

In silico and *in vitro* Evaluation of Microwave-Assisted Synthesized Isoxazole Chalcone Derivatives of Selected Phytoconstituents for Antidiabetic Activity

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ABSTRACT

Background: Diabetes is a major chronic health problem worldwide that has acquired significant attention from the scientific community. One approach to managing glucose synthesis and reducing the risk of diabetes is through the inhibition of specific enzymes. Interestingly, heterocyclic compounds have been shown to effectively inhibit key enzymes involved in glucose metabolism, making them promising candidates for diabetes treatment. **Materials and Methods:** In this study, we aimed to dock the α -glucosidase enzyme with 100 naturally derived inhibitors using PyRx software. Among these, 6 compounds were synthesized and evaluated for their pharmacological activity. **Results:** In brief, from the initial screening, 50 compounds with a binding affinity greater than -7.2 kcal/mol were selected for further interaction analysis to aid in compound design. Notably, compounds SP2 and SP4-1-{3-nitro-5-[5-(quinolin-3-yl)-4,5-dihydrofuran-3-yl]phenyl}ethan-1-one and (4-chlorophenyl){3-[5-(quinolin-3-yl)-4,5-dihydrofuran-3-yl]phenyl}methanone, respectively exhibited significantly higher α -amylase and α -glucosidase inhibitory activity showing 90.57% inhibition in the α -amylase assay and an 83.23% inhibition in the α -glucosidase assay to be comparable to acarbose, indicating strong therapeutic relevance. **Conclusion:** Overall, the study demonstrated that the newly synthesized molecules possess medicinal potential against diabetes mellitus while adhering to Lipinski's rule of five.

Keywords: α -glucosidase Inhibitors, Diabetes Mellitus, *In silico*, α -amylase, Pharmacological Activity.

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INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder described by prominent blood glucose levels due to insulin deficiency or resistance. In most developed countries, it primarily affects middle-aged individuals and children.¹ Diabetes can lead to severe complications, including obesity, stroke, and heart failure. According to the International Diabetes Federation, the number of people with diabetes is projected to rise from 415 million in 2015 to 640 million by 2040. Type I Diabetes (T1DM) accounts for 5-10% of cases, while Type II Diabetes (T2DM) is far more prevalent, affecting over 90% of diabetic

individuals. The development of DM is influenced by complex dysfunctions in organs, proteins, and enzymes.^{2,3} Given the multifactorial nature of diabetes, no single experimental model or treatment can fully address its complexity. Effective disease management involves a combination of a balanced diet, regular exercise, and the use of synthetic or natural therapeutics. In diabetes, α -amylase and α -glucosidase enzymes play a decisive role in breaking down carbohydrates, leading to postprandial blood sugar spikes.⁴ Various cellular and metabolic targets have been explored to expand pancreatic function, augment insulin secretion, and optimize insulin activity at target sites. Consequently, α -amylase and α -glucosidase inhibitors serve as essential therapeutic markers for diabetes treatment.⁵⁻⁷ However, the clinical application of many existing diabetes therapeutics is limited due to gastrointestinal side effects, metallic taste, and constipation, loss of appetite, poor bioavailability, and short circulation half-life. These challenges highlight the urgent need for the development of novel anti-diabetic agents. Heterocyclic



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compounds have emerged as promising candidates for managing diabetes effectively.^{8,9}

Molecular docking is an *in silico* approach used to predict the most stable ligand-receptor interaction. It evaluates the binding affinity of specific ligands to the active site of a receptor protein. Docking efficiency is assessed based on docking scores and interaction types, such as hydrogen bonding, ionic interactions, van der Waals forces, and π - π stacking. A more negative binding energy indicates stronger ligand-receptor interactions, signifying better docking performance.¹⁰⁻¹²

Several herbs that are used as medicines have been reported to help prevent/treat diabetes at different stages. For example, curcumin may be used to treat pre-diabetes in order to prevent Type 2 (T2) diabetes from developing, while cinnamon may help treat diabetes patients with both hypertension and diabetes. Additionally, aloe vera leaf gel has been shown to increase insulin levels and restore pancreatic beta cells¹³ in doses of 300 mg/kg. Multiple α -glucosidase inhibitors are used for the treatment of T2 diabetes (e.g., voglibose, acarbose, miglitol, etc.) and several studies are ongoing looking for potential natural products that can be effectively used to manage diabetes; some plants, such as aster, cinnamon, mistletoe fig and bitter oleander have potential inhibitory effects on α -glucosidase. In addition, several medicinal herbs including *Camellia sinensis*, *Aloe vera*, and basil also have anticlavitic effects due to their ability to inhibit amylase.¹⁴

Alpha-Glucosidase inhibitors (acarbose, miglitol, and voglibose) inhibit lysosomal withdraw α -glucosidase activity by structurally resembling saccharides (i.e., carbohydrate binding sites on α -glucosidase). Therefore, the carbohydrate binding region of α -glucosidase interacts with all of these forms of inhibitor. However, many of the common adverse effects associated with the use of prescribed anti-diabetic agents include liver disorders including lactic acidosis; gastrointestinal symptoms including abdominal cramping, flatulence, and diarrhea. Current medication's negative side effects have resulted in efforts to develop alternative forms of lysosomal α -glucosidase inhibitor technology with reduced adverse reactions. Therefore, in this study, phytochemicals from Indian plant species were selected on the basis of literature survey based on their potential to inhibit α -glucosidase. The ligand-receptor interactions were analyzed using *in silico* methods. Ligand structures were optimized using Chem Sketch, and the receptor-ligand interactions were visualized using Discovery Studio Visualizer.^{15,16}

MATERIALS AND METHODS

Materials

Databases, including pdb, DrugBank, PubChem, as well as using PyRx and Discovery Studio Visualizer as our software tools. The PDB (Protein Data Bank) started as a single repository for storage of Structure Data for macromolecules and opened its doors at

BNL in 1971 to create one central location on the planet that contained all the Structural Data on all the macromolecules found till today. All of the X-ray Crystallography determined Structural Data and six other types of Structural Data can be found in the PDB. ChemSketch is the leading all-purpose Chemical Drawing and Graphics Software Package from ACD/Labs for Chemists to Draw Molecules, Drawing and/or Writing Reactions, creating (proof Calculating) Professional Presentations and Reports. In addition to creating drawings of compounds, ChemSketch can convert structures from SMILES notation to structure and vice versa. In our research we used Discovery Studio Visualizer to visualize the interaction between receptor protein and ligand. We also used FT-IR and HNMR Spectroscopy to characterize the compounds from synthetic procedures.

Methods

In silico studies (Docking Studies)

Protein and Ligand preparation

We have conducted an analysis using molecular docking in order to determine how the molecules interact with the active site of human diabetic α -glucosidase, which is a type of lysosomal α -glucosidases (PDB ID: 5KZX) having a 2.0 Å resolution (high quality). The Three Dimensional (3D) crystal structure for α -glucosidases was downloaded from the RCSB Protein Data Bank (PDB, RCSB) (<https://www.rcsb.org/>). The crystallographic inhibitor, ions (Mg^{+} , K^{+}) and the water molecules further than the active site were deleted and assigned bond orders, polar hydrogens were added, Checked and repaired missing atoms in the protein crystal structure, edited histidine hydrogens, assigned Radii, added Kolmann charges (-480.326), Gasteiger charges (-22.4268) using AutoDock Vina.

Phytochemicals are selected from a plant source that has enzyme inhibition properties for alpha-Glucosidase. Previously reported 100 phytochemicals possessing α -glucosidase enzyme inhibitory activity were chosen. The SDF files of respective phytochemicals were retrieved from the PubChem database. All SDF files of ligands were imported into PyRx. The ligands were then converted to PDBQT format using PyRx's open babel plugin and used for molecular docking.

Molecular docking

The purpose of molecular docking is to predict the position, contact, and conformation of a ligand molecule within a target molecule binding site, which is typically a large macromolecule. A molecular docking study was performed using Autodock vina of PyRx 0.8 to check the binding interactions with residues at the active site of the target protein (PDB:5KZX). In PyRx's Vina workspace, the grid box for PDB: 5KZX was selected to cover the binding site residues with centers X:1.3148, Y:18.1751, Z:17.7808, and dimensions X:95.7864, Y:92.5980, Z:107.2510. The level of exhaustiveness was set to default 8. BIOVIA Discovery Studio

2020 was used to visualize docking interactions as well as analyze saved poses.

Drug likeliness and pharmacokinetic prediction

The loss of drugs due to poor pharmacokinetics in drug development procedures results in higher development costs. Screening of potential drugs has been improved by the accessibility of *in silico* pharmacokinetic tools. A molecule's physicochemical properties, which are crucial factors that influence a molecule's effectiveness, safety, or metabolism, can be predicted using Lipinski's rule of five. A drug must have $MW < 500$, $\log P < 5$, $HBA \leq 10$, $HBD \leq 5$. The features of a molecule's ADME (absorption, distribution, metabolism, and excretion) are that determine its Pharmacokinetic profile.

Synthesis of selected compounds

All of the reagents used were reagent-grade and purified as necessary. Melting points were determined with a melting point apparatus (digital), Lab India. The FTIR (Fourier Transform Infrared Spectroscopy) spectra for all test compounds were taken by Shimadzu using FTIR spectroscopy. The Nuclear Magnetic Resonance (NMR) spectra for all test compounds were obtained on a Bruker DRX-300 and used Dimethyl Sulfoxide (DMSO) as the solvent and Tetramethylsilane (TMS) as the standard for calibration. The mass spectrometric data for all test compounds were obtained by Shimadzu using LCMS 2010A. The thin-layer chromatography results were obtained using methodology.

Procedure for the microwave-assisted synthesis of isoxazole chalcone derivatives (SP1-SP6)

The synthetic scheme (Figure 1) was developed after designing the six target molecules (SP1-SP6). Following the design, we synthesized these compounds according to the proposed synthetic route. Initially, Dimethylformamide (DMF) (2.5 mol) was added to a thoroughly dried Round-Bottom Flask (RBF) at room temperature, followed by the dropwise addition of Phosphoryl Chloride ($POCl_3$) (7 mol) under continuous stirring for 15 min. Subsequently, N-phenylacetamide was introduced at room temperature, and the reaction mixture was heated at 75°C for 2 hr. Upon completion, the reaction mixture was poured into ice-cold water, and the resulting precipitate was collected by filtration. In a separate round-bottom flask, six ketones (bromoacetophenone, 4-chlorobenzophenone, p-nitroacetophenone, 3-methyl-1-phenylpyrazole-5-one, dichloroacetophenone, and 3-nitroacetophenone) were dissolved in 95% ethanol (20 mL) along with substituted aromatic aldehyde (0.001 mol). To this, 10 mL of 60% aqueous sodium hydroxide solution was added dropwise. The reaction mixture was stirred at 5-10°C for 2 hr, then poured over crushed ice, and acidified with dilute Hydrochloric Acid (HCl). The resulting solid was collected, washed, and dried. Next, a mixture of chalcone (0.01 mol), hydroxylamine hydrochloride (0.01 mol), and sodium acetate in

ethanol (25 mL) was subjected to microwave irradiation at 210 W for 10-5 min at 90-100°C. The reaction mixture was then poured into ice-cold water, and the precipitate was collected by filtration, washed, and dried for further characterization.

In vitro analysis of anti-diabetic activity using α -amylase enzyme inhibition assay

In this step, 500 μ L of the test compound (1 mM) was mixed with 500 μ L of 0.1 M phosphate buffer (pH 6.9) containing α -amylase enzyme (fungal diastase, 0.5%). The reaction mixture was incubated at 25°C for 10 min. Subsequently, 500 μ L of a 1% starch solution in 0.1 M phosphate buffer (pH 6.8) was added, and the mixture was further incubated at 25°C for 10 min. For the control, the same procedure was followed, except 500 μ L of enzyme was replaced by an equal volume of buffer. After the incubation period, 1000 μ L of di-nitrosalicylic acid reagent was added to both the test (concentration 1mg/mL) and control mixtures. The reaction mixtures were then placed in a boiling water bath for 10 min and allowed to cool. The absorbance of each sample was measured at 540 nm using a spectrophotometer. The percentage inhibition of α -amylase enzyme was calculated using the following formula:

$$\text{Inhibition(\%)} = \frac{\text{Abs 540(control)} - \text{Abs 540 (synthesized product)}}{\text{Abs 540(control)}} \times 100$$

In vitro analysis of anti-diabetic activity using α -glucosidase inhibition activity

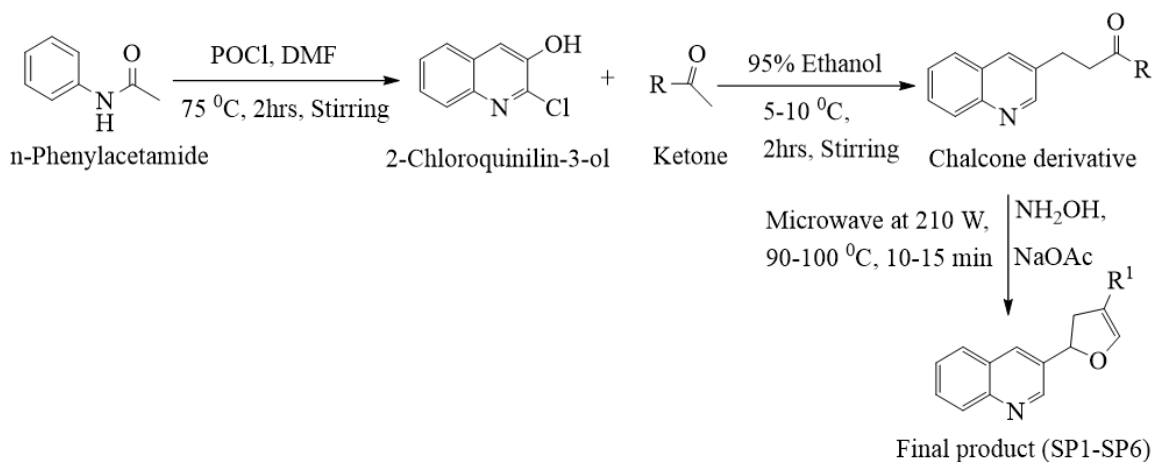
The α -glucosidase inhibitory potential of the compounds was evaluated using a standard method with minor modifications. Briefly, 500 μ L of the test compounds, 50 μ L of glutathione, and 10 μ L of α -glucosidase in phosphate buffer were added to a 96-well plate and incubated at 37°C for 15 min. After incubation, 20 μ L of P-Nitrophenyl- α -D-Glucopyranoside (P-NPG) (5 mM) was introduced, and the mixture was maintained at 37°C for an additional 15 min. The reaction was terminated by adding 50 μ L of Na_2CO_3 (0.1 M) to the reaction mixture. A blank sample, which lacked α -glucosidase, was prepared similarly to the test samples (concentration 1mg/mL). To serve as a control, the experiment was conducted in triplicate ($n=3$) without the test compound. The absorbance of the resulting solutions was measured at 400 nm. The α -glucosidase activity was quantified by measuring the amount of p-nitrophenol produced, with acarbose used as a positive control.

$$\text{Inhibition (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

RESULTS AND DISCUSSION

In silico studies

All the synthesized derivatives were docked against target proteins and compared with phytochemicals. The phytochemicals exhibited docking scores ranging from -7.2 to -9.8 kcal/mol. Similarly, the synthesized derivatives showed docking scores



SP1	SP2	SP3	SP4	SP5	SP6

Figure 1: Synthetic scheme for the synthesis of final compounds (SP1- SP6).

within the range of -8.1 to -9.2 kcal/mol. The interactions of phytochemicals with amino acid residues are detailed in Table 1. Overall, the docking scores of the synthesized derivatives with the target proteins were higher, indicating a significant affinity between the synthesized compounds and the target proteins (Tables 2 and 3).

Drug likeliness and pharmacokinetic prediction

The drug likeliness and pharmacokinetic properties of the synthesized compounds (SP1-SP6) were predicted using the free Swiss ADME server online (<http://www.swissadme.ch>). As reference drugs, all of the examined compounds (SP1-SP6) do not deviate from the Lipinski rule of 5 (MW < 500, logP < 5, HBA ≤ 10, HBD ≤ 5). Since their values lie within the normal range (Table 4). Thus, these compounds could be considered lead compounds.

Physical characteristics of synthesized compounds

SP1 (2-bromo-1-[4-[5-(quinolin-3-yl)-4,5-dihydrofuran-3-yl]phenyl]ethan-1-one)

S Yield: 86%; Yellowish white solid; mp. 98-100°C; R_f : 0.72 (chloroform and DMSO); FTIR (KBr, ν_{max} , cm^{-1}): 3233 (N-H), 1666 (C=O), 1594 (C-C Stretch), 1306 (C-N), 1008 (C-O), 760 (C-Br); $^1\text{H-NMR}$ spectrum (400MHz, CDCl_3): δ 2.278 (s, 2H, CH_2Br), 7.266, 7.402, 7.422, 7.510, 7.503, 7.487, and 7.481 aromatic protons in structures (Figures 2 and 3).

SP2 (1-[3-nitro-5-[5-(quinolin-3-yl)-4,5-dihydrofuran-3-yl]phenyl]ethan-1-one)

Yield: 88%; Greyish solid; mp. 90-92°C; R_f : 0.78 (chloroform and DMSO); FTIR (KBr, ν_{max} , cm^{-1}): 3234 (N-H), 1668 (C=O), 1523 (C-C Stretch), 1349 (C-N); $^1\text{H-NMR}$ spectrum (400MHz, CDCl_3): δ 2.168 (C-H alkyl), 10.345, indicative of the acid group, with aromatic protons at δ =7.729, 7.529, 7.510, and 7.489 aromatic protons in structures.

SP3 (1-[2,4-dichloro-3-[5-(quinolin-3-yl)-4,5-dihydrofuran-3-yl]phenyl]ethan-1-one)

Yield: 84%; Yellowish white; mp. 72-74°C; R_f : 0.82 (chloroform and DMSO); FTIR (KBr, ν_{max} , cm^{-1}): 3210 (N-H stretch), 1596 (C=O), 1309 (C-N stretch), 1484 (C-C), 1054 (C-O), and 754 (C-Cl); $^1\text{H-NMR}$ spectrum (400MHz, CDCl_3): δ 2.254 (C-H alkyl), 9.378 (3=CH, 1-benzene, 2-substituted benzimidazole), aromatic protons at δ =7.493, 7.488, 7.472, and 7.467 (present on the phenyl ring).

SP4 (4-chlorophenyl){3-[5-(quinolin-3-yl)-4,5-dihydrofuran-3-yl]phenyl}methanone

Yield: 78%; Greyish white; mp. 90-92°C; R_f : 0.85 (chloroform and DMSO); FTIR (KBr, ν_{max} , cm^{-1}): 3295 (N-H), 1652 (C=O), 1311 (C-N), 914 (C-O), 687 (C-Cl); $^1\text{H-NMR}$ spectrum (400MHz, CDCl_3): δ 7.701, 7.683, 7.454, 7.466, and 7.673 (on the phenyl ring), and amide protons at δ =9.459 and 9.067 (3=CH, 1-benzene, 2-substituted benzimidazole), absence of H from alkyl C-H

confirm the absence of alkyl hydrogen that can be seen in SP2 and SP3 (due to presence of alkyl CH₂ attached to C=O).

SP5 (1-{4-nitro-2-[5-(quinolin-3-yl)-4,5-dihydrofuran-3-yl]phenyl}ethan-1-one)

Yield: 72%; Greyish black; mp. 210-214°C; R_f: 0.87 (chloroform and DMSO); FTIR (KBr, $\nu_{\max}$, cm⁻¹): 1297 (C-N), 3304 (N-H), 1670 (C-C), 1520 (C-NO₂). ¹H-NMR spectrum (400MHz, CDCl₃): δ 2.153-2.170 (C-H alkyl), 7.123, 7.265, 7.384, 7.556, 7.683, 7.779 (aromatic protons) 8.037, corresponding to the 4=CH group (benzene of basic benzimidazole).

α -amylase enzyme inhibition assay

The % α -amylase enzyme inhibition was calculated using an α -amylase inhibitory assay, with Acarbose used as the standard, exhibiting a 93.47% inhibition. The inhibition values of the

synthesized compounds were compared with acarbose. Among all the synthesized compounds, SP2 and SP4 demonstrated the highest inhibition, both showing 90.57%. SP5 and SP6 exhibited 89.13% and 89.85% inhibition, respectively. The lowest inhibition was observed with SP3, which showed an inhibition of 88.40% (Table 5).

α -glucosidase inhibition assay

The α -glucosidase inhibitory potential of synthesized compounds was determined in comparison to the standard Acarbose. In brief, the % inhibition shown by the entire compound was observed in the range of 88.4 to 90.6% Table 5. The α -glucosidase % of compound SP2 and SP4 was found to be more which was comparable to the acarbose (93.47 $\pm$ 6.8%).

Figure 4 represents the histogram showing the significance level for the data as **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, ns $p > 0.05$.

Table 1: Binding affinity of top 50 phytochemicals along with their interactions.

Phytochemicals	Binding affinity (Kcal/mol)	Interacting residues	Interaction type
Gitogenin	-9.9	ALA97, ALA93, LYS96, PRO94, PRO545, VAL544, GLY546, TYR543, ARG275, ARG331, MET122, ASP91.	Vander Waals
		ASP319	Conventional H bond
		ILE98	C-H bond
		TRP126	Pi-sigma
		TYR110	Pi-Alkyl
Spergulin B	-9.3	TYR110, PRO125, ILE98, ASP91, ALA93, ASP319, ARG275, TYR543, LYS96, ASP95, GLY546, ALA97, GLY123.	Vander Waals
		ARG331	Conventional H bond
		MET122, PRO94	C-H bond
		TRP126	Pi-sigma
		VAL544	Unfavorable acceptor
Alizarin	-9.2	LEU641, PRO669, LEU706	Vander Waals
		HIS717, PHE362, MET363, HIS714, THR711, HIS708, TYR710.	Conventional H bond
		VAL723, HIS708	Carbon H bond
		VAL723	Pi-sigma
		LEU369	Pi-Pi T-shaped
		PRO364, PRO640	Pi-alkyl
Hippomanin A	-8.9	THR833, TRP367, GLU895, GLY896, GLU863, ASP861, GLN827, LEU899, TRP859.	Vander Waals
		THR834, GLN838, ARG837, ASP860, ALA897, GLY898, SER894.	Conventional H bond
		ARG437	Unfavorable donor

Phytochemicals	Binding affinity (Kcal/mol)	Interacting residues	Interaction type
Apigenin	-8.9	PRO669, ARG702	Vander Waals
		PHE362, THR711, TYR710, HIS708, LEU706, TYR668.	Conventional H bond
		HIS714, MET363	Carbon H bond
		TRP746, PRO640, LEU641, PRO364.	Pi-Pi T-shaped
		LEU369	Pi-alkyl
		VAL723	Pi-sigma
Scirpusin A	-8.9	SER523, PHE525, ARG600, ASP404, TRP516, ILE441, TRP613.	Vander Waals
		ASP282, TRP481, HIS674, ASP616, LEU677, SER676, LEU678, TRP618.	Conventional H Bond
		ASP518	Pi-anion
		MET519	Pi-sulfur
		TRP376	Pi-pi stacked
		PHE649, ALA284	Pi-Pi T-shaped
		LEU650	Pi-alkyl
Scirpusin B	-8.7	PHE525, ARG600, LEU405, ASP404, ILE441, TRP516, HIS674, SER523.	Vander Waals
		TRP481, TRP613, ASP616, LEU677, SER676, LEU678, TRP618.	Conventional H bond
		ASP282, ASP518	Pi-anion
		LEU650	Pi-sigma
		MET519	Pi-sulfur
		TRP376	Pi-pi stacked
		PHE649	Pi-Pi T-Shaped
Isoflavone	-8.6	ALA284	Pi-Alkyl
		PHE362, TYR710, HIS708.	Vander Waals
		PRO669, LEU706, HIS714.	Conventional H Bond
		VAL723	Pi-Sigma
		TRP746	Pi-Pi T-Shaped
Spergulin A	-8.6	PRO364, LEU369, PRO640, LEU641.	Pi-Alkyl
		PRO595, ARG594, GLU869, LEU865, PHE362, HIS717, ARG608, TYR360, GLY359, ASP356, PRO198.	Vander Waals
		HIS584, LEU868, VAL867, SER864, GLU866.	Conventional H Bond
		MET363	Unfavorable donor
Urosolic acid	-8.6	PRO545, ASP319, TYR543, ARG275, ARG331, PRO94, MET122, ALA93, ASP91, ILE98, CYS127, TRP126.	Van der Waals
		VAL544	Conventional H Bond
Genistein	-8.6	TRP746, HIS708, LEU641, THR711, LEU706, PRO707.	Vander Waals
		TYR710, HIS714, MET363.	Conventional H bond
		LEU369, VAL723	Pi-Sigma
		PRO640, PRO364, PRO669.	Pi-Alkyl

Phytochemicals	Binding affinity (Kcal/mol)	Interacting residues	Interaction type
Scirpusin C	-8.4	TRP481, PHE525, SER523, ASP404, ILE441, TRP516, PHE649, HIS674, TRP613, SER676.	Vander Waals
		ASP282, ASP616, ARG600.	Conventional H bond
		ALA284	Pi-Alkyl
		ASP518	Pi-anion
		LEU650	Pi-sigma
		MET519	Pi-sulfur
		TRP376	Pi-Pi T-Shaped
		TRP618	Unfavorable donor
Cassigarol E	-8.3	SER894, GLU895, GLN838, LEU899, GLY898, THR834, GLY435, LEU431, ARG436, GLU863, ASP861.	Vander Waals
		ARG837, GLY896, HIA432.	Conventional H Bond
		AR437	Pi-cation
		TRP859	Pi-Pi T-Shaped
Mahanine	-8.2	PRO4, VAL544, GLY546, PRO545, TYR543, ARG275, ARG331, ASP91, PRO125, GLY123.	Vander Waals
		CYS127, ALA97, LYS96, ASP319.	Conventional H bond
		MET122	Pi-sulfur
		TYR110, ILE98, TRP126	Alkyl
		ALA93	Pi-Alkyl
Kompasinol A	-8.2	PHE362, PRO595, ARG594, GLU869, LEU868, PHE858, MET363, SER864.	Vander Waals
		ARG608, GLU866	Conventional H bond
		HIS717	Unfavorable donor
		ARG585, TYR609, HIS584, VAL867.	Pi-donor H bond
		LEU865	Pi-sigma
		HIS717	Pi-Pi T-shaped
		VAL588	Pi-alkyl
Emodin	-8.1	THR99, ALA93, ASP91, CYS127	Vander Waals
		ILE98, TYR110, GLY123	Conventional H Bond
		TRP126, GLN124, MET122.	Pi-donor H bond
		TRP126	Pi-Alkyl
		TRP126	PI-Pi T-shaped
Ergosterol	-8.1	ASP616, ASP519, ARG600, TRP613, ILE441, ASP404, TRP516, HIS674, ILE283.	Vander Waals
		ARG281, ASP282, PHE525, SER523, MET519, TRP481	Conventional H bond
		TRP376, PHE649, ALA555	Alkyl, Pi-Alkyl

Phytochemicals	Binding affinity (Kcal/mol)	Interacting residues	Interaction type
Psoralidin	-8	PHE649	Vander Waals
		ASP616, ARG686	Conventional H Bond
		ASP282	Unfavorable acceptor
		ASP282	Pi-anion
		TRP376	Pi-sigma
		TRP481, PHE525	Pi-pi stacked, Pi-Pi T shaped
		LEU405	Alkyl, Pi-alkyl
Isoquercitrin	-7.9	ASP356, TYR360, GLYA59, VAL358, PHE362, HIS717.	Van der Waals
		ARG608, GLU196, THR197, VAL357, LEU355.	Conventional H Bond
		ASP356, VAL358	Carbon H Bond
		TYR360	Pi-Pi stacked
		ARG608	Pi-Alkyl
Flavonol	-7.9	HIS584, PRO595, PHE362.	Vander Waals
		GLU866, SER864, MET363	Conventional H Bond
		PRO595	C-H bond
		LEU865	Pi-sigma
		VAL588, ARG594, HIS717	Pi-Pi T-shaped
		VAL867, LEU868	Pi-alkyl
Chondrillasterol	-7.9	MET363, PHE362, ARG594, PRO595, HIS584, LEU865, GLU866, ASP356, TYRA360, GL359.	Vander Waals
		TYR360, HIS717, ARG608	C-H bond
		ARG608, HIS717	Pi-sigma
		TYR360	Alkyl, Pi-alkyl
Hespiretin	-7.9	GLN715, GLU721, THR722, HIS714, GLY747, LEU369, TYR710.	Vander Waals
		GLU748, ARG725, PRO669, HIS708, THR711, GLY855, ARG854.	Conventional H Bond
		GLU856	Pi-anion
		VA723, TR746	Pi-sigma
		VAL723	Alkyl, Pi-alkyl
Isovetixin	-7.8	PRO545, VAL544, MET122, PRO125, CYS127, ALA93, ILE98, LYS96.	Vander Waals
		VAL547, ARG331, TYR543, PRO94, GLN124, TRP126.	Conventional H Bond
		VAL544, GLY550, ALA97	C-H bond
		ASP91	Unfavorable acceptor
		ARG275	Pi-cation
		GLY546	Pi-sigma

Phytochemicals	Binding affinity (Kcal/mol)	Interacting residues	Interaction type
Kuranidin	-7.8	TYR110, ILE98, ALA93, CYS127, PRO125, MET122, ARG275, VAL544, PRO545.	Vander Waals
		ALA97, PRO94, TYR543, GLY546, ASP319.	Conventional H Bond
		ASP91, GLY123	C-H bond
		ARG331	Unfavorable donor
		ASP91	Pi-anion
		TRP126	Pi-alkyl
Cyanidin-3-galactoside	-7.8	PHE362, LEU865, SER864, VAL867, MET363.	Vander Waals
		GLU869, LEU868, GLU866, ARG594, TYR360, ARG585.	Conventional H Bond
		HIS584	Pi-sigma
		ARG608	Pi-Pi T-shaped
		VAL588	Pi-alkyl
Coryfolin	-7.8	VAL193, ALA604, GLY605, TYR609, PHE490, LEU496, THR491, THR578, LEU574.	Vander Waals
		VAL358	Unfavorable acceptor
		LEU577	Pi-sigma
		PRO493, LEU565, PHE564, LEU195, PRO194, ILE581.	Alkyl, Pi-Alkyl
Procyanidin	-7.7	TYR292, LEU283, ARG600, ALA555, MET519, TRP36, PHE649, LEU650.	Vander Waals
		ASP616, ARG281, ASP282	Conventional H Bond
		PHE525, TRP481	Pi-Pi stacked; Pi-Pi T shaped
Ganoderol B	-7.7	GLY546, ALA93, LYS96, ALA9, ILE98, TYR110, THR99, PRO125, MET122, ASP91, PRO94, ARG275, ARG331, TYR543, PRO545.	Vander Waals
		VAL544	Conventional H Bond
		ASP319	Unfavorable acceptor
		TRP126	Pi-Alkyl
Biacalein	-7.7	TYR110, LYS96, ALA97, PRO94, GLY123	Vander Waals
		ILE98, ALA93, ASP91, GLN124.	Conventional H Bond
		PRO125, MET122, ILE98	Pi-donor H bond
		TRP126	Pi-Pi stacked
Flavanone	-7.6	HIS584, ARG594, MET363, SER864.	Vander Waals
		GLU866	Conventional H Bond
		PRO595, PHE362	C-H bond
		LEU865	Pi-Sigma
		HIS717	Pi-Pi T shaped
		VAL588, VAL867, LEU868	Pi-Alkyl

Phytochemicals	Binding affinity (Kcal/mol)	Interacting residues	Interaction type
Kurarinone	-7.6	HIS64, TRP516, ILE441, ASP616, TRP481, LEU650, SER676.	Vander Waals
		ASP404	Unfavorable acceptor
		ASP518	Pi-anion
		PHE649	Pi-Pi stacked
		PH525, TRPA376, LEU677, LEU678.	Alkyl, Pi-alkyl
Norkurarinol	-7.6	ILE441, TRP516, HIS674, TRP613, TYR292, PHE649, LEU650, TRP376, LEU677, LEU678, SER676	Vander Waals
		ASP404, ARG600, ASP616, ASP282, MET519, PHE525.	Conventional H Bond
		ASP518	Pi-anion
		TRP481	Pi-Pi T shaped
Vitexin	-7.5	HIS432, GLU863, THR593, HIS507, GLN429.	Vander Waals
		ARG436, GLY435, ARG437.	Conventional H Bond
		ARG591, ASP513	Pi-cation, pi-anion
		PRO511	Pi-Alkyl
Swretisin	-7.5	GLY335, GLY334, ASN316, TYR569, SER566.	Vander Waals
		ARG189, ARG190, LYS162, ASN536.	Conventional H Bond
		ASP243	Unfavorable donor
		TYR191	Unfavorable acceptor
		LEU538	Pi-sigma, Pi-alkyl
2,3-di-o-galloyl-1,5-anhydro-D-glucitol	-7.5	LEU899, GLY862, GLU895.	Vander Waals
		GLN900, TRP859, GLN858, GLY898, ARG837, ASP861, ALA897, GLY896, SER894.	Conventional H Bond
		ASP862	Unfavorable acceptor
		GLN858	Pi-Sigma
Flavone	-7.5	PHE362, SER864, HIS584	Vander Waals
		GLU866	Conventional H Bond
		PRO595	C-H bond
		LEU865	Pi-Sigma
		HIS717	Pi-Pi T shaped
		VAL588, ARG594, VAL86, MET363, LEU868.	Pi-Alkyl
Cyanidin	-7.4	LYS96, PRO94, MET122	Vander Waals
		ALA93, ASP91, GLY123, PRO125, GLN124, CYS127, ILE98, TYR110, THR99.	Conventional H Bond
		ASP91	Unfavorable acceptor
		TRP126	Pi-Pi stacked

Phytochemicals	Binding affinity (Kcal/mol)	Interacting residues	Interaction type
<i>Piperumbellactam B</i>	-7.4	THR99, TYR110, ILE98, ASP91, PRO125, TRP126, GLY123.	Vander Waals
		CYS127	Pi-donor H bond
		TRP126	Pi-Pi stacked
		ALA93	Pi-alkyl
8-demethylsideroxylin	-7.4	VAL867, ARG594, PRO595, PHE362, HIS584, TYR609.	Van der Waals
		MET363, LEU868, GLU866, GLU869, HIS717.	Conventional H Bond
		ARG608	Alkyl
		LEU868	Pi-alkyl
1-hydroxy-2-methylanthraquinone	-7.4	ALA93, ILE98, TYR110, PRO125, GLY123, MET122, GLN124.	Vander Waals
		ASP91	Unfavorable donor
		CYS127	Unfavorable acceptor
		TRP126	Pi-Pi stacked
Physcion	-7.4	GLN776, VAL778, PRO779, THR700, GLU781.	Vander Waals
		THR700	C-H Bond
		ILE780	Pi-Sigma
		LYS697	Alkyl
		LEU775, LEU701	Pi-alkyl
Morin	-7.4	PHE506, PRO457, ASP413, TYR455, SER454.	Vander Waals
		ASN417, ASP409, LYS418, PHE416, GLN509, ARG456, GLU505, PHE414.	Conventional H Bond
		PRO457	C-H Bond
		ARG412	Pi-cation
		THR415	Pi-alkyl
8-demethylsideroxylin	-7.4	VAL867, ARG594, PRO595, PHE362, HIS584, TYR609.	Vander Waals
		MET363, LEU868, GLU866, GLU869, HIS717.	Conventional H Bond
		ARG608	Alkyl, Pi-alkyl
Flavon-3-ol	-7.3	SER864, ARG594	Van der Waals
		PRO595, HIS584, GLU866.	Conventional H Bond
		HIS865, LEU868	Pi-Sigma
		VAL867	Pi-Pi T shaped
		VAL588	Pi-alkyl
Fisetin	-7.3	ARG608, PHE362, MET363, PRO595.	Vander Waals
		GLU869, VAL867, GLU866, LEU865, SER864, ARG594, HIS584.	Conventional H Bond
		MET363	Pi-donor H bond
		HIS717	Pi-Pi T shaped
		LEU868	Pi-alkyl

Phytochemicals	Binding affinity (Kcal/mol)	Interacting residues	Interaction type
Catachin	-7.3	LEU496, ALA582, THR578, ARG608, VAL358.	Vander Waals
		PHE490, LEU565, LEU574, VAL193, PHE503, ALA604, GLY605.	Conventional H Bond
		PRO194, ILE581, LEU577.	Pi-Alkyl
(Z)-Guggulsterone	-7.3	ASP518, ASP282, MET519, TRP481, TRP376, PHE649, LEU650, ASP616.	Vander Waals
		ARG600	Conventional H Bond
		PHE525	Pi-Alkyl
Crysophanol	-7.2	VAL775, THR700	Van der Waals
		GLU781, ILE780, PRO779, THR777, GLN776.	Conventional H Bond
		LYS697, LEU701, LEU775	Alkyl, Pi-Alkyl
Epigallocatechin	-7.2	TYR543, ARG275, VAL321, ARG331, PRO94, ASP91, PRO125, TRP126.	Van der Waals
		ILE98, ALA97, LYS96, ASP95, MET122.	Conventional H Bond
		ALA93, CYS127	Unfavorable acceptor-acceptor
		ALA93	Pi-Alkyl

Table 2: Binding affinity (Kcal/mol) and interactions of designed molecules with residues at the active site of target protein alpha-glucosidase (PDB: 5KZX).

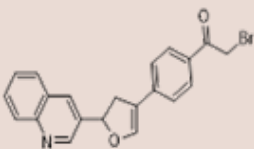
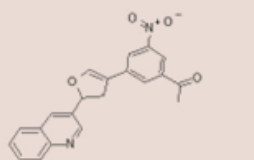
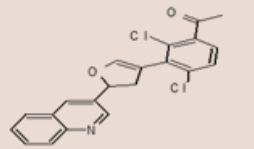
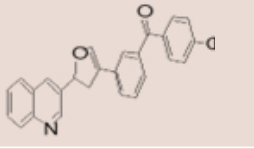
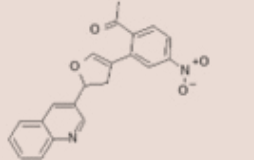
Chemical code	Structure	Binding affinity (Kcal/mol)	Interacting residues	Interaction type
SP1		-8.1	PRO89, LEU831, LYS697, LEU701	Pi-Pi stacked
SP2		-8.9	PHE525 PHE525, TRP481, TRP376.	H Bond Pi-Pi stacked
SP3		-8.8	ARG854 TRP746, LEU369, VAL723 HIS708	H Bond Pi-Pi stacked Pi-Alkyl
SP4		-8.7	ASP616 PHE525, TRP481, PHE649, TRP376	H Bond Pi-Pi stacked
SP5		-9.2	HIS584, ARG608, TYR360 HIS717, ARG594	H Bond Pi-Pi stacked

Table 3: Interactions of designed molecules (TOP 4).

Compound	3D interaction diagram	2D interaction diagram
SP2		
SP3		
SP4		
SP5		

CONCLUSION

This study successfully used a multi-faceted approach of *in silico*, synthetic and *in vitro* methods to create and examine new isoxazole chalcone derivatives (SP1-SP6) as α -glucosidase and α -amylase anti-diabetic agents. Molecular docking studies provided evidence that the compounds synthesized during this investigation had strong binding affinity and positive interactions with the active site residues of the target protein PDB ID: 5KZX that were similar or greater in affinity to the selected phytochemicals. Overall, the predicted pharmacokinetics of the compounds indicate they pass all aspects of Lipinski's rule of five and are therefore good drug-like candidates for further oral formulation as anti-diabetic agents.

All derivatives were synthesized, the successful formation of which was confirmed through FTIR and ^1H -NMR spectroscopy, which established the compounds' structural integrity as well as existed confirmatory functional groups. *In vitro* enzyme assays were also

in line with computational modeling and exhibited significant inhibition of both α -amylase and α -glucosidase activity of SP2 (1-{3-nitro-5-[5-(quinolin-3-yl)-4,5-dihydrofuran-3-yl] phenyl} ethan-1-one) and SP4 (4-chlorophenyl){3-[5-(quinolin-3-yl)-4,5-dihydrofuran-3-yl] phenyl} methanone in a manner comparable to systematic drug acarbose. Overall, these results suggest that the new synthetic compounds, in particular SP2 and SP4, have great potential as potent inhibitors that may modulate postprandial hyperglycemia.

In summary, the use of computational modeling, rational design, and biological assessment has enabled us to identify promising lead candidates with potential use in type 2 diabetes mellitus. Further studies including detailed mechanistic investigation, *in vivo* efficacy, and toxicity are needed for further evaluation of their therapeutic utility. This study ultimately lays the groundwork for the generation of structurally novel and biologically active anti-diabetic agents derived from an isoxazole chalcone scaffold.

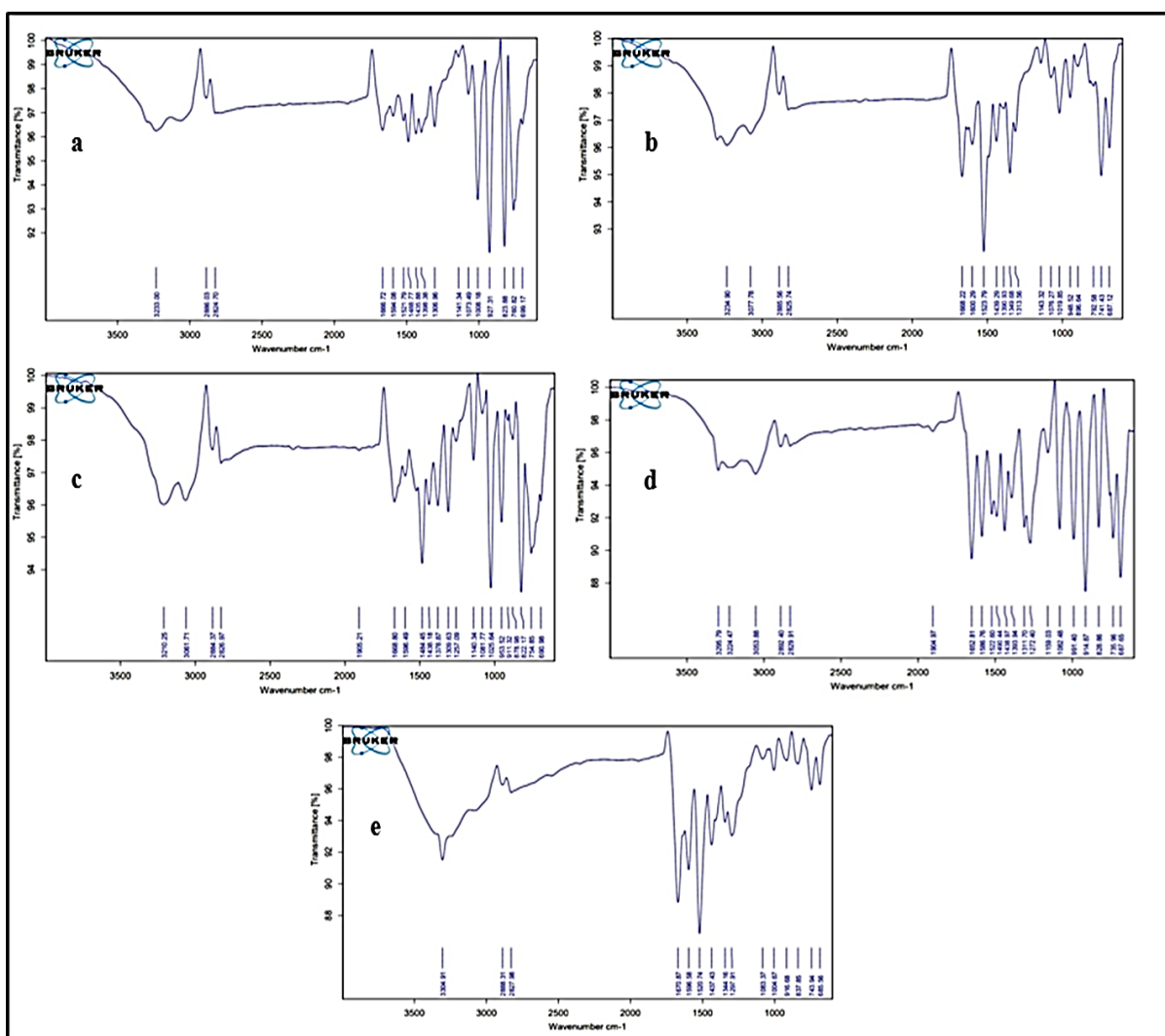


Figure 2: FTIR analysis of synthesized compounds.

Table 4: Drug-likeness of designed molecules.

Sl. No.	Code	Molecular Formula	Lipinski's Rule of 5					Lipinski's Viol.	Lipinski's Rule
			MW (g/mol)	mLogP	HBA	HBD	MR		
1.	SP1	$C_{21}H_{16}BrNO_2$	394.26	3.06	3	0	102.97	0	Yes
2.	SP2	$C_{21}H_{16}N_2O_4$	360.36	1.72	5	0	103.92	0	Yes
3.	SP3	$C_{21}H_{15}Cl_2NO_2$	384.26	3.70	3	0	105.12	0	Yes
4.	SP4	$C_{26}H_{18}ClNO_2$	411.88	4.05	3	0	119.79	0	Yes
5.	SP5	$C_{21}H_{16}N_2O_4$	360.36	1.72	5	0	103.92	0	Yes

Table 5: The % inhibition of α -amylase and α -glucosidase by synthesized compounds.

Samples	Concentration (mg/mL)	% Inhibition $\pm$ SD (α -amylase)	% Inhibition $\pm$ SD (α -glucosidase)
Standard (Acarbose)	1	93.47 $\pm$ 6.8	88.23 $\pm$ 5.4
SP2	1	90.57 $\pm$ 7.2	86.27 $\pm$ 4.8
SP3	1	88.40 $\pm$ 5.9	63.39 $\pm$ 5.9
SP4	1	90.57 $\pm$ 6.3	83.00 $\pm$ 3.8
SP5	1	89.13 $\pm$ 7.8	73.85 $\pm$ 7.8

n=3.

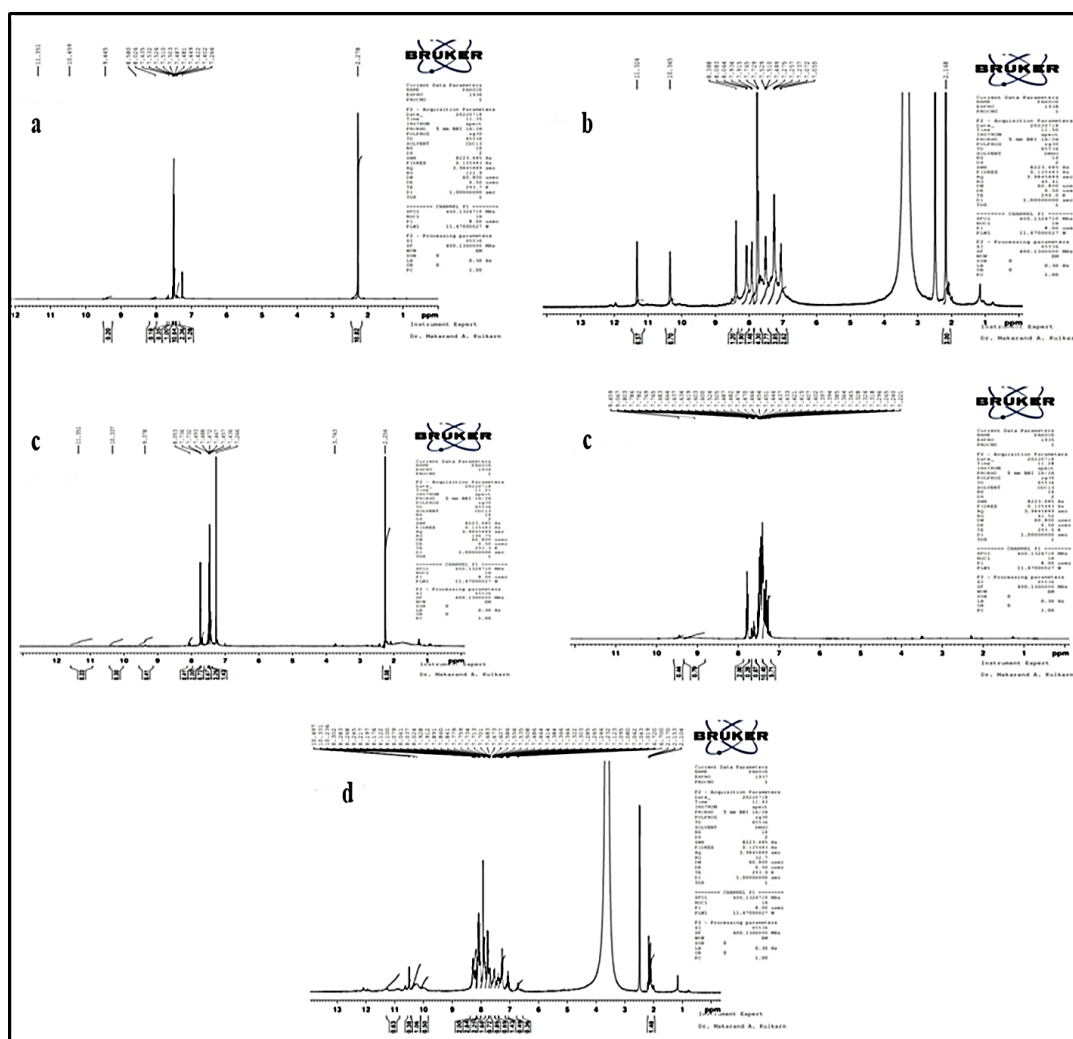


Figure 3: NMR analysis of synthesized compounds.

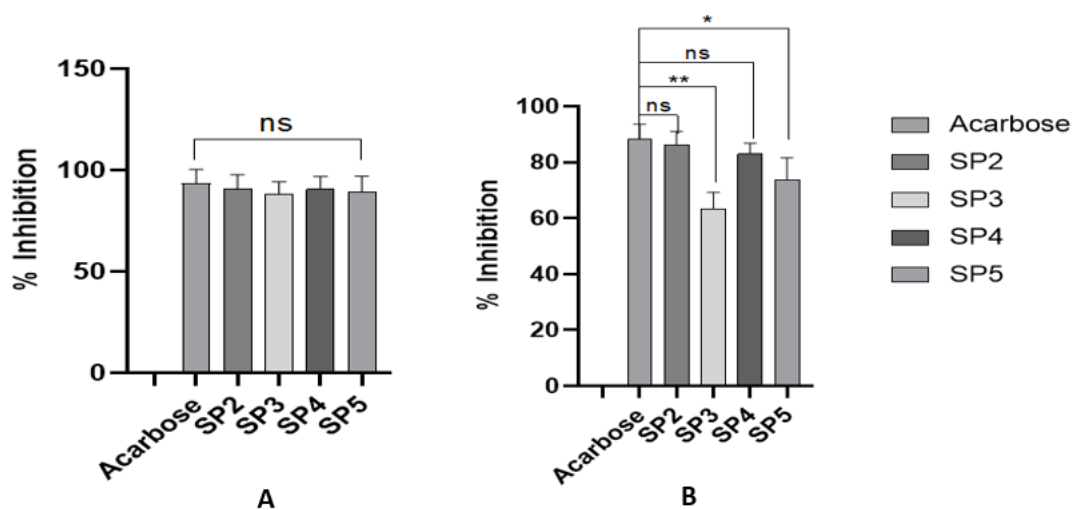


Figure 4: Histogram of effect of synthesized compounds on % Inhibition of (A) α -amylase (B) α -glucosidase enzyme. All the data are the mean of three replicates ($n=3$) and vertical bars show Standard Errors ($\pm$ SE). The significance level for the data was **** $p<0.0001$, *** $p<0.001$, ** $p<0.01$, ns $p>0.05$.

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ABBREVIATIONS

DM: Diabetes mellitus; **SP:** Synthesized Product; **ADME:** Absorption, Distribution, Metabolism, and Excretion; **DMSO:** Dimethylsulfoxide; **NMR:** Nuclear Magnetic Resonance; **FTIR:** Fourier Transform Infrared Spectroscopy; **SAR:** Structure-Activity Relationship; **P-NPG:** p-nitrophenyl- α -D-glucopyranoside.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This study employed a combined *in silico*, synthetic, and *in vitro* approach to develop novel isoxazole chalcone derivatives (SP1-SP6) as potential anti-diabetic agents targeting α -glucosidase and α -amylase enzymes. Molecular docking studies showed strong binding affinity of the synthesized compounds with the target protein PDB ID: 5KZX, indicating promising inhibitory potential. The predicted pharmacokinetic properties satisfied Lipinski's rule of five, suggesting good drug-likeness for oral administration. Structural confirmation of the synthesized derivatives was achieved using FTIR and ^1H -NMR spectroscopy. *In vitro* enzyme inhibition studies demonstrated significant activity, with SP2 and SP4 showing inhibition comparable to the standard drug acarbose. Overall, the findings suggest that these compounds, particularly SP2 and SP4, may serve as promising candidates for further development as anti-diabetic agents to control postprandial hyperglycemia.

REFERENCES

- Holst JJ, Vilsbøll T, Deacon CF. The incretin system and its role in type 2 diabetes mellitus. *Molecular and cellular endocrinology*. 2009;297(1-2):127-36.
- Mbanya JC, Assah FK, Saji J, Atanga EN. Obesity and type 2 diabetes in Sub-Saharan Africa. *Current diabetes reports*. 2014;14(7):1-8.
- Scheen AJ. Is there a role for α -glucosidase inhibitors in the prevention of type 2 diabetes mellitus?. *Drugs*. 2003;63:933-51.
- Coughlan KA, Valentine RJ, Ruderman NB, Saha AK. AMPK activation: a therapeutic target for type 2 diabetes?. *Diabetes, metabolic syndrome and obesity: targets and therapy*. 2014: 241-53.
- Chao EC. Dapagliflozin: an evidence-based review of its potential in the treatment of type-2 diabetes. *Core Evidence*. 2012: 21-8.
- Shi G, Sun C, Gu W, Yang M, Zhang X, Zhai N, *et al.*, Free fatty acid receptor 2, a candidate target for type 1 diabetes, induces cell apoptosis through ERK signaling. *J Mol Endocrinol*. 2014;53(3):367-80.
- Rondinone CM, Wang LM, Lonnroth P, Wesslau C, Pierce JH, Smith U. Insulin receptor substrate (IRS) 1 is reduced and IRS-2 is the main docking protein for phosphatidylinositol 3-kinase in adipocytes from subjects with non-insulin-dependent diabetes mellitus. *Proceedings of the National Academy of Sciences*. 1997;94(8):4171-5.
- Qi Y, Xu Z, Zhu Q, Thomas C, Kumar R, Feng H, *et al.*, Myocardial loss of IRS1 and IRS2 causes heart failure and is controlled by p38 α MAPK during insulin resistance. *Diabetes*. 2013;62(11):3887-900.
- Lim AK, Nikolic-Paterson DJ, Ma FY, Ozols E, Thomas MC, Flavell RA, Davis RJ, Tesch GH. Role of MKK3-p38 MAPK signalling in the development of type 2 diabetes and renal injury in obese db/db mice. *Diabetologia*. 2009;52(2):347-58.
- Tamrakar AK, Maurya CK, Rai AK. PTP1B inhibitors for type 2 diabetes treatment: a patent review (2011-2014). *Expert opinion on therapeutic patents*. 2014;24(10):1101-15.
- Zhang S, Zhang ZY. PTP1B as a drug target: recent developments in PTP1B inhibitor discovery. *Drug discovery today*. 2007;12(9-10):373-81.
- Chao EC, Henry RR. SGLT2 inhibition-a novel strategy for diabetes treatment. *Nature reviews drug discovery*. 2010;9(7):551-9.
- Somlak Chuengsamarn, SutheeRattanamongkolgul, Rataya Luechapudiporn, Chada Phisalaphong, SiwanonJirawatnotai; Curcumin Extract for Prevention of Type 2 Diabetes. *Diabetes Care*. 2012;35(11): 2121-7
- Pivari F, Mingione A, Brasacchio C, Soldati L. Curcumin and Type 2 Diabetes Mellitus: Prevention and Treatment. *Nutrients*. 2019;8: 11(8):1837
- Citro A, Cantarelli E, Piemonti L. The CXCR1/2 pathway: involvement in diabetes pathophysiology and potential target for T1D interventions. *Current Diabetes Reports*. 2015;15(10):1-9.
- Saddique FA, Ahmad M, Ashfaq UA, Ahmad MN, Anjum MN, Mohsin NU, Aslam S. Alpha-glucosidase inhibition and molecular docking studies of 1, 2-benzothiazine 1, 1-dioxide based carbohydrazides. *Pakistan journal of pharmaceutical sciences*. 2019;32:2829.

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