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α-Glucosidase inhibitory antihyperglycemic activity of substituted chromenone derivatives

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ABSTRACT

Series of 3,4- and 3,6-disubstituted chromenones including new chromenone derivatives were synthesized applying various synthetic strategies including Pechmann condensation, Knoevenagel condensation, Reimer–Tiemann reaction and Suzuki coupling in very good yields. Synthesized compounds (**4a–z**) were screened for in vitro α-glucosidase inhibitory and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activities. Majority of compounds displayed varying degrees of α-glucosidase inhibitory and DPPH scavenging activity. Compound **4x** emerged as the most potent α-glucosidase inhibitor in present series of compounds owing to the presence of 3-acetyl-6-(6-methoxy-3-pyridyl) group on chromenone; however, it could not display DPPH scavenging activity and was found to be mixed non-competitive type inhibitor of rat intestinal α-glucosidase. When tested in vivo for antihyperglycemic activity in starch loaded Wistar rats, it displayed significant antihyperglycemic property. This is the first report assigning rat intestinal α-glucosidase inhibitory property for this class of new chromenones and presents new family of compounds possessing α-glucosidase inhibitory activities and antihyperglycemic property. Compound **4x** may serve as an interesting new compound for the development of therapeutics targeted against diet-induced hyperglycemia in diabetes.

1. Introduction

With the admission of the fact that 'diabetes is a cardiovascular disease' (CVD), rise in CVD is being cautioned due to the world wide increase in prevalence of diabetes.[1] Until recently, fasting hyperglycemia has been the main therapeutic target for diabetes mellitus. On the other hand, in recent years importance of postprandial hyperglycemia (PPHG) has been recognized as an important cardiovascular risk factor.[1e] Furthermore, PPHG is also identified as one of earliest detectable abnormalities expressed in diabetes,[2] better predictor of progression of diabetes[3] and has been implicated in inducing oxidative stress[4] that is recognized as a major pathophysiological link between CVD and diabetes.[5] Therefore, agents that hold the capacity of slowing down postprandial hyperglycemic excursion (PPHGE) and resultant oxidative stress may become therapeutics of colossal importance.

Food starches constitute a major source of energy in the diet of many mammalian species, including humans. Slowing down of digestion and absorption of dietary starches either by means of dietary manipulation with low glycemic-index food or by inhibition of starch digesting enzyme α-glucosidase present at the intestinal brush borders, have shown promise in reducing PPHGE, hyperinsulinemia, burden of oxidative stress, and CVD.[6] The α-glucosidase inhibitors appear better therapeutic in controlling PPHGE in Asian people[7] presumably because of their specific food habits[8] particularly Indians who demonstrate higher glycemic response to all foods[9] and present increased prevalence of PPHG[10] than other ethnic groups around the world.

In the course of our efforts in discovery and identification of traditional Indian medicinal plants[11] and development of synthetic library of compounds[12] possessing intestinal α-glucosidase inhibitory and relevant antihyperglycemic activity, we observed α-glucosidase inhibitory potential in substituted new chromenone derivatives. Natural as well as synthetic chromenones (2*H*-1-benzopyran-2-ones) have become important class of oxygenated heterocycles[13] mainly due to their broad spectrum biological activities such as antibiotics, antibacterial, antitumor, antiviral, anticoagulants, antipsoriatic, and as anti-HIV.[14] Compounds derived from 4-hydroxycoumarin protect liver cells from damage by peroxides.[15]

In this study we report synthesis of number of substituted new chromenone derivatives applying various synthetic strategies, their rat intestinal α-glucosidase inhibitory potentials and DPPH free radical scavenging activity as well as discuss the structure–activity

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relationship in relation to various substitutions and α-glucosidase inhibition. Furthermore, we also report antihyperglycemic activity of most potent α-glucosidase inhibitor in starch-induced hyperglycemia in normal Wistar rats.

2. Results and discussion

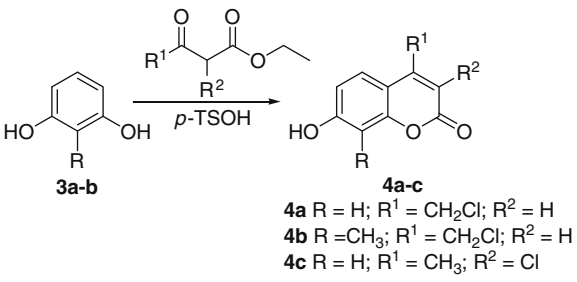
2.1. Chemistry

In continuation of our efforts on synthesis and development of synthetic methodologies for heterocycles[16] we herein report the synthesis of substituted chromenone derivatives applying various synthetic strategies. Scheme 1 illustrates synthesis of 4-substituted chromenones **4a–c** by the Pechmann condensation of resorcinol **3a** and **b** with β-ketoesters in presence of *p*-TSOH (20 mol %) in dry toluene under reflux conditions. Scheme 2 illustrates the synthesis of 3-substituted chromenones **4d–j** by Knoevenagel condensation of salicylaldehydes **3c–e** with β-ketoesters in presence of piperidine in acetonitrile and 3,6-disubstituted-2*H*-2-chromenones **4k–z** were synthesized from 4-bromophenol **1** as the starting material. The 4-bromophenol **1** was subjected for Reimer–Tiemann reaction to give 5-bromosalicylaldehyde **2**. Compound **2** was then subjected for Suzuki coupling with various phenylboronic acids in presence of $Pd(PPh_3)_4$ to give 5-substituted salicylaldehydes **3g–n**. Application of Suzuki coupling for the synthesis of **3f**, **3h–n** was made for the first time in preparation of substituted salicylaldehyde intermediates. Knoevenagel reaction of substituted salicylaldehydes **3g–n** with various β-ketoesters in presence of piperidine in acetonitrile solvent under mild conditions proceeded efficiently to give 3,6-disubstituted-2*H*-2-chromenones **4k–z** in very good yields (Scheme 3). Thus synthesized compounds (**4a–z**) were characterized by spectral data and results are presented in Table 1.

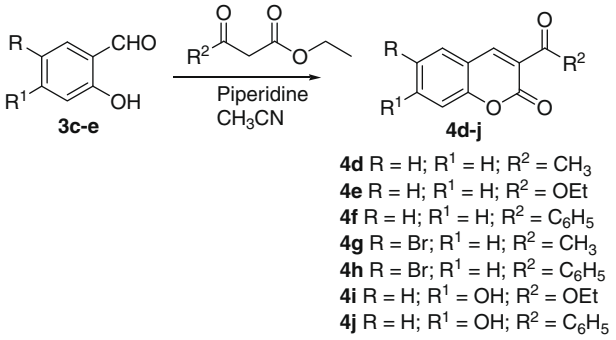
2.2. Biological activity

All the synthesized compounds were evaluated in vitro for their potential for DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging and rat intestinal α-glucosidase inhibitory activity. Values are presented in Table 1, the compounds **4b**, **4c**, **4f**, **4l**, and **4z** could display mild DPPH scavenging activity at primary screening concentration of 25 μg/mL. However, except compound **4m**, all other exhibited varying degrees of α-glucosidase inhibitory activity at primary screening concentration of 100 μg/mL. Compounds that displayed more than 60% α-glucosidase inhibitory activity were selected for concentration dependent activity evaluation and calculation of IC_{50} values.

Based on IC_{50} values we could observe some structure–activity relationship. Though, 3-substituted chromenone derivatives (**4d–f**) displayed moderate enzyme inhibition, substitution of bromine at sixth position on aryl moiety reduced the activity (**4g** and **4h**). In case of 7-hydroxy substituted chromenones (**4a–c**, **4i**, and **4j**), compound **4b** with methyl group at eighth position displayed better activity than 4-chloromethyl (**4a**), 3-chloro-4-methyl (**4c**), ethyl-3-carboxylate (**4i**) and 3-benzoyl substituted chromenone (**4j**).



Scheme 1.

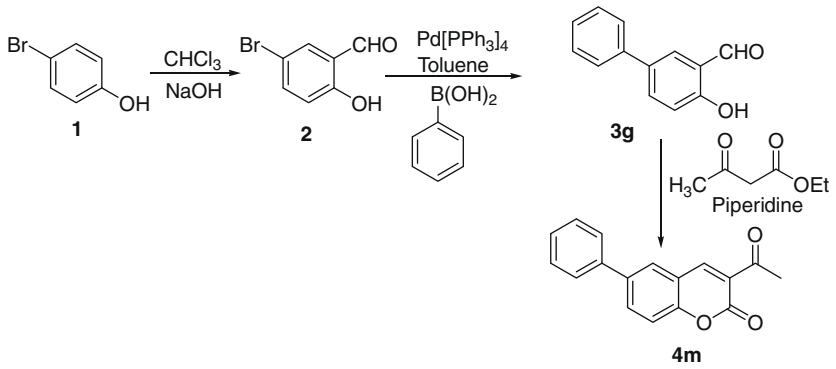


Scheme 2.

The 3-acetyl-6-phenylchromenone (**4m**) could not display α-glucosidase inhibitory activity. However, introduction of tolyl group at sixth position significantly added enzyme inhibitory capacity (**4p**). On the contrary, ethyl-3-carboxy group (**4q**) reduced the activity potential, whereas 3-benzoyl substitution (**4o**) enhanced the activity level when compared with **4n** bearing ethyl-3-carboxylate group. It appears therefore that substitution of 6-tolyl (**4p**) and 3-benzoyl (**4o**) has added advantage in improving α-glucosidase inhibitory activity than that of ethyl-3-carboxylate (**4n** and **4q**). Introduction of 4-fluorophenyl group (**4s**) was observed advantageous over 4-chlorophenyl (**4r**) in improving enzyme inhibitory potential. Substitution of ethyl-3-carboxylate (**4t**) further increases the activity of 6-(4-fluorophenyl)-3-acetylchromenone (**4s**). Morpholinophenyl group on 6-aryl (**4z**) was no more advantageous over 6-(4-fluorophenyl)-3-acetylchromenone (**4s**).

Compound **4x** emerged as the most potent α-glucosidase inhibitor in present series of compounds owing to the presence of 3-acetyl-6-(6-methoxy-3-pyridyl) group on chromenone, however substitution of ethyl-3-carboxylate group drastically reduced the activity (**4y**). 3-Acetyl-6-(6-methoxy-3-pyridyl) substitution (**4x**) had advantage over 3-acetyl-6-(4-pyrimidinyl) chromenones (**4v** and **4w**). Furthermore, 3-acetyl-6-(6-methoxy-3-pyridyl) in compound **4x** was found an important substitution in improving the activity when compared to **4u**. The fact that 6-(6-methoxy-3-pyridyl) group improves α-glucosidase inhibition, is also evident from the activity potential of **4l** over **4k**.

Therefore, compound **4x** was selected for the study of its mechanism of enzyme inhibition and evaluation of its potential in mitigating starch-induced hyperglycemia in normal Wistar rats. Figure 1 presents Lineweaver–Burk plot which states that the slope for **4x** crosses slope of the control left to the intercept above *x*-axis.[17] Therefore, it may be designated as mixed non-competitive inhibitor of rat intestinal α-glucosidase enzyme. The prior oral administration of **4x** in overnight fasted rats before inducing hyperglycemia by starch feeding showed that it slowed down digestion of starch significantly (Fig. 2). Calculation of area under the curve (AUC)[18] further disclosed the fact that it has strong ability of reducing starch-induced hyperglycemic burden (Fig. 3). Though it appears from Figures 2 and 3 that **4x** has better antihyperglycemic activity than a commercial antihyperglycemic drug acarbose with tetrasaccharide structure, it is important to note that the dose of **4x** in this study was 10 times higher than that of the acarbose. The blood glucose level in some of the rats tested with **4x** decreased below their basal value ('0' h value) indicates that compound may possess other mode of antihyperglycemic activity also.



Scheme 3.

Table 1
Synthesis of chromenone derivatives and their activity profiles

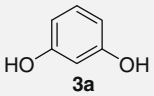
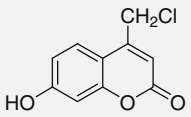
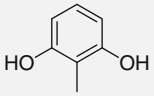
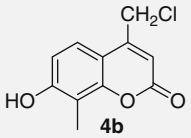
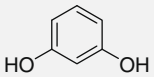
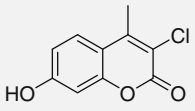
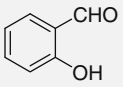
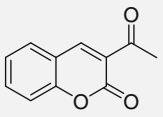
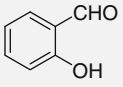
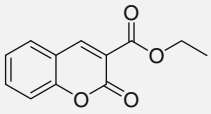
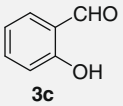
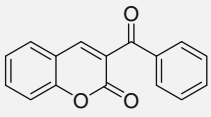
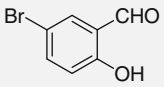
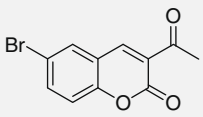
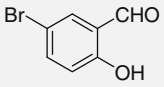
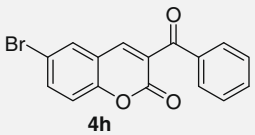
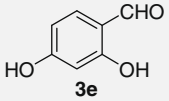
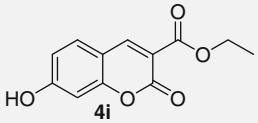
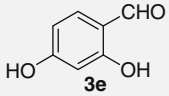
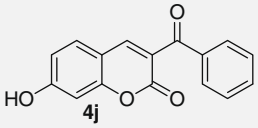
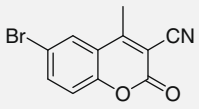
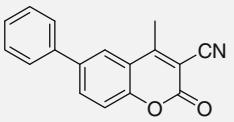
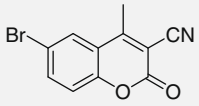
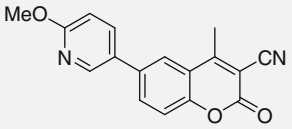
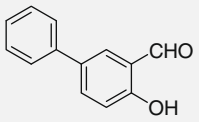
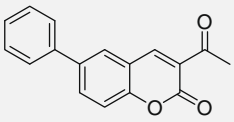
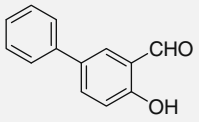
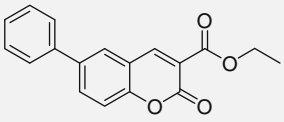
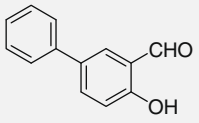
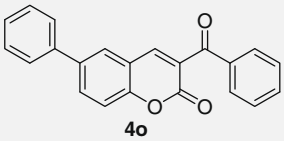
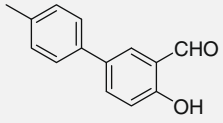
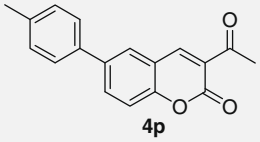
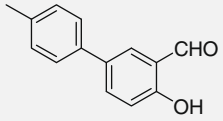
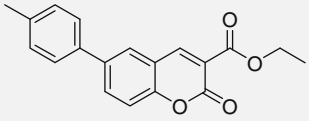
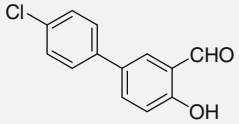
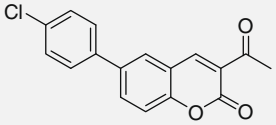
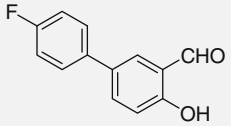
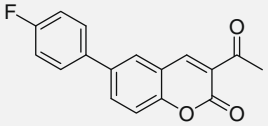
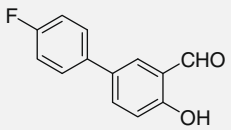
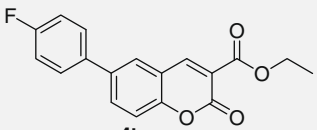
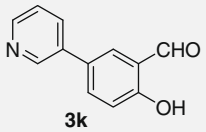
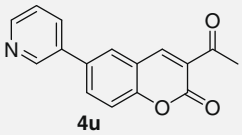
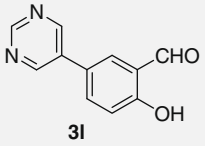
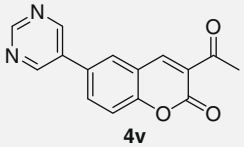
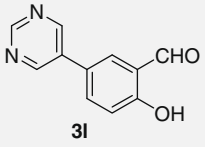
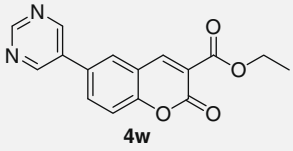
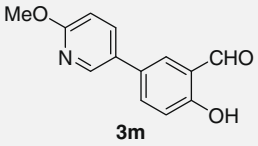
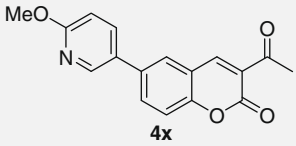
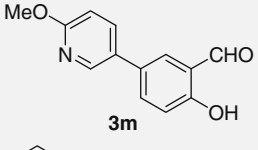
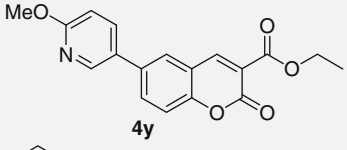
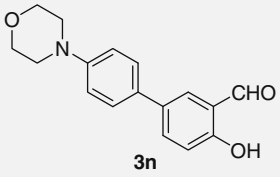
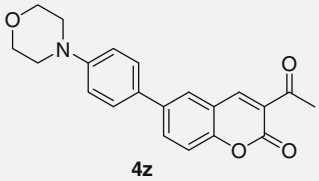
Entry	Substrate (**3**)	Compound (**4**)	Yield* (%)	% DPPH scavenging	% AGH inhibition (IC_{50}, μM)
1	 **3a**	 **4a**	65	1.32	78.34 (242.8)
2	 **3b**	 **4b**	72	28.47	72.7 (68.7)
3	 **3a**	 **4c**	56	20.75	77.82 (128.3)
4	 **3c**	 **4d**	90	0.83	53.19
5	 **3c**	 **4e**	88	1.82	46.95
6	 **3c**	 **4f**	90	30.8	44.38
7	 **3d**	 **4g**	86	1.52	31.95
8	 **3d**	 **4h**	88	2.22	18.14
9	 **3e**	 **4i**	65	NA	77.6 (91.0)

Table 1 (*continued*)

Entry	Substrate (**3**)	Compound (**4**)	Yield* (%)	% DPPH scavenging	% AGH inhibition (IC_{50}, μM)
10	 **3e**	 **4j**	62	NA	53.71
11	 **3f**	 **4k**	70	10.39	95.5 (52.90)
12	 **3f**	 **4l**	72	34.98	93.06 (44.5)
13	 **3g**	 **4m**	95	19.56	NA
14	 **3g**	 **4n**	85	4.80	77.06 (185.90)
15	 **3g**	 **4o**	90	2.45	69.95 (52.20)
16	 **3h**	 **4p**	82	NA	86.47 (52.60)
17	 **3h**	 **4q**	75	NA	65.89 (111.50)
18	 **3i**	 **4r**	76	12.54	76.89 (96.40)
19	 **3j**	 **4s**	82	15.55	74.26 (53.10)
20	 **3j**	 **4t**	86	NA	66.72 (37.60)
21	 **3k**	 **4u**	80	3.72	96.40 (58.10)

(*continued on next page*)

Table 1 (*continued*)

Entry	Substrate (**3**)	Compound (**4**)	Yield* (%)	% DPPH scavenging	% AGH inhibition (IC_{50}, μM)
22	 **3l**	 **4v**	85	NA	83.52 (37.20)
23	 **3l**	 **4w**	72	NA	80.39 (40.50)
24	 **3m**	 **4x**	85	NA	85.98 (28.20)
25	 **3m**	 **4y**	72	NA	76.92 (104.60)
26	 **3n**	 **4z**	88	20.50	85.06 (49.00)

% DPPH scavenging activity is based on values obtained with primary screening concentration of 25 μg/mL and that for α-glucosidase inhibition concentration was 100 μg/mL. NA: not active. Values in parentheses represent μM IC_{50} value for the respective compound. AGH; α-glucosidase.

* Isolated and unoptimised yields.

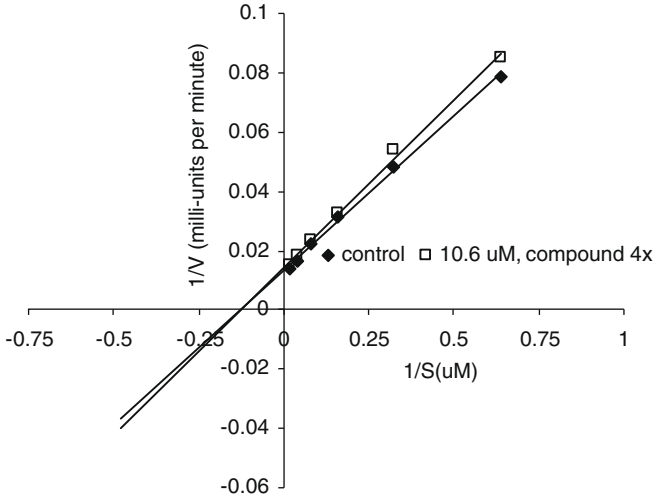


Figure 1. Lineweaver–Burk plot for the hydrolysis of substrate *p*-nitrophenyl-α-D-glucopyranoside (*p*-NPG) by rat intestinal α-glucosidase. Experimental procedures remain same as described earlier except that the V_{max} kinetics was recorded with 10 s lag time after addition of the substrate for 10 min at the interval of 30 s, with or without pre-incubation of **4x** with different dilutions of substrate concentration. It is evident from the figure that the slope for compound **4x** at 10.6 μM concentration crosses slope of the control left to the intercept of *x*-axis. Therefore, compound **4x** might be inhibiting enzyme α-glucosidase by mixed non-competitive mechanism.

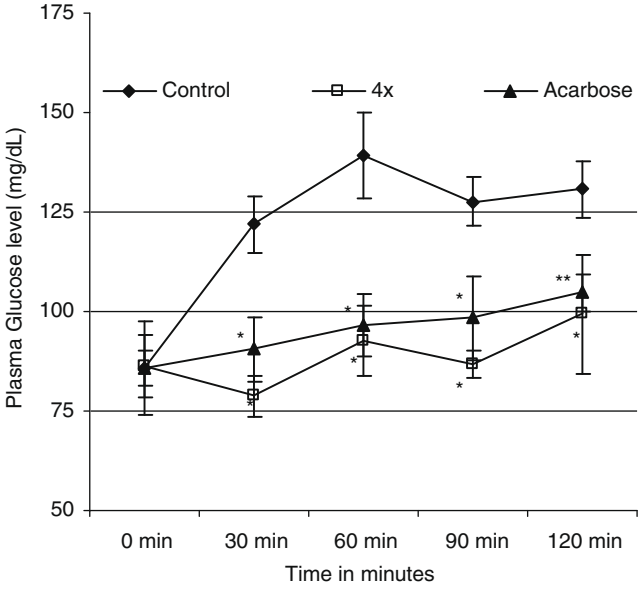


Figure 2. Antihyperglycemic activity of test compound **4x** in rats. Plasma glucose levels of rats after starch tolerance test in various groups of animals at different time points. One way ANOVA analysis followed by Bonferroni's multiple comparison tests was applied to find difference between the groups. Values represent mean ± SD, $n = 6$, $*$ and $**$ represent statistical comparisons between control and test groups. $^{*}p < 0.001$, $^{**}p < 0.01$. The dose of compound **4x** was selected 100 mg/kg body weight and for standard drug acarbose 10 mg/kg body weight as per our previous reports.

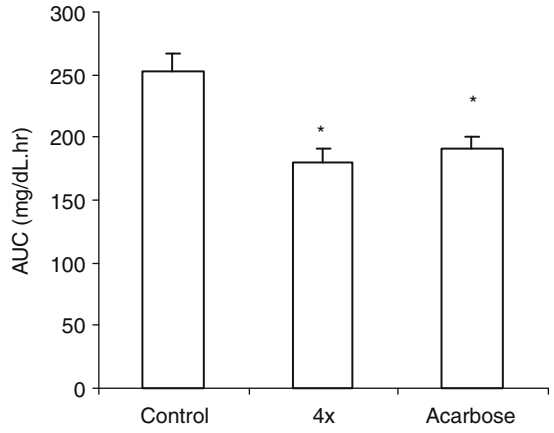


Figure 3. Area under the curve (AUC) represents influence on blood glucose level per hour. It was calculated applying following formula:[18] AUC (mg/dL h) = $(BG_0 + BG_{30}) \times 0.5/2 + (BG_{30} + BG_{60}) \times 0.5/2 + (BG_{120} + BG_{60}) \times 1/2$, where BG_0, BG_{30}, BG_{60}, and BG_{120} represent blood glucose level at 0, 30, 60 and 120 min after starch feeding to the rats. One way ANOVA analysis followed by Bonferroni's multiple comparison tests was applied to find difference between the groups. *p <0.001 in comparison with control.

3. Conclusion

Compounds **4k**–**z** were synthesized applying new synthetic approaches. Suzuki coupling for the synthesis of compounds **3h**–**n** has been applied for the first time in synthesizing chromenone derivatives. Though compounds **4a**–**g**, **4i**, and **4j** are known in the literature along with the synthetic methodology applied in this report, the assignment of the α-glucosidase inhibitory activity to all the chromenone derivatives in this report is new aspect. Compound **4x** is new and being reported in this study to possess intestinal α-glucosidase inhibitory as well as antihyperglycemic activity for the first time. Therefore, it may serve as a model compound for design and development of therapeutic based on α-glucosidase inhibitory antihyperglycemic activity. This study further presents chromenone derivatives as new class of α-glucosidase inhibitors.

4. Experimental

4.1. Chemistry

^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Gemini 200 MHz and Avance 300 MHz spectrometer in $CDCl_3$ using TMS as internal standard. IR spectra were recorded on a Nicollet 740 FTIR spectrometer. Mass spectra were obtained on Agilent LCMS instrument. Melting points were determined in open glass capillary tubes on a Metler FP 51 melting point apparatus and are uncorrected. All reactions were monitored by thin layer chromatography (TLC) on pre-coated Silica Gel 60 F_{254} (mesh); spots were visualized under UV light. Merck silica gel (60–120; 100–200 mesh) was used for chromatography. All the reactions were carried out using reagent-grade solvents, and the reagents were purchased from Sigma–Aldrich.

4.1.1. General procedure for the synthesis of 3-substituted chromenones (4a–c)

The resorcinol (1 mmol), ethyl 4-chloro acetoacetate (1.1 mmol) and *p*-TSOH (10 mol %) in dry toluene refluxed for 20 min. The crude compound on flash chromatography using silica gel gave 4-(chloromethyl)-7-hydroxy-2*H*-2-chromenone (**4a**) in 65% yield. Similarly the compounds **4b** and **4c** were prepared by using the corresponding β-ketoesters.

4.1.1.1. 4-(Chloromethyl)-7-hydroxy-8-methyl-2H-2-chromenone (4b). Solid, mp 254–256 °C. IR (KBr): 3275, 2923, 2853, 1703, 1660, 1574, 1507, 1376, 1315, 1222, 1154, 1089, 975 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 9.92 (s, 1H, OH), 7.34 (d, 1H, J = 8.2 Hz, aromatic), 6.92 (d, 1H, J = 8.2 Hz, aromatic), 6.36 (s, 1H, aromatic), 4.64 (s, 2H, CH_2Cl), 2.28 (s, 3H, CH_3). Mass (LC–MS): *m/z* 225 [M+1].

4.1.1.2. 3-Chloro-4-methyl-7-hydroxy-2H-2-chromenone (4c). Solid, ^{1}H NMR ($CDCl_3$): δ 7.45 (d, 1H, J = 8.0 Hz, aromatic), 6.80 (d, 1H, J = 8.2 Hz, aromatic), 6.75 (s, 1H, aromatic), 2.54 (s, 3H, CH_3). Mass (LC–MS): *m/z* 211 [M+1].

4.1.2. General procedure for the synthesis of 5-substituted salicylaldehydes (3g–n)

$Pd(PPh_3)_4$ (10 mol %) was added to a stirred solution of 5-bromosalicylaldehyde (**2**, 1 mmol), phenylboronic acid (1 mmol), K_2CO_3 (2.5 mmol) in dry toluene (5 mL) and ethanol (1 mL) at room temperature under N_2 atmosphere and the contents were stirred at the same temperature for 20 min. The reaction mixture was slowly heated to 75–80 °C for 2 h. After completion of the reaction (TLC), the solvents were removed under reduced pressure, the reaction mixture was extracted with ethylacetate (2 × 20 mL) and washed with water, the organic layer was separated, dried over sodium sulfate, solvent removed under reduced pressure and the obtained crude product was purified by flash chromatography using silica gel (60–120) with hexane/ethylacetate (98:2) as eluent to give 2-hydroxy-5-phenylbenzaldehyde (**3g**) in 78% yield. Yellow solid, mp 95–97 °C. IR (KBr): 3056, 2850, 1654, 1586, 1473, 1420, 1373, 1296, 1233, 1176, 898, 767 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 10.98 (s, 1H, OH), 10.00 (s, 1H, CHO), 7.75 (d, J = 8.2 Hz, 2H, aromatic), 7.50 (d, J = 8.4 Hz, 2H, aromatic), 7.42–7.38 (m, 2H, aromatic), 7.32–7.30 (m, 1H, aromatic), 7.08 (d, J = 8.8 Hz, 1H, aromatic). Mass (LC–MS): *m/z* 197 [M−1].

4.1.2.1. 2-Hydroxy-5-(4-methylphenyl)-benzaldehyde (3h). Yield 65%. Yellow solid, mp 74–76 °C, IR (KBr): 3418, 2922, 2854, 1651, 1591, 1478, 1262, 1169, 815, 723 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 10.88 (s, 1H, OH), 9.88 (s, 1H, CHO), 7.68–7.62 (m, 2H, aromatic), 7.33 (d, J = 8.2 Hz, 2H, aromatic), 7.15 (d, J = 8.4 Hz, 2H, aromatic), 6.97 (d, J = 8.0 Hz, 1H, aromatic), 2.32 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.67, 160.73, 137.19, 136.41, 135.58, 133.25, 131.57, 129.66, 126.39, 120.66, 118.02, 21.05. Mass (LC–MS): *m/z* 211 [M−1].

4.1.2.2. 2-Hydroxy-5-(4-chlorophenyl)-benzaldehyde (3i). Yield 76%. Yellow solid, mp 85–87 °C. IR (KBr): 3031, 2848, 2362, 1656, 1588, 1476, 1394, 1266, 1172, 1092, 816 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 10.98 (s, 1H, OH), 9.98 (s, 1H, CHO), 7.74–7.66 (m, 2H, aromatic), 7.48–7.36 (m, 4H, aromatic), 7.06 (d, J = 8.4 Hz, 1H, aromatic). Mass (LC–MS): *m/z* 231, 233 [M−1].

4.1.2.3. 2-Hydroxy-5-(4-fluorophenyl)-benzaldehyde (3j). Yield 82%. Yellow solid, mp 91–93 °C. IR (KBr): 3449, 3062, 2852, 1659, 1592, 1478, 1221, 1170, 904, 829 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 11.00 (s, 1H, OH), 9.96 (s, 1H, CHO), 7.74–7.69 (m, 2H, aromatic), 7.53–7.47 (m, 2H, aromatic), 7.18–7.05 (m, 3H, aromatic). Mass (LC–MS): *m/z* 217 [M+1].

4.1.3. General procedure for the synthesis of 3,6-disubstituted chromenone derivatives (4d–z)

Piperidine (5 mol %) was added to a stirred solution of 5-phenyl-2-hydroxybenzaldehyde (**3g**, 1 mmol), ethylacetoacetate (1.1 mmol) in CH_3CN (4 mL) at room temperature. The contents were stirred for 4 h at the same temperature. After completion of the reaction (TLC), the solvent was removed under reduced pressure and the crude product was subjected for column chromatography purification using silica gel (60–120) with hexane/

ethylacetate (8:2) as eluent to give 3-acetyl-6-phenyl-2*H*-2-chromenone (**4m**) in 95% yield as yellow solid, mp 198–200 °C. IR (KBr): 3067, 2922, 1743, 1679, 1565, 1483, 1359, 1208, 1109, 970, 841, 765 cm^{-1}, ^1H NMR ($CDCl_3$): δ 8.54 (s, 1H, aromatic), 7.85–7.81 (m, 2H, aromatic), 7.53 (d, *J* = Hz, 2H, aromatic), 7.45–7.38 (m, 4H, aromatic), 2.72 (s, 3H, CH_3). Mass (LC–MS): *m/z* 265 [M+1].

4.1.3.1. 3-Benzoyl-2H-2-chromenone (4f). Solid, mp 132–134 °C. IR (KBr): 3283, 3081, 2985, 2948, 1687, 1626, 1447, 1387, 1281, 1234, 1136, 1042, 916 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.05 (s, 1H, aromatic), 7.84 (d, 2H, *J* = 7.16 Hz, aromatic), 7.65–7.55 (m, 3H, aromatic), 7.49–7.26 (m, 4H, aromatic). ^{13}C NMR ($CDCl_3$): 191.60, 154.69, 145.38, 136.14, 133.77, 133.59, 129.53, 128.55, 126.92, 124.93, 118.10, 116.87. Mass (LC–MS): *m/z* 251 [M+1].

4.1.3.2. 3-Benzoyl-7-hydroxy-2H-2-chromenone (4j). Solid, mp 217–219 °C. IR (KBr): 3191, 1688, 1651, 1589, 1514, 1446, 1373, 1221, 1149, 930 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.12 (s, 1H, aromatic), 7.86–7.82 (m, 2H, aromatic), 7.65–7.56 (m, 1H, aromatic), 7.52–7.44 (m, 3H, aromatic), 6.86–6.78 (m, 2H, aromatic). Mass (LC–MS): *m/z* 267 [M+1].

4.1.3.3. 4-Methyl-2-oxo-6-phenyl-2H-chromene-3-carbonitrile (4k). Yellow solid, mp 185–187 °C. IR (KBr): 2922, 2852, 2224, 1721, 1607, 1561, 1481, 1448, 1378, 1288, 1249, 1084, 759 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.92–7.84 (m, 2H, aromatic), 7.62–7.58 (m, 2H, aromatic), 7.56–7.40 (m, 4H, aromatic), 2.84 (s, 3H, CH_3). Mass (LC–MS): *m/z* 262 [M+1].

4.1.3.4. 6-(6-Methoxy-3-pyridyl)-4-methyl-2-oxo-2H-chromene-3-carbonitrile (4l). Yellow solid, mp 199–201 °C. IR (KBr): 2921, 2851, 2226, 1731, 1604, 1563, 1483, 1368, 1283, 1082, 997, 825, 763 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.32 (s, 1H, heteroaromatic), 7.82–7.72 (m, 3H, heteroaromatic + aromatic), 7.46 (d, 1H, *J* = 8.2 Hz, aromatic), 6.82 (d, 1H, *J* = 8.2 Hz, aromatic), 3.98 (s, 3H, OCH_3), 2.84 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 145.13, 137.25, 133.58, 127.82, 123.52, 118.35, 111.32, 53.73, 29.66. Mass (LC–MS): *m/z* 293 [M+1].

4.1.3.5. 3-Benzoyl-6-phenyl-2H-2-chromenone (4o). Yellow solid, mp 158–160 °C. IR (KBr): 3415, 2922, 2854, 1715, 1664, 1572, 1477, 1254, 1210, 1153, 924, 759 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.12 (s, 1H, aromatic), 7.90–7.82 (m, 3H, aromatic), 7.76 (d, 1H, *J* = 2.0 Hz, aromatic), 7.64–7.35 (m, 9H, aromatic). ^{13}C NMR (75 MHz, $CDCl_3$): δ 145.47, 133.84, 132.56, 129.59, 129.11, 128.61, 128.04, 127.19, 127.03, 117.29. Mass (LC–MS): *m/z* 327 [M+1].

4.1.3.6. 3-Acetyl-6-(4-methylphenyl-2H-2-chromenone (4p). Yellow solid, mp 205–207 °C. ^1H NMR ($CDCl_3$): δ 8.52 (s, 1H, aromatic), 7.84, 7.82 (dd, 1H, *J* = 8.2 Hz, 2.6 Hz, aromatic), 7.78 (d, 1H, *J* = 2.6 Hz, aromatic), 7.46–7.38 (m, 3H, aromatic), 7.26 (d, 2H, *J* = 8.0 Hz, aromatic), 2.78 (s, 3H, $COCH_3$), 2.40 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 195.68, 159.47, 154.67, 147.79, 138.60, 138.26, 136.15, 133.43, 130.05, 128.05, 127.05, 124.92, 118.66, 117.20, 30.77, 21.32. Mass (LC–MS): *m/z* 279 [M+1].

4.1.3.7. 3-Acetyl-6-(4-chlorophenyl)-2H-2-chromenone (4r). Yellow solid, mp 232–234 °C. IR (KBr): 3426, 2919, 2850, 1725, 1681, 1612, 1562, 1230, 1123, 811, 768 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.60 (s, 1H, aromatic), 7.88–7.82 (m, 2H, aromatic), 7.56–7.42 (m, 5H, aromatic), 2.60 (s, 3H, CH_3). Mass (LC–MS): *m/z* 299 [M+1].

4.1.3.8. 3-Acetyl-6-(4-fluorophenyl)-2H-2-chromenone (4s). Yellow solid, mp 196–198 °C. IR (KBr): 3064, 2925, 1727, 1681, 1566, 1513, 1401, 1230, 1160, 974, 814 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.56 (s, 1H, aromatic), 7.85–7.76 (m, 2H, aromatic), 7.57–7.51 (m, 2H, aromatic), 7.45 (d, 1H, *J* = 8.4, aromatic), 7.22–7.15 (m, 2H, aromatic), 2.75 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 195.37, 164.44, 154.52, 147.38, 137.42, 133.16, 128.72, 128.61, 127.96, 124.83, 118.45, 117.11, 116.21, 115.92. Mass (LC–MS): *m/z* 283 [M+1].

4.1.3.9. Ethyl-6-(4-fluorophenyl)-2-oxo-2H-chromene-3-carboxylate (4t). Yellow solid, mp 180–182 °C. IR (KBr): 3061, 2983, 2923, 2852, 1742, 1710, 1575, 1482, 1265, 1161, 1033, 975, 830, 791 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.56 (s, 1H, aromatic), 7.81, 7.78 (d,d, 1H, *J* = 2.0, *J* = 8.6, aromatic), 7.22 (d, 1H, *J* = 2.0, aromatic), 7.55–7.49 (m, 2H, aromatic), 7.42 (d, 1H, *J* = 8.4, aromatic), 7.20–7.12 (m, 2H, aromatic), 4.43 (q, 2H, OCH_2), 1.43 (t, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 148.50, 133.08, 128.73, 128.62, 127.29, 117.24, 116.21, 115.92, 62.07, 29.62, 14.21. Mass (LC–MS): *m/z* 313 [M+1].

4.1.3.10. 3-Acetyl-6-(3-pyridyl)-2H-2-chromenone (4u). Yellow solid, mp 200–202 °C. IR (KBr): 3063, 2922, 2852, 1735, 1676, 1561, 1361, 1207, 967, 802 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.81 (d, 1H, *J* = 2.2 Hz, heteroaromatic), 8.68, 8.66 (dd, 1H, *J* = 8.0 Hz, 2.0 Hz, heteroaromatic), 8.58 (s, 1H, aromatic), 7.94–7.83 (m, 3H, aromatic + heteroaromatic), 7.48 (d, 1H, *J* = 8.2 Hz, aromatic), 7.46–7.40 (m, 1H, aromatic), 2.78 (s, 3H, $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.05, 159.40, 155.44, 148.23, 147.70, 147.17, 135.94, 134.72, 133.51, 128.85, 125.38, 124.78, 119.09, 117.90, 30.60. Mass (LC–MS): *m/z* 266, 288 [M+1, M+Na].

4.1.3.11. 3-Acetyl-6-(4-pyrimidinyl)-2H-2-chromenone (4v). Yellow solid, mp 250–252 °C. Yield: 85%. IR (KBr): 3437, 3044, 2923, 2852, 1737, 1681, 1563, 1420, 1206, 975 cm^{-1}. ^1H NMR ($CDCl_3$): δ 9.80 (s, 1H, heteroaromatic), 8.98 (s, 2H, heteroaromatic), 8.55 (s, 1H, aromatic), 7.88–7.83 (m, 2H, aromatic), 7.56 (d, 1H, *J* = 8.2 Hz, aromatic), 2.74 (s, 3H, $COCH_3$). Mass (LC–MS): *m/z* 267 [M+1].

4.1.3.12. Ethyl-2-oxo-6-(4-pyrimidinyl)-2H-chromene-3-carboxylate (4w). Yellow solid, mp 194–196 °C. IR (KBr): 3508, 3061, 2925, 2854, 1742, 11709, 1625, 1400, 1269, 1025, 848 cm^{-1}. ^1H NMR ($CDCl_3$): δ 9.30 (s, 1H, heteroaromatic), 9.00 (s, 2H, heteroaromatic), 8.60 (s, 1H, aromatic), 7.89–7.80 (m, 2H, aromatic), 7.56 (d, 1H *J* = 8.0 Hz, aromatic), 4.45 (q, 2H, OCH_2), 1.48 (t, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 162.91, 158.23, 156.23, 156.23, 155.65, 155.02, 147.92, 132.83, 131.47, 127.87, 119.84, 118.88, 118.35, 62.45, 14.43. Mass (LC–MS): *m/z* 297 [M+1].

4.1.3.13. 3-Acetyl-6-(6-methoxy-3-pyridyl)-2H-2-chromenone (4x). Yellow solid, mp 204–206 °C. IR (KBr): 2957, 1932, 1739, 1679, 1608, 1566, 1481, 1360, 1288, 1236, 1022, 973, 823 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.52 (s, 1H, heteroaromatic), 8.34 (s, 1H, aromatic), 7.82–7.70 (m, 3H, heteroaromatic + aromatic), 7.44 (d, 1H, *J* = 8.0 Hz, aromatic), 6.82 (d, 1H, *J* = 8.0 Hz, aromatic), 3.98 (s, 3H, OCH_3), 2.74 (s, 3H, $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 195.33, 164.07, 159.06, 154.57, 147.28, 145.02, 137.19, 135.20, 132.78, 127.86, 127.57, 124.95, 118.62, 117.33, 111.26, 53.68, 30.55. Mass (LC–MS): *m/z* 296 [M+1].

4.1.3.14. Ethyl-6-(6-methoxy-3-pyridyl)-2-oxo-2H-chromene-3-carboxylate (4y). Yellow solid, mp 161–163 °C. IR (KBr): 3432, 3060, 2979, 2930, 1741, 1710, 1481, 1289, 1264, 1022, 834, 793 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.56 (s, 1H, heteroaromatic), 8.34 (s, 1H, aromatic), 7.82–7.68 (m, 3H, aromatic + heteroaromatic), 7.42 (d, 1H, *J* = 8.2 Hz, aromatic), 6.82 (d, 1H, *J* = 8.0 Hz, aromatic), 4.48 (q, 2H, OCH_2), 3.98 (s, 3H, OCH_3), 1.44 (t, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 164.03, 162.94, 156.51, 154.41, 148.35, 144.99, 137.19, 135.02, 132.69, 127.91, 126.87, 118.83, 118.24, 117.42, 111.21, 62.05, 53.66, 14.19. Mass (LC–MS): *m/z* 326 [M+1].

4.1.3.15. 3-Acetyl-6-(4-morpholinophenyl)-2H-2-chromenone (4z). Yellow solid, mp 158–160 °C. IR (KBr): 3056, 2923, 2853, 2366, 1745, 1709, 1622, 1573, 1476, 1365, 1268, 1029, 758 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.55 (s, 1H, aromatic), 7.86, 7.82 (dd, 1H, J = 8.0 Hz, 2.0 Hz, aromatic), 7.78 (d, 1H, J = 2.0 Hz, aromatic), 7.52 (d, 2H, J = 8.0 Hz, aromatic), 7.42 (d, 1H, J = 8.2 Hz, aromatic), 6.98 (d, 2H, J = 8.2 Hz, aromatic), 3.94–3.88 (m, 4H, $O(CH_2)_2$), 3.28–3.20 (m, 4H, $N(CH_2)_2$), 2.74 (s, 3H, $COCH_3$). Mass (LC–MS): m/z 350 [M+1].

4.2. Biological activity evaluation

4.2.1. DPPH free radical scavenging activity

Assay for the scavenging of stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was done as reported earlier.[11] Briefly, in a 96-well microplate, 25 μL of test sample dissolved in DMSO (1 mg/mL), 125 μL of 0.1 M Tris–HCl buffer (pH 7.4) and 125 μL of 0.5 mM DPPH solution dissolved in absolute ethyl alcohol were added. The reaction mixture was shaken well and incubated in dark for 30 min and read at 517 nm spectrophotometrically (Spectra Max plus384, Molecular Devices Corporation, Sunnyvale, CA, USA). Percentage of DPPH scavenging was calculated as $(1 - B/A) \times 100$, where A represents absorbance of control without test samples, and B represents absorbance in presence of test samples.

4.2.2. α-Glucosidase inhibitory assay

α-Glucosidase inhibitory activities were determined as per earlier reported methods.[11,12] Rat intestinal acetone powder in normal saline (100:1; w/v) was sonicated properly and the supernatant was used as a source of crude intestinal α-glucosidase after centrifugation. In brief, 20 μL of test samples (5 mg/mL DMSO solution) were reconstituted in 100 μL of 100 mM-phosphate buffer (pH 6.8) in 96-well microplate and incubated with 50 μL of crude intestinal α-glucosidase for 5 min before 50 μL substrate (5 mM, p-nitrophenyl-α-D-glucopyranoside prepared in same buffer) was added. Release of p-nitrophenol was measured at 405 nm spectrophotometrically (Spectra Max plus 384), Molecular Devices Corporation, Sunnyvale, CA, USA) 5 min after incubation with substrate. Individual blanks for test samples were prepared to correct background absorbance where substrate was replaced with 50 μL of buffer. Control sample contained 10 μL DMSO in place of test samples. Percentage of enzyme inhibition was calculated as $(1 - B/A) \times 100$, where [A] represents absorbance of control without test samples, and [B] represents absorbance in presence of test samples. For calculation of 50% enzyme inhibitory activity ($IC_{50\%}$) more than five dilutions of primary screening concentration (5 mg/mL DMSO solution) of test compounds were prepared. The IC_{50} values were calculated applying logarithmic regression analysis.

4.2.3. Animal experiment

Antihyperglycemic activity study was done according to the method reported earlier.[11,12] Wistar rats of either sex weighing between 195 and 215 g were obtained from National Institute of Nutrition (CPCSEA Reg. No. 154, Government of India), Hyderabad. The animals were housed in standard polyvinyl cages. The room temperature was maintained at 22 ± 1 °C with an alternating 12 h light dark cycle. Food and water were provided ad libitum. Experiments were performed as per the Institutional Animal Ethical Committee norms. The rats were divided into various groups' viz. control, test compound **4x** group (100 mg/kg body weight) and a group with standard drug acarbose (10 mg/kg body weight). Five rats in each group were taken. All the animals were kept for overnight fasting. Next day forenoon blood was collected from retro orbital plexus in EDTA containing tubes, and plasma glucose levels for basal ('0' h) value were measured by glucose–oxidase test method using auto blood analyzer instrument (Bayer EXPRESS PLUS). Test compound **4x** and standard drug acarbose were suspended in normal saline and administered orally through gastric intubation. The control group of animals were treated sham with normal saline. Fifteen minutes after treatment, animals were fed with soluble-starch dissolved in normal saline at a dose of 2 g/kg body weight. Thereafter, blood was collected at intervals of 30, 60, 90, and 120th min post starch feeding. Plasma was separated out for glucose measurement as described above.

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