COMPUTATIONAL DESIGN AND TOXICITY PREDICTION OF OXAZOLE DERIVATIVES TARGETING PPARγ AS POTENTIAL THERAPEUTICS FOR DIABETES MELLITUS IN COMPARE TO ROSIGLITAZONE AND PIOGLITAZONE

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ABSTRACT

The goal of this research is to investigate new oxazole derivative from designed series (A1-7; B1-8 & C1-8) in order to find new drug molecules for treatment of Diabetes Mellitus (DM). The PPAR receptor was chosen as the target of molecular docking investigations, which were executed using PyRx software. In silico analyses, including physicochemical properties, drug score, drug likeness, solubility, and toxicity prediction, were conducted using software such as Swiss ADME, Osiris property explorer, Lipinski filter and Toxtree method. All molecules passed the Lipinski rule with the zero violations and synthetic score was also found to be in the easy limit. All ligands showed drug score values ranging from 0.11 to 0.9 (no negative value). Compounds A6, C2, C5, C6, C7 and C8 were shown drug score from 0.91 to 0.80, which is closer to 1 and therefore considered as druggable ligands, when compared with the standard drug, Rosiglitazone and Pioglitazone also found non-toxic. All compounds shown logP values between -0.25 to 4.58. The RMSD value of receptor and receptor-ligand complexes was analyzed, and it revealed the stability of binding interactions and remained stable throughout the simulation. Compound C8 was found highest RMSD score (67.34Å) in compare to other compounds and standard drug Rosiglitazone (64.31Å). The TPSA were found within the range 35.26 to 128.60 and MR also were in the range 32.21-113.62. Compounds were found to be non-substrate for p glycoprotein except C4, high GIA% (>90%), also displayed negative permeability across the BBB, and most of compounds were found inhibitor of CYP 1A2 and CYP 2C19 and non-inhibitor of CYP 2C9, CYP 2D6 and CYP 3A4. Compound C5 was exhibited higher drug score (0.91), bioactivity score and revealed good drug relevant properties, ADME and no toxicity profile in compared to other ligands and standard drugs. The most active compound of the series was found C5 and C8 therefore further studies on this compound continue in our research laboratory to acquire more information about SAR and QSAR. Finally, it is conceivable that further derivatization of these compounds could result in obtaining more selective lead compounds.

Keywords: PPARγ, Oxazole, Binding Site, Computer Aided Design, Drug Discovery, Diabetes Mellitus.

INTRODUCTION

Diabetes Mellitus (DM) refers to a class of metabolic diseases that, if uncontrolled, result in hyperglycemia. The World Health Organization (WHO) estimates that 422 million adults worldwide had diabetes in 2014, with 70 million cases being reported from India alone. By 2045, 629 million people worldwide are expected to have diabetes, according to the International Diabetes Federation (IDF). These disorders arise from deficiency in insulin action, insulin secretion, or both, and disrupt the metabolism of fats, carbohydrates, and proteins. The global incidence and mortality rates related to diabetes are on the rise, necessitating comprehensive planning, monitoring, and inspecting global precautions for the effective management of this fatal non-contagious disease[1-2]. The expansion of diabetes includes various pathogenic mechanisms. There are mainly three major types of diabetes mellitus: Type 1, which is an insulin-dependent chronic autoimmune disorder where the pancreas synthesizes little or no insulin; Type 2, a chronic illness also known as insulin-independent diabetes mellitus that affects blood glucose regulation; and Type 3, known as gestational diabetes, diagnosed during pregnancy and arising due to glucose intolerance [3-4].

Currently, there is an extensive anti-diabetic drug accessible in the market, such as Biguanide, Thiazolidinedione, GLP-1 receptor agonists, Sulfonylureas, Meglitinide analogues, DPP-4 inhibitors, and alpha glucosidase and alpha amylase inhibitors. In spite of this these medicines have displayed efficacy in managing diabetes, they are also associated with several side effects, including nephropathy, cardiovascular issues, kidney complications, chronic joint inflammation, hypoglycemia, liver dysfunction, diarrhea, and digestive discomfort[5-7]. Consequently, there is a requirement to develop diabetes treatment alternatives that are safer and better tolerated. Several targeted receptors have been identified for the managing type 2 DM, including glucokinase, PPARγ, aldose reductase, glycogen phosphorylase, insulin receptor, protein tyrosine phosphate 1-β, alpha-glucosidase, and Dipeptidyl peptidase-4 (DPP-4). Peroxisome proliferator-activated receptor gamma (PPAR) is a glitazone receptor and one of these targets[8-9].

It is essential for regulating genes involved in cell differentiation and multiple metabolic processes, including lipid and glucose homeostasis. Stimulation of the PPARγ receptor promotes insulin sensitization and increases glucose metabolism. In adipocytes, activation of PPARγ enhances the production of insulin mediators in peripheral tissues. A DNA-binding domain, an agonist-independent initiation domain, and an agonist-dependent stimulation domain make up the PPARγ structure. Drugs that bind to the PPARγ receptor heterodimerize with the retinoid X receptor, which then activates target protein genes by interacting with the PPRE response component[10-12].

Oxazole moiety is comprised of a five-membered ring with oxygen at the 1 position and nitrogen at the 3rd position. It is an aromatic compound with weak base properties and possesses three active substitutions at positions C-2, C-4 and C-5. Oxazole has demonstrated therapeutic activity and exhibits various biological activities, including antibacterial, antifungal, anti-inflammatory, anticancer, antidepressant, and hypoglycemic effects. Molecular docking is a computational modeling method employed in bioinformatics to study the interaction among molecules or ligands, resulting in the formation of stable complexes [13-14]. These computational tools are significant for anticipating the 3D structure of the complex and depend on the binding properties between the ligand and target proteins. Molecular docking techniques enable the identification of important factors such as binding affinity, free energy, prediction of active sites, and stability of the complexes[14].

Computer-aided drug design (CADD) is a rapid and cost-effective method employed in the discovery of novel drugs. Computational studies have gained prominence in pharmaceutical development, particularly in predicting the ADMET properties of compounds. By investigating the ADMET properties, researchers can identify potential drug candidates and prioritize them based on the "Lipinski rule of 5," which improves the likelihood of success in higher-class drug selection. Molecular docking plays a crucial part in drug design and development by determining the interactions between lead molecules and target receptor sites, along with evaluating their binding energies[15-16]. This research

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utilizes the Swiss ADME online software (http://www.swissadme.ch/), which assists in the development and discovery of drugs with varying molecular sizes and structures. The software facilitates the prediction of ADME studies, physicochemical properties, pharmacokinetics, drug-likeness, molecular weight, water solubility, and permeability, providing valuable insights into the potential of drug candidates [17].

With the increasing average cost of developing new drugs, which currently stands at around 2.8 billion U.S dollars, there is a need for extensive screening of lead molecules in the initial phases of drug development to ensure safety and efficacy, while also managing costs. Computational technique has become preferred by scientists due to their benefits of being less laborious, accurate, and cost-effective, enabling the screening of a larger number of compounds. In our research, we have utilized two important online freeware tools, namely OSIRIS and Toxtree. OSIRIS property explorer, which can be freely downloaded online (https://www.organic-chemistry.org/prog/peo/), allows the prediction of toxicity risks and calculation of physicochemical properties for novel lead molecules. To use this software, a Java platform needs to be created, providing an easy way to assess toxicity risks associated with the compounds. Toxtree (v3.1.0), available online as freely accessible software (http://toxtree.sourceforge.net/), employs a decision tree method to estimate the toxic hazards of molecules. The results obtained from Toxtree include information on carcinogenicity, genotoxicity, skin irritation, mutagenicity, sensitization, and biodegradability of the compounds[18-20].

PyRx software is automated docking software available online that facilitates the exploration of protein-ligand interactions by predicting docking modes. This software specifically reads PDB format files, which accelerates the calculations of binding energy. In our study, we employed the PyRx software to examine the interactions of oxazole as ligands with the target receptor 1PRG. The docking results obtained from PyRx software were thoroughly analyzed and discussed in our manuscript. PyRx software is widely utilized by industries, biopharmaceutical companies, researchers, academic institutions, and other laboratories for the discovery of novel medicines. In our research, we conducted molecular docking studies using PyRx software with the receptor protein PPARγ (1PRG) to evaluate the anti-diabetic activity of 25 novel oxazole derivatives. We compared the binding of these compounds with the standard drug rosiglitazone, which exhibited a binding affinity of -9.1. After analyzing the drug-receptor interactions, we further examined the compounds with the lowest binding energy in comparison to the standard drug [21-22]. The structures of these selected compounds were visualized using the online freeware Discovery Studio Visualization (https://discover.3ds.com/discovery-studio-visualizer-download).

MATERIAL AND METHOD

We employed OSIRIS, Toxtree, PyRx software, ChemDraw3D 15.1, Open Babel, Swiss ADME, Discovery studio visualizer, and other bioinformatics tools in our present investigation.

Selection of ligand

We identified 25 distinctive oxazole derivatives, and Chem Draw 3D was used to develop the 2D molecular structures. The structures were then stored in PDB file format. After that, a number of physicochemical parameters of the chosen derivatives were determined and optimized. These values, which were created for molecular docking studies, were then contrasted with the standard drug rosiglitazone and pioglitazone. The selection process involved obtaining Rosiglitazone and Pioglitazone, two standard drugs, from PubChem. The compounds were downloaded in SDF format and subsequently converted to PDB format files are listed in Figure 1.

Preparation of ligands

Using the free PyRx software, the ligand was created by altering ionization, torsion, removing water molecules, adding polar hydrogen, and adding Kollman charges. The ligand was after then saved in PDBQT file format [22-23].

Target Molecule: Selection and Preparation

The crystal structure of target protein PPARγ were downloaded from protein database https://www.rcsb.org/ (1 PRG), DOI: 10.2210/pdb1PRG/pdb and saved as PDB format. Their solution of protein structure is ranging from 2.1to 2.20Å. Each ligand was individually docked into the receptor using ligand-protein interactions as a basis [24-25]. The 3D structure of the receptor was visualized using Discovery Studio Visualizer, as depicted in Figure 1.

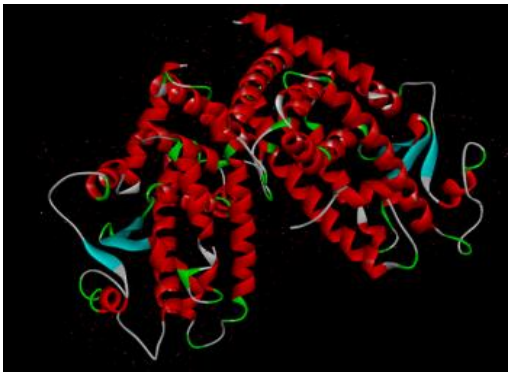

Fig 1: 3D structure of PPARγ (PDB ID: 1PRG) drawn in discovery studio visualizer software.

Prediction of Physicochemical Properties

The physicochemical properties of the ligands were determined, and the selected ligands were screened using Lipinski's Rule of Five. This rule is employed to evaluate lipophilicity, polar surface area, hydrogen bond acceptors, hydrogen bond donors, water solubility, and refractivity, which are important factors[26]. Table 1 presents the corresponding values obtained.

Prediction of ADME properties

Swiss ADME web-based tool is free online software used for screening of pharmacokinetic properties like absorption, distribution, metabolism and excretion. We have also predicted the oral bioavailibity, lipophilicity and solubility of ligand molecules.[27] Structures were drawn in the screen of software which is then converted into SMILES format as input file. As we know that absorption of drugs relies on water solubility, skin permeation (log Kp), P-glycoprotein, Gastro-Intestinal absorption and permeability and distribution is influenced by blood-brain barrier (BBB).[28] Different CYP models are used to evaluate the distribution and metabolism of oxazole derivatives specifically CYP1AC2 inhibitor, CYP2C19 inhibitor, CYP2C9 inhibitor, CYP2D6 inhibitor and CYP3A4 inhibitor. Lastly, excretion is influenced by the total clearance. Table 2 shows the predicted results of all the 25 derivatives and standard drug.[29] Swiss ADME software also provides a graphical representation for orally available bioactive drug.

Drug score and toxicity prediction of selected ligands

Drug discovery and development of novel drug molecule by using computational drug designing the essential role is to provide drug with low toxicity for use of oral administration. The drug marketed for oral use must be non-toxic should possess good absorption and dissolution in gastro intestinal tract to reach the blood. Therefore, drug solubility and dissolution (logS) are an important factor for drug likeness predictions.[30] Afterwards, these compounds 1 to 25 were also simulated for solubility, toxicity, drug score and drug likeness using OSIRIS tool. The properties of these derivatives like mutagenic, tumorigenic, skin irritation, and reproductive effect are coded in colors. High toxicity is indicated by red color, yellow indicates standard drug and green shows no toxicity risk. Table 3 shows OSIRIS data of selected 25 compounds, blue color indicates the importance of standard ligand. Over all the compounds ligand C_3, C_5, C_7, C_8 and A_6 highlighted and have excellent drug score and possess low toxicity. We have also predicted toxicity through online available software Toxtree (v3.1.0version) for comparison, which is used to identify, analyze and estimate the toxic hazard using decision tree approach. It is done by entering SMILES of the ligand molecule as an input file, and the results collected from Toxtree freeware.

Molecular docking study using PyRx

Molecular docking of all the selected oxazole derivatives was done by using PyRx software. PyRx used ligand and protein structure in PDB format. Anti-

diabetic activity of these molecules was predicted by evaluating the binding energy score and binding affinity when the selected ligand fit to a target receptor. All the parameters were evaluated and show that the drug with lowest binding energy gives the excellent interactions.[31-32] In our study rosiglitazone is used as standard drug for comparison purpose was docked with PPARγ receptor recognizing the predicted data. The docking studies reveal that the molecules with lowest negative binding energies are known to be best-docked oxazole derivative with PPARγ. The target protein was prepared in discovery studio by removing water and hetero atoms. Then addition of polar hydrogens and kollman charges was done. Grid was generated using grid box for binding at specific amino acid at the receptor site. Then docking was done after ligand and protein preparation using PyRx.

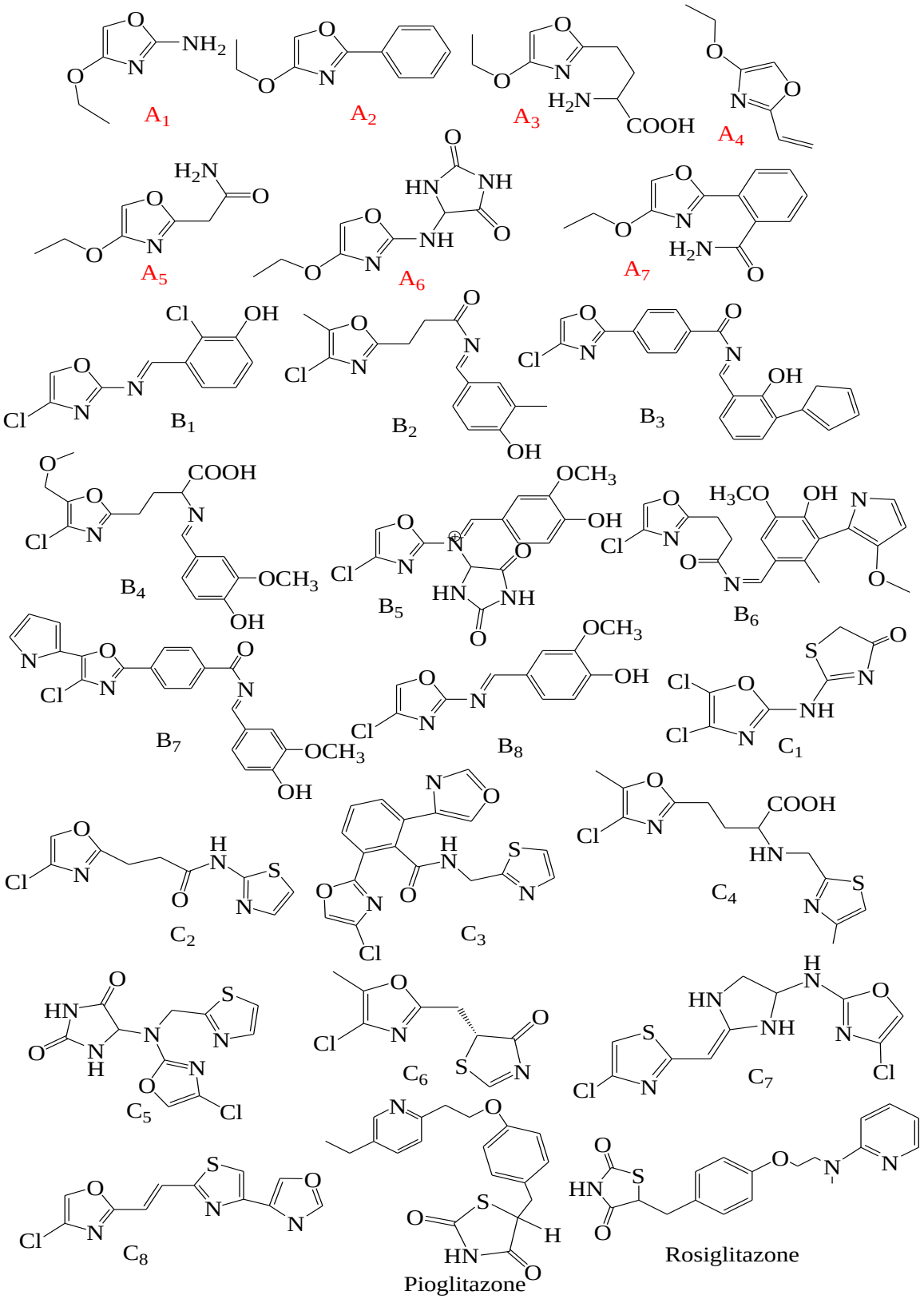

Fig 2: Displaying 25 oxazole derivatives, and standard drugs Rosiglitazone and Pioglitazone.

Table 1: Physicochemical properties of the selected ligands.

Ligands	Molecular formula	MW g/mol	CLogP	HBD	HBA	LV	MR	TPSA (Å)
A1	$C_5H_8N_2O_2$	128	0.59	1	3	0	32.21	61.28
A2	$C_{11}H_{11}NO_2$	189.21	2.53	0	3	0	53.24	35.26
A3	$C_9H_{14}N_2O_4$	214.22	0.38	2	6	0	51.67	96.58
A4	$C_7H_9NO_2$	139.15	1.61	0	3	0	37.89	35.26
A5	$C_7H_{10}N_2O_3$	170.12	0.22	1	4	0	40.48	78.35
A6	$C_8H_{10}N_4O_4$	226.19	-0.25	3	5	0	58.83	105.4
A7	$C_{12}H_{12}N_2O_3$	232.24	1.67	1	4	0	61.33	78.35
B1	$C_{10}H_6Cl_2N_2O_2$	257.07	2.94	1	4	0	62.24	58.62
B2	$C_{15}H_{15}ClN_2O_3$	306.74	3.05	1	5	0	81.23	75.69
B3	$C_{22}H_{15}ClN_2O_3$	390.82	4.58	1	5	0	109.26	75.69
B4	$C_{17}H_{19}ClN_2O_6$	382.80	2.27	2	8	0	95.02	114.38
B5	$C_{14}H_{12}ClN_4O_5$	351.72	-0.13	3	6	0	89.14	116.70
B6	$C_{20}H_{20}ClN_3O_5$	417.84	3.23	2	7	0	109.03	109.94
B7	$C_{22}H_{16}ClN_3O_4$	421.83	4.08	2	6	0	113.62	100.71
B8	$C_{11}H_9ClN_2O_3$	252.65	2.44	1	5	0	63.72	67.85
C1	$C_6H_3Cl_2N_3O_2S$	252.08	1.71	1	4	0	58.13	92.79
C2	$C_9H_8ClN_3O_2S$	257.70	1.76	1	4	0	60.79	96.26
C3	$C_{17}H_{11}ClN_4O_3S$	386.81	2.84	1	6	0	95.60	122.29
C4	$C_{13}H_{16}ClN_3O_3S$	329.80	1.48	2	6	0	80.37	116.49
C5	$C_{10}H_8ClN_5O_3S$	313.72	0.60	2	5	0	77.60	128.60
C6	$C_8H_7ClN_2O_2S$	230.67	1.84	0	4	0	58.55	80.76
C7	$C_{10}H_9Cl_2N_5OS$	318.18	2.13	3	3	0	82.05	103.25
C8	$C_{11}H_6ClN_3O_2S$	279.70	2.62	0	5	0	68.05	93.19
Rosiglitazone	$C_8H_{19}N_3O_3S$	357.43	2.28	4	7	0	101.63	96.83
Pioglitazone	C19H20N2O3S	356.44	2.61	1	4	0	102.17	93.59

LV: Lipinski's violation, MW: Molecular weight, CLogP: Lipophilicity, HBD: Number of hydrogen bond donor, HBA: Number of hydrogen bond acceptor, MR: Molar refractivity, TPSA: Topological polar surface area in Å.

Table 2: Prediction of absorption, distribution, metabolism and excretion parameters of selected oxazole derivatives using Swiss ADME.

Ligands	GI	BBB	P-gp substrate	CYP 1A2 inhibitor	CYP 2C19 inhibitor	CYP 2C9 inhibitor	CYP 2D6 inhibitor	CYP 3A4 Inhibitor	Log Kp (cm/s)
A1	High	No	No	No	No	No	No	No	-6.61
A2	High	Yes	No	Yes	Yes	No	No	No	-5.56
A3	High	No	No	No	No	No	No	No	-8.06
A4	High	Yes	No	Yes	No	No	No	No	-5.86
A5	High	No	No	No	No	No	No	No	-5.55
A6	High	No	No	No	No	No	No	No	-7.54
A7	High	No	No	Yes	No	No	No	No	-6.62
B1	High	Yes	No	Yes	Yes	No	No	No	-5.59
B2	High	Yes	No	Yes	Yes	Yes	No	No	-5.93
B3	High	No	No	Yes	Yes	Yes	No	No	-5.19
B4	High	No	Yes	Yes	Yes	No	No	No	-6.99
B5	High	No	Yes	No	No	No	No	No	-7.43
B6	High	No	No	Yes	Yes	Yes	No	Yes	-6.61
B7	High	No	No	No	Yes	Yes	No	Yes	-5.86
B8	High	Yes	No	Yes	Yes	No	No	No	-6.03
C1	High	No	No	Yes	No	No	No	No	-6.15
C2	High	No	No	Yes	Yes	No	No	No	-6.62
C3	High	No	No	Yes	Yes	Yes	Yes	Yes	-6.61
C4	High	No	Yes	No	Yes	No	No	No	-8.15
C5	High	No	No	No	Yes	No	No	No	-7.38
C6	High	No	No	Yes	Yes	No	No	No	-6.19
C7	High	No	No	Yes	Yes	Yes	No	Yes	-5.49
C8	High	No	No	Yes	Yes	Yes	Yes	No	-6.03
Rosiglitazone	High	No	No	No	Yes	Yes	Yes	Yes	-6.27
Pioglitazone	High	No	No	Yes	Yes	Yes	Yes	Yes	-5.81

GI: Gastro intestinal absorption, BBB: blood brain barrier permeation, P-gp: p-glycoprotein substrate, CYP1AC2: Cytochrome P450 family 1 subfamily A member 2 (PDB:2HI4), CYP2C19: Cytochrome P450 family 2 subfamily C member 19 (PDB:4GQS), CYP2C9: Cytochrome P450 family 2 subfamily C member 9 (PDB:1OG2), CYP2D6: Cytochrome P450 family 2 subfamily D member 6 (PDB:5TFT), CYP3A4: Cytochrome P450 family 3 subfamily A member 4 (PDB:4K9T), Log Kp: skin permeation in cm/s.

Table 3: Drug score and toxicity studies of selected compounds using OSIRIS freeware.

Ligands	Log S	Drug likeness	Drug score	Toxicity			
				Mutagenic	Tumorigenic	Reproductive effect	Skin irritant
A1	-1.33	-2.43	0.52	No	No	No	No
A2	-3.08	-0.65	0.55	No	No	No	No
A3	-1.3	-1.30	0.49	No	No	No	No
A4	-2.0	-6.70	0.23	No	Yes	No	No
A5	-0.85	-0.94	0.63	No	No	No	No
A6	-1.33	1.17	0.83	No	No	No	No
A7	-2.47	-2.51	0.21	No	No	No	No
B1	-3.82	0.32	0.64	No	No	No	No
B2	-3.33	-3.97	0.25	No	Yes	No	No
B3	-6.01	-3.39	0.11	Yes	Medium	No	No
B4	-2.54	-1.59	0.40	No	Medium	No	No
B5	-2.07	-1.33	0.35	No	No	No	No
B6	-3.8	-0.5	0.49	No	No	No	No
B7	-5.76	-1.44	0.23	No	Medium	No	No
B8	-3.1	1.04	0.62	No	Medium	No	No
C1	-3.6	-1.05	0.26	No	Yes	No	Medium
C2	-2.33	1.40	0.83	No	No	No	No
C3	-6.29	3.43	0.61	No	No	No	No
C4	-2.06	-2.63	0.49	No	No	No	No
C5	-2.62	6.43	0.91	No	No	No	No
C6	-1.79	0.75	0.80	No	No	No	No
C7	-4.38	2.48	0.73	No	No	No	No
C8	-2.93	2.39	0.86	No	No	No	No
Rosiglitazone	-3.67	9.14	0.80	No	No	No	No
Pioglitazone	-4.23	8.98	0.70	No	No	No	No

Table 4: Prediction of toxicity for ligands using Toxtree freeware.

Ligands	Carmmer's rule	KroesTTC	Carcinogenicity	Skin sensitization	Protein binding
A_1	High class	Low risk	Yes	No	No
A_2	Low class	Low risk	No	No	No
A_3	Low class	Low risk	No	No	Yes
A_4	Low class	low risk	No	No	No
A_5	High class	Low risk	No	No	Yes
A_6	High class	Low risk	No	No	Yes
A_7	Low class	Low risk	No	No	No
B1	High class	Low risk	Yes	No	Yes
B2	High class	Low risk	No	No	Yes
B3	High class	Low risk	No	Yes	Yes
B4	High class	Low risk	No	Yes	Yes
B5	High class	Low risk	No	Yes	Yes
B6	High class	Low risk	Yes	No	Yes
B7	High class	Low risk	No	Yes	Yes
B8	High class	Low risk	No	No	Yes
C1	High class	Low risk	No	No	Yes
C2	High class	Low risk	No	No	Yes
C3	High class	Low risk	No	No	Yes
C4	High class	Low risk	Yes	No	Yes
C5	High class	Low risk	No	No	Yes
C6	High class	Low risk	No	No	Yes
C7	High class	Low risk	No	No	Yes
C8	High class	Low risk	No	No	Yes
Rosiglitazone	High class	Low risk	No	No	Yes
Pioglitazone	High class	Low risk	No	No	Yes

Table 5: The docking minimum binding energies of ligands with PPARγ and interacting residues.

Ligands	Minimum Binding energy (Kcal/mol)	RMSD score (Å)	Interacting residues
A1	-5.0	60.12	Met364, Cys285, Ile281, Ile281, Phe264,
A2	-6.0	61.15	Cys285, Arg288, Ile341, Sre342, His449, Phe282
A3	-5.8	41.35	Leu431, Phe432, Leu421, Lys422, Ser428, His425
A4	-5.2	40.51	Glu369, Ser289, Pro366, Leu469, Tyr473, Cys285
A5	-5.6	61.52	Met364, His449, Ser289, Cys285, Arg288, His449, Glu291, Glu343
A6	-7.0	64.71	Pro227, Glu343, Val339, Cys285, Arg288, Ile241, Glu295, Leu228
A7	-7.0	59.82	Pro227, Glu295, Ala292, Leu333, Leu340, Glu291
B1	-6.9	61.48	Glu343, Glu295, Arg288, Val339, Leu228, Met329
B2	-9.3	64.46	Met364, Cys285, His449, Phe363, Phe282, Ser289
B3	-11.1	65.22	Glu343, Glu291, Phe264, Cys285, His266, Arg280, Arg288, Leu228, Leu255, Leu333, Ile341
B4	-7.3	56.13	Glu295, Ala292, Pro227, Met329, Leu333
B5	-7.6	59.49	Met329, Ile325, Arg288, Ala292, Ser289
B6	-8.5	45.29	Leu421, Leu431, Phe432, His425, Lys422, Ser429
B7	-10.1	62.71	His449, Arg288, Cys285, Phe282, Met364, Leu356, Ile281
B8	-7.1	57.21	Glu291, Glu295, Leu333, Leu228, Pro227, Arg288, Lys265
C1	-6.1	59.42	Arg288, Leu228, Glu295, Pro227, Glu295, Leu333, Ala292, Ile326, Met329
C2	-6.8	65.80	Phe282, Phe363, His449, Met364, Cys285, Ile281
C3	-8.4	63.51	Glu295, Leu475, Arg288, Cys285, Lys265, Leu228
C4	-6.8	59.36	Leu421, Leu431, His334, Arg288, Ile326
C5	-6.6	61.34	Met329, Ala292, Arg288, Ile341, Val339, Leu340, Glu295
C6	-6.0	61.19	Tyr473, Gln286, Asp475, His449, Cys285
C7	-7.1	63.24	Glu295, Leu340, Arg288, Ile341, Leu333, Phe264
C8	-7.3	67.34	Cys285, Ile341, Leu340, Ser342, Ala292,Gly291, Arg288
Rosiglitazone	-9.1	64.31	Arg280, Ser342, The264, Ilu341, Gly284, Arg288

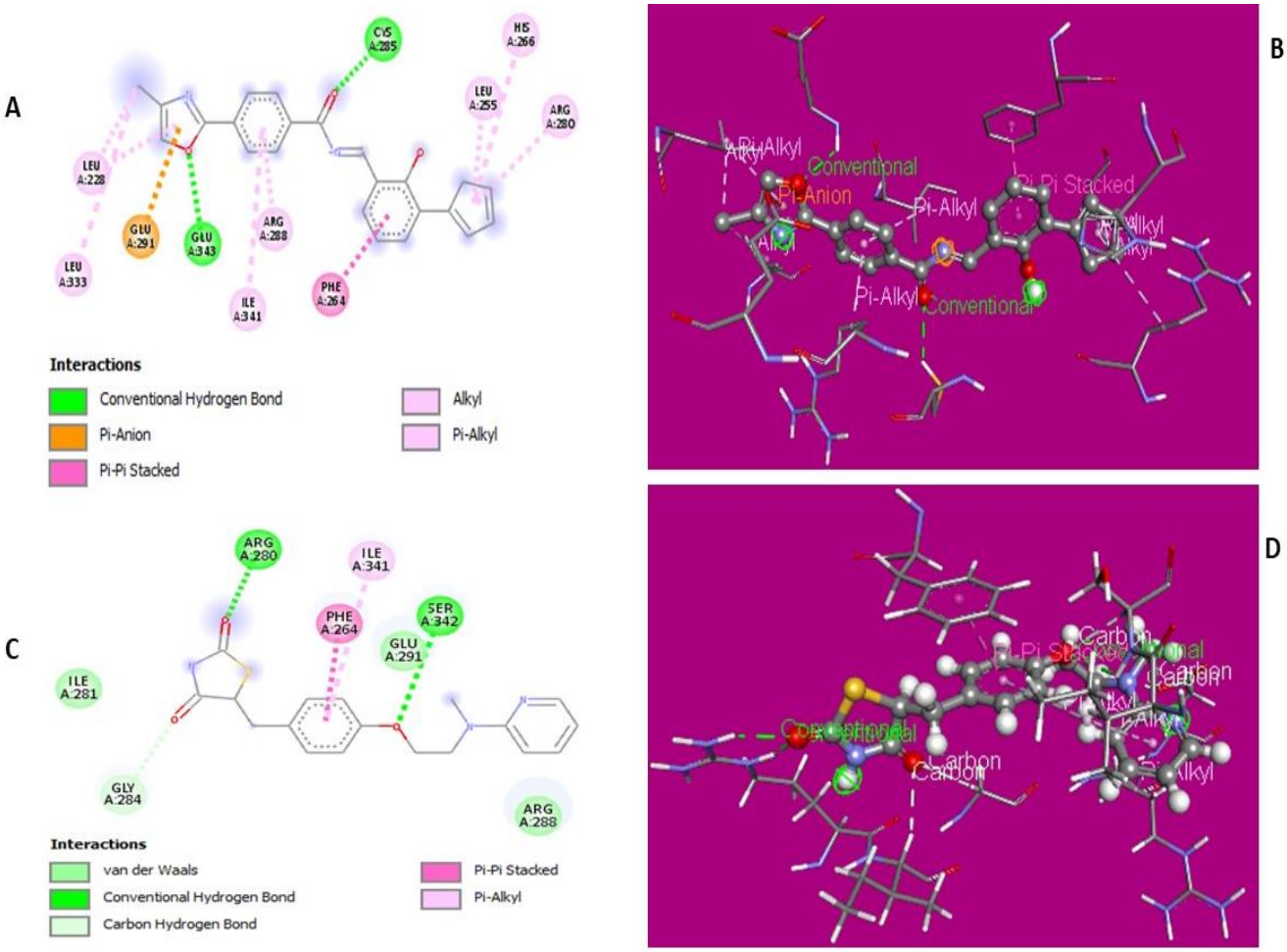

Fig. 3: Illustration of 2D and 3D bind pose of B3 and Rosiglitazone. (A) & (B) 2D & 3D poses of B3 with 1PRG, (C) & (D) 2D & 3D poses of Rosiglitazone with 1PRG.

RESULT AND DISCUSSIONS

Predicted Physicochemical Properties of Ligand

Structure of oxazole derivatives are given in Figure 1. The physical parameters of selected oxazole derivatives, such as hydrogen Bond Donor (HBD), hydrogen Bond Acceptor (HBA), log P, molar refractivity, molecular weight and Lipinski violation are listed in Table 1. In drug discovery and drug design, the main aim is to predict that the selected molecules should be safe, non-toxic and biologically active. Thus, ligands and standard drug molecules have been investigated for their toxicity and drug likeness. The physicochemical properties of different derivatives are listed in Table 1. All the compounds are obeying Lipinski's rule of five and Veber's approach, which is an essential rule for analyzing drug likeness and developing a molecule that enhances its oral activity and bioavailability.[33-35] All the screened compounds follow this rule and indicate high oral absorption and permeation of compounds. Also, Veber's rule is applied to predict oral bioavailability. The rule states that the polar surface area (PSA) should be less than or equal to 140 Å and number of rotatable bonds should be less than 12; indicative of good absorption and permeability. Among these lead compounds ligand B2, B3, B6, and B7 show log P more than 3 due to presence of bulky aromatic ring substituted in oxazole moiety. Lipophilicity (log P) values should be range in between 0 to 3 which shows excellent bioavailability, solubility and permeability. All the 23 compounds show logP values between -0.25 to 4.58. The data is provided in Table 1.

In-silico ADME prediction using Swiss ADME

The predicted ADME properties of oxazole derivatives using Swiss ADME, an online freely available tool (http://www.swissadme.ch/), are listed in Table 2. The total polar surface area (TPSA) of all 25 ligands ranges from 26-105 Å. The result shows that all ligands obey the Lipinski rule, that is, total polar surface area (TPSA) less than 150Å, predicting polarity with effective oral absorption and strong membrane permeation. Compound absorption can be easily predicted by analyzing the Gastrointestinal Absorption (GIA) and P-glycoprotein substrate. The results of GIA reveal that all the ligand molecules have high oral absorption. For BBB permeability, except ligand number A2, A4, B1, B2, B8 all other ligand molecules possess a low BBB permeability level. Results reveal that P-gp substrate or inhibitor is an essential parameter to protect the central nervous system and to prevent multidrug-resistant cancer due to stimulation of P-gp substrate in cancer cells. The ligand B4, B5, and C4 show high P-gp expression and can be carcinogenic. Literature reveals method of estimation of log P, and the values obtained for the selected ligands ranges from 4.58 to -0.25.[19] Compound A2, A4, A7, B1, B4, B8, C3, C7, and C8 shows highest Clog P values, this indicates good bioavailability, on the other hand decreased ClogP between -0.13 to 0.60 indicates high skin permeation.

Interaction of ligands with cytochrome (CYP) P450 enzyme is crucial for metabolism of ligands in liver. Cytochrome P450 enzyme is the standard mechanism derived for metabolism-based drug-drug interactions in pharmacokinetics, this includes several isoenzyme inhibitors such as CYP1AC2, CYP2C19, CYP3A4, CYP2C9 and CYP2D6.[35-37] From the result shown in Table 2 many ligands act as inhibitor of CYP1AC2 and CYP2C19. Compound B2, B3, B6, B7, C3, C7, and C8 inhibit CYP2D6, CYP3A4 and CYP2C9. Thus, among the 25 screened ligands several ligands might be metabolized in the liver. Finally, elimination and excretion of drug molecules can be predicted by solubility and molecular weight of compounds. The results revealed that all the screened molecules follow Lipinski rule of five are said to be drug-like candidates.

Drug Score and Toxicity Prediction

The dissolution of drug can be monitored by drug solubility (log S) analysis which plays an important role to know aqueous solubility of drug in gastrointestinal tract and can cross blood brain barrier easily. The dissolution of drug depends mainly on surface area of the compound. Therefore, aqueous solubility (log S) of the drug considered to be higher than -6.00 which affect drug absorption. The drug score is used to analyze all essential parameters such as drug likeness, molecular weight, Clog P value, and toxicity prediction. If any of the 25 selected ligand molecules shows zero or negative value of drug score, it would be rejected and not considered as drug-like while if the score is greater than zero, it is known to be drug like molecule[37-38].

Toxicity is the pivotal parameter to analyze whether the ligand is toxic or non-toxic. Toxicity of the ligands has been predicted using online available tool OSIRIS and Toxtree. OSIRIS model is used to predict drug score, log S, drug likeliness and toxicity. *In-vitro* and *in-vivo* toxicity studies are considered to be tedious and costly. So, *in-silico* toxicity and drug-likeliness studies of compounds has been effectively studied without animal use. The OSIRIS software predicts several toxicity parameters such as tumorigenic effect, mutagenicity, reproductive effect, and irritant effect ofcompounds.[39-40] Drug can show toxicity with no risk, medium risk, and high risk. The selected ligands are effective and cause no toxicity. The ligand A1, B1, B3, B4, B5, and C4 show high toxicity risk as presented in Table 4.

The standard drug, Rosiglitazone and Pioglitazone (Figure 1), is used to validate the software, which shows drug-likeness with the drug score 0.80 and 0.70, and non-toxicity risk. All ligands show drug score values ranging from 0.11 to 0.9 (no negative value). Compound A6, C2, C5, C6, C7, and C8 show drug score from 0.91 to 0.8, which is closer to 1 and therefore considered as druggable compound, also these are non-toxic (Table 3 and 4). Toxtree prediction also conforms the compounds are druggable with no toxicity. Compound A6, C2, C5, C6, C7, and C8 possess no toxicity risk and high drug score as compared to standard drugs as estimated by Toxtree method. Cramer's rule indicates that all the above compounds are in high class and Kroes thresholds of toxicological concern (TTC) decision tree estimates the toxicity nature of compounds. It could be concluded that among 25 oxazole derivatives, the ligands A6, C2, C5, C6, C7, and C8 are druggable ligands when compared with the standard drug, Rosiglitazone and Pioglitazone.

Molecular Docking Analysis

Among all the oxazole derivatives, ligand B3 exhibited the strongest docking interactions with the highest binding energy value of -11.1 kcal/mol against the 1PRG receptor, when compared to the standard drug rosiglitazone. Figure 3 displays the pictures of the docking interactions between the 1PRG receptor and ligand B3. These results indicate that compound B3, which demonstrated the highest binding energy, shows promising potential as an anti-diabetic compound without any observed toxicity.[23-24 & 31]

The 2D amino acid interactions of rosiglitazone involved Arg280, Ser342, Thr264, Ile341, Gly284, and Arg288 (Figure 3C). On the other hand, the docking of ligand B3 with the target protein PPARγ receptor revealed amino acid residues Glu343, Glu291, Phe264, Cys285, His266, Arg280, Arg288, Leu228, Leu255, Leu333, and Ile341, represented by green color, indicating hydrogen bonding. Additionally, orange color indicates pi-cation interactions, pink color indicates pi-pi interactions, and blue color represents pi donor hydrogen bonds (Figure 3A). These findings suggest that ligand B3, due to its strong binding energy and favorable interactions with the receptor, holds potential as a viable candidate for anti-diabetic therapy.

CONCLUSION

The new series (A1-7; B1-8 & C1-8) of oxadiazole ring were design with an intention to search new lead compound against the Diabetes Mellitus. Among the designed series (A1-7; B1-8 & C1-8), the C1-8 series were found more effective and safer in compare to others. Swiss ADME, OSIRIS and Toxtree were also used to assess the pharmacokinetics, oral bioavailability, toxicity and safety endpoints of molecules. Compound A6, C2, C5, C6, C7, and C8 possess no toxicity risk and high drug score as compared to standard drugs. It could be concluded that among oxazole derivatives, the ligands A6, C2, C5, C6, C7, and C8 are druggable ligands when compared with the standard drug, Rosiglitazone and Pioglitazone. Compound C5 and C8 showed highest drug score (0.91 and 0.86 respectively) among the new derivative and standard drug Rosiglitazone and Pioglitazone (0.80 and 0.70 respectively), and do not exhibit any end point toxicity. Molecules C5 and C8 were also displayed negative permeability across the BBB and found higher GIA %. Results indicated that the designed oxazole derivatives possessed favorable physicochemical properties for oral bioavailability and exhibited excellent binding affinity to the target site of PPARγ (1PRG). The compounds C5 are found inhibitor of CYP 2C19 and non-inhibitor of CYP 1A2, CYP 2C9, CYP 2D6 and CYP 3A4. The compounds C8 are found inhibitor of CYP 2C19, CYP 1A2, CYP 2C9, CYP 2D6 and non-inhibitor of CYP 3A4. Compound A2, A4, A7, B1, B4, B8, C3, C7, and C8 shows highest Clog P values, this indicates good bioavailability, on the other

hand decreased ClogP between -0.13 to 0.60 indicates high skin permeation. The amino acid interactions of rosiglitazone involved Arg280, Ser342, Thr264, Ile341, Gly284, and Arg288. On the other hand, the docking of ligand C5 with the target protein PPARγ receptor revealed amino acid residues Met329, Ala292, Arg288, Ile341, Val339, Leu340 and Glu295, represented by green color, indicating hydrogen bonding. Additionally, orange color indicates pi-cation interactions, pink color indicates pi-pi interactions, and blue color represents pi donor hydrogen bonds. The RMSD value of receptor and receptor-ligand complexes was calculated, and it showed that the compounds were found to be stable and remained stable throughout the simulation study. All ligands show drug score values ranging from 0.11 to 0.9 (no negative value). The current study sheds new light on the significance of oxadiazoles derivatives and molecules C5 and C8 were identified as potential leads compounds (highest drug score, GIA%, no end point toxicity, no carcinogenicity and skin sensitization) for further research for a variety of medical applications.

AUTHOR CONTRIBUTIONS

Study design, Investigation, Supervision and Manuscript writing-original draft preparation: Asif Husain, Mohammad Ajmal, Mohammad Rashid; Methodology implementation, Data Analysis and Organizational support: Mohammad Rashid, Mausin Khan, Sweta Joshi and Alka N. Choudhary; Review, Data collection and assembly of data in a tabular form: Sana Hashmi, Alka N. Choudhary; Manuscript text editing and final approval of manuscript: All participating authors.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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