Supporting Information

Synthesis and evaluation of anticancer activity of novel andrographolide derivatives

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Instrumentation and chemicals

All non aqueous reactions were carried out under N₂ atmosphere in flame-dried glassware. All reagents were procured from Sigma Aldrich and were used as received. Dichloromethane was freshly distilled from CaH₂. Reactions were monitored by TLC using Kieselgel 60 F₂₅₄ (E. Merck) silica plates and visualised by UV detection (at 254 nm) and staining with 5 % sulphuric acid in methanol. Melting points were measured using A. KRUSS OPTRONIC and are uncorrected. NMR spectra were recorded on Bruker Avance 300/400 MHz in CDCl₃ and DMSO-d₆ using TMS as internal standard. IR data are given only for compounds with significant functions (OH, C=O) and were recorded as neat or KBr plate and are reported in wave number (cm⁻¹).
Synthesis and characterization of compounds

General experimental procedure for compounds 3-8 (Scheme 1):
Andrographolide 1 (100 mg, 0.28 mmol) and alcohol (1.428 mmol) were taken in acetonitrile (10 mL). To this solution, CAN (313 mg, 0.57 mmol) in minimum amount of acetonitrile was added slowly using dropping funnel. The reaction mixture was stirred at room temperature for 24 h then concentrated to half volume and aquified with 15 ml of water and then extracted with ethyl acetate (3 × 20 ml). The separated organic layers were combined, washed with brine solution, dried over Na₂SO₄ and evaporated under reduced pressure to get crude residue. The residue was purified through silica gel (100-200 mesh) column chromatography (ethyl acetate-petroleum ether) to afford the desired products 3/4/5/6/7/8.

3,19-O-ethylideneandrographolide (3) Yield: 96 mg (90%), white solid, m.p. 198–200 °C; IR (KBr): 3412, 2941, 1744, 1673, 1403, 1108 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ (ppm) 6.92 (t, J = 6.3 Hz, 1H), 4.97 (m, 2H), 4.88 (s, 1H), 4.59 (s, 1H), 4.44 (dd, J = 10.5 Hz, 6.3 Hz, 1H), 4.23 (d, J = 10.5 Hz, 1H), 4.03 (d, J = 11.1 Hz, 1H), 3.47–3.38 (m, 2H), 2.83 (br s, 1H), 2.56–2.48 (m, 2H), 2.41–2.0 (m, 3H), 1.81 (m, 3H), 1.69–1.66 (m, 1H), 1.35 (s, 3H), 1.27–1.06 (br m, 6H), 0.85 (s, 3H); ¹³C NMR (75 MHz, CDCl₃): δ (ppm) 170.18, 148.90, 146.51, 127.99, 109.12, 92.19, 80.0, 74.42, 68.90, 66.09, 55.70, 54.58, 38.79, 37.54, 36.65, 36.02, 25.94, 24.70, 22.71, 21.77, 21.23, 15.22; ESI-MS: positive ion mode: m/z 399.41 [M+Na]⁺, observed for C₂₂H₃₂O₅.

3,19-O-n-propylideneandrographolide (4) Yield: 84 mg (76%), yellow solid, m.p. 176–178 °C; IR (KBr): 3403, 2937, 1726, 1674, 1401, 1102 cm⁻¹; ¹H NMR (300 MHz,
CDCl$_3$: $\delta$ (ppm) 6.97 (t, $J = 6.9$ Hz, 1H), 5.03 (d, $J = 5.4$ Hz, 1H), 4.90 (s, 1H), 4.74 (t, $J = 4.8$ Hz, 1H), 4.60 (s, 1H), 4.46 (dd, $J = 10.5$ Hz, 6.3 Hz, 1H), 4.26 (dd, $J = 10.5$ Hz, 1.8 Hz, 1H), 4.03 (d, $J = 11.1$ Hz, 1H), 3.51–3.42 (m, 2H), 2.58–2.52 (m, 2H), 2.43–1.16 (br m, 12H), 1.36 (s, 3H), 0.92 (t, $J = 7.5$ Hz, 3H), 0.81 (s, 3H); ESI-MS: positive ion mode: $m/z$ 391.07 [M+H]$^+$, observed for C$_{23}$H$_{34}$O$_5$.

3,19-O-\textit{n}-butylideneandrographolide (5) Yield: 86 mg (75%), light yellow solid, m.p. 132–134 °C; $\delta$ (ppm) 6.95 (t, $J = 6.6$ Hz, 1H), 5.03 (d, $J = 6$ Hz, 1H), 4.89 (s, 1H), 4.80 (t, $J = 5.1$ Hz, 1H), 4.60 (s, 1H), 4.46 (dd, $J = 10.5$ Hz, 6.3 Hz, 1H), 4.26 (dd, $J = 10.5$ Hz, 2.1 Hz, 1H), 4.03 (d, $J = 11.4$ Hz, 1H), 3.51–3.41 (m, 2H), 2.63–1.16 (br m, 16H), 1.36 (s, 3H), 0.91 (m, 3H), 0.81 (s, 3H); ESI-MS: positive ion mode: $m/z$ 405.34 [M+H]$^+$, 427.40 [M+Na]$^+$, observed for C$_{24}$H$_{36}$O$_5$.

3,19-O-\textit{n}-pentylideneandrographolide (6) Yield: 72 mg (61%), yellow solid, m.p. 162–164 °C; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ (ppm) 6.94 (t, $J = 6.9$ Hz, 1H), 5.02 (d, $J = 5.1$ Hz, 1H), 4.89 (s, 1H), 4.84 (t, $J = 5.1$ Hz, 1H), 4.61 (s, 1H), 4.45 (dd, $J = 10.5$ Hz, 6.1 Hz, 1H), 4.26 (d, $J = 10.5$ Hz, 1H), 4.03 (d, $J = 11.4$ Hz, 1H), 3.41–3.51 (m, 2H), 2.56–2.27 (m, 4H), 2.04–0.90 (br m, 17H), 1.36 (s, 3H), 0.81 (s, 3H); ESI-MS: positive ion mode: $m/z$ 440.94 [M+Na]$^+$, observed for C$_{25}$H$_{38}$O$_5$.

3,19-O-\textit{n}-hexylideneandrographolide (7) Yield: 43 mg (35 %), white solid, m.p. 181–183 °C; IR (KBr): 3411, 2940, 1744, 1674, 1400, 1081 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ (ppm) 6.96 (t, $J = 6.9$ Hz, 1H), 5.03 (d, $J = 5.1$ Hz, 1H), 4.90 (s, 1H), 4.79 (t, $J = 4.8$ Hz, 1H), 4.60 (s, 1H), 4.46 (dd, $J = 10.5$ Hz, 6.3 Hz, 1H), 4.26 (dd, $J = 10.5$ Hz, 2.1 Hz, 1H), 4.03 (d, $J = 11.1$ Hz, 1H), 3.49–3.41 (m, 2H), 2.57–2.52 (m, 2H), 2.45–0.90 ( br m, 21H), 1.36 (s, 3H), 0.81 (s, 3H); $^{13}$C NMR (75
MHz, CDCl₃): δ (ppm) 170.17, 148.91, 146.60, 128.04, 109.14, 95.31, 79.94, 74.43, 68.99, 66.15, 55.77, 54.76, 38.85, 37.61, 36.94, 36.08, 35.17, 31.77, 25.99, 24.75, 23.60, 22.76, 22.61, 21.79, 15.28, 14.04; ESI-MS: positive ion mode: m/z = 433.40 [M+H]+, 455.38 [M+Na]+, observed for C₂₆H₄₀O₅.

3,19-O-benzylideneandrographolide (8)

Yield: 111 mg (86 %), yellow solid, m.p. 142–144 °C; ¹H NMR (300 MHz, CDCl₃): δ (ppm) 7.34–7.50 (m, 5H), 6.94 (t, J = 6.0 Hz, 1H), 5.76 (s, 1H), 5.02 (d, J = 3.9 Hz, 1H), 4.91 (s, 1H), 4.63 (s, 1H), 4.42 (dd, J = 10.5 Hz, 6.3 Hz, 1H), 4.26–4.19 (m, 2H), 3.67 (dd, J = 12.6 Hz, 4.5 Hz, 1H), 3.59 (d, J = 11.4 Hz, 1H), 2.75 (s, 1H), 2.58–2.53 (m, 2H), 2.50–2.37 (m, 2H), 2.06–2.04 (m, 1H), 1.98–1.78 (br m, 4H), 1.49 (s, 3H), 1.34–1.21 (br m, 3H), 0.87 (s, 3H); ESI-MS: positive ion mode: m/z = 439.16 [M+H]+, 461.08 [M+Na]+, observed for C₂₇H₃₄O₅.

Experimental procedure and characterization for 14-acetyl-3,19-O-ethylideneandrographolide (3a): Compound 3 (100 mg, 0.2659 mmol) and TEA (0.5 mL) and acetic anhydride (1 mL) were taken in to CH₂Cl₂ (10 mL) and stirred at RT for 30 min. The completion of the reaction monitored by TLC, The reaction mixture was concentrated and poured into cold water, then filtered and dried. It was crystallized in CH₂Cl₂:MeOH (9:1) at RT to get desired compound 3a. Yield: 105 mg (95 %), white crystalline solid, m.p. 150–152 °C; IR (KBr): 3490, 2984, 1754, 1740, 1674, 1403, 1072 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ (ppm) 6.96 (t, J = 6.9 Hz, 1H), 5.89 (d, J = 5.4 Hz, 1H), 4.93 (q, J = 4.8 Hz, 1H), 4.84 (s, 1H), 4.53–4.48 (m, 2H), 4.20 (dd, J = 11.1 Hz, 1.5 Hz, 1H), 3.99 (d, J = 11.4 Hz, 1H), 3.45–3.35 (m, 2H), 2.41–2.36 (m, 2H), 2.24–1.12 (br m, 12H), 2.08 (s, 3H), 1.33
(s, 3H), 0.74 (s, 3H); $^{13}$C NMR (300 MHz, CDCl$_3$): $\delta$ (ppm) 170.84, 169.36, 150.65, 147.11, 124.34, 109.36, 92.58, 80.35, 71.93, 69.28, 68.16, 56.02, 55.10, 39.16, 37.93, 37.06, 36.42, 26.35, 25.62, 23.09, 22.18, 21.66, 21.08, 15.22; ESI-MS: positive ion mode: $m/z$ = 419 [M+H]$^+$, 441 [M+Na]$^+$, 859 [2M+Na]$^+$, observed for C$_{24}$H$_{34}$O$_6$.

Crystal data for compound 3a: C$_{24}$H$_{34}$O$_6$, $M$ = 418.51, colourless needle, 0.15 × 0.08 × 0.06 mm$^3$, orthorhombic, space group $P2_12_12_1$ (No. 19), $a$ = 11.987(3), $b$ = 12.551(3), $c$ = 14.454(4) Å, $V$ = 2174.7(9) Å$^3$, $Z$ = 4, $D_c$ = 1.278 g/cm$^3$, $F_{000}$ = 904, CCD Area Detector, MoK$\alpha$ radiation, $\lambda$ = 0.71073 Å, $T$ = 294(2)K, $2\theta_{\text{max}}$ = 50.0º, 21307 reflections collected, 2185 unique ($R_{\text{int}}$ = 0.0341). Final $\text{GooF} = 1.123$, $R_I = 0.0450$, $wR_2 = 0.1114$, $R$ indices based on 1977 reflections with $I>2\sigma(I)$ (refinement on $F^2$), 275 parameters, 0 restraints, $\mu = 0.091$ mm$^{-1}$. CCDC 937572 contains supplementary crystallographic data for the structure.

Experimental procedure and characterization of 14-deoxy-11,12-didehydro-3,19-O-ethyldene andrographolide (3b): Compound 3 (100 mg, 0.26 mmol) was dissolved in anhydrous pyridine (2 mL), and then activated alumina (0.2 g) was added and it was stirred at 100–110 °C for 12 h. The completion of the reaction monitored by TLC, it was cooled and filtered, washed with CH$_2$Cl$_2$ (3 × 10 mL), and evaporated to obtain a residue. It was crystallized from 2-propanol:water (1:4) to yield compound 3b. Yield: 85.6 mg (90%), white solid, m.p. 120–122 °C; IR (KBr): 2936, 1758, 1624, 1451, 1086 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ (ppm) 7.16 (s, 1H), 6.91 (dd, $J$ = 10.2 Hz, 15.6 Hz, 1H), 6.12 (d, $J$ = 15.9 Hz, 1H), 4.97 (q, $J$ = 4.5 Hz, 1H), 4.85–4.79 (m, 3H), 4.55 (s, 1H), 4.09 (d, $J$ = 10.8 Hz, 1H),
3.50–3.40 (m, 2H), 2.51–1.10 (br m, 13H), 1.38 (s, 3H), 0.93 (s, 3H); \(^{13}\text{C NMR}\) (75 MHz, CDCl\(_3\)): \(\delta\) 172.68, 148.37, 143.55, 136.23, 129.60, 121.68, 109.88, 92.69, 80.89, 70.03, 69.43, 61.95, 54.52, 38.92, 37.79, 37.19, 36.75, 26.30, 22.39, 22.13, 21.64, 16.42; ESI-MS: positive ion mode: \(m/z = 381.29\) [M+Na]\(^+\), observed for C\(_{22}\)H\(_{30}\)O\(_4\).

**Experimental procedure and characterization for 14-deoxy-14,15-dehydro-3,19-O-ethylidene andrographolide (3c):** Compound 3a (100 mg, 0.239 mmol) and DMAP (0.25 mmol) were taken into CH\(_2\)Cl\(_2\) (5 mL) and stirred at RT. After completion of reaction (1 h, TLC monitoring), diluted with water (20 ml) and extracted with CH\(_2\)Cl\(_2\) (2 \times 20 mL). The combined organic layers were washed with water (5 mL), brine solution (5 mL), dried over Na\(_2\)SO\(_4\) and evaporated under reduced pressure. The residue was purified through silica gel (100-200 mesh) column chromatography (in ethyl acetate-petroleum ether) to afford the desired product 3c. Yield: 76 mg (72 %), light yellow solid, m.p. 208-210 °C; IR (KBr): 3435, 2936, 1767, 1649, 1442, 1040 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) (ppm) 6.99 (s, 1H), 6.69 (t, \(J = 6.6\) Hz, 1H), 6.15 (d, \(J = 3.0\) Hz, 1H), 4.98–4.95 (m, 2H), 4.43 (s, 1H), 4.06–4.02 (d, \(J = 12.0\) Hz, 1H), 3.51–3.39 (m, 2H), 2.52–1.19 (br m, 15H), 1.37 (s, 3H), 0.81 (s, 3H); \(^{13}\text{C NMR}\) (CDCl\(_3\), 75 MHz): \(\delta\) (ppm) 168.29, 147.11, 145.85, 144.48, 126.20, 109.35, 105.35, 92.63, 80.41, 69.34, 56.25, 55.13, 39.33, 37.98, 37.11, 36.54, 30.08, 26.40, 26.37, 23.13, 22.23, 21.67, 15.72; ESI-MS: positive ion: \(m/z = 381.29\) [M+Na]\(^+\), observed for C\(_{22}\)H\(_{30}\)O\(_4\).

**General experimental procedure and characterization for Compound 3d, 3e, and 3f:** Compounds 3/3a/3b (0.2659 mmol) and mCPBA (0.39 mmol) in CH\(_2\)Cl\(_2\)
were stirred for 4 h. After completion of the reaction (TLC monitoring), the mixture was washed with aq. Na$_2$CO$_3$, brine and water successively. The CH$_2$Cl$_2$ phase was dried over Na$_2$SO$_4$, filtered and concentrated. The concentrated residue was purified over silica gel (100-200 mesh) column chromatography (in ethyl acetate-petroleum ether) to afford the desired products 3d/3e/3f.

**8,17-epoxy-3,19-O-ethylideneandrographolide (3d)** Yield: 74 mg (68 %), white solid, m.p. 138–140 °C; IR (KBr): 3423, 2944, 1753, 1674, 1403, 1108 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ (ppm) 6.79 (t, $J = 5.7$ Hz, 1H), 4.98 (m, 2H), 4.38 (dd, $J = 10.2$ Hz, 6.0 Hz, 1H), 4.24 (d, $J = 10.2$ Hz, 1H), 4.03 (d, $J = 11.1$ Hz, 1H), 3.48–3.43 (m, 2H), 2.87 (s, 1H), 2.87–2.65 (m, 2H) 2.29–1.18 (br m, 14H), 1.39 (s, 3H), 0.94 (s, 3H); $^{13}$C NMR (300 MHz, CDCl$_3$): $\delta$ (ppm) 170.52, 146.49, 129.47, 92.68, 80.06, 74.09, 69.10, 65.64, 60.41, 54.59, 53.72, 51.49, 40.05, 36.89, 36.33, 36.16, 26.31, 23.02, 21.63, 21.39, 20.86, 15.8; ESI-MS: positive ion mode: $m/z$ 415.19 [M+H]$^+$, observed for C$_{22}$H$_{32}$O$_6$.

**14-acetyl-8,17-epoxy-3,19-O-ethylideneandrographolide (3e)** Yield: 80 mg (70 %), yellow solid, m.p.: 121-123 °C; IR (KBr): 3463, 2949, 1753, 1740, 1674, 1404, 1105 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ (ppm) 6.93 (t, $J = 6.8$ Hz, 1H), 5.83 (d, $J = 6$ Hz, 1H), 4.99 (q, $J = 4.4$ Hz, 1H), 4.55 (dd, $J = 10.8$ Hz, 6 Hz, 1H), 4.23 (dd, $J = 11.2$ Hz, 1.2 Hz, 1H) 3.98 (d, $J = 11.2$ Hz, 1H), 3.39 (m, 2H), 2.67 (d, $J = 3.2$ Hz, 1H), 2.30–2.26 (m, 1H), 2.16–2.12 (m, 1H), 2.05 (s, 3H), 1.88–1.86 (m, 1H), 1.79–1.74 (m, 3H), 1.66–1.63 (d, $J = 13.2$ Hz, 1H), 1.48–1.44 (m, 1H), 1.33–1.30 (m, 2H), 1.25 (s, 3H), 1.21–1.11 (m, 6H), 0.88 (s, 3H); $^{13}$C NMR (125 MHz, DMSO-d$_6$); $\delta$ (ppm) 170.11, 168.95, 150.53, 123.04, 91.39,
Crystal data for compound 3e: C_{24}H_{34}O_{7}, M = 434.51, colorless plate, 0.18 × 0.17 × 0.09 mm³, orthorhombic, space group P2₁2₁2₁ (No. 19), a = 12.4798(16), b = 12.7207(17), c = 14.3452(19) Å, V = 2277.3(5) Å³, Z = 4, D_c = 1.267 g/cm³, F₀₀₀ = 936, CCD Area Detector, MoKα radiation, λ = 0.71073 Å, T = 294(2)K, 2θ_{max} = 50.0º, 21934 reflections collected, 2280 unique (R_{int} = 0.0219). Final GooF = 1.053, R₁ = 0.0339, wR₂ = 0.0931, R indices based on 2171 reflections with I>2σ(I) (refinement on F²), 285 parameters, 0 restraints, μ = 0.092 mm⁻¹. CCDC 943354 contains supplementary Crystallographic data for the structure.

14-deoxy-11,12-didehydro-8,17-epoxy-3,19-O-ethylideneandrographolide (3f)

Yield: 64 mg (65 %) light yellow solid, m.p. 79–81 °C; IR (KBr): 3451, 2942, 1753, 1403, 1097 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ (ppm) 7.14 (s, 1H), 6.56 (dd, J = 9.0 Hz, 3.0 Hz, 1H), 4.98–4.78 (m, 3H), 4.11 (m, 1H), 3.45 (m, 2H), 2.79 (s, 1H), 2.54 (d, J = 3.0 Hz, 1H), 2.28–0.94 (br m, 14H) 1.38 (s, 3H), 0.91 (s, 3H); ¹³C NMR (CDCl₃, 300 MHz): δ (ppm) 172.47, 144.49, 131.20, 129.07, 124.53, 92.70, 80.62, 69.94, 69.31, 59.45, 58.40, 54.15, 51.51, 39.24, 37.48, 37.05, 35.75, 26.35, 21.64, 21.55, 20.79, 16.46; ESI-MS: positive ion mode: m/z 375.40 [M+H]⁺, observed for C_{22}H_{30}O_{5}.

Cytotoxicity evaluation

The cytotoxicity of the compounds was determined using MTT assay. 1×10⁶ cells/well were seeded in 200 µl DMEM, supplemented with 10 % FBS in each well of
96-well micro culture plates and incubated for 24 h at 37 °C in a CO₂ incubator. Compounds, diluted to the desired concentrations in culture medium, were added to the wells with respective vehicle control. After 48 h of incubation, 10 µl MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (5 mg/ml) was added to each well and the plates were further incubated for 4 h. Then the supernatant from each well was carefully removed, formazon crystals were dissolved in 100 µl of DMSO and absorbance at 540 nm wavelength was recorded.

Molecular modeling parameters and energy minimization

To find the possible interactions of compound 1 with anticancer target, we docked all compounds at target protein αβ-tubulin for HeLa cell and epidermal growth factor receptor (EGFR) for human lung cancer. Sybyl X 2.0 interfaced with Surflex-Dock module was used for molecular docking. Program automatically docks ligand into binding pocket of a target protein by using protomol based algorithm and empirically produced scoring function. The X-ray crystallographic structures of αβ-tubulin complex with ligand (PDB: 1JFF) and EGFR (PDB: 2ITW) was taken from the protein data bank (PDB) and modified for docking calculations. Since there are no PDB ID available in protein data bank (www.rcsb.org) of these target i.e. ACHN (renal cell carcinoma), B-16 (melanoma) and IEC-6 (small intestine) we are not perform docking study. Co-crystallized ligand was removed from the structure, water molecules were removed, H atoms were added and side chains were fixed during protein preparation. Protein structure minimization was performed by applying Tripos force field and partial atomic charges were calculated by Gasteiger-Huckel method. In reasonable binding pocket, all the compounds were docked into the binding pocket and 20 possible active docking
conformations with different scores were obtained for each compound. During the docking process, all of the other parameters were assigned their default values.$^{1-3}$

**X-ray method**

X-ray data of compound 3a and 3e were collected at room temperature using a Bruker Smart Apex CCD diffractometer with graphite monochromated MoKα radiation ($λ = 0.71073$ Å) with ω-scan method.$^4$ Preliminary lattice parameters and orientation matrices were obtained from four sets of frames. Unit cell dimensions were determined using 5607 reflections for compound 3a and 5435 reflections for compound 3e. Integration and scaling of intensity data were accomplished using SAINT program.$^4$ The structures were solved by Direct Methods using SHELXS97$^5$ and refinement was carried out by full-matrix least-squares technique using SHELXL97.$^5$ Anisotropic displacement parameters were included for all non-hydrogen atoms. All H atoms were positioned geometrically and treated as riding on their parent C atoms, with C-H distances of 0.93--0.96 Å, and with $U_{iso}(H) = 1.2U_{eq}(C)$ or $1.5U_{eq}$ for methyl atoms. In the absence of significant anomalous scattering efforts, Friedel pairs were merged. The absolute configuration of the procured material was known in advance.
Suppl. Table 2. Comparison of binding affinity of 3, 3a, 3d, 3e, 7 and the standard drug doxorubicin against anticancer target EGFR kinase (PDB: 21TW).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total Score</th>
<th>Binding site amino acid residues (with in 4Å)</th>
<th>Amino acid in H-bond</th>
<th>Length of H-bond (Å)</th>
<th>No. of H-bonds</th>
</tr>
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<tr>
<td>3a</td>
<td>5.7828</td>
<td>LEU-718, GLY-719, PHE-723, VAL-726, LYS-728, ALA-743, LYS-745, THR-790, LEU-792, MET-793, PRO-794, GLY-796, CYS-797, ASN-842, LEU-844, LYS-852, THR-854, ASP-855</td>
<td>THR-854</td>
<td>2.1</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>5.3047</td>
<td>PHE-723, VAL-726, ALA-743, LYS-745, GLU-762, MET-766, CYS-775, LEU-788, THR-790, GLN-791, LEU-792, MET-793, CYS-797, ASP-837, ARG-841, ASN-842, LEU-844, THR-854, ASP-855, ASP-857</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>
**Suppl. Table 3.** Comparison of binding affinity of 1, 3a, 3e, 7 and standard drug doxorubicin against anticancer target αβ-tubulin (PDB ID: 1JFE).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total Score</th>
<th>Binding site amino acid residues (with in 4Å)</th>
<th>Amino acid in H-bond</th>
<th>Length of H-bond (Å)</th>
<th>No. of H-bonds</th>
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</table>
**Suppl. Table 4.** Compliance of active 3,19-O-acetal derivatives of andrographolide to the computational parameters of pharmacokinetics (ADME).

<table>
<thead>
<tr>
<th>Compound</th>
<th>log S for aqueous solubility</th>
<th>LogP</th>
<th>log Khsa for Serum Protein Binding</th>
<th>log BB for blood-brain barrier penetration</th>
<th>No. of metabolic reactions</th>
<th>Predicted CNS Activity</th>
<th>log hERG for K+ Channel Blockage</th>
<th>Apparent Caco-2 Permeability (nm/sec)</th>
<th>Apparent MDCK Permeability (nm/sec)</th>
<th>Polar SA (PSA)</th>
<th>log Kp for skin permeability</th>
<th>% Human Oral Absorption in GI (+/- 20%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-3.049</td>
<td>2.113</td>
<td>-0.208</td>
<td>254.165</td>
<td>112.551</td>
<td>[97.854 -3.857]</td>
<td>78.516</td>
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<td>1243.682</td>
<td>[86.463 -2.291]</td>
<td>100</td>
<td>761.68</td>
<td>74.909</td>
<td>-2.231</td>
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<td>3.001</td>
<td>-0.107</td>
<td>1333.982</td>
<td>675.495</td>
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<td>92.172</td>
<td>696.958</td>
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<td>3d</td>
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<td>1.784</td>
<td>-0.401</td>
<td>1041.237</td>
<td>516.796</td>
<td>[103.907 -3.021]</td>
<td>90.439</td>
<td>215.962</td>
<td>74.909</td>
<td>-2.231</td>
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<td></td>
</tr>
<tr>
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<td>1.913</td>
<td>-0.73</td>
<td>1490.726</td>
<td>761.68</td>
<td>[74.909 -2.231]</td>
<td>100</td>
<td>0.716</td>
<td>215.962</td>
<td>-7.957</td>
<td>20</td>
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<tr>
<td>7</td>
<td>-5.003</td>
<td>4.629</td>
<td>0.404</td>
<td>1373.148</td>
<td>696.958</td>
<td>[72.651 -2.139]</td>
<td>100</td>
<td>215.962</td>
<td>74.909</td>
<td>-2.231</td>
<td>100</td>
<td></td>
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<tr>
<td>8</td>
<td>-5.442</td>
<td>4.551</td>
<td>0.554</td>
<td>1041.237</td>
<td>516.796</td>
<td>[103.907 -3.021]</td>
<td>90.439</td>
<td>0.716</td>
<td>215.962</td>
<td>-7.957</td>
<td>20</td>
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<td>Doxorubicin</td>
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<td>0.188</td>
<td>-0.543</td>
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<td>74.909</td>
<td>[74.909 -2.231]</td>
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<td>215.962</td>
<td>74.909</td>
<td>-2.231</td>
<td>100</td>
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</tr>
</tbody>
</table>

Footnote: * For 95% of known drugs, based on –Qikprop software (Schrödinger, USA). hERG= Human Ether-a-go-go-Related Gene, Caco-2= a human colon carcinoma cell line, MDCK= Madin-Darby canine kidney cell line, PSA= Polar surface area.
References and notes


5. G. M. Sheldrick, SHELXS97 and SHELXL97, Programs for crystal structure solution and refinement; University of Gottingen: Germany, (1997).
$^1$H NMR SPECTRA of 3 (300 MHz, CDCl$_3$):

$^{13}$C NMR SPECTRA of 3 (75 MHz, CDCl$_3$):
DEPT 135 of 3 (75 MHz, CDCl₃):

ESI-MASS SPECTRA of 3:

V081219 6 (0.222) Cm (4/16-(23+23))

Current Data Parameters
NAME: CANET - CMAP
EXPNO: 3
PROCNO: 1

F2 - Acquisition Parameters
Date: 20110329
Time: 12:50
INSTRUM: spect
PROBHD: 5 mm Multinuclear
PULPROG: dept135
TD: 65536
SOLVENT: CDCl3
NS: 512
DS: 4
SWH: 17985.611 Hz
FIDRES: 0.274438 Hz
AQ: 1.8219508 sec
RG: 16384
DW: 27.300 usec
DE: 6.00 usec
TE: 298.15 K
CNST2: 146.000000 sec
D1: 2.00000000 sec
d2: 0.00344828 sec
d12: 0.00032000 sec
DELTA: 0.00001464 sec
tD0: 1

== CHANNEL f1 =
NUC1: 13C
P1: 11.50 usec
d2: 23.00 usec
PL1: -600 dB
SFO1: 75.472950 MHz

== CHANNEL f2 =
CPDPRG2: wait16
NUC2: 1H
P3: 6.75 usec
P4: 13.50 usec
CPDPRG2: 120.500 sec
PL2: 4000 dB
PL12: 39.05 dB
SFO2: 300.1312000 MHz
IR SPECTRA of 3 (KBr):

![IR Spectra of 3](image)

$^1$H NMR SPECTRA of 4 (300 MHz, CDCl$_3$):

![$^1$H NMR Spectra of 4](image)
ESI-MASS SPECTRA of 4:

IR SPECTRA of 4 (KBr):
$^1$H NMR SPECTRA of 5 (300 MHz, CDCl$_3$):

ESI-MASS SPECTRA of 5:

V031245 6 (0.222) Cm (5:7-(16+18))
$^1$H NMR SPECTRA of 6 (300 MHz, CDCl$_3$):

![H NMR Spectrum Image]

ESI-MASS SPECTRA of 6:

![ESI-MASS Spectrum Image]
$^1$H NMR SPECTRA of 7 (300 MHz, CDCl$_3$):

$^{13}$C NMR SPECTRA of 7 (75 MHz, CDCl$_3$):
ESI-MASS SPECTRA of 7:

IR SPECTRA of 7 (KBr):
$^1$H NMR SPECTRA of 8 (300 MHz, CDCl$_3$):

ESI-MASS SPECTRA of 8:
$^1$H NMR SPECTRA of 3a (300 MHz, CDCl$_3$):

$^{13}$C NMR SPECTRA of 3a (75 MHz, CDCl$_3$):
ESI mass SPECTRA of 3a:

IR SPECTRA of 3a (KBr):
$^1$H NMR SPECTRA of 3b (300 MHz, CDCl$_3$):

$^{13}$C NMR SPECTRA of 3b (75 MHz, CDCl$_3$):
ESI-MASS SPECTRA of 3b:

IR SPECTRA of 3b (KBr):
$^1$H NMR SPECTRA of 3c (300 MHz, CDCl$_3$):

$^{13}$C NMR SPECTRA of 3c (75 MHz, CDCl$_3$):
ESI-MASS SPECTRA of 3c:

IR SPECTRA of 3c (KBr):
$^1$H NMR SPECTRA of 3d (300 MHz, CDCl$_3$):

$^{13}$C NMR SPECTRA of 3d (75 MHz, CDCl$_3$):
ESI-MASS SPECTRA of 3d:

IR SPECTRA of 3d (KBr):
$^1$H NMR SPECTRA of 3e (400 MHz, DMSO-d6):

$^{13}$C NMR SPECTRA of 3e (125 MHz, DMSO-d6):
ESI-MASS SPECTRA of 3e:

IR SPECTRA of 3e (KBr):
$^1$H NMR SPECTRA of 3f (300 MHz, CDCl$_3$):

$^{13}$C NMR SPECTRA of 3f (75 MHz, CDCl$_3$):
ESI-MASS SPECTRA of 3f:

IR SPECTRA of 3f (KBr):