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*Semisynthesis and cytotoxic activities of andrographolide analogues

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Abstract

Andrographolide **1,** a diterpenoid lactone of the plant *Andrographis paniculata*, known to possess antitumour activity in *in vitro* and *in vivo* breast cancer models was subjected to semisynthesis leading to the preparation of a number of novel compounds. These compounds exhibited *in vitro* antitumour activity with moderate to excellent growth inhibition against MCF-7 (breast) and HCT-116 (colon) cancer cells. Compounds 3,19-(2-chlorobenzylidene)andrographolide(**5**), 3,19-(3-chlorobenzylidene)andrographolide(**6**), 3,19-(3-fluorobenzylidene)andrographolide(**7**), 3,19-(4-fluorobenzylidene)andrographolide(**8**), 3,19-(2-fluorobenzylidene)andrographolide(**10**), 3,19-(2-chloro-5-nitrobenzylidene)andrographolide (**21**), 3,19-(4-chlorobenzylidene)andrographolide(**30**) and 3,19-(2-chloro-4-fluorobenzylidene) andrographolide(**31**) were also screened against 60 NCI (National Cancer Institute, USA) human tumour cell lines derived from nine cancer cell types.

Keywords: *Andrographolide, semisynthesis, cytotoxicity, National Cancer Institute (NCI), MTT assay*

Introduction

Andrographis paniculata Nees (Acanthaceae), also known commonly as 'king of bitters', is a well-known herb in India, China and Southeast Asia. Extracts of *A. paniculata* has been shown to possess antiinflammatory, antiviral, immunostimulatory, hypoglycemic, hypotensive and anticancer activities [1–8]. The main components of *A. paniculata* are diterpenes, flavonoids and stigmasterols [8] with the major constituents being the labdane diterpenoids [9,10]. Andrographolide **1** (Figure 1) is the major diterpenoid of *A. paniculata* extract and chemically designated as 3-{2-[decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylene-1-napthalenyl]ethylidene}dihydro-4-hydroxy-2(3*H*)-furanone. Andrographolide is found in the whole plant but is mostly concentrated in the leaves. Anticancer activity of andrographolide in human cancer cells and immunomodulatory activities in human immune cells has been reported recently [11]. However, andrographolide lacks selectivity and potency towards several tumour cell lines. To improve upon its selectivity and potency as an anticancer agent, several attempts have been made to chemically modify the molecule so as to improve its structure-activity relationships (SAR). Here, a series of novel analogues of andrographolide were synthesised and evaluated for *in vitro* antitumour activity against different cancer cell lines. The majority of the analogues demonstrated good *in vitro* antitumour activity.

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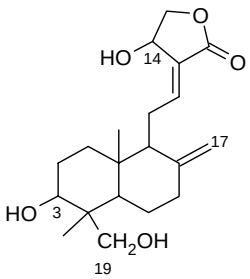


Figure 1. Andrographolide (**1**)
Note: please refer to appendix

In a previous report, it was showed that an intact γ-butyrolactone ring, double bonds at C-12 and C-13, C-8 and a C-17, and hydroxyl group at C-14 of andrographolide are responsible for its cytotoxic activity [12]. In order to determine the importance of the hydroxyl groups present at C-3 and C-19 of andrographolide for cytotoxic activity, we synthesised andrographolide analogues by coupling the two hydroxyl groups present at C-3 and C-19. Here, we report the semisynthesis of andrographolide analogues and their *in vitro* anticancer activities in pre-screen and 60 NCI cancer cell lines.

Experimental

Chemistry

Silica gel 60 (0.040–0.063 mm) and 20 cm × 20 cm silica gel 60 F_{254}-coated TLC plates were obtained from Merk (Darmstadt, Germany). Melting points were recorded using an Electrothermal melting point apparatus and are uncorrected. IR spectra were measured in KBr on a Perkin Elmer RX I FT-IR spectrometer. ^{1}H NMR was recorded on a Bruker ARX 250 instrument at 250 MHz and a Bruker ARX 400 instrument at 400 MHz. All NMR spectra were obtained in deuterated solvents; chemical shift values δ are expressed in parts per million (ppm), and coupling constants (J) are in hertz (Hz). Mass spectra were recorded on a Micromass Platform spectrometer, an AEI MS-902 (nominal mass).

*Preparation of andrographolide analogues (**2**–**10**)*

*3,19-(4-bromobenzylidene)andrographolide (**2**)*. A mixture of andrographolide (0.5 g, 1.42 mmol), 4-bromobenzaldehyde (0.132 g, 0.714 mmol) and $ZnCl_2$ (80 mg) in DMSO (1 ml) was stirred at room temperature for 5–6 h. After completion of the reaction (checked by TLC), the contents were diluted with water and extracted with dichloromethane. The organic layer was dried over anhydrous Na_2SO_4 and purified by column chromatography (chloroform: methanol = 99:1) to afford 3,19-(4-bromobenzylidene)andrographolide. IR: 3447 (OH), 2926, 1755, 1669 (C = O), 1217, 899 cm^{-1}; ^{1}H NMR (250 MHz; $CDCl_3$): δ 0.87 (3H, s, H-20), 1.30–1.22 (3H, m), 1.47 (3H, s, H-18), 1.61 (3H, s), 2.18 (1H, d, J = 6.3 Hz, H-11), 2.48–2.40 (2H, m), 2.61–2.55 (2H, m), 3.59 (1H, d, J = 11.4 Hz, H-19b), 3.67 (1H, dd, J = 4.3 and 11.5 Hz, H-19a), 4.30–4.23 (1H, m), 4.51–4.44 (1H, m), 4.63 (1H, s, H-17b), 4.93 (1H, s, H-17a), 5.05 (1H, bs), 5.73 (1H, s, H-14), 7.00 (1H, t, J = 6.6 Hz, H-12), 7.37 (2H, m), 7.5 (2H, m); EI-MS *m/z* 516 (M^+– H) $C_{27}H_{33}BrO_5$.

Preparation of compounds **3**–**10** was carried out by a method similar to that described for 3,19-(4-bromobenzylidene)andrographolide by the reaction of the corresponding aromatic aldehydes (0.714 mmol) with andrographolide (1.42 mmol).

*3,19-(2-bromobenzylidene)andrographolide (**3**)*. IR: 3448, 2936, 1739, 1668, 1103, 973 cm^{-1}; ^{1}H NMR (250 MHz; $CDCl_3$): δ 0.84 (3H, s, H-20), 1.31 (3H, s, H-18), 1.49 (3H, s), 2.62–2.44 (4H, m), 3.54 (1H, d, J = 11.3 Hz, H-19b), 3.62 (1H, dd, J = 4.3 and 11.2 Hz, H-19a), 4.50–4.43 (1H, m), 4.92 (1H, s, H-17a), 5.03 (1H, d, J = 5.6 Hz), 6.06 (1H, s, H-14), 6.98 (1H, m, H-12), 7.24–7.18 (1H, m), 7.36 (1H, t, J = 7.5 Hz), 7.53 (1H, d, J = 7.8 Hz), 7.74 (1H, dd, J = 1.4 and 7.7 Hz); EI-MS *m/z* 516 (M^+– H) $C_{27}H_{33}BrO_5$.

*3,19-(3-bromobenzylidene)andrographolide (**4**)*. IR: 3420, 2941, 1748, 1660, 1262, 1106, 902 cm^{-1}; ^{1}H NMR (250 MHz; $CDCl_3$): δ 0.87 (3H, s, H-20), 1.35–1.19 (4H, m), 1.47 (3H, s, H-18), 1.98–1.79 (5H, m), 2.48–2.39 (2H, m), 2.61–2.55 (2H, m), 3.59 (1H, d, J = 11.2 Hz, H-19b), 3.68 (1H, dd, J = 4.5 and 12.5 Hz, H-3), 4.30–4.23 (2H, m), 4.51–4.44 (1H, m), 4.62 (1H, s, H-17b), 4.92 (1H, s, H-17a), 5.04 (1H, d, J = 5.9 Hz), 5.73 (1H, s, H-14), 6.98 (1H, t, J = 6.8 Hz, H-12), 7.22 (1H, m), 7.48–7.37 (3H, m), 7.66 (1H, m); EI-MS *m/z* 516 (M^+–H) $C_{27}H_{33}BrO_5$.

*3,19-(2-chlorobenzylidene)andrographolide (**5**)*. IR: 3439, 2942, 1742, 1671, 1275, 1103, 759 cm^{-1}; ^{1}H NMR (250 MHz; $CDCl_3$): δ 0.83 (1H, s, H-20), 1.19 (3H, s, H-18), 1.61-1.51 (4H, m), 1.81 (3H, s), 2.91-2.74 (4H, m), 4.06–3.89 (2H, m), 4.62 (1H, s, H-17b), 4.75 (1H, dd, J = 6.0 and 10.4 Hz, H-15a), 4.94 (1H, s, H-17a), 5.22 (1H, s), 5.32 (1H, d, J = 5.8 Hz, H-14), 6.43 (1H, s), 7.13 (1H, t, J = 7.1 Hz, H-12), 7.66–7.57 (4H, m), 8.05 (1H, m); EI-MS *m/z* 456 (M^+– OH) $C_{27}H_{33}ClO_5$.

*3,19-(3-chlorobenzylidene)andrographolide (**6**)*. IR: 3422, 2944, 1747, 1663, 1390, 1200, 1106, 990 cm^{-1}; ^{1}H NMR (250 MHz; $CDCl_3$): δ 0.87 (3H, s, H-20), 1.47 (3H, s, H-18), 1.92–1.85 (4H, m), 2.08 (1H, s), 2.48–2.38 (2H, m), 2.61–2.55 (2H, m), 3.49 (1H, s), 3.60 (1H, d, J = 11.4 Hz, H-19a), 3.68 (1H, dd, J = 4.3 and 12.8 Hz, H-3), 4.3–4.23 (2H, m), 4.47 (1H, dd, J = 6.0 and 10.4 Hz, H-15a), 4.62 (1H,

s, H-17b), 4.92 (1H, s, H-17a), 5.05 (1H, bs), 5.74 (1H, s, H-14), 6.98 (1H, td, $J = 1.5$ and 6.9 Hz, H-12), 7.38-7.29 (3H, m), 7.51 (1H, s); EI-MS *m/z* 473 (M^+) $C_{27}H_{33}ClO_5$.

3,19-(3-fluorobenzylidene) andrographolide (7). IR: 3402, 2942, 1752, 1676, 1451, 1103, 910 cm^{-1}; ^1H NMR (250 MHz; $CDCl_3$): δ 0.87 (3H, s, H-20), 1.30–1.10 (4H, m), 1.47 (3H, s, H-18), 2.00–1.70 (5H, m), 3.70–3.40 (3H, m), 4.26 (2H, m), 4.45 (1H, dd, $J = 6.0$ and 10.4 Hz, H-15a), 4.63 (1H, s, H-17b), 4.92 (1H, s, H-17a), 5.01 (1H, bs, H-14), 5.75 (1H, s, H-12), 7.00 (2H, m), 7.30–7.20 (3H, m); EI-MS *m/z* 456 (M^+) $C_{27}H_{33}FO_5$.

3,19-(4-fluorobenzylidene) andrographolide (8). IR: 3422, 2942, 1747, 1672, 1384, 1223, 1101, 915 cm^{-1}; ^1H NMR (250 MHz; $CDCl_3$): δ 0.86 (3H, s, H-20), 1.25 (4H, m), 1.47 (3H, s, H-18), 1.92 (7H, m), 2.48 (5H, m), 2.98 (1H, d, $J = 5.7$ Hz, H-19a), 3.58 (1H, d, $J = 10.4$ Hz, H-15b), 3.66 (1H, dd, $J = 4.5$ and 12.5 Hz, H-3), 4.43 (1H, dd, $J = 6.1$ and 10.4 Hz, H-15a), 4.63 (1H, s, H-17b), 4.91 (1H, s, H-17a), 4.98 (1H, bs), 5.74 (1H, s, H-14), 6.93 (1H, t, $J = 6.1$ Hz, H-12), 7.04 (3H, t, $J = 8.6$ Hz), 7.47 (3H, m); EI-MS *m/z* 457 ($M^+ + H$) $C_{27}H_{33}FO_5$.

3,19-(3-chloro-4-fluorobenzylidene) andrographolide (9). IR: 3448, 2943, 1751, 1501, 1259, 1102, 901 cm^{-1}; ^1H NMR (250 MHz; $CDCl_3$): δ 0.85 (3H, s, H-20), 1.24 (4H, m), 1.44 (3H, s, H-18), 1.92 (6H, m), 2.46 (4H, m), 3.19 (1H, bs), 3.57 (1H, d, $J = 11.3$ Hz, H-19b), 3.66 (1H, dd, $J = 4.6$ and 12.6 Hz, H-3), 4.23 (1H, d, $J = 11.3$ Hz, H-19a), 4.47 (1H, dd, $J = 6.1$ and 10.4 Hz, H-15b), 4.63 (1H, s, H-17b), 4.91 (1H, s, H-17a), 4.99 (1H, bs), 5.71 (1H, s, H-14), 6.92 (1H, t, $J = 6.4$ Hz, H-12), 7.12 (1H, t, $J = 8.6$ Hz), 7.34 (1H, m), 7.56 (1H, dd, $J = 1.7$ and 7.1 Hz); EI-MS *m/z* 491 (M^+) $C_{27}H_{33}ClFO_5$.

3,19-(2-fluorobenzylidene) andrographolide (10). IR: 3448, 2928, 1752, 1228, 1113, 1015, 920 cm^{-1}; ^1H NMR (250 MHz; $CDCl_3$): δ 0.88 (3H, s, H-20), 1.26 (3H, s, H-18), 1.51 (3H, s), 1.67 (6H, m), 2.53 (5H, m), 3.40 (1H, d, $J = 10.3$ Hz, H-15a), 3.68 (1H, dd, $J = 4.7$ and 12.7 Hz, H-3), 4.27 (2H, m), 4.46 (1H, dd, $J = 6.0$ and 10.4 Hz, H-15b), 4.63 (1H, s, H-17b), 4.92 (1H, s, H-17a), 5.03 (1H, d, $J = 5.7$ Hz), 6.10 (1H, s, H-14), 7.00 (1H, s, H-12), 7.18 (1H, m), 7.33 (2H, m), 7.68 (1H, dd, $J = 1.6$ and 7.3 Hz); EI-MS *m/z* 456 (M^+) $C_{27}H_{33}FO_5$.

*Preparation of andrographolide analogues (**11**–**31**)*

*3,19-(3,4-dimethoxybenzylidene) andrographolide (**11**)*. Andrographolide (0.5 g, 1.42 mmol), 3,4-dimethoxybenzaldehyde (0.119 g, 0.714 mmol) and pyridinium *p*-toluenesulphonate (few crystals) were dissolved in DMSO (1 ml) and stirred at 70°C for 2–3 h. After completion of the reaction (checked by TLC), the contents were cooled to room temperature and triethylamine (0.5 ml) was added to quench the remaining catalyst. The reaction mixture was diluted with water and extracted with ethylacetate. The organic layer was dried over anhydrous Na_2SO_4 and purified by column chromatography (chloroform:methanol = 99:1) to afford 3,19-(3,4-dimethoxybenzylidene)andrographolide. IR: 3438, 2940, 1756, 1673, 1264, 1100, 912 cm^{-1}; ^1H NMR (250 MHz; $CDCl_3$): δ 0.87 (3H, s, H-20), 1.38–1.21 (5H, m), 1.49 (3H, s, H-18), 1.98–1.71 (5H, m), 2.61–2.43 (5H, m), 3.59 (1H, d, $J = 10.4$ Hz, H-15b), 3.68 (1H, dd, $J = 4.3$ and 11.4 Hz, H-3), 3.89 (1H, d, $J = 10.5$ Hz, H-15a), 4.26 (2H, d, $J = 10.7$ Hz, H-19b), 4.49–4.43 (1H, m), 4.63 (1H, s, H-17b), 4.92 (1H, s, H-17a), 5.03 (1H, bs), 5.73 (1H, s, H-14), 6.85 (1H, d, $J = 8.0$ Hz, H-12), 7.05–6.94 (3H, m); EI-MS *m/z* 498 (M^+) $C_{29}H_{38}O_7$.

Preparation of compounds **12**–**31** was carried out by a method similar to that described for 3,19-(3,4-dimethoxybenzylidene)andrographolide by the reaction of corresponding aromatic aldehydes (0.714 mmol) with andrographolide (1.42 mmol).

*3,19-(3,5-dimethoxybenzylidene) andrographolide (**12**)*. IR: 3432, 2945, 1734, 1677, 1204, 1152, 1053, 915 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$): δ 0.88 (3H, s, H-20), 1.32–1.26 (3H, m), 1.48 (3H, s, H-18), 1.92–1.83 (4H, m), 2.3 (1H, d, $J = 6.5$ Hz, H-11), 2.48–2.44 (2H, m), 2.6–2.55 (1H, m), 3.59 (1H, dd, $J = 1.0$ and 10.6 Hz, H-15b), 3.68 (1H, dd, $J = 4.7$ and 12.6 Hz, H-3), 3.8 (7H, s), 4.25 (2H, dd, $J = 6.1$ and 10.5 Hz, H-15a), 4.46 (1H, d, $J = 10.4$ Hz, H-19b), 4.63 (1H, s, H-17b), 4.93 (1H, s, H-17a), 5.03 (1H, bs), 5.7 (1H, s, H-14), 6.44 (1H, t, $J = 2.3$ Hz), 6.66 (2H, d, $J = 2.3$ Hz), 6.97 (1H, t, $J = 3.4$ Hz, H-12); EI-MS *m/z* 498 (M^+) $C_{29}H_{38}O_7$.

*3,19-(3-hydroxy-4-methoxybenzylidene) andrographolide (**13**)*. IR: 3409, 2946, 1725, 1279, 1101, 1025, 891 cm^{-1}; ^1H NMR (250 MHz; DMSO-D_6): δ 0.59 (3H, s, H-20), 1.14-0.94 (7H, m), 1.88–1.55 (4H, m), 3.8 (1H, dd, $J = 4.2$ and 12.3 Hz, H-3), 3.84 (1H, dd, $J = 1.8$ and 9.9 Hz, H-15b), 3.96 (1H, d, $J = 11.1$ Hz, H-19b), 4.2 (1H, dd, $J = 6.0$ and 9.9 Hz, H-15a), 4.46 (1H, s), 4.64 (1H, s, H-17b), 4.73 (1H, s, H-17a), 5.48 (1H, s), 5.56 (1H, d, $J = 6.0$ Hz, H-14), 6.43 (1H, t, $J = 6.3$ Hz, H-12), 6.65–6.54 (3H, m), 8.78 (1H, s); EI-MS *m/z* 484 (M^+) $C_{28}H_{36}O_7$.

*3,19-(2-hydroxy-3-methoxybenzylidene) andrographolide (**14**)*. IR: 3382, 2927, 1749, 1473, 1245, 1064, 914 cm^{-1}; ^1H NMR (400 MHz; DMSO-D_6): δ 0.81 (3H, s, H-20), 1.39 (3H, s, H-18), 1.93 (1H, d, $J = 9.7$ Hz), 3.42 (1H, d, $J = 11.3$ Hz, H-19a), 3.55 (1H, dd, $J = 4.6$ and 12.4 Hz, H-3), 3.78 (3H, s), 4.05 (1H, dd, $J = 1.9$ and 10.0 Hz, H-15b), 4.16 (1H,

d, $J = 11.2$ Hz, H-19b), 4.41 (1H, dd, $J = 6.1$ and 10.0 Hz, H-15a), 4.67 (1H, s, H-17b), 4.86 (1H, s, H-17a), 4.94 (1H, d, $J = 5.8$ Hz), 5.78 (1H, bs), 6.08 (1H, s, H-14), 6.64 (1H, t, $J = 6.1$ Hz), 6.75 (1H, t, $J = 8.0$ Hz, H-12), 6.90 (1H, dd, $J = 1.2$ and 8.0 Hz), 7.01 (1H, dd, $J = 1.5$ and 7.8 Hz), 8.67 (1H, bs); EI-MS *m/z* 483 ($M^+ - H$) $C_{28}H_{36}O_7$.

3,19-(2-ethoxy-4-hyodroxybenzylidene) andrographolide (***15***). IR: 3256, 2942, 1731, 1281, 1172, 1103, 920 cm^{-1}; ^1H NMR (400 MHz; DMSO-D_6): δ 0.80 (3H, s, H-20), 1.36 (3H, s, H-18), 1.93 (1H, d, $J = 9.2$ Hz), 2.00 (1H, td, $J = 4.7$ and 12.8 Hz), 2.37 (1H, d, $J = 13.1$ Hz, H-11), 2.54 (2H, s), 3.43 (1H, d, $J = 11.1$ Hz, H-19a), 3.55 (1H, dd, $J = 4.6$ and 12.4 Hz, H-3), 3.98 (2H, dd, $J = 7.0$ and 13.9 Hz), 4.05 (1H, dd, $J = 1.9$ and 10.0 Hz, H-15b), 4.17 (1H, d, $J = 11.3$ Hz, H-19b), 4.41 (1H, dd, $J = 6.1$ and 10.0 Hz, H-15a), 4.67 (1H, s, H-17b), 4.85 (1H, s, H-17a), 4.94 (1H, d, $J = 4.8$ Hz), 5.70 (1H, s), 5.77 (1H, bs), 6.65 (1H, t, $J = 6.3$ Hz), 6.73 (1H, d, $J = 7.8$ Hz), 6.80 (1H, dd, $J = 1.4$ and 8.3 Hz), 6.92 (1H, d, $J = 1.4$ Hz, H-12), 8.97 (1H, bs); EI-MS *m/z* 498 (M^+) $C_{29}H_{38}O_7$.

3,19-(3,4-dihydroxybenzylidene) andrographolide (***16***). IR: 3330, 2962, 1727, 1647, 1167, 877 cm^{-1}; ^1H NMR (400 MHz; CD_3COCD_3-D_6): δ 0.73 (1H, s, H-20), 0.90 (3H, s), 1.22 (1H, s), 1.43 (3H, s, H-18), 2.10 (7H, s), 2.70–2.62 (4H, m), 3.51 (1H, dd, $J = 1.1$ and 10.2 Hz, H-15b), 3.60 (1H, dd, $J = 4.7$ and 12.4 Hz, H-3), 4.17 (1H, dd, $J = 2.0$ and 10.1 Hz, H-15a), 4.74 (1H, d, $J = 0.9$ Hz, H-17b), 4.90 (1H, s, $J = 1.1$ Hz, H-17a), 5.17 (1H, d, $J = 6.1$ Hz), 5.72 (1H, s, H-14), 6.80–6.76 (3H, m), 7.01 (4H, d, $J = 7.8$ Hz), 7.38–7.35 (7H, m); EI-MS *m/z* 469 ($M^+ - H$) $C_{27}H_{34}O_7$.

3,19-(2,5-dimethoxybenzylidene) andrographolide (***17***). IR: 3434, 2940, 1755, 1672, 1278, 1094, 910 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$): δ 0.89 (3H, s, H-20), 1.32–1.23 (2H, m), 1.51 (3H, s, H-18), 1.92–1.80 (4H, m), 2.31(1H, d, $J = 6.7$ Hz, H-11), 2.48 (1H, m), 2.65–2.55 (1H, m), 3.57 (1H, dd, $J = 1.7$ and 10.3 Hz, H-15b), 3.66 (1H, dd, $J = 4.7$ and 12.5 Hz, H-3), 3.80 (6H, d, $J = 2.8$ Hz), 4.27–4.23 (2H, m), 4.46 (1H, dd, $J = 6.0$ and 10.4 Hz, H-15a), 4.63 (1H, s, H-17b), 4.92 (1H, s, H-17a), 5.03 (1H, t, $J = 6.0$ Hz), 6.13 (1H, s), 6.86–6.82 (1H, m), 6.97 (1H, td, $J = 1.6$ and 6.9 Hz, H-12), 7.23 (1H, d, $J = 2.8$ Hz); EI-MS *m/z*(M^+) $C_{29}H_{38}O_7$.

3,19-(4-nitrobenzylidene) andrographolide (***18***). IR: 3447, 2924, 1755, 1670, 1217, 1015, 900 cm^{-1}; ^1H NMR (400 MHz; DMSO-D_6): δ 0.82 (3H, s, H-20), 1.37 (3H, s, H-18), 1.94 (1H, d, $J = 9.2$ Hz), 2.01 (1H, td, $J = 4.7$ and 12.8 Hz), 3.52 (1H, d, $J = 11.2$ Hz, H-19b), 3.64 (1H, dd, $J = 4.8$ and 12.6 Hz, H-3), 4.05 (1H, dd, $J = 1.9$ and 9.9 Hz, H-15b), 4.27 (1H, d, $J = 11.3$ Hz, H-19a), 4.41 (1H, dd, $J = 6.1$ and 10.0 Hz, H-15a), 4.68 (1H, s, H-17b), 4.86 (1H, s, H-17a), 4.95 (1H, d, $J = 5.8$ Hz), 5.77 (1H, bs), 6.01 (1H, s, H-14), 6.65 (1H, t, $J = 6.3$ Hz, H-12), 7.69 (2H, d, $J = 8.7$ Hz), 8.20 (2H, d, $J = 8.7$ Hz); EI-MS *m/z* 466 ($M^+ - H$) $C_{27}H_{33}NO_7$.

3,19-(2-nitrobenzylidene) andrographolide (***19***). IR: 3422, 2942, 1751, 1530, 1105, 1017, 904 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$): δ 0.88 (3H, s, H-20), 1.43 (3H, s, H-18), 2.46 (1H, d, $J = 12.9$ Hz), 2.62–2.57 (2H, m), 3.67–3.60 (2H, m), 4.46 (1H, dd, $J = 6.0$ and 10.4 Hz, H-15b), 4.64 (1H, s, H-17b), 4.93 (1H, s, H-17a), 5.07 (1H, d, $J = 5.9$ Hz), 6.37 (1H, s, H-14), 6.97 (1H, td, $J = 1.5$ and 6.8 Hz, H-12), 7.48 (1H, td, $J = 1.2$ and 7.7 Hz), 7.63 (1H, td, $J = 1.1$ and 7.6 Hz), 7.84 (1H, dd, $J = 1.1$ and 8.1 Hz), 7.93 (1H, dd, $J = 1.2$ and 7.8 Hz); EI-MS *m/z* 466 ($M^+ - H$) $C_{28}H_{33}NO_7$.

3,19-(3-nitrobenzylidene) andrographolide (***20***). IR: 3388, 2940, 1727, 1677, 1221, 1102, 1021, 905 cm^{-1}; ^1H NMR (400 MHz; DMSO-D_6): δ 0.82 (3H, s, H-20), 1.37 (3H, s, H-18), 1.94 (1H, d, $J = 8.7$ Hz, H-11), 2.01 (1H, td, $J = 4.7$ and 12.8 Hz), 2.37 (1H, d, $J = 11.2$ Hz), 3.52 (1H, d, $J = 11.2$ Hz, H-19b), 3.65 (1H, dd, $J = 4.8$ and 12.6 Hz, H-3), 4.05 (1H, dd, $J = 1.9$ and 10.1 Hz, H-15b), 4.25 (1H, dd, $J = 6.1$ and 10.0 Hz, H-15a), 4.27 (1H, d, $J = 11.2$ Hz, H-19a), 4.68 (1H, s, H-17b), 4.86 (1H, s, H-17a), 4.95 (1H, d, $J = 4.8$ Hz), 5.77 (1H, bs), 6.03 (1H, s, H-14), 6.65 (1H, t, $J = 6.3$ Hz, H-12), 7.7–7.67 (1H, m), 7.87 (1H, d, $J = 7.8$ Hz), 8.24 (2H, m); EI-MS *m/z* 483 (M^+) $C_{28}H_{33}NO_7$.

3,19-(2-chloro-5-nitrobenzylidene) andrographolide (***21***). IR: 3415, 2944, 1751, 1665, 1186, 910 cm^{-1}; ^1H NMR (250 MHz; $CDCl_3$): δ 0.89 (3H, s, H-20), 1.49 (3H, s, H-18), 2.04–1.9 (6H, m), 2.47 (2H, d, $J = 13.2$ Hz), 2.61 (2H, d, $J = 6.3$ Hz), 3.75–3.62 (2H, m), 4.33–4.29 (2H, m), 4.48 (1H, dd, $J = 6.0$ and 10.4 Hz, H-15a), 4.64 (1H, s, H-17b), 4.93 (1H, s, H-17a), 5.06 (1H, s), 6.09 (1H, s, H-14), 6.99 (1H, t, $J = 6.7$ Hz, H-12), 7.52 (1H, d, $J = 8.7$ Hz), 8.14 (1H, dd, $J = 2.7$ and 8.7 Hz), 8.6 (1H, d, $J = 2.6$ Hz); EI-MS *m/z* 517 (M^+) $C_{27}H_{32}ClNO_7$.

3,19-(2-hydroxy-5-nitrobenzylidene) andrographolide (***22***). IR: 3504, 2894, 1729, 1599, 1288, 1105, 912 cm^{-1}; ^1H NMR (400 MHz; DMSO-D_6): δ 0.81 (3H, s, H-20), 1.40 (3H, s, H-18), 1.84 (1H, d, $J = 13.1$ Hz), 1.94 (1H, d, $J = 9.7$ Hz), 2.01 (1H, td, $J = 4.5$ and 12.6 Hz), 2.54 (1H, s), 3.38 (3H, bs), 3.62 (1H, dd, $J = 4.8$ and 12.2 Hz, H-3), 4.05 (1H, dd, $J = 1.7$ and 10.0 Hz, H-15b), 4.21 (1H, d, $J = 11.2$ Hz, H-19b), 4.40 (1H, dd, $J = 6.1$ and 10.0 Hz, H-15a), 4.68 (1H, s, H-17b), 4.86 (1H, s, H-17a), 4.93 (1H, d, $J = 5.8$ Hz), 5.76 (1H, bs, H-14), 6.08 (1H, s), 6.65 (1H, t, $J = 6.1$ Hz, H-12), 6.98

(1H, d, $J = 9.2$ Hz), 8.10 (1H, dd, $J = 2.9$ and 8.7 Hz), 8.27 (1H, d, $J = 2.9$ Hz); EI-MS *m/z* 464 [M^+– (2 OH)] $C_{27}H_{33}NO_8$.

3,19-(4-methylbenzylidene) andrographolide (***23***). IR: 3390, 2940, 1731, 1102, 1021, 905 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$): δ 0.78 (3H, s, H-20), 1.39 (3H, s, H-18), 1.79–1.73 (4H, m), 2.24 (3H, s), 2.46–2.35 (1H, m), 2.5–2.47 (1H, m), 3.48 (1H, d, $J = 11.3$ Hz, H-19b), 3.56 (1H, dd, $J = 4.7$ and 12.5 Hz, H-3), 4.17–4.12 (2H, m), 4.36 (1H, dd, $J = 6.1$ and 10.4 Hz, H-15b), 4.53 (1H, s, H-17b), 4.82 (1H, s, H-17a), 4.91 (1H, d, $J = 4.9$ Hz), 5.64 (1H, s, H-14), 6.86 (1H, td, $J = 1.6$ and 6.9 Hz, H-12), 7.07 (1H, d, $J = 7.8$ Hz), 7.16 (1H, s), 7.28 (1H, s); EI-MS *m/z* 452 (M^+) $C_{28}H_{36}O_5$.

3,19-(3-methylbenzylidene) andrographolide (***24***). IR: 3392, 2938, 1727, 1676, 1221, 1162, 904 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$): δ 0.82 (3H, s, H-20), 1.27–1.20 (2H, m), 1.44 (3H, s, H-18), 1.83–1.75 (4H, m), 1.97 (1H, s), 2.22 (1H, d, $J = 6.6$ Hz, H-11), 2.30 (3H, s), 2.47–2.37 (1H, m), 2.56–2.49 (1H, m), 3.53 (1H, dd, $J = 1.1$ and 11.3 Hz, H-19b), 3.62 (1H, dd, $J = 4.7$ and 12.5 Hz, H-3), 4.22–4.17 (2H, m), 4.38 (1H, dd, $J = 6.1$ and 10.4 Hz, H-15a), 4.57 (1H, s, H-17b), 4.87 (1H, s, H-17a), 4.97 (1H, bs), 5.68 (1H, s, H-14), 6.92 (1H, td, $J = 1.6$ and 6.9 Hz, H-12), 7.10-7.08 (1H, m), 7.21–7.17 (2H, m), EI-MS *m/z* 452 (M^+) $C_{28}H_{36}O_5$.

3,19-(2-methylbenzylidene) andrographolide (***25***). IR: 3489, 2935, 1741, 1670, 1281, 1096, 820 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$): δ 0.91 (3H, s, H-20), 1.34 (3H, s, H-18), 1.63 (1H, s), 1.95–1.83 (3H, m), 2.31 (1H, d, $J = 3.9$ Hz, H-11), 2.41 (3H, s), 3.63 (1H, d, $J = 11.7$ Hz, H-3), 3.70 (1H, dd, $J = 4.8$ and 12.7 Hz), 4.29–4.26 (2H, m), 4.47 (1H, dd, $J = 6.1$ and 10.4 Hz, H-15a), 4.65 (1H, s, H-17b), 4.95 (1H, s, H-17a), 5.04 (1H, bs), 5.95 (1H, s, H-14), 6.99 (1H, t, $J = 6.8$ Hz, H-12), 7.26–7.23 (2H, m), 7.65 (1H, dd, $J = 3.6$ and 5.1 Hz); EI-MS *m/z* 452 (M^+) $C_{28}H_{36}O_5$.

3,19-(4-methoxy-3-methylbenzylidene) andrographolide (***26***). IR: 3434, 2947, 1740, 1257, 1131, 805 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$): δ 0.86 (3H, s, H-20), 1.48 (3H, s, H-18), 1.88–1.84 (4H, m), 2.2 (3H, s, OCH_3), 2.37 (1H, d, $J = 3.4$ Hz, H-11), 2.47-2.41 (2H, m), 2.59–2.53 (1H, m), 3.57 (1H, d, $J = 11.3$ Hz, H-19b), 3.65 (1H, dd, $J = 4.7$ and 12.6 Hz, H-3), 3.80 (3H, s), 4.23 (2H, dd, $J = 1.8$ and 8.5 Hz), 4.42 (1H, dd, $J = 6.1$ and 10.4 Hz, H-15a), 4.61 (1H, s, H-17b), 4.91 (1H, s, H-17a), 5.00 (1H, bs), 5.69 (1H, s, H-14), 6.78 (1H, d, $J = 8.1$ Hz), 6.98 (1H, dd, $J = 1.5$ and 6.9 Hz, H-12), 7.25 (1H, s); EI-MS *m/z* 482 (M^+) $C_{29}H_{38}O_6$.

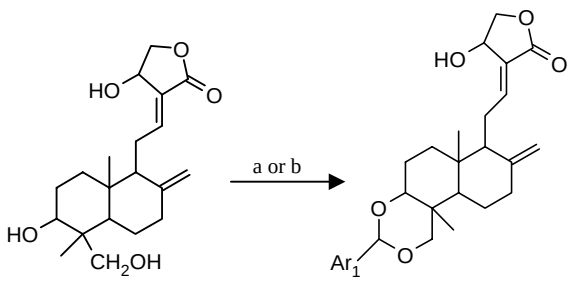
3,19-(3-fluoro-4-methoxybenzylidene) andrographolide (***27***). IR: 3414, 2942, 1734, 1669, 1275, 1124, 994 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$): δ 0.87 (3H, s, H-20), 1.47 (3H, s, H-18), 2.28 (1H, d, $J = 7.0$ Hz, H-11), 2.60–2.55 (1H, m), 3.58 (1H, d, $J = 11.3$ Hz, H-19b), 3.66 (1H, dd, $J = 4.7$ and 12.6 Hz, H-3), 3.88 (3H, s), 4.27–4.23 (2H, m), 4.45 (1H, dd, $J = 6.0$ and 10.4 Hz, H-15a), 4.63 (1H, s, H-17b), 4.93 (1H, s, H-17a), 5.03 (1H, t, $J = 6.4$ Hz), 5.70 (1H, s, H-14), 6.99-6.92 (2H, m), 7.24–7.20 (1H, m); EI-MS *m/z* 485 (M^+– H) $C_{28}H_{35}FO_6$.

3,19-(3-bromo-4-methoxybenzylidene) andrographolide (***28***). IR: 3512, 2941, 1724, 1268, 1102, 907 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$): δ 0.87 (3H, s, H-20), 1.47 (3H, s, H-18), 1.59 (1H, s), 2.20 (1H, d, $J = 7.0$ Hz), 2.45–2.39 (2H, m), 2.61–2.55 (1H, m), 3.58 (1H, dd, $J = 1.1$ and 10.3 Hz, H-15b), 3.68 (1H, dd, $J = 4.8$ and 12.6 Hz, H-3), 3.89 (1H, s), 4.28–4.23 (2H, m), 4.45 (1H, dd, $J = 6.1$ and 10.4 Hz, H-15a), 4.63 (1H, s, H-17b), 4.93 (1H, s, H-17a), 5.03 (1H, t, $J = 6.4$ Hz), 5.70 (1H, s, H-14), 6.88 (1H, d, $J = 8.5$ Hz), 6.97 (1H, td, $J = 1.6$ and 6.9 Hz, H-12), 7.39 (1H, dd, $J = 2.0$ and 8.4 Hz), 7.70 (1H, d, $J = 2.0$ Hz); EI-MS *m/z* 547 (M^+) $C_{28}H_{35}BrO_6$.

3,19-(3-chloro-4-methoxybenzylidene) andrographolide (***29***). IR: 3512, 2944, 1726, 1676, 1270, 1195, 1021, 820 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$): δ 0.87 (3H, s, H-20), 1.47 (3H, s, H-18), 1.60 (1H, s), 2.27 (1H, d, $J = 7.0$ Hz, H-11), 2.48–2.39 (1H, m), 2.60–2.55 (1H, m), 3.58 (1H, dd, $J = 1.0$ and 11.3 Hz, H-15b), 3.66 (1H, dd, $J = 4.7$ and 12.6 Hz, H-3), 3.87 (3H, s), 4.27–4.23 (2H, m), 4.45 (1H, dd, $J = 6.1$ and 10.4 Hz, H-15a), 4.63 (1H, s, H-17b), 4.93 (1H, s, H-17a), 5.43 (1H, t, $J = 6.3$ Hz), 5.70 (1H, s, H-14), 6.91 (1H, d, $J = 8.5$ Hz), 6.97 (1H, td, $J = 1.6$ and 7.3 Hz, H-12), 7.34 (1H, dd, $J = 2.0$ and 8.5 Hz), 7.53 (1H, d, $J = 2.0$ Hz); EI-MS *m/z* 503 (M^+) $C_{28}H_{35}ClO_6$.

3,19-(4-chlorobenzylidene) andrographolide (***30***). IR: 3447, 2951, 1755, 1670, 1217, 1114, 899 cm^{-1}; ^1H NMR (250 MHz; $CDCl_3$): δ 0.87 (3H, s, H-20), 1.46 (3H, s, H-18), 2.22 (1H, d, $J = 7.1$ Hz, H-11), 2.48–2.39 (1H, m), 3.59 (1H, d, $J = 11.4$ Hz, H-19b), 3.64 (1H, dd, $J = 4.6$ and 12.6 Hz, H-3), 4.29–4.24 (2H, m), 4.44 (1H, dd, $J = 6.0$ and 10.5 Hz, H-15a), 4.62 (1H, s, H-17b), 4.92 (1H, s, H-17a), 5.05 (1H, t, $J = 6.3$ Hz), 5.74 (1H, s, H-14), 6.96 (1H, td, $J = 1.5$ and 6.8 Hz, H-12), 7.45–7.32 (4H, m); EI-MS *m/z* 473 (M^+) $C_{27}H_{33}ClO_5$.

3,19-(2-chloro-4-fluorobenzylidene) andrographolide (***31***). IR: 3464, 2951, 1751, 1242, 1098, 901 cm^{-1}; ^1H NMR (250 MHz; $CDCl_3$): δ 0.88 (3H, s, H-20), 1.35–1.26 (3H, m), 1.48 (3H, s, H-18), 2.08–1.83 (5H, m), 2.21 (1H, d, $J = 7.0$ Hz, H-11), 2.55–2.38 (2H, m), 2.62 (2H, s), 3.71–3.58 (2H, m), 4.27 (1H, dd, $J = 2.0$ and 10.4 Hz, H-15b), 4.47 (1H, dd,



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Scheme 1. Synthesis of andrographolide derivatives. Reagents and conditions: (a) $ZnCl_2$/DMSO/RT/5-6 h (b) pyridinium *p*-toluenesulphonate/DMSO/60 – 70°C/2-3 h.

$J = 6.0$ and 10.5 Hz, H-15a), 4.63 (1H, s, H-17b), 4.92 (1H, s, H-17a), 5.05 (1H, t, $J = 6.2$ Hz), 6.06 (1H, s, H-14), 7.11–6.97 (3H, m), 7.73 (1H, dd, $J = 6.2$ and 8.6 Hz); EI-MS *m/z* 490 ($M^+ - H$) $C_{27}H_{32}ClFO_5$.

Biology

MCF-7 (human breast cancer) and HCT-116 (human colon cancer) cells were purchased from American Type Cell Collection (ATCC, Rockville, MD, USA) and were used for *in vitro* pre-screen anticancer activity. Cells were cultured in RPMI 1640 medium with L-glutamine, supplemented with 10% heat inactivated (55°C for 1 h) foetal bovine serum (FBS), 200 U/ml of penicillin and 200 μg/ml of streptomycin, at 37°C in an atmosphere of 5% CO_2 and 95% air.

In vitro *cytotoxicity assay*. This assay was carried out based on the method by Mosmann [14]. Briefly, cells were plated in 96-well flat-bottomed tissue culture plates at initial seeding densities of between 3000 to 5000 cells per well in a volume of 180 μl of culture media. Cells were incubated at 37°C (5% CO_2 and 95% air) overnight to allow attachment to the wells. Compounds were diluted serially with concentrations ranging from 1 μM to 1000 μM and 20 μl of each compound dilution was added into the appropriate wells in four replicates. Following incubation at 37°C in an atmosphere of 5% CO_2 and 95% air for 72 h, 50 μl of MTT (2 mg/ml in PBS) was added into each well containing the cells in 200 μl medium. The plates were re-incubated for 4 h to allow metabolism of MTT by cellular mitochondrial dehydrogenases. Following the incubation period, the suspension of MTT and medium was aspirated from each well and 150 μl of DMSO:glycine buffer (0.1 M glycine/0.1 M NaCl/ pH 10.5) (4:1) was added into each well. The plates were then shaken to dissolve the formazan crystals and the absorbance of formazan was read at 550 nm on an Anthos microplate reader (Anthos Labtec Instruments GmbH, Lagerhausstr, Austria). The absorbance of the purple formazan at 550 nm is proportional to the number of viable cells. For both the cell lines, 0-day cell densities (by MTT end point) were also determined for both cell lines in order to obtain percentage growth of cells post-treatment. The results were analysed using Deltasoft 3 computer program (BioMetallics Inc., Princeton, NJ, USA). From the semi-log dose-response curves (percentage of growth vs concentration), three response parameters (GI_{50}, TGI and LC_{50}) were determined.

NCI in vitro *screen*. The synthesized compounds 3,19-(2-chlorobenzylidene)andrographolide(**5**), 3,19-(3-chlorobenzylidene)andrographolide(**6**), 3,19-(3-fluorobenzylidene)andrographolide(**7**), 3,19-(4-fluorobenzylidene)andrographolide(**8**), 3,19-(2-fluorobenzylidene)andrographolide(**10**), 3,19-(2-chloro-5-nitrobenzylidene)andrographolide(**21**), 3,19-(4-chlorobenzylidene)andrographolide(**30**) and 3,19-(2-chloro-4-fluorobenzylidene)andrographolide(**31**) were also evaluated for antitumour activity at the National Cancer Institute (NCI) at Bethesda, MD, USA, following the known *in vitro* disease-oriented antitumour screening program against a panel of ~60 human tumour cell lines derived from various cancer types (lung, colon, melanoma, brain, prostate, renal, ovarian and leukaemia) [16,17]. The percentage growth was evaluated spectrophotometrically versus controls that were not treated with test agents. A 48 h continuous exposure protocol was followed and a sulphorhodamine B (SRB) protein assay was used to estimate cell viability. Furthermore, a mean graph midpoint (MG_MID) was calculated for each of the mentioned parameters, giving an averaged activity parameter over all the cell lines.

Results and discussion

Chemistry

Andrographolide is a diterpene containing a γ-lactone ring connected to a decalin ring system *via* an unsaturated C-2 moiety and contains three hydroxyls at C-3, C-14, and C-19. The hydroxyl groups at C-3 and C-19 are secondary and primary, respectively, and the one at C-14 is allylic in nature. The derivatives of andrographolide were synthesised by coupling of two hydroxyl groups at C-3 and C-19. Coupling of hydroxyl groups were carried out by reacting andrographolide with different types of aromatic aldehydes having different kinds of functional groups (Scheme 1). The corresponding analogues synthesised from andrographolide were evaluated for their *in vitro* antitumour activities against the MCF-7 (breast) and HCT-116 (colon) cell lines.

Table I. Dose response parameters of andrographolide and its derivatives in *in vitro* anticancer pre-screen cell lines[1].

Compounds	GI_{50}		TGI		LC_{50}	
	MCF-7	HCT-116	MCF-7	HCT-116	MCF-7	HCT-116
1	6.4 ± 2.1	5.1 ± 0.1	64.7 ± 12.2	15.5 ± 9.3	81.1 ± 17.5	39.5 ± 9.1
2	5.3 ± 0.3	4.8 ± 0.2	9.7 ± 0.3	9.2 ± 0.1	32.3 ± 2.1	9.6 ± 0.1
3	4.2 ± 2.8	3.8 ± 3.5	8.8 ± 1.1	8.1 ± 1.6	9.4 ± 0.3	8.6 ± 1.2
4	5.3 ± 1.6	4.7 ± 0.3	43.2 ± 3.1	9.0 ± 0.5	56.6 ± 4.4	9.6 ± 0.1
5	5.0 ± 1.1	5.2 ± 1.2	27.5 ± 18.0	9.2 ± 0.3	43.7 ± 3.3	9.6 ± 0.1
6	4.9 ± 2.1	3.9 ± 1.2	29.5 ± 15.9	19.2 ± 2.3	42.3 ± 11.2	15.5 ± 6.6
7	6.8 ± 0.4	5.7 ± 1.6	66.6 ± 8.8	28.6 ± 3.3	82.8 ± 4.9	43.8 ± 5.5
8	3.5 ± 1.5	2.3 ± 2.2	9.1 ± 0.3	8.8 ± 2.6	9.5 ± 0.1	10.6 ± 1.8
9	5.2 ± 1.8	3.8 ± 1.7	9.1 ± 1.5	8.7 ± 0.2	9.5 ± 0.3	9.4 ± 0.1
10	5.5 ± 0.4	4.5 ± 2.2	9.4 ± 1.5	9.0 ± 0.9	9.9 ± 3.3	9.5 ± 2.1
11	6.3 ± 1.2	4.5 ± 1.3	61.0 ± 28.0	9.2 ± 0.1	80.1 ± 14.3	9.7 ± 0.1
12	4.1 ± 0.4	4.1 ± 0.4	8.7 ± 0.3	9.4 ± 0.2	9.5 ± 0.1	20.2 ± 18.4
13	7.5 ± 1.0	7.1 ± 0.7	8.8 ± 11.3	66.0 ± 5.6	89.2 ± 6.6	82.9 ± 2.9
14	7.1 ± 1.4	12.4 ± 5.4	69.3 ± 2.5	76.7 ± 6.6	86.8 ± 5.0	87.8 ± 4.1
15	3.6 ± 1.2	4.5 ± 0.9	11.6 ± 5.9	9.1 ± 0.2	25.3 ± 7.4	16.8 ± 8.6
16	18.0 ± 10.1	10.4 ± 4.1	74.9 ± 5.2	75.6 ± 7.3	90.6 ± 5.2	8.5 ± 3.5
17	3.6 ± 0.6	7.7 ± 3.1	8.7 ± 0.4	9.0 ± 0.1	9.5 ± 0.2	9.5 ± 0.1
18	0.7 ± 0.1	2.6 ± 3.3	86.1 ± 6.1	81.5 ± 9.3	96.6 ± 5.7	95.1 ± 8.3
19	4.6 ± 2.8	3.9 ± 1.2	30.5 ± 2.2	12.2 ± 5.3	32.3 ± 10.5	18.2 ± 3.1
20	3.5 ± 0.2	4.9 ± 1.1	8.8 ± 1.1	9.2 ± 0.2	11.1 ± 3.3	9.5 ± 0.1
21	2.3 ± 1.8	5.3 ± 3.8	8.0 ± 0.5	8.5 ± 2.8	10.2 ± 2.2	9.2 ± 0.2
22	4.9 ± 0.4	5.9 ± 0.6	22.4 ± 13.6	37.9 ± 6.3	51.3 ± 16.6	67.5 ± 13.4
23	3.9 ± 0.1	6.6 ± 2.4	8.8 ± 0.8	9.2 ± 0.4	14.1 ± 8.2	19.2 ± 16.8
24	5.1 ± 0.3	6.9 ± 0.7	44.3 ± 4.9	55.6 ± 15.5	72.5 ± 11.2	77.5 ± 7.4
25	4.7 ± 0.6	5.2 ± 0.3	19.9 ± 18.4	14.6 ± 4.7	40.5 ± 4.5	42.7 ± 2.5
26	4.8 ± 1.2	5.8 ± 1.2	79.3 ± 12.2	66.9 ± 6.6	96.8 ± 5.9	87.7 ± 4.9
27	7.1 ± 1.8	5.8 ± 2.6	19.7 ± 6.3	9.2 ± 10.1	12.3 ± 2.1	19.6 ± 9.1
28	7.8 ± 3.1	6.4 ± 1.5	18.8 ± 1.4	8.6 ± 5.2	12.4 ± 9.3	18.8 ± 7.3
29	10.1 ± 1.9	7.0 ± 1.8	41.2 ± 3.9	9.5 ± 10.5	56.6 ± 11.6	9.8 ± 11.1
30	4.7 ± 2.8	5.9 ± 3.5	32.5 ± 10.2	12.2 ± 3.3	37.3 ± 13.5	16.2 ± 7.1
31	2.2 ± 1.3	2.5 ± 1.0	8.0 ± 2.7	8.4 ± 0.2	8.6 ± 0.5	9.1 ± 5.1

[1] Human colon (HCT-116) and breast (MCF-7) cancer cell lines were treated for 72 h with four different concentrations of compounds ranging from 0.1 to 100 μM. MTT [14] assay was used to calculate GI_{50}, TGI and LC_{50} values (expressed in μM). Values are mean of 3 separate experiments and errors represent the SD values.

In vitro *anticancer screening*

When potential anticancer compounds are discovered or synthesised, they are commonly subjected to testing in a panel of cell lines to determine their level of potency and selectivity. Potency is usually expressed as GI_{50} (concentration that produces 50% growth inhibition), TGI (total growth inhibition or cytostatic effect) and LC_{50} (−50% growth: lethal concentration or "net cell killing" or cytotoxicity parameter) values [13].

The anticancer activity of andrographolide derivatives were evaluated in MCF-7 human breast cancer and HCT-116 colon cancer cell lines. The growth inhibitory and cytotoxic properties of these compounds were determined by using a 72 h MTT cell viability assay [14]. The majority of the derivatives showed similar activity as the parent compound against both MCF-7 and HCT-116 cell lines except compound 3,19-(4-nitrobenzylidene)andrographolide(**18)** (Table I). The later was approximately 9-fold and 2-fold more potent than andrographolide against MCF-7 and HCT-116 cell lines, respectively. It was also approximately 4-fold more selective towards MCF-7 cells compared to HCT-116 cells.

With respect to TGI and LC_{50} values, selectivity and potency of the derivatives varied when compared to that of the parent compound. Derivatives 3,19-(4-bromobenzylidene)-, 3,19-(2-bromobenzylidene)-, 3,19-(4-fluorobenzylidene)-, 3,19-(3-chloro-4-fluorobenzylidene)-, 3,19-(2-fluorobenzylidene)-, 3,19-(3,5-dimethoxybenzylidene)-, 3,19-(2-ethoxy-4-hydroxybenzylidene)-, 3,19-(2,5-dimethoxybenzylidene)-, 3,19-(2-nitrobenzylidene)-, 3,19-(3-nitrobenzylidene)-, 3,19-(2-chloro-5-nitrobenzylidene)-, 3,19-(4-methylbenzylidene)-, 3,19-(3-fluoro-4-methoxybenzylidene)-, 3,19-(3-bromo-4-methoxybenzylidene)- and 3,19-(2-chloro-4-fluorobenzylidene) andrographolide showed improvement in antitumour activities compared to the parent compound.

The results of *in vitro* anticancer evaluation in 60 NCI cancer cell lines are illustrated in Table II, where for each compound the growth inhibitory activity (GI_{50}) and the mean graph midpoints (MG_MID) values were considered. The mean graph midpoint

Table II. Inhibition GI_{50} values of *in vitro* cancer cell lines by andrographolide derivatives[1].

Panel cell line	1[2]	5	6	7	8	10	21	30	31
Leukaemia									
CCRF-CEM	3.98	0.281	1.93	2.12	nt	0.333	2.17	0.518	0.244
HL-60(TB)	15.84	9.36	3.05	1.03	0.626	0.918	nt	>100	2.35
K-562	3.16	6.72	3.46	5.87	2.45	4.26	2.14	3.41	2.19
MOLT-4	25.11	14.00	2.95	1.21	0.648	2.17	nt	2.71	1.27
RPMI-8226	5.01	nt	nt	nt	nt	nt	nt	nt	nt
SR	1.00	nt	nt	nt	nt	nt	nt	>100	nt
Non-small cell lung cancer									
A549/ATCC	25.11	3.43	2.72	3.77	4.09	3.72	1.40	1.62	2.19
EKVX	31.62	21.50	14.40	19.10	10.60	21.80	11.51	3.13	3.69
HOP-62	31.62	18.10	22.70	25.30	2.35	18.80	1.94	15.20	2.60
HOP-92	25.11	0.172	1.59	0.793	0.674	1.28	15.30	1.31	0.307
NCI-H226	39.81	1.80	1.99	2.30	2.05	2.20	1.51	1.29	1.66
NCI-H23	19.95	16.80	9.58	15.10	1.81	18.90	2.32	3.40	2.18
NCI-H322M	31.62	11.90	11.00	14.80	2.98	14.60	2.46	2.13	2.53
NCI-H460	19.95	19.40	18.60	16.50	3.08	16.80	1.69	4.35	1.99
NCI-H522	10.00	1.86	nt	0.242	2.62	1.29	3.36	nt	0.368
Colon cancer									
COLO 205	15.84	1.91	1.43	1.37	1.81	1.76	18.10	0.626	1.56
HCC-2998	25.11	11.00	4.74	4.84	2.00	16.90	2.58	3.11	2.21
HCT-116	15.84	12.70	5.16	21.80	1.91	5.86	1.25	1.97	1.53
HCT-15	15.84	20.40	10.30	14.20	2.39	16.90	2.18	2.34	1.47
HT29	0.501	3.19	2.27	2.55	2.91	4.16	3.02	0.976	1.75
KM12	15.84	2.18	3.58	7.09	2.83	6.84	1.80	1.37	1.92
SW-620	10.00	4.27	3.48	4.21	3.57	4.34	3.71	0.924	2.78
CNS cancer									
SF-268	25.11	20.50	20.40	17.30	3.29	16.90	1.76	10.20	1.92
SF-295	19.95	5.58	12.30	12.60	2.49	11.70	12.90	2.28	2.23
SF-539	19.95	2.47	2.19	2.37	2.79	2.92	3.32	2.36	2.24
SNB-19	50.11	4.00	2.72	11.50	2.99	5.08	18.90	2.63	1.91
SNB-75	15.84	1.85	2.11	3.26	2.29	3.45	3.27	1.08	1.15
U251	10.00	18.00	2.30	14.00	1.69	10.70	1.57	1.72	1.46
Melanoma									
LOX IMVI	12.58	3.97	2.04	7.81	1.78	2.90	1.51	1.74	1.25
MALME-3M	15.84	nt	nt	nt	nt	nt	nt	nt	nt
M14	15.84	16.00	25.80	27.90	2.60	17.10	1.66	3.07	3.91
SK-MEL-2	15.84	2.31	3.86	2.03	2.77	13.9	4.93	2.23	2.82
SK-MEL-28	12.58	2.04	2.12	2.93	2.89	3.16	2.49	1.92	1.87
SK-MEL-5	15.84	14.30	3.12	15.50	10.50	17.00	7.13	1.82	1.79
UACC-257	19.95	14.20	2.91	11.60	11.70	10.60	1.84	1.93	1.17
UACC-62	19.95	2.78	1.97	5.91	2.50	3.80	nt	1.53	1.77
Ovarian cancer									
IGROV1	19.95	2.55	1.31	2.93	2.10	5.33	3.97	0.318	1.85
OVCAR-3	10.00	1.39	2.22	2.46	1.50	2.77	22.40	1.65	1.23
OVCAR-4	10.00	2.94	2.14	9.56	2.50	6.31	nt	1.32	1.71
VCAR-5	15.84	19.00	13.40	16.60	2.80	21.40	2.73	13.70	2.09
OVCAR-8	10.00	3.07	3.48	3.33	2.71	4.61	0.093	0.824	3.03
SK-OV-3	25.11	14.80	15.10	15.80	10.50	13.60	17.60	4.36	3.69
Renal cancer									
786-0	5.01	18.10	14.20	19.40	1.92	6.11	1.82	4.17	1.76
A498	31.62	23.90	15.80	25.50	22.00	21.00	23.50	2.97	2.45
ACHN	31.62	18.40	11.00	16.90	1.95	14.80	1.60	2.09	1.65
RXF 393	19.95	Nt	nt	nt	nt	0.013	nt	1.91	nt
CAKI-1	nt	2.43	9.46	10.00	1.62	4.45	19.00	1.25	1.63
SN12C	50.11	3.43	1.68	2.62	1.73	2.37	1.67	0.751	1.68
K-10	15.84	11.80	3.71	19.00	4.39	17.90	2.84	1.75	2.19
UO-31	10.00	1.62	1.93	2.70	2.39	14.60	3.18	1.13	2.65
Prostate cancer									
PC-3	19.95	0.631	3.10	4.07	1.62	3.71	7.83	2.22	1.35
DU-145	39.81	10.60	4.14	12.00	3.77	7.52	8.44	1.68	2.05
Breast cancer									
MCF-7	15.84	26.30	5.53	13.60	2.89	7.63	0.513	3.38	1.72
NCI/ADR-RES	25.11	7.85	3.14	3.44	1.81	12.9	1.84	3.14	1.92
MDA-MB-231/ATCC	25.11	2.25	2.06	3.00	2.11	2.37	1.91	1.81	1.80
HS 578T	39.81	28.00	7.33	34.60	3.92	20.20	6.85	3.11	2.75

Table II – *continued*

Panel cell line	1[2]	5	6	7	8	10	21	30	31
MDA-MB-435	10.00	2.35	4.31	40.13	2.00	3.22	4.15	1.98	1.85
BT-549	7.94	10.20	3.07	9.99	2.90	7.77	3.82	2.16	2.25
T-47D	25.11	1.69	1.30	1.88	1.85	2.26	23.90	0.309	1.14
MG_MID	16.59	5.49	4.26	6.16	2.63	5.49	3.46	2.08	1.77

[1] Data obtained from the NCI's *in vitro* disease-oriented human tumour cells screen [15]. Values (GI_{50}) are expressed in μM. nt: not tested. Mean panel values (mean graph midpoints MG-MID) of the response parameter were obtained by averaging the individual values for each cell line.
[2] The values for andrographolide are based on previous screens conducted by the NCI where the highest concentration tested was also 100 μM.

values were obtained by averaging the individual values for each cell line. From the reported data, it appears that all the compounds possess antiproliferative activity. All eight compounds that were tested in 60 NCI cancer cell lines were more potent compared to that of the parent compound.

In this study we observed that the presence of an electron rich rings (e.g. 3,19-(3,4-dimethoxybenzylidene)andrographolide), electron deficient rings (e.g. 3,19-(2-chlorobenzylidene)andrographolide), inductive effective groups (e.g. 3,19-(4-bromobenzylidene) andrographolide) at C-3 and C-19 of andrographolide did not show variations in the antitumour activities. All the compounds synthesised by the coupling reaction showed similar GI_{50} values in pre-screen cell lines, except for compound 3,19-(4-nitrobenzylidene)andrographolide. However, these compounds differed in their (TGI values) cytostatic and cytocidal (LC_{50} values) effects. This suggests that the presence of alkyl or aryl moieties at C-3 and C-19 may also play an important role in promoting anticancer activities.

Conclusion

In conclusion, in our search for development of antitumour agents from medicinal plants, we have synthesised a number of andrographolide analogues possessing potent *in vitro* antitumour activity against different cancer cell lines. The mechanisms of the antitumour activity and further variations of the parent structure are currently being investigated to improve both potency and selectivity and to obtain more detailed information on the structure activity relationship (SAR).

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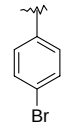
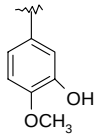
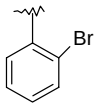
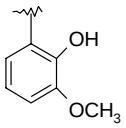
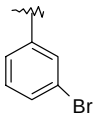
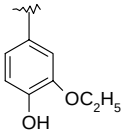
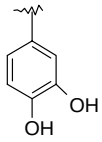
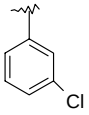
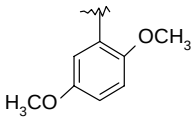
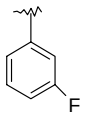
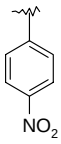
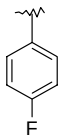
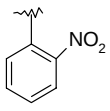
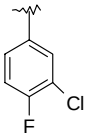
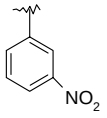
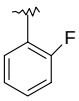
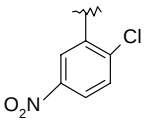
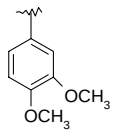
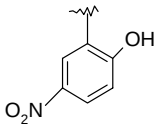
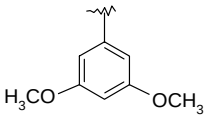
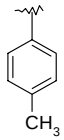
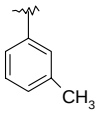
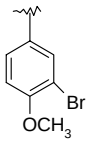
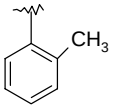
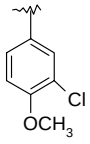
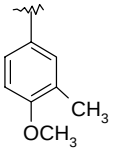
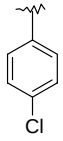
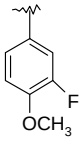
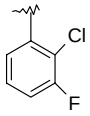
Appendix

Scheme 1. Synthesis of andrographolide derivatives. Reagents and conditions: (a) $ZnCl_2$/DMSO/RT/5–6 h (b) pyridinium p-toluene-sulphonate/DMSO/60–70°C/2–3 h.

Compound No.	Ar_1	M.p. (°C)	Yield (%)	Compound No.	Ar_1	M.p. (°C)	Yield (%)
2		212–213	62	13		227–228	30
3		228–229	64	**14**		165–166	91
4		168–169	92	**15**		172–173	98
5		206–207	67	**16**		190	77
6		192	32	**17**		223–224	72
7		179–180	82	**18**		196–197	74
8		115–116	34	**19**		185–186	17
9		103–104	89	**20**		165–166	70
10		206–208	84	**21**		184–185	81

Appendix – *continued*

Compound No.	Ar_1	M.p. (°C)	Yield (%)	Compound No.	Ar_1	M.p. (°C)	Yield (%)
11		119–120	68	**22**		169–170	95
12		125–126	99	**23**		193–194	71
24		143–144	77	**28**		227–228	85
25		239–240	91	**29**		226–227	81
26		193–194	65	**30**		225–228	91
27		219–220	81	**31**		215–216	78