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RESEARCH ARTICLE

12-Amino-andrographolide analogues: synthesis and cytotoxic activity

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Abstract Andrographolide, a diterpenoid lactone of the plant *Andrographis paniculata*, has been shown to be cytotoxic against various cancer cells in vitro. In the present study, a series of β-amino-γ-butyrolactone analogues has been synthesized from naturally occurring andrographolide via one pot tandem aza-conjugate addition–elimination reaction. By using economic procedure without any base or catalyst at room temperature, the products obtained were in fair to excellent yields with high stereoselectivity. The cytotoxicity of all new amino analogues were evaluated against six cancer cell lines and revealed their potential for being developed as promising anticancer agents.

Keywords 12-Amino-andrographolide · Cytotoxic activity · *Andrographis paniculata* · Tandem aza-conjugate addition–elimination

Introduction

Andrographis paniculata Nees is a traditional medicinal herb in many Asian countries which is known to possess a variety of pharmacological activities (Matsuda et al. 1994; Sharma et al. 1992). Andrographolide, the major labdane diterpene from this herb has been implicated to be responsible for the treatment quality of *A. paniculata.* Andrographolide exhibits various pharmacological properties such as antibacterial (Singha et al. 2003), antihepatotoxic (Handa and sharma 1990), anti-HIV (Calabrese et al. 2000), anticancer (Matsuda et al. 1994; Reddy et al. 2005; Siripong et al. 1992), hypoglycemic and hypotensive activities (Kumar et al. 2004; Dai et al. 2006; Kameda et al. 1984). Improving the biological activity of andrographolide with high efficacy and potency by structural modification has been received attention in these recent years (Xu et al. 2007; Li et al. 2007; Jiang et al. 2009). The synthetic andrographolide analogues have been reported to exhibit various biological activities, especially with regard to cytotoxic activity (Wang et al. 2010, 2011; Nanduri et al. 2004a, b; Jada et al. 2006, 2009; Das et al. 2010; Xu and Wang 2011; Fan et al. 2010a, b; Sirion et al. 2012). Some of the synthetic analogues were reported previously by our group (Sirion et al. 2012), possess diverse cytotoxic activities with variety of substitutions. C-19-silyl and trityl-analogues of andrographolide showed a significant high activity (Fig. 1). These analogues are simply obtained through synthesis in only 1 or 2 steps in excellent yields. In fact, a few andrographolide analogues have been reported to possess potent activity over anticancer drug in clinic. In our continuing efforts to search for potent synthetic compounds from naturally occurring source, we have developed new andrographolide-based compounds with potent biological activity to improve the therapeutic anticancer potential of andrographolide.

In the present study, we explored the modification of andrographolide via tandem aza-conjugate addition–elimination reaction leading to 12-amino-andrographolide

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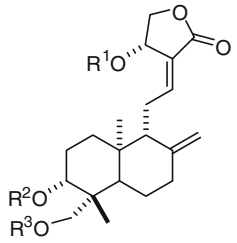


R^1= H, R^2 = H, R^3 = CPh_3 ; potent cytotoxicity against P-388 and LU-1 cell lines
R^1= H, R^2 = H, R^3 = TBS ; potent cytotoxicity against P-388 and ASK cell lines

Fig. 1 The synthetic andrographolide-based compounds possess diverse cytotoxic activities with variety substitutions (Sirion et al. 2012)

analogues (Fan et al. 2010a, b). These synthesized compounds were evaluated for their in vitro cytotoxic activities. Various anilines with different substitution groups were introduced into the andrographolide analogue **3** to afford new C-12-amino andrographolide analogues **4a–o** in order to investigate their preliminary structure–activity relationships.

Materials and methods

Plant material, extraction and isolation

Andrographolide (**1**) was isolated according to the reported procedure (Matsuda et al. 1994) from dried aerial part of *A. paniculata* plant which harvested from Pak Tho district, Ratchaburi province, Thailand. The structure of andrographolide obtained from the plant extract was identified based on ^{1}H-NMR spectral data comparing with commercial andrographolide (purchased from Sigma-Aldrich, CAS No. 5588-58-7).

Synthesis

All chemical reagents were obtained from chemical companies and used without further purification. Proton NMR spectra were recorded on a Bruker AVANC (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 parts per million (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.

General procedure for the synthesis of amino andrographolide analogues (**4a–o**)

To a stirred solution of 19-TBS-3,14-Ac-andrographolide (**3**) (0.091 mmol) in methanol (1.0 mL) was added amine compound (0.137 mmol) at room temperature. After the stirring was continued at room temperature for 0.5–72 h, the reaction mixture was quenched carefully with cooled NH_4Cl(aq) (10 mL), extracted with EtOAc (3 × 10 mL). The combined organic layer was washed with water (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc/*n*-hexane) to afford the corresponding product (**4a–o**).

*12-(Phenyl)amino-19-TBS-3-Ac-14-deoxy andrographolide (**4a**)*

According to the general procedure, 19-TBS-3,14-Ac-andrographolide (**3**) (53.9 mg, 0.098 mmol) with aniline (18.8 mg, 0.202 mmol) was stirred at room temperature for 4 h. The residue was purified by column chromatography (30 % EtOAc/*n*-hexane) to afford the corresponding product **4a** in 79 % yield (45.4 mg) as a brown solid, R_f 0.56 (30 % EtOAc/*n*-hexane). Mp 77–81 °C; IR (neat, ν_{max}): 3108, 2946, 1745, 1604, 1504, 1400, 1252, 1094, 849 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.20 (1H, brs, H-14), 7.13 (2H, dd, J = 8.0, 7.5 Hz, Ar–H), 6.70 (1H, t, J = 7.5 Hz, Ar–H), 6.54 (2H, d, J = 8.0 Hz, Ar–H), 4.94 (1H, brs, H-17b), 4.81 (1H, d, J = 18.0 Hz, H-15b), 4.74 (1H, dd, J = 18.0, 1.5 Hz, H-15a), 4.72 (1H, brs, H-17a), 4.57–4.51 (1H, m, H-3), 4.30 (1H, dd, J = 9.5, 5.5 Hz,

H-12), 3.81 (1H, d, $J = 10.5$ Hz, H-19b), 3.57 (1H, d, $J = 10.5$ Hz, H-19a), 2.42 (1H, dm, $J = 14.0$ Hz), 2.14 (1H, dd, $J = 14.0$, 9.5 Hz), 2.04 (3H, s, $COCH_3$), 1.91–1.78 (5H, m), 1.73-1.64 (3H, m), 1.51 (1H, d, $J = 10.0$ Hz), 1.21–1.09 (2H, m), 0.92 (3H, s, H-18), 0.88 (9H, s, $SiC(CH_3)_3$), 0.77 (3H, s, H-20), 0.01 (6H, s, $Si(CH_3)_2$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 172.63, 170.75, 148.56, 146.54, 134.32, 129.24, 118.11, 113.82, 107.10, 80.16, 70.09, 63.64, 55.61, 53.69, 50.43, 42.49, 39.37, 38.77, 37.02, 28.50, 25.85, 25.62, 24.30, 23.03, 21.26, 18.19, 14.49, −5.66, −5.74; HRMS (ESI) *m/z* calcd for $C_{34}H_{51}NO_5SiNa$ $[M+Na]^+$ 604.3434, found 604.3389.

*12-((2-Fluoro)phenyl)amino-19-TBS-3-Ac-14-deoxyandrographolide (**4b**)*

According to the general procedure, 19-TBS-3,14-Ac-andrographolide (**3**) (52.1 mg, 0.095 mmol) with 2-fluoroaniline (16.0 mg, 0.144 mmol) was stirred at room temperature for 48 h. The residue was purified by column chromatography (20 % EtOAc/*n*-hexane) to afford the corresponding product **4b** in 96 % yield (54.9 mg) as a brown solid, R_f 0.48 (20 % EtOAc/*n*-hexane). Mp 73–75 °C; IR (neat, ν_{max}): 2952, 1746, 1624, 1516, 1250, 1094, 846 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.22 (1H, brs, H-14), 6.98–6.87 (2H, m, Ar–H), 6.66–6.58 (1H, m, Ar–H), 6.55–6.46 (1H, m, Ar–H), 4.95 (1H, brs, H-17b), 4.82 (1H, d, $J = 18.0$ Hz, H-15b), 4.76 (1H, dd, $J = 18.0$, 1.5 Hz, H-15a), 4.71 (1H, brs, H-17a), 4.57–4.50 (1H, m, H-3), 4.33–4.25 (1H, m, H-12), 3.81 (1H, d, $J = 10.5$ Hz, H-19b), 3.57 (1H, d, $J = 10.5$ Hz, H-19a), 2.42 (1H, dm, $J = 14.0$ Hz), 2.17 (1H, dd, $J = 14.0$, 9.5 Hz), 2.04 (3H, s, $COCH_3$), 1.94–1.77 (5H, m), 1.74–1.60 (3H, m), 1.55 (1H, d, $J = 10.0$ Hz), 1.28–1.10 (2H, m), 0.92 (3H, s, H-18), 0.88 (9H, s, $SiC(CH_3)_3$), 0.77 (3H, s, H-20), 0.01 (6H, s, $Si(CH_3)_2$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 172.50, 170.73, 151.75, 148.63, 146.40, 135.00, 134.71, 124.41, 117.54, 114.78, 113.30, 107.03, 80.20, 70.10, 63.65, 55.65, 53.68, 50.20, 42.53, 39.43, 38.71, 37.06, 28.91, 25.86, 25.62, 24.32, 23.05, 21.23, 18.21, 14.49, −5.56, −5.57; HRMS (ESI) *m/z* calcd for $C_{34}H_{50}FNO_5SiNa$ $[M+Na]^+$ 622.3340, found 622.3286.

*12-((3-Fluoro)phenyl)amino-19-TBS-3-Ac-14-deoxyandrographolide (**4c**)*

According to the general procedure, 19-TBS-3,14-Ac-andrographolide (**3**) (52.5 mg, 0.096 mmol) with 3-fluoroaniline (15.5 mg, 0.139 mmol) was stirred at room temperature for 7 h. The residue was purified by column chromatography (30 % EtOAc/*n*-hexane) to afford the corresponding product **4c** in 67 % yield (38.7 mg) as a brown solid, R_f 0.46 (30 % EtOAc/*n*-hexane). Mp 73–75 °C; IR (neat, ν_{max}): 3119, 1742, 1403, 1243, 1093, 846 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.22 (1H, brs, H-14), 7.05 (1H, td, $J = 7.5$, 7.0 Hz, Ar–H), 6.38 (1H, td, $J = 7.0$, 2.0 Hz, Ar–H), 6.31 (1H, dd, $J = 7.5$, 1.5 Hz, Ar–H), 6.22 (1H, dt, $J = 11.2$, 1.5 Hz, Ar–H), 4.95 (1H, brs, H-17b), 4.84 (1H, d, $J = 18.0$ Hz, H-15b), 4.76 (1H, dd, $J = 18.0$, 1.5 Hz, H-15a), 4.71 (1H, brs, H-17a), 4.57–4.51 (1H, m, H-3), 4.26 (1H, dd, $J = 9.5$, 5.5 Hz, H-12), 3.81 (1H, d, $J = 10.5$ Hz, H-19b), 3.57 (1H, d, $J = 10.5$ Hz, H-19a), 2.42 (1H, dm, $J = 14.0$ Hz), 2.14 (1H, dd, $J = 14.0$, 9.5 Hz), 2.04 (3H, s, $COCH_3$), 1.91–1.78 (5H, m), 1.74–1.64 (3H, m), 1.49 (1H, d, $J = 10.4$ Hz), 1.21–1.08 (2H, m), 0.92 (3H, s, H-18), 0.87 (9H, s, $SiC(CH_3)_3$), 0.77 (3H, s, H-20), 0.01 (6H, s, $Si(CH_3)_2$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 172.45, 170, 163.93, 148.48, 148.40, 146.68, 133.83, 130.38, 109.62, 107.11, 104.52, 100.35, 80.11, 70.12, 63.65, 55.62, 53.67, 50.41, 42.48, 39.35, 38.77, 37.03, 28.33, 25.84, 25.62, 24.28, 23.03, 21.25, 18.18, 14.50, −6.45, −6.65; HRMS (ESI) *m/z* calcd for $C_{34}H_{50}FNO_5SiNa$ $[M+Na]^+$ 622.3340, found 622.3279.

*12-((4-Fluoro)phenyl)amino-19-TBS-3-Ac-14-deoxyandrographolide (**4d**)*

According to the general procedure, 19-TBS-3,14-Ac-andrographolide (**3**) (51.8 mg, 0.094 mmol) with 4-fluoroaniline (16.1 mg, 0.144 mmol) was stirred at room temperature for 7 h. The residue was purified by column chromatography (30 % EtOAc/*n*-hexane) to afford the corresponding product **4d** in 78 % yield (44.2 mg) as a brown solid, R_f 0.51 (30 % EtOAc/*n*-hexane). Mp 69–71 °C; IR (neat, ν_{max}): 3138, 1750, 1627, 1519, 1404, 1252, 1095, 848 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.17 (1H, brs, H-14), 6.79–6.86 (2H, m, Ar–H), 6.44–6.51 (2H, m, Ar–H), 4.94 (1H, brs, H-17b), 4.82 (1H, d, $J = 18.0$ Hz, H-15b), 4.73 (1H, dd, $J = 18.0$, 1.5 Hz, H-15a), 4.71 (1H, brs, H-17a), 4.53 (1H, dd, $J = 10.5$, 6.0 Hz, H-3), 4.22 (1H, dd, $J = 9.5$, 5.0 Hz, H-12), 3.81 (1H, d, $J = 10.5$ Hz, H-19b), 3.57 (1H, d, $J = 10.5$ Hz, H-19a), 2.41 (1H, dm, $J = 14.0$ Hz), 2.10 (1H, dd, $J = 14.0$, 9.5 Hz), 2.03 (3H, s, $COCH_3$), 1.91–1.77 (5H, m), 1.73–1.63 (3H, m), 1.48 (1H, d, $J = 10.0$ Hz), 1.21–1.04 (2H, m), 0.91 (3H, s, H-18), 0.87 (9H, s, $SiC(CH_3)_3$), 0.77 (3H, s, H-20), 0.01 (6H, s, $Si(CH_3)_2$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 172.54, 170.75, 156.14, 148.38, 146.72, 142.91, 134.10, 115.66, 115.06, 107.08, 80.10, 70.09, 63.62, 55.60, 53.64, 51.32, 42.45, 39.30, 38.76, 37.02, 28.40, 25.82, 25.60, 24.27, 23.01, 21.23, 18.16, 14.47, −6.40, −6.60; HRMS (ESI) *m/z* calcd for $C_{34}H_{50}FNO_5SiNa$ $[M+Na]^+$ 622.3340, found 622.3288.

*12-((4-Bromo)phenyl)amino-19-TBS-3-Ac-14-deoxyandrographolide (**4e**)*

According to the general procedure, 19-TBS-3,14-Ac-andrographolide (**3**) (52.1 mg, 0.095 mmol) with 4-bromoaniline (24.2 mg, 0.140 mmol) was stirred at room temperature for 4 h. The residue was purified by column chromatography (20 % EtOAc/*n*-hexane) to afford the corresponding product **4e** in 61 % yield (38.0 mg) as a yellow solid, R_f 0.48 (20 % EtOAc/*n*-hexane). Mp 81–83 °C; IR (neat, ν_{max}): 3100, 2962, 1747, 1598, 1403, 1254, 1089, 1023, 839, 437 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.20 (2H, d, $J = 9.0$ Hz, Ar–H), 7.18 (1H, brs, H-14), 6.41 (2H, d, $J = 9.0$ Hz, Ar–H), 4.94 (1H, brs, H-17b), 4.83 (1H, d, $J = 18.0$ Hz, H-15b), 4.74 (1H, dd, $J = 18.0$, 1.5 Hz, H-15a), 4.70 (1H, brs, H-17a), 4.54 (1H, dd, $J = 10.0$, 6.5 Hz, H-3), 4.28-4.20 (1H, m, H-12), 3.81 (1H, d, $J = 10.5$ Hz, H-19b), 3.57 (1H, d, $J = 10.5$ Hz, H-19a), 2.42 (1H, dm, $J = 13.5$ Hz), 2.11 (1H, dd, $J = 13.5$, 9.5 Hz), 2.04 (3H, s, COCH_3), 1.90–1.78 (5H, m), 1.73–1.63 (3H, m), 1.49 (1H, d, $J = 10.0$ Hz), 1.21–1.07 (2H, m), 0.91 (3H, s, H-18), 0.87 (9H, s, SiC(CH_3)$_3$), 0.77 (3H, s, H-20), 0.01 (6H, s, Si(CH_3)$_2$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 172.49, 170.77, 148.51, 146.69, 145.61, 133.92, 131.95, 115.45, 109.78, 107.08, 80.12, 70.12, 63.64, 55.62, 53.68, 50.56, 42.47, 39.35, 38.77, 37.03, 28.38, 25.84, 25.61, 24.28, 23.03, 21.25, 18.18, 14.49, −5.66, −5.75; HRMS (ESI) *m/z* calcd for $C_{34}H_{50}BrNO_5Si$ $[M+H]^+$ 662.2612, found 622.2618.

*12-((4-Methoxy)phenyl)amino-19-TBS-3-Ac-14-deoxyandrographolide (**4f**)*

According to the general procedure, 19-TBS-3,14-Ac-andrographolide (**3**) (50.6 mg, 0.092 mmol) with 4-methoxyaniline (18.7 mg, 0.152 mmol) was stirred at room temperature for 6 h. The residue was purified by column chromatography (30 % EtOAc/*n*-hexane) to afford the corresponding product **4f** in 79 % yield (53.4 mg) as a brown solid, R_f 0.40 (30 % EtOAc/*n*-hexane). Mp 73–75 °C; IR (neat, ν_{max}): 3474, 2942, 2863, 1743, 1630, 1520, 1465, 1244, 1093, 1023, 841 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.16 (1H, brs, H-14), 6.71 (2H, d, $J = 9.0$ Hz, Ar–H), 6.52 (2H, d, $J = 9.0$ Hz, Ar–H), 4.92 (1H, brs, H-17b), 4.80 (1H, d, $J = 18.0$ Hz, H-15b), 4.72 (1H, dd, $J = 18.0$, 1.5 Hz, H-15a), 4.71 (1H, brs, H-17a), 4.53 (1H, dd, $J = 10.5$, 6.0 Hz, H-3), 4.20 (1H, dd, $J = 9.5$, 5.0 Hz, H-12), 3.80 (1H, d, $J = 10.5$ Hz, H-19b), 3.71 (3H, s, OCH_3), 3.56 (1H, d, $J = 10.5$ Hz, H-19a), 2.40 (1H, dm, $J = 13.5$ Hz), 2.10 (1H, dm, $J = 13.5$ Hz), 2.04 (3H, s, COCH_3), 1.92–1.76 (5H, m), 1.74–1.63 (3H, m), 1.47 (1H, d, $J = 11.0$ Hz), 1.20–1.06 (2H, m), 0.90 (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 172.62, 170.71, 152.69, 148.33, 146.65, 145.00, 134.38, 115.77, 114.76, 107.11, 80.14, 70.05, 63.62, 60.33, 55.63, 53.65, 51.74, 42.47, 39.29, 38.76, 37.03, 28.44, 25.82, 25.60, 24.29, 23.00, 21.22, 18.16, 14.47, −5.70, −5.80; HRMS (ESI) *m/z* calcd for $C_{35}H_{53}NO_6SiNa$ $[M+Na]^+$ 634.3540, found 634.3480.

*12-((4-Hydroxy)phenyl)amino-19-TBS-3-Ac-14-deoxyandrographolide (**4g**)*

According to the general procedure, 19-TBS-3,14-Ac-andrographolide (**3**) (50.0 mg, 0.091 mmol) with 4-hydroxyl aniline (16.0 mg, 0.147 mmol) was stirred at room temperature for 3 h. The residue was purified by column chromatography (30 % EtOAc/*n*-hexane) to afford the corresponding product **4g** in 87 % yield (48.8 mg) as a brown solid, R_f 0.17 (30 % EtOAc/*n*-hexane). Mp 93–95 °C; IR (neat, ν_{max}): 3127, 1744, 1408, 1222, 1060, 794 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.14 (1H, brs, H-14), 6.63 (2H, d, $J = 9.0$ Hz, Ar–H), 6.46 (2H, d, $J = 9.0$ Hz, Ar–H), 4.92 (1H, brs, H-17b), 4.78 (1H, brd, $J = 18.0$ Hz, H-15b), 4.71 (1H, brs, H-17a), 4.68 (1H, brd, $J = 18.0$ Hz, H-15a), 4.53 (1H, dd, $J = 11.0$, 5.5 Hz, H-3), 4.23–4.15 (1H, m, H-12), 3.80 (1H, d, $J = 10.5$ Hz, H-19b), 3.57 (1H, d, $J = 10.5$ Hz, H-19a), 2.39 (1H, dm, $J = 13.5$ Hz), 2.11–2.02 (1H, m), 2.04 (3H, s, COCH_3), 1.92–1.76 (5H, m), 1.78–1.60 (3H, m), 1.46 (1H, d, $J = 10.5$ Hz), 1.35–1.05 (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 172.90, 171.03, 148.24, 147.04, 145.35, 140.28, 134.28, 116.13, 115.22, 107.16, 80.32, 70.18, 63.58, 55.57, 53.59, 51.85, 42.48, 39.27, 38.74, 37.00, 28.35, 25.83, 25.57, 24.29, 23.00, 21.26, 18.17, 14.48, −5.70, −5.80; HRMS (ESI) *m/z* calcd for $C_{34}H_{51}NO_6Si$ $[M+H]^+$ 598.3486, found 598.3480.

*12-((4-Methanol)phenyl)amino-19-TBS-3-Ac-14-deoxyandrographolide (**4h**)*

According to the general procedure, 19-TBS-3,14-Ac-andrographolide (**3**) (49.7 mg, 0.091 mmol) with (4-aminophenyl)methanol (16.8 mg, 0.136 mmol) was stirred at room temperature for 3 h. The residue was purified by column chromatography (30 % EtOAc/*n*-hexane) to afford the corresponding product **4h** in 79 % yield (44.0 mg) as a yellow solid, R_f 0.13 (30 % EtOAc/*n*-hexane). Mp 89–91 °C; IR (neat, ν_{max}): 3121, 3026, 1745, 1636, 1403, 1253, 1097, 1027, 849 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.18 (1H, brs, H-14), 7.12 (2H, d, $J = 8.5$ Hz, Ar–H), 6.51 (2H, d, $J = 8.5$ Hz, Ar–H), 4.94 (1H, brs, H-17b), 4.80 (1H, brd, $J = 18.0$ Hz, H-15b), 4.72 (1H, dd, $J = 18.0$, 1.5 Hz, H-15a), 4.71 (1H, brs, H-17a), 4.57–4.49 (1H, m, H-3), 4.51 (2H, s,

CH_2OH), 4.29 (1H, dd, J = 9.0, 5.5 Hz, H-12), 3.80 (1H, d, J = 10.5 Hz, H-19b), 3.57 (1H, d, J = 10.5 Hz, H-19a), 2.42 (1H, dm, J = 13.5 Hz), 2.12 (1H, dd, J = 13.5, 9.5 Hz), 2.03 (3H, s, COCH_3), 1.90–1.77 (5H, m), 1.73–1.63 (3H, m), 1.51 (1H, d, J = 10.0 Hz), 1.21–1.08 (2H, m), 0.91 (3H, s, H-18), 0.87 (9H, s, SiC(CH_3)$_3$), 0.77 (3H, s, H-20), 0.01 (6H, s, Si(CH_3)$_2$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 172.58, 170.75, 148.54, 146.57, 146.29, 134.24, 130.52, 128.73, 113.79, 107.07, 80.16, 70.07, 65.17, 63.62, 55.61, 53.67, 50.47, 42.48, 39.34, 38.75, 37.02, 28.50, 25.82, 25.59, 24.28, 23.01, 21.20, 18.16, 14.47, −5.69, −5.76; HRMS (ESI) *m/z* calcd for $C_{35}H_{53}NO_6Si$ $[M+H]^+$ 612.3676, found 612.3760.

*12-((4-Carboxyl)phenyl)amino-19-TBS-3-Ac-14-deoxyandrographolide (**4i**)*

According to the general procedure, 19-TBS-3,14-Ac-andrographolide (**3**) (50.0 mg, 0.091 mmol) with 4-aminobenzoic acid (19.0 mg, 0.139 mmol) was stirred at room temperature for 96 h. The residue was purified by column chromatography (50 % EtOAc/*n*-hexane) to afford the corresponding product **4i** in 61 % yield (33.4 mg) as a white solid, R_f 0.44 (50 % EtOAc/*n*-hexane). Mp 97–101 °C; IR (neat, ν_{max}): 3786, 3102, 1743, 1602, 1404, 1267, 1088, 848 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.88 (2H, d, J = 9.0 Hz, Ar–H), 7.23 (1H, brs, H-14), 6.50 (2H, d, J = 9.0 Hz, Ar–H), 4.96 (1H, s, H-17b), 4.86 (1H, d, J = 18.0 Hz, H-15b), 4.78 (1H, d, J = 18.0 Hz, H-15a), 4.71 (1H, s, H-17a), 4.54 (1H, dd, J = 10.0, 6.5 Hz, H-3), 4.39 (1H, dd, J = 10.5, 4.5 Hz, H-12), 3.81 (1H, d, J = 10.5 Hz, H-19b), 3.57 (1H, d, J = 10.5 Hz, H-19a), 2.43 (1H, dm, J = 13.0 Hz), 2.22–2.14 (1H, m), 2.04 (3H, s, COCH_3), 1.92–1.77 (5H, m), 1.75–1.62 (3H, m), 1.51 (1H, d, J = 10.0 Hz), 1.28–1.09 (2H, m), 0.91 (3H, s, H-18), 0.87 (9H, s, SiC(CH_3)$_3$), 0.77 (3H, s, H-20), 0.01 (6H, s, Si(CH_3)$_2$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 172.43, 171.81, 170.82, 150.99, 148.55, 146.80, 133.51, 132.34, 132.27, 118.11, 113.75, 112.26, 107.09, 80.12, 70.20, 63.63, 55.59, 53.65, 49.78, 42.47, 39.38, 38.75, 37.03, 28.30, 25.83, 25.61, 24.27, 23.03, 21.23, 18.18, 14.49, −5.67, −5.76; HRMS (ESI) *m/z* calcd for $C_{35}H_{51}NO_7SiNa$ $[M+Na]^+$ 648.3332, found 648.3356.

*12-(Naphthyl)amino-19-TBS-3-Ac-14-deoxyandrographolide (**4j**)*

According to the general procedure, 19-TBS-3,14-Ac-andrographolide (**3**) (50.0 mg, 0.091 mmol) with 1-naphthylamine (20.0 mg, 0.140 mmol) was stirred at room temperature for 24 h. The residue was purified by column chromatography (30 % EtOAc/*n*-hexane) to afford the corresponding product **4j** in 89 % yield (51.5 mg) as a silver solid, R_f 0.44 (30 % EtOAc/*n*-hexane). Mp 83–85 °C; IR (neat, ν_{max}): 3122, 1746, 1587, 1403, 1253, 1091, 846 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.83–7.50 (2H, m, Ar–H), 7.46–7.42 (2H, m, Ar–H), 7.28–7.23 (2H, m, Ar–H), 7.21 (1H, brs, H-14), 6.41 (1H, dd, J = 7.2, 1.0 Hz, Ar–H), 5.07 (1H, brs, H-17b), 4.90 (1H, brs, H-17a), 4.81 (1H, d, J = 18.0 Hz, H-15b), 4.74 (1H, dd, J = 18.0, 1.5 Hz, H-15a), 4.59–4.53 (1H, m, H-3), 4.48–4.41 (1H, m, H-12), 3.83 (1H, d, J = 10.5 Hz, H-19b), 3.59 (1H, d, J = 10.5 Hz, H-19a), 2.46 (1H, dm, J = 14.0 Hz), 2.30 (1H, dd, J = 14.0, 8.0 Hz), 2.04 (3H, s, COCH_3), 2.02–1.76 (5H, m), 1.76–1.62 (4H, m), 1.28–1.24 (2H, m), 0.92 (3H, s, H-18), 0.89 (9H, s, SiC(CH_3)$_3$), 0.81 (3H, s, H-20), 0.03 (6H, s, Si(CH_3)$_2$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 172.84, 170.65, 149.76, 146.20, 141.87, 134.69, 134.42, 128.60, 126.27, 125.78, 124.94, 123.72, 120.07, 117.88, 107.16, 105.51, 80.21, 70.15, 63.68, 55.64, 54.26, 51.27, 42.54, 39.65, 38.80, 37.03, 28.63, 25.86, 25.66, 24.34, 23.03, 21.21, 18.21, 14.54, −5.66, −5.73; HRMS (ESI) *m/z* calcd for $C_{38}H_{53}NO_5Si$ $[M+H]^+$ 632.3693, found 632.3630.

*12-((3,4-Dimethoxy)phenyl)amino-19-TBS-3-Ac-14-deoxyandrographolide (**4k**)*

According to the general procedure, 19-TBS-3,14-Ac-andrographolide (**3**) (50.6 mg, 0.092 mmol) with 3,4-dimethoxyaniline (22.1 mg, 0.144 mmol) was stirred at room temperature for 1.5 h. The residue was purified by column chromatography (30 % EtOAc/*n*-hexane) to afford the corresponding product **4k** in 79 % yield (46.9 mg) as a red solid, R_f 0.20 (30 % EtOAc/*n*-hexane). Mp 73–75 °C; IR (neat, ν_{max}):3779, 3120, 1744, 1606, 1502, 1404, 1249, 1093, 1038, 846 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.16 (1H, brs, H-14), 6.67 (1H, d, J = 8.5 Hz, Ar–H), 6.19 (1H, d, J = 2.5 Hz, Ar–H), 6.07 (1H, dd, J = 8.5, 2.4 Hz, Ar–H), 4.93 (1H, brs, H-17b), 4.81 (1H, brd, J = 18.0 Hz, H-15b), 4.73 (1H, brd, J = 18.0 Hz, H-15a), 4.72 (1H, brs, H-17a), 4.53 (1H, dd, J = 10.5, 6.0 Hz, H-3), 4.22 (1H, dd, J = 9.5, 5.0 Hz, H-12), 3.81 (1H, d, J = 10.5 Hz, H-19b), 3.79 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 3.57 (1H, d, J = 10.5 Hz, H-19a), 2.41 (1H, dm, J = 13.0 Hz), 2.10–2.01 (1H, m), 2.03 (3H, s, COCH_3), 1.92–1.77 (5H, m), 1.73–1.62 (3H, m), 1.47 (1H, d, J = 10.5 Hz), 1.20–1.06 (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 172.69, 170.74, 149.90, 148.33, 146.54, 142.10, 141.26, 134.43, 112.96, 107.08, 105.14, 100.38, 80.15, 70.09, 63.62, 56.53, 55.75, 55.62, 53.58, 51.34, 42.47, 39.29, 38.77, 37.04, 28.53, 25.82, 25.60, 24.28, 23.01, 21.22, 18.16, 14.48, −5.70, −5.80; HRMS (ESI) *m/z* calcd for $C_{36}H_{55}NO_7Si$ $[M+H]^+$ 642.3781, found 642.3871.

*12-((3,5-Dichloro-4-hydroxy)phenyl)amino-19-TBS-3-Ac-14-deoxyandrographolide (**4l**)*

According to the general procedure, 19-TBS-3,14-Ac-andrographolide (**3**) (50.1 mg, 0.091 mmol) with 4-amino-2,6-dichlorophenol (25.2 mg, 0.142 mmol) was stirred at room temperature for 5 h. The residue was purified by column chromatography (30 % EtOAc/*n*-hexane) to afford the corresponding product **4l** in 34 % yield (20.8 mg) as a brown solid, R_f 0.58 (30 % EtOAc/*n*-hexane). Mp 117–119 °C; IR (neat, ν_{max}):3786, 3121, 2959, 1744, 1598, 1473, 1394, 1253, 1085, 843, 758 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.23 (1H, brs, H-14), 6.51 (2H, s, Ar–H), 5.43 (1H, brs, OH), 4.95 (1H, brs, H-17b), 4.86 (1H, brd, J = 18.0 Hz, H-15b), 4.78 (1H, brd, J = 18.0 Hz, H-15a), 4.69 (1H, brs, H-17a), 4.54 (1H, dd, J = 10.5, 6.0 Hz, H-3), 4.17–4.11 (1H, m, H-12), 3.80 (1H, d, J = 10.5 Hz, H-19b), 3.56 (1H, d, J = 10.5 Hz, H-19a), 2.42 (1H, dm, J = 12.0 Hz), 2.14–2.05 (1H, m), 2.04 (3H, s, $COCH_3$), 1.88–1.77 (5H, m), 1.73–1.61 (3H, m), 1.45 (1H, d, J = 10.5 Hz), 1.20–1.02 (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 172.38, 170.79, 148.25, 147.22, 140.65, 133.44, 129.62, 121.62, 114.47, 107.18, 80.11, 70.21, 63.66, 55.64, 53.63, 51.57, 42.49, 39.32, 38.78, 37.06, 28.21, 25.84, 25.61, 24.28, 23.04, 21.23, 18.18, 14.50, −5.50, −5.60; HRMS (ESI) *m/z* calcd for $C_{34}H_{49}Cl_2NO_6Si$ $[M+H]^+$ 666.2740, found 666.2796.

*12-Morpholinyl-19-TBS-3-Ac-14-deoxyandrographolide (**4m**)*

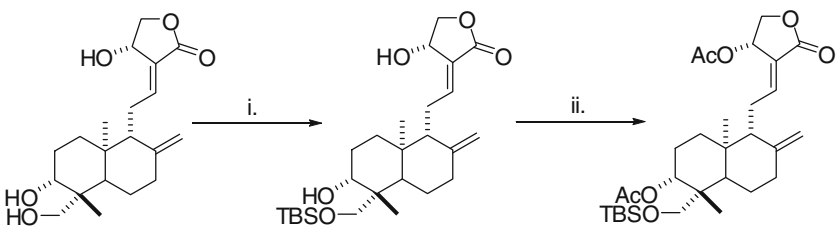
According to the general procedure, 19-TBS-3,14-Ac-andrographolide (**3**) (49.6 mg, 0.091 mmol) with morpholine (15.4 mg, 0.177 mmol) was stirred at room temperature for 0.5 h. The residue was purified by column chromatography (50 % EtOAc/*n*-hexane) to afford the corresponding product **4m** in 57 % yield (29.8 mg) as a brown solid, R_f 0.40 (50 % EtOAc/*n*-hexane). Mp 93–95 °C; IR (neat, ν_{max}):2937, 2870, 1742, 1622, 1508, 1464, 1254, 1086, 1026, 841 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.22 (1H, brs, H-14), 4.92–4.88 (2H, m, H-15), 4.88 (1H, brs, H-17b), 4.69 (1H, s, H-17a), 4.51 (1H, dd, J = 12.0, 5.5 Hz, H-3), 3.77 (1H, d, J = 10.5 Hz, H-19b), 3.69–3.64 (4H, m, $(CH_2)_2O$), 3.57 (1H, d, J = 10.5 Hz, H-19a), 3.59 (1H, dd, J = 12.0, 3.0 Hz, H-12), 2.52–2.43 (4H, m, $(CH_2)_2N$), 2.35 (1H, dm, J = 12.0 Hz), 2.03 (3H, s, $COCH_3$), 1.87–1.59 (8H, m), 1.34 (1H, d, J = 10.5 Hz), 1.17–1.02 (2H, m), 0.90 (3H, s, H-18), 0.87 (9H, s, $SiC(CH_3)_3$), 0.78 (3H, s, H-20), 0.01 (6H, s, $Si(CH_3)_2$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 173.76, 170.86, 147.89, 147.39, 131.86, 107.22, 80.29, 69.88, 67.19, 63.54, 58.29, 55.59, 53.03, 49.79, 42.46, 39.32, 38.60, 37.18, 25.84, 25.47, 25.26, 24.34, 23.13, 21.25, 18.19, 14.51, −5.68, −5.74; HRMS (ESI) *m/z* calcd for $C_{32}H_{53}NO_6Si$ $[M+H]^+$ 576.3676, found 576.3727.

*12-Pyrrolidinyl-19-TBS-3-Ac-14-deoxyandrographolide (**4n**)*

According to the general procedure, 19-TBS-3,14-Ac-andrographolide (**3**) (51.7 mg, 0.094 mmol) with pyrrolidine (9.0 mg, 0.13 mmol) was stirred at room temperature for 1.0 h. The residue was purified by column chromatography (30 % EtOAc/*n*-hexane) to afford the corresponding product **4n** in trace as yellow oil, R_f 0.40 (30 % EtOAc/*n*-hexane). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.14 (1H, brs, H-14), 4.90 (1H, brs, H-17b), 4.82 (1H, dd, J = 18.0, 1.5 Hz, H-15b), 4.76 (1H, dd, J = 18.0, 1.5 Hz, H-15a), 4.68 (1H, brs, H-17a), 4.53 (1H, dd, J = 11.5, 6.0 Hz, H-3), 3.78 (1H, d, J = 10.5 Hz, H-19b), 3.57 (1H, d, J = 10.5 Hz, H-19a), 3.19–3.10–3.19 (1H, m, H-12), 2.89 (2H, dd, J = 18.0, 7.0 Hz, NCH_2-a), 2.69 (2H, dd, J = 18.0, 6.5 Hz, NCH_2-b), 2.41–2.33 (1H, m), 2.03 (3H, s, $COCH_3$), 1.94–1.51 (12H, m), 1.36 (1H, d, J = 11.5 Hz), 1.32–0.98 (2H, m), 0.90 (3H, s, H-18), 0.87 (9H, s, $SiC(CH_3)_3$), 0.74 (3H, s, H-20), 0.01 (6H, s, $Si(CH_3)_2$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 173.10, 170.77, 147.14, 146.90, 135.22, 107.42, 80.27, 70.03, 63.62, 55.73, 54.01, 47.14, 42.56, 39.14, 38.83, 37.16, 30.98, 30.39, 26.77, 25.87, 25.61, 24.39, 23.08, 21.25, 18.21, 14.55, −5.65, −5.70.

Evaluation of cytotoxic activity

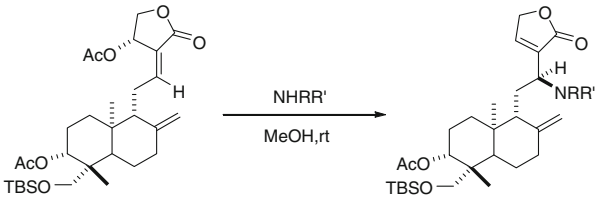
The cytotoxic activities of extracts and compounds were determined using the standard sulforhodamine B (SRB)



Scheme 1 i. TBS-Cl, Py. ii. Ac_2O

andrographolide 1 **2** **3**

Table 1 Reactions of 19-TBS-3,14-Ac-andrographolide **3** with amines



3 **4a-o**

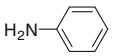
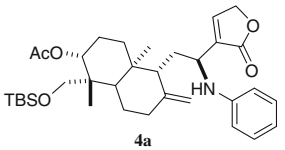
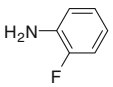
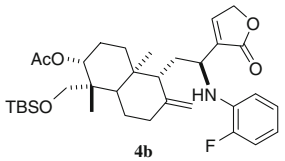
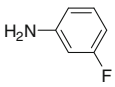
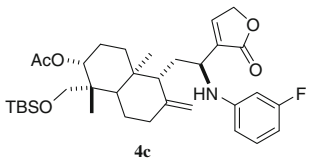
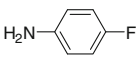
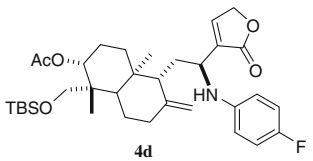
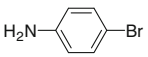
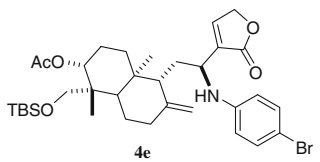
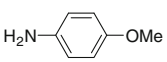
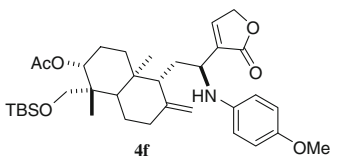
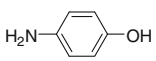
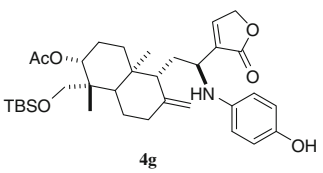
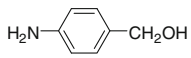
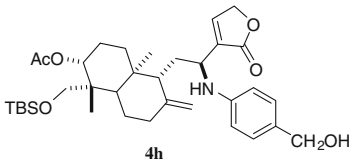
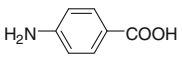
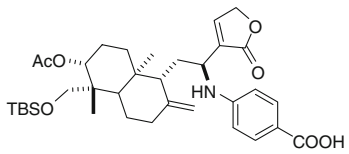
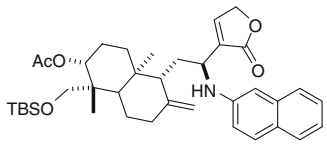
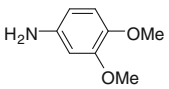
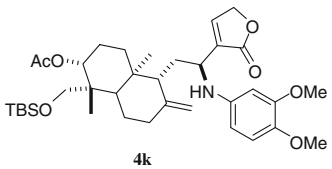
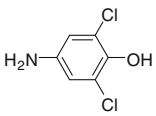
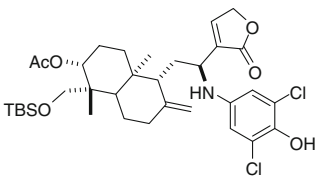
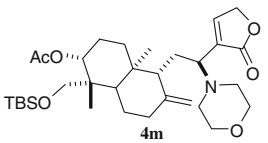
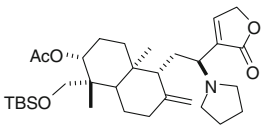
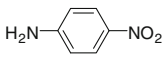
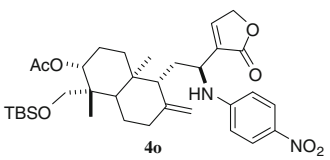
Entry	HNRR'	Time/h	β-amino andrographolide	%Yield
1		4	 **4a**	79
2		48	 **4b**	96
3		7	 **4c**	67
4		7	 **4d**	78
5		24	 **4e**	61
6		6	 **4f**	79
7		3	 **4g**	87

Table 1 continued

Entry	HNRR'	Time/h	β-amino andrographolide	%Yield
8		3	 **4h**	59
9		96	 **4i**	61
10		24	 **4j**	89
11		1.5	 **4k**	79
12		5	 **4l**	34
13		0.5	 **4m**	57
14		1.0	 **4n**	trace
15		72	 **4o**	no. rxn.

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).

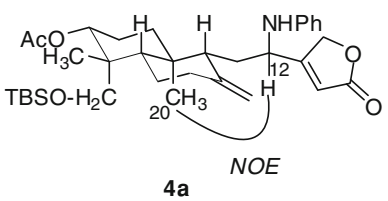
Results and discussion

The target amino-andrographolide analogues were synthesized by using the natural available andrographolide as starting material as shown in Scheme 1 and Table 1. The precursor **3**, which was prepared according to the reported procedure (Sirion et al. 2012) was protected with TBS at C-19 followed by protection with acetyl groups at C-3 and C-14. *N*-alkylation at C-12 was conducted via tandem conjugate addition–elimination (CAE) reaction with various anilines resulted in amino-andrographolide in low to good yields ranging from trace to 96 %. The results are summarized in Table 1.

The CAE reaction was first conducted with **3** and aniline in methanol without any base or catalyst. The reaction proceeded smoothly, selectively and efficiently at room temperature to give the product **4a** in good yield. The stereochemistry at C-12 of **4a** was determined by NOE experiments. NOE was observed between the H-12 and H-20 of the methyl group (Fig. 2).

Various anilines bearing halogenated groups were investigated under the present condition to give the corresponding 12-amino-andrographolides **4b-4e** in fair to excellent yields (Table 1, entries 1–5). In case of 2-fluoro- and 4-bromo-aniline, the reactions were performed as long as 1–2 days to complete the reaction (entries 2 and 5). In addition, the anilines bearing 3-methoxy, hydroxy and hydroxymethyl proved to be good nucleophiles providing the corresponding products in shorter time (entries 6–8). Aniline bearing 4-COOH, the CAE reaction proceeded very sluggishly and required 4 days reaction time (entry 9).

Extension of this reaction to other aniline nucleophiles such as naphthyl (entry 10) furnished the corresponding product in high yield, while aniline bearing electron withdrawing as nitro group did not provide the desired product (entry 15). Reaction of di-substituents electron-donating aniline with **3** furnished the corresponding product **4k** in 79 % (entry 11), while tri-substituents dichlorohydroxyl aniline led to the dramatic decrease in the yield (34 %) due to the increased steric hindrance of aniline (entry 12). Reaction of morpholine gave the corresponding product in 57 % yield in short reaction time. Trace of product was observed when pyrrolidine was used as nucleophile (entries 13–14).



4a

Fig. 2 NOE correlation for compound **4a**

The cytotoxic activity of andrographolide, the parent compound **1** and its synthetic amino-analogues were evaluated in vitro against six cancer cell lines which 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) using SRB assay. All tested analogues were dissolved in DMSO (0.1 %). Ellipticine which is a potent anti-cancer agent was used as a positive control. It exhibits several modes of mechanisms against cancer cells (Auclair 1987). As shown in Table 2, the obtained results were expressed as ED_{50} values (drug concentration causing 50 % growth inhibition) in μM.

Interestingly, an introduction of an aniline group to C-19-TBS-andrographolide analog **3** via tandem CAE reaction afforded a new compound **4a** whose cytotoxic activity was markedly increased. Particularly the cytotoxicity of analog **4a** on ASK cell was increased to ~20-folds over the natural andrographolide. Comparison of the cytotoxic activities of compounds **4b–d** revealed the importance of the substituent position of fluoro group on aniline ring of amino-andrographolide. Compounds **4b** and **c** contained fluoro group at 2-, and 3- position respectively having similar activities, which are 5 to tenfold more active than 4-fluoro derivatives **4d** (see Table 2). The effects of the C-4 halogenated substituent on the aniline ring were demonstrated by comparison of the 4-fluoro derivatives **4d** and 4-bromo derivative **4e.** Attachment of the bromo group at the C-4 position led to a dramatic increase in the cytotoxic activity relative to derivative **4d**.

Moreover, the effect of C-4 substituent of the aniline ring on cytotoxicity were observed on both P-388 and ASK cells by comparison of the 4-methoxy derivative **4f** (ED_{50} = 5.48, 5.52 μM, respectively) and the 4-hydroxy derivative **4g** (ED_{50} = 0.49, 0.88 μM, respectively). Attachment of a more electron-donating group such as the methoxy group at the C-4 position led to a dramatic decrease in the cytotoxic activity. Changing the hydroxy group to hydroxymethyl derivatives such as **4h** (ED_{50} = 4.80–5.88 μM) resulted in a dramatic decrease in activity, but when changing to 4-carboxylic derivative **4i**, the compound exhibited more potent cytotoxicity against both P-388 and ASK cells than ellipticine. However, the electronic effect of the C-4 substituent of the aniline

Table 2 Cytotoxic activities of andrographolide analogues against cancer cell lines

Compound	ED_{50} (μM)[a]					
	P-388	KB	COL-2	MCF-7	LU-1	ASK
1	2.25 ± 0.19	27.37 ± 1.05	13.6 ± 0.41	15.4 ± 0.21	12.98 ± 0.15	16.18 ± 0.27
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
4a	0.66 ± 0.02	4.29 ± 0.13	3.35 ± 0.13	4.35 ± 0.07	5.08 ± 0.14	0.77 ± 0.00
4b	0.57 ± 0.02	4.41 ± 0.08	4.07 ± 0.15	4.39 ± 0.03	5.30 ± 0.02	0.81 ± 0.00
4c	0.61 ± 0.03	4.40 ± 0.07	4.52 ± 0.09	4.50 ± 0.06	5.37 ± 0.01	0.87 ± 0.01
4d	5.42 ± 0.11	24.65 ± 1.20	22.10 ± 5.58	28.91 ± 1.21	>50 ± 2.87	6.72 ± 0.86
4e	0.55 ± 0.01	4.10 ± 0.04	4.14 ± 0.13	4.36 ± 0.01	4.72 ± 0.11	0.84 ± 0.00
4f	5.48 ± 0.10	6.62 ± 0.39	5.35 ± 0.04	5.24 ± 0.12	6.33 ± 0.25	5.52 ± 0.04
4g	0.49 ± 0.04	4.76 ± 0.02	5.02 ± 0.05	4.43 ± 0.03	5.79 ± 0.05	0.88 ± 0.02
4h	4.80 ± 0.16	4.94 ± 0.03	5.17 ± 0.03	4.87 ± 0.10	5.88 ± 0.08	5.10 ± 0.10
4i	0.60 ± 0.02	4.60 ± 0.07	4.06 ± 0.29	4.31 ± 0.07	5.72 ± 0.03	0.80 ± 0.01
4j	5.01 ± 0.06	5.82 ± 0.02	5.90 ± 0.05	7.08 ± 0.04	7.91 ± 0.06	5.50 ± 0.04
4k	0.47 ± 0.02	4.38 ± 0.03	2.27 ± 0.30	4.16 ± 0.01	5.42 ± 0.02	0.77 ± 0.01
4l	4.46 ± 0.25	14.15 ± 2.09	7.51 ± 0.13	11.67 ± 3.04	9.28 ± 0.90	5.90 ± 0.05
4m	0.60 ± 0.08	5.05 ± 0.03	5.07 ± 0.01	4.73 ± 0.03	5.84 ± 0.01	3.27 ± 0.26
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] Each value represents mean ± SE from three different experiments performed in triplicate. 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 (Ellipt) was used as a positive control. The results were expressed as ED_{50} values (drug concentration causing 50 % growth inhibition) in μM

ring on amino-andrographolide did not alter cytotoxic activity to the other cell lines.

Comparing to **1**, the naphthyl amino and tri-substituents dichloro-hydroxyl aniline groups on 12-amino-andrographolide led to a moderate increase in activity, except on P-388 cell. With the di-substituted methoxyl-aniline, compound **4k** showed the best activities over other analogues on five cell lines and better than ellipticine on P-388 and ASK cancer cells. Comparing to **3**, amino-andrographolide analogues **4a–c**, **4e**, **4g**, **4i** and **4k** exhibited more potent cytotoxicity against ASK cell than the andrographolide precursor **3**.

In summary, we have demonstrated a protocol for stereoselective synthesis of amino-andrographolide based compounds which were conducted via tandem aza-conjugate addition–elimination reaction of andrographolide with various aniline nucleophiles using economic procedure without any base or catalyst at room temperature. An evaluation of the cytotoxicity of the synthetic C-19 TBS-amino-andrographolide analogues revealed that introduction of aniline derivatives to andrographolide enhanced the cytotoxic activity of the natural parent compound. The type of the mono-substituent on the C-4 aniline moiety of amino-analogues is important for the cytotoxic activity, with the 4-hydroxy and 4-COOH derivatives showing the highest activity on P-388 cell and ASK respectively. The position of fluoro group on the aniline moiety was also played an important role on the activity. Among the synthesized compounds, 3,4-dimethoxyphenylamino derivative **4k** was found to be the most active. It exhibited threefolds more potent than ellipticine with ED_{50} values of 0.47 and 0.77 μM on P-388 and ASK cancer cells and could be simply obtained under mild condition with high yield and short reaction time. This compound might serve as a starting point of building block for future structure modification against cancer cells.

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