A new thiophene and two new monoterpenoids from Xanthium sibiricum

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Three new compounds (1–3), together with six known compounds (4–9), were isolated from the fruits of Xanthium sibiricum. The structures and the absolute configurations of sibiricumthionol (1), (+)-(5Z)-6-methyl-2-ethenyl-5-hepten-1,2,7-triol [(+)–2], (−)-(5Z)-6-methyl-2-ethenyl-5-hepten-1,2,7-triol [(−)–2], (2E,4E,1’S, 2R, 4’S, 6’R)-dihydrophaseic acid (3), (+)-xanthienopyran [(+)–4] and (−)-xanthienopyran [(−)–4] were established by extensive spectroscopic analyses, X-ray crystallographic analysis, ECCD analysis and ECD calculations. Caffeic acid (7) and caffeic acid ethyl ester (8) weekly inhibited α-glucosidase enzymatic activity by 44.5% and 40.2%, respectively, at 40 µM. Protocatechuic acid (9) selectively exhibited cytotoxicity against HepG2 cell lines, with an IC₅₀ value of 2.92 µM.

Keywords: Xanthium sibiricum; thiophene; monoterpenoid; sesquiterpene

1. Introduction

The fruits of Xanthium sibiricum L. have been used as traditional Chinese medicine for hundreds of years to treat leucoderma, fever, scrofula, sinusitis, headache, herpes, and cancer [1–4]. In a previous study [5], we have reported the isolation and structural elucidation of a pair of rare racemic spirodienone sesquineolignans from the extract of this medicinal plant. As part of a program to systematically study the chemical diversity of traditional Chinese medicines and their biological effects, the remaining fractions of the EtOAc extract were investigated, leading to the isolation of a new thiophene, two new monoterpenoids, together with six known compounds. The isolation and structural elucidation of three new compounds 1, (+)-2, and (−)-2, the absolute configurations of compounds 3, (+)-4, and (−)-4, and the bioactivities of the isolated compounds are reported in this paper.

2. Results and discussion

Compound 1 was isolated as a yellow amorphous powder and was assigned a molecular formula of C₉H₁₁NO₂S based on the HRESIMS (m/z 198.0581 [M + H]⁺; calcld 198.0583) and NMR data. The IR spectrum indicated the presence of hydroxyl (3371 cm⁻¹) and carbonyl (1651 cm⁻¹) groups. The ¹H NMR data (Table 1) revealed one methyl signal at δH 2.53 (3H, br s), one olefinic proton at δH 6.81 (1H, br s), and two directly connected methylene signals at δH 3.58 (2H, t, 5.5) and 3.70 (2H, t, 5.5). The ¹³C NMR and DEPT data (Table 1) revealed nine carbon resonances, including one amide carbonyl group (δC 167.5),
one methyl group (δC 16.4), four olefinic carbon atoms (δC 154.8, 152.8, 133.5, 121.3), and three methylene groups (δC 61.7, 51.9, 46.9). HMBC correlations (Figure 2) from H-3 to C-2/C-4/C-5 indicated a fragment of C(2)–C(3)H–C(4)–C(5). HMBC correlations from H-3 to C-11 and from Me-11 to C-2/C-3 indicated that C-11 and C-2 were directly connected. The HMBC correlation from Me-11 to C-5 and the chemical shifts of C-2 (δC 152.8) and C-5 (δC 133.5) together with the molecular formula of C9H11NO2S showed that C-2 was connected with C-5 through a sulfur atom to form ring A. The signal intensity of HMBC correlation from Me-11 to C-5 is weaker than HMBC correlations from Me-11 to C-2 and from Me-11 to C-3, which indicated the four-bond correlation from Me-11 to C-5 is reasonable. The HMBC correlation from H-8 to C-6 and the chemical shifts of H-8 (δH 4.41) and C-8 (δC 51.9) showed that C-8 was connected with C-6 through a nitrogen atom. Analyses of the degrees of unsaturation of compound 1 and the HMBC correlations from H-8 to C-4/C-5 indicated that another five-membered ring (ring B) was fused to ring A at C-4 and C-5. The fragment of C(9)H2–C(10)H2 confirmed by 1H–1H COSY correlation (Figure 2) was connected with N-7 by HMBC correlations from H2-9 to C-6/C-8. In addition, one hydroxyl group, which was determined by IR data (3370 cm⁻¹) was located at C-10 by the chemical shifts of C-10 (δC 61.7) and H2-10 (δH 3.70).

Thus, the structure of compound 1 was characterized as shown in Figure 1 and named sibiricumthionol.

Compound 2 was isolated as a white amorphous powder and was assigned a molecular formula of C10H18O3 based on the HRESIMS (m/z 209.1153 [M + Na]+; calcd 209.1148) and NMR data. The IR spectrum indicated the presence of a hydroxyl group (3359 cm⁻¹). The 1H NMR data (Table 2) revealed one methyl signal at δH 1.76 (3H, s), four olefinic protons at δH 5.30 (t, 3.0), 5.89 (1H, dd, 18.0, 12.0), 5.29 (dd, 18.0, 1.0), and 5.17 (1H, dd, 12.0, 1.0). The 1H NMR, 13C NMR, and HSQC spectra suggested the presence of ten carbon resonances, including one methyl (δC 21.8), four olefinic carbons (δC 142.8, 135.9, 129.2, 114.7), and three oxygenated carbons (δC 76.9, 69.9, 61.6). The NMR and IR data together with analysis of the degrees of unsaturation showed that compound 2 was a mono-terpenoid possessing three hydroxyl groups. The fragments of CH(9)–CH2(10) and CH2(3)–CH2(4)–CH(5) were confirmed by 1H–1H COSY and HSQC spectra. C-1 was connected to C-2 by HMBC correlations from H2-10/H-9 to C-2, from H2-1 to C-2/C-3, and from H2-3 to C-2/C-9. C-5, C-7 and C-8 were connected

| No. | Type     | δC   | δH (J in Hz) |
|-----|----------|------|--------------|
| 2   | C        | 152.8|              |
| 3   | CH       | 121.3| 6.81 br s    |
| 4   | C        | 154.8|              |
| 5   | C        | 133.5|              |
| 6   | C        | 167.5|              |
| 8   | CH2      | 51.9 | 4.41 br s    |
| 9   | CH2      | 46.9 | 3.58 (t, 5.5)|
| 10  | CH2      | 61.7 | 3.70 (t, 5.5)|
| 11  | CH3      | 16.4 | 2.53 br s    |

Table 2. 1H NMR(600 MHz) and 13C NMR (150 MHz) spectral data of compound 2 in CD3OD.

| No. | Type     | δC   | δH (J in Hz) |
|-----|----------|------|--------------|
| 1   | CH2      | 69.9 | 3.42 (dd, 12.0, 12.0) |
| 2   | C        | 76.9 |              |
| 3   | CH2      | 38.4 | 1.55(1.57 m) |
| 4   | CH       | 22.9 | 2.09(2.11 m) |
| 5   | CH       | 129.2| 5.30 (t, 3.0) |
| 6   | C        | 135.9|              |
| 7   | CH2      | 61.6 | 4.06 s       |
| 8   | CH3      | 21.8 | 1.76 s       |
| 9   | CH       | 142.8| 5.89 (dd, 18.0, 12.0) |
| 10a | CH2      | 114.7| 5.29 (dd, 18.0, 1.0) |
| 10b |         |      | 5.17 (dd, 12.0, 1.0) |
to C-6 by HMBC correlations from H$_2$-7 to C-5/C-6, from Me-8 to C-5/C-6, and from H-5 to C-6/C-7. The NOESY and NOE correlations of H$_2$-4/H$_2$-7 and H-5/Me-8 indicated the cis configuration of the double bond.

**Figure 3.** It is worth noting that compound 2 lacks optical activity, indicating a racemic nature. Compound 2 was successfully separated by HPLC using a CHIRALPAK AD-H column (250 x 5 mm, 5 µm) and a flow rate of 1.0 ml/min to obtain its two optically pure enantiomers, (+)-2 and (-)-2 (Supporting Information, Figure S20). Each enantiomer was obtained with enantiomeric excess (ee) ≥ 99%. The first peak eluted from the HPLC was determined as the (+)-enantiomer {[α]$^D_{25}$ + 17.0 (c 0.10, MeOH)} followed by the (-)-enantiomer {[α]$^D_{25}$ - 17.0 (c 0.10, MeOH)}. The components (+)-2 and (-)-2 possessed identical NMR spectra with compound 2, indicating the successful resolution of enantiomers. Thus, the structures of (+)-2 and (-)-2 were established as shown in **Figure 1** and named (+)-(5Z)-6-methyl-2-ethenyl-5-hepten-1,2,7-triol and (-)-(5Z)-6-methyl-2-ethenyl-5-hepten-1,2,7-triol, respectively.

Compound 3 was obtained as yellow crystals (in CH$_3$OH–H$_2$O) and had a molecular formula of C$_{15}$H$_{22}$O$_5$ based on HRESIMS (m/z 281.1398 [M–H]$^-$; calcd 281.1394) and NMR data. The IR spectrum indicated the presence of hydroxyl (3400 cm$^{-1}$) and carbonyl (1685 cm$^{-1}$) groups. The $^1$H NMR spectrum (Table 3) showed three methyl groups [δ$_H$ 2.31 (3H, s), 1.10 (3H, s) and 0.90 (3H, s)], three olefinic protons [δ$_H$ 5.86 (1H, s), 6.66 (1H, d, 15.0) and 6.54 (1H, d, 15.0) and 6.54 cm$^{-1}$] and two hydroxylic protons [δ$_H$ 3.80 (1H, d, 12.0) and 3.70 (1H, d, 12.0)].

**Table 3.** $^1$H NMR (600 MHz) and $^{13}$C NMR (150 MHz) spectral data of compound 3 in CD$_3$OD.

| NO. | type | $\delta_C$ | $\delta_H$ (J in Hz) |
|-----|------|------------|----------------------|
| 1   | C    | 170.3      |                      |
| 2   | CH   | 121.1      | 5.86 (s)             |
| 3   | C    | 152.9      |                      |
| 4   | CH   | 137.7      | 6.66 (d, 15.0)       |
| 5   | CH   | 133.9      | 6.54 (d, 15.0)       |
| 6   | CH$_3$ | 14.1 | 2.31 (s)             |
| 1'  | C    | 83.4       |                      |
| 2'  | C    | 87.8       |                      |
| 3'a | CH$_2$ | 45.9 | 2.03 (dd, 13.5, 6.5) |
| 3'b |       |           | 1.73 (dd, 13.5, 10.5) |
| 4'  | CH   | 65.9       | 4.00 (4.11 (1H, m)   |
| 5'a | CH$_2$ | 44.5 | 1.80 (dd, 12.5, 6.5) |
| 5'b |       |           | 1.63 (dd, 12.5, 8.5) |
| 6'  | C    | 49.0       |                      |
| 7'  | CH$_3$ | 19.6 | 1.10 (3H, s)         |
| 8'a | CH$_2$ | 77.3 | 3.80 (1H, d, 12.0)   |
| 8'b |       |           | 3.70 (1H, d, 12.0)   |
| 9'  | CH$_3$ | 16.3 | 0.90 (3H, s)         |
[67x633]three methylene groups including an oxygen-bearing isolated methylene group [δH 2.03 (1H, dd, 13.5, 6.5) and 1.73 (1H, dd, 13.5, 10.5), δH 1.80 (1H, dd, 12.5, 6.5) and 1.63 (1H, dd, 12.5, 8.5), δH 3.80 (1H, d, 12.0) and 3.70 (1H, d, 12.0)] and an oxymethine group [δH 4.09–4.11 (1H, m)]. The 13C NMR and DEPT spectra displayed five nonprotonated carbon atoms including an olefinic group (δC 152.9), a carbonyl group (δC 170.3), a quaternary (δC 49.0), and two oxygenated quaternary (δC 83.4 and 87.8) carbon atoms together with ten protonated carbon atoms. According to the above features, the NMR spectroscopic data of compound 3 were very similar to those of 6-hydroxy-dihydrophaseic acid [6] except for the absence of an oxygen-bearing isolated methylene group and the presence of a signal for a tertiary methyl group [δH 2.31 (s)], which indicated that the hydroxy methylene group at C-3 in 6-hydroxy-dihydrophaseic acid was replaced by a methyl group in compound 3. This was further confirmed by HMBC correlations from Me-6 to C-2/C-3/C-4 (Figure 2).

The relative configuration of compound 3 was deduced from a NOESY experiment, in which the correlations of Me-6/H-5 and H-2/H-4 verified that the conjugated diene was 2E,4E-configuration. The cross-peaks between H-4′ and H-3′a/H-5′a indicated that H-4′, H-3′a, and H-5′a were on the same face of the six-membered ring. Moreover, cross-peaks were observed for H-5/Me-7′ and H-3′b, H-5/Me-9′, and H-5′b indicated that bond C1′–C5, Me-7′, Me-9′, H-3′b and H-5′b were on the opposite face of the six-membered ring as shown in Figure 3. According to the established relative configuration of compound 3, the possible absolute configurations of compound 3 were proposed to be (1S, 2R, 4′S, 6′R) (3a) or (1′R, 2′S, 4′R, 6′S) (3b). The calculated ECD spectrum of 3a displayed a CD curve similar to the experimental spectrum of compound 3 (Figure 4). Meanwhile, the absolute configuration of compound 3 was also supported by the single crystal that was obtained for X-ray diffraction. The crystal structure (Figure 5) obtained by anomalous scattering of CuKα radiation allowed the unambiguous assignment of the absolute configuration of compound 3 (1′S, 2′R, 4′S, 6′R). Thus, the structure of compound 3 was characterized as shown in Figure 1 and named (2E,4E,1′S, 2′R, 4′S, 6′R)-dihydrophaseic acid. Compound 3 has been detected and the structure was assigned on the basis of LC-MSn [7]. However, no spectral NMR data were reported earlier. In this paper,
the NMR spectral data of compound 3 were presented in Table 3, and the relative and absolute configurations were determined for the first time.

Compound 4 possessed identical NMR data with (+)-xanthienopyran [8] and (−)-xanthienopyran [9]. Compound 4 was optically inactive, [α]_D^{25} = 0 (c 0.3, CH₃OH), indicating that it was obtained as a racemate. Subsequent HPLC separation of compound 4 on a chiral column yielded two compounds whose NMR data were identical to those of compound 4 prior to HPLC separation. However, the isolated compounds showed opposite optical rotation, and their CD spectra displayed mirror curves. This confirmed the successful separation of enantiomers (+)-4 and (−)-4. Each enantiomer was obtained with enantiomeric excess (ee) ≥ 99% (Supporting Information, Figure S35). The absolute configuration of the stereogenic

Figure 3. Key NOESY correlations of compounds 2 and 3.

Figure 4. Comparison of the experimental and calculated ECD spectra of compound 3.
center at C-8 position in (+)-4 was assigned on the basis of circular dichroism (CD) spectroscopic evidence. In exciton-coupled circular dichroism (ECCD) \[10,11\], the interaction between two strong electronic transition dipoles leads to “split” Cotton effects (CE), the signs of which are directly related to the chiral twist between the corresponding chromophores. The positive helicity observed for (+)-4 is in agreement with observed positive exciton-coupled CD experimental data showing a positive Cotton effect at 255 nm ($\Delta\epsilon + 4.92$) and negative effect at 225 nm ($\Delta\epsilon - 4.50$). For (−)-4, the CD curve showed opposite signs at 255 nm ($\Delta\epsilon - 6.84$) and 225 nm ($\Delta\epsilon + 6.44$) (Supporting Information, Figures S38 and S39). Thus, the absolute configurations of (+)-4 [(+)-xanthienopyran] and (−)-4 [−(−)-xanthienopyran] were assigned to be 8R and 8S, respectively.

The known compounds were identified as caffeic acid (7) [12], caffeic acid ethyl ester (8) [13] and protocatechuic acid (9) [14], by NMR analysis and comparison with literature data.

Compounds 7 and 8 weakly inhibited $\alpha$-glucosidase enzymatic activity by 44.5% and 40.2% at concentration of 40 $\mu$M, respectively (acarbose was used as a positive control, inhibition rate of 99.6%).

The cytotoxic activities of the isolated compounds were tested \textit{in vitro} against five human cell lines (HCT-116, HepG2, BGC-823, NCI-H1650, and A2780) using the MTT method. However, only compound 9 selectively exhibited cytotoxicity against HepG2 cell lines, with an IC$_{50}$ value of 2.92 $\mu$M. The other compounds were inactive (IC$_{50} > 10$ $\mu$M) against the cell lines tested (taxol was used as a positive control, IC$_{50} = 3.0 \times 10^{-2}$ $\mu$M).

3. Experimental

3.1. General experimental procedures

Optical rotation was measured on a Jasco P-2000 automatic digital polarimeter (JASCO, Tokyo, Japan). UV spectra were measured on a JASCO V650 spectrophotometer (Jasco, Tokyo, Japan). CD spectra were recorded on a JASCO J-815 spectropolarimeter (Jasco, Easton, MD). IR spectra were recorded on a 5700 FT-IR spectrometer (Nicolet, Madison, WI, USA). HRESIMS data were recorded
on a 6250 Accurate-Mass Q-TOF LC/MS spectrometer (Agilent, Santa Clara, CA, USA). NMR spectra were recorded on Inova 500 (Varian, Inc, Palo Alto, CA, USA), Agilent-NMR-VNMRS600 (Agilent, Santa Clara, CA, USA), or AVANCE III 800 (Bruker, Ettlingen, Germany) for 1D and 2D NMR. Preparative HPLC was performed on an LC-6AD instrument (Shimadzu, Kyoto, Japan) equipped with an SPD-10A detector (Shimadzu, Kyoto, Japan) using an YMC-Pack ODS-A column (250 × 20 mm, 5 μm). Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China).

3.2. Plant material
The fruits from Xanthium sibiricum (100 kg) were collected at Helen town in Heilongjiang Province of China during September 2012 and identified by associate Prof. Lin Ma from the Institute of Materia Medica at the Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (ID-S-2444) was deposited at the herbarium of the Institute of Materia Medica at the Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.

3.3. Extraction and isolation
The extraction and isolation procedures were reported previously [3]. Fraction ED (20.8 g) was chromatographed over RP-C_{18} silica gel (3.5 × 40 cm) and eluted with MeOH/H_{2}O (30:70, 50:50, 70:30, and 100:0) to give fractions ED1 to ED15. ED12 (120.0 mg, MeOH/H_{2}O, v/v 30:70) was purified by RP-preparative HPLC (20% MeOH/H_{2}O, 5.0 ml/min) to obtain compounds 3 (10.0 mg, t_R = 25.0 min) and 4 (12.5 mg, t_R = 30.0 min). (+)-4 (5.0 mg, t_R = 18.0 min) and (-)-4 (5.0 mg, t_R = 38.0 min) were subsequently separated from each other by chiral-semi-preparative HPLC (25% isopropyl alcohol in hexane, 2.0 ml/min). EG (110.0 g) was separated by flash chromatography over MCI gel (5 × 60 cm), eluted with a gradient of increasing CH_{3}OH (0–100%) in H_{2}O to give fractions EGA – EGE. EGA (10.0 g, MeOH/H_{2}O, v/v 20:80) was subjected to RP flash CC (10 – 95% MeOH in H_{2}O) to give subfractions EGA1 – EGA20. EGA-15 (50.0 mg, MeOH/H_{2}O, v/v 30:70) was subjected to RP-semi-preparative HPLC (20% MeOH in H_{2}O) to yield compound 1 (8 mg, t_R = 26.5 min). EGA-16 (350.0 mg, MeOH/H_{2}O, v/v 30:70) was initially purified by RP-semi-preparative HPLC (20% MeOH in H_{2}O, 5.0 ml/min), and then compounds 2 (10.0 mg, t_R = 10.0 min), 7 (15.0 mg, t_R = 12.0 min), 9 (8.0 mg, t_R = 15.0 min), and 8 (5.0 mg, t_R = 20.0 min) were obtained. (+)-2 (4.0 mg, t_R = 18.0 min) and (-)-2 (4.0 mg, t_R = 20.0 min) were subsequently separated from each other by chiral-semi-preparative HPLC (15% isopropyl alcohol in hexane, 1.0 ml/min).

3.3.1. Sibiricumthionol (1)
White amorphous powder; UV (MeOH) λ_{max} (log ε) 203 (4.17), 268 (3.91) nm; IR ν_{max} 3371, 2939, 1651, 1512, 1454, 1266 cm^{-1}; ^{1}H NMR, and ^{13}C NMR spectral data see Table 1; HR-ESIMS: m/z 198.0581 [M + H]^+ (calcd for C_{9}H_{12}NO_{2}S, 198.0583).

3.3.2. 6-Methyl-2-ethenyl-5-hepten-1,2,7-triol (2)
White amorphous powder; (+)-2: [α]_{D}^{25} + 17.0 (c 0.1, CH_{3}OH); (-)-2: [α]_{D}^{25} - 17.0 (c 0.1, CH_{3}OH). UV (MeOH) λ_{max} (log ε) 203 (2.71) nm; IR ν_{max} 3359, 2920, 2851, 1659, 1454, 1266 cm^{-1}; ^{1}H NMR and ^{13}C-NMR spectral data see Table 2; HR-ESIMS: m/z 209.1153 [M + Na]^+ (calcd for C_{10}H_{18}O_{3}Na, 209.1148);
3.3.3. (2E,4E,1 S, 2 R, 4 S, 6 R)-Dihydropaphaseic acid (3)
Colorless crystals; [α]D²⁵ = −36.8 (c 0.3, CH₃OH); IR νmax 3400, 2938, 1685, 1244, 1178, 1073 cm⁻¹; CD (MeOH) Δε260nm −5.87; ¹H NMR and ¹³C NMR spectral data see Table 3; HRESIMS: m/z 281.1398 [M − H]⁻ (calcd for C₁₅H₂₁O₅, 281.1394).

3.3.4. (+)-Xanthienopyran [(+)-4]
[α]D²⁵ + 50.3 (c 0.3, CH₃OH); CD (MeOH) Δε₂₂₅nm = 4.50, Δε₂₅₅nm = 4.92.

3.3.5. (−)-Xanthienopyran [(−)-4]
[α]D²⁵ − 51.0 (c 0.3, CH₃OH); CD (MeOH) Δε₂₂₅nm = 6.44, Δε₂₅₅nm = −6.84.

3.4. Crystallographic data for compound 3
C₁₅H₂₄O₆, M = 300.34, yellow plate (MeOH−H₂O), 0.50 × 0.40 × 0.07 mm³, tetragonal space group P₄₁₂₁₂; a = 7.56684(18) Å, b = 7.56684(18) Å, c = 54.2674(14) Å, V = 3107.19(13) Å³, T = 97(5) K, Z = 8, ρcalcd = 1.284 g/cm³, μ = 0.821 mm⁻¹, F (000) = 1296, 3052 reflections in h (-6/6), k (0/9), l (0/67), measured in the range 6.52° ≤ θ ≤ 114.2°, completeness θmax = 99.9%, 3052 independent reflections, Rint = 0.0000, 196 parameters, 0 restraints, GOF = 1.085. Final R indices: R₁ = 0.0415, wR₂ = 0.1053. R indices (all data): R₁ = 0.0427, wR₂ = 0.1066, and largest difference peak and hole: 0.304 and −0.322 e Å⁻³. The absolute structure was determined using a Flack parameter of −0.08 (19). Crystallographic data have been deposited at The Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 1038601. The data can be obtained free of charge via www.ccdc.cam.ac.uk/products/csd/request.

3.5. In vitro cytotoxicity assays
The isolates were tested for their cytotoxic activity against five human cell lines (HCT-116, HepG2, BGC-823, NCI-H1650, and A2780) by established colorimetric MTT assay protocols [15]. Taxol was used as the positive control.

3.6. α-Glucosidase inhibitory activity assays
Rat small intestinal brush border membrane vesicles were prepared and its suspension in 0.1 M phosphate buffer (pH 6.0) was used as the small intestinal α-glucosidase of maltase, sucrase, isomaltase, and trehalase. The enzyme suspension was diluted to hydrolyse sucrose to produce D-glucose in the following reaction. Reaction was performed in a 96-well plate. The substrate (sucrose: 100 mg/dl), test compound and the enzyme in 0.1 M phosphate buffer (pH 6.0, 0.2 ml) were incubated together at 37°C. After 30 min of incubation, the plate was immediately heated to 80–85°C for 3 min to stop the reaction, and then cooled. Glucose concentration was determined by the glucose-oxidase method. The assay was performed in triplicate with five different concentrations around the IC₅₀ values. The IC₅₀ values were calculated from the dose–response curves thus obtained in the experiments [16]. Acarbose was used as the positive control.

Disclosure statement
No potential conflict of interest was reported by the authors.

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References

[1] F.L. Hsu, Y.C. Chen, and J.T. Cheng, *Planta Med.* 66, 228 (2000). doi:10.1055/s-2000-8561.

[2] K.C. Huang, *The pharmacology of Chinese herbs drugs* (CRC Press, Boca Raton, 1993), p. 160.

[3] J.A. Marco, J.F. Sanz-Cervera, J. Corral, M. Carda, and J. Jakupovic, *Phytochemistry* 34, 1569 (1993). doi:10.1016/S0031-9422(00)90847-1.

[4] A.H. Nsari and K.S. Dubey, *Asian J. Chem.* 12, 521 (2000).

[5] Y.S. Shi, Y.B. Liu, Y. Li, L. Li, J. Qu, S.G. Ma, and S.S. Yu, *Org. Lett.* 16, 5406 (2014). doi:10.1021/ol502649a.

[6] J. Bai, H. Chen, Z.F. Fang, S.S. Yu, W.J. Wang, Y. Liu, S.G. Ma, Y. Li, J. Qu, S. Xu, J.H. Liu, F. Zhao, and N. Zhao, *Phytochemistry* 80, 137 (2012). doi:10.1016/j.phytochem.2012.05.015.

[7] G.C. Martin, F.G. Dennis Jr., J. MacMillan, and P. Gaskin, *J. Am. Soc. Hortic. Sci.* 102, 16 (1978).

[8] A.A. Mahmoud, A.A. Ahmed, M. Inuma, T. Tanaka, Y. Takahashi, and H. Naganawa, *Terrahedron Lett.* 36, 8985 (1995). doi:10.1016/0040-4039(95)01966-L.

[9] C. Lee, P. Huang, P. Hsieh, T. Hwang, Y. Hou, F. Chang, Y. Wu, C.L. Lee, P.C. Huang, and P.W. Hsieh, *Planta Med.* 74, 1276 (2008). doi:10.1055/s-2008-1081295.

[10] N. Harada and K. Nakanishi, *Circular Dichroic Spectroscopy-exciton Coupling In Organic Stereochemistry* (university science books, Mill Valley, CA, 1983), pp. 364–370.

[11] K. Nakanishi and N. Berova, in *Circular Dichroism-Principles and Application*, edited by K. Nakanishi, N. Berova, R. W. Woody (VCH Publishers, New York, 1994), pp. 361–375.

[12] Y.Q. Yin, Z.B. Shen, and L.Y. Kong, *J. Chin. Med. Mater.* 31, 1501 (2008).

[13] D.B. Zhao, W. Zhang, M.J. Li, and X.H. Liu, *China J. Chin. Mater. Med.* 31, 1869 (2006).

[14] W. Wang, C.R. Yang, and Y.J. Zhang, *Acta Bot. Yunnan.* 31, 284 (2009). doi:10.3724/SP.J.1143.2009.09031.

[15] S.G. Ma, W.Z. Tang, Y.X. Liu, Y.C. Hu, S.S. Yu, Y. Zhang, X.G. Chen, J. Qu, J.H. Ren, Y.B. Liu, S. Xu, J. Liu, Y.Y. Liu, Y. Li, H.N. Lü, and X.F. Wu, *Phytochemistry* 72, 115 (2011). doi:10.1016/j.phytochem.2010.10.021.

[16] Y.M. Kim, M.H. Wang, and H.I. Rhee, *Carbohydr. Res.* 339, 715 (2004). doi:10.1016/j.carres.2003.11.005.