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Design Modulation of some Novel Green NanoPd Complexes as Potential Anti-Cancer Agent

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Abstract : Prediction of anticancer activity of newly synthesized palladium (II) metal nano complexes from aqueous extract of *Macrotyloma uniflorum*(horse gram) and *Vigna unguiculata* (Lobia) seeds. The newly prepared nano particles were characterized by UV-visible spectrophotometer and FTIR. The reduced Pdnano particles were analysed with SEM analysis. FTIR spectra confirmed the involvement of diverse functional groups participated. The morphology and size of the Pd nanoparticles were examined by SEM analysis, which showed most of the nano particles were nearly spherical with nano range size. The newly synthesized complexes have revealed significant *in vitro* cytotoxic activity against human Cervical, Lung and MCF-7 breast adenocarcinoma cancer cell lines with cell death largely caused by apoptosis. The result concludes that *Macrotyloma uniflorum*(horsegram) and *Vigna unguiculata* (Lobia) seeds aqueous extract bio-reduced Pd micro particle to nanoparticles, possess potential anticancer property. They may have wide applications in medicine and pharmaceutical fields.
Key Words: Green Pdnano particles, UV & IR spectra, SEM, *Macrotyloma uniflorum*, Cervical, Lung and MCF-7 breast cancer cell lines.

Introduction:

There is a tremendous growth in the field of Nano science and Nanotechnology over the past two decades. The *nanoscale* defined as the "length of a particle approximately from 1 nm to 100 nm" but technically the nanoscopic scale is the size at which fluctuations in the averaged properties instigate to have a major effect on the performence of a system, and must be analyzed. This incorporates both *nanostructured materials*, which have internal or surface structure on the nanoscale and *nano-objects*, which are discrete piece of material, a nanomaterial or nanoparticle may be a member of both these categories[1]. Nanotechnology is characterized by size is normally very broad, includingfields of science as diverse as surface chemistry, organic-chemistry, Biotechnology, energy storage, microfabrication[2], molecular engineering techniques etc[3]. Nanotechnology is able to create many new engineering materials and devices with a wide range of applications, such as medicine, electronics, biomaterials energy production, and consumer products in nano scale. Nanotechnology raises many issues which includes concerns about the toxicity and environmental impact of nanomaterials,[4] and their potential effects on global economy.Metal-containing compounds have been utilized throughout history to treat a wide variety of disorders [5]. Organic chemistry- metal complexes have

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dominated in medicinal chemistry as diagnostic tools and anticancer agents[6]. The Accidental discovery of cisplatin, *cis*-$[Pt^{II}(NH_3)_2Cl_2]$stimulated research in anti cancer agents. However, Cisplatin clinical use is restricted due to dose-dependent toxicity and resistance coupled with a narrow spectrum of activity[7]. So these boundaries have prompted scientists to explore for platinum and palladium-based compounds that show a broader spectrum of activity withhigher selectivity as well as with low toxicity [8]. The compounds known as carboplatin and oxaliplatin. Over the past 3 decades, platinum-based drugs, notably cisplatin and carboplatin, have dominated the treatment of various cancers .

Though more than thirty platinum antineo plastic agents examined in clinical trials, only carboplatin has been accepted worldwide [9].

Because of the considerable biological activity of palladium (II) metal complexes have attracted the researchers a lot and lower side effects along with higher lipophilicity or solubility as compared to cisplatin, made these compounds very promising. [10-11]. Few scientists reviewed several research publications on antiviral, antibacterial, and antifungal activity of Pd (II) complexes with different types of ligands having sulfur and nitrogen donor ligands and different drugs as ligands [12]. Nanoparticles synthesis is done by processing of bulk material into nanostructure particles. Various methods for synthesis of nanoparticles use described here as under. Various different methods have been employed to prepare silver nanoparticles with different sizes and shapes, such as UV irradiation, photochemical method, electron irradiation, and so no-electrochemical method. Physical method used for the synthesis of nanoparticles is mechanical and Vapour deposition [13].Chemical reduction of nanoparticles is the most commonly applied method for the preparation of stable, colloidal dispersion in water or organic solvents[14].Three types of biological methods are used for the synthesis for manufacturing of low-cost, energy-efficient and non-toxic metal nanoparticle[15].The biological components used for the synthesis processor are:

1. By using microorganism like fungi, yeasts (eukaryotes) or bacteria, actinomycetes (prokaryotes).
2. Utilization of plant extracts or enzymes.
3. The various methods involved in biosynthesis of nanoparticles are the preparation of reducing agent at different condition, analysis and characterization.

Metal nanoparticles have been synthesized for a long time, but modern developments show the significant role of microorganisms and biological systems in invention of metal nanoparticles other than physical and chemical method. The recent use of microorganisms instead of chemicals in this area is rapidly developing due to their growing success and simplicity of formation of nanoparticles. Furthermore, biosynthesis of metal nano particles is an ecofriendly method (green chemistry) without use of harsh, toxic and expensive chemicals[16,17].

The quality and kind of nanoparticle synthesized using green chemistry are significantly influenced by length of time for which the reaction medium is incubated [18].

The to improve nanoparticles production without the use of harsh, toxic, and expensive chemicals generally used in conventional physical and chemical processes, currently biological synthesis via Nano biotechnology processes which have a considerable potential have been used [19-20]. Biosynthesis of nanoparticles by means of plants or plant based extracts are safe, have relatively short production times, and a lesser fabrication cost compared to other natural systems.

Several techniques can be used to characterized nanoparticle, including ultraviolet- (UV-) visible spectroscopy, atomic force microscopy (AFM), transmission electron microscopy (TEM), scanning electron microscopy (SEM), dynamic light scattering (DLS), X-ray photoelectron spectroscopy (XPS), powder X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR),nuclear magnetic resonance (NMR), nanoparticle tracking analysis (NTA) for evaluation of Brownian motion, and particle size analysis [21].Cancer is still one of the most difficult diseases to treat and was responsible for 13% (7.4 million) of all deaths worldwide [22]. An increase in cancer death rates to 12 million is calculated by 2030. Surgery, radiotherapy, hormone and chemotherapy are the most widely used therapies for cancer treatment[23].

Material and method:

Analytical methods & Physical measurements:

All the chemicals used were of analytical grade. N and S were estimated by the kjeldhal's and Messenger's method respectively. Pd was estimated gravimetrically, Chloride is estimated volumetrically by Volhard's method. Molecular weight is determined by Rastcampher method.UV recorded on SHINADZU 1800 spectrophotometer. IR by FT-IR spectrophotometer. H^1 and ^{13}C NMR recorded on ECS-400 MHz NMR, in DMSO-d6using TMS as internal standard.

Synthesis of ligands:

The new hydrazinecarbothioamide (L^1H) of 2-hydroxy-1-naphthaldehyde were synthesized by the condensation of 2- hydroxy-1-naphthaldehyde with thiosemicarbazide in 1:1 molar ratio using ethanol as a solvent. The contents were refluxed for about 3-5 h andwasseparated out as crystalline solid. The solid was dried and purified by recrystallisationby the same solvent.

The isonicotinylhydrazide of 2-floroacetophenone (L^2H), p-hydroxyacetophenone(L^3H) and 1-acetonaphthone (L^4H) were prepared by the condensation of the carbonyl compounds with isoniazid in 1:1 ratio using ethanol as solvent. Refluxing was done for 3-5 hours. The ligands were separated out as crystalline solids. They were dried & purified.

2-Hydroxy-1-naphthalenylmethylene hydrazine carbothioamide(L^1H) ($C_{12}H_{11}N_3OS$):

Colour Light Brown, M.P. 270°C. Yield 78%.Mol. Wt. 45.08 (245.30). Elemental analysis (%):calc.: C, 58.32; H, 4.43; N, 17.05; S, 12.97%. Found : C, 58.78; H, 4.54; N, 17.13; S, 13.02%. IR (KBr, cm^{-1}): ν(C=N): 1620.^{1}H NMR (500 MHZ, $CDCl_3$) δ 9.25 (s, 1 H); 8.28 (s, 1H); 11.33 (s, 1H); 3.22 (s, 1H); 7.15-8.45 (m, 6 H). ^{13}C NMR(125 MHZ, $CDCl_3$) δ153.83 (1 C), 165.45 (1 C), 153.7, 109.5, 136.8, 126.3, 126.4, 123.5, 127.7, 129.0, 130.3, 120.3 (10 C).

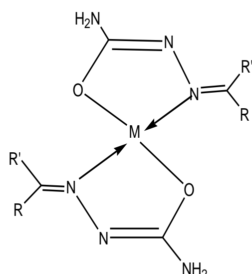
Preparation of $[Pd(L^n)_2]$ (substitution) complexes

A methanolic solution of $PdCl_2$ was mixed with ethanolic solution of the ligands in 1:2 molar ratio. Aqueous NH_4OH was added drop wise to the reaction mixture until it was weakly alkaline (pH ca. 8.0). The mixture was heated under reflux for about 1 h. On cooling, the complexes**$[Pd(L^1)_2]$**and **$[Pd(L^2)_2]$**were separated out and were filtered off, washed off with methanol and dried in vacuum.

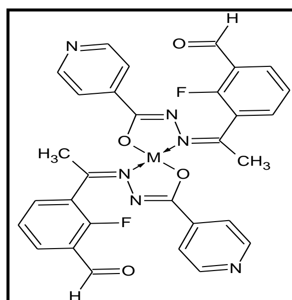
$[Pd(L^1)_2]$Colour Dark Brown, M.P., 310°C, Mol. Wt. 560.79 (562.87), Elemental analysis (%): calc.: C, 51.07; H, 3.44; N, 14.79; Pd, 18.74%. Found: C, 51.13; H, 3.56; N, 14.90; Pd, 18.81%. IR (KBr, cm^{-1}): ν(C=N): 1638; ν(Pd←N): 364; ν(Pd-O): 416.^{1}H NMR (500 MHZ, $CDCl_3$) δ 8.52 (s, 1H); 11.33 (s, 1H); 3.22 (s, 1H); 7.87-8.48 (m, 6 H).

Preparation of $[Pd(L^n)_2]$ (addition)complexes

The methonolic solution of $PdCl_2$ was mixed with the methanolic solution of the ligands (L^3H, L^4H) in 1:2 molar ratios. With the help of magnetic stirrer the reaction mixture was stirred for 2-3 hrs in presence of few drops of concentrated HCl to form complexes of the type of $[M(L^nH)_2]Cl_2$.

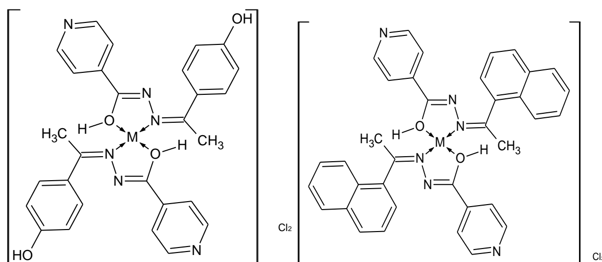


(Fig.1)



(Fig.2)

Substitution Pd complexes with ligands (L^1H), (L^2H) where, M= Pd andR = 2-hydroxynapthaldehyde, R'= H.

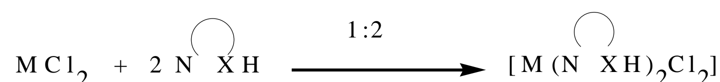


(Fig.3) **(Fig.4)**

Addition Pd complexes with ligands (L^3H), (L^4H) where, M= Pd

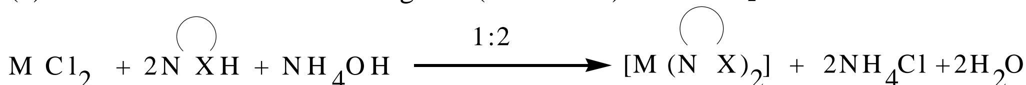
Addition reactions

The reactions of ligands (L^1H& L^2H) with metal chloride, $PdCl_2$, yield complex of different compositions depending on the mode of preparation. MCl_2 reacts with ligands in 1:2 molar ratio in presence of few drops of concentrated HCl. The refluxing medium used are methanol for Pd.



Though, in the presence of aqueous ammonium hydroxide the two chlorine atoms are replaced as indicated below:

(b) **Substitution reaction with** ligands (L^3H& L^4H) with $PdCl_2$



(Where, NX is the donor system of ligands; X = O/S and M= Pd.)

The coloured product so obtained is quite stable and insoluble in many organic solvents but considerable soluble in DMF and DMSO. All the complexes are diamagnetic in nature as projected for the square planar d^8 metal complexes. The complexes are monomers as shown by their molecular weight determinations. The data from analytical and physicochemical studies were correlated to explain the properties and nature of the complexes.

Synthesis of Palladium nano-particles

Black coated horse gram (*Macrotyloma uniflorum*) and Lobia*(Vigna unguiculata)*seeds were collected and the mature seeds were used in the synthesis. The next step is germination for which seeds were washed with $KMnO_4$ solution. After washing, 100 gm seeds were soaked in distilled water for about 12 h (Fig.5).

The seeds were allowed to germinate for 3 to 4 days at room temperture and they were moistened with distilled water regularly for every 10 h. The germinated seeds freeze dried and were grounded to 60 mesh size in 60 ml of acetone to remove lipid content. The mixture obtained was filtered through double layer Whatmann filter paper-1. The homogenate was dried in a tray drier overnight at 32˚C and were further coarsely grounded using motar and pestle and the powder is obtained. To the 10 gm of dry powder, 20 ml of phosphate buffer solution of pH 8.0 is added. The mixture (buffer and powder) is stirred in 2M NaCl and incubated for 24 hours at room temperature. This mixture was further filtered and centrifuged at 10000 rpm for 8 minutes below 4˚C and the 5-7 ml supernatent was collected. Enzyme, protease collected from supernatant by using g $(NH_4)_2SO_4$ precipitation[24,25]. The enzyme concentration of crude extract ranged between 2.15 to 2.30 mg/ml.

For preparation of nanoparticles, 3 ml of collected enzyme is added to the 30 ml solution of 0.01 M of Pd complexes, stirred and kept at room temperature. After 2-3 days metal complex were converted into black coloured nano particles.

Fig 5: Germinated Seeds

Result and Discussion:

Scanning electron microscope

The nano particles of Palladium are scanned by the scanning electron microscopy, SEM (FEI Quanta 200 Hv model). Figures show the SEM images of Pd nanoparticles. The results show that the particles are converted to micro and nano size range between 75-95nm.

A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals, contains information of sample'ssurface topography and composition.

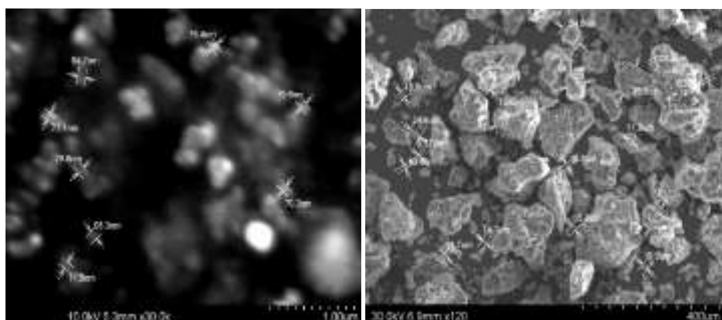


Fig 6: SEM images of Nano-particles

Anti Cancer Activity

Maintenance of cell line:

The cell lines were purchased from NCCS, Pune. The cells were maintained in DMEM supplemented with 10-12 % FBS and the antibiotics penicillin/streptomycin (0.5 mL^{-1}), in the atmosphere of 5% CO_2 /95% air at 35-37˚C. For MTT assay, Each Test compounds were weighed separately and dissolved in DMSO. With media make up the final concentration to 1 mg/mL and the cells were treated with series of concentrations from 10 to 100 µg/ mL.

Cell viability:

Cell viability was estimated by the MTT Assay with triplicate experiments of six concentrations of compounds (5, 10, 25, 50, 75 and 100 µg).cells were trypsinized and perform the tryphan blue assay to know viable cells in cell suspension. Cells were counted by haemocytometer and seeded at density of 5.0 X 10^{3} cells / well in 100 µL media in 96 well plate culture medium and incubated overnight at 37 ^{0}C. After incubation, the old media was taken offand 100 µL fresh media was added with different concentrations of test compound in representative wells in 96 plate. After 48 h, the drug solution was discarded and the fresh media with MTT

solution (0.5 mg / mL$^{-1)}$ was added to each well and plates were incubated at 37 ^{0}C for 3 hrs. At the end of incubation time, precipitate is formed which is result of the reduction of the MTT salt to chromophoreformazan crystals by the cells having metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 560 nm on a micro plate reader.

Calculation of % Viable cells

From the all samples the absorbance reading of the blank should be deducted and absorbance readings from test samples must be divided by those of the control and multiplied by 100 to give percentage cell viability or proliferation the formula isgiven below. Cell viability would be designated by the greater absorbance valuesthan the control, while lower values would suggest cell death or inhibition of proliferation.

$$\% \text{ viable cells} = \frac{(abs_{sample} - abs_{blank})}{(abs_{control} - abs_{blank})} \times 100$$

Test Result

Result: HeLa Cell Line (Cervical Cancer Cell line)

Compound 1

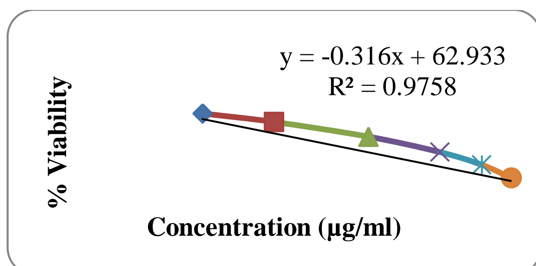
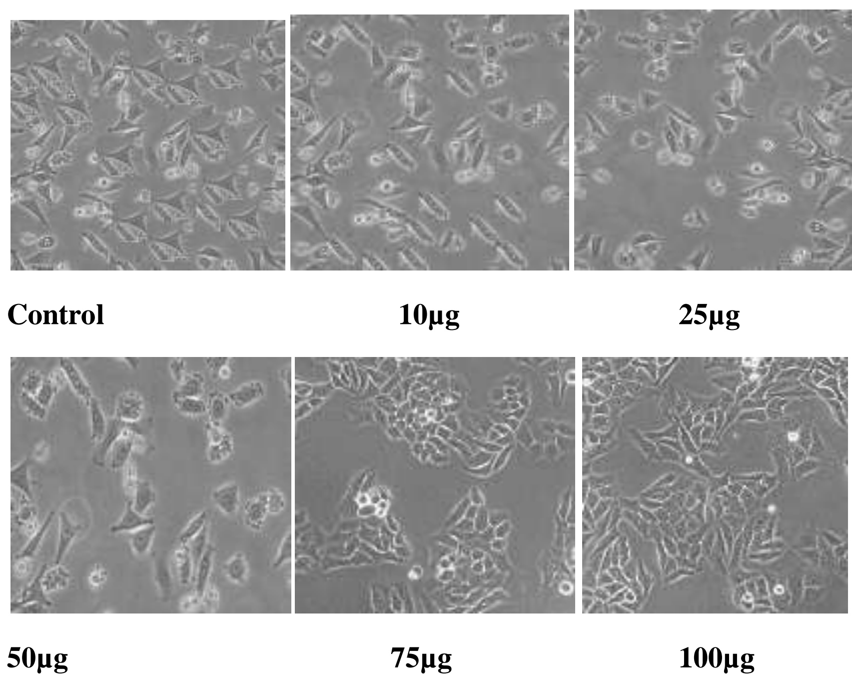


Figure 7: Cytotoxic Effect of *Compound 1* on HeLa Cell Line

Table 1: Cytotoxicity Properties of *Compound 1* against HeLa Cell Line

Concentration(µg/ml)	**Absorbance (570nm)**			**Average**	**Average-Blank**	**% Viability**	**IC_{50} (µg/ml)**
100	0.423	0.425	0.426	0.424	0.416	32.755	40.917
75	0.503	0.505	0.507	0.505	0.497	39.133	
50	0.582	0.584	0.585	0.583	0.575	45.275	
25	0.675	0.679	0.68	0.678	0.67	52.755	
10	0.768	0.77	0.771	0.769	0.761	59.921	
5	0.82	0.822	0.823	0.821	0.813	64.015	
Untreated	1.278	1.279	1.278	1.278	1.27	100	
Blank	0.008	0.009	0.008	0.008	0		



Compound 2: HeLa Cell Line (Cervical Cancer Cell line)

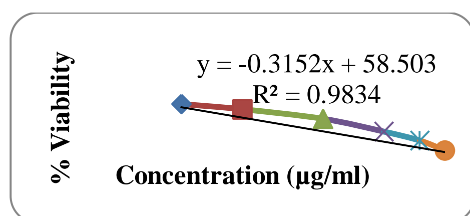
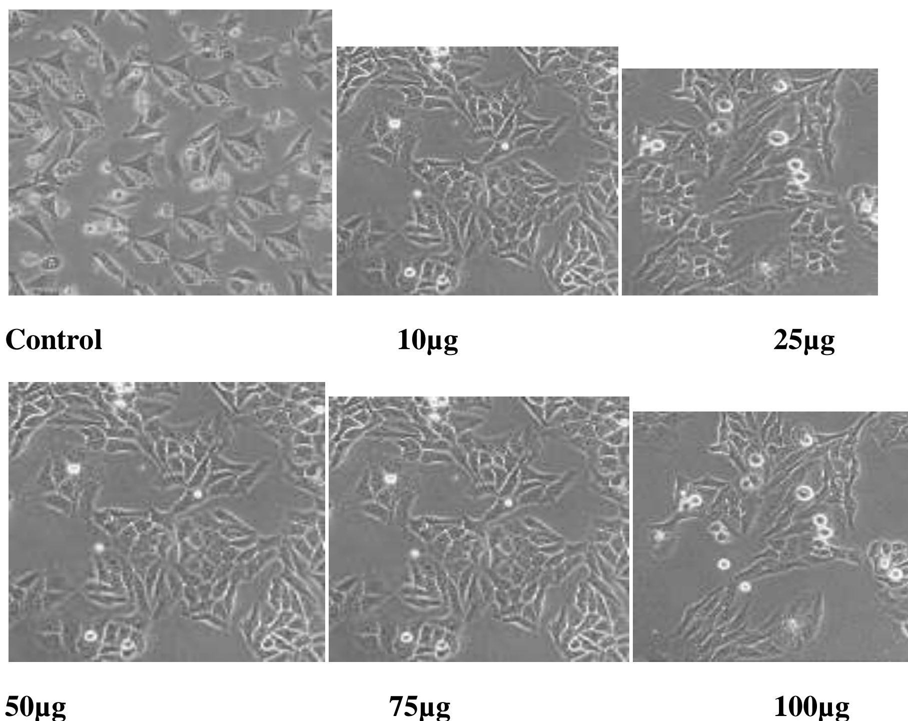


Figure 8: Cytotoxic Effect of Compound 2 on HeLaCell Line

Table 2: Cytotoxicity Properties of *Compound 2* against HeLa Cell Line

Concentration(µg/ml)	**Absorbance (570nm)**			**Average**	**Average-Blank**	**% Viability**	**IC_{50} (µg/ml)**
100	0.37	0.371	0.373	0.371	0.363	28.282	26.984
75	0.448	0.45	0.452	0.45	0.442	34.803	
50	0.524	0.526	0.527	0.525	0.517	40.708	
25	0.631	0.633	0.635	0.633	0.625	49.212	
10	0.712	0.713	0.715	0.713	0.705	55.511	
5	0.756	0.757	0.759	0.757	0.749	58.976	
Untreated	1.278	1.279	1.278	1.278	1.27	100	
Blank	0.008	0.009	0.008	0.008	0		



Result: A549 Cell Line (Lung Cancer Cell line)

Compound 1 :

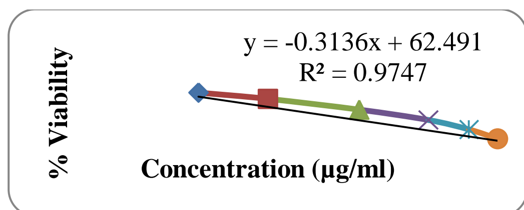
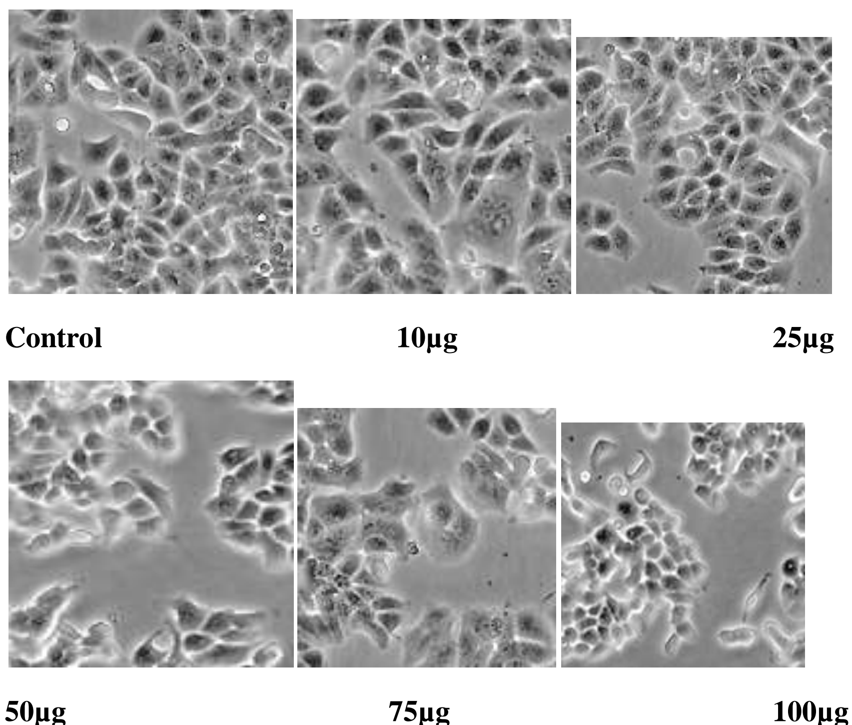


Figure 9: Cytotoxic Effect of *Compound 1* on A549 Cell Line

Table 3: Cytotoxicity Properties of *Compound 1*against A549 Cell Line

Concentration(µg/ml)	Absorbance (570nm)			Average	Average-Blank	% Viability	IC_{50} (µg/ml)
100	0.425	0.427	0.428	0.426	0.419	32.531	39.904
75	0.506	0.507	0.509	0.507	0.5	38.819	
50	0.587	0.589	0.591	0.589	0.582	45.186	
25	0.678	0.679	0.681	0.679	0.672	52.173	
10	0.771	0.773	0.775	0.773	0.766	59.472	
5	0.826	0.828	0.829	0.827	0.82	63.664	
Untreated	1.295	1.296	1.295	1.295	1.288	100	
Blank	0.007	0.008	0.007	0.007	0		



Compound 2: A549 Cell Line (Lung Cancer Cell line)

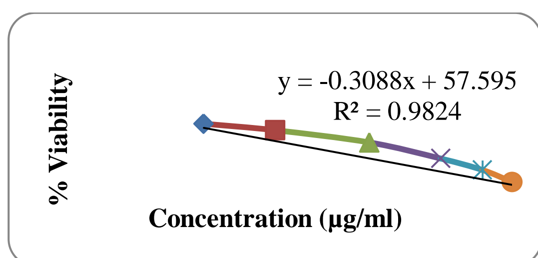
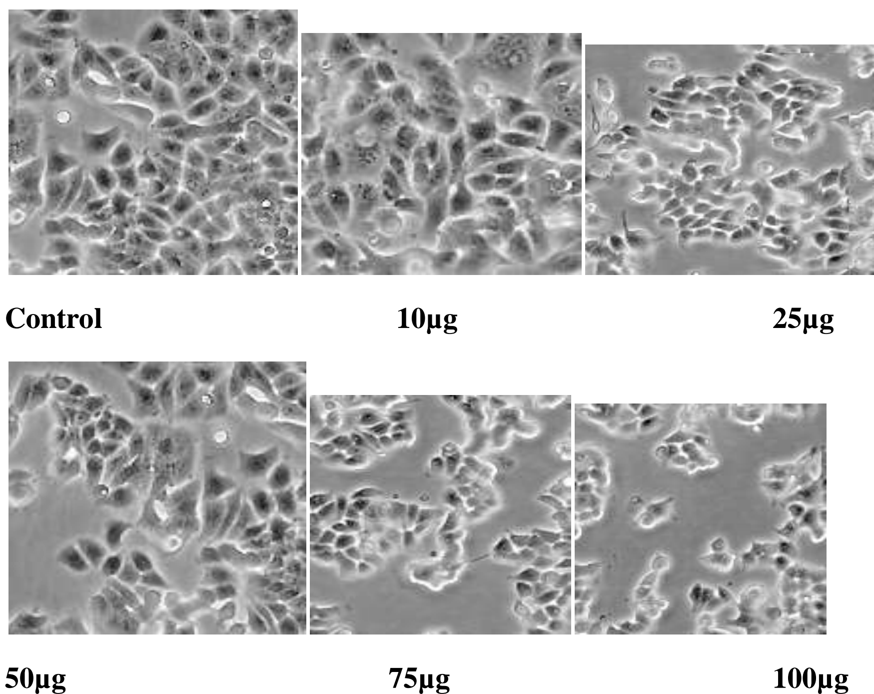


Figure 10: Cytotoxic Effect of Compound 2 **on A549 Cell Line**

Table 4: Cytotoxicity Properties of *Compound 2* against A549 Cell Line

Concentration(µg/ml)	Absorbance (570nm)			Average	Average-Blank	% Viability	IC_{50} (µg/ml)
100	0.369	0.37	0.372	0.37	0.362	28.105	24.642
75	0.448	0.45	0.451	0.449	0.441	34.239	
50	0.524	0.525	0.527	0.525	0.517	40.139	
25	0.631	0.633	0.634	0.632	0.624	48.447	
10	0.711	0.713	0.715	0.713	0.705	54.736	
5	0.755	0.757	0.758	0.756	0.748	58.074	
Untreated	1.295	1.296	1.295	1.295	1.288	100	
Blank	0.007	0.008	0.007	0.007	0		



Result: MCF-7 Cell Line (Breast Cancer Cell line)

Compound 1 :

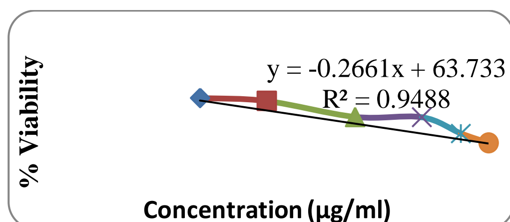
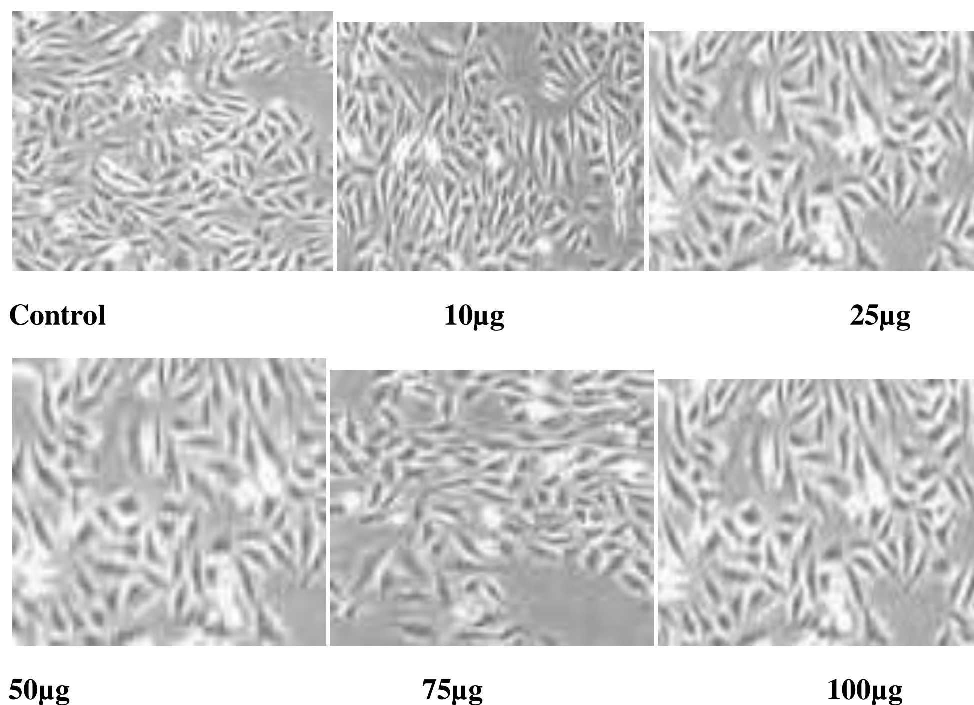


Figure 11: Cytotoxic Effect ofCompound 1 on MCF-7 Cell Line

Table 5: Cytotoxicity Properties of*Compound 1*against MCF-7 Cell Line

Concentration(µg/ml)	Absorbance (570nm)			Average	Average-Blank	% Viability	IC_{50} (µg/ml)
100	0.462	0.464	0.465	0.463	0.457	37.489	51.616
75	0.529	0.531	0.533	0.531	0.525	43.068	
50	0.645	0.647	0.649	0.647	0.641	52.584	
25	0.648	0.649	0.651	0.649	0.643	52.748	
10	0.762	0.764	0.765	0.763	0.757	62.1	
5	0.783	0.785	0.787	0.785	0.779	63.904	
Untreated	1.225	1.226	1.225	1.225	1.219	100	
Blank	0.006	0.007	0.006	0.006	0		



Compound 2: MCF-7 Cell Line (Breast Cancer Cell line)

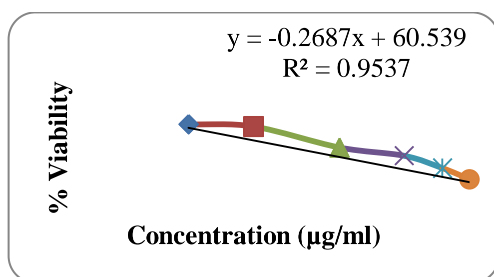
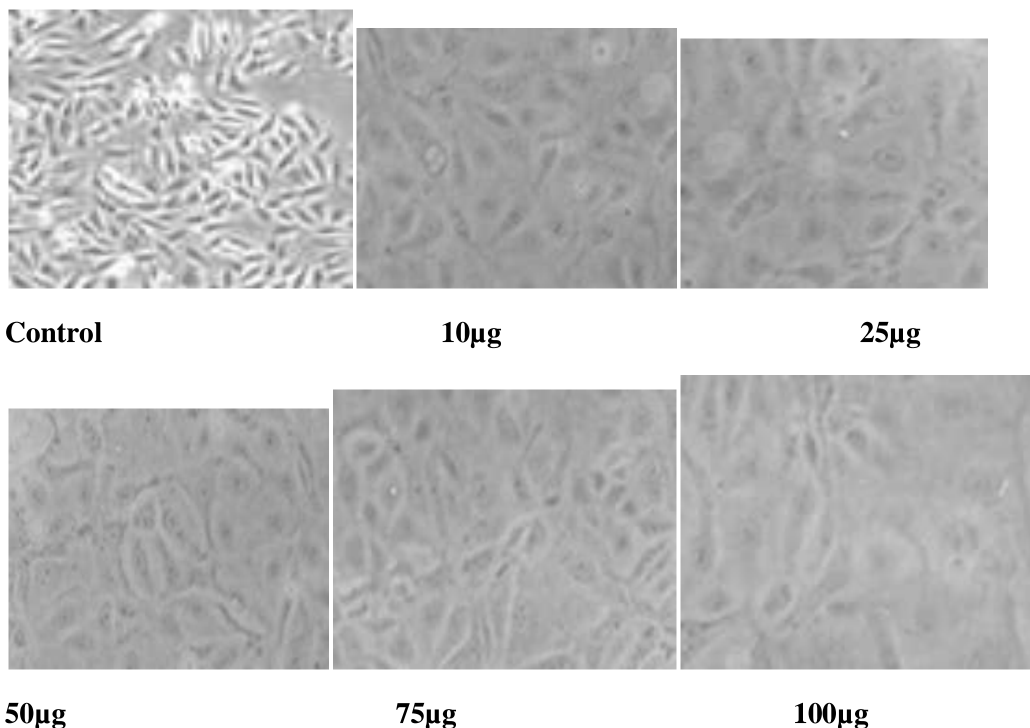


Figure 12: Cytotoxic Effect of *Compound 2* on MCF-7 Cell Line

Table 6: Cytotoxicity Properties of *Compound 2*against MCF-7 Cell Line

Concentration(µg/ml)	Absorbance (570nm)			Average	Average-Blank	% Viability	IC_{50} (µg/ml)
100	0.431	0.433	0.435	0.433	0.427	35.028	39.291
75	0.495	0.497	0.498	0.496	0.49	40.196	
50	0.566	0.568	0.569	0.567	0.561	46.021	
25	0.613	0.615	0.617	0.615	0.609	49.959	
10	0.733	0.735	0.736	0.734	0.728	59.871	
5	0.747	0.749	0.751	0.749	0.743	60.951	
Untreated	1.225	1.226	1.225	1.225	1.219	100	
Blank	0.006	0.007	0.006	0.006	0		



Result : HeLa Cell Line (Cervical Cancer Cell line)

Compound 3:

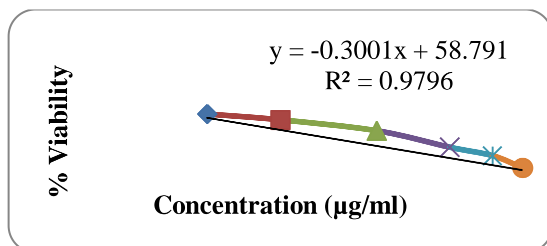
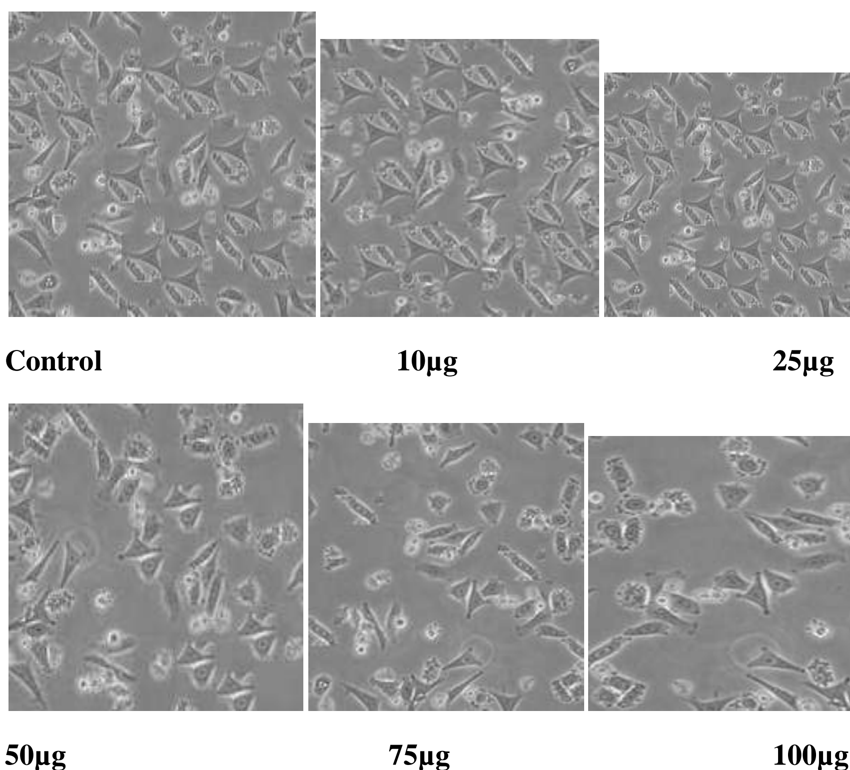


Figure 13: Cytotoxic Effect of*Compound 3* on HeLaCell Line

Table 7: Cytotoxicity Properties of*Compound 3*against HeLa Cell Line

Concentration(µg/ml)	Absorbance (570nm)			Average	Average-Blank	% Viability	IC_{50} (µg/ml)
100	0.387	0.389	0.391	0.389	0.381	30	29.3
75	0.472	0.474	0.475	0.473	0.465	36.614	
50	0.529	0.531	0.533	0.531	0.523	41.181	
25	0.643	0.645	0.647	0.645	0.637	50.157	
10	0.719	0.72	0.722	0.72	0.712	56.063	
5	0.758	0.76	0.762	0.76	0.752	59.212	
Untreated	1.278	1.279	1.278	1.278	1.27	100	
Blank	0.008	0.009	0.008	0.008	0		



HeLa Cell Line (Cervical Cancer Cell line)

Compound 4:

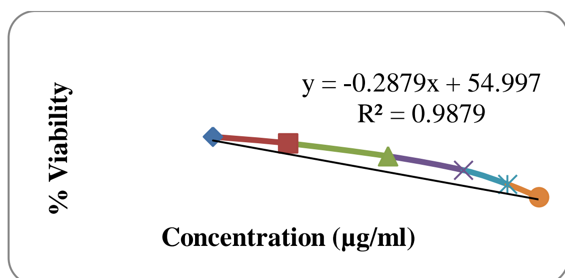
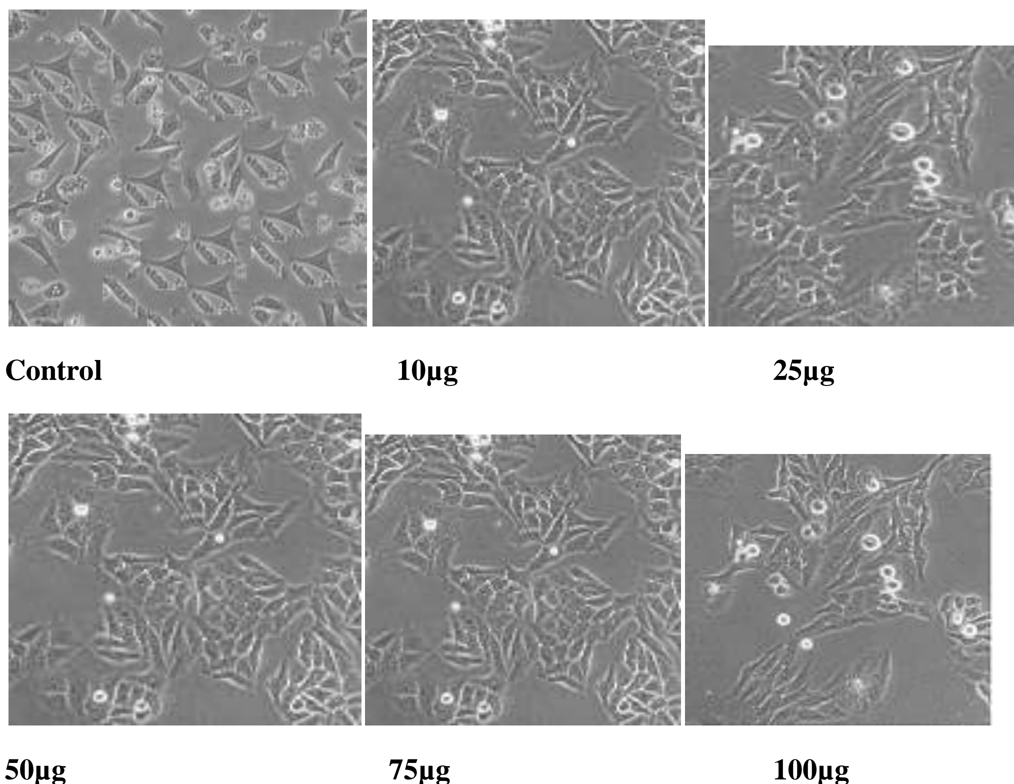


Figure 14: Cytotoxic Effect of*Compound 4*on HeLaCell Line

Table 8: Cytotoxicity Properties of*Compound 4*against HeLa Cell Line

Concentration(µg/ml)	Absorbance (570nm)			Average	Average-Blank	% Viability	IC_{50} (µg/ml)
100	0.352	0.354	0.355	0.353	0.345	27.165	17.386
75	0.427	0.429	0.431	0.429	0.421	33.149	
50	0.511	0.513	0.514	0.512	0.504	39.685	
25	0.593	0.595	0.597	0.595	0.587	46.22	
10	0.67	0.671	0.673	0.671	0.663	52.204	
5	0.709	0.711	0.712	0.71	0.702	55.275	
Untreated	1.278	1.279	1.278	1.278	1.27	100	
Blank	0.008	0.009	0.008	0.008	0		



Result : A549 Cell Line (Lung Cancer Cell line)

Compound 3:

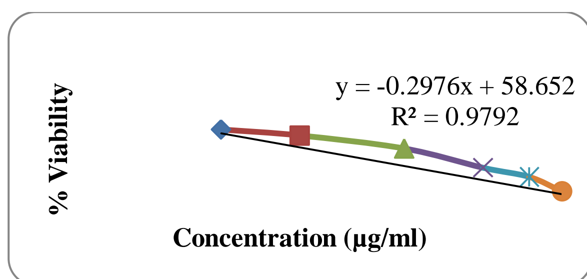
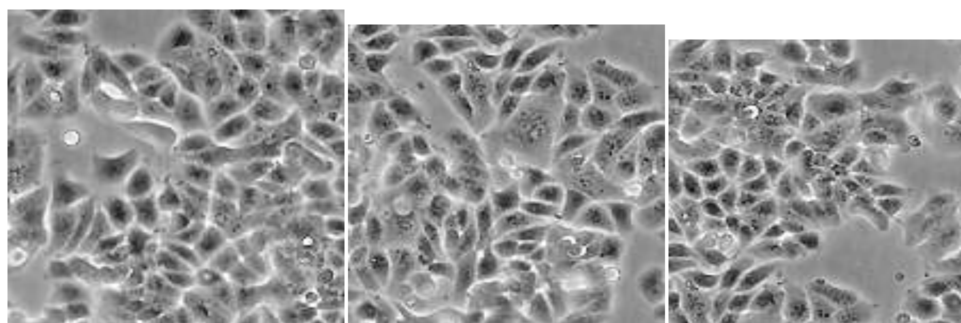


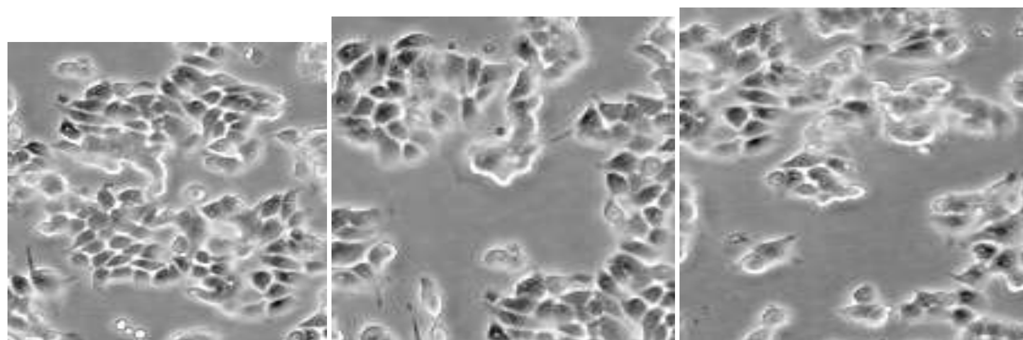
Figure 15: Cytotoxic Effect of*Compound 3* on A549 Cell Line

Table 9: Cytotoxicity Properties of *Compound 3*against A549 Cell Line

Concentration(µg/ml)	Absorbance (570nm)			Average	Average-Blank	% Viability	IC_{50} (µg/ml)
100	0.394	0.395	0.397	0.395	0.387	30.046	29.124
75	0.481	0.483	0.484	0.482	0.474	36.801	
50	0.536	0.537	0.539	0.537	0.529	41.071	
25	0.652	0.654	0.655	0.653	0.645	50.077	
10	0.729	0.731	0.733	0.731	0.723	56.133	
5	0.766	0.768	0.769	0.767	0.759	58.928	
Untreated	1.295	1.296	1.295	1.295	1.288	100	
Blank	0.007	0.008	0.007	0.007	0		



Control **10µg** **25µg**



50µg **75µg** **100µg**

A549 Cell Line (Lung Cancer Cell line)

Compound 4:

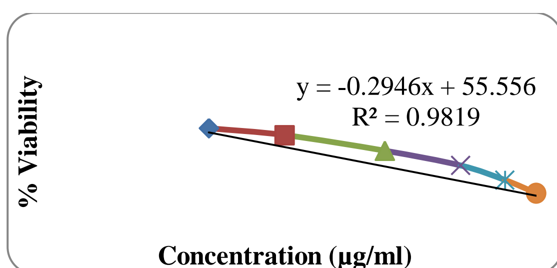
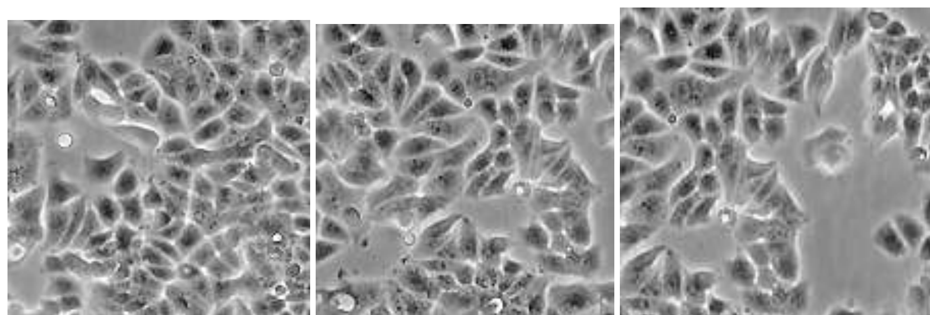


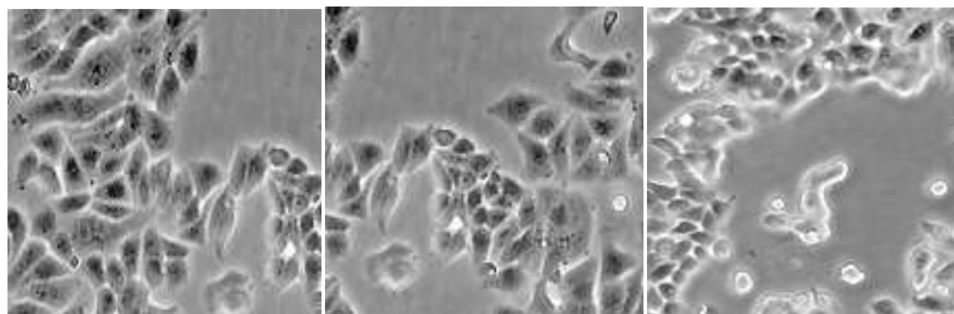
Figure 16: Cytotoxic Effect of *Compound 4* on A549Cell Line

Table 10: Cytotoxicity Properties of *Compound 4* against A549 Cell Line

Concentration(µg/ml)	Absorbance (570nm)			Average	Average-Blank	% Viability	IC_{50} (µg/ml)
100	0.357	0.359	0.361	0.359	0.352	27.329	18.877
75	0.433	0.435	0.436	0.434	0.427	33.152	
50	0.516	0.517	0.519	0.517	0.51	39.596	
25	0.599	0.601	0.603	0.601	0.594	46.118	
10	0.689	0.691	0.692	0.69	0.683	53.028	
5	0.728	0.73	0.731	0.729	0.722	56.055	
Untreated	1.295	1.296	1.295	1.295	1.288	100	
Blank	0.007	0.008	0.007	0.007	0		



Control **10µg** **25µg**



50µg **75µg** **100µg**

Result : MCF-7 Cell Line (Breast Cancer Cell line)

Compound 3:

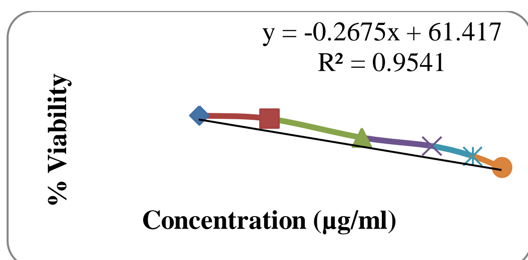
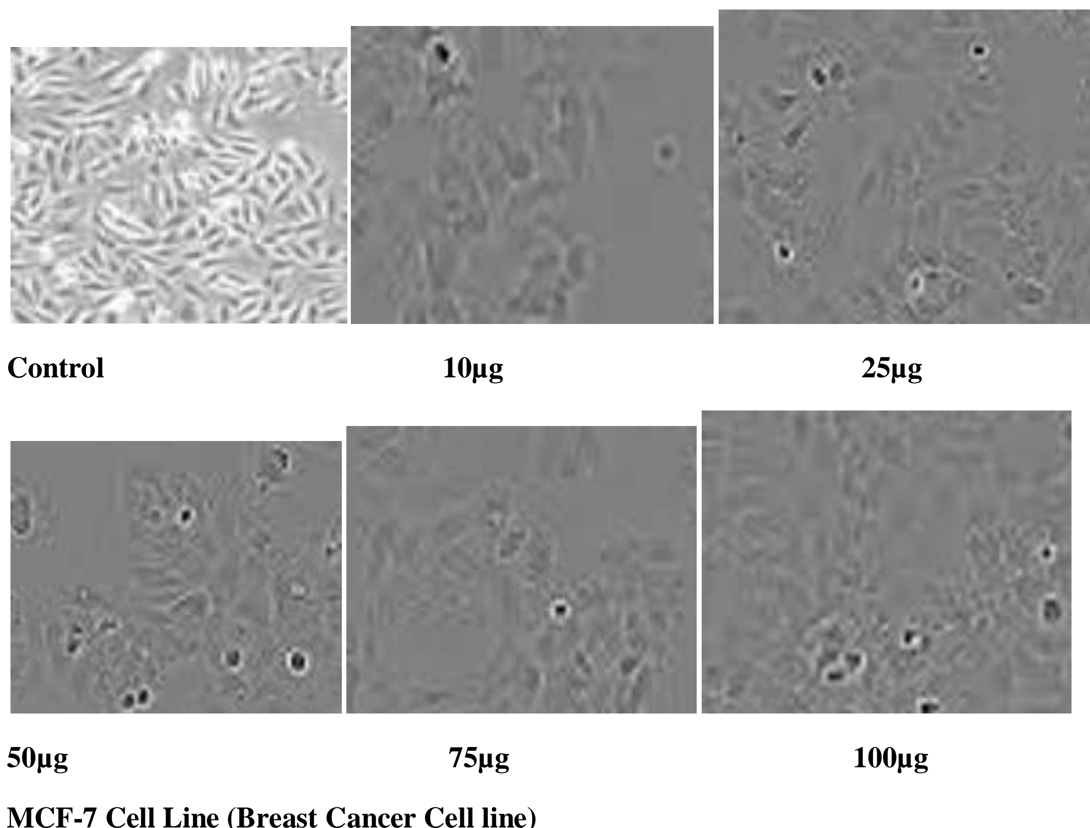


Figure 17: Cytotoxic Effect ofCompound 3 on MCF-7 Cell Line

Table 11: Cytotoxicity Properties of*Compound 3*against MCF-7 Cell Line

Concentration(µg/ml)	Absorbance (570nm)			Average	Average-Blank	% Viability	IC_{50} (µg/ml)
100	0.442	0.443	0.446	0.443	0.437	35.849	42.734
75	0.511	0.513	0.515	0.513	0.507	41.591	
50	0.573	0.575	0.577	0.575	0.569	46.677	
25	0.625	0.627	0.629	0.627	0.621	50.943	
10	0.742	0.744	0.745	0.743	0.737	60.459	
5	0.762	0.763	0.765	0.763	0.757	62.1	
Untreated	1.225	1.226	1.225	1.225	1.219	100	
Blank	0.006	0.007	0.006	0.006	0		



MCF-7 Cell Line (Breast Cancer Cell line)

Compound 4:

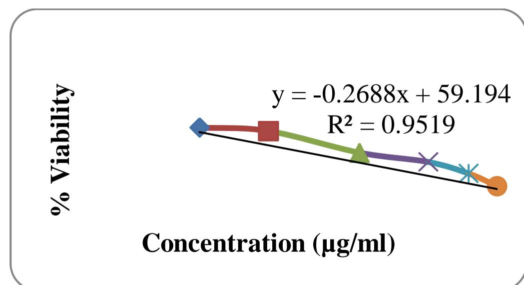


Figure 18: Cytotoxic Effect of*Compound 4* on MCF-7Cell Line

Table 12: Cytotoxicity Properties of*Compound 4*against MCF-7 Cell Line

Concentration(µg/ml)	**Absorbance (570nm)**			**Average**	**Average-Blank**	**% Viability**	**IC_{50} (µg/ml)**
100	0.413	0.415	0.417	0.415	0.409	33.552	34.291
75	0.483	0.485	0.486	0.484	0.478	39.212	
50	0.545	0.547	0.549	0.547	0.541	44.38	
25	0.596	0.598	0.6	0.598	0.592	48.564	
10	0.715	0.717	0.719	0.717	0.711	58.326	
5	0.735	0.737	0.738	0.736	0.73	59.885	
Untreated	1.225	1.226	1.225	1.225	1.219	100	
Blank	0.006	0.007	0.006	0.006	0		

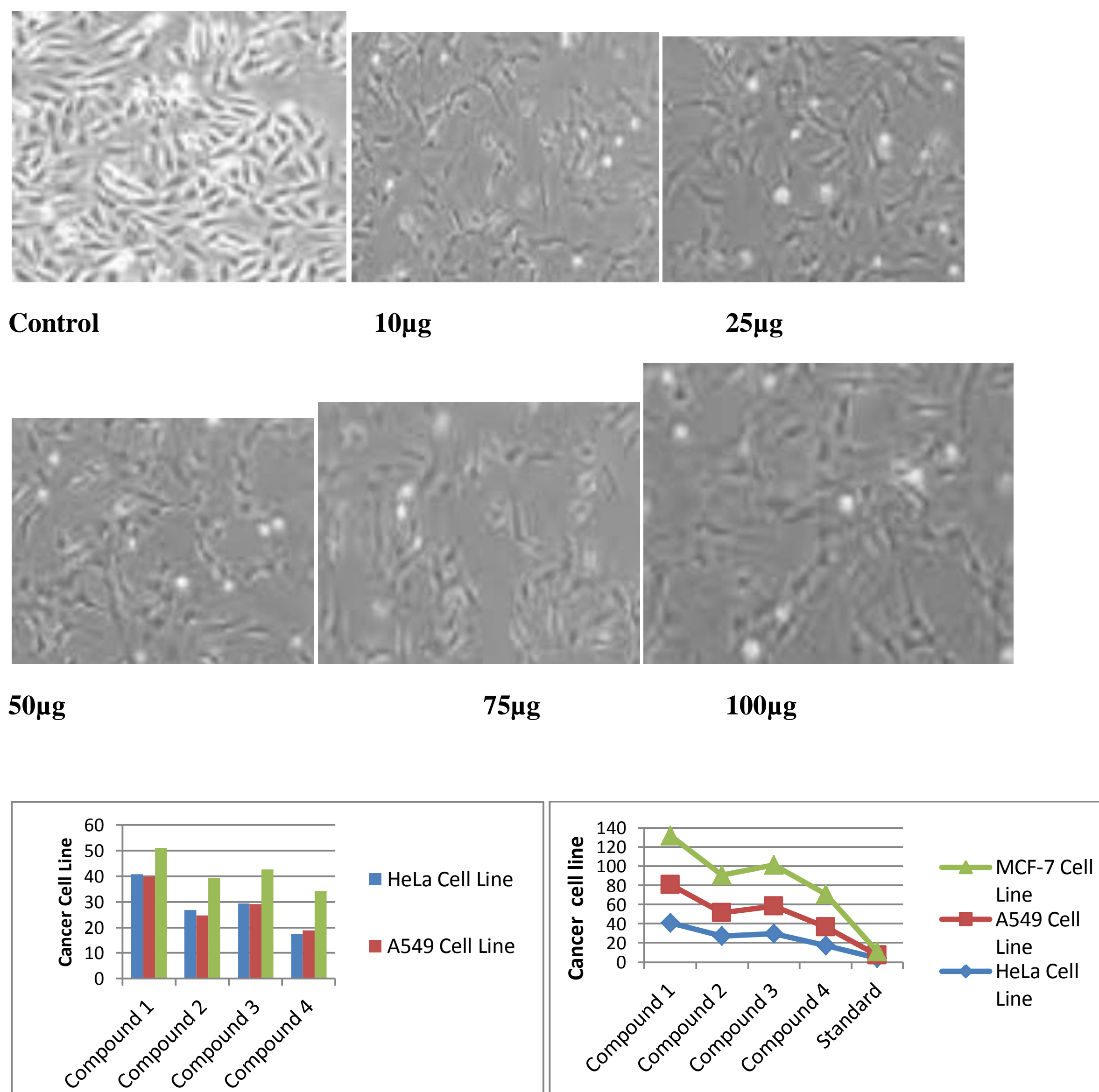


Fig 19 : Comparison of IC_{50} values of all Pd compounds with Standard drug

Conclusion

A lot of potential research has been carried out in the therapeutic application of metal-based nano-complexes as growing demand in cancer therapy. The compounds which are synthesized recently have exhibited great cytotoxic effects in *invitro*studies are examined closily. Some of the compounds exhibit good activity towards cancerous cells.The biological route for Synthesizing nanoparticles offers a clean, nontoxic and environment-friendly method of synthesizing nanoparticles with a wide range of sizes, shapes, compositions, and physicochemical properties. In comparison with microorganisms, the plant approach is more advantageous to convert micro to nano, since it does not need any special complex, and multi-step Materials procedures such as isolation, culture preparation, and culture maintenance. In addition, synthesis through plants or plant parts tends to be faster than microorganisms, is more cost-effective and relatively easy to scale up for the production of large quantities of nanoparticles. This development put to rest the fear of toxicity associated with many organometallics since the drugs are delivered to the cancer cells in nano- fraction leaving behind healthy cells unharmed.

After scrutinizing the results, all compounds are showing high activity on all the tested three cancer cell lines. Out of which greater response is exhibited by addition compounds over substitution compounds predominantly on Hela cancer cell line.

References:

1. J.H. Schiller, D. Harrington, C.P.Belani, C. Langer, A. Sandler, J. Krook, J. Zhu and D.H. Johnson, *N. Engl. J. Med*.,346, 92–98, 2002.
2. M.Cao , M.Seike , C.Soeno , H.Mizutani , K.Kitamura , Y.Minegishi , R.Noro,A.Yoshimura, L.Cai and A.Gemma, *Int. J. Oncol*., 4, 869–875,2012.
3. A. Bindoli , M. Pia, G. Scutari, C. Gabbiani, A. Casini and L. Messori, *Coord. Chem. Rev*., 253,1692–1707, 2009.
4. C. Buzea, I.I. Pacheco and K. Robbie, *Biointerphases,* **2** (4), 17–71, 2007.
5. U. Ndagi, N. Mhlongo and M.E. Soliman, *Drug. Des.Devel.Ther.***,** 11, 599-616, 2017.
6. *D.* Chen ,V. Milacic , M. Frezza and Q.P. Dou,*Curr. Pharm. Des.*,15,777–91, 2009.
7. I. Kostova, *Curr. Med. Chem.,* 13(9), 1085–1107, 2006.
8. U. Jungwirth, C.R. Kowol, B.K. Keppler, C.G. Hartinger, W. Berger and P. Heffeter, *Antioxid. Redox Signal*,15(4),1085–1127, 2011.
9. M.A.Jakupec, M. Galanski and B.K.Keppler,*Rev. Physiol. Biochem. Pharmacol.*,146, 1–53 2003.
10. R.A. Saito,T.Watabe, K. Horiguchi, T. Kohyama, M. Saitoh,T. Nagase and K.Miyazono *Cancer Res.*, 69, 2783–2791, 2009.
11. V. Mourino, J.P. Cattalini and A.R. Boccaccini.*J. R. Soc. Interface.*,9(68), 401–419, 2012.
12. A. Bhargava and U.N.Vaishampayan,*Expert Opin. Investig. Drugs*, 8(11),1787–1797, 2009.
13. F. Mafune, J.Y. Kohno, Y.Takeda,T. Kondow, *J. Phys. Chem.*, 106(31), 7575-7577, 2002.
14. A. Soltermann,V. Tischler,S. Arbogast, J. Braun, N. Probst-Hensch,W.Weder, H. Moch and G. Kristiansen,*Clin. Cancer Res.*, 14,7430–7437, 2008.
15. N.Kaushik,M.S. Thakkar,S.Snehit,M.S.Mhatre,Y.Rasesh and M.S. Parikh, *Nanomedicines*,6, 257-262, 2010.
16. M.K. Biyala, K. Sharma, M. Swami, N. Fahmi, and R.V. Singh, *Transition Metal Chemistry,* 33(3), 377–381, 2008.
17. A. Ahmad, P. Mukherjee, S. Senapati, D. Mandal, M.I. Khan, R. Kumar and M.Sastry,*Colloids Surf.*, 28, 313– 318,2003.
18. M. Darroudi, M.B. Ahmad, R. Zamiri, A.K. Zak, A.H. Abdullah, and N.A. Ibrahim, *International Journal of Nanomedicine,*6(1), 677–681, 2011.
19. V.Vidhu,K.Aromal, and D.Philip, SpectrochimicaActa Part A: Molecular and *Biomolecular Spectroscopy,* 83(1), 392-397,2011.
20. M. Akhtaruzzaman, N.H.M. Rubel Mozumder, R. Jamal, A. Rahman and T. Rahman, *Agric. Sci. Res. J.,* 2(8), 434-440, 2012.
21. G.B. Khomutov and S.P. Gubin, *Materials Science and Engineering* , 22(2), 141–146, 2002.
22. I.Kostova, *Curr. Med. Chem*.,13(9), 1085–1107, 2006.
23. R. Gupta, Q.K. Beg and P. Lorenz, *Appl. Microbiol. and Biotechnology*, 59(1), 15-32, 2002.
24. M. Arredondo and M.T. Núñez, *Mol. Aspects Med.*, 26(4–5), 313–327, 2005.
25. R. Gupta, Q.K. Beg and P. Lorenz, *Appl. Microbiol. and Biotechnology*, 59(1), 15-32,2002.

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