Synthesis of salidroside analogues and their ability of DPPH radical scavenging activity

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Salidroside is a phenylpropanoid glycoside isolated from Rhodiolarosea L., a traditional Chinese medicinal plant, and has displayed a broad spectrum of pharmacological properties. In this paper, about 22 novel glycosides have been synthesized and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of each glycoside has been evaluated. 2-(3,4,5-Trihydroxyphenyl)ethyl β-D-galactopyranoside and 3-(3,4,5-trihydroxyphenyl)propyl β-D-glucopyranoside exhibit significant activity prior to salidroside and Vitamin C with EC50 values of 35.85 µM and 36.71 µM, respectively. The results indicate that the phenolic hydroxyl group of these compounds is important for radical scavenging activity and phenyl ring substitution by electron-donating substituents lead to increased antioxidant activity.

Keywords: Salidroside, analogues, Koenigs-Knorr method, DPPH

Salidroside (2-(4-hydroxyphenyl)ethyl β-D-glucopyranoside), which is isolated from the root of Rhodiolarosea L. and has been used as an adaptogen in traditional Tibetan medicine, has been reported to possess various pharmacological activities including resisting anoxia, eliminating fatigue, anti-oxidation, resist radiation, neuroprotection, memory enhancing, anti-ageing and hepatoprotection activities. Salidroside showed a moderate DPPH radical scavenging activity at high concentration, which may be synthesized through glucosylation of tyrosol or 4-O-acetylphenylethanol by Koenigs-Knorr method. More and more phenylethanoid glycosides are being isolated from medicinal plants. 2-(3,4-dihydroxyphenyl)ethyl β-D-allopyranoside and 2-(3,4-dihydroxyphenyl)ethyl β-D-glucopyranoside have been isolated from liverwort or syringe velutina. 2-(3,4-dihydroxyphenyl)ethyl β-D-glucopyranoside has shown the most potent cytotoxic effect on several tumor cell lines (P-388, L-201, SNU-5, and HL-60) among the compounds extracted from syringe velutina.

Considerable effort has been devoted to the isolation and assessment of pharmacological properties, synthesis and structure modification of salidroside. In this study, in order to search for more active compounds, 22 salidroside analogues have been synthesised by Koenigs-Knorr method. The antioxidant properties on DPPH radical scavenging activity of these compounds have also been investigated.

Results and Discussion

Synthesis of salidroside analogues

The synthesis starts with cheap available methyl 3,4,5-trihydroxy benzoate 4 and tyrosol (Scheme I and Scheme II). Protection of the phenolic groups of methyl 3,4,5-trihydroxy benzoate 4 as benzyl ether 5w (benzyl chloride, K2CO3, DMF, 94% yield) followed by reduction with LiAlH4 gave the compound 6w in 95% yield. Chlorination with SOCl2 gave benzyl chloride 7w (89% yield) that was converted into phenylacetonitrile 8w (93% yield) with NaCN. The phenylacetonitrile 8w was hydrolyzed to the 3,4,5-trihydroxyphenylacetic acid 9w (KOH, EtOH/H2O, 88%) and then reduced to compound 10w (yield 85%) with LiAlH4. Compound 10x was prepared from 4 by the similar method for the preparation of 10w. As Scheme I shows,
alkylation of diethylmalonate with 7 yielded the substituted diethyl benzylmalonate (11w 88%, 11x 81%), while subsequent deethoxycarbonylation of 11 with sodium chloride in wet DMSO lead to 12w in 95% yield and 12x in 92% yield. In the final step, 12 were converted to 13w in 92% yield and 13x in 90% yield by LiAlH₄ reduction in THF under reflux conditions.

As shown in Scheme II, starting from the cheaply available tyrosol 14, bromination with NBS was carried out to produce 15y in 65% yield and 15z in 91% yield and then treated with benzyl chloride to give the product 16 in 96% yield. 17 and 18 were obtained in 80-90% yield by displacement of aryl bromide with methoxide or ethoxide in presence of CuBr.
The structure of DPPH

Glycosides were prepared as shown in Scheme III. Glycosylation of alcohol with Koenigs-Knorr method in the presence of Ag$_2$CO$_3$, and then the direct deacetylation with NaOMe-MeOH and hydrogenation with Pd/C gave the desired products.

**DPPH radical scavenging activity**

DPPH (Figure 1) is a stable, commercially available radical, characterized by an absorption band at 517 nm. DPPH is frequently used to evaluate antioxidant activities of natural as well as synthetic compounds. In order to assess the radical scavenging potential of the glycosides tested, the reactivity toward the stable free radical DPPH was measured at 517 nm and absorbance decrease of ethanol solution of DPPH containing glycosides tested. A 96-well plate was used to generate a quantitative measure of radical scavenging activities of glycosides. For each compound, five concentrations were employed and the percentage of remaining DPPH was determined after 30 min at 37°C. The antioxidant activity was expressed as a percentage of scavenging activity on DPPH radical: $\%S = \left[1 - \frac{\text{absorbance of sample}}{\text{absorbance of control}}\right] \times 100$. The mean

![Figure 1 — The structure of DPPH](image-url)
values were obtained from quintuplicate experiments. The EC50 value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, was calculated from the results. Analytical grade Vitamin C (VC) constituted the positive control.

Some of these compounds were able to scavenge the DPPH radical. 3g-l were similarly used with Vitamin C. 2a-f, 2s-t showed no activity and 2o-r and salidroside showed slightly less activity, while 3m-n, 3u-v showed the best activity of scavenging DPPH radical. EC50 is shown in Table I.

According to the data, glycosides of many phenolic hydroxyl groups lend a marked antioxidant activity to these molecules, and polyphenols 3m-n and 3u-v were especially efficient in the DPPH assay.

Experimental Section

Melting points were obtained on X-5 melting point detector and are uncorrected. 1H NMR spectra were recorded on a Bruker Avance instrument at 500 or 300 MHz with TMS as the internal standard in CDCl3, CD3OD or D2O. Elemental analysis (C and H) were performed on Elementar VarioEL III analyzer (German). IR was recorded on AVATAR-370 FT-IR Thermo Nicolet with KBr disk. Flash column chromatography was performed over silica gel (200-300 mesh) (Hai Yang Chemical Factory, Qingdao, Shangdong, P.R. China). All reactions and chromatographic separations were monitored by TLC. All reagents and solvents were commercially available. 2-Pentafluorophenylethanol was obtained from J&K CHEMICA.

3,4,5-Tribenzyloxybenzyl cyanide, 8w

3,4,5-Tribenzyloxybenzyl cyanide 8w was prepared from methyl 3,4,5-trihydroxybenzoate 4 in 74% yield17-19. m.p. 94-96°C. 1H NMR (CDCl3): δ 3.64 (s, 2H, PhCH2), 5.04 (s, 2H, PhCH2O), 5.10 (s, 4H, 2 × PhCH2O), 6.59 (s, 2H, ArH), 7.27-7.40 (m, 15H, 15 × PhH).

3,4,5-Tribenzyloxyphenylacetic acid, 9w

3,4,5-Tribenzyloxybenzyl cyanide 8w (11.0 g, 25.3 mmol) and potassium hydroxide (11.3 g, 202 mmol) were dissolved in a mixture of ethanol (70 mL) and water (50 mL). The mixture was heated at reflux for 15 hr. After cooling, the solution was poured into water (600 mL) and the mixture was extracted with ether (100 mL). The aqueous layer was acidified (pH=2) with a solution of 2 M HCl. The suspension was extracted with AcOEt (3 × 100 mL). The combined organic layers were washed with saturated aq. NaCl (3 × 50 mL) and dried over anhydrous Na2SO4. After removal of the solvent under reduced pressure, a yellow solid was obtained. Recrystallization from ethyl acetate and light petroleum gave 10.1 g (88%) of 9w as a white solid. m.p. 111-113°C. 1H NMR (CDCl3): δ 3.55 (s, 2H, PhCH2), 5.02 (s, 2H, PhCH2O), 5.09 (s, 4H, 2 × PhCH2O), 6.61 (s, 2H, ArH), 7.24-7.43 (m, 15H, 15 × PhH).

2-(3,4,5-Tribenzyloxyphenyl)ethanol, 10w

A suspension of LiAlH4 (1.52 g, 40 mmol) in dry THF (25 mL) was stirred and then a solution of 3,4,5-tribenzyloxyphenylacetic acid 9w (9.2 g, 20.3 mmol) in dry THF (40 mL) was added dropwise at RT within 0.5 hr. The reaction mixture was then refluxed for 3 hr. After cooling, the mixture was quenched by pouring in 1 M HCl and was extracted with AcOEt (4 × 50mL). Organic layer was washed with brine (3 × 30mL), dried over anhydrous Na2SO4 and evaporated. The crude product was rapidly purified by short column chromatography over a silica gel eluting with a 1:30 mixture of MeOH and CH2Cl2. The white solid was obtained in 85% yield (7.6 g). m.p. 67-69°C. 1H NMR (CDCl3): δ 2.73 (t, J = 6.4 Hz, 2H, PhCH2), 3.76 (t, J = 6.4 Hz, 2H, CH2OH), 5.03 (s, 2H, OCH2Ph), 5.09 (s, 4H, 2 × OCH2Ph), 6.50 (s, 2H, 2 × ArH), 7.24-7.49 (m, 15H, 15 × PhH).

2-(3,4,5-Trimethoxyphenyl) ethanol, 10x

2-(3,4,5-Trimethoxyphenyl) ethanol 10x was prepared from 4 by the similar method for the preparation of 10w. 1H NMR (CDCl3): δ 2.80 (t, J = 6.4 Hz, 2H, PhCH2), 3.78-3.92 (m, 11H, 3 × OCH3, CH2OH), 6.45 (s, 2H, 2 × ArH).

Diethyl 2-(3,4,5-tribenzyloxybenzyl) propane -1,3-dioate, 11w

In a mixed solution of diethyl malonate (3.84 g, 24 mmol), dry DMSO (60 mL) and K2CO3 (2.52 g, 40 mmol), 3,4,5-tribenzyloxy benzyl chloride 7w (8.46 g, 20 mmol) was added, the reaction was carried out at 50°C for 6 hr, then poured into ice-water (300 mL), neutralized with 3 M aqueous HCl and extracted with AcOEt (4×40 mL). Organic layer was washed with brine (3×30 mL), dried over anhydrous Na2SO4 and concentrated, and the residue was recrystallized from petroleum ether to give compound 11w (10.0 g, 88%).
m.p. 79-81°C. 1H NMR (CDCl3): δ 1.25 (m, 6H, 2 ×OCH2CH2), 3.12 (d, J = 7.5 Hz, 2H, PhCH2), 3.65 (t, J = 7.5 Hz, 1H, CH-COOEt), 4.14-4.24 (m, 4H, 2 × OCH2CH3), 5.02 (s, 2H, CH2OPh), 5.04 (s, 4H, 2 × OCH2Ph), 6.48 (s, 2H, 2 × ArH). 7.24-7.40 (m, 15H, 15 × PhH).

Diethyl 2-(3,4,5-trimethoxybenzyl) propane-1,3-dioate, 11x

Diethyl 2-(3, 4, 5-trimethoxybenzyl) propane-1,3-dioate 11x was prepared in 81% yield from 7x by the similar method for the preparation of 11w. m.p. 77°C. 1H NMR (CDCl3): δ 1.21-1.25 (m, 6H, 2 × OCH2CH3), 3.16 (d, J = 7.7 Hz, 2H, PhCH2), 3.62 (t, J = 7.8 Hz, 1H, PhCH2CH2), 3.81 (s, 3H, OCH3), 3.82 (s, 6H, 2 ×OCH2), 4.13-4.22 (m, 4H, 2 × OCH2CH3), 6.42 (s, 2H, 2 × ArH).

Ethyl β-(3,4,5-tribenzoxylphenyl)-propionate, 12w

Compound 11w (5.68 g, 10 mmol), water (0.18 g, 10 mmol) and NaCl (1.17 g, 20 mmol) were dissolved in DMSO (24 mL). The mixture was heated to reflux with stirring for 5 hr. The reaction mixture was poured into water (100 mL), and extracted with ether (3 × 40 mL). The combined extracts were washed with brine (3 × 20 mL), dried over anhydrous MgSO4, and the solvent removed in vacuo. The resulting yellow solid was purified by short column chromatography on silica gel eluting with a mixture of AcOEt and light petroleum to give 12w (4.7g, 95%). 1H NMR (CDCl3): δ 1.24 (t, J = 7.1 Hz, 3H, CH3), 2.62 (t, J = 7.7 Hz, 2H, CH2COO), 2.84 (t, J = 7.6 Hz, 2H, PhCH2), 4.14 (q, J = 7.1 Hz, OCH2CH3), 5.02 (s, 2H, OCH2Ph), 5.09 (s, 4H, 2 × OCH2Ph), 6.49 (s, 2H, 2 × ArH), 7.23-7.41 (m, 15H, 15 × PhH).

Ethyl β-(3,4,5-trimethoxybenzyl)-propionate, 12x

12x was prepared in 92% yield from 11x by the similar method for the preparation of 12w. 1H NMR (CDCl3): δ 1.24 (t, J = 7.1 Hz, 3HLOCH2CH3), 2.61 (t, J = 7.8 Hz, 2H, PhCH2), 2.89 (t, J = 7.8 Hz, 2H, CH2CO), 3.81 (s, 3H, OCH3), 3.84 (m, 6H, 2 ×OCH2), 4.13 (q, J = 7.1 Hz, 2H, OCH2CH3), 6.42 (s, 2H,2 × ArH).

3-(3,4,5-Tribenzyloxyphenyl)-1-propanol, 13w

A suspension of LiAlH4 (0.76 g, 20 mmol) in dry THF (12 mL) was stirred and then a solution of 12w (4.96 g, 10.0 mmol) in dry THF (20 mL) was added dropwise at RT within 0.5 h. The reaction mixture was then refluxed for 2 hr. After cooling, the mixture was quenched by pouring in 1 M HCl and was extracted with AcOEt (3 × 25 mL). Organic layer was washed with brine (3 × 15 mL), dried over anhydrous Na2SO4, and concentrated. The crude product was rapidly purified by short column chromatography over silica gel eluting with a mixture of AcOEt and light petroleum (v/v = 1:2). The white solid was obtained in 92% yield (4.18 g). m.p. 54-55°C. 1H NMR (CDCl3): δ 1.78-1.84 (m, 2H, CH2CH2OH), 2.60 (t, J = 9.0 Hz, 2H, PhCH2), 3.58 (t, J = 6.5 Hz, 2H, CH2OH), 5.03 (s, 2H, OCH2Ph), 6.48 (s, 2H, 2 × ArH), 7.25-7.42 (m, 15H, 15 × PhH).

3-(3,4,5-Trimethoxyphenyl)-1-propanol, 13x

13x was prepared in 90% yield from 12x by the similar method for the preparation of 13w. 1H NMR (CDCl3): δ 1.84-1.93 (m, 2H, CH2CH2CH2OH), 2.65 (t, J = 7.6 Hz, 2H, PhCH2), 3.69 (t, J = 6.4 Hz, 2H, CH2OH), 3.82 (s, 3H, OCH3), 3.84 (s, 6H, 2 × OCH3), 6.42 (s, 2H, 2 × ArH).

General procedure for the synthesis of 15 (Ref 22)

NBS (3.7 g, 21 mmol or 1.8 g 10 mmol) was added portion wise to tyrosol 14 (1.39 g, 10 mmol) in CH2Cl2 (30 mL) at 0°C until the reaction was complete as indicated by TLC. The reaction mixture was quenched by pouring in 1 M HCl and was extracted with AcOEt (3 × 25 mL). Organic layer was washed with brine (3 × 15 mL), dried over anhydrous Na2SO4, and concentrated. The crude product was rapidly purified by short column chromatography over silica gel eluting with a mixture of AcOEt and light petroleum (v/v = 1:2). The white solid was obtained in 92% yield (4.18 g). m.p. 54-55°C. 1H NMR (CDCl3): δ 1.78-1.84 (m, 2H, CH2CH2OH), 2.60 (t, J = 9.0 Hz, 2H, PhCH2), 3.58 (t, J = 6.5 Hz, 2H, CH2OH), 5.03 (s, 2H, OCH2Ph), 6.48 (s, 2H, 2 × ArH), 7.25-7.42 (m, 15H, 15 × PhH).

2-Bromo-4-(2-hydroxyethyl)phenol, 15y

White solid, m.p. 88-89°C (Lit. 91-93°C). 1H NMR (CDCl3): δ 2.78 (t, J = 6.5 Hz, 2H), 3.82 (t, J = 6.5 Hz, 2H), 6.94 (d, J = 8.0 Hz, 1H), 7.07 (dd, J = 1.5 Hz, 8.0 Hz, 1H), 7.33 (d, J = 1.5 Hz, 1H).

2,6-Dibromo-4-(2-hydroxyethyl)phenol, 15z

White solid, m.p. 99-100°C (Lit. 93°C). 1H NMR (CDCl3): δ 2.76 (t, J = 6.0 Hz, 2H, PhCH2), 3.83 (t, J = 6.0 Hz, 2H, CH2OH), 7.32 (s, 2H, 2 × ArH).

General procedure for the synthesis of 16

To a solution of 15 (48 mmol), dry acetone (100 mL) and potassium carbonate (8.28 g, 60 mmol), was added benzyl chloride (6.38 g, 50.4 mmol). The
mixture was then refluxed for one day under nitrogen with vigorous stirring. Then cooled and concentrated and extracted with AcOEt. The organic extracted was washed with brine and dried over anhydrous Na₂SO₄. Filtration and concentration of the filtrate under reduced pressure gave the product.

2-(4-Benzylxy-3-bromophenyl)ethanol, 16y
Colorless oil. ¹H NMR (CDCl₃): δ 2.76 (t, J = 6.6 Hz, 2H, PhCH₂), 3.80 (t, J = 6.6 Hz, 2H, CH₂OH), 5.12 (s, 2H, OCH₂Ph), 6.81-7.42 (m, 8H, 8 ×ArH).

2-(4-Benzylxy-3,5-dibromophenyl)ethanol, 16z
White solid. m.p. 66-66.5°C. ¹H NMR (CDCl₃): δ 2.80 (t, J = 6.4 Hz, 2H, PhCH₂), 3.86 (t, J = 6.4 Hz, 2H, CH₂OH), 5.01 (s, 2H, OCH₂Ph), 7.26-7.42 (m, 8H, 8 ×ArH).

General procedure for the methoxylation of aromatic bromines
The protocol was improved with respect to that previously reported. A 25% solution of MeONa in MeOH (8.0 mL, 35 mmol) was mixed at RT with CuBr (250 mg, 1.74 mmol) and DMF (0.8 mL). After 1 hour, acetobrome-sugar (8.75 mmol) in DMF (8 mL) stirring at 110°C, which was added dropwise over 1 hour to a solution of 16 (8.75 mmol) in DMF (8 mL) stirring at 110°C. After 1.5 hour, the mixture was quenched with ice water and extracted with EtOAc. The organic layers were washed with brine, dried over anhydrous Na₂SO₄ and the solvent evaporated. This method is also suitable for the ethoxylation of aromatic bromides.

2-(4-Benzylxy-3-methoxyphenyl)ethanol, 17y
White solid, m.p. 65-66°C (lit. 65-69°C). ¹H NMR (CDCl₃): δ 2.80 (t, J = 6.4 Hz, 2H, PhCH₂), 3.83 (t, J = 6.4 Hz, 2H, CH₂OH), 3.89 (s, 3H, OCH₃), 5.14 (s, 2H, PhCH₂O), 6.70 (dd, J = 2.0 Hz, 8.1 Hz, 1H), 6.78 (d, J = 2.0 Hz, 1H), 6.83 (d, J = 8.1 Hz, 1H), 7.29-7.39 (m, 3H), 7.40-7.45 (m, 2H).

2-(4-Benzylxy-3,5-methoxyphenyl)ethanol, 17z
Yellowish oil. ¹H NMR (CDCl₃): δ 2.80 (t, J = 6.4 Hz, 2H, PhCH₂), 3.81-3.88 (m, 8H, 2 ×OCH₃, CH₂OH), 4.94 (s, 2H, PhCH₂O), 6.44 (s, 2H, 2 ×ArH), 7.25-7.50 (m, 5H, 5 ×PhH).

2-(4-Benzylxy-3-ethoxyphenyl)ethanol, 18
Yellowish solid. ¹H NMR (CDCl₃): δ 1.45 (t, J = 7.0 Hz, 3H, OCH₂CH₃), 2.78 (t, J = 6.3 Hz, 2H, PhCH₂), 3.81 (t, J = 6.3 Hz, 2H, CH₂OH), 4.10 (q, J = 7.0 Hz, 2H, PhOCH₂CH₃), 5.10 (s, 2H, PhCH₂O), 6.67-6.85 (m, 3H, 3 ×ArH), 7.28-7.45 (m, 5H, 5 ×ArH).

General procedure for the synthesis of glycosides 2a-v
A solution of corresponding alcohol (4 mmol) in dry ether-dichloromethane (1:1, 25 mL) was stirred, and Ag₂CO₃ (1.1 g, 4 mmol) and powdered molecular sieves (4 Å, 2 g) were added to it at RT under N₂ atmosphere. After 1 hour, acetobrome-sugar 1a or 1b (2.46 g, 6 mmol) and Ag₂CO₃ (0.55 g, 2 mmol) were added rapidly to the mixture and stirring was continued for 14 hours in the dark. The reaction mixture was filtered and evaporated under reduced pressure. The residue was purified by short column chromatography over silica gel eluting with a 1:2 mixture of AcOEt and light petroleum to afford a colorless gummy mass.

The colorless gummy mass dissolved in MeOH, NaOCH₃ (10.8 mg, 0.2 mmol) was added, then stirred for 2 hours at RT. After neutralization of the mixture by adding Amberlite IRA-120, the resin was filtered and washed with MeOH. The filtrate was concentrated in vacuo and recrystallized from ether to give the desired product in yield 55%-75% as white solid.

3,4,5-Trimethoxybenzyl β-d-glucopyranoside, 2a
m.p. 118-20°C. MS: m/z 361 [M+H]+; ¹H NMR (D₂O): δ 3.35 (t, J = 8.5 Hz, 1H), 3.40-3.45 (m, 2H), 3.49 (t, J = 8.5 Hz, 1H), 3.74-3.76 (m, 1H), 3.79 (s, 3H), 3.88 (s, 6H), 3.92-3.96 (m, 1H), 4.49 (d, J = 7.5 Hz, 1H), 4.70 (d, J = 12 Hz, 1H), 4.85 (d, J = 12 Hz, 1H), 6.83 (s, 2H); IR (KBr): 3376, 2941, 1592, 1508, 1416, 1362, 1335, 1240, 1130, 1053 cm⁻¹. Anal. Calcld for C₁₆H₂₂O₉: C, 53.33; H, 6.71. Found: C, 53.38; H, 6.75%.

3,4,5-Trimethoxybenzyl β-d-galactopyranoside, 2b
MS: m/z 361 [M+H]+; ¹H NMR (D₂O): δ 3.54-3.61 (m, 2H), 3.65-3.69 (m, 1H), 3.70-3.82 (m, 5H), 3.89 (s, 6H), 3.91-3.95 (m, 1H), 4.43 (d, J = 7.2 Hz, 1H), 4.73 (d, J = 12 Hz, 1H), 4.88 (d, J = 12 Hz, 1H), 6.87 (s, 2H); IR (KBr): 3390, 2942, 1594, 1504, 1416, 1362, 1335, 1240, 1130, 1053 cm⁻¹. Anal. Calcld for C₁₆H₂₂O₉: C, 53.33; H, 6.71. Found: C, 53.29; H, 6.70%.

Pentafluorophenyl ethyl β-d-glucopyranoside, 2c
m.p. 134-36°C. MS: m/z 375 [M+H]+; ¹H NMR (CD₃OD): δ 3.06 (t, J = 6.9 Hz, 2H), 3.13 (t, J = 8.4
Pentafluorophenyl ethyl β-D-galactopyranoside, 2d
m.p. 110-113°C. MS: m/z 375 [M+H]+; 1H NMR (CD3OD): δ 3.06 (t, J = 7.0 Hz, 2H), 3.43- 3.50 (m, 3H), 3.65-3.70 (m, 2H), 3.73-3.81 (m, 2H), 4.01-4.09 (m, 1H), 4.20 (d, J = 7.6 Hz, 1H); IR (KBr): 3406, 2940, 1507, 1120, 1094, 1037 cm⁻¹. Anal. Calcd for C14H15F5O6: C, 44.93; H, 4.04. Found: C, 44.90; H, 4.01%.

2-(3,4,5-Trimethoxyphenyl) ethyl β-D-glucopyranoside, 2e
m.p. 117-119°C. MS: m/z 375 [M+H]+; 1H NMR (D2O): δ 2.93 (t, J = 6.5 Hz, 2H), 3.26 (t, J = 8.4 Hz, 1H), 3.37-3.51 (m, 3H), 3.69-3.73 (m, 1H), 3.77 (s, 3H), 3.87 (s, 6H), 3.93-3.97 (m, 2H), 4.13-4.18 (m, 1H), 4.47 (d, J = 7.8 Hz, 1H), 6.73 (s, 2H); IR (KBr): 3384, 2935, 1506, 1501, 1462, 1424, 1366, 1240, 1130, 1038 cm⁻¹. Anal. Calcd for C17H20O6: C, 54.54; H, 7.00. Found: C, 54.58; H, 7.03%.

2-(3,4,5-Trimethoxyphenyl) ethyl β-D-galactopyranoside, 2f
MS: m/z 375 [M+H]+; 1H NMR (D2O): δ 2.94 (t, J = 6.0 Hz, 2H), 3.50 (t, J = 8.3, 1H), 3.63-3.67 (m, 2H), 3.77 (s, 5H), 3.87 (s, 6H), 3.93-3.98 (m, 2H), 4.13-4.16 (m, 1H), 4.41 (d, J = 7.6 Hz, 1H), 6.73 (s, 2H); IR (KBr): 3375, 2932, 1598, 1513, 1467, 1423, 1336, 1240, 1130, 1053 cm⁻¹. Anal. Calcd for C17H20O6: C, 54.54; H, 7.00. Found: C, 54.51; H, 7.00%.

2-(4-Benzyloxy-3-methoxyphenyl) ethyl β-D-galactopyranoside, 2g
m.p. 122-24°C. 1H NMR (CD3OD): δ 2.86 (t, J = 7.2 Hz, 2H), 3.20 (t, J = 7.8 Hz, 1H), 3.25-3.27 (m, 2H), 3.35-3.37 (m, 1H), 3.62-3.68 (m, 1H), 3.70-3.77 (m, 1H), 3.83 (s, 3H), 3.84-3.87 (m, 1H), 4.03-4.11 (m, 1H), 4.29 (d, J = 7.7 Hz, 1H), 5.05 (s, 2H), 6.73 (dd, d J = 2.0 Hz, 8.3 Hz, 1H), 6.87 (d, J = 8.3 Hz, 6.92 (d, J = 2.0 Hz, 1H), 7.27-7.36 (m, 3H), 7.40-7.43 (m, 2H).

2-(4-Benzyloxy-3-methoxyphenyl) ethyl β-D-galactopyranoside, 2h
m.p. 139-42°C. 1H NMR (CD3OD): δ 2.87 (t, J = 7.0 Hz, 2H), 3.45 (dd, d J = 3.3 Hz, 9.7 Hz, 1H), 3.48 - 3.53 (m, 2H), 3.72-3.77 (m, 3H), 3.81-3.83 (m, 1H), 3.84 (s, 3H), 4.03-4.10 (m, 1H), 4.25 (d, J = 7.6 Hz, 1H), 5.05 (s, 2H), 6.74 (dd, d J = 1.8 Hz, 8.2 Hz, 1H), 6.87 (d, J = 8.2 Hz, 1H), 6.93 (d, J = 1.8 Hz, 1H), 7.28 (t, J = 7.3 Hz, 1H), 7.34 (t, J = 7.3 Hz, 2H), 7.42 (d, J = 7.3 Hz, 2H).

2-(4-Benzyloxy-3-ethoxyphenyl) ethyl β-D-glucopyranoside, 2i
m.p. 128-30°C. 1H NMR (CD3OD): δ 1.39 (t, J = 7.0 Hz, 3H), 2.86 (t, J = 7.1 Hz, 2H), 3.18 (t, J = 8.4 Hz, 1H), 3.26-3.29 (m, 2H), 3.30-3.35 (m, 1H), 3.66 (dd, J = 5.2 Hz, 11.8 Hz, 1H), 3.72-3.74 (m, 1H), 3.86 (dd, J = 1.9 Hz, 12.1 Hz, 1H), 4.05-4.08 (m, 3H), 4.29 (d, J = 7.8 Hz, 1H), 5.06 (s, 2H), 6.73-6.91 (m, 3H), 7.26-7.48 (m, 5H).

2-(4-Benzyloxy-3,5-dimethoxyphenyl) ethyl β-D-glucopyranoside, 2k
m.p. 46-48°C. 1H NMR (CD3OD): δ 2.89 (t, J = 7.1 Hz, 2H), 3.19 (t, J = 8.3 Hz, 1H), 3.26-3.28 (m, 2H), 3.34-3.39 (m, 1H), 3.62-3.68 (m, 1H), 3.79 (s, 6H), 3.84-3.89 (m, 2H), 4.06-4.11 (m, 1H), 4.31 (d, J = 7.7 Hz, 1H), 4.90 (s, 2H), 6.59 (s, 2H), 7.27-7.46 (m, 5H).

2-(4-Benzyloxy-3,5-dimethoxyphenyl) ethyl β-D-galactopyranoside, 2l
m.p. 125-27°C. 1H NMR (CD3OD): δ 2.89 (t, J = 7.2 Hz, 2H), 3.46 (dd, d J = 3.4 Hz, 9.7 Hz, 1H), 3.49-3.54 (m, 2H), 3.73-3.79 (m, 3H), 3.80 (s, 6H), 3.83 (dd, J = 0.9 Hz, 3.4 Hz, 1H), 4.04-4.12 (m, 1H), 4.27 (d, J = 7.6 Hz, 1H), 4.90 (s, 2H), 6.59 (s, 2H), 7.28-7.46 (m, 5H).

2-(3,4,5-Tribenzyloxyphenyl)ethyl β-D-glucopyranoside, 2m
m.p. 78-80°C. 1H NMR (CD3OD): δ 2.89 (t, J = 7.0 Hz, 2H), 3.22 (t, J = 8.0 Hz, 1H), 3.29-3.31 (m, 2H), 3.39 (t, J = 8.8 Hz, 1H), 3.67-3.72 (m, 1H), 3.75-3.79 (m, 1H), 3.91 (dd, d J = 1.4 Hz, 10.8 Hz, 1H), 4.08 - 4.11 (m, 1H), 4.33 (d, J = 7.8 Hz, 1H), 4.96 (s, 2H), 5.12 (s, 4H), 6.73 (s, 2H), 7.24-7.47 (m, 15H).

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2-(3,4,5-Tribenzyloxyphenyl)ethyl β-D-galactopyranoside, 2n
m.p. 144-46°C. 1H NMR (CD3OD): δ 2.87 (t, J = 6.9 Hz, 2H), 3.44 - 3.57 (m, 3H), 3.74-3.77 (m, 3H), 3.83-3.85 (m, 1H), 4.04-4.10 (m, 1H), 4.26 (d, J = 7.4 Hz, 1H), 4.93 (s, 2H), 5.09 (s, 4H), 6.70 (s, 2H), 7.24-7.47 (m, 15H).

2-(3-Bromo-4-hydroxyphenyl) ethyl β-D-glucopyranoside, 2o
MS: m/z 379 [M+H]+; 1H NMR (D2O): δ 2.90 (t, J = 6.6 Hz, 2H), 3.26 (t, J = 8.5 Hz, 1H), 3.38 -3.53 (m, 3H), 3.72 (dd, J = 5.4 Hz, 12.3 Hz, 1H), 3.87-3.94 (m, 2H), 4.06-4.13 (m, 1H), 4.46 (d, J = 7.8 Hz, 1H), 6.98 (d, J = 8.1 Hz, 1H), 7.21 (d, J = 8.1 Hz, 1H), 7.55 (s, 1H); IR (KBr): 3421, 2927, 1613, 1509, 1421, 1368, 1283, 1224, 1077 cm⁻¹. Anal. Calcd for C19H19BrO7; C, 44.34; H, 5.05. Found: C, 44.38; H, 5.03%.

2-(3-Bromo-4-hydroxyphenyl) ethyl β-D-galactopyranoside, 2p
MS: m/z 379 [M+H]+; 1H NMR (D2O): δ 2.88 (t, J = 6.5 Hz, 2H), 3.48 (t, J = 8.9 Hz, 1H), 3.60 -3.66 (m, 2H), 3.75 (s, 2H), 3.86-3.91 (m, 2H), 4.09-4.13 (m, 1H), 4.39 (d, J = 7.7 Hz, 1H), 6.97 (d, J = 8.1 Hz, 1H), 7.19 (d, J = 8.1 Hz,1H), 7.54 (s, 1H); IR (KBr): 3395, 2930, 1609, 1508, 1418, 1372, 1284, 1222, 1079 cm⁻¹. Anal. Calcd for C19H19BrO7; C, 44.34; H, 5.05. Found: C, 44.32; H, 5.02%.

2-(3,5-Dibromo-4-hydroxyphenyl) ethyl β-D-glucopyranoside, 2q
MS: m/z 458 [M+H]+; 1H NMR (CD3OD): δ 2.82 (t, J = 6.8 Hz, 2H), 3.18 (t, J = 8.4 Hz, 1H), 3.26-3.39 (m, 3H), 3.61-3.73 (m, 2H), 3.87 (dd, J = 1.5 Hz, 11.7 Hz, 1H), 4.00-4.07 (m, 1H), 4.29 (d, J = 7.7 Hz, 1H), 7.40 (s, 2H); IR (KBr): 3411, 2929, 1717, 1561, 1478, 1407, 1240, 1158, 1028 cm⁻¹. Anal. Calcd for C19H19BrO7; C, 36.71; H, 3.96. Found: C, 36.75; H, 3.93%.

2-(3,5-Dibromo-4-hydroxyphenyl) ethyl β-D-galactopyranoside, 2r
MS: m/z 458 [M+H]+; 1H NMR (D2O): δ 2.87 (t, J = 6.0 Hz, 2H), 3.48 (t, J = 8.6 Hz, 1H), 3.60 -3.64 (m, 2H), 3.75 (s, 2H), 3.87-3.91 (m, 2H), 4.07-4.11 (m, 1H), 4.39 (d, J = 7.4 Hz, 1H), 7.53 (s, 2H); IR (KBr): 3383, 2887, 1711, 1555, 1479, 1407, 1240, 1159, 1065 cm⁻¹. Anal. Calcd for C19H19BrO7; C, 36.71; H, 3.96. Found: C, 36.73; H, 3.95%.

3-(3,4,5-Trimethoxyphenyl)propyl β-D-glucopyranoside, 2s
MS: m/z 389 [M+H]+; 1H NMR (D2O): δ 1.89-1.99 (m, 2H), 2.69 (t, J = 7.8 Hz, 2H), 3.29 (t, J = 8.5 Hz, 1H), 3.36-3.52 (m, 3H), 3.67-3.73 (m, 1H), 3.76 (s, 3H), 3.87 (s, 6H), 3.91-4.1 (m, 3H), 4.43 (d, J = 7.9 Hz, 1H), 6.68 (s, 2H); IR (KBr): 3417, 2941, 1590, 1508, 1461, 1423, 1331, 1242, 1126, 1077, 1031 cm⁻¹. Anal. Calcd for C18H25O6; C, 55.66; H, 7.27. Found: C, 55.69; H, 7.32%.

3-(3,4,5-Trimethoxyphenyl)propyl β-D-galactopyranoside, 2t
m.p. 101-103°C. MS: m/z 389 [M+H]+; 1H NMR (CD3OD): δ 1.91-1.99 (m, 2H), 2.70 (t, J = 7.8 Hz, 2H), 3.52 - 3.54 (m, 1H), 3.64-3.68 (m, 3H), 3.77 (s, 5H), 3.87 (s, 6H), 3.92-3.96 (m, 2H), 4.36 (d, J = 7.7 Hz, 1H), 6.70 (s, 2H); IR (KBr): 3409, 2939, 1590, 1508, 1460, 1421, 1331, 1242, 1125, 1057 cm⁻¹. Anal. Calcd for C18H25O6; C, 55.66; H, 7.27. Found: C, 55.62; H, 7.25%.

3-(3,4,5-Tribenzyloxyphenyl)propyl β-D-glucopyranoside, 2u
m.p. 58-60°C. 1H NMR (CD3OD): δ 1.85-1.90 (m, 2H), 2.64-2.68 (m, 2H), 3.22 (t, J = 7.8 Hz, 1H), 3.25-3.28 (m, 2H), 3.36 (t, J = 8.7 Hz, 1H), 3.46-5.50 (m, 1H), 3.66 (dd, J = 11.9 Hz, 5.5 Hz, 1H), 3.86 (dd, J = 11.8 Hz, 2.2 Hz, 1H), 3.87-3.92 (m, 1H), 4.23 (d, J = 7.8 Hz, 1H), 4.94 (s, 2H), 5.09 (s, 4H), 6.63 (s, 2H), 7.22-7.36 (m, 15H).

3-(3,4,5-Tribenzyloxyphenyl)propyl β-D-galactopyranoside, 2v
m.p. 111-13°C. 1H NMR (CD3OD): δ 1.84-1.90 (m, 2H), 2.62-2.69 (m, 2H), 3.45-3.49 (m, 3H), 3.55 (t, J = 8.6 Hz, 1H), 3.74 (dd, J = 1.8 Hz, 6.6 Hz, 1H), 3.75 (m, 1H), 3.84 (dd, J = 0.8 Hz, 3.4 Hz, 1H), 3.87-3.91 (m, 1H), 4.19 (d, J = 7.7 Hz, 1H), 4.94 (s, 2H), 5.09 (s, 4H), 6.63 (s, 2H), 7.22-7.46 (m, 15H).

General procedure for the synthesis of glycosides 3g-n and 3u-v
A suspension of 2g (2.6 g, 6.2 mmol) and 5% Pd/C (0.26 g) in methanol (50 mL) was stirred under hydrogen at RT overnight. The mixture was filtered through celite and the filtrate concentrated under reduced pressure. The crude product was purified by flash chromatography over silica gel eluting with a 6:1 mixture of CHCl₃ and MeOH to afford the desired product 3g (1.9 g, 94%) as a white solid. The
glycosides 3h–n and 3u–v were prepared from corresponding substrate in the same manner just as described above.

2-(4-Hydroxy-3-methoxyphenyl) ethyl β-D-glucopyranoside, 3g

White solid, m.p. 153-55°C. MS: m/z 331 [M+H]+; 
1H NMR (D2O): δ 2.90 (t, J = 6.7 Hz, 2H), 3.50 (t, J = 8.8 Hz, 2H), 3.34-3.50 (m, 3H), 3.71 (dd, J = 5.3 Hz, 12.2 Hz, 1H), 3.87 (s, 3H), 3.89-3.92 (m, 2H), 4.09-4.16 (m, 1H), 4.39 (d, J = 7.8 Hz, 1H), 6.82-6.89 (m, 2H), 7.00 (s, 1H); IR (KBr): 3514, 3434, 3147, 2967, 1603, 1523, 1454, 1374, 1253, 1091, 1050 cm⁻¹. Anal. Calcd for C15H22O5: C, 54.54; H, 6.71. Found: C, 54.48; H, 6.69%.

2-(4-Hydroxy-3-methoxyphenyl) ethyl β-D-galactopyranoside, 3h

White solid, m.p. 153-56°C. MS: m/z 331 [M+H]+; 
1H NMR (D2O): δ 1.39 (t, J = 7.0 Hz, 3H), 2.88 (t, J = 6.9 Hz, 2H), 3.25 (t, J = 8.6 Hz, 1H), 3.36-3.49 (m, 3H), 3.71 (dd, J = 5.7 Hz, 12.3 Hz, 1H), 3.87-3.92 (m, 2H), 4.11-4.16 (m, 3H), 4.46 (d, J = 8.0 Hz, 1H), 6.84 (d, J = 8.0 Hz, 1H), 6.90 (J = 8.0 Hz, 1H), 7.01 (s, 1H). IR (KBr): 3404, 2985, 1614, 1519, 1437, 1361, 1272, 1160, 1079, 1043 cm⁻¹. Anal. Calcd for C16H24O5: C, 55.81; H, 7.02. Found: C, 55.79; H, 7.01%.

2-(4-Hydroxy-3-ethoxyphenyl) ethyl β-D-glucopyranoside, 3i

White solid, m.p. 170-73°C. MS: m/z 361 [M+H]+; 
1H NMR (D2O): δ 2.89 (t, J = 6.9 Hz, 2H), 3.49 (dd, J = 7.9 Hz, 9.9 Hz, 1H), 3.62 (dd, J = 3.4 Hz, 9.9 Hz, 1H), 3.65-3.67 (m, 1H), 3.74-3.77 (m, 2H), 3.85 (s, 6H), 3.89-3.91 (m, 2H), 4.12 (m, 1H), 4.39 (d, J = 7.9 Hz, 1H), 6.70 (s, 2H); IR (KBr): 3512, 3410, 2937, 1612, 1522, 1463, 1335, 1225, 1126, 1086, 1047 cm⁻¹. Anal. Calcd for C16H25O6: C, 53.33; H, 6.71. Found: C, 53.32; H, 6.70%.

2-(4-Hydroxy-3,5-dimethoxyphenyl) ethyl β-D-galactopyranoside, 3j

White solid, m.p. 153-55°C. MS: m/z 345 [M+H]+; 
1H NMR (D2O): δ 2.71 (t, J = 6.9 Hz, 2H), 3.18 (t, J = 8.5 Hz, 1H), 3.28 (t, J = 4.7 Hz, 2H), 3.40 (t, J = 8.9 Hz, 1H), 3.65 (dd, J = 12.4 Hz, 5.4 Hz, 1H), 3.75 – 3.85 (m, 2H), 3.97-4.03 (m, 1H), 4.38 (d, J = 7.9 Hz, 1H), 6.40 (s, 2H); IR (KBr): 3384, 2935, 1614, 1537, 1453, 1328, 1198, 1018 cm⁻¹. Anal. Calcd for C14H25O6: C, 50.60; H, 6.07. Found: C, 50.59; H, 6.09%.

2-(3,4,5-Trihydroxyphenyl)ethyl β-D-glucopyranoside, 3k

Greenish solid, MS: m/z 333 [M+H]+; 1H NMR (D2O): δ 2.79 (t, J = 7.0 Hz, 2H), 3.49 (t, J = 8.8 Hz, 1H), 3.61-3.67 (m, 2H), 3.76-3.78 (m, 2H), 3.82-3.92 (m, 2H), 4.02-4.12 (m, 1H), 4.38 (d, J = 7.7 Hz, 1H), 6.47 (s, 2H); IR (KBr): 3396, 2931, 1615, 1539, 1452, 1328, 1200, 1035 cm⁻¹. Anal. Calcd for C14H25O6: C, 50.60; H, 6.07. Found: C, 50.64; H, 6.02%.
3-(3,4,5-Trihydroxyphenyl)propyl β-D-glucopyranoside, 3u

Greenish solid, MS: m/z 347 [M+H]+; 1H NMR (D2O): δ 1.87 (m, 2H), 2.54 (t, J = 7.3 Hz, 2H) 3.28 (t, J = 8.5 Hz, 1H), 3.38-3.52 (m, 3H), 3.67-3.73 (m, 2H), 3.89-3.92 (m, 2H), 4.42 (d, J = 7.9 Hz, 1H), 6.43 (s, 2H); IR (KBr): 3331, 2929, 1616, 1540, 1452, 1334, 1210, 1026 cm−1. Anal. Calcd for C18H22O6: C, 52.02; H, 6.40. Found: C, 52.04; H, 6.42%.

3-(3,4,5-Trihydroxyphenyl)propyl β-D-galactopyranoside, 3v

Greenish solid, MS: m/z 347 [M+H]+; 1H NMR (D2O): δ 1.83-1.92 (m, 2H), 2.54 (t, J = 7.5 Hz, 2H), 3.53 (t, J = 8.8 Hz, 1H), 3.62-3.67 (m, 3H), 3.75-3.79 (m, 2H), 3.88-3.93 (m, 2H), 4.36 (d, J = 7.8 Hz, 1H), 6.43 (s, 2H); IR (KBr): 3418, 2926, 1614, 1542, 1454, 1334, 1210, 1026 cm−1. Anal. Calcd for C18H22O6: C, 52.02; H, 6.40. Found: C, 52.05; H, 6.44%.

DPPH radical scavenging capacity assay

Although a large number of antioxidant assays are available, the DPPH free radical is very stable and thus allows for easy handling and manipulation. In addition, its stability implies that a potential antioxidant will react with other well-known free radical entities which are more unstable and therefore more reactive. This assay was performed according to the method of Zhang et al.30 with slight modifications. In a 96-well plate, 100 µL of synthetic glycoside, salidroside (purchased from Aladdin) or Vitamin C (positive control, purchased from Aladdin) at varying concentrations in methanol was added to 100 µL of 0.25 mM DPPH in ethanol solution. Samples were prepared in quintuplicate, and five different concentrations were employed. The plate was shaken for 2 min and stored in the dark for an additional 30 min at 37°C. The absorbance was measured at 517 nm with an EIX-800 Microelisa Reader (Bio-Tek Inc., USA). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The EC50 value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, was calculated from the results.

Conclusions

The convenient synthesis of salidroside analogues has been reported, all derived from cheap available starting material, tyrosol and methyl 3,4,5-trihydroxy benzoate. Their ability of DPPH radical scavenging activity has been investigated. Some of these compounds have displayed good activity with IC50 values ranging from 35.85 µM to 87.5 µM, such as 3g-n, 3u-v. A beneficial effect of an electron-donating group at the 3 and 5-position of the phenol moiety is observed.

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