Supporting Information

New cytotoxic cucurbitacins from *Wilbrandia ebracteata* Cogn.

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Spectroscopic data of compounds 3-6

**Isoecurbitacin R (3):** C$_{30}$H$_{46}$O$_{7}$; $^1$H NMR (500 MHz, CDCl$_3$): δ = 5.94 (1H, m, H-6), 4.3 (1H, ddd, $J = 7.5$ Hz, H-16), 3.90 (1H, dd, $J = 1.4$, 5.0 Hz, H-3), 3.12 (1H, d, $J = 14.5$ Hz, H-12a), 2.90 (1H, m, H-23a), 2.74 (1H, m, H-10), 2.64 (1H, d, $J = 14.5$ Hz, H-12b), 2.61 (1H, m, H-23b), 2.57 (1H, d, $J = 7.1$ Hz, H-17), 2.48 (1H, dddd, $J = 2.0$, 4.0, 8.0, 20.0 Hz, H-7b), 2.40 (1H, dd, $J = 5.0$, 13.0, Hz, H-1a), 2.23 (1H, ddd, $J = 1.4$, 13.0, 14.0 Hz, H-1b), 2.04 (1H, m, H-7a), 2.0 (1H, d, $J = 8.0$ Hz, H-8), 1.82 (1H, m, H-15a), 1.80 (2H, m, H-24), 1.42 (1H, m, H-15a), 1.41 (3H, s, H-21), 1.33 (3H, s, H-29), 1.24 (3H, s, H-27), 1.21 (3H, s, H-26), 1.18 (3H, s, H-30), 1.02 (3H, s, H-19), 0.96 (3H, s, H-18), 0.80 (3H, s, H-18) and $^{13}$C NMR data (125 MHz, CDCl$_3$): δ = 215.5 (C-22), 211.9 (C-11), 210.6 (C-2), 138.2 (C-5), 121.8 (C-6), 80.2 (C-3), 79.2 (C-20), 71.0 (C-16), 70.4 (C-25), 57.8 (C-17), 50.6 (C-14), 48.6 (C-12), 48.3 (C-13), 48.2 (C-9), 46.7 (C-4), 45.4 (C-15), 42.7 (C-8), 42.3 (C-8), 36.1 (C-1), 36.9 (C-24), 36.3 (C-10), 30.9 (C-23), 29.8 (C-27), 28.8, (C-26), 24.5 (C-21), 24.1 (C-29), 23.8 (C-7), 20.9 (C-28), 20.1 (C-19), 19.8 (C-18), 18.9 (C-30). ESI-MS (positive mode) m/z 541.3146 [M + Na]$^+$ (calcd. for C$_{30}$H$_{46}$O$_{7}$Na 541.3136).

**Cucurbitacin B (4):** C$_{32}$H$_{46}$O$_{8}$; $^1$H-NMR (500 MHz, CDCl$_3$): δ = 7.06 (1H, d, $J = 15.6$ Hz, H-24), 6.47 (1H, d, $J = 15.6$, H-23), 5.79 (1H, m, H-6), 4.42 (1H, dd, $J = 5.2$, 13 Hz, H-2), 4.36 (1H, t, $J = 7.5$ Hz, H-16), 3.25 (1H, d, $J = 14.5$ Hz, H-12a), 2.74 (1H, m, H-10), 2.69 (1H, d, $J = 14.5$ Hz, H-12b), 2.50 (1H, d, $J = 7.1$ Hz, H-17), 2.41 (1H, m, H-7a), 2.31 (1H, ddd, $J = 3.6$, 6.1, 13.0, Hz, H-1), 2.01 (CH$_3$CO$_2$, s), 1.99 (1H, m, H-7b), 1.97 (1H, d, $J = 8.0$ Hz, H-8), 1.88 (1H, dd, $J = 9.0$, 13.0 Hz, H-15b), 1.57 (3H, s, H-27), 1.54 (3H, s, H-26), 1.44 (3H, s, H-21), 1.43 (1H, d, $J = 13.0$ Hz, H-15a), 1.35 (3H, s, H-30), 1.34 (3H, s, H-28), 1.28 (3H, s, H-29), 1.24 (1H, ddd, $J = 13.0$, 13.0, 13.0 Hz, H-1b), 1.07 (3H, s, H-19), 0.97 (3H, s, H-18); $^{13}$C NMR (125 MHz, CDCl$_3$): δ =
213.1 (C-3), 212.2 (C-11), 202.4 (C-22), 151.9 (C-24), 140.5 (C-5), 120.4 (C-6), 120.3 (C-23), 79.3 (C-25), 78.2 (C-20), 71.6 (C-2), 71.2 (C-16), 58.2 (C-17), 50.6 (C-14), 50.2 (C-4), 48.6 (C-12), 48.4 (C-9), 48.1 (C-13), 45.3 (C-15), 42.3 (C-8), 35.9 (C-1), 33.7 (C-10), 29.4 (C-29), 26.4 (C-27), 25.8 (C-26), 23.9 (C-21), 23.8 (C-7), 21.3 (C-28), 20.0 (C-19), 19.8 (C-18), 18.8 (C-30), CH₃CO₂ (170.3, 21.9); ESI-MS (negative mode) m/z 557.3124 [M - H] - (calcd. for C₃₂H₄₅O₈ 557.3119).

23,24-dihydrocucurbitacin B (5): C₃₂H₄₈O₈; ¹H NMR (500 MHz, CDCl₃): δ = 5.79 (1H, m, H-6), 4.41 (1H, dd, J = 5.2, 11.5 Hz, H-2), 4.31 (1H, t, J = 7.5 Hz, H-16), 3.26 (1H, d, J = 14.5 Hz, H-12a), 2.82 (1H, m, H-23a), 2.73 (1H, m, H-10), 2.70 (1H, d, J = 14.5 Hz, H-12b), 2.53 (1H, m, H-23b), 2.55 (1H, m, H-17), 2.41 (1H, m, H-7a), 2.32 (1H, ddd, J = 3.6, 6.1, 13.0 Hz, H-1), 2.06 (2H, m, H-24), 1.97 (1H, d, J = 8.0 Hz, H-8), 1.98 (1H, m, H-7b), 1.85 (1H, dd, J= 9.0, 13.0 Hz, H-15a), 1.46 (3H, s, H-27), 1.44 (3H, s, H-26), 1.43 (3H, s, H-21), 1.40 (1H, m, H-15b), 1.37 (3H, s, H-30), 1.35 (3H, s, H-28), 1.29 (3H, s, H-29), 1.27 (1H, ddd, J= 13.0, 13.0, 13.0 Hz, H-1b), 1.08 (3H, s, H-19), 0.98 (3H, s, H-18); ¹³C NMR (125 MHz, CDCl₃): δ = 213.9 (C-22), 213.1 (C-3), 212.1 (C-11), 140.6 (C-5), 120.5 (C-6), 81.3 (C-25), 78.9 (C-20), 71.7 (C-2), 71.1 (C-16), 57.8 (C-17), 50.7 (C-14), 48.7 (C-12), 48.4 (C-13), 48.4 (C-9), 45.5 (C-15), 42.3 (C-8), 36.1 (C-1), 34.8 (C-24), 33.8 (C-10), 30.7 (C-23), 29.4 (C-29), 26.2 (C-26), 25.8 (C-27), 24.5 (C-21), 23.9 (C-7), 21.3 (C-28), 20.1 (C-19), 19.8 (C-18), 18.8 (C-30), CH₃CO₂ (170.4, 22.4); ESI-MS (negative mode) m/z 559.3314 [M - H] - (calcd. for C₃₂H₄₇O₈ 559.3276).

Cucurbitacin R (6): C₃₀H₄₆O₇; ¹H NMR (500 MHz, CDCl₃): δ = 5.78 (1H, m, H-6), 4.42 (1H, dd, J = 6.0, 11.5 Hz, H-2), 4.31 (1H, t, J = 7.5 Hz, H-16), 3.25 (1H, d, J = 14.5, H-12a), 2.96 (1H, dt, J = 18.0, 6.5 Hz, H-23), 2.74 (1H, m, H-10), 2.68 (1H, m, H-12b), 2.65 (1H, m, H-23), 2.62 (1H, d, J = 7.0 Hz, H-17), 2.40 (1H, m, H-7a), 2.32 (1H, ddd, J = 4.0, 6.5, 16.0 Hz, H-1a), 2.00 (1H, m, H-7b), 1.96 (1H, d, J = 8.0 Hz, H-8), 1.83 (2H, m, H-24), 1.83 (1H, m, H-15a), 1.40 (1H, m, H-15b), 1.43 (3H, s, H-21), 1.38 (3H, s, H-30), 1.35 (3H, s, H-29), 1.28 (3H, s, H-28), 1.25 (3H, s, H-26), 1.23 (3H, s, H-27), 1.23 (1H, ddd, J = 13.0, 13.0, 13.0 Hz, H-1b), 1.07 (3H, s, H-19), 0.98 (3H, s, H-18); ¹³C NMR (125 MHz, CDCl₃): δ = 215.50 (C-22), 213.4 (C-11), 212.1 (C-3), 140.4 (C-5), 120.4 (C-6), 79.2 (C-20), 71.6 (C-2), 70.9 (C-16), 70.3 (C-25), 57.8 (C-17), 50.7 (C-
**Determination of cytotoxicity**

The human embryo rhabdomyosarcome (RD) and the human epidermoid carcinoma of the nasopharynx (KB) cells were obtained from Adolfo Lutz Institute, São Paulo, SP, Brazil. The human ileocecal adenocarcinoma (HCT-8) and the human non-small cell lung cancer (A549) were kindly provided by Dr. George Di Giovanni from Texas A & M University System, El Paso, TX, USA, and by Dr. Rosina Gironès from the Microbiology Department of the University of Barcelona, Spain, respectively. RD, KB, and A549 cells were grown in minimal essential medium MEM, and HCT-8 cells in RPMI-1640 medium. All cell lines were supplemented with 10% fetal bovine serum, 100 U/mL penicillin G, 100 µg/mL streptomycin, and 25 µg/mL amphotericin B. Cell cultures were kept in tissue culture flasks in a humidified atmosphere of 5% CO₂ at 37°C. The effect of the samples treatment on proliferation of RD, KB, HCT-8, and A549 cells was measured by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay [15]. Approximately 10⁴ cells were plated per well in 96-well plates and treated with different concentrations of each sample. After 72h at 37°C, the medium was removed, 50 µL of MTT reagent (1 mg/mL) was added to each well, and cells were further incubated at 37°C for 4 h. The MTT solution was removed, 100 µL of dimethyl sulfoxide was added to each well to dissolve formazan crystals, and the plates were gently shaken, whereby crystals were completely dissolved. The absorbances were read on a multiwell spectrophotometer at 540 nm. The 50% cytotoxic concentration (CC₅₀) of each sample was defined as the concentration that reduced cell viability by 50% when compared to untreated controls. Doxorubicin (Zodiac) and paclitaxel (Glenmark) (both at 0 to 10 µM) were used as positive controls (purity > 98%).

**Statistical analysis**
The mean ± standard deviations are representative of three independent experiments. For determination of CC\textsubscript{50} values non-linear regressions of concentration-response curves were used.