CYTOTOXICITY OF PHENYL PYRIMIDINE NANO PARTICLES ON MCF-7 CANCER CELL LINES

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Abstract

Evaluation of anticancer activity of some newly synthesized phenyl pyrimidine compounds, prepared by Biginelli condensation and converted to nano compounds. The structural characterization was confirmed by the multiple techniques, such as, analytical, spectral (UV, FTIR, NMR etc), XRD, microscopy and light scattering i.e. SEM, in order to show the presence of nanoparticles and their size and shape. The compounds and their nano particles are tested on MCF-7 breast cancer c ell lines to demonstrate their cytotoxicity by cell viability tests.

Key words: Biginelli compounds, nano particles, SEM, Cancer cell cycle and cytotoxicity.

INTRODUCTION

Nano scale materials are defined as a set of substances where at least one dimension is less than approximately 100 nanometers. A nanometer is one millionth of a millimeter - approximately 100,000 times smaller than the diameter of a human hair. . A nanometer (nm) is one billionth of a meter, or 10 to the power of (-9) meter. One nanometer is approximately the length equivalent to 10 hydrogen or 5 silicon atoms aligned in a line. Nano materials are of interest because at this scale unique optical, magnetic, electrical, and other properties emerge. These emergent properties have the potential for great impacts in electronics, medicine, and other fields.

Antioxidants protect cells against cell damage causing molecules known as free radicals. The development of nanoparticles used as therapeutic agents has introduced new opportunities for the improvement of medical treatment. Oxidation of biomolecules for the regulation of oxidative chain reaction and plant plays an effective role in nanoparticle synthesis as they are free from toxic chemicals as well as provide natural capping agents.

Antifungal properties of Zno nanoparticles, synthesized with and without the use of surfactants, under different reaction conditions, on strains of *Fusarium* sp. The antifungal activity of these NPs was compared with the fungus subjected to traditional antifungal treatment using copper sulphate. The results indicated that the antifungal activity of the Zno NPs depended on the concentration and size of the NP. The latter characteristic was determined by the different reaction conditions used during their synthesis.

The nanotechnology-based drug-delivery system (NDDS) targeted specifically towards cancer cells has several advantages over conventional therapies, such as longer shelf life, improvement in bio-distribution of cancer drugs, and administration of both hydrophilic and hydrophobic substances, through oral, nasal, parenteral, and intraocular routes.[1]

Nanotechnology is an interdisciplinary research field developed with an amalgamation of chemistry, engineering, biology, and medicine, and has various useful applications in cancer biology, such as early detection of tumors, discovery of cancer biomarkers, and development of novel treatments.

Anticancer drugs, such as paclitaxel, doxorubicin, 5-fluorouracil, and dexamethasone, have been successfully formulated using nanomaterials. [2]

NP probes, Nano cantilever, nanowire, and nanotube arrays are expected to solve the problem of early detection of different types of cancer. [3]

Nano cantilevers, nanowires, and nanotubes also have many applications in biology and medicine

because of their unique structures and properties. Use of these devices facilitates the transition from single-biomarker to multiple-biomarker cancer diagnostics, prognostics, and treatment.

Pyrimidine nucleus exhibited remarkable pharmacological activities. Pyrimidine derivatives form a component in a number of useful drugs and are associated with many biological and therapeutical activities. Nitrogen containing heterocyclic ring such as pyrimidine is a promising structural moiety for drug design. Condensed pyrimidine derivatives have been reported as anti-microbial[4], analgesic, anti-viral, anti-inflammatory[5], anti-HIV[6], anti-tubercular[7], anti-tumour[8], anti-neoplastic[9], anti-malarial[10], diuretic[11], cardiovascular[12] agents. Pyrimidine compounds are also used as hypnotic drugs for the nervous system.

In medicinal chemistry pyrimidine derivatives have been very well known for their therapeutic applications. The presence of a pyrimidine base in thymine, cytosine and uracil, which are the essential binding blocks of nucleic acids, DNA and RNA is one possible reason for their activity. The literature indicated that the compounds having pyrimidine nucleus possess broad range of biological activities.

A pyrimidine has many properties in common with pyridine, as the number of nitrogen atoms in the ring increases the ring pi electrons become less energetic and electrophilic aromatic substitution gets more difficult while nucleophilic aromatic substitution gets easier.

In 1893, Pietro Biginelli reported the first synthesis of 3,4-dihydroprimidin-2(1H)ones (DHPM) by a very simple one-pot condensation reaction of an aromatic aldehyde, urea and ethyl acetoacetate in ethanolic solution. This efficient approach to partly reduced pyrimidines, termed the Biginelli reaction or condensation, was largely ignored in the following years, and therefore, also the synthetic potential of these multi-functionalized dihydropyrimidines remained unexplored. In recent years, however, interest in these compounds has increased rapidly, and the scope of the original cyclocondensation reaction has been widely extended by variation of all three components.

Pyrimidinones or Dihydropyrimidinones (DHPMs) are well known for their wide range of bioactivities and their applications in the field of drug research have stimulated the invention of a wide range of synthetic methods for their preparation and chemical transformations. Out of the five major bases in Nucleic acids three are pyrimidine derivatives which comprises of Cytosine [13] which is found in DNA and RNA, Uracil [14] in RNA and Thymine [15] in DNA. Because of their involvement as bases in DNA and RNA, they have become very important in the world of synthetic organic chemistry. Aryl-substituted 3, 4-dihydropyrimidin-2(1H)-one and their derivatives are an important class of substances in organic and medicinal chemistry.

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. These contrast with benign tumors, which do not spread to other parts of the body. Possible signs and symptoms include a lump, abnormal bleeding, prolonged cough, unexplained weight loss, and a change in bowel movements. While these symptoms may indicate cancer, they may have other causes. [16]

Tobacco use is the cause of about 22% of cancer deaths. Another 10% are due to obesity, poor diet, lack of physical activity, and excessive drinking of alcohol. Other factors include certain infections, exposure to ionizing radiation and environmental pollutants. [17]

In the developing world nearly 20% of cancers are due to infections such as hepatitis B, hepatitis C and human papillomavirus infection. [18] These factors act, at least partly, by changing the genes of a cell. Typically many genetic changes are required before cancer develops. Approximately 5–10% of cancers are due to inherited genetic defects from a person's parents. Cancer can be detected by certain signs and symptoms or screening tests. It is then typically further investigated by medical imaging and confirmed by biopsy.

Many cancers can be prevented by not smoking, maintaining a healthy weight, not drinking too much alcohol, eating plenty of vegetables, fruits and whole grains, vaccination against certain infectious diseases, not eating too much processed and red meat, and avoiding too much sunlight exposure. Early detection through screening is useful for cervical and colorectal cancer. The benefits of screening in breast cancer are controversial. Cancer is often treated with some combination of radiation therapy, surgery, chemotherapy, and targeted therapy. [19]

PROCEDURE

i.Crushed using china dish

The Pyrimidine complexes are grinded in a china dish thoroughly.

ii. Sonication

Complexes are sonicated is for evenly dispersing nanoparticles in liquids.

After grinding, the pyrimidine complexes are dissolved in a methanol solvent and sonicated for about 20 minutes in a sonicator. This process is repeated for 2 times.

Characterization

IR Study

Fourier transforms infrared spectroscopy (FTIR) spectroscopic study of the sample was performed by Shimadzu, Japan, using KBr as a reference. 0.25 g dried nano particle sample was mixed with KBr (sample/KBr ratio was 1/100) and were pressed into the transparent thin pellet.

A FTIR spectrum of carbon materials was obtained in the range on 3242 to 781 cm^{-1}.

The FTIR spectrum of prepared dried nano particles indicates the presences of some functional groups of hydrocarbon, nitrogen and oxygen. In the spectrum, peak at 3242cm^{-1} (m) is for N-H stretch, peak at 1722 cm^{-1} is for C=O stretch, peaks at 1645&1600 cm^{-1} is for C=C stretch in the alkene or in aromatic compounds. A very weak peak at 1630 is for C=C aromatic stretch. Peaks at 1462, 1388 and 2980 cm^{-1} are for the C-H bend in CH_3 &CH_2 , peak at 1091 cm^{-1} is for C-O-C of ethyl and pyrimidine ringand the peak at781 is for C-Cl stretch.

UV-Visible:

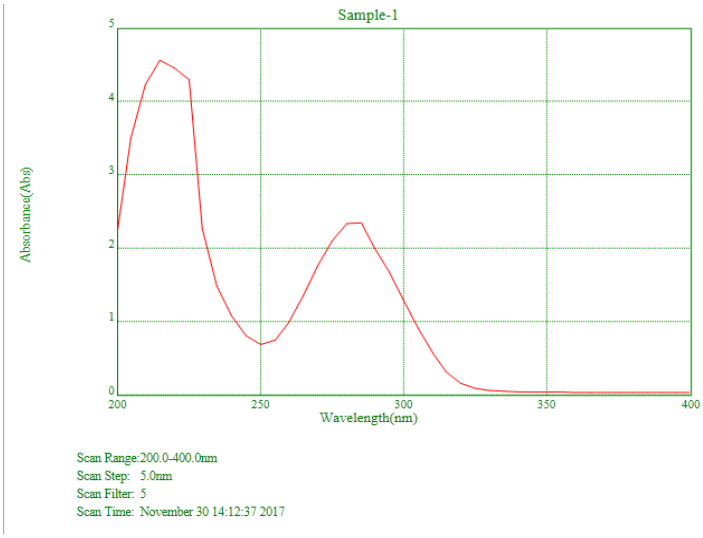
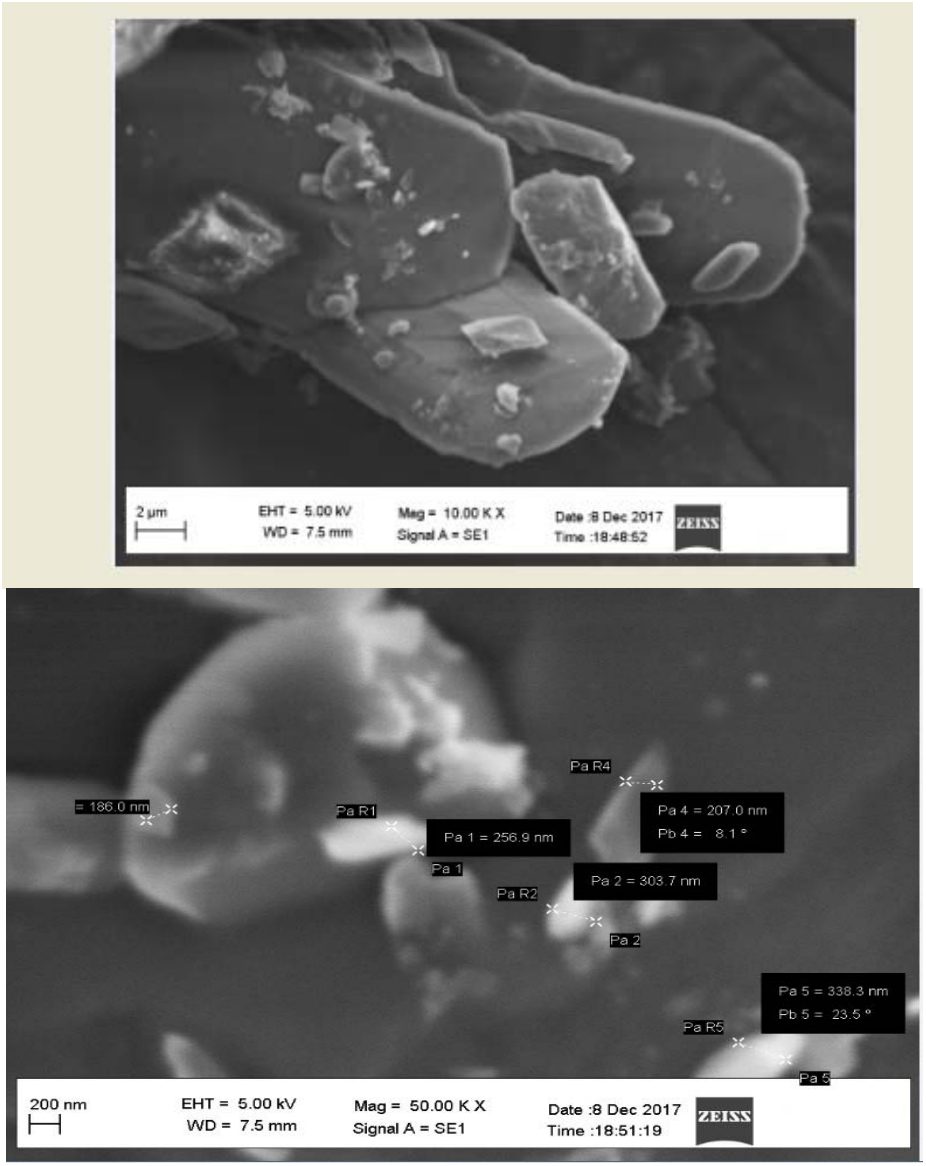


Fig 7. UV-Visible absorption spectra of Compound 2

Fig 7 shows the UV-Visible absorption spectra of B10 compound shows a band due to the >C=N chromophore in the spectrum of the compound at 285 nm shifts to a higher

wavelength. Such a shift in n- π * transition band is probably due to the donation of a lone pair of electrons by the nitrogen. Further, two bands at 260 nm and 305 nm are due to π- π * transitions, these are assigned to the benzenoid ring and (NH) band of the ( ) group respectively. The K band π- π * showed a red shift due to the increase in conjugation and the B-band undergoes a hypsochromic shift.

SEM



The nano particles are formed as shown by the scanning electron microscopy, SEM (*FEI Quanta 200 Hv model*) images of Phenyl pyrimidine complexes in the range between 180-300nm.

Result and Discussion

ANTICANCER ACTIVITY OF COMPOUNDS

1. INTRODUCTION:

Measurement of cell viability and proliferation forms the basis for numerous in vitro assays of a cell population's response to external factors. The MTT Cell Proliferation Assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability.

1.1 Aim:

To determine the Anti-cancer activity of test compounds in vitro by MTT Assay.

1.2 MATERIALS AND METHODS:

DMEM, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], trypsin, EDTA Phosphate Buffered Saline (PBS) and were purchased from Sigma Chemicals Co.

(St. Louis, MO) and Fetal Bovine Serum (FBS) were purchased from Gibco. 25 cm ^{2} and 75 cm^2 flask and 96 well plated purchased from eppendorf India.

1.2.1 Maintenance of Cell Line:

The MCF-7 Breast cancer cell line were purchased from NCCS, Pune and the cells were maintained in RPMI – 1640 medium supplemented with 10 % FBS and the antibiotics penicillin/streptomycin (0.5 mL^{-1}), in atmosphere of 5% CO_2 /95% air at 37 ^{0} C.

1.2.2 Preparation of Test Compound:

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.

1.3 MCF – 7 CELL VIABILITY BY MTT ASSAY:

1.3.1 Principle:

MTT Assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The assay depends both on the number of cells present and on the assumption that dead cells or their products do not reduce tetrazolium. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, dark purple colored formazan crystals. The cells are then solubilized with a DMSO and the released, solubilized formazan reagent is measured spectrophotometrically at 570 nm.

1.3.2 Procedure:

Cell viability was evaluated by the MTT Assay with three independent experiments with six concentrations of compounds in triplicates. MCF-7 cells were trypsinized and preform 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, take off the old media and add fresh media 100 µl with different concentrations of test compound in represntive wells in 96 plates. After 48 hrs., Discard the drug solution and add the fresh medic 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, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50 % values is generated from the dose-response curves for each cell line using with origin software.

$$\% \text{ Inhibition} = \frac{100\ (\text{Control} - \text{Treatment})}{\text{Control}}$$

Results:

1. **Compound 1:**

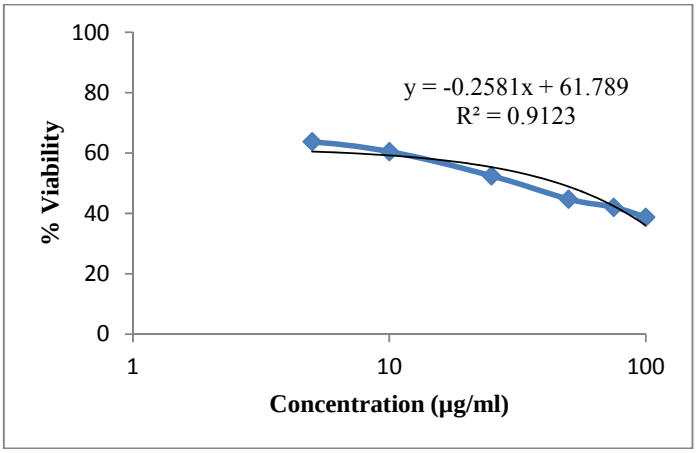


Figure 1: Cytotoxic Effect of the compound (1) on MCF-7 Cell Line

Concentration(µg/ml)	Absorbance at 570nm			Average	Average-Blank	% Viability	IC_{50} (µg/ml)
100	0.695	0.697	0.699	0.697	0.695	38.761	45.658
75	0.754	0.756	0.758	0.756	0.754	42.052	
50	0.802	0.804	0.806	0.804	0.802	44.729	
25	0.941	0.943	0.945	0.943	0.941	52.481	
10	1.085	1.087	1.089	1.087	1.085	60.513	
5	1.145	1.147	1.148	1.146	1.144	63.803	
Untreated	1.795	1.796	1.795	1.795	1.793	100	
Blank	0.002	0.003	0.002	0.002	0		

Table 1: Cytotoxic Properties of compound (1) on MCF-7 Cell Line

2. Compound 2

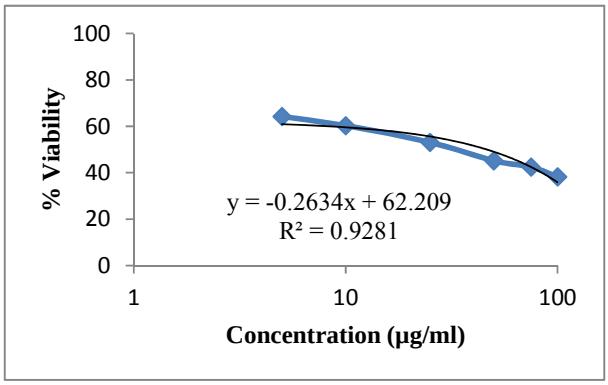


Figure 2: Cytotoxic Effect of the compound (2) on MCF-7 Cell Line

Concentration(µg/ml)	Absorbance at 570nm			Average	Average-Blank	% Viability	IC_{50} (µg/ml)
100	0.685	0.687	0.689	0.687	0.685	38.204	46.387
75	0.761	0.763	0.765	0.763	0.761	42.442	
50	0.81	0.812	0.814	0.812	0.81	45.175	
25	0.952	0.954	0.956	0.954	0.952	53.095	
10	1.081	1.083	1.085	1.083	1.081	60.29	
5	1.152	1.154	1.156	1.154	1.152	64.249	
Untreated	1.795	1.796	1.795	1.795	1.793	100	
Blank	0.002	0.003	0.002	0.002	0		

Table 2: Cytotoxic Properties of compound (2) on MCF-7 Cell Line

Invitro Cytotoxicity

S. NO	SAMPLE NAME	MCF 7
1	Compound 1	45.65
2	Compound 2	46.38
3	Doxorubicin	4.35

Conclusion

Evaluation of antifungal and anti bacterial activities, of phenyl pyrimidine complexes exhibit significant cytotoxicity. The complexes were further tested against MCF-7 breast cancer cell line as no information is available in literature on the antimicrobial and cytotoxicity of the reported bioactive compounds. Both the complexes have been proved potent against cytotoxicity as percent inhibition is 65 and 68 revealed by the graph and the table.

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