Supporting Information

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Cytotoxic activity of *Laserpitium latifolium* L. extract and its daucane and phenylpropanoid constituents

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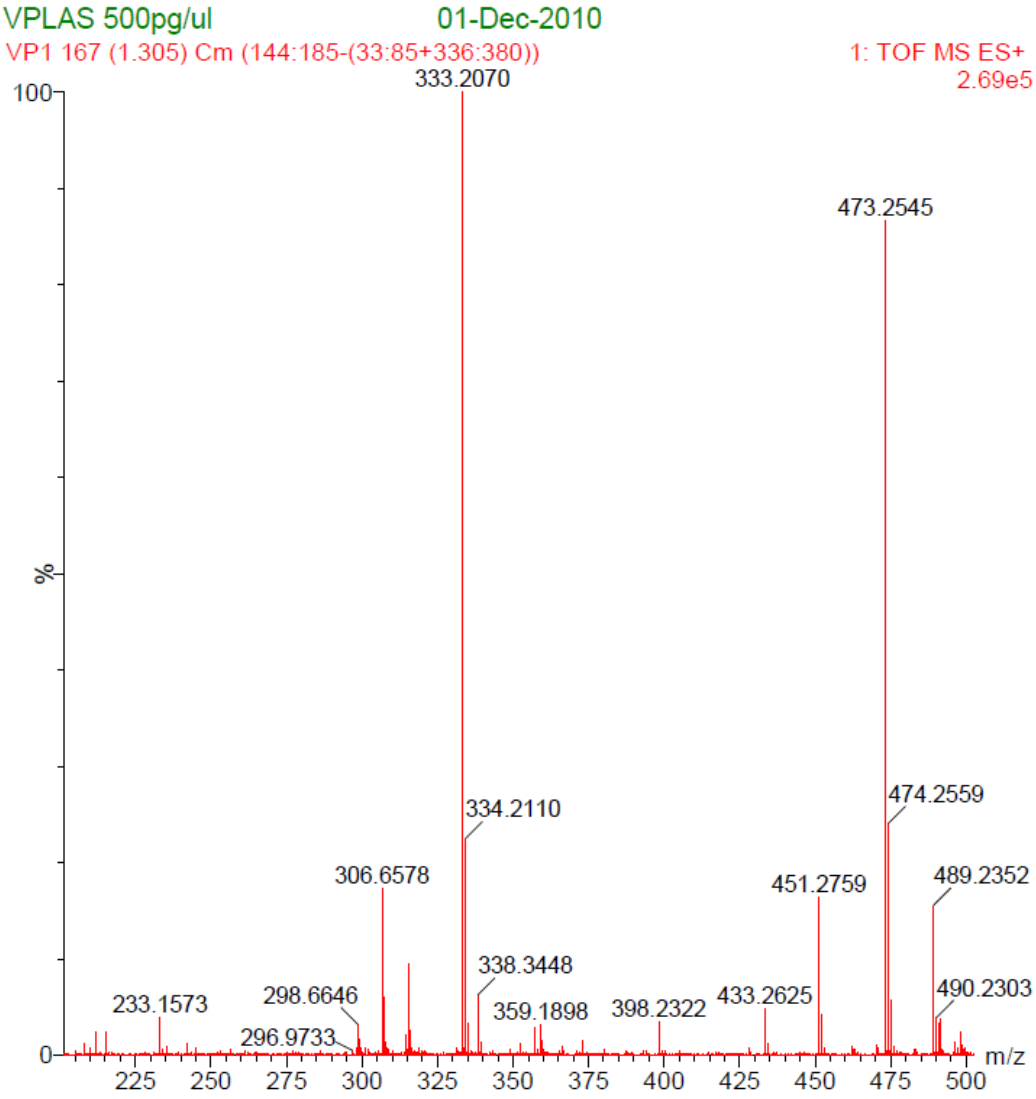
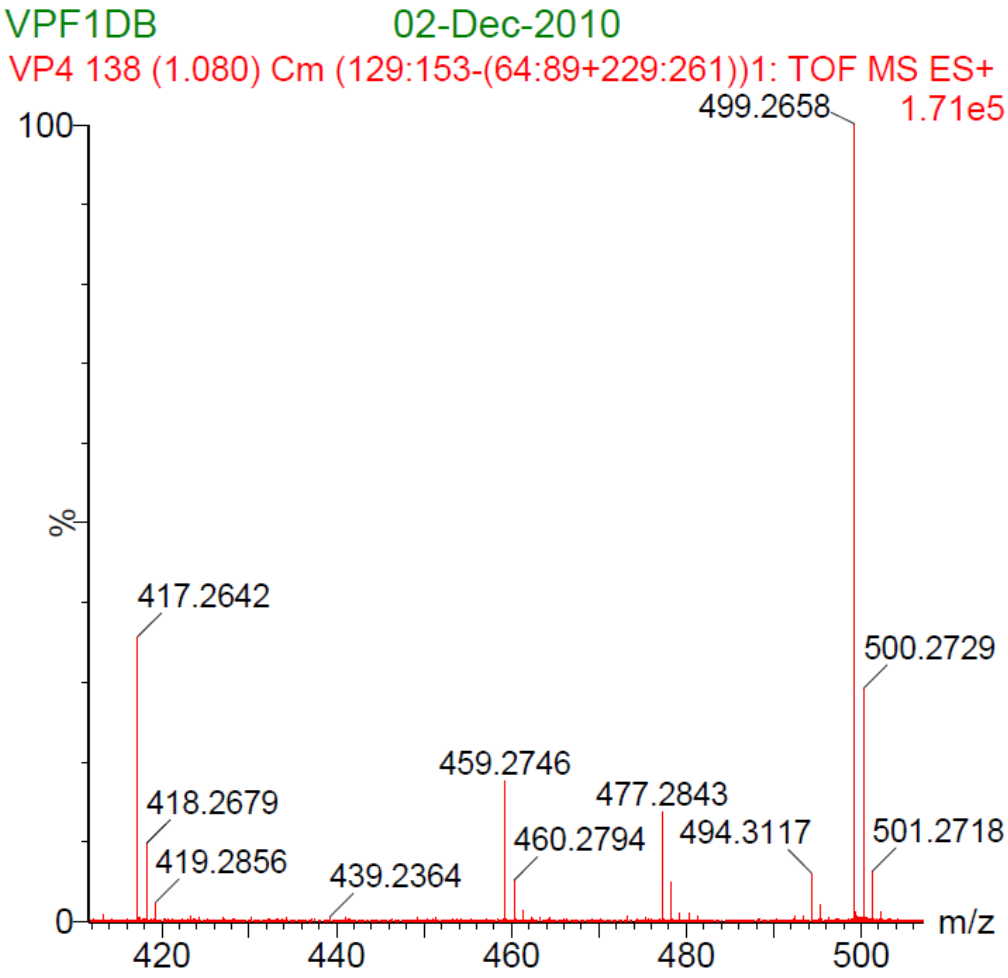
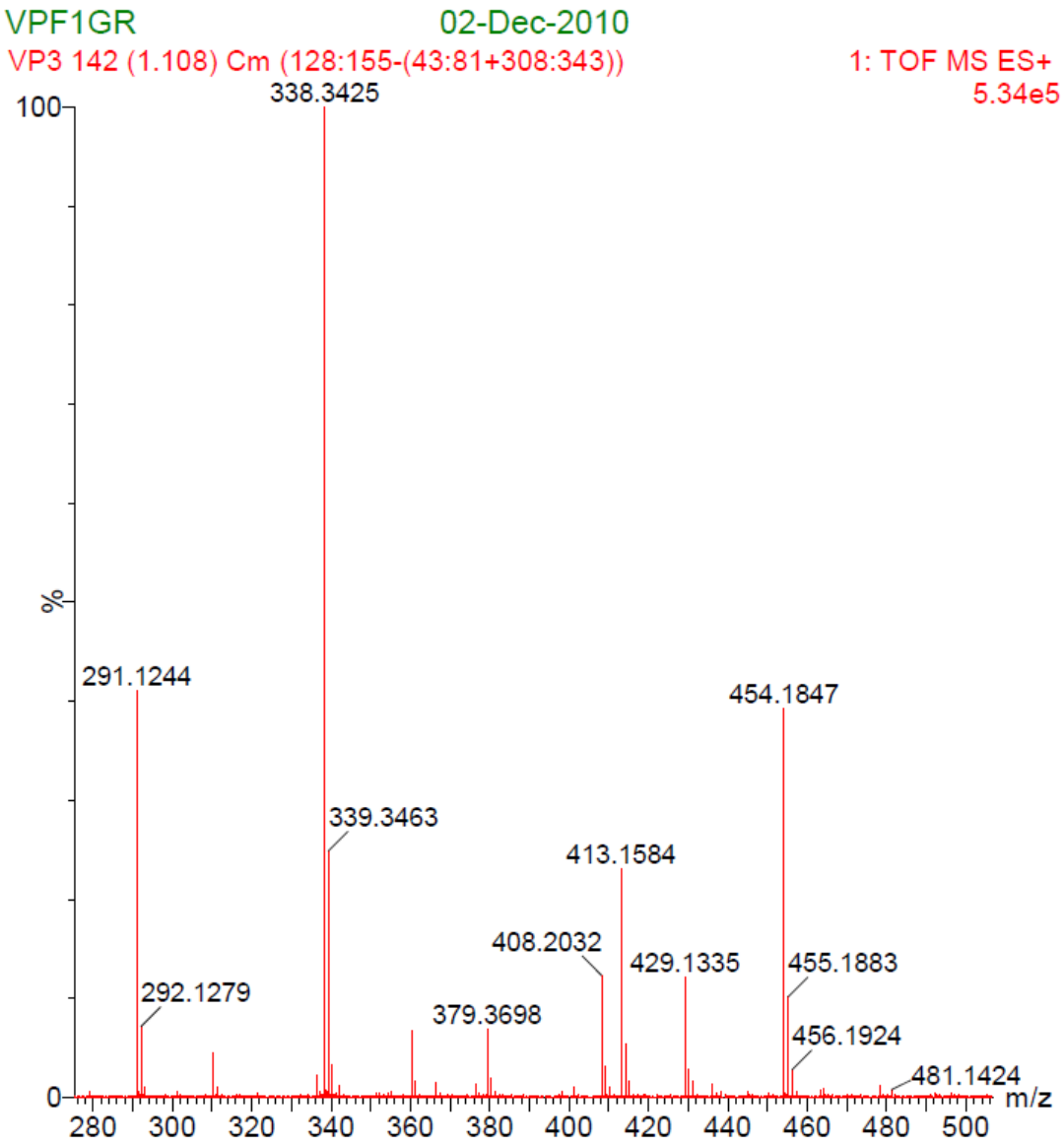


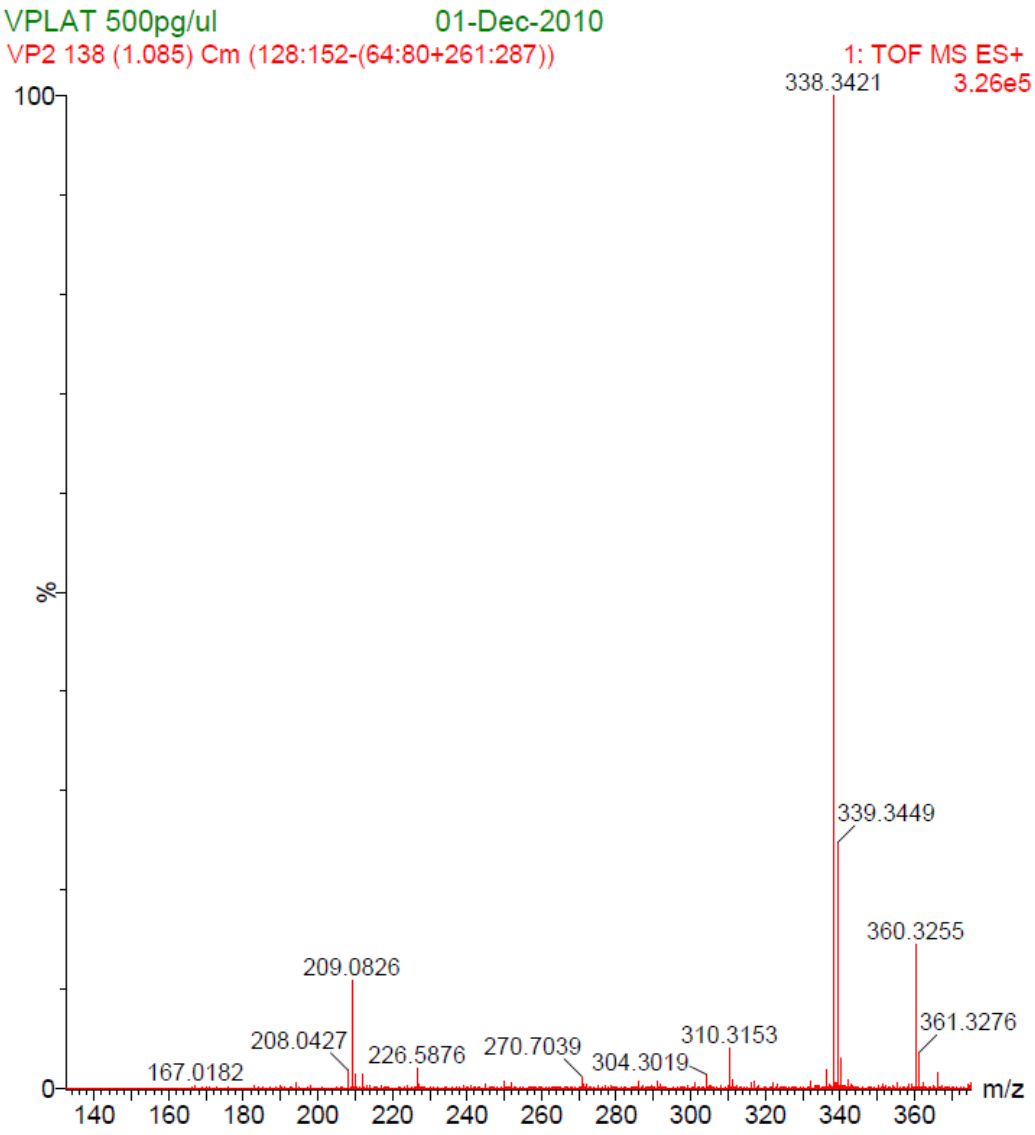
S1: HR-MS Spectrum of Laserpitin



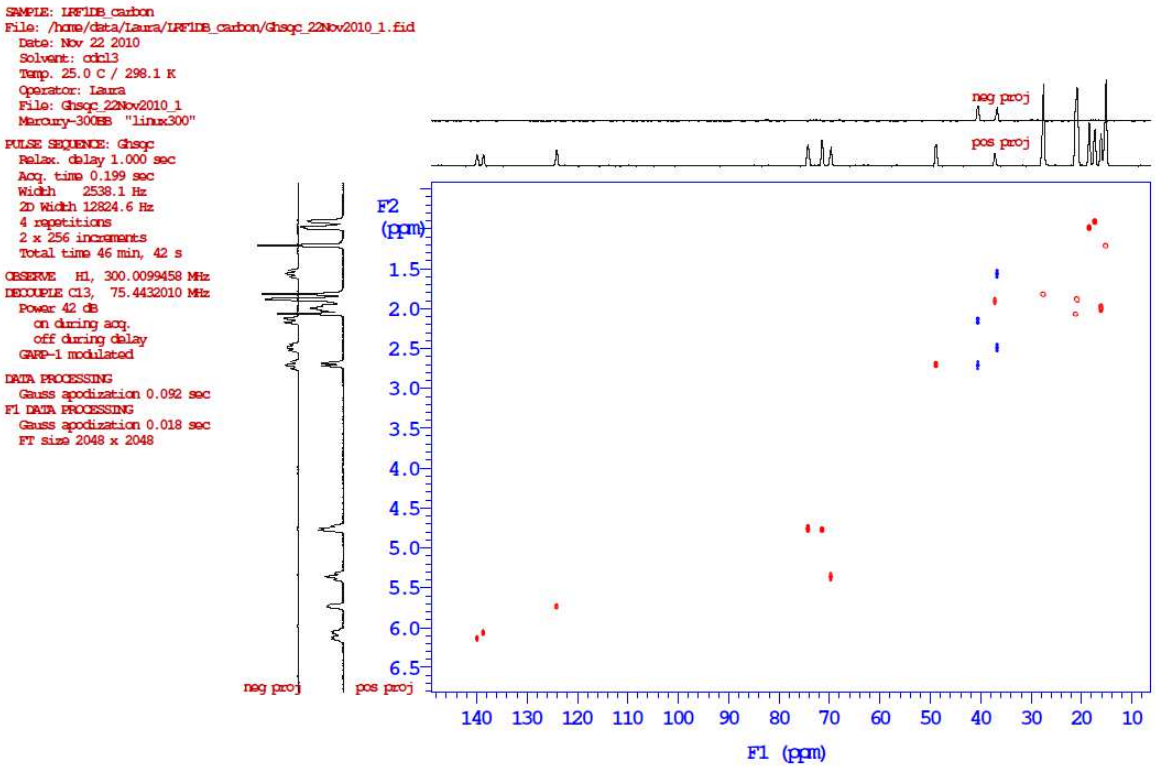
S2: HR-MS Spectrum of Acetyldesoxodehydrolaserpitin



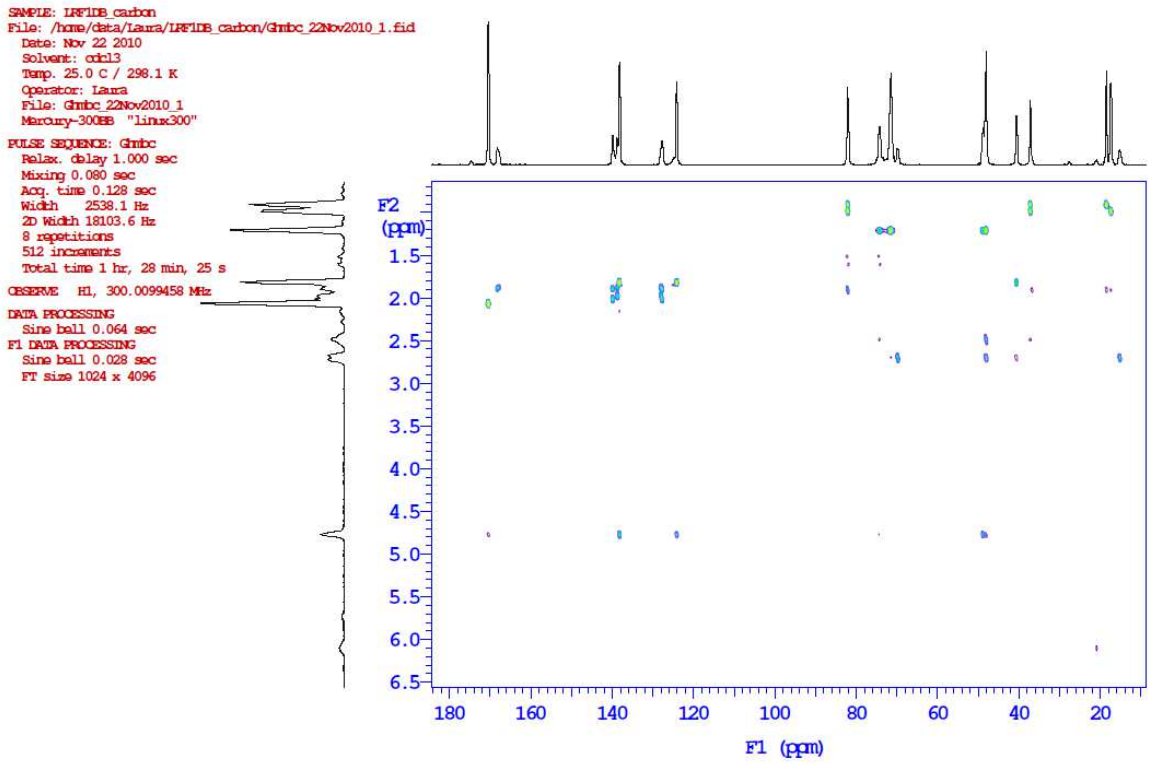
S3: HR-MS Spectrum of Laserin



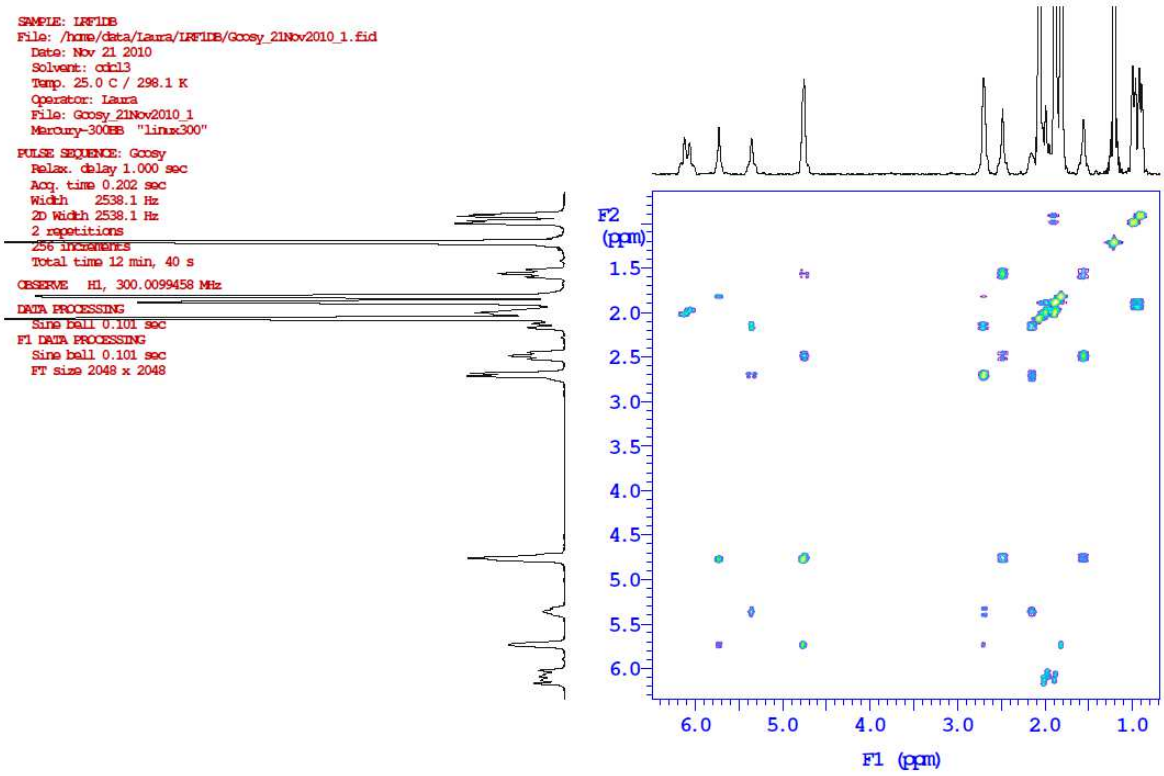
S4: HR-MS Spectrum of Latifolon



S5: gHSQC Spectrum of Acetyldesoxodehydrolaserpitin



S6: gHMBC Spectrum of Acetyldesoxodehydrolaserpitin



S7: COSY Spectrum of Acetyldesoxodehydrolaserpitin

S8: General experimental procedures

General experimental procedures. 1D (^{1}H NMR, ^{13}C NMR) and 2D (gHSQC, gHMBC and COSY) NMR experiments were performed using a Varian Mercury 300 spectrometer. The samples were dissolved in deuterochloroform ($CDCl_3$) and TMS (Sigma-Aldrich, Bornem, Belgium) was used as internal standard. High-Resolution Mass Spectra (HR-MS) were obtained on a Waters LCT Premier XE orthogonal acceleration time of flight mass spectrometer. HPLC analyses were carried out by HPLC-UV using a Varian Omnispher column C_{18} (250 × 4.6 mm, 5 μm) in combination with a Waters 2695 Alliance separations module and a 996 photodiode array detector (Waters, Milford, MA). Kieselgel Merck, 230–400 mesh, 60A (Merck, Germany) was used for flash chromatography separations. Celite 545 was obtained from Acros Organics (Geel, Belgium). Purification by semipreparative chromatography was conducted on a Varian Omnisphere C_{18} column (250 × 21.4 mm, 10 μm, Varian, St. Katelijne-Wawer, Belgium) using a Gilson 322 Pump (Gilson, Middleton, United States) with a Gilson UV-vis 156 detector and a Gilson 206 Fraction Collector. Separation was followed by TLC analyses on silica gel 60 F254 (Merck, Germany) precoated plates. Solvents used were of analytical reagent grade or of the highest quality commercially available and were purchased from Biosolve (Valkenswaard, The Netherlands). Vanillin and sulphuric acid used for TLC detection of terpenoids were purchased form Sigma-Aldrich (Bornem, Belgium). In cytotoxic assays, absorbance was measured using an ELISA plate reader (Safire2TM, Tecan, Männedorf, Switzerland) on transparent flat-bottomed 96 well microtiter plates (Nunc A/S, Roskilde, Denmark).

S9: Cell cultures

Cell cultures. The human breast adenocarcinoma cell lines MCF 7/6 and MCF 7/AZ, an invasive and a non-invasive type, respectively, were provided by the laboratory of Experimental Cancer Research (Ghent University Hospital, Belgium). The cells were cultivated in DMEM/F12 (Ham's) (1/1) (Invitrogen, Merelbeke, Belgium) containing L-glutamine and supplemented with 10% FBS (Greiner Bio-One, Wemmel, Belgium), 50 IU/mL penicillin and 50 μg/mL streptomycin (Invitrogen, Merelbeke, Belgium). The cell cultures were maintained at 37 °C in a 5% CO_2 atmosphere with 95% humidity. Subconfluent cells (80%) were passaged with a solution containing 1% trypsin and 0.02% EDTA. Cells were used up to 10 passages and after that tests were restarted from the original cultures.

S10: MTT assay

MTT assay. A variation of the MTT assay described by Mosmann was used (Cited n references as 12). The cells were seeded in densities of 5×10^3 cell/well in flat-bottomed 96 well plates (Nunc A/S, Roskilde, Denmark). 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyl tetrazolium bromide (MTT) reagent was purchased from Sigma-Aldrich (Bornem, Belgium). The total extract or compound at the specified contentration in fresh medium was added to the wells after incubation of the cells at 37 °C for 48 h. Vinblastine sulfate (Sigma-Aldrich, Bornem, Belgium) was used as a reference compound. After incubation for 48 h, the medium was removed and 100 μL of MTT (5 mg/mL in PBS) was added to wells. The plates were incubated for a further 2 h. After removal of the medium, the formazan crystals were dissolved in 100 μL of DMSO and the absorbance was measured using an ELISA plate reader (Safire2TM, Tecan, Männedorf, Switzerland) at 570/560 nm. The absorbance was considered to be directly correlated with the number of actively metabolizing and, thus, living cells. Concentrations of test materials that caused a 50% reduction in the number of cells versus negative controls (IC_{50}) were estimated from the dose-viability curves.

S11: SRB assay

SRB assay. The assay performed in this investigation was a variation of the test used by Skehan et al. (cited in manusctript as reference 13). Sulforhodamine sodium salt (SRB), glacial acetic acid, trichloroacetic acid (TCA) and trizma base were purchased from Sigma-Aldrich (Bornem, Belgium). The cells were seeded in densities of 5×10^3 cell/well in flat-bottomed 96 well plates (Nunc A/S, Roskilde, Denmark). The extract or compound at the specified concentration in the fresh medium was added to the well after 48 h of incubation of the cells at 37 °C. Vinblastine sulfate (Sigma-Aldrich, Bornem, Belgium) was used as a reference compound in concentration range 0.1 to 40 nM. After addition of extract/compounds or reference compound, cultures were further incubated for 48 h. Next, the medium was removed and 50 μL of a 50% TCA solution was added, for the selective fixation of cellular proteins, and the plates were placed 1 h in a fridge. Then, plates were washed 5 times with distilled water, in order to remove non-fixed proteins and TCA. After drying the plates in an oven, 200 μL of a 0.4% SRB in 1% acetic acid solution was added to each plate and kept in the dark for next 30 minutes. After removing excess SRB dye by four consecutive washings (200 μL of glacial acetic acid each), plates were dried and 200 μL of Tris base (10 mM) was added. The optical density was read using an ELISA reader (Safire2TM, Tecan, Männedorf, Switzerland) at 570/650 nm. The measured absorbance was considered to be directly correlated to cellular protein content. Concentrations of test materials that caused a 50% reduction in the number of cells versus negative controls (IC_{50}) were estimated from the dose-viability curves.

S12: Statistical and numerical analyses

Statistical and numerical analyses. Statistical differences were determined using ANOVA analysis with Bonferroni correction for multiple comparisons (SPSS 15.0). Statistical significance of inhibition was determined in comparison to control groups at significance level * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. Correlation between the number of cells and the measured ELISA absorbance was estimated from the cell growth curves using software for numerical non-linear analyses (Wolfram Matematica 7.0).