Assessment of some synthesized novel 9-substituted tetrahydroacridine derivatives in diabetic disease management in rats

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Background and objective
9-Substituted 1,2,3,4-tetrahydroacridine derivatives, especially those bearing substituted aliphatic or aromatic amino groups at the position 9, have been widely used for treating some chronic diseases because of their role as acetylcholine esterase inhibitors. Therefore, some new substituted tetrahydroacridine derivatives were synthesized to investigate their efficiency as antidiabetic agents *in vivo*.
Materials and methods
Some new substituted tetrahydroacridine derivatives hybridized at their position 9 with nitrogen, oxygen, and/or sulfur heterocycles or sulfa drugs starting from the known intermediate compound 9-chlorotetrahydroacridine were synthesized. Biologically, these compounds were investigated for antidiabetic potentials using Wistar rats. Diabetes was induced using streptozotocin (45 mg/kg) and then biochemical assays and histopathological examinations were applied to assess the therapeutic efficiency and safety margins of these compounds.
Results and discussion
Biochemical and histopathological examinations demonstrated the efficiency and safety margins of these compounds *in vivo*. Different biochemical analyses and histopathological examinations were estimated in diabetic and treated groups as compared with the healthy one. Data listed in this study showed an acceptable improvement percentage in glucose level and α-amylase, liver function enzymes (glutamic pyruvic transaminase and glutamic oxaloacetic transaminase), and lipid profile (triglyceride, low-density lipoprotein, total cholesterol) parameters after treatment with new synthetic compounds. Histopathological examination showed regeneration of treated groups. 9-Sulfadiazine-tetrahydroacridine (9-SDTHA) derivative (**2b**) was the most safe and efficient compound. The superiority of this compound may be because of the presence of a pyrimidine ring, which is the main constituent of DNA and RNA structure. These compounds could be suggested as antidiabetic agents.

Keywords:
anti-diabetic agent, hyperglycemia, tetrahydroacridine (THA) derivatives, type 2 diabetes

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Introduction

Type 2 diabetes or non-insulin-dependent diabetes mellitus is a disease that causes hyperglycemia as a result of glucose overproduction at hepatic level because of the presence of abnormal cells of the β-islets of Langerhans function or insulin resistance at target cells [1]. The problem of diabetes dispersion in the world is due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity [1]. The prevalence of diabetes was estimated to be nearly 2.8% and expected to increase to 4.4% by the year 2030 [2]. According to WHO (2011), more than 80% of diabetes deaths take place in low-income and middle-income countries. Currently available synthetic antidiabetic drugs produce serious side effects such as hypoglycemic coma and fail to significantly alter the course of diabetic complications [3]. Diverse biological activities describe tetrahydroacridine derivatives as local anesthetics, anti-inflammatory [4], antimicrobial agents [5], and as acetylcholinesterase inhibitors [6–8]. Some members of this class of compounds are used as memory-enhancing agents for treating Alzheimer's disease [9,10]. Acetylcholine is associated with stimulation of parasympathetic nervous system and leads to a decrease in heart rate and blood pressure, constriction of bronchi, increased salivation, promotion of digestion, and increase in intestinal peristalsis besides its role in blood glucose control [11,12]. Acetylcholinesterase is an enzyme that hydrolyzes the

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ester bond in acetylcholine, leading to release of acetic acid and choline and termination of the action of acetylcholine. Tacrine(1,2,3,4-tetrahydro-9-acridinamine) is an acetylcholinesterase inhibitor that is considered a drug that is effective for the treatment of mental disturbances and Alzheimer's disease [11]. 9-[*N*,*N*-(diethylamino acetyl)amino]-1,2,3,4-tetrahydroacridine (I) is a strong acetylcholinesterase inhibitor besides its strong local anesthetic action at comparatively low toxicity and potent antidepressant properties [1]. In addition, it has been reported that several functionalized tetrahydroacridin-9-yl phenyl amines protected neuronal HT22 cell from glutamate-induced cell death by reducing intracellular levels of free-radical species [12].

Diabetes was found to be an important cause of liver disease in which patients with diabetes were found to have different liver diseases, beginning with abnormal liver enzymes, nonalcoholic fatty liver disease, cirrhosis, hepatocellular carcinoma, and end with acute liver failure [13]. In the current study, the most famous liver enzymes 'glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT)', lipid profile, serum glucose, and α-amylase, which are mostly altered in diabetics, have been estimated for further indication of the efficiency of novel synthetic compounds. The objective of the present study is directed to investigate novel 9-substituted tetrahydroacridine derivatives as antidiabetic agents with more efficiency and biosafety margins.

Materials and methods

Synthesis

General procedure for the synthesis of compounds

All melting points were uncorrected and were recorded on open glass capillaries using Electrothermal IA 9000 (Electrothermal, Germany) digital melting points apparatus. Analytical data were obtained from the microanalytical Unit (Cairo University, Egypt). Infrared (IR) spectra (KBr discs) were recorded on a Perkin Elmer 1430 spectrophotometer. ^{1}H nuclear magnetic resonance (NMR) spectra were measured with Joel 270 MHz in dimethyl sulfoxide-d_6 (DMSO-d_6), and the chemical shifts were recorded in ppm relative to TMS. The mass spectra were recorded on GcMC-Qp 1000 EX Shimadzu Gas chromatography MS spectrometer (E.I. 70 eV; Japan). Follow-up of the reactions and checking the purity of the compounds were made by TLC on silica-gel-precoated aluminum sheets (type 60F254; Merck, Darmstadt, Germany) and the spots were detected by exposure to ultraviolet lamp for a few seconds.

*4-(1,2,3,4-Tetrahydroacridin-9-ylamino)benzene sulfonamide (**2a**)*

A mixture of 9-chlorotetrahydroacridine (**1**) [10] (0.01 mol) and sulfanilamide or the appropriate sulfa drug (0.01 mol) in 20 ml of absolute ethanol with a few drops of dilute HCl was refluxed for 12 h. The reaction mixture was concentrated *in vacuo*, cooled, and poured onto ice water. The resulting precipitate was recrystallized from methanol to give compound **2a**, melting point (m.p.): 280–282C in 65% yield; IR (KBr, cm^{-1}): 3420–3210 (broad NH), 1617 (C=N), 1336 and 1145 (N-SO_2); ^{1}H NMR (DMSO-d_6, ppm): 1.8 (m, m, ^{4}H, CH_2, CH_2), 2.4 (t, ^{2}H, CH_2), 3.25 (t, ^{2}H, CH_2), and 7.1–8.43 (m, ^{8}H, aromatic protons); MS, *m*/*z* (%): 353 (5). Analysis: for $C_{19}H_{19}N_3O_2S$ (353.2); calculated: C: 64.51, H: 5.38, N: 11.88%; found: C: 65.01, H: 5.50, N: 12.11%.

*4-[(1,2,3,4-Tetrahydroacridin-9-ylamino)-N-(pyrimidin-2-yl)] benzene sulfonamide (**2b**)*

Seventy percent yield; m.p.: 218–219°C from ethanol; IR (KBr, cm^{-1}): 3380–3200 (broad NH), 1620 (C=N), 1340 and 1145 (N-SO_2); MS, *m*/*z* (%): 433 (8). Analysis: for $C_{23}H_{21}N_5O_2S$ (432.4); calculated: C: 63.89, H: 5.10, N: 16.21%; found: C: 64.11, H: 4.88, N: 15.87%.

*4-[(1,2,3,4-Tetrahydroacridin-9-ylamino)-N-(pyridine-2-yl)] benzene sulfonamide (**2c**)*

Sixty-eight percent yield; m.p.: 192–4°C from ethanol; ^{1}H NMR (DMSO-d_6, ppm): 1.8 (m, m, ^{4}H, CH_2, CH_2), 2.5 (t, ^{2}H, CH_2), 3.25 (t, ^{2}H, CH_2), and 7.8–8.5 (^{12}H, aromatic protons). Analysis: for $C_{24}H_{22}N_4O_2S$ (430.3); calculated: C: 66.94, H: 5.12, N: 13.00%; found: C: 67.22, H: 4.88, N: 12.56%.

*4-[(1,2,3,4-Tetrahydroacridin-9-ylamino)-N-(thiazol-2-yl)] benzene sulfonamide (**2d**)*

Fifty-five percent yield; m.p.: 216°C; IR (KBr, cm^{-1}): 3375–3180 (broad NH), 1620 (C=N), 1340 and 1145 (N-SO_2). Analysis: for $C_{22}H_{20}N_4O_2S_2$ (436.2); calculated: C: 60.55, H: 4.58, N: 12.84%; found: C: 61.05, H: 5.00, N: 12.44%.

*9-(4-Aminophenoxy)-1,2,3,4-tetraahydroacridine (**3**)*

A mixture of compound **1** [10] (2.2 g; 0.01 mol), 4-aminophenol (2.18 g; 0.02 mol), and 5 g of potassium carbonate in 25 ml of DMF was heated under reflux for 8 h. Then the solvent was evaporated under vacuum, cooled, poured onto ice/cold water, and the formed

precipitate was filtered off, washed with cold water, and recrystallized from aqueous ethanol to give 82% yield of **3**; m.p.: 280°C; IR (KBr, cm^{-1}): 3375–3380 (NH_2), 1620 (C=N). Analysis: for $C_{19}H_{18}N_2O$ (290.4); calculated: C: 78.51, H: 6.19, N: 9.64%; found: C: 78.33, H: 5.80, N: 10.11.

*9-[4-N-[(4-methoxybenzylidene)imino]phenoxy]-1,2,3,4-tetrahydroacridine (**4a**)*

A mixture of the appropriate aromatic aldehyde, namely *p*-anisaldehyde and/or furan-2-carboxaldehyde (10 mmol), and compound **3** (10 mmol) in ethanol (30 ml containing a few drops of acetic acid) was refluxed for 3 h. Then, the hot mixture was filtered off. After cooling, the filtrate was diluted with water and the formed precipitate was filtered off and recrystallized from ethanol to give compound **4a**, m.p.: 203–5°C in 70% yield; ^{1}H NMR (DMSO-d_6, ppm): 1.8 (m, m, ^{4}H, CH_2, CH_2), 2.4 (t, ^{2}H, CH_2), 3.25 (t, ^{2}H, CH_2), 3.9 (s, ^{3}H, OCH_3), 6.4 (s, ^{1}H, CH=N), and (m, ^{12}H, aromatic protons). Analysis: for $C_{27}H_{24}N_2O_2$ (408.2); calculated: C: 79.49, H: 5.88, N: 6.86%; found: C: 78.90, H: 6.11, N: 7.21% [14].

*9-[4-N-(furan-2-ylmethylene)imino]phenoxy]1,2,3,4-tetrahydroacridine (**4b**)*

Pale brown powder M.p. 185–6°C from ethanol, 55% yield, MS, *m/z* (%): M+ at 369 (5). Analysis: for $C_{24}H_{20}N_2O_2$ (368.2); calculated: C: 78.26, H: 5.43, N: 7.60%; found: C: 77.77, H: 5.66, N: 7.11%.

*2-Aryl-[4-(1,2,3,4-tetrahydroacridin-9-yloxy)phenyl] thiazolidin-4-ones (**5a,b**)*

A solution of the proper Schiff base **4a** or **4b** (10 mmol) and thioglycolic acid (2 ml, 20 mmol) in dioxane (20 ml) was stirred at room temperature for 2 days and then warmed at 50°C on a water bath for 1 h. The solvent was evaporated under vacuum and the residue was washed with 4 N sodium carbonate solution and with water until it was carbonate free; next, it was washed with cold ethanol and dried under vacuum at room temperature to give compound **5a,b** [15].

*p-Methoxyphenylthiazolidinone derivative (**5a**)*

Yellow crystals, M.p. 160–2°C (ethanol) 60% yield, IR (KBr, cm^{-1}): 1705 (C=O) and 1617 (C=N); MS, *m/z* (%): M+1 at 483 (8). Analysis: for $C_{29}H_{26}N_2O_3S$ (482.2); calculated: C: 72.21, H: 5.39, N: 5.80%; found: C: 71.76, H: 4.95, N: 5.75%.

*Furan-2-ylthiazolidinone derivatives (**5b**)*

Pale brown powder, M.p. 140–3°C (ethanol), 55% yield, ^{1}H NMR (DMSO-d_6, ppm): 1.8 (m, m, ^{4}H, CH_2, CH_2), 2.4 (t, ^{2}H, CH_2), 3.25 (t, ^{2}H, CH_2), 3.38 (s, ^{2}H, CH_2 thiazolidinone ring), 5.9 (s, H, CH, thiazolidinone), 7.3–8.5 (m, ^{11}H aromatic protons). Analysis: for $C_{26}H_{22}N_2O_3S$(442.2), Calcd., 70.58; H, 4.97; N, 6.33%, Found, C, 70.90, H, 4.76; N, 6.66%.

*3-Methyl-1-(1,2,3,4-tetrahydroacridin-9-yl)-1-Hpyrazolin-5-one (**7a**)*

A mixture of 9-hydrazino-1,2,3,4-tetrahydroacridine (**6**) (0.01 mol; 2.13 g) [prepared by reaction of a solution of compound **1** (2.17 g) and hydrazine hydrate (0.15 g) in 30 ml butanol under reflux for 5 h to give 80% yield of **6**, m.p.: 280°C], and ethyl acetoacetate or the appropriate diketone, namely, diethylmalonate or acetylacetone (0.015 mol) with few drops of piperidine in ethanol (50 ml). The mixture was poured onto ice/water and the precipitate was filtered off and recrystallized from aqueous ethanol to give compound **7a**, m.p.: 125–8°C; IR (KBr, cm^{-1}): 1700 (C=O) and 1615 (C=N); MS, *m/z* (%): M+ at 279 (10). Analysis: for $C_{17}H_{17}N_3O$ (279.2); calculated: C: 73.11, H: 6.34, N: 15.05; found: C: 72.78, H: 6.50, N: 14.70%.

*3-Ethoxy-1-(1,2,3,4-tetrahydroacridin-9-yl)-1H-pyrazolin-5-one (**7b**)*

The foregoing method was applied using diethylmalonate instead of ethyl acetoacetate to give **7b**, m.p.: 128–9°C in 70% yield; ^{1}H NMR (DMSO-d_6, ppm): 1.7 (t, ^{3}H, CH_3), 1.82 (m, m, ^{4}H, CH_2, CH_2 of THA), 2.5 (t, ^{2}H, CH_2), 3.25 (t, ^{2}H, CH_2), 3.38 (s, ^{2}H, CH_2 of pyrazoline), 4.1 (q, ^{2}H, CH_2 of ethyl), and at 7.2–8 (m, ^{4}H aromatic protons). Analysis: for $C_{18}H_{19}N_3O$ (283.2); calculated: C: 76.27, H: 6.70, N: 14.83%; found: C: 75.75, H: 6.55, N: 15.32%.

*3,5-Dimethyl-1-(1,2,3,4-tetrahydroacridin-9-yl)-1H-pyrazole (**7c**)*

Acetylacetone is used here as a diketone to give **7c**, m.p.: 85–7°C in 65% yield; MS, *m/z* (%): M+ 276 (8). Analysis: for $C_{18}H_{19}N_3O_2$(309.2) Calcd, C, 70.00; H, 6.14, N, 13.59%, Found, C, 70.15, H, 6.55; N, 13.32%.

Preparation of the Mannich bases 8

*4-Diethylaminomethyl-3-methyl-1-(1,2,3,4-tetrahydroacrin-9-yl)-1H-pyrazolin-5-one (**8a**)*

To an ethanolic solution (20 ml) of 0.001 mol of compound **7a** was added to a 0.002 mol of the appropriate secondary amine either diethylamine or morpholine dissolved in which (0.002 mol) of ethanolic paraformaldehyde. The reaction mixture was refluxed for 8 h, cooled, and filtered off. The

formed precipitate was recrystallized from aqueous ethanol to give the Mannich base **8a**, m.p.: 220–2°C in 55% yield; MS, *m/z* (%): M+ 365 (5). Analysis: for $C_{22}H_{28}N_4O$ (364.4); calculated: C: 72.00, H: 7.69, N: 15.38%; found: C: 71.55, H: 7.33; N: 14.66%.

*4-Morpholinomethyl-3-methyl-1-(1,2,3,4-tetrahydroacridin-9-yl)-1H-pyrazolin-5-one (**8b**)*

Pale brown powder, M.p. 140–3°C (ethanol), 55% yield, m.p.: 210°C; IR (KBr, cm^{-1}): 1700 (C=O) and 1615 (C=N); ^{1}H NMR (DMSO-d_6, ppm): 1.8 (m, m, ^{4}H, CH_2, CH_2), 1.5, 2.1 (t, t, ^{8}H, $^2CH_2$, $^2CH_2$ morpholino), 2.4 (t, ^{2}H, CH_2), 3.26 (t, ^{2}H, CH_2), 3.6 (s, ^{3}H, CH_3), 4.2 (s, ^{2}H, CH_2) and 7.3–8 (m, ^{4}H, aromatic protons of THA). Analysis: for $C_{22}H_{26}N_4O_2$ (378.02); calculated: C: 69.84, H: 6.87, N: 14.81%; found: C: 70.21, H: 7.23, N: 15.32%.

Biology

A total of 40 adult, male, Wistar rats (body weight 200±25 g) were brought from the animal house, National Research Centre (Cairo, Egypt). Animals were housed in special cages at room temperature (25°C) with a 12 : 12-h light : dark cycle and had free access to water and chow diet for 1 week to be acclimatized. Physiological characteristic features of the experimental animals were recorded twice a week.

Diabetes induction

Rats were divided into three main groups. The first group (G1: five rats) served as the healthy control, whereas the second group was divided into three subgroups each with five rats (G3, G4, and G5) to study toxicity assay of the tested compounds. The third group was subjected to diabetes induction [16] as follows: streptozotocin (STZ; Sigma, St. Louis, Missouri, USA) was injected by a single intraperitoneal dose of 45 mg/kg body weight that is dissolved in 0.05 mol/l citrate buffer, pH 4.5. All groups that were injected with STZ were administered 5 ml of 40% glucose orally after 2 h of STZ injection and 20% glucose in drinking water overnight to avoid hypoglycemia. After 1 week of diabetes induction, glucose level was measured in blood using OneTouch instrument. Rats that showed a glucose level more than 300 mg/dl were considered diabetic. Diabetic groups is subdivided into four subgroups (five rats each): G2, which is considered as a positive control, and G6, G7, and G8, which were treated with new synthesized compounds **7c**, **2c**, and **2b**, respectively, for 28 days at a dose of 10 mg/kg body weight orally.

Biochemical assay

After 4 weeks, animals were fasted overnight and then killed to collect blood samples into sterilized tubes, and the samples were allowed to stand for 10 min to clot. Serum was separated by centrifugation at 3000 rpm for 10 min and preserved at −20°C for biochemical analysis. Serum glucose, α-amylase, GPT, GOT, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglyceride were measured.

Histopathological examination

Brain, liver, and pancreas samples were fixed in 10% formalin and embedded in paraffin. Samples were prepared for sectioning at 4-μm thickness. Slides were stained with hematoxylin and eosin (H&E) and examined by light microscopy [17].

Statistical analysis

All data were expressed as mean±SD. Statistical analysis was carried out by one-way analysis of variance, using the SPSS software computer program (IBM Corporation: downloaded from ibm.com/marketplace/cloud/statistical-analysis-and-reporting Website).

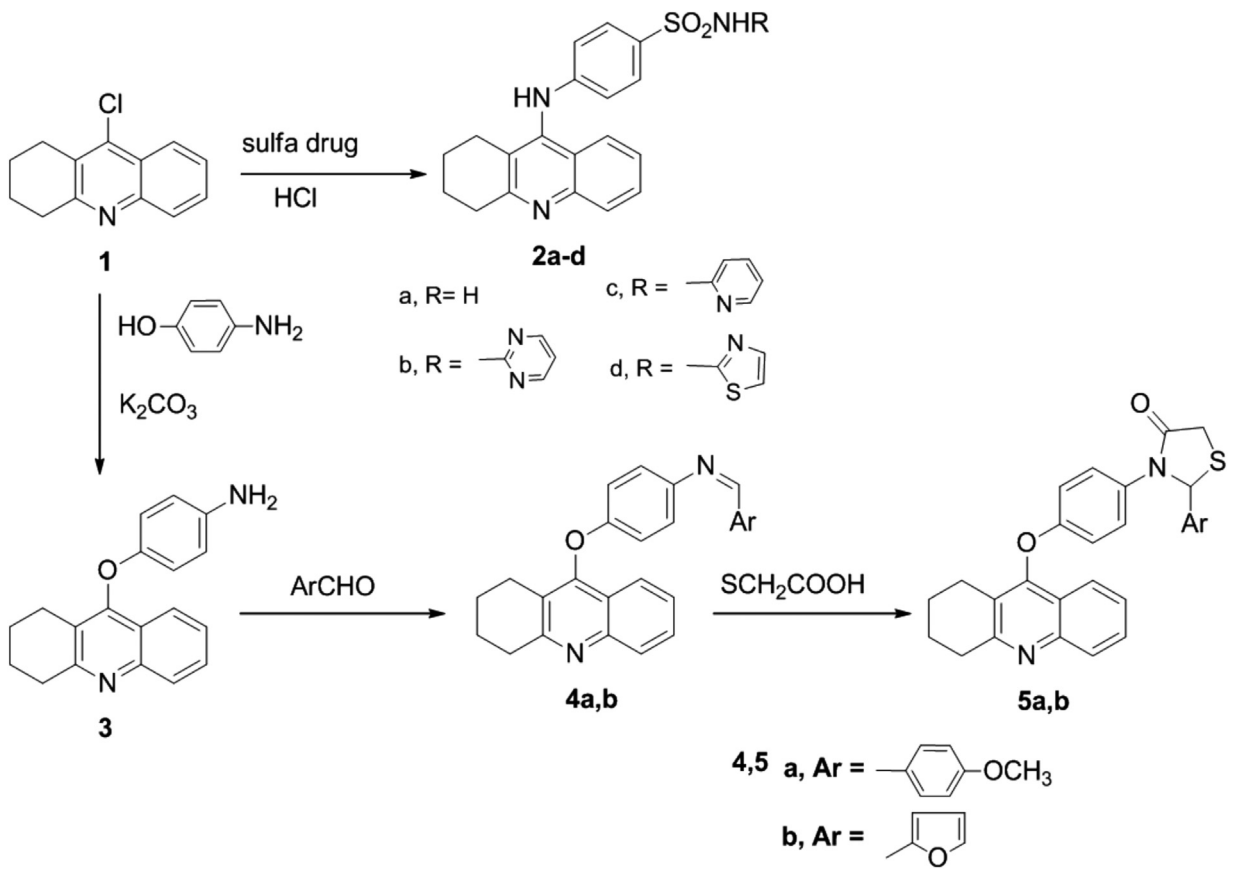
Results and discussion

Synthesis

The present study deals with the synthesis of new chemical compounds and investigated biologically for antidiabetic potentials. The known starting intermediate of choice is 9-chloro-1,2,3,4-tetrahudroacridine [18], which was allowed to react with sulfanilamide and other sulfa drugs, namely sulfadiazine, sulfapyridine, sulfamethoxazole, and/or sulfathiazole, in the presence of dilute HCl to give the corresponding 9-[4-(sufonamido)anilino]-1,2,3,4-tetrahydroacridine and the 9-[4-(pyrimidin-2-ylamino)-sulphonylanilino]-9-[4-(pyridine-2-ylamino)sulphonylanilino], and/or 9-[4-(thiazol-2-ylamino)sulfonylanilino]-1,2,3,4-tertahydroacridine derivatives (**2a–d**), respectively (Fig. 1).

On the other hand, reaction of **1** with *p*-aminophenol in the presence of potassium carbonate afforded the corresponding 9-(*p*-aminophenoxy)-1,2,3,4-tetrahydroacridine (**3**), which upon reaction with aromatic aldehydes, namely *p*-anisaldehyde and/or furfural in ethanol, according to a reported method [19], afforded the corresponding Schiff bases, namely 9-[*p*-arylidine-iminophenoxy]-1,2,3,4-tetrahydroacridines (**4a,b**), respectively. It has been reported that pioglitazone, (*RS*)-5-[4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl)thiazolidine-2,4-dione, is a potent antidiabetic and antihyperglycemic agent of choice because of the presence of a thiazolidinone moiety with its S-C=N toxophoric unit [12,13]. It works by lowering blood sugar by

Figure 1



Synthesis of 2a–d, 3, 4a, 4b, 5a, 5b.

making the cells of the body more sensitive to the action of insulin [20,21]. Therefore, it is of interest to incorporate the tetrahydroacridine moiety with a thiazolidinone moiety in one molecule hoping to develop the antidiabetic activity of the resulting structure. Accordingly, reaction of compound **4** with thioglycolic acid in dry benzene afforded the corresponding thiazolidinone derivatives **5a** and **5b**, respectively (Fig. 1).

Further, it has been reported that several pyrazoline derivatives and other pyrazolinones–tetrahydroacridines possess a considerably wide spectrum of biological activity, especially as acetylcholinesterase inhibitors [22]. This study was designed to synthesize some new tetrahydroacridine–pyrazole and pyrazolone derivatives for antidiabetic potentials. Thus, heating of **1** with hydrazine hydrate in butanol under reflux for 8 h afforded the known 1,2,3,4-tetrahydroacridin-9-yl-hydrazine derivative (**6**) [15,23]. Reaction of compound **6** with different β-diketones, namely ethyl acetoacetate, diethylmalonate, and/or acetylacetone, according to a reported method [24], afforded the corresponding pyrazolones or dimethylpyrazole derivatives **7a–c**, respectively. Furthermore, compound **7a** underwent a Mannich reaction with paraformaldehyde and a secondary amine, namely diethylamine and/or morpholine, to give the corresponding Mannich bases **8a and 8b**, respectively. The presence of this Mannich side chain may overcome the insolubility problem of such compounds through the formation of hydrochloride salts, which may increase its absorption and the biological activity [25] (Fig. 2).

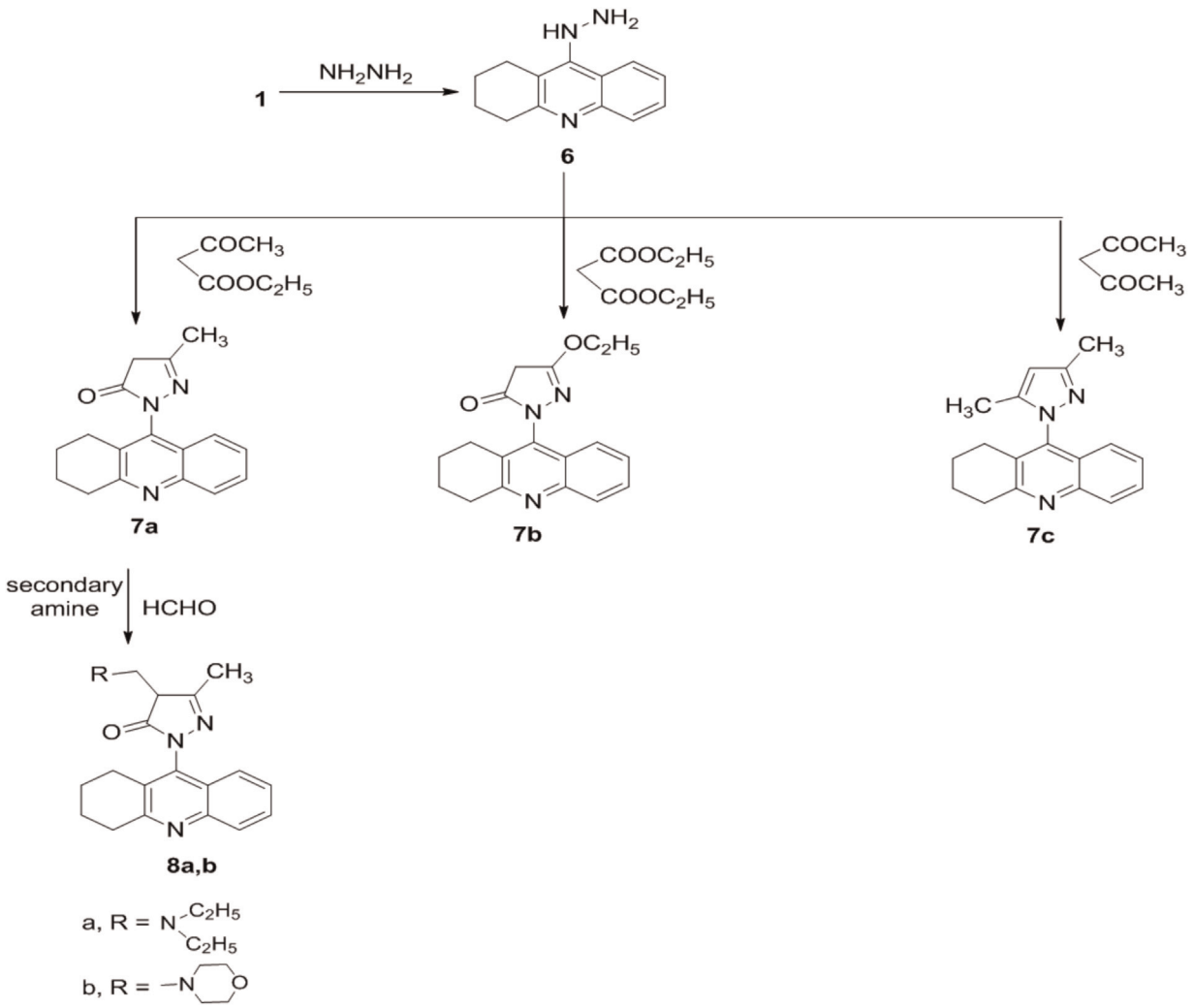
Biology

Diabetes mellitus is a worldwide chronic metabolic disorder that affects ~150 million and is expected to rise to almost 300 million by the year 2025 [26,27].

Biochemical examination

The present study pointed out that biochemical parameters such as glucose, α-amylase, GPT, GOT, cholesterol, LDL, and triglyceride levels were not affected by the newly tested compounds, as shown in Fig. 3. However, alterations in these biochemical markers due to induction of diabetes in rats mostly returned back to normal after treatment with the desired new compounds (Table 1). Determination of glucose in serum in the diabetic group showed a

Figure 2



Synthesis of 7a, 7b, 7c, 8a, 8b.

Table 1 Some biochemical parameter values in diabetic rats compared with control and treated groups in addition to determination of the parameters to detect toxicity of novel substituted tetrahydroacridine derivatives

Groups	Glucose (control±SD) (mg/dl)	α-Amylase (U/l)	GOT (mg/dl)	GPT (mg/dl)	Cholesterol (mg/dl)	LDL (mg/dl)	Triglyceride (mg/dl)
Control	109.34±9.519[a]	126.69±2.39[a]	35.4478±2.61[a]	35.39±5.85[a]	54.5100±9.57[a]	93.1504±6.37[a]	133.53±8.09[a]
Diabetic rates	471.91±97.47[b]	68.205±2.39[b]	236.99±20.95[b]	340.76±18.24[b]	99.5720±18.58[b]	198.15±19.74[b]	282.32±16.28[b]
7c Tox	167.94±56.54[a]	118.21±2.51	87.69±3.87[a]	66.87±8.24[a]	67.4820±9.69[a]	108.11±5.23[a]	160.83±6.55[a]
2c Tox	104.33±1.34[a]	114.73±1.87	80.88±1. 99[a]	42.83±5.72[a]	55.64±11.94[a]	100.26±7.05[a]	163.13±4.52[a]
2b Tox	116.84±12.96[a]	121.01±1.70[a]	45.78±3.31[a]	36.96±4.86[a]	48.6320±6.09[a]	92.626±6.45[a]	137.89±7.88[a]
7c treated	152.43±5.65[a]	115.69±1.36	207.37±12.95[a]	43.65±9.43[a]	65.6060±8.73[a]	160.63±8.29[a]	210.45±17.38[a]
2c treated	166.61±21.11[a]	108.52±7.63	202.39±8.66[a]	70.93±7.21[a]	73.0180±8.16[a]	107.06±13.90[a]	186.36±11.79[a]
2b treated	111.39±41.62[a]	117.56±1.15	121.57±3.87[a]	38.49±6.04[a]	51.6100±5.86[a]	102.61±10.86[a]	177.45±19.21[a]

GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; LDL, low-density lipoprotein. [a,b]The values more significant in relation to positive control value.

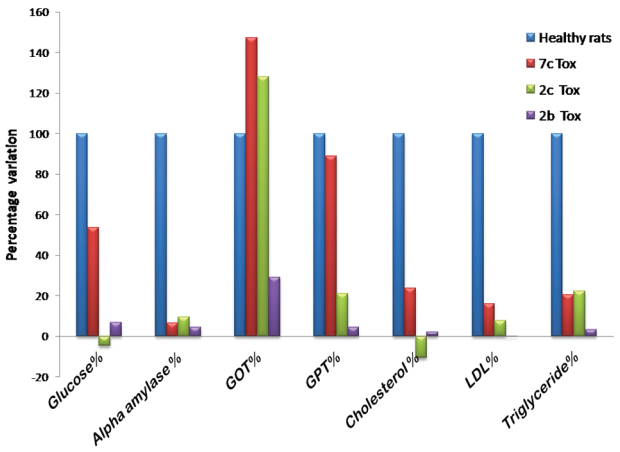
highly significant value as compared with healthy groups, and this value declined and returned back to normal in pretreated rats with compounds **2b**, **2c**, and **7c**. The most efficient compounds to reduce hyperglycemia were **2b**, **2c**, and **7c**, respectively. In contrast, the results demonstrate that α-amylase declined in diabetic groups as compared with the healthy one. After treatment by the selected compounds, the enzyme value increased to be near negative controls. Furthermore, **2b** was more efficient than **2c** and **7c**. GOT and GPT were highly significantly increased in diabetic rats as compared with healthy controls. Furthermore, these enzymes tend to decrease in treated groups. Data

recorded declared that the most efficient and safe of new the synthetic compounds was **2b**, which had slight changes in GPT and GOT. In the present study, lipid profile (cholesterol, LDL, HDL, and triglyceride) was estimated. In the diabetic group, a highly significant increase in cholesterol, LDL, and triglyceride values and, in contrast, a highly significant decrease in HDL were recorded. In the treated groups, lipid profile tends to be near normal value as compared with healthy groups. Data recorded an acceptable improvement percentage in all tested biochemical parameters after treatment with new synthetic compounds. Furthermore, the results indicated that compound **2b** was the most safe and efficient compound in the treatment of diabetes (Figs. 3 and 4).

Histopathological examination

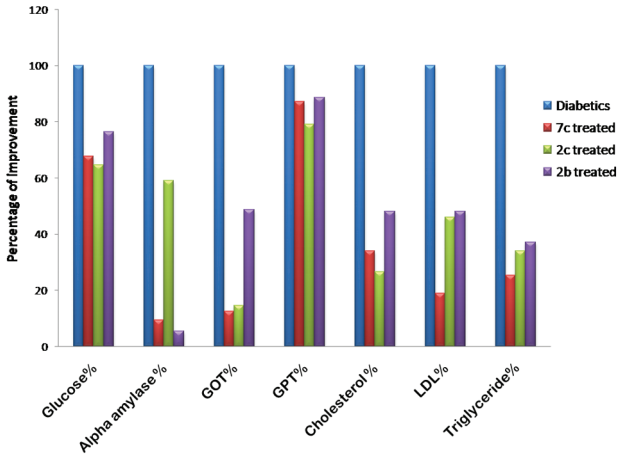
Histopathological examination of liver samples from different groups (H&E, ×200, ×400) where (1) liver section from the negative control group (healthy groups) showed hepatic tissue with normal structure and architecture; (2) liver section from positive control group showed preserved (intact) lobular hepatic architecture, hepatocyte with ballooning (black arrow), binucleated hepatocytes (yellow arrow), microsteatotic (3) changes (red arrow), and central vein congestion (black arrow); (4) liver section from **2c**-treated group showed hepatic tissue with mild hydropic degeneration (black arrow) and interlobular lymphocytes (yellow arrow); and (5) liver section from **2b**-treated group showed almost normal hepatic tissue formed of thin plates of hepatocytes with congested sinusoids (black

Figure 3



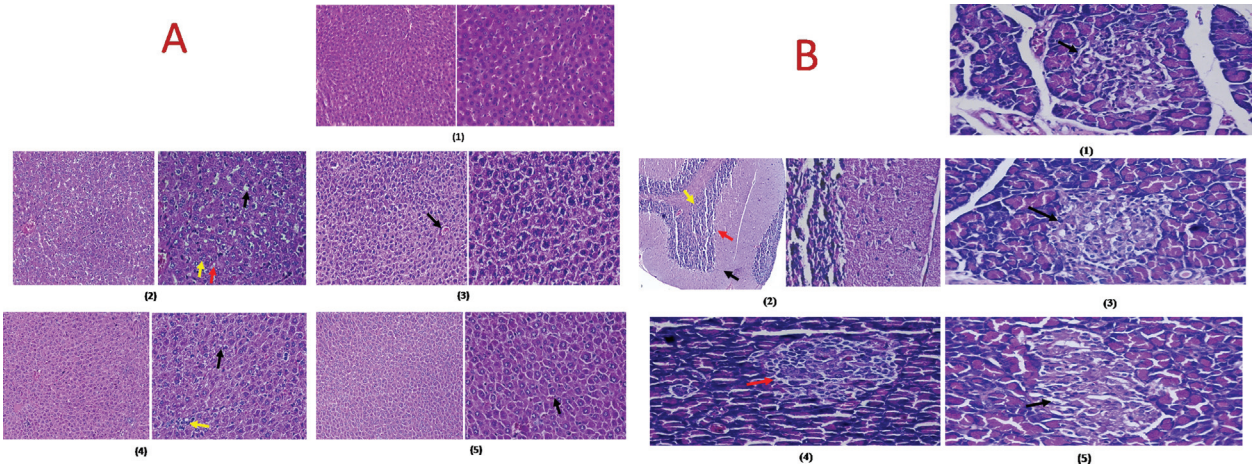
Variation percentage in biochemical parameters after injection of healthy animals with new synthetic compounds.

Figure 4



Improvement percentage of diabetic groups after treatment by new selected synthetic compounds.

Figure 5



(a) Histopathological examination of liver samples from different groups (H&E, ×200, ×400). (b) Histopathological examination of pancreas samples from different groups (H&E, ×200).

arrow). Furthermore, Fig. 5b showed histopathological examination of pancreas samples from different groups (H&E, ×200) where (1) pancreatic section the from negative control group showed normal size of β cells of islets of Langerhans, shown by arrow; (2) pancreatic section from positive control group showed small-size, atrophic pancreatic islets of Langerhans, shown by arrow (H&E, ×200, ×400); (3) pancreatic section from **7c**-treated group showed pancreatic islets of Langerhans of almost normal size, shaped regularly, and arranged evenly, shown by arrow; (4) pancreatic section from the **2c**-treated group showed pancreatic islets of Langerhans of normal size, shaped regularly, and arranged evenly, shown by arrow; and (5) pancreatic section from **2b**-treated group showed pancreatic islets of hypertrophic Langerhans cells and were shaped regularly and arranged evenly, shown by arrow. It is well known that the activity of β cells is already regulated by many neurotransmitters and hormones [28]. Pancreatic islets are abundantly innervated by parasympathetic nerves, which contain acetylcholine, which is considered the major neurotransmitter of parasympathetic nervous system and can enhance glucose-stimulated insulin secretion from pancreatic β-islets of Langerhans [29]. Acetylcholine esterase is an enzyme that hydrolyzes the ester bond in acetylcholine, leading to release of acetic acid and choline and termination of the action of acetylcholine. Here, novel chemical compounds were synthesized and designed to inhibit acetylcholine esterase that regulates B-cell function, leading to glucose regulation. In the current study, nonsignificant alternation of different biochemical estimations was recorded on healthy rats that were treated only with new chemical compounds. This finding declares safety of these compounds. Low α-amylase activity in the diabetic groups as compared with that in healthy ones was recorded in this study. This deficiency in amylase may due to the presence of a higher amount of carbohydrates, which need an excess of amylase to catalyze starch [30,31]. Previous studies indicated negative correlation between amylase and diabetes [32,33]. No significant changes were found in all healthy groups that were injected with new compounds, which suggests the safety of these compounds in pancreatic amylase secretion. Previous studies have demonstrated a positive relationship between type 2 diabetes and liver enzymes [34–36]. Furthermore, Ahn *et al.* [37] suggested that the association of liver enzymes and type 2 diabetes may be a risk factor for type 2 diabetes. In the current study, diabetic groups have a higher incidence of liver function test abnormalities than negative control groups. Elevations of GPT and GOT often reflect underlying insulin resistance. Generally, after treatment with chemical compounds, liver enzyme levels decrease as well. Diabetes is considered a multifactorial disorder; apart from pancreatic hormones, interaction of other factors plays an important role in the carbohydrate, protein, and lipid metabolisms. High abnormal levels of cholesterol, LDL, and triglycerides are mostly observed with a high significant degree of the diabetic control [38]. Diabetes may alter the lipoproteins to a form that promotes atherogenesis. Dyslipidemia raised triglycerides, cholesterol, and LDL [39,40]. In a previous study, diabetes mellitus has an effect on lipid profile balance in diabetic patients [41]. In the current study, by induction of diabetes high levels of triglycerides, cholesterol, and LDL was observed as compared with healthy groups. Furthermore, by treatment of diabetic groups with new chemical compounds, these parameters were altered to be normal again, which indicates the efficiency of these compounds in regulation of hyperglycemia and further other physiological regulations of different biochemical metabolic actions.

Histopathological examination of pancreas and liver of healthy, diabetic, and treated groups showed regenerative power of the new synthetic compounds of interest and therefore signified that they are antidiabetic. Furthermore, it is declared that compound **2b** has regenerative power, followed by **2c** and finally **7c**.

Conclusion

The current study investigated three novel tetrahydroacridine derivatives that have antidiabetic activity. The study demonstrated the efficiency and safety margin of these compounds *in vivo*. The most effective compound was **2b**, because of the presence of a pyrimidine ring, which is the main composition of DNA and RNA. These compounds could be suggested as antidiabetic agents.

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Conflicts of interest

There are no conflicts of interest.

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