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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and cytotoxic activity of 14-deoxy-12-hydroxyandrographolide analogs

Uthaiwan Sirion[1] · Teerapich Kasemsuk[1] · Pawinee Piyachaturawat[2] · Kanoknetr Suksen[2] · Apichart Suksamrarn[3] · Rungnapha Saeeng[1]

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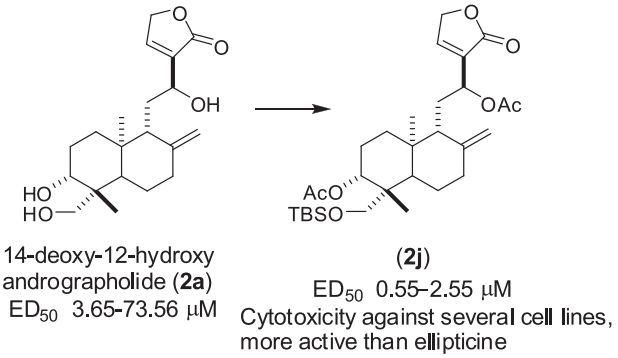
Abstract Important anticancer cytotoxicological properties are reported from synthetic analogs of 14-deoxy-12-hydroxy-andrographolide, a compound that occurs in *Andrographis paniculata*, an indigenous Southeast Asia plant. Cytotoxicities of 15 synthesized 14-deoxy-12-hydroxy-andrographolide analogs were evaluated against a panel of cancer cell lines with some exhibiting much greater than the parent compound. For example analog **2j** was 6–35 times more potent than the parent compound and the anticancer agent, ellipticin, on all cell lines except LU-1 cancer cells. With promising cytotoxicities and simple preparation, this compound has significant potential in the treatment of cancer.

Graphical Abstract



Keywords 14-deoxy-12-hydroxyandrographolide · Cytotoxic activity · *Andrographis paniculata*

Introduction

Andrographis paniculata (Brum. f.) Nees, is a herbaceous plant used widely in traditional medicine in Southeast Asia for treatment of dyspepsia, dysentry, malaria, respiratory infections, and as an antidote for snakebites (Misra et al. 1992). Many ent-labdane diterpenoids have been isolated from *A. paniculata* (Balmain and Connolly 1973; Chen et al. 2008; Kleipool 1952; Shen et al. 2006) but the major constituent, andrographolide **1**, is known for its diverse biological activities including its antihepatoxic potential in the treatment of liver damage (Handa and Sharma 1990), HIV (Calabrese et al. 2000; Reddy et al. 2005), cancer (Kumar et al. 2004; Siripong et al. 1992) and as a bacter-icide (Singha et al. 2003). Structure-related diterpenoids,

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✉ Rungnapha Saeeng
rungnaph@buu.ac.th

[1] Department of Chemistry and Center for Innovation in Chemistry, Faculty of Science, Burapha University, Chonburi 20131, Thailand

[2] Department of Physiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

[3] Department of Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand

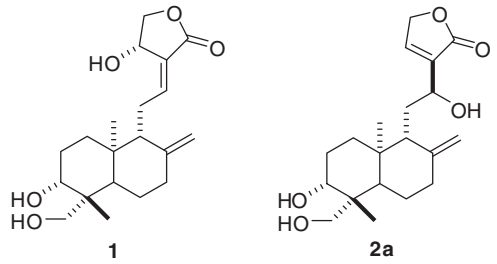


Fig. 1 Structures of andrographolide **1** and 14-deoxy-12-hydroxyandrographolide **2a**

14-deoxy-12-hydroxyandrographolide **2a** and andrographolide **1**, (Fig. 1), show similar inducibility and cytostatic activities (Matsuda et al. 1994), and serve as precursors for structural modification generating new and interesting compounds. A large number of andrographolide derivatives now have been synthesized from A. *paniculata* and screened for their bioactivity (Chen et al. 2013; Dai et al. 2006; Das et al. 2010; Fan et al. 2010; Jada et al. 2006, 2007; and others). However, the development of therapeutic derivatives from the uniquely structured 14-deoxy-12-hydroxy andrographolide is numerically limited. This substance with its ent-labdane skeleton was first isolated in 0.0004% yields from the aerial portions of *A. paniculata* as a new diterpenoid therapeutic agent (Matsuda et al. 1994).

Compound **2a** can be prepared from andrographolide **1** via the rearrangement of allylic hydroxyl at C-14 using pyridinium dichromate (Thunguntla et al. 2004). Synthesis of these diterpenoid derivatives is attractive because of their potential bioactivities such as α-glucosidase inhibition (Xu et al. 2007), LPS-induced inflammatory cytokines TNF-α, interleukin-6 (Li et al. 2007), anti-HIV (Wang et al. 2011) and anticancer properties (Nateewattana et al. 2013).

Recently, we reported enhanced cytotoxic activity on P-388 and LU-1 cancer cells by C-19 modified andrographolide analogs (Sirion et al. 2012; Kasemsook et al. 2013). In the present study we report more extensive cytotoxicity studies on new analogs of the 14-deoxy-12-hydroxyandrographolide-scaffold (**2a**; Fig. 1). These new analogs are obtained through chemical modification of 14-deoxy-12-hydroxyandrographolide **2a** using cheap reagents, convenient procedures and are attractive for process scale synthesis.

Materials and methods

General

All chemical reagents were obtained from chemical companies and used without further purification. Proton NMR spectra were recorded on a Bruker Avance (400 MHz). All spectra were measured in $CDCl_3$ solvent and chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane (δ 0.00) or $CDCl_3$ (δ 7.26) as internal standard. Data reported were chemical shift (multiplicity, integrated intensity or assignment, coupling constant in Hz, assignment). Carbon NMR spectra were recorded on a Bruker AVANC (100 MHz). All spectra were measured in $CDCl_3$ solvent and chemical shifts are reported as δ values in ppm relative to $CDCl_3$ (δ 77.0) as internal standard. High-resolution mass spectra (HRMS) data were obtained with a Finnigan MAT 95. Infrared spectra were determined on a Perkin Elmer FT/IR-2000S spectrophotometer and are reported in wave number (cm^{-1}). Analytical thin-layer chromatography (TLC) was conducted on precoated TLC plates; silica gel 60F-254 (E. Merck, Darmstadt, Germany). Silica gel columns for open-column chromatography utilized silica gel 60 (0.040–0.063 mm) (E. Merck, Darmstadt, Germany). Melting points were measured using a melting point apparatus (Griffin) and are uncorrected.

Synthesis of 14-deoxy-12-hydroxyandrographolide analogs

Synthesis of 19-Tr-14-deoxy-12-hydroxyandrographolide (**2b**) To a stirred solution of 14-deoxy-12-hydroxyandrographolide (**2a**) (41.9 mg, 0.119 mmol) in pyridine (500 µL) was added triphenylmethyl chloride (TrCl) (164 mg, 0.59 mmol), then the reaction mixture was heated at 80 °C for 6 h. The reaction mixture was quenched with NH_4Cl and extracted with EtOAc (3 × 20 mL). The combined organic layer was washed with brine (10 mL), dried over sodium sulfate anhydrous, and then the solution was concentrated under reduced pressure. The residue was purified by column chromatography (40% EtOAc/*n*-hexane) to give 19-Tr-14-deoxy-12-hydroxyandrographolide (**2b**) in 72% yield (50.9 mg) as a white solid, R_f 0.45 (60% EtOAc/*n*-hexane).

19-Tr-14-deoxy-12-hydroxyandrographolide (**2b**): Mp 111–112 °C; IR (Neat): 3218, 2933, 1751, 1446, 1046, 749, 704 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.44–7.40 (6 H, m, PhH), 7.33–7.28 (6H, m, PhH), 7.27–7.21 (3H, m, PhH), 7.22 (1H, brs, H-14), 4.82–4.79 (3H, m, H-15, H-17b), 4.63 (1H, brs, H-17a), 4.42–4.49 (1H, m, H-12), 4.09 (1H, d, $J = 8.5$ Hz, OH), 3.23 (1H, d, $J = 9.5$ Hz, H-19b), 3.16–3.23 (1H, m, H-3), 3.10 (1H, d, $J = 9.5$ Hz, H-19a), 2.50–2.57 (1H, m, OH), 2.32 (1H, dm, $J = 13.0$ Hz, H-7b), 1.95–1.81 (2H, m), 1.80–1.70 (2H, m), 1.65–0.86 (7H, m), 1.53 (3H, s, H-18), 0.12 (3H, s, H-20); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.90, 148.08, 145.02, 143.40 (3×C), 136.04, 128.44 (6×C), 128.05 (6×C), 127.24 (3×C), 107.48, 87.60, 80.14, 70.40, 67.35, 64.79, 55.80, 53.23, 42.75, 39.02, 38.08, 36.90, 30.16, 28.11, 24.03, 23.30, 15.03; HRMS (ESI) *m/z* calcd for $C_{39}H_{44}O_5Na$ $[M+Na]^+$ 615.3087, found 615.3070.

Synthesis of 3,12-Ac-19-Tr-14-deoxyandrographolide (**2c**) The stirred solution of 19-Tr-14-deoxy-12-hydroxyandrographolide (**2b**) (20.6 mg, 0.035 mmol) in acetic anhydride (500 µL) was refluxed to 145 °C for 3 h. The reaction mixture was diluted with EtOAc (20 mL) and quenched with saturated $NaHCO_3$. The solution mixture was extracted with EtOAc (3 × 20 mL). The combined organic layer was washed with brine (10 mL), dried over sodium sulfate anhydrous, and then the solution was concentrated under reduced pressure. The residue was purified by column chromatography (30% EtOAc/*n*-hexane) to give 3,12-Ac-19-Tr-14-deoxyandrographolide (**2c**) in 80% yield (18.7 mg) as a white solid, R_f 0.58 (40% EtOAc/*n*-hexane).

3,12-Ac-19-Tr-14-deoxyandrographolide (**2c**): Mp 84–87 °C; IR (Neat): 2932, 1760, 1738, 1370, 1237, 1029, 750 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.48–7.43 (6H, m, PhH), 7.32–7.25 (7H, m, H-14, PhH), 7.24–7.19 (3H, m, PhH), 5.61 (1H, dd, $J = 9.5$, 4.5 Hz, H-12), 4.83 (1H, brs, H-17b), 4.82 (2H, brs, H-15), 4.68 (1H, brs, H-17a), 4.52 (1H, dd, $J = 12.0$, 4.0, H-3), 3.31 (1H, d, $J = 10.0$ Hz, H-19b), 3.05 (1H, d, $J = 10.0$ Hz, H-19a), 2.32 (1H, dm, $J = 12.0$ Hz, H-7b), 2.15–2.02 (2H, m), 2.04 (3H, s, $COCH_3$), 2.00 (3H, s, $COCH_3$), 1.89–1.75 (4H, m), 1.67–1.02 (5H, m), 1.13 (3H, s, H-18), 0.22 (3H, s, H-20); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.41, 170.81, 169.97, 148.23, 146.59, 144.24 (3 × C), 132.59, 128.86 (6 × C), 127.25 (6 × C), 126.81 (3 × C), 107.71, 86.84, 80.26, 70.03, 68.23, 62.22, 55.65, 52.14, 42.53, 38.94, 38.17, 36.73, 26.99, 24.92, 24.19, 22.91, 21.33, 21.08, 14.25; HRMS (ESI) *m/z* calcd for $C_{43}H_{48}O_7Na$ $[M + Na]^+$ 699.3298, found 699.3356.

General procedure for synthesis of compounds (**2d–2f**) Method A. The stirred solution of 14-deoxy-12-hydroxyandrographolide (**2a**) (20.3 mg, 0.058 mmol) in acetic anhydride (1.0 mL) was heated at 70 °C for 2 h. The reaction mixture was diluted with EtOAc (20 mL) and quenched with saturated $NaHCO_3$. The solution mixture was extracted with EtOAc (3 × 20 mL). The combined organic layer was washed with brine (10 mL), dried over sodium sulfate anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography (50% EtOAc/*n*-hexane) to give 19-Ac-14-deoxy-12-hydroxyandrographolide (**2d**) in 24% yield (5.50 mg) as a white solid, R_f 0.22 (70% EtOAc/*n*-hexane), 3,19-Ac-14-deoxy-12-hydroxyandrographolide (**2e**) in 29% yield (7.20 mg) as a white solid, R_f 0.56 (70% EtOAc/*n*-hexane), and 3,12,19-Ac-14-deoxyandrographolide (**2f**) in 18% yield (5.10 mg) as a white solid, R_f 0.69 (50% EtOAc/*n*-hexane).

Method B. The stirred solution of 14-deoxy-12-hydroxyandrographolide (**2a**) (23.6 mg, 0.067 mmol) in acetic anhydride (1.0 mL) was heated at 145 °C for 1.5 h. The reaction mixture was diluted with EtOAc (20 mL) and quenched with saturated $NaHCO_3$. The solution mixture was extracted with EtOAc (3 × 20 mL). The combined organic layer was washed with brine (10 mL), dried over sodium sulfate anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography (30% EtOAc/*n*-hexane) to give 3,12,19-Ac-14-deoxyandrographolide (**2f**) in 80% yield (25.7 mg) as a white solid, R_f 0.22 (30% EtOAc/*n*-hexane).

19-Ac-14-deoxy-12-hydroxyandrographolide (**2d**): Mp 177–190 °C; IR (Neat): 3142, 1738, 1404, 1259, 1047 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.30 (1H, brs, H-14), 4.95 (1H, brs, H-17b), 4.85 (2H, brs, H-15), 4.77 (1H, brs, H-17a), 4.55 (1H, t, $J = 7.0$ Hz, H-12), 4.32 (1H, d, $J =$ 11.5 Hz, H-19b), 4.13 (1H, d, $J = 11.5$ Hz, H-19a), 3.32 (1H, dd, $J = 12.0$, 4.0 Hz, H-3), 2.60 (1H, brs, OH), 2.46 (1H, ddd, $J = 12.5$, 3.5, 2.0 Hz, H-7b), 2.06 (3H, s, $COCH_3$), 2.09–2.02 (1H, m), 2.00–1.80 (4 H, m), 1.75–1.58 (4H, m), 1.52–1.17 (2H, m), 1.15 (3H, s, H-18), 0.70 (3H, s, H-20); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.93, 170.98, 148.10, 145.21, 136.11, 107.92, 78.86, 70.46, 67.59, 64.91, 55.41, 53.25, 42.45, 39.37, 38.24, 36.98, 30.27, 27.79, 24.45, 22.45, 21.09, 14.91; HRMS (ESI) *m/z* calcd for $C_{22}H_{32}O_6Na$ $[M+Na]^+$ 415.2097, found 415.2102.

3,19-Ac-14-deoxy-12-hydroxyandrographolide (**2e**): Mp 183–185 °C; IR (Neat): 3134, 1738, 1404, 1249, 1039, 894 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.27 (1H, brs, H-14), 4.93 (1H, brs, H-17b), 4.85 (2H, brs, H-15), 4.76 (1H, s, H-17a), 4.57 (1H, dd, $J = 11.5$, 5.0 Hz, H-3), 4.54–4.49 (1H, m, H-12), 4.34 (1H, d, $J = 11.5$ Hz, H-19b), 4.09 (1H, d, J = 11.5 Hz, H-19a), 2.63 (1H, brd, $J = 6.0$ Hz, OH), 2.43 (1H, dm, H-7b), 2.03 (6H, s, 2 × $COCH_3$), 2.07–1.98 (1H, m), 1.95–1.82 (4H, m), 1.78–1.47 (4H, m), 1.31–1.15 (2H, m), 0.99 (3H, s, H-18), 0.72 (3H, s, H-20); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.95, 170.85, 170.57, 147.75, 145.21, 135.99, 107.90, 79.76, 70.45, 67.24, 64.70, 55.40, 53.03, 41.28, 39.25, 38.32, 36.81, 30.32, 24.83, 24.19, 22.61, 21.16, 21.05, 14.63; HRMS (ESI) *m/z* calcd for $C_{24}H_{34}O_7Na$ $[M+Na]^+$ 457.2203, found 457.2237.

3,12,19-Ac-14-deoxyandrographolide (**2f**): Mp 138–142 °C; IR (Neat): 2943, 1735, 1372, 1237, 1030 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.35 (1H, brs, H-14), 5.66 (1H, dd, J = 9.0, 4.5 Hz, H-12), 4.92 (1H, brs, H-17b), 4.84 (2H, brs, H-15), 4.78 (1 H, brs, H-17a), 4.57 (1H, dd, $J = 12.0$, 5.0 Hz, H-3), 4.34 (1H, d, $J = 11.5$ Hz, H-19b), 4.08 (1H, d, J = 11.5 Hz, H-19a), 2.39 (1H, dm, $J = 10.5$ Hz, H-7b), 2.19 (1H, ddd, $J = 13.0$, 9.0, 1.0 Hz), 2.06 (3H, s, $COCH_3$), 2.03 (6H, s, 2 × $COCH_3$), 1.95 (1H, ddd, $J = 13.0$, 11.0, 4.5 Hz), 1.90–1.80 (3H, m), 1.79–1.57 (2H, m), 1.54–1.45 (2H, m), 1.26 (1H, dm, $J = 9.0$ Hz), 1.18 (1H, td, $J = 13.0$, 4.5 Hz), 0.99 (3H, s, H-18), 0.71 (3H, s, H-20); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.47, 170.89, 170.56, 170.00, 148.32, 146.35, 132.73, 108.12, 79.74, 70.09, 68.23, 64.73, 55.34,

52.19, 41.30, 39.15, 38.20, 36.72, 27.24, 24.81, 24.22, 22.60, 21.18, 21.09 (2×C), 14.59; HRMS (ESI) *m/z* calcd for $C_{26}H_{36}O_8Na$ $[M+Na]^+$ 499.2308, found 499.2353.

General procedure for synthesis of compounds (**2g–2i**) To a stirred solution of 14-deoxy-12-hydroxyandrographolide (**2a**) in pyridine was added silyl chloride [TBSCl, triisopropyl chloride (TIPSCl) or TBDPSCl] at room temperature. After the stirring was continued at room temperature for 1–8 h, the reaction mixture was quenched with NH_4Cl, and extracted with EtOAc (3 × 20 mL). The combined organic la yer was washed with brine (10 mL), dried over sodium sulfate anhydrous, and then the solution was concentrated under reduced pressure. The residue was purified by column chromatography to give the desired products (**2g–2i**).

Synthesis of 19-TBS-14-deoxy-12-hydroxyandrographolide (**2g**) According to the general procedure, 14-deoxy-12-hydroxyandrographolide (**2a**) (40.8 mg, 0.116 mmol) with *tert*-butyldimethylsilyl chloride (TBDMSCl) (91.7 mg, 0.58 mmol) in pyridine (500 µL) was stirred at room temperature for 1 h. The residue was purified by column chromatography (60% EtOAc/*n*-hexane) to afford the corresponding product (**2g**) in 80% yield (43.0 mg) as a white solid, R_f 0.54 (60% EtOAc/*n*-hexane).

19-TBS-14-deoxy-12-hydroxyandrographolide (**2g**): Mp 188–190 °C; IR (Neat): 3366, 2931, 1748, 1260, 1055, 750 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.28 (1H, brd, J = 1.5 Hz, H-14), 4.90 (1H, brs, H-17b), 4.84 (2H, brs, H-15), 4.74 (1H, brs, H-17a), 4.52 (1H, dd, J = 13.0, 6.5 Hz, H-12), 4.33 (1H, d, J = 7.0 Hz, OH), 4.17 (1H, d, J = 10.5 Hz, H-19b), 3.36 (1H, d, J = 10.5 Hz, H-19a), 3.29 (1H, ddd, J = 11.0, 7.0, 4.0 Hz, H-3), 2.61 (1H, d, J = 6.5 Hz, OH), 2.41 (1H, ddd, J = 13.5, 4.0, 2.5 Hz, H-7b), 2.03 (1H, ddd, J = 13.5, 8.0, 1.5 Hz), 1.98–1.76 (5H, m), 1.74–1.55 (2H, m), 1.20 (3H, s, H-18), 1.31–1.15 (2H, m), 1.10 (1H, td, J = 13.0, 3.5 Hz), 0.88 (9H, s, $SiC(CH_3)_3$), 0.65 (3H, s, H-20), 0.06 (3H, s, $SiCH_3$), 0.05 (3H, s, $SiCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.96, 148.18, 145.07, 136.08, 107.72, 80.11, 70.43, 67.39, 65.17, 55.38, 53.23, 42.53, 39.26, 38.23, 36.94, 30.28, 28.56, 25.76 (3×C), 24.08, 23.12, 18.05, 15.59, −5.17, −5.18; HRMS (ESI) *m/z* calcd for $C_{26}H_{44}O_5SiNa$ $[M+Na]^+$ 487.2856, found 487.2895.

Synthesis of 19-TBDPS-14-deoxy-12-hydroxyandrographolide (**2h**) According to the general procedure, 14-deoxy-12-hydroxyandrographolide (**2a**) (41.9 mg, 0.119 mmol) with *tert*-butyldiphenylsilyl chloride (TBDPSCl) (150 µL, 0.57 mmol) in pyridine (500 µL) was stirred at room temperature for 1 h. The residue was purified by column chromatography (60% EtOAc/*n*-hexane) to afford the corresponding product (**2h**) in 82% yield (57.6 mg) as a white solid, R_f 0.51 (50% EtOAc/*n*-hexane).

19-TBDPS-14-deoxy-12-hydroxyandrographolide (**2h**): Mp 71–73 °C; IR (Neat): 2933, 2859, 1753, 1054, 750 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.68–7.61 (4H, m, PhH), 7.48–7.37 (6H, m, PhH), 7.24 (1H, brs, H-14), 4.83 (2H, brs, H-15), 4.81 (1H, brs, H-17b), 4.65 (1H, brs, H-17a), 4.58 (1H, dd, J = 12.5, 6.5 Hz, H-12), 4.40 (1H, d, J = 7.5 Hz, OH), 4.15 (1H, d, J = 10.0 Hz, H-19b), 3.33 (1H, d, J = 10.0 Hz, H-19a), 3.36–3.29 (1H, m, H-3), 2.54 (1H, d, J = 6.5 Hz, OH), 2.29 (1H, ddd, J = 13.0, 3.5, 2.5 Hz, H-7b), 2.02–1.71 (5H, m), 1.68–1.59 (2H, m), 1.51 (1H, d, J = 10.0 Hz), 1.30 (3H, s, H-18), 1.18–0.94 (3H, m), 1.04 (9H, s, $SiC(CH_3)_3$), 0.39 (3H, s, H-20); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.94, 148.05, 145.07, 136.05, 135.70 (2 × C), 135.54 (2 × C), 132.38, 132.05, 129.99 (2 × C), 127.88 (2 × C), 127.81 (2 × C), 107.64, 80.29, 70.42, 67.38, 65.89, 55.40, 53.19, 42.83, 39.17, 38.12, 36.88, 30.21, 28.49, 26.80 (3 × C), 23.81, 23.11, 19.07, 15.38; HRMS (ESI) *m/z* calcd for $C_{36}H_{48}O_5SiNa$ $[M + Na]^+$ 611.3169, found 611.3221.

Synthesis of 19-TIPS-14-deoxy-12-hydroxyandrographolide (**2i**) According to the general procedure, 14-deoxy-12-hydroxyandrographolide (**2a**) (51.0 mg, 0.145 mmol) with TIPSCl (0.30 mL, 1.40 mmol) in pyridine (500 µL) was stirred at room temperature for 8 h. The residue was purified by column chromatography (60% EtOAc/*n*-hexane) to afford the corresponding product (**2i**) in 67% yield (49.1 mg) as a white solid, R_f 0.47 (60% EtOAc/*n*-hexane).

19-TIPS-14-deoxy-12-hydroxyandrographolide (**2i**): Mp 95–98 °C; IR (Neat): 3380, 2942, 2867, 1750, 1460, 1056, 883 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.27 (1H, brs, H-14), 4.90 (1H, brs, H-17b), 4.84 (2H, brs, H-15), 4.73 (1H, brs, H-17a), 4.56–4.49 (1H, m, H-12), 4.47 (1H, d, J = 7.0 Hz, OH), 4.29 (1H, d, J = 10.0 Hz, H-19b), 3.47 (1H, d, J = 10.0 Hz, H-19a), 3.30 (1H, ddd, J = 13.0, 7.0, 5.0 Hz, H-3), 2.62 (1H, brd, J = 5.0 Hz, OH), 2.41 (1H, dm, J = 13.0 Hz, H-7b), 2.03 (1H, ddd, J = 14.0, 8.0, 2.0 Hz), 1.96–1.76 (5H, m), 1.74–1.62 (1H, m), 1.57 (1H, d, J = 10.0 Hz), 1.27 (3H, s, H-18), 1.26–1.00 (6H, m), 1.07 (18H, d, J = 5.0 Hz, $Si(CH(CH_3)_2)_3$), 0.64 (3H, s, H-20); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.98, 148.11, 145.11, 136.07, 107.72, 80.20, 70.44, 67.31, 65.65, 55.32, 53.22, 42.74, 39.23, 38.21, 36.91, 30.27, 28.58, 24.05, 22.95, 17.91 (6 × C), 15.64, 11.63 (3 × C); HRMS (ESI) *m/z* calcd for $C_{29}H_{50}O_5SiNa$ $[M + Na]^+$ 529.3326, found 529.3370.

General procedure for synthesis of compounds (**2j–2l**) To a stirred solution of 19-silylether-14-deoxy-12-hydroxyandrographolide (**2g**, **2h** or **2i**) in acetic anhydride (500 µL) was refluxed to 145 °C for 1–2.5 h. The reaction mixture was diluted with EtOAc (20 mL) and quenched with

saturated $NaHCO_3$. The solution mixture was extracted with EtOAc (3×20 mL). The combined organic layer was washed with brine (10 mL), dried over sodium sulfate anhydrous, and then the solution was concentrated under reduced pressure. The residue was purified by column chromatography to give the desired products (**2j–2l**).

Synthesis of 19-TBS-3,12-Ac-14-deoxyandrographolide (**2j**) According to the general procedure, 19-TBS-14-deoxy-12-hydroxyandrographolide (**2g**) (20.1 mg, 0.043 mmol) was refluxed at 145 °C for 1.15 h. The residue was purified by column chromatography (40% EtOAc/*n*-hexane) to afford the corresponding product (**2j**) in 81% yield (19.1 mg) as a white solid, R_f 0.42 (40% EtOAc/*n*-hexane).

19-TBS-3,12-Ac-14-deoxyandrographolide (**2j**): Mp 104–107 °C; IR (Neat): 2934, 1756, 1738, 1254, 1028, 752 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.35 (1H, brs, H-14), 5.66 (1H, dd, $J = 9.5$, 4.5 Hz, H-12), 4.90 (1H, brs, H-17b), 4.83 (2H, brs, H-15), 4.75 (1H, brs, H-17a), 4.56–4.53 (1H, m, H-3), 3.79 (1H, d, $J = 10.5$ Hz, H-19b), 3.57 (1H, d, $J = 10.5$ Hz, H-19a), 2.36 (1H, dm, $J = 12.0$ Hz, H-7b), 2.20 (1H, ddd, $J = 13.5$, 9.0, 1.0 Hz), 2.06 (3H, s, $COCH_3$), 2.04 (3H, s, $COCH_3$), 1.94 (1H, $J = 13.5$, 11.0, 5.0 Hz), 1.85–1.74 (3H, m), 1.73–1.63 (3H, m), 1.47 (1H, d, $J =$ 10.0 Hz), 1.21–1.11 (2H, m), 0.91 (3H, s, H-18), 0.87 (9H, s, $SiC(CH_3)_3$), 0.76 (3H, s, H-20), 0.01 (6H, s, $Si(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.44, 170.77, 170.00, 148.26, 147.05, 132.63, 107.56, 80.17, 70.04, 68.31, 63.59, 55.61, 52.31, 42.51, 39.25, 38.59, 37.06, 27.11, 25.85 ($3 \times$ C), 25.53, 24.34, 23.06, 21.27, 21.12, 18.20, 14.43, −5.17, −5.18; HRMS (ESI) *m/z* calcd for $C_{30}H_{48}O_7SiNa$ $[M + Na]^+$ 571.3067, found 571.3115.

Synthesis of 19-TBDPS-3,12-Ac-14-deoxyandrographolide (**2k**) According to the general procedure, 19-TBDPS-14-deoxy-12-hydroxyandrographolide (**2h**) (29.3 mg, 0.050 mmol) was refluxed at 145 °C for 2 h. The residue was purified by column chromatography (40% EtOAc/*n*-hexane) to afford the corresponding product (**2k**) in 70% yield (23.5 mg) as a white solid, R_f 0.49 (30% EtOAc/*n*-hexane).

19-TBDPS-3,12-Ac-14-deoxyandrographolide (**2k**): Mp 71–73 °C; IR (Neat): 2953, 1761, 1734, 1372, 1237, 1081, 1028, 704 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.70–7.63 (4H, m, PhH), 7.45–7.33 (6H, m, PhH), 7.26 (1H, brs, H-14), 5.65 (1H, dd, $J = 9.5$, 4.5 Hz, H-12), 4.90 (1H, brs, H-17b), 4.83 (2H, brs, H-15), 4.75 (1H, brs, H-17a), 4.53 (1H, dd, $J = 11.5$, 4.5 Hz, H-3), 3.77 (1H, d, $J = 10.5$ Hz, H-19b), 3.71 (1H, d, $J = 10.5$ Hz, H-19a), 2.38 (1H, dm, $J =$ 12.5 Hz, H-7b), 2.22–2.13 (1H, m), 2.06 (3H, s, $COCH_3$), 1.90 (3H, s, $COCH_3$), 1.95–1.70 (5H, m), 1.66–1.43 (3H, m), 1.22–1.11 (2H, m), 1.04 (9H, s, $SiC(CH_3)_3$), 1.00 (3H, s, H-18), 0.62 (3H, s, H-20); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.42, 170.73, 169.99, 148.27, 146.80, 135.80 ($2 \times$ C), 135.73 ($2 \times$ C), 133.54, 133.50, 132.62, 129.58, 129.52, 127.57 ($2 \times$ C), 127.49 ($2 \times$ C), 107.74, 80.07, 70.04, 68.27, 63.57, 55.54, 52.20, 43.07, 39.16, 38.39, 36.83, 27.05, 26.90 ($3 \times$ C), 25.36, 24.24, 22.71, 21.15, 21.11, 19.26, 14.62; HRMS (ESI) *m/z* calcd for $C_{40}H_{52}O_7SiNa$ $[M + Na]^+$ 695.3380, found 695.3406.

Synthesis of 19-TIPS-3,12-Ac-14-deoxyandrographolide (**2l**) According to the general procedure, 19-TIPS-14-deoxy-12-hydroxyandrographolide (**2i**) (20.8 mg, 0.041 mmol) was refluxed at 145 °C for 2.5 h. The residue was purified by column chromatography (40% EtOAc/*n*-hexane) to afford the corresponding product (**2l**) in 63% yield (15.2 mg) as a white solid, R_f 0.74 (40% EtOAc/*n*-hexane).

19-TIPS-3,12-Ac-14-deoxyandrographolide (**2l**): Mp 156–160 °C; IR (Neat): 3464, 2946, 1738, 1240, 1097, 752 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.35 (1H, brs, H-14), 5.66 (1H, dd, $J = 9.5$, 4.5 Hz, H-12), 4.91 (1H, brs, H-17b), 4.84 (2H, brs, H-15), 4.75 (1H, brs, H-17a), 4.55 (1H, dd, $J = 11.0$, 5.5 Hz, H-3), 3.85 (1H, d, $J = 10.5$ Hz, H-19b), 3.78 (1H, d, $J = 10.5$ Hz, H-19a), 2.37 (1H, dm, $J =$ 13.0 Hz, H-7b), 2.20 (1H, ddd, $J = 13.0$, 9.0, 1.0 Hz), 2.07 (3H, s, $COCH_3$), 2.03 (3H, s, $COCH_3$), 1.94 (1H, ddd, $J =$ 13.5, 11.0, 4.5 Hz), 1.87–1.76 (3H, m), 1.71–1.55 (3H, m), 1.47 (1H, d, $J = 10.0$ Hz), 1.22–0.93 (5H, m), 1.06 (18H, d, $J = 4.5$ Hz, $Si(CH(CH_3)_2)_3$), 0.98 (3H, s, H-18), 0.74 (3H, s, H-20); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.46, 170.84, 170.02, 148.31, 146.93, 132.61, 107.66, 80.18, 70.06, 68.30, 63.41, 55.58, 52.24, 43.07, 39.21, 38.44, 36.94, 27.06, 25.33, 24.32, 22.72, 21.25, 21.12, 18.07 ($6 \times$ C), 14.73, 11.91 ($3 \times$ C); HRMS (ESI) *m/z* calcd for $C_{33}H_{54}O_7SiNa$ $[M+Na]^+$ 613.3537, found 613.3516.

Synthesis of 14-deoxy-12-hydroxy-3,19-isopropylideneandrographolide (**2m**) To a stirred solution of 14-deoxy-12-hydroxyandrographolide (**2a**) in acetone (45.0 mL) was added 2,2-dimethoxypropane (1.26 mL, 10.3 mmol) and followed by addition of pyridinium *p*-toluenesulfonate (PPTS) (14.3 mg, 0.0571 mmol). After the stirring was continued at room temperature for 2 h, the solvent was removed by evaporator. The residue was diluted with EtOAc (50 mL), and quenched with saturated $NaHCO_3$ and H_2O. The solution mixture was extracted with EtOAc ($3 \times$ 50 mL). The combined organic layer was washed with brine (20 mL), dried over sodium sulfate anhydrous, and then the solution was concentrated under reduced pressure. The residue was purified by column chromatography (30% EtOAc/*n*-hexane) to give 14-deoxy-12-hydroxy-3,19-isopropylideneandrographolide (**2m**) in 82% yield (65.6 mg) as a white solid, R_f 0.50 (70% EtOAc/*n*-hexane).

14-Deoxy-12-hydroxy-3,19-isopropylideneandrographolide (**2m**): Mp 102–104 °C; IR (Neat): 3258, 2946, 1745, 1405, 1034 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.30 (1H,

brs, H-14), 4.94 (1H, brs, H-17b), 4.85 (2H, brs, H-15), 4.77 (1H, brs, H-17a), 4.55 (1H, brs, H-12), 4.15–4.10 (1H, m, OH), 3.96 (1H, d, $J = 11.5$ Hz, H-19b), 3.48 (1H, d, $J = 8.0$ Hz, H-3), 3.17 (1H, d, $J = 11.5$ Hz, H-19a), 2.43 (1H, d, $J = 12.5$ Hz, H-7b), 2.07–1.87 (4H, m), 1.81–1.69 (3H, m), 1.42 (3H, s, H-21), 1.37 (3H, s, H-22), 1.32–1.14 (4H, m), 1.20 (3H, s, H-18), 0.96 (3H, s, H-20); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.02, 148.61, 145.32, 136.09, 107.98, 99.07, 76.44, 70.49, 67.60, 63.84, 53.18, 52.44, 38.71, 38.09, 37.87, 34.30, 30.45, 27.27, 26.07, 25.28, 25.02, 23.46, 16.32.

Synthesis of 12-TBS-14-deoxy-3,19-isopropylideneandrographolide (**2n**) To a stirred solution of 14-deoxy-12-hydroxy-3,19-isopropylideneandrographolide (**2m**) (388 mg, 0.994 mmol) in DMF (10.0 mL) was added TBDMSCl (225 mg, 1.49 mmol) and followed by addition of imidazole (203 mg, 2.98 mmol). After the stirring was continued at room temperature for 6 h, the reaction mixture was diluted with EtOAc (50 mL) and quenched with saturated NH_4Cl and H_2O. The solution mixture was extracted with EtOAc (3 × 50 mL). The combined organic layer was washed with brine (20 mL), dried over sodium sulfate anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography (20% EtOAc/*n*-hexane) to give 12-TBS-14-deoxy-3,19-isopropylideneandrographolide (**2n**) in 57% yield (285 mg) as a white solid, R_f 0.64 (20% EtOAc/*n*-hexane).

12-TBS-14-deoxy-3,19-isopropylideneandrographolide (**2n**): Mp 118–120 °C; IR (Neat): 2944, 1753, 1456, 1074, 842 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.27 (1H, brs, H-14), 4.81 (1H, brs, H-17b), 4.77 (1H, brs, H-15b), 4.74 (1H, s, H-15a), 4.65–4.60 (1H, m, H-12), 4.59 (1H, brs, H-17a), 3.93 (1H, d, $J = 11.5$ Hz, H-19b), 3.47 (1H, dd, $J = 8.5$, 3.5 Hz, H-3), 3.14 (1H, d, $J = 11.5$ Hz, H-19a), 2.35 (1H, dm, $J = 13.0$ Hz, H-7b), 2.00–1.65 (7H, m), 1.40 (3H, s, H-21), 1.36 (3H, s, H-22), 1.25–1.15 (4H, m), 1.17 (3H, s, H-18), 0.89 (9H, s, SiC(CH_3)$_3$), 0.88 (3H, s, H-20), 0.07 (3H, s, SiCH_3), −0.02 (3H, s, SiCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.75, 148.33, 145.22, 138.42, 107.25, 99.15, 76.15, 70.29, 66.93, 63.99, 52.15, 51.18, 38.43, 38.03, 37.96, 33.86, 31.77, 26.93, 25.94, 25.80 (3×C), 25.18, 24.77, 23.51, 18.06, 16.75, −4.79, −4.87.

Synthesis of 12-TBS-14-deoxyandrographolide (**2o**) 12-TBS-14-deoxy-3,19-isopropylideneandrographolide (**2n**) (43.0 mg, 0.085 mmol) was dissolved in a solution of CH_3COOH/H_2O (7:3) (1 mL). The reaction was stirred at room temperature for 15 min. After the reaction was completed conversion, identified by TLC, the reaction mixture was diluted with EtOAc (10 mL) and quenched with saturated $NaHCO_3$. The solution mixture was extracted with EtOAc (3 × 20 mL). The combined organic layer was washed with brine (10 mL), dried over sodium sulfate anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography (20% EtOAc) to give 12-TBS-14-deoxyandrographolide (**2o**) in 72% yield (28.6 mg) as a white solid, R_f 0.07 (20% EtOAc/*n*-hexane).

12-TBS-14-deoxyandrographolide (**2o**): Mp 146–148 °C; IR (Neat): 3139, 1746, 1404, 1248, 1045, 890 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26 (1H, d, $J = 1.0$ Hz, H-14), 4.80 (1H, brs, H-17b), 4.77 (1H, t, $J = 1.5$ Hz, H-12), 4.75 (1H, brs, H-17a), 4.57 (2H, brs, H-15), 4.14 (1H, d, $J = 11.0$ Hz, H-19b), 3.43 (1H, dd, $J = 10.0$, 5.0 Hz, H-3), 3.28 (1H, d, $J = 11.0$ Hz, H-19a), 2.36 (1H, dm, $J = 12.0$ Hz, H-7b), 1.91–1.74 (7H, m), 1.60 (1H, t, $J = 5.0$ Hz), 1.30–1.02 (3H, m), 1.22 (3H, s, H-18), 0.87 (9H, s, SiC(CH_3)$_3$), 0.59 (3H, s, H-20), 0.06 (3H, s, SiCH_3), −0.04 (3H, s, SiCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.62, 147.93, 145.32, 138.48, 107.15, 80.66, 70.21, 67.01, 64.15, 55.41, 51.49, 42.92, 39.07, 38.07, 36.78, 31.88, 28.24, 25.78 (3 × C), 24.03, 22.59, 18.02, 15.32, −4.71, −4.80.

Synthesis of 3,19-Ac-12-TBS-14-deoxyandrographolide (**2p**) To a stirred solution of 12-TBS-14-deoxyandrographolide (**2o**) (15.0 mg, 0.032 mmol) in acetic anhydride (1.0 mL) was refluxed to 145 °C for 1 h. The reaction mixture was diluted with EtOAc (10 mL) and quenched with saturated $NaHCO_3$. The solution mixture was extracted with EtOAc (3 × 10 mL). The combined organic layer was washed with brine (10 mL), dried over sodium sulfate anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography (20% EtOAc/*n*-hexane) to give 3,19-Ac-12-TBS-14-deoxyandrographolide (**2p**) in 94% yield (16.5 mg) as a white solid, R_f 0.40 (20% EtOAc/*n*-hexane).

3,19-Ac-12-TBS-14-deoxyandrographolide (**2p**): Mp 108–110 °C; IR (Neat): 3132, 2959, 1743, 1402, 1248, 1043, 846 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28 (1H, d, $J = 1.0$ Hz, H-14), 4.84 (1H, brs, H-17b), 4.80–4.77 (2H, m, H-15), 4.61 (1H, brs, H-17a), 4.59–4.56 (1H, m, H-12), 4.55 (1H, dd, $J = 11.0$, 5.0 Hz, H-3), 4.33 (1H, d, $J = 11.5$ Hz, H-19b), 4.07 (1H, d, $J = 11.5$ Hz, H-19a), 2.37 (1H, dm, $J = 12.5$ Hz, H-7b), 2.03 (3H, s, COCH_3), 2.02 (3H, s, COCH_3), 1.92–1.64 (7H, m), 1.62–1.13 (4H, m), 0.99 (3H, s, H-18), 0.87 (9H, s, SiC(CH_3)$_3$), 0.68 (3H, s, H-20), 0.06 (3H, s, SiCH_3), −0.04 (3H, s, SiCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.63, 170.82, 170.53, 147.72, 145.44, 138.58, 107.16, 79.96, 70.24, 66.76, 64.73, 55.46, 51.53, 41.32, 39.14, 38.18, 36.80, 32.26, 25.77 (3×C), 24.90, 24.23, 22.55, 21.15, 21.02, 18.01, 14.69, −4.71, −4.79.

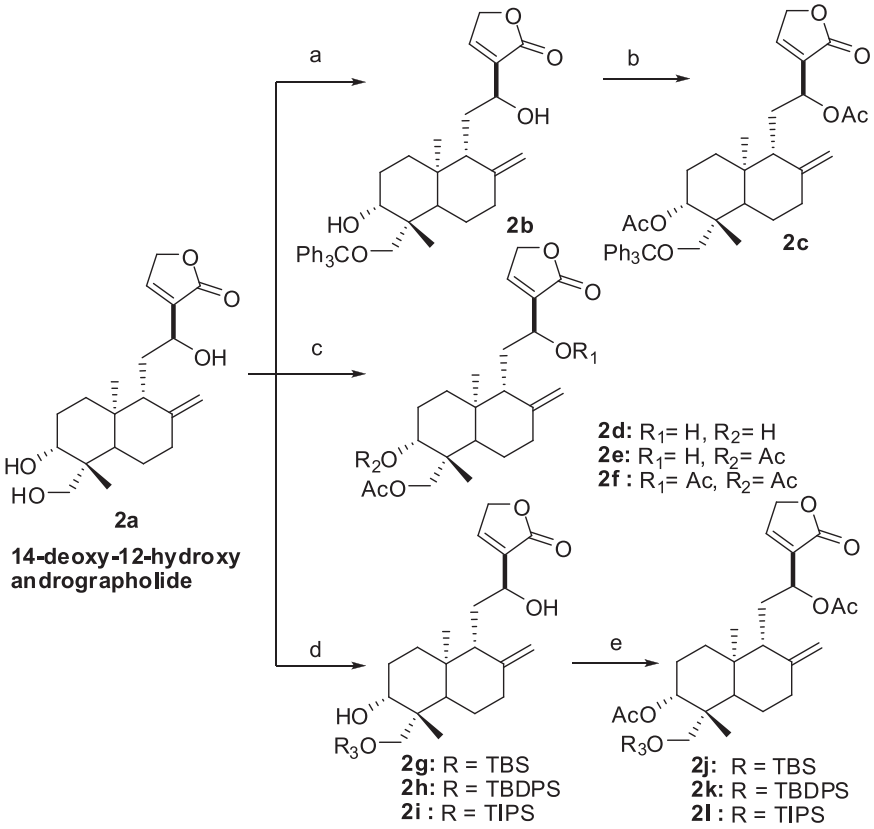
Evaluation of cytotoxic activity The cytotoxic activities of extracts and compounds were determined using the standard sulforhodamine B (SRB) assay in 96-well microtiter plates.

Ellipticine was used as a positive control. Six cell lines were employed, including P-388 (mouse lymphoid neoplasma), KB (human epidermoid carcinoma in the mouth), Col-2 (human colon cancer), MCF-7 (human breast cancer), Lu-1 (human lung cancer) and ASK (rat glioma).

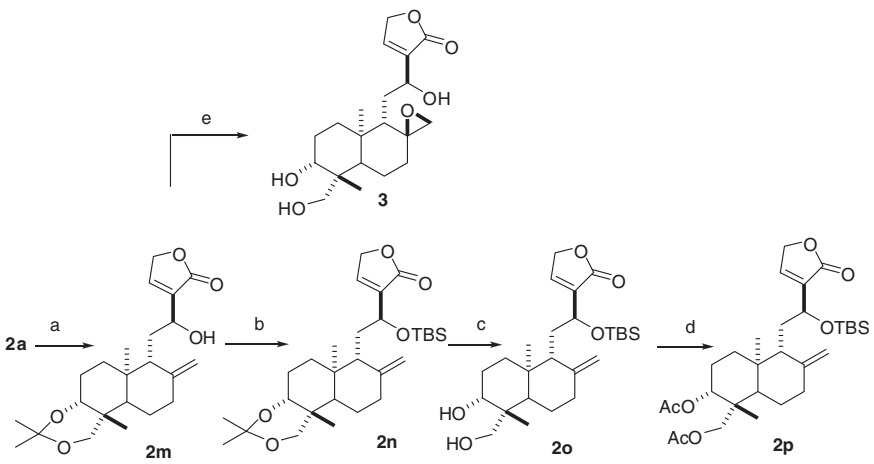
Results and discussion

Trityl (triphenylmethyl), acetyl or silyl groups such as *tert*-butyldimethylsilyl (TBS), *tert*-butyldiphenylsilyl (TBDPS) and triisopropylsilyl (TIPS) were introduced to the 14-deoxy-12-hydroxyandrographolide analog **2a** as shown in Scheme 1. Compounds **2b**, **2d** and **2g–2i** were obtained in moderate to high yields from reactions of compound **2a** and pyridine in the presence of TrCl, acetic anhydride and trialkylsilyl-chloride. Acetylation of the resulting analogs of hydroxyl groups at C-3 and/or C-12 led to the new compounds **2c**, **2e**, **2f** and **2j–2l** in high yields (Kandanur et al. 2015).

Compounds **2m–2p** were prepared from **2a** (Scheme 2). Conversion of hydroxyl groups at C-3 and C-19 with

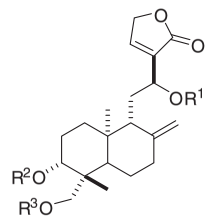


Scheme 1 Reagents and conditions: (**a**) TrCl, pyridine, 80 °C, 6 h, 72%; (**b**) Ac_2O, 145 °C, 3 h, 80%; (**c**) Ac_2O, 70 °C, 2 h, total 71%; (**d**) TBSCl, pyridine, rt, 1 h, (**2g**) 80%; TBDPSCl; pyridine, rt, 1 h, (**2h**) 82%; TIPSCl, pyridine, rt, 8 h, (**2i**) 67%; (**e**) Ac_2O, 145 °C, (**2j**) 81%; (**2k**) 70%; (**2l**) 63%



Scheme 2 Reagents and conditions: (**a**) 2,2-dimethoxypropane, PPTS, acetone, rt, 2 h, 82%; (**b**) TBSCl, imidazole, DMF, rt, 6 h, 57%; (**c**) AcOH/H_2O (7:3), rt, 15 min, 72%; (**d**) Ac_2O, 145 °C, 1 h, 94%; (**e**) mCPBA, CH_2Cl_2/MeOH, 60%

Table 1 Cytotoxic activity of 14-deoxy-12-hydroxyandrographolide analogs as μM causing 50% growth inhibition against six human cancer cells



Compd.				ED_{50} (μM) (SRB assay)						ED_{50} ratios relative to the **2a**
no.	R^1	R^2	R^3	P-388	KB	COL-2	MCF-7	LU-1	ASK	
2a	14-deoxy-12-hydroxyandrographolide			3.65 ± 0.18	68.37 ± 0.08	41.92 ± 3.12	73.56 ± 1.67	40.96 ± 1.26	71.26 ± 2.12	1
3	Epoxy-14-deoxy-12-hydroxyandrographolide			>100	>100	>100	>100	>100	>100	–
2b	H	H	CPh_3	2.51 ± 0.07	3.30 ± 0.01	3.74 ± 0.03	4.51 ± 0.09	3.22 ± 0.01	3.19 ± 0.01	1.4–22.3
2c	Ac	Ac	CPh_3	0.74 ± 0.04	3.98 ± 0.24	3.33 ± 0.07	8.26 ± 0.13	5.93 ± 0.05	4.77 ± 0.12	4.9–17.2
2d	H	H	Ac	1.91 ± 0.03	52.06 ± 0.44	36.69 ± 1.02	59.64 ± 2.83	38.44 ± 0.69	69.08 ± 5.35	1.0–1.9
2e	H	Ac	Ac	0.34 ± 0.01	41.26 ± 1.94	17.95 ± 0.05	18.53 ± 0.54	15.07 ± 0.28	34.24 ± 3.00	1.7–4.0
2f	Ac	Ac	Ac	5.80 ± 0.38	>100	77.86 ± 2.33	79.78 ± 0.06	61.35 ± 0.61	59.96 ± 1.29	0–1.2
2g	H	H	TBS	>100	>100	>100	>100	>100	29.71 ± 0.61	–
2h	H	H	TBDPS	2.63 ± 0.02	3.99 ± 0.03	6.01 ± 0.14	5.18 ± 0.03	3.36 ± 0.02	3.28 ± 0.007	1.4–21.7
2i	H	H	TIPS	2.68 ± 0.05	4.36 ± 0.05	5.12 ± 0.05	5.48 ± 0.14	3.55 ± 0.09	3.39 ± 0.04	1.4–21.0
2j	Ac	Ac	TBS	0.55 ± 0.03	2.35 ± 0.03	2.00 ± 0.18	2.13 ± 0.04	2.09 ± 0.02	1.98 ± 0.04	6.6–35.0
2k	Ac	Ac	TBDPS	2.24 ± 0.19	6.80 ± 0.05	4.75 ± 0.09	8.58 ± 0.04	5.55 ± 0.10	9.62 ± 0.01	1.6–10.1
2l	Ac	Ac	TIPS	1.75 ± 0.12	6.85 ± 0.03	4.77 ± 0.17	9.13 ± 0.001	6.87 ± 0.03	7.55 ± 0.02	2.1–10.1
2m	H			3.35 ± 0.27	66.80 ± 1.04	43.6 ± 6.00	55.47 ± 6.54	42.81 ± 2.78	60.81 ± 2.37	1.0–1.2
2n	TBS			11.39 ± 0.61	58.79 ± 1.07	>100	83.30 ± 1.60	47.49 ± 1.10	>100	–
2o	TBS	H	H	15.38 ± 0.47	>100	>100	>100	>100	0.14	–
2p	TBS	Ac	Ac	2.36 ± 0.04	7.25 ± 0.33	15.45 ± 0.15	11.73 ± 0.17	8.73 ± 0.04	0.5–3.8	1.5–9.4
1	Andrographolide			2.25 ± 0.19	27.37 ± 1.05	13.6 ± 0.41	15.4 ± 0.21	12.98 ± 0.15	16.18 ± 0.27	1.6–4.8
Ellipticine				2.44 ± 0.17	2.46 ± 0.11	2.72 ± 0.07	2.71 ± 0.21	1.62 ± 0.01	3.56 ± 0.37	

[a] Cell lines used are P-388 (murine leukemia cell line); KB (human epidermoid carcinoma of the mouth); COL-2 (human colon cancer); MCF-7 (human breast cancer); LU-1 (human lung cancer); and ASK (rat glioma). Ellipticine was used as a positive control. Results are expressed as ED_{50}, μM. Each value represents mean ± SE from three different experiments performed in triplicate

acetonide afforded **2m** in high yields. Silylation of the hydroxyl group at C-12 generated TBS-ether **2n**, which was then treated with acetic acid solution for deprotection of acetonide to produce **2o**, followed by acetylation to generate compound **2p**. Epoxidation and deprotection of acetonide group of **2m** with MCPBA produced epoxy derivative **3**.

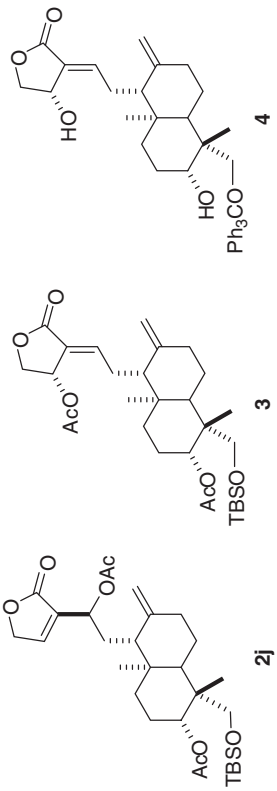
Cytotoxic activity of andrographolide, the parent compound **1**, 14-deoxy-12-hydroxyandrographolide **2a** and its synthetic analogs were evaluated in vitro against six cancer cell lines; P-388 (murine leukemia cell line), KB (human epidermoid carcinoma of the mouth), COL-2 (human colon cancer), MCF-7 (human breast cancer), LU-1 (human lung cancer) and ASK (rat glioma) using SRB assay. All tested analogs were dissolved in DMSO (0.1%). Ellipticine, a potent anti-cancer agent was used as a positive control.

Comparative cytotoxicities of 14-deoxy-12-hydroxyandrographolide **2a** and andrographolide **1**, found **2a** to have the lower activity in terms of the concentration, μM, causing 50% growth inhibition (ED_{50}; Table 1). Significant improvement in cytotoxic activity of **2a** occurred when a trityl or silyl-ether group such as TBDPS and TIPS was introduced at C-19 of **2a**. Interestingly, analogs **2b**, **2h** and **2i** demonstrated 9–20- fold increases in cytotoxicity in all cell lines except on P-388 cancer cells, relative to that of the parent **2a**. Moreover, these three compounds exhibited greater toxicity against ASK cancer cells than the reference drug, ellipticin. These results suggested that substitution at C-19 played a critical role in the enhanced activity and might be an effective moiety to improve cytotoxic potency of the scaffold. Epoxidation of **2a** to obtain **3** led to a dramatic increase in cytotoxicity.

Introduction of acetyl group to the C-19 position of **2a** to generate **2d** lowered cytotoxicity relative to those with trityl-analogs and silyl-analogs, but demonstrated better selectivity on P-388 cancer cells than other analogs in these series. Addition of acetyl group to C-3 to produce **2e**, gave similar activity to that of **2d**. Analog **2g** with TBS-ether at C-19 showed almost no activity in all cell lines in accord with **2o**, which contained TBS-ether at C-14. Interestingly, when acetyl groups were introduced at the remaining hydroxyl groups of **2g** and **2o** to give analogs **2j** and **2p**, there was significant improvement in cytotoxic activity. Cytotoxicity of **2j** was highest in this series, indeed 6–35 times greater than that of the corresponding parent **2a** and much stronger than that of ellipticin in all cell lines except LU-1. Interestingly, introduction of two acetyl groups at C-3 and C-14 of trityl-analog **2b** to give analog **2c**, silyl analog **2h** and **2i** to generate analogs **2k** and **2l**, did not improve cytotoxicity compared to those without acetyl in contrast to the response to **2j**. Further, acetonide moiety on **2m** and **2n** dramatically decreased cytotoxicity for all cell lines.

Our previous work (Sirion et al. 2012), indicates 14-deoxy-12-hydroxyandrographolide analog **2j** showed better

Table 2 Comparison of cytotoxic activities of andrographolide (**1**) and 14-deoxy-12-hydroxyandrographolide (**2a**) analogs against six human cancer cell lines



Compd. no.	ED_{50} (μM)[a] (SRB assay)					
	P-388	KB	COL-2	MCF-7	LU-1	ASK
2j	0.55 ± 0.03	2.35 ± 0.03	2.00 ± 0.18	2.13 ± 0.04	2.09 ± 0.02	1.98 ± 0.04
3	0.34 ± 0.01	3.62 ± 0.14	2.23 ± 0.05	2.84 ± 0.17	2.92 ± 0.08	1.22 ± 0.14
4	0.45 ± 0.01	2.42 ± 0.03	2.73 ± 0.03	2.72 ± 0.01	0.88 ± 0.002	2.86 ± 0.02
Ellipticine	2.44 ± 0.17	2.46 ± 0.11	2.72 ± 0.07	2.71 ± 0.21	1.62 ± 0.01	3.56 ± 0.37

[a] Cell lines used are P-388 (murine leukemia cell line); KB (human epidermoid carcinoma of the mouth); COL-2 (human colon cancer); MCF-7 (human breast cancer); LU-1 (human lung cancer); and ASK (rat glioma). Ellipticine was used as a positive control. Results are expressed as ED_{50}, μM. Each value represents mean ± SE from three different experiments performed in triplicate

Table 3 The lipophilicity calculation data of 14-deoxy-12-hydroxyandrographolide analogs

Comp.	R^1	R^2	R^3	Lipophilicity LogP[a]
2a	14-deoxy-12-hydroxyandrographolide			0.81
3	Epoxy-14-deoxy-12-hydroxyandrographolide			0.13
2b	H	H	CPh_3	6.26
2c	Ac	Ac	CPh_3	7.67
2d	H	H	Ac	1.51
2e	H	Ac	Ac	2.21
2f	Ac	Ac	Ac	2.92
2g	H	H	TBS	4.13
2h	H	H	TBDPS	6.00
2i	H	H	TIPS	3.64
2j	Ac	Ac	TBS	5.54
2k	Ac	Ac	TBDPS	7.41
2l	Ac	Ac	TIPS	5.05
2m	H			2.70
2n	TBS			6.03
2o	TBS	H	H	4.13
2p	TBS	Ac	Ac	5.54
1	Andrographolide			1.05

[a] Lipophilicity was calculated using the Molinspiration online property calculation toolkit (http://www.molinspiration.com)

activity over the same substituted andrographolide analog (**3**) in all human cancer cell line except P-388 and ASK (Table 2). Compared with the most potent C-19, trityl-andrographolide analog (**4**) in the previous work, **2j** exhibited greater cytotoxicity than that of compound (**4**) in all human cancer cell line except on P-388 and LU-1.

The results of this study indicated that modification of hydroxyl groups of the 14-deoxy-12-hydroxyandrographolide scaffold in vitro yielded greater cancer toxicity potential than modifications of andrographolide scaffold **3** with the same substitutions. The presence of the *exo*-alkene between C-12 and C-13 of α-alkylidene-χ-butyrolactone moiety of andrographolide was not important to cytotoxicity contrary to earlier reports (Das et al. 2010; He et al. 2003). Cytotoxicity found for the major andrographolide and minor analogs **2a** seems directly dependent on their lipophilicity by introducing suitable substitution groups at C-3, C-14 and C-19 of andrographolide based compounds. As shown in Table 3, lipophilicity calculations suggested that activity of high activity compounds such as **2b**, **2h** and **2i** increased directly with LogP.

In summary, we have synthesized a series of 14-deoxy-12-hydroxyandrographolide analogs and examined the cytotoxicity of this scafford as compared to the corresponding parent compound and the reference anti cancer agent, ellipticin. An evaluation of the synthetic analogs indicated the position and the types of substitutions at C-19, C-3 and C-14 of 14-deoxy-12-hydroxyandrographolide-scaffold that played an important role in cytotoxic activity. Increasing lipophilicity by introducing of *tert*-butyl-dimethylsilyl group at C-19 and acetyl at C-3 and C-14 led to compound (**2j**) which is the most active compound in this series. It exhibited the highest activity, 6–35 times higher than that of the natural parent compound and much stronger than that of ellipticin for all cell lines except LU-1. With elevated cytotoxities and simple preparation, the new synthetic 14-deoxy-12-hydroxyandrographolide derivatives of the minor component from *A. paniculata* may well serve as a new class of anticancer compounds.

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Conflict of interest The authors declare that they have no conflict of interest.

References

Balmain A, Connolly JD (1973) Minor diterpenoid constituents of *Andrographis paniculata Nees*. J Chem Soc Perkin Trans 1:1247–1251

Calabrese C, Berman SH, Babish JG, Ma X, Shinto L, Dorr M, Wells K, Wenner CA, Standish LJ (2000) A phase I trial of andrographolide in HIV positive patients and normal volunteers. Phytother Res 14:333–338

Chen D, Song Y, Lu Y, Xue X (2013) Synthesis and in vitro cytotoxicity of andrographolide-19-oic acid analogues as anti-cancer agents. Bioorg Med Chem Lett 23:3166–3169

Chen L, Zhu H, Wang R, Zhou K, Jing Y, Qiu F (2008) ent-Labdane diterpenoid lactone stereoisomers from *Andrographis paniculata*. J Nat Prod 71:852–855

Dai GF, Xu HW, Wang JF, Liu FW, Liu HM (2006) Studies on the novel α-glucosidase inhibitory activity and structure–activity relationships for andrographolide analogues. Bioorg Med Chem Lett 16:2710–2713

Das B, Chowdhury C, Kumar D, Sen R, Roy R, Das P, Chatterjee M (2010) Synthesis, cytotoxicity, and structure–activity relationship (SAR) studies of andrographolide analogues as anti-cancer agent. Bioorg Med Chem Lett 20:6947–6950

Fan QQ, Wang QJ, Zeng BB, Wu YL, Ji H (2010) Synthesis and antitumor effect of novel andrographolide derivative. J China Pharm Univ 41:326–332

Handa SS, Sharma A (1990) Hepatoprotective activity of andrographolide against galactosamine and paracetamol intoxication in rats. Ind J Med Res 92:276–283

He X, Li J, Gao H, Qiu F, Hu K, Cui X, Yao X (2003) Four new andrographolide metabolites in rats. Tetrahedron 59:6603–6607

Jada SR, Hamzah AS, Lajis NH, Saad MS, Stevens MFG, Stanslas J (2006) Semisynthesis and cytotoxic activities of andrographolide analogues. J Enzyme Inhib Med Chem 21:145–155

Jada SR, Subur GS, Matthews C, Hamzah AS, Lajis NH, Saad MS, Stevens MFG, Stanslas J (2007) Semisynthesis and in vitro anticancer activities of andrographolide analogues. Phytochemistry 68:904–912

Kandanur SGS, Golakoti NR, Nanduri S (2015) Synthesis and in vitro cytotoxicity of novel C-12 substituted-14-deoxy-andrographolide derivatives as potent anti-cancer agents. Bioorg Med Chem Lett 25:5781–5786

Kasemsook S, Sirion U, Suksen K, Piyachaturawat P, Suksamrarn A, Saeeng R (2013) 12-Amino-andrographolide analogues: synthesis and cytotoxic activity. Arch Pharm Res 36:1454–1464

Kleipool RJC (1952) Constituenis of *Andrographis paniculata* Nees. Nature 169:33–34

Kumar RA, Sridevi K, Kumar NV, Nanduri S, Rajagopal S (2004) Anticancer and immunostimulatory compounds from *Andrographis paniculata*. J Pharmacol 92:291–295

Li J, Huang W, Zhang H, Wang X, Zhou H (2007) Synthesis of andrographolide derivatives and their TNF-γ and IL-6 expression inhibitory activities. Bioorg Med Chem Lett 17:6891–6894

Matsuda T, Kuroyanaki M, Sukiyama S, Umehara K (1994) Cell differentiation-inducing diterpenes from *Andrographis paniculata Nees*. Chem Pharm Bull 42:1216–1225

Misra P, Pal NL, Guru PY, Katiyar JC, Srivastava V, Tandon JS (1992) Antimalarial activity of *Andrographis paniculata* (kalmegh) against *Plasmodim berghei* NK 65 in *Mastomys natalensis*. Int J Pharmacogn 30:263–274

Nateewattana J, Saeeng R, Kasemsook S, Suksen K, Dutta S, Jariyawat S, Chairoungdua A, Suksamrarn A, Piyachaturawat P (2013) Inhibition of topoisomerase II α activity and induction of apoptosis in mammalian cells by semi-synthetic andrographolide analogues. Invest New Drugs 31:320–332

Reddy VL, Reddy SM, Ravikanth V, Krishnaiah P, Goud TV, Rao TP, Ram TS, Gonnade RG, Bhadbhade M, Venkateswarlu Y (2005) New bis-andrographolide ether from *Andrographis paniculata Nees* and evaluation of anti-HIV activity. Nat Prod Res 19:223–230

Shen YH, Li RT, Xiao WL, Xu G, Lin ZW, Zhao QS, Sun HD (2006) ent-Labdane diterpenoids from *Andrographis paniculata*. J Nat Prod 69:319–322

Singha PK, Roy S, Dey S (2003) Antimicrobial activity of *Andrographis paniculata*. Fitoterapia 74:692–694

Sirion U, Kasemsook S, Suksen K, Piyachaturawat P, Suksamrarn A, Saeeng R (2012) New substituted C-19-andrographolide analogues with potent cytotoxic activities. Bioorg Med Chem Lett 22:49–52

Siripong P, Kongkathip B, Preechanukool K, Picha P, Tunsuwan K, Taylor WC (1992) Cytotoxic diterpenoid constituents from Andrographis paniculata, Nees leaves. J Sci Soc Thailand 18:187–194

Thunguntla SSR, Nyavanandi VK, Nanduri S (2004) A facile route for the synthesis of limonidilactone analogues from andrographolide. Tetrahedron Lett 45:9357–9360

Wang B, Li J, Huang WL, Zhang HB, Qian H, Zheng YT (2011) Synthesis and biological evaluation of andrographolide derivatives as potent anti-HIV agents. Chin Chem Lett 22:781–784

Xu HW, Dai GF, Liu GZ, Wang JF, Liu HM (2007) Synthesis of andrographolide derivatives: a new family of alpha-glucosidase inhibitors. Bioorg Med Chem 15:4247–4255