RESEARCH PAPER

Rational Design, In Silico studies, Synthesis, and Biological Evaluation of Azetidine-Based Compounds for Antidiabetic Activity

Mohini Patidar[1], Nitin Deshmukh[1], Gourav Trivedi[1], Prabhat Kumar Das[2], Ruchita Raghuvanshi[3], Ayushi Patud[1*] and Sujit Pillai[1]

[1]*Department of Pharmaceutical Chemistry, GRY Institute of Pharmacy, Borawan, Khargone, MP-451228*
[2]*Department of Pharmacology, GRY Institute of Pharmacy, Borawan, Khargone, MP-451228*
[3]*Department of Pharmaceutics, GRY Institute of Pharmacy, Borawan, Khargone, MP-451228*
***Corresponding Author:** Ayushi Patud*
aayushipatud0@gmail.com

Received: 27th Feb, 2026; Revised: 20th March 2026; Accepted: 8th April, 2026; Available Online: 20th April, 2026

ABSTRACT
Diabetes mellitus remains a significant global health concern, necessitating the discovery of novel therapeutic agents with improved efficacy and safety profiles. The present study focused on the design and evaluation of azetidine derivatives as potential antidiabetic agents. A total of 40 azetidine derivatives were designed and screened using in-silico tools to assess their physicochemical and pharmacokinetic properties. Molecular docking was performed against the target protein DPP4 (PDB ID: 5Y7J) to evaluate their binding affinity, using metformin as the reference standard. Compounds F40, F33, F07, F21, and F39 showed enhanced binding interactions, with F40 exhibiting the most favourable binding energy (–8.56409 kcal/mol). The top-performing candidates were synthesized via cyclization of Schiff bases using chloroacetyl chloride, and their structures were confirmed through IR, NMR, and mass spectrometry. In vivo antidiabetic studies demonstrated that compound F40 significantly improved glycemic control and lipid profile parameters in treated animals, without observable toxicity at doses up to 1000 mg/kg. Our study reveals that azetidine pharmacophore are one of the important pharmacophores to develop antidiabetic potential compounds.
Keywords: *Azetidine derivatives, Diabetes mellitus, DPP-4 inhibition, Molecular docking, Antidiabetic activity*
How to cite this article: Patidar M, Deshmukh N, Trivedi G, Das PK, Raghuvanshi R, Patud A, Pillai S. Rational Design, in Silico Studies, Synthesis, and Biological Evaluation of Azetidine-Based Compounds for Antidiabetic Activity. Int J Drug Deliv Technol. 2026;16(31s):123-132. DOI: 10.25258/ijddt.16.31s.15

Source of support: Nil.
Conflict of interest: None

INTRODUCTION

Diabetes mellitus is a prevalent metabolic condition affecting populations globally, marked by elevated blood sugar levels due to issues with insulin secretion, insulin function, or both. Persistent hyperglycemia, a frequent outcome of uncontrolled diabetes, leads to long-term harm, dysfunction, and failure of various organs including the kidneys, eyes, nerves, heart, and blood vessels. [1] The World Health Organization defines diabetes mellitus as a metabolic disorder with multiple causes, characterized by chronic high blood sugar levels and disturbances in the metabolism of carbohydrates, fats, and proteins, stemming from defects in insulin secretion, action, or both. Heterocyclic compounds have consistently been an intriguing focus in the field of chemistry. The distinctive structure of Azetidinone plays a crucial role in medicinal chemistry. [2] The distinctive structure of azetidinone is significant in the field of medicinal chemistry. Azetidine acts as the base ring for azetidinones, and the incorporation of a carbonyl group at position-2 increases reactivity. [3]

Azetidin-2-one is recognized as the β-lactam ring of a four-membered cyclic amide, named for the nitrogen atom attached to the β carbon adjacent to the carbonyl group [1]. The origins of azetidine compounds trace back to 1907, when the Schiff base reaction [2] involving aniline and aldehyde was introduced through a cycloaddition process. The chemistry surrounding this compound plays a significant role in organic synthesis [3], particularly following Alexander Fleming's discovery of Penicillin and the increasing demand for more effective agents against bacterial and fungal infections due to the resistance observed in microbial species [4, 5].When used to process enzymes of the growing peptidoglycan layer, the antibiotic's molecular activity is highly selective and irreversible inhabitation [6]. This four-membered structure is very strong and widely used for a variety of micro-activities by bacteria or viruses that affect human vital cells [8]. Azetidine-2-one is a very important type of synthetic chemical structure with very wide bands of biological activities such as anti-bacterial [7], anti-inflammatory [9], CNS activity, and anti-cancer activity [10].

**Author for Correspondence: aayushipatud0@gmail.com*

In this research, we will analyse the newly synthesized compounds (HL1, HL2, and 1–12) using a variety of characterization methods, including FT-IR (Fourier transform infrared), NMR (nuclear magnetic resonance), and mass spectrometry. We will also explore the potential of these synthesized compounds as antidiabetic and antioxidant agents. The increase in blood glucose levels leading to diabetes has become a significant issue in today's world. Therefore, scientists aim to develop treatments or medications that can effectively manage diabetes by regulating blood sugar levels. In this contemporary era, there have been substantial advancements in the methods for treating diabetes, addressing both hyperglycaemia and hypoglycaemia. [11-12]

Despite these advancements, all treatment methods and medications still have associated side effects, paving the way for further research. An antioxidant is a substance that prevents the oxidation of cellular molecules. The activity of antioxidants is thought to rely on their capacity to capture positively charged electrophilic species, eliminate oxygen radicals, and/or bind metals to create inactive complexes. [13] We will juxtapose the experimental results with theoretical analyses such as molecular docking (MD) and density functional theory (DFT) simulations. Additionally, the current investigation will be expanded to generate ADME/T scores using the SwissADME prediction website to evaluate the pharmacological profile of the developed drugs.

COMPUTATIONAL STUDY

In-silico screening

In-silicoscreening is acomputational techniqueused in drug discovery anddevelopment topredict thebinding affinity of small molecules to a target protein or receptor. It involves the use of computer algorithms and molecular modelling techniques to virtually screen large libraries of chemical compounds and identify potential drug candidates.

In-silicoscreening of all proposed structures of novel Azetidine-2-one derivatives were carried out using various computational chemistrysoftware's suchas ACD Lab/ChemSketch 12.0, Molinspiration, PreADMETand Molegro Virtual docker 6.0. [14]

A computational technique for determining the architecture of compounds produced by two or more different molecules is called molecular docking. Predicting the intended three-dimensional structures is the aim of docking investigations. In order to predict a small molecule's affinity and activity, docking is frequently utilized to predict how therapeutic small molecules would fit with their protein targets. A key component of logical drug design is docking. The process of positioning molecules in the best possible configurations to engage with a receptor is known as docking. When molecules are joined to form a sustained complex, a mechanism known as "docking" occurs within a cell. [15]

EXPERIMENTAL SECTION

Materials

All reagents and solvents used in this work were of synthetic grade obtained from Oxford Laboratory and Loba-chemiePvt. Ltd. Melting points were determined by open tube capillary method and areuncorrected (˚C). Progress of the reactions was monitored by TLC on silica gel-G in solvent system chloroform -methanol 8:2 and the spots were located under iodine vapours and UV light. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in DMSO-d6 solution using TMS as an internal reference standard on a Bruker-Avance-III 500 NMR spectrometer of 500 MHz. GC-MS spectra were recorded on a JEOL GC MATE II GC-MS1000 X spectrometer. All reagents used were analytical grade.

Designing of compound:

On the basis of reported structure activity relationship, 40 compounds were designed using ChemDraw ultra 8.0 as potential antidiabetic agents with different substitutions shown in **Table 1**

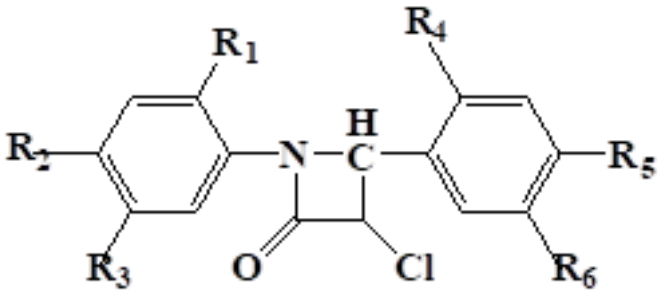


Figure 1: Designed Pharmacophore

Synthesis of compounds: On the basis of docking result, compounds F40, F33, F07, F21 and F39 were synthesized using synthetic Scheme **(Fig 2).**

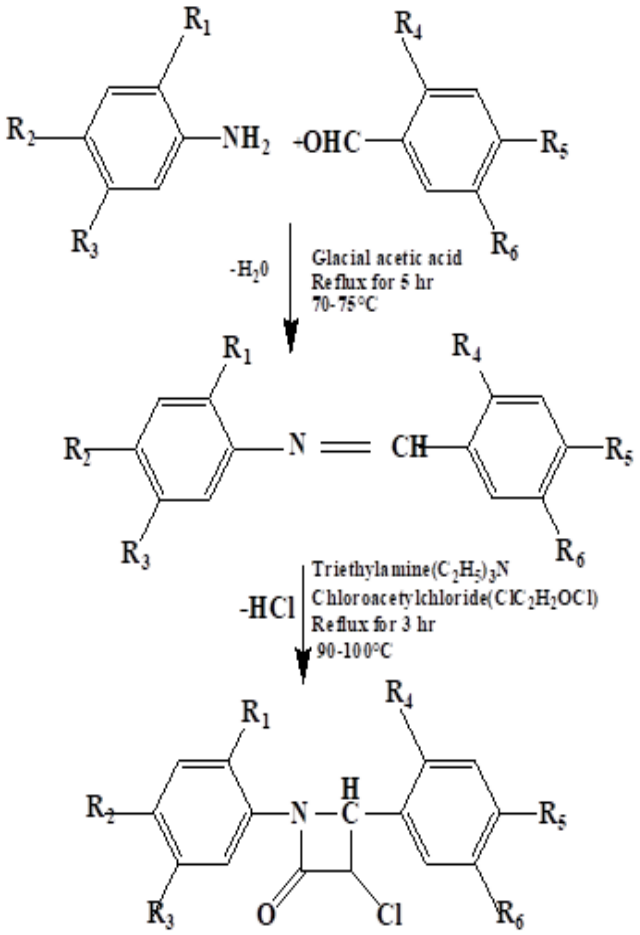


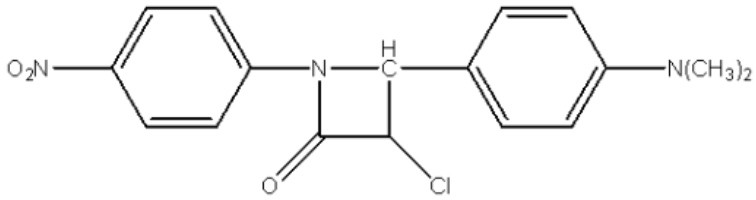
Figure 2: Scheme of Synthesis

Step-I: - General Procedure for the Synthesis of Schiff base

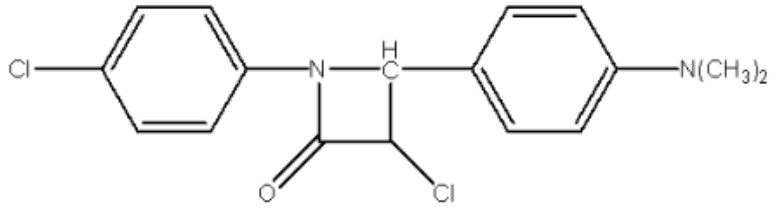
Aromatic amine (0.01 M) was weighed and dissolved in 30 mL of ethanol in round bottom flask. Benzaldehyde (0.02 M) was then added to the solution, followed by the addition of 3–5 drops of glacial acetic acid. The reaction mixture was refluxed at 70–75 °C for 5–6 hours. After completion, the mixture was poured into ice-cold water. The precipitated solid was filtered, washed with cold water and recrystallized from ethanol. [16,17]

Step-II: - General Procedure for the Synthesis of Azetidinederivatives

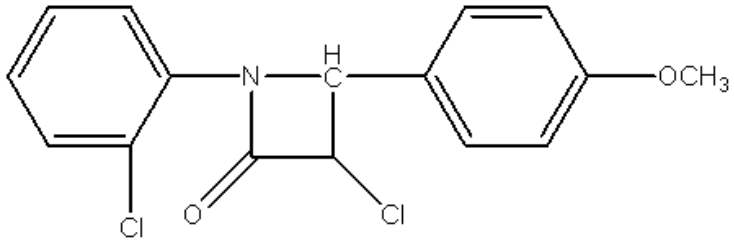
The Schiff base (0.01 M) obtained in the previous step was weighed and dissolved in 20 ml of ethanol in a round-bottom flask. Triethylamine (1 ml) was added to the solution, followed by the dropwise addition of chloroacetyl chloride (1.13 ml) with vigorous stirring. The reaction mixture was refluxed at 90–100 °C for up to 3 hours. The resulting solid was filtered, washed with distilled water several times, and recrystallized from ethanol.[16]The synthesizedpharmacophore with chemical structure and their compound ID are shown in **Fig 3.** [18, 19]



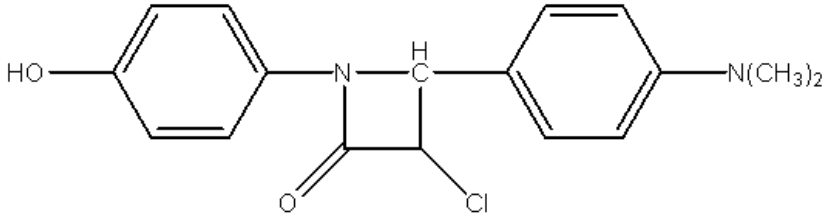
Compound ID: F40



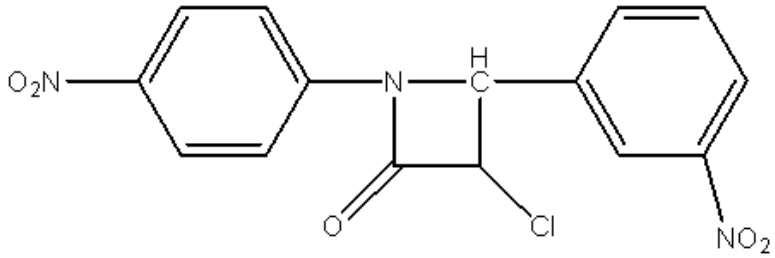
Compound ID: F33



Compound ID: F07



Compound ID: F21



CompoundID: F39

Figure 3: Structures of synthesized molecules

Physicochemical Characterization Procedure

The synthesized azetidine derivatives were subjected to physicochemical characterization using standard experimental methods. The melting point of each compound was determined using a digital melting point apparatus and recorded in °C without correction. The Rf values were evaluated by TLC using pre-coated silica gel plates (silica gel 60 F254) and an appropriate solvent system (e.g., ethyl acetate:hexane). The developed plates were visualized under UV light, and Rf values were calculated as the ratio of the distance traveled by the compound to that of the solvent front. The appearance (color and physical state) of each compound was recorded by visual inspection. Molecular weight and molecular formula were confirmed using mass spectrometry. All experiments were performed under controlled laboratory conditions, and the obtained data were used to confirm the purity and identity of the synthesized compounds. [20,21]

Pharmacological Anti-diabetic activity

Adult female Wistar rats (150–180 g) were procured and acclimatized for one week under standard laboratory conditions (temperature 22 ± 2 °C, relative humidity 55 ± 5%, and a 12 h light/dark cycle) with free access to standard pellet diet and water. Type 2 diabetes mellitus was induced by feeding the animals a high-fat diet for 4 weeks, followed by a single intraperitoneal injection of alloxan monohydrate (120 mg/kg body weight). After 72 h of alloxan administration, FBG levels were measured using a glucometer, and animals with FBG ≥ 200 mg/dL were considered diabetic and included in the study.The diabetic rats were randomly divided into five groups (n = 6): Group I (normal control), Group II (diabetic control), Group III (standard, metformin 100 mg/kg), Group IV (test compound F33, 100 mg/kg), and Group V (test compound F40, 100 mg/kg). All treatments were administered orally once daily for 28 consecutive days. Throughout the experimental period, blood glucose levels and other relevant biochemical parameters were monitored at predetermined intervals to evaluate the antidiabetic efficacy of the test compounds. [22,23]

DPP-4 Enzyme Inhibition Assay

The DPP-4 inhibitory activity of the synthesized azetidine derivatives (F40, F33, F07, F21, and F39) was evaluated using a colorimetric assay. Briefly, the reaction mixture consisted of Tris–HCl buffer (pH 8.0), DPP-4 enzyme solution, and varying concentrations of the test compounds (10–100 µM). The mixture was pre-incubated at 37 °C for 10 min, followed by the addition of Gly-Pro-p-nitroanilide substrate to initiate the reaction. After incubation for 30 min at 37 °C, the release of p-nitroaniline was measured at 405 nm using a microplate reader. Sitagliptin was used as the reference standard. All experiments were performed in triplicate. The percentage inhibition was calculated, and IC_{50} values were determined from dose–response curves. [24,25]

GLP-1 Estimation Study

To elucidate the mechanism of antidiabetic action, serum glucagon-like peptide-1 (GLP-1) levels were estimated using a commercially available ELISA kit. Blood samples were collected from experimental animals at the end of the treatment period, and serum was separated by centrifugation. The assay was performed according to the manufacturer's protocol, and absorbance was measured at 450 nm. GLP-1 concentrations were calculated using a standard calibration curve. [26, 27]

RESULTS

In - silico prediction of Synthesized derivatives:

The molecular properties of the designed azetidine derivatives were evaluated using MolinspirationCheminformatics software to assess their drug-likeness and oral bioavailability. All compounds complied with Lipinski's Rule of Five (MW ≤ 500 Da, logP ≤ 5, hydrogen bond donors ≤ 5, and acceptors ≤ 10), with TPSA values within acceptable limits (≤ 140 Å²), indicating favorable pharmacokinetic profiles and good oral bioavailability potential. Furthermore, bioactivity prediction using Molinspiration revealed that the selected compounds (F40, F33, F07, F21, and F39) exhibited promising interactions with key biological targets, including enzymes, ion channels, GPCRs, kinases, and proteases, with favorable bioactivity scores indicating their ability to effectively bind and modulate these targets, thereby supporting their potential as lead candidates for therapeutic development **Table 1**.

Table 1 Result of Molinspiration and PreADMET study

Results of Molinspiration properties									
Code	**Properties**								
	mi LogP	**TPSA**	**n Atoms**	**MW**	**n OH**	**n OHNH**	**n Violations**	**n Rotb**	**Volume**
F40	3.46	69.37	24	345.79	6	0	0	4	292.81
F33	4.18	23.55	22	335.23	3	0	0	3	283.01
F07	4.08	29.54	21	332.19	3	0	0	3	262.65
F21	3.02	43.77	22	316.79	4	1	0	3	277.49
F39	3.29	111.96	24	347.71	8	0	0	4	270.24

Result of Biological activity						
Code	**GPCR ligand**	**Ion channel modulator**	**Kinase inhibitor**	**Nuclear receptor ligand**	**Protease inhibitor**	**Enzyme inhibitor**
F40	-0.38	-0.13	-0.19	-0.21	-0.23	-0.19
F33	-0.21	-0.37	-0.10	-0.24	-0.42	-0.18
F07	-0.28	-0.29	-0.24	-0.24	-0.28	-0.28
F21	-0.21	-0.21	-0.17	-0.38	-0.21	-0.34
F39	-0.24	-0.24	-0.06	-0.34	-0.42	-0.12

Result of ADME properties					
Properties	**F40**	**F33**	**F07**	**F21**	**F39**
BBB	0.24	0.47	0.23	0.36	0.26
CaCo2	17.76	56.66	54.94	35.22	17.42
HIA	98.58	100.00	100.00	96.12	93.07
MDCK	2.77	3.60	22.91	7.29	2.41
Plasma Protein Binding	94.37	92.18	96.94	88.32	94.02
Skin Permeability	-2.96	-2.84	-2.94	-3.15	-2.94

Molecular Docking Analysis

Molecular docking studies using Molegro Virtual Docker (MVD) evaluated the binding affinity of azetidine derivatives (F40, F33, F07, F21, and F39) against DPP-4 (PDB ID: 5Y7J). All compounds were successfully docked within the active site, with F40 exhibiting the lowest MolDock score (−125.29 kcal/mol), indicating the strongest binding affinity. The ligands formed key hydrogen bond interactions with amino acid residues such as Ser630, Ile107, Arg125, Lys463, Asp709, and Asn710, along with additional hydrophobic interactions that stabilized the complexes. These findings suggest favorable binding characteristics and support their potential as antidiabetic agents **Figure 3, Table 2.**

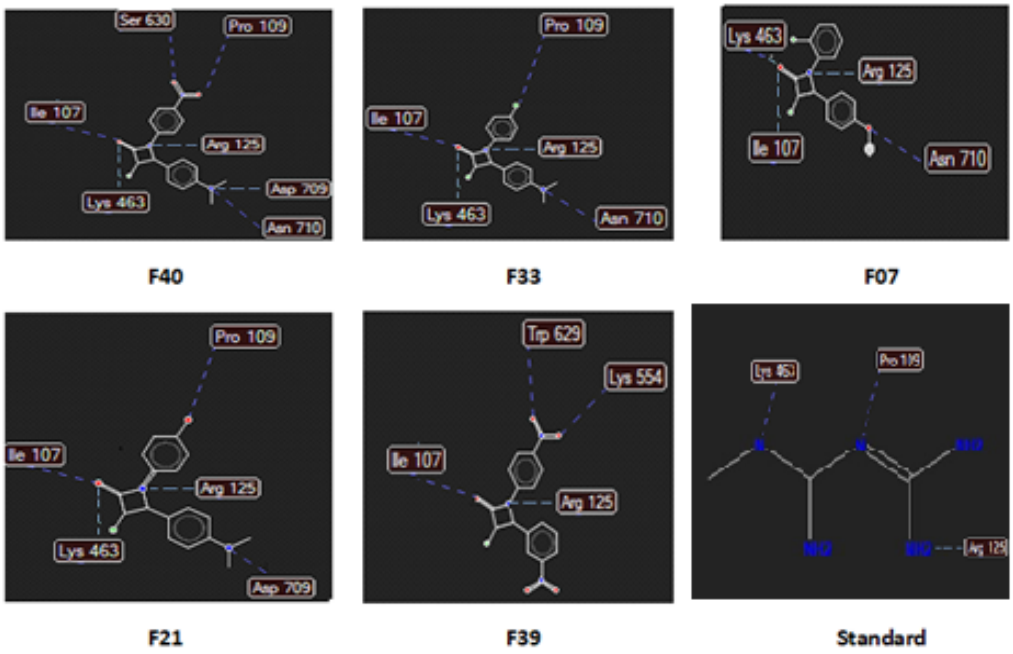


Figure 4: Hydrogen bond interaction of compound

Table 2: Results of docking analysis

Comp. Code	Mol Dock Score	H-bond Interaction	Interaction
F40	-125.297	-8.56409	Ser 630, Pro 109, lle107, Arg125, Lys 463, Asp 709, Asn710
F33	122.481	-7.138249	Pro109, lle107, Arg125, Lys 463, Asn710
F07	-120.327	-7.629504	Lys 463, Arg 125, lle107, Asn 710
F21	-116.385	-7.98494	Pro109, lle107, Arg125, Lys 463, Asp 709
F39	-114.728	-8.00885	lle107, Arg125, Lys 554, Arg125

Physicochemical Characterization

The physicochemical characterization of the synthesized azetidine derivatives (F40, F33, F07, F21, and F39) confirmed their successful synthesis and purity, as indicated by their crystalline nature and distinct coloration. The molecular weights (320.77–345.7 g/mol) fall within the acceptable range for drug-like molecules. Rf values (0.4–0.6) suggest moderate polarity, supporting favorable membrane permeability. The melting points (160–200 °C) indicate good thermal stability, with F07 showing the highest stability. Additionally, the presence of chloro substituents may enhance lipophilicity and metabolic stability. Overall, these properties support their suitability for further biological evaluation **Table 3**.

Table 3: Physical Data of synthesised compound

Comp. code	Mol. Formula	MW (g/mol)	Appearance	Rf value	Melting point
F40	$C_{16}H_{16}ClN_3O_3$	333.77	Brown crystalline solid	0.4	180 °C
F33	$C_{16}H_{15}Cl_2N_2O$	337.21	Brown crystalline solid	0.6	160 °
F07	$C_{16}H_{13}Cl_2NO_2$	338.19	Yellow to light orange crystalline solid	0.5	200 °
F21	$C_{16}H_{17}ClN_2O_2$	320.77	Brown crystalline solid	0.6	170 °C
F39	$C_{15}H_9ClN_3O_5$	345.7	Yellow to light orange crystalline solid	0.4	170 °C

Spectral Analysis of synthesized Compounds

F40 λ Max 222.00 nm, IR (KBr, cm-1) 1719.84 (β-lactam C=O), 1491.80 (Aromatic C=C), 720.93 (C-Cl stretch),1619.39 (NO2 group stretch), 1246.37 (C-N stretch),2628 (C-H stretch) .H1 NMR (CDCl3, 400MHz): δ – 7.989 (Ar NO2 group stretch), 6.984 (Ar-N(CH_3)$_2$ rings stretch), 2.892 (N(CH_3)$_2$ proton stretch), 5.490 (CHCl),C13 NMR (CDCl3 300MHz); δ – 160.79(C=O of β-lactam carbonyl group), 127.79 (C-C of Ar), 62.79 C-Cl stretch, 42.45 (N(CH_3)$_2$ stretch) LC/MS: m/z 345.1(M+) .

F33 λ Max 262.00 nm, IR (KBr, cm-1) 1723.14 (β-lactam C=O), 1481.15(Aromatic C=C), 719.96 (C-Cl stretch),1619.39 (NO2 group stretch), 1235.39 (C-N stretch),2576.57 (C-H stretch) .H1 NMR (CDCl3, 400MHz): δ –6.984 (Aromatic protons (two para-Substituted phenyls, 2.942 (N(CH_3)$_2$ proton stretch),

*Author for Correspondence: aayushipatud0@gmail.com

5.490 (CHCl),5.762 (CH_2 onβ-lactam ring stretch) ,C13 NMR (CDCl3 300MHz); δ – 155.16(C=O of β-lactam carbonyl group), 127.70 (C-C ofAr), 62.76 (C-Cl stretch), 41.28 ($N(CH_3)_2$ stretch) LC/MS: m/z 335.4(M+).

F07 λ Max 298.00 nm, IR (KBr, cm-1) 1787.90 (β-lactam C=O), 1491.16 (Aromatic C=C), 793.81 (C-Cl stretch), 1236.66 (C=O stretch),2948.91 (C-H stretch) .H1 NMR (CDCl3, 400MHz): δ – 7.148 (2 chloro phenyl stretch 6.984 (4 methoxyphenyl stretch), 5.490 (CHCl stretch),5.189(Other β-lactam CH stretch) C13 NMR (CDCl3 300MHz); δ – 160.19(C=O of β-lactam carbonyl group), 127.70 (C-C of Ar), 63.19 (C-Cl stretch), 76.76 (OCH_3 carbon stretch) LC/MS: m/z 322.4(M+)

F21 λ Max 308.00 nm, IR (KBr, cm-1) 1786.06 (β-lactam C=O), 1531.58 (Aromatic C=C), 743.67 (C-Cl stretch), 3415.86 (OH stretch),1308.46 (C-N stretch), 2949.99 (N(CH3)2 C-H stretch).H1 NMR (CDCl3, 400MHz): δ – 7.346 (Aromatic proton), 6.9845(OH stretch), 5.772 (CHCl stretch),5.090(Other β-lactam CH stretch) C13 NMR (CDCl3 300MHz); δ – 154.59(C=O of β-lactam carbonyl group), 133.89(C-C of Ar), 63.26 (C-Cl stretch), 134.28 (Phenolic carbon stretch),40.28 ($N(CH_3)_2$) LC/MS: m/z 316.2(M+)

F39 λ Max 273.00 nm, IR (KBr, cm-1) 1787.84 (β-lactam C=O), 1527.68[NO2 (asymmetric)] ,1321.61 (NO2 (symmetric)),1595.08 (Aromatic C=C stretch), 701.22 (C-Cl stretch),.H1 NMR (CDCl3, 400MHz): δ – 7.945 (4 Nitrophenyl stretch), 7.964 (3 nitrophenyl stretch), 5.168 (CHCl stretch),5.154(Other β-lactam CH stretch) C13 NMR (CDCl3 300MHz); δ – 172.06(C=O of β-lactam carbonyl group), 132.62 (C-C of Ar), 62.76 (C-Cl stretch),LC/MS: m/z 347.4(M+)

Anti-diabetic activity

Fasting Blood Glucose (FBG)

Diabetic rats showed persistently high glucose (≥ 320 mg/dL). Metformin and test compounds significantly ($p < 0.01$) reduced FBG. Compound 40 showed maximum effect, approaching near-normal levels by day 21 **Figure 5**.

Oral Glucose Tolerance Test (OGTT)

Diabetic rats exhibited impaired glucose clearance. Treatment with Metformin and test compounds improved glucose tolerance, with compound 40 showing results comparable to the standard **Figure 6**.

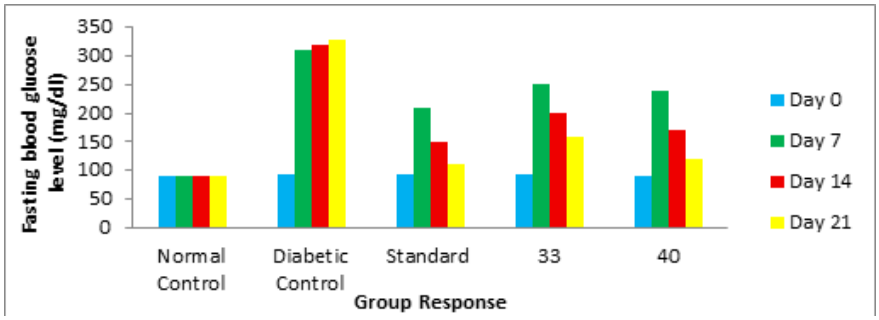


Figure 5: Result of FBG Levels (mg/dL)

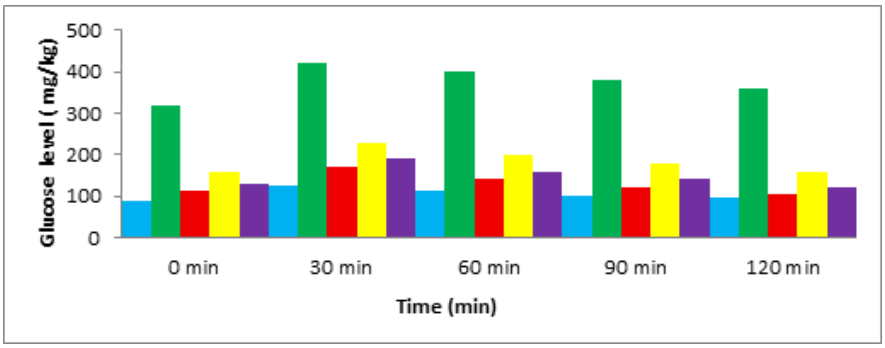


Figure 6: Result of OGTT (mg/dL) on Day 21

Serum Biochemical Parameters (Day 28)

- Diabetic rats showed decreased insulin (6.2 µIU/mL), increased TC, TG, LDL, and reduced HDL.
- Compound 40 significantly improved insulin (12.5 µIU/mL) and normalized lipid profile, close to standard treatment **Figure 7**.

Acute Toxicity Study

No mortality was observed up to 1000 mg/kg. Mild reversible signs (lethargy, salivation) appeared at higher doses. Both compounds were considered safe at therapeutic doses.

Result of DPP-4 Enzyme Inhibition Assay

The synthesized compounds exhibited concentration-dependent inhibition of DPP-4 enzyme activity. Among the tested derivatives, compound F40 demonstrated the most potent inhibitory activity, with the lowest IC_{50} value, followed by F33 and F07. The enhanced activity of F40 may be attributed to favorable interactions within the DPP-4 active site, as observed in molecular docking

studies. The correlation between docking scores and enzyme inhibition results supports the reliability of the computational predictions. Overall, the findings suggest that the azetidine scaffold plays a significant role in DPP-4 inhibition and may serve as a promising lead for antidiabetic drug development **Table 4**.

Result of GLP-1 Estimation Study

The treatment with azetidine derivatives resulted in a significant increase in serum GLP-1 levels compared to the diabetic control group. Notably, compound F40 exhibited the highest elevation in GLP-1 concentration, comparable to the standard drug. This increase suggests effective inhibition of DPP-4 activity, thereby preventing GLP-1 degradation and enhancing insulin secretion. The results are in agreement with the in silico docking and in vitro enzyme inhibition findings, confirming that the antidiabetic activity of the synthesized compounds is mediated, at least in part, through the DPP-4/GLP-1 pathway.

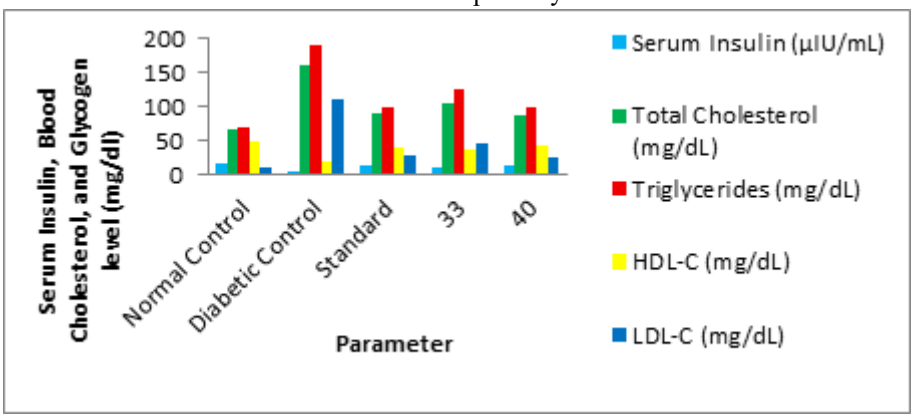


Figure 7: Result of Serum Biochemical Parameters (Day 21)

Table 3: DDP-4 inhibitory activity of synthesized compounds

Compound Code	IC_{50} (µM)
F40	18.5
F33	25.3
F07	32.1
F21	40.7
F39	45.2
Standard (Sitagliptin)	12.0

DISCUSSION

Test compounds, particularly compound 40, significantly reduced blood glucose, improved glucose tolerance, increased serum insulin, and normalized lipid parameters in diabetic rats. Toxicity studies confirmed safety up to 1000 mg/kg. These findings suggest compound 40 has potent antidiabetic potential comparable to metformin.

CONCLUSION

In this study, a series of azetidine derivatives were synthesized and evaluated for their antidiabetic potential. Based on physicochemical properties, molecular docking, and in-silico prediction studies, five derivatives (F40, F33, F07, F21, and F39) were successfully synthesized. The compounds were characterized using IR spectroscopy, NMR, and mass spectrometry. Ligand-binding interactions of the synthesized analogues with the target protein were analyzed through molecular docking studies.Docking results demonstrated good to moderate interactions between the compounds and the protein receptor 5Y7J, involving hydrogen bond and steric bond interactions. Among the tested compounds, F40 exhibited the highest binding affinity, with a binding energy of -8.56409 kcal/mol, which was superior to the standard drug Metformin (-6.01594 kcal/mol).The antidiabetic effects of all compounds ranged from moderate to excellent. Test compound 40 exhibits potent antidiabetic activity, significantly improving glycemic control and lipid profile in diabetic rats, with an efficacy nearly equivalent to metformin. Additionally, it possesses a favorable safety profile with no lethal or severe toxic effects observed up to 1000 mg/kg. These findings suggest that compound 40 holds substantial promise as a potential therapeutic agent for managing Type 2 Diabetes.Our findings suggest that the azetidine scaffold is a promising pharmacophore for the development of antidiabetic agents. Further QSAR studies are required to elucidate the essential structural features responsible for activity.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the GRY Institute ofPharmacy, Borawan, for providing the research facilitiesNMR center instrument facility IISERBhopal, for IR ,NMR and Mass Spectroscopy.

CONFLICT OF INTEREST

The authors declare no conflict of interest

REFERENCES

1. J.T. Lowe, M.D. Lee IV, L.B. Akella, E. Davoine, E.J. Donckele, L. Durak, L.A. Marcaurelle, "Synthesis andprofiling of a diverse collection of azetidine-based scaffolds for the development of CNS-focused lead-like libraries" The Journal of Organic Chemistry **77**, (2012): 7187.
2. M. Han, C. Song, N. Jeong, H.G. Hahn, "Exploration of 3-aminoazetidines as triple reuptake inhibitors by bioisosteric modification of 3-α-oxyazetidine" ACS Medicinal Chemistry Letters **5**, (2014): 999.
3. D.R. Parmar, J.Y. Soni, R. Guduru, R.H. Rayani, R.V. Kusurkar, A.G. Vala, "Azetidines of pharmacological interest" Archiv der Pharmazie **354**, (2021): 2100062.
4. A. Haredi Abdelmonsef, M. Eldeeb Mohame, M. El-Naggar, H. Temairk, A. Mohamed Mosallam, "Novel quinazolin-2,4-dione hybrid molecules as possible inhibitors against malaria: synthesis and in silico molecular docking studies" Frontiers in Molecular Biosciences **7**, (2020): 105.
5. D. Ding, J.R. Nickell, A.G. Deaciuc, N.R. Penthala, L.P. Dwoskin, P.A. Crooks, "Synthesis and evaluation of novel azetidine analogs as potent inhibitors of vesicular [3H] dopamine uptake" Bioorganic & Medicinal Chemistry **21**, (2013): 6771.
6. A. Fawcett, A. Murtaza, C.H. Gregson, V.K. Aggarwal, "Strain-release-driven homologation of boronic esters: application to the modular synthesis of azetidines" Journal of the American Chemical Society **141**, (2019): 4573.
7. S.C. Schmid, I.A. Guzei, J.M. Schomaker, "A stereoselective [3+1] ring expansion for the synthesis of highly substituted methylene azetidines" Angewandte Chemie International Edition **56**, (2017): 12229.
8. E.A. Kim, C.H. Cho, J. Kim, H.G. Hahn, S.Y. Choi, S.J. Yang, S.W. Cho, "The azetidine derivative KHG26792 protects against ATP-induced activation of NFAT and MAPK pathways through P2X7 receptor in microglia" Neurotoxicology **51**, (2015): 198.
9. F. Couty, B. Drouillat, G. Evano, O. David, "2-Cyanoazetidines and azetidinium ions: scaffolds for molecular diversity" European Journal of Organic Chemistry (2013): 2045.
10. M. Andresini, S. De Angelis, A. Uricchio, A. Visaggio, G. Romanazzi, F. Ciriaco, N. Corriero, L. Degennaro, R. Luisi, "Azetidine–borane complexes: synthesis, reactivity, and stereoselective functionalization" The Journal of Organic Chemistry **83**, (2018): 10221.
11. B. Chandrakar, A. Jain, S. Roy, V.R. Gutlapalli, S. Saraf, A. Suppahia, A. Verma, A. Tiwari, M. Yadav, A. Nayarisseri, "Molecular modeling of acetyl-CoA carboxylase (ACC) from Jatropha curcas and virtual screening for identification of inhibitors" Journal of Pharmaceutical Research **6**, (2013): 913–918.
12. D. Ashok, M.G. Devulapally, V.K. Aamate, S. Gundu, S. Adam, S. Murthy, S. Balasubramanian, B. Naveen, T. Parthasarathy, "Novel pyrano[3,2-b]xanthen-7(2H)-ones: synthesis, antimicrobial, antioxidant and molecular docking studies" Journal of Molecular Structure **1177**, (2019): 215–228.
13. S.S. Shah, D. Shah, I. Khan, S. Ahmad, U. Ali, A. Rahman, "Synthesis and antioxidant activities of Schiff bases and their complexes: an updated review" Biointerface Research in Applied Chemistry **10**, (2020): 6936–6963.
14. V. Vani, S. Sreeja, N. Fathimathul, A. Harshad, M.M. Mahima Krishnan, N. Naisam, S. Sneha, "In-silico design of thiadiazole derivatives as anticonvulsant agents" Asian Journal of Pharmaceutical Research and Development **12**, (2024): 85–91.
15. S. Liu, L. Ma, X. Ren, W. Zhang, D. Shi, Y. Huo, Y. Ba, Y. Bai, N. Cheng, "A new model of type 2 diabetes mellitus induced by high-fat diet and streptozotocin" Life Sciences **286**, (2021): 120062.
16. E.H. Attrill, O. Scharapow, S. Perera, et al., "Controlled induction of type 2 diabetes using high-fat diet and streptozotocin in rodents" Scientific Reports **15**, (2025): 8812.
17. H. Li, Y. Wang, W. Zheng, et al., "High-fat diet–induced metabolic dysfunction and its role in diabetes progression" Lipids in Health and Disease **24**, (2025): 313.
18. Y. Zhang, X. Liu, J. Wang, et al., "Discovery of novel DPP-4 inhibitors: design, synthesis, biological evaluation, and molecular docking studies" Bioorganic Chemistry **115**, (2021): 105205.
19. H. Patel, P. Patel, S. Shah, et al., "Design, synthesis, and evaluation of new antidiabetic agents as DPP-4 inhibitors" Journal of Molecular Structure **1265**, (2022): 133401.
20. R. Kaur, S. Arora, B. Singh, et al., "Synthesis and biological evaluation of heterocyclic derivatives as potent DPP-4 inhibitors" European Journal of Medicinal Chemistry **245**, (2023): 114898.
21. A. Sharma, R. Gupta, S. Kumar, et al., "DPP-4 inhibition and GLP-1 enhancement: a promising strategy for type 2 diabetes management" Life Sciences **280**, (2021): 119705.
22. N. Li, H. Zhou, H. Wu, et al., "Role of GLP-1 in glucose homeostasis and therapeutic implications in diabetes" Frontiers in Endocrinology **13**, (2022): 897845.
23. V. Singh, P. Bansal, G. Kaur, et al., "Mechanistic insights into antidiabetic activity of novel compounds

targeting DPP-4/GLP-1 pathway" Biomedicine & Pharmacotherapy **170**, (2024): 115994.

24. K. Raval et al., "Basics, types and applications of molecular docking: a review" IP International Journal of Comprehensive and Advanced Pharmacology **7**, (2022): 12–16.
25. O.S. Hashim et al., "Synthesis, characterization of azetidine derivative and studying the antioxidant activity" Journal of Medicinal and Chemical Sciences **6**, (2023): 553–558.
26. M. Patidar, R. Dubey, S. Minz, M. Pradhan, N. Deshmukh, "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" Journal of Applied Pharmaceutical Research **13**, (2025): 194–203.
27. M. Patidar, R. Dubey, N. Deshmukh, "Exploring 1,3,4-oxadiazole derivatives for hepatocellular carcinoma: synthesis and bioactivity evaluation" Journal of Applied Pharmaceutical Research **13**, (2025): 127–138.