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Design, Synthesis and Pharmacological Evaluation of Benzoyl Hydrazone Derivative as Potential Multitarget Antidiabetic Agent: Molecular Docking, Biological Evaluation and ADME Profiling

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Diabetes is a chronic disease marked by increased blood glucose levels, often associated with oxidative stress, leading to severe complications. Current antidiabetic therapies often target single pathways, but a multitarget approach may enhance efficacy and reduce complications. Benzoyl hydrazone derivatives, known for diverse pharmacological activities, offer promise as multifunctional antidiabetic agents. The study focuses on the synthesis and biological evaluation of novel benzoyl hydrazone derivatives against three critical diabetic targets *viz*., aldose reductase, α-glucosidase and peroxisome proliferator-activated receptor-gamma (PPAR-γ). Eighteen derivatives were designed and subjected to molecular docking, with compound 4-hydroxy-3,5-dimethoxybenzaldehyde 4-hydroxybenzoylhydrazone) (**HB18**) exhibiting the strongest binding energies: –9.33 kcal/mol (aldose reductase), –6.34 kcal/mol (α-glucosidase) and –6.49 kcal/mol (PPAR-γ). ADME predictions indicated the favourable drug-like properties, high gastrointestinal absorption, compliance with Lipinski's rule and low toxicity risk. Compound **HB18** was synthesized with 80% yield and structurally confirmed by infrared R, nuclear magnetic resonance and mass spectroscopic analysis. Acute oral toxicity testing in rats (2000 mg/kg) revealed no mortality or adverse effects. *In vitro* antioxidant assays showed potent DPPH (IC_{50} = 18.7 µg/mL) and scavenging of hydrogen peroxide activities in comparison to vitamin C. *In vivo*, compound **HB18** significantly reduced fasting blood glucose from 126.21 to 287.62 mg/dL in streptozotocin-induced diabetic rats at 400 mg/kg ($p < 0.01$), with improved lipid profiles. This integrated *in silico*, *in vitro* and *in vivo* approach demonstrates compound **HB18** as a promising multitarget antidiabetic candidate with antioxidant potential, warranting further pharmacological development.

Keywords: Benzoyl hydrazone, Antidiabetic, Molecular docking, α-Glucosidase, PPAR-γ, Aldose reductase, Antioxidant, ADME.

INTRODUCTION

Diabetes is a clinical condition caused due to a defect in insulin secretion or defective insulin binding or insulin action, indicative of high blood glucose levels, is a well-known global health burden among households [1]. The escalating occurrence of both type 1 and type 2 diabetes poses considerable socio-economic challenges across the globe [2]. Beyond elevated blood glucose levels, diabetes mellitus is a complex metabolic disorder associated with a range of long-term complications affecting the microvasculature (retinopathy, nephropathy, neuropathy) and microvasculature (cardiovascular disease, stroke) [3]. The pathogenesis of diabetes mellitus is multifactorial, involving genetic predisposition, lifestyle factors and environmental influences [4]. Notably, free radicals plays a critical role in the development and progression of diabetic complications [5]. Chronic hyperglycaemia leads to impaired antioxidant defence mechanisms and increased production of reactive oxygen species (ROS) contributing to cellular damage and tissue dysfunction [6].

Given the complex and multifactorial nature of diabetes, a therapeutic strategy targeting multiple pathways involved in glucose homeostasis and the development of complications holds significant promise [7]. Traditional antidiabetic agents

often focus on a single target, such as increasing insulin secretion or improving insulin sensitivity. However, the intricate interplay of various metabolic pathways suggests that agents with the ability to modulate multiple targets could offer superior efficacy and potentially address the diverse pathological mechanisms underlying diabetes mellitus [8]. This multitarget approach can lead to synergistic effects, improved glycaemic control and a reduction in the risk of long-term complications [9].

Benzoyl hydrazone derivatives (–C(=O)–NH–N=) have garnered considerable attention in medicinal chemistry due to their potential as multifunctional agents with diverse pharmacological activities [10]. Benzoyl hydrazones, a specific class of hydrazones containing a benzoyl group, have demonstrated a wide spectrum of biological effects, including anticancer, anti-inflammatory, antimicrobial and antidiabetic properties [11,12]. The structural versatility of benzoyl hydrazones allows for the facile synthesis of many analogues with varying substitution patterns, enabling the modulation of their pharmacological profiles and target interactions [13]. Several studies have reported the potential of benzoyl hydrazone derivatives to exhibit antidiabetic activity, including inhibition of key enzymes involved in carbohydrate metabolism [14,15].

As mentioned earlier, it is a significant factor in the pathogenesis and progression of diabetes and its complications [5,6]. The imbalance between the production of ROS and the capacity of the antioxidant defence system leads to cellular damage and contributes to insulin resistance and β-cell dysfunction [16]. Therefore, agents with antioxidant properties can play a crucial role in mitigating the adverse effects of oxidative stress in diabetic patients [17]. The search for antidiabetic compounds with inherent antioxidant activity is a promising field for developing more effective and potentially disease modifying therapies [18].

Furthermore, the development of an effective therapeutic agent hinges not only on its pharmacological potency but also on its pharmacokinetic and toxicological properties, collectively known as ADME (absorption, distribution, metabolism and excretion) [19]. To streamline drug discovery, computational tools such as molecular docking and ADME prediction have become essential for early-stage screening, enabling the identification of lead compounds with optimal binding affinities and favourable drug-like profiles [20]. When integrated with chemical synthesis and subsequent *in vitro* and *in vivo* evaluations, these *in silico* strategies enhance the efficiency and reliability of discovering promising drug candidates with minimized toxicity risks [21].

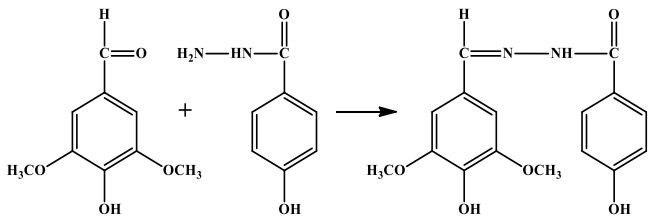
The current study explores the designing and docking of a series of 18 benzoyl hydrazone derivatives (Fig. 1) against three key diabetic targets, for example, α-glucosidase, aldose reductase and PPAR-γ. Compound 4-hydroxy-3,5-dimethoxy benzaldehyde 4-hydroxybenzoyl hydrazine (**HB18**) was identified as a lead based on its strong binding affinity and favourable ADME profile. This compound was synthesized and structurally characterized with infrared spectroscopy (IR), nuclear magnetic resonance (NMR) and mass spectrometry (MS). Acute toxicity was assessed in rats to evaluate the safety profile of the compound. Its antidiabetic efficacy was tested in streptozotocin induced diabetic rats and compared with conventional therapies. Furthermore, its antioxidant activity was evaluated using DPPH and hydrogen peroxide scavenging assays.

EXPERIMENTAL

Analytical grade chemicals and reagents were used in this study and purchased from reputable different suppliers such as Merck Life Sciences Pvt. Ltd. and Sigma-Aldrich. These included methanol, 4-hydroxybenzohydrazide, carbinol and streptozotocin (STZ), all of which were used without further purification unless otherwise stated. Solvents employed for synthesis and purification were distilled prior to use. Standard reference drugs such as glibenclamide and ascorbic acid were utilized for the comparative evaluations in antidiabetic and antioxidant assays, respectively.

Infrared analysis (Shimadzu FTIR-84005 instrument) was performed using KBr pellet method and used to identify the characteristic functional groups. ^{13}C and ^{1}H and NMR spectra were recorded in DMSO-d_6 using a Bruker Avance III spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts (δ) are reported in ppm with tetramethyl silane (TMS) as an internal standard. The electrospray ionization mass spectrometry (ESI-MS) was obtained on a Waters Q-TOF Micro mass spectrometer equipped with an ESI source operated in positive ion mode was employed to verify the molecular mass of the compound, supporting its proposed molecular formula.

Synthesis of compound HB18: Compound **HB18** was synthesized by refluxing a mixture of 1.82 g of 4-hydroxy 3,5-dimethoxy benzaldehyde and 1.52 g of 4-hydroxy benzhydrazide, dissolved in 25 mL of carbinol, for 4 h. The contents were allowed to cool and the solid product was separated by filtration. The resultant product was recrystallized twice from hot methanol (**Scheme-I**). Pure light greenish coloured crystals of 4-hydroxy 3,5-dimethoxy benzaldehyde 4-hydroxy benzoyl hydrazone (**HB18**, $C_{16}H_{16}N_2O_5$, yield: 80%, m.p. 292-294 ºC, R_f: 0.64) was obtained. This compound is found to be soluble in DMF and methanol.



Scheme-I: Synthesis of benzoyl hydrazone derivative **HB18**

Molecular docking studies: A total of 18 benzoyl hydrazone derivatives were designed and subjected to molecular docking studies using Auto Dock 4.2 software. Three target proteins *viz.* α-glucosidase (PDB ID: 5NN4), aldose reductase (PDB ID: 4LBS) and PPAR-γ (PDB ID: 6MS7) were obtained from the Protein Data Bank. By removing the water molecules and adding polar hydrogens the structures were prepared. The ligands were energy-minimized and converted into PDBQT format. Docking was performed using the lamarckian genetic algorithm and binding energies and interactions were analyzed using Auto Dock Tools and protein ligand interaction profiler.

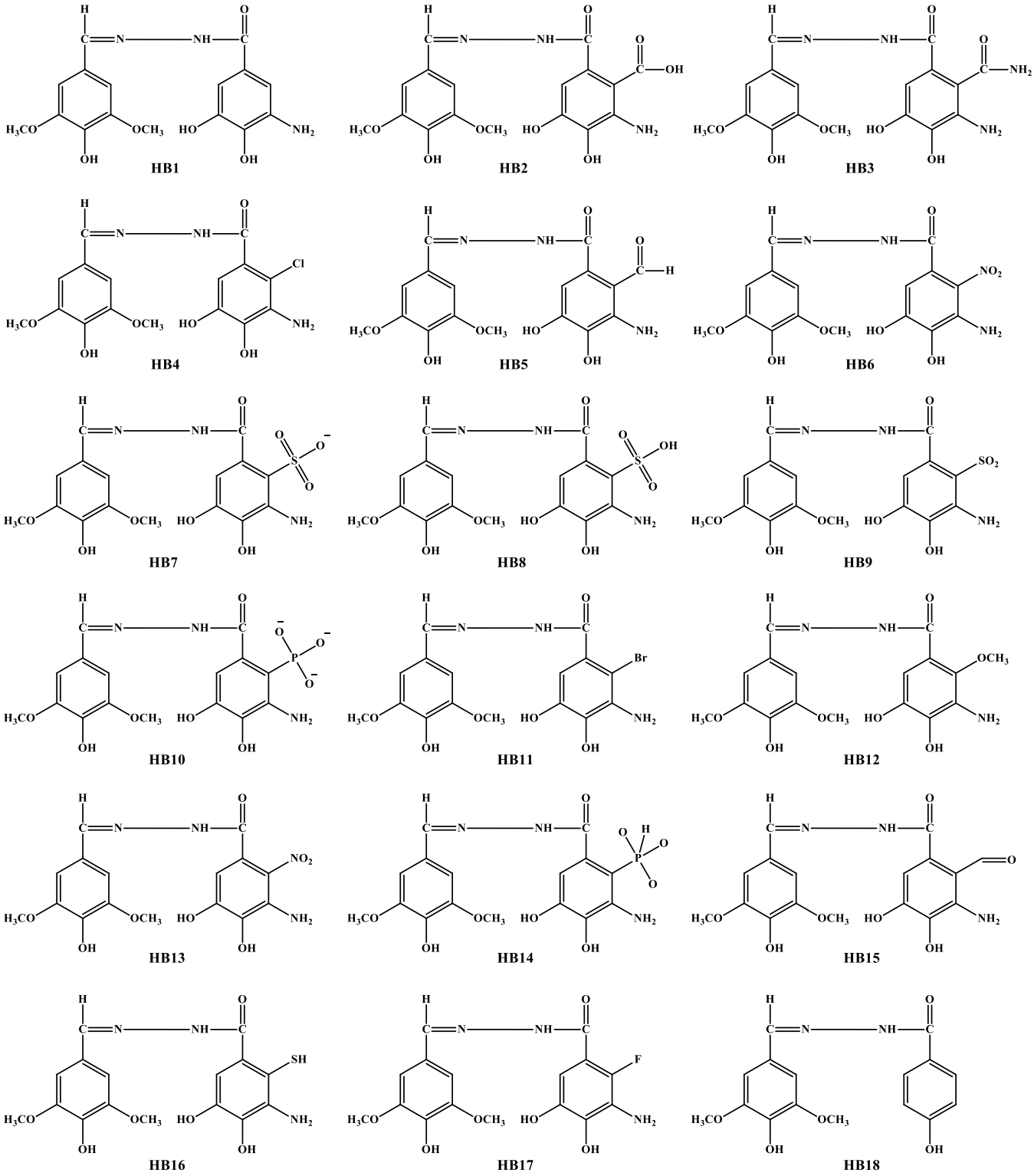


Fig. 1. Structures of the compounds **HB1-HB18**

ADME analysis: The ADME properties of all 18 benzoyl hydrazone derivatives were predicted using the Swiss ADME web server. Key pharmacokinetic parameters such as gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, cytochrome P450 enzyme (particularly CYP2D6) inhibition, hepatotoxicity, aqueous solubility, intestinal absorption and plasma protein binding (PPB) were assessed. Furthermore, drug-likeness was evaluated based on Lipinski's rule of five and bioavailability scores were calculated to determine the potential of the compounds as orally active drug candidates.

Animals: Adult healthy Albino Wistar rats of either sex, weighing between 180-200 g, were obtained from SV Animal Home, Bangalore, India. These animals were used for eval-

uating acute toxicity and antidiabetic activity. Prior to the experiments, the rats were acclimatized for 1 week and housed in polypropylene cages under standard laboratory conditions like at room temperature, 60% relative humidity and a 12 h light/dark cycle. They were provided with a standard pellet diet and had free access to water throughout the study period. All animals were handled with care to minimize stress and avoid excessive adrenal stimulation. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) under approval number SJCP/PCOL/AD2025-10/002 and all procedures were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), with registration no. 1519/PO/Re/S/11/CPCSEA. Animals were divided into 5 groups (n = 6) of six animals each. Group 1 were treated as normal; group 2 are diabetic control, group 3 include glibenclamide treatment and group 4 and 5 receive **HB18** at 200 mg/kg and 400 mg/kg, respectively. Over a 28-day period, the treatment was continuous.

Acute toxicity studies: Acute oral toxicity was assessed according to OECD guideline 423 in healthy Wistar rats [22]. The synthesized compound was administered orally at a dose of 2000 mg/kg body weight and animals were observed for behavioural changes, signs of toxicity and mortality for 14 days.

***In vitro* antioxidant assays:** The *in vitro* antioxidant potential of the synthesized compound was assessed using two standard assays. In the DPPH radical scavenging assay, various concentrations of the compound were incubated with DPPH solution and the reduction in absorbance was measured at 517 nm to determine free radical scavenging activity. In the hydrogen peroxide scavenging assay, the compound's ability to neutralize hydrogen peroxide was evaluated by measuring absorbance at 230 nm. In both assays, ascorbic acid was used as the reference standard and IC_{50} values were calculated to compare the antioxidant efficacy of compound **HB18**.

***In vivo* antidiabetic studies:** For compound **HB18**, *in vivo* antidiabetic activity was evaluated in diabetic Wistar rats by inducing streptozotocin (STZ). Diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg) prepared in citrate buffer (pH 4.5) and rats with fasting blood glucose levels above 250 mg/dL after 72 h were considered diabetic. The animals were divided into five groups (n = 6) comprising a diabetic control, standard treatment group receiving metformin (100 mg/kg), normal control and test groups treated with the synthesized compound at 200 and 400 mg/Kg body weight. Treatments were administered orally once daily for 28 days. Blood glucose levels were monitored at regular intervals using a glucometer. At the end of the treatment period, blood samples were collected and serum was analyzed for biochemical parameters including fasting blood glucose, total cholesterol, triglycerides and HDL levels using standard enzymatic assay kits.

Statistical analysis: The mean ± SEM was used to express all results. GraphPad Prism software was used to analyze the data using a one-way ANOVA and then Tukey's post hoc test. Statistical significance was defined as a *p*-value of less than 0.05.

RESULTS AND DISCUSSION

The lead compound **HB18**, was successfully synthesized *via* a condensation reaction and purified to obtain light greenish crystals with a melting point of 292-294 ºC and an overall yield of 80%. Compound **HB18** was structurally characterized using IR, ^{1}H NMR and mass spectrometry. In IR spectrum, a medium absorption band at 1642 cm^{-1} is attributed to >C=O stretching and a strong band at 1609 cm^{-1} corresponding to >C=N- stretching. Additional bands at 3529 cm^{-1} and 3075 cm^{-1} were assigned to >NH and –OH stretching, respectively, supporting the presence of hydrazone and phenolic functional groups. The ^{1}H NMR spectrum recorded in DMSO-d_6 exhibited a singlet at δ 11.54 ppm for the NH proton, a singlet at δ 10.13 ppm for the phenolic OH and a singlet at δ 8.1 ppm representing the –N=CH proton. Aromatic protons appeared as doublets and multiplets in the δ 6.84-7.80 ppm range, while methoxy protons showed a singlet at δ 6.63 ppm (6H). In ^{13}C NMR, compound exhibited 16 distinct carbons, for example, two methoxy groups, an azomethine carbon (C=N), a benzoyl carbonyl, two aromatic rings (one *para*-substituted, one multi-substituted with OMe and OH). The ^{13}C NMR showed peaks for carbonyl (~170 ppm), C=N (~155–160 ppm), aromatic C–O (~150–162 ppm), quaternary aromatic carbons (~135–145 ppm), aromatic CH carbons (~115–132 ppm) and methoxy carbons (~55–62 ppm). Symmetry in the *para* ring reduces the number of unique aromatic signals on that ring. The mass spectrum of compound **HB18** show M+1 peak at 316.1 (*m/z*) corresponding to its molecular weight.

Molecular docking and ADME analysis: Among the designed 18 benzoyl hydrazone derivatives, 4-hydroxy-3,5-dimethoxybenzaldehyde 4-hydroxybenzoyl hydrazone (**HB18**) exhibited the most favourable binding energies across all targets (Table-1), suggesting strong potential as a multi-target antidiabetic agent. The binding interactions revealed the hydrogen

TABLE-1
SUMMARY OF BENZOYL HYDRAZINE DERIVATIVES, THEIR ASSIGNED CODES AND CORRESPONDING BINDING ENERGIES

Compound code	Binding energies		
	4LBS	5NN4	6MS7
HB1	-8.03	-7.03	-7.62
HB2	-6.15	-5.12	-8.52
HB3	-7.10	-5.49	-7.85
HB4	-7.52	-5.02	-7.33
HB5	-7.89	-5.31	-7.78
HB6	-7.05	-5.62	-8.32
HB7	-7.03	-6.21	-7.70
HB8	-7.81	-4.25	-7.69
HB9	-6.21	-4.82	-7.81
HB10	-5.16	-4.26	-7.47
HB11	-7.47	-5.70	-7.33
HB12	-8.09	-5.74	-7.32
HB13	-6.87	-6.25	-7.27
HB14	-4.25	-5.04	-7.71
HB15	-7.69	-4.72	-7.35
HB16	-7.33	-5.24	-6.76
HB17	-7.76	-5.36	-7.07
HB18	-9.33	-6.34	-6.49

bonding and hydrophobic interactions within the active sites of the enzymes, supporting the predicted inhibitory activity. ADMET predictions using the SwissADME web tool indicated that compound **HB18** possessed the desirable pharmacokinetic properties. The compound complied with Lipinski's rule of five, exhibited high gastrointestinal absorption, low risk of CYP450 inhibition and good oral bioavailability [23]. The predicted non-toxic nature and acceptable plasma protein binding capacity further supported its drug-likeness and suitability for *in vivo* studies.

Binding analysis with HB18 aldose reductase: The binding of compound **HB18** to three key antidiabetic targets *viz*. aldose reductase, α-glucosidase and PPAR-γ involves the synergistic hydrophobic and hydrogen bonding interactions. In aldose reductase, aromatic residues such as TRP20, TYR48 and TYR209 form a hydrophobic pocket stabilized by van der Waals and π-π interactions, while THR19, ASP43, ASN160 and SER210 contribute to a dense hydrogen bond network for strong, specific binding. In α-glucosidase, PHE90 and PHE129 engage **HB18** molecule *via* both π-stacking and hydrogen bonds, supported by hydrophobic VAL236 and hydrogen bonding from PRO131, resulting in moderate affinity driven by aromatic complementarity and polar anchoring. In PPAR-γ, hydrophobic residues ALA292 and LEU330 enclose the ligand, while key hydrogen bonds with ARG288, GLU295 and SER342, alongside backbone-mediated interactions from ILE326 and LEU330, enhance specificity and stability.

Molecular docking studies confirmed the strong affinity of molecule HB18, with binding energies of –9.33 kcal/mol for α-glucosidase, –6.34 kcal/mol for aldose reductase and –6.49 kcal/mol for PPAR-γ, outperforming many derivatives. The interactions included multiple hydrogen bonds and hydrophobic contacts critical for enzyme inhibition. ADME predictions indicated that molecule **HB18** complies with Lipinski's rule of five, with high gastrointestinal absorption, low CYP450 inhibition risk and favourable pharmacokinetics, supporting its potential as a safe, orally bioavailable antidiabetic candidate (Tables 2 and 3).

Docked pose of molecule **HB18** showing hydrogen bond interaction with amino acids in the binding site are shown in Fig. 2.

***In vitro* antioxidant activity:** One of the main causes to the pathophysiology of diabetes and its complications is the uncontrolled activity of free radicals. Hydrogen peroxide scavenging and DPPH assays were used to assess the antioxidant activity of compound **HB18**. This compound showed a concentration-dependent increase in free radical scavenging, with an IC_{50} of 22.13 ± 0.41 μg/mL, comparable to the standard antioxidant ascorbic acid (IC_{50} = 21.26 ± 1.58 μg/mL). At 120 μg/mL, compound **HB18** exhibited 83.27 ± 0.19% inhibition, indicating potent antioxidant activity that may complement its antidiabetic effects by mitigating oxidative damage in diabetic patients. The results are depicted in Table-4.

Acute toxicity studies: Acute oral toxicity studies [22] in Wistar rats revealed no signs of toxicity or mortality at the tested dose levels. The animals remained healthy and active throughout the observation period, suggesting the safety of compound **HB18** for the further pharmacological evaluations. Acute oral toxicity studies in Wistar rats demonstrated that compound **HB18** was non-toxic at tested doses, with no mortality or adverse effects observed over 14 days (Table-5). Rats maintained the normal body weight (143 ± 9.89 g) and behaviour, confirming the safety profile and suitability of compound **HB18** for further pharmacological testing

***In vivo* antidiabetic activity:** The antihyperglycemic efficacy of HB18 was assessed in streptozotocin (STZ) induced diabetic rats. Administration of compound **HB18** at doses of 200 and 400 mg/kg significantly reduced the fasting blood

TABLE-2
ASSESSMENT OF DRUG-LIKENESS BASED ON LIPINSKI'S AND VEBER'S RULES FOR THE BENZOYL HYDRAZINE DERIVATIVES

Compound codes	Lipinski rule of five						Veber's rule	
	Log P (<5)	m.w. (<500 Da)	Molar refractivity	H-bond acceptors (<10)	H-bond donors (<5)	Violations	Total polar surface area (Å^2) (<140 Å^2)	No. of rotatable Bonds (<10)
HB1	1.67	347.32	91.07	7	5	Yes	146.63	6
HB2	1.25	391.33	98.03	9	6	No	183.93	7
HB3	1.23	390.35	99.16	8	6	No	189.72	7
HB4	2.02	381.77	98.06	7	5	Yes	146.63	6
HB5	1.82	375.33	96.46	8	5	Yes	163.70	7
HB6	2.34	406.35	104.36	9	4	No	155.71	8
HB7	3.14	441.41	105.40	10	5	No	198.38	8
HB8	-0.00	428.31	104.28	10	6	No	209.38	7
HB9	-80.35	427.39	100.93	10	6	No	209.38	7
HB10	0.00	428.31	104.28	10	8	No	220.91	7
HB11	1.57	361.35	96.03	7	5	Yes	146.63	6
HB12	1.90	377.35	97.56	8	5	Yes	155.86	7
HB13	0.24	392.32	95.04	9	5	No	166.71	7
HB14	0.00	429.32	105.67	10	8	No	220.9	7
HB15	1.82	375.33	96.46	8	5	Yes	163.70	7
HB16	1.38	379.39	98.32	7	5	Yes	185.43	6
HB17	1.83	365.31	365.31	8	5	Yes	146.63	6
HB18	2.16	316.31	84.64	6	3	Yes	100.38	6

TABLE-3
DRUG-LIKENESS AND ADME PROFILING OF SYNTHESIZED BENZOYL HYDRAZONE DERIVATIVES

Compound codes	Pharmacokinetics								
	GI abs.	BBB pen.	P-gp sub.	Inhibitor					Log Kp (skin permeation, cm/s)
				CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4	
HB1	Low	No	No	Yes	No	No	No	No	-7.69
HB2	Low	No	No	Yes	No	No	No	No	-7.90
HB3	Low	No	No	Yes	No	No	No	No	-8.36
HB4	Low	No	No	Yes	No	Yes	No	No	-7.45
HB5	Low	No	No	Yes	No	No	No	No	-7.85
HB6	Low	No	No	Yes	No	Yes	No	No	-7.55
HB7	Low	No	No	Yes	No	No	No	No	-8.53
HB8	Low	No	No	Yes	No	No	No	No	-8.67
HB9	Low	No	No	Yes	No	No	No	No	-8.67
HB10	Low	No	No	Yes	No	No	No	No	-9.13
HB11	Low	No	No	Yes	No	No	No	No	-7.52
HB12	Low	No	No	Yes	No	No	No	No	-7.89
HB13	Low	No	No	Yes	No	Yes	No	No	-7.69
HB14	Low	No	No	Yes	No	Yes	No	No	-9.13
HB15	Low	No	No	Yes	No	Yes	No	No	-7.85
HB16	Low	No	No	Yes	No	No	No	No	7.78
HB17	Low	No	No	Yes	No	No	No	No	-7.73
HB18	High	No	No	No	No	No	No	No	-6.77

Compound codes	Drug-likeness					
	Ghose	Egan	Muegge	Bioavailability score	Lead-likeness	Synthetic accessibility
HB1	Yes	No	Yes	0.55	Yes	2.78
HB2	Yes	No	No	0.11	No	3.00
HB3	Yes	No	No	0.17	No	3.04
HB4	Yes	No	Yes	0.55	No	2.90
HB5	Yes	No	No	0.55	No	2.92
HB6	Yes	No	No	0.55	No	3.36
HB7	Yes	No	No	0.11	No	3.54
HB8	Yes	No	No	0.11	No	3.42
HB9	Yes	No	No	0.11	No	3.39
HB10	No	No	No	0.17	No	3.42
HB11	Yes	No	Yes	0.55	No	2.90
HB12	Yes	No	No	0.55	No	3.06
HB13	Yes	No	No	0.55	No	3.22
HB14	No	No	No	0.17	No	3.46
HB15	Yes	No	No	0.55	No	2.92
HB16	Yes	No	No	0.55	No	2.95
HB17	Yes	No	Yes	0.55	No	2.91
HB18	Yes	Yes	Yes	0.55	Yes	2.61

(GI: Gastrointestinal; BBB: Blood brain barrier; CYP: Cytochrome P enzymes)

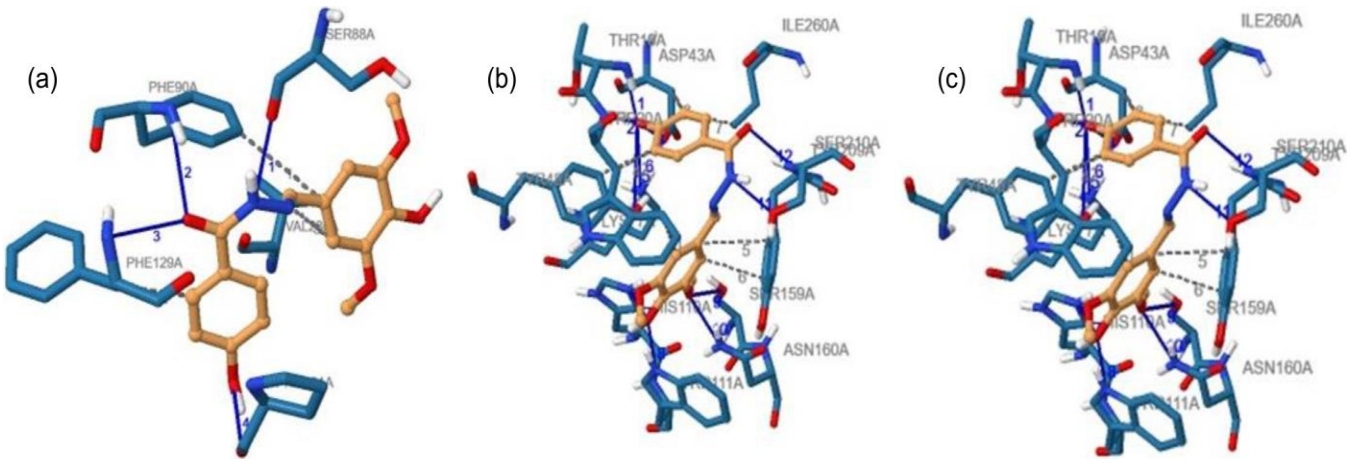


Fig. 2. Docked pose of HB18 with amino acids in the binding site with: (a): 5NN(b): 4LBS (c): 6MS7

TABLE-4
RESULTS OF *in vitro* ANTIOXIDANT ACTIVITY

Concentration (µg/mL)	Inhibition (%)	
	HB18	Ascorbic acid
20	29.68 ± 0.51	30.47 ± 0.22
40	45.32 ± 0.29	50.63 ± 0.87
60	58.91 ± 0.48	63.14 ± 0.42
80	63.75 ± 0.33	70.89 ± 0.68
100	70.84 ± 0.65	81.72 ± 0.61
120	83.27 ± 0.19	90.45 ± 0.39
IC_{50}	22.13 ± 0.41	21.26 ± 1.58

*All values shown as mean ± SD (n = 6); Values are given as mean ± SEM (n = 6)

glucose levels over 28 days, compared to diabetic controls (312.01 ± 3.12 mg/dL). The 400 mg/kg dose lowered glucose to 126.21 ± 3.79 mg/dL, while the 200 mg/kg dose reduced it to 187.95 ± 2.77 mg/dL by day 28, both effects being statistically significant ($p < 0.05$). Although the reduction was slightly less than the standard drug glibenclamide (118.47 ± 2.68 mg/dL), HB18 showed promising dose-dependent antihyperglycemic activity.

Moreover, compound **HB18** improved the lipid profiles, which is important as diabetes is often accompanied by dyslipidemia. The compound significantly lowered total cholesterol (131.88 ± 2.67 mg/dL at 400 mg/kg *vs.* 212.89 ± 2.61 mg/dL in diabetic controls), triglycerides (77.53 ± 4.12 *vs.* 140.12 ± 3.67 mg/dL), VLDL and LDL levels, while increasing the HDL levels (25.69 ± 2.71 mg/dL *vs.* 8.79 ± 0.95 mg/dL in diabetic controls). This indicates that compound **HB18** not only regulates glucose but also mitigates diabetic dyslipidemia, contributing to the cardiovascular protection. The results of *in vivo* antidiabetic activity and lipid profiles of Albino Wistar rats treated with compound **HB18** are shown in Tables 6 and 7, respectively.

Conclusion

This study designed, synthesized and evaluated a series of benzoyl hydrazone derivatives as potential multi-target antidiabetic agents, identifying 4-hydroxy-3,5-dimethoxybenzaldehyde 4-hydroxybenzoyl hydrazone (**HB18**) as the lead compound. Compound **HB18** showed the strong binding affinities toward α-glucosidase, aldose reductase and PPAR-γ, along with favourable pharmacokinetic and drug-likeness

TABLE-5
ACUTE ORAL TOXICITY STUDIES OF COMPOUND **HB18**

Compound	Body weight (g) Rat (n = 5)	Mortality (animal died)			Toxicity profile
		After 24 h	After 7 days	After 14 days	
HB18	143 ± 9.89	0	0	0	Safe

TABLE-6
In vivo ANTIDIABETIC POTENTIAL OF COMPOUND **HB18**

Group	Treatment	Blood glucose (mg/dl) levels on different days				
		0	7	14	21	28
Normal control	Vehicle (normal saline)	95.13 ± 1.28	96.42 ± 1.21	94.11 ± 0.42	**95.23 ± 0.41**	97.14 ± 0.39
Diabetic control	STZ 60 mg/kg, i.p.	284.76 ± 0.34[a]	312.54 ± 2.48[a]	309.78 ± 1.56[a]	**316.45 ± 2.68[a]**	312.01 ± 3.12[a]
Glibenclamide (5 mg/kg, po)	STZ 60 mg/kg, i.p + glibenclamide at 5 mg/kg	289.41 ± 2.67[a]	221.67 ± 2.79[b]	181.26 ± 3.65[b]	**147.92 ± 3.57[b]**	118.47 ± 2.68[b]
HB18-200	STZ 60 mg/kg, i.p + **HB18** at 200 mg/kg	293.57 ± 1.89[a]	237.34 ± 3.63[b]	191.94 ± 2.71[b]	**172.16 ± 0.49[b]**	147.95 ± 2.77[b]
HB18-400	STZ 60 mg/kg, i.p + **HB18** at 400 mg/kg	287.62 ± 3.48[a]	223.41 ± 3.87[b]	185.18 ± 3.92[b]	**155.34 ± 3.88[b]**	126.21 ± 3.79[b]

*All results are shown as mean ± SD (n = 6); [a]$p \leq 0.05$ in comparison to animals treated with a vehicle (Group 1); [b]$p \leq 0.05$ in comparison to animals with diabetes (group 2).

TABLE-7
LIPID PROFILES OF ALBINO WISTAR RATS TREATED WITH COMPOUND **HB18**

Group	Treatment	Lipid profile (mg/dL)*				
		Total cholesterol	Triglycerides	VLDL	LDL	HDL
Normal control	Vehicle (normal saline)	81.22 ± 3.45	54.47 ± 2.38	15.87 ± 0.52	46.12 ± 1.54	39.15 ± 2.67
Diabetic control	STZ 60 mg/kg, i.p.	212.89 ± 2.61	140.12 ± 3.67	66.34 ± 2.03	112.07 ± 0.31	8.79 ± 0.95
Glibenclamide (5 mg/kg, po)	STZ 60 mg/kg, i.p + glibenclamide at 5 mg/kg	113.57 ± 1.24[c]	90.21 ± 2.83[c]	24.15 ± 1.77[c]	53.09 ± 3.02[c]	31.02 ± 2.55[c]
HB18-200	STZ 60 mg/kg, i.p + **HB18** at 200 mg/kg	137.26 ± 3.49[c]	83.45 ± 3.76[c]	32.71 ± 4.59[c]	65.47 ± 2.75[c]	19.48 ± 3.81[c]
HB18-400	STZ 60 mg/kg, i.p + **HB18** at 400 mg/kg	131.88 ± 2.67[c]	77.53 ± 4.12[c]	27.04 ± 1.22[c]	52.11 ± 2.96[c]	25.69 ± 2.71[c]

*All values shown as mean ± SD (n = 6); Values are given as mean ± SEM (n = 6); [a]$p < 0.01$, [b]$p < 0.01$, [c]$p < 0.001$ compared to diabetic control (one-way ANOVA followed by a Dunnett's t test). (VLDL: very low-density lipoproteins; LDL: low density lipoproteins; HDL: High density lipoproteins).

profiles, including compliance with Lipinski's rule of five, high gastrointestinal absorption and low predicted toxicity. Structural characterization confirmed successful synthesis, *in vitro* antioxidant assays revealed potent free radical scavenging activity comparable to ascorbic acid and acute toxicity studies demonstrated safety at tested doses in Wistar rats. *In vivo* evaluation in streptozotocin-induced diabetic rats showed significant reductions in fasting blood glucose and improved lipid profiles in a dose-dependent manner, comparable to standard glibenclamide. These results highlight the potential of compound **HB18** as a safe and multifunctional antidiabetic agent. However, further studies on chronic toxicity, comparative efficacy, structure-activity relationships and clinical validation are necessary to fully establish its therapeutic value.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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