# Xanthone Glycosides from *Swertia bimaculata* with α-Glucosidase Inhibitory Activity

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**Key words**
- *Swertia bimaculata*
- Gentianaceae
- xanthone glycosides
- α-glucosidase inhibition

## Abstract

Seven new xanthone glycosides (**1**–**7**) were isolated from the *n*-butanol extract of *Swertia bimaculata*, together with six known compounds (**8**–**13**). Their structures were elucidated on the basis of extensive spectroscopic analyses (1D- and 2D-NMR, HRESIMS, UV, and IR) and comparison with data reported in the literature. All the compounds were evaluated for their α-glucosidase inhibitory activities *in vitro*, and compounds **3, 4**, and **7** exhibited significant activities to inhibit α-glucosidase. Meanwhile the effects of different substitutions on the α-glucosidase inhibitory activity of xanthone glycosides from *S. bimaculata* are also discussed.

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## Introduction

The medicinal plants from the genus *Swertia* (Gentianaceae) are used as traditional Asian medicines for the treatment of various digestive diseases [1–3]. For example, *Swertia mileensis*, known as "Qing-Ye-Dan", has been documented in the Chinese Pharmacopoeia (1977–2010 editions) to cure hepatitis clinically [1]. Previous work has also found that *Swertia. chirayita* [4], *Swertia japonica* [5], *Swertia punicea* [6], *Swertia kouitchensis* [7], *Swertia macrosperma* [8], and *Swertia bimaculata* [9] can alleviate the hyperglycemic status in diabetic animals. Phytochemical investigations have indicated that the main constituents from this genus are xanthones [10], triterpenoids [11], secoiridoid glucosides [12], and flavonoids.

*S. bimaculata* (Siebold & Zuccarini), a well-known ethnomedicine to cure hepatitis and dyspepsia [13], was recorded as Zhang Yacai in Jiuhuang Bencao by the Chinese Ming Dynasty 600 years ago. According to our previous work, the *n*-butanol fraction of the ethanol extract from the whole plant of *S. bimaculata* showed an antidiabetic effect *in vitro* and *in vivo* [9]. However, the active constituents from the *n*-butanol fraction of *S. bimaculata* remained unclear. Therefore, a systematic study was initiated to investigate the chemical constituents isolated from the *n*-butanol fraction of *S. bimaculata*. As shown in **Fig. 1**, here we report the isolation of seven new xanthone glycosides (**1**–**7**), along with six known ones (**8**–**13**). The α-glucosidase inhibitory activity and the structure-activity relationships of all isolated compounds were also evaluated.

## Results and Discussion

The *n*-butanol extract of the whole herbs of *S. bimaculata* was chromatographed over silica gel, Sephadex LH-20, Toyopearl HW-40C, and ODS, and further purified by semipreparative HPLC to yield seven new xanthone glycosides (**1**–**7**), together with six known ones (**8**–**13**) (**Fig. 1**).

Compound **1** was isolated as a yellow amorphous powder. Its molecular formula was deduced to be $C_{28}H_{34}O_{17}$ on the basis of HRESIMS (*m/z* 641.1748 $[M-H]^-$, calcd. for $C_{28}H_{33}O_{17}$, 641.1723). After acid hydrolysis, compound **1** gave D-xylose and D-glucose (see Acid hydrolysis and sugar analysis). The UV spectrum of compound **1** in MeOH displayed the characteristic absorption at $\lambda_{max}$ 247, 278, and 345 nm for the xanthones. The IR spectrum of compound **1** suggested the presence of hydroxyl groups (3417 $cm^{-1}$), hydrogen-bonded ketone (1649 $cm^{-1}$), and the aromatic rings (1606, 1581, and 1486 $cm^{-1}$) [14]. The $^1$H-NMR spectrum displayed signals for four methoxy

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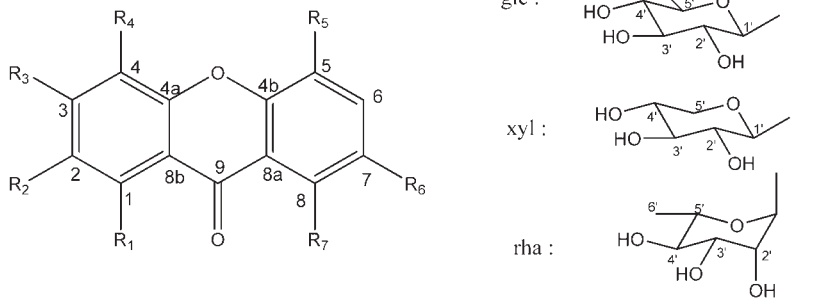


**Fig. 1** Chemical structures of compounds **1–13**.

| | R1 | R2 | R3 | R4 | R5 | R6 | R7 |
|---|---|---|---|---|---|---|---|
| **1** | O-glc(6-1)-xyl | OMe | OMe | OMe | OMe | H | OH |
| **2** | OH | OMe | OMe | OMe | OMe | H | O-glc(6-1)-xyl |
| **3** | O-glc(6-1)-xyl | H | OMe | OMe | OMe | H | OH |
| **4** | OH | H | OMe | OMe | OMe | H | O-glc(6-1)-xyl |
| **5** | O-glc(6-1)-xyl | H | OH | OMe | OMe | H | OH |
| **6** | O-glc(6-1)-glc | H | OH | OMe | OMe | H | OH |
| **7** | O-glc(4-1)-glc | H | OH | OMe | OMe | H | OH |
| **8** | OH | H | O-glc | OMe | OMe | H | H |
| **9** | O-glc | H | OH | OMe | OMe | H | OH |
| **10** | OH | H | O-glc | H | OMe | H | OH |
| **11** | OH | H | OMe | H | OH | H | O-glc |
| **12** | OH | H | OMe | H | H | O-xyl(2-1)-rha | OH |
| **13** | OH | H | OH | H | OH | H | O-glc |

groups ($\delta_H$ 4.07, 3.99, 3.90, and 3.84) and two *ortho*-coupled protons ($\delta_H$ 7.44 and 6.70, $J$ = 8.4 Hz) (**Table 1**). The characteristic peak at $\delta_H$ 12.26 permitted the assignment of a hydroxyl group at C-1 or C-8. Two *ortho*-coupled protons at H-6 ($\delta_H$ 7.44, $J$ = 8.4 Hz) and H-7 ($\delta_H$ 6.70, $J$ = 8.4 Hz) were confirmed unambiguously through the HMBC experiment. In the $^1$H-NMR spectrum (**Fig. 36S**) of **1a** (the xanthone aglycone of compound **1**), two protons at $\delta_H$ 11.28 and 11.76 permitted the assignment of two hydroxyl groups at C-1 and C-8 [15]. This indicated that the glucose residue of compound **1** was located at C-1 or C-8. The glucosyl group was proposed at C-1 by an HMBC correlation from H-1′ ($\delta_H$ 5.02, $J$ = 7.6 Hz) to C-1 ($\delta$c 144.3). In the HMBC spectrum, the correlation of H-1″ ($\delta_H$ 3.91) with C-6′ ($\delta$c 68.2) suggested that the xylose moiety was attached to C-6′ of the glucosyl group. The relative configurations of two glucose residues were deduced to be $\beta$ by the coupling constants ($J$) of 7.6 Hz and 7.4 Hz [16]. The positions of the four methoxys were confirmed by HMBC and HSQC spectra. In the HMBC spectrum of **1**, $\delta_H$ 3.84, 4.07, 3.99, and 3.90 correlated with C-4 ($\delta$c 137.0), C-3 ($\delta$c 152.9), C-2 ($\delta$c 142.4), and C-5 ($\delta$c 139.5), respectively (**Fig. 2**). Thus, the structure of compound **1** was defined as 1-O-[$\beta$-D-xylopyranosyl-(1 → 6)-$\beta$-D-glucopyranosyl]-8-hydroxy-2,3,4,5-tetramethoxyxanthone.

Compound **2** was isolated as a yellow amorphous powder. Its molecular formula was established as $C_{28}H_{34}O_{17}$ on the basis of HRESIMS (*m/z* 641.1710 [M – H]$^-$, calcd. for $C_{28}H_{33}O_{17}$, 641.1723). After acid hydrolysis, compound **2** gave D-xylose and D-glucose. The xanthone aglycone (**2a**) of **2** (**Fig. 37S**) was the same as **1a** by comparison with the $^1$H-NMR spectrum (**Fig. 36S**). This indicated that the glucose residue of compound **2** should be located at C-1 or C-8. In the HMBC spectrum, the glucosyl group was proposed at C-8 by the correlations from H-1′ ($\delta_H$ 4.86, $J$ = 7.6 Hz) and H-6 ($\delta_H$ 7.49, $J$ = 8.4 Hz) to C-8 ($\delta$c 150.3), and the correlation of H-1″ ($\delta_H$ 4.21) with C-6′ ($\delta$c 68.3) suggested that the xylose moiety was attached to C-6′ of the glucosyl group. The relative configurations of two glucose residues were also deduced to be $\beta$ by the coupling constants ($J$) of 7.6 and 7.4 Hz. Thus, the structure of compound **2** was defined as 8-O-[$\beta$-D-xylopyranosyl-(1 → 6)-$\beta$-D-glucopyranosyl]-1-hydroxy-2,3,4,5-tetramethoxyxanthone.

Compound **3** was isolated as a yellow amorphous powder, and its molecular formula was deduced to be $C_{27}H_{32}O_{16}$ from HRESIMS at *m/z* 647.1432 [M + Cl]$^-$ (calcd. for $C_{27}H_{32}O_{16}Cl$, 647.1384). After acid hydrolysis, compound **3** also gave D-xylose and D-glucose. Its $^1$H-NMR spectra data (**Table 1**) were similar to those of compound **1**, except for a methoxy group ($\delta_H$ 3.99) that was substituted by a singlet aromatic proton ($\delta_H$ 6.90). The proton at $\delta_H$ 6.90 was assigned to H-2 due to the long-range coupling correla-

**Table 1** NMR data of compounds **1–4** (in DMSO-$d_6$, $\delta$ in ppm and $J$ in Hz).

| Position | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|
| | $\delta_H$ | δc | $\delta_H$ | δc | $\delta_H$ | δc | $\delta_H$ | δc |
| 1 | | 144.3 | 12.79, s | 149.9 | | 154.6 | 12.90, s | 158.2 |
| 2 | | 142.4 | | 134.9 | 6.90, s | 96.8 | 6.55, s | 95.2 |
| 3 | | 152.9 | | 153.5 | | 158.3 | | 159.5 |
| 4 | | 137.0 | | 131.8 | | 130.8 | | 128.0 |
| 4a | | 147.2 | | 144.5 | | 150.3 | | 147.8 |
| 4b | | 144.3 | | 146.2 | | 144.4 | | 146.2 |
| 5 | | 139.5 | | 143.1 | | 139.4 | | 143.2 |
| 6 | 7.44, d (8.4) | 120.9 | 7.49, d (8.4) | 118.7 | 7.42, d (8.4) | 120.8 | 7.47, d (8.4) | 118.8 |
| 7 | 6.70, d (8.4) | 108.6 | 7.27, d (8.4) | 111.4 | 6.68, d (8.4) | 108.8 | 7.28, d (8.4) | 111.9 |
| 8 | 12.26, s | 153.6 | | 150.3 | 12.40, s | 153.8 | | 150.4 |
| 8a | | 108.8 | | 111.3 | | 108.7 | | 111.4 |
| 8b | | 110.6 | | 105.0 | | 105.3 | | 103.0 |
| 9 | | 181.2 | | 181.6 | | 181.0 | | 181.0 |
| Glc-1′ | 5.02, d (7.6) | 103.5 | 4.86, d (7.6) | 102.3 | 5.09, d (7.6) | 101.3 | 4.84, d (7.6) | 102.6 |
| 2′ | 3.44, m | 74.1 | 3.41, m | 73.4 | 3.43, m | 73.4 | 3.40, m | 73.3 |
| 3′ | 3.24, m | 76.3 | 3.29, m | 76.1 | 3.32, m | 76.2 | 3.30, m | 76.0 |
| 4′ | 3.13, m | 69.9 | 3.18, m | 69.8 | 3.24, m | 69.9 | 3.20, m | 69.8 |
| 5′ | 2.87, m | 76.4 | 3.08, m | 76.6 | 3.07, m | 76.7 | 3.09, m | 76.7 |
| 6′ | 3.86, d (9.4) | 68.2 | 4.01, d (9.4) | 68.3 | 4.01, d (9.4) | 69.0 | 4.01, d (9.4) | 68.4 |
| | 3.44, m | | 3.56, m | | 3.59, m | | 3.56, m | |
| Xyl-1″ | 3.91, d (7.4) | 103.6 | 4.21, d (7.4) | 103.9 | 4.15, d (7.4) | 104.2 | 4.22, d (7.4) | 104.0 |
| 2″ | 2.82, m | 73.1 | 3.01, m | 73.3 | 2.93, m | 73.2 | 3.01, m | 73.4 |
| 3″ | 3.31, m | 76.3 | 3.56, m | 76.3 | 3.66, m | 76.0 | 3.56, m | 76.3 |
| 4″ | 3.17, m | 69.4 | 3.27, m | 69.6 | 3.17, m | 69.6 | 3.18, m | 69.6 |
| 5″ | 3.53, m | 65.3 | 3.67, m | 65.6 | 3.68, m | 65.7 | 3.67, m | 65.6 |
| | 2.72, m | | 2.96, m | | 2.99, m | | 2.96, m | |
| 2-OMe | 3.99, s | 61.6 | 3.80, s | 60.6 | | | | |
| 3-OMe | 4.07, s | 61.4 | 4.05, s | 61.5 | 3.98, s | 56.5 | 3.92, s | 56.6 |
| 4-OMe | 3.84, s | 61.4 | 3.92, s | 61.4 | 3.85, s | 60.9 | 3.81, s | 60.9 |
| 5-OMe | 3.90, s | 57.5 | 3.95, s | 57.0 | 3.90, s | 57.4 | 3.94, s | 57.0 |

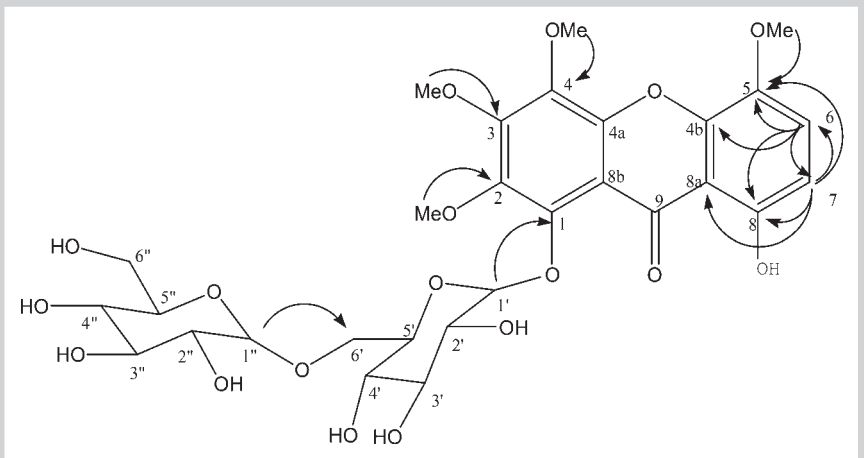


**Fig. 2** Key HMBC (H→C) correlations of compound **1**.

tions with C-4 (δc 130.8), C-3 (δc 158.3), C-1 (δc 154.6), and C-8b (δc 105.3). The glucosyl group was also proposed at C-1 by an HMBC correlation from H-1′ ($\delta_H$ 5.09, $J$ = 7.6 Hz) to C-1 (δc 154.6). Thus, the structure of compound **3** was defined as 1-O-[β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl]-8-hydroxy-3,4,5-trimethoxyxanthone.

Compound **4** was isolated as a yellow amorphous powder. The molecular formula of **4** was established as $C_{27}H_{32}O_{16}$ by its HRESIMS (*m/z* 647.1388 $[M + Cl]^-$; calcd. for $C_{27}H_{32}O_{16}Cl$, 647.1384). After acid hydrolysis, compound **4** also gave D-xylose and D-glucose. The xanthone aglycone (4a) of **4** was identified as 1,8-dihydroxy-3,4,5-trimethoxyxanthone by comparison with the $^{13}$C-NMR data reported in the literature (**Fig. 3S**) [17]. This indicated that the glucose residue of compound **4** should be located at C-1 or C-8. The HMBC spectrum of **4** showed that H-1′ ($\delta_H$ 4.84, $J$ = 7.6 Hz) and H-6 ($\delta_H$ 7.47, $J$ = 8.4 Hz) had a correlation with C-8 (δc 150.4), which indicated that the glucose residue should be located at C-8 of the aglycone. The correlation of H-1″ ($\delta_H$ 4.22) with C-6′ (δc 68.4) suggested that the xylose moiety was attached to C-6′ of the glucosyl group. The relative configurations of

**Table 2** NMR data of compounds **5**–**7** (in DMSO-$d_6$, $\delta$ in ppm and $J$ in Hz).

| Position | 5 | | 6 | | 7 | |
|---|---|---|---|---|---|---|
| | $\delta_H$ | $\delta$c | $\delta_H$ | $\delta$c | $\delta_H$ | $\delta$c |
| 1 | | 154.4 | | 154.4 | | 154.7 |
| 2 | 6.82, s | 101.3 | 6.85, s | 101.0 | 6.75, s | 102.3 |
| 3 | | 158.0 | | 157.6 | | 159.8 |
| 4 | | 130.1 | | 130.0 | | 130.5 |
| 4a | | 151.0 | | 151.1 | | 151.0 |
| 4b | | 144.2 | | 144.2 | | 144.2 |
| 5 | | 139.4 | | 139.4 | | 139.3 |
| 6 | 7.40, d (8.4) | 120.6 | 7.41, d (8.4) | 120.6 | 7.40, d (8.4) | 120.4 |
| 7 | 6.66, d (8.4) | 108.6 | 6.67, d (8.4) | 108.6 | 6.66, d (8.4) | 108.5 |
| 8 | 12.51, s | 153.9 | 12.49, s | 153.9 | 12.54, s | 153.9 |
| 8a | | 108.7 | | 108.8 | | 108.6 |
| 8b | | 104.4 | | 104.6 | | 103.6 |
| 9 | | 180.7 | | 180.7 | | 180.5 |
| Glc-1′ | 4.88, d (7.6) | 102.0 | 4.91, d (7.6) | 101.9 | 4.97, d (7.6) | 101.9 |
| 2′ | 3.39, m | 73.4 | 3.44, m | 73.7 | 3.43, m | 73.1 |
| 3′ | 3.30, m | 76.1 a | 3.31, m | 76.1 | 3.48, m | 74.5 |
| 4′ | 3.30, m | 69.6 | 3.25, m | 69.4 | 3.50, m | 79.5 |
| 5′ | 3.10, t (8) | 76.3 | 3.59, m | 76.0 | 3.54, m | 75.3 |
| 6′ | 3.99, m | 68.0 | 4.03, m | 68.2 | 3.73, m | 60.0 |
| | 3.63, m | | 3.66, m | | 3.67, m | |
| Glc-2/Xyl | | | | | | |
| 1″ | 4.21, d (7.4) | 104.0 | 4.25, d (7.4) | 103.4 | 4.33, d (7.4) | 103.1 |
| 2″ | 3.01, m | 73.3 | 2.97, m | 73.3 | 3.03, m | 73.3 |
| 3″ | 3.53, m | 76.0 a | 3.17, m | 76.5 | 3.19, m | 76.5 |
| 4″ | 3.27, m | 69.3 | 3.05, m | 70.1 | 3.08, m | 70.0 |
| 5″ | 3.69, m | 65.6 | 3.07, m | 76.9 | 3.21, m | 76.8 |
| | 2.99, m | | | | | |
| 6″ | | | 3.66, m | 61.0 | 3.73, m | 61.0 |
| | | | 3.46, m | | 3.37, m | |
| 4-OMe | 3.90, s | 60.7 | 3.89, s | 60.8 | 3.90, s | 60.7 |
| 5-OMe | 3.86, s | 57.3 | 3.86, s | 57.3 | 3.85, s | 57.3 |

a: Assignments may be interchanged

two glucose residues were also deduced to be $\beta$ by the coupling constants ($J$) of 7.6 Hz and 7.4 Hz. Thus, the structure of compound **4** was defined as 8-O-[$\beta$-D-xylopyranosyl-(1 → 6)-$\beta$-D-glucopyranosyl]-1-hydroxy-3,4,5-trimethoxyxanthone.

Compound **5** was obtained as a yellow amorphous powder. The HRESIMS spectrum of **5** exhibited an [M – H]$^-$ ion at *m/z* 597.1446 (calcd. for $C_{26}H_{29}O_{16}$, 597.1461), corresponding to the molecular formula $C_{26}H_{30}O_{16}$. After acid hydrolysis, compound **5** gave D-xylose and D-glucose. The $^1$H-NMR (**Table 2**) data of **5** was similar to those of 1-O-$\beta$-D-glucopyranosy-3,8-dihydroxy-4,5-dimethoxyxanthone (**9**), except for one additional proton at $\delta_H$ 4.21 ($J$ = 7.4 Hz) and a group of glycosyl protons between $\delta_H$ 3.69 and $\delta_H$ 2.99. The $^{13}$C-NMR spectrum displayed five carbon signals ($\delta$c 104.0, 73.3, 76.0, 69.3, and 65.6) attributed to a xylose moiety, and C-6′ of the glucosyl group shifted from $\delta$c 60.6 to 68.0. In the HMBC spectrum, the correlation of H-1″ ($\delta_H$ 4.21) with C-6′ ($\delta$c 68.0) suggested that the xylose moiety was attached to C-6′ of the glucosyl group. The relative configurations of two glucose residues were deduced to be $\beta$ by the coupling constants ($J$) of 7.6 Hz and 7.4 Hz. Thus, the structure of compound **5** was defined as 1-O-[$\beta$-D-xylopyranosyl-(1 → 6)-$\beta$-D-glucopyranosyl]-3,8-dihydroxy-4,5-dimethoxyxanthone.

Compound **6** was also isolated as a yellow amorphous powder. Its molecular formula was assigned as $C_{27}H_{32}O_{17}$ on the basis of HRESIMS (*m/z* 627.1567 [M – H]$^-$; calcd. for $C_{27}H_{31}O_{17}$, 627.1567). The NMR (**Table 2**) data of **6** were similar to those of compound **5**, except that the signals for a xylose residue were replaced by signals associated with a glucose residue. Its HMBC spectrum displayed the correlation between the proton at the anomeric center of Glc-2 ($\delta_H$ 4.25) and C-6′ ($\delta$c 68.2) of Glc-1. This suggested that the Glc-2 residue was linked to the Glc-1 residue by a (1 → 6) linkage [10]. The coupling constant of the proton at the anomeric center of Glc-2 was found to be 7.4 Hz. Therefore, the structure of compound **6** was defined as 1-O-[$\beta$-D-glucopyranosyl-(1 → 6)-$\beta$-D-glucopyranosyl]-3,8-dihydroxy-4,5-dimethoxyxanthone.

Compound **7** was isolated as a yellow amorphous powder. Its molecular formula was deduced to be $C_{27}H_{32}O_{17}$ on the basis of HRESIMS (*m/z* 627.1602 [M – H]$^-$; calcd. for $C_{27}H_{31}O_{17}$, 627.1567). The NMR (**Table 2**) data of **7** were also similar to those of compound **9**, except for a group of signals from an additional glucose residue and the downfield shift of C-4′ of Glc-1 ($\delta$c from 69.4 to 79.5). This suggested that the Glc-2 residue was located at C-4′ of Glc-1 [18]. This assumption was supported by the HMBC spectrum, in which the proton at the anomeric center of Glc-2 ($\delta_H$ 4.33) showed a correlation with C-4′ of Glc-1 ($\delta$c 79.5). The relative configurations of two glucose residues were deduced to be $\beta$ by the coupling constants of the protons at the anomeric center. Accordingly, the structure of compound **7** was defined as 1-O-[$\beta$-D-xylopyranosyl-(1 → 4)-$\beta$-D-glucopyranosyl]-3,8-dihydroxy-4,5-dimethoxyxanthone.

| Compound | $IC_{50}$ μM | Compound | $IC_{50}$ μM |
|---|---|---|---|
| **1** | 442 ± 47 | **8** | > 1000 |
| **2** | > 1000 | **9** | 578 ± 39 |
| **3** | 142 ± 17 | **10** | > 1000 |
| **4** | 136 ± 14 | **11** | 389 ± 23 |
| **5** | 417 ± 32 | **12** | 765 ± 54 |
| **6** | 478 ± 45 | **13** | 679 ± 58 |
| **7** | 258 ± 19 | acarbose | 426 ± 45 |

[a] Each value represents the mean ± SD (n = 3)

**Table 3** Inhibitory effects of compounds **1–13** and acarbose against α-glucosidase[a].

Comparisons of NMR data reported in the literature led to the identification of the known compounds as 3-O-β-D-glucopyranosyl-1-hydroxy-4,5-dimethoxyxanthone (**8**) [19], 1-O-β-D-glucopyranosyl-3,8-dihydroxy-4,5-dimethoxyxanthone (**9**) [20,21], 3-O-β-D-glucopyranosyl-1,8-dihydroxy-5-methoxyxanthone (**10**) [22], swertianolin (**11**) [23,24], 7-O-[α-L-rhamnopyranosyl-(1 → 2)-β-D-xylopyranoyl-1,8-dihydroxy-3-methoxyxanthone (**12**) [25], and norswertianolin (**13**) [26].

All isolated compounds were evaluated for α-glucosidase inhibitory activities. The $IC_{50}$ values, defined as the compound concentration that inhibits α-glucosidase activity by 50%, are summarized in **Table 3**. Among them, compounds **3, 4**, and **7** exhibited more potent α-glucosidase inhibitory activities ($IC_{50}$s of 142 μM, 136 μM, and 258 μM, respectively) than the positive control acarbose ($IC_{50}$ 426 μM). It was observed that the methoxy group located at C-2 (compounds **1** and **2**) produced steric hindrance and lowered the inhibitory activity. The presence of an O-glc-(4–1)-glc residue (compound **7**) resulted in relatively more effective inhibitory activity than other diglycoside units (compounds **5** and **6**). But the reason why the glycoside units located at C-1 (compound **1**) exhibited more potent inhibitory activity than the units located at C-8 (compound **2**) is still unclear. Thus, further experiments will be performed to evaluate the effects of different substitutions on the α-glucosidase inhibitory activity of xanthone glycosides.

## Materials and Methods

### General experimental procedures

Optical rotations were measured on an AA10R digital polarimeter. UV spectra were run on a Carry-50 UV-vis spectrophotometer. IR spectra were recorded on an Avatar-360 FT-IR spectrophotometer with KBr pellets. 1D- and 2D-NMR spectra were recorded on a Bruker AV-400 spectrometer (400 MHz for $^1H$ and 100 MHz for $^{13}C$, respectively). Chemical shifts are expressed in δ (ppm) and are referenced to the solvent peaks at $\delta_H$ 2.50 and δc 39.5 for DMSO-$d_6$. HRESIMS were measured by an Agilent 6520 Q-TOF LC-MS mass spectrometer. Column chromatography was performed with macroporous resin (Diaion AB-8; Mitsubishi Chemical Crop.), polyamide resin (30–60 mesh, Mitsubishi Chemical Corp.), ODS gel (50 μm, YMC), Sephadex LH-20 (GE Healthcare), Toyopearl HW-40C (Tosoh), and silica gel (200–300 mesh; Qingdao Marine Chemical, Inc.). Semipreparative HPLC was performed on a Hitachi Spectra Series HPLC system equipped with an L-2130 pump and a UV L-2400 detector in a YMC-ODS column (10 mm × 250 mm, 5 μm; flow rate at 1.5 mL/min; wavelength detection at 254 nm). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates after spraying with 10% $H_2SO_4$ in EtOH. GC analysis was carried out on an Agilent 7820A GC system using an HP-5 (30 m × 0.32 mm × 0.25 μm) column; detection FID; carrier gas $N_2$; injection temperature 250 °C, detection temperature 250 °C, and column temperature 180 °C. Preparative HPLC methanol and chemicals of analytical grade, such as methanol, dichloromethane, and ethyl acetate, were purchased from Sinopharm Chemical Reagent Co., Ltd.

### Plant materials

The whole plant of *S. bimaculata* was collected in Enshi, Hubei Province, People's Republic of China, in October 2010. The plant material was identified by Jia-chun Chen, Tongji School of Pharmaceutical Sciences, Huazhong University of Science and Technology. A voucher specimen (No. 20101010) has been deposited at the Department of Pharmacognosy of the same University.

### Extraction and isolation

The air-dried whole plants of *S. bimaculata* (23 kg) were powdered and extracted successively with 95% EtOH (2 × 200 L, under reflux, each for 2 h) and 50% EtOH (2 × 200 L, under reflux, each for 2 h) at 60 °C. The obtained EtOH extracts were combined and concentrated in vacuum to give a residue (5 kg), which was suspended in $H_2O$ and partitioned with petroleum ether, $CH_2Cl_2$, EtOAc, and *n*-butanol, respectively.

After removal of the solvent in vacuum, the *n*-butanol fraction (900 g) was separated on an AB-8 macroporous resin column (120 × 20 cm i.d., eluted with a mixture of $H_2O$ and EtOH from 1 : 0 to 0 : 1) to give seven fractions. Fr. B5 (200 g, eluted with $H_2O$ : EtOH 1 : 1) was separated on a silica gel column (80 × 10 cm i.d., 200–300 mesh, 3000 g; solvent system: $CHCl_3$ -MeOH, 1 : 0 → 0 : 1, v/v; flow rate: 50 mL/min) to give 9 fractions (Fr. B5-1→B5–9). Fr. B5-2 (2 g, eluted with $CHCl_3$:MeOH 10 : 1) was separated on Toyopearl HW-40C (130 × 4 cm i.d.; solvent system: $CHCl_3$-MeOH, 1 : 1; flow rate: 5 mL/min) and then purified by Sephadex LH-20 (120 × 2.5 cm i.d., eluted with MeOH) to yield compound **8** (40 mg). Fr. B5-3 (20 g, eluted with $CHCl_3$:MeOH 8 : 1) was separated on a polyamide resin column (50 × 8 cm i.d., 30–60 mesh, 1200 g, solvent system: MeOH-$H_2O$, 0 : 10 → 10 : 0, v/v, flow rate: 20 mL/min) to give 5 fractions. Fr. B5-3C (200 mg, eluted with MeOH : $H_2O$ 30 : 70) was then subjected to Sephadex LH-20 (120 × 2.5 cm i.d., eluted with MeOH) to yield compound **9** (150 mg). Fr. B5-4 (30 g, eluted with $CHCl_3$: MeOH 7 : 1) was separated on an ODS column (40 × 5 cm i.d., 350 g; solvent system: MeOH-$H_2O$, 0 : 10 → 10 : 0, v/v) to give 9 fractions. Fr. B5-4C (2 g, eluted with MeOH : $H_2O$ 35 : 65) was then subjected to Toyopearl HW-40C (130 × 4 cm i.d.) and eluted with MeOH to give 9 fractions (Fr. B5-4C1→B5-4C9). Fr. B5-4C3 (200 mg) was fractioned by semipreparative HPLC on a YMC C18 column (MeOH–$H_2O$–$CH_3COOH$, 55 : 45 : 0.1, v/v, flow rate: 1.5 mL/min) to yield compounds **1** (50 mg, $t_R$ 30.2 min) and **2** (60 mg, $t_R$ 25.8 min), while Fr. B5-4C4 (200 mg) was purified by the same way using

MeOH-$H_2O$-$CH_3COOH$ (55 : 45 : 0.1) as the eluent to afford compounds **3** (10 mg, $t_R$ 23.4 min) and **4** (15 mg, $t_R$ 28.7 min). Fr. B5-4C5 (150 mg) was fractioned on Sephadex LH-20 and eluted with MeOH to yield compound **10** (30 mg). Fr. B5-4C6 (100 mg) was subjected to semipreparative HPLC on a YMC C18 column (MeOH–$H_2O$–$CH_3COOH$, 65 : 35 : 0.1, v/v, flow rate: 1.5 mL/min) to yield compounds **11** (30 mg, $t_R$ 21.8 min) compound **12** (15 mg, $t_R$ 16.5 min).

Fr. B3 (80 g, eluted with $H_2O$ : EtOH 3 : 7) was also separated on a silica gel column (60 × 8 cm i.d., 100–200 mesh, 1500 g; solvent system: CHCl3–MeOH, 1 : 0 → 0 : 1, v/v; flow rate: 50 mL/min) to give 7 fractions (Fr. B3-1→B3–7). Fr. B3–5 (6 g, eluted with $CHCl_3$ : MeOH 10 : 1) was then subjected to Toyopearl HW-40C and eluted with MeOH to give 5 fractions (Fr. B3–5A→B3–5E). Fr. B3–5C (200 mg) was fractioned on Sephadex LH-20 and eluted with MeOH to yield compound **13** (10 mg). Fr. B3–5D (270 mg) was subjected to Sephadex LH-20 using MeOH as the solvent, followed by semipreparative HPLC on a YMC C18 column (MeOH–$H_2O$–$CH_3COOH$, 55 : 45 : 0.1, v/v, flow rate: 1.5 mL/min) to give compounds **5** (30 mg, $t_R$ 18.7 min), **6** (20 mg, $t_R$ 24.6 min), and **7** (25 mg, $t_R$ 14.8 min). The purity (≥ 95%) of all isolated compounds was measured by HPLC analyses.

## Isolates

*1-O-[β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl]-8-hydroxy-2,3,4,5-tetramethoxyxanthone* (**1**): yellow amorphous powder; $[\alpha]_D^{20}$ − 72.3 (0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 204 (4.38), 260 (4.32), 279 (4.13), 326 (3.78) nm; IR (KBr) $\nu_{max}$ 3396, 2915, 1649, 1588, 1485, 1464, 1406, 1278, 1064, 814 $cm^{-1}$; $^1$H-NMR and $^{13}$C-NMR data, see **Table 1**; HRESIMS: *m/z* 641.1748 $[M - H]^-$ (calcd. for $C_{28}H_{33}O_{17}$, 641.1723).

*8-O-[β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl]-1-hydroxy-2,3,4,5-tetramethoxyxanthone* (**2**): yellow amorphous powder; $[\alpha]_D^{20}$ − 69.2 (0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 205 (4.33), 262 (4.27), 275 (4.13), 328 (3.70) nm; IR (KBr) $\nu_{max}$ 3417, 2930, 1645, 1609, 1584, 1494, 1463 1421, 1280, 1064, 814 $cm^{-1}$; $^1$H-NMR and $^{13}$C-NMR data, see **Table 1**; HRESIMS: *m/z* 641.1710 $[M - H]^-$ (calcd. for $C_{28}H_{33}O_{17}$, 641.1723).

*1-O-[β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl]-8-hydroxy-3,4,5-trimethoxyxanthone* (**3**): yellow amorphous powder; $[\alpha]_D^{20}$ − 28.6 (0.04, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 215 (4.24), 251 (3.78), 335 (3.32) nm; IR (KBr) $\nu_{max}$ 3356, 2921, 1738, 1644, 1606, 1542, 1475, 1314, 1074, 827 $cm^{-1}$; $^1$H-NMR and $^{13}$C-NMR data, see **Table 1**; HRESIMS: *m/z* 647.1432 $[M + Cl]^-$ (calcd. for $C_{27}H_{32}O_{16}Cl$, 647.1384).

*8-O-[β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl]-1-hydroxy-3,4,5-trimethoxyxanthone* (**4**): yellow amorphous powder; $[\alpha]_D^{20}$ − 41.9 (0.06, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 213 (4.21), 252 (3.73), 335 (3.30) nm; IR (KBr) $\nu_{max}$ 3420, 2924, 1649, 1606, 1578, 1520, 1482, 1423, 1382, 1026, 1001, 826 $cm^{-1}$; $^1$H-NMR and $^{13}$C-NMR data, see **Table 1**; HRESIMS: *m/z* 647.1388 $[M + Cl]^-$ (calcd. for $C_{27}H_{32}O_{16}Cl$, 647.1384).

*1-O-[β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl]-3,8-dihydroxy-4,5-dimethoxyxanthone* (**5**): yellow amorphous powder; $[\alpha]_D^{20}$ − 68.8 (0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 204 (4.42), 232 (4.34), 253 (4.40), 279 (4.02), 330 (3.93) nm; IR (KBr) $\nu_{max}$ 3397, 2924, 1649, 1606, 1581, 1515, 1486, 1423, 1249, 1197, 1060, 954 $cm^{-1}$; $^1$H-NMR and $^{13}$C-NMR data, see **Table 2**; HRESIMS: *m/z* 597.1446 $[M - H]^-$ (calcd. for $C_{26}H_{29}O_{16}$, 597.1461).

*1-O-[β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl]-3,8-dihydroxy-4,5-dimethoxyxanthone* (**6**): yellow amorphous powder; $[\alpha]_D^{20}$ − 65.9 (0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 203 (4.38), 231 (4.31), 253 (4.34), 278 (4.04), 332 (3.92) nm; IR (KBr) $\nu_{max}$ 3338, 2922, 1738, 1648, 1603, 1518, 1447, 1418, 1381, 1180, 1060, 828 $cm^{-1}$; $^1$H-NMR and $^{13}$C-NMR data, see **Table 2**; HRESIMS: *m/z* 627.1566 $[M - H]^-$ (calcd. for $C_{27}H_{31}O_{17}$, 627.1567).

*1-O-[β-D-xylopyranosyl-(1 → 4)-β-D-glucopyranosyl]-3,8-dihydroxy-4,5-dimethoxyxanthone* (**7**): yellow amorphous powder; $[\alpha]_D^{20}$ − 54.2 (0.08, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 204 (4.48), 231 (4.31), 253 (4.50), 278 (4.01), 330 (3.98) nm; IR (KBr) $\nu_{max}$ 3390, 2924, 1739, 1648, 1602, 1523, 1418, 1339, 1253, 1064, 813 $cm^{-1}$; $^1$H-NMR and $^{13}$C-NMR data, see **Table 2**; HRESIMS: *m/z* 627.1602 $[M - H]^-$ (calcd. for $C_{27}H_{31}O_{17}$, 627.1567).

## Acid hydrolysis and sugar analysis

Compound **1** (2 mg) was dissolved in 0.1 N $CF_3C00H$ (2 mL) and heated at 70 °C for 8 h. The reaction mixture was extracted with $CH_2Cl_2$. The aqueous layer was evaporated under vacuum and compared with references D-glucose and D-xylose (Sigma-Aldrich) by TLC (silica gel with $CHCl_3$-MeOH-$H_2O$, 6 : 4 : 1). The residue was then dissolved in pyridine (1 mL). Then 600 µL of HMDS-TMCS (hexamethydisilazane-trimethylchlorosilane, 2 : 1) was added, and the mixture was stirred at 60 °C for 30 min. The supernatant was analyzed by GC under the following conditions: HP-5 (30 m × 0.32 mm × 0.25 µm) column; detection FID; carrier gas $N_2$; injection temperature 250 °C, detection temperature 250 °C, and column temperature 180 °C. From the acid hydrolysis of **1**, D-glucose and D-xylose were confirmed by comparison of the retention times of their derivatives with those of authentic sugars derivatized in a similar way, which showed retention times of 7.15 and 13.30 min, respectively [27, 28]. The constituent sugars of compounds **2**–**7** were identified using the same method as for **1**.

## α-Glucosidase inhibitory assay

α-Glucosidase (from *Saccharomyces cerevisiae*; Sigma-Aldrich) inhibitory activities were determined by using p-nitrophenyl-α-D-glucopyranoside (PNPG; Sigma-Aldrich) as the substrate, according to a reported method with minor modifications [29, 30]. Briefly, 20 µL of enzyme solution [0.4 U/mL α-glucosidase in 0.1 M potassium phosphate buffer (pH 6.8)] and 120 µL of the test compound (purity ≥ 95%) in water containing 0.5% DMSO (Sigma-Aldrich) were mixed and incubated for 30 min at 37 °C. After incubation, 20 µL of PNPG solution [5.0 µM PNPG in 0.1 M potassium phosphate buffer (pH 6.8)] was added into each well and incubated together for 30 min at 37 °C. Then 80 µL 0.2 M $Na_2CO_3$ in 0.1 M potassium phosphate buffer was added to each well to stop the reaction. The absorbance of PNP released was measured on a UV max kinetic microplate reader (Bio Tek, Synergy 2) at 405 nm. Blank readings (no enzyme) were substracted from each well and the results were compared to the control (no sample). The pharmacological inhibitor acarbose (purity ≥ 99%; Sigma-Aldrich) was used as a positive control. Inhibition (%) was obtained by the following formula:

$$\text{Inhibition (\%)} = [(A_{(control)} - A_{(sample)})/A_{(control)}] \times 100$$

## Supporting information

$^1$H, $^{13}$C, 2D-NMR, and HRESIMS spectra for compounds **1**–**7**, as well as of 1D-NMR spectra for compounds **1a, 2a**, and **4a** are available as Supporting Information.

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## Conflict of Interest

There is no conflict of interest among all authors.

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