Research Article

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Exploration of ketone derivatives of succinimide for their antidiabetic potential: *In vitro* and *in vivo* approaches

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Abstract: Diabetes mellitus (DM) is one of the most challenging diseases among all the other diseases in the recent era, and it is a life-threatening disorder. The best enzymes to target for treating DM are α-glucosidase and α-amylase. For this purpose, we explored numerous succinimides with ketone functionalities. First, we explored these compounds for their *in vitro* analysis. Compounds 1 and 4 exhibited excellent inhibition of both enzymes in *in vitro* studies. These compounds displayed excellent activity with IC_{50} values of 3.69 and 1.526 $\mu g \cdot mL^{-1}$ against the α-glucosidase enzyme. In the α-amylase inhibitory assay, compound 1 has shown excellent potential with an IC_{50} value of 1.07 $\mu g \cdot mL^{-1}$ and compound 4 with an IC_{50} value of 0.115 $\mu g \cdot mL^{-1}$. Based on the *in vitro* analysis, the potent compounds were further subjected to their *in vivo* analysis. Before the *in vivo* analysis, the toxicity profile was checked, and it was confirmed that the compounds were safe at 1,500 $\mu g \cdot kg^{-1}$. Then, these compounds were subjected for their *in vivo* anti-diabetic potential in a mouse model of diabetes. Various concentrations of compounds 1 and 4 were explored by *in vivo* analysis using glibenclamide as a standard drug. The blood glucose level of the tested and control groups was measured at 0 to 15 days accordingly. Similarly, we also explored compounds 1 and 4 for the oral glucose tolerance test at 0–120 min using glibenclamide as the standard drug. Hence, the succinimide having ketone moiety displayed excellent potential against diabetes.

Keywords: ketone, derivatives, succinimide, biological screening

1 Introduction

A higher blood glucose level is the primary symptom of the heterogeneous collection of disorders known as diabetes mellitus (DM) [1]. The number of diabetic patients sharply amplified globally from 108 million in 1980 to around four times in 2014 [2]. The estimation of the global pervasiveness of diabetes in percentage showed awestruck results. There was a clear increment in diabetes from 4.7% in 1980 to 8.5% in 2014, with prevalence increasing or, at best, remaining stable in all countries over this period [3]. In 2017, the number of diabetic patients was 451 million. In 2045, the number may have increase up to 693 million. The main problem is that half of the total population, 49.7, suffering from diabetes is not diagnosed properly. Moreover, the number of people with impaired glucose tolerance test was estimated to be 374 million, and nearly 21.3 million live births to women were predicted to have been affected by some form of hyperglycemia in pregnancy [4]. Currently, Pakistan is at the top of this index in diabetes.

DM is a condition of defective protein, lipid, and carbohydrate metabolism that causes low insulin production or resistance to insulin action, which results in persistent hyperglycemia [5]. DM has two main types, namely, type 1 and 2. Type 2 DM is the most typical form of the disease. This type of diabetes is more common in adults. It accounts for 90% of all diabetes cases. Complications of DM are vast and the main cause of mortality and morbidity [6]. Chronic diabetes causes the failure of many vital organs, including

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the heart, kidneys, nerves, and eyes. The symptoms of DM are loss of vision, excessive hunger, excessive thirst, increased urination, and polyphagia [7]. Occasionally, hyperglycemic patients are accompanied by certain growth-impairing abnormalities and infections. If diabetes becomes uncontrolled, it will cause hypoglycemia, including non-ketotic hyperglycemia and occasionally ketoacidosis. Other complications of DM are retinopathy resulting from vision loss, nephropathy that leads to kidney failures, sexual dysfunction, and peripheral neuropathy resulting in foot ulcers, heart diseases, genitourinary, and vascular complications [8]. Furthermore, increased blood pressure and changes in lipoprotein metabolism are the two most prevalent illnesses in people with DM. To lower postprandial hyperglycemia, the therapeutic option for non-insulin-dependent DM is to decrease the absorption of sugar from the intestinal tract. This is typically accomplished by suppressing important enzymes involved in the metabolism of simple monosaccharides' carbohydrates. The most important enzyme in this process, glucosidase, is found in a wide variety of plants, animals, and microorganisms [9].

A membrane-bound gut enzyme called glucosidase plays a major role in the breakdown of complex sugars and the release of glycol from their non-reducing side, which facilitates sugar absorption. Amylase is primarily responsible for glycol metabolism and spreads widely in plants, microorganisms, and animals. Breaking the bonds of 1,4-glucan, the main enzyme linked with type 2 DM can catalyze the breakdown of starch and other carbohydrate polymers, leading to postprandial hyperglycemia in T2DM patients [10]. Currently, oral hypoglycemic agents and insulin are used in the treatment of diabetes. These agents include biguanide, pioglitazone, glibornuride, bromocriptine, glitazone, bezafibrate, glipizide, pioglitazone, rosiglitazone, saroglitazar, and metformin. Due to various adverse effects of the aforementioned drugs such as skin, gastrointestinal problems, hematological, hypoglycemic coma, and kidney and liver dysfunction [11], the side effect-free treatment of diabetes is still a challenge for the medical system. Additional research for the treatment of diabetes is now being done. In this study, we explored succinimide derivatives for their *in vitro* and *in vivo* anti-diabetic potentials based on the previous literature survey [12–18]. The current study's objective was to look into the synthetic compounds' *in vitro* anti-diabetic potential. Animal models have also been used to examine these substances.

2 Materials and method

2.1 Chemicals

The chemicals listed in Table 1 were used in the current investigation.

Table 1: List of chemicals and reagents

Sr.#	Chemicals	CAS No.
1.	Phenyl maleimide	(941-69-5)
2.	L-Isoleucine	(73-32-5)
3.	Potassium hydroxide	(1310-58-3)
4.	Creatinine	(60-27-5)
5.	8-Hydroxyquinoline	(148-24-3)
6.	TLC silica gel 60 F254	(105554)
7.	Silica gel powder	(7631-86-9)
8.	*N*-hexane	(110-54-3)
9.	Methanol	(67-56-1)
10.	Ethyl acetate	(141-78-6)
11.	Chloroform	(67-66-3)
12.	Hydrochloric acid	(7647-01-0)
13.	Dimethyl-sulfoxide	(67-68-5)
14.	Alcohol	(64-17-5)
16.	α-Glucosidase	9001-42-7
17.	α-Amylase	9000-90-2
18.	Acarbose	56180-94-0
19.	Aloxane	ALX2244-11-3
20.	Glibenclamide	Donated by Sanofi Aventis Pharma (Pvt.) Ltd, Pakistan

2.2 Synthesis

In this research, ketone was added to maleimide in the presence of potassium hydroxide and creatinine in an appropriate container, and the reaction was started until the reaction was completed. Thin-layer chromatography (TLC) was used to keep track of the reaction's progress. After completion of reaction workup was done with the help of distilled water. A separating funnel was used to separate the aqueous layer from the organic layer. Two layers were formed, and the aqueous layer was removed and discarded. Following separation, the organic layer was added to a rotary evaporator to dry the organic layer while operating at a low vacuum. The unpurified reaction mixture was then adsorbed on silica gel and loaded into a column for multipolar solvent purification [19].

2.3 Characterization

Structural details were elucidated using ^{1}H NMR and ^{13}C NMR.

2.4 *In vitro* anti-diabetic assay

2.4.1 α-Glucosidase assay

Different quantities of the synthesized compounds (31.25, 62.5, 125, 250, and 500 μg·mL^{-1}, respectively) were prepared

according to the previously prescribed procedure [20]. In all, 1,200 µL of buffer solution and 200 µL of substrate solution glucopyranoside (15 mg/10 mL distilled water) were prepared and then added to this solution to the above concentrations to produce samples for *in vitro* glucosidase activity. The aforementioned mixture also contained 0.5 µg·mL^{-1} of the glucosidase enzyme in distilled water. The reaction mixture was then prepared and incubated at 37°C for 20 min. After allowing the mixture to settle for a few minutes, HCl was added to stop the reaction. A spectrophotometer was used to measure the color's intensity at 540 nm, and the percentage inhibition was calculated using the following formula:

$$\text{Percent inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.4.2 α-Amylase assay

According to the previously described methodology [21], the α-amylase activity was measured. Amylase solution of 250 µL and phosphate buffer solvent of 250 µL were added to the mixture of the compounds of different concentrations (31.25, 62.5, 125, 250, and 500 µg·mL^{-1}). Then, starch solution of 250 µL was added to that combination after incubation for 20 min, and the reaction mixture that had been created was then held in a water bath at 100°C for some time. The microplate reader used 656 nm to gauge the color's intensity. The % inhibition was calculated using the following formula:

$$\text{Percent inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.5 *In vivo* anti-diabetic assay

2.5.1 Test animals

For the *in vivo* phase of this study, albino mice that were 3–4 weeks old and weighed 20–27 g were used. They were acquired from the National Institutes of Health in Islamabad, Pakistan, and kept in sterile containers in isolated lab facilities at a temperature of 25°C + 2°C, humidity of 50% + 5%, and a period of 12 h of light to 12 h of darkness. They were also fed a mouse nutrient tape and water mixture as directed.

2.5.2 Acute toxicity studies

To establish the toxicity tests of the newly synthesized chemicals, the test animals were divided into six groups of four animals (n = 5). The synthetic substances were given intraperitoneally (i.p.) at doses ranging from 100 to 1,500 mg·kg^{-1} body weight. The animals were critically observed for the first 24 h, then the observation time was exceeded to 72 h. Any aberrant reactions were observed 3 days after the drugs were given to the animals [22].

2.6 Diabetes induction and experiment design

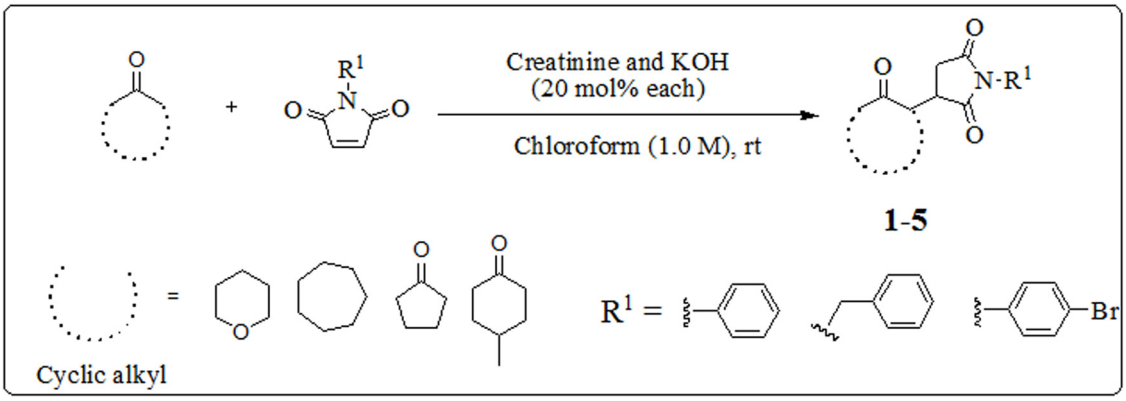
According to the claimed method of producing DM, alloxan was employed. Animals that had been fasting for 16 h were given a single injection of freshly manufactured alloxan (ALX) intraperitoneally at a concentration of 150 mg·kg^{-1}. Following the administration of ALX, the animals' glucose levels were checked to keep an eye out for the onset of diabetes. Only animals with diabetes and incident blood glucose levels over 200 mg·dL^{-1} were chosen for the investigations. The hypoglycemic effects of the synthesized succinimide derivatives were studied in 30 animals. Six test animals were placed in each of the five groups (n = 5). Group II was labeled the control group and received only I/P with normal saline. During the induction of diabetes, Group III received a conventional medication (glibenclamide), while Group I served as the diabetic control group and simply received alloxan. Groups IV and V each received a unique dosage of the tested samples. The blood glucose level of each animal was noted on days 0, 4, 7, 10, and 15 of the experiment [23].

2.7 Biochemical assay

Blood samples were collected from the retro-orbital plexus of each animal under mild anesthesia for 15 days following pure given compounds for various biochemical alterations [24].

2.8 Oral glucose tolerance test (OGTT)

The OGTT, which measures the ability to adequately respond to a glucose challenge, is performed 5 days before the experiment's end on the 15th day. The OGTT was performed on overnight-fasted mice, comprising control and treatment



Scheme 1: Synthesis of succinimide derivatives (Compounds 1–5).

mice. As an alternative to glibenclamide, glucose was given orally at a dose of 2 g·kg^{-1}. To assess the effect of exogenously provided D-glucose on treated mice, the blood glucose level was measured at intervals of 0, 30, 60, and 120 min after the administration of glucose [25].

Ethical approval: The research related to animals' use has been complied with all the relevant national regulations and institutional policies for the care and use of animals. The Ethics Committee, Bacha Khan University Charsadda, Department of Pharmacy, approved the experimental protocol with ethical approval no. Phrm-22/05S.

3 Results

3.1 Procedure for the synthesis of succinimide derivatives

Various N-substituted maleimides (equiv. 1 mmole), creatinine, and 20 µmol% KOH were added to a well-mixed solution of ketones (equiv. 2 mmole) in chloroform at room temperature. When the reaction was finished, a sufficient amount of water was added to quench it (15 mL). A separatory funnel was used to isolate the chloroform component. Three separate separations of the organic layer were carried out (15 mL each). Following separation, the organic layer was dried using a rotary evaporator under a low vacuum. After being adsorbed onto silica gel, the reaction mixture was then put onto the purification column. *N*-hexane and ethyl acetate were used as solvents in the column chromatography. From the pure product, the yield of the end product was estimated in Scheme 1 and Figure 1.

3.2 Characterization of the synthesized compounds

3.2.1 (4-Oxo-tetrahydro-pyran-3yl)-1-succinimide (compound 1)

This reaction was completed in 19 h, and the color of the obtained product is white with 74% yield. The R_f value in methanol and chloroform (1:6) was calculated as 0.42. ^{13}C NMR (100 MHz, $CDCl_3$) (ppm): 174.96, 129.89, 129.21, 128.73, 126.31, 70.22, 69.01, 66.62, 53.11, 51.23, 43.14, 41.68, 36.97, 32.30, 31.47. ^{1}H NMR (400 MHz, $CDCl_3$) (ppm): 7.25 to 7.48 (m; 5H), 4.26 to 4.75 (m; 2H), 3.52 to 3.78 (m; 2H), 2.71 to 3.19 (m; 5H), 2.33 to 2.41 (m; 1H); HPLC purity: 98.4%, TR: 6.3 min. LC-MS: C15H15NO4 (*m*/*z*): 274.1 [M + H]. Analysis calculated in percentage: N, 5.13; H, 5.53; C, 65.92; Found (%): N, 5.15; H, 5.52; C, 65.73 (Figures S1 and S2).

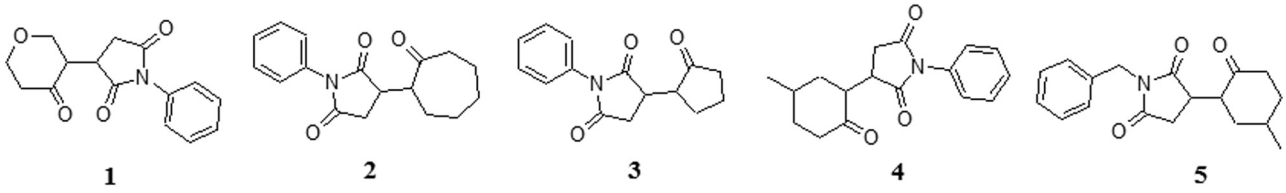


Figure 1: Structures of the synthesized compounds.

3.2.2 (2-Oxo-cyclo-heptyl)-1-succinimde (compound 2)

The reaction was finished in 18 h, and the color was white with 78–80% yield. The R_f value in ethyl acetate and *n*-hexane (1:4) was measured as 0.52.

^{13}C NMR (100 MHz, $CDCl_3$) (ppm): 214.03, 213.86, 177.27, 175.12, 174.91, 134.61, 133.66, 130.15, 129.77, 128.21, 127.30, 127.06, 54.14, 53.19, 38.64, 34.11, 33.55, 33.39, 31.70, 31.06, 30.66, 30.51, 30.31, 28.94, 25.22. ^{1}H NMR (400 MHz; $CDCl_3$) (ppm): 7.45 to 7.50 (m, 2H), 7.37 to 7.41 (m, 1H), 7.32 to 7.35 (m, 1H), 7.24 to 7.27 (m, 1H), 3.42 to 3.55 (m, 1H), 3.22 to 3.38 (m, 1), 2.75 to 3.01 (m, 2H), 2.61 to 2.75 (m, 2H), 2.00 to 2.27 (m, 2H), 1.23 to 1.99 (m, 6H); HPLC purity: 97.1%, TR: 13.5 min. LC-MS for $C_{17}H_{19}NO_3$ (*m*/*z*): 286.1 [M + H]. Analysis calculated in percentage: N, 4.91; H, 6.71; C, 71.56; Found (%): N, 4.88; H, 6.72; C, 71.70 (Figures S3 and S4).

3.2.3 (2-Oxocyclopentyl)-1phenylsuccinimide (compound 3)

This reaction was finished in 24 h, and the color was yellowish with 75% yield. The R_f value in solvent ethyl acetate in *n*-hexane (1:2) was measured as 0.41. ^{13}C NMR (100 MHz, $CDCl_3$) (ppm): 216.19, 179.82, 176.88, 132.78, 129.81, 128.92, 127.07, 51.08, 40.01, 37.96, 30.68, 25.59, 23.69; ^{1}H NMR (400 MHz, $CDCl_3$) (ppm): 7.44 to 7.49 (m, 2H), 7.36 to 7.41 (m, 1H), 7.23 to 7.27 (m, 2H), 3.45 (dd, *J*: 8.43, 5.26, & 3.17 Hz; 1H), 2.99 (dd, *J*: 9.66 & 18.39 Hz; 1H), 2.82 to 2.89 (m, 1H), 2.95 (dd, *J:* 5.26 & 18.39 Hz; 1H), 2.36 to 2.45 (m, 1H), 2.18 to 2.26 (m, 2H), 2.06 to 2.17 (m, 2H), 1.82 to 1.95 (m, 2H); HPLC purity: 97.3%, TR: 9.1 min. LC-MS for $C_{15}H_{15}NO_3$ (*m*/*z*): 258.1 [M + H]. Analysis calculated in percentage: N, 5.44; H, 5.88; C, 70.02; Found (%): N, 5.47; C, 70.21; H, 5.86 (Figures S5 and S6).

3.2.4 3-(5-Methyl-2-oxocyclohexyl)-1-phenylsuccinimide (compound 4)

The synthesis of this compound was finished in 24 h. The color was white solid. The isolated yield was 78%. R_f value was 0.47 in ethyl acetate and *n*-hexane (1: 4). ^{13}C NMR (100 MHz; $CDCl_3$) (ppm): 210.85, 210.45, 178.74, 178.64, 175.88, 175.83, 132.54, 132.25, 129.29, 128.74, 126.93, 126.78, 126.76, 51.30, 47.47, 46.15, 41.34, 41.22, 41.02, 38.18, 37.41, 37.18, 37.13, 35.72, 35.24, 34.92, 33.60, 33.37, 32.40, 32.00, 26.93, 26.88, 21.37, 21.35, 17.70, 17.67. ^{1}H NMR (400 MHz; $CDCl_3$) (ppm): 7.38 to 7.41 (m, 2H), 7.19 to 7.27 (m, 2H), 7.31 to 7.33 (m, 1), 3.03 to 3.15 (m, 1H), 2.94 to 2.97 (m, 1H), 2.43 to 2.81 (m, 2H), 2.16 to 2.36 (m, 2H), 1.87 to 2.03 (m, 2H), 1.61 to 1.83 (m, 1H), 1.26 to 1.44 (m, 1H), 1.14 to 1.20 (m, 3H), 0.95 to 0.96 (m, 1H); HPLC purity: 96.5%, TR: 11.2 min. LC-MS for $C_{17}H_{19}NO_3$ (*m*/*z*): 286.5 [M + H]. Analysis calculated in percentage: N, 4.91; H, 6.69; C, 71.56; Found (%): N, 4.93; H, 6.71; C, 71.76 (Figures S7 and S8).

3.2.5 1-Benzyl-3(5-methyl-2-oxo-cyclohexyl)succinimide (compound 5)

This reaction was finished in 24 h. The color was yellowish with a total yield of 63%. The R_f value was calculated as 0.45 in ethyl acetate in *n*-hexane (1:4). ^{13}C NMR (100 MHz; $CDCl_3$) (ppm): 212.54, 210.18, 179.43, 176.54, 176.42, 136.07, 128.83, 128.77, 128.69, 128.63, 128.05, 127.84, 100.07, 99.54, 50.56, 46.76, 45.52, 42.56, 41.38, 41.17, 41.04, 39.94, 37.42, 37.24, 35.22, 34.97, 32.92, 32.71, 32.32, 31.79, 26.95, 26.82, 21.30, 21.21, 17.83, 17.77. ^{1}H NMR (400 MHz; $CDCl_3$) (ppm): 7.23 to 7.39 (m, 5H), 4.62 to 4.73 (m, 2H), 2.63 to 3.08 (m, 1H), 2.24 to 2.36 (m, 3H), 1.83 to 2.02 (m, 4H), 1.32 to 1.45 (m, 2H), 1.16 to 1.26 (m, 1H), 1.00 (d; *J*: 6.58 Hz, 3H); HPLC purity: 95.7%, TR: 12.1 min. LC-MS for $C_{18}H_{21}NO_3$ (*m*/*z*): 300.2 [M + H]. Analysis calculated in percentage: N, 4.68; H, 7.07; C, 72.22; Found (%): N, 4.71, H, 7.09, C, 72.01 (Figures S9 and S10).

3.3 α-Glucosidase inhibitory result

In the α-glucosidase inhibition test, compound 1 at concentrations of 500, 250, 125, 62.50, and 31.25 $\mu g \cdot mL^{-1}$ showed a percent inhibition of 84.62 ± 0.56, 79.35 ± 0.22, 74 36 ± 0.81, 71.62 ± 0.21, and 56.16 ± 0.18 with an IC_{50} of 3.69 $\mu g \cdot mL^{-1}$. Compound 2 performed similarly at doses of 500, 250, 125, 62.50, and 31.25 $\mu g \cdot mL^{-1}$ showed a percent inhibition of 80.30 ± 0.70, 75.70 ± 0.80, 61.91 ± 0.88, 47.80 ± 0.90, and 43.00 ± 0.60, respectively, with an IC_{50} of 45.19 $\mu g \cdot mL^{-1}$. Similarly, compound 3 displayed a percentage inhibition of 71.36 ± 0.57, 66.85 ± 2.24, 61.08 ± 0.47, 56.90 ± 0.96, and 46.35 ± 0.51, respectively, and an estimated IC_{50} of 22.45 $\mu g \cdot mL^{-1}$. In this assay, compound 4 showed a percent inhibition of 91.90 ± 0.96, 85.08 ± 0.47, 77.40 ± 0.20, 71.61 ± 0.43, and 65.45 ± 0.90, respectively, and an IC_{50} of 1.526 $\mu g \cdot mL^{-1}$. Compound 5 at doses of 500, 250, 125, 62.50, and 31.25 $\mu g \cdot mL^{-1}$ showed a percentage inhibition of 78.91 ± 1.30, 65.00 ± 0.30, 58 .76 ± 0.58, 52.67 ± 0.61, and 43.74 ± 0.61, respectively, and an IC_{50} of 39.89 $\mu g \cdot mL^{-1}$. The standard drug acarbose was used in this assay, which had a percent inhibition of 93.56 ± 1.06, 90.31 ± 0.88, 87.56 ± 1.21, 85.44 ± 0.22, and 83.30 ± 1.20, respectively, at the same concentration as IC_{50} of 0.50 $\mu g \cdot mL^{-1}$, as shown in Table 2. Compound 1 and compound 4 were the most active compared to the others in this assay (Table 2).

Table 2: α-Glucosidase inhibitory activity of the synthesized compounds

Compound	Conc. (μg·mL^{-1})	Percent inhibitions (±SEM)	IC_{50} (μg)
1	500	84.62 ± 0.56***	3.69
	250	79.35 ± 0.22***	
	125	74.36 ± 0.81***	
	62.50	71.62 ± 0.21***	
	31.25	56.16 ± 0.18***	
2	500	80.30 ± 0.70***	45.19
	250	75.70 ± 0.80***	
	125	61.91 ± 0.88***	
	62.50	47.80 ± 0.90***	
	31.25	43.00 ± 0.60**	
3	500	71.36 ± 0.57***	22.45
	250	66.85 ± 2.24***	
	125	61.08 ± 0.47***	
	62.50	56.90 ± 0.96***	
	31.25	46.35 ± 0.51***	
4	500	91.90 ± 0.96 ns	1.526
	250	85.08 ± 0.47*	
	125	77.40 ± 0.20**	
	62.50	71.61 ± 0.43***	
	31.25	65.45 ± 0.90***	
5	500	78.91 ± 1.30***	39.89
	250	65.00 ± 0.30***	
	125	58.76 ± 0.58***	
	62.50	52.67 ± 0.61***	
	31.25	43.74 ± 0.61***	
Acarbose (Std)	500	93.56 ± 1.06	0.50
	250	90.31 ± 0.88	
	125	87.56 ± 1.21	
	62.50	85.44 ± 0.22	
	31.25	83.30 ± 1.20	

All values are taken as mean ± SEM (n = 3). Two-way ANOVA followed by the Bonferroni test was followed. Values significantly differ from the standard drug, i.e.,* = P < 0.05, ** = P < 0.01, *** = P < 0.001, and ns = not significant.

3.4 α-Amylase inhibitory assay

In the amylase inhibitory test, compound 1 at concentrations of 500, 250, 125, 62.50, and 31.25 μg·mL^{-1} showed a percentage inhibition of 88.90 ± 1.16, 82.27 ± 0.58, 79.36 ± 0.57, 71.34 ± 0.98, and 67.70 ± 1.25, respectively, with an IC_{50} of 1.07 μg·mL^{-1}. Similarly, compound 2 showed percent inhibition of 85.62 ± 0.56, 74.35 ± 0.21, 71.36 ± 0.82, 65.62 ± 0.27, and 51.16 ± 0.18, respectively, with an IC_{50} 11.16 of μg·mL^{-1}. Likewise, compound 3 with doses of 500, 250, 125, 62.50, and 31.25 μg·mL^{-1} showed percent inhibition of 80.90 ± 0.00, 73.08 ± 46, 67.40 ± 0.22, 62.61 ± 0.42, and 53.45 ± 0.92 with an IC_{50} of 7.17 μg·mL^{-1}. Compound 4 showed a percentage inhibition of 95.45 ± 0.49, 91.75 ± 0.58, 87.79 ± 0.62, 83.61 ± 0.53, and 78.75 ± 0.63 and an IC_{50} of 0.115 μg·mL^{-1}. In compound 5, with doses of 500, 250, 125, 62.50, and 31.25 μg·mL^{-1} showed a percent inhibition of 87.90 ± 0.92, 83.08 ± 0.45, 80.40 ± 0.22, 75.61 ± 0.45, and 71.45 ± 0.92, each with an IC_{50} of 15.43 μg·mL^{-1}. In the amylase inhibition test, the standard drug acarbose showed a percentage inhibition of 95.66 ± 0.88, 92.32 ± 0.52, 89.50 ± 0.44, 87.27 ± 0.57, and 86.44 ± 0.58, respectively, with an IC_{50} of 0.090 μg·mL^{-1}. In this assay, compounds 1 and 4 again are the most active compounds, and the second most active compound is fourth compared to others (Table 3).

3.5 Acute toxicity results

Based on the acute toxicity series findings of lethal dosage (LD0 to LD100), the dose range of Compounds 1 and 4

Table 3: α-Amylase inhibition activity of the synthesized compounds

Compound	Conc. (μg·mL^{-1})	Percent inhibitions (± SEM)	IC_{50} (μg)
1	500	88.90 ± 1.16**	1.07
	250	82.27 ± 0.58***	
	125	79.36 ± 0.57*	
	62.50	71.34 ± 0.98*	
	31.25	67.70 ± 1.25*	
2	500	85.62 ± 0.56***	11.16
	250	74.35 ± 0.21***	
	125	71.36 ± 0.82***	
	62.50	65.62 ± 0.27***	
	31.25	51.16 ± 0.18***	
3	500	80.90 ± 0.00*	7.17
	250	73.08 ± 0.46**	
	125	67.40 ± 0.22**	
	62.50	62.61 ± 0.42**	
	31.25	53.45 ± 0.92**	
4	500	95.45 ± 0.49ns	0.115
	250	91.75 ± 0.58ns	
	125	87.79 ± 0.62ns	
	62.50	83.61 ± 0.53ns	
	31.25	78.75 ± 0.63**	
5	500	77.90 ± 0.92***	10.79
	250	73.08 ± 0.45***	
	125	60.40 ± 0.22***	
	62.50	55.61 ± 0.45***	
	31.25	51.45 ± 0.92***	
Acarbose	500	95.66 ± 0.88	0.090
	250	92.32 ± 0.52	
	125	89.50 ± 0.44	
	62.50	87.27 ± 0.57	
	31.25	86.44 ± 0.58	

All values are taken as mean ± SEM (n = 3). Two-way ANOVA followed by the Bonferroni test were followed. Values were significantly different compared to the standard drug, i.e., *P < 0.05, **P < 0.01, ***P < 0.001, and ns = not significant.

Table 4: Acute-toxicity studies with tested synthesized compounds

Groups	Animals	Tested compounds 1 and 4 (mg·kg^{-1})
1	5	100
2	5	200
3	5	400
4	5	500
5	5	1,000
6	5	1,500

n = 5 per group.

employed for acute toxicity was 100–1,500 mg·kg^{-1} body weight. The entire dosing schedule for synthetic compounds is shown in Table 4. Each animal was individually and often observed during the first 24 h of the acute toxicity research test for behavioral abnormalities and general toxicity alterations. After that, 3 days of daily observations were done. In this toxicological study, the newly synthesized chemicals were tested for harmful or toxic effects, but no anomalous effects were discovered. The medicine was proven to be safe, up to 1,500 mg. The compounds' LD_{50} in mice was roughly 1,500 mg·kg^{-1}. In mice given 1,000 mg·kg^{-1} of body weight of 1 and 4 synthesized compounds, no abnormalities were seen in the nasal or ocular system, respiration, coat and skin, sweat, urinary incontinence, defecation incontinence, salivation, hair loss, blood pressure and heart rate, or CNS abnormalities like gait, drowsiness, ptosis, and convulsions (Table 4).

3.6 *In vivo* anti-diabetic study results

Based on their potential *in vitro* anti-diabetic study results, we evaluated two synthetic drugs for anti-diabetic efficacy in this assay. The experiment was conducted using the common medication glibenclamide. Compound 1 demonstrated a reduction in blood glucose levels over 15 days to 120, 201, 48, 34, and 36 mg·dL^{-1} at strengths of 500, 250, 125, 62.5, and 31.25 µg·kg^{-1}, respectively, while compound 4 demonstrated a reduction in blood glucose levels of 202, 109, 69, 64, and 37 mg·dL^{-1} at doses of 500, 250, 125, 62.5, and 31.25 µg·kg^{-1}, respectively, compared to the most active glibenclamide, both compounds 1and 4 were able to significantly reduce blood glucose levels (Table 5).

3.7 Biochemical assays

In this research, the values of serum glutamate oxaloacetate (SGOT), serum glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (ALP) were assessed in alloxan-

Table 5: *In vivo* results of synthesized compounds against the standard drug

Sr.#	Groups		Dose (µg·kg^{-1})	Blood glucose level (mg·dL^{-1})					Decrease in blood glucose after 15 days (mg·dL^{-1})	Change in body weight (g)
				0 day	4th day	7th day	10th day	15th day		
1	Diabetic control		0.35 mL	476	481	501	512	524	−48	−13.4
2	Normal control saline		—	123***	109***	101***	94***	92***	31	—
3	Glibenclamide	1	500	473***	303***	257***	212***	198***	275	+10.3
		2	250	431***	342***	310***	270***	201***	230	+8.1
		3	125	446***	405***	380***	361***	334***	112	+6.3
		4	62.5	381***	348***	304***	230***	285***	96	+5.7
		5	31.25	406***	381***	345***	328***	317***	89	+2.9
4	Compound 1	1	500	410***	386***	373***	315***	290***	120	+7.3
		2	250	418***	396***	347***	215***	217***	201	+5.5
		3	125	443*	435*	416*	401*	395*	48	+4.1
		4	62.5	380***	371***	365***	351***	346***	34	+4.4
		5	31.25	414**	406**	397**	380**	378**	36	+2.3
5	Compound 4	1	500	459***	403***	360***	302***	257***	202	+7.1
		2	250	465^{ns}	467^{ns}	445^{ns}	407^{ns}	356^{ns}	109	+3.5
		3	125	438*	432*	420*	394*	369*	69	−3.3
		4	62.5	389***	357***	349***	336***	325***	64	−4.2
		5	31.25	454 ^{ns}	446 ^{ns}	432 ^{ns}	423 ^{ns}	417 ^{ns}	37	−6.2

Data were analyzed by two-way ANOVA followed by the Bonferroni test, $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, ns = not significant ($P > 0.05$). All the data were compared to the diabetic control group.

Table 6: Biochemical tests results

Treatment	Route	Biochemical changes after 15 days			
		SGOT (IU)	SGPT (IU)	ALP (IU)	Serum creatinine (mg·dL^{-1})
Group I diabetic control	IP	19.1 ± 0.37	14.1 ± 0.32	184.1 ± 0.33	0.60 ± 0.27
Group II normal control	IP	45.1 ± 0.55$^{\#\#\#}$	53.1 ± 0.57$^{\#\#\#}$	290.7 ± 0.69$^{\#\#\#}$	2.7 ± 0.36$^{\#}$
Group III GB	IP	19.26 ± 0.61***	17.8 ± 0.26***	178.4 ± 0.78***	0.53 ± 0.47^{ns}
Group IV Compound 1	IP	22.6 ± 0.63***	32.5 ± 0.95***	180.3 ± 0.89***	0.77 ± 0.67^{ns}
Group V Compound 4	IP	26.7 ± 0.85***	38.3 ± 0.9***	183.3 ± 0.73***	0.80 ± 0.42^{ns}

Two-way ANOVA followed by the Bonferroni post-test. Group I compared with Group II. After that group II compared with group (III–VII). Data are represented as changes in LFTs and serum creatinine (mean ± SEM of n = 3). $^{***}P < 0.001$. $^{\#\#\#}P < 0.001$, $^{\#}P < 0.05$ which means the comparison of normal control to the diabetic control. ns; not significant.

triggered diabetic mice. Table 6 represents the effect of tested compounds on the liver enzymes. It was found that with the administration of compounds 1 and 4 there was no significant rise in the level of SGPT, ALP, and SGOT that was checked against the glibenclamide as standard. Moreover, the tested compounds were also evaluated for serum creatinine changes. Compounds 1 and 4 showed no rise in serum creatinine above the normal range.

3.8 OGTT results

Overnight fasting animals were used for the OGTT, comprising control and treatment mice. Glucose was given orally at a dose of 2 g·kg^{-1} instead of the usual medication, glibenclamide. To assess the impact of exogenously delivered D-glucose on treated mice, blood glucose levels were measured at intervals of 0, 30, 60, and 120 min after administering glucose. After 120 min of glucose administration, mice treated with compound 1 demonstrated excellent results of 158.2 mg·dL^{-1}, followed by compound 4 (152.8 mg·dL^{-1}), in comparison to the usual glibenclamide (138.4 mg·dL^{-1}) (Table 7).

Table 7: OGTT results

Treatment	OGTT (mg·dL^{-1})			
	0 min	30 min	60 min	120 min
Group-I (Tween 80)	210.5	228.3	253.7	296.9
Group II (GB)	151.1***	173.7***	215.4***	138.4***
Compound 1	163.4***	195.8***	212.2***	158.2***
Compound 4	161.3***	186.2***	211.4***	152.8***

Data were analyzed by two-way ANOVA followed by the Bonferroni test, $^{***}P < 0.001$. All the data were compared to the tween-80 group.

4 Discussion

The *in vitro* anti-diabetic activity of the synthesized compounds was investigated. First, all α-amylase inhibitory properties were assessed. This α-amylase enzyme, which is present in saliva and pancreatic juice, is responsible for breaking down large polysaccharides into smaller ones. However, the small intestine may contain α-glucosidase, which converts disaccharides into monosaccharides. The α-glucosidase and α-amylase slow down the absorption of carbohydrates at the intestinal level and increase postprandial blood sugar levels. The succinimide moiety of synthetic heterocyclic compounds has been used to inhibit carbohydrate metabolic enzymes. The succinimide derivatives support an inhibitory effect on the enzyme, resulting in a decrease in blood glucose levels. The result of the experimental work showed that succinimide derivatives are useful in treating postprandial hyperglycemia. The α-amylase inhibitory activity of compound 1 at the highest concentration showed excellent potential, with an IC_{50} value of 1.07 μg·mL^{-1}, and compound 4 showed 0.115 μg·mL^{-1}. Similarly, in the α-glucosidase assay, compound 1 demonstrated excellent percent inhibition, as shown in Table 1 and 2. Before the *in vivo* evaluation, the toxicity profile of the synthesized compounds was also verified for consistency with the safety profile of the compounds, and it was concluded that our synthesized compounds were safe up to 1,500 mg·kg^{-1}. In the OGTT, compounds 1 and 4 showed a significant decline in blood glucose levels from 30 min to group 1's. The ability of the samples being investigated to lower postprandial glucose levels can be ascribed to several factors, including decreased glycogenolysis and gluconeogenesis, increased peripheral glucose utilization, and restricted glucose uptake. This shows that the tested samples have the potential to improve regulatory processes, implying that the compounds may be useful in reducing complications of diabetes associated with hyperglycemia [26].

Body weight is a sensitive indicator of an experimental animal's health, and a decline in body weight is correlated with metabolic abnormalities caused by poisoning [27]. One of the potential causes of weight loss is a diabetic condition caused by specific harmful chemicals. In diabetic animals, a lack of insulin inhibits the body from delivering blood glucose to the cells for energy, causing the cells to start burning fat and muscle instead, lowering body weight. In most previous investigations, body weights of diabetic animals compared to their control peers were mainly studied in adults and especially male animals, although only a few cases included animals of both sexes. In the present study, however, the body weights of alloxan-induced diabetic young, adult, and old mice of both sexes were estimated and compared to the control animals of the same sex and age group. Loss of body weight in diabetic animals such as rabbits, dogs, mice, or rats compared to the control animal has been reported by several previous investigators. Several experimental studies have shown that administration of alloxan or streptozotocin after their intracellular accumulation selectively causes the destruction of the pancreatic cell membrane and cytotoxicity, leading to a reduction in pancreatic islets, depletion of cells, and an overall reduction in the number of cells; this can lead to insufficient insulin secretion. With a lack of insulin in the blood, sugar cannot enter the cell, which leads to a hyperglycemic state. In such conditions, the body attempts to eliminate extra sugar by excreting it through the urine. A decrease in body water due to frequent urination may result in a reduction in body weight. However, weight loss may also be caused by an excessive breakdown of muscle proteins, which can be used as a substitute for the energy that would otherwise be provided by glucose since insulin is not present in the blood and the glucose entry into the cell is largely unsuccessful. The body weight loss in mice with alloxan diabetes seen in the current investigation lends support to the aforementioned concept. The animals' pancreatic islets of Langerhans are destroyed by the beta-cytotoxin agent alloxan. This results in a decrease in insulin production, which raises blood sugar levels. When the mice were given continuous treatment with the produced compounds 1 and 4, there was a striking decrease in BGL. For experiments, a dosage of 500–31.25 $\mu g \cdot kg^{-1}$ was employed. The reduction in BGL was observed from 120 to 36 $mg \cdot dL^{-1}$ and from 202 to 37 $mg \cdot dL^{-1}$ by compounds 1 and 4, respectively. At a dose of 500–31.25 $\mu g \cdot kg^{-1}$ each, reduction in BGL plays a significant role in reducing BGL in alloxan-induced hyperglycemia in animals when compared with standard drug glibenclamide. Synthesized ketone derivatives' pharmacological actions have demonstrated that they can fulfill the criteria for being approved as medicines. Additionally, the SAR analyses of these variants can be used to improve them.

5 Conclusion

In this research work, we synthesized various ketone derivatives and investigated their anti-diabetic potential. Among these five compounds, only compounds 1 and 4 had an anti-hyperglycemic activity in *in vitro* activities. Acute *in vivo* toxicity research did not reveal any unique symptoms. During the *in vivo* assay, both compounds 1 and 4 displayed a very excellent effect on diabetic mice compared to the reference drug glibenclamide. Based on the aforementioned findings, we are working on designing further studies like SAR and mechanistic studies in the near future.

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