APPENDIX–1

The detailed procedure for MTT assay and apoptosis analysis:

Collection and culture of cell lines

The Human skin cancer cells (A431 and A375) and Human embryonic kidney cells (HEK-293) were purchased from the National Centre for Cell Sciences (NCCS), Pune, India.

The cancer cells were maintained in Dulbecco's Modified Eagles Medium (DMEM). It is supplemented with L-glutamine (2 mM) and Balanced Salt Solution (BSS) adjusted to contain Na_2CO_3 (1.5 g/L), nonessential amino acids (0.1 mM), sodium pyruvate (1 mM), glucose (1.5 g/L), 4-(2-hydroxyethyl)-1-piperazine ethane sulphonic acid (10 mM) and fetal bovine serum (10%). Penicillin and streptomycin (100 IU/100 µg) were adjusted to 1 mL/L. The cells were maintained at 37°C with CO_2 (5%) in a humidified CO_2 incubator.

Evaluation of MTT assay

The inhibitory concentration (IC_{50}) value was evaluated using an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Normal and cancer cells were grown (1×10^5 cells/well) in a 96-well plate for 48h into 80% confluence. The medium was replaced and fresh medium containing serially diluted synthesized compounds, and the cells were further incubated for 48h. The culture medium was removed and 100 µL of the MTT [3-(4,5-dimethylthiozol-2-yl)-3,5-diphenyl tetrazolium bromide] (Hi-Media) solution was added to each well and incubated at 37°C for 4h. After removing the supernatant, 50 µL of DMSO was added to each of the wells and incubated for 10 min to solubilize the formazan crystals. An ELISA multi-well plate reader (Thermo Multiskan EX, USA) measured the optical density (OD) at 620 nm. The OD value was used to calculate the percentage of viability using the following formula.

$$\text{Cell viability (\%)} = \frac{\text{OD value of experimental sample}}{\text{OD value of experimental control}} \text{ X } 100$$

Acridine Orange/Ethidium Bromide Staining

Acridine orange (100 mg/mL) and Ethidium bromide (100 mg/mL) was mixed in distilled water to get the dye mixture. Cell suspensions (1×10^5 cells/mL) (9 mL) were mixed with dye mixture (1 µL) and taken in a clean microscope cover slip. The cancer cells were thoroughly washed with saline solution (phosphate-buffered (PBS) at pH 7.2. Then it was stained with AO/EtBr (1 mL) and incubated for 2 min. Again, the cancer cells were cleaned with saline solution (PBS) and seen under a (Nikon Eclipse, Inc, Japan model) fluorescence microscope at 20X magnification with an excitation filter at 520 nm.

Propidium Iodide (PI) Staining

Propidium iodide (100 mg/mL) was mixed in distilled water to get the dye mixture. Cell suspensions (1×10^5 cells/mL) (9 mL) were mixed with dye mixture (1 µL) and was taken in a clean microscope cover slip. The cancer cells were thoroughly washed with saline solution (phosphate-buffered (PBS)) at pH 7.2. Then it was stained with PI (1 mL) and incubated for 2 min. Again, the cancer cells were cleaned with saline solution (PBS) and seen under a (Nikon Eclipse, Inc, Japan model) fluorescence microscope at 20X magnification with an excitation filter at 480 nm.