Influence of 2-mercapto Benzimidazole Derivative and its Copper (II) Complexes on the Growth Activity of *Oncidium* Gower Ramsey

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Abstract

Copper(II) complexes of 2-((1-methyl-1H-imidazol-2-ylthio)methyl)-5-chloro-1H-benzo[d]imidazole were synthesized using 2-mercapto benzimidazole derivative and Copper(II) salts. The conductance measurements confirmed the non-electrolyte nature of the synthesized complexes. While IR and magnetic susceptibility studies indicates distorted tetrahedral geometry around the metal ion. Plant growth activity of synthesized Copper(II) complexes studied using Protocorm like Bodies (PLBs) of *Oncidium* Gower Ramsey showed that the compounds enhanced the production of embryos/shoots from in vitro cultured sections of PLBs.

Keywords: *Benzimidazole derivative, 2-mercapto-N-methyl imidazole, plant growth regulators, Oncidium*

1.0 Introduction

Among organic compounds, benzimidazole derivatives are found to exhibit variety of biological activities like antiviral and antibacterial [1-2] anticancer [3], anticandidal [4-5], increased seed germination percentage in wheat [6-7] and enhanced production of somatic embryos/plantlets in *Oncidium* [8]. Biological activity of benzimidazole derivatives was found to enhance the formation of complexes with transition metal ions [9-10]. *Oncidium* Gower Ramsey is an important orchid grown worldwide for its long inflorescence. In vitro propagation of *Oncidium* Gower Ramsey is influenced by many factors including major and minor nutrients, carbon source, growth adjuvants, growth regulators, etc [11–15]. We have shown earlier that 2-mercapto-N-methyl imidazole substituted benzimidazole derivatives have plant growth regulator activities [16].

In continuation of our research work, we have reported in this paper, the synthesis of Copper(II) complexes of 2-((1-methyl-1H-imidazol-2-

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ylthio)methyl)-5-chloro-1H-benzo[d]imidazole (ligand) and studies on their plant growth activity.

2.0 Experimental

2.1 Material and Methods

The analytical grade chemicals were used in the study. Magnetic susceptibility study of the synthesized complexes was done on Sherwood scientific magnetic susceptibility balance. CHN analysis and conductance measurement were carried using Carlo-Erba analyzer and Elico model-180 conductivity meter respectively.

2.2 Synthesis of 2-((1-methyl-1H-imidazol-2-ylthio)methyl)-5-chloro-1H-benzo[d]imidazole (heterocycle)(Ligand)

Titled ligand was prepared according to procedure available in the literature [17-18]. A mixture of 1 mmol each of precursor 2-mercapto-N-methyl imidazole and NaOH in alcohol medium (10 mL) was stirred for about 45 min. To this, 1 mmol of methanol solution (10 mL) of 6-chloro-2-(chloromethyl)-1H-benzimidazole was added slowly and kept reflux for about 6-8 hrs. Result compound was purified by chromatographic technique.

2.3 Synthesis of Copper(II) complexes

Chloride and bromide salts of Copper(II) were made to react with synthesized heterocycle in 1:1 molar ratio in alcohol medium at refluxing temperature to yield brown colored complexes of the composition $[CuLX_2]$, where X= Cl^- or Br^-.

Physical properties and analytical data of the synthesized heterocycle and Copper(II) complexes are given in Table-1.

Table 1. Physical properties and analytical data of chloro and bromo complexes of Copper(II) containing Ligand

Complex	Color	M P (°C)	μ^a_{eff} (BM)	Λ^b_M ($\Omega^{-1}cm^2mol^{-1}$)	Analytical data (%)*		
					C	H	N
Ligand (L)	White	132	-	-	51.70 (51.28)	3.98 (3.61)	20.10 (20.43)
$[CuLCl_2]$	Brown	225	1.94	24	32.23 (32.05)	2.78 (2.46)	12.32 (12.46)
$[CuLBr_2]$	Brown	205	1.90	16	28.55 (28.67)	2.01 (2.20)	11.29 (11.15)

[a] Faraday method, [b] Molar conductance of Ca. 10^{-3} M solution in DMF around 25 °C

* Calculated values in parenthesis

2.4 Plant growth study

The PLBs of *Oncidium* 'Gower Ramsey' were initiated using inflorescence stalk and maintained on modified Murashige and Skoogs (MS) nutrient medium as reported in the literature [13]. PLBs sections were cultured on Murashige and Skoogs medium and which are supplemented with synthesized ligand and Copper(II) complexes at different concentrations (2µM or 5µM). In each culture bottle, five PLBs sections were placed, three replicates were maintained for different concentration and each experiment repeated twice. The cultures were maintained under controlled environmental conditions and structural changes were observed with respect to number of explants responded on nutrient MS medium and quantity of shoots developed from each PLBs section were recorded.

3.0 Results and Discussion

3.1 FT IR study

The Infrared spectra of the Copper(II) complexes (Fig. 1) were recorded as KBr pellets. Spectral peaks of heterocycle are compared with the metal complexes confirmed the coordination between them through metal ion. Copper(II) complexes have shown an absorption band around 3100-3200 cm^{-1} agreeing to $\nu_{(N-H)}$ stretching vibrations, which is around 3300 cm^{-1} in uncoordinated heterocycle. The absorption bands around 1620 cm^{-1} corresponding to of $\upsilon_{C=N/\ C=C}$ vibrations are slightly shifted to lower frequency in the case of Copper(II)complexes indicating the binding of imine nitrogen of the heterocycle [19-21]

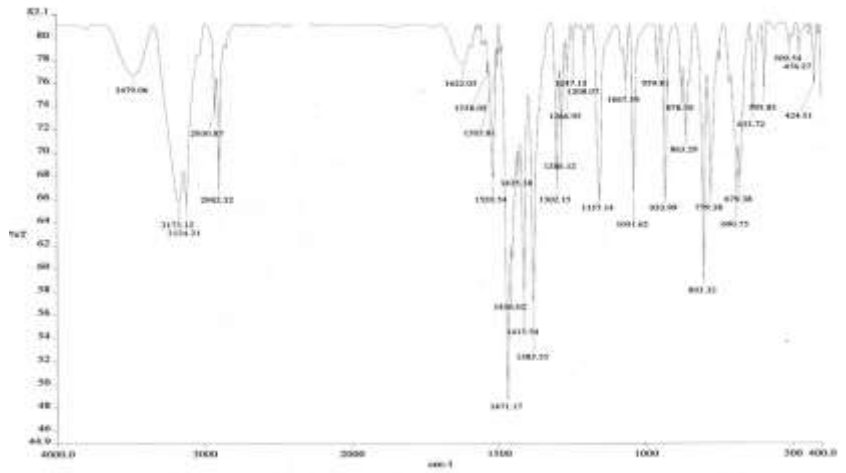

Fig. 1. FT IR spectrum (in KBr) of $CuLCl_2$

The Far-IR spectra of the halide complexes (Fig. 2) have been recorded in polyethylene powder and bands corresponding to terminal halides stretches have been identified [22]. The bands correspond to ν_{Cu-Cl} and ν_{Cu-Br} were recorded at 296 and 258 cm^{-1} respectively.

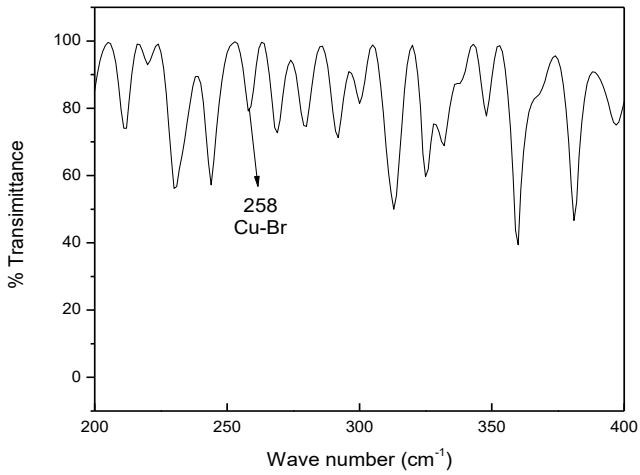

Fig. 2. Far IR spectrum (in polyethene) of $CuLBr_2$

3.2 Magnetic susceptibility measurements

The magnetic susceptibility of the Copper(II) compounds was measured at ambient temperature. The complexes showed magnetic moment values in the range 1.90-1.94 BM. The obtained values are nearer to typical tetrahedral geometry [23].

3.3 Influence of Ligand and Copper(II) complexes on plant let production

The cultured PLBs sections were enlarged and produced globular embryos in 3 to 4 weeks on modified MS nutrient medium and also on modified MS supplemented with synthesized ligand and Copper(II) complexes at different concentrations(2µM or 5µM). The globular embryos subsequently developed into plantlets (Fig. 3). The effect of plant growth regulators and adjuvants were studied on the induction and development of embryos/PLBs/shoots in *Oncidium* Gower Ramsey as per the standard procedure [11-15]. In the present study, the induction of embryos/shoots were improved with the addition of heterocycle or Copper(II) complexes (Table-2). The modified MS medium supplemented with 5µM of Ligand

showed induction of maximum number (84) of shoots. Among the two complexes studied in the present investigation, the MS medium added with chloro complex produced more number of embryos/shoots compared to bromo complex (Table-2). Similar observations were reported earlier in *Oncidium* Gower Ramsey with the addition of 2-mercapto-N-methyl imidazole substituted benzimidazole derivative [15]. As ligand concentration direct influence on explants, it not so much expected in Copper(II) complexes at higher concentration [24].

Table 2. Effects of Ligand and Copper(II) complexes on plant let production

Compounds	**Concentration (µM)**	**No. of explants showing (out of 5)**	**Survival percentage**	**Average no. of shoots per explant**
Control	-	4	80	20
Ligand (L)	2	3	60	39
	5	4	80	84
$[CuLCl_2]$	2	3	60	64
	5	2	20	78
$[CuLBr_2]$	2	3	60	55
	5	2	20	65

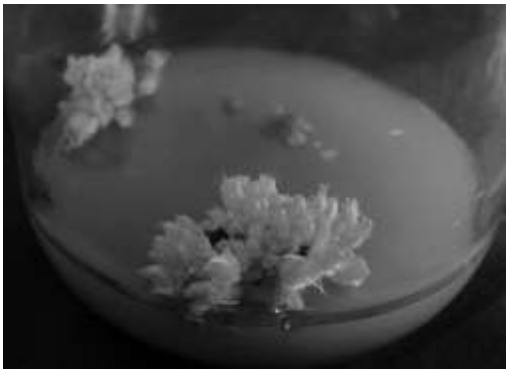

Fig. 3. Production of embryos/shoots from PLBs sections on modified MS medium supplemented with 5 µM Ligand.

4.0 Conclusion

The IR and electronic spectra studies and magnetic moment data confirm the coordination of imine nitrogens of benzimidazole and imidazole rings to the metal ion leading to a tetrahedral geometry (Fig. 4). Plant growth studies revealed that use of two different halogens on the ring did not give prominent result compared to heterocycle. Copper(II) complexes have

shown appreciable enhancement in the average number of shoots. Among the halogens, chloro complexes shows better response due more electronegativity, but further studies are required to confirm the results [25].

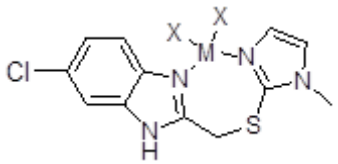

Fig. 4. Tetrahedral structure of $[CuLX_2]$

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