

The Biological Evaluations of Chemically Synthesized HETEROLEPTIC and HOMOLEPTIC Compounds can be a Good Source of Drug has been Examined by these Antibacterial, Antifungal, Antidiabetic and Anticancer Activity

Atrejee Majumder

M.Tech. Scholar, Department of Genetic Engineering,
School of Bioengineering, SRM Institute of Science and
Technology, Chennai, TN, India.

Dr. S. SHOBANA

Assistant Professor, Department of Genetic Engineering,
School of Bioengineering, SRM Institute of Science and
Technology, Chennai, TN, India

Abstract— A series of Heteroleptic metal (II) complexes of formulation $[M(Tm)(di-imine)](ClO_4)$, $[Tm = \text{hydrotris(methimazolyl)borate, di-imine} = 2,2'-bipyridyl \text{ or } 1,10\text{-phenanthroline and } M = \text{Mn (II), Ni (II), Cu (II) or Zn (II)}]$ have been synthesized and characterized by spectroscopic methods. Antimicrobial activity of the synthesized Heteroleptic complexes were evaluated against Gram (–ve) (*Escherichia coli.*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and Gram (+ve) (*Bacillus subtilis.*) bacterial, and three Fungal (*Candida sp.*, *Penicillium sp.*, *Aspergillus sp.*) strains with respect to the standard drugs Tetracycline and Anti-diabetic activity with respect to the standard drugs Acarbose. The cytotoxic activity of copper (II) complexes 1 and 3 against “MDA-MB-231” adenocarcinoma cell line was assessed by MTT assay, which showed exponential responses toward increasing concentration of complexes. A series of Homoleptic bis(terpyridine)copper (II) complexes of the type $[Cu(L1-5)_2]Cl_2$ (1-5), where L1-5 = 4'-(4-substituted)-2,2':6',2''-terpyridines have been synthesized and characterized. All the complexes bring about hydroxyl radical mediated DNA cleavage in the presence of H_2O_2 . In vitro cytotoxicity of the complexes (1-3) was tested against three cancerous cells such as human breast adenocarcinoma (MDA-MB-231) cell line by MTT reduction assay. DNA binding and cleavage are two critical events for gene mutation and carcinogenesis in biological systems. DNA targeting to the small ligands of the development play important roles in life process by serving as essential cofactors, fulfilling cellular functions that cannot met by organic molecules that frequently incorporated into pharmaceuticals for diagnostic or therapeutic purposes. Studies to complement the anticancer drugs the copper (II) complexes are considered the best alternatives to cisplatin because of their bio-friendly nature as copper plays a significant role in biological systems. Heteroleptic copper (II) complexes with Terpyridine and Homoleptic complex drug we have been interested in exploring the theoretical and biological properties of copper (II) complexes.

Keywords—Heteroleptic, Homoleptic compound, Zone of Inhibition, Sodium Phosphate buffer, MDA-MB-231 MDA, Tetracycline, Acarbose.

I. INTRODUCTION

(i) A series of “Nanoparticles” called Heteroleptic metal (II) complexes of formulation $[M(Tm)(di-imine)](ClO_4)$ (1-8), $[Tm = \text{hydrotris(methimazolyl)borate, diamine} = \text{bipyridyl or } 1,10\text{ phenanthroline and } M = \text{Mn (II), Ni (II), Cu (II) or Zn (II)}]$ was first reported by Reglinski [1]. The Heteroleptic transition metal (II) complexes containing N, O donor bioactive ligands have received much attention in analytical, industrial, biological and therapeutic applications. Bipyridyl and 1,10 phenanthroline cost, non-toxicity, favorable absorption and excited state properties [2]. Further, they have played vital role in the development of coordination chemistry and wide range of biological activates play important role in DNA binding affinity. Heteroleptic metal (II) complexes derived from hydrotris(methimazolyl) borate ligand and diamines as ancillary ligands together with their spectral characterization by employing spectroscopic techniques.[3] These complexes show different molecular geometry, DNA structural selectivity and DNA binding affinity. Among the co ligands, Heteroleptic complexes containing the strong field classic chelating N donor bidentate ligands such as 1,10 phenanthroline where The Heteroleptic metal (II) complexes containing N, O donor bioactive ligands have received much attention in analytical, medical biological and therapeutic applications.[4] (ii) Homoleptic bis (terpyridine)copper (II) complexes of the type $[Cu(L1-5)_2]Cl_2$ (1-5), where L1-5 = 4'-(4-2,2':6',2''-terpyridines “Nanoparticles” have been DNA binding and cleavage are two critical events for gene mutation and carcinogenesis in biological systems.[1] The combined effects between the metal and the ligands could result in enhancement of biological activities or activate new action mechanisms. In the new chemotherapeutic agents, inorganic complexes that function by intercalation of their aromatic ligands into the base pair of DNA which are of intense interest. Many properties of metal complexes such as size, oxidation state, geometry and chirality that could influence the binding mode to DNA for

diagnostic or therapeutic purposes.^[2] The bis(terpyridine) complexes bind strongly to DNA and exhibit prominent anticancer activities and others. Methods electrophoresis techniques have been used to probe the DNA interaction of the synthesized copper (II) complexes compounds. It has been demonstrated that copper accumulates in tumors due to the selective permeability of cancer cell membranes because their anti-proliferative activities through ligand exchange and inhibit the proliferation of tumor cells. The terpyridine complexes are well documented for their high DNA affinity through intercalation, DNA nuclease activity and cytotoxicity.^[3] The variety of copper complexes and several copper (II) terpyridine complexes have shown considerable nuclease and antitumor activity in physiologically relevant conditions. The bis(terpyridine) complexes bind strongly to DNA and exhibit prominent anticancer activities by inducing cell death. These compounds play a major role in biological and therapeutic applications.^[4]

II. OBJECTIVE

The Anti-bacterial activity was studied against *Bacillus sp.*, *E. coli*, *P. aeruginosa*, *Klebsiella sp.* on Muller Hinton agar medium. The Anti-fungal activity was studied against *Aspergillus sp.*, *Candida sp.* and *Penicillium sp.* on SDB medium. The Anti-diabetic activity was studied by α glucosidase inhibitory assay. Anti-cancer activity and cell proliferation assay for checking the viability% and inhibition% study will be studied by MTT assay.

III. MATERIALS AND METHODS:

Anti-bacterial: The Anti-bacterial activity of the Heteroleptic and Homoleptic compound (10,20,30,40,50,60 μ g/ml) were studied against *Bacillus sp.*, *E. coli*, *P. aeruginosa*, *Klebsiella sp.* on Muller-Hinton agar First compounds(1mg/100 μ l) should be melted in DMSO (Di-Methyl Sulphoxide). Sterilized Petri plates should be taken and Muller-Hinton Agar(250ml) should pour and should be solidified This whole process should be happened in a sterile condition means, in the environment of Laminar Air Flow. In the solidified Agar the bacterial culture should be pour (25ml/plate) and spread with L-rod. Make 6 holes by the gel puncture. Compounds should be added by 10ul micro tips. Now keep those plates in normal temperatures for 5-10minites and put Para film on the plates and keep those plates in normal incubator for 24h without contamination. Next day we have to check the Zone of Inhibition (a round spot and patches can see outside the puncture). We have to measure the zones and count the inhibition against control and tetracycline.

Anti-fungal: The Anti-fungal activity of the Heteroleptic and Homoleptic compound (10,20,30,40,50,60 μ g/ml) were studied against *Aspergillus sp.*, *Candida sp.* and *Penicillium sp.* on SDB agar medium. First compounds (1mg/100 μ l) should be melted in DMSO (Di-Methyl Sulphoxide). Sterilized Petri plates should be taken and SDB Agar (250ml) should pour and should be solidified. This whole process should be happened in a sterile condition means, in the environment of Laminar Air Flow. In the solidified Agar the bacterial culture should be pour (25ml/plate) and spread with L-rod. Make 6 holes by the gel puncture. Compounds should be added by 10ul micro tips. Now keep those plates in normal temperatures for 5-10minites and put Para film on the plates and keep those plates in normal incubator for 24h without contamination. Next day we will see

the Zone of Inhibition (a round spot and patches can see outside the puncture). We have to measure the zones and count the inhibition against control and tetra cyclin.

Anti-diabetic: The anti-diabetic activity of Heteroleptic and Homoleptic Compound (10,20,30,40,50, 60 μ g/ml) were studied by α -glucosidase inhibitory assay. Add 200 μ l Sodium Phosphate buffer in a test tubes. Add 20 μ l enzyme (α amylase) in all test tubes. Add compound extract in concentration range of μ g/ml i.e. 10, 20, 30 (3-1, 3-2, 3-3) and 10, 20, 30 (1-1, 1-2, 1-3) against standard drug (Acarbose). Add Acarbose in concentration range of 30 μ g/ml. Incubate: 10 min, room temperature. Add 400 μ l DNS, Water bath (boiling water) for 5 min. Cool down, 15ml distilled water. Inhibition% at Absorbance: 540nm.

Anti-cancer: Cell Line: - MDA-MB-231: - This adenocarcinoma cell line is an epithelial, human breast cancer cell line. Media: - DMEM: - Dulbecco's Modified Eagle's medium is a modification of Basal Medium Eagle (BME) Anti-cancer activity of the Heteroleptic and Homoleptic Compound for Cell Proliferation Assay and the other compounds (1,1; 1,2; 1,3) and (3,1 ;3,2 ;3,3) will be studied by MTT assay. Remove Media from each well. Wash each well with 0.3 PBS then remove it. Add 0.7 Media, Resuspend cells in each well. Transfer the suspension to 1.5 ml Eppendorf tube. Take 10 μ l of suspension buffer, add 10 μ l of Trypan Blue, Leave for 2 minutes. Count cells using Hemocytometer. MTT Assay: (Day1): cells grown in monolayer. Adherent cells should be released from their substrate by trypsinization. Pellet the cells by centrifugation at 500 rpm for 5min at 2-8 $^{\circ}$ c. Remove the supernatant by aspiration. Centrifugation spread adjusted individual cell types. Wash cells by re-suspending by sterile PBS. Pellet cells by centrifugation at 500 rpm for 5 minutes at 2-8 $^{\circ}$ c. Carefully remove supernatant. Resuspend the cells in 1ml sterile PBS. Count and remove cells and record it. Serial dilution, PBS add and remove, incubate cells for 6-12 hrs. 37 $^{\circ}$ c. (DAY 2): Add 10 μ l of MTT to each well. Incubate in CO₂ Incubator for 2-4 hrs. Add 100 μ l of solubilizing solution each well. Leave plate covered in dark at 37 $^{\circ}$ c in CO₂ incubator for at least 2 hrs. Samples may be read after 2hrs. View cells periodically for the appearance at the time 24 hrs. and 48 hrs.

IV. REVIEW OF LITERATURE

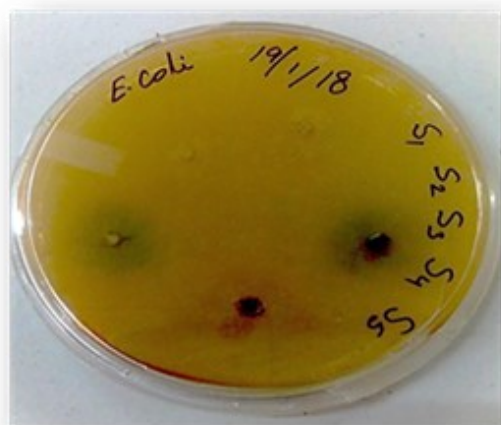
According to Allahverdiyev AM1, Kon KV, Abamor ES, Bagirova M, Rafailovich M. in the journal "Coping with Antibiotic Resistance: Combining Nanoparticles with Antibiotics and Other Antimicrobial Agents," the global escalation of bacterial resistance to conventional medical antibiotics is a serious concern in the modern medicine area of drug discovery. The high incidence of multidrug-resistant germs among bacteria-based illnesses reduces the efficiency of existing therapies, resulting in thousands of fatalities. As a result, new advances in current therapeutic approaches and creative tactics are urgently required to address this issue. In the publication "Antibiotic-loaded biomaterials and the risks of antibiotic resistance spread after prophylactic and therapeutic clinical use." Campoccia D, Montanaro L, Speziale P, Arciola CR. Biomaterials 2010, 31:6363–6377. Antibiotic-loaded biomaterials, including medicines, are now used in conventional medical procedures for local treatment and prevention of implant infections. Significant amounts of active medications are administered directly to the site of

infection, avoiding or lowering the dangers of systemic effects that are now required. According to the another journal "Systemic distribution, nuclear entry and cytotoxicity of amorphous Nano silica following topical application by Nabeshi H1, Yoshikawa T, Matsuyama K, Nakazato Y, Matsuo K, Arimori A, Isobe M, Tochigi S, Kondoh S, Hirai T, Akase T." Nanomaterials (NMs) with particle sizes less than 100 nm are now being used effectively in a variety of industrial and medical applications. NMs are difficult in terms of triggering a toxicological impact due to their tiny size. However, biological and/or cellular reactions to NMs are sometimes unpredictable and even contradictory, therefore medicines are being investigated.

V. RESULTS:

The Significance my work that the Biological Evaluations of the Chemically Synthesized HETEROLEPTIC and HOMOLEPTIC compounds is relevant because same properties and concentration of compounds have the value of the effect of antibacterial, antifungal, antidiabetic, anticancer effect that can giving a good result against their substitute Drugs that I can show in my work done, as follows-

1. Anti-bacterial activity: A.



B.
C



D.



Figure 1: Anti-bacterial activity: A) *Bacillus* sp. B) *E. coli* C) *Pseudomonas* sp. D) *Klebsiella* sp.

	ZONE OF INHIBITION (mm)			
	Gram Positive	Gram Negative		
	<i>Bacillus</i>	<i>E. coli.</i>	<i>Pseudomonas</i>	<i>Klebsiella</i>
3,1(10µg/ml)	10	11	13	12
3,2(20µg/ml)	12	13	11	10
3,3(30µg/ml)	14	13	16	15
1,1(40µg/ml)	15	15	18	16
1,2(50µg/ml)	18	23	25	24
1,3(60µg/ml)	20	25	27	26
Control	---	---	---	---
Tetracycline (30µg/ml)	25	30	34	38

Table 1: Zone of Inhibitions for Anti-bacterial activities.

Here In between Gram positive and Gram negative anti-bacterial activity the different concentration of compounds I have taken against Tetracycline (30µg/ml) where (control is 0): [3, 1 (10µg/ml) Pseudomonas has more zone of inhibition. 3, 2 (20µg/ml) E. coli has more Zone of Inhibition. 3, 3(30µg/ml)

again *Pseudomonas* has more ZOI. In 1, 1(40µg/ml) for *Pseudomonas*; 1,2 (50µg/ml) has also *Pseudomonas* and 1, 3(60µg/ml) for *Pseudomonas* has more zone of inhibition.] So, this compound mostly effects in *Pseudomonas*.

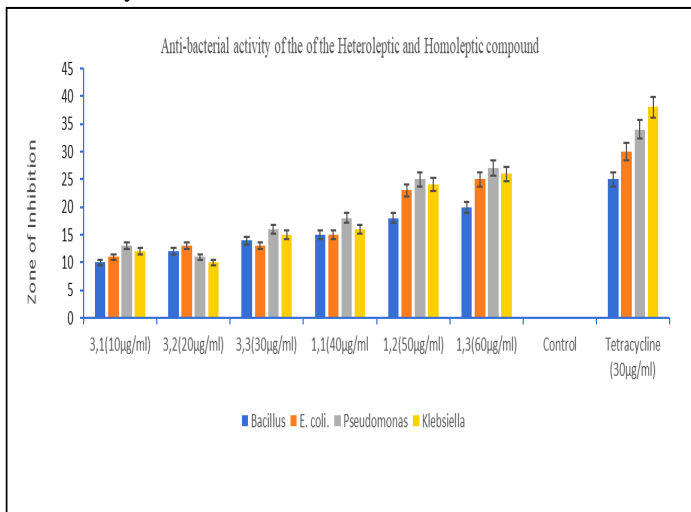
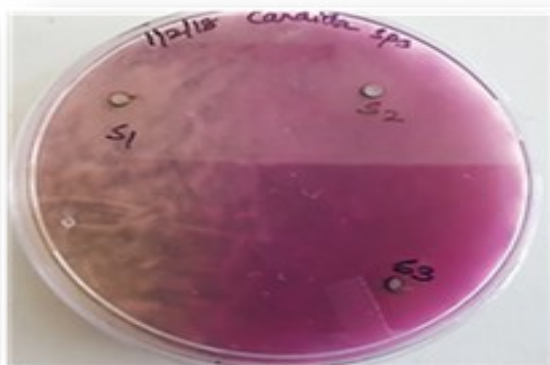
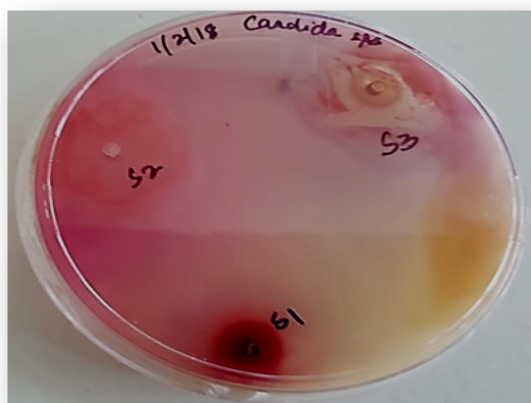


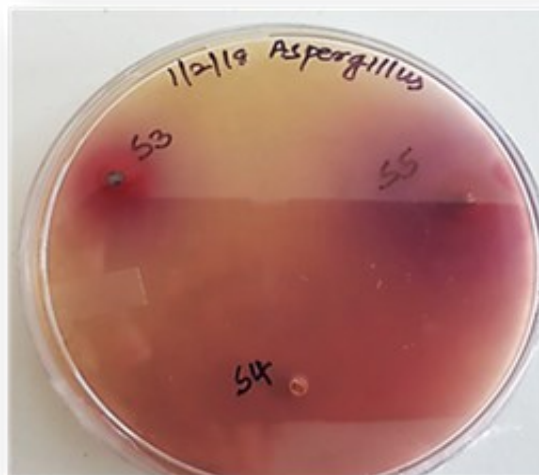
Figure A: Graphical chart view of the Zone of Inhibition for Anti-bacterial activity also shows the very good activity in *Pseudomonas*.

2. Anti-fungal activity:

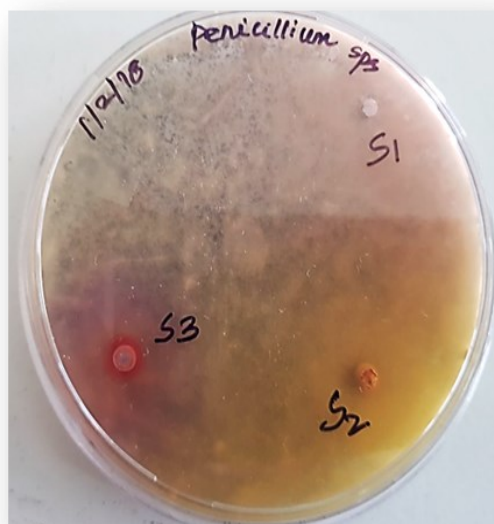
A.



B.



C.



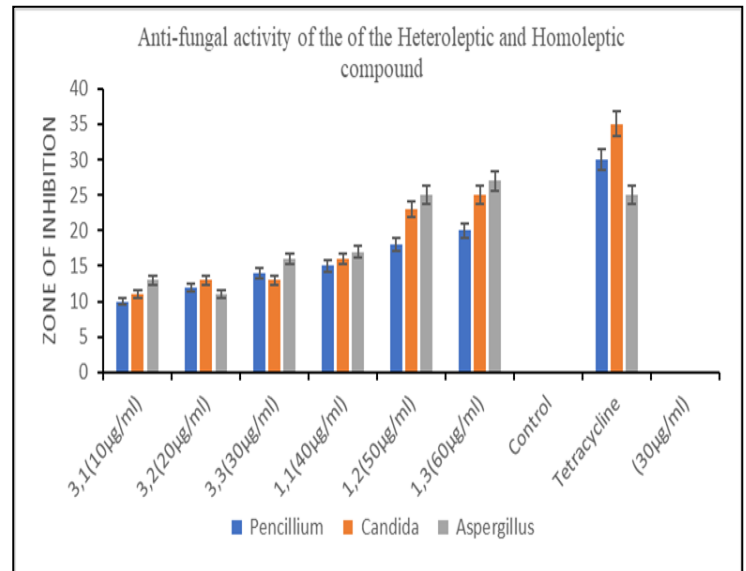
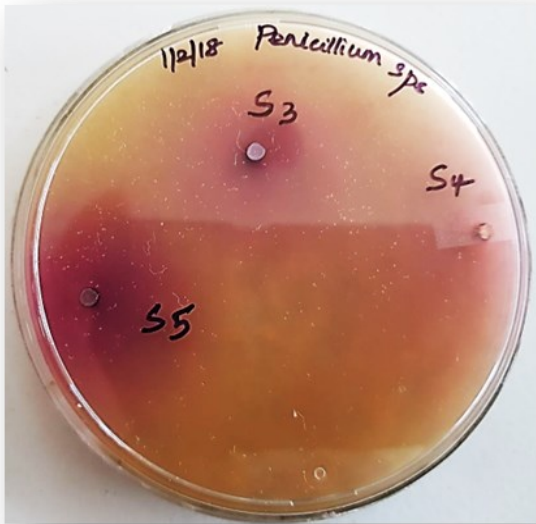


Figure 2: Anti-fungal activity: A) *Candida* sp. B) *Aspergillus* sp. C) *Penicillium* sp.

Figure B: Graphical chart view of the Zone of Inhibition for Anti-fungal activity also shows the very good activity in *Aspergillus* sp.

ZONE OF INHIBITION (mm)			
	<i>Penicillium</i>	<i>Candida</i>	<i>Aspergillus</i>
3,1(10µg/ml)	10	11	13
3,2(20µg/ml)	12	13	11
3,3(30µg/ml)	14	13	16
1,1(40µg/ml)	15	16	17
1,2(50µg/ml)	18	23	25
1,3(60µg/ml)	20	25	27
Control	-----	-----	-----
Tetracycline (30µg/ml)	30	35	25

3. Anti-diabetic activity:

Table 2: Zone of Inhibitions for anti-fungal activities



Here In between anti-fungal activity the different concentration of compounds I have taken against Tetracycline (30µg/ml) where: [3, 1 (10µg/ml) *Aspergillus* has more zone of inhibition. 3, 2 (20µg/ml) *Candida* has more Zone of Inhibition. 3, 3(30µg/ml) again *Aspergillus* has more ZOI. In 1, 1(40µg/ml) for *Aspergillus*; 1,2(50µg/ml) has also *Aspergillus* and 1, 3(60µg/ml) for *Aspergillus* has more zone of inhibition.] So, this compound mostly effects in *Aspergillus*.

Figure 3: α -glucosidase inhibitory assay

NAME OF THE COMPOUND	CONCENTRATION (µg/ml)	INHIBITION%
3,1(10µg/ml)	10	65.4±0.3
3,2(20µg/ml)	20	78.8±0.33
3,3(30µg/ml)	30	82.3±0.39
1,1(40µg/ml)	10	87±0.31
1,2(50µg/ml)	20	92±0.52
1,3(60µg/ml)	30	93±0.52
Acarbose	30	95±0.42

Table 3: The % of the inhibition in different concentrations for anti-diabetic activity. The inhibitory activity expressed as a percentage of the control sample without inhibitors and the Data are presented as the mean ± SD values of triplicate determinations.

Here 3, 1 (10µg/ml) inhibition% 65; 3,2 (20µg/ml) inhibition% 78; 3,3(30µg/ml) inhibition% 82 and 1,1(10µg/ml) inhibition%87; 1,2(20µg/ml) inhibition% 92; 1,3(30µg/ml) inhibition% 93, against Acarbose (30µg/ml)95. So, here concentration is increasing with good inhibition activity.

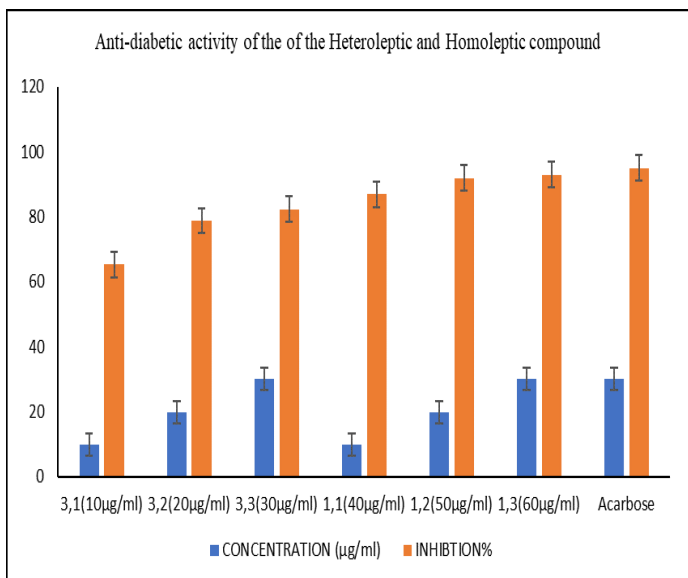


Figure C: Graphical chart view of the concentration is increasing with good inhibition activity.

4. Anti-Cancer activity:

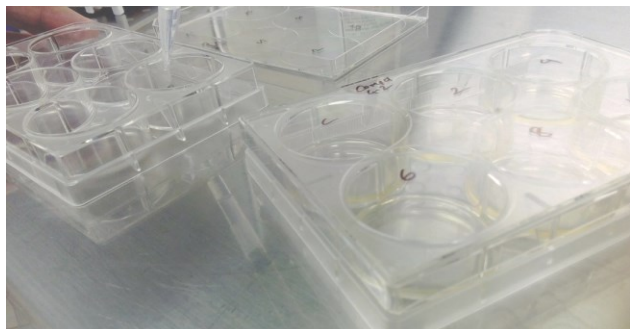


Figure 4: In Anti-Cancer activity, the cell proliferation assay for checking the viability% of the cell. Cell Line: - MDA-MB-231. Media: -DMEM.

$$\text{Cell Viability\%} = \frac{\text{Total live cells} \times 100}{\text{Total number of cells}}$$

Concentration	Viability%					
	Control	2	4	6	8	10
4,1	90	75	60	52	45	30
4,2	90	66	59	55	33	26

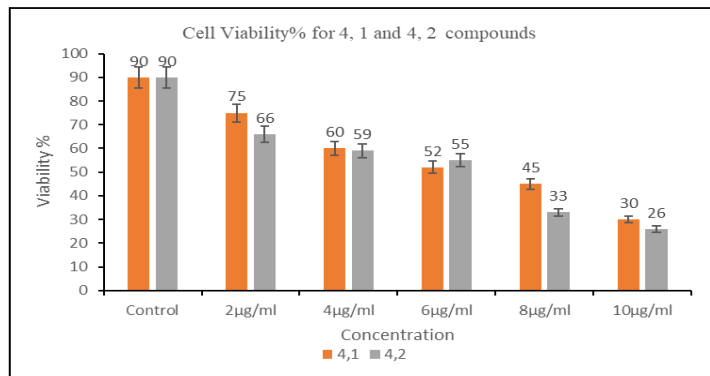
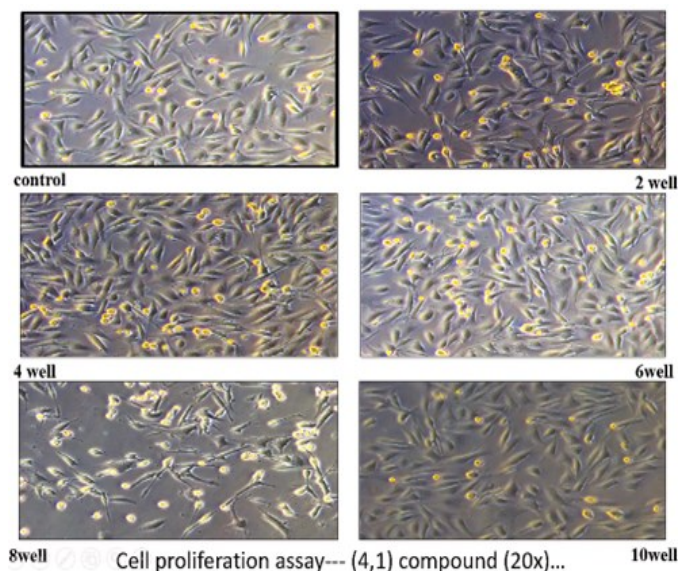
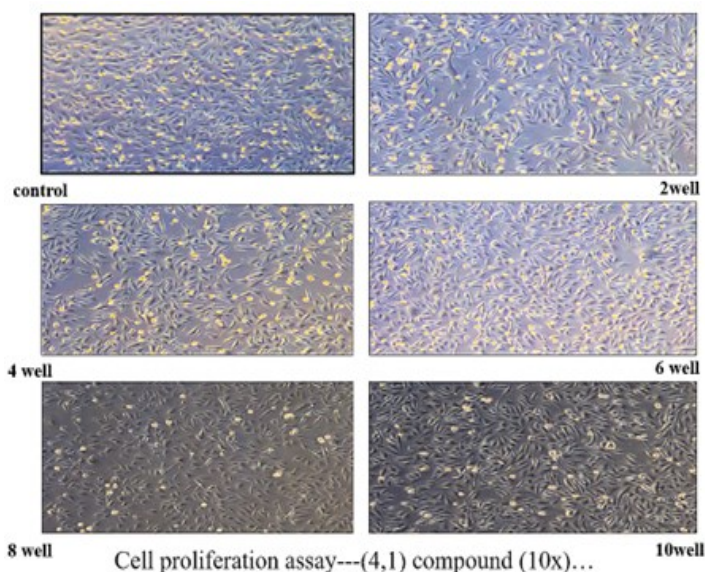
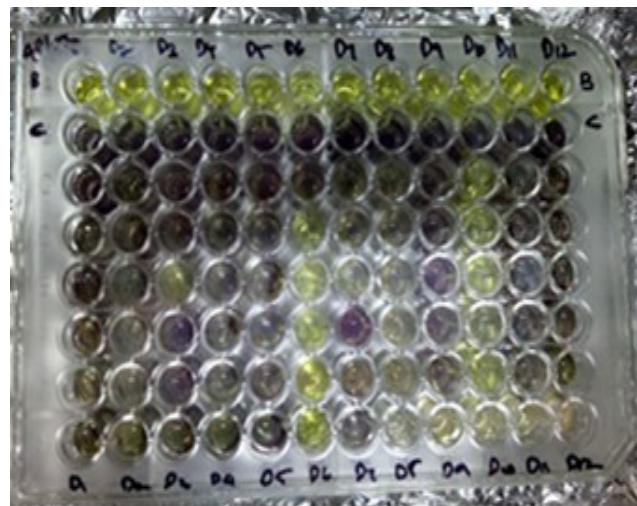
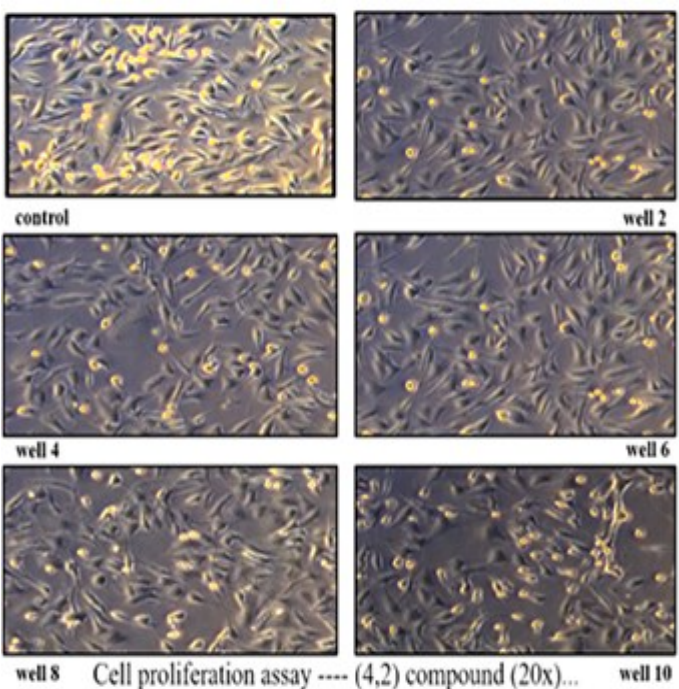
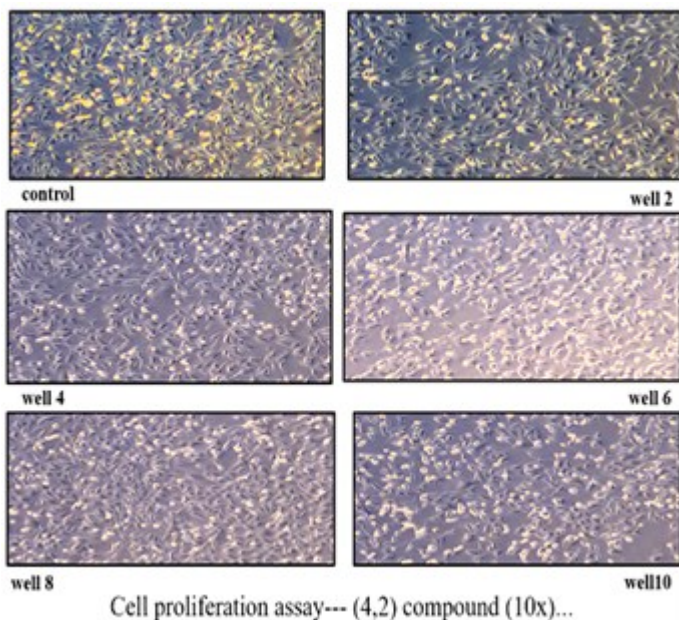


Figure D: Graphical chart view of control against concentrations 4,1 and 4,2.

Here cell proliferation assay of both compounds' viability% has been checked. In Haemocytometer result: Here both control is same it means there is no dead cells and all live cells in there, it has good viability but for well- 2,4,6,8,10 in 5 different concentration viability% decrease means not all live cells also some dead cells also in there.





individual cell types. Wash cells by re-suspending by sterile PBS. Pellet cells by centrifugation at 500 rpm for 5 minutes at 2-8°C. Carefully remove supernatant. Resuspend the cells in 1ml sterile PBS. Count and remove cells and record it. Serial dilution, PBS add and remove, incubate cells for 6-12 hrs. 37°C.

(DAY 2): Add 10µl of MTT to each well. Incubate in CO₂ Incubator for 2-4 hrs. Add 100 µl of solubilizing solution each well. Leave plate covered in dark at 37°C in CO₂ incubator for at least 2 hrs. Samples may be read after 2hrs. View cells periodically for the appearance at the time 24 hrs. and 48 hrs.

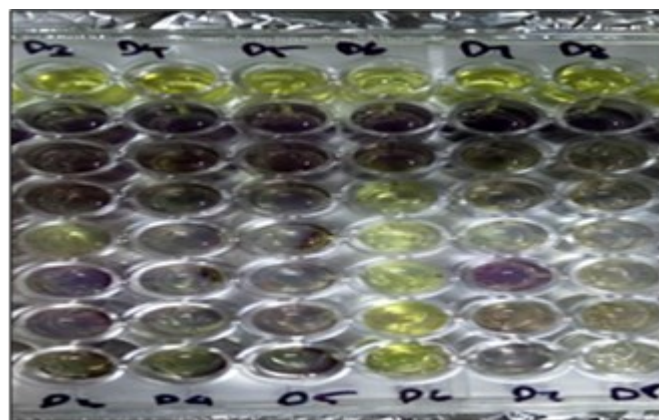


Figure 5: These pictures show the different cell proliferation assay in the different cell well plates compound 4,1 and 4,2.

A. MTT ASSAY: MTT(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). The MTT assay is a colorimetric assay for assessing cell metabolic activity. NAD(P)H-dependent cellular oxidoreductase enzymes may reflect the number of viable cells present.

(Day1): Cells grown in monolayer: Adherent cells should be released from their substrate by trypsinization. Pellet the cells by centrifugation at 500 rpm for 5min at 2-8°C. Remove the supernatant by aspiration. Centrifugation spread adjusted

B. In 96 Well plates I have done the assay in D3,D4, D5,D6, D7,D8 wells. In the D3 well I have added 3,1 compound; in D4 well 3,3 compound; D5 well 3,4 compound;D6 well 1,1 compound; D7 well 1,2 compound and in D8 well 1,3 compound. The purple color is the indication.

Calculate the cell density and percentage cell viability% using the following formula. Cell density =OD Sample-OD Blank. Calculate the average cell density of triplets:

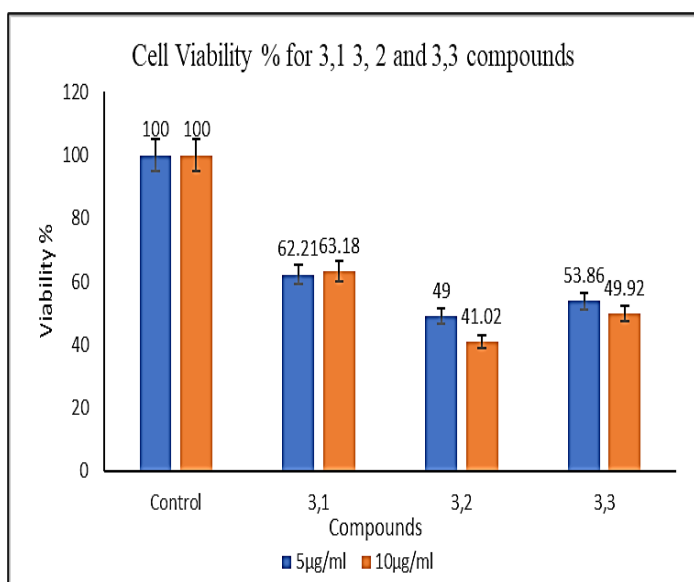
$$\text{Percentage of cell viability (\%)} = \frac{\text{OD Sample} \times 100}{\text{OD Control}}$$

If the absorbance values of the experimental samples are higher than the control cells, this indicates an increase in cell proliferation. Alternatively, if the absorbance rates of

the experimental samples are lower than the controls; this indicates a reduction in the rate of cell proliferation or a reduction in overall cell viability.

$$\text{Cell Viability\%} = \frac{\text{OD Sample}}{\text{OD Control}} \times 100$$

Viability % for 48 hours...				
Concentration	Control	3,1	3,2	3,3
5µg/ml	100	62.21	49.00	53.86
10µg/ml	100	63.18	41.02	49.92



Viability % for 48 hours...				
Concentration	Control	1,1	1,2	1,3
5µg/ml	100	33.46	37.78	39.77
10µg/ml	100	29.94	33.46	36.81

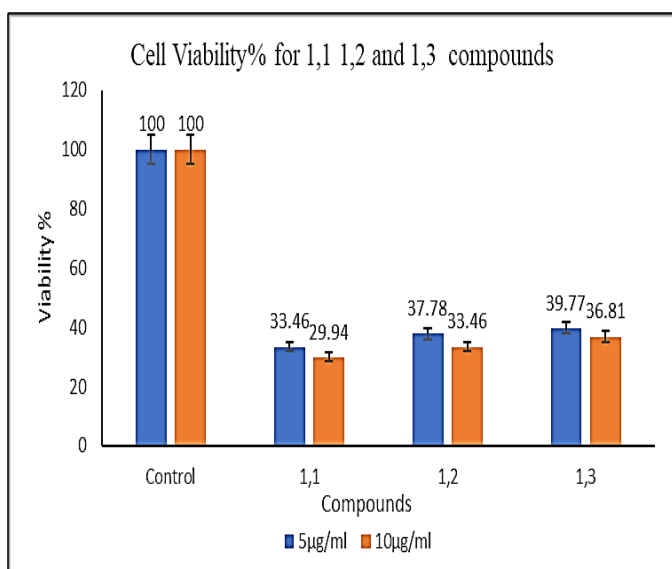
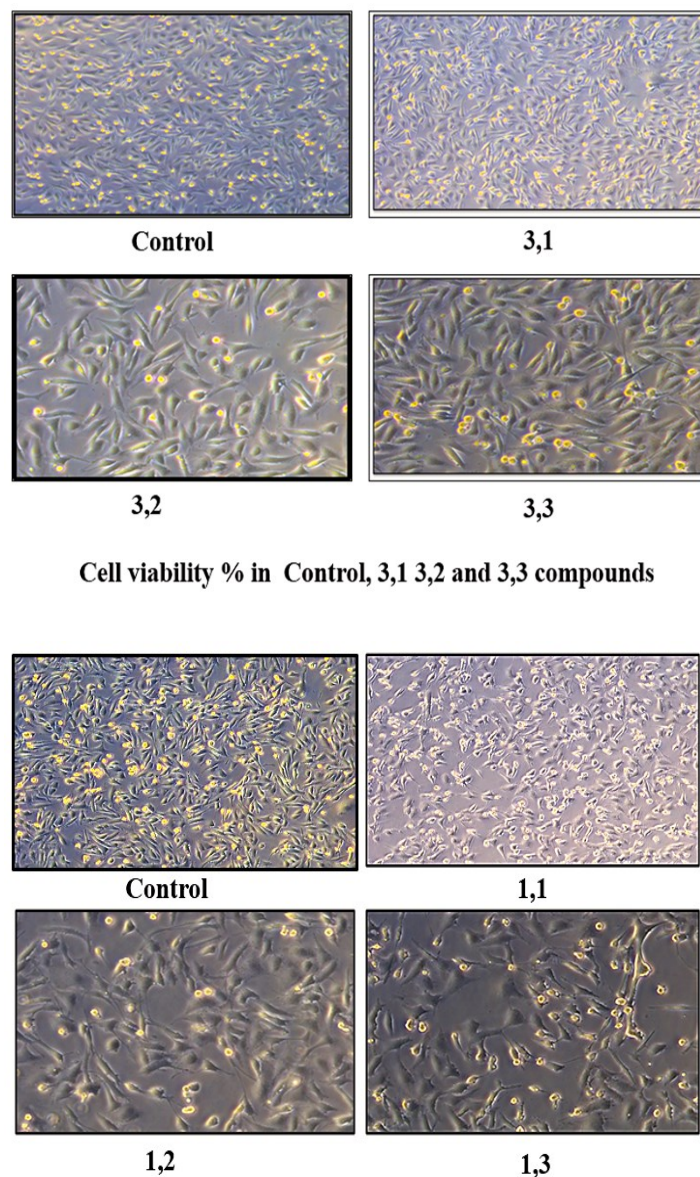


Figure E: Graphical chart view of control against concentrations 3,1 3,2 and 3,3 and the comparison with graphical chart view of 1,1 1,2 and 1,3.



Cell viability % in Control, 3,1 3,2 and 3,3 compounds

Cell viability % in Control, 1,1 1,2 and 1,3 compounds

Figure 6: These pictures show the different cell viability % assay in different compound in 3,1 ,3,2 ,3,3 and 1,1 1,2 1,3 the different cell well plates.

Here, we can see 3, 1 in 5µg/ml and 10µg/ml viability% is mostly good or best where 3, 2; 3,3 also has a good viability% than 1,1;1,2;1,3 compounds.

VI. CONCLUSIONS

In my work done at the Genetic Engineering Lab of SRM Institute of Science and Technology, I have found that in between the Homoleptic and Heteroleptic compound; Where in Antibacterial assay 3, 1 compound does better work against than Tetracycline. So, it is good as a drug for antibacterial assay. In Antifungal assay also 3,1 compound does better work against than Tetracycline. So, it is good as a drug for antifungal assay. Even in the Antidiabetic assay also 3, 1 compound does better work against than Acarbose. So, it is good as a drug for antidiabetic assay. And also, in Anticancer: 3, 1 compound do better work of cell viability% in MTT Assay is good. So, it is good as a drug anticancer assay. So, it concluded that 3, 1 Heteroleptic metal (II) compound work best in between other compound.

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