmes.

ANALYSIS OF DRUG LIKENESS PROPERTIES

Structures of the compound were drawn by means of Chemsketch and the SMILE notations are generated.

Molinspiration software is used to calculate the "LIPINSKI RULE OF FIVE" and drug likeness analysis. According to LIPINSKI RULE OF FIVE, none of the compounds shows the violation of the rule. The results are presented in Table 01.

Table 01: Lipinski Rule of Five Analysis.

Si. No	Compound Code	Ar	Log P	MW	nOH	nOHNH	No: VIOLATIONS
1	F1	Phenyl	3.43	307.38	5	2	0
2	F2	2 Chloro Phenyl	4.06	341.83	5	2	0
3	F3	3 Chloro Phenyl	4.08	341.83	5	2	0
4	F4	4 Chloro Phenyl	4.10	3.41.83	5	2	0
5	F5	2 bromo phenyl	4.19	386.28	5	2	0
6	F6	3 bromo phenyl	4.21	386.28	5	2	0
7	F7	4 bromo phenyl	4.24	386.28	5	2	0
8	F8	2 methyl Phenyl	3.83	321.41	5	2	0
9	F9	3 methyl Phenyl	3.85	321.41	5	2	0
10	F10	4 methyl Phenyl	3.87	321.41	5	2	0
11.	F11	2 nitro Phenyl	3.34	352.38	8	2	0
12	F12	3 nitro Phenyl	3.36	352.38	8	2	0
13	F13	4 nitro Phenyl	3.38	352.38	8	2	0
14	F14	2 Fluro phenyl	3.54	325.37	5	2	0
15	F15	3 Fluro phenyl	3.56	325.37	5	2	0
16	F16	4 Fluro phenyl	3.59	325.37	5	2	0
17	F17	2 hydroxy Phenyl	3.16	323.38	6	3	0
18	F18	3 hydroxy Phenyl	2.92	323.38	6	3	0
19	F19	4 hydroxy Phenyl	2.95	323.38	6	3	0
20	F20	2 isopropyl Phenyl	4.42	349.46	5	2	0
21	F21	3 isopropyl Phenyl	4.91	349.46	5	2	0
22	F22	4 isopropyl Phenyl	4.94	349.46	5	2	0
23	F23	2 methoxy phenyl	3.44	337.41	6	2	0
24	F24	3 methoxy phenyl	3.46	337.41	6	2	0
25	F25	4 methoxy phenyl	3.48	337.41	6	2	0

ADME AND TOXICITY PREDICTION

ADME properties are evaluated by means of SWISS ADME software. It examined every individual ADME behaivours of molecule under investigation. The predictions for passive human gastrointestinal absorption (HIA) are carried out BOILED-Egg model17. The binary classification models, which focus on the propensity for a given small molecule to be substrate or inhibitor of proteins governing important pharmacokinetic behaviours. The bioavailability score of the proposed derivatives were also calculated. The proposed derivatives are able to estimate important ADME

behaviours for pharmacokinetics optimization and evaluation of small molecules. All the 25 derivatives showed high gastrointestinal absorption, CYP inhibitor, and good bioavailability score. Thus, the 25 proposed derivatives were taken for further toxicity studies.

Table 02: ADME prediction by Swiss ADME Software.

SI. NO	COMPOUND CODE	GI ABSORPTION	CYP1A2 INHIBITOR	BIOAVALABILITY SCORE
1	F1	HIGH	YES	0.55
2	F2	HIGH	YES	0.55
3	F3	HIGH	YES	0.55
4	F4	HIGH	YES	0.55
5	F5	HIGH	YES	0.55
6	F6	HIGH	YES	0.55
7	F7	HIGH	YES	0.55
8	F8	HIGH	YES	0.55
9	F9	HIGH	YES	0.55
10	F10	HIGH	YES	0.55
11	F11	HIGH	YES	0.55
12	F12	HIGH	YES	0.55
13	F13	HIGH	YES	0.55
14	F14	HIGH	YES	0.55
15	F15	HIGH	YES	0.55
16	F16	HIGH	YES	0.55
17	F17	HIGH	YES	0.55
18	F18	HIGH	YES	0.55
19	F19	HIGH	YES	0.55
20	F20	HIGH	YES	0.55
21	F21	HIGH	YES	0.55
22	F22	HIGH	YES	0.55
23	F23	HIGH	YES	0.55
24	F24	HIGH	YES	0.55
25	F25	HIGH	YES	0.55

The 25 proposed derivatives are taken for toxicity studies by using online preADMET program and studied the toxicity properties. All the 25 proposed derivatives showed “NO RISK” of carcinogenicity and mutagenicity.

Table 03: Toxicity prediction by preADMET Programme.

COMPOUND CODE	TOXICITY	
	MUTAGENICITY	CARCINOGENICITY
F1	NO RISK	NO RISK
F2	NO RISK	NO RISK
F3	NO RISK	NO RISK
F4	NO RISK	NO RISK
F5	NO RISK	NO RISK
F6	NO RISK	NO RISK
F7	NO RISK	NO RISK
F8	NO RISK	NO RISK
F9	NO RISK	NO RISK
F10	NO RISK	NO RISK
F11	NO RISK	NO RISK
F12	NO RISK	NO RISK
F13	NO RISK	NO RISK
F14	NO RISK	NO RISK
F15	NO RISK	NO RISK
F16	NO RISK	NO RISK

F17	NO RISK	NO RISK
F18	NO RISK	NO RISK
F19	NO RISK	NO RISK
F20	NO RISK	NO RISK
F21	NO RISK	NO RISK
F22	NO RISK	NO RISK
F23	NO RISK	NO RISK
F24	NO RISK	NO RISK
F25	NO RISK	NO RISK

MOLECULAR DOCKING STUDIES

The 25 derivatives were docked with 3TOP by means of Schrodinger 12.5 software in GLIDE interface. The 25 prepared ligands are docked to the protein (PDB ID: 3TOP CHAIN A). The results are compared with the standard drug ACARBOSE. The Compounds such as F18, F25, F3, F5, F16, F20 showed high docking score. The docking interaction reveals that the compound F18 is linked with the ILE 1814, MET 1778, ASN 1776 of the amino acid residue of the receptor and the compounds F25, F3, F5 and F15 are linked with ILE 1814, MET 1778 of the amino acid residues of the receptor.

Table 04: Molecular docking with Glide.

COMPOUND CODE	DOCKING SCORE
F1	-6.3
F2	-4.5
F3	-7.5
F4	-4.8
F5	-8.0
F6	-4.7
F7	-5.2
F8	-6.0
F9	-6.2
F10	-4.3
F11	-4.1
F12	-2.4
F13	-5.0
F14	-4.5
F15	-6.5
F16	-7.5
F17	-6.3
F18	-8.4
F19	-8.0
F20	-7.5
F21	-4.5
F22	-4.4
F23	-8.2
F24	-6.2
F25	-8.2
STANDARD(ACARBOSE)	-8.5

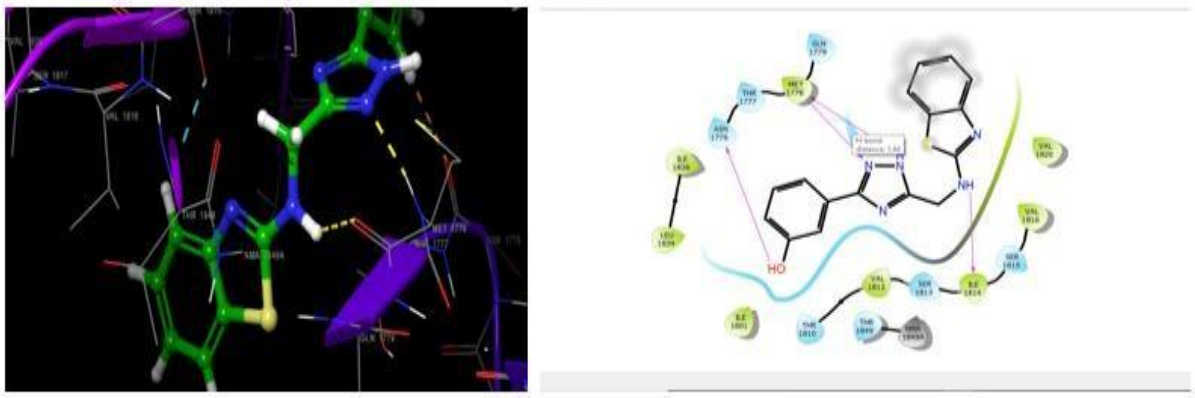

Fig 02: 2D and 3D binding interactions of Compound F18 on 3TOP.

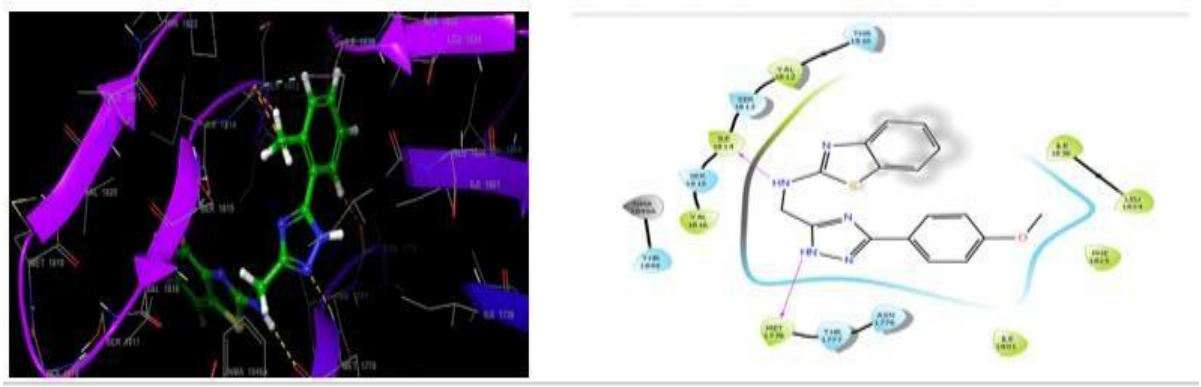

Fig 03: 2D and 3D binding interactions of Compound F25 on 3TOP.

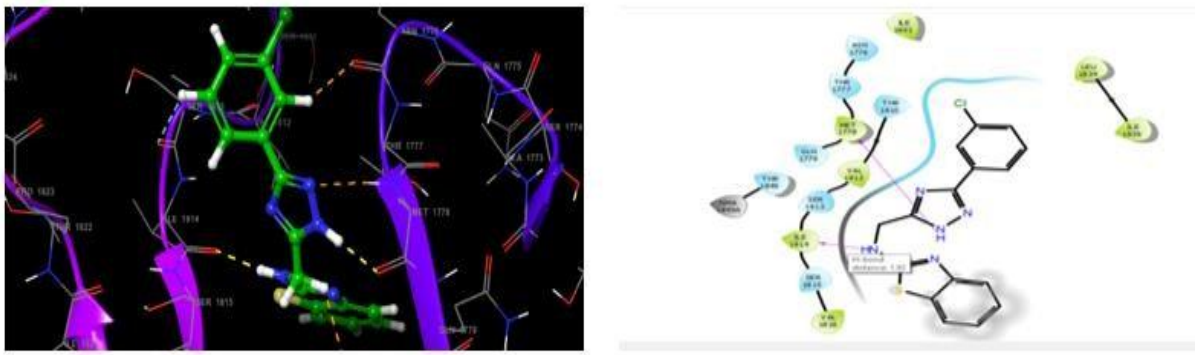

Fig 04: 2D and 3D interactions of Compound F3 on 3TOP.

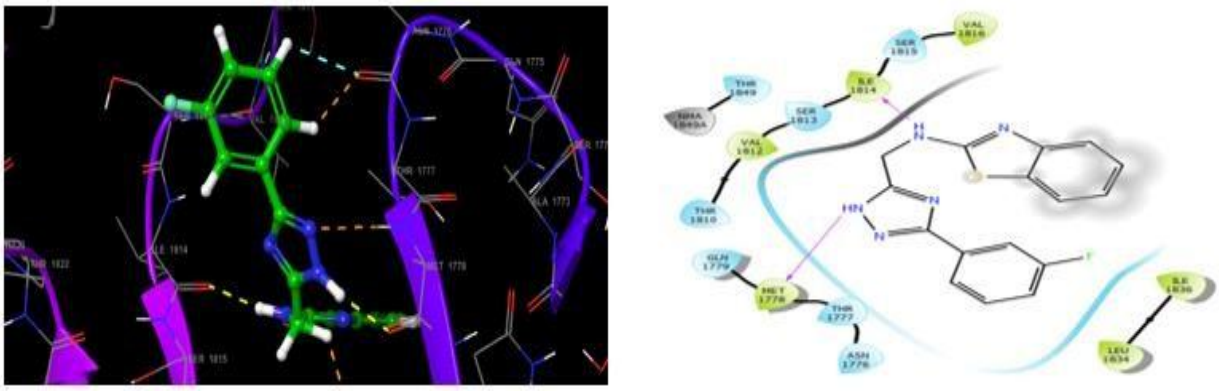

Fig 05: 2D and 3D interactions of Compound F15 on 3TOP.

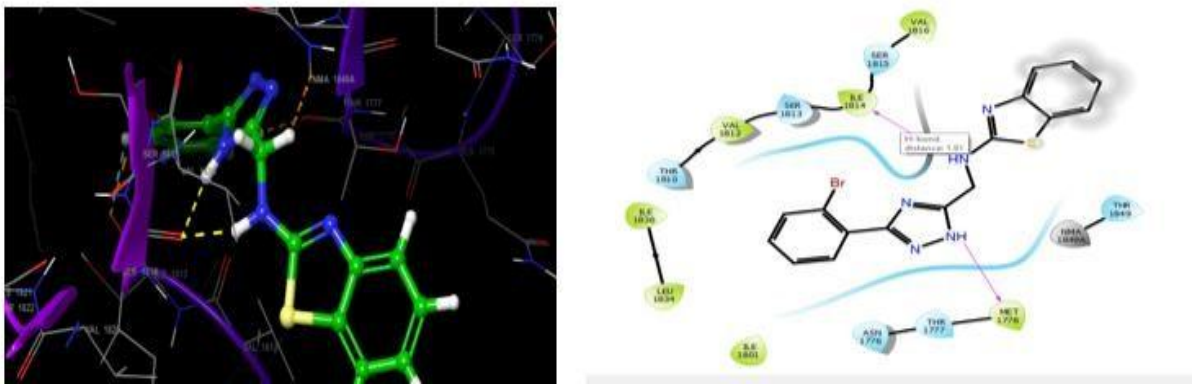

Fig 06: 2D and 3D interactions of Compound F5 on 3TOP.

CONCLUSION

The research work was an attempt to explore the antidiabetic potential of benzothiazole scaffold in drug development through *in silico* studies. An in-silico study was carried out on 25 Benzothiazole derivatives as anti-diabetic compounds. The present work involved in the preliminary *in silico* screening of various novel analogues for quantifying their drug likeness using Molinspiration software. The ADMET studies are carried out by Swiss ADME and preADMET software. All the proposed derivatives were docked with protein obtained from PDB with PDB ID: 3TOP, using Schrödinger maestro 12.5 software's glide programme. These compounds may eventually lead to the identification of potential pharmacologically active compounds. Molecular Docking for this study revealed that ligand F18, F25, F3, F5 and F15 are the most active compounds having the highest docking scores. Ligand F18 being among the ligands with the highest docking scores form 3 interactions. From these studies, it was found that, it was found that in future these newly synthesized derivatives of substituted Benzothiazole can be considered as a lead molecule in antidiabetic drug discovery process.

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