Synthesis, Biological Activity of Salidroside and Its Analogues

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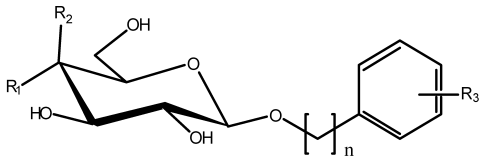
Salidroside is a phenylpropanoid glycoside isolated from *Rhodiola rosea* L., a traditional Chinese medicinal plant, and has displayed a broad spectrum of pharmacological properties. In this paper, about 18 novel salidroside analogues were prepared through Koenigs–Knorr method, the effects of these compounds over PC12 was assessed with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. The novel compounds differ in the substituents attached to the benzene ring or in the glycosyl donor. According to the data, compounds (3,5-dimethoxyphenyl)methyl β-D-glucopyranoside and (3,5-dimethoxyphenyl)methyl β-D-galactopyranoside with methoxy group at 3 and 5-positions of the benzene ring were the most viability at concentration of 300 μmol/l and 60 μmol/l, respectively.

Key words salidroside; analogue; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method; hypoglycemia and serum limitation; PC12 cell

Salidroside, a traditional Chinese medicine, was known as the main bioactive component in the root of *Rhodiola sachalinensis* A. BOR., and was reported to have many pharmacological properties, such as resisting anoxia,[1)] anti-radiation and antifatigue,[2,3)] improving oxygen lack and postponing ageing.[4)] In recent years, salidroside has been used in such special posts as diver, astronaut, pilot and mountaineer to enhance the ability for survival in adverse environment.[5)] Furthermore, many researches revealed that it has the activities of preventing cardiovascular disease[6)] and anti-tumor.[7)] However, the resource of wild *R. sachalinensis* A. BOR. was on the edge of exhaustion.

Salidroside is a 2-(4-hydroxyphenyl)ethyl β-D-glucopyranoside (Fig. 1, **5e**) which may be synthesized by glucosylation of tyrosol.[8,9)] Considerable effort has been devoted to the isolation and assess of pharmacological properties, synthesis and structure modification of salidroside is very few. In order to search for more activity compounds, 20 compounds (Fig. 1, **4a**—**d**, **5e**—**f**, **4g**—**t**) were successfully produced by Koenigs–Knorr method.



	R_1	R_2	n	R_3		R_1	R_2	n	R_3
4a	OH	H	1	H	**4k**	OH	H	1	2-CH_3O
4b	H	OH	1	H	**4l**	H	OH	1	2-CH_3O
4c	OH	H	2	H	**4m**	OH	H	1	4-CH_3O
4d	H	OH	2	H	**4n**	H	OH	1	4-CH_3O
5e	OH	H	2	4-OH	**4o**	OH	H	1	2,4-Dichloro
5f	H	OH	2	4-OH	**4p**	H	OH	1	2,4-Dichloro
4g	OH	H	1	4-OH	**4q**	OH	H	1	3,4-Dimethoxy
4h	H	OH	1	4-OH	**4r**	H	OH	1	3,4-Dimethoxy
4i	OH	H	1	4-Cl	**4s**	OH	H	1	3,5-Dimethoxy
4j	H	OH	1	4-Cl	**4t**	H	OH	1	3,5-Dimethoxy

Fig. 1. Structure of Salidroside and Its Analogues

The synthesis of the compounds **3a**—**t** was completed, which starting from the corresponding acetobromo-sugars (D-glucose, D-galactose) with the suitable substituted aromatic alcohol. The reaction was conducted in dry ethyl ether and dichloromethane for 10 h under dark with the presence of Ag_2CO_3. The process was optimized in the presence of 4 Å MS. Powdered MS 4 Å was dried at 140 °C under reduced pressure for 1 d before use. The designed target compounds **4a**—**4t** were obtained from **3a**—**4t** by direct deacetylation with CH_3ONa/CH_3OH in good yields (Chart 1, over 90% to almost in quantitative). The catalytic hydrogenation of **4e** and **f** to remove the protection of benzyl in the phenol hydroxy group is operated by refluxed with 5% Pd/C and $HCOONH_4$ in anhydrous methanol (Chart 1).

According to 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, after the PC12 cells were exposed to hypoglycemia and serum limitation for 24 h, it was found that hypoglycemia and serum limitation induced significant decreases in cell viability of cultured PC12 cells, as compared to that of control group. Exposure to hypoglycemia and serum limitation for 24 h was used to induce PC12 cell injury in all experiments unless otherwise stated. The cell viability of PC12 for each compound provide a quantitative comparison of salidroside and its analogues. MTT assay confirmed that the exposure to hypoglycemia and serum limitation caused the cell viability loss in cultured PC12 cells, and further showed that pretreatment with salidroside and its analogues at different concentrations (60, 300, 1000 μmol/l) significantly attenuated the cell viability loss evoked by hypoglycemia and serum limitation except **4i** and **4j**, with 60 μmol/l **4t** and 300 μmol/l **4s** yielding an attenuating effect close to that of control group. Overall, the data indicated that **4s** and **4t** are more active in the assay, which provided some insights into the design of new salidroside analogue.

Experimental

General Commercial reagents were used without further purification unless otherwise stated. All melting points are uncorrected. ^{1}H-NMR spectra

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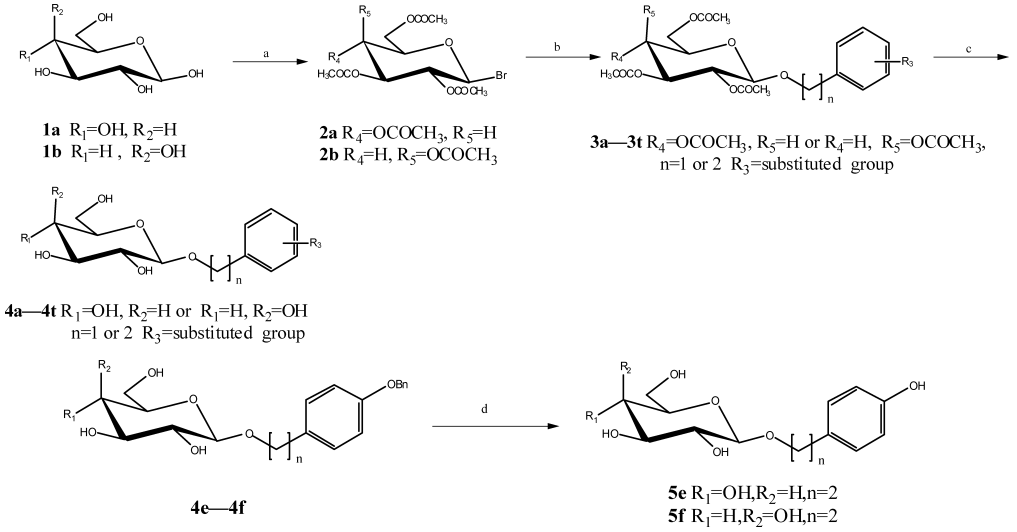

Reagents: a) P, Br_2, Ac_2O; b) Ag_2CO_3, CH_2Cl_2, Et_2O, rt, 10 h; c) NaOMe, MeOH, rt, 3 h; d) $HCOONH_4$, 5% Pd/C, CH_3OH, reflux, 6 h.

Chart 1. Synthetic Route for Salidroside and Its Analogues

Table 1. Experiment Data of Compounds

Compound No.	Yield (%)	Compound No.	Yield (%)	mp (°C)	δ (C1-H)	Compound No.	Yield (%)	Compound No.	Yield (%)	mp (°C)	δ (C1-H)
3a	52	**4a**	100	120—121	4.18 (J=7.8 Hz)	**3k**	70	**4k**	96	121—122	4.5 (J=7.85 Hz)
3b	62	**4b**	96	80—82	4.18 (J=7.7 Hz)	**3l**	72	**4l**	91	142—143	4.46 (J=7.9 Hz)
3c	66	**4c**	92	96—97	4.17 (J=7.8 Hz)	**3m**	68	**4m**	92	149—150	4.5 (J=8.05 Hz)
3d	61	**4d**	94	97—98	4.13 (J=7.2 Hz)	**3n**	58	**4n**	95	130—131	4.45 (J=7.85 Hz)
3e	58	**5e**	91	159—160	4.15 (J=7.8 Hz)	**3o**	55	**4o**	90	160—161	4.52 (J=7.89 Hz)
3f	60	**5f**	100	55—57	4.10 (J=7.25 Hz)	**3p**	62	**4p**	96	—	4.46 (J=7.47 Hz)
3g	61	**4g**	100	164—165	4.38 (J=7.32 Hz)	**3q**	35	**4q**	94	117—118	4.51 (J=8.0 Hz)
3h	64	**4h**	100	137—139	4.43 (J=7.56 Hz)	**3r**	42	**4r**	93	146—148	4.45 (J=8.0 Hz)
3i	55	**4i**	96	136—138	4.53 (J=7.2 Hz)	**3s**	63	**4s**	95	146—148	4.51 (J=8.0 Hz)
3j	52	**4j**	92	138—140	4.45 (J=6.85 Hz)	**3t**	65	**4t**	92	150—152	4.45 (J=8.0 Hz)

were recorded on a Bruker AC 400 instrument at 500 MHz, and the following abbreviations are used for the description of the patterns: s=singlet, d=doublet, t=triplet, m=multiplet. The ^{1}H-NMR spectra were recorded with tetramethylsilane (TMS, δ 0.00) as the internal standard and were run in dimethyl sulfoxide (DMSO) or D_2O, J values are expressed in Hz. Flash chromatography was performed on silica gel (200—300 mesh) (Hai Yang Chemical Factory, Qingdao, Shangdong, P. R. China). Compounds **2a** and **b** were prepared through literature.[10]

General Procedure for Compounds 4a—t A mixture of **2a** or **2b** (2.05 g, 5 mmol) and alcohol (4 mmol) was dissolved in dry dichloromethane–ether (2 : 1, 15 ml) and 2 g 4 Å MS was added, then stirred at room temperature under N_2 atmosphere for 20 min. After that Ag_2CO_3 (1.4 g, 5 mmol) was added rapidly to the mixture and stirring continued for 10 h avoid light. The reaction mixture was filtered and evaporated under reduced pressure. The crude product was purified by column chromatography using 1 : 3 or 1 : 2 EtOAc–petroleum ether to afford a colourless gummy mass **3a**—**t** (yield shown in Table 1). To a solution of compounds **3a**—**t** (2.5 mmol) in MeOH (10 ml) was added NaOMe (10 mg), and the reaction mixture was stirred at room temperature for 2 h. After that diluted with MeOH and acidified with Amberlite IRA-120 (H+) resin to pH=7. The reaction mixture was filtered and evaporated to dryness under reduced pressure afford **4a**—**t** as white solid[11] (Table 1).

Procedure for Compounds 5e and f 2-(4-Hydroxyphenyl)ethyl β-D-Glucopyranoside (**5e**): A mixture of **4e** (180 mg, 460 μmol), 5% Pd/C (208 mg) and ammonium formate (0.15 g, 2.3 mmol) in MeOH (6 ml) was stirred at refluxing for 6 h. Then filtered, concentrated and purified by column chromatography using 8 : 1 $CHCl_3$–MeOH to give 125 mg (91%) of **5e**. The physical constants were in agreement with the literature.[13]

2-(4-Hydroxyphenyl)ethyl β-D-Galactopyranoside (**5f**): **5f** was prepared from **4f** in the same manner just as described above, white solid: ^{1}H-NMR (DMSO) δ: 9.12 (1H, s), 7.02 (2H, d, J=8.35 Hz), 6.66 (2H, d, J=6.55 Hz), 4.75 (1H, s), 4.62 (1H, s), 4.51 (1H, s), 4.28 (1H, s), 4.10 (1H, d, J=7.25 Hz), 3.82—3.87 (1H, m), 3.62 (1H, s), 3.45—3.58 (3H, m), 3.27—3.32 (3H, m), 2.70—2.74 (2H, m). IR (KBr) cm^{-1}: 3268, 1630, 1614, 1246, 1079, 1020. *Anal.* Calcd for $C_{14}H_{20}O_7$: C, 55.99; H, 6.71. Found: C, 56.08; H, 6.77.

Cell Culture Rat PC-12 cells, obtained from the American Type Culture Collection (Manassas, VA, U.S.A.), were plated and maintained in high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% horse serum, 5% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 U/ml streptomycin in 5% CO_2/air at 37 °C. After being pretreated with 60, 300, or 1000 μmol/l compounds synthesized above for 24 h, the cells were subjected to hypoglycemia and serum limitation by replacing the culture medium with the glucose-free DMEM supplement with 1% horse serum and 1% FBS, 100 U/ml penicillin, and 100 U/ml streptomycin, and in the presence of compounds at original concentrations for another 24 h incubation.[14] At the end of incubation, the MTT solution was added and further incubated at 37 °C for 4 h followed by the addition of DMSO to dissolve the resulting formazan. The absorbance (optical density (OD)) values were measured by spectrophotometry at 570 nm with an EIX-800 Microelisa reader (Bio-Tek Inc., U.S.A.).

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11) Benzyl β-D-galactopyranoside (**4b**): ^{1}H-NMR (DMSO) δ: 7.38—7.40 (2H, d, J=6.95 Hz), 7.30–7.35 (2H, m), 7.25–7.28 (1H, m), 4.89 (1H, d, J=4.85 Hz), 4.79 (1H, d, J=12.30 Hz), 4.65 (1H, d, J=5.45 Hz), 4.53–4.58 (2H, m), 4.33 (1H, d, J=4.70 Hz), 4.18 (1H, d, J=7.70 Hz), 3.64 (1H, t, J=3.65 Hz), 3.49–3.57 (2H, m), 3.25–3.27 (1H, m). IR (KBr) cm^{-1}: 3412, 2943, 1650, 1454, 1418, 1211, 1141, 1077, 1040. *Anal.* Calcd for $C_{13}H_{18}O_6$: C, 57.77; H, 6.71. Found: C, 57.68; H, 6.80. Phenethyl β-D-glucopyranoside (**4c**): ^{1}H-NMR (DMSO) δ: 7.25—7.29 (4H, m), 7.17—7.20 (1H, m), 3.91—3.96 (1H, m), 3.62—3.68 (2H, m), 3.40—3.45 (1H, m), 4.91 (1H, d, J=4.95 Hz), 4.86 (1H, d, J=4.86 Hz), 4.83 (1H, d, J=5.10 Hz), 4.43 (1H, t, J=6.0 Hz), 4.17 (1H, d, J=7.80 Hz), 3.11—3.15 (1H, m), 3.07—3.10 (1H, m), 3.01—3.05 (1H, m), 2.93—2.98 (1H, m), 2.83—2.87 (2H, m). IR (KBr) cm^{-1}: 3362, 1940, 1610, 1515, 1210, 1072, 1030. *Anal.* Calcd for $C_{14}H_{20}O_6$: C, 59.14; H, 7.09. Found: C, 58.98; H, 7.12. 2-(4-Benzyloxyphenyl)ethyl β-D-glucopyranoside (**4e**): mp 96—98 °C. ^{1}H-NMR datum was in agreement with the literature.[12] IR (KBr) cm^{-1}: 3419, 2913, 2865, 1513, 1453, 1251, 1160, 1076, 1027. *Anal.* Calcd for $C_{21}H_{26}O_7$: C, 64.6; H, 6.71. Found: C, 64.72; H, 6.58. 2-(4-Benzyloxyphenyl)ethyl β-D-galactopyranoside (**4f**): mp 127—128 °C. ^{1}H-NMR (DMSO) δ: 7.43 (2H, d, J=6.8 Hz), 7.36—7.39 (2H, m), 7.30—7.33 (1H, m), 7.17 (2H, d, J=8.7 Hz), 6.91 (2H, d, J=8.7 Hz), 5.06 (2H, s), 4.75 (1H, d, J=4.50 Hz), 4.61 (1H, d, J=5.10 Hz), 4.50 (1H, t, J=5.30 Hz), 4.27 (1H, d, J=4.65 Hz), 4.11 (1H, d, J=7.25 Hz), 3.84—3.89 (1H, m), 3.56—3.62 (2H, m), 3.46—3.53 (2H, m), 3.30—3.33 (1H, m), 3.25—3.28 (2H, m), 2.77—2.79 (2H, m). IR (KBr) cm^{-1}: 3429, 2934, 1614, 1514, 1453, 1252, 1145, 1058. *Anal.* Calcd for $C_{21}H_{26}O_7$: C, 64.6; H, 6.71. Found: C, 64.52; H, 6.80. 2-(4-Hydroxyphenyl)methyl β-D-galactopyranoside (**4h**): ^{1}H-NMR (D_2O) δ: 7.35 (2H, d, J=8.37 Hz), 6.91 (2H, d, J=8.37 Hz), 4.85 (1H, d, J=11.28 Hz), 4.65 (1H, d, J=11.28 Hz), 4.43 (1H, d, J=7.56 Hz), 3.91 (1H, d, J=3.03Hz), 3.73—3.85 (2H, m), 3.59—3.69 (2H, m), 3.49—3.55 (1H, m). IR (KBr) cm^{-1}: 3438, 1250, 1162, 1040. *Anal.* Calcd for $C_{13}H_{18}O_7$: C, 54.54; H, 6.34. Found: C, 54.62; H, 6.48. (4-Chlorophenyl)methyl β-D-glucopyranoside (**4i**): ^{1}H-NMR (D_2O) δ: 7.47 (4H, s), 4.93 (1H, d, J=11.55 Hz), 4.53 (1H, d, J=7.20 Hz), 3.93 (1H, d, J=11.55 Hz), 3.76 (2H, s), 3.41—3.46 (4H, m). IR (KBr) cm^{-1}: 3355, 2930, 1599, 1492, 1466, 1208, 1078, 1042. *Anal.* Calcd for $C_{13}H_{17}ClO_6$: C, 51.24; H, 5.62. Found: C, 51.12; H, 5.68. (3,5-Dimethoxyphenyl)methyl β-D-glucopyranoside (**4s**): ^{1}H-NMR (D_2O) δ: 6.73 (2H, s), 6.58 (1H, s), 4.89 (1H, d, J=12 Hz), 4.74 (1H, d, J=12.5 Hz), 4.51 (1H, d, J=8 Hz), 3.94 (1H, d, J=12 Hz), 3.84 (6H, s), 3.70—3.75 (1H, m), 3.38—3.49 (3H, m), 3.31 (1H, t, J=8.5Hz). IR (KBr) cm^{-1}: 3377, 2938, 2836, 1596, 1472, 1210, 1160, 1038, 1012. *Anal.* Calcd for $C_{15}H_{22}O_8$: C, 54.54; H, 6.71. Found: C, 54.72; H, 6.80. (3,5-Dimethoxyphenyl)methyl β-D-galactopyranoside (**4t**): ^{1}H-NMR (D_2O) δ: 6.74 (2H, s), 6.59 (1H, s), 4.90 (1H, d, J=12 Hz), 4.75 (1H, d, J=12.5 Hz), 4.45 (1H, d, J=8 Hz), 3.92 (1H, s), 3.85 (6H, s), 3.77—3.81 (2H, m), 3.61—3.69 (2H, m), 3.59 (1H, t, J=8 Hz). IR (KBr) cm^{-1}: 3384, 2937, 2878, 1601, 1473, 1208, 1157, 1048, 1017. *Anal.* Calcd for $C_{15}H_{22}O_8$: C, 54.54; H, 6.71. Found: C, 54.43; H, 6.58.
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