DESIGN, SYNTHESIS, CHARACTERIZATION AND *IN-VITRO* EVALUATION OF CYTOTOXIC ACTIVITY OF NOVEL COPPER COMPLEXES OF SUBSTITUTED 1H-BENZIMIDAZOLES

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Abstract

In recent years, there has been a rapid expansion in research and development of novel metal-based anticancer drugs to improve clinical effectiveness and to reduce general toxicity and also to broaden the spectrum of activity. Hence,in this studies, an attempt has been made to Design, Synthesize, Characterize novel Copper complexes of substituted 1H-Benzimidazoles and their pharmacological evaluation for *in vitro* Cytotoxic activity by Brine shrimp lethality bioassay. Thereafter, results were estimated in terms of LC50 as compared with the standard drug Cisplatin.. All complexes showed significant cytotoxic activity. Among all complexes, complex (4c) showed more cytotoxic activity and it is more cytotoxic than Cisplatin. Complex (4e) was found to possess least cytotoxic activity. Complexes (4b, 4d and 4h) showed moderate cytotoxic activity.

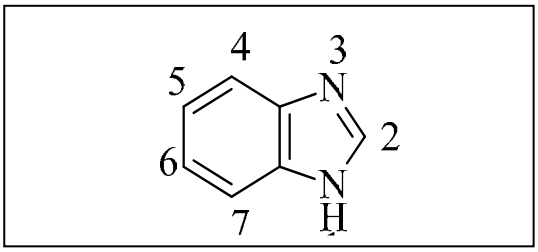
Keywords: Benzimidazole-Copper Complexes, Cytotoxicity, Brine shrimp lethality bioassay, Reactive oxygen species.

1. Introduction

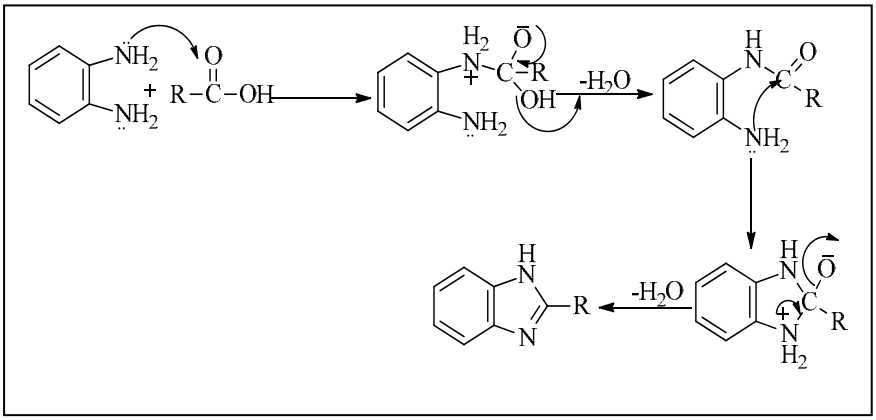
Cancer is the second most frequent cause of death in the world. [1] Discovery of antitumor activity of cisplatin began a search for other metal complexes with cytotoxic properties against cancer cells. One of the transition metals whose complexes extensively tested for antitumor application is copper. Copper is a trace element essential for human life. It is building element of several important enzymes. e. g. Superoxide dismutase, cytochrome oxidase, tyrosinase. & it regulates the intracellular redox potential while its complexes possess antibacterial, antifungal, antiviral, anti-inflammatory & anticancer properties as potential as. Currently, anticancer drugs are extensively studied, mainly complexes of copper. There are only few complexes of copper in this article, and among them some complexes are showing very strong cytotoxic activity against tumor cells in Vitro. [2]

The benzimidazole ring system is an important pharmacophore in medicinal chemistry and modern drug exploration. 2-Substituted benzimidazoles have been found to possess anti-inflammatory. [3] Antispasmodic, [4] antihistamines, [5] antimicrobial, [6] [7] [8], antitumor, [9] anticancer [10] and Cyclo-Oxyganase inhibitor activities. [11] Recently, different authors universally have recite antispasmodic, antiproliferative or anticancer potential of benzimidazole. [12-15] Benzimidazole is a heterocyclic aromatic organic compound. This compound is bicycling in nature which comprises of the liquefaction of benzene and imidazole. This important group of centers has found practical applications in a number of fields. Benzimidazole is isoteric with indole and purine nuclei, which are instant in a number of fundamental cellular components and bioactive compounds.[16] This heterocycle may represent a kind of priviledged substructure, which may interact with dissimilar proteins and enzymes.indeed a number of important drugs used in different therapeutic areas contain benzimidazoles**.**[17] In times of 1950's, was an important period concerning the biological consequences of benzimidazoles and the closely related purines; the mortal role of purines in the biological system was established and it was discovered that 5,6-dimethyl-1-(D-ribofuranosyl) benzimidazole is an integral part of

structure of Vit.B12. And several other kinds of benzimidazole moieties are still investigational therapeutic agents. [18-19]



Scheme 1: Synthetic pathways of benzimidazole with reaction mechanism:[20]



Mechanism of anticancer activity of copper.

The anticancer activity of copper complex compounds is related to their ability to produce reactive oxygen species (ROS). Copper (I) ions can reduce hydrogen peroxide to hydroxyl radical. Copper (II) ions may in alter be reduced to Cu (I) by superoxide anion (O^{2}•-), or glutathione. Therefore, it can be inferred that the production of reactive oxygen species such as OH• are driven by the copper, regardless of the form in which it is initially introduced into the body– Cu^{+}, or Cu^{2+}. [2, 21]

$Cu^{2+} + O_2$▪ ⟶ $Cu^{2+} + O_2$

$Cu^{++} + H_2O_2$ ⟶ $Cu^{2+} + OH^{\bullet} + OH^{-}$

Superoxide anion (O2•-) is the product of reduction of the molecular oxygen that appear in many biological processes. It is converted into hydrogen peroxide through dismutation. Both of these forms of ROS leads to the formation of another type of reactive oxygen species – the hydroxyl radical ($OH^{\bullet}$). It happens in a retroaction cause to catalyze by cuprous ions. This radical is believed to be the principle element causing DNA detriment in cells under oxidative stress. [22-23]

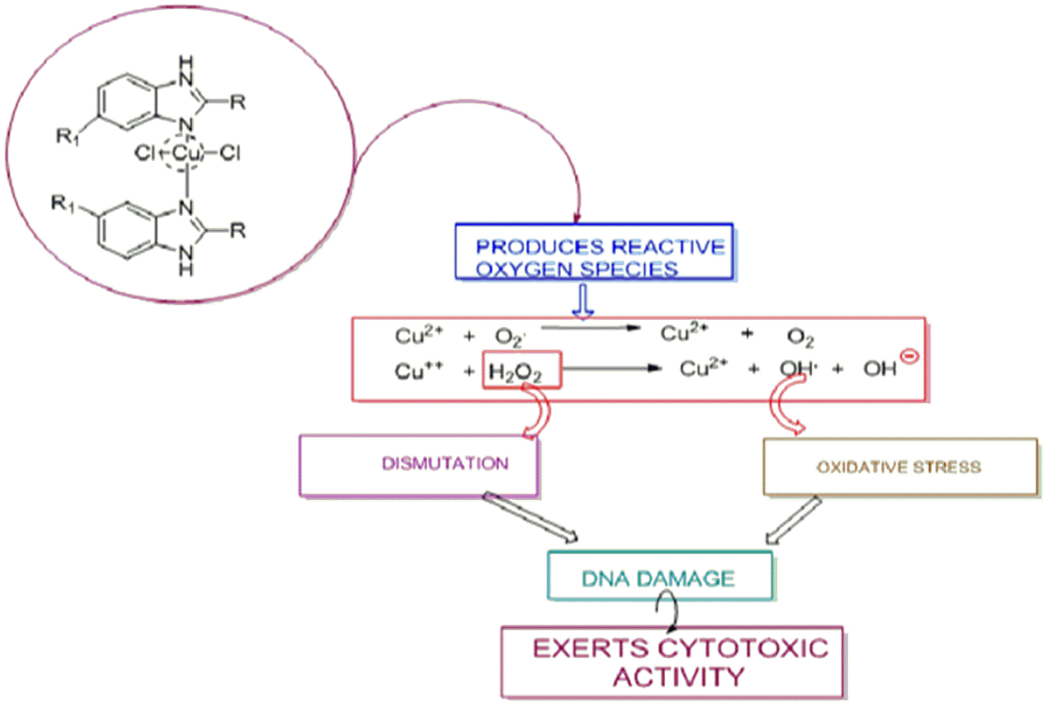
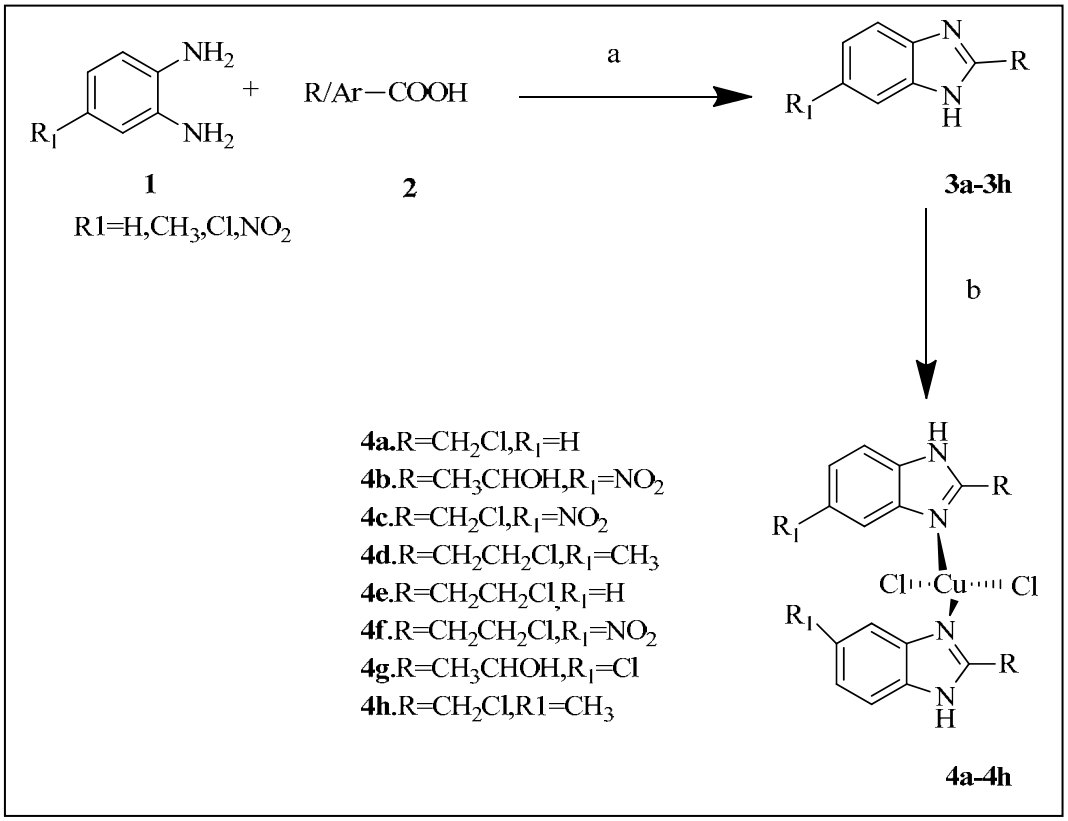


Figure 1. Mechanism of anticancer activity of Benzimidazole-Copper complexes in a pictorial form.



Scheme 2 : General synthetic scheme for synthesis of benzimidazole-copper complexes.(4a-4h)

Reaction conditions: a-25ml 4N HCl, reflux for 8hrs, b-Ethanol,$CuCl_2.2H_2O$, Stirring for 4hrs.

2. 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 as at the end for completion of the reaction. The spots were visualized in UV chamber or by iodine vapors in an enclosed chamber.All the melting points were determined with thiel'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 (H^1 NMR) were recorded on Bruker 400MHz spectrophotometer using d6-DMSO[Dimethyl sulphoxide] as a solvent and chemical shifts were expressed in parts per million (δ ppm), downfield from TMS as an internal standard.Mass spectra (MS) were recorded on LCMS instrument with APCI as well as on 4000Q – TRAP MS/MS System.Cisplatin Injection BP was used as control drug to study Cytotoxic activity sold under the brand name 'CYTOPLATIN-10' containing Cisplatin 0.5 mg/mL. Mfg. by Cipla Ltd.

Experimental section:

General procedure for synthesis of substituted benzimidazoles [3a-3h].

Various substituted benzimidazoles were synthesized by using substituted O-phenylenediamine [1], and various alkyl/aryl acids [2], and 25ml of 4N hydrochloric acid [a] were taken in a RBF and refluxed for 8hrs at 70^0C. Hydrochloric acid was added during the synthesis as a condensation reagent according to the well-known Phillips method. The reaction was monitored by TLC. A test portion was dumped into the water and basified with ammonia solution. After completion of the 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 is then recrystallized by using chloroform. After that MP, % yield is calculated. [24-25]

Table 1. Structures of substituted benzimidazoles (3a-3h) acting as ligands given with structures of substituted OPD & alkyl/aryl acids as starting materials in synthesis.

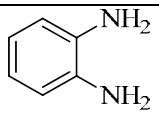
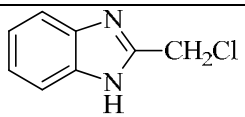
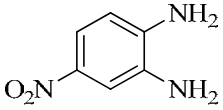
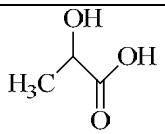
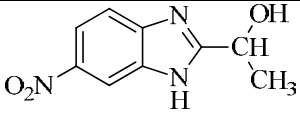
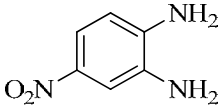
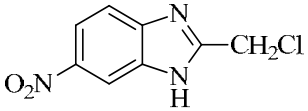
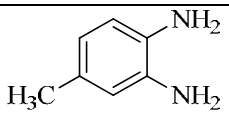
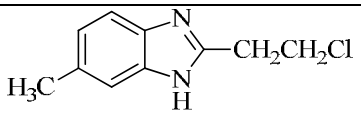
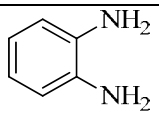
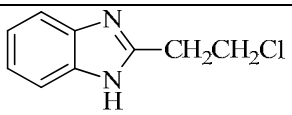
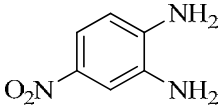
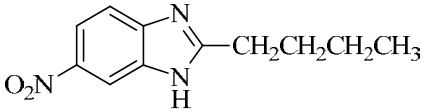
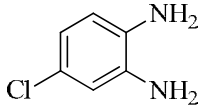
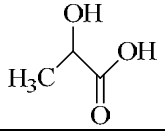
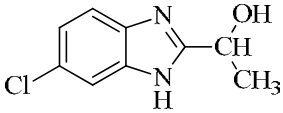
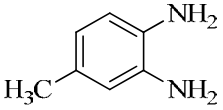
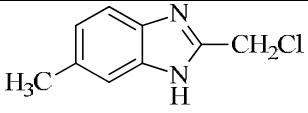
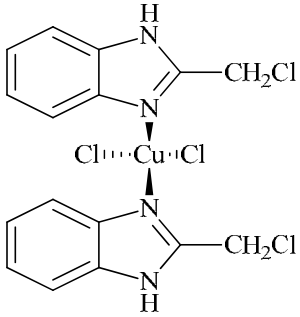
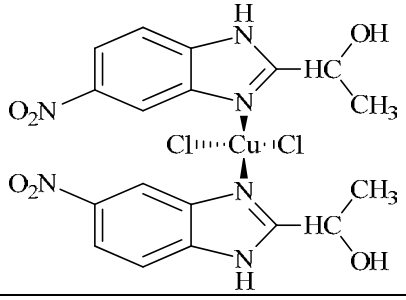
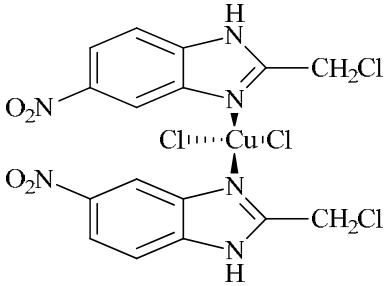
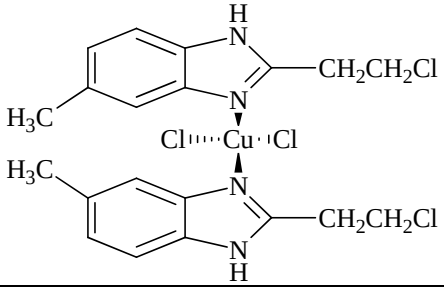
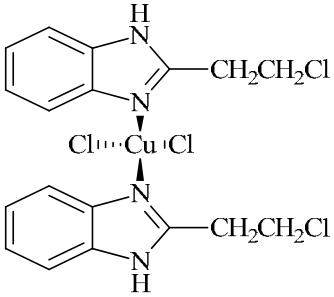
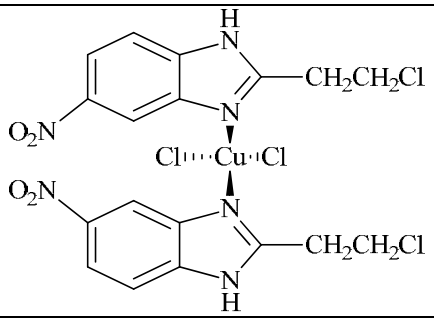
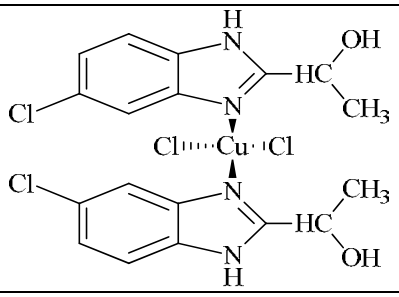
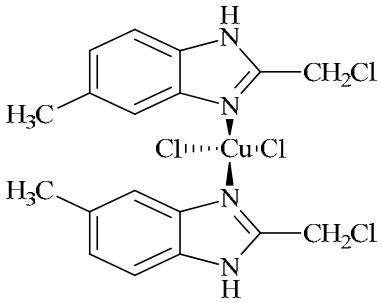
Sr.No	Substituted OPD	Alkyl/Aryl acids	Substituted benzimidazoles
3a		$ClCH_2COOH$	
3b			
3c		$ClCH_2COOH$	
3d		$Cl\text{-}CH_2CH_2COOH$	
3e		$Cl\text{-}CH_2CH_2COOH$	
3f		$CH_3CH_2CH_2CH_2COOH$	
3g			
3h		$ClCH_2COOH$	

Table 2. Structures and nomenclature of substituted Benzimidazole -copper complexes.(4a-4h)

Sr.no	Chemical Structures of 1H-Benzimidazole Copper complexes	IUPAC Nomenclature of 1H-Benzimidazole Copper complexes
4a		Bis(2-(chloromethyl)-1H-benzo[d]imidazole-1-yl)copper(IV)chloride
4b		Bis1-(6-nitro-1H-benzo(d)imidazole-2yl)ethanol-1-yl)copper (IV)chloride
4c		Bis-(2-chloromethyl)-6-nitro-1-benzo(d)imidazole-1-yl)copper(IV)chloride
4d		Bis2-(2-chloroethyl)-6-methyl-1H-benzo(d)imidazole-1-yl)copper(IV) chloride
4e		Bis(2-(2-chloroethyl)-1H-benzo[d]imidazole-1-yl)copper(IV)chloride

4f		Bis2-(2-chloroethyl)-6-nitro-1H-benzo(d)imidazole-1-yl)copper(IV)chloride
4g		Bis1-(6-chloro-1H-benzo(d)imidazole-2-yl)ethanol-1-yl)copper (IV)chloride
4h		Bis(2-chloromethyl)-6-methyl-1H-benzo[d]imidazole-1-yl)copper(IV) chloride

General procedures for synthesis of benzimidazole-copper complexes.(4a-4h)

Synthesis of Bis (2-(chloromethyl)-1H-benzo[d]imidazole-1-yl) copper (IV) chloride (4a)

To a stirred solution of 2-(chloromethyl)-1H-benzo(d)imidazole (0.10 mol) (3a) in an ethanol, an alcoholic solution of $CuCl_2.2H_20$ (0.05mol) (b) was added, dropwise over 30 minutes at room temperature., the reaction mixture protected from light, was stirred at room temperature for 4 hrs. The resulting crude precipitate was filtered off and purified by repeated washing with small portions of water, ethanol and diethylether and dried. [6]. M.P 228-230,%yield-62.70%,IR [KBr] (cm^{-1}) 3039 (C-H stretching),1620& 1462(C=C),2949(-CH_2),3265(N-H stretching),1317(C-N vibration),738(C-Cl),433(Cu-N) .H^1NMR 4.-4.5[s,-CH_2(Ha)],4.5-5[s,-NH(Hb)],7-7.5[m-Ar-] .C^{13}NMR 137.9-141.5[s(-C)],115.2-123.0[(s(-CH)],36.8[s(-CH2)] MS:MS:$C_{16}H_{12}C_{l4}CuN_4$, m/e470, $C_{15}H_{10}Cl_3CuN_4$.m/e41 Composition C 41.09,H 3.02,Cl 30.32,Cu 13.59,N 11.98.

Synthesis of Bis 1-(6-nitro-1H-benzo (d) imidazole-2yl) ethanol-1-yl) copper (IV) chloride (4b)

To a stirred solution of 1-(6-nitro-1H-benzo(d)imidazole (0.10 mol) (3b) in an ethanol, an alcoholic solution of $CuCl_2.2H_2O$ (0.05 mol) (b) was added drop wise over 30 minutes 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. [6].M.P 234--236,%yield-55.30, IR [KBr] (cm^{-1}) 3026 (C-Hstretching),1606 & 1483 (C=C),3265 (N-H stretching),1317 (C-N vibration),432(Cu-N),1536(-NO_2) .H^1NMR: 1.49[d(-CH3)(Ha)] 5.0[s(N-H(Hb)],7.66-8.19[m(Ar-H(Hc)] C^{13}NMR :138.2-144.3[s(-C)],111.4-116.1[s(-CH)],22.5[s(-CH3)] MS:MS:$C_{18}H_{18}Cl_2CuN_6O_6$,m/e548.99,$C_9H_8Cl_3N_3O_3$, m/e207.06,$C_9H_9Cl_2CuN_3O_3$,m/e341.93. Composition:C 39.39,H 3.31 Cl 12.92 Cu 11.58 O 17.19.

Synthesis of Bis-(2-chloromethyl)-6-nitro-1-benzo(d)imidazole -1-yl)copper(IV)chloride (4c)

To a stirred solution of 2-chloromethyl-6-nitro-1-benzo(d)imidazole (0.10 mol) (3c) in an ethanol, an alcoholic solution of $CuCl_2.2H_2O$ (0.05 mol) (b) was added drop wise over 30 minutes 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. [6].M.P 240-242,%purity-54.36,IR[KBr](cm-1)3016(C-Hstretching),1618&1458(C=C),2916(CH_2),3057(N-H),1288(C-Nvibration),785(C-Cl),433(Cu-N).1531(-NO2) H^1NMR 7.66-8.19[m (-CH)] 5.0[s(N-H)],4.26 [s (-

CH_2)] C^{13} NMR 111.4-144.3[m(-C)],141.5[s(-CH)] MS:MS $C_{16}H_{12}Cl_4CuN_6O_4$ m/e556.89,$C_8H_6ClCuN_3O_2$ m/e211.01,$C_8H_6Cl_3N_3O_2$ m/e 345.88: Composition: C 34.45,H 2.17,Cl 25.43,Cu 11.40,N 15.07,O 11.48.

Synthesis of Bis 2-(2-chloroethyl)-6-methyl-1H-benzo(d)imidazole-1-yl)copper(IV)chloride (4d)

To a stirred solution of 2-(2-chloroethyl)-6-methyl-1H-benzo(d)imidazole (0.10 mol) (3d) in an ethanol, an alcoholic solution of $CuCl_2.2H_2O$ (0.05 mol) (b) was added 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. [6]. M.P 220-222,%yield-62.50,IR [KBr] (cm-1) 3003 (C-Hstretching),1602&1450(C=C),2802(CH_2),3226(N-H),740(C-Cl),437(Cu-N). H^1NMR 7.12-7.54[m]CH(Ha)],5.0[s(N-H(Hb)]2.83-3.71[t(-CH_2)], C^{13}NMR41.1[s(-CH2)],135.9-141.5[s(-C)],21.3[s(-CH3)] MS:MS C m/e522.99,$C_{10}H_{11}Cl_3CuN_2$ m/e328.93,$C_{10}H_{11}ClN_2$ m/e 194.06. Composition:C 34.46,H 2.17,Cl 25.43,Cu 11.40,N 15.07,O 11.48.

Synthesis of Bis(2-(2-chloroethyl)-1H-benzo[d]imidazole-1-yl)copper(IV)chloride (4e)

To a stirred solution of 2-(2-chloroethyl)-1H-benzo[d]imidazole-1-yl (0.10 mol) (3e) in an ethanol, an alcoholic solution of $CuCl_2.2H_2O$ (0.05 mol) (b) was added 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 [6]. M.P 260-262, % Purity-54.90, IR [KBr] (cm-1) 3013 (C-H stretching), 1600&1455 (C=C), 2799 (CH_2), 3230 (N-H), 744 (C-Cl), 437 (Cu-N). H^1MR 7.12-7.54[m(C-H),2.83-3.71[t (-CH_2)], 5.0 [s (-NH)], 2.34[d(-CH_3)].C^{13}NMR 137.9-141.5[s(C)],115.1-123 [s(CH)],33-41.1[s(-CH_2)] MS: MS $C_{18}H_{18}Cl_4CuN_4$ m/495.72, $C_{19}H_9Cl_3CuN_2$ m/e 314.93.$C_9H_9ClN_2$ m/e180.05. Composition:C 43.61,H 3.66,Cl 28.61,Cu 12.82,N 11.30.

Synthesis of Bis2-(2-chloroethyl)-6-nitro-1H-benzo(d)imidazole-1-yl)copper(IV)chloride (4f)

To a stirred solution of 2-(2-chloroethyl)-6-nitro-1H-benzo(d)imidazole (0.10 mol) (3f) in an ethanol, an alcoholic solution of $CuCl_2.2H_2O$ (0.05 mol) (b) was added 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. [6] M.P 254-256 ,%yield-64.90,IR cm-1 3005 (C-Hstretching),1598&1450(C=C),2800(CH_2),3234(N-H),744(C-Cl),434(Cu-N),734(C-Cl).H^1NMR7.66-8.19[m(-CH)],5.0[s(-NH)],2.83-3.71[t(-CH_2) C^{13}NMR 138.8-145.0[s(-C)],111.4-118.6[s(C-H)],33-41.1[s(C-H_2)]MS:MS $C_{18}H_{16}Cl_4CuN_6O_4$ m/e 584.93,$C_9H_9ClN_2$ m/e180.05,$C_9H_9Cl_3CuN_2$ m/e314.91.. Composition:C 36.91,H 2.75,Cl 24.21,Cu 10.85,N 14.35,0 10.93.

Synthesis of Bis 1-(6-chloro-1H-benzo(d)imidazole-2-yl)ethanol-1-yl)copper (IV)chloride (4g)

To a stirred solution of 1-(6-chloro-1H-benzo(d)imidazole ((0.10 mol (3g) in an ethanol, an alcoholic solution of $CuCl_2.2H_2O$ (0.05 mol) (b) was added drop wise over 30 minutes 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. [6] M.P 262-264, %yield-60.32,IR[KBr] (cm-1) 3019 (C-Hstretching),1600&1456(C=C),2800(CH_2),3236(N-H),742(C-Cl),436(Cu-N),3504-3704(O-H), H^1NMR1.49-2.34[q(-CH_3)],7.14-8.36[m(-CH)],5.0[s(-NH)],3.65[d(-OH)] C^{13} NMR 129.2-141.5[s(-C)],65-124.1[s(C-H)],22.5[s(C-H_2)]MS:MS $C_{18}H_{18}Cl_4CuN_4O_2$ m/e 526.95,$C_9H_9Cl_3N_2O$ m/e 328.91,$C_9H_9CuN_2O$ m/e 196.04. Composition:C 40.97,H 3.44,Cl 26.87, Cu 12.04,N 10.62,0 6.06.

Synthesis of Bis(2-chloromethyl)-6-methyl-1H-benzo[d]imidazole-1-yl)copper(IV) chloride (4h)

To a stirred solution of 2-chloromethyl)-6-methyl-1H-benzo[d]imidazole (0.10 mol) (3h) in an ethanol, an alcoholic solution of $CuCl_2.2H_2O$ (0.05 mol) (b) was added drop wise over 30 minutes 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 [6]. M.P 236-238,%yield-60.12,IR[KBr] (cm-1) 3020 (C-H stretching),1601&1454(C=C),2802(CH_2),3232(N-H),744(C-Cl),432(Cu-N).H^1NMR 4.26-7.54[s (-CH_2)],2.34[q(-CH_3)],5.0[s(-NH)].C^{13}NMR 21.3[s(-CH3)],115.1-141.5[s(-CH)],36.8[s(-CH_2)MS:MS $C_{18}H_{18}Cl_4CuN_4$ 494.2,$C_9H_9Cl_3CuN_2$ 314.91,$C_9H_9ClN_2$ 180.05.Composition:C 43.61,H 3.66, Cl 28.61,Cu 12.82,N 11.30.

Table 3. Physicochemical constants of benzimidazole-copper complexes. (4a-4h)

Sr.No.	R	R^1	Molecular formula	Molecular weight	% yield	M.P (^{O}c)	Mobile phase composition	R_f value
[4a]	CH_2Cl	H	$C_{16}H_{12}Cl_4CuN_4$	467.67	62.70	228-230	EtA:nH 2:3	0.51
[4b]	CH_2CHOH	NO_2	$C_{18}H_{18}Cl_2CuN_6O_6$	548.82	55.30	234-236	EtA:nH 2:3	0.57
[4c]	CH_2Cl	NO_2	$C_{16}H_{12}Cl_4CuN_6O_4$	557.66	54.36	240-242	EtA:nH 2:3	0.52
[4d]	CH_2CH_2Cl	CH_3	$C_{16}H_{12}Cl_4CuN_6O_4$	523.77	62.50	220-222	EtA:nH 1:1	0.52
[4e]	CH_2CH_2Cl	H	$C_{18}H_{18}Cl_4CuN_4$	495.72	54.90	260-262	EtA:nH 1:1	0.53
[4f]	CH_2CH_2Cl	NO_2	$C_{18}H_{16}Cl_4CuN_6O_4$	585.72	64.90	254-256	EtA:nH 1:1	0.48
[4g]	CH_3CHOH	Cl	$C_{18}H_{18}Cl_4CuN_4O_2$	527.72	60.32	262-264	EtA:nH 1:1	0.44
[4h]	CH_2Cl	CH_3	$C_{18}H_{18}Cl_4CuN_4$	495.72	60.12	236-238	EtA:nH 1:1	0.41

3. Pharmacological Evaluation

Brine shrimp lethality bioassay

Standard: Cisplatin

Solvent: DMSO

Test samples: 4a-4h

Brine Shrimp Lethality Bioassay

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of synthesis compound. Brine shrimp lethality bioassay is easily mastered, costs little and it utilizes small amount of test compound. This provides a front line screen that can be backed up by more specific and expensive bioassay.

This *in vitro* lethality test has been successfully used as a preliminary study of antitumor agents. [26]

Preparation of brine solution

38 g of iodised 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 marine 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 each of compounds were dissolved in 10 ml of DMSO to obtain the stock concentration of 1000 µg/ml and then stock solution was diluted to various concentrations 100, 10, 1 µg/mL. In order to prevent the toxicity results from possible false effect originated from DMSO's toxicity, stock solutions of the compounds were prepared according to suggested volume range by dissolving in DMSO. Pure DMSO was used as a positive control for the toxicity assay. [27]

Application of test solution and larvae to the test tubes

About 5 ml of brine solution was taken into each test tube. Suitable dilutions of the test substance were made as per the concentration. The 0.05 ml diluted test solution was added to the test tubes.

- 30 active shrimps (larvae) were added into each test tube
- The solution should be mixed thoroughly
- The surviving (larvae) shrimps were counted after 24 hours and lethality concentration LC_{50} was assessed.

4. Results and Discussion

Chemistry:

Synthetic pathways of benzimidazole is outlined in scheme 1 in a typical experimental procedure substituted o-phenylenediamine was allowed to react with alkyl/aryl acids derivative in presence of 4N HCl to give benzimidazole..wherein,lone pair of nitrogen attacks on the positively charged carbon atom of carboxylic acid (nucleophillic substitution).acid catalyst protonates one of the OH group, making it good leaving group as H_2O.Then lone pair from oxygen will form carbonyl and removal of H_2O molecule takes place.second NH_2 molecule from OPD will get involved in formation of 2^{nd} aromatic ring.finally,actual formation of aromatic ring

& lone pair from nitrogen will push into the ring & H_2O molecule will remove as a biproduct.this benzimidazole get complexated with copper to form benzimidazole-copper complexes and evaluated for their cytotoxic activity. [28] Synthetic pathways of benzimidazole- copper complexes [4a-4h] depicted in scheme 2 .in a typical experimental

Procedure, To a stirred solution of corresponding substituted benzimidazole(0.10mol) in an ethanol, an alcoholic solution of $CuCl_2.2H_2O$ (0.05 mol)(b) was added drop wise over 30 min at room temperature. The reaction mixture protected from light was stirred at room temperature for 4hrs.the purity of all compounds was checked by TLC.the structure of all synthesized compounds was established by IR,^{1}H,^{13}C NMR & mass spectral analysis. [28-30] Results of elemental analysis of synthesized compounds was in agreement with theoretical values. the outcome of in vitro cytotoxic studies of newly synthesized compounds revealed that these compounds have significant cytotoxic activity.

Biology:

Brine shrimp lethality bioassay was performed in laboratory. Complexes has solubility problem so it should be dissolved in DMSO for the preparation of drug solution. Following results were obtained by which LC_{50} was calculated. These results were compared with standard drugs i.e. cisplatin. The positive control was done with DMSO.

1. 30 active shrimps (larvae) were added into each test tube
2. No. of brine shrimps taken: 30

Table 4. Brine Shrimp Lethality Bioassay of benzimidazole-copper complexes.(4a-4h)

Comp.	Conc. Ppm	Mortality of shrimps			% mean mortality	Lc_{50} µg/Ml
		I	II	III		
4a	1000	15	16	15	51.15	910.09
	100	12	13	13	42.23	
	10	6	6	7	21.13	
	1	1	3	3	7.79	
4b	1000	24	21	24	76.69	611.99
	100	15	16	18	54.45	
	10	13	11	12	40.10	
	1	7	5	8	22.25	
4c	1000	26	25	27	86.70	541.79
	100	18	19	18	61.13	
	10	11	11	13	38.91	
	1	8	6	6	22.24	
4d	1000	18	19	18	61.14	765.69
	100	13	14	14	45.58	
	10	7	8	9	26.70	
	1	3	5	5	14.47	
4e	1000	14	15	15	48.91	959.69
	100	11	10	11	35.60	
	10	6	5	5	17.80	
	1	4	5	4	14.47	
4f	1000	14	14	13	45.60	1022.49
	100	11	10	11	35.56	
	10	8	7	3	26.70	

	1	2	3	4	10.11	
4g	1000	15	15	17	52.25	892.85
	100	11	12	14	41.15	
	10	6	7	4	18.85	
	1	4	2	2	8.85	
4h	1000	18	17	19	60.15	772.79
	100	16	14	15	50.10	
	10	11	10	8	32.24	
	1	6	5	6	18.90	
Cisplatin	1000	21	24	21	73.35	642.67
	100	14	15	16	50.00	
	10	9	8	9	27.80	
	1	6	5	4	17.80	

Positive control with DMSO has shown mortality of 2 shrimps.

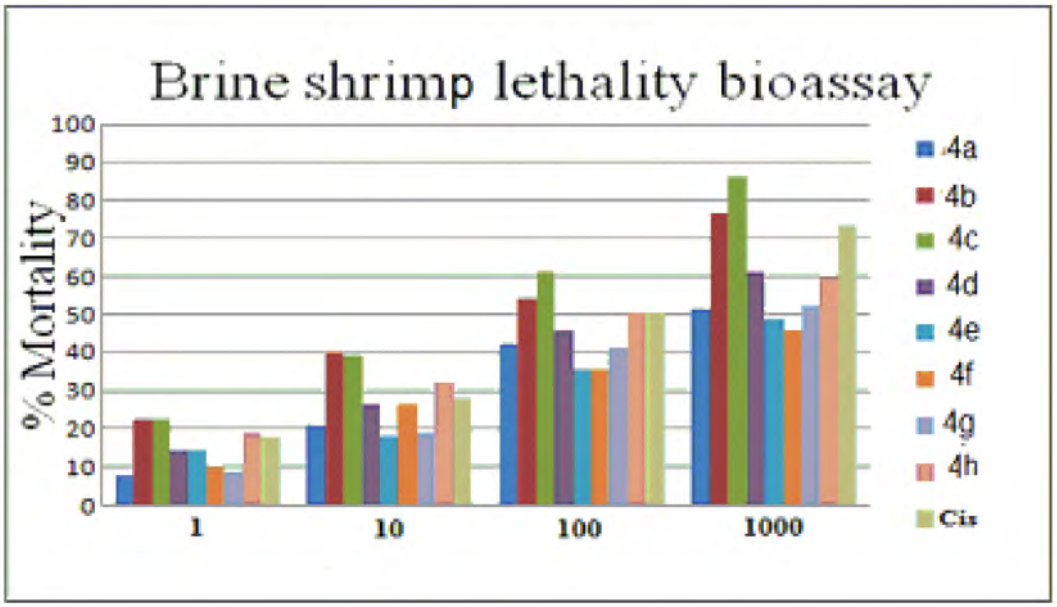


Figure 2. Graphical representation showing activity of Benzimidazole-Copper complexes with respect to Cisplatin.

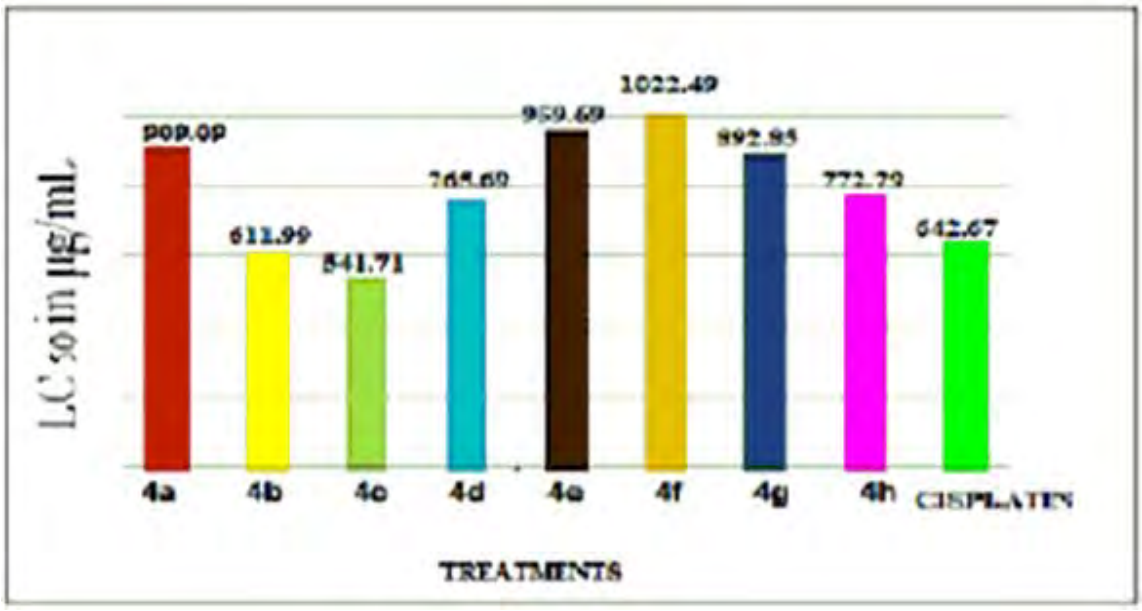


Figure 3. Graphical representation showing LC_{50} values of Benzimidazole Copper complexes with respect to Cisplatin.

5. Conclusion:

The substituted 1H-Benzimidazoles as a ligand were synthesized and exposed to complexation with copper. All synthesized complexes were appraised for cytotoxic activity by Brine shrimp lethality bioassay. Cytotoxicity was calculated in terms of LC_{50}. In conclusion, it was found that unsubstituted o-phenylenediamine gives more % yield than substituting one. All complexes compounds showed significant cytotoxic activity. Among all complexes, complex (4c) showed more cytotoxic activity and it is more cytotoxic than Cisplatin. Complex (4e) was found to possess least cytotoxic activity. Complexes (4b) and (4d) showed moderate cytotoxic activity.

The synthesized complexes were characterized by IR, H^1 NMR, MS and DSC. The synthesized compounds, i.e. benzimidazole copper complexes were adequately confirmed by H^1 NMR., IR spectra of complexes show the vibration at 395-455 cm-1 for Cu-N. From DSC graph provided in the supplementary files for complex (4a), melting point of this complex is observed at 228-230^oC and Hence it is concluded that the complex (4a) had been synthesized successfully.

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