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Synthesis and antihyperglycemic activity of phenolic C-glycosides ☆

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ABSTRACT

Various phenolic C-glycosides were evaluated for their in vitro and in vivo antihyperglycemic activity employing glucose uptake by rat muscle cell lines (L-6) and low dosed-streptozotocin-induced diabetic rats, respectively. Some of phenolic *C*-glycosides were isolated from *Pterocarpus marsupium* and *Ulmus wallichiana* and other were synthesized by unprotected sugar and phloroacetophenone using $Sc(OTf)_3$ in aqueous ethanol. Eight among tested compounds showed significant lowering of blood glucose level on low dosed-streptozotocin-induced diabetic rats. The compound **24** lowered the blood glucose levels by 34.9% and 33.6% during 0–5 h and 0–24 h, respectively, at the dose of 25 mg/kg body weight which is comparable to standard antidiabetic drug metformin.

Diabetes mellitus, often simply referred to as diabetes is a metabolic disorder in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not properly use insulin, a hormone that is required to convert sugar, starches, and other food into energy. Human body has to maintain the threshold blood glucose level which may be done with insulin or glucagon depending on the condition. Patients with diabetes are 4 times more likely to have coronary heart disease and stroke. The chronic hyperglycemia of this disease is associated with long-term dysfunction, damage, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels.[1] One of the hallmarks of type II diabetes is decreased sensitivity of muscle and adipose cells to insulin. Compounds that increase the sensitivity of muscle and adipose to insulin may be useful in the treatment of diabetes and its complications. Upon insulin treatment, insulin receptor is phosphorylated which activates insulin signal transduction pathway leading to increased glucose uptake by glucose transporter 4 (GLUT4) in adipocytes (fat) or myocytes (muscle). Therefore, measuring glucose uptake by these cells provides the most relevant end point assay for insulin sensitivity.[2]

Despite the fact that many oral therapeutic agents which increase the insulin sensitivity, such as metformin, sulfonylureas, thiazolidinedione and DPPIV inhibitors, have already been used in clinical situations, it is still difficult to tightly control plasma glucose and prevent diabetic complications.[3–5] The discovery of new lead structure has become an important area of research in medicinal chemistry as new chemical entity is continue to fall and toxicity of the present drugs becomes a dilemma. In continuation to our antidiabetic drug discovery program, a series of C-glycosides were isolated from terrestrial plants, derivatives prepared and evaluated for their glucose uptake enhancing activity and in vivo antihyperglycemic activity potential on low dosed-streptozotocin-induced diabetic male albino rats of Sprague–Dawley strain. Streptozotocin is a powerful alkylating agent that induces multiple DNA strand breaks. A single large dose of streptozotocin can produce type 1 diabetes mellitus in rodents by disrupting β-cell of pancreas.[6]

Naturally-occurring aryl C-glycosides[7–9] exhibit interesting biological activities especially *C*-glycosyl flavonoids which show a variety of bioactivities such as antiviral,[10] cytotoxic,[11] DNA binding[12] and hypotensive[13] activities and their derivatives that contain a trans-caffeoyl group show cytotoxic activity against P388 lymphocytic leukemia cells. Biological importance of aryl C-glycoside over aryl O-glycoside may be due to replacement of the exocyclic carbon–oxygen bond at the anomeric center with a carbon–carbon bond which creates a hydrolytically stable carbohydrate mimetic with many possible biological applications.[14] For instance, examination of carbohydrate–protein interactions and cell surface carbohydrate signaling is possible by conjugation of oligosaccharides to molecular probes through a C-glycoside tether.[15]

C-Glycosides **1–7** were isolated from an aqueous extract of the heartwood of *Pterocarpus marsupium* Roxb. (Leguminaceae).[16] It has been used in the treatment of diarrhea, toothache, fever, urinary tract and skin infections. The wooden glass made up of heartwood of this plant is being used for drinking water to control blood sugar in Ayurvedic system of medicine.[17,18] The aqueous extract of the heartwood of *P. marsupium* has been

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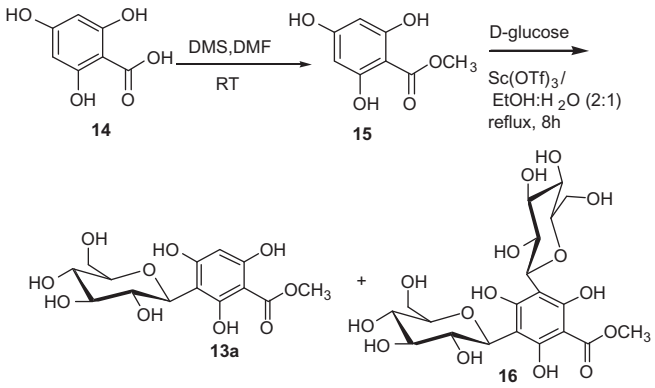
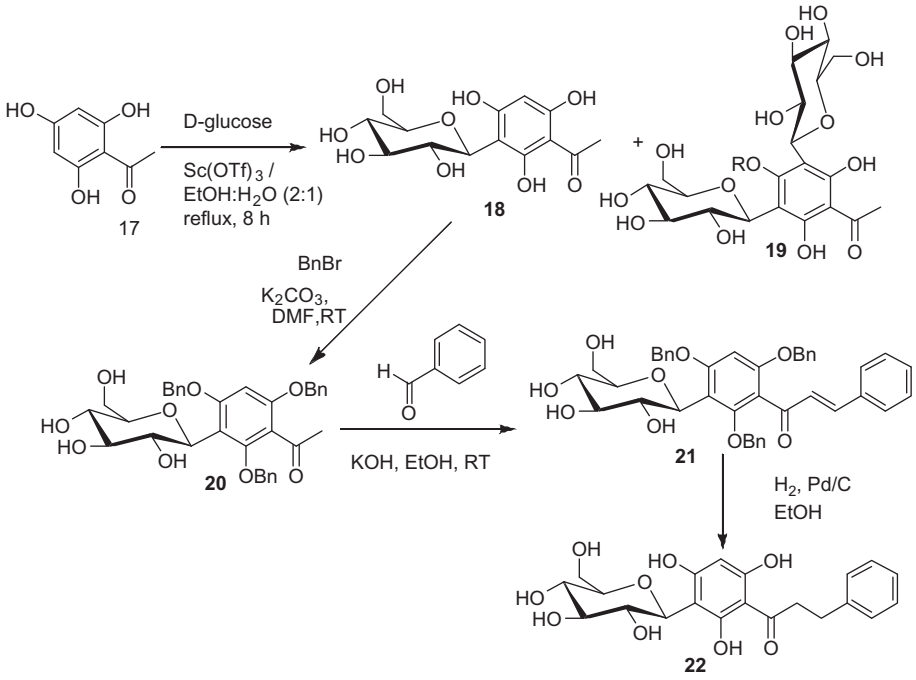


Scheme 1. Synthesis of compounds **13** and **16**.

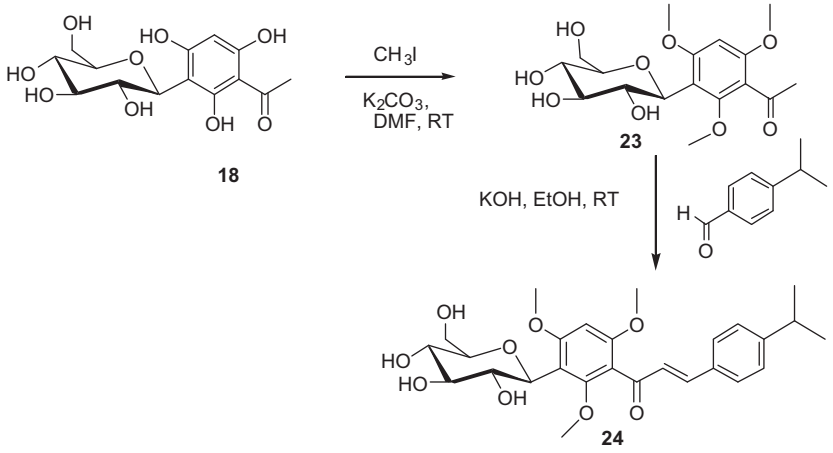
tested clinically and found effective in non-insulin dependent diabetes mellitus patients.[19]

Flavonoid C-glucosides **8–13** were isolated from ethanolic extract of stem bark of *Ulmus wallichiana*.[20] To get the diversity in the C-glycosides for SAR study, compound **13**, **16**, **19**, **22** and **24** were synthesized using an elegant method developed by Sato et al.[21] for C-glycosylation using $Sc(OTf)_3$ with unprotected sugars in aqueous media. The structure of 3-*C*-β-D-glucopyranoside-2,4,6-trihydroxymethylbenzoate (**13**) isolated from *U. wallichiana* was also verified by its synthesis.

The esterification of 2,4,6-trihydroxybenzoic acid (**14**) in the presence of dimethyl sulfate and K_2CO_3 in DMF afforded esters **15**. Unprotected D-glucose and ester **15** were reacted in the presence of a catalytic amount of $Sc(OTf)_3$ in an aqueous ethanol, lead to the formation of the 3-*C*-β-D-glucopyranosylphloroacetophenone **13** in 40% yield, along with the di-*C*-β-D-glucoside **16** in 39% yield (Scheme 1).



Scheme 2. Synthesis of compound **18**, **19** and **22**.



Scheme 3. Synthesis of compound **24**.

Table 1
In vivo antihyperglycemic activity of phenolic C-glycosides on the blood glucose levels of streptozotocin-induced diabetic rats

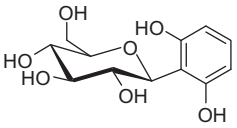
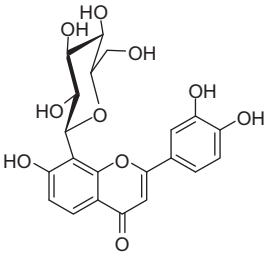
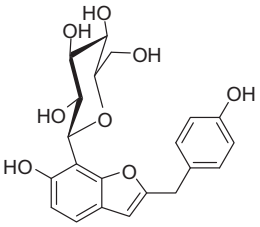
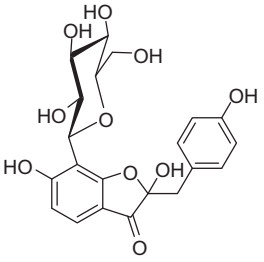
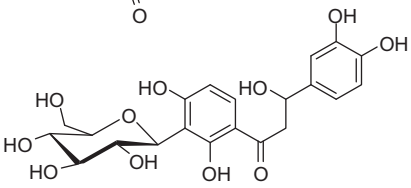
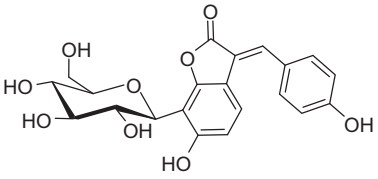
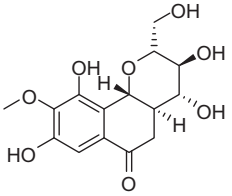
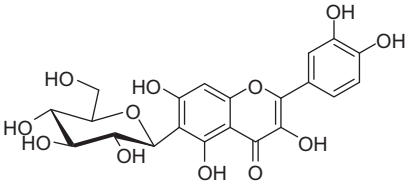
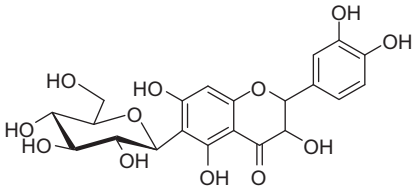
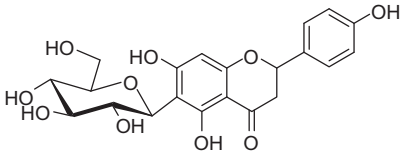
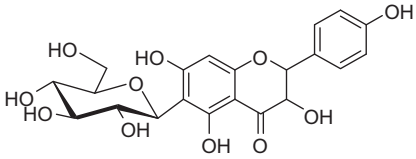
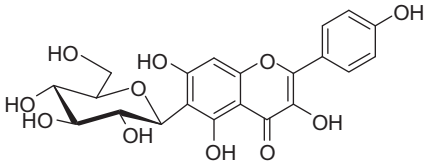
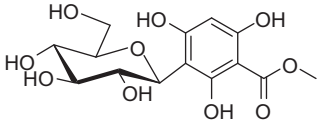
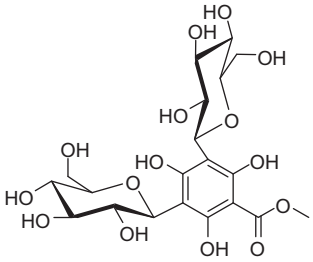
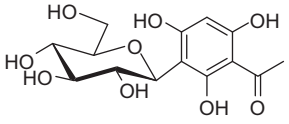
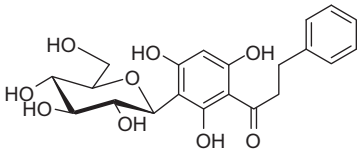
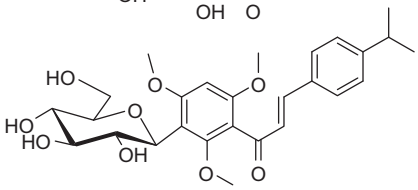
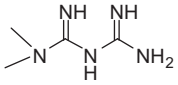
Compounds	Structure	% Antihyperglycemic activity		% Increase in glucose uptake by L-6 muscle cells
		0–5 h AUC	0–24 h AUC	
1		12.7	22.3	Nil
2		14.3*	16.1*	Nil
3		9.78	11.2	ND
4		1.99	7.90	Nil
5		20.0***	19.5***	ND
6		17.2***	29.0***	16.0
7		9.04	10.5	Nil
8		15.6	8.56	ND

Table 1 (*continued*)

Compounds	Structure	% Antihyperglycemic activity		% Increase in glucose uptake by L-6 muscle cells
		0–5 h AUC	0–24 h AUC	
9		17.6*	12.8*	10.2
10		8.41	15.1	12.2
11		23.8***	23.8***	ND
12		16.7*	23.5*	12.2
13		7.27	2.90	ND
16		11.5	7.78	10.8
19		18.0**	20.9**	15.3
22		22.3***	26.5***	11.6
24		34.9***	33.6***	ND
Metformin		27.2***	24.1***	19.4

Values are mean ± SEM, $n = 5$.

* $p < 0.05$ versus vehicle treated control.

** $p < 0.01$ versus vehicle treated control.

*** $p < 0.001$ versus vehicle treated control.

The ^{1}H NMR of **13** showed one singlet at δ_H 6.00 for an aromatic proton and one as doublet at δ_H 4.83 (J = 9.9 Hz) for anomeric proton. Configuration of the sugar moiety of **13** was determined to be β as $J_{1'2'}$ = 9.9 Hz. The spectral data of synthesized 3-*C*-β-D-glucopyranoside-2,4,6-trihydroxymethylbenzoate was identical with the natural compound **13** isolated from *U. wallichiana* stem bark.[19] The ^{1}H NMR spectrum of **16**, showed the absence of aromatic signal around at δ_H 6.00 and the presence of one doublet at δ_H 4.67 (2H, d, J = 9.6 Hz, H-1′, 1″) integrating for two protons corresponding to two anomeric protons, indicated the presence of two glucose moiety in the molecule. The anomeric configuration of both glucose moieties of **16** is due to the large coupling constant (J = 9.6 Hz). The anomeric carbons were resonated at δ_C 74.6 showed that both sugar moieties were connected with aglycone moiety by C–C linkage. Similarly 3-C-β-D-glucopyranosylphloroacetophenone (**18**) and 3,5-di-*C*-β-D-glucopyranosylphloroacetophenon[21] (**19**) were synthesized by unprotected D-glucose and phloroacetophenonere (**17**) (Scheme 2).

3-*C*-β-D-Glucopyranosylphloroacetophenone (**18**) was subjected to aromatic benzylation with 3.5 equiv of benzyl bromide in the presence of potassium carbonate in DMF followed by Aldol condensation with benzaldehyde in EtOH and then subsequent hydrogenolysis with H_2, Pd/C led to the formation of 3′-*C*-β-D-glucopyranosyldihydrochalcone (**22**).[22] 3-*C*-β-D-Glucopyranosylphloroacetophenon (**18**) was subjected to methylation with methyl iodide in the presence of K_2CO_3, in DMF followed by Aldol condensation with 4-isopropylbenzaldehyde led to the formation of compound **24**[23] is a new compounds. All these synthesized compounds were characterized with the help of ^{1}H and ^{13}C NMR, mass spectroscopy and elemental analysis (Scheme 3).

These compounds were examined for their effect on glucose uptake enhancing activity. The effect was measured using L-6 rat muscle cells according to Hwang et al.[24,25] with some minor modifications. Skeletal muscle is the main tissue involved in insulin-induced stimulation of glucose uptake. Insulin increases glucose uptake in skeletal muscle by increasing functional glucose transport molecules in the plasma membrane. Glucose transport in skeletal muscle can also be stimulated by contractile activity. The maximal effects of insulin and contractile activity on glucose transport are additive. In skeletal muscle, both insulin and contractile activity stimulate translocation of glucose transporter GLUT-4 protein from an intracellular membrane pool to the plasma membrane. Resistance to this stimulatory effect of insulin is a major pathological feature of diabetes. In the tested compounds only compound **6**, **19** and **22** enhance the glucose uptake by 16.0%, 15.3% and 11.6%, respectively, over control and results were compared with metformin which were used as the standard anti diabetic drugs.

All the seventeen compounds were evaluated for their in vivo antihyperglycemic activity on low dosed streptozotocin-induced diabetic rats.[26,27] Metformin was taken as positive control. Among the seventeen screened compounds, eight compounds (**2**, **5**, **6**, **11**, **12**, **19**, **22** and **24**) demonstrated moderate to excellent antihyperglycemic activity to the tune of 14–35% on the STZ-induced diabetic rats at 25 mg/kg oral dose (Table 1, Figs. 1–4).

The structure–activity profile revealed that flavonoids with hydroxy group at position 3 and 4′, showed good inhibitions compare to without hydroxyl at these position. Activity data also revealed that the absence of double bond in flavonoids showed an appreciable increase in activity and while an additional hydroxyl group at 3′ position of flavonoids reduces the activity as the compounds **8** and **12** are less active than **9** and **11**. Compound **11** is most active in this series because it has hydroxyl group at 4′ position and does not contain double bond in ring C, hydroxyl group at 3′ position. It is evident from the activity data that if C-ring of flavanoids is changed into five membered ring as in compound **3** and **4** activity decreases whereas isoaurone **6** showed good activity. In chalcone it seems that aromatic ring having more lipophilic groups are more active as **24** is more active than compounds **22** and **5**. Among all these compounds, chalcones are found most active

In conclusion, the C-glycosides exhibited moderate to excellent in vivo antihyperglycemic activity ranging from 19.9% to 34.9% in

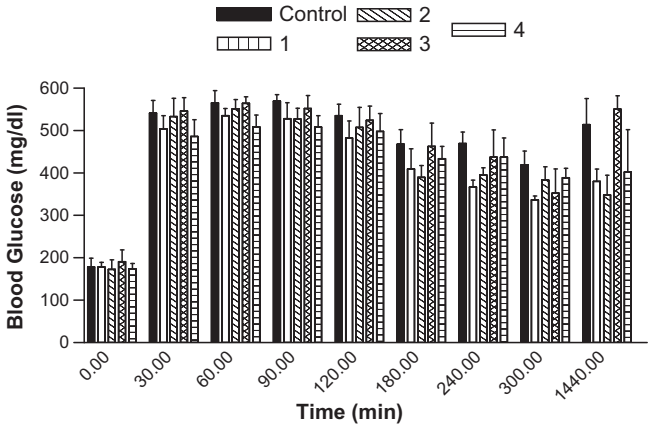


Figure 1. Effect of compounds **1**–**4** (at 25 mg/kg) on the blood glucose levels of the streptozotocin-induced diabetic rats at various time intervals.

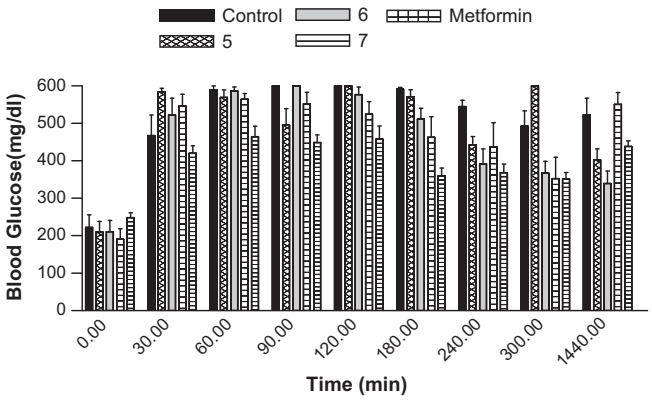


Figure 2. Effect of compounds **5**–**7** (at 25 mg/kg) on the blood glucose levels of the streptozotocin-induced diabetic rats at various time intervals.

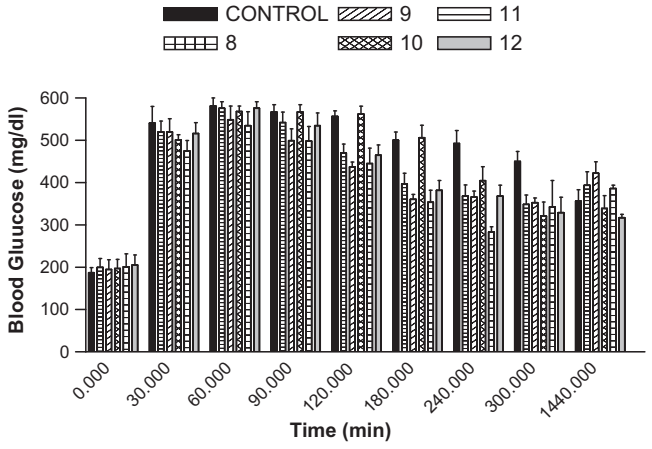


Figure 3. Effect of compounds **8**–**12** (at 25 mg/kg) on the blood glucose levels of the streptozotocin-induced diabetic rats at various time intervals.

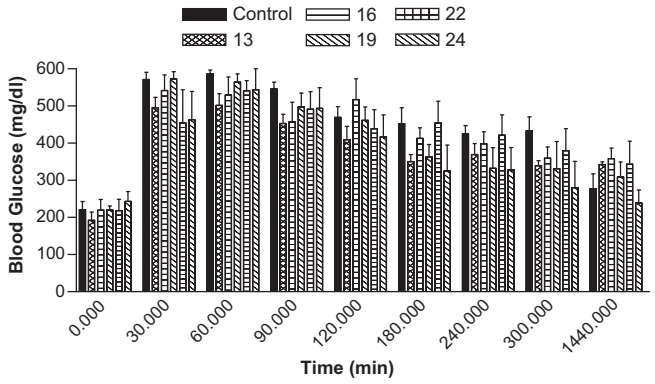


Figure 4. Effect of compounds **13**, **16**, **19**, **22** and **24** (at 25 mg/kg) on the blood glucose levels of the streptozotocin-induced diabetic rats at various time intervals.

STZ model. Compounds **11**, **22** and **24** lowered the blood glucose to around 23.8%, 26.5% and 33.6% after 24 h on STZ model, which is comparable to standard drug metformin. Further study on lead optimization and mechanism of action is in progress.

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22. *Spectral data of* **22**: ^{1}H NMR: (CD_3OD, 300 MHz) δ_H 7.22 (4H, m, H-2, 3, 5, 6), 7.15 (1H, m, H-4), 5.94 (1H, s, H-5′), 4.87 (1H, d, J = 9.0 Hz, H-1″), 3.98 (1H, t, J = 8.9 Hz, H-2″), 3.39 (1H, m, H-3″), 3.40 (2H, m, H-4″, 5″), 3.83 (1H, m, H-6″a), 3.70 (1H, m, H-6″b), 3.34 (2H, m, H-α), 2.94 (2H, t, J = 7.2 Hz, H-β).[13]C NMR: (CD_3OD, 75 MHz) δ_C 206.5 (C=O), 165.9 (C-4′), 165.0 (C-2′), 164.1 (C-6′), 143.2 (C-1), 129.6 (C-2,6), 129.6 (C-3, 5), 126.9 (C-4), 105.5 (C-1′), 104.4 (C-3′), 96.0 (C-5′), 82.6 (C-3″), 80.0 (C-5″), 76.1 (C-1″), 73.1 (C-2″), 71.6 (C-4″), 62.6 (C-6″), 47.1 (C-α), 32.2 (C-β). ESIMS: m/z 421 $[M+Na]^+$.
23. *Spectral data of* **24**: ^{1}H NMR: (CD_3OD, 300 MHz) δ_H 7.83 (1H, d, J = 16.0 Hz, H-β), 7.62 (2H, d, J = 7.2 Hz), 7.42 (1H, d, J = 16.0 Hz, H = α), 7.39 (2H, d, J = 7.2 Hz, H-3, 5), 6.12 (1H, s, H-5′), 4.85 (1H, d, J = 9.0 Hz, H-1″), 3.97 (1H, t, J = 8.9 Hz, H-2″), 3.37 (1H, m, H-3″), 3.40 (2H, m, H-4″, 5″), 3.83 (1H, m, H-6″a), 3.81 (9H, s, $3OCH_3$) 3.70 (1H, m, H-6″b), 2.98 (1H, s, H-1‴), 1.33 (6H, d, J = 6.9 Hz, H-2‴, 3‴). ESIMS: m/z 525 $[M+Na]^+$.
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25. *Methodology of glucose uptake*
L6 cell culture: Stock culture of L6 myoblasts were maintained in DMEM supplemented with 10% (v/v) FBS, streptomycin (200 μg/ml), and penicillin G (100 μg/ml) under an atmosphere of 5% CO_2/95% humidified air at 37 °C. For differentiation into myotubes, cells were reseeded in 24-well plate (approx. 35,000 cells/well) containing DMDE media (10% FBS) for overnight culture. When cells were nearly confluent, 10% FBS containing DMDE media were replaced with DMDE containing 2% FBS and cells were maintained for seven to eight days for differentiation into myotubes. Medium was changed every 48 h prior to use in experiments.
Glucose uptake assay by L6 rat muscle cells
Measurements of radio labeled 2-deoxyglucose uptake were carried out with some modification in previously described method of Hwang et al.[23] Differentiated L6 mature myotubes cultured on 24-well plates were treated with the desired concentration of test compounds (10 μM or 50 μM) for 18 h. After that cells washed three-times with Krebs–Ringer *N*-(2-hydroxye thyl)piperazine-*N*-2-ethanesulfonic acid (HEPES) buffer saline. For glucose uptake measurement, cells were incubated in HEPES buffer saline (HBS) containing 3 μCi radio-labeled 2-deoxy-[3*H*]-D-glucose and 20 μM unlabeled 2-deoxyglucose for 15 min. The reaction was terminated by three quick washes with ice-cold HEPES buffer saline. Non-specific uptake was determined in the presence of 10 μM cytochalasin B. Cells were lysed in 0.1 NaOH and cell-associated radioactivity was determined by liquid scintillation counter and results were expressed as cpm/well as compared to controls.
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27. Evaluation for antihyperglycemic activity in streptozotocin-induced diabetic rats Male albino rats of Sprague–Dawley strain of blood glucose level between 60 and 80 mg/dl (8–10 weeks of age body weight 140 ± 20 g) were selected for this study. Streptozotocin was dissolved 0.1 M citrate buffer pH 4.5, and calculated amount of the fresh solution was injected to overnight fasted rats (60 mg/kg) intraperitoneally. Blood glucose was checked 48 h later by glucometer by using glucostrips and animals showing blood glucose values between 140 and 270 mg/dl were selected and divided into groups of five animals each. Rats of experimental groups were administered suspension of the desired test samples orally (made in 1.0% gum acacia) at a dose of 25 mg/kg body weight. Animals of control group were given an equal amount of 1.0% gum acacia. Control group was taken as 100%. A sucrose load of 2.5 g/ kg of body weight was given after 30 min of drug administration. After 30 min of post-sucrose load, blood glucose level was again checked at 1, 2, 3, 4, 5, 6 and at 24 h, respectively. Comparing the AUC of experimental and control groups determined the percent antihyperglycemic activity. Statistical analysis was made by Dunnett's test (Prism Software).