Research Article

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Antioxidant and antidiabetic potentials of methoxy-substituted Schiff bases using *in vitro*, *in vivo*, and molecular simulation approaches

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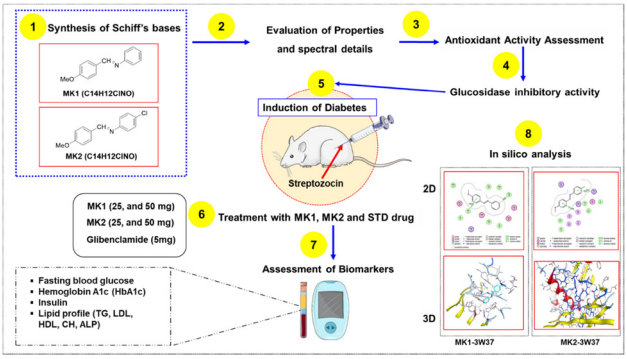
Abstract: The current study attempted to synthesize methoxy-substituted Schiff's bases, namely MK1 and MK2, and evaluate their antidiabetic effects using *in vitro*, *in vivo*, and molecular docking studies. Experimental animals (rat model) received the synthetic compounds, MK1 and MK2, orally in doses of 25 and 50 mg/kg body weight, respectively. When comparing compound MK2 at the tested doses to glibenclamide on day 28, the diabetic rats' blood glucose levels were nearly normal (139.02 and 121.23 mg/dL at 25 and 50 mg/kg body weight doses). The IC_{50} for MK1 against α-glucosidase inhibitory potential was found to be 281.29 μg/mL, while for MK2, it is reported to be 204.69 μg/mL. Furthermore, the acute toxicity, lipid profile, and its effect on blood biochemical parameters were also examined. In addition, through *in silico* analysis, the binding of MK1 and MK2 was elucidated with α-glucosidase enzyme, showcasing its antidiabetic mechanism at molecular levels. The *in silico* studies also predicted the two compounds to be inactive toward the human hERGs cardiac potassium channel, which indicates no potential risk of cardiac toxicity. Overall, the toxicity predictions suggest that compounds MK1 and MK2 are non-toxic and non-carcinogenic.



Graphical abstract

Keywords: Schiff's bases, antidiabetic potential, diabetes, glibenclamide

1 Introduction

Diabetes mellitus (DM), one of the metabolic illnesses, is characterized by a persistent increase in the level of blood glucose [1]. This is associated with the interruption of the metabolism of carbohydrates, proteins, and lipids. This variation in metabolism arises due to the low amount of insulin production from the pancreas or because the body does not use the insulin produced [2]. It ranks among the top ten causes of adult mortality, accounting for an estimated four million deaths globally in 2023 [3]. The biomarkers of diabetes include fasting blood sugar, hemoglobin A1c (HbA1c), insulin, β-cell function markers (e.g., HOMA-B), triglycerides (TG), lipid profile, and inflammatory

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biomarkers such as C-reactive protein [4,5]. These markers are essential for the diagnosis, monitoring, and management of diabetes, providing insights into blood sugar levels, insulin production, and overall metabolic health [6]. The most common types of DM include type 1 DM, type 2 DM, and gestational DM [7]. To effectively manage diabetes, oxidative stress and hyperglycemia must be addressed, as these conditions worsen diabetic complications. For the onset and progression of diabetes, oxidative stress, which is characterized by an excess of reactive oxygen species (ROS) generation and a deficiency of antioxidant defense, is essential [8]. By neutralizing ROS, antioxidants can reduce oxidative stress and prevent damage to biological components. While designing treatment plans to combat diabetes, combinations with antioxidant and antidiabetic properties are crucial [9].

The main groups of antidiabetic drugs are thiazolidinedione, dipeptidyl peptidyl 4 inhibitors, meglitinide, biguanides, sulfonylureas, and sodium-glucose cotransporter inhibitors [10]. However, the management of diabetes without experiencing negative side effects, even with the development of various synthetic oral medications, poses a major challenge [11]. Research on complementary medicines, synthesis, and natural therapies has expanded, and the search for more potent synthetic or semisynthetic compounds with fewer side effects has sparked a renewed wave of scientific interest in standard approaches [12]. Chemical compounds (imines) having a hydrocarbyl group on the nitrogen atom R2C = NR (R ≠ H) are known as Schiff bases. These Schiff bases can be interchangeable with azomethines. They are known as auxiliary ligands instead of reactive ligands because Schiff bases can modify the structure and reactivity of the transition metal ion in the center of the complex without undergoing any irreversible changes of their own [13]. To date, various pharmacological activities are documented, showcasing their role in many clinical applications, for instance, antibacterial [14], antifungal [15], herbicidal [16], anticancer [17], anthelmintic [18], antiviral [19], anti-HIV [20], antiprotozoal [21], anticonvulsant [22], and anti-inflammatory activity [23]. Because of their potential for functional modification, ease of synthesis, and diversity of structures, they are a dynamic option in medicinal chemistry. Methoxy substitution in Schiff bases is important because the electron-donating nature of the methoxy group influences their antioxidant potential [24]. Schiff bases of pioglitazone have shown improved antidiabetic and antioxidant properties and potential hints for dual therapeutics [25]. Likewise, Schiff bases of chitosan-quinoline have also significant increase in their antidiabetic and antioxidant potential [26]. Multiple studies have shown the antidiabetic potential of different types of Schiff bases [27]. Conversely, not much is known about the combined antidiabetic and antioxidant properties of methoxy-substituted Schiff bases [28]. The discovery of methoxy-substituted Schiff bases having both properties could lead to the development of new diabetes treatments. Molecular simulations can further provide detailed insights into the mechanism of action of these Schiff bases. A study comprising a comprehensive assessment of combined antioxidant and antidiabetic potential and their therapeutic relevance is necessary through *in vitro*, *in vivo*, and molecular docking analyses.

Keeping in view the immense potential of Schiff bases, in the present study, we have attempted to formulate methoxy Schiff bases and evaluated them for their antioxidant and antidiabetic activities. The antioxidant potential was evaluated *in vitro,* and their antidiabetic potential was evaluated *in vivo* by assessing their effect in the management of hyperglycemia in streptozocin (STZ)-induced diabetic rats. The synthesized compounds were designated as (*E*)-*N*-(4-methoxybenzylidene) benzenamine (MK1) and (*E*)-*N*-(4-methoxybenzylidene)-4-chlorobenzenamine (MK2), which were characterized by physicochemical characteristics as defined in the literature. These compounds were then used for testing their antidiabetic potential. The synthesized compounds and standard drug glibenclamide were orally administered to different groups of rats, including some groups with induced diabetic rats. Furthermore, the acute toxicity, lipid profile, and its effect on blood biochemical parameters were also examined. In addition, through *in silico* analysis, the binding of MK1 and MK2 was elucidated with α-glucosidase enzyme, showcasing its antidiabetic mechanism at molecular levels.

2 Materials and methods

2.1 Synthesis of (*E*)-*N*-(4-methoxybenzylidene) benzenamine (MK1) and (*E*)-*N*-(4-methoxybenzylidene)-4-chlorobenzenamine (MK2)

Schiff's bases share the azomethine group, which has the generic formula RHC = N-R1, where R and R1 are variously substitutable cycloalkyl, heterocyclic, alkyl, or aryl groups [29]. These substances are sometimes referred to as azomethines, imines, or anils. Several studies have demonstrated the significant chemical and biological significance of a single pair of electrons in a sp2 hybridized orbital of the nitrogen atom in the azomethine group [30]. Schiff bases are generally good chelating agents due to their unique C═N group characteristic, relative ease of

synthesis, and synthetic flexibility, particularly when a functional group such as −OH or −SH is close to the azomethine group so that it can combine with the metal ion to form a five- or six-membered ring [31]. Keeping in view the above nature, Schiff bases MK1 were synthesized by treating 4-methoxy benzaldehyde with aniline in the presence of sodium hydroxide at a temperature of 70°C in ethanol by reflux condensation for 5–12 h. For the synthesis of MK2, 4-methoxy benzaldehyde was treated with 4-chloroaniline in the presence of sodium hydroxide at a temperature of 70°C in ethanol by reflux condensation for 5–12 h [32,33].

2.2 *In vitro* antioxidant activity

Following the published protocol, the antioxidant potentials of the MK1 and MK2 were evaluated utilizing 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals. To make a 0.04 mg/mL methanolic DPPH solution, 4 mg of DPPH was dissolved in 100 mL of methanol. Methanol was used to produce stock solutions of MK1 and MK2 samples, as well as standard solution (ascorbic acid). About 03 mL of methanolic DPPH was added to each concentration of stock solutions of samples and standard solution in different concentrations (50, 100, 150, and 200 µg/mL); the mixture was then incubated for 30 min at room temperature in a dark setting. A spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) was used to detect the absorbance at 517 nm. An ascorbic acid standard was employed, while methanol served as the blank. A methanol auto-zero was developed for the UV spectrophotometer [34]. Standard tocopherol and its synthetic analogs (MK1 and MK2) were combined with the DPPH solution at various concentrations (µg/mL). The free radical scavenging activity was evaluated, and the IC_{50} values were determined.

2.3 α-Glucosidase inhibitory activity

The α-glucosidase inhibitory activity of the compounds (MK1 and MK2) was evaluated by completely mixing 20 µL of α-glucosidase (0.5 unit/mL), 120 µL of 0.1 M phosphate buffer at pH 6.9, and 10 µL of MK1 and MK2 at varying doses. Following that, the mixture was maintained at 37°C for 15 min in an incubator. The combination solution was then mixed with 20 µL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer at pH 6.9. This made it possible for the enzymatic reaction to start. The absorbance was calculated at 405 nm. Following the measurement of α-glucosidase activity, the estimated IC_{50} values were determined [35].

2.4 *In vivo* studies

2.4.1 Animals

Rats weighing between 165 and 190 g were brought to the University of Malakand from the NIH Islamabad. Mice and rats were housed in a 25°C ± 2 environment with a light/dark (16/8) cycle. As stated in the literature, the process was conducted in accordance with the UK's Animal Scientific Process Act (1986) [36].

2.4.2 Acute toxicity study

MK1 and MK2 rats (07 groups, each comprising four animals) were used to determine acute toxicity. Following the techniques of Hussain et al., with some modifications, the tests were carried out in two stages. During each phase, the experimental groups were given MK1 and MK2 orally at doses of 25 and 50 mg/kg body weight, respectively, whereas the control group received normal saline [34].

2.4.3 Administration of STZ

Before inducing diabetes, each animal was fasted for the entire night. Fresh STZ was produced in 0.1 mole of pH 4.5 citrate buffer. A single intraperitoneal dose of 50 mg/kg body weight of STZ was administered. When the animals' blood glucose level exceeded 250 mg/dL, they were classified as diabetic [35].

2.4.4 Animals grouping and dosing

The animal groups constituted and doses given are summarized in Table 1.

Table 1: Animal's grouping and dosing details

S. no.	Group	No. of rats	Treatment	Dose (mg)	Route of drug administration
1	Control	04	Normal saline	10	PO
2	Diabetic	04	Normal saline	10	PO
3	Diabetic	04	MK-1	25	PO
4	Diabetic	04	MK-1	50	PO
5	Diabetic	04	MK-2	25	PO
6	Diabetic	04	MK-2	50	PO
7	Diabetic	04	Standard drug (glibenclamide)	5	PO

2.4.5 Blood profile

Assessment of glucose level and body weight was carried out on the 29th day of the experimental cycle. Animals were sacrificed with the help of isoflurane given through the cardiac puncture for the collection of blood samples for the evaluation of *ex-vivo* parameters.

2.4.6 Assessment of serum profile

Many biochemical parameters, for example, low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC), alkaline phosphatase (ALP), TG, and insulin level were determined.

2.5 *In silico* studies

Molecular docking is a computational technique used to understand the complex structures formed by the interaction of multiple molecules. Its main goal is to predict the three-dimensional (3D) configuration of a ligand within the binding site of a target receptor protein. This method plays a crucial role in drug discovery and has quickly become an essential tool in modern drug design [37]. For our docking study, we selected the α-glucosidase enzyme as a receptor. We used the crystal structure of sugar beet α-glucosidase (PDB: 3W37), which was retrieved from the Protein Data Bank (https://www.rcbs.org). The protein was prepared using Molecular Operating Environment (MOE) software 2022 [38]. Initially, water molecules and attached ligands were removed using the MOE SEQ window. Missing amino acid residues were identified and repaired using Modeler software [39]. Any residual structural deficiencies were adjusted through the MOE structural preparation window. The 3D structures were protonated, and partial charges were assigned by the Gasteiger (PEOE) forcefield. The energy of the protein was minimized using the Amber14 forcefield precisely designed for amino acids and proteins. Active sites were recognized by applying the site finder tool integrated into MOE software. After preparation, the protein was obtained in a ready-to-dock format. Similarly, using ChemDraw software, structures of methoxy-substituted Schiff bases (MK1 and MK2) were drawn and stored in mol format [40]. Later, MOE was used to convert these structures to PDB format. They underwent an energy minimization process prior to molecular docking analysis, and the MOE then transformed them into sdf format.

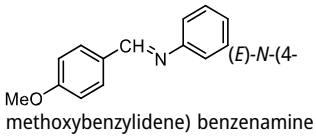
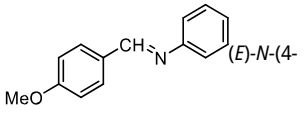
Part of the drug development process involves predicting a possible drug candidate's physicochemical, pharmacological, toxicological, and pharmacokinetic characteristics. A compound's druggability can be estimated using these factors. There are predictions regarding the possible drug candidates' toxicity, water solubility, lipophilicity, compliance with Lipinski's criteria, topological polar surface area (TPSA), and blood–brain barrier (BBB) penetration [41]. *In silico* models were utilized to predict the toxicity and absorption, distribution, metabolism, and excretion (ADME) of the chosen methoxy-substituted Schiff bases (MK1 and MK2). The SwissADME service predicted pharmacokinetics, drug-likeness (Lipinski's rules), physicochemical characteristics, lipophilicity (log Po/*w*), water solubility (log *S*), and pharmacokinetics [42]. Toxicity was predicted online by the pkCSM [43] and ProTox 3.0 online servers [44].

3 Results and discussion

3.1 Properties and spectral details of the synthesized compounds

In order to make Schiff's bases MK1, 4-methoxy benzaldehyde was treated with aniline in the presence of sodium hydroxide at a temperature of 70°C in ethanol by reflux condensation for 05–12 h. In order to produce MK2, 4-methoxy benzaldehyde was reflux-condensed for 05–12 h at a temperature of 70°C in ethanol with the presence of 4-chloroaniline. The synthesized compounds MK1 and MK2 were obtained with a good yield and purity. Their physical properties, such as appearance, solubility, melting point, and r.f., are detailed in Table 2. The spectral data for MK1 and MK2 match previous literature reports, confirming their identity. The reported ^{1}H NMR ($CDCl_3$, 400 MHz) for MK1 is 8.37 (s, 1H, N═CH), 7.72 (s, 2H, Ar-H), 7.23–7.70 (m, 7H, Ar-H), 3.84 (s, 3H) and MK2 is 8.35 (s, 1H, CH═N), 7.85–7.83 (d, J = 8.8 Hz, 2H, Ar-H), 7.35–7.33 (d, J = 8.8 Hz, 2H, Ar-H), 7.14–7.12 (*d*, J = 8.7 Hz, 2H, Ar-H), 7.00–6.97 (*d*, J = 8.8 Hz, 2H, Ar-H), 3.88 (s, 3H, OCH_3) [45,46]. In the literature, various methods are followed to synthesize Schiff bases; for instance, the synthesis of two Schiff bases, 2-[(2-hydroxynaphthalen-1-ylmethylene)-amino], has been described by Neelofar et al. They reacted 2-hydroxy-1-naphthaldehyde and L-histidine combine to produce 3-(1*H*-imidazol-4-yl)-propionic acid (HNLH), and 4-[(2-hydroxynaphthalen-1-ylmethylene)-amino] *N*-(4methyl-pyrimidin-2-yl)-benzenesulfonamide (HNSM) is produced when 2-hydroxy-1-naphthaldehyde and sulfamethazine condensed [47]. The Schiff bases SP-5 (benzylidene aniline) and SP-18 (benzylidene urea) were produced by Wahab et al. by condensation of benzaldehyde with aniline and urea in the presence of natural acid that was derived from tamarind and lemon [48].

Table 2: Physical parameters of the synthesized compounds

Comp	Structure	Mol. formula	Appearance	Molecular weight.	Yield%	Solubility	m.p. (°C)	r.f.	Ref.
MK1	 (*E*)-*N*-(4-methoxybenzylidene) benzenamine	$C_{14}H_{13}NO$	Yellowish white solid	211.26	768.1	Chloroform, methanol	64–68	0.57	[51,52]
MK2	 (*E*)-*N*-(4-methoxybenzylidene)-4-chlorobenzenamine	$C_{14}H_{12}ClNO$	White solid	245.71	74.2	Chloroform, methanol	119–121	0.66	[53]

The condensation reaction of chitosan/O-CMC with nitro- and chloro-substituted salicyl aldehydes has been reported to yield six distinct chitosan-based Schiff bases [49]. Another study attempted to synthesize Schiff base (cobalt(III) chelate with tridentate azomethine ligand containing a benzimidazole moiety) by the reaction of *o*-aminophenol with 2-acetylbenzimidazole and used to prepare the cobalt(III) complex $[CoL_2]_2(ClO_4)_2{\cdot}3H_2O$ (I) [50].

3.2 Antioxidant activity

The antioxidant potentials of MK1 and MK2 were assessed through the DPPH scavenging assay. The IC_{50} for MK1 against DPPH potential was found to be 131.67 µg/mL, while for MK2, it is reported to be 112.04 µg/mL (Table 3). The inhibitory activity of tocopherol in terms of IC_{50} is 15.92 µg/mL. MK2 indicates higher antioxidant activity compared to MK1. When the relevant literature is examined, it can be observed that Schiff bases exhibit antioxidant potentials. For example, a study evaluated the antioxidant activity of several resveratrol analogs, such as *p*-(*N*,*N*-dimethyl)aminobenzylidene-2-aminothiophenol, *p*-nitrobenzylidene-2-aminothiophenol, and 4′-hydroxyphenyl-benzo[d]thiazole [54]. These analogs were synthesized, characterized, and their antioxidant activity was assessed. It has been found that chitosan derivatives with Schiff bases have better antifungal and antioxidant properties. Many chitosan derivatives containing Schiff bases were produced in a recent study. This study assessed the antioxidant properties of chloracetyl chitosan oligosaccharide derivatives grafted with pyridine-4-aldehyde Schiff bases against O^{2-} and the $DPPH^{\cdot}$ radical by performing structural characterization [55]. A study employed various techniques, such as the FRAP and CUPRAC reduction methods and the ABTS and DPPH radical scavenging methods, to demonstrate the effective antioxidant activity of the Co^{2+} and Fe^{2+} complexes of Schiff bases [56]. Profound antioxidant properties were also demonstrated by certain Schiff base ligands ((*E*)-6-methyl-2-(2,3,4-trimethoxybenzylideneamino)-4,5,6,7-tetrahydrobenzo[b]-thiophene-3-carbonitrile) and associated Co^{2+} and Pd^{2+} complexes [57].

3.3 Glucosidase inhibitory activity

The antidiabetic potentials of MK1 and MK2 are summarized in Table 4. The IC_{50} for MK1 against α-glucosidase inhibitory potential was found to be 281.29 µg/mL, while for MK2, it is reported to be 204.69 µg/mL. The inhibitory activity of α-glucosidase for acarbose in terms of IC_{50} is 46.81 µg/mL. Studies have shown that the Schiff base's structural backbone determines the extent to which

Table 3: Antioxidant potentials of the synthesized compounds in terms of their IC_{50} values

Sample	DPPH IC_{50} (µg/mL)
MK1	131.67
MK2	112.04
Tocopherol	15.92

Table 4: Anti-glucosidase potentials of the synthesized compounds in terms of their IC_{50} values

Sample	Glucosidase IC_{50} (µg/mL)
MK1	281.23
MK2	204.69
Acarbose	46.81

substitution inhibits α-glucosidase. In comparison to their unsubstituted derivatives, the enzymatic inhibition improved to varying degrees upon substitution of the Schiff bases by either the hydroxy or nitro group [58]. The hydroxylation reaction is most likely the cause of the extremely low IC50 values that the *o*-hydroxyl derivatives (2, 5, and 8) showed when it came to the inhibition of α-amylase and α-glucosidase. Comparing the *p*-nitro Schiff bases (7–9) to their unsubstituted counterparts, the inhibitory actions of the former demonstrated increased activity against the digestive enzymes, primarily due to glycosylation of the functional unit. The only deviation was Schiff base (1)'s decreased inhibitory efficacy against α-amylase, which may have resulted from its stereochemical arrangement [59]. Due to the presence of a sulfonic acid moiety that altered the enzymes' ideal operating pH, Schiff bases (7–9) exhibited the least inhibitory action (IC_{50} values 4.20 × 102 ± 0.34 to 9.61 × 102 ± 0.84), as reported in the study by Ahmed et al. [60]. The 89.8% suppression of α-amylase and the 88.81% inhibition of hemoglobin in the niosomal formulation's *in vitro* antidiabetic potential were both significantly higher than the levels attained with acarbose, indicating antidiabetic effectiveness. Protein structural changes in hemoglobin and α-amylase were discovered by interaction binding tests between phytoniosomes and extract. Based on the polyphenol analysis in the study by Imtiaz et al., *Tradescantia pallida* leave extract and niosomes contained well-established antidiabetic active substances [61].

3.4 *In vivo* antidiabetic studies

3.4.1 Effect on the blood glucose level

In the *in vivo* experiments, 25 and 50 mg/kg body weight of the complexes were utilized, which was 1/10th of the maximal acceptable dose according to OCED guidelines, since the acute toxicity research results showed that 500 mg/kg body weight was classified as non-toxic with no discomfort. Table 5 makes it clear that, immediately following 30, 60, 90, and 120 min after the compounds in tested quantities were administered, there is no apparent distinction between the blood glucose levels of the treatment group and the normal control group.

After oral delivery, the glucose level in the MK1- and MK2-treated groups appeared to be lower than in the control group. The blood glucose levels of group 3 and 4 animals were recorded as 463.67 and 459.98 mg/dL after 07 days of receiving a dosage of MK1 at a rate of 25 and 50 mg/kg body weight (bw). A notable drop in the glucose level was noted on day 14 (323.7 and 291.47 mg/dL), 21 (236.65, 201.18 mg/dL), and 28 (175.47 and 146.82 mg/dL). The total drop in the blood glucose level by MK1 (25 and 50 mg/kg) from day 01 to 28 was 288.2 and 313.16 mg/dL, respectively. Similarly, the blood glucose levels of group 05 and 06 animals receiving MK2 at doses of 25 and 50 mg/kg bw were recorded for 28 days. On days 7 and 14, the blood glucose level was recorded as 379.15 and 298.17 mg/dL (receiving MK2 at a dose of 25 mg/kg), while 359.95 and 294.93 mg/dL (receiving MK2 at a dose of 25 mg/kg), respectively. The total drop in blood glucose level from day 01 to 28 was 332.03 (receiving MK2 at a dose of 25 mg/kg) and 343.1 mg/dL (receiving MK2 at a dose of 50 mg/kg). Group 7 received glibenclamide (standard drug) at a dosage of 5 mg/kg bw, and blood glucose level dropped to 328.42 mg/dL after 07 days. After 14 and 21 days, blood glucose level was lowered to 265.25 and 156.12 mg/dL, with a total reduction of 304.36 mg/dL after 21 days. After the initiation of the experiment, the blood glucose level dropped to 11.67 mg/dL, indicating a total reduction of 348.81 mg/dL since the administration of the standard drug.

Overall, glibenclamide and MK2 have shown the best activity among these compound-based blood glucose lowering effects in tested models with a total reduction of

Table 5: Blood glucose levels (mg/dL) of rats treated with various drug agents at different concentrations

Days		NC	DC	MK1 (25 mg)	MK1 (50 mg)	MK2 (25 mg)	MK2 (50 mg)	STD
Day 1	Mean	103.32	459.15	463.67	459.98	471.05	464.33	460.48
	SEM	2.18	7.19	7.32	6.61	5.84	7.00	6.31
Day 7	Mean	98.62	477.13	406.37	365.83	379.15	359.95	328.42
	SEM	2.57	6.20	4.24	5.23	5.33	4.80	4.41
Day 14	Mean	102.62	469.05	323.77	291.47	298.17	294.93	265.25
	SEM	2.36	6.26	5.32	4.06	4.04	3.53	3.47
Day 21	Mean	103.83	461.57	236.65	201.18	202.70	177.53	156.12
	SEM	3.60	6.46	3.65	3.16	3.65	2.13	2.99
Day 28	Mean	100.55	454.63	175.47	146.82	139.02	121.23	111.67
	SEM	2.09	6.34	4.24	3.47	4.76	4.20	2.82

Table 6: Effect of various drug agents at different concentrations on body weight of STZ-induced diabetic rats

Days		NC	DC	MK1 (25 mg)	MK1 (50 mg)	MK2 (25 mg)	MK2 (50 mg)	STD
Day 1	Mean	179.72	180.65	175.43	176.42	177.98	175.42	176.08
	SEM	2.56	2.02	3.78	2.13	2.94	1.92	2.91
Day 28	Mean	174.72	149.50	165.15	164.98	167.32	168.13	170.53
	SEM	2.62	2.88	3.37	2.41	3.61	2.69	2.16

332.03 (receiving MK2 at a dose of 25 mg/kg), 343.1 mg/dL (receiving MK2 at a dose of 50 mg/kg), and 348.81 mg/dL (receiving glibenclamide as standard drug).

3.4.2 Effects on body weight

Table 6 presents the impact of diabetes on body mass and the contribution of chemical compounds to its recovery. The animals in the diabetic group showed a notable reduction in weight. However, in comparison to the standard group, the MK1 and MK2 sets have significantly helped the body recover from the weight loss associated with diabetic conditions. After 14 days of analysis, the diabetic group's weight was 180.65 g, indicating a weight loss due to STZ activity, compared to 179.72 g for the control group. At the first day, the weight of the JA1-cured animals was set at 175.43 and 176.42 g, with quantities of 25 and 50 mg/kg bw; these weights were rather close to the animals in the normal control group. MK2 has regularized the body weight to 177.98 and 175.42 g for the experimental group. The body weight of 176.08 g was normalized with glibenclamide, a standard antidiabetic drug, prescribed at a dose of 5 mg/kg bw.

According to the results obtained on the 28th day, the animals in the normal group weighed an average of 174.72 g, while the animals in the diabetic group weighed an average of 149.50 g. This indicates that diabetes has caused a significant reduction in body weight, which has been controlled by the MK2, MK1, and standard drugs. Similarly, MK2 at specified doses has increased the animals' weight by roughly 167.32 and 168.13 g, as noted for groups 5 and 6. Group 7 (glibenclamide group) weights were recorded as 170.53 g. Following dosages of 25 and 50 mg/kg body weight daily for 28 days (165.15 and 164.98 g, respectively), MK1 contributed to an increase in the weight of group 3 and 4 animals. Similarly, MK2 at specified doses has increased the animals' weight by roughly 167.32 and 168.13 g, as noted for groups 5 and 6. Group 7 (glibenclamide group) weights were recorded as 170.53 g.

3.4.3 Antihyperlipidemic effects

Table 7 lists the lipid profile of the STZ-induced diabetic animals after receiving various drug agents at different concentrations. TG, cholesterol, and LDL levels in the animals with diabetes are comparatively high compared to the corresponding normal group; however, there is a noticeable decrease in HDL in the diabetic group. MK1, MK2, and glibenclamide (standard drug) have normalized the levels of TG, cholesterol, and LDL and HDL. In STZ diabetic rats, MK1 and MK2 at doses of 25 and 50 mg/kg bw reduced the levels of CH, TG, and LDL, while at the same time, they improved the levels of HDL at the specified doses. The distinct effects on lipid profile factors have been pronounced per the given facts.

Table 7: Effects of various drug agents at different concentrations on the lipid profile of STZ-induced diabetic rats

Biomarker		NC	DC	MK1 (25 mg)	MK1 (50 mg)	MK2 (25 mg)	MK2 (50 mg)	STD
HDL	Mean	38.40	118.33	85.62	76.53	66.39	61.12	47.29
	SEM	1.77	5.87	4.79	4.20	2.94	3.69	4.00
LDL	Mean	66.22	37.23	44.04	49.33	47.58	48.89	58.19
	SEM	3.95	2.25	2.22	2.51	2.35	2.92	3.78
TG	Mean	105.30	215.92	145.15	129.01	130.05	118.15	109.73
	SEM	3.31	7.80	3.67	5.75	5.23	4.12	4.07
CH	Mean	59.25	205.72	102.46	91.94	87.21	74.42	64.28
	SEM	2.64	8.23	2.75	3.27	3.06	2.52	4.64
ALP	Mean	115.45	237.03	146.65	132.78	145.92	134.49	123.82
	SEM	5.11	11.15	4.79	3.79	3.37	2.49	5.53

3.4.3.1 Effects on blood cholesterol level

Blood cholesterol levels were reported to be 59.25 mg/dL in the control group and 205.72 mg/dL in the diabetes group (Table 7). The lower values experienced in distinction for the diabetic group would demonstrate the enhancing effects of the compounds, according to the groups given the compounds at the same time and also experiencing diabetes. Blood cholesterol levels were dropped to 102.46 mg/dL in group 3 after receiving MK1 treatment at a dose of 20 mg/kg bw, while group 4 received a higher dose of 50 mg/kg bw, which elevated blood cholesterol levels to 91.94 mg/dL. While the standard group's level was recorded at 64.28 mg/dL, MK2 in groups 5 and 6 reduced the CH level to 87.21 and 74.42 mg/dL at the tested levels. It was noted that MK2 had demonstrated notable activities among the validated compounds based on the values of lowering cholesterol levels in STZ-induced diabetic rat models.

3.4.3.2 Effects on LDL

In both the normal control and diabetic control groups, the LDL level rises with diabetes and is significantly higher than expected (66.22 mg/dL in group 1 and 37.23 mg/dL in group 2, Table 7). The hypolipidemic effects of MK1 therapy have been demonstrated at 44.04 md/dL and 49.33 mg/dL in groups 3 and 4, respectively, at doses of 25 and 50 mg/kg bw. MK2 nearly decreased in identical amounts, with values in group 5 and 6 animals being 47.58 mg/dL and 48.89 mg/dL, respectively. The level for the glibenclamide-treated group was found to be 58.19 mg/dL. MK2 appeared as the most potent compound among all of the tested drugs.

3.4.3.3 Effects on TG

Excessive intake of oil and DM are associated with elevated TG levels. At this point, Table 7 makes it clear that the animal in the normal control group has a TG level of 105.30 mg/dL, which has increased almost twofold in advance to the onset of diabetes (215.92 mg/dL). Following treatment with MK1 at dosages of 25 and 50 mg/kg body weight, groups 3 and 4 experienced an increase in TG levels to 145.15 and 129.01 mg/dL, respectively. The TG levels in Groups 5 and 6 increased to 130.05 and 118.15 mg/dL after receiving the same amounts of MK2. Glibenclamide-treated group (group 7) experienced a notable decrease in TG levels with a value of 109.73 mg/dL.

3.4.3.4 Effects on HDL

Diabetes typically causes a reduction in HDL levels, which are utilized as a diagnosis indicator. Table 7 shows that the HDL level in the normal control group is 38.40 mg/dL; however, in group 2 animals, the STZ booster caused the HDL level to drop to 118.33 mg/dL. Following treatment, the STZ diabetic rats in groups 3 and 4 showed improvement in this level, reaching 85.62 and 76.53 mg/dL, respectively. MK1 only had a small impact on improving the abnormal parameters. The HDL levels of the MK2-treated animals were increased to 66.39 and 61.12 mg/dL, respectively, as observed for the animals in groups 5 and 6. After receiving glibenclamide, the HDL level of the group 7 animals reached 47.29 mg/dL.

3.5 Effect on the level of HBA1c

According to Table 8, the HBA1c value for the normal control group was 4.54; however, the diabetes control group's value was 14.76. MK1 at doses of 25 and 50 mg was administered to groups 3 and 4, respectively. For 25 mg, the reported HBA1c level was 9.67 mmol/mol, whereas for 50 mg, it was 7.92 mmol/mol. Likewise, following treatment of groups 5 and 6 with MK2 at doses of 25 and 50 mg, respectively, the HBA1c levels were recorded as 7.98 and 7.51, and 5.07 mmol/mol for the standard. Lastly, for group 7 receiving the standard drug, the HBA1c levels were recorded as 5.07 mmol/mol.

3.6 Discussion on the key results obtained from *in vivo* studies

In this study, we investigated the antidiabetic effects of the administration of the two compounds MK1 and MK2 on glucose levels, body weight, lipid profile, and HBA1c homeostasis in the presence and absence of drug intervention in

Table 8: Effects of various drug agents at different concentrations on the HBA1c in STZ diabetic rats

Days		NC	DC	MK1 (25 mg)	MK1 (50 mg)	MK2 (25 mg)	MK2 (50 mg)	STD
HBA1c	Mean	4.54	14.76	9.67	7.92	7.98	7.51	5.07
	SEM	0.22	1.44	0.45	0.41	0.53	0.42	0.18

the STZ pre-diabetic rat model. Compounds MK1 and MK2 were given to diabetic rats at 25 and 50 mg/kg body weight in the *in vivo* antidiabetic trials. Over the course of 28 days, the rats' blood glucose levels significantly decreased. MK2 demonstrated a total reduction of 343.1 mg/dL, especially at 50 mg/kg, which was almost identical to the reduction of 348.81 mg/dL seen with the standard drug glibenclamide. Additionally, the compounds improved diabetes-induced body weight loss; groups treated with MK2 showed the strongest recovery. Besides, MK1 and MK2 showed strong antihyperlipidemic effects by considerably lowering cholesterol, TG, and LDL levels while raising HDL levels. Additionally, both substances successfully reduced HbA1c levels, demonstrating their general effectiveness in the management of diabetes and related metabolic disorders. Elevated HbAc1 levels are a long-term marker of pre-diabetes, a disease that frequently occurs before type 2 diabetes [62]. The current study supports previous research showing that, when compared to a conventional diet, the consumption of Schiff bases significantly decreased the incidence of diabetes and calorie intake in rats. The reduction in blood glucose and body fat remained subsequently maintained [25,63]. Reduced calorie intake and potential antidiabetic effect in mice models can be attributed to the observed decrease in body weight [64]. Another study found that administering the ruthenium complex in conjunction with dietary modification decreased calorie intake, indicating that this complex may have an activity via improving caloric intake [65]. Additionally, research indicates that metformin, a frequently prescribed drug for pre-diabetes, can lower calorie intake in obese pre-diabetic individuals, hence restoring their body weight. These were further supported by the findings of a recent study published in the literature [66]. In comparison to normal healthy mice, the diabetic control mice had remarkably higher levels of TC, TG, HDL, LDL, and VLDL, according to the biochemical examination of lipids. However, the treated groups exhibited reduced damage, particularly those treated with MK2, whose profile was comparable to that of the standard treated group. Insulin stimulates the lipoprotein–lipase enzyme, which facilitates the breakdown of TG into glycerol and fatty acids, consequently initiating the metabolism of lipids [25,63]. These fats are essential because they give the body energy and serve as a transporter for stored energy. Hypertriglyceridemia in diabetes is caused by the inactivation of the lipoprotein–lipase enzyme by insulin shortage or resistance. The movement of cholesterol from the liver to other tissues that may develop coronary heart disease is the cause of elevated LDL. Although HDL serves as a valuable lipoprotein and excellent cholesterol, it protects atherosclerosis by delivering endogenous cholesterol and cholesterol esters to the liver and steroidogenic cells. When compared to extract- and acarbose-treated mice, the study by Imtiaz et al. showed that niosome-treated alloxan-induced diabetic mice had normal lipid values [67].

3.7 Molecular docking studies

In the present study, the inhibition potential of the synthesized Schiff bases against α-amylase and α-glucosidase was evaluated through molecular docking to elucidate their mechanism of action and role in controlling hyperglycemia and management of diabetes. Compound MK1 exhibited a docking score of −5.22 kcal/mol and formed H–pi and pi–H interactions with residues TRP329 and ALA234, respectively, with 3W37. The two-dimensional (2D) and 3D interactions of MK1-3W37 are shown in Figures 1 and 2.

The digestive enzymes that catalyze the breakdown of carbohydrates into blood sugar can be identified as α-amylase and α-glucosidase. The small intestine's brush surface contains the enzyme α-glucosidase, which breaks down polysaccharides, whereas the salivary gland and pancreas release α-amylase, which converts starch and oligosaccharides into sugar [68]. By reducing the metabolism of carbohydrates, these enzymes' inhibition plays an essential role in regulating blood glucose levels. Nowadays, a number of antidiabetic medications, including acarbose, voglibose, and miglitol, are used in clinical practice. These medications work by decreasing the activity of the α-glucosidase enzyme, which interferes with the metabolism of carbohydrates and lowers the risk of postprandial hyperglycemia and hyperinsulinemia [69]. A recently reported study unveiled the presence of syringic acid, *p*-coumaric acid, morin, and catechin in *Tradescantia pallia* leaves and confirmed that these compounds exhibited significant antidiabetic potential by inhibiting α-amylase enzyme and non-enzymatic glycosylation of hemoglobin [70]. In several research attempts, the *in vitro* antidiabetic potential of the compounds is assessed using α-amylase and non-enzymatic glycosylation of hemoglobin protein assays. A mechanistic insight into interactions between the chemical compound, human α-amylase, and hemoglobin protein is scrutinized by employing the molecular docking method [71]. Additionally, hyperglycemia can cause non-enzymatic glycosylation of proteins, particularly hemoglobin, which is a diagnostic marker for type 2 diabetes. To control hyperglycemia associated with diabetes, researchers aim to inhibit α-amylase. A recent study by Imtiaz et al. attempted to assess the antidiabetic activity of phenolic compounds obtained from *T. pallida* through α-amylase enzyme inhibition assay. According to the study findings, syringic acid and *p*-coumaric acid exhibited 61 and

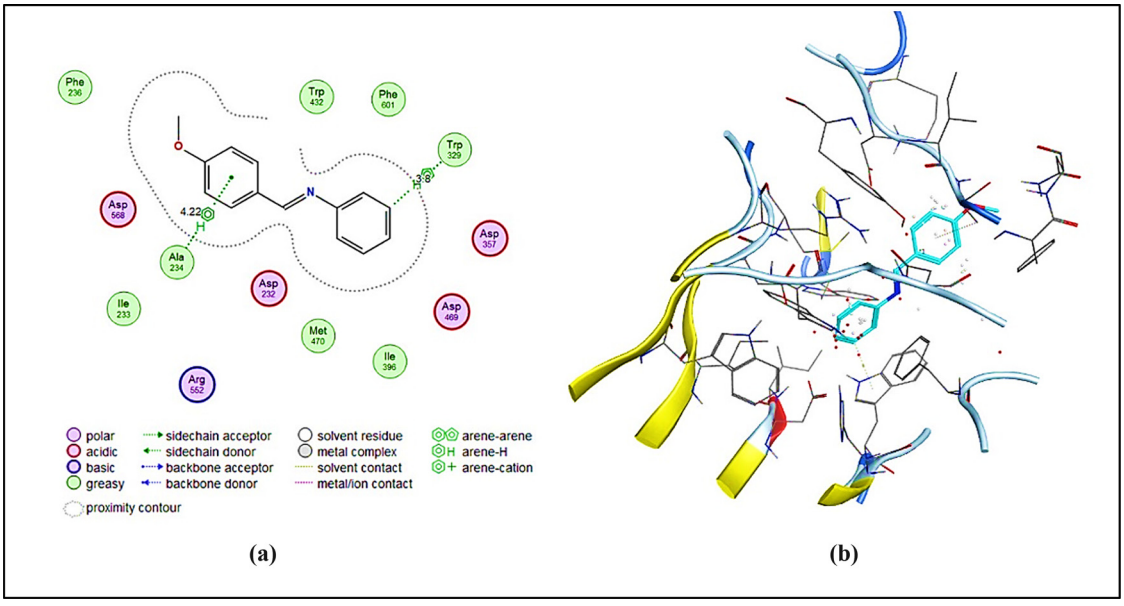


Figure 1: (a) 2D and (b) 3D interactions of MK1-3W37.

45% inhibition of α-amylase at 1 mg/mL, while morin and catechin showed 73.59 and 67.52% inhibition of α-amylase at 750 µg/mL [72]. By offering comprehensive insights into receptor–ligand interactions, energy compatibility, and binding modalities, molecular docking facilitates the identification of promising compounds and simplifies the drug development process [70].

Compound MK2 exhibited a binding energy of −5.73 kcal/mol and formed two interactions with 3W37. A pi–cation interaction was established between the aromatic ring of compound MK2 and the cation of residue ARG670 of the 3W37 enzyme. Similarly, a pi–H interaction was established between the second aromatic ring of compound MK2 and the hydrogen atom of the residue GLU792 of the 3W37 enzyme. The 2D and 3D interactions of MK2-3W37 are shown in Figure 2.

By forming interactions in the vicinity of the active site of the α-glucosidase enzyme, compounds MK1 and MK2 can hinder its normal function. The docking results suggest that the two compounds can act as possible α-glucosidase inhibitors by suppressing the absorption of carbohydrates from the small intestine (Table 9). Consequently, they might be assessed as antihyperglycemic drugs, which could

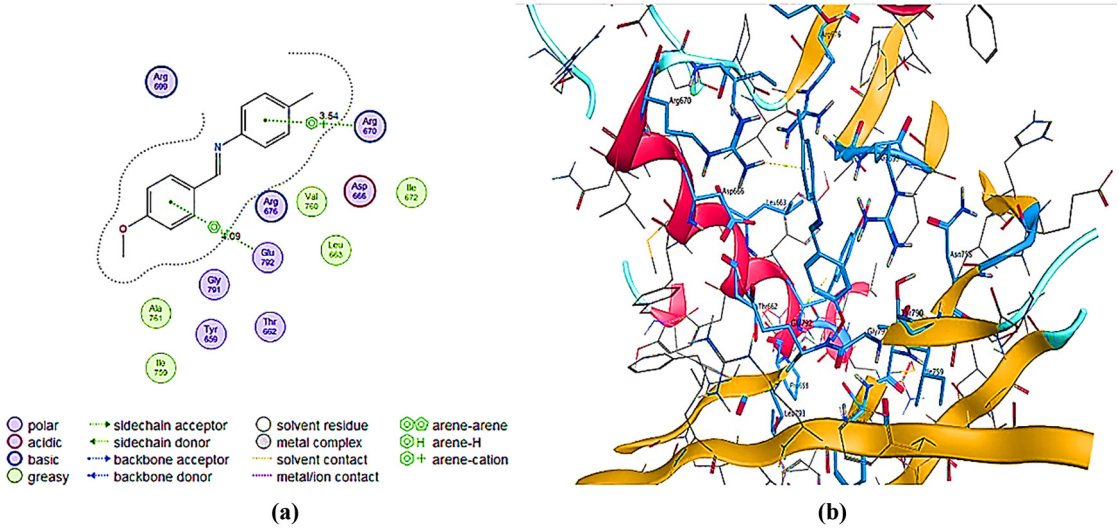


Figure 2: (a) 2D and (b) 3D interactions of MK2-3W37.

Table 9: Binding scores, RMSD values, receptor interactions, distances, and energies of methoxy-substituted Schiff bases (MK1 and MK2)

Ligand	Docking score (kcal/mol)	RMSD	Receptor interaction	Distance (Å)	*E* (kcal/mol)
MK1	−5.22	1.29	TRP329/H–pi	3.80	−1.3
			ALA234/pi–H	4.22	−0.5
MK2	−5.73	2.04	ARG670/pi–cation	3.54	−0.8
			GLU792/pi–H	4.09	−0.9

reduce blood sugar by delaying the breakdown and absorption of complex carbohydrates [73].

The predicted ADME results indicate that the two compounds obey Lipinski's rule of 5, as they did not show any violation of the rule. A molecule with a molecular mass less than 500 Da, no more than five hydrogen bond donors, no more than ten hydrogen bond acceptors, and a log *P* (lipophilicity measure) not greater than 5 are the requirements for an orally active drug candidate according to Lipinski's rule of 5 [74]. The TPSA values of both compounds were recorded to be in the optimum range (21.59 $Å^2$). According to the Veber rule, the optimal range of TPSA values is 0–140 $Å^2$ [75]. A key step in drug absorption is the breakdown of the capsule or tablet and its subsequent dissolution. Poor aqueous solubility is unfavorable to decent and thorough oral absorption. Therefore, the initial determination of water solubility is of great significance in drug discovery [76]. The predicted water solubility of a potential drug candidate is given as the logarithm of the molar concentration (log mol/L) and is denoted by log *S*. Drugs with aqueous solubility values in the range of −4 to 0.5 log mol/L are regarded as soluble [43]. In this context, the *in silico* results also confirmed favorable log *S* values of −3.26 and −3.80 for MK1 and MK2, respectively. Moreover, the gastrointestinal absorption of both compounds was predicted to be high.

The toxicity results indicate that both compounds were found to be inactive against hepatotoxicity, carcinogenicity, cardiotoxicity, nephrotoxicity, respiratory toxicity, nutritional toxicity, and clinical toxicity. However, both compounds were predicted to be active for skin sensitization. The main cause of drug cancellation in drug discovery is cardiac toxicity. Compounds that bind to the cardiac potassium channel encrypted by the human ether-a-go-go-related gene (hERG) are commonly known to cause cardiotoxicity. This can result in an extended QT syndrome, which can lead to fatal ventricular arrhythmias and sudden death [76]. The *in silico* studies also predicted the two compounds to be inactive toward the human hERGs cardiac potassium channel, which indicates no potential risk of cardiac toxicity. The median lethal dose weight (LD_{50}) was predicted to be 500 mg kg^{-1} for both compounds, categorizing them as class 4 toxic. Overall, the toxicity predictions suggest that compounds MK1 and MK2 are non-carcinogenic and non-toxic. The Bioavailability Radar of the compounds

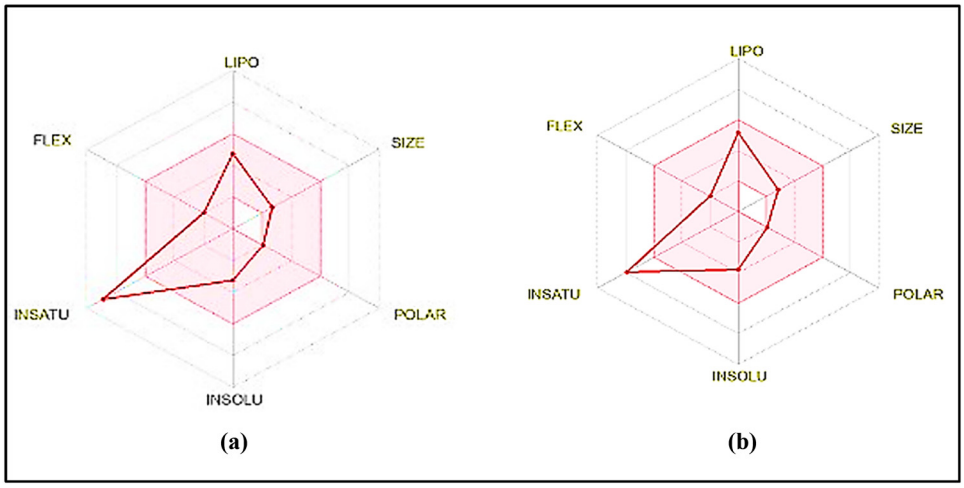


Figure 3: Radar for bioavailability of compounds (a) MK1 and (b) MK2. The pink area denotes the ideal range for each property: size: TPSA between 0.00 and 140 $Å^2$ (based on the Veber rule), flexibility: no more than nine rotatable bonds, solubility: log *S* not higher than 6, lipophilicity: XLOGP3 between −0.7 and +5.0, saturation: fraction of carbons in the sp3 hybridization not less than 0.25.

Table 10: ADME and toxicity predictions of compounds MK1 and MK2

Properties	MK1	MK2
Molecular weight (Da)	211.26	225.29
Number of rotatable bonds	3	3
Number of hydrogen bond donors	0	0
Number of hydrogen bond acceptors	2	2
TPSA ($Å^2$)	21.59	21.59
Lipophilicity: XLOGP3 (log Po/*w*)	2.79	3.56
Lipophilicity: consensus (log Po/*w*)	3.15	3.58
Gastrointestinal absorption	High	High
Water solubility (log *S*) (calculated with the ESOL model)	−3.26	−3.80
BBB permeation	Yes	Yes
Lipinski's rules (violations)	0	0
Clinical toxicity	No	No
Predicted median lethal dose (LD_{50}) (mg/kg)	500	500
Max. tolerated dose in humans (Log mg/kg/day)	0.799	0.761
hERG I inhibitor	No	No
hERG II inhibitor	No	No
Carcinogenicity	No	No
Cardiotoxicity	No	No
Hepatotoxicity	No	No
Nephrotoxicity	No	No
Nutritional toxicity	No	No
Respiratory toxicity	No	No
Skin sensitization	Yes	Yes

MK1 and MK2 is presented in Figure 3 for a swift assessment of drug-likeness. The overall ADME and toxicity results are provided in Table 10.

4 Conclusions

According to the study findings, the chemical compounds MK1 and MK2 significantly reduce blood glucose levels, improve body weight, and restore normal lipid profiles in STZ-induced diabetic rats. For instance, MK2 showed significant antihyperlipidemic advantages in addition to strong glucose-lowering effects that were almost on the scale with the standard drug glibenclamide. MK2 demonstrated a total glucose reduction of 343.1 mg/dL, especially at 50 mg/kg, which was almost identical to the reduction of 348.81 mg/dL seen with the standard drug glibenclamide. Additionally, the compound improved diabetes-induced body weight loss; groups treated with MK2 showed the strongest recovery. The compounds exhibited a significant decrease in HbA1c levels, suggesting their potential for long-term diabetes control. *In silico* analysis confirmed the binding of MK1 and MK2 with α-glucosidase enzyme, showcasing its antidiabetic mechanism at molecular levels. The in silico studies also predicted that the resulting compounds possess no potential risk of cardiac toxicity. These results imply that MK1 and MK2 may be good options for the development of novel antidiabetic medications. Accordingly, MK-2 might be made available for *in vivo* evaluation in multiple animal models in order to extend and validate its potential use as a diabetic drug for humans in the near future.

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Ethical approval: The conducted research is not related to either human or animals use.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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