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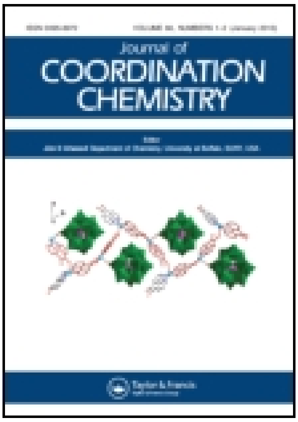

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Versatile ligational behavior of azopyrazolone derived from hydralazine

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Versatile ligational behavior of azopyrazolone derived from hydralazine

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Five complexes of 3-methyl-4 -(phenylhydrazono)-1-phthalazinyl-2-pyrazolin-5-one (LH) were synthesized. The ligand and complexes were characterized using elemental analyses and various analytical techniques such as IR, UV, NMR, mass spectra, magnetic susceptibility measurements, and thermal decomposition studies such as TG/DTG. The geometries of the complexes with special emphasis on the versatile ligational behavior of LH are discussed. All five complexes have octahedral geometry. In four of the complexes [M(LH)Cl_3] where M = Fe(III)/Cr(III) and [M(LH)$(OAc)_2H_2O$] and M = Cu(II)/Ni(II), the ligand was neutral and tridentate. Another copper complex [CuL(OAc)$(H_2O)_2$] in which the ligand is tridentate, mono-anionic one was also obtained in an excess of aqueous solution. Antifungal studies of the compounds are also carried out.

Keywords: Hydralazine; 3-Methyl-4 -(phenylhydrazono)-1-phthalazinyl-2-pyrazolin-5-one; Coordination compounds; Antifungal studies

1. Introduction

1-Phthalazinyl hydrazine (hydralazine) is an antihypertensive under the trade name apresoline, the first medicinal compound containing a hydrazino group. Druey and Marxes [1] attributed the antihypertensive action of hydralazine and its derivatives to the presence of NH_2–NH–CR=N–N=C<. The ligand (LH) has been synthesized by diazo coupling aniline with methyl acetoacetate and then cyclizing the product with hydralazine. The ligational behavior of LH has not been reported yet and hence its complexes with Cr(III), Fe(III), Ni(II), and Cu(II) have been synthesized and characterized in the present investigation. Pyrazolones and their complexes exhibit antimicrobial [2] properties. Therefore, the antifungal studies of the compounds against *Aspergillus niger* are also conducted.

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2. Experimental

2.1. *Reagents and apparatus*

All the chemicals used for syntheses were of Analar grade. Solvents such as methanol, acetic acid, acetone, dichloromethane and chloroform were purchased from E Merck and used as received. Commercial ethanol was purified by distillation. Metal salts used for synthesis of complexes were acetates of Co(II), Ni(II), and Cu(II) and chlorides of Cr(III) and Fe(III). N-1 phthalazinylazopyrazolone, (LH) was synthesized as reported [3]. UV-Visible spectra were recorded using $10^{-3}\,\mathrm{mol\,L^{-1}}$ solutions of ligands and complexes in methanol. The solvent, methanol was taken as a reference and scanning was from 200 to 800 nm. Infrared (IR) spectra were recorded by making thin KBr discs with the compound. The scanning range was $4000–400\,\mathrm{cm^{-1}}$. Standardization of the instrument was done with PS film. Fast atom bombardment (FAB) mass spectra were obtained using Argon as the FAB gas, accelerating voltage, 10 kV, and *m*-nitrobenzyl alcohol as matrix. Electrospray ionization (ESI) mass spectra were measured by dissolution of the sample in methanol with ESI capillary set at 3.5 kV and voltage 40 V. Nuclear magnetic resonance (NMR) spectra were obtained using $CDCl_3$ with TMS as internal standard. Magnetic susceptibilities were determined at room temperature on a Sherwood Magway susceptibility instrument which was calibrated using $Hg[Co(NCS)_4]$. Thermogravimetric studies, TG/DTG were carried out in nitrogen with a heating rate of $10^{\circ}\mathrm{C\,min^{-1}}$. Elemental analyses of C, H, and N were carried out on a Vario EL III CHNS analyser. Some of the metal percentage analyses were determined using an ICP-AES spectrometer using argon as the conductor for plasma state at a core temperature of 9000 K. The other metal percentages were determined by adopting standard procedure [4] as outlined below. The complex was digested with a mixture of conc. HNO_3 and 70% perchloric acid till all the white fumes subsided. It was then made to a known volume and used for estimation of metal ions. The metals, copper (as CuCNS), cobalt (as $Hg[Co(NCS)_4]$), nickel (as nickel dimethyl glyoxime), and iron (as Fe_2O_3) were estimated by gravimetric methods. Chromium was estimated spectrophotometrically [5] after oxidizing to dichromate. Chloride has been estimated by Volhard's method by fusion with Na_2CO_3 followed by decomposition in HNO_3.

2.2. *Synthesis*

2.2.1. Synthesis of methyl-2-phenylhydrazono-3-oxobutyrate. Aniline (0.01 mol) was diazotized by dissolving in 1 : 1 HCl and adding $NaNO_2$ (0.01 mol) solution keeping the temperature at ~0–5°C. This diazonium chloride solution was added dropwise with stirring to a well-cooled ethanolic solution (0.01 mol) of methyl acetoacetate. The pH was kept at 7. The orange oily liquid was stirred for 0.5 h keeping the temperature low and the solidified product was filtered, washed with water, dried and recrystallized from methanol. This product was dried over anhydrous $MgSO_4$ and verified for purity (TLC).

2.2.2. Synthesis of LH. Methyl-2-phenylhydrazono-3-oxobutyrate (phenylazo methyl acetoacetate) solution (0.003 mol) was prepared in glacial acetic acid. Hydralazine hydrochloride (0.003 mol) in minimum distilled water was added dropwise to the solution. One drop of conc. H_2SO_4 was also added and the mixture was kept under reflux for 10 h on a steam bath. The product was checked for cyclization using neutral $FeCl_3$ solution which imparts a deep red color indicating the presence of pyrazolone (aromatic-OH) moiety. The ligand thus obtained (figure 1) was filtered, dried, purified by recrystallization from methanol, and kept in a desiccator over anhydrous $MgSO_4$.

2.2.3. Synthesis of metal complexes. The metal complexes were synthesized by mixing solutions of metal salts and ligand in methanol in 1 : 2 or 1 : 3 molar ratio for divalent and trivalent metals, respectively. To a boiling methanolic solution of the ligand (0.02 mol) an aqueous solution of the metal ion (0.01 mol) was added dropwise. The resultant mixture was refluxed on a steam bath for 3 h. The precipitated complexes were filtered while warm, washed with water and acetic acid successively, and dried over anhydrous $MgSO_4$ in a desiccator. Copper(II) gave two complexes, different in color, stoichiometry, spectral, and TG data [6]. The brown complex was obtained when the solvent for the ligand was diluted to a 25% solution with water. Both copper complexes were analyzed and characterized. All the complexes were paramagnetic.

2.3. *Antifungal studies*

The antifungal activities of the compounds were evaluated by the well diffusion method against the fungus *A. niger*, cultured on potato dextrose agar. The stock solution (10^{-2} mol L^{-1}) was prepared by dissolving the compounds in ethanol and the solutions were serially diluted to find minimum inhibitory concentration values. The concentration of the compounds used in this study was 2000 ppm. In a typical procedure, a well was made on the agar medium inoculated with microorganisms. The well was filled with the test solution using a micropipette and the plate was incubated 24 h for the fungus at 30°C. During this period, the test solution diffused and growth of the inoculated

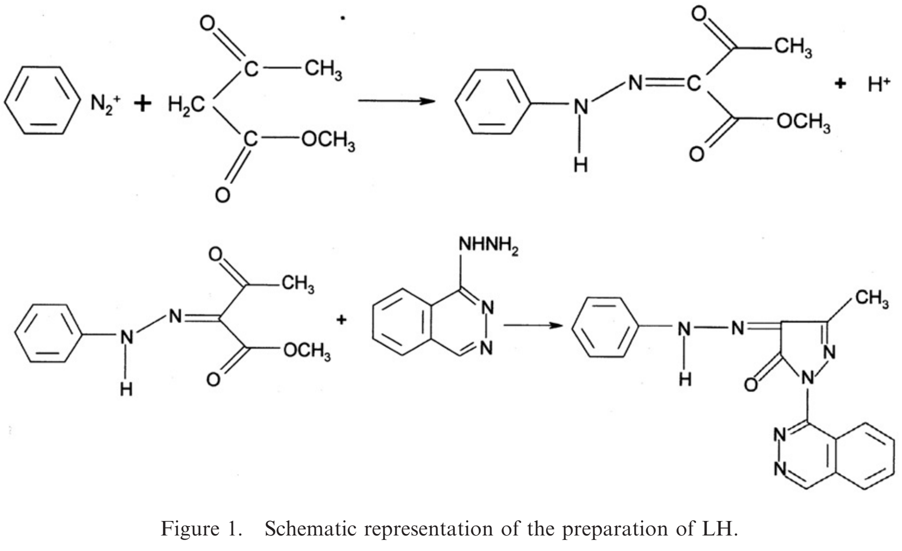

Figure 1. Schematic representation of the preparation of LH.

microorganisms was affected. The inhibition zone was developed and the diameter measured. The experiment was done in triplicate for accuracy.

3. Results and discussion

Arylazo pyrazolin-5-ones are shown [7] to exist in the hydrazone form. On the basis of detailed investigations, LH was also assigned a hydrazone structure. Elemental percentages of the compound were in good agreement with the molecular formula $C_{18}N_6OH_{14}$. The analytical and electronic spectral details of the compounds are shown in table 1.

3.1. *Molar conductance*

The molar conductances of the metal complexes in methanol were below 14.00 $\text{Ohm}^{-1}\,\text{cm}^2\,\text{mol}^{-1}$ showing that they were non-electrolytes. A value of 60–115 $\text{Ohm}^{-1}\,\text{cm}^2\,\text{mol}^{-1}$ is expected [8] for a 1 : 1 electrolyte.

3.2. *Electronic spectral studies and magnetic behavior*

The electronic spectrum of the ligand does not have an absorption band at ~270–280, characteristic of an azo structure. Hence it must exist in the hydrazone form. Electronic spectra of the compounds predict geometry to some extent. The extinction coefficients are given in $(\text{mol L}^{-1})^{-1}\,\text{cm}^{-1}$. The spectrum of the ligand was characterized by two bands; an intense absorption at 308 nm (€ ~ 10^4) due to π–π^* transition and a weak to moderate absorption band at 380 nm (€ ~ 600) due to the n–π^* transition. The complexes were paramagnetic with μ_{eff} values expected for the proposed geometry. Cr(III) complex was dark green. The magnetic moment of 3.72 B.M. suggests an octahedral geometry [9] and electronic spectral bands at 400 nm (€ = 54) due to $^4A_{2g}(P) \rightarrow {}^4T_{1g}(F)$ and 576 nm (€ = 15,000) due to $^4A_{2g}(F) \rightarrow {}^4T_{2g}(F)$ transitions [10] gave additional support to octahedral complex. Fe(III) complex showed spectral bands which overlapped with CT bands. Bands at 630 nm (€ = 55) could be due to $^6A_{1g} \rightarrow {}^4T_{1g}$ transition and that at 412 nm (€ = 50) may be assigned [11] to $^6A_{1g} \rightarrow {}^4T_{2g}$. The magnetic moment value of 5.91 B.M. indicated high-spin octahedral geometry. The spectrum of Ni(II) complex had bands at 386 nm (€ = 550) and 676 nm (€ = 175) due to $^3A_{2g} \rightarrow {}^3T_{1g}(P)$ and $^3A_{2g} \rightarrow {}^3T_{1g}(F)$ transitions, respectively. The $\nu 2/\nu 1$ ratio for the chelate is 1.73, in the usual range (1.6–1.82) for octahedral Ni(II). The magnetic moment value of 2.95 B.M. also pointed to octahedral geometry for Ni(II) complexes. Assignments of Cu(II) d–d transitions are more complicated because of the relatively low symmetry (i.e., less than cubic). However, in cubic environment, the d^9 configuration makes Cu(II) subject to Jahn–Teller distortions, giving an unsymmetrical band. Three transitions, $^2B_{1g} \rightarrow {}^2A_{1g,}$ $^2B_{1g} \rightarrow {}^2B_{2g,}$ and $^2B_{1g} \rightarrow {}^2E_{g,}$ are possible in octahedral Cu(II), but only two transitions are observed at 514 nm and 314 nm for $[\text{Cu(LH)(OAc)}_2\text{H}_2\text{O}]$. The electronic spectra and magnetic moment suggest octahedral Cu(II). The probable assignments are 514 nm (€ = 240) a charge transfer band and 314 nm (€ = 360), probably a ligand-based transition. The magnetic moment of the

Table 1. Physico-chemical data of the compounds.

Compound	Color	Yield (%)	m.p. (°C)	Elemental analysis found (Calcd) (%)					Magnetic moment μ (B.M.)	Electronic spectral data λ_{max} (nm)
				C	H	N	M	X		
LH	Yellow	50	110	65.95 (65.45)	4.92 (4.20)	25.86 (25.45)	–	–		286, 308, 380, 512
[Cr(LH)Cl$_3$]	Dark green	40	200	43.90 (44.30)	2.88 (2.87)	17.97 (17.20)	10.04 (10.87)	20.62 (21.85)	3.72	286, 312, 410, 576, 686
[Fe(LH)Cl$_3$]	Yellowish brown	40	170	43.83 (43.96)	2.92 (2.65)	17.59 (17.10)	11.56 (11.37)	21.06 (21.68)	5.91	288, 314, 412, 514, 630
[Ni(LH)(OAc)$_2$(H$_2$O)]	Brown	60	>300	50.58 (50.39)	3.51 (3.81)	16.61 (16.01)	11.01 (11.18)	–	2.95	286, 310, 386, 676
[CuL(OAc)(H$_2$O)$_2$]	Dark brown	40	190	49.31 (49.18)	3.09 (3.89)	17.68 (17.21)	13.26 (13.02)	–	2.1	280, 316, 512, 680
[Cu(LH)(OAc)$_2$H$_2$O]	Pista green	50	215	50.15 (50.00)	3.81 (3.98)	15.81 (15.91)	12.32 (12.03)	–	1.76	288, 314, 514

Table 2. Important IR spectral bands (cm^{-1}) and their assignments.

Compound	ν(OH)water	ν(NH)	νC=O	νC=N	νM–N	νM–O
LH		3177b	1698s	1454m		
$[Cr(LH)Cl_3]$		3175b	1556s	1498m	557m	507m
$[Fe(LH)Cl_3]$		3167b	1554s	1507m	578m	510m
$[Ni(LH)(OAc)_2H_2O]$	3419b	3180b	1550s	1499m	581m	509m
$[CuL(OAc)(H_2O)_2]$	3420b	nil	1547s	1498m	582m	490m
$[Cu(LH)(OAc)_2H_2O]$	3422b	3174b	1547s	1498m	582m	507m

s, sharp; b, broad; m, medium.

complex was 1.76 B.M. The brown $CuL(OAc)(H_2O)_2$ exhibited normal magnetic moment of 2.1 B.M., slightly higher than spin only value (1.73 B.M.), indicating the distorted octahedral geometry [12]. This complex shows broad asymmetric bands in the region 680 nm (€ = 50) and at 512 nm (€ = 250) assignable to $^2B_{1g} \rightarrow {}^2A_{1g}$ and charge transfer transition, respectively. The former band may be due to $^2E_g \rightarrow {}^2T_{2g}$ due to Jahn–Teller effect, suggesting a distorted octahedral geometry for these complexes. The value of transition ratio ν2/ν1 is 1.32.

3.3. *IR spectral studies*

The IR spectrum showed a broad band at 3177 cm^{-1} assigned to NH stretch (hydrogen bonded) as observed in phenylhydrazono ketones. Two strong bands at 1698 cm^{-1} indicated [13] C=O (exocyclic) conjugated with C=N. The band at 1546 cm^{-1} could be attributed to >C–NH–N = (not cyclic) [14]. In the IR spectra of the complexes, the band at ~1550 cm^{-1} is a clear indication of chelated C=O, suggesting involvement of carbonyl oxygen of pyrazolone in coordination. The band at 1698 cm^{-1} in the ligand spectrum disappears in spectra of the complexes. The bands around 1400 cm^{-1} indicate C=N; lower bands at 580 and 520 cm^{-1} were due to νM–N and νM–O, respectively [15]. The important IR spectral bands and their assignment are entered in table 2.

3.4. *^{1}H NMR spectrum*

The ^{1}H NMR spectrum of LH shows a low-field signal at ~14 ppm for acidic proton. Other signals were at 7.47, 7.45, 8 ppm due to phthalazinyl protons as shown in the spectrum, very much in accord with reported [16] values. Other resonances were at 7.25–7.30 ppm (unsymmetrical pattern – phenyl) and 2.5 ppm ($-CH_3$). The absence of a signal at ~6.48 ppm (C-4 proton) [17] also indicated a hydrazone structure.

3.5. *Mass spectra*

The mass spectrum of LH showed all the expected peaks. The molecular ion peak was present at *m/z* 330, though with a low-percentile value. It is expected to have a low value due to the bulky pendant-like phthalazinyl ($C_8H_5N_2$) which gets easily detached. All the other expected peaks were present in the spectrum at *m/z* 93 (b.p., $C_6H_8N^+H_2$), 77

($C_6H_5^+$), 128 ($C_8H_5N_2^+$), 203 $(M–C_8H_5N_2)^+$, etc. The base peak and *m*/*z* 93 and the absence of ($M–N_2$) peak clearly point to a hydrazone [18] structure rather than an azo structure. Based on the mass spectrum, the structure of LH has been confirmed. Mass spectra of the two copper complexes showed the parent ion at 488 and 528 for the brown colored and green colored complexes, respectively. Both spectra showed fragment peaks due to the detachment of $C_8H_5N_2$(phthalazinyl) and $C_4N_2OH_3$(pyrazolonyl). The mass spectrum of the brown colored copper complex agreed well with the structural formula [CuL(OAc)(H_2O)$_2$]. The values of prominent *m*/*z* peaks were 488 (M^+), 469 ($M–H_2O$), 450 (M–twoH_2O), 301 [M–($C_8H_5N_2$ + OAc)], 279 ($M–C_8H_5N_2$–OAc–H_2O), 203 [M–($C_8H_5N_2 + C_4N_2OH_3$ + OAc)], 243 [M–($C_8H_5N_2 + C_5N_2H_3–H_2O$)] – base peak, 185 [M–($C_8H_5N_2 + C_5N_2H_3 + H_2O$–OAc)], 92 ($C_6H_5N^+H$), 77 ($C_6H_5^+$), etc. Additional M + 1, M + 2, etc. peaks were also present. The mass spectrum of green copper complex agreed well with the structural formula [CuLH(OAc)$_2$$H_2O$]. The prominent *m*/*z* values are 528 (M^+), 474 ($M–CH_3COO$), 416 (M–2xCH_3COO), 154-Basepeak [M–(2x$CH_3COO + H_2O + C_8N_2H_5$ (phthalazinyl) +$C_6H_5N_2$], 107 ($C_6H_5N_2^+$), 78$(C_6H_5)^+$.

3.6. *Thermal data*

The decomposition patterns of [CuL(OAc)(H_2O)$_2$] and [Cu(LH)(OAc)$_2$$H_2O$] are summarized in table 3. As can be observed from the TG traces, both Cu(II) complexes are stable to 200°C. In both cases, the coordinated H_2O and the CH_3COO^- were lost at about same temperature, ~200°C. The complex in which LH was a mono-anionic ligand (L) retained this ligand to a temperature of 900°C. This was expected because of the stronger covalent attachment of L to Cu(II) in the former than a dative bond made by LH to Cu(II) in the latter. The decomposition patterns agree with the structural formulas suggested for the Cu(II) complexes.

3.7. *Antifungal activity*

All the compounds synthesized have been tested for their antifungal activity against *A. niger*. *Aspergillus niger* is less likely to cause human disease than some other *Aspergillus* species, but, if large amounts of spores are inhaled, a serious lung disease, aspergillosis, can occur. *Aspergillus niger* is also one of the most common causes of otomycosis, a fungal ear infection, which can cause pain, temporary hearing loss, and, in severe cases, damage to the ear and tympanic membrane. Currently, the available drug [19] is amphotericin B. In this study, amphotericin B has been used as control. Amphotericin B is also commonly used in tissue cultures to prevent fungi from contaminating cell cultures. Mammalian and fungal membranes both contain sterols, a primary membrane target for amphotericin B. Because mammalian and fungal membranes are similar in structure and composition, this is one mechanism by which amphotericin B causes cellular toxicity. The objective of the present antifungal study was to find the possibility of another potential drug comparable with the control but less toxic. The results are presented in table 4. All the synthesized compounds exhibited some antifungal activity. All the complexes, except that of Ni(II), showed more activity than ligand. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism. The

Table 3. Decomposition data of copper complexes.

Complex	Color	Formula weight	Decomposition stage	Decomposition temp (°C) DTG	Mass loss % from TG (Calcd)	Decomposition pattern
[CuL(OAc)(H_2O)$_2$]	Brown	488	Stage 1	200.7	19.25 (19.47)	loss of $CH_3COO +$ two H_2O
			Stage 2	364.4	54.3 (48.9)	loss of ($CH_3COO +$ two $H_2O +$ phthalazinyl $+ CH_3$)
			Stage 3	400.6	61.3 (64.5)	loss of ($CH_3COO +$ two $H_2O +$ phthalazinyl $+ CH_3 + C_6H_5$)
			Stage 4	900.6	78 (83.6)	Final residue CuO
[Cu(LH)(OAc)$_2$$H_2O$]	Green	528	Stage 1	215.8	72.9 (78.5)	(loss of two $CH_3COO +$ $H_2O +$ phthalazinyl $+ C_6H_5N_2 + CH_3$)
			Stage 2	364.5	90.7 (84.5)	Final residue CuO

Table 4. Antifungal activities of the compounds against *A. niger*.

	Inhibition zone (mm)		
Compound	Trial 1	Trial 2	Trial 3
LH	19	20	20
[Cr(LH)Cl$_3$]	23	22	22
[Fe(LH)Cl$_3$]	26	25	26
[Ni(LH)(OAc)$_2$(H$_2$O)]	15	16	14
[CuL(OAc)(H$_2$O)$_2$]	20	21	20
[Cu(LH)(OAc)$_2$H$_2$O]	35	35	36
Amphotericin B	30	30	31
Ethanol (solvent)	20	21	20

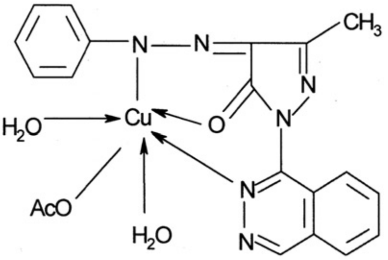

Figure 2. Tentative structure of the Cu(II) complex with L.

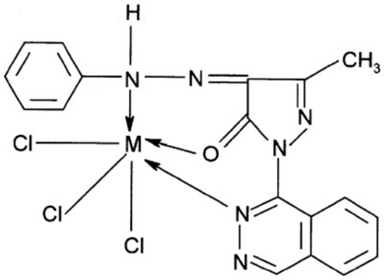

Figure 3. Tentative structures of M = Fe(III)/Cr(III) complexes.

green copper chelate, [Cu(LH)(OAc)$_2$H$_2$O], was better than the control medicine against the fungal strain.

4. Conclusion

From the above discussion, the versatile nature of LH is obvious. Phenylazo pyrazolones can be tridentate when N-1 of the pyrazolone has a substituent with suitable hetero atom. Thus in the present case nitrogen of phthalazinyl takes part in

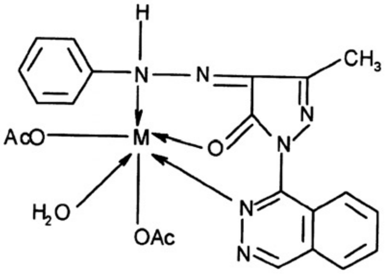

Figure 4. Tentative structures of M = Ni(II)/Cu(II) complexes.

coordination making it a tridentate neutral ligand. One exception has been observed in the case of brown copper(II) complex [CuL(OAc)(H_2O)$_2$] in which it acts as a tridentate mono-anionic ligand. Both structures have been confirmed by mass spectral and TG analyses. Therefore, based on the different physico-chemical evidence, the complexes are assigned octahedral geometry (figures 2–4). The green copper chelate [Cu(LH)(OAc)$_2$$H_2O$] has more activity than the current drug against *A. niger*. *In vivo* studies can be performed to develop the pharmaceutical compatibility.

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