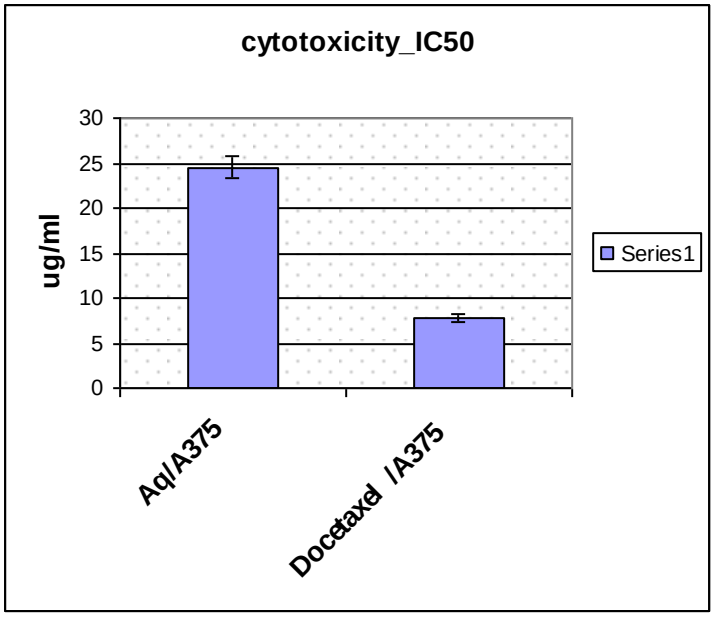
Electronic Supplementary Material (ESI) for RSC Advances.

Researcher	: Dr.Marwa elhawary email: Marwaelhawary527@yahoo.com mob.
Assay	: MTT cytotoxicity assay
Samples	: 01 compounds.
Cell lines	: ---
Ref.	: ---
Date	: 28-07-2020
Reader	: BIOLINE ELIZA READER wl 450 nm
Kit used	: SIGMA chemicals
Solvent	: DMSO

Lab REPORT

* Cytotoxicity results

Ser			Cytotoxicity IC50	
	Sample		A375	
	code	MW	ug/ml	
1	Aq/A375		24.5±1.9	
2	Docetaxel		7.73±0.6	



Assay Protocol

Cell Line cells were obtained from American Type Culture Collection , cells were cultured using DMEM (Invitrogen/Life Technologies) supplemented with 10% FBS (Hyclone,), 10 ug/ml of insulin (Sigma), and 1% penicillin-streptomycin. All of the other chemicals and reagents were from Sigma, or Invitrogen.
Plate cells (cells density 1.2 – 1.8 × 10,000 cells/well) in a volume of 100µl complete growth medium + 100 ul of the tested compound per well in a 96-well plate for 24 hours before the MTT assay .

Cell cuture protocol

1. Remove culture medium to a centrifuge tube.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin 0.53 mM EDTA solution to remove all traces of serum which contains Trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Transfer the cell suspension to the centrifuge tube with the medium and cells from step 1, and centrifuge at approximately 125 xg for 5 to 10 minutes. Discard the supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels.
7. Incubate cultures at 37°C for 24 hrs.

8-After treatment of cells with the serial concentrations of the compound to be tested incubation is carried out for 48 h at 37°C ,then the plates are to be examined under the inverted microscope and proceed for the MTT assay

MTT – Cytotoxicity assay protocol

The MTT method of monitoring in vitro cytotoxicity is
well suited for use with multiwell plates. For best results, cells in the log phase of growth should be employed and final cell number should not exceed 106 cells/cm2. Each test should include a blank containing complete medium without cells.

1. Remove cultures from incubator into laminar
flow hood or other sterile work area.

2. Reconstitute each vial of MTT [M-5655] to be used with 3 ml of medium or balanced salt solution without phenol red and serum. Add reconstituted MTT in an amount equal to 10% of the culture medium volume.

3. Return cultures to incubator for 2-4 hours depending on cell type and maximum cell density. (An incubation period of 2 hours is generally adequate but may be lengthened for low cell densities or cells with lower metabolic activity.) Incubation times should beconsistent when making comparisons.

4. After the incubation period, remove cultures from incubator and dissolve the resulting formazan crystals by adding an amount of MTT Solubilization Solution [M-8910] equal to the original culture medium volume.

5. Gentle mixing in a gyratory shaker will enhance dissolution. Occasionally, especially in dense cultures, pipetting up and down [trituration] may be required to completely dissolve the MTT formazan crystals.

6. Spectrophotometrically measure absorbance at a wavelength of 450 nm. Measure the background absorbance of multiwell plates at 690 nm and subtract from the 450 nm measurement. Tests performed in multiwell plates can be read using the appropriate type of plate reader or the contents of individual wells may be transferred to appropriate size cuvets for spectrophotometric measurement.

researcher	assay	Date	cells
Dr.Marwa Elhawary	MTT	28-Jul	A375

	Blank	CC	Sample No. Aq/A375					Sample No. Docetaxel/A375				
	1	2	3	4	5	6	7	8	9	10	11	12
A	B	C	100ug	25ug	6.25ug	1.56ug	0.39ug	100ug	25ug	6.25ug	1.56ug	0.39ug
B	B	C	100ug	25ug	6.25ug	1.56ug	0.39ug	100ug	25ug	6.25ug	1.56ug	0.39ug
C	B	C	100ug	25ug	6.25ug	1.56ug	0.39ug	100ug	25ug	6.25ug	1.56ug	0.39ug

ROBONIK P2000 eia reader

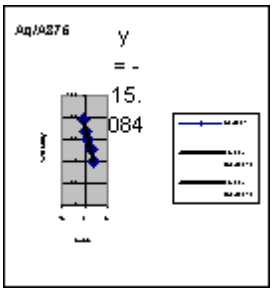
Wave length: 560450 nm

Reference: 630 nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	0.471	0.196	0.241	0.288	0.319	0.362	0.162	0.194	0.243	0.292	0.345
B	0.001	0.493	0.188	0.239	0.291	0.325	0.371	0.154	0.205	0.245	0.297	0.339
C	0.001	0.467	0.191	0.238	0.286	0.308	0.375	0.157	0.187	0.242	0.284	0.338
mean	0.0004	0.477	0.1917	0.239	0.2883	0.3173	0.369	0.158	0.1953	0.2433	0.291	0.3407
%		`	40.182	50.17	60.447	66.527	77.43	33.05	40.95	51.013	61.01	71.419

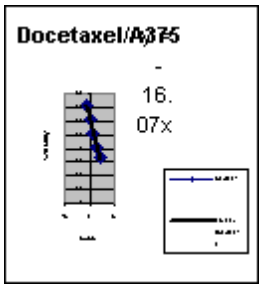
Aq/A375

log conc.	% viability
2	40.182
1.398	50.175
0.796	60.447
0.193	66.527
-0.41	77.428



Docetaxel/A375

log conc.	% viability
2	33.054
1.398	40.95
0.796	51.013
0.193	61.006
-0.409	71.419



IC50=

IC50=