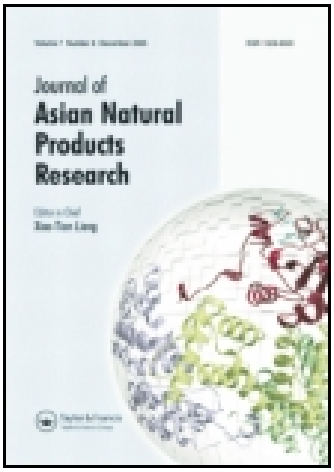

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Synthesis and anti-nociceptive and anti-inflammatory effects of gaultherin and its analogs

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Synthesis and anti-nociceptive and anti-inflammatory effects of gaultherin and its analogs

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The synthesis of gaultherin (**1**) and its analogs was carried out to provide 11 glycosides under phase-transfer catalytic conditions. The activities of all synthesized compounds were evaluated by nitric oxide production inhibitory assay *in vitro*. Methyl 2-*O*-(4-*O*-β-D-galactopyranosyl)-β-D-glucopyranosylbenzoate (**5f**) showed significantly anti-nociceptive and anti-inflammatory effects by the evaluation *in vivo*. Structure–activity relationships within these compounds were discussed.

Keywords: gaultherin; analogs; phase-transfer catalytic method; anti-nociceptive; anti-inflammatory

1. Introduction

Gaultherin (**1**; Figure 1) is a glycoside of methyl salicylate with a disaccharide, which was obtained from the plant of *Gaultheria yunnanensis* Rehd [1]. It is well known that drugs containing salicylic acid are key members of nonsteroidal anti-inflammatory drugs, clinically used as antipyretic, anti-inflammatory, antirheumatic, antithrombotic, and antitumor agents [2]. However, various side effects come out in some of the patients who had taken these drugs, such as inciting the gastrointestinal tract, preventing grume, and injuring liver, kidney and central nervous system. The most concerned adverse effect is inciting the gastrointestinal tract with common symptoms of indigestion, stomach ulcers, bleeding, and perforation [3]. Recently, it was found that gaultherin from the natural product had the activities of antipyretic, anti-inflammatory, antirheumatic, and antithrombotic, with prolonged effects and reduced adverse effects [1]. Pharmacokinetic study showed that the release of salicylic acid would be slowed down in patients who had taken gaultherin [1]. Given the significant biological superiority, it was highly desirable to synthesize and biologically evaluate gaultherin and its analogs, which could contribute to the structure–activity relationships study and the new drug discovery.

Previously, Robertson reported the synthesis of monotropitoside (gaultherin) with dry active silver oxide and ammonia as catalyst, respectively [4]. Zang *et al.* reported the synthesis and bioactivity of salicylic acid saccharide carboxylate as inducer in plants [5]. Here, we reported the synthesis of gaultherin by phase-transfer catalytic method catalyzed by tetrabutyl ammonium bromide (TEAB) without the limitation of anhydrous condition, which was facile for the industrial production.

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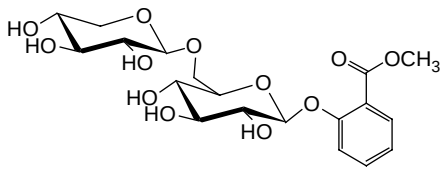


Figure 1. The structure of gaultherin (**1**).

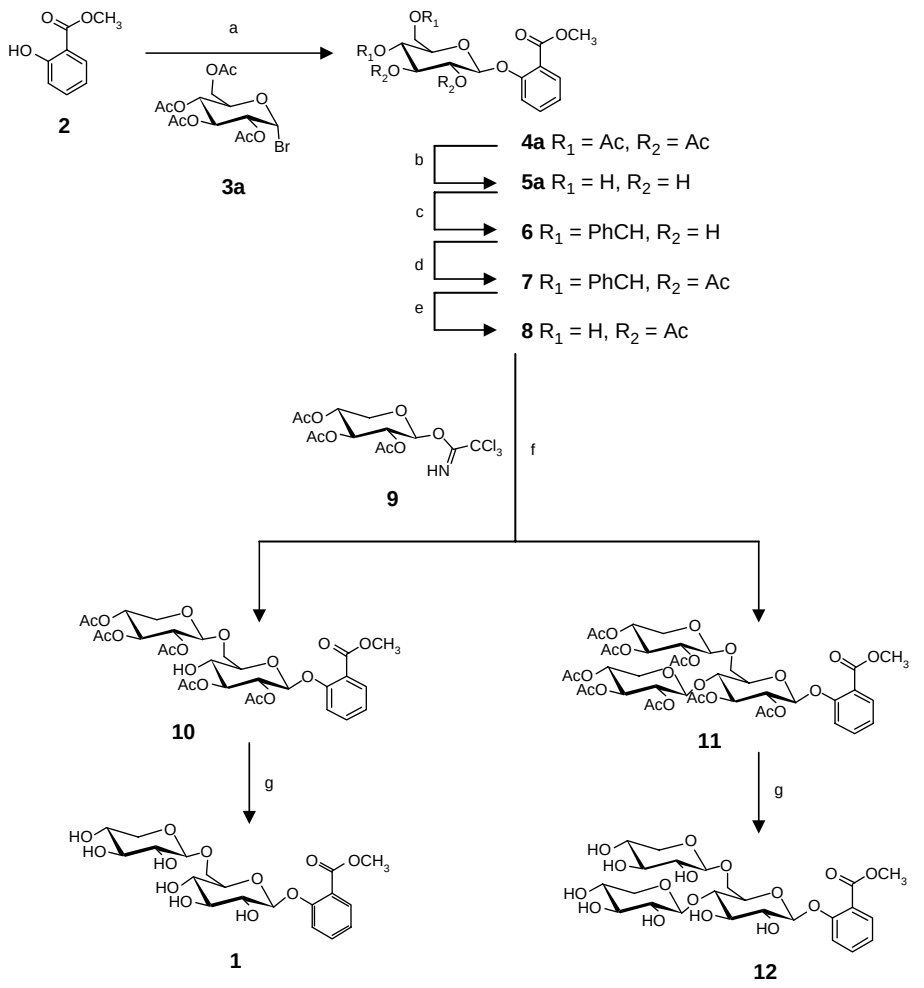
To study the structure–activity relationship of glycosides of salicylate, we synthesized a series of analogs by introducing different sugar moieties in 2-OH of salicylate by phase-transfer catalytic method, and their anti-nociceptive and anti-inflammatory effects were evaluated *in vitro* and *in vivo*.

2. Results and discussion

After analyzing the structure of gaultherin, we designed the method and prepared the target compound (**1**) successfully in 11 steps from methyl salicylate, D-glucose, and D-xylose. First, **4a** was afforded when methyl salicylate reacted with acetobromoglucose (**3a**) under promotion with phase-transfer catalyst TEAB [6–9]. Consequently, the removal of the acetyl of **4a** through ester exchange in NaOMe–MeOH afforded glycoside **5a** [10]. Treatment of **5a** with protective groups such as benzaldehyde and acetic anhydride produced the key intermediate **8** [10,11]. The glucopyranosyl of **8** had two hydroxyls (4-OH and 6-OH) with different activations. It is well known that the reaction activity of 6-OH was better than 4-OH, and thus we supposed to get **10** when **8** was reacted with equal trichloroacetimidate (**9**) under promotion with boron trifluoride ether solution. When the reaction was brought into practice, the target compound **10** was obtained as supposed along with the by-product **11**, which was the glycoside of methyl salicylate bearing trisaccharide [10]. Finally, removal of acetyl groups by NaOMe–MeOH afforded **1** and **12** (Scheme 1). The structures of **1** and **12** were determined by the spectral data.

To investigate the influence of different sugar moieties and to compare the anti-inflammatory and analgesic effects of the glycosides, we used a series of monosaccharides and disaccharides such as D-glucose, D-galactose, D-mannose, D-xylose, L-rhamnose, maltose, and lactose as glycosyl donors. So far, few anti-inflammatory and analgesic activities of methyl salicylate glycosides bearing these sugar moieties have been reported [5,12,13]. According to the same procedure described for **5a**, compounds **5b**–**5g** were prepared (Scheme 2). We also used salicylic acid as the receptor to afford saccharide carboxylate **13a**–**13b**, according to the literature [5,13]. The configuration of the glucosidic bond was determined from the ^{1}H NMR spectra of the products. The stereochemistry of the glycosides was assigned on the basis of coupling constants (Table 1).

Gaultherin and all the analogs were screened for their nitric oxide (NO) production inhibitory effects at 1×10^{-6} M *in vitro*. The inhibition ratio of the compounds is listed in Table 2. Gaultherin (**1**), **5f**, **12**, and **13b** exhibited remarkable NO production inhibitory activities with an average inhibition ratio of 40.2%. The results indicated that the activities of glycosides with disaccharides were better than those with monosaccharides. Based on the evaluation *in vitro* and the benefit of industrial production, we prepared the glycoside with disaccharide (**5f**) to evaluate the activity *in vivo*. The anti-inflammatory effect of **5f** was evaluated by croton oil-induced ear edema in mice (Table 3). The anti-nociceptive effect of **5f** by acetic acid-induced writhing response in mice was observed (Table 4). The known drug aspirin was included in the assay as a positive control. As a result, the anti-inflammatory effect of **5f** (400 mg/kg) was similar to aspirin (200 mg/kg), and the anti-nociceptive



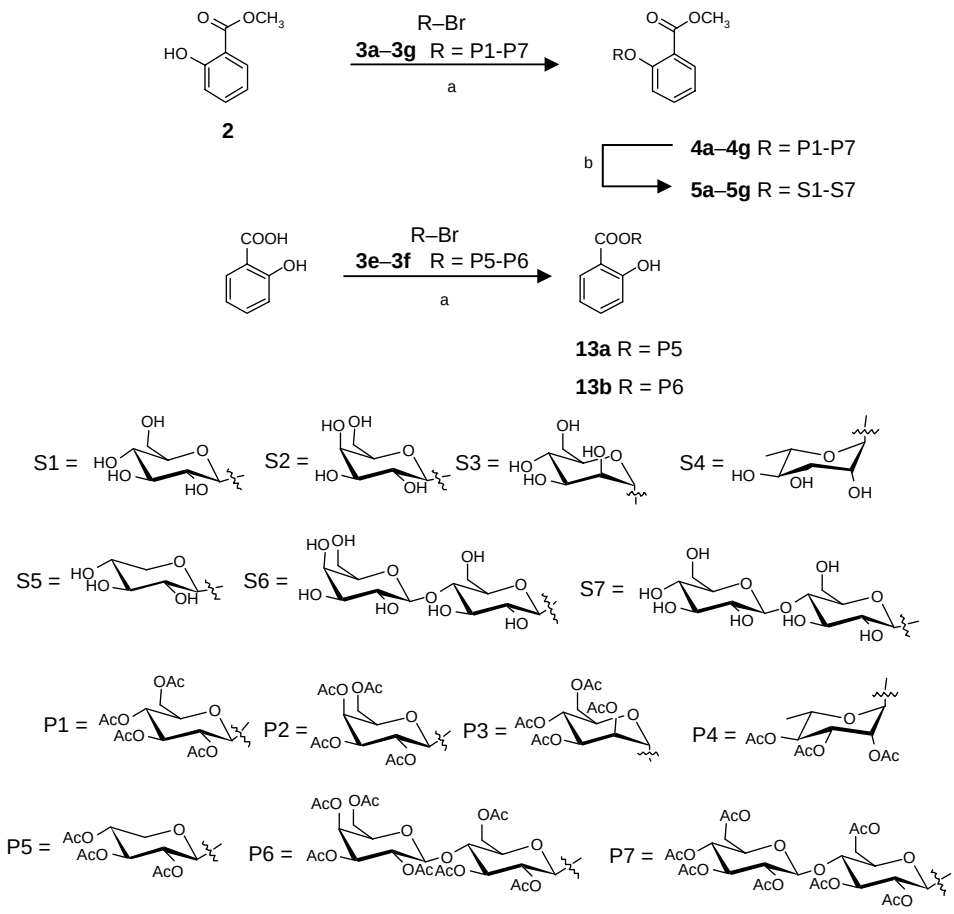
Scheme 1. The synthetic route of **4a**–**12**. Reagents and conditions: (a) TEAB, NaOH, CH_2Cl_2, 40°C, 4 h, 66%; (b) NaOMe, MeOH, r.t., 3 h, 45%; (c) $ZnCl_2$, PhCHO, 12 h, r.t., 49%; (d) pyridine, Ac_2O, 10 h, r.t., 75%; (e) 80% HOAc, 80°C, 3 h, 79%; (f) BF_3–Et_2O, CH_2Cl_2, 4 Å MS, N_2, r.t., 20 h; (g) NaOMe, MeOH, r.t., 3 h, 45%.

effect of **5f** (800 mg/kg) was similar to aspirin (200 mg/kg).

3. Experimental

3.1 General experimental procedures

The optical rotations were determined on a Perkin-Elmer digital polarimeter. UV spectra were taken on a Shimadzu UV-300 spectrophotometer. IR spectra were recorded on a Nicolet 5700 spectrometer as KBr pellets. ^1H and ^{13}C NMR spectra were run on INOVA-500 spectrometer with solvent peaks as references. ESIMS were obtained on an Agilent 1100 series LC/MSD Trap SL mass spectrometer. Column chromatography was carried out on silica gel (100–200, 200–300 mesh, Qingdao Marine Chemistry Company, Qingdao, China). Silica gel GF254 (Qingdao Marine Chemistry Company) was used to make silica gel plates.



Scheme 2. The synthetic route of **5a**–**5 g** and **13a**–**13b**. Reagents and conditions: (a) TEAB, NaOH, CH_2Cl_2, 40°C, 4 h, 66%; (b) NaOMe, MeOH, r.t., 3 h, 45%.

3.2 General procedures for the target compounds

*3.2.1 Methyl 2-O-β-D-glucopyranosylbenzoate (**5a**)*

According to the reported method [6–9], methyl 2-*O*-(2,3,4,6-tetra-*O*-acetyl)-β-D-glucopyranosylbenzoate (**4a**) was obtained as a white amorphous powder from starting material D-glucose and methyl salicylate, yielding 66% for two steps. Compound **5a** was obtained as a white amorphous powder from **4a** after the removal of acetyl groups

Table 1. Configuration of the glucosidic bond of compounds.

Compounds	$J_{(1',2')}$(Hz)	Configuration	Compounds	$J_{(1',2')}$ (Hz)	Configuration
1	7.6	β	**5e**	7.0	β
12	7.5	β	**5f**	8.0	β
5a	6.5	β	**5 g**	7.5	β
5b	8.0	β	**13a**	6.5	β
5c	1.5	α	**13b**	8.0	β
5d	–	α			

Table 2. Inhibitory effects of NO production of compounds[a].

Groups	($\bar{x} \pm s$ ($n = 3$))	Inhibition (%)
Normal	0.306 ± 0.062	–
Control	3.235 ± 0.062	–
1	0.306 ± 0.007	39.8**
12	0.307 ± 0.058	39.4*
5a	3.190 ± 0.040	1.5
5b	2.956 ± 0.090	9.5**
5c	0.334 ± 0.014	24.8*
5d	0.334 ± 0.009	24.70*
5e	0.340 ± 0.007	21.5*
5f	3.287 ± 0.068	38.5*
5 g	3.188 ± 0.108	1.6
13a	0.356 ± 0.028	13.0
13b	0.300 ± 0.027	43.0**

Notes: [a]Results are expressed as means ± SD.
*$p < 0.05$, **$p < 0.01$, significantly different from control by Student's *t*-test.

according to the reported method [10], yielding 45%. m.p. 126.1–127.8°C; $[\alpha]_D^{20}$ −28.7 ($c = 0.09$, MeOH); UV (MeOH) λ_{max}: 234, 286 nm; IR (KBr) ν_{max}: 3426, 2906, 1723, 1602, 1491, 1450, 1437, 1364, 1264, 1081, 747 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ_H: 7.26 (1H, d, $J = 8.5$ Hz, H-3), 7.51 (1H, m, H-4), 7.08 (1H, t, $J = 8.5$ Hz, H-5), 7.62 (1H, dd, $J = 8.5$, 1.5 Hz, H-6), 3.80 (3H, s, H-8), 4.89 (1H, d, $J = 6.5$ Hz, H-1′), 3.0 ~ 5.0 (H-2′, 3′, 4′, 5′, 6′a, 6′b); ESI-MS *m/z*: 337 $[M + Na]^+$.

3.2.2 *Compounds **5b**–**5 g** were prepared according to the same procedure described for **5a***

*3.2.2.1 Methyl 2-O-β-D-galactopyranosylbenzoate (**5b**).* m.p. 171.3–172.9°C; $[\alpha]_D^{20}$ −22.0 ($c = 0.10$, MeOH); UV (MeOH) λ_{max}: 230, 286 nm; IR (KBr) ν_{max}: 3469, 3453, 2944, 1685, 1605, 1491, 1454, 1432, 1316, 1238, 1086, 753 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ_H: 7.26 (1H, br.d, $J = 8.5$ Hz, H-3), 7.50 (1H, m, H-4), 7.07 (1H, t, $J = 8.0$ Hz, H-5), 7.62 (1H, dd, $J = 8.0$, 1.5 Hz, H-6), 3.79 (3H, s, H-8), 4.84 (1H, d, $J = 8.0$ Hz, H-1′), 3.0 ~ 4.0 (H-2′, 3′, 4′, 5′, 6′a, 6′b); ESI-MS *m/z*: 337 $[M + Na]^+$.

*3.2.2.2 Methyl 2-O-α-D-mannopyranosylbenzoate (**5c**).* $[\alpha]_D^{20}$ + 52.6 ($c = 0.10$, MeOH); UV (MeOH) λ_{max}: 212, 229, 287 nm; IR (KBr) ν_{max}: 3405, 2949, 1718, 1601, 1489, 1455, 1435, 1304, 1255, 1087, 757 cm^{-1}; ^{1}H-NMR (CD_3OD, 500 MHz) δ_H: 7.37 (1H, d, $J = 8.5$ Hz, H-3), 7.43 (1H, m, H-4), 7.03 (1H, t, $J = 8.5$ Hz, H-5), 7.68 (1H, dd, $J = 8.5$, 1.5 Hz, H-6), 3.82 (3H, s, H-8), 5.48 (1H, d, $J = 1.5$ Hz, H-1′), 3.0 ~ 4.0 (H-2′, 3′, 4′, 5′, 6′a, 6′b); ESI-MS *m/z*: 337 $[M + Na]^+$.

*3.2.2.3 Methyl 2-O-α-L-rhamnopyranosylbenzoate (**5d**).* $[\alpha]_D^{20}$ −78.9 ($c = 0.10$, MeOH); UV (MeOH) λ_{max}: 233, 287 nm; IR (KBr) ν_{max}: 3360, 2940, 1713, 1601, 1490, 1455, 1435, 1304, 1254, 1087, 756 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ_H: 7.30 (1H, d, $J = 8.5$ Hz, H-3), 7.52 (1H, m, H-4), 7.10 (1H, m, H-5), 7.77 (1H, dd, $J = 7.5$, 1.5 Hz, H-6), 3.90 (3H, s, H-8), 5.51 (1H, s, H-1′), 1.24 (3H, d, $J = 6.5$ Hz, H-6′), 3.0 ~ 4.5 (H-2′, 3′, 4′, 5′); ESI-MS *m/z*: 298 $[M + Na]^+$.

Table 3. Anti-inflammatory effect of **5f** by croton oil-induced ear edema in mice[a].

Groups	Dose (mg/kg)	*n*	Swelling value (mg)	Inhibition (%)
Control	0	10	19.3 ± 1.3	–
Aspirin[b]	200	10	11.6 ± 1.6	45***
5f	400	10	12.8 ± 1.8	39***

Notes: [a]Results are expressed as means ± SD.
[b]Positive control substance.
***$p < 0.001$, significantly different from control by Student's *t*-test.

Table 4. Anti-nociceptive effect of **5f** by acetic acid-induced writhing response in mice[a].

Groups	Dose (mg/kg)	*n*	Writhing response (times)	Inhibition (%)
Control	0	10	29.04 ± 3.05	–
5f	200	10	16.72 ± 4.45	45.84*
5f	400	10	15.73 ± 3.56	49.36**
5f	800	10	9.83 ± 2.60	69.62***
Aspirin[b]	200	10	7.67 ± 2.12	77.07***

Notes: [a] Results are expressed as means ± SD.
[b] Positive control substance.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, significantly different from control by Student's *t*-test.

*3.2.2.4 Methyl 2-O-β-D-xylopyranosyl-benzoate (**5e**).* $[\alpha]_D^{20}$ −87.6 ($c = 0.10$, MeOH); UV (MeOH) λ_{max}: 209, 230, 286 nm; IR (KBr) ν_{max}: 3393, 2894, 1713, 1601, 1490, 1452, 1435, 1262, 1088, 1048, 761 cm^{-1}; ^{1}H-NMR (CD_3OD, 500 MHz) δ_H: 7.24 (1H, d, $J = 8.0$ Hz, H-3), 7.52 (1H, dt, $J = 8.0$, 1.5 Hz, H-4), 7.07 (1H, t, $J = 8.0$ Hz, H-5), 7.73 (1H, dd, $J = 8.0$, 1.5 Hz, H-6), 3.83 (3H, s, H-8), 4.90 (1H, d, $J = 7.0$ Hz, H-1′), 3.0 ~ 4.0 (H-2′, 3′, 4′, 5′a, 5′b); ESI-MS *m/z*: 307 $[M + Na]^+$.

*3.2.2.5 Methyl 2-O-(4-O-β-D-galacto-pyranosyl)-β-D-glucopyranosylbenzoate (**5f**).* m.p. 177.1–178.6°C; $[\alpha]_D^{20}$ −71.2 ($c = 0.10$, MeOH); UV (MeOH) λ_{max}: 209, 230, 286 nm; IR (KBr) ν_{max}: 3382, 2921, 2882, 1717, 1602, 1491, 1458, 1319, 1250, 1077, 1049, 761 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ_H: 7.26 (1H, d, $J = 8.0$ Hz, H-3), 7.52 (1H, m, H-4), 7.09 (1H, d, $J = 7.5$ Hz, H-5), 7.62 (1H, dd, $J = 7.5$, 2.0 Hz, H-6), 3.79 (3H, s, H-8), 5.00 (1H, d, $J = 8.0$ Hz, H-1′), 4.51 (1H, d, $J = 7.0$ Hz, H-1″), 3.3 ~ 5.0 (H-2′, 3′, 4′, 5′, 6′a, 6′b, 2″, 3″, 4″, 5″, 6″a, 6″b). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ_C: 121.7 (C-1), 156.0 (C-2),116.4 (C-3), 133.3 (C-4), 121.4 (C-5), 130.3 (C-6), 166.4 (C-7), 103.8 (C-1′), 100.4 (C-1″), 80.0, 75.5, 75.0, 74.7, 73.3, 73.1, 70.6, 68.2, 60.4, 60.1 (C-2′, 3′, 4′, 5′, 6′, 2″, 3″, 4″, 5″, 6″), 52.0 (C-8); ESI-MS *m/z*: 499 $[M + Na]^+$.

*3.2.2.6 Methyl 2-O-(4-O-β-D-gluco-pyranosyl)-β-D-glucopyranosylbenzoate (**5g**).* m.p. 178.0–180.0°C; $[\alpha]_D^{20}$ + 25.4 ($c = 0.10$, MeOH); UV (MeOH) λ_{max}: 231, 287 nm; IR (KBr) ν_{max}: 3491, 2929, 2911, 2868, 1677, 1603, 1491, 1460, 1438, 1322, 1248, 1068, 762 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ_H: 7.27 (1H, d, $J = 8.0$ Hz, H-3), 7.52 (1H, m, H-4), 7.10 (1H, d, $J = 8.0$ Hz, H-5), 7.64 (1H, dd, $J = 8.0$, 1.5 Hz, H-6), 3.80 (3H, s, H-8), 4.98 (1H, d, $J = 7.5$ Hz, H-1′), 3.0 ~ 6.0 (H-2′, 3′, 4′, 5′, 6′a, 6′b, 1″, 2″, 3″, 4″, 5″, 6″a, 6″b); ESI-MS *m/z*: 499 $[M + Na]^+$.

*3.2.2.7 1-(2,3,4-Tri-O-acetyl)-β-D-xylo-pyranosyl-2-hydroxybenzoate (**13a**).* m.p. 140.1–144.5°C; $[\alpha]_D^{20}$ −81.7 ($c = 0.15$, $CHCl_3$); UV ($CHCl_3$) λ_{max}: 249, 313 nm; IR (KBr) ν_{max}: 2948, 1754, 1687, 1614, 1483, 1369, 1245, 1221, 1063, 763 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ_H: 7.00 (1H, d, $J = 8.5$ Hz, H-3), 7.54 (1H, m, H-4), 6.96 (1H, m, H-5), 7.71 (1H, dd, $J = 8.0$, 1.5 Hz, H-6), 10.19 (1H, s, -OH), 6.09 (1H, d, $J = 6.5$ Hz, H-1′), 3.6 ~5.5 (4H, H-2′, 3′, 4′, 5′), 2.04 (3H, s, -OAc), 2.03 (3H, s, -OAc), 2.02 (3H, s, -OAc); ESI-MS *m/z*: 419 $[M + Na]^+$.

*3.2.2.8 1-[2,3,6-Tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)]-β-D-glucopyranosyl-2-hydroxybenzoate (**13b**).* $[\alpha]_D^{20}$ −26.1 ($c = 0.10$,

$CHCl_3$); UV ($CHCl_3$) λ_{max}: 244, 313 nm; IR (KBr) ν_{max}: 2965, 1754, 1696, 1486, 1370, 1229, 1074, 762 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ_H: 7.00 (1H, d, $J = 8.5$ Hz, H-3), 7.52 (1H, m, H-4), 6.94 (1H, t, $J = 8.0$ Hz, H-5), 7.61 (1H, d, $J = 8.0$ Hz, H-6), 10.18 (1H, s, -OH), 6.12 (1H, d, $J = 8.0$ Hz, H-1′), 3.0 ~ 5.5 (H-2′, 3′, 4′, 5′, 6′a, 6′b, 1″, 2″, 3″, 4″, 5″, 6″a, 6″b), 2.15 (3H, s, -OAc), 2.06 (3H, s, -OAc), 2.00 (9H, s, -OAc × 3), 1.95 (3H, s, -OAc), 1.89 (3H, s, -OAc); ESI-MS *m/z*: 779 $[M + Na]^+$.

3.2.3 *Methyl 2-O-(2,3-di-O-acetyl-4,6-O-benzylidene)-β-D-glucopyranosyl benzoate (**7**)*

When selective shelter of hydroxyl groups at C-2, C-3, C-4, and C-6 of the glucose residue on **5a** was successfully carried out using benzaldehyde and acetic anhydride, compound **7** was afforded, yielding 37% for two steps. m.p. 180.0–185.0°C; $[\alpha]_D^{20}$ −153.6 ($c = 0.10$, $CHCl_3$); UV ($CHCl_3$) λ_{max}: 242, 282 nm; IR (KBr) ν_{max}: 2949, 2882, 1752, 1732, 1715, 1604, 1490, 1455, 1373, 1307, 1251, 1232, 1096, 1082, 1062, 1031, 757, 700 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 7.12 (2H, m, H-3, 4), 7.45 (1H, m, H-5), 7.77 (1H, d, $J = 7.6$ Hz, H-6), 3.86 (3H, s, H-8), 7.36 ~ 7.49 (5H, m, H-2′, 3′, 4′, 5′, 6′), 5.54 (1H, s, H-7′), 5.21 (1H, d, $J = 6.8$ Hz, H-1″), 5.38 (2H, m, H-2″, 3″), 4.43 (1H, dd, $J = 10.4$, 4.4 Hz, H-6″a), 3.71 ~ 3.83 (3H, m, H-4″, 5″, 6″b), 2.07 (6H, s, -OAc × 2); ESI-MS *m/z*: 425 $[M + K]^+$.

3.2.4 *Gaultherin (**1**)*

The removal of the protective group on **7** in 80% HOAc at 80°C for 3 h afforded **8**, yielding 79% [11]. When xylose as a donor attached to the C-6 of the glucopyranosyl of **8** under promotion with BF_3–Et_2O, **1** was afforded. m.p. 104.0–105.0°C; $[\alpha]_D^{20}$ −86.0 ($c = 0.10$, MeOH); UV (MeOH) λ_{max}: 231, 287 nm; IR (KBr) ν_{max}: 3391, 2891, 1708, 1601, 1491, 1451, 1437, 1309, 1266, 1074, 1043, 762 cm^{-1}; ^{1}H-NMR (CD_3OD, 400 MHz) δ_H: 7.40 (1H, d, $J = 8.0$ Hz, H-3), 7.51 (1H, m, H-4), 7.07 (1H, d, $J = 7.6$ Hz, H-5), 7.70 (1H, dd, $J = 7.6$, 1.6 Hz, H-6), 3.83 (3H, s, H-8), 4.81 (1H, d, $J = 7.6$ Hz, H-1′), 4.27 (1H, d, $J = 7.6$ Hz, H-1″), 3.0 ~ 4.1 (H-2′, 3′, 4′, 5′, 6′a, 6′b, 2″, 3″, 4″, 5″a, 5″b); ^{13}C-NMR (CD_3OD, 100 MHz) δ_C: 122.3 (C-1), 158.6 (C-2), 119.2 (C-3), 135.4 (C-4), 123.7 (C-5), 132.0 (C-6), 168.5 (C-7), 52.8 (C-8), 103.9 (C-1′), 105.5 (C-1″), 77.7, 77.6, 77.4, 75.0, 74.9, 71.3, 71.2, 69.9, 66.9 (C-2′, 3′, 4′, 5′, 6′, 2″, 3″, 4″, 5″); ESI-MS *m/z*: 469 $[M + Na]^+$.

3.2.5 *Methyl 2-O-[4-(O-β-D-xylopyranosyl)-6-(O-β-D-xylopyranosyl)]-β-D-glucopyranosylbenzoate (**12**)*

Compound **12** was prepared according to the same procedure described for **1**. m.p. 130.0–132.0°C; $[\alpha]_D^{20}$ −67.5 ($c = 0.10$, MeOH); UV (MeOH) λ_{max}: 233, 285 nm; IR (KBr) ν_{max}: 3392, 2892, 1708, 1602, 1491, 1451, 1310, 1267, 1075, 1043, 763 cm^{-1}; ^{1}H-NMR (CD_3OD, 400 MHz) δ_H: 7.29 (1H, d, $J = 8.0$ Hz, H-3), 7.45 (1H, m, H-4), 7.04 (1H, d, $J = 8.0$ Hz, H-5), 7.67 (1H, dd, $J = 8.0$, 1.6 Hz, H-6), 3.79 (3H, s, H-8), 4.86 (1H, d, $J = 7.5$ Hz, H-1′), 4.43 (1H, d, $J = 8.0$ Hz, H-1″), 4.27 (1H, d, $J = 7.2$ Hz, H-1‴), 3.0 ~ 4.2 (H-2′, 3′, 4′, 5′, 6′a, 6′b, 2″, 3″, 4″, 5″a, 5″b, 2‴, 3‴, 4‴, 5‴a, 5‴b); ^{13}C-NMR (CD_3OD, 100 MHz) δ_C: 122.5 (C-1), 158.5 (C-2), 119.1 (C-3), 135.2 (C-4), 123.8 (C-5), 132.1 (C-6), 168.5 (C-7), 52.9 (C-8), 103.7 (C-1′), 105.4 (C-1″), 105.2 (C-1‴), 79.9, 77.9, 77.8, 75.6, 75.5, 74.9, 74.8, 74.7, 71.2, 71.1, 68.6, 67.1, 67.0 (C-2′, 3′, 4′, 5′, 6′, 2″, 3″, 4″, 5″, 2‴, 3‴, 4‴, 5‴); ESI-MS *m/z*: 601 $[M + Na]^+$.

3.3 *Anti-nociceptive and anti-inflammatory assay*

3.3.1 *Acetic acid-induced writhing test*

A slight modification of the acetic acid-induced writhing method was used [14].

One hour after administering **5f** (200–800 mg/kg), aspirin (200 mg/kg), and the vehicle orally to groups of 10 mice, 0.6% aqueous solution of acetic acid was administered intraperitoneally to each mouse at a dose of 10 ml/kg body weight. Each animal was placed in a transparent observation cage 5 min after the acetic acid injection, and the number of writhes per mouse was counted over a 10 min period. Writhing was defined as a contraction of the abdominal muscles together with a stretching of the hind limbs.

3.3.2 *Croton oil-induced ear edema test*

Aspirin (200 mg/kg), **5f** (400 mg/kg), and the vehicle were administered orally for 3 days before the topical application of 1 mg croton oil dissolved in 50 μl acetone (20 μl/ear) to the right ear of mice. The activity was evaluated using the following process: the animals were sacrificed by deep ether anesthesia 3 h after the topical treatment. Eight millimeter diameter disks were removed from each ear and weighed on a balance [14,15].

3.3.3 *NO assay*

Murine macrophage RAW264.7 cells were cultured at 37°C in RPMI1640, containing 10% fetal bovine serum, in a humidified atmosphere containing 5% CO_2. The cells were pretreated with all the synthetic compounds (1×10^{-6} M) 1 h before being treated with 0.5 μg/ml LPS for 24 h. The nitrite concentration in the culture medium was measured as an indicator of NO production based on the Griess reaction. A total of 70 μl of each supernatant was mixed with the same volume of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride in water), and the resulting mixture was then incubated at room temperature for 10 min. The absorbance at 540 nm was then measured using a microplate reader. Freshly cultured medium was used as the blank in all experiments [14,16].

3.3.4 *Statistical analysis*

All values were expressed as ± SD. The Student's *t*-test for unpaired observations between normal or control and tested samples was carried out to identify statistical differences; p values less than 0.05 were considered as significantly different.

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