

ORIGINAL ARTICLE

Synthesis and blood glucose lowering activity of some novel benzenesulfonylthiourea derivatives substituted with 4-aryl-1-oxophthalazin-2(1H)yl-ones

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Abstract

Some new benzenesulfonylthiourea derivatives substituted with phthalazones (**2a–q**) were synthesized by refluxing the appropriate 4-aryl-1-oxophthalazin-2(1H)yl benzenesulfonamides with isothiocyanate in dry acetone over anhydrous K₂CO₃. All the synthesized compounds were characterized on the basis of IR, ¹H NMR, MS data and elemental analysis. These synthesized compounds (**2a–q**) at the dose of 20 mg/kg were tested for antihyperglycemic activity in the glucose-fed hyperglycemic normal rat model and among these compounds **2f** and **2m** showed modest antihyperglycemic activity.

Keywords

Antihyperglycemic, diabetes, phthalazone, sulfonylthiourea

History

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Introduction

Non-Insulin Dependent Diabetes Mellitus (NIDDM) is a multifactorial metabolic disease characterized by abnormalities at multiple organ sites. These defects include insulin resistance and insulin deficiency^{1,2}. The former is primarily represented by decreased insulin-stimulated glucose uptake in skeletal muscle, augmented endogenous glucose production (predominately in the liver) and enhanced lipolytic activity in fat³. The latter is an apparent progressive process with both functional defects in islet cell function and, eventually, apparent loss of β-cell mass^{4,5}. These defects are intimately linked with derangements in one system exacerbating those in the others⁶. NIDDM is the most common form of diabetes constituting nearly 90% of the diabetic population in any country.

Despite recent advances both in chemistry and molecular pharmacology of antidiabetic drugs, diabetes still remains as a life-threatening disease, which tends to spread all over the world. The clinical profile of diabetic subjects is often worsened by the presence of several long-term complications, namely neuropathy, nephropathy, retinopathy and cataract.

Over the last 40 years, oral therapy for type 2 Diabetes Mellitus (DM) has focused on sulfonylureas (SU) and biguanides⁷. SU drugs improve glucose levels by stimulating insulin secretion by the pancreatic β-cells⁸. The SU were discovered accidentally; some sulfonamides were demonstrated to induce hypoglycaemia in experimental animals. This observation led to the development of a group of substituted arylsulfonylureas

differing at the para-position of the benzene ring at one nitrogen residue of the urea moiety. Carbutamide (1-butyl-3-sulfonylurea) became the first drug in the diabetic therapy later withdrawn due to its adverse effect on bone marrow. However, this compound initiated the synthesis of many thousands of analogs. Two generations have been differentiated among them, according to their substitution patterns. In contrast to the first generation (tolbutamide, acetohexamide, tolazamide, chlorpropamide), the aliphatic side chain is substituted with a cyclohexyl ring in the second generation (glibenclamide, glipizide, glimepiride) of these oral hypoglycemic agents.

Side effects of SUs include weight gain^{9–11} and hypoglycemia^{10,12}. Hypoglycemia risk becomes a more important issue as patients' overall glucose control approaches the normal range. During the past several years, the cardiology community has become disquieted because of the potential effect of SUs on myocardial ischemic preconditioning¹³. The actual importance of this issue in clinical practice remains unclear, but it has likely been exaggerated.

Obesity and weight gain are also contributors to development of diabetes and Coronary Heart Disease (CHD). The risk of diabetes for obese adults can be more than 90-fold the risk for slender adults. Small increase in physical activity and sustained weight losses of 5% of initial body weight can reduce risk for developing diabetes by 58%. Thus, an emphasis on weight management may be the most important therapeutic task¹⁴. Obesity, the principle cause of type 2 diabetes, remains an important target for possible drug therapy. It is assumed that weight loss agents will likely play an increasingly important role in the future therapy of obese type 2 diabetic patients¹⁵.

Chronic diabetes is accompanied by complications, such as neuropathy, nephropathy, cataracts and retinopathy, which are practically not controlled by insulin. These complications are

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considerately caused by an accumulation of sorbitol, which is produced from glucose by Aldose Reductase (AR) in polyol pathway. AR converts glucose to sorbitol only at high glucose levels in plasma and tissue in diabetes. The difficulty in obtaining normalization of blood glucose values has underlined the importance of the search for new and effective Aldose Reductase Inhibitors (ARIs) to control the consequences of elevated glucose levels and thereby delaying the onset and retarding the progression of diabetic complications, such as neuropathy, nephropathy, retinopathy and cataract. A large number of AR inhibitors have been prepared synthetically and some of them are used therapeutically¹⁶. Recently compounds containing pyridazine nucleus have been reported as AR inhibitors^{17–19}. Zopolrestat is a phthalazinone derivative that has been in clinical trials; it inhibits AR and has potential use in the prevention of retinopathy, neuropathy and cataract formation in diabetes²⁰.

The levels of serum lipids are usually elevated in DM and such an elevation represents a risk factor for CHD²¹. The abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots mainly due to the action of insulin, since under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic state, lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridaemia²¹.

In addition, insulin has an inhibitory action on 3-hydroxy-3-methyl glutaryl Co A (HMG- Co A) reductase, a key rate-limiting enzyme responsible for the metabolism of cholesterol-rich LDL particles²². Recently two compounds derived from phthalazones have been reported as HMG- Co A reductase inhibitors²³. Azelastine, a phthalazone derivative, has shown the best plasma glucose lowering activity as well as vasorelaxant activity²⁴.

Since the phthalazone nucleus have been reported to have lipid lowering and AR inhibitory activity, it was considered to synthesize SU substituted with 4-aryl-1-oxophthalazin-2(1H)-ones (Figure 1).

In the present study, 17 new target compounds (**2a–q**) were synthesized by condensing appropriate 4-aryl-1-oxophthalazin-2(1H)-yl benzenesulfonamides with isothiocyanate (Scheme 1) and these compounds were assessed for oral antihyperglycemic effects in glucose-fed hyperglycemic normal rats.

Experimental

Melting points were determined by open capillary tubes and are uncorrected. Infrared (IR) spectra were recorded (in KBr) on a BIO-RAD FTS-135 spectrophotometer (Waltham, MA) and ν_{\max} values are given in cm^{-1} . ^1H NMR spectra were recorded on a Bruker Spectrospin DPX 300-MHz/400-MHz spectrometer (Fällanden, Switzerland) using deuterated DMSO or CDCl_3 as

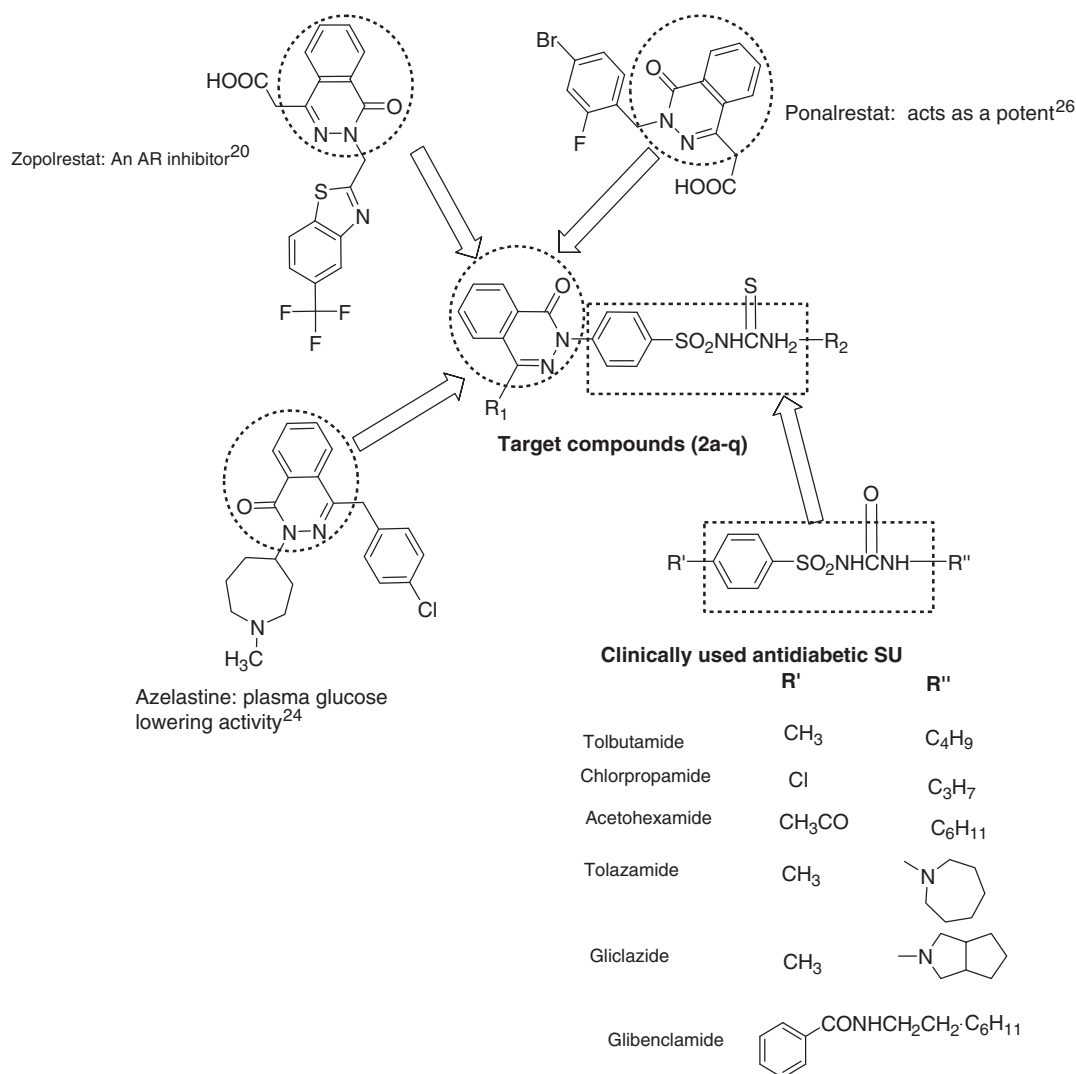
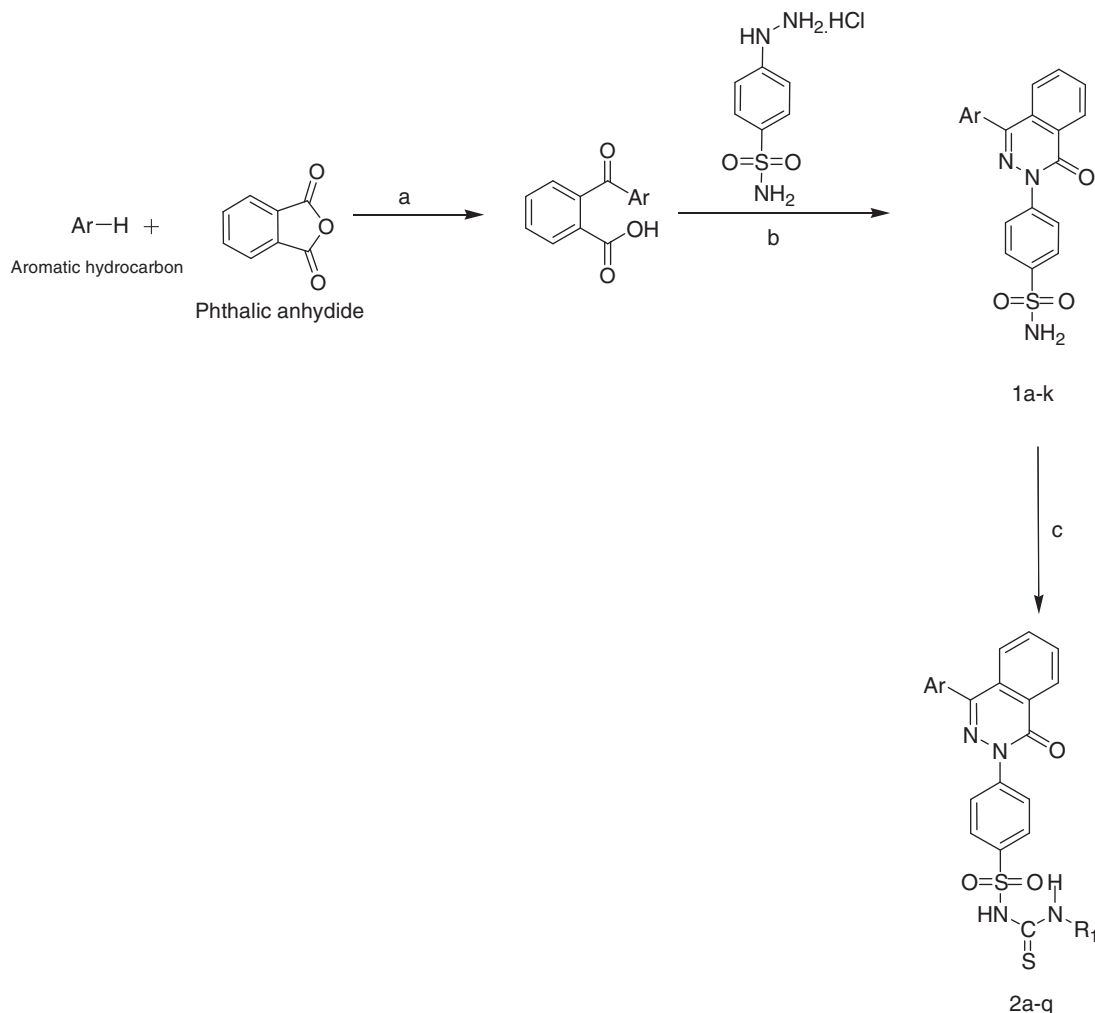


Figure 1. Structure of biologically active agent of benzenesulfonylthiourea's as blood glucose lowering and rationally designed template for targeted compound.



Scheme 1. Reagents and conditions: (a) anhydrous AlCl_3 , room temperature; (b) absolute alcohol, reflux; (c) isothiocyanate, K_2CO_3 , dry acetone, reflux 24–72 h. **1a, 2a, 2f, 2j, 2o**: Ar = Phenyl; **1b, 2b, 2g, 2k, 2p**: Ar = 4-Methylphenyl **2a–2e**: $\text{R}_1 = -\text{CH}_2\text{C}_6\text{H}_5$; **1c, 2c, 2h, 2l**: Ar = 4-Chlorophenyl; **1d, 2d, 2i, 2m, 2q**: Ar = 4-Chloro-3-methylphenyl; **1e, 2e, 2n, 2k**: Ar = 2-Chloro-5-methyl; **2f–2i**: $\text{R}_1 = -\text{C}_3\text{H}_7$; **2j–2n**: $\text{R}_1 = -\text{C}_4\text{H}_9$; **2o–2q**: $\text{R}_1 = -\text{C}_6\text{H}_{11}$.

solvent and tetramethyl silane (TMS) as an internal standard. Chemical shifts are given in δ (ppm) scale and coupling constants (J values) are expressed in Hertz. Mass spectra (MS) were scanned by using ESI Bruker Esquire 3000 (Billerica, MA). The m/z values of the more intense peaks are mentioned. Purity of the compounds was checked on TLC plates (silica gel G), which were visualized by exposing to iodine vapors. Elemental analysis was carried out on a CHNS Elementar (Vario EL III, Hanau, Germany).

General procedure for the synthesis of sulfonylthioureas (2a–q)

A solution of appropriate 4-arylphthalazone (1 mmol) in dry acetone was refluxed over anhydrous K_2CO_3 for 1–1.5 h. At this temperature, a solution of isothiocyanate (1.2 mmol) in dry acetone of 5 mL was added in a drop-wise manner. Refluxing was continued for 24–72 h. On completion of the reaction, acetone was removed by distillation method under reduced pressure and the residue was suspended in water. The suspension was acidified with acetic acid. It was filtered, washed and dried residue was crystallized by ethyl alcohol.

N-(benzylcarbamothioyl)-4-(1-oxo-4-phenylphthalazin-2(1H)-yl)benzenesulfonamide (**2a**)

Yield = 25%, m.p. 231–232 °C, $R_f = 0.30$ (toluene:ethyl acetate:formic acid, 7.5: 2: 0.5). IR ν_{max} (KBr, in cm^{-1}): 3368

and 2892 (NH of thiouriedo group), 1570 ($\text{C}=\text{S}$ of thiourea), 1664 (cyclic carbonyl), 1592 ($\text{C}=\text{N}$), 1340 and 1134 cm^{-1} (SO_2N). ^1H NMR (300 MHz, DMSO, δ): 4.52 (2H, d, $J = 6.3$ Hz, $-\text{CH}_2$ of benzyl unit), 7.16–7.29 (5H, m, benzyl unit protons), 7.58–7.98 (13H, m, H-4', H-3', H-5', H-2', H-6', H-6, H-7, H-8/H-5, NHCSNHCH_2 , H-3'', H-5'', H-2'', H-6''), 8.46–8.47 (1H, m, H-5/H-8). ESI-MS (m/z): 526 [M^+], 527 [$\text{M}^+ + 1$]. Anal. Calcd. for $\text{C}_{28}\text{H}_{22}\text{N}_4\text{O}_3\text{S}_2$: C = 63.86, H = 4.21, N = 10.64, S = 12.18. Found: C = 63.88, H = 4.18, N = 10.60, S = 12.16.

N-(benzylcarbamothioyl)-4-(1-oxo-4-*p*-tolylphthalazin-2(1H)-yl)benzenesulfonamide (**2b**)

Yield = 72%, m.p. 218–219 °C, $R_f = 0.46$ (toluene:ethyl acetate:formic acid, 7.5:2:0.5). IR ν_{max} (KBr, in cm^{-1}): 3358 and 2853 (NH of thiouriedo group), 1503 ($\text{C}=\text{S}$ of thiourea group), 1656 (cyclic $\text{C}=\text{O}$), 1591 ($\text{C}=\text{N}$), 1372 and 1137 cm^{-1} (SO_2N). ^1H NMR (400 MHz, CDCl_3 , δ): 2.45 (3H, s, CH_3), 4.52 (2H, s, $-\text{CH}_2$ of benzyl unit), 7.13–7.19 (5H, m, benzyl unit protons), 7.25 (2H, d, $J = 6.8$ Hz, H-3', H-5'), 7.45 (2H, d, $J = 7.2$ Hz, H-2', H-6'), 7.63–7.89 (8H, m, H-6, H-7, H-8/H-5, H-3'', H-5'', H-2'', H-6'' and NHCSNHCH_2), 8.59 (1H, s, H-5/H-8). ESI-MS (m/z): 541 [$\text{M}^+ + 1$], 563 [$\text{M}^+ + \text{Na}$]. Anal. Calcd. for $\text{C}_{29}\text{H}_{24}\text{N}_4\text{O}_3\text{S}_2$: C = 64.42, H = 4.47, N = 10.36, S = 11.86. Found: C = 64.44, H = 4.44, N = 10.37, S = 11.85.

N-(benzylcarbamothioyl)-4-(4-(4-chlorophenyl)-1-oxophthalazin-2(1*H*)-yl)benzenesulfonamide (**2c**)

Yield = 40%, m.p. 204–206 °C, R_f = 0.66, (toluene:ethyl acetate:formic acid, 7.5:2.0:0.5). IR ν_{\max} (KBr, in cm^{-1}): 3342 and 2862 (NH of thiourea group), 1531 (C=S of thiourea), 1662 (cyclic carbonyl), 1584 (C=N), 1382 and 1106 (SO_2N). ^1H NMR (400 MHz, DMSO, δ): 4.64 (2H, d, J = 5.1 Hz, $-\text{CH}_2$ of benzyl unit), 7.07–7.21 (5H, m, benzyl unit protons), 7.50 (2H, d, J = 8.4 Hz, H-3', H-5'), 7.61 (2H, d, J = 8.0 Hz, H-2', H-6'), 7.69–7.72 (1H, m, H-8/H-5), 7.79–7.87 (2H, m, H-6, H-7), 7.89 (2H, d, J = 8.8 Hz, H-3'', H-5''), 7.96 (2H, d, J = 8.0 Hz, H-2'', H-6''), 8.45 (1H, *ortho*–*meta* coupled dd, J = 3.2 Hz, J = 5.6 Hz, H-5/H-8), 8.58–8.59 (1H, m, NHCSNHCH_2 protons). ESI-MS (m/z): 562 [$\text{M}^+ + 2$]. Anal. Calcd. for $\text{C}_{28}\text{H}_{21}\text{ClN}_4\text{O}_3\text{S}_2$: C = 59.94, H = 3.77, N = 9.99, S = 11.43. Found: C = 60.00, H = 3.75, N = 10.00, S = 11.43.

The characterization data of compounds (**2d–q**) can be obtained online as supplementary data.

Effect of synthesized compounds on oral glucose tolerance in normal rats

Albino rats (either sex) of Wistar strain weighing 150–200 g were fasted overnight and divided into groups of six animals each. Animals of group I received only vehicle (10 ml/kg) orally to serve as control, while animals of group II were given gliclazide 20 mg/kg orally, suspended in the vehicle (10 ml/kg). The test compounds in the dose of 20 mg/kg suspended in the vehicle (10 ml/kg) were administered orally to respective groups. All the animals were given glucose (3 g/kg, p.o.) 30 min after dosing. Blood samples were collected from retro-orbital plexus just prior to and 60 min after the glucose loading and blood glucose levels were measured with an autoanalyzer by using AccuCheck Advantage II glucose kit (Roche, West Sussex, UK).

Results and discussion**Synthesis of compounds**

The synthetic route used to synthesize title compounds (**2a–q**) is outlined in Scheme 1. The 4-aryl-phthalazone-substituted benzenesulfonamide derivatives (**1a–k**) synthesized through reported method²⁵ were converted to SU by refluxing with appropriate isothiocyanates in dry acetone containing K_2CO_3 . Purity of the compounds was checked on TLC plates (Merck, Darmstadt, Germany) (silica gel G) which were visualized by exposing to iodine vapors.

Structures of the synthesized compounds (**2a–q**) were established on the basis of elemental analysis and various spectroscopic methods, viz. IR, ^1H NMR and MS. Elemental analysis (C, H, N and S) data were within $\pm 0.4\%$ of the theoretical values. In the IR spectra of **2a–q** three bands characteristic of SU moiety out of which one band for thiourea group at (1503 – 1570 cm^{-1}) and two bands for NH at (3320 – 3371 cm^{-1} and 2850 – 2960 cm^{-1}) were observed. Apart from these a band in the region 1578 – 1594 cm^{-1} corresponding to C=N stretching, a band at 1647 – 1712 cm^{-1} for cyclic carbonyl group (C=O) and two bands at 1340 – 1387 and 1080 – 1183 cm^{-1} for $>\text{SO}_2\text{NH}$ group were also observed. The structure was further established by proton NMR spectral data. The H-5/H-8 proton of phthalazone ring appeared in the NMR spectra at δ 8.41–8.62.

Antihyperglycemic activity

In the present study, oral antihyperglycemic effects of 17 compounds (**2a–q**) were assessed in glucose-fed hyperglycemic normal rats. The marketed SU drug gliclazide was selected as

Table 1. Antihyperglycemic activity of SU derivatives (**2a–q**) and the standard drug gliclazide in glucose-fed (3 g/kg) hyperglycemic normal rats.

Treatment	Dose per kg b.w. (mg)	% Activity	Significance
2a	20	4.79	$p < 0.05$
2b	20	8.57	$p < 0.05$
2c	20	4.61	$p > 0.05$
2d	20	6.22	$p < 0.05$
2e	20	11.66	$p > 0.05$
2f	20	16.0	$p < 0.05$
2g	20	9.05	$p < 0.05$
2h	20	11.44	$p < 0.05$
2i	20	3.27	$p > 0.05$
2j	20	1.0	$p < 0.05$
2k	20	1.92	$p < 0.05$
2l	20	8.40	$p > 0.05$
2m	20	17.53	$p < 0.01$
2n	20	9.25	$p > 0.05$
2o	20	11.09	$p < 0.05$
2p	20	3.18	$p > 0.05$
2q	20	12.18	$p > 0.05$
Gliclazide	20	35.0	$p < 0.01$

positive control. Quantitative glucose tolerance of each animal was calculated by the area under curve (AUC) method by using the Prism software (Irvine, CA). Comparing AUC of experimental and control groups determined the percentage of antihyperglycemic activity. Samples showing significant inhibition ($p < 0.05$) were considered as active samples. Statistical comparison was made by Dunnett's test.

The results showed that two compounds namely **2f** and **2m** were found to be in abundance. The compounds **2f** and **2m** significantly inhibited the rise in postprandial hyperglycemia to the tune of 16.0% ($p < 0.05$) and 17.53% ($p < 0.01$), respectively. The standard drug Gliclazide caused nearly 35% ($p < 0.01$) inhibition. With regard to the SAR, it seems impossible to extract an obvious structure–activity relationship from the data shown in Table 1.

Supplementary material

The characterization data of compounds (**2d–q**) can be obtained online as supplementary data. Supplementary data associated with this article can be found, in the online version.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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