Supporting Information

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Antibacterial and Cytotoxic Activities of Diterpenoids Isolated from Indian *Plectranthus coesta*

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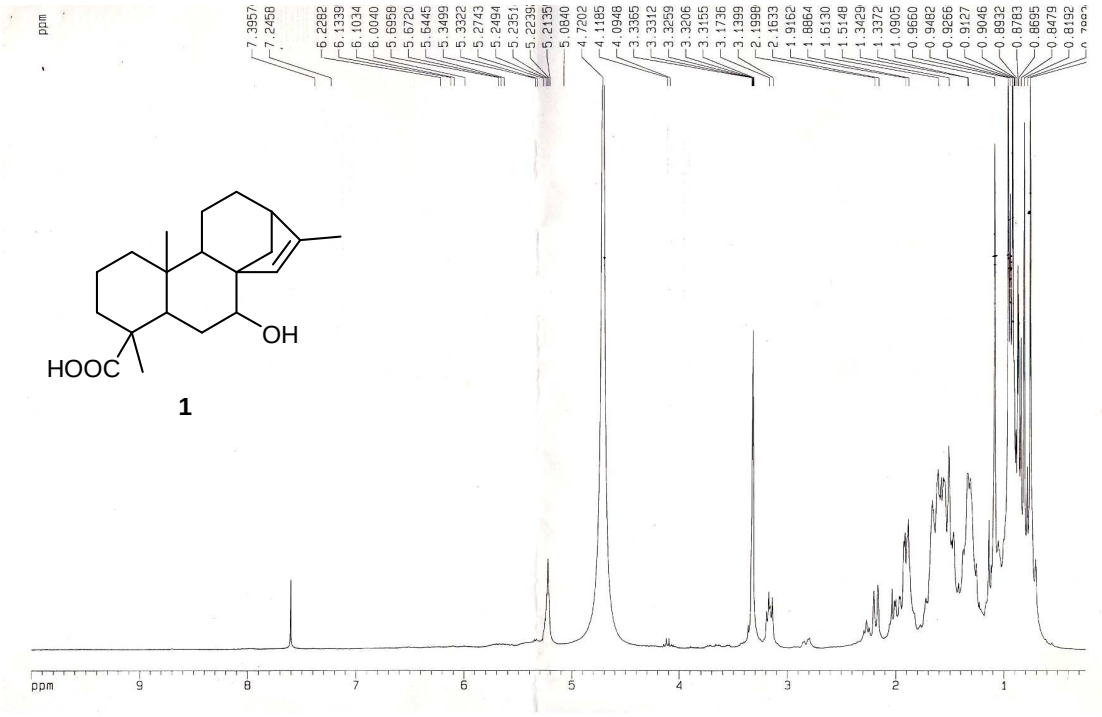
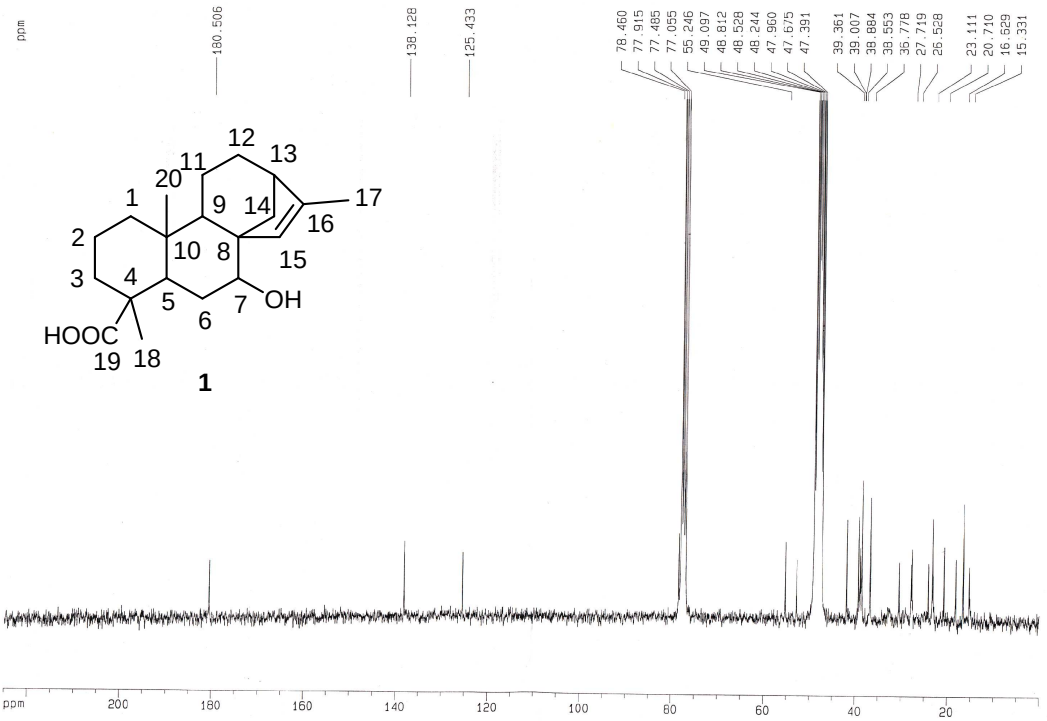


S1: ^{1}H-NMR (300 MHz, $CDCl_3$+MeOD, 40 ^{o}C,) Spectrum of *Ent-7-Hydroxykaur-15,16-en-19-oic acid* (**1**)

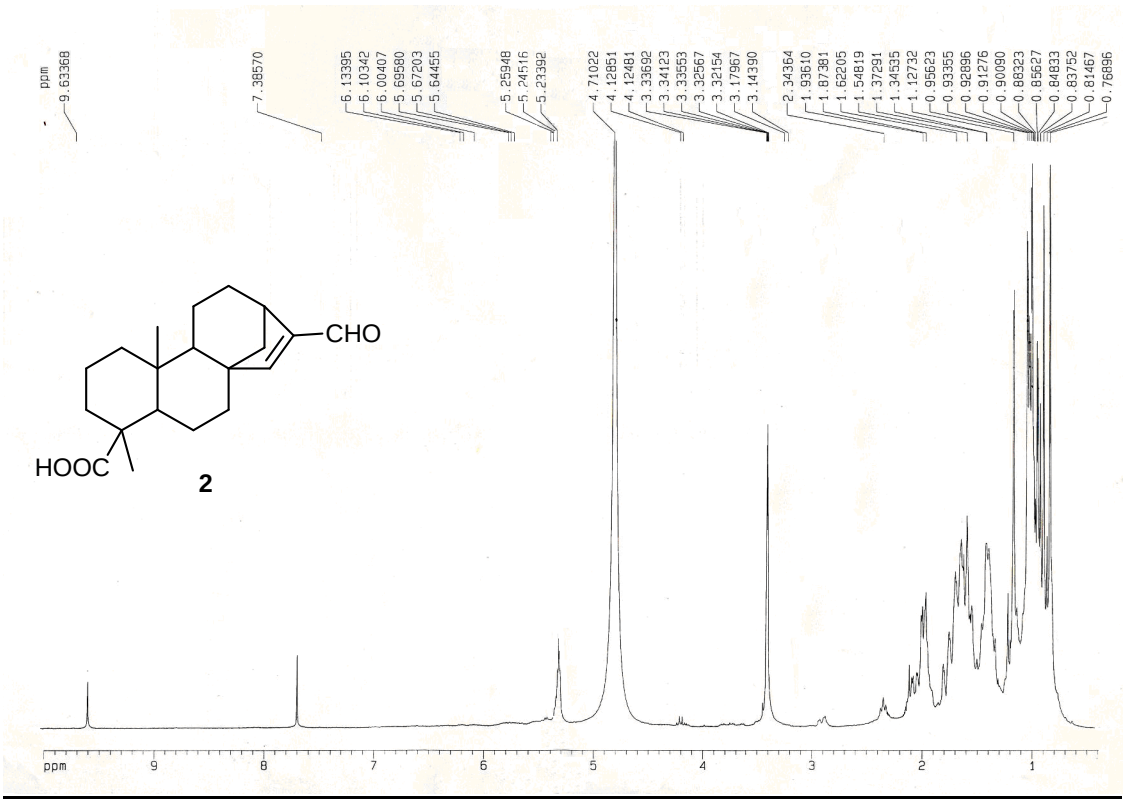


S2: ^{13}C-NMR (75.5 MHz, $CDCl_3$+MeOD, 40 ^{o}C) Spectrum of *Ent-7-Hydroxykaur-15,16-en-19-oic acid* (**1**)

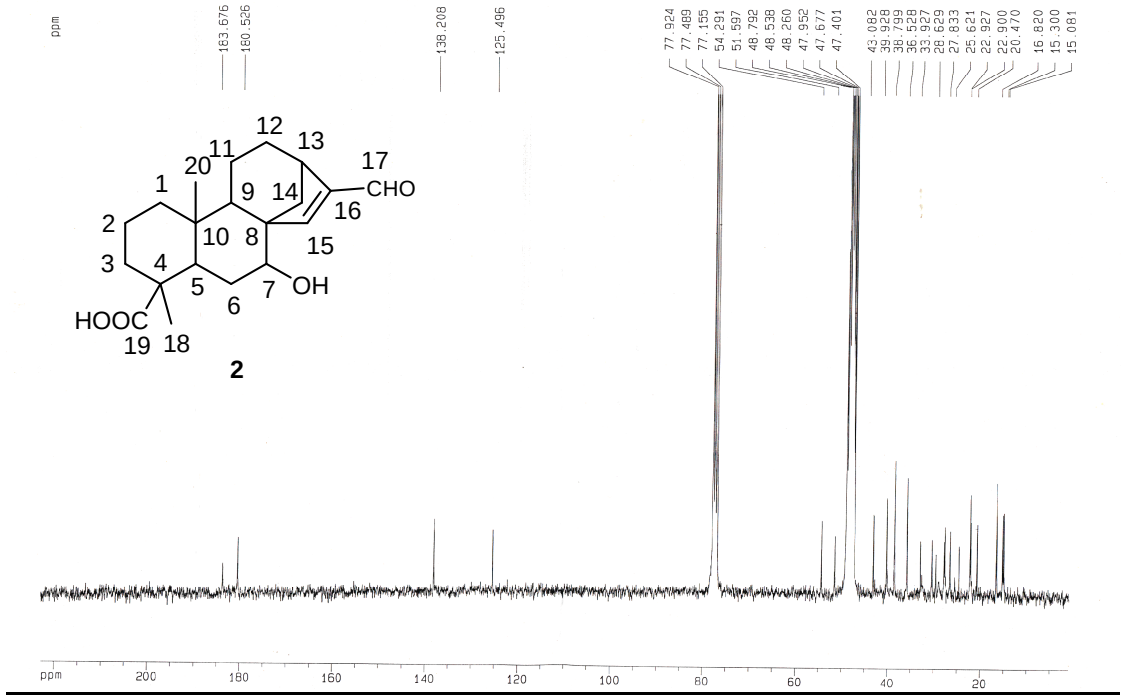
S3: Analytical data of *Ent-7-Hydroxykaur-15,16-en-19-oic acid* (**1**)

Yield: 21%; White solid powder; m.p. 264-266 °C; IR ν_{max} (KBr) cm^{-1}: 3410 (OH), 2870 (C–H), 2922 (C–H), 1712 (C=O), 1620 (C=C), 1280 (C–O); ^{1}H-NMR (300 MHz, $CDCl_3$+MeOD, 40 ^{o}C, TMS) δ: 17.56* (1H, s, br, H-19), 5.21 (1H, s, H-15), 3.17 (1H, m, H-7), 2.19 (1H, m, H-13), 1.91 (2H, dd, H-6), 1.88 (1H, t, H-5), 1.70 (2H, t, H-3), 1.51 (3H, s, H-17), 1.45 (1H, t, H-9), 1.40 (2H, m, H-2), 1.34 (2H, t, H-1), 1.34 (2H, m, H-12), 1.34 (2H, m, H-11), 1.33 (2H, d, H-14), 0.94 (3H, s, H-18), 0.90 (3H, s, H-20); ^{13}C-NMR (75.5 MHz, $CDCl_3$+MeOD, 40 ^{o}C, TMS) δ: 180.50 (C-19), 138.12 (C-16), 125.43 (C-15), 78.46 (C-7), 55.24 (C-8), 53.00 (C-9), 43.00 (C-5), 39.36 (C-4), 39.00 (C-13), 38.88 (C-1), 36.77 (C-3), 29.00 (C-14), 26.00 (C-12, C-6), 27.71 (C-10), 26.52 (C-11), 23.11 (C-17), 20.71 (C-18), 16.62 (C-2), 15.33 (C-20); EIMS *m/z* (rel. int.): 319[M^+], 341[M^++Na]; Anal. calcd. for $C_{20}H_{30}O_3$: C, 75.68; H, 9.54. Found: C, 75.71; H, 9.50.

*^{1}H-NMR peak was not shown for COOH group proton in ^{1}H-NMR Spectra. Thus, it twas taken directly from the NMR instrument due to broad range peak.



S4: ^{1}H-NMR (300 MHz, $CDCl_3$+MeOD, 40 ^{o}C) Spectrum of *17-Oxokaur-15,16-en-19-oic acid* (**2**)

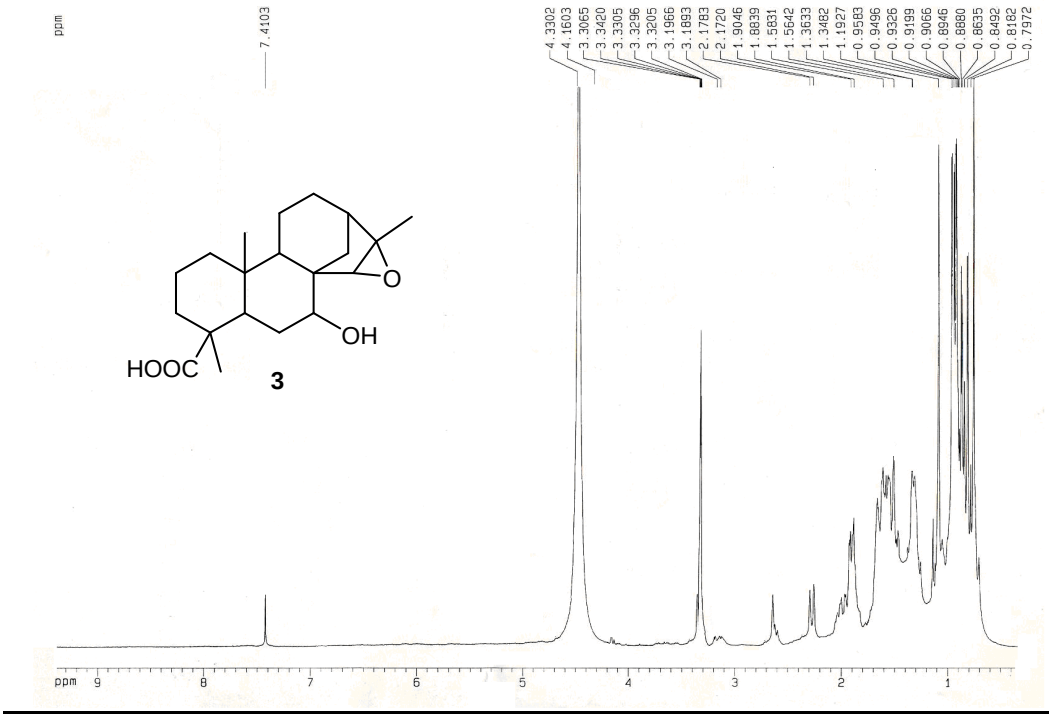


S5: ^{13}C-NMR (75.5 MHz, $CDCl_3$+MeOD, 40 ^{o}C) Spectrum of *17-Oxokaur-15,16-en-19-oic acid* (**2**)

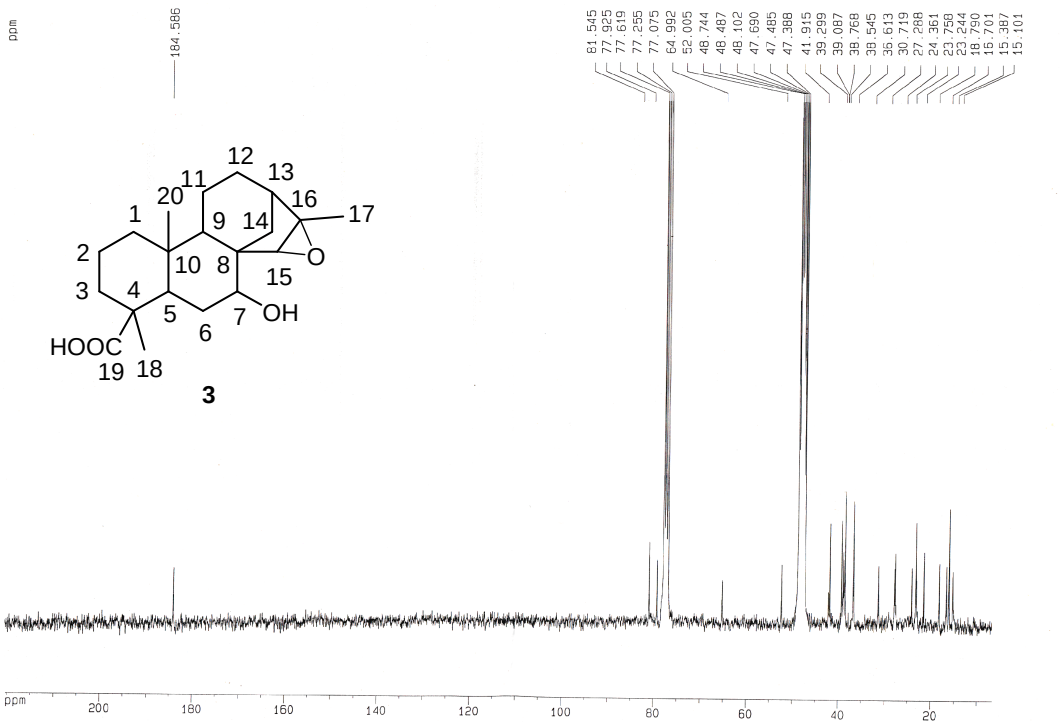
S6: Analytical data of *17-Oxokaur-15,16-en-19-oic acid* (**2**)

Yield: 19%; Off white solid powder; m.p. 101-105 °C; IR ν_{max} (KBr) cm^{-1}: 2930 (C–H), 2850 (C–H), 2730 (C–H), 1715-1680 (br, C=O), 1605 (C=C), 1301 (C–O); ^{1}H-NMR (300 MHz, $CDCl_3$+MeOD, 40 ^{o}C, TMS) δ: 17.85* (1H, s, br, H-19), 9.63 (1H, s, H-17), 5.25 (1H, s, H-15), 1.93 (1H, t, H-5), 1.93 (1H, m, H-13), 1.87 (2H, t, H-3), 1.62 (2H, m, H-12), 1.54 (1H, t, H-9), 1.42 (2H, m, H-2), 1.40 (2H, t, H-1), 1.34-137 (8H, m, H-6, H-7, H-11, H-14 (2H each)), 0.95 (3H, s, H-18), 0.92 (3H, s, H20); ^{13}C-NMR (75.5 MHz, $CDCl_3$+MeOD, 40 ^{o}C, TMS) δ: 183.67 (C-17), 180.52 (C-19), 138.20 (C-16), 125.49 (C-15), 54.29 (C-9), 51.59 (C-5), 43.08 (C-4), 39.92 (C-8), 38.79 (C-1), 36.52 (C-7), 33.92 (C-3), 28.62 (C-14), 27.83 (C-10), 25.62 (C-13), 25.00 (C-12), 22.92 (C-11), 20.47 (C-18), 16.82 (C-20), 15.30 (C-2), 15.08 (C-6); EIMS *m/z* (rel. int.): 316 [M^{+}]; Anal. calcd. for $C_{20}H_{28}O_3$: C, 75.81; H, 8.72. Found: C, 75.86; H, 9.75.

*^{1}H-NMR peak was not shown for COOH group proton in ^{1}H-NMR Spectra. Thus, it twas taken directly from the NMR instrument due to broad range peak.



S7: ^{1}H-NMR (300 MHz, $CDCl_3$+MeOD, 40 ^{o}C) Spectrum of *Ent-7-Hydroxy-15,16-epoxykauran-19-oic acid* (**3**)



S8: ^{13}C-NMR (75.5 MHz, $CDCl_3$+MeOD, 40 ^{o}C) Spectrum of *Ent-7-Hydroxy-15,16-epoxykauran-19-oic acid* (**3**)

S9: Analytical data of *Ent-7-Hydroxy-15,16-epoxykauran-19-oic acid* (**3**)

Yield: 18%; Off white solid powder; m.p. 282-284 °C; IR ν_{max} (KBr) cm^{-1}: 3430 (OH), 2930 (C–H), 2870 (C–H), 1705 (C=O), 1622 (C–O), 1127 (C–O–C). ^{1}H-NMR (300 MHz, $CDCl_3$+MeOD, 40 ^{o}C, TMS) δ: 17.70* (1H, s, br, H-19), 3.19 (1H, m, H-7), 2.70 (1H, s, H-15), 2.00 (2H, dd, H-6), 1.90 (1H, t, H-5),1.88 (1H, m, H-13), 1.70 (2H, t, H-3), 1.50 (2H, m, H-2), 1.40 (2H, t, H-1), 1.40 (1H, t, H-9), 1.36 (2H, d, H-14), 1.36 (2H, m, H-12), 1.34 (2H, m, H-11), 0.95 (3H, s, H-17), 0.93 (3H, s, H-18), 0.89 (3H, s, H-20). ^{13}C-NMR (75.5 MHz, $CDCl_3$+MeOD, 40 ^{o}C, TMS) δ: 184.58 (C-19), 81.54 (C-7), 77.92 (C-15), 64.99 (C-16), 52.00 (C-8), 41.91 (C-5), 39.29 (C-4), 38.08 (C-13), 38.76 (C-9), 36.61 (C-1), 30.71 (C-3), 27.28 (C-10, C-12), 24.36 (C-6), 23.75 (C-11), 23.24 (C-20), 18.79 (C-17), 16.70 (C-18), 15.38 (C-2), 15.10 (C-14); EIMS *m/z* (rel. int.): 357.1 [M^{+}+Na]; Anal. calcd. for $C_{20}H_{30}O_4$: C, 71.68; H, 9.12; Found: C, 71.71; H, 8.18.

*^{1}H-NMR peak was not shown for COOH group proton in ^{1}H-NMR Spectra. Thus, it twas taken directly from the NMR instrument due to broad range peak.

S10: Biological Assay: *In vitro* antibacterial activity bioassay

All the diterpenoids **1-3** had screened *in vitro* against *Pseudomonas aeruginosa* (MTCC 1688), *Escherichia coli* (BL21), and *Klebsiella planticola* (MTCC 2272), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (WHO 6) strains. Various methods are available for the evaluation of the antibacterial activity of different types of drugs. However, the most widely used method, which consists of determining the antibacterial activity of the drug is to add it in known concentrations to the cultures of the test organisms, was employed. Different concentrations of the test compound in 200 mL of culture medium were prepared in 96-well flat-bottomed microculture plates (Nunc, Nunclon) by double dilution method. The wells were prepared in triplicate for each concentration. Each well was inoculated with 10.0 μL of bacterial suspension containing 10^6 cells/mL. The plates were incubated at 37 °C for 16 hr and the OD was measured at a 600 nm of the suspension to assess the inhibition of cell growth due to treatment with compounds. All the tests were repeated up to three times.

S11: Biological Assay: Human cell lines

Human cerebral glioma tumor U-87, cervical cancer Hela and normal HEK cells used in the present studies. Both carcinoma and normal HEK cell lines were purchased from National Center for Cell Sciences (NCCS, Pune, India). All three cell lines were cultured in 75 cm^2 culture flasks (Corning, USA) using Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1% nonessential amino acids, 1% glutamine, penicillin (100 IU/mL) and streptomycin (100 mg/mL) (all from Euroclone, UK). All cultures were maintained at 37 °C, 95% relative humidity and 5% CO_2. The growth medium was changed every other day until the time of use. U87 cells of passage numbers 54-60, Hela cells of passage numbers 31-34 and HEK cells passage number 47-49 were used in the cytotoxicity tests. Prior to each cytotoxicity test, the cells were harvested using trypsin-ethylenediamine tetraacetic acid (EDTA)-PBS solution (Hela and HEK with 0.25% trypsin-0.05 mM EDTA and U87 with 0.25% trypsin-0.8 mM EDTA, according to the distributor's instructions) and diluted at a density of 5×10^5 cells/mL in MTT assays. Stock cultures were passaged every third day after harvesting the cells with 0.05% trypsin and seeding 8×10^3 cells/cm^2 in tissue culture flasks to maintain the cells in the exponential phase. All experiments were carried out in exponentially growing cells. The cell suspension was seeded into 96-well plates (Corning, USA) at 100 µL/well, and incubated for approximately 24 hr before tests in order to reach confluency. Before the cells were seeded into 96-well plates, the plates were treated with 0.01% poly-D-lysine solution (Sigma-Aldrich, Germany).

S12: Biological Assay: *In vitro* cell growth inhibition bioassay

Cells were seeded in 96-well plates at a concentration of 1×10^4 cells/well in 200 μL of complete media and incubated for 24, 48 and 72 hrs at 37 °C in 5% CO_2 atmosphere to allow for cell adhesion. Stock solutions (2 mg/mL) of the compounds made in DMSO were filter-sterilized, then further diluted up to 0.45 µg/mL incomplete media for treatment against U87, HeLa and HEK cell lines. A 100 μL solution of compound was added to a 100 μL solution of fresh medium in wells to give final concentrations of 1000-0.45 µg/mL. All assays were performed in two independent sets of quadruplicate tests. Control group containing no drug, was run in each assay. Following 24, 48 and 72 hrs exposure of cells to drug, each well was carefully rinsed with 200 μL PBS buffer. Cytotoxicity was assessed using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). 20 μL MTT solutions (5 mg/mL dd H_2O) along with 200 μL of fresh, complete media were added to each well and plates were incubated for 4 hr. Following incubation, the medium was removed and the purple formazan

precipitate in each well was sterilized in 200 μL DMSO. Absorbance was measured using Techan microplate reader (molecular device) at 570 nm and results are expressed as IC_{50} which is directly proportional to growth inhibition and metabolic active cell number.

S13: General

Melting points were determined on a Glassco melting point apparatus (Cat. no. 514.303.01). The melting points were determined in open capillaries on an electrically heated melting point apparatus and are uncorrected. IR (KBr) spectra were recorded using Perkin-Elmer 1710 FT-IR spectrophotometer and the values are expressed as ν_{max} cm^{-1}. Mass spectral data were recorded on a Jeol (Japan) JMS-DX303 and micromass LCT, Mass Spectrometer/Data system. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker Spectrospin spectrometer at 300 MHz and 75.5 MHz, respectively using $CDCl_3$+CD_3OD (1 drop) as a solvent and TMS as an internal standard. Elemental analyses for all compounds were performed on a Carlo Erba Model EA-1108 elemental analyzer and data of C, H and N is within ±0.4% of calculated values. Column chromatography (CC) performed by using silica gel (SiO_2; 60-120, 100-200 mesh) and solvent were distilled prior to use. SiO_2-GF_{254} glass plate (Merk) were used for thin layer chromatography.