Research Article

Synthesis and *In-Vitro* Cytotoxic Anti cancer Activity of Novel Copper Complexes of Substituted 1H-Benzimidazole

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ABSTRACT

Synthesis and in vitro cytotoxic activity of novel copper complexes of substituted 1H-Benzimidazole. Promising entities for target-specific next-generation anticancer therapeutics are copper-based compounds. Instead, because of their large range of possible functionalization and coordination modes, benzimidazole scaffolds play an important role. Recently, metal complexes are being hybridized with various moieties to discover new drugs because of their beneficial attributes. Copper is good for synthesizing a metal complex among metals because of its endogenous, redox and DNA cleavage capacity, anti-cancer efficacy and selective permeability reported for cancer cells. In this study, first we synthesized a new series of benzimidazole ligands. Then we have synthesized a series of bidentate copper(Ⅱ)Benzimidazole complex then characterized by various techniques such as Infrared spectroscopy, Nuclear magnetic resonance spectroscopy. Synthesized compounds further investigated for invitro anticancer activity on the human cell line by brine shrimp lethality bioassay. Cytotoxicity was calculated in terms of LC50. Cisplatin was taken as standard for the biological evaluation. In this study it was found that from synthesized 8 complexes the C6 complexes was more cytotoxic than cisplatin, where C3 was least toxic in comparison with standard drug cisplatin. Where complex C1, C2, C4, C5, C7, C8 were moderately cytotoxic as compared to cisplatin.

Keywords: Cancer, Copper Complex, Benzimidazole, Cytotoxicity, Cisplatin.

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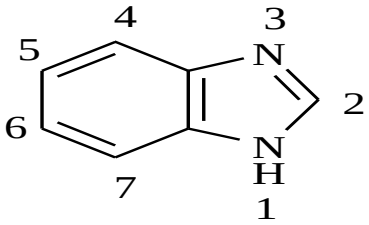
INTRODUCTION

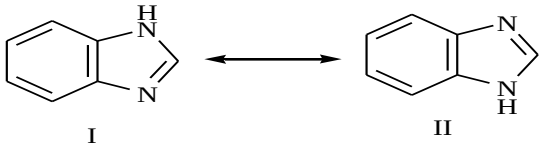
Chemistry of benzimidazole

A cancerous tumor is mass of abnormal cells that could invade or spread to other parts of the body, in contrast to a benign tumor, which doesn't spread. Possible signs and symptoms include a lump, abnormal bleeding, coughing that is prolonged, weight loss, and bowel movements that change unexpectedly. These symptoms may be indicative of cancer, but also may indicate other diseases. Over 100 types of cancers affect humans. Benzimidazole is a heterocyclic aromatic organic compound. Bicyclic in nature, this compound is the product of the fusion of benzene and imidazole. This important class of substances finds practical applications in a number of fields. Benzimidazole is isosteric with indole and purine nuclei, which are present in a number of fundamental cellular components and bioactive compounds. The heterocycle may act as a privileged substructure that interacts with proteins and enzymes. In early 1950's, was an important period regarding the biological significance of benzimidazoles and the closely related purines; the vital role of purines in the biological system was established and 5,6-dimethyl-1-(D-ribofuranosyl) benzimidazole is found in Vit.B12.[1-2]



Discovery of novel effective anticancer drugs with less side effects is very important. Since the discovery of the activity of one of the most successful anticancer compound cis-diaminedichloroplatinum (II) (cisplatin) thousands of platinum complexes have been synthesized and evaluated for their anticancer activity. A few of these complexes which entered clinical trials of five are currently approved: cisplatin and carboplatin world-wide, and oxaliplatin, in a few countries, nedaplatin in Japan, Lobaplatin in China and heptaplatin in South Korea [3,4].

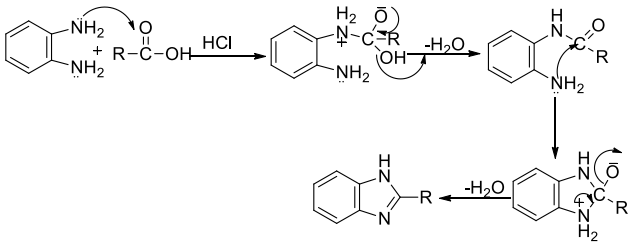
Benzimidazoles containing a hydrogen atom attached to nitrogen in the 1-position readily tautomerize. As with imidazoles and amidines, this tautomerism is anal ogous. This may be depicted as follows:



I II

Although benzimidazole is depicted as (I) possessing the proton at N1, there exist a rapid exchange between –NH and =N- nitrogen atoms. Tautomerism (I) and (II) occur through intermolecular process involving two or more benzimidazole molecules or through interaction with a protic solvent such as water. The groups at position C5 and C6 in the ring system are chemically equivalent.

Reaction mechanism of synthesis of benzimidazole:



Anticancer activity of platinum containing drugs

Compounds those having cis-geometry block the cell growth are class of platinum coordinate compound confirmed by SAR study, the most active complex is cisplatin[5,6].

It is generally believed that passive diffusion is the main mechanism through which cisplatin enters the cell, although facilitated or active transport mechanisms may contribute to the cellular uptake as well[7,8]. Within the cell, chloride concentrations are only 4mM and the hydrolysis of cisplatin takes place so that one or both chlorine atoms are replaced by water molecules. The most important species is the $[PtCl(H2O)(NH3)2]^+$ cation [9,10,11]. This cation is the most reactive, because H2O is a much better leaving group than chloride.

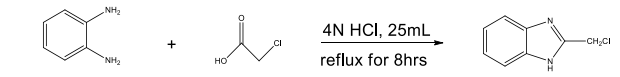
Before cisplatin enters the cell it may bind to phospholipids and phosphatidylserine in the cell membrane [12,13]. In the cytosol many potential platinum-binding sites are also available, including RNA and sulphur-containing proteins and peptides [14,15]. it is found than LT 1% of the cisplatin molecules that enter the cell bind to nuclear DNA [16]. Proteins like Glutathione and other thiols bind rapidly and irreversibly to platinum metal [17]. The deactivated platinum-GSH adducts are excreted from the cell by the glutathione S-conjugate pump [18] . The non-specific toxic effects which induced by binding to the non-DNA targets. It is known, for instance, that the reaction with cisplatin blocks the enzymatic functions of several proteins [19].

MATERIALS AND METHODS

- All chemicals used were of Sigma Aldrich, SD Fine Chemicals and Thomas baker.
- All solvents used were of reagent grade and ordered from Sigma Aldrich, SD Fine Chemicals and Thomas baker.
- Thin-layer chromatography (TLC) was performed on 60 F254 precoated silica gel plates (Merck) to establish identity of reactants and products monitored in between reactions as well at the end for completion of reaction. The spots were visualized in UV chamber or by iodine vapors in an enclosed chamber.
- All the melting points were determined with thiol's tube melting/boiling point apparatus and are uncorrected.
- IR spectra were recorded on KBr pellets on a shimadzu 1000 FTIR spectrometer in the range of 4000-200 cm-1, Resolution 2.0 with No. of scan- 45. Apodization; Happ-Genzel.
- Proton resonance magnetic spectra (1H NMR) were recorded on Bruker 400MHz spectrophotometer using d6-DMSO as a solvent and chemical shift were expressed in parts per million (δ ppm), downfield from TMS as an internal standard.
- As well as LCMS withAPCI and the 4000Q TRAP system , Mass spectra (MS) were recorded.
- As a control drug, we used Cisplatin Injection BP, man ufactured by Cipla Ltd. and offered under the brand name CYTOPLATIN-10 containing Cisplatin 0.5 mg/mL.

Synthesis of Ligands

Procedure for Synthesis of 2-chloro methyl 1H-benzimidazole.

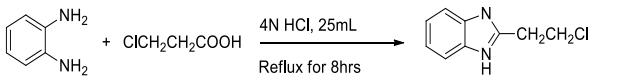


O-phenylenediamine | Mono chloro acetic acid | 2-(chloromethyl)-1H-benzo(d)imidazole

Procedure

O-phenylenediamine (0.13mol), monochloroacetic acid(0.104mol), and 25mL of 4N hydrochloric acid were taken in an RBF and refluxed for 8hrs at 700C. According to the well-known Phillips method, hydrochloric acid was added during the synthesis as a condensation reagent. Reaction was monitored by TLC. A test portion was dumped in water and basified with ammonia solution. The reaction mixture was poured into ice-cold water after it was finished. It was then basified with conc. Ammonia solution. The solid precipitated was filtered and dried. The solid are then recrystallized by using chloroform. After that MP, % yield is calculated.[20]

Procedure for Synthesis of 2-chloro-ethyl-benzimidazole:



OPD | 3-chloropropanoic acid | 2-Chloro-ethyl-benzimidazole

Procedure

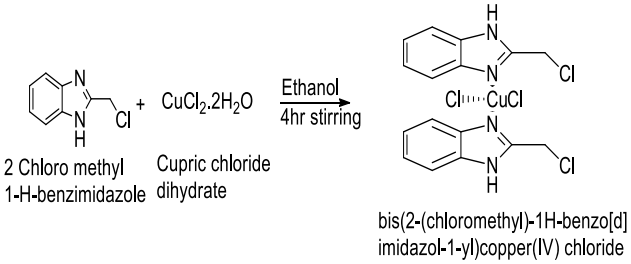
O-phenylenediamine (0.13mol), 3-chloropropionic acid (0.104mol), and 25mL of 4N hydrochloric acid were taken

in an RBF and refluxed for 8hrs at 700C. Hydrochloric acid was added during the synthesis as a condensation reagent according to the well-known Phillips method. Reaction was monitored by TLC. A test portion was dumped in water and basified with ammonia solution. After completion of reaction, the reaction mixture was poured in ice-cold water. It was then basified with conc. Ammonia solution. The solid precipitated was filtered and dried. The solid are then recrystallized by using chloroform. After that MP, % yield is calculated. [20]

Synthesis of complexes

Procedure for Synthesis of complex 2 chloromethyl-1H-benzimidazole copper (IV)

To a stirred solution of 2-chloromethyl-1H-benzimidazole (0.10 mol) in an ethanol was added an alcoholic solution of CuCl2.2H2O (0.05 mol) drop wise over 30 min at room temperature. The reaction mixture protected from light was stirred at room temperature for 4hrs. The resulting crude precipitate was filtered off and purified by repeated washing with small portions of water, ethanol, and diethyl ether and dried. [21-23]



RESULTS AND DISCUSSION

Spectral analysis of ligands

The ligands were synthesized according to the literature procedure using substituted o-phenylenediamine and various acids. Total 8 ligands were synthesized by condensation of o-phenylenediamine and acids in presence of 4N HCl %yield, melting point and Rf values (TLC) were calculated for all 8 ligands. The ligands were characterized by IR, ^{1}H NMR and MS.

Ligand 1] 2-(chloromethyl)-1H-benzo(d)imidazole

yield-62.47%; MP-167-169°C; IR (KBr) Cm^{-1}: (3057) CH, (1600 and 1475) C=C, (3086) NH, 744 (C-Cl), (1315) C-N. ^{1}H NMR δ(DMSO)- 4.25 (S,2H)Ha; 5.10(S,H)Hb; 7.54(d,H)Hc; 7.20(d,H)Hd; 7.24(s,3H)He; 7.54(d,H)Hf.

Ligand2] 2-(2-chloroethyl)-1H-benzo(d)imidazole

Yield-58.19%, MP- 135-137°C, IR (KBr) Cm^{-1}: (3016) CH, (1600 and 1475) C=C, (3226) NH, 729 (C-Cl), (1354) C-N. ^{1}H NMR δ(DMSO)- 3.69 (s,2H)Ha; 2.80 (s,2H)Hb; 5.0(s,H)Hc; 7.58(d,H)Hd; 7.22(d,H)He; 7.22(d,H)Hf; 7.58(s,H)Hg.

Ligand 3] 6-chloro-2-(chloromethyl)-1H-benzo(d)imidazole

Yield-76.41%, MP- 200-202°C, IR (KBr) Cm^{-1}: (3026) CH, (1616 and 1492) C=C, (3226) NH, 709 (C-Cl), (1288) C-N. ^{1}H NMR δ(DMSO)- 4.22(s,2H)Ha; 4.5(s,H)Hb; 7.55(d,H)Hc; 7.14(d,H)He; 8.36(s,H)Hf.

Ligand 4] (1H-benzo(d)imidazol-2-yl)(phenyl)methanol

Yield-66.50%, MP- 268-270°C, IR (KBr) Cm^{-1}: (3024) CH, (1622 and 1456) C=C, (3221) NH, 740 (C-Cl), (1290) C-N. ^{1}H NMR δ(DMSO)- 5.60(s,H)Ha, 7.36(d,H)Hb; 7.38(d,H)Hc; 7.38(d,H)Hd; 7.36(d,H)He; 5.2(s,H)Hf; 7.50(d,H)Hg; 7.22(d,H)Hh; 7.22(d,H)Hi; 7.50(d,H).

Ligand 5] 2-butyl-1H-benzo(d)imidazole

Yield-69.37%, MP- 160-162°C, IR (KBr) Cm^{-1}: (3057) CH, (1606 and 1471) C=C, (3421) NH, 742 (C-Cl), (1285) C-N. ^{1}H NMR δ(DMSO)- 0.97(s,3H)Ha, 1.27(m,2H)Hb; 1.57(m,2H)Hc; 2.89(t,2H)Hd; 4.5(s,H)He; 7.55(d,H)Hf; 7.26(d,H)Hg; 7.26(d,H); 7.55(d,H).

Ligand 6] 1-(6-methyl-1H-benzo(d)imidazol-2-yl) ethanol

Yield-57.89%, MP- 208-210°C, IR (KBr) Cm^{-1}: (3050) CH, (1671 and 1460) C=C, (3420) NH, 760 (C-Cl), (1280) Cu-N. ^{1}H NMR δ(DMSO)- 4.70(t,H)Ha, 5.0(s,H)Hb; 7.10(d,H)Hc; 7.55(d,H)Hd; 2.34(s,3H)He; 7.12(d,H)Hf; 3.60(s,H)Hg; 1.50(s,3H)Hh.

Ligand 7] (6-methyl-1H-benzo(d)imidazol-2-yl) (phenyl)methanol

Yield-44.85%, MP- 289-291°C, IR (KBr) Cm^{-1}: (3045) CH, (1600 and 1480) C=C, (3415) NH, 730 (C-Cl), (1282) Cu-N. ^{1}H NMR δ(DMSO)- 5.79(s,H)Ha, 5.05(s,H)Hb; 7.12(d,H)Hc; 7.54(d,H)Hd; 2.34(s,3H)He; 7.40(d,H)Hf; 7.36(d,H)Hg; 7.38(d,H)Hh; 6.43(s,H)Hi.

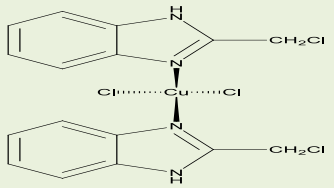
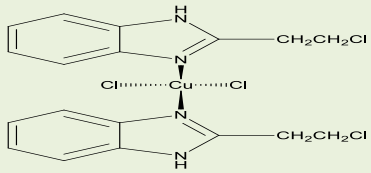
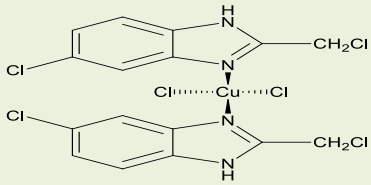
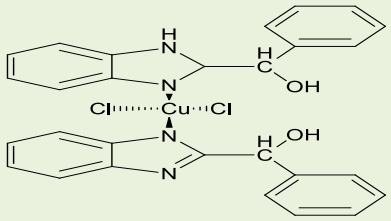
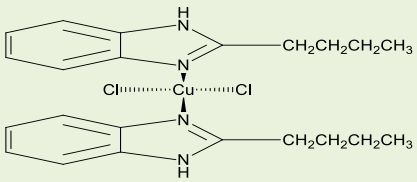
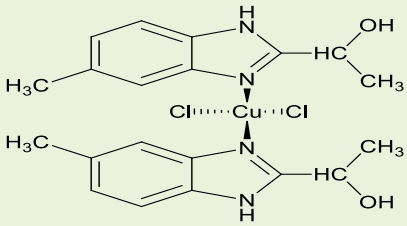
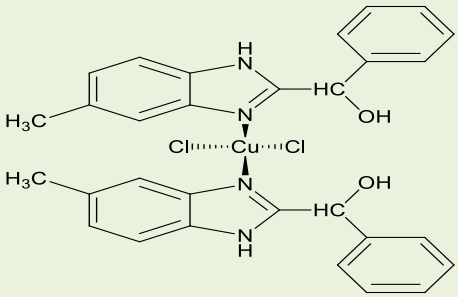
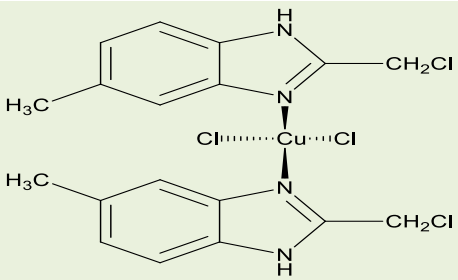
Ligand 8] 2-(chloromethyl)-6-methyl-1H-benzo(d)imidazole

Yield-53.04%, MP- 182-184°C, IR (KBr) Cm^{-1}: (3053) CH, (1610 and 1470) C=C, (3440) NH, 735 (C-Cl), (1213) Cu-N. ^{1}H NMR δ(DMSO)- 4.30 (S,2H)Ha; 5.07(S,H)Hb; 7.20(d,H)Hc; 7.55(d,H)Hd; 2.35(s,3H)He; 7.43(d,H)Hf.

Spectral analysis of Complexes

The complexes were synthesized according to the literature procedure using synthesized ligands and Cupric chloride dihydrate. Total 8 complexes were synthesized by condensation of synthesized ligands and cupric chloride dihydrate in presence of ethanol. %yield, melting point and Rf values (TLC) were calculated for all 8 complexes. The ligands were characterized by IR, ^{1}H NMR.

Table 1: Structures and nomenclature of the complexes.

Compound code	Structure	Name
C1		bis(2-(chloromethyl)-1H-benzo[d]imidazole-1-yl)copper(IV)chloride
C2		bis(2-(2-chloroethyl)-1H-benzo[d]imidazole-1-yl)copper(IV)chloride
C3		bis(6-chloro-2-(chloromethyl)-1H-benzo[d]imidazole-1-yl)copper(IV)chloride
C4		bis(2-hydroxy(phenyl)methyl)-1H-benzo[d]imidazole-1-yl)copper(IV)chloride
C5		bis(2-butyl-1Hbenzo[d]imidazole-1-yl)copper(IV)chloride
C6		bis(2-(1-hydroxyethyl)-6-methyl-1H-benzo[d]imidazole-1-yl)copper(IV)chloride
C7		bis(2-(hydroxyl(phenyl) methyl-1Hbenzo[d]imidazole-1-yl)copper(IV)chloride
C8		bis(2-chloromethyl)-6-methyl-1H-benzo[d]imidazole-1-yl)copper(IV) chloride

Complex 1] Bis(2-(chloromethyl)-1H-benzo[d]imidazole-1-yl) copper (IV)chloride

Yield-58.60%, MP-225-230°C, IR (KBR) cm^{-1}: (3039) CH, (1620 and 1462) C=C, (3265) NH, (738) C-Cl, (433) Cu-N. ^{1}H NMR δ(DMSO)- 4.26 (s,2H)Ha; 5.0(s,H)Hb; 7.59(d,H)Hc; 7.25(d,H)Hd; 7.25(d,H)He; 7.59(d,H)Hf.

Complex 2] Bis(2-(2-chloroethyl)-1H-benzo[d]imidazole-1-yl) copper IV) chloride

Yield-45.30%, MP- 232-237°C, IR (KBr) Cm^{-1}: (3057) CH, (1606 and 1471) C=C, (3421) NH, 742 (C-Cl), (432) Cu-N. ^{1}H NMR δ(DMSO)- 3.71 (s,2H)Ha; 2.83 (s,2H)Hb; 5.0(s,H)Hc; 7.58(d,H)Hd; 7.22(d,H)He; 7.22(d,H)Hf; 7.58(s,H)Hg.

Complex 3] Bis(6-chloro-2-(chloromethyl)-1H-benzo[d]imidazole-1-yl) copper (IV) chloride

Yield-35.36%, MP-248-253°C, IR(KBr) Cm^{-1}: (3016) CH, (1618 and 1458) C=C, (3057) NH, (785) C-Cl, (433) Cu-N. ^{1}H NMR δ(DMSO)- 4.26(s,2H)Ha; 5.0(s,H)Hb; 7.55(d,H)Hc; 7.14(d,H)He; 8.36(s,H)Hf.

Complex 4] Bis(2-hydroxy(phenyl)methyl)-1H-benzo[d]imidazole-1-yl) copper (IV)chloride

Yield-42.11%, MP- >280°C, IR (KBr) Cm^{-1}: (3003) CH, (1602 and 1450) C=C, (3226) NH, (740) C-Cl, (437) Cu-N. ^{1}H NMR δ(DMSO)- 5.20(s,H)Ha, 7.36(s,H)Hb; 7.38(s,H)Hc; 7.38(d,H)Hd; 7.36(d,H)He; 3.8(s,H)Hf; 5.0(s,H)Hg; 6.58(d,H)Hh; 6.50(d,H)Hi.

Complex 5] bis(2-(1-hydroxyethyl)-6-methyl-1H-benzo[d]imidazole-1-yl) copper (IV)chloride

Yield-42.11%, MP- >280°C, IR (KBr) Cm^{-1}: (3012) CH, (1606 and 1454) C=C, (3210) NH, (725) C-Cl, (435) Cu-N. ^{1}H NMR δ(DMSO)- 0.90(s,3H)Ha, 1.31(m,2H)Hb; 1.59(m,2H)Hc; 2.87(t,2H)Hd; 5.0(s,H)He; 7.59(d,H)Hf; 7.22(d,H)Hg; 7.22(d,H); 7.59(d,H).

Complex 6] Bis(2-(1-hydroxyethyl)-6-methyl-1H-benzo[d]imidazole-1-yl) copper (IV)chloride

Yield-42.11%, MP- >280°C, IR (KBr) Cm^{-1}: (3028) CH, (1615 and 1430) C=C, (3225) NH, (745) C-Cl, (433) Cu-N. ^{1}H NMR δ(DMSO)- 4.68(t,H)Ha, 5.0(s,H)Hb; 7.12(d,H)Hc; 7.54(d,H)Hd; 2.34(s,3H)He; 7.43(d,H)Hf; 3.65(s,H)Hg; 1.49(s,3H)Hh.

Complex 7] Bis(2-(hydroxyl(phenyl) methyl-1Hbenzo[d]imidazole-1-yl) copper (IV)chloride

Yield-42.11%, MP- >280°C, IR (KBr) Cm^{-1}: (3000) CH, (1605 and 1450) C=C, (3230) NH, (760) C-Cl, (440) Cu-N. ^{1}H NMR δ(DMSO)- 5.79(s,H)Ha, 5.0(s,H)Hb; 7.12(d,H)Hc; 7.54(d,H)Hd; 2.34(s,3H)He; 7.43(d,H)Hf; 7.36(d,H)Hg; 7.38(s,H)Hh; 6.43(s,H)Hi.

Complex 8] Bis(2-chloromethyl)-6-methyl-1H-benzo[d]imidazole-1-yl) copper (IV) chloride

Yield-42.11%, MP- >280°C, IR (KBr) Cm^{-1}: (3010) CH, (1600 and 1440) C=C, (3225) NH, (740) C-Cl, (430) Cu-N. ^{1}H NMR δ(DMSO)- 4.26 (S,2H)Ha; 5.0(S,H)Hb; 7.12(d,H)Hc; 7.54(d,H)Hd; 2.34(s,3H)He; 7.43(d,H)Hf.

Table 2: Properties of complexes

Sr. no.	Molecular Formula	Mol. Wt. gm/mol	% Yield	M.P. (0C)
C1	C16H12Cl2CuN4	465.65	58.60	225-230
C2	C18H16Cl2CuN4	493.70	45.30	232-237
C3	C16H10Cl2CuN4	534.54	35.36	248-253
C4	C28H22Cl2CuN4O2	580.95	42.11	> 280
C5	C22H26Cl2CuN4	480.92	60.55	220-225
C6	C20H22Cl2CuN4O2	484.87	53.89	261-266
C7	C30H26Cl2CuN4O2	609.01	61.22	270-275
C8	C18H16Cl4CuN4	493.70	58.45	240-245

Pharmacological evaluation

Brine shrimp lethality bioassay

The cytotoxicity of the synthesized compounds were investigated using a brine shrimp lethality test. The brine shrimp lethality bioassay is simple to use, inexpensive, and only requires a little amount of test substance. This serves as a preliminary screening tool that can be supplemented by a more specialized and costly bioassay. [24-25]

Preparation of brine solution

38 g of iodize sodium chloride was weighed, dissolved in 1000 ml of distilled water and filtered to obtain a clear solution.

Hatching of Artemia salina shrimps

Brine shrimp (*Artemia salina*) were hatched using brine shrimp eggs in a vessel filled with artificial sea water under constant aeration for 48 hours. The active shrimps (nauplii, larvae) were collected and used for the assay.

Preparation of sample solution

10 mg of each chemical was dissolved in 10 mL DMSO to provide a stock solution with a concentration of 1000 g/mL, which was subsequently diluted to 100, 10, and 1 g/mL. Stock solutions of the compounds were generated according to the prescribed volume range by dissolving in DMSO to prevent the toxicity results from being tainted by

the toxicity of DMSO. Pure DMSO was used as a positive control for the toxicity assay. [26]

Application of test solution and larvae to the test tubes

Each test tube received around 5 mL of brine solution. According to the concentration, appropriate dilutions of the test material were made. The test tubes were filled with the 0.05 mL diluted test solution.

RESULTS

In the lab, a brine shrimp lethality test was performed. Because complexes have a low solubility, they must be dissolved in DMSO before being used to make a medication solution. The LC50 was computed using the following results. The results were compared against those of a typical medication, cisplatin. DMSO was used as a positive control.

- 30 active shrimps (larvae) were added into each test tube.
- The surviving (larvae) shrimps were counted after 24 hours and lethality concentration LC50 was assessed.

Brine shrimp lethality bioassay

Table 3: Pharmacological evaluation

Comp.	Conc. Ppm	Mortality of shrimps			% mean mortality	Lc50 µg/mL
		I	II	III		
1	1000	15	16	15	51.11	909.09
	100	12	13	13	42.22	
	10	6	6	7	21.11	
	1	1	3	3	7.78	
2	1000	24	21	24	76.67	611.99
	100	15	16	18	54.44	
	10	13	11	12	40.00	
	1	7	5	8	22.22	
3	1000	26	25	27	86.67	541.79
	100	18	19	18	61.11	
	10	11	11	13	38.89	
	1	8	6	6	22.22	
4	1000	18	19	18	61.11	765.69
	100	13	14	14	45.56	
	10	7	8	9	26.67	
	1	3	5	5	14.44	
5	1000	14	15	15	48.89	959.69
	100	11	10	11	35.56	
	10	6	5	5	17.78	
	1	4	5	4	14.44	
6	1000	14	14	13	45.55	1022.49
	100	11	10	11	35.55	
	10	8	7	3	26.66	
	1	2	3	4	10.00	
7	1000	15	15	17	52.22	892.85
	100	11	12	14	41.11	
	10	6	7	4	18.88	
	1	4	2	2	8.88	
8	1000	18	17	19	60.00	772.79
	100	16	14	15	50.00	
	10	11	10	8	32.22	
	1	6	5	6	18.89	
Cisplatin	1000	21	24	21	73.33	642.67
	100	14	15	16	50.00	
	10	9	8	9	27.78	
	1	6	5	4	17.78	

Positive control with DMSO has shown mortality of 2 shrimp.

No. of shrimps taken- 30.

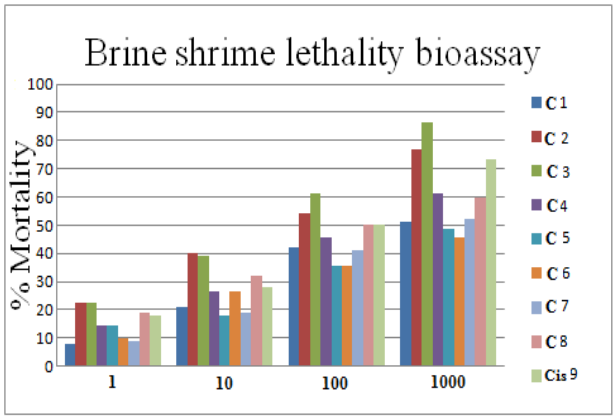


Figure 1: Graphical representation showing activity of complexes with respect to cisplatin

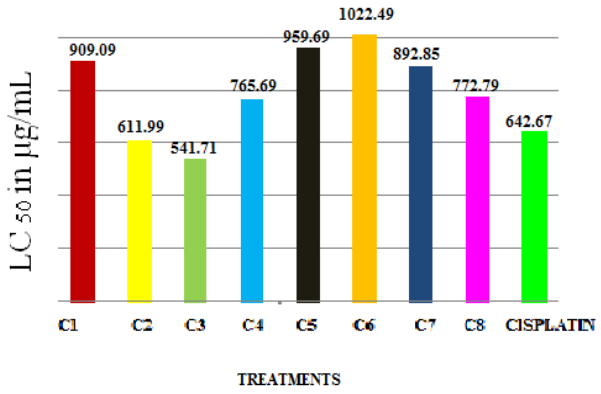


Figure 2: Graphical representation showing LC50 values of complexes and cisplatin.

DISCUSSIONS

It's critical to find new anticancer medications that are effective and have fewer adverse effects. One of the greatest discoveries in pharmaceutical science has been the use of metals such as platinum in the treatment of cancer, which has led to the synthesis of many platinum-based compounds and the evaluation of their anticancer activity. Platinum compounds have strong anticancer properties, with less mild to moderate side effects. As a result, synthesizing metal compound derivatives with anticancer properties could be useful in the treatment of a variety of cancers. Copper metal could be useful in anticancer compounds because it is already used in several therapies, such as Wilson disease. This derivatives may have certain disadvantages, just as it has numerous positives.

CONCLUSION

The cytotoxic activity of all eight produced complexes was tested using a brine shrimp lethality bioassay. The LC50 was used to calculate cytotoxicity. For the biological examination, cisplatin was used as the gold standard. 1H NMR was used to confirm the produced chemical the vibration of Cu-N can be seen in the IR spectra of complexes at 435 cm-1 When compared to ligands, the proton value changes to the down field in 1H NMR. Compound complex 6 has the highest cytotoxicity of any compound, and standard drug Cisplatin where complex 3 shows less cytotoxicity than any other complexes.

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