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Two new noroleanane-type triterpenoid saponins from the stems of *Stauntonia chinensis*

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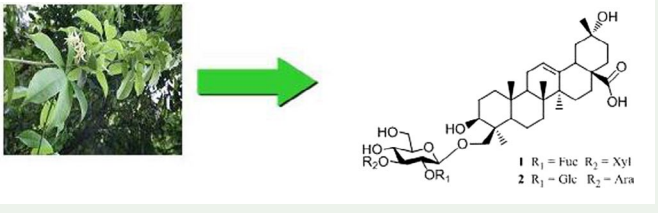
ABSTRACT

Two new noroleanane-type triterpenoid saponins, 3*β*,20*α*,24-trihydroxy-29-norolean-12-en-28-oic acid 24-*O*-*β*-L-fucopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranoside (**1**) and 3*β*,20*α*,24-trihydroxy-29-norolean-12-en-28-oic acid 24-*O*-*β*-D-glucopyranosyl-(1→2)-[*α*-L-arabinopyranosyl-(1→3)]-*β*-D-glucopyranoside (**2**) were isolated from the stems of *Stauntonia chinensis* DC., together with three known compounds, brachyantheraoside B_2 (**3**), eupteleasaponin VIII (**4**) and fargoside B (**5**). Their structures were elucidated by spectroscopic and chemical methods. The cytotoxic activities of compounds **1** and **2** were evaluated against five human tumor cell lines (HCT-116, HepG2, BGC-823, NCI-H1650, and A2780). Compounds **1** and **2** showed moderate cytotoxic activities toward the tested cell lines with IC_{50} values ranging from 12.71 to 32.04 μM.



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Stauntonia chinensis; Lardizabalaceae; noroleanane-type triterpenoids; cytotoxic activity

1. Introduction

Stauntonia chinensis DC., belonging to the family Lardizabalaceae, grows in southern China, including Jiangxi, Guangdong, and Guangxi provinces (Shanghai Scientific and Technical Publishers Shanghai, China 2006). Its stems are used as a traditional Chinese medicine known as 'Ye Mu Gua' for anti-nociceptive and anti-inflammatory effects (Gao et al. 2008). Previous

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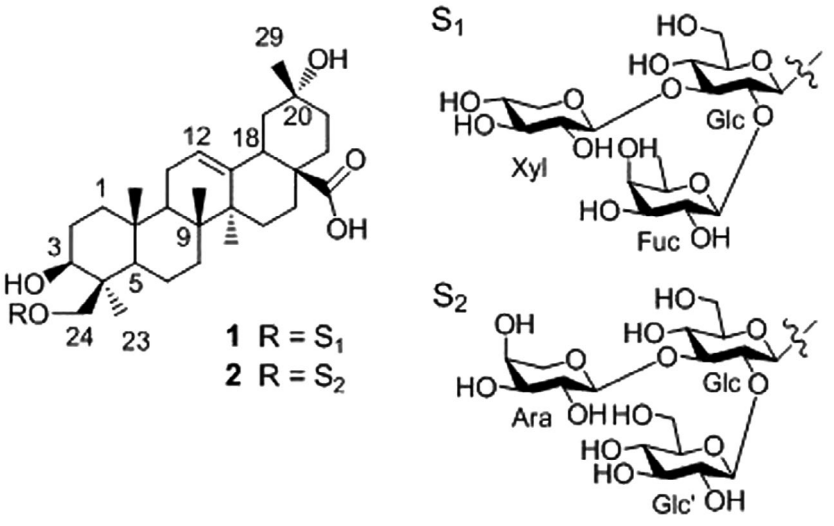

Figure 1. Chemical structures of compounds **1** and **2**.

investigation on *S. chinensis* showed its rich content of triterpenoid saponins with anticancer and anti-inflammatory activities (Wang et al. 1989; Gao et al. 2008). As part of a program to search for anticancer properties plant resources has led to the isolation of two new compounds, 3*β*,20*α*,24-trihydroxy-29-norolean-12-en-28-oic acid 24-*O*-*β*-L-fucopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranoside (**1**), 3*β*,20*α*,24-trihydroxy-29-norolean-12-en-28-oic acid 24-*O*-*β*-D-glucopyranosyl-(1→2)-[*α*-L-arabinopyranosyl-(1→3)]-*β*-D-glucopyranoside (**2**), and three known compounds, brachyantheraoside B_2 (**3**), eupteleasaponin VIII (**4**) and fargoside B (**5**) from the stems of *S. chinensis*. Reported herein are the isolation and structure elucidation and biological activity of these compounds.

2. Results and discussion

The H_2O extract of the stems of *Stauntonia chinensis* DC. was partitioned with $CHCl_3$, EtOAc, and *n*-BuOH, successively. The *n*-BuOH-soluble portion was separated by a combination of silica gel, repeated medium-pressure liquid chromatography and preparative HPLC to afford two new compounds (**1** and **2**) (see Figure 1) and three known compounds **3**–**5**.

Compound **1** was obtained as a white amorphous powder. Its molecular formula was determined as $C_{46}H_{74}O_{18}$ according to HR-ESI-Q-TOF-MS at *m/z* 913.4851 $[M–H]^-$ and supported by the NMR spectroscopic data. The ^1H NMR spectrum of **1** in pyridine-d_5 showed five tertiary methyls signals at δ_H 0.80, 0.94, 1.21, 1.53, and 1.59, an olefinic proton at δ_H 5.54 (1H, brs), two oxymethylene protons at δ_H 4.13 and 4.21 (1H each, d, J = 10.2 Hz). In addition, the ^1H and ^{13}C NMR signals for **1** showed three anomeric proton signals at δ_H 4.85 (1H, d, J = 7.8 Hz), 5.24 (1H, d, J = 7.8 Hz) and 5.62 (1H, d, J = 7.8 Hz) with the corresponding carbon resonances at δ_C 103.9, 105.7 and 103.9, respectively (see Experimental section). The ^{13}C NMR spectrum of **1** displayed 46 carbon signals, including a pair of olefinic carbons at δ_C 122.5 and 144.1, typical for a double bond at C-12(13) in olean-12-ene skeleton (Mehta et al. 2010; Achouri et al. 2017), and a carboxy carbonyl carbon at δ_C 180.6. However, direct bond and long-range correlation data suggested that one of the geminal methyl groups at

C-20 had been replaced by hydroxyl group, forming noroleanolic acid. The NMR data (see Experimental section) analysis of **1** indicated that it has the same aglycone as 3*β*,20*α*,24-trihydroxy-29-norolean-12-en-28-oic acid (Liu et al. 2017). Acid hydrolysis of **1** with 2 M HCl afforded D-glucose, L-fucose and D-xylcose, which were identified by GC analysis of their trimethylsilyl L-cysteine derivatives (Zhang et al. 1996). The *β*-anomeric configurations for glucose, xylcose, and fucose were determined from their coupling constants [J = 7.8 Hz (glc), 7.8 Hz (xyl), 7.8 Hz (fuc), respectively].

The linkages of these sugars were determined respectively through their HMBC spectra. In the HMBC spectrum of **1** (see Figure S1), the long-range correlations of H-1 (δ_H 5.62) of the fucose with C-2 (δ_C 79.1) of the glucose, H-1 (δ_H 5.24) of the xylcose with C-3 (δ_C 87.8) of the glucose, H-1 (δ_H 4.85) of the glucose with C-24 (δ_C 72.9) of the aglycone, and H-24 (δ_H 4.14, 4.21) of the aglycone with C-1 (δ_C 103.9) of the glucose obtain the linking sequence of the sugar chains. The relative configuration of **1** was indicated by the NOESY spectrum (see Figure S2), which showed NOE correlations between the following proton pairs: H-3/H-5, H-3/H_3-23, and H-18/H_3-30. On the basis of the above data, compound **1** was elucidated as 3*β*,20*α*,24-trihydroxy-29-norolean-12-en-28-oic acid 24-*O*-*β*-L-fucopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranoside.

Compound **2** was obtained as a white, amorphous powder, with the molecular formula $C_{46}H_{74}O_{19}$, as deduced from the $[M-H]^-$ peak at *m/z* 929.4777 by HR-ESI-Q-TOF-MS. The NMR spectroscopic data of compounds **2** and **1** were almost identical except that the fucose and xylose in **1** was replaced by a glucose (δ_C 104.2, 79.2, 78.5, 76.1, 70.2, 61.8) and a arabinose (δ_C 105.6, 75.2, 73.2, 70.0, 68.3) in **2**, respectively (see Experimental section). The connectivity for the sugar residues was further confirmed from the following HMBC correlations (see Figure S3): Glc-H-1 (δ_H 4.87)/aglycone-C-24 (δ_C 72.8), Glc′-H-1 (δ_H 5.67)/Glc-C-2 (δ_C 79.4), and Ara-H-1 (δ_H 5.27)/Glc-C-3 (δ_C 88.3). Thus, compound **2** was established as 3*β*,20*α*,24-trihydroxy-29-norolean-12-en-28-oic acid 24-*O*-*β*-D-glucopyranosyl-(1→2)-[*α*-L-arabinopyranosyl-(1→3)]-*β*-D-glucopyranoside.

Compounds **1** and **2** were evaluated for cytotoxic activities against five human cell lines (HCT-116, HepG2, BGC-823, NCI-H1650, and A2780) with paclitaxel as a positive control (see Table 1). As shown in Table 1, Compounds **1** and **2** showed moderate cytotoxic activities toward the tested cell lines with IC_{50} values ranging from 12.71 to 32.04 μM.

3. Experimental

3.1. General experimental procedures

Melting points were determined on an X-4 digital micro-melting point apparatus without correction (Beijing Taike Apparatus Co. Ltd., Beijing, China). Optical rotations were measured on an Autopol IV-T/V (Rudolph Research Analytical, New Jersey, USA). UV spectra were recorded in MeOH on a Jasco V650 spectrophotometer (JASCO, Inc., Easton, Maryland, USA). The ^1H (600 MHz), ^{13}C (150 MHz), and 2D NMR spectra were recorded on a Bruker AVANCE III 600 instrument using TMS (Tetramethylsilane) as an internal reference (Bruker Company, Massachusetts, USA). HR-ESI-Q-TOF-MS data were obtained on a Waters Xevo E2Q-Tof mass spectrometer (Waters, Massachusetts, USA). Medium pressure liquid chromatography (MPLC) purification was performed on a Büchi chromatography system (Büchi Corp., Flawil, Switzerland). Preparative HPLC (high performance liquid chromatography) was conducted with an Angilent Technologies 1200 series instrument with a MWD detector using a

Table 1. Cytotoxicity of compounds **1** and **2**.

	IC_{50} (μM)				
Compound	HCT-116	HepG2	BGC-823	NCI-H1650	A2780
1	23.46 ± 0.47	26.27 ± 0.46	25.45 ± 0.41*	21.67 ± 0.59	12.72 ± 0.36
2	24.64 ± 0.75	32.04 ± 0.53	28.94 ± 0.62	17.28 ± 0.67	31.76 ± 0.92
Paclitaxel [a]	$(5.24 \pm 0.22) \times 10^{-2}$	$(6.01 \pm 0.73) \times 10^{-3}$	$(3.33 \pm 0.25) \times 10^{-3}$	$(4.25 \pm 0.37) \times 10^{-3}$	$(8.44 \pm 0.95) \times 10^{-3}$

[a]Positive control. $n = 3$.
*$p < 0.001$ vs. Paclitaxel.

YMC-pack ODS (Octadecylsilyl)-A column (5 μm, 250 × 20 mm). Column chromatography was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, China) and Develosil ODS (50 μm, Nomura Chemical Co. Ltd., Osaka, Japan). TLC (thin layer chromatography) was carried out with glass precoated with silica gel GF_{254} plates (Qingdao Marine Chemical Ltd., Qingdao, China). Spots were visualized under UV light or by spraying with 10% sulfuric acid in EtOH followed by heating.

3.2. Plant material

The stems of *Stauntonia chinensis* DC. were collected from Ganzhou, Jiangxi province of China, in October 2013. The plant was identified by Prof.. Yong Liu at Jiangxi University of Traditional Chinese Medicine, China. A voucher specimen (No. 20131022) is deposited in the Herbarium of Jiangxi Provincial Institute for Drug Control.

3.3. Extraction and isolation

The stems of *Stauntonia chinensis* DC. (40 kg) were first cut into 2 cm segments and then extracted with H_2O (400 L × 2) at reflux for 2 × 3 h. The aqueous residue partitioned with $CHCl_3$ (5000 mL × 3), EtOAc (5000 mL × 3), and *n*-BuOH (8000 mL × 4), successively. After removing solvent, part of *n*-BuOH-soluble portion (120 g) was fractionated via silica gel column chromatography, eluting with $CHCl_3$–MeOH (2:1), to afford ten fractions A_1–A_{10} on the basis of TLC analysis. Fraction A_4 (12.2 g) was subjected to silica gel CC and eluted with $CHCl_3$–MeOH (2:1 → 1:1, v/v) to afford eight fractions (A_{4-1} – A_{4-8}). Fraction A_{4-2} (3.9 g) was chromatographed by MPLC over an ODS column, eluted with CH_3CN–H_2O gradient mixtures (25:75 → 35:65, v/v) at a flow rate of 20.0 mL/min to afford fifteen fractions (B_1–B_{15}) on the basis of HPLC analysis. Fraction B_2 (161 mg) was purified by preparative HPLC (YMC-ODS-A 5 *μ*m, 250 mm × 20 mm, detection at 210 nm) using 22% CH_3CN in H_2O (7 mL/min) containing 0.1% TFA (Trifluoroacetic acid) as mobile phase to yield compound **1** (3.4 mg, t_R 83 min), **2** (7.8 mg, t_R 135 min), and **4** (6.2 mg, t_R 115 min). Fraction B_3 (68 mg) was subjected to preparative HPLC (YMC-ODS-A 5 *μ*m, 250 mm × 20 mm, detection at 210 nm) using 22% CH_3CN–H_2O (7 mL/min) containing 0.1% TFA as mobile phase to yield compounds **3** (5.3 mg, t_R 156 min) and **5** (7.2 mg, t_R 175 min).

3.3.1. 3β,20α,24-trihydroxy-29-norolean-12-en-28-oic acid 24-O-β-L-fucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranoside (1)

$C_{46}H_{74}O_{18}$, white amorphous powder; mp 232–234 °C; $[\alpha]_D^{20}$ + 16.5 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 206 (2.27) nm; IR ν_{max} 3411, 2929, 2362, 1687, 1447, 1382, 1258, 1205, 1138, 1065, 1007, 941, 841, 802, 723, 632, 569 cm^{-1}; ^{1}H-NMR (C_5D_5 N, 600 MHz) Aglycone δ_H: 0.80 (3H, s, H-25), 0.88 (1H, brd, *J* = 12.0 Hz, H-5), 0.94 (3H, s, H-26), 1.21 (3H, s, H-27), 1.53 (3H, s, H-23), 1.59 (3H, s, H-29), 3.35 (1H, dd, *J* = 13.8, 3.0 Hz, H-18), 3.47 (1H, dd, *J* = 9.8, 6.2 Hz, H-3), 4.13 (1H, d, *J* = 10.2 Hz, H-24a), 4.21 (1H, d, *J* = 10.2 Hz, H-24b), 5.54 (1H, brs, H-12); Sugars: Glc δ_H: 3.78 (1H, m, H-5), 4.07 (1H, m, H-4), 4.19 (1H, m, H-3), 4.24 (1H, m, H-2), 4.27 (1H, dd, *J* = 12.0, 5.4 Hz, H-6b), 4.43 (1H, dd, *J* = 12.0, 1.8 Hz, H-6a), 4.85 (1H, d, *J* = 7.8 Hz, H-1); Fuc δ_H: 1.73 (1H, d, *J* = 6.0 Hz, H-6), 3.68 (1H, m, H-2), 3.83 (1H, m, H-3), 4.15 (1H, m, H-4), 4.23 (1H, m, H-5), 5.62 (1H, d, *J* = 7.8 Hz, H-1); Xyl δ_H: 3.76 (1H, m, H-5b), 4.11 (1H, m, H-2), 4.16 (1H, m, H-3), 4.23 (1H, m, H-5a), 4.47 (1H, m, H-4), 5.24 (1H, d, *J* = 7.8 Hz, H-1). ^{13}C-NMR (C_5D_5 N, 150 MHz) Aglycone

δ_C: 39.2 (C-1), 28.6 (C-2), 79.6 (C-3), 43.4 (C-4), 56.9 (C-5), 19.6 (C-6), 33.8 (C-7), 40.0 (C-8), 48.3 (C-9), 37.5 (C-10), 24.3 (C-11), 122.5 (C-12), 144.1 (C-13), 42.4 (C-14), 28.4 (C-15), 24.2 (C-16), 47.1 (C-17), 44.7 (C-18), 48.4 (C-19), 70.2 (C-20), 36.5 (C-21), 35.4 (C-22), 23.8 (C-23), 72.9 (C-24), 16.0 (C-25), 17.6 (C-26), 26.3 (C-27), 180.6 (C-28), 26.0 (C-29) ; Sugars: Glc δ: 103.9 (C-1), 79.1 (C-2), 87.8 (C-3), 69.8 (C-4), 78.5 (C-5), 62.5 (C-6) ; Fuc δ: 103.9 (C-1), 73.5 (C-2), 77.1 (C-3), 76.5 (C-4), 70.0 (C-5), 18.9 (C-6) ; Xyl δ: 105.7 (C-1), 75.2 (C-2), 78.9 (C-3), 73.2 (C-4), 68.2 (C-5); HR-ESI-Q-TOF-MS *m/z* 913.4851 $[M - H]^-$ (calcd for $C_{46}H_{73}O_{18}$, 913.4797), 713.4760 [M – H – Fuc – $3H_2O]^-$.

3.3.2. 3β,20α,24-trihydroxy-29-norolean-12-en-28-oic acid 24-O-β-D-glucopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→3)]-β-D-glucopyranoside (2)

$C_{46}H_{74}O_{19}$, white amorphous powder; mp 226–228 °C; $[\alpha]_D^{20}$ + 20.1 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 206 (2.20) nm; IR ν_{max} 3414, 2931, 1690, 1456, 1380, 1262, 1204, 1078, 937, 909, 839, 800, 722, 631, 561 cm^{-1}; ^{1}H-NMR (C_5D_5 N, 600 MHz) Aglycone δ_H: 0.69 (3H, s, H-25), 0.81 (1H, brd, *J* = 12.0 Hz, H-5), 0.88 (3H, s, H-26), 1.18 (3H, s, H-27), 1.48 (3H, s, H-23), 1.59 (3H, s, H-29), 3.34 (1H, dd, *J* = 11.2, 4.6 Hz, H-18), 3.44 (1H, dd, *J* = 9.8, 6.0 Hz, H-3), 4.15 (1H, d, *J* = 10.2 Hz, H-24a), 4.20 (1H, d, *J* = 10.2 Hz, H-24b), 5.52 (1H, brs, H-12); Sugars: Glc δ_H: 3.88 (1H, m, H-5), 4.12 (1H, m, H-4), 4.19 (1H, m, H-2), 4.28 (1H, m, H-3), 4.31 (1H, dd, *J* = 12.0, 5.4 Hz, H-6b), 4.49 (1H, dd, *J* = 12.0, 3.0 Hz, H-6a), 4.87 (1H, d, *J* = 7.8 Hz, H-1); Glc′ δ_H: 3.55 (1H, m, H-5), 4.01 (1H, m, H-2), 4.26 (1H, m, H-3), 4.28 (1H, m, H-6b), 4.41 (1H, dd, *J* = 12.0, 1.8 Hz, H-6a), 4.58 (1H, m, H-4), 5.67 (1H, d, *J* = 8.4 Hz, H-1); Ara δ_H: 3.77 (1H, brd, *J* = 12.0 Hz, H-5b), 4.11 (1H, m, H-3), 4.25 (1H, m, H-4), 4.26 (1H, m, H-5a), 4.50 (1H, m, H-2), 5.27 (1H, d, *J* = 7.8 Hz, H-1). ^{13}C-NMR (C_5D_5 N, 150 MHz) Aglycone δ_C: 39.1 (C-1), 28.5 (C-2), 80.5 (C-3), 42.9 (C-4), 56.9 (C-5), 19.1 (C-6), 33.7 (C-7), 40.0 (C-8), 48.4 (C-9), 37.3 (C-10), 24.2 (C-11), 122.8 (C-12), 144.6 (C-13), 42.3 (C-14), 28.5 (C-15), 24.1 (C-16), 47.1 (C-17), 44.7 (C-18), 48.4 (C-19), 70.0 (C-20), 36.5 (C-21), 35.4 (C-22), 23.7 (C-23), 72.8 (C-24), 16.4 (C-25), 17.5 (C-26), 26.2 (C-27), 180.3 (C-28), 26.0 (C-29); Sugars: Glc δ: 102.6 (C-1), 79.4 (C-2), 88.3 (C-3), 69.8 (C-4), 78.6 (C-5), 62.4 (C-6) ; Glc′ δ: 104.2 (C-1), 76.1 (C-2), 79.2 (C-3), 70.2 (C-4), 78.5 (C-5), 61.8 (C-6) ; Ara δ: 105.6 (C-1), 73.2 (C-2), 75.2 (C-3), 70.0 (C-4), 68.3 (C-5); HR-ESI-Q-TOF-MS *m/z* 929.4777 $[M - H]^-$ (calcd for $C_{46}H_{73}O_{19}$, 929.4746), 713.4760 [M – H – Glc – $3H_2O]^-$.

3.4. Determination of absolute configurations of the sugar moieties in 1 and 2

Based on the reported procedure (Zhong et al. 2013), each 2 mg of compounds **1** and **2** was dissolved in 2 M HCl (dioxane:H_2O, 1:1 v/v) and refluxed for 10 h. After removal of the HCl by evaporation and extraction with EtOAc, the H_2O extract was again evaporated and dried *in vacuo* to furnish a monosaccharide residue. The residue was dissolved in pyridine (1 mL) to which 2 mg L-cysteine methyl ester hydrochloride was added. The mixture was kept at 60 °C for 2 h, evaporated under an N_2 stream, and dried *in vacuo*, then trimethylsilylated with N-trimethylsilylimidazole (0.2 mL) for 2 h. The mixture was partitioned between *n*-hexane and H_2O (2 mL each), and the *n*-hexane extract was analyzed by gas chromatography (GC) under the following conditions: capillary column, HP-5 (30 m × 0.25 mm × 0.25 μm; Dikma); FID detector with a temperature of 280 °C; injection temperature 250 °C; initial temperature 160 °C, then raised to 280 °C at 5 °C/min, final temperature maintained for 10 min; carrier gas, N_2; carrier gas flow rate 3 mL/min; split ratio 10:1. The standard sugars underwent the same reaction and GC conditions. The retention times of persilylated D-xylose, L-arabinose,

D-glucose, and L-fucose were found to be 15.705 min, 16.232 min, 19.035 min, and 19.418 min, respectively.

3.5. Cytotoxicity assay

The cytotoxic activity of compounds **1** and **2** were evaluated using human lung cancer (NCI-H1650), human gastric cancer (BGC-823), human hepatoma cancer (HepG2), human colon cancer (HCT-116), and human ovarian cancer (A2780) cell lines. Paclitaxel was used as a positive control. Following 72 h of continuous treatment of the cells with the samples, the supernatant was doffed off and 0.1 mL of MTT (0.5 mg/mL in RPM1640) was added after each well had been carefully washed with RPM1640. The cell growth was measured with an MTT assay procedure (Carmichael et al. 1987) and the IC_{50} values were calculated from a dose-dependent curve from NCI-H1650, BGC-823, HepG2, HCT-116, and A2780 cell lines.

3.6. Statistical analysis

The results of the cytotoxicity were expressed as means ± SD. Student's *t* test was used to determine statistical comparisons between the data-sets. $p < 0.05$ was considered to be significant.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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