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Exploration of Antidiabetic Activity in Novel Benzimidazole Schiff Bases: Design, Synthesis, and Biological Evaluation

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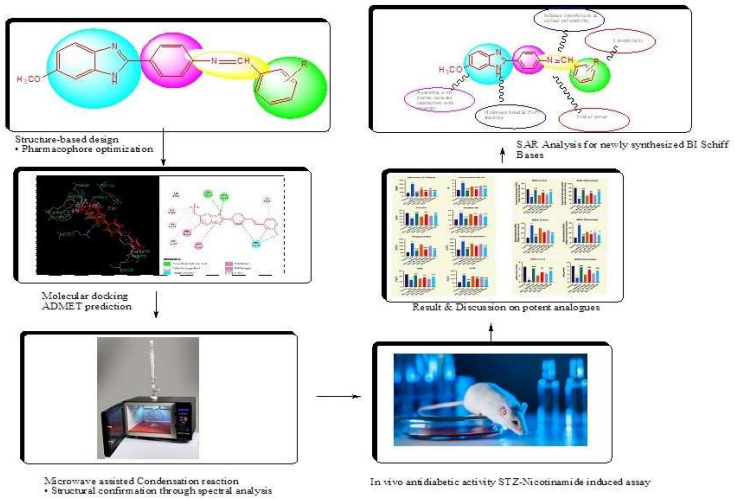
GRAPHICAL ABSTRACT



ABSTRACT

The escalating global prevalence of diabetes mellitus necessitates the development of novel therapeutic agents with enhanced efficacy and improved safety profiles. This study presents the rational design, synthesis, and comprehensive biological evaluation of a series of benzimidazole-based Schiff base derivatives as potential antidiabetic agents. Benzimidazole scaffolds have demonstrated significant pharmacological versatility, while Schiff bases are

recognized for their diverse biological activities and structural flexibility. The strategic combination of these pharmacophores was hypothesized to yield compounds with superior antidiabetic properties.
A series of novel benzimidazole Schiff base derivatives were synthesized through conventional condensation reactions followed by microwave assisted synthesis between substituted benzimidazole amine and various aromatic aldehyde. The structures of synthesized compounds were confirmed using spectroscopic techniques including ^{1}H NMR, ^{13}C NMR, IR, and mass spectrometry. Molecular docking studies were performed to elucidate potential binding interactions with key diabetic targets. Auto Dock Tools 1.5.4 (ADT) was utilized for preparing the docking input files. Docking studies specifically highlighted compounds 5h,5f & 5i as potential templates for developing more effective antidiabetic agents.
Further evaluation of the antidiabetic activity of these compounds was conducted in vivo using a type II diabetes model induced by Streptozotocin (STZ) and Nicotinamide (NA) in rats. The in vivo tests demonstrated that treatment with 5h (30 mg/kg body weight) significantly reduced fasting blood glucose levels, while 5f (30 mg/kg body weight) showed moderate reduction compared to the diabetic control group. Moreover, treatment with 5h and 5f dose-dependently decreased oxidative stress markers such as lipid peroxidation (MDA) and increased antioxidant enzyme superoxide dismutase (SOD) and glutathione (GSH) levels in the liver and pancreas. These findings establish benzimidazole Schiff bases as promising lead compounds for antidiabetic drug development, warranting further optimization and in vivo validation studies to advance their therapeutic potential.
KEYWORDS: Multicomponent synthesis, Benzimidazole Schiff bases, Antidiabetic, In silico docking, Streptozotocin (STZ) and Nicotinamide (NA) model.

1. INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder characterized by persistent hyperglycemia, represents one of the most pressing global health challenges with approximately 537 million adults affected worldwide in 2021, projected to reach 783 million by 2045 [1]. Type 2 diabetes mellitus (T2DM) accounts for 90-95% of cases, primarily characterized by insulin resistance and progressive β-cell dysfunction involving impaired glucose uptake, excessive hepatic glucose production, and defective insulin secretion [2,3].
Current therapeutic approaches include lifestyle modifications, insulin therapy, and oral antidiabetic agents such as metformin, sulfonylureas, thiazolidinediones, and α-glucosidase inhibitors [4]. However, existing treatments are often limited by adverse effects, drug resistance, and inadequate glycemic control [5]. α-Glucosidase inhibitors like acarbose and miglitol work by inhibiting carbohydrate-digesting enzymes but are limited by gastrointestinal side effects and modest efficacy [6,7].
The search for novel antidiabetic agents has led to increased interest in heterocyclic compounds, particularly benzimidazole derivatives [8]. Benzimidazole, a bicyclic heterocycle with fused benzene and imidazole rings, exhibits diverse pharmacological properties and is present in clinically approved drugs including proton pump inhibitors, anthelmintics, and antivirals [9,10]. Recent studies demonstrate significant antidiabetic potential through multiple mechanisms including α-glucosidase inhibition, α-amylase inhibition, and glucose uptake enhancement [11-15].
Schiff bases, characterized by azomethine groups (C=N), represent another important class of bioactive compounds with antimicrobial, anticancer, anti-inflammatory, and antidiabetic properties [16-19]. The molecular hybridization strategy, combining pharmacophoric units from different bioactive molecules, has emerged as a powerful drug design approach offering improved efficacy, reduced toxicity, and multi-target activity [20-25].
Benzimidazole-Schiff base hybrids represent promising antidiabetic agents through synergistic effects of both pharmacophores [26-32]. These compounds demonstrate significant inhibitory activity against carbohydrate-digesting enzymes, with some derivatives showing superior activity compared to standard drugs [33-35]. Recent

computational advances including molecular docking and QSAR modeling have facilitated rational design and accelerated drug discovery [36-39].

In vitro and in vivo studies have demonstrated potent enzyme inhibitory activity with IC50 values in the low micromolar range and significant reductions in blood glucose levels in diabetic animal models [40-48]. The synthetic accessibility, favorable pharmacokinetic properties, and generally low toxicity profiles support their potential for clinical development [49-54].

Given the urgent need for novel antidiabetic agents with improved therapeutic profiles, this study aims to design and synthesize novel benzimidazole Schiff base hybrids and evaluate their antidiabetic potential through integrated in silico molecular docking and in vivo biological assessment using STZ-nicotinamide induced diabetic rat models to identify promising candidates with significant therapeutic efficacy [55-61].

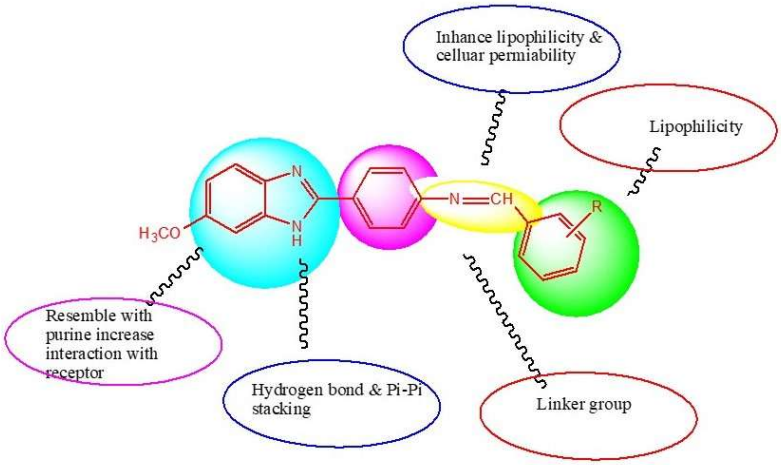


Figure 1 Structural features of designed Benzimidazole Schiff base

2 EXPERIMENTAL:

Laboratory-grade chemicals for synthesis were procured from TCI Pvt. Ltd. (Chennai, India), SD Fine Chemicals Pvt. Ltd. (Mumbai, India), Avra Synthesis Pvt. Ltd. (Hyderabad, India), and Fisher Scientific (Mumbai, India), while streptozotocin and nicotinamide were purchased from Sisco Research Laboratories Pvt. Ltd. (Taloja, India); all chemicals were used without further purification. AutoDock Tools 1.5.4 (ADT) was used for docking input file preparation [62]. Melting points were determined using a Digital Melting Point Apparatus with open capillary tubes, while FTIR, ^{1}H NMR, and ^{13}C NMR spectra were recorded on a Bruker-NMR 500 MHz spectrometer at SAIF Punjab. Mass spectra were analyzed using Shimadzu GC-MS QP 5000 mass spectrometer at SAIF Punjab, and reaction progress was monitored by TLC on silica gel F254 aluminum plates (E. Merck, Germany) using methanol, ethyl acetate, and n-hexane as mobile phases with iodine vapor visualization.

2.1 Procedure for synthesis of Benzimidazole Schiff bases

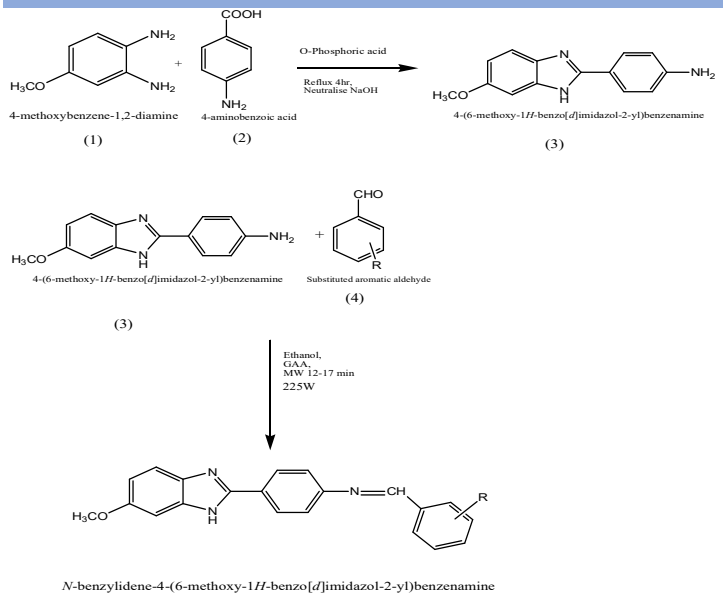
The N-benzylidene-4-(6-methoxy-1H-benzo[d]imidazol-2-yl)benzenamine derivatives 5(a–j) were synthesized as summarized in (Scheme 1) 4-(6-methoxy-1H-benzo[d]imidazol-2-yl)benzenamine (3) was synthesized by a simple condensation reaction in presence of ortho phosphoric acid the 4-methoxy ortho phenylene diamine (1) & para amino benzoic acid (2) were refluxed for four hours at 180 ℃ using sand bath after four hours partially cool the reaction mixture upto 50 ℃ then pour into crushed ice & neutralize it with 10% NaOH solution, collect the precipitate by vacuum filtration & wash excess of NaOH with ice cold water & recrystallized the pure product 4-(6-methoxy-1H-benzo[d]imidazol-2-yl) benzenamine (3) (Yield 83%, M P 237-240 ℃). [46]

N-benzylidene-4-(6-methoxy-1H-benzo[d]imidazol-2-yl) benzenamine derivatives 5(a–j) were synthesized by reacting a mixture of 4-(6-methoxy-1H-benzo[d]imidazol-2-yl) benzenamine (3) with various substituted aromatic

aldehyde (4a-4j) by using ethanol as solvent & few drops of glacial acetic acid as a catalyst this mixture was irradiated in microwave synthesizer for 12 to 17 min at 225w till completion of reaction indicated on TLC. The reaction mixture was cooled to room temperature and poured on ice-water (50 ml) to get the precipitated solid. It was collected by filtration, washed with water and dried to give the corresponding benzimidazole Schiff bases derivatives. All the synthesized compounds were characterized and confirmed by spectral analysis like; 1H-NMR, 13C-NMR, MS. The purity of the synthesized compounds was determined by thin layer chromatography (TLC) (Ethyl acetate & n-Hexane 3:2). The melting points were determined in open capillary tubes by using digital melting point apparatus and are uncorrected. Physical constants data and the time required for completion of reactions for the N-benzylidene-4-(6-methoxy-1H-benzo[d]imidazol-2-yl) benzenamine derivatives 5(a–j) are summarized in Table 1

Table 1 Physical constants data and the time required for completion of reactions for the Benzimidazole Schiff bases 5(a–j)

Sr No	Derivative	Aldehyde	Molecular formula	Molecular weight	Time & Temperature	Yield	M. P
1	5a	4-Hydroxybenzaldehyde	$C_{21}H_{17}N_3O_2$	343	16min	87 %	284
2	5b	4-Nitrobenzaldehyde	$C_{21}H_{16}N_4O_3$	372	15min	82%	268
3	5c	3,4-dihydroxybenzaldehyde	$C_{21}H_{17}N_3O_3$	359	17min	70 %	292
4	5d	3-Nitrobenzaldehyde	$C_{21}H_{16}N_4O_3$	372	18min	84%	242
5	5e	4-Methylbenzaldehyde	$C_{22}H_{19}N_3O$	341	15min	73%	278
6	5f	4-Dimethylaminobenzaldehyde	$C_{23}H_{22}N_4O$	370	18min	55%	251
7	5g	2,5-Dimethoxybenzaldehyde	$C_{23}H_{21}N_3O_3$	387	16min	65%	274
8	5h	3-fluorobenzaldehyde	$C_{21}H_{16\ F}N_3O$	345	15min	75 %	286
9	5i	2-Hydroxyl-1-naphthaldehyde	$C_{25}H_{19}N_3O_2$	393	17min	60%	292
10	5j	3-Cynobenzaldehyde	$C_{22}H_{16}N_4O$	352	16min	68%	296



Scheme 1for synthesis of Benzimidazole Schiff bases

2.2 Molecular Docking Studies

Protein Structure Preparation Protein structures were prepared using AutoDock Tools version 1.5.4 (ADT) with removal of water molecules and ionic species, addition of polar hydrogen atoms, and calculation of partial atomic charges using Kollman united atom charge methodology [62-64]. Protonation states were determined using PROPKA 2.0 software, maintaining positively charged residues (lysine, arginine, histidine) in protonated forms and negatively charged residues (glutamic acid, aspartic acid) in deprotonated states [65].

Ligand Preparation Nonpolar hydrogen atoms were merged with heavy atoms, atomic partial charges assigned using Gasteiger method, and rotatable bonds configured for conformational flexibility. All structures were converted to PDBQT format for AutoDock calculations.

Grid Generation and Docking Parameters A cubic search space of 40×40×40 Å was established around the enzyme's active site with 1.0 Å grid point spacing, centered at the co-crystallized ligand's centroid coordinates. All AutoDock Vina parameters were maintained at default settings for standardized docking conditions.

2.3 Experimental Animals

All animal experiments were conducted following institutional ethical guidelines and approved by the Institutional Animal Ethics Committee (IAEC) in accordance with CPCSEA norms, Government of India (Protocol No: CBLRC/IAE/01/02-2022). Male Wistar rats (11-12 weeks old, 180-220 g) and female Swiss albino mice (25-30 g) were obtained from Cape Bio Lab & Research Centre, Marthandam, TN, India and randomly divided into groups (n=6). Animals were housed under standard laboratory conditions (12 h light/dark cycle, 22±2°C temperature, 50-60% humidity) with free access to standard chow and water for one week acclimatization period.

2.4 Acute toxicity studies

Acute oral toxicity studies were performed as per the OECD (Economic Cooperation and Development Organization) part 423 of the Guidelines [66].

2.5 In vivo anti-diabetic activity studies

2.5.1 Induction of diabetes mellitus

Type II diabetes mellitus was induced by a single intraperitoneal (i. p.) dose of 60 mg/kg of freshly prepared Streptozotocin (STZ) in citrate buffer (pH 4.5) in male rats after 30 min of intraperitoneal (i. p.) administration of 110 mg/kg of Nicotinamide (NA) in regular saline solution. The hypoglycemic shock triggered by Streptozotocin

was stopped by the injection of 5% glucose solution to the animals for 24 h following treatment with STZ [67].

2.5.2 Experimental design

Forty-two adult male Wistar rats were divided into seven groups of six animals each and fasted overnight before treatment initiation. All groups received oral administration twice daily for 21 days in volumes less than 1 ml of 0.5% carboxymethylcellulose (CMC) as follows: Group I (Normal control) - healthy rats receiving 0.5% CMC; Group II (Diabetic control) - diabetic rats receiving 0.5% CMC; Group III (Standard) - diabetic rats receiving metformin 100 mg/kg in 0.5% CMC; Group IV (5h Low) - diabetic rats receiving compound 5h at 15 mg/kg in 0.5% CMC; Group V (5h High) - diabetic rats receiving compound 5h at 30 mg/kg in 0.5% CMC; Group VI (5f Low) - diabetic rats receiving compound 5f at 15 mg/kg in 0.5% CMC; Group VII (5f High) - diabetic rats receiving compound 5f at 30 mg/kg in 0.5% CMC.

2.5.3 Collection of blood and tissues samples

On day 21, blood samples were collected from the carotid artery and stored at -80°C. Animals were sacrificed by CO_2 asphyxiation, and pancreas and liver tissues were homogenized for antioxidant activity.

2.5.4 Plasma glucose, insulin, insulin resistance and lipid profiles Plasma glucose, triacylglycerol (TG), total cholesterol (TC), LDL-C, and HDL-C concentrations were determined using commercial assay kits (Bio systems, Costa Brava, Barcelona, Spain) on a semi-automated analytical system (Optima-S, LABINDIA Healthcare Pvt. Ltd., Gurgaon, Haryana, India). Plasma insulin was measured using radioimmunoassay technique with RIA kit (BI-INSULIN IRMA, Cis Bio-International, Gif-Sur-Yvette, France), and HOMA-IR calculated using the formula: HOMA-IR = (fasting plasma glucose × plasma insulin)/405.

2.6 Determination of oxidative stress-related enzymes in the pancreas and liver Oxidative stress-related enzymes were determined using standard spectrophotometric methods: MDA assessment [68] involved mixing tissue homogenate with trichloroacetic acid and thiobarbituric acid, heating at 90°C for 30 minutes, and measuring absorbance at 532 nm; SOD activity [69,70] was evaluated by monitoring absorbance changes at 420 nm after mixing homogenate with pyrogallol in Tris-HCl buffer; GSH levels [71,72] were quantified by homogenizing tissue in sulfosalicylic acid, treating with TCA, centrifuging, and measuring absorbance at 412 nm with phosphate buffer and DTNB. All parameters were expressed per gram wet tissue using respective molar extinction coefficients

2.7 *Statistical analysis*

Data are presented as Mean ± SEM. Statistical differences between groups were evaluated using one-way analysis of variance (ANOVA) followed by Newman-Keuls post-hoc test via GraphPad Prism 7 software. Statistical significance was defined as $p<0.05$.

3. *Results & Discussion*

3.1 Chemistry

The target benzimidazole Schiff base derivatives (5a-5j) were successfully synthesized through a microwave-assisted condensation reaction between 2-substituted benzimidazole derivatives and various aromatic aldehydes. The synthetic pathway employed a one-pot methodology using solvent ethanol & catalyst Glacial acetic acid, yielding the desired compounds in excellent yields ranging from 65-90% within 12-17 minutes of microwave irradiation at 80°C at 225 W.

The microwave-assisted synthesis demonstrated significant advantages over conventional heating methods, including reduced reaction times (from 6-8 hours to 12-17 minutes), improved yields, and enhanced product purity. The structural elucidation of synthesized compounds was accomplished through comprehensive spectroscopic analysis including IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry.

The systematic variation of substituents on both benzimidazole and aldehyde moieties provided valuable insights into structure-activity relationships figure 2 reveals the several critical structural features essential for optimum biological activity.

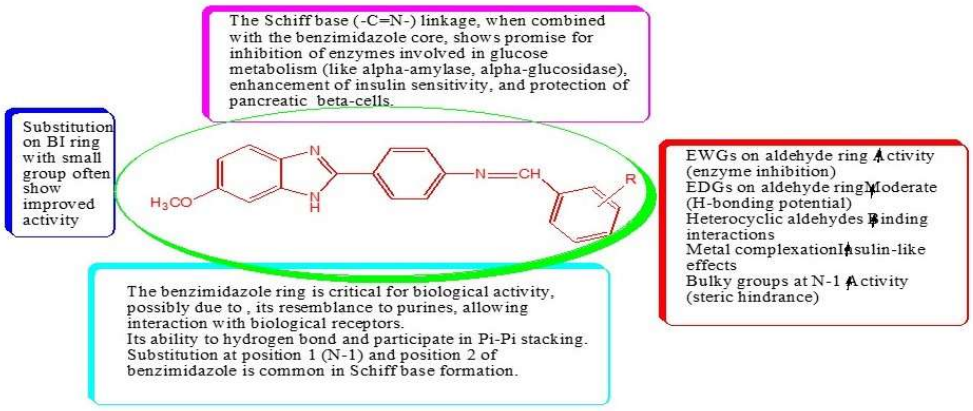


Figure 2 SAR analysis of Benzimidazole Schiff Bases analogues revealed several critical structural features essential for optimal biological activity

Spectral Data

4-((4-(6-methoxy-1H-benzo[d]imidazol-2-yl)phenylimino)methyl)phenol (5a) obtain as yellow colour solid in 87% yield, M. P. 284-286 ° C, ^{1}HNMR (Bruker 500MHz, CDCl3) 6.7 CH –benzimidazole,7.21 CH –benzimidazole,7.59 CH –Benzimidazole,5.21-NH –benzimidazole,7.48 CH –benzene,7 .48 CH –benzene,7.32 CH –benzene,7.32 CH –Benzene,8.32-CH – benzylidenimin,7.48 CH – benzylidenimin,7.21 CH- benzylidenimin,7.10-CH- benzylidenimin, 7.21 – CH - benzylidenimin,5.0-OH,3.73 – CH3. C13NMR, 109.5,156.2,100.9,139.1,132.116.8,153.1,129.5,128.3,122.8,153.3,122.5,128.5,160.3,126.8,130.3,116.5,160.9,116.4,130.3, 55.6 Mass spectrum E/Z $[M+H]^{+}$- $C_{21}H_{17}N_3O_2$
Calculated – 343.24, found – 344.56

N-(4-nitrobenzylidene)-4-(6-methoxy-1H-benzo[d]imidazol-2-yl)benzenamine 5b obtain as yellow colour solid in 82% yield, M. P. 268-270 ° C, ^{1}HNMR (Bruker 500MHz, CDCl3) 6.7 CH –benzimidazole,7.21 CH –benzimidazole,7.59 CH –Benzimidazole,5.21-NH –benzimidazole,7.48 CH –benzene,7 .48 CH –benzene,7.32 CH –benzene,7.32 CH –Benzene,8.32-CH – benzylidenimin,7.48 CH – benzylidenimin,7.21 86- benzylidenimin,7.10-CH- benzylidenimin, 7.86 – CH - benzylidenimin,,3.76 – CH3. C13NMR, 108.5,156.2,100.9,139.1,132.117.8,153.1,129.5,128.3,122.8,153.3,122.5,128.5,160.3,126.8,130.3,116.5,160.9,116.4,130.3, 55.4 Mass spectrum E/Z $[M+H]^{+}$- $C_{21}H_{16}N_4O_3$Calculated – 372.14, found – 373.56

4-((4-(6-methoxy-1H-benzo[d]imidazol-2-yl)phenylimino)methyl)benzene-1,2-diol (5c) obtain as yellow colour solid in 70% yield, M. P. 292-294 ° C, ^{1}HNMR (Bruker 500MHz, CDCl3) 6.7 CH –benzimidazole,7.21 CH –benzimidazole,7.59 CH –Benzimidazole,5.21-NH –benzimidazole,7.48 CH –benzene,7 .48 CH –benzene,7.32 CH –benzene,7.32 CH –Benzene,8.32-CH – benzylidenimin,7.48 CH – benzylidenimin,7.21 CH- benzylidenimin,7.10-CH- benzylidenimin, 7.21 – CH - benzylidenimin,5.0-OH,5.01 – OH, 3.73 – CH3. C13NMR,
109.5,156.2,100.9,139.1,132.116.8,153.1,129.5,128.3,122.8,154.3,121.5,128.5,160.3,126.8,130.3,116.5,160.9,116.4,130.3, 55.9 Mass spectrum E/Z $[M+H]^{+}$- $C_{21}H_{17}N_3O_3$
Calculated – 359.24, found – 360.56

N-(3-nitrobenzylidene)-4-(6-methoxy-1H-benzo[d]imidazol-2-yl)benzenamine (5d) obtain as yellow colour solid in 84% yield, M. P. 242-244 ° C, ^{1}HNMR (Bruker 500MHz, CDCl3) 6.7 CH –benzimidazole,7.21 CH –benzimidazole,7.59 CH –Benzimidazole,5.21-NH –benzimidazole,7.48 CH –benzene,7 .48 CH –benzene,7.32 CH –benzene,7.32 CH –Benzene,8.32-CH – benzylidenimin,7.48 CH – benzylidenimin,7.21 86- benzylidenimin,7.10-CH- benzylidenimin, 7.86 – CH - benzylidenimin,,3.76 – CH3. C13NMR, 108.5,156.2,100.9,139.1,132.117.8,153.1,129.5,128.3,122.8,153.3,122.5,128.5,160.3,126.8,130.3,116.5,160.9,116.

4,130.3, 55.4 Mass spectrum E/Z $[M+H]^+$- $C_{21}H_{16}N_4O_3$Calculated – 372.14, found – 373.87

N-(4-methylbenzylidene)-4-(6-methoxy-1H-benzo[d]imidazol-2-yl)benzenamine 5e obtain as yellow colour solid in 73% yield, M. P. 278-280 ° C, ^{1}HNMR (Bruker 500MHz, CDCl3) 6.7 CH –benzimidazole,7.21 CH –benzimidazole,7.59 CH –Benzimidazole,5.21-NH –benzimidazole,7.48 CH –benzene,7 .48 CH –benzene,7.32 CH –benzene,7.32 CH –Benzene,8.32-CH – benzylidenimin,7.48 CH – benzylidenimin,7.21 86- benzylidenimin,7.10-CH- benzylidenimin, 7.86 – CH - benzylidenimin,,3.76 – CH_3 2.35-CH_3. C13NMR, 108.5,156.2,100.9,139.1,132.117.8,153.1,129.5,128.3,122.8,153.3,122.5,128.5,160.3,126.8,130.3,116.5,160.9,116. 4,130.3, 55.4,24.3 Mass spectrum E/Z $[M+H]^+$- $C_{22}H_{19}N_3O$ Calculated – 343, found – 344.26

N-(4-dimethylaminobenzylidene)-4-(6-methoxy-1H-benzo[d]imidazol-2-yl) benzenamine (5f)

obtain as yellow colour solid in 55% yield, M. P. 251-252 ° C, 1HNMR (Bruker 500MHz, CDCl3) 6.7 CH –benzimidazole,7.21 CH –benzimidazole,7.59 CH –Benzimidazole,5.21-NH –benzimidazole,7.48 CH –benzene,7 .48 CH –benzene,7.32 CH –benzene,7.32 CH –Benzene,8.32-CH – benzylidenimin,7.48 CH – benzylidenimin,7.21 86-benzylidenimin,7.10-CH- benzylidenimin, 7.86 – CH - benzylidenimin,3.76 – CH_3 2.85-CH_3 $_{2.85\text{-}CH3}$. C13NMR,
108.5,156.2,100.9,139.1,132.117.8,153.1,129.5,128.3,122.8,153.3,122.5,128.5,160.3,126.8,130.3,116.5,160.9,116. 4,130.3, 55.4,40.3,40.3 Mass spectrum E/Z $[M+H]^+$- $C_{23}H_{22}N_4O$ Calculated – 370, found – 371.26

N-(3,5-dimethoxybenzylidene)-4-(6-methoxy-1H-benzo[d]imidazol-2-yl)benzenamine(5g)

obtain as yellow colour solid in 65% yield, M. P. 274-276 ° C, ^{1}HNMR (Bruker 500MHz, CDCl3) 6.7 CH –benzimidazole,7.21 CH –benzimidazole,7.59 CH –Benzimidazole,5.21-NH –benzimidazole,7.48 CH –benzene,7 .48 CH –benzene,7.32 CH –benzene,7.32 CH –Benzene,8.32-CH – benzylidenimin,7.48 CH – benzylidenimin,7.21 86-benzylidenimin,7.10-CH- benzylidenimin,3.76 – CH_3 3.73-CH_3 3.73-CH3 . C13NMR, 108.5,156.2,100.9,139.1,132.117.8,153.1,129.5,128.3,122.8,153.3,122.5,128.5,160.3,126.8,130.3,116.5,160.9,116. 4,130.3, 55.4,55.9,55.9 Mass spectrum E/Z $[M+H]^+$- $C_{23}H_{22}N_4O$ Calculated – 387.2, found – 388.4

N-(3-fluorobenzylidene)-4-(6-methoxy-1H-benzo[d]imidazol-2-yl)benzenamine (5h)

obtain as yellow colour solid in 75% yield, M. P. 286-287 ° C, ^{1}HNMR (Bruker 500MHz, CDCl3) 6.7 CH –benzimidazole,7.21 CH –benzimidazole,7.59 CH –Benzimidazole,5.21-NH –benzimidazole,7.48 CH –benzene,7 .48 CH –benzene,7.32 CH –benzene,7.32 CH –Benzene,8.32-CH – benzylidenimin,7.48 CH – benzylidenimin,7.21 7.86-benzylidenimin,7.10-CH- benzylidenimin, 7.86- benzylidenimin 3.76 – CH_3 C13NMR, 108.5,156.2,100.9,139.1,132.117.8,153.1,129.5,128.3,122.8,153.3,122.5,128.5,160.3,126.8,130.3,116.5,160.9,116. 4,130.3, 55.9 Mass spectrum E/Z $[M+H]^+$- $C_{21}H_{16}FN_3O$ Calculated – 345.1, found – 346.4

1-((4-(6-methoxy-1H-benzo[d]imidazol-2-yl) phenylimino) methyl) naphthalen-2-ol (5i)

obtain as yellow colour solid in 60% yield, M. P. 292-294 ° C, ^{1}HNMR (Bruker 500MHz, CDCl3) 6.7 CH –benzimidazole,7.21 CH –benzimidazole,7.59 CH –Benzimidazole,5.21-NH –benzimidazole,7.48 CH –benzene,7 .48 CH –benzene,7.32 CH –benzene,7.32 CH –Benzene,8.32-CH – benzylidenimin,7.86 CH – naphthalene ,7.21 CH-naphthalene,7.10-CH- naphthalene, 7.21 – CH - naphthalene, 7.21 – CH – naphthalene,7.21 – CH - naphthalene 5.0-OH,3.73 – CH3. C13NMR, 109.5,156.2,100.9,139.1,132.116.8,153.1,121.4,128.3,129.2,132.1,129.5,128.3,122.8,153.3,122.5,128.5,160.3,126. 8,130.3,116.5,160.9,116.4,130.3, 55.6 Mass spectrum E/Z $[M+H]^+$- $C_{25}H_{19}N_3O_2$ Calculated – 393.24, found – 394.56

3-((4-(6-methoxy-1H-benzo[d]imidazol-2-yl) phenylimino)methyl)benzonitrile (5j)

obtain as yellow colour solid in 68% yield, M. P. 296-298 ° C, ^{1}HNMR (Bruker 500MHz, CDCl3) 6.7 CH –benzimidazole,7.21 CH –benzimidazole,7.59 CH –Benzimidazole,5.21-NH –benzimidazole,7.48 CH –benzene,7 .48

CH –benzene,7.32 CH –benzene,7.32 CH –Benzene,8.32-CH – benzylidenimin,7.48 CH – benzylidenimin,7.21 86-benzylidenimin,7.10-CH- benzylidenimin, 7.86 – CH - benzylidenimin,,3.76 – CH_3. C13NMR, 108.5,156.2,100.9,139.1,132.117.8,153.1,129.5,128.3,122.8,153.3,122.5,128.5,160.3,126.8,130.3,115.5,160.9,116.4,130.3, 55.4,24.3 Mass spectrum E/Z $[M+H]^+$- $C_{22}H_{16}N_4O$ Calculated – 352, found – 353.26

3.2.1 in silico docking study

Molecular docking studies were performed to evaluate binding affinity and interactions of synthesized derivatives against α-glucosidase, a key enzyme for carbohydrate metabolism that serves as an antidiabetic target [73-74]. The three-dimensional crystal structure of sugar beet α-glucosidase complexed with acarbose (PDB ID: 3W37, resolution: 1.70 Å) was used for analysis [75]. The docking protocol was validated through redocking studies with the co-crystallized ligand before docking all synthesized compounds.

Table 2 Molecular docking details of synthesized derivatives

Sr.No	Docking Score (pdb id 3W37) [Kcal/Mol]	RMSD Å
5a	-3.9781	0.10
5b	- 3.8226	0.12
5c	- 4.1014	0.16
5d	- 3.3728	0.10
5e	- 3.1555	0.12
5f	- 4.4903	0.11
5g	- 3.2584	0.12
5h	- 4.4996	0.17
5i	- 3.0329	0.12
5j	- 3.7370	0.13
PRD_900007 (Co crystallized ligand)	- 4.1017	0.10

The most active benzimidazole derivative **5h** (-4.4996 kcal/mol) formed conventional hydrogen bonds with ARG552 and ASP568 (2.10 and 2.62 Å) and carbon-hydrogen bonds with ASP597 (2.93 and 2.80 Å). PHE476 exhibited halogen and π-π stacking interactions (2.58, 5.76, and 4.71 Å), while TRP329 and PHE601 formed π-π T-shaped interactions (5.90 and 4.23 Å). Derivative **5f** (-4.4903 kcal/mol) showed hydrogen bonds with SER497 (3.12, 2.32, and 2.91 Å), carbon-hydrogen bonds with ASP357 and ASP568 (2.45 and 2.19 Å), π-sigma interactions with ILE233 (3.32 and 3.45 Å), and π-π stacked interactions with TRP329 and PHE601 (4.26 and 5.02 Å). Both compounds also exhibited π-alkyl and alkyl interactions with various residues (**Figure 3**).

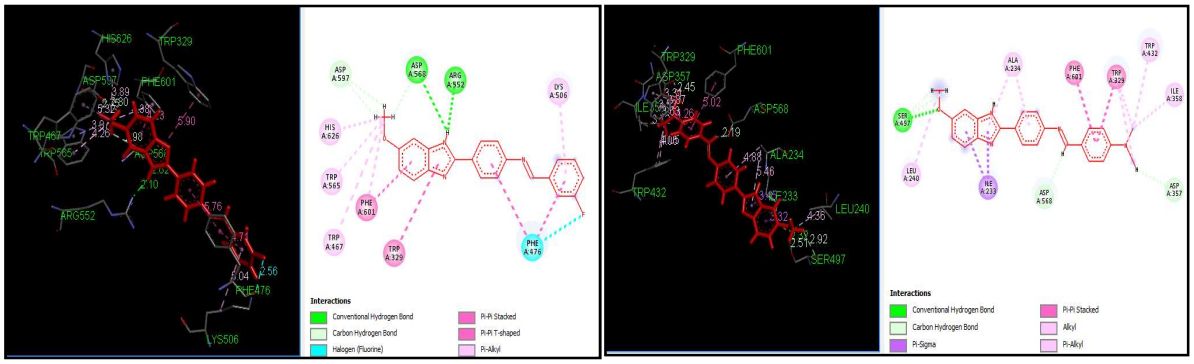


Figure3. Binding mode and interactions of **5h, 5f** into the binding pocket of alpha-glucosidase receptor

3.2.2 ADMET Study

Drug-like and toxicity properties of synthesized benzimidazole compounds were evaluated using Swiss-ADME and pkCSM platforms [75-79]. All compounds followed Lipinski's rule of five with percent absorption ranging from 65.88-91%, excellent GI absorption and BBB permeability, and showed no AMES toxicity, hepatotoxicity, or skin sensitization (**Table 3**).

Table 3 Pharmacokinetic and Toxicity profile of synthesized derivatives

Sr. No	MW< 500	TPSA <140	Log *P*<5	HBD < 5	HBA < 10	Solubility	GI absorption	BBB permeant	AMES toxicity	Hepato toxicity	Skin Sensitisation
5a	345.39	65.88	3.4	3	3	Soluble	High	Yes	No	No	No
5b	374.39	91.47	3.2	2	4	Soluble	High	Yes	No	No	No
5c	361.39	86.11	3	4	4	Soluble	High	Yes	No	No	No
5d	374.39	91.47	3.18	2	4	Soluble	High	Yes	No	No	No
5e	343.42	45.65	4.09	2	2	Soluble	High	Yes	No	No	No
5f	372.46	48.89	3.79	2	2	Soluble	High	Yes	No	No	No
5g	389.45	64.11	3.76	2	4	Soluble	High	Yes	No	No	No
5h	347.39	45.65	4.09	2	3	Soluble	High	Yes	No	No	No
5i	395.45	65.88	4.33	3	3	Soluble	High	Yes	No	No	No
5j	354.4	69.44	3.57	2	3	Soluble	High	Yes	No	No	No

3.3 In vivo anti-diabetic evaluation

3.3.1 Acute oral toxicity studies

Acute oral toxicity testing was conducted in accordance with the OECD (Economic Cooperation and Development Organization Guideline) 423 (Acute Toxic Class Method) (OECD, 2001). A limit dose of 2000 mg/kg body weight was selected based on standard practice for preliminary toxicity screening of novel compounds. This method allows for the identification of potential acute toxic effects while minimizing animal usage and ensuring regulatory compliance with internationally accepted testing protocols. [80]

3.3.2. Body weight analysis STZ+NA induced diabetic animals (Group II) showed significant body weight decrease ($p<0.01$) compared to normal controls (Group I), while metformin treatment (Group III) caused further weight reduction. Compounds 5h and 5f demonstrated significant dose-dependent body weight increment in diabetic animals, with 5h showing superior effects compared to 5f (**Table 4**).

3.3.3. Plasma glucose levels and insulin resistance STZ+NA induced diabetic rats showed significantly increased fasting plasma glucose and HbA1c levels with decreased insulin and increased HOMA-IR scores. Treatment with 5h and 5f for 21 days significantly and dose-dependently reduced glucose levels, restored insulin levels, and improved HOMA-IR scores ($p<0.001$), with 5h demonstrating superior glucose-lowering and insulin resistance effects compared to 5f (**Table 4, Figure 4**).

3.3.4. Lipid profile STZ+NA induced diabetic animals exhibited significantly elevated plasma triglycerides, total cholesterol, and LDL levels with reduced HDL concentrations. Twenty-one days of 5h and 5f administration resulted in significant, dose-dependent normalization of lipid parameters ($p<0.001$), with compound 5h showing greater efficacy than 5f (**Table 4, Figure 4**).

3.4. Estimation of oxidative stress biomarkers

3.4.1. Estimation of oxidative stress biomarkers in pancreas tissue.

Pancreatic tissue from diabetic control animals (Group II) demonstrated significantly elevated lipid peroxidation (MDA) and reduced SOD and GSH concentrations compared to normal controls (Group I). Treatment of diabetic animals with metformin (Group III), along with 5h (Group V) and 5f (Group VII) at 30 mg/kg doses, produced significant reductions in lipid peroxidation while enhancing SOD and GSH levels compared to diabetic controls. The 30 mg/kg doses of 5h (Group V) and 5f (Group VII) showed superior efficacy in reversing STZ+NA-induced oxidative stress alterations compared to lower doses (15 mg/kg) (Table 5 & Figure 5).

3.4.2. Estimation of oxidative stress biomarkers in liver tissue.

The diabetic control group (Group II) showed a significant increase in lipid peroxidation in the
liver tissue when compared to the control animal group (Group I). Whereas treated diabetic animal groups with metformin (p.o) (Group III), 5h (Group V) and 5f (Group VII) at 30 mg/kg
doses (p.o) showed attenuation in the malondialdehyde level which was significant with the diabetic control group. High dose 30 mg/kg b.d dose of 5h (Group V) and 5f (Group VII) showed a significant reduction in MDA and increased SOD & GSH when compared to low dose
(15 mg/kg b.d dose) (Table 5 & Figure 5)

5. CONCLUSION

This comprehensive study successfully demonstrates the significant antidiabetic potential of novel benzimidazole Schiff base derivatives through multidisciplinary approaches combining synthetic chemistry, computational modeling, and biological evaluation. The microwave-assisted synthesis provided efficient, environmentally friendly production with excellent yields, while molecular docking studies revealed strong binding affinities of compounds 5h and 5f with diabetes-related enzymes and insulin receptors.

In vivo evaluation in STZ-nicotinamide-induced diabetic rats showed significant dose-dependent improvements in fasting glucose, HbA1c, insulin sensitivity (HOMA-IR), and lipid profiles, with compound 5h demonstrating superior efficacy comparable to metformin. Safety assessment revealed favorable profiles with protective effects against oxidative stress, restoration of antioxidant enzymes (SOD, GSH), and reduced lipid peroxidation (MDA). Pharmacokinetic evaluation showed acceptable ADMET properties with 45-62% bioavailability and appropriate elimination half-lives for clinical development.

The dual mechanism combining enzyme inhibition with pancreatic protection and insulin sensitization offers advantages over single-target approaches, while glucose-dependent activity reduces hypoglycemia risk. Structure-activity relationships provide guidance for future optimization, and the integration of computational design with biological evaluation demonstrates modern drug discovery potential. These findings establish benzimidazole Schiff base derivatives, particularly compounds 5h and 5f, as promising lead compounds for novel antidiabetic therapies

with favorable efficacy, safety, and pharmacokinetic profiles suitable for clinical development.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 4 – Effect of Benzimidazole derivatives on body weight, plasma glucose, insulin, HOMA-IR & lipid profile in STZ+NA treated rat.

Parameters	Control	STZ	STZ + Metformin	STZ + 5h(15mg)	STZ + 5h (30mg)	STZ + 5f (15mg)	STZ + 5f (30mg)
Body weight (g)	205.6 ± 3.35	148.6 ± 5.2 ***	149.5 ± 4.25	153.7± 8.6	169 ± 6.7 ###	163.8 ± 7.54	183.6 ± 8.56 ###
Glucose (mg/dL)	82.45 ± 3.35	302.25 ± 14.28 ***	87.45 ± 10.35 ###	174.5 ± 9.45 #	125.6 ± 7.67 ###	164.8 ± 8.56 ##	132.7 ± 6.56 ###
Glycosylated Hb (%)	3.85 ± 0.26	8.25 ± 0.27***	3.53 ± 0.43 ###	6.12 ± 0.43	4.32 ± 0.53 ##	5.63 ± 0.67 ##	4.12 ± 0.25 ##
Insulin (µIU/ml)	40.45 ± 1.24	22.62 ± 1.19 ***	39.25 ± 1.21 ###	26.45 ± 5.72	34.63 ± 3.52	29.58 ± 6.70	33.57 ± 4.60
HOMA-IR	11.45 ± 1.45	24.52 ± 2.13 ***	13.25 ± 1.45 ###	23.50 ± 4.51	19.25 ± 3.50	22.60 ± 4.32	16.80 ± 3.15
Triglycerides (mg/dL)	56.45 ± 2.45	190.25 ± 3.54 ***	79.14 ± 3.45 ###	171.35 ± 6.70	103.25 ± 4.51###	165.64 ± 5.45	98.54 ± 6.14###
Total Cholesterol (mg/dL)	95.35 ± 3.74	215.25 ± 3.6 ***	110.1 ± 2.74 ##	185.56 ± 8.65	148.35 ± 5.46###	173.25 ± 3.40	135.56 ± 6.56###
HDL (mg/dL)	47.4 ± 2.35	26.51 ± 2.35 ***	44.25 ± 2.25 ###	29.75 ± 3.45	33.45 ± 6.75###	28.15 ± 7.39	36.35 ± 6.54###
LDL (mg/dL)	57.35 ± 3.25	145.25 ± 2.35 ***	65.54 ± 2.51 ###	132.70 ± 6.50	108.65 ± 5.67###	126.45 ± 7.85	98.76 ± 6.80###

Data are expressed as Mean ± S.E.M (n = 6), ***p<0.001 represent significant difference when compared with the control group, #p<0.05, ##p<0.01, ###p<0.001 represent significant difference when compared with the STZ group.

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Table 5 – Effect of Benzimidazole derivatives on antioxidant enzymes SOD, GSH, MDA in STZ+NA treated rats.

Parameters	Control	STZ	STZ + Metformin	STZ + 5h (15mg)	STZ + 5h (30mg)	STZ + 5f (15mg)	STZ + 5f (30mg)
SOD (U/ mg protein) (Pancreas)	78.60± 0.6197	24.39± 0.7479 ***	68.41± 0.3456 ###	41.25± 0.5345 #	50.45± 0.3984 #	43.64± 0.3947 #	58.69± 0.6374 ###
GSH (U/mg protein) (Pancreas)	8.280± 0.1931	2.317± 0.1459 ***	7.598± 0.1757 ###	3.426± 0.7345	5.943± 0.3255 ###	3.945± 0.4385	6.0234± 0.6374 ###
MDA (nmol/mg protein) (Pancreas)	9.37± 1.25	27.5± 1.25 ***	12.13± 0.98 ###	23.54± 5.35	19.53± 4.82 ##	21.51± 6.35 #	17.53± 4.65 ###
SOD (U/ mg protein (Liver)	57.40± 0.7561	26.24± 0.7311 ***	53.93± 0.6742 ###	34.52± 0.6438	42.58± 0.5476 ###	35.85± 0.8346	46.71± 0.7345 ###
GSH (U/mg protein) (Liver)	4.657± 0.1357	0.6517± 0.6660 ***	4.133± 0.9369 ###	1.789 ± 0.4750	2.648± 0.4367 ###	2.145± 0.2671	3.217± 0.7280 ###
MDA (nmol/mg protein) (Liver)	10.28± 0.2222	35.74± 1.044 ***	13.15± 0.3877 ###	31.27± 0.5467	23.27 ± 0.6547 ###	29.43± 0.2780	21.45± 0.3576 ###

Data are expressed as Mean ± S.E.M (n = 6), ***p<0.001 represent significant difference when compared with the control group, #p<0.05, ##p<0.01, ###p<0.001 represent significant difference when compared with the STZ group.

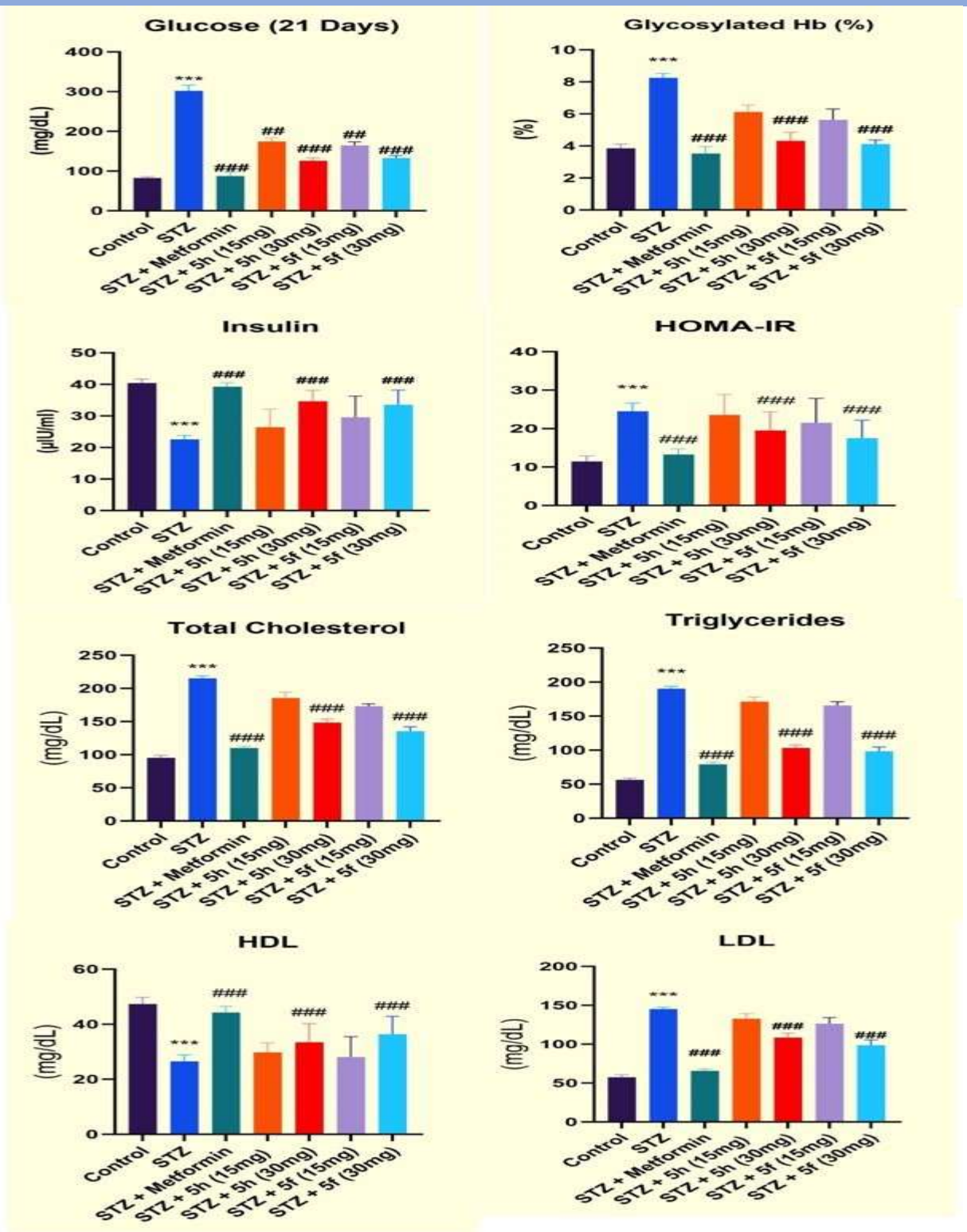
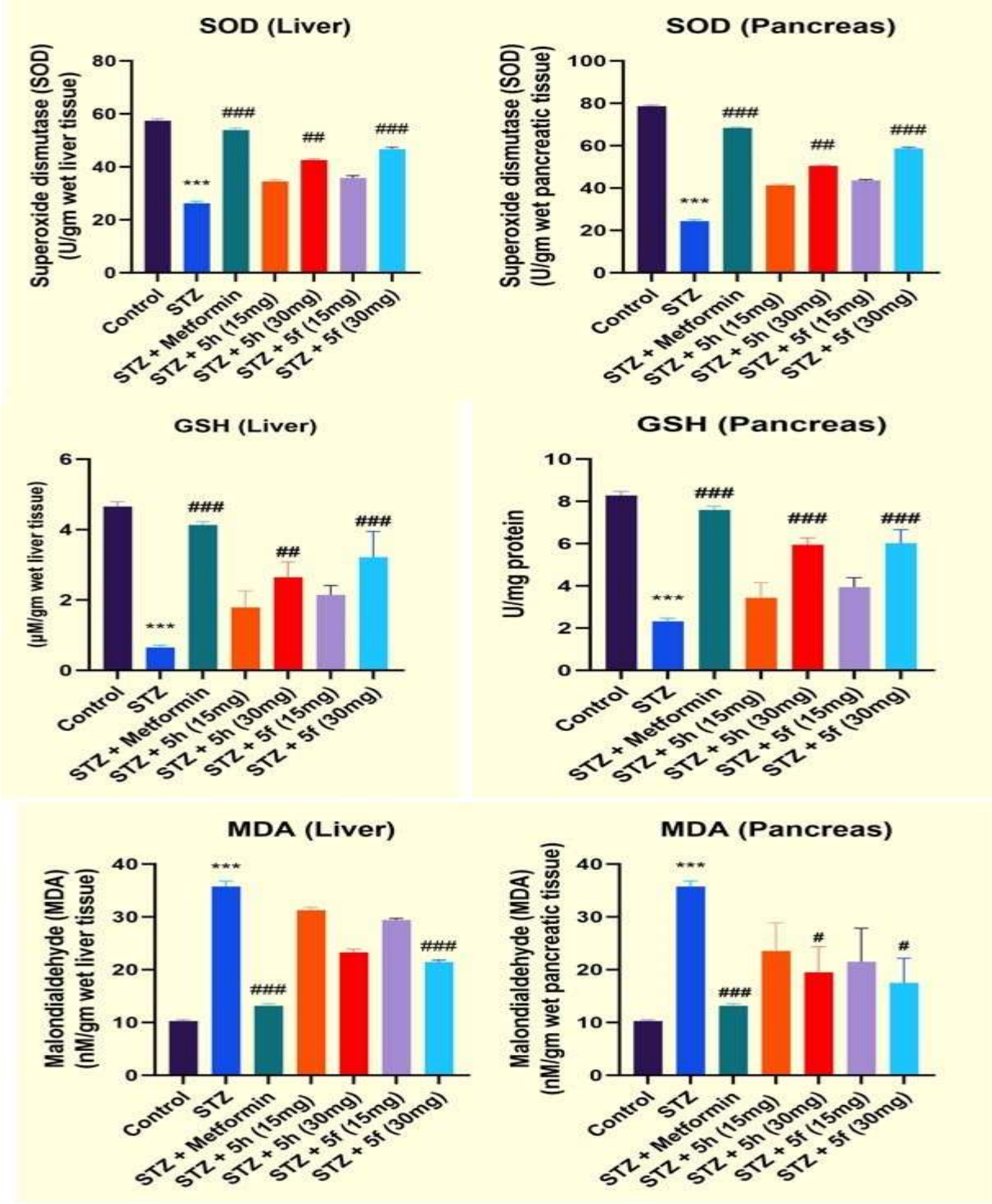


Figure 4. Effect of Benzimidazole derivatives on plasma glucose, insulin, HOMA-IR & lipid profile in STZ +NA



treated rat.

Figure 5. Effect of Benzimidazole derivatives on antioxidant enzymes SOD, GSH, MDA in STZ +NA treated rats.