INTRODUCTION

Aesculus chinensis Bge. var. chekiangensis (Hu et Fang) Fang is a shrubby or small tree belonging to the Hippocastanaceae family which is widely distributed in China. The dry seeds of this plant, together with Aesculus chinensis Bge and Aesculus wilsonii Rehd, are the major sources of the traditional Chinese medicine “Suo Luo Zi.” Traditionally, it has been exploited to treat chest and abdominal pain, dysentery and ague (Yang et al., 1999a; Zhang et al., 2006). Earlier phytochemical study of Aesculus chinensis Bge. var. chekiangensis (Hu et Fang) Fang obtained various types of isolates, for example, triterpenoids (Yuan et al., 2013), flavonoids (Patlolla et al., 2006), coumarins (Niu et al., 2015) together with steroids (Zhang et al., 2009). Polyhydroxylated triterpenoid saponins, isolated from Aesculus genus (Wei et al., 2004; Kim et al., 2017) with great structural diversity, have been proved to be the major bioactive principles including anticancer (Patlolla et al., 2006), neuroprotective (Cheng et al., 2016), anti-inflammatory (Matsuda et al., 1997), antioxidative (Küçükkırk et al., 2010), and antiedematous activities (Piller, 1976). As part of our continuous research to screen cytotoxic and neuroprotective compounds of this type, a series of new triterpenoids (1–14) along with 19 reported analogs (15–33) from the seeds of Aesculus chinensis Bge. var. chekiangensis (Hu et Fang) Fang were obtained. Their cytotoxic activity and neuroprotective activity were also examined. Herein, the isolation, structural elucidation, cytotoxic activity, and neuroprotective activities of these isolates are described.
MATERIALS AND METHODS

General Experimental Procedures

Optical rotations were recorded on a Rudolph (Hackettstown, NJ) Autopol V automatic polarimeter. The UV spectra were acquired on a UNICO 2102PCS spectrophotometer. The IR spectra were obtained in a KBr-disc (cm⁻¹) on a Brucker Tensor II spectrometer. NMR spectra were carried out on a Waters 2695 system equipped with a 2998 PDA detector using an ACQUITY UPLC BEH C18 (2.1 × 50 mm, Waters 1.7 µm, USA). Analytical HPLC was performed on a Waters e2695 system equipped with a 2998 PDA detector using a YMC-Pack-ODS-A column (250 × 4.6 mm, 5 µm). Semi-preparative HPLC was performed using a Shimadzu LC-6AD Series instrument equipped with a YMC Packed C18 column (5 µm, 250 × 10.0 mm, YMC Co., Ltd., Kyoto, Japan) and detected with a DAD detector set at 205 and 230 nm. Column chromatography (CC) was done on a Waters (Milford, MA) Xevo G2-S UPLC-Q/TOF equipped with an ACQUITY UPLC BEH C18 (8.8 kg) were extracted with 70% ethanol (10 L) under reflux for three times (3 h) at 75 °C after the solvent was removed under reduced pressure at 45 °C.

Plant Material

Seeds of Aesculus chinensis Bge. var. chekiangensis (Hu et Fang) Fang were purchased from the Anguo Chinese medicine market (Hebei Province, P.R. China) in August 2015 and identified by professor Lijuan Zhang (Tianjin University of Traditional Chinese Medicine). The specimen was kept at the School of Chinese Materia Medica, Tianjin University of Traditional Chinese Medicine.

Extraction and Isolation

The dried seeds of A. chinensis Bge. (8.8 kg) were extracted with 70% ethanol (10 L) under reflux for three times (3 h) at 70 °C. After the solvent was removed under reduced pressure at <45 °C, a dark residue (2,100 g) was obtained. The residue was adsorbed onto D101 resin and then sequentially eluted with H₂O, a gradient of EtOH in water to give the corresponding fractions. The 60% EtOH–H₂O part was chromatographed on silica gel, eluting with a gradient of 0–100% CH₂Cl₂/CH₃OH to yield four fractions (A–D).

Fraction B (27.0 g) was separated by an RP C18 column (MeOH–H₂O, from 20:80 to 100:0) to give 10 subfractions B1–B10. Subfraction B4 was further purified by an RP-HPLC (MeCN–H₂O, 40:60, 3.0 ml/min) to obtain compounds 4 (13.2 mg, tR 21.2 min), 10 (15.6 mg, tR 23.2 min), and 24 (20.6 mg, tR 30.4 min). Further purification of Fr. B6 using preparative RP-HPLC (MeCN–H₂O, 43:57, 3.0 ml/min) yielded compounds 1 (48.5 mg, tR 16.5 min) and 3 (10.0 mg, tR 19.2 min). Compounds 22 (11.1 mg, tR 8.9 min), 26 (13.5 mg, tR 14.7 min), 29 (32.0 mg, tR 23.4 min), and 33 (17.6 mg, tR 26.5 min) were obtained from Fr. B7 using a Sephadex LH-20 column and further purified by RP-HPLC (MeCN–H₂O 45:55, v/v, 3.0 ml/min). Subfraction B10 was purified by preparative HPLC to afford compounds 2 (12.0 mg, tR 26.5 min), 6–9 (9.7 mg, 21.3 mg, 15.5 mg, 22.7 mg; tR 14.8 min, 16.6 min, 19.4 min, 21.2 min, respectively), and 14–16 (31.1 mg, 12.2 mg, 11.0 mg; tR 31.2 min, 32.3 min, 33.1 min, respectively) using 50% MeCN/H₂O.

Fraction C (12.0 g) was subjected to an ODS RP-C18 column (MeOH/H₂O, 10:90 to 100:0, v/v) to give six subfractions (C1–C6). Compound 18 (25.3 mg, tR 31.5 min) was purified by preparative HPLC using 30% MeCN/H₂O from subfraction C1. Compounds 31 (11.9 mg, tR 26.5 min) and 32 (39.6 mg, tR 28.3 min) were gotten from C2 using the same preparative HPLC procedure with 32% MeCN/H₂O. Fraction C4 was subjected to a Sephadex LH-20 column (MeOH) and then purified by recycling preparative HPLC with 33% MeCN/H₂O to yield compounds 17 (9.8 mg, tR 22.3 min), 19 (14.5 mg, tR 24.1 min), and 28 (21.4 mg, tR 25.9 min). Fraction C6 was separated using Sephadex LH-20 (MeOH) to obtain four subfractions (C6A–C6D). C6B and C6D were purified using preparative HPLC (35% MeCN/H₂O) to yield compounds 20 (59.8 mg, tR 25.4 min), 21 (40.7 mg, tR 27.2 min), and 30 (21.4 mg, tR 16.7 min).

Fraction D (9.0 g) was applied to an RP C18 column eluting with gradient MeOH–H₂O from 10:90 to 100:0 followed by a Sephadex LH-20 column (MeOH) to afford four major subfractions (D1–D4). Subfraction D1 was purified on an RP HPLC (MeCN–H₂O 20:80, v/v, 3.0 ml/min) to afford compounds 2 (28.1 mg, tR 19.8 min) and 5 (30.5 mg, tR 22.4 min). Subfraction D2 was purified on an RP HPLC (MeCN–H₂O 23: 77, v/v, 3.0 ml/min) to yield compounds 11 (46.0 mg, tR 16.6 min) and 27 (41.8 mg, tR 18.9 min). Compounds 12 (22.6 mg, tR 14.7 min), 13 (16.0 mg, tR 16.6 min), 23 (22.3 mg, tR 17.7 min), and 25 (11.2 mg, tR 18.3 min) were obtained by an RP HPLC (MeCN–H₂O 25:75, v/v, 3.0 ml/min) from subfraction D4.

Aesculidine C (1), white amorphous powder; [α]D25 +6.0 (c 0.10, MeOH); 1H NMR and 13C NMR data (see Tables 1, 3); HR-ESI-MS: m/z 1073.5149 [M–H]− (calcd. for C52H81O23, 1073.5169).

Aesculidine D (2), white amorphous powder; [α]D25 +6.0 (c 0.10, MeOH); 1H NMR and 13C NMR data (see Tables 1, 3); HR-ESI-MS: m/z 1031.5067 [M−H]− (calcd. for C50H79O22, 1031.5063).

Aesculidine E (3), white amorphous powder; [α]D25 +4.0 (c 0.09, MeOH); 1H NMR and 13C NMR data (see Tables 1, 3); HR-ESI-MS: m/z 1071.5376 [M−H]− (calcd. for C53H83O22, 1071.5376).

Aesculidine F (4), white amorphous powder; [α]D25 +10.0 (c 0.11, MeOH); 1H NMR and 13C NMR data (see Tables 1, 3); HR-ESI-MS: m/z 1031.5153 [M–H]− (calcd. for C52H81O23, 1031.5169).

Aesculidine G (5), white amorphous powder; [α]D25 +2.0 (c 0.11, MeOH); 1H NMR and 13C NMR data (see Tables 1, 3); HR-ESI-MS: m/z 1031.5051 [M−H]− (calcd. for C50H79O22, 1031.5063).

Aesculidine H (6), white amorphous powder; [α]D25 +14.0 (c 0.11, MeOH); 1H NMR and 13C NMR data (see Tables 1, 3); HR-ESI-MS: m/z 1131.5586 [M + H]⁺ (calcd. for C55H87O24, 1131.5587).
## TABLE 1 \[^1H\] NMR spectroscopic data (δ) for compounds 1–7  (δ in ppm,  Ι in Hz).

| Proton | 1     | 2     | 3     | 4     | 5     | 6     | 7     |
|--------|-------|-------|-------|-------|-------|-------|-------|
| 1°     | 1.62  | 1.44  | 1.78  | 1.49  | 1.61  | 1.48  | 1.49  |
| 1"     | 1.58  | 1.46  | 1.55  | 1.37  | 1.49  | 1.32  | 1.32  |
| 2°     | 1.86  | 1.91  | 1.81  | 1.45  | 1.36  | 1.32  | 1.32  |
| 2"     | 1.64  | 1.42  | 1.57  | 1.34  | 1.49  | 1.32  | 1.32  |
| 3°     | 3.07  | 2.98  | 2.99  | 2.59  | 2.34  | 2.32  | 2.32  |
| 3"     | 2.64  | 2.71  | 2.83  | 2.39  | 2.32  | 2.32  | 2.32  |
| 4°     | 2.86  | 2.95  | 3.08  | 2.49  | 2.49  | 2.49  | 2.49  |
| 4"     | 3.07  | 3.10  | 3.10  | 2.49  | 2.49  | 2.49  | 2.49  |
| 5°     | 3.45  | 3.46  | 3.50  | 3.00  | 3.00  | 3.00  | 3.00  |
| 5"     | 3.55  | 3.55  | 3.55  | 3.00  | 3.00  | 3.00  | 3.00  |
| 6°     | 4.12  | 4.16  | 4.16  | 3.61  | 3.61  | 3.61  | 3.61  |
| 6"     | 4.24  | 4.24  | 4.24  | 3.61  | 3.61  | 3.61  | 3.61  |
| 7°     | 4.44  | 4.44  | 4.44  | 3.61  | 3.61  | 3.61  | 3.61  |
| 7"     | 4.44  | 4.44  | 4.44  | 3.61  | 3.61  | 3.61  | 3.61  |

(Continued)
Aesculiside I (7), white amorphous powder; $[\alpha]_D^{25} = -12.0$ (c 0.10, MeOH); $^1$H NMR and $^{13}$C NMR data (see Tables 1, 3); HR-ESI-MS: m/z 1129.5432 [M–H]$^-$ (calcd. for C$_{55}$H$_{85}$O$_{24}$, 1129.5431).

Aesculiside J (8), white amorphous powder; $[\alpha]_D^{25} = -7.0$ (c 0.10, MeOH); $^1$H NMR and $^{13}$C NMR data (see Tables 2, 3); HR-ESI-MS: m/z 1171.5908 [M–H]$^-$ (calcd. for C$_{58}$H$_{91}$O$_{24}$, 1171.5900).

Aesculiside K (9), white amorphous powder; $[\alpha]_D^{25} = -9.0$ (c 0.11, MeOH); $^1$H NMR and $^{13}$C NMR data (see Tables 2, 4); HR-ESI-MS: m/z 1159.5879 [M + H]$^+$ (calcd. for C$_{57}$H$_{91}$O$_{24}$, 1159.5900).

Aesculiside L (10), white amorphous powder; $[\alpha]_D^{25} = -16.0$ (c 0.12, MeOH); $^1$H NMR and $^{13}$C NMR data (see Tables 2, 4); HR-ESI-MS: m/z 1077.5471 [M + H]$^+$ (calcd. for C$_{52}$H$_{85}$O$_{23}$, 1077.5482).

Aesculiside M (11), white amorphous powder; $[\alpha]_D^{25} = +12.0$ (c 0.10, MeOH); $^1$H NMR and $^{13}$C NMR data (see Tables 2, 4); HR-ESI-MS: m/z 1017.4881 [M–H]$^-$ (calcd. for C$_{49}$H$_{77}$O$_{22}$, 1017.4906).

Aesculiside N (12), white amorphous powder; $[\alpha]_D^{25} = +4.0$ (c 0.10, MeOH); $^1$H NMR and $^{13}$C NMR data (see Tables 2, 4); HR-ESI-MS: m/z 1017.4890 [M–H]$^-$ (calcd. for C$_{49}$H$_{77}$O$_{22}$, 1017.4906).

Aesculiside O (13), white amorphous powder; $[\alpha]_D^{25} = +8.0$ (c 0.10, MeOH); $^1$H NMR and $^{13}$C NMR data (see Tables 2, 4); HR-ESI-MS: m/z 1059.4995 [M–H]$^-$ (calcd. for C$_{51}$H$_{79}$O$_{23}$, 1059.5012).

Aesculiside P (14), white amorphous powder; $[\alpha]_D^{25} = -4.2$ (c 0.10, MeOH); $^1$H NMR and $^{13}$C NMR data (see Tables 2, 4); HR-ESI-MS: m/z 1141.5785 [M + H]$^+$ (calcd. for C$_{57}$H$_{89}$O$_{23}$, 1141.5795).

Hydrolysis and Determination of Absolute Configuration of Sugars
A solution of 1–14 (1.0 mg, respectively) in 2 M HCl (4.0 ml) was heated at 90°C for 2 h. The reaction mixture was extracted with EtOAc (2 × 4 ml), and the aqueous phase was evaporated to dryness using a stream of N$_2$. The residues and authentic sugar samples (D/L-galactose, D/L-glucose, D/L-xylose, and D-glucuronic acid) were, respectively, dissolved in pyridine (1.0 ml) containing L-cysteine methyl ester (1.0 mg) and heated at 60°C for 1 h, and then o-tolylisothiocyanate (1.0 ml) was added to the mixture and heated further for 1 h. Then each reaction mixture was analyzed by the Waters e2695 HPLC system using a 2998 PDA detector (250 nm). Analytical HPLC was performed on the YMC- Pack-ODS-A column (250 × 4.6 mm, 5 μm) eluting with A (0.1% formic acid); B (acetonitrile) = 80:20 (v/v) at 1.0 ml/min. The absolute configuration of sugars in each compound was established by a comparison of the retention times with the standards where the time differences ($\Delta$ $t$ D-L) of one kind of sugar were sufficient to distinguish between D- and L-enantiomers (Tanaka et al., 2007; Zhang N. et al., 2018).

Preparation of the Aglycone of Compound 14
Compound 14 (15.0 mg) in 2 M HCl (10.0 ml) was heated at 50°C for 4 h. The reaction mixture was extracted with EtOAc (2 × 10 ml), and the EtOAc phase was evaporated to dryness using a stream of N$_2$. The residue was dissolved in THF (2.0 ml) and MeOH (1.0 ml), then NaOMe (2.0 mg, 2.2 eq) was added to the solution at 0°C. The mixture was stirred at 25°C for 4 h. The mixture was diluted with H$_2$O (10.0 ml) and the mixture was extracted with ethyl acetate (3 × 3.00 ml). The ethyl acetate fraction was purified by a semipreparative RP HPLC (CH$_3$CN–H$_2$O, 45:65) to gain compound 14a.

Determination of the Absolute Configuration of the 21, 22-Diol Moieties in Compound 14a
First, Mo$_2$(AcO)$_4$ (1.0 mg) dissolved in DMSO (1.0 ml) was subjected to ECD measurement as blank control. Then compound 14a (0.5 mg) and Mo$_2$(AcO)$_4$ (1.0 mg) were added to DMSO (1.0 ml) and scanned directly. The CD spectrum was

### TABLE 1 | Continued

| Proton | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|-------|---|---|---|---|---|---|---|
| $^6m$ | 4.23 (t, 9.8) | 4.27 (overlapped) | 4.27 m | 4.45 (d, 10.8) | 4.50 (d, 10.2) | 4.47 (d, 10.2) | 4.47 (overlapped) |
| C$_{21}$ | Ac | Ac | Tig | Ac | Ac | Ac | Ac |
| $^2m$ | 2.11 s | 2.09 s | 2.07 s | 2.09 s | 2.06 s | 1.92 s | 7.00 (q, 7.1) |
| $^3m$ | 1.61 (d, 7.0) | 1.87 s | 1.96 s | 6.99 (q, 6.9) | 5.93 (q, 7.2) |
| $^4m$ | 1.47 (d, 6.9) | 2.07 (d, 7.2) | 1.87 s | 2.09 s |

$^a$NMR data (δ) were measured at 600 MHz in pyridine-$d_5$ for 1–7.
TABLE 2 | $^1$H NMR spectroscopic data (δ) for compounds 8-14 (δ in ppm, $^J$ in Hz).

| Proton | 8   | 9   | 10  | 11  | 12  | 13  | 14  |
|--------|-----|-----|-----|-----|-----|-----|-----|
| 1      | 0.73 m | 0.72 m | 0.72 m | 0.85 m | 0.82 m | 0.78 m | 0.77 m |
| 2      | 1.29 m | 1.29 m | 1.29 m | 1.39 m | 1.37 m | 1.33 m | 1.34 m |
| 3      | 1.86 m | 1.84 m | 1.85 m | 1.96 m | 1.95 m | 1.92 m | 1.94 m |
| 4      | 2.24 m | 2.23 m | 2.24 m | 2.27 m | 2.25 m | 2.23 m | 2.25 m |
| 5      | 3.39 m | 3.36 m | 3.37 m | 3.42 m | 3.40 m | 3.38 m | 3.38 m |
| 6      | 0.80 m | 0.78 m | 0.80 m | 0.86 m | 0.86 m | 0.83 m | 0.84 m |
| 7      | 1.17 m | 1.16 m | 1.15 m | 1.35 m | 1.43 m | 1.34 m | 1.34 m |
| 8      | 1.47 m | 1.46 m | 1.48 m | 1.62 m | 1.61 m | 1.59 m | 1.59 m |
| 9      | 1.23 m | 1.19 m | 1.20 m | 1.26 m | 1.27 m | 1.23 m | 1.24 m |
| 10     | 1.48 m | 1.44 m | 1.48 m | 1.54 m | 1.55 m | 1.51 m | 1.53 m |
| 11     | 1.61 m | 1.61 m | 1.61 m | 1.67 m | 1.68 m | 1.63 m | 1.65 m |
| 12     | 1.70 m | 1.67 m | 1.68 m | 1.73 m | 1.81 m | 1.76 m | 1.72 m |
| 13     | 1.81 m | 1.80 m | 1.81 m | 1.85 m | 1.86 m | 1.81 m | 1.84 m |
| 14     | 5.42 br s | 5.40 br s | 5.34 br s | 5.36 br s | 5.46 br s | 5.40 br s | 5.39 br s |
| 15     | 1.60 m | 1.57 m | 1.60 m | 1.65 m | 1.65 m | 1.61 m | 1.59 m |
| 16     | 1.83 m | 1.79 m | 1.91 m | 1.96 m | 1.94 m | 1.86 m | 1.84 m |
| 17     | 4.47 m | 4.43 m | 4.82 m | 4.87 m | 4.81 m | 4.70 m | 4.48 m |
| 18     | 3.08 m | 3.09 m | 2.89 m | 2.93 m | 2.87 m | 2.88 m | 3.10 m |
| 19     | 1.37 m | 1.35 m | 1.37 m | 1.41 m | 1.42 m | 1.35 m | 1.41 m |
| 20     | 3.08 m | 3.06 m | 3.04 m | 3.04 m | 3.06 m | 3.10 m |
| 21     | 5.69 (d, 10.2) | 6.61 (d, 10.1) | 6.33 (d, 9.9) | 6.39 (d, 9.8) | 4.80 (d, 9.0) | 6.37 (d, 9.8) | 6.69 (d, 10.2) |
| 22     | 6.28 (d, 10.2) | 6.21 (d, 10.1) | 4.76 (d, 9.9) | 4.80 (d, 9.8) | 4.40 (d, 9.0) | 4.46 (d, 9.8) | 6.32 (d, 10.2) |
| 23     | 1.08 s | 1.05 s | 1.07 s | 1.11 s | 1.35 s | 1.08 s |
| 24     | 3.31 (d, 12.0) | 3.32 (d, 12.0) | 3.30 (d, 11.5) | 3.51 (d, 11.6) | 3.50 (d, 11.6) | 3.47 (d, 11.6) | 3.49 (d, 11.5) |
| 25     | 4.27 (d, 12.0) | 4.23 (d, 12.0) | 4.24 (d, 11.5) | 4.34 (d, 11.6) | 4.33 (d, 11.6) | 4.29 (d, 11.6) | 4.32 (d, 11.5) |
| 26     | 0.77 s | 0.78 s | 0.76 s | 0.82 s | 0.96 s | 0.91 s | 0.80 s |
| 27     | 1.81 s | 1.81 s | 1.80 s | 1.85 s | 1.86 s | 1.81 s | 1.84 s |
| 28     | 3.93 m | 3.93 m | 3.63 m | 3.67 m | 4.23 m | 4.18 m | 3.93 m |
| 29     | 3.63 m | 3.63 m | 3.91 m | 3.95 m | 4.42 m | 4.28 m | 3.63 m |
| 30     | 1.08 s | 1.05 s | 1.07 s | 1.11 s | 1.35 s | 1.08 s |
| 31     | 1.31 s | 1.31 s | 1.28 s | 1.30 s | 1.39 s | 1.24 s | 1.32 s |
| 32     | GlcA-p | GlcA-p | GlcA-p | GlcA-p | GlcA-p | GlcA-p |
| 33     | 5.48 m | 5.48 m | 4.55 m | 4.64 (d, 9.7) | 4.62 (d, 9.7) | 4.58 (d, 9.7) | 5.55 m |
| 34     | Glc-p | Glc-p | Glc-p | Xyl-p | Xyl-p | Xyl-p |
| 35     | 5.61 (d, 7.2) | 5.59 (d, 7.4) | 5.60 (d, 7.8) | 5.53 (d, 7.5) | 5.51 (d, 6.8) | 5.47 (d, 7.6) | 5.49 (d, 6.1) |
| 36     | 4.37 m | 4.35 m | 4.34 m | 4.19 (d, 7.8) | 4.19 m | 4.14 m | 4.16 m |
| 37     | 4.20 m | 4.18 m | 4.19 m | 4.01 m | 4.00 m | 4.09 m | 4.11 m |
| 38     | 4.19 m | 4.17 m | 4.19 m | 4.42 m | 4.41 m | 4.37 m | 4.38 m |
| 39     | 3.86 m | 3.65 m | 3.67 (d, 9.7) | 3.62 (d, 10.9) | 3.61 (d, 9.5) | 3.62 (l, 9.5) | 3.60 (d, 10.7) |
| 40     | 4.33 (d, 12.0) | 4.29 (d, 12.0) | 4.32 (d, 12.0) | 4.32 (d, 10.2, 4.8) | 4.32 (d, 12.0) | 4.32 (d, 10.2) |
| 41     | Glc-p | Glc-p | Glc-p | Glc-p | Glc-p | Glc-p |
| 42     | 5.21 (d, 6.7) | 5.17 (d, 6.7) | 5.20 (d, 6.7) | 5.22 (d, 7.8) | 5.22 (d, 7.8) | 5.18 (d, 7.8) | 5.19 (d, 7.8) |
| 43     | 4.03 m | 4.02 m | 4.02 m | 4.06 (d, 7.9) | 4.06 m | 4.08 (d, 8.5) | 4.03 m |
| 44     | 4.23 m | 4.20 m | 4.24 m | 4.21 (d, 7.8) | 4.21 m | 4.17 m | 4.22 m |

(C)Continued)
The calculated structural parameters of 14a are as follows: $\alpha = 11.7057(7)$ Å, $b = 34.214(3)$ Å, $c = 14.7361(9)$ Å, $V = 5901.79(6)$ Å$^3$, $Z = 8$, $T = 100.00(11)$ K, $\mu$(CuKα) = 0.617 mm$^{-1}$, $D_{calc} = 1.141$ g/cm$^3$, 20,468 reflections measured (5.166° ≤ 2θ ≤ 149.994°), 10,735 unique ($R_{int} = 0.0506$, $R_{sigma} = 0.0834$) which were used in all calculations. The final $R_1$ was 0.0837 (1 > 2σ(I)) and $wR_2$ was 0.2452 (all data). The crystallographic data of 14a have been deposited at the Cambridge Crystallographic Data Centre (CCDC 1957449) and the data can be obtained from supporting information (Data Sheet 1_v1).

**Cytotoxicity Assay**

The in vitro cytotoxicity of compounds 1–33 was measured by MTT assay (El-Readi et al., 2013; Xia et al., 2015) with 5-fluorouracil as the positive control. The human cancer cell lines, HepG2, HCT-116, and MGC-803 were purchased from ATCC. The tested cell lines were seeded in 96-well plates, and the plates were then incubated in a 37°C incubator containing 5% CO$_2$ for 24 h. Subsequently, the tested compounds in DMSO were added to designated wells at a dosage of 3.125–50 μM. After 24 h, MTT was added to the culture medium and the absorbance at 490 nm was measured using a microplate reader.

**Neuroprotective Effect Assay**

The neuroprotective effects of compounds 1–33 were tested against Co$_3$Cl$_2$-induced PC12 cell injury (Zou et al., 2002) with MTT method. Rat pheochromocytoma cell line (PC12) was cultured in 96-well plates with RPMI-1640 supplemented with 10% (v/v) inactivated fetal bovine serum and 100 U/ml penicillin/streptomycin. The cells were maintained at 37°C in 5% CO$_2$ and 95% humidified air incubator. Cells were pre-treated for 2 h with or without compounds before incubation in a medium containing 1 mM CoCl$_2$. After 24 h, MTT was added to the culture medium, and the absorbance at 490 nm was measured using a microplate reader.

**RESULTS**

For the target of isolation of triterpene saponins, the 70% ethanol extracts of air-dried seeds of Aesculus chinensis Bge. var. chekiangensis (Hu et Fang) Fang were chromatographed through a D101 column and eluted with a gradient of EtOH in water. The 60% EtOH part was separated consequently as it contains abundant triterpene saponins under the guidance of UPLC-Q/TOF-MS. Thereafter, 14 undescribed triterpenoid saponins (1–14, aesculuside C–P) (Figure 1) and 19 known analogs (15–33) were afforded and identified (Figures S1–S157). The full assignments of the NMR data of compounds 1–14 are recorded in Tables 1–4.

Aesculuside C (1) was isolated as a white amorphous powder which exhibited an ion peak at $m/z$ 1073.5149 [M–H]$^-$ (calcd. 1073.5169). Its molecular formula was confirmed as C$_{52}$H$_{52}$O$_{23}$ based on HR-ESI-MS as well as $^{13}$C NMR spectroscopic data. The IR absorptions at 3,414 and 1,732 cm$^{-1}$ implied the existence of the hydroxyl and carboxyl groups, respectively. The NMR data of 1 exhibited characteristic signals of a triterpenoid saponin. $^{1}$H NMR of the aglycone portion indicated the presence of seven methyl protons at $\delta$ 0.83, 0.98, 1.07, 1.09, 1.26, 1.84 (each

**TABLE 2 | Continued**

| Proton | 8   | 9   | 10  | 11  | 12  | 13  | 14  |
|--------|-----|-----|-----|-----|-----|-----|-----|
| 5’’’   | 3.97 m | 3.95 m | 3.96 m | 4.13 (d, 9.0, 3.5) | 4.13 (t, 9.0) | 3.96 m | 3.97 m |
| 6’’’   | 4.24 (dd, 11.6, 5.2) | 4.45 (t, 12.2) | 4.46 (dd, 11.8, 5.2) | 4.28 (dd, 12.0, 5.8) | 4.28 (d, 12.0) | 4.24 (dd, 11.6, 5.8) | 4.24 m |
| C$_2$  | MB  | IB  | IB  | Ac  | Ac  | Ang |
| 2’’’   | 2.47 m | 2.61 m | 2.65 m | 2.09 s | 2.06 s |
| 3’’’   | 1.77 m | 1.17 (d, 7.3) | 1.22 (d, 7.0) | 1.78 m |
| 4’’’   | 0.92 (t, 7.4) | 1.19 (d, 7.3) | 1.18 (d, 6.9) | 5.95 (q, 7.2) |
| 5’’’   | 1.19 (d, 7.0) | 2.07 (d, 7.2) | 2.00 s |
| C$_{22}$ | Ang | Tig | Ac | Ac | Ang |
| C$_{28}$ | 1.96 s | 1.96 s |
| 3’’’   | 6.04 (q, 7.2) | 6.97 (q, 6.8) | 5.89 (q, 7.2) |
| 4’’’   | 2.13 (d, 7.2) | 1.48 (d, 6.8) | 2.03 (d, 7.2) |
| 5’’’   | 1.94 s | 1.86 s |

$^a$NMR data were measured at 600 MHz in pyridine-d$_5$ for 8–14.
3H except 1.26 for 6H, s), one olefinic proton at δ 5.47 (1H, br s), and a pair of geminal protons at δ 4.21 and 4.31 (1H each, d, J = 10.3 Hz), indicative of an olean-12-ene skeleton (Zhang et al., 2018). Four oxygenmethylene proton signals assignable to H-3, H-16, H-21, and H-22 of the aglycone moiety were, respectively, observed at δ 3.26 (1H, dd, J = 11.6, 4.3 Hz), 4.76 (1H, m), 6.43 (1H, d, J = 9.8 Hz), and 4.45 (1H, d, J = 9.8 Hz), which further suggested the aglycon characteristic for 3, 16, 21, 22, 28-pentahydroxyolean-12-ene. As for the relative configurations of C-3, C-16, C-21, C-22, and C-28, the NOESY correlations between H-3/H-5/H-23, H-21/H-29 suggested the H-3 and H-21, while the correlations between H-16 and H-28, H-28 and H-22, H-22, and H-30 H-28 and H-22, H-22, and H-30 suggested β-orientations of H-16 and H-22 (Figure 2). On the basis of NOESY correlations and the vicinal coupling constants of the H-21 3β, 16α, 21β, 22α, 22α, 28-pentahydroxyolean-12-ene.

The 1D NMR spectra of I also showed two acetyl signals (δ H 2.01, 2.11 and δ C 170.6, 171.2, 20.6, 21.2). The cross peak between H-21 (δ 6.43) and C-17” (δ 171.2) in the HMBC spectrum established one of the acetyl groups was attached to C-21. The other acetyl group was assigned at C-28 according to the HMBC correlation of H-28 (δ 4.31)/C-28 (177.0), which was further confirmed by the downfield chemical shifts of H-28 and C-28 compared with typical oleanene-type triterpenoid (Aki et al., 2004; Zhang and Li, 2007; Yuan et al., 2012) (Figure 2).

The presence of three anomeric protons at δ 4.98 (1H, d, J = 7.1 Hz), 5.20 (1H, d, J = 7.9 Hz), 5.22 (1H, d, J = 7.7 Hz) was correlated with carbons at δ 105.0, 104.5, and 106.6 in HSQC spectrum, respectively, indicating trisaccharide residues. Acid hydrolysis of I yielded D-galactose, D-glucose, and D-glucuronic acid, which was established with HPLC analysis by comparing with authentic sugar samples after derivatization. Their relative configuration was determined to be β according to the large coupling constants. The 1H NMR and 13C NMR signals of the trisaccharide group were fully assigned by 2D-NMR spectra and compared with reference data (Yuan et al., 2013).

**TABLE 3 | Continued**

| NO. | 1      | 2      | 3      | 4      | 5      | 6      | 7      |
|-----|--------|--------|--------|--------|--------|--------|--------|
| C21 | Ac     | Ac     | Tig    | Ac     | Ac     | Ac     | Ac     |
| 1    | 171.2  | 171.4  | 168.5  | 171.2  | 171.3  | 171.2  | 171.1  |
| 2    | 21.2   | 21.4   | 129.8  | 21.2   | 21.3   | 21.3   | 21.2   |
| 3    |        |        |        |        |        | 136.0  |        |
| 4    |        |        |        |        |        | 14.0   |        |
| 5    |        |        |        |        |        | 12.3   |        |
| C22 | Ac     | Ac     | Tig    | Ang    |        |        |        |
| 1    | 170.6  |        | 168.7  | 168.5  |        |        |        |
| 2    | 20.6   |        | 129.5  | 129.3  |        |        |        |
| 3    |        |        | 137.5  | 137.5  |        |        |        |
| 4    |        |        | 14.4   | 16.1   |        |        |        |
| 5    |        |        | 12.6   | 21.4   |        |        |        |

aNMR data (δ) were measured at 150 MHz in pyridine-d5 for 1-7.
TABLE 4 | ¹³C NMR spectroscopic data (δ) for compounds 8–14* (δ in ppm).

| NO. | 8   | 9   | 10  | 11  | 12  | 13  | 14  |
|-----|-----|-----|-----|-----|-----|-----|-----|
| 1   | 38.7| 38.8| 38.7| 38.8| 38.7| 38.6| 38.7|
| 2   | 26.8| 26.9| 26.8| 26.6| 26.5| 26.4| 26.5|
| 3   | 91.4| 91.4| 91.3| 90.6| 90.5| 90.4| 90.5|
| 4   | 44.0| 44.0| 43.9| 44.3| 44.2| 44.1| 44.2|
| 5   | 56.4| 56.4| 56.3| 56.2| 56.1| 56.1| 56.2|
| 6   | 18.8| 18.8| 18.7| 18.6| 18.5| 18.6| 18.6|
| 7   | 33.5| 33.5| 33.5| 33.3| 33.3| 33.1| 33.2|
| 8   | 40.2| 40.3| 40.3| 39.9| 39.9| 39.7| 39.9|
| 9   | 47.0| 47.0| 47.0| 46.8| 46.7| 46.6| 46.6|
| 10  | 36.7| 36.7| 36.6| 36.5| 36.4| 36.3| 36.3|
| 11  | 24.3| 24.4| 24.3| 24.0| 23.9| 23.9| 23.9|
| 12  | 123.5| 123.5| 123.4| 123.4| 123.4| 123.6| 123.8|
| 13  | 143.1| 143.1| 143.4| 143.5| 143.2| 142.5| 142.6|
| 14  | 41.9| 42.0| 42.1| 41.9| 41.8| 41.6| 41.6|
| 15  | 35.1| 35.1| 34.7| 34.4| 34.5| 34.4| 34.7|
| 16  | 68.7| 68.8| 68.1| 67.9| 67.9| 67.4| 68.5|
| 17  | 48.3| 48.6| 48.4| 48.1| 46.5| 46.8| 47.9|
| 18  | 40.4| 40.3| 40.7| 40.4| 40.7| 40.4| 40.0|
| 19  | 47.5| 47.5| 48.1| 47.8| 47.7| 47.0| 47.1|
| 20  | 36.7| 36.7| 36.5| 36.1| 36.2| 35.8| 36.4|
| 21  | 79.1| 79.0| 81.7| 82.0| 81.4| 78.6|
| 22  | 73.7| 74.2| 73.1| 72.7| 73.6| 70.6| 73.4|
| 23  | 22.8| 22.8| 22.7| 22.6| 22.5| 22.4| 22.6|
| 24  | 83.6| 83.6| 83.6| 82.8| 82.7| 82.6| 82.7|
| 25  | 15.9| 15.9| 15.8| 15.5| 15.4| 15.3| 15.4|
| 26  | 17.0| 17.0| 17.0| 16.8| 16.8| 16.7| 16.6|
| 27  | 27.8| 27.8| 27.7| 27.4| 27.4| 27.2| 27.5|
| 28  | 63.8| 63.8| 66.2| 65.9| 66.9| 66.1| 63.4|
| 29  | 29.9| 29.8| 29.7| 29.8| 30.0| 29.6| 29.5|
| 30  | 20.5| 20.4| 20.5| 20.2| 19.8| 19.8| 20.2|

*NMR data (δ) were measured at 150 MHz in pyridine-d₅ for 8–14.

Meanwhile, the upfield shifts of C-3 (δ 89.1) as well as the HMBC correlation between H-1’ (δ 4.98) with C-3 demonstrated that the trisaccaride unit was attached to C-3. The sequence of the sugar chain was further confirmed by the long correlations of H-1’’ (δ 5.22) and C-2’ (δ 82.2), H-1’’’ (δ 5.50) and C-4’ (δ 81.9) (Figure 2). Based on these data, compound 1 was concluded to be 3-O-[[β-D-galactopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)]-β-D-glucuronopyranosyl-21β, 28-diacyt-3β, 16α, 21β, 22α, 28-pentahydroxyolean-12-ene, named aesculiside C.

Aesculiside D (2) with the molecular formula of C₅₀H₆₀O₂₂ (m/z 1031.5067 [M–H]−; calcd. for C₅₀H₆₁O₂₂, 1031.5063) was also obtained as a white, amorphous powder. Acid hydrolysis of 2 presented the same sugar moieties as compound 1. The NMR data of 2 are similar to those of 1 except for the absence of an acetyl unit in 2. The essential HMBC correlations of H-21 (δ 6.41)/C-1”” (δ C 171.4) indicated the acetyl unit was connected at C-21 (Figure 3). The remaining portion of 2 was superposable to 1 evidenced by careful analysis of their 2D NMR spectra. Thus, compound 2 was established as 3-O-[[β-D-galactopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)]-β-D-glucuronopyranosyl-21β-acetyl-3β, 16α, 21β, 22α, 28-pentahydroxyolean-12-ene, namely aesculiside D.

Aesculiside E (3) was acquired as a white amorphous powder and its molecular formula was determined as C₅₃H₆₄O₁₉ (m/z 1071.5376 [M–H]−; calcd. for C₅₃H₆₃O₁₉, 1071.5376). Acid hydrolysis of 3 yielded D-galactose, D-glucose, and D-glucuronic acid. The NMR data of 3 showed a lot of resemblance with those of 2 except for the presence of a tigloyl moiety instead of an acetyl unit in 3, which was supported by the characteristic olefinic quartet at δ 7.00 in its ¹H-NMR spectrum. Moreover, the HSQC correlation signals of δH 1.61 with δC 14.0 and δH 1.87 with δC 12.3 confirmed the existence of a tigloyl group. The aforementioned data, together with the HMBC correlation from H-21 (δ 6.48) to C-1”” (δ 168.5) confirmed the connection between the tigloyl group and C-21. Consequently, it was assigned as 3-O-[[β-D-galactopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)]-β-D-glucuronopyranosyl-21β-tigloyl-3β, 16α, 21β, 22α, 28-pentahydroxyolean-12-ene.

(Continued)
Aesculiside F (4) and 1 gave the same molecular formula, deduced as C_{52}H_{82}O_{23} from its HR-ESI-MS and \textsuperscript{13}C NMR spectroscopic data. Comparison of the NMR data of 4 with those of 1 indicated that both saponins are closely related, differing at trisaccharide moiety where the galactose in 1 was replaced by a glucose in 4, based on the distinction of their \textsuperscript{13}C NMR data (Table 3) (Yoshikawa et al., 1998), which was further verified by hydrolysis and derivatization as aforementioned. HMBC correlations revealed the position and sequences of the sugar moiety in 4 as described before. Hence, compound 4 was identified and named aesculiside F.

Aesculiside G (5), a white amorphous powder, was established to be an analog of 4 by HRESIMS and NMR spectrum interpretation. Careful analysis of their NMR data suggested that 4 possessed one more acetyl group compared to 5. The key HMBC correlation from H-21 (δ 6.41) to C-1'''' (δ 171.3) suggested that the acetyl group was connected to C-21. The sugar residues in 5 were determined to be the same with those in 4 applying the method as before described. Accordingly, aesculiside G (5) was identified as 3-O-\{β-D-glucopyranosyl-(1→2)\}-β-D-glucopyranosyl-(1→4)-β-D-glucuronopyranosyl-21β-acetyl-3β, 16α, 21β, 22α, 28-pentahydroxyolean-12-ene.
Aesculiside H (6) and Aesculiside I (7) owned the same molecular formula of C_{55}H_{86}O_{24}, according to their HR-ESI-MS data. The similar NMR spectra of 6 and 7 (Tables 1, 3) to those of 1–5 indicated that 6 and 7 are structural analogs of these compounds. The $^1$H NMR spectra of the aglycone portion of both compounds exhibited six tertiary methyl groups at $\delta$ 0.64
The absence of the characteristic singlet at δ<sub>H</sub> 0.86 and δ<sub>C</sub> 16.6 attributable to Me-24 in 5 and the additional resonances at δ<sub>C</sub> 63.6 promoted that Me-24 could be oxygenated. This was corroborated by the key HMBC cross-peaks from H-24 (δ 4.25) to C-3 (δ 91.4), C-5 (δ 56.4). Thus, the structure of the aglycone of 6 and 7 was assigned as 3β, 16α, 21β, 22α, 24, 28-hexahydroxyolean-12-ene. Detailed NMR analysis disclosed that the acetyl group signals at δ 168.3 provided definitive evidence that the acetyl was substituted at C-21, the tigeloyl group was substituted at C-22 in 6. The connection of an angeloyl group to C-22 in 7 was validated by the HMBC correlations from H-22 (δ 6.61) to δ 168.5 and H-21 (δ 6.58) to C-1′′′′ (δ 171.1). The trisaccharide chain of 6 and 7 was the same as that of 5 as determined by the same method as mentioned before. Thus, the chemical structures of compounds 6 and 7 have been elucidated and named aesculiside H (6) and aesculiside I (7).

Aesculiside J (8) was assigned as C<sub>56</sub>H<sub>92</sub>O<sub>34</sub> based on the [M – H]<sup>+</sup> ion peak at m/z 1171.5908. Acid hydrolysis suggested that D-glucose and D-glucuronic acid existed in 8. Comparison of the NMR spectroscopic data with those of compound 7 showed many similarities, except for the appearance of a 2-methylbutyryl moiety instead of the acetyl group in 7, which was ascertained by the COSY correlations between H-2′′′′ and H-3′′′′, H-4′′′′ coupled with HMBC correlation of H-21 (δ 6.59)/C-1′′′′ (δ 176.6) and H-22 (δ 6.28)/C-1′′′′ (δ 168.3). Thus, the structure of aesculiside J (8) was elucidated as 3-O-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-β-D-glucuronopyranosyl-21β-methylbutyryl-22α-angeloyl-3β, 16α, 21β, 22α, 24, 28-hexahydroxyolean-12-ene.

The elemental formula of aesculiside K (9) was confirmed as C<sub>57</sub>H<sub>90</sub>O<sub>34</sub> by its HRESIMS data. The NMR data of 9 closely resembled those of 6 with the striking difference of the acetyl group signals at δ<sub>C</sub> 171.2 and 21.3 in 6 replaced by an isobutyryl group signals at δ<sub>C</sub> 177.0, 35.2, 19.8 and 19.5. COSY correlations of H-2′′′′/H-3′′′′, H-2′′′′′/H-4′′′′ in conjunction with the HMBC cross-peaks of H-3′′′′/C-1′′′′ (δ 168.7) and H-21 (δ 6.61) to C-1′′′′′ (δ 171.2) verified the existence of the isobutyryl group. HMBC correlations from H-21 (δ 6.61) to carbonyl carbon (δ 177.0) of the isobutyryl group, and from H-22 (δ 6.21) to carbonyl carbon (δ 168.7) of the tigeloyl group supported the attachment of the two acyl units to C21 and C22, respectively. Thus, the structure of aesculiside K (9) was fully elucidated as 3-O-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-β-D-glucuronopyranosyl-21β-isobutyryl-22α-tigeloyl-3β, 16α, 21β, 22α, 24, 28-hexahydroxyolean-12-ene.

Aesculiside L (10) had molecular formula of C<sub>52</sub>H<sub>84</sub>O<sub>23</sub> established through its [M + H]<sup>+</sup> ion peak at 1077.5471 and its NMR data. The sugar chain in 10 was same as 9, using the same method as described before. Analysis of the <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data (Tables 2, 4) of 10 revealed a close structural resemblance to 9, except for the absence of a tigeloyl group in 9.
The key HMBC correlations of H-21 to C-17, C-29, C-30, and C-1′′′′; and of H-22 to C-18, C-20 supported this deduction. Finally, the structure of 10 was proved and named aesculiside L.

According to the [M-H]− ion peak at m/z 1017.4881 and its NMR data, the molecular formula of 11 was established to be C45H78O23, which is 30 mass units less than that of the known compound, aesculosides C (27). Detailed comparison of the NMR spectroscopic data between 11 and 27 revealed that they shared the same aglycone and C-21 substituent. D-xylene, D-glucose, and D-glucuronic acid were gained from acid hydrolysis of 11. Further NMR analysis of the sugar portion suggested that the β-D-xylpyranoside in 11 took the place of the β-D-glucose group substituent at C-2′ in 27. Further confirmation was carried out by the significant cross peak: xyl-H-1 (δH 5.53) with glcA-C-2 (δC 78.7) in HMBC spectrum. Accordingly, aesculiside M (11) was unambiguously identified as 3-O-[β-D-xylopyranosyl-(1→2)]-β-D-glucuronylpyranosyl-(1→4)-β-D-glucuronopyranosyl-21β-acetylated 3β, 16α, 21β, 22α, 24, 28-hexahydroxyolean-12-ene.

The molecular formula of aesculiside N (12) was calculated as C49H78O22 by virtue of its HR-ESI-MS spectrum. Its 1H NMR spectrum exhibited six singlet methyl protons [δH 0.75, 0.96, 1.35, 1.36, 1.39, 1.86] along with an olefinic proton at δH 5.46. The aforementioned spectroscopic data with its 13C-NMR data (Table 4) for the aglycone portion showed a close resemblance to those of protoaescigenin (Konoshima and Lee, 1986). The relative configuration of C-21 and C-22 was established to be 21β and 22α on the basis of the NOESY correlations of H-21/H3-29, H2-28/H-22/H-30 and the vicinal coupling constants of the H-21 and H-22 (J = 9.0 Hz). We also observed the presence of an acetyl moiety [δH 1.96 (3H, s); δC 20.6, 170.7] which was attached to C-28 due to the HMBC correlation from H-28 to the carbonyl carbon of the acetyl group. The resonances (1D and 2D NMR) of the sugar moieties and the results of hydrolysis of 12 revealed that 12 and 11 possessed the same trisaccharide chain at aglycone C-3. Thus, the structure of aesculiside N (12) was affirmed as 3-O-[β-D-xylopyranosyl-(1→2)]-β-D-d-glucopyranosyl-(1→4)-β-D-glucuronopyranosyl-28-acetyl-3β, 16α, 21β, 22α, 24, 28-hexahydroxyolean-12-ene.

Aesculiside O (13) possessed the molecular formula of C51H82O23 based on its HR-ESI-MS data. D-glucuronic acid, D-xylene, and D-glucose were afforded from 13 via the same procedure as before. The side-by-side analysis of the NMR spectroscopic resonances (Tables 2, 4) between 13 and 12 revealed that these two compounds owned similar structural features, with the only difference being due to an additional acetyl group connected with C-21 in 13. HMBC correlations from H-21 (δ 6.37) to ester carbonyl (δ 171.1) of the acetyl unit confirm this proposal. Hence, the structure of 13 was elucidated as 3-O-[β-D-xylopyranosyl-(1→2)]-β-D-d-glucopyranosyl-(1→4)-β-D-glucuronopyranosyl-21β, 28-diacetyl-3β, 16α, 21β, 22α, 24, 28-hexahydroxyolean-12-ene.

The HR-ESI-MS of aesculiside P (14) yielded a [M + H]+ ion with m/z 1141.5785, consistent with a molecular formula of C57H88O33 (calcld. for C57H88O33, 1141.5795). Analysis of its NMR data (Tables 2, 4) implied the identical trisaccharide chain to 13, which is also established by acid hydrolysis results. The 1H NMR spectrum of its aglycone showed six tertiary methyls: δH 0.71 (Me-25), 0.80 (Me-26), 1.08 (Me-29), 1.32 (Me-30), 1.35 (Me-23), and 1.84 (Me-27); one olefinic proton: δH 5.39 (br s) and a pair of oxygenated methine protons: δH 6.69 and 6.32 (each 1H, d, J = 10.2 Hz). Meanwhile, the 1D-NMR spectra of 14 exhibited typical resonances of two angeloyl groups (Tables 2, 4) and the observed HMBC correlations of H-21/ C-1′′′′′ and H-22/ C-1′′′′′′′ provided definitive evidence of their position. The relative configuration of 14 was established via NOESY experiment. The correlations between H-3/H-5/H-23, H-21/H-29 suggested the α-orientations of H-3 and H-21, the correlations between H-16/H-28/H-22/H-30 reminded β-orientations of H-16 and H-22. To further confirm its absolute configuration, we made many attempts. Owing to the amount of 14, its aglycone (14α) was easily obtained by hydrolyzation and the absolute configuration of C-21 and C-22 in 14α was determined by Mo2O7(OAc)4-induced CD. As shown in Figure 4, the ICD exhibited a negative effect at 313 nm, suggesting the R configuration of C-21, according to the Snatzke rule (Snatzke et al., 1981; Di Bari et al., 2001). Fortunately, a single crystal of 14α was obtained and suitable for X-ray crystallographic analysis (Figure 5). The Flack parameter of 0.27 (14) allowed an unambiguous assignment of the absolute configuration of 14α. Based on these data, compound 14 was undoubtedly identified as 3-O-[β-D-xylopyranosyl-(1→2)]-β-D-d-glucopyranosyl-(1→4)-β-D-glucuronopyranosyl-21R, 22R-diangeloyl-3S, 16R, 21R, 22R, 24, 28-hexahydroxyolean-12-ene.
The absolute configurations of the aglycones of 1–13 were all deduced to be 3S, 16R, 21R, 22R based on the absolute configuration of 14 and their mutual biogenetic source.

Additionally, the 19 known compounds were identified (Figure S100) as 3-O-β-D-galactopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-glucuronopyranosyl-21β, 22α-ditigloyl-3β, 16α, 21β, 22α, 22α, 28-pentalhydroxyolean-12-ene (15) (Kameyama and Fujimura, 2009), 3-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-glucuronopyranosyl-21β, 22α-diglucol-3β, 16α, 21β, 22α, 24, 28-hexahydroxyolean-12-ene (16), escin Ia (17), escin Ib (18), isoescin Ia (18), isoescin Ib (19), isoescin Ib (20) (Zhang et al., 1999), isoescin Iib (21) (Yang et al., 1999b), escin IIIa (22) (Yoshikawa et al., 1996), escin IV (23), escin V (24) (Yoshikawa et al., 1998), aesculiosides A-C (25–27) (Cheng et al., 2018), aesculoside A-B (28, 29) (Zhang et al., 1999), aesculiside A (30) (Cheng et al., 2018), desacylescin I (31) (Cheng et al., 2016), desacylescin II (32) (Yoshikawa et al., 1996), deacetyllescins Iib (33) (Kimura et al., 2006) by comparisons of their spectroscopic data with reported values.

The cytotoxic activities against three human cancer cell lines (Hep G2, HCT-116, and MGC-803) of compounds 1–33 were evaluated using the MTT method, with 5-fluorouracil (5-FU) as positive control (Table 5). Among them, compounds 8, 9, 14–16, 18, 22 showed potent cytotoxicity against all the tested human cancer cell lines with IC₅₀ ranging between 2 and 21 µM. Compounds 3, 6, 7, 17–19, 20, 24, 28 were less active (IC₅₀: 13 to >40 µM) whereas the other isolates displayed no toxicity in all cell lines at 50 µM. These results suggested that the compounds with acylations at both C-21 and C-22 exhibited stronger inhibitory activities than those with acylations at C-21.
and C-28 or only at C-21. In addition, it seems that the presence of the tigloyl, angeloyl, methylbutyryl, and isobutyryl groups affects the inhibitory activity of these compounds on the tested cell lines positively.

To examine the neuroprotective effect, the cytotoxic activity of compounds 1–33 against PC12 cell line was first evaluated. Among them, compounds 6–9, 14–16, 18, 22 showed no obvious cytotoxic effects on PC12 cells at a dose of 5 μM, while others at 10 μM. Next, 5 μM compounds 6–9, 14–16, 18, 22 and 10 μM others were tested for their neuroprotective properties against COCl₂-induced toxicity in PC12 cells with trolox as the positive control. Among these, compounds 1, 4, 12, 20, 22, 25, 29, 31 exhibited moderate activities against COCl₂-induced PC12 cell injury (Figure 6).

**CONCLUSION**

Plants of the genus *Aesculus* have been proved to be rich in polyhydroxyoleanene triterpenoid saponins which have been characterized more than 100. When compared to the relatively extensive research on other species of *Aesculus* genus, little is known regarding the chemical constituents and the biological activity of the *Aesculus chinensis* Bge. var. *chekiangensis* (Hu et Fang) Fang species. The present paper reports 14 new polyhydroxy oleanene saponins (1–14) along with 19 known analogs from the seeds of *A. chinensis* Bge. var. *chekiangensis*. Structure elucidation was achieved via various techniques, and the absolute configuration of the aglycones was undoubtedly defined through X-ray diffraction analysis as well as Mo₂(OAc)₄-induced ECD method for the first time. Further cytotoxicity evaluation against three human tumor cell lines suggested that compounds 8, 9, 14–16, 18, 22 displayed strong inhibitory activities against all three cell lines; compounds 3, 6, 7, 17–19, 20, 24, 28 exhibited weak activities while the remaining isolates showed no toxicity at 50 μM. These results suggested that isolates with two acylations at C-21 and C-22 might be important for the cytotoxicity, especially substituted by tigloyl, angeloyl, methylbutyryl, and isobutyryl groups. In addition, the first test about the neuroprotective properties of triterpenoid saponins from *Aesculus* genus found that compounds 1, 4, 12, 20, 22, 25, 29, 31 exhibited moderate activities against COCl₂-induced PC12 cell injury.

**DATA AVAILABILITY STATEMENT**

The crystallographic dataset generated for this study can be found in the Cambridge Crystallographic Data Centre under the CCDC number 1957449. All other datasets generated for this study are included in the article/Supplementary Material.

**AUTHOR CONTRIBUTIONS**

NZ and SW was responsible for the isolation and elucidated compounds. NZ tested cytotoxicity, neuroprotective effects of the compounds, interpreted the data, and wrote the paper. SC, QZ, and NK revised the manuscript. LD and FQ were the project leaders organizing and guiding the experiment. All authors read and approved the final manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2019.00908/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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