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A MECHANISM-DRIVEN STRATEGY FOR IN-SILICO PREDICTION, MOLECULAR DOCKING, SYNTHESIS, AND BIOLOGICAL ASSESSMENT OF SUBSTITUTED 1,3,4-OXADIAZOLE DERIVATIVES AS NOVEL ANTIDIABETIC AGENTS

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ABSTRACT
Background: Diabetes mellitus is a long-standing and debilitating metabolic condition that imposes a substantial global health burden, leading to severe and widespread complications. **Objectives**: This study aims to predict physicochemical properties of 1,3,4-oxadiazole derivatives using in-silico methods and molecular docking simulations to explore their potential as α-glucosidase inhibitors for diabetes management. Furthermore, this study aims to experimentally synthesize and characterize these derivatives to validate their inhibitory activity. **Methods**: In silico drug-likeness, pharmacokinetic, and toxicity profiling of substituted oxadiazole derivatives were performed using the Molinspiration and PreADMET web tools. Molecular docking simulations were conducted with the target protein alpha-glucosidase (PDB ID: 3WY1) to assess its anti-diabetic potential. This study suggests that oxadiazole has the potential to be a novel anti-diabetic agent. **Results**: Compound 3a1 formed 5 significant hydrogen bonds with Gly228, Thr226, Leu227, Tyr235, Glu271 with docking scores of -156.118 and re-rank scores of -91.600 comparable to the standard drug Miglitol, which formed 6 hydrogen bonds Val380, Asp401, Lys398, Gly399, Glu377, Asp379 but had lower docking and re-rank scores (-69.4415 and -95.887). Based on docking results, five oxadiazole derivatives were synthesized via Mannich base cyclization, yielding 62.2 – 79.9%. They showed moderate to excellent anti-diabetic activity, with compounds 3a1 and 3a3 demonstrating no toxicity or mortality at 40 mg/kg oral dose. **Conclusion**: Our study highlights that the oxadiazole pharmacophore is a key structural motif for the development of potential anti-diabetic compounds.

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INTRODUCTION

Diabetes mellitus is a persistent metabolic disorder characterized by prolonged hyperglycemia, arising from dysregulated insulin secretion, reduced insulin sensitivity, or both. According to the WHO, diabetes affects approximately 422 million people globally**,** establishing it as a critical public health issue with widespread implications [1]. The incidence of diabetes has escalated considerably in recent decades, primarily driven by changes in lifestyle, increasing urbanization, and a growing aging population. If not properly managed, diabetes can result in serious and debilitating complications, such as cardiovascular disease, stroke, kidney failure, blindness, and lower limb amputation. These complications greatly diminish quality of life and significantly burden healthcare systems worldwide [2]. The 1,3,4-oxadiazole scaffold has attracted considerable interest recently due to its versatile biological activities and promising potential as a pharmacophore in drug development. Several commercially available drugs, including raltegravir (an antiviral agent) [3], fenadiazole (a hypnotic drug) [4], zibotentan (an anticancer agent) [5], ataluren (a treatment for Duchenne muscular dystrophy) [6], and tiodazosin (an alpha-1 adrenergic receptor antagonist) [7], feature the 1,3,4-oxadiazole ring in their structure (Figure 2). Furthermore, literature reports have demonstrated that the 1,3,4-oxadiazole scaffold exhibits a broad range of biological activities, including antibacterial [8,9], antidiabetic [10], antimicrobial [11,12], anti-inflammatory [13], anticancer [14–16], antioxidant [17,18], and antiparasitic properties [19]. Notably, minor structural modifications to the 1,3,4-oxadiazole moiety can significantly impact its quantitative and qualitative biological activity. To develop novel 1,3,4-oxadiazole ether derivatives with enhanced antioxidant and anticancer activities, while minimizing toxicity, we synthesized a series of compounds incorporating phenacyl, coumarin, and N-phenylacetamide moieties. Molecular docking is a computational approach designed to simulate the preferred binding conformation of a ligand within its target protein, enabling the prediction of stable ligand-protein interactions [20]. This method facilitates atomic-level modeling of interactions between small molecules and proteins, providing insights into ligand dynamics within target binding sites and advancing the understanding of key biochemical mechanisms [21]. Molecular docking follows a two-step process: (1) predicting the optimal conformation, spatial orientation, and positioning of the ligand within the binding site, and (2) assessing its binding affinity**.** The interaction strength between molecules is quantified using scoring functions, which aid in evaluating their compatibility. Deciphering molecular binding dynamics is pivotal for structure-based drug design and for unraveling essential biochemical mechanisms [22, 23]. Workspaces are the core components of Molegro Virtual Docker. The main element is the workspace, which displays all of the user's information in terms of molecules (such as proteins, ligands, cofactors, water molecules, and poses), user-defined constraints (shown as small spheres), cavities (shown as a grid mesh), different graphical objects (such as labels, backbone visualizations, molecular surfaces, etc.) and interactions (Hydrogen, Hydrophobic and steric interaction) [24,25]. Molecular docking is a valuable tool in drug discovery, enabling rapid virtual screening and prediction of binding modes. However, it has limitations, including scoring function inaccuracies, neglect of protein flexibility and solvation effects, and potential false results, necessitating experimental validation [26, 27]. This study presents the rational design, synthesis, and biological assessment of a novel series of 1,3,4-oxadiazole derivatives as potential α-glucosidase inhibitors. This study aims to leverage in silico techniques to evaluate the ADMET properties of these compounds, shedding light on their inhibitory mechanisms and serving as a basis for future refinement and therapeutic advancement. Furthermore, this study evaluates the selectivity and safety profiles of the synthesized derivatives through in vitro assays, offering valuable insights into their therapeutic potential for managing related diseases.

MATERIAL AND METHOD

All reagents and solvents were of synthetic grade, sourced from Oxford Laboratory and Lobachemie Pvt. Ltd. Reaction progress was tracked via TLC (hexane-ethyl acetate, 1:1) with detection under iodine vapors and UV light. IR spectra were recorded on an FTIR-1800 Shimadzu, while ^{1}H NMR, ^{13}C NMR, and mass spectra were analysed by Bruker's AVANCE-III 400MHz FT NMR spectrometers. Based on an extensive literature review and reported structure-activity relationships, 30 compounds were designed as potential anti-diabetic agents with various substitutions using ChemDraw Ultra 8.0.

COMPUTATIONAL STUDY

Molinspiration-based drug-likeness and biological activity prediction

Molinspiration is a web-based cheminformatics platform that provides software tools for processing and manipulating

molecules. This platform offers a range of functionalities, including calculating physicochemical properties such as logP, molecular weight, topological polar surface area (TPSA), and hydrogen bond donors/acceptors. Additionally, Molinspiration enables the prediction of bioactivity using a Bayesian algorithm-based model, which employs a fragment-based approach to calculate a bioactivity score.

PreADMET Analysis

Pre-ADMET analyses facilitate the optimization of lead compounds, reduce the likelihood of late-stage failures, and streamline the drug development pipeline by predicting key parameters using the PreADMET server, such as intestinal permeability, plasma protein binding, metabolic stability, and potential toxicity (e.g., hepatotoxicity or hERG channel inhibition). In this study, pre-ADMET predictions were performed using the preADMET software, accessible through an online server (link unavailable) [22, 23].

Docking Study

To investigate the binding interactions of ligand molecules within their target site, a molecular docking study was performed on 30 compounds with the target protein α-glucosidase (PDB ID: 3WY1) using Molegro Virtual Docker (MVD 6.0) software by 64 bit operating system under window 7 with an Lenovo Intel Core i3 - 4 Core 12th Gen. 5 compounds were selected on the basis of good docking score and their interaction with the receptor. The X-ray crystallography structures of alpha-glucosidase (PDB ID: 3WY1) chemical name- (3R,5R,7R)-octane-1,3,5,7-tetracarboxylic acid was retrieved from RCSB protein data bank. Computational ligand-target binding software Molegro Virtual Docker (MVD) was employed in analysing these complexes of crystal structure of alpha-glucosidase (PDB ID: 3WY1) as target with selected designed derivatives in order to interpret structural basis of target protein specificity. The interaction energy of compounds with target enzyme is assigned, "grid point". Finally, these ligands were docked with the potential active sites of target molecules. By the end of this process with numerous runs, binding energies were evaluated. Some of the parameters were set as default. Docking results were clustered and lowest binding energy cluster was considered as representative binding state. The minimum binding energies showed that target protein was docked successfully with ligand molecules. Docking scores in Molegro Virtual Docker are considered a rough estimate of binding affinity, as they are based on simplified scoring functions that may not fully capture the complexities of protein-ligand interactions. Experimental validation is often necessary to confirm predicted binding affinities.

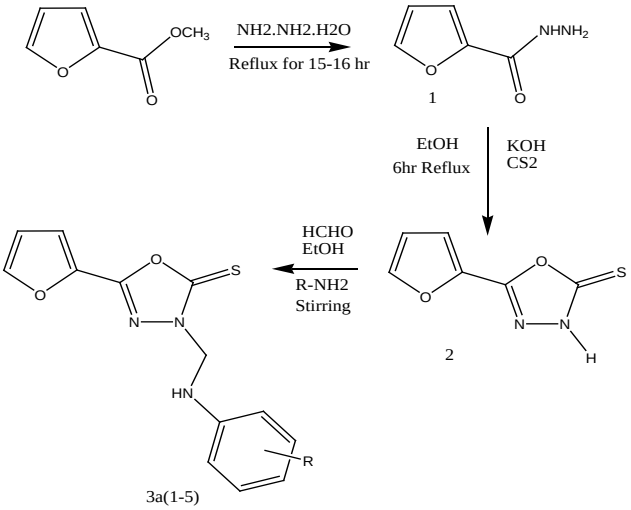


Figure 1: General synthesis scheme of derivative

Standard Method for the Synthesis of Furan-2-Carbohydrazide

A mixture of 1-(furan-2-yl) ethan-1-one (0.1 mole) and hydrazine hydrate (0.2 mole) was refluxed in 50 mL ethanol for 15 hours. The reaction mixture was then concentrated, cooled, and poured onto crushed ice. The resulting solid was filtered, dried, and recrystallized from ethanol to obtain the purified product Figure 1.

Standard Method for the Synthesis of 5-(furan-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione

A mixture of furan-2-carbohydrazide (1.52 g, 0.01 mole), KOH (0.56 g, 0.01 mole), and carbon disulfide (10 mL) was refluxed in 50 mL of 95% ethanol for 12–12.5 hours. The reaction mixture was then concentrated, cooled to room temperature, and acidified with dilute HCl. The resulting crude product was filtered and recrystallized from ethanol to obtain the purified compound.

Standard Method for the Synthesis of 3-[(Substitutedanilino) methyl]-5-(furan-2-yl)

A mixture of 5-(furan-2-yl)-1,3,4-oxadiazole-2(3H)-thione (0.97 g, 0.005 mol) and 0.005 mol of various aromatic amines was refluxed in 25 mL of pyridine for 4 hours. The reaction mixture was then cooled and poured onto crushed ice,

precipitating a solid product. The resulting solid was filtered, dried, and recrystallized from ethanol to obtain the purified compound. The synthesized pharmacophores, their chemical structures, and compound IDs are presented in Figure 3.

RESULT AND DISCUSSION

Result of In-silico drug-likeness, pharmacokinetic, and toxicity properties

Compounds 3a1–3a5 met Lipinski's Rule of Five and showed promising bioactivity against therapeutic targets, including nuclear receptors, GPCRs, ion channels, and enzymes, as analyzed by Molinspiration software Table 1. The Lipinski's rule of five properties of 1,3,4-Oxadiazole is within the acceptable range. The molecular weight being less than 500 Daltons falls within the acceptable range for drug-likeness. Additionally, hydrogen bond donor (less than 5), hydrogen bond acceptor (less than 10), and logP (less than 5) properties follow the RO5 Table 1. The Molinspiration analysis provided key parameter values critical for assessing the compound's potential. The LogP value ranging from 1,51 to 2.33 indicates that all the derivatives possess moderate to high lipophilicity, which favors membrane permeability. The TPSA, calculated as <110Å², suggests the compound will likely exhibit favorable absorption and solubility characteristics. The bioactivity scores include 0.03 for GPCR ligand activity, suggesting moderate interaction potential with G-protein-coupled receptors. 0 rotation bond value indicates that derivatives have flexibility. These parameter values collectively provide a comprehensive understanding of optimizing its drug-likeness and therapeutic potential, aiding in developing more effective and safer therapeutic agents.

Table 1: Results of Molinspiration, drug-likeness, pharmacokinetic, and toxicity properties

Results of Molinspiration properties

Code	Properties								
	mi LogP	**TPSA**	**n Atoms**	**MW**	**n OH**	**n OHNH**	**n Violations**	**n Rotb**	**Volume**
3a1	2.12	147.78	25	363.31	11	1	1	6	274.94
3a2	2.19	93.43	22	317.33	7	2	0	5	255.27
3a3	1.51	96.59	21	305.31	7	3	0	4	244.31
3a4	2.31	65.37	21	303.34	6	1	0	5	253.82
3a5	2.33	65.37	**21**	303.34	6	1	0	5	253.82

Result of Biological activity

Code	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
3a1	0.03	-0.29	-0.17	0.01	0.29	0.32
3a2	-0.37	-0.63	-0.31	-0.35	0.09	-0.34
3a3	0.03	-0.29	-0.23	0.03	0.26	0.28
3a4	-0.23	-0.43	-0.16	-0.27	-0.22	0.18
3a5	-0.39	-0.66	-0.31	-0.35	0.09	-0.32

Result of Drug Likeness of synthesized compounds

Drug Likeness		Compounds
CMC_like_Rule	Qualified	3a1,3a2,3a3,3a4,3a5
	Not qualified	-
MDDR_like_ Rule	Mid Structure	3a1,3a2,3a3,3a4,3a5
	Drug Like	-
Rule_of_Five	Suitable	3a1,3a2,3a3,3a4,3a5
	Not Suitable	-

Result of ADME properties

Properties	Range	Features	3a1	3a2	3a3	3a4	3a5
BBB(Blood Brain Barrier)	More than 1	CNS active compounds	--	--	--	1.276	--
	Less than 1	CNS inactive compounds	0.0947	0.2591	0.4264	--	0.7683

HIA (Human Intestinal Absorption)	0-20%	Poor absorption	--	--	--	--	--
	20-70%	Moderate absorption	60.74	--	--	--	--
	70-100%	Higher absorption	--	94.29	90.44	97.64	97.64
PPB (Plasma Protein Binding)	More than 90%	Strongly bounded	100%	99.9%	----	95.06	94.19
	Less than 90%	Weakly bounded	----	----	89.1%	----	----
Caco-2 Permeability	Less than 4	Lower	----	----	----	----	----
	4-70	Moderate	20.59	20.89	20.61	33.72	33.49
	More than 70	Higher	--	--	--	--	--
CYP2D6	Non-inhibitor	Acceptance Yes	Non	Non	Non	Non	Non
	Inhibitor	Acceptance No	--	--	--	--	--
MDCK (Madin-Darby Canine Kidney)	Less than 25	Lower	0.128	2.81	--	--	--
	25-500	Moderate	--	--	33.05	15.89	81.33
	More than 500	Higher	--	--	--	--	--
P-gp_ Inhibition	Non-inhibitor	Acceptance No			No	No	
	Inhibitor	Acceptance Yes	Yes	Yes			Yes

Result of Toxicity studies

Toxicity		**Compounds**
Ames_test	Mutagen	2,3,6,12,14
	Non-Mutagen	
Carcino_Mouse	Negative	2,6,12,14
	Positive	3
Carcino_Rat	Negative	2,3,6,12
	Positive	14
hERG_inhibition	Ambiguous	
	Medium Risk	3,6,12,14
	Low-risk	2

ADMET Analysis

The PreADMET results were analysed to evaluate the selected compounds' pharmacokinetic properties and toxicity profiles. These results provide a comprehensive understanding of the ADMET properties and properties under Five: drug-likeness. The 1,3,4-Oxadiazole derivative have high bioavailability along with good solubility and cellular permeability, low BBB permeability (less than 1), high predicted Human intestinal absorption (70 to 100%), plasma protein binding (Strongly bounded more than 90%), moderate Caco-2 Permeability (4 to 70), potential for CYP2D6 non inhibitor and P-gp inhibitor. Additionally, toxicity assessments, including non-mutagenicity, carcinogenicity, and acute toxicity, were examined to predict the safety profile of the compounds. Results are shown in Table 1. Compounds 3a1 to 3a5 successfully passed the in-silico computational prediction screening, demonstrating good ADMET properties and favorable pharmacokinetic and toxicity profiles.

Molecular Docking

The target compound exhibited strong activity, supported by high docking scores and favourable binding patterns. It indicates its ability to interact with key amino acids in the binding site of alpha-glucosidase (PDB ID: 3WY1). Compounds 3a1, 3a3, and 3a4 showed superior inhibitory potential, with docking scores of -156.118, -138.098, and -138.172, respectively, and re-rank scores of -91.600, -91.207, and -117.52 kcal/mol. These scores were higher than the co-crystallized ligand, which had docking

scores of -91.6144 and re-rank scores of -93.688 kcal/mol. Compound 3a1 demonstrated 5 significant hydrogen bonding interactions Gly228, Thr226, Leu227, Tyr235, Glu271 which is notably comparable to standard drug Miglitol, which exhibited only 6 hydrogen bond Val380, Asp401, Lys398, Gly399, Glu377, Asp379 with a docking score and re-rank score of -69.4415 and -95.887 respectively (Table 2 & Figure 2 and 3). The validation study showed that the RMSD value for the dock orientation was 1.24 A^0, which is lower than the crystal resolution of the 3WY1 protein structure (1.91 A^0) reported in the protein data bank. The molecular docking results suggest that these newly designed compounds could be potent alpha-glucosidase inhibitors.

Physical Characterization

The physical characterization of compounds 3a1–3a5 reveals distinct property variations. The molecular weight ranges from 303 to 363 g/mol, with 3a1 being the heaviest and 3a4 and 3a5 having the lowest molecular weights (303 g/mol). The compounds exhibit different appearances, varying from light yellow (3a1) and pale yellow (3a2, 3a4) to white (3a3) and brown (3a5). Their Rf values range from 0.68 to 0.78, indicating differences in polarity and mobility. The melting points also vary significantly, from 110–112°C (3a4) to 285–288°C (3a2), suggesting structural differences affecting thermal stability. All compounds demonstrate good solubility in ethanol and ethyl acetate Table 3.

STRUCTURAL ANALYSIS OF COMPOUNDS

Compound 3a1 λ Max 222.00 nm, **IR (**KBr, cm-1) 3246 (str. NH), 2925 (CH str. of methylene), 1616 (CN oxadiazole ring str.), 1144 (CS str. of thione), **H^1 NMR** (CDCl3, 400MHz): δ - 14.2 (1H, s, N-H of oxadiazole ring), 9.2 (1H, s, N-H of aniline ring), 8.3 (1H, d, C-H of furan ring), 7.8-8.2 (1H, d, C-H), 4.8 (2H, s, methylene group), **C^{13} NMR** (CDCl3 300MHz); δ - 178.2 (C=S of thione group), 165.2, 159.4 (C=N of oxadiazole), 154.2 (C-O of oxadiazole), 144.2-149.2 (C-N Ar.), 134.2 ,124.2(C-C of furan ring), 126.2-128.2 (C-C of Ar), 42.2 (CH_2), **LC/MS: m/z** 363.09 (M+)

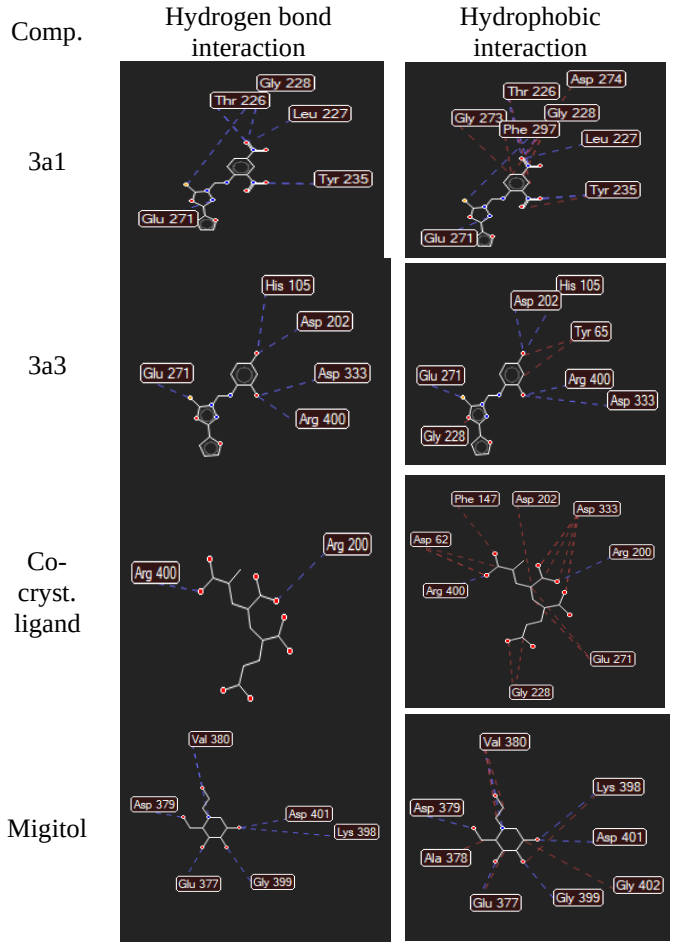
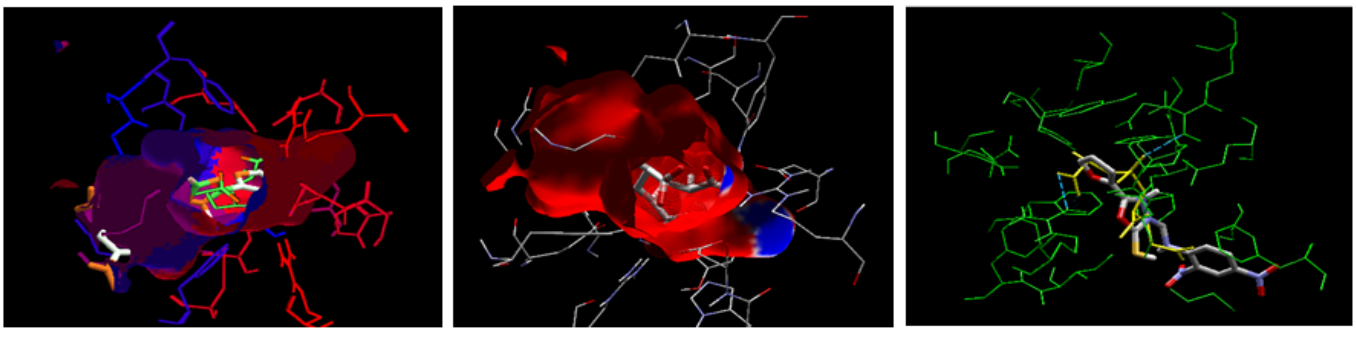


Figure 2: Docking Interactions of 3a1 and 3a2, Co-crystallized ligand and standard drug Miglitol on PDB 3WY1



A **B** **C**

Figure 3: Binding modes of the most active compounds within the active site: A Hydrophobic site view, B. Electrostatic interaction view. C. Hydrogen bond interaction

Table 2: Results of Moldock score

S.No	Ligand	Mol Dock Score	Rerank score	H-bond Interaction	Steric interaction
1	3a1	-156.118	-118.227	Gly228, Thr226, Leu227,Tyr235, Glu271	Gly228, Thr226, Gly273, Phe297, Asp274, Leu227,Tyr235, Glu271
2	3a2	-135.717	-110.029	Tyr399,Asp333, Arg400, Gly228	Ile146, Asn301, Ala229, Tyr399,Asp333, Arg400, Gly228
3	3a3	-138.098	-113.408	Asp333, Arg400,Glu271, Asp202, His105	Tyr65, Gly228, Asp333, Arg400,Glu271, Asp202, His105
4	3a4	-138.172	-105.88	Asp274, Gly228, Thr226, Glu271, Arg400	Phe297, Gly273, Gly228, Asp274, Gly228, Thr226, Glu271, Arg400
5	3a5	-138.68	-113.058	Gly228, Arg400, Asn301, Tyr389	Ile146, Gly228, Arg400, Asn301, Tyr389
6	Co-Crystallized Ligand	-91.6144	-87.1658	Arg400, Arg200	Phe147, Asp202, Asp333, Asp62, Glu271, Gly228, Arg400, Arg200
7	Miglitol (Standard)	-69.4415	-95.887	Val380, Asp401Lys398, Gly399, Glu377, Asp379	Ala378, Gly402, Val380, Asp401, Lys398, Gly399, Glu377, Asp379

All compounds were reviewed in detail to retrieve their binding interaction information, which can be highly decisive for inhibiting targets. Binding interaction diagrams were obtained using MVD Virtual Docker 6.0.

Table 3. Physical Data of the Synthesised Compound

Property	3a1	3a2	3a3	3a4	3a5
Molecular weight (g/mol)	363	317	305	303	303
Appearance	Light yellow	Pale yellow	White	Pale yellow	brown
Rf value	0.68	0.76	0.68	0.71	0.78
Melting point (^{o}C)	269-272	285-288	270-272	110-112	223-224
Solubility	Ethanol, Ethyl acetate	Ethanol, Ethyl acetate	Ethanol, Ethyl acetate	Ethanol, Ethyl acetate	Ethanol, Ethyl acetate

Compound 3a2 λ Max 240.20 nm, **IR (KBr, cm-1)** 3423 (O-H stretching of carboxylic acid), 3285 (N-H str.), 2923 (C-H str. CH_2), 1693 (C=O str.), 1614 (C=N stretching of oxadiazole), 1521 (N-H bending), 1256 (C-S str. of thione), 1016 (C-H bending Ar.) - **H^1 NMR** (CDCl3, 400MHz): δ 12.9 (1H, s, O-H of carboxylic acid), 9.3 ppm (1H, s, N-H of amine group), 6.5-8.3 (1H, d, C-H of furan ring), 7.4-7.9(2H, d, C-H of benzene ring), 4.8 (2H, s, CH_2), **C^{13} NMR** (CDCl3 300MHz); δ - 172.2 (C=O of carboxylic acid), 159.6-165.4 (C=N of oxadiazole ring), 124.6-128.3 (C-C of furan ring), 118.2-121.2 (C-C of benzene ring), 42.4(CH_2), **LC/MS: m/z** 317.2 (M+)

Compound 3a3 λ Max 262.42 nm, **IR (KBr, cm-1);** 3465 (O-H str.), 3362 (N-H str.), 2923 (C-H str. of CH_2), 1256 (C-S str. of thione), 1016 (C-H bending Ar.), **H^1 NMR** (CDCl3, 400MHz): δ - 10.3, 9.6 (1H, s, O-H of phenol group), 6.9-7.8 (1H, d, C-H of furan ring), 8,3-8.6 (1H, d, C-H of benzene ring), 4.8 (2H, s, CH_2 group), **C^{13} NMR** (CDCl3 300MHz); δ - 178.2 (C=S of thione group), 163.2-165.4(C=N of oxadiazole ring), 154.2 (C-O of oxadiazole ring), 126.4-129.6 (C-C of benzene ring), 42.8 (CH_2 group), **LC/MS: m/z** 305.04 (M+)

Compound 3a4 λ Max 285.12 nm, **IR (KBr, cm-1);** - 3421 (N-H str.), 3123 (C-H str.), 2923 (C-H str. of CH_2), 2835 (C-H str. of OCH_3), 1256 (C-S stretching of thione group), 1016 (C-H bending Ar.), **H^1 NMR** (CDCl3, 400MHz): δ 9.3 (1H, s, N-H), 6.64-7.14 (1H, d, C-H of Ar.), 4.8 (2H, s, CH_2 group), 3.8 (3H, s, OCH_3), **C^{13} NMR** (CDCl3 300MHz); δ 178.6 (C=S of thione), 165.2-167.4 (C=N of oxadiazole ring), 126.4-128.2 (C-C of Ar.), 55.2 (OCH_3), 42.2 (CH_2 group), **LC/MS: m/z** 303.09 (M+)

Compound 3a5 λ Max 288.22 nm, **IR (KBr, cm-1);** - 3422 (N-H str.), 3120 (C-H str.), 2912 (C-H str. of CH_2), 2829 (C-H str. of OCH_3), 1246 (C-S stretching of thione group), 1021 (C-H bending Ar.), **H^1 NMR** (CDCl3, 400MHz): δ 9.1 (1H, s, N-H), 6.52-7.24 (1H, d, C-H of Ar.), 4.9 (2H, s, CH_2 group), 3.6 (3H, s, OCH_3), **C^{13} NMR** (CDCl3 300MHz); δ 177.6 (C=S of thione), 164.2-166.4 (C=N of oxadiazole ring), 123.4-127.2 (C-C of Ar.), 54.2 ppm (OCH_3), 41.2 (CH_2 group), **LC/MS: m/z** 303.09 (M+)

Effect of substitution on activity

The different substituents (OH, NO2, OCH3, CH3, Cl, etc.) on the compounds can significantly impact their activity by altering physicochemical properties, such as electronegativity, lipophilicity, and steric hindrance. These properties, in turn, influence protein-ligand interactions, binding affinity, and bioavailability, ultimately affecting the compound's potency and efficacy. Oxadiazole derivatives with NO_2 substitution have shown significant binding with protein and a high docking score. Further, the antidiabetic activity of nitro-substituted oxadiazole derivatives is comparable to that of the standard drug Miglitol.

Pharmacological Anti-diabetic activity

Alloxan is a diabetogenic compound that triggers diabetes in experimental animals by specifically destroying pancreatic beta cells, causing insulin deficiency and prolonged elevated blood glucose levels. It is a well-established model for studying type 1 diabetes and assessing new anti-diabetic treatments. In this study, 21-day administration of 3a1 and 3a3 significantly improved diabetic symptoms in alloxan-induced rats, showcasing their potential as promising therapeutic agents. The acute toxicity study confirmed that 3a1 and 3a3, administered orally at 400 mg/kg, exhibited no toxicity or mortality in treated animals, indicating their safety and viability for further investigation. Our study demonstrates that 3a1 and 3a3 effectively reduce triglyceride and total cholesterol levels while elevating HDL in diabetic rats, suggesting their role in lipid metabolism regulation by limiting free fatty acid release. Notably, HDL levels increased after 21 days of treatment. In vitro studies confirm that alloxan induces β-cell destruction by generating reactive oxygen species, triggering cell necrosis and a cytosolic calcium surge. Metformin treatment promoted β-cell regeneration, a similar effect observed with 3a1 and 3a3, as shown in Table 4 and Figure 3. Compound 3a1 formed 5 significant hydrogen bonds with Gly228, Thr226, Leu227, Tyr235, Glu271 with docking scores of -156.118 and re-rank scores of -118.227 comparable to the standard drug Miglitol, which formed 6 hydrogen bonds Val380, Asp401, Lys398, Gly399, Glu377, Asp379 but had lower docking and re-rank scores (-69.4415 and -95.887). On the basis of the docking result, a novel series of 1,3,4-oxadiazole derivatives was synthesized and tested for antidiabetic activity. During in vivo and in vitro studies, compounds 3a1 and 3a3 dramatically lowered blood glucose levels. Furthermore, 3a1 and 3a3 effectively reduce triglyceride and total cholesterol levels while elevating HDL in diabetic rats, suggesting their role in lipid metabolism regulation by limiting free fatty acid release. Notably, HDL levels increased after 21 days of treatment. The results show that 3a1 and 3a2 may be potential targets for inhibiting the alpha-glucosidase inhibitor.

Table 4: Effect of Drugs on the serum lipid profiles in Alloxan-Diabetic rats after 21 days of treatment

Exp. Group	Treatment	Blood glucose concentration (mg/dl) (mean ± S.E.M.)		
		HDL (mg/ dl)	VLDL (mg/dl)	LDL (mg/dl)
1	Normal control	54.32± 1.1	24± 2.8	23.2± 1.8
2	Diabetic Control	38.26± 2.9	39.28± 2.3	36.68± 1.2
3	Miglitol (15mg/kg)	42± 1.8	29.3± 2.5	24.25± 3.1
4	3a1 (40mg/kg)	49.58± 0.4	39.8± 3.1	34.36± 2.5
5	3a3 (40mg/kg)	49.32±0.9	33.48±2.2	27.20±3.2

Exp. Group	Treatment	Blood glucose concentration (mg/dl) (mean ± S.E.M.)	
		TG (mg/dl)	Total Cholesterol (mg/dl)
1	Normal control	71.75± 2.4	55.35± 2.2
2	Diabetic Control	128± 3.4	88.3± 4.2
3	Miglitol (15mg/kg)	84± 2.7	59.35± 1.9
4	3a1 (40mg/kg)	113± 3.1	75.30± 1.3
5	3a3 (40mg/kg)	107± 2.8	72.85±2.1

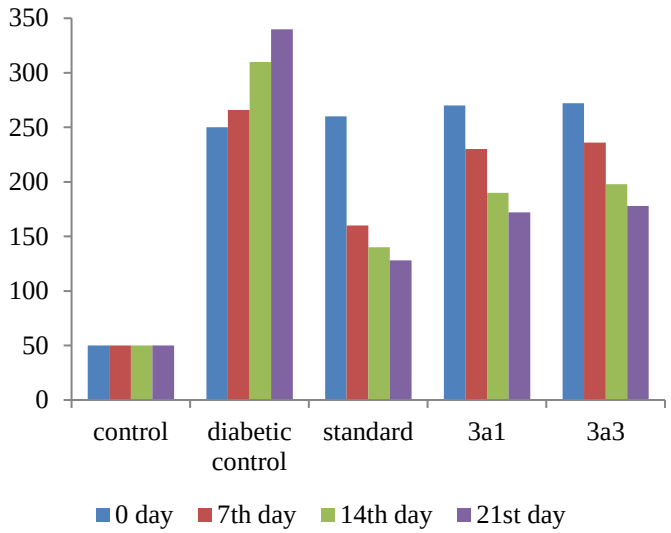


Figure 3. Effect of Drugs on the blood glucose levels in Alloxan induced diabetic rats (Multi-dose treatment /sub-acute study).

CONCLUSION

In conclusion, this study demonstrates the promising anti-diabetic potential of 1,3,4-oxadiazole derivatives, particularly compound 3a1, which exhibited the highest binding affinity (–156.118 kcal/mol) and key interactions Gly228, Thr226, Leu227, Tyr235, Glu271 which is notably comparable to standard drug Miglitol, which exhibited only 6 hydrogen bond Val380, Asp401, Lys398, Gly399, Glu377, Asp379 with a docking score and re-rank score of -69.4415 and -95.887 respectively. Utilizing physicochemical analysis, molecular docking, and in silico predictions, five derivatives (3a1, 3a2, 3a3, 3a4, and 3a5) were successfully designed, synthesized, and assessed. IR spectroscopy, NMR, and mass spectrometry characterized the synthesized compounds.

The compounds exhibited moderate to good anti-diabetic activity, with nitro substitution enhancing pharmacological activity. The strong ligand-protein interactions observed, coupled with favorable physicochemical properties and absence of acute toxicity at 40 mg/kg for key candidates, highlight these compounds as strong leads for further development. These findings lay a solid foundation for future research, particularly QSAR modeling and in vivo efficacy studies, to optimize the structural features responsible for enhanced biological activity and to advance these derivatives toward clinical evaluation. Our findings suggest that oxadiazole derivatives show promise as potential anti-diabetic agents and warrant further investigation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTION

Nitin Deshmukh designed the oxadiazole derivatives and conducted an in silico computational study. Based on the computational results, Mohini Patidar synthesized oxadiazole derivatives, and Sunita Minz designed and guided pharmacological Anti-diabetic activity assays. Raghvendra Dubey evaluated and interpreted the results. Nitin Deshmukh and Madhulika Pradhan drafted and finalized the manuscript. All authors reviewed and approved the final draft of the manuscript.

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