

## Copper (II) Complexes of Bishydrazone Derivatives: Synthesis, Characterization and Urease Inhibition Studies

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**Abstract:** Six new Copper(II) complexes with bishydrazone derived from the condensation of 5-chloro-isatin monohydrazone with different substituted benzaldehyde, have been synthesized and characterized by using various spectroscopic and physicochemical techniques including FT-IR, EI-mass, elemental analysis, UV-Visible, magnetic susceptibility and conductivity measurements. The magnetic and electronic data indicate square planer geometry. These synthesized complexes were also screened for urease inhibition activity. The result shows that the urease inhibition activity enhanced with the complexation.

**Key words:** Bishydrazone • 5-Chloro-isatin monohydrazone • Urease inhibition activity • Complexation

### INTRODUCTION

Synthesis of new ligands is the most effective approach to develop metal complexes having unique properties and novel reactivity. Bishydrazone belongs to one of important class of organic compounds that can behave as ligands which are medically of prime importance [1]. A number of biological activities were studied with the isatin derivative of bishydrazone, including anticancer [2], anti-inflammatory [3], anticonvulsant, antidepressant, antimicrobial [4], antifungal [5] and antiglycation [4-6].

Biological activities of the ligands that contained various donor atoms (N, O, S, etc.) can be enhanced or altered due to the coordination of ligands with the metal ions [1, 7, 8]. Thus a substantial rising interest in the design of coordination complexes as drugs and diagnostic agents is currently perceived in the area of scientific investigations. Coordination complexes of bishydrzones also exhibit a number of pharmacological activities, such as anticancer, antiviral, antibacterial, antifungal and antimalarial [8-15].

Urease is one of the nickel containing metallo enzyme that is used to hydrolyze urea into  $\text{NH}_3$  and  $\text{CO}_2$ . The

resulting carbamate spontaneously decomposes to yield a second molecule of ammonia and carbon dioxide. Reactions catalyzed by urease may cause high concentration of ammonia and pH elevation which have important negative implication in medicine and agriculture. These negative effects can be counterbalanced by urease inhibitors, which control the activity of urease [15].

Recently urease inhibitors have played a vital role in the treatment of the infections caused by urease producing bacteria [16]. Urease inhibitors can be broadly classified into two fields: (1) organic compounds, such as acetohydroxamic acid, humic acid and 1,4-benzoquinone, (2) heavy metal ions, such as  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Pd}^{2+}$  and  $\text{Cd}^{2+}$  [17]. Some of these inhibitors are prevented from use in vivo because of their toxicity or instability. Thus, interest has been developed to synthesize new urease inhibitors either organic or metal complexes with good bioavailability and low toxicity [18-19].

Copper is one of the important elements that are present in very trace amounts but vital to the health of living organisms. Due to its important biological behavior, many copper complexes with bishydrzones have been reported [8]. In the present work, we aimed to synthesize the Cu(II) complexes with the bishydrazone ligands to

study the coordination behavior and also investigate its urease inhibition activities in complexes and uncomplexed bishydrazones.

## MATERIALS AND METHODS

**Physical Measurements:** Proton NMR spectrum were recorded on Avance Bruker AM 300 MHz. Carbon, hydrogen and nitrogen analysis was performed on Perkin Elmer 2400 series II CHN/S analyzer. Infrared spectrum of the bishydrazone and its complexes were recorded through KBr disc on Shimadzu IR-prestige-21. Metal contents of the synthesized complexes were analyzed using Perkin Elmer series 200 atomic absorption spectrometer. Molar conductance measurements were made at room temperature in DMSO using JENAY 4701 conductivity meter. Electronic spectrum of the ligands and complexes were recorded on Perkin-Elmer Lambda-5 UV/Vis spectrometer and magnetic moment was carried out by using Sherwood MSB Mk1. Enzyme inhibition activities was performed on Spectramax plus 384 Molecular Device-USA.

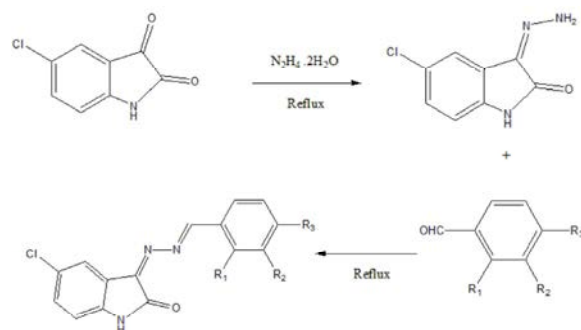
**Materials:** All the chemicals used were of analytical grade (BDH and Sigma-Aldrich) and used without further purification. All the solvents were distilled before use.

**Synthesis of Bishydrazone Derivatives:** The 5-chloro-isatin monohydrazone was prepared by refluxing together 0.5M solutions of 5-Chloro isatin and hydrazine hydrate in methanol for 2-3 hours at 60°C to 70°C. On cooling the resulting yellow color crystals of monohydrazone was recrystallized using methanol.

Resultant crystals of monohydrazone were further treated with the different substituted aromatic aldehyde in 1:1 ratio; the reaction mixture was reflux for 3-4 hours in methanol. The solid product was filtered after cooling at room temperature and washed with  $\text{CCl}_4$ . The product was recrystallized with methanol and dried in vacuum to furnish the required bishydrazone [6]. *Scheme 01* representing the synthesis of bishydrazone derivatives.

**Synthesis of Copper(II) Complexes:** Copper(II) complexes of the bishydrazone were prepared by refluxing together the solution of 0.2M  $\text{CuCl}_2$  (25ml) and 0.4M (30ml) solution of bishydrazone in methanol for 2 hours at steam bath. The resulting solution was concentrated, filtered and washed with  $\text{CCl}_4$ . Resulting microcrystalline powder of Copper(II) complexes were obtained with slow evaporation from methanolic solution.

**Urease Inhibition Activity Experiments:** Urease inhibition activity was determined by modified indophenol method based on measurement of product (ammonia) formed



Scheme 1: Synthesis of Bishydrazones from 5-Chloro isatin

Bishydrazone	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
BH-1	-H	-H	-OCH <sub>3</sub>
BH-2	-H	-H	-N(CH <sub>3</sub> ) <sub>2</sub>
BH-3	-H	-OCH <sub>3</sub>	-OCH <sub>3</sub>
BH-4	-H	-OH	-OH
BH-5	-OCH <sub>3</sub>	-H	-H
BH-6	-H	-H	-OH

during the reaction [20-21]. Phosphate buffer of pH 8.2 (0.01M  $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ , 1-mM EDTA and 0.01 M  $\text{LiCl}$ ) was taken in 96 well plates marked as Blank, Control and Inhibitor. Urease (Jack bean) solution (10  $\mu\text{L}$ ) was added and mixed with the 5  $\mu\text{L}$  of test compound of concentration ranging from 5  $\mu\text{M}$  to 500  $\mu\text{M}$  and incubated at 30°C for 15 min. 50  $\mu\text{L}$  each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70  $\mu\text{L}$  of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to wells. Increase in absorbance was measured after 50 min at 630 nm on micro plate reader. All reactions were performed in triplicates. The standard used in this assay was thiourea and percentage inhibitions were calculated by formula:

$$\text{Inhibition (\%)} = \frac{(\text{OD control} - \text{OD test comp})}{\text{OD control}} \times 100$$

The  $\text{IC}_{50}$  values were then calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc. Amherst, MA, USA).

## RESULT AND DISCUSSION

The results of the elemental analysis of the prepared bishydrazone ligands and their Copper(II) complexes were reported in Table 1 together with the respective molar conductivity and magnetic moment data. The results suggested that the bishydrazones acted as neutral bidentate ligands to form the complexes at 1:2 metal to ligand molar ratio, all the complexes being 1:2 electrolytes.

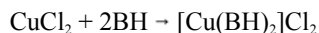
Table 1: Elemental analysis of bishydrazones and its Cu(II) complexes along with the molar conductance and magnetic moment data.

Compounds	Empirical formula Formula mass (g/mol)	%Yield	Cu%		C%		H%		N%		Conductance ( $\mu$ s)	$\mu_{\text{eff}}$ (BM)
			Obs	Cal	Obs	Cal	Obs	Cal	Obs	Cal		
BH-1	$\text{C}_{16}\text{H}_{12}\text{ClN}_3\text{O}_2$ 313.0618	77	-	-	61.09	61.25	3.69	3.86	13.18	13.39	---	---
$[\text{Cu}(\text{BH}-1)_2]\text{Cl}_2$	$[\text{Cu}(\text{C}_{32}\text{H}_{14}\text{Cl}_2\text{N}_3\text{O}_4)_2]\text{Cl}_2$ 759.0009	64	8.21	8.34	50.81	50.44	3.02	3.17	10.91	11.03	163	1.88
BH-2	$\text{C}_{17}\text{H}_{15}\text{ClN}_4\text{O}$ 326.0934	86	-	-	62.34	62.48	4.49	4.63	16.94	17.15	---	---
$[\text{Cu}(\text{BH}-2)_2]\text{Cl}_2$	$[\text{Cu}(\text{C}_{34}\text{H}_{30}\text{Cl}_2\text{N}_8\text{O}_2)_2]\text{Cl}_2$ 785.0642	67	8.16	8.06	51.51	51.82	3.69	3.84	13.93	14.22	158	1.89
BH-3	$\text{C}_{17}\text{H}_{14}\text{ClN}_3\text{O}_3$ 343.0724	72	-	-	59.52	59.40	3.98	4.10	11.98	12.22	---	---
$[\text{Cu}(\text{BH}-3)_2]\text{Cl}_2$	$[\text{Cu}(\text{C}_{34}\text{H}_{28}\text{Cl}_2\text{N}_6\text{O}_6)_2]\text{Cl}_2$ 819.0220	73	6.62	7.73	49.47	49.68	3.52	3.43	10.34	10.22	171	1.91
BH-4	$\text{C}_{15}\text{H}_{10}\text{ClN}_3\text{O}_3$ 315.0411	71	-	-	60.07	60.11	3.14	3.36	14.31	14.02	---	---
$[\text{Cu}(\text{BH}-4)_2]\text{Cl}_2$	$[\text{Cu}(\text{C}_{30}\text{H}_{20}\text{Cl}_2\text{N}_6\text{O}_6)_2]\text{Cl}_2$ 762.9594	68	8.49	8.66	49.37	49.10	2.62	2.75	11.22	11.45	157	1.83
BH-5	$\text{C}_{16}\text{H}_{12}\text{ClN}_3\text{O}_2$ 313.0618	82	-	-	61.33	61.25	3.88	3.86	13.16	13.39	---	---
$[\text{Cu}(\text{BH}-5)_2]\text{Cl}_2$	$[\text{Cu}(\text{C}_{32}\text{H}_{24}\text{Cl}_2\text{N}_6\text{O}_4)_2]\text{Cl}_2$ 759.0009	62	8.41	8.34	50.69	50.44	3.04	3.17	10.88	11.03	154	1.85
BH-6	$\text{C}_{15}\text{H}_{10}\text{ClN}_3\text{O}_2$ 299.0462	85	-	-	47.38	47.07	2.98	3.19	13.07	13.31	---	---
$[\text{Cu}(\text{BH}-6)_2]\text{Cl}_2$	$[\text{Cu}(\text{C}_{30}\text{H}_{20}\text{Cl}_2\text{N}_6\text{O}_4)_2]\text{Cl}_2$ 730.9696	68	8.17	8.30	47.08	47.05	2.69	2.63	10.63	10.97	169	1.89

Table 2: Proton NMR spectra of Bishydrazones

Compounds	Chemical shift (ppm)				
	Phenolic Ar-OH	Indole Ring-NH	Azomethine -CH-N	Indole Ring-H	Aromatic Ar-H
BH-1	---	10.90	8.75	7.5-8.8	6.99-7.85
BH-2	---	10.84	8.61	7.4-8.7	6.88-7.89
BH-3	---	10.97	8.67	7.5-8.9	6.92-7.58
BH-4	10.9	9.60	8.57	6.8-7.4	6.90-7.25
BH-5	---	10.83	8.81	7.6-8.8	7.03-7.97
BH-6	11.8	10.87	8.74	6.9-7.7	7.00-7.60

Thus, the general formula of the synthesized complexes was found to be  $[\text{Cu}(\text{BH})_2]\text{Cl}_2$  and the complexes were formed according to the equation:



Complexes are stable and non-hygroscopic with very little solubility in organic solvents but highly soluble in DMSO and DMF. The results shown in Table-1 coincided well with the expected complexes composition.

**<sup>1</sup>H NMR Study of Bishydrazones:** In the Proton NMR spectrum of the six different bishydrazones derivatives, characteristics signal due to its azomethine proton (-CH-N) was observed around 8.57-8.81. The -NH of indole ring exhibits its signals at around 9.60-10.97.

Phenolic proton of BH-4 and BH-6 gives their peaks at 10.9 and 11.80 respectively; this indicates its involvements in H-bonding. Multiplet signals around 6.88-7.97 were ascribed to aromatic protons (Ar-H). Summarized data of the proton NMR is presented in Table 2.

**R Spectral Studies:** Bishydrazones derivatives shows strong band in the range of 3171-3273  $\text{cm}^{-1}$  attributed to indole ring (N-H) while lactonyl carbon (C=O) exhibits its characteristics band at around 1724-1745  $\text{cm}^{-1}$ . In addition to these bands, strong bands were also observed in the region of 1591-1614  $\text{cm}^{-1}$  attributed to aldimine HC-N group. IR spectrum of bishydrazones derivatives also exhibited some other strong bands like 1668-1681  $\text{cm}^{-1}$ , 1273-1309  $\text{cm}^{-1}$ , 1002-1024  $\text{cm}^{-1}$  and 808-835  $\text{cm}^{-1}$  which were assigned to ketimine (C=N), phenolic (C-O), hydrazinic (N-N) and C-Cl groups respectively.

All the Copper(II) complexes exhibits significant positive and negative shifts in bands (1574-1584  $\text{cm}^{-1}$ ) originating from aldimine HC-N stretching frequency as compare to free ligands of bishydrazones. This indicates the coordination of nitrogen with the Copper(II) ion.

Another considerable 23-41  $\text{cm}^{-1}$  shift in lactonyl carbon were also observed in the Copper(II) complexes, confirming the binding of carbonyl oxygen with the metal ion.

Table 3: Important IR spectral bands of the bishydrazone ligands and its Copper(II) complexes (cm<sup>-1</sup>)

Compounds	Indol			Ketimine	Aldimine	Phenolic	Hydrazinic			
	$\nu$ (OH)	$\nu$ (NH)	$\nu$ (C=O)	$\nu$ (C=N)	$\nu$ (HC=N)	$\nu$ (C-O)	$\nu$ (N-N)	$\nu$ (C-Cl)	$\nu$ (Cu-N)	$\nu$ (Cu-O)
BH-1	--	3192	1745	1681	1591	1274	1022	810	---	---
[Cu(BH-1) <sub>2</sub> ]Cl <sub>2</sub>	---	3187	1704	1677	1574	1271	1017	813	411	527
BH-2	---	3190	1735	1681	1600	---	1002	810	---	---
[Cu(BH-2) <sub>2</sub> ]Cl <sub>2</sub>	---	3188	1698	1697	1577	---	1005	808	417	516
BH-3	---	3174	1743	1681	1591	1273	1022	808	---	---
[Cu(BH-3) <sub>2</sub> ]Cl <sub>2</sub>	---	3171	1702	1682	1577	1274	1019	815	416	526
BH-4	3390	3271	1724	1675	1614	1309	1024	813	---	---
[Cu(BH-4) <sub>2</sub> ]Cl <sub>2</sub>	3382	3267	1697	1675	1584	1306	1019	819	412	523
BH-5	---	3273	1737	1670	1608	1296	1024	835	---	---
[Cu(BH-5) <sub>2</sub> ]Cl <sub>2</sub>	---	3268	1702	1664	1579	1291	1027	827	411	526
BH-6	3292	3180	1724	1668	1608	1286	1010	812	---	---
[Cu(BH-6) <sub>2</sub> ]Cl <sub>2</sub>	3290	3184	1701	1664	1582	1289	1006	807	419	531

Table 4: IC<sub>50</sub> values against Urease inhibition activity

Compound	IC <sub>50</sub> (μM)		Compound	IC <sub>50</sub> (μM)	
	Mean	+ SEM		Mean	+ SEM
BH-1	NA		CuBH-4	46.30	± 0.61
CuBH-1	31.25	± 0.59	BH-5	>100	
BH-2	NA		CuBH-5	32.05	± 0.41
CuBH-2	49.21	± 0.69	BH-6	>100	
BH-3	>100		CuBH-6	44.64	± 0.31
CuBH-3	37.71	± 0.15	Thiourea	21.2	± 0.08
BH-4	NA				

SEM (standard error of mean)

NA (not active)

\* Standard Urease inhibition activity

Stretching frequencies of N-H (indol ring) and C-N (ketimine) in all the Copper(II) complexes remain unaltered indicating that these groups were not involved in coordination. The new bands in the region of 516-531 cm<sup>-1</sup> and 411-419 cm<sup>-1</sup> in the spectra of the complexes were assigned to stretching frequencies of Cu-O and Cu-N bonds respectively.

Thus the Infrared spectral results provide strong evidences for the complexation of bishydrazone derivatives with Cu (II) ions in bidentate mode.

The major infrared absorption bands of the bishydrazone and its copper (II) complexes are summarized in Table 3.

**Electronic Spectral and Magnetic Studies:** The electronic spectrum of bishydrazone derivatives exhibited two absorption bands in the region of 320-360nm, which were assigned to n-π\* transitions of the aldimine and ketimine moieties, respectively [22-24].

In the electronic spectrum of the Copper(II) complexes, the n-π\* transitions were slightly shifted

towards lower wavelength suggesting that structure of ligands after chelation remain unaltered. The broad band at 710-740nm is attributed to the <sup>2</sup>B<sub>1g</sub> → <sup>2</sup>A<sub>1g</sub> transitions, which is well-suited with a square planer geometry around the Copper(II) ion in the coordination complexes [22-24].

Magnetic moment values of the complexes vary in the range of 1.83 to 1.91 BM, is slightly higher than spin only value (1.73) suggesting consistency with the square planer geometry and also confirm the absence of metal-metal interaction in the complexes [22].

The proposed structure of Cu(II) complexes of bishydrazone derivatives is given in Fig. 01.

**Urease Inhibition Assay:** Urease inhibition ability of the bishydrazone derivatives and its Copper(II) complexes were studied by calculating the IC<sub>50</sub> values of the material tested against jack bean urease. The results of urease inhibitory activity have been summarized in Table 4.

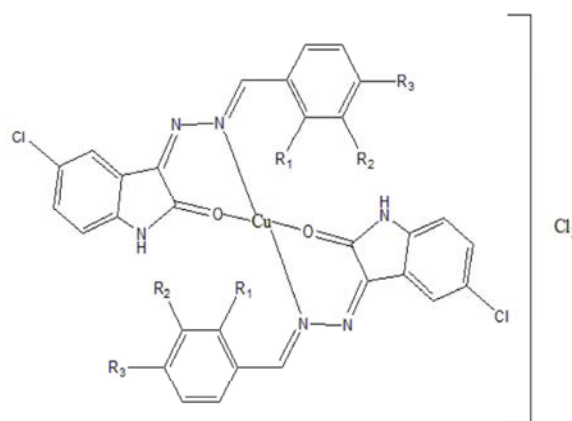


Fig. 1: Structure of Cu(II) complexes with bishydrazones.

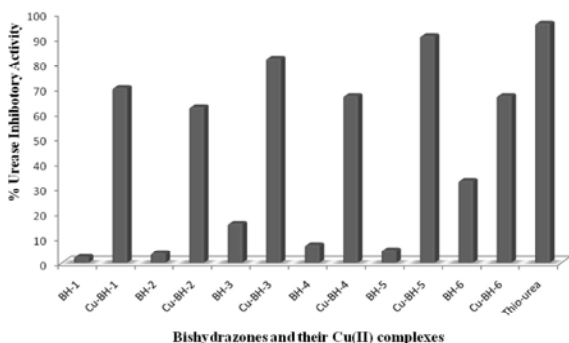


Fig. 2: Urease Inhibitory Activity of Bishydrazones and their Cu(II) complexes /100 $\mu$ M

As an enzyme inhibitor, ligand had no ability to inhibit urease, there  $IC_{50}$  values are much higher than the standard i.e. thiourea ( $IC_{50} = 21.2 \pm 0.08$  iM). On the other hand, Copper(II) complexes had much stronger urease inhibitory activities with  $IC_{50}$  values in the range of 31.25-49.21 iM. This shows that inhibitory efficiency toward urease increase on coordination with metal ion. This pronounced increase in the activity toward urease, perhaps due to the strong Lewis acid properties of Cu(II) ions or may be due to the formation of chelate (a less polar form of the metallo element) [18].

It was also clear from the Fig. 02 that CuBH-1 and CuBH-5 shows stronger ability to inhibit urease than others complexes and both contains  $-OCH_3$  group in their structure at aromatic ring at para and ortho positions respectively, suggesting the importance of substitution on these positions in the inhibitory action against urease.

## CONCLUSION

In this study, the Copper(II) complexes with bishydrazones derived from 5-chloro-isatin-mono-hydrazones and different substituted aromatic aldehydes were synthesized and characterized. These bishydrazones act as bidentate ligand. On the basis of different techniques, the structure of the complexes is proposed (Fig. 1). Bishydrazones were found to be moderate antioxidant while its Copper(II) complexes exhibit substantial urease inhibitory activity.

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