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# Microwave assisted synthesis, spectroscopic characterization and biological aspects of some new chromium(III) complexes derived from N^O donor Schiff bases

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The present study is structured to address the development of green microwave processes in the manufacture of a new series of biologically potent (N^O) donor Schiff bases and their Cr(III) complexes. These have been characterized by elemental analysis, melting point and molecular weight determinations, IR, UV-vis and EPR spectral analysis, and X-ray powder diffraction studies. The bioefficient compounds have been synthesized by thermal as well as microwave assisted methods. The azomethine ligands 1-(2-pyridyl) ethanone isonicotinoyl hydrazone ($L^1H$) and 1-(2-naphthyl) ethanone isonicotinoyl hydrazone ($L^2H$) have been synthesized by the condensation of 1-(2-pyridyl) ethanone and 1-(2-naphthyl) ethanone with isonicotinic acid hydrazide in a 1 : 1 molar ratio, using ethanol as a solvent. The Cr(III) complexes have been prepared by mixing $CrCl_3{\cdot}6H_2O$ in 1 : 1 and 1 : 2 molar ratios with ligands $L^1H$ and $L^2H$ in methanol. The synthesized ligands and their new metal complexes have been screened *in vitro* for antimicrobial activity and *in vivo* for antifertility activity on male albino rats. The spectral data suggested a hexa-coordinated environment around the central metal ion. Physicochemical studies of the ligands and their Cr(III) complexes aided the construction of their proposed structures.

## 1. Introduction

The use of microwave irradiation in chemical synthesis has become increasingly popular within the pharmaceutical and academic arenas, because it is a new enabling technology for drug discovery and development[1] and due to certain advantages, particularly shorter reaction times and rapid optimization of chemical reactions.[2] Presently, thermally driven chemical synthesis takes place by one of two ways: conventional heating or microwave accelerated heating. For the conventional heating method, reactants are slowly activated by a conventional external heat source.[3] Heat is driven into the substance, passing first through the walls of the vessel in order to reach the solvent and reactants. This is a slow and inefficient method for transferring energy into the reacting system. For the microwave heating method, microwaves couple directly with the molecules of the entire reaction mixture, leading to a rapid rise in temperature. Since the process is not limited by the thermal conductivity of the vessel, the result is an instantaneous localized superheating of any substance that will respond to either dipole rotation or ionic conduction.[4] Many of the metal complexes of Schiff base ligands containing N^O donor atoms, such as semicarbazones[5,6] and thiosemicarbazones,[7,8] isonicotinoyl hydrazones and their metal chelates, have been found to exhibit fungicidal, bactericidal, antiviral, and antitubercular activities. Thus the metal complexes of such type of Schiff base ligands are continuing to attract the interest of scientists and the coordination of these ligands with transition metals has been explored more thoroughly [9–11] than their coordination with non-transition metals. Isonicotinic acid hydrazide (INH) is a drug of proven therapeutic importance and is used against a wide spectrum of bacterial ailments.[12] In the past Agarwal and Sarin[13] have investigated the coordinating ability of INH-derivatives with metal ions. Hydrazones derived from the condensation of isonicotinic acid hydrazide with heterocyclic aldehydes have been found to show better antitubercular activity than INH. Because of the wide range of medicinal applications of isonicotinic acid hydrazides[14,15] and their abilities to coordinate with the transition metal ions,

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it is highly desirable to synthesize and characterize transition metal complexes with such Schiff base ligands. Keeping all these facts under consideration, during the present investigations we have synthesized, characterized and screened the biologically potent ligands and their chromium complexes against a variety of pathogenic fungal and bacterial strains. Further, the complexes were also tested for their antifertility activity in male albino rats and the results were indeed positive.

## 2. Experimental

All chemicals and reagents used were of AR grade and dried and distilled before use. The $CrCl_3{\cdot}6H_2O$ was purchased from Alfa Aesar. All the reagents were dried and distilled before use. 1-(2-pyridyl) ethanone and 1-(2-naphthyl) ethanone were purchased and used as such. Apparatus fitted with Quickfit interchangeable joints were used to carry out the reactions.

### 2.1. Preparation of the ligands

**2.1.1. Preparation of the azomethine ligands ($L^1H$ and $L^2H$).** Two different routes were used for the synthesis of ($L^1H$ and $L^2H$) ligands (Scheme 1).

(A) **Microwave method**: In the microwave assisted method, 1-(2-pyridyl) ethanone isonicotinoyl hydrazone ($L^1H$) and 1-(2-naphthyl) ethanone isonicotinoyl hydrazone ($L^2H$) were formed by the condensation of 1-(2-pyridyl) ethanone (0.05 mol) and 1-(2-naphthyl) ethanone (0.05 mol) with isonicotinic acid hydrazide (0.05 mol) in a 1 : 1 molar ratio using a microwave oven, with 5 mL ethanol as solvent. The reactions were complete in a short period of 10–12 min. The solution was then concentrated under reduced pressure, which upon cooling gave dark yellow crystalline precipitates. The products were washed in alcohol and recrystallized in the same solvent.

(B) **Thermal method**: For comparison purposes, the above ligands were also synthesized by the thermal method, where instead of a few drops of alcohol, a hot ethanolic solution (25 mL) of 1-(2-pyridyl) ethanone (0.01 mol) and 1-(2-naphthyl) ethanone (0.01 mol) was mixed with a hot ethanolic solution (30 mL) of isonicotinic acid hydrazide (0.01 mol) in a 1 : 1 molar ratio. The contents were refluxed for about 3–4 hours in a water bath. The solution was then concentrated under reduced pressure, which upon cooling gave dark yellow crystalline precipitates. The products were washed in alcohol and recrystallized in the same solvent. The structures of ligands ($L^1H$ and $L^2H$) are shown in (Fig. 1).

A comparison between the thermal method and microwave method is given in (Table 1).

### 2.2. Preparation of the complexes

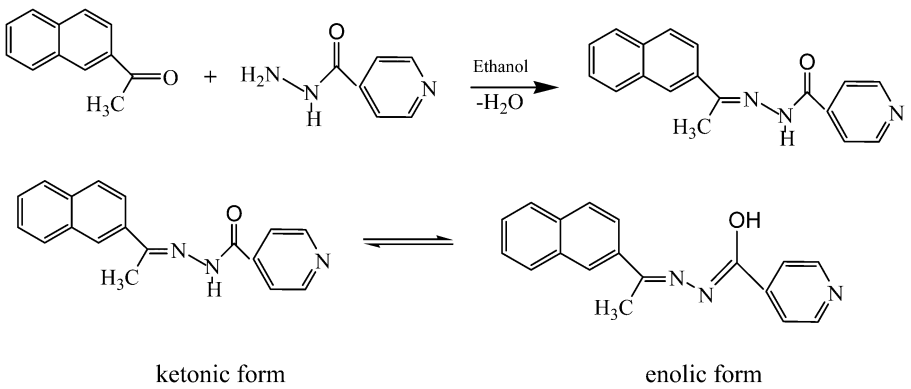
For comparison purposes, two different routes were employed for the synthesis of the metal complexes with each of the ligands ($L^1H$ and $L^2H$). A comparison between the thermal method and microwave method has been given in Table 1.

(A) **Microwave method**: The complexes were prepared by irradiating a reaction mixture of $CrCl_3{\cdot}6H_2O$ and the respective ligand ($L^1H$ and $L^2H$) in an appropriate stoichiometric proportion in 3–5 mL dry methanol using NaOH. The dry products were recovered from the microwave oven and dissolved in ~5 mL of dry methanol, where the sodium chloride precipitate (formed during the course of the reaction) was removed by filtration and the filtrate was then concentrated under reduced pressure. The resulting compounds were washed with cyclohexane and recrystallized with methanol.

(B) **Thermal method**: The complexes were also synthesized by the thermal method, where instead of 10–14 min, reactions were completed in 14–16 hours and the yield of the products was also less than that obtained by the microwave assisted synthesis. In this method a methanolic solution of $CrCl_3{\cdot}6H_2O$ was added to a methanolic solution of ligand ($L^1H$ and $L^2H$) in 1 : 1 and 1 : 2 molar ratios using NaOH in appropriate stoichiometric proportions. The resulting mixture was heated under reflux for 14–16 hours, filtered to remove NaCl and the solvent was concentrated under reduced pressure. The product was dried in a vacuum. The resulting compounds were washed with cyclohexane and recrystallized with methanol.

### 2.3. Physical measurements and analytical methods

The molecular weights were determined by the Rast Camphor method.[16] The metal contents were analysed gravimetrically. Sulfur and nitrogen were determined by Messenger's[17] and Kjeldahl's methods,[18] respectively. Carbon and hydrogen analyses




**Scheme 1** Synthesis of ligand $L^2H$ and its tautomeric form.

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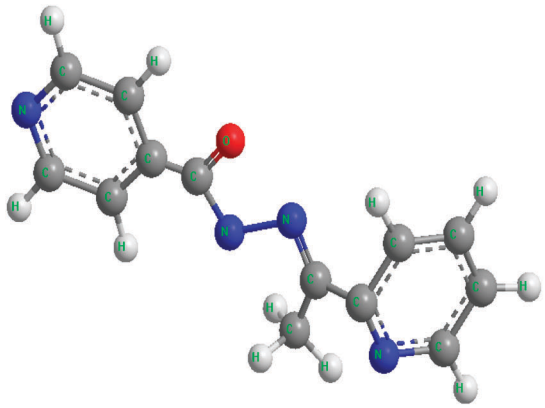
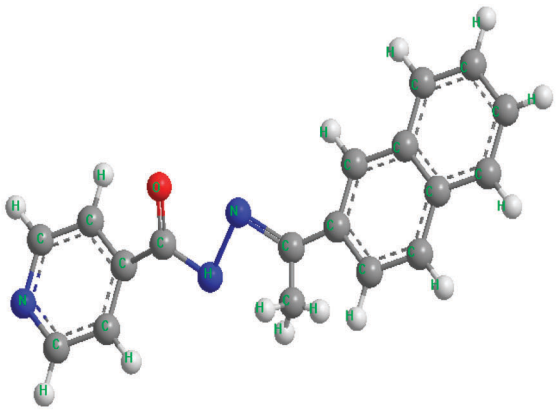


1-(2-pyridyl) ethanone isonicotinoyl hydrazone

($L^1H$)



1-(2-naphthyl) ethanone isonicotinoyl hydrazone

($L^2H$)

**Fig. 1** Structures of the azomethine ligands ($L^1H$ and $L^2H$).

**Table 1** Comparison between the microwave and thermal method

| | Yield % | | Solvent (mL) | | Time | |
|---|---|---|---|---|---|---|
| Compounds | Thermal | Microwave | Thermal | Microwave | Thermal (h) | Microwave (m) |
| $L^1H$ | 65 | 82 | 100 | 5 | 3 | 10 |
| $L^2H$ | 73 | 90 | 100 | 5 | 4 | 12 |
| $[CrCl_2(L^1)(H_2O)_2]$ | 73 | 83 | 50 | 3 | 15 | 10 |
| $[CrCl(L^1)_2(H_2O)]$ | 66 | 87 | 45 | 5 | 14 | 10 |
| $[CrCl_2(L^2)(H_2O)_2]$ | 70 | 80 | 40 | 3 | 16 | 12 |
| $[CrCl(L^2)_2(H_2O)]$ | 77 | 84 | 50 | 3.5 | 14 | 14 |

were performed at the CDRI, Lucknow. Infrared spectra were recorded on a Nicolet Megna FTIR-550 spectrophotometer using KBr pellets. The electronic spectra were recorded on a Varian–Cary/5E spectrophotometer at SAIF, IIT, Madras, Chennai. EPR spectra of the complexes were monitored on Varian E-4X band spectrometer at SAIF, IIT, Madras, Chennai.

### 2.4. Antimicrobial studies

**2.4.1. Antifungal studies.** The bioefficiencies of the ligands and their metal complexes, synthesized by thermal as well as microwave methods, were checked *in vitro*. The antifungal activities of the ligands and their complexes have been evaluated against two pathogenic fungi, *Candida albicans* and *Aspergillus niger*, by the agar plate technique. The potato dextrose agar (PDA) medium was prepared in the laboratory to maintain the fungal growth. For PDA preparation, 20 g potato was extracted with distilled water (100 mL) at 100 °C for 1 h and was filtered off using a cotton filter. The potato juice was then mixed with 2 g dextrose and 1.5 g agar and finally the pH of the prepared PDA media was adjusted to 7. Solutions of the test compounds in methanol at 100 and 200 ppm concentrations were prepared and mixed with the medium. The medium was then poured into Petri plates and the spores of the fungi were placed on the medium with the help of an inoculum needle. These Petri plates were wrapped in polythene bags containing a few drops of alcohol and placed in an incubator at 25 ± 2 °C. The activity was determined after 96 h of incubation at room temperature (25 °C). The controls were also run and three replicates were used in each case. The linear growth of the fungus was obtained by measuring the diameter of the fungal colony after four days and the percentage inhibition was calculated as $100 \times (C - T)/C$, where $C$ = diameter of the fungus colony in the control plate after 96 h and $T$ = diameter of the fungal colony in the test plates after the same period. The antifungal screening data of compounds were compared with the standard (Flucanazone).

**2.4.2. Antibacterial studies.** The antibacterial activity of the ligands and their chromium complexes were evaluated against two bacteria including Gram-positive (*Bacillus subtilis*), and Gram-negative (*Escherichia coli*). The nutrient agar medium [composition: peptone (5 g), beef extract (5 g), NaCl (5 g), agar–agar (20 g) and distilled water (1000 mL)] was pipetted into the Petri dish. When it solidified, 5 mL of warm seeded agar was applied. The seeded agar was prepared by cooling the molten agar to 40 °C and then adding 10 mL of the bacterial suspension. The compounds were dissolved in methanol in 500 and 1000 ppm concentrations. Paper discs of Whatman No. 1 filter paper, with diameters of 5 mm, were soaked in the

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solutions of varied concentrations. The discs were dried and placed on the medium previously seeded with organisms in Petri plates at a suitable distance. The Petri plates were stored in an incubator at 28 ± 2 °C for 24 hours. The diameters of the zone of inhibition produced by the compounds were compared with the standard antibiotic (streptomycin). The zone of inhibition thus formed around each disc containing the test compounds was measured accurately in mm.

### 2.5. Antifertility activity

**Material and methods.** Fertile healthy adult male rats of the *Wistar Strain*, with an average body weight between 200–230 g, were used for the present study. The animals were housed in plastic cages and were maintained at a room temperature (22° ± 2 °C) and uniform light (14 : 10 : L : D). The rats had a diet of standard commercial pelleted feed (Ashirwad Food Industries Ltd., Chandigarh, India) and fresh water *ad libitum* throughout the study.

**Arrangement of animals for the study.** The animals were divided into seven groups of six animals. The animals in group A served as a control group and received the vehicle (0.5 mL olive oil) through oral insterbation at the same time every day. The animals in groups B and C received ligands ($L^1H$ and $L^2H$), whereas the animals in groups D, E, F and G received chromium(III), complexes of ligands ($L^1H$ and $L^2H$), respectively. The animals were maintained under perfect supervision and in accordance to the guidelines of committee for the purpose of control and supervision of experiment on animals (CPCSEA) for the regulations of scientific experiments on animals. The experimental protocol has approval of the institutional ethical committee Dept of zoology UOR Jaipur.

**Mode of administration of the compound.** In groups B and C, the ligands (20 mg $kg^{-1}$ b.wt) were dissolved in 0.5 mL olive oil and given orally by a pearl point needle for a period of 60 days. The animals in groups D, E, F and G, received same doses of the respective compounds for the same period. No rat mortality occurred during the study period. The mating exposure test was done on day 55 of the experiment. The rats were sacrificed after 24 h of the last dose (61st day) to perform various tests.

**Fertility test.** The mating exposure test of all the animals was performed. They were cohabited with pro-estrous females in the ratio of 1 : 3. The vaginal plug and presence of sperms in the vaginal smear was checked for positive mating. Females were separated and the resulting pregnancies were noted, when dams gave birth. Fertility was calculated in the control as well as in the treated groups. At the end of the experimental period, the animals were weighed and autopsied under light ether anesthesia.

**Parameters studied**

*Body and organ weights.* The initial and final weights of the testes, epididymis, seminal vesicle and ventral prostate were recorded and processed for biochemical estimations.

*Sperm dynamics.* Sperm density and sperm motility were assessed by standard methods.

*Biochemical analysis.* Testicular cholesterol, testicular glycogen, protein and sialic acid in the testes, epididymis, ventral prostate and seminal vesicle were estimated by standard laboratory methods.

*Serum testosterone.* Testosterone in the serum was measured by the radioimmuno assay.[19] Differences between the control and treated animals were evaluated statistically using the Student's "*t*" test. A *t*-test is any statistical hypothesis test in which the test statistic follows a Student's *t* distribution if the null hypothesis is supported. It is most commonly applied when the test statistic would follow a normal distribution if the value of a scaling term in the test statistic was known. When the scaling term is unknown and is replaced by an estimate based on the data, the test statistic (under certain conditions) follows a Student's *t* distribution.

## 3. Results and discussion

The elemental analysis and spectral data are consistent with the formulation of compounds $[CrCl_2(L)(H_2O)_2]$ and $[(CrCl(L)_2\text{-}(H_2O)]$. The resulting chromium complexes are green solids, soluble in MeOH, DMF and DMSO. Molecular weight determinations indicate their monomeric nature. The reactions of $CrCl_3{\cdot}6H_2O$ with the ligands ($L^1H$ and $L^2H$) were carried out in unimolar and bimolar ratios in methanol solution with the formation of M←N and M–O bonds yielding the substitution products. The reactions proceed as shown in Scheme 1 and 2.

$$CrCl_3 \cdot 6H_2O + LH + NaOH \xrightarrow[MeOH]{1:1} [CrCl_2(L)(H_2O)_2] + NaCl + 5H_2O \quad (1)$$

$$CrCl_3 \cdot 6H_2O + 2LH + 2NaOH \xrightarrow[MeOH]{1:2} [CrCl(L)_2(H_2O)] + 2NaCl + 7H_2O \quad (2)$$

Metal complexes were prepared by the microwave method, in addition to the conventional method. The reason for synthesizing the metal complexes by the microwave method is due to its ecofriendly nature. The reaction time is brought from hours to seconds and a small amount of solvent is required for completion of the reaction. An enhancement of the yield of the resulting products is observed.

The physical properties and analytical data of the ligands and their metal complexes, synthesized by a green chemical approach as well as conventional method are detailed in Tables 2 and 3.

### 3.1. Infrared spectral data

The significant IR bands of the ligands and their metal complexes, along with their tentative assignments, are reported in Table 4, which were used for the establishment of the mode of coordination of the bidentate ligands towards the metal ion. The IR spectra of the ligands ($L^1H$ and $L^2H$) show a strong band at 1615–1610 $cm^{-1}$, due to the >C═N group, which shifts to a lower wave number (~15–10 $cm^{-1}$) in the chromium(III) complexes, indicating the

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**Table 2** Analytical data and physical properties of the ligands and their complexes – synthesized by conventional heating

| Compounds | Colour | Melting point (°C) | Found (calculated) (%) C | H | N | Cl | M | Mol. wt. found (calculated) |
|---|---|---|---|---|---|---|---|---|
| $L^1H$ | Dark yellow | 140–150 | 63.97 (64.98) | 4.53 (5.33) | 22.61 (23.31) | — | — | 238.30 (240.26) |
| $L^2H$ | Dark yellow | 130–142 | 73.70 (74.72) | 13.82 (14.52) | 4.74 (5.22) | — | — | 287.23 (289.33) |
| $[CrCl_2(L^1)(H_2O)_2]$ | Green | 260–265 (d[a]) | 38.18 (39.21) | 3.19 (3.79) | 13.87 (14.13) | 16.21 (17.80) | 12.28 (13.05) | 397.06 (398.18) |
| $[CrCl(L^1)_2(H_2O)]$ | Green | 220 (d[a]) | 52.46 (53.47) | 3.64 (4.14) | 18.94 (19.18) | 5.46 (6.07) | 8.20 (8.90) | 581.42 (583.97) |
| $[CrCl_2(L^2)(H_2O)_2]$ | Green | 266–270 (d[a]) | 47.30 (48.32) | 3.98 (4.05) | 8.78 (9.39) | 15.12 (15.85) | 10.94 (11.62) | 445.57 (447.30) |
| $[CrCl(L^2)_2(H_2O)]$ | Green | 223–225 (d[a]) | 62.35 (63.38) | 4.19 (4.43) | 11.91 (12.32) | 4.33 (5.19) | 6.87 (7.62) | 679.32 (682.11) |

[a] d = decomposition.

**Table 3** Analytical data and physical properties of the ligands and their complexes – synthesized by microwave heating

| Compounds | Colour | Melting point (°C) | Found (calculated) (%) C | H | N | Cl | M | Mol. Wt. found (calculated) |
|---|---|---|---|---|---|---|---|---|
| $L^1H$ | Dark yellow | 140–150 | 64.20 (64.98) | 5.01 (5.33) | 22.82 (23.31) | — | — | 239.06 (240.26) |
| $L^2H$ | Dark yellow | 130–142 | 74.15 (74.72) | 14.20 (14.52) | 4.98 (5.22) | — | — | 288.13 (289.33) |
| $[CrCl_2(L^1)(H_2O)_2]$ | Green | 260–265 (d[a]) | 38.86 (39.21) | 3.54 (3.79) | 13.92 (14.13) | 16.90 (17.80) | 12.88 (13.05) | 397.32 (398.18) |
| $[CrCl(L^1)_2(H_2O)]$ | Green | 220 (d[a]) | 53.09 (53.47) | 3.78 (4.14) | 18.95 (19.18) | 5.65 (6.07) | 8.34 (8.90) | 582.12 (583.97) |
| $[CrCl_2(L^2)(H_2O)_2]$ | Green | 266–270 (d[a]) | 47.91 (48.32) | 3.98 (4.05) | 8.98 (9.39) | 15.32 (15.85) | 11.06 (11.62) | 446.18 (447.30) |
| $[CrCl(L^2)_2(H_2O)]$ | Green | 223–225 (d[a]) | 62.96 (63.38) | 4.23 (4.43) | 11.96 (12.32) | 4.84 (5.19) | 7.02 (7.62) | 680.41 (682.11) |

[a] d = decomposition.

**Table 4** IR ($cm^{-1}$) spectral data of the ligands and their metal complexes

| Compound | IR spectral data ($cm^{-1}$) $\nu$(NH) | $\nu$(OH) | $\nu$(C=N) | $\nu$(M–O) | $\nu$(M←N) |
|---|---|---|---|---|---|
| $L^1H$ | 3244 | — | 1615 | — | — |
| $L^2H$ | 3250 | — | 1610 | — | — |
| $[CrCl_2(L^1)(H_2O)_2]$ | — | 3465 | 1602 | 588 | 435 |
| $[CrCl(L^1)_2(H_2O)]$ | — | 3452 | 1605 | 585 | 423 |
| $[CrCl_2(L^2)(H_2O)_2]$ | — | 3467 | 1600 | 600 | 440 |
| $[CrCl(L^2)_2(H_2O)]$ | — | 3460 | 1595 | 598 | 428 |

coordination of the azomethine nitrogen to the metal atom. The IR spectra of the ligands show bands at 3244–3250 $cm^{-1}$, due to $\nu$(NH) group, which has disappeared from the spectra of the complexes. The medium intensity bands in the region 1675–1690 $cm^{-1}$, due to $\nu$(C=O) vibrations in the ligands ($L^1H$ and $L^2H$), shifted to a lower frequency in the spectra of their complexes, which indicates amide–imidol tautomerism and their subsequent coordination through the imidol oxygen. In the spectra of all the chromium(III) complexes, a band is observed in the range 880–876 $cm^{-1}$, which may be attributed to the coordinated water molecule. Further, a broad band at around 3467–3452 $cm^{-1}$ may be due to the $\nu$(O–H) of the water molecule. The single band observed at *ca.* 320–315 $cm^{-1}$ is due to $\nu$(Cr–Cl), suggesting that all the complexes have a *trans* structure. The presence of non-ligand bands, due to $\nu$(Cr←N) and $\nu$(Cr–O), in the spectra of the chromium(III) complexes around 440–421 $cm^{-1}$ and 600–585 $cm^{-1}$, further confirm the complexation of the ligands to the metal ion.

### 3.2. Electronic spectral analysis

The nature of the ligand field around the metal ion and the geometry of the metal complexes have been deduced from the electronic spectra of the chromium(III) complexes that were recorded in DMSO. In the case of the chromium(III) complexes, the three d–d transitions are expected and are also observed experimentally. Three bands at 16556–17513, 22780–24038 and 30211–33000 $cm^{-1}$ were observed due to the $^4A_2g \rightarrow {}^4T_2g$ ($\nu_1$), $^4A_2g \rightarrow {}^4T_1g$ ($\nu_2$) and $^4A_2g \rightarrow {}^4T_1g$ (P) ($\nu_3$) transitions, respectively, suggesting an octahedral geometry around the $Cr^{3+}$ ion.[20] Various ligand field parameters like *D*q, *B* and $\beta$ have been calculated and given in Table 5. The energy of the first spin-allowed transition [$^4A_2g$ (F) $\rightarrow {}^4T_2g$ (F)] directly gives the value of 10*D*q. The electronic repulsion parameter is expressed in terms of Racah parameter and '*B*' has been evaluated during these studies. The nephelauxetic ratio $\beta$ indicates that the complexes have appreciable covalent character.

**Table 5** Electronic spectral data ($cm^{-1}$) of the chromium(III) complexes[a]

| Compounds | Transitions | Spectral bands ($cm^{-1}$) | *D*q | $B^\circ$ | $\beta = B/B^\circ$ | $\nu_2/\nu_1$ |
|---|---|---|---|---|---|---|
| $[CrCl_2(L^1)(H_2O)_2]$ | $^4A_{2g}$ (F) → $^4T_{2g}$(F) | (16 863) | 1684 | 636 | 0.69 | 1.38 |
| | $^4A_{2g}$(F) → $^4T_{1g}$(F) | (23 310) | | | | |
| | $^4A_{2g}$(F) → $^4T_{1g}$(P) | (30 959) | | | | |
| $[CrCl(L^1)_2(H_2O)]$ | $^4A_{2g}$ (F) → $^4T_{2g}$(F) | (17 241) | 1721 | 607 | 0.66 | 1.36 |
| | $^4A_{2g}$(F) → $^4T_{1g}$(F) | (23 474) | | | | |
| | $^4A_{2g}$(F) → $^4T_{1g}$(P) | (32 786) | | | | |
| $[CrCl_2(L^2)(H_2O)_2]$ | $^4A_{2g}$ (F) → $^4T_{2g}$(F) | (16 556) | 1654 | 605 | 0.65 | 1.37 |
| | $^4A_{2g}$(F) → $^4T_{1g}$(F) | (22 780) | | | | |
| | $^4A_{2g}$(F) → $^4T_{1g}$(P) | (32 362) | | | | |
| $[CrCl(L^2)_2(H_2O)]$ | $^4A_{2g}$ (F) → $^4T_{2g}$(F) | (17 391) | 1738 | 618 | 0.67 | 1.36 |
| | $^4A_{2g}$(F) → $^4T_{1g}$(F) | (23 752) | | | | |
| | $^4A_{2g}$(F) → $^4T_{1g}$(P) | (33 000) | | | | |

[a] *B* = complex, $B^\circ$ = free ion.

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### 3.3. $^1$H-NMR Spectra

The $^1$H-NMR spectra of the ligands were recorded in DMSO-$d_6$ using TMS as the internal standard. The $^1$H-NMR spectra of the ligands show a signal at $\delta$ 10.08–11.03 ppm, which is due to the NH proton. A singlet observed at 8.84–8.90 ppm may be assigned to the α-protons of the pyridine ring. The multiplet observed at 6.97–8.28 ppm may be assigned to the β-protons of the pyridine ring and the protons of the aromatic ring. The spectra also exhibit signals due to the methyl protons attached to the azomethine group at $\delta$ 2.33–2.50 ppm.

### 3.4. ESR spectral analysis and magnetic moments

The ESR spectra of the 1 : 1 and 1 : 2 chromium(III) complexes, synthesized by different routes, were recorded at room temperature. These consist of a single broad peak in each case and from which the Lande splitting factor ('*g*' values) has been calculated and given in Table 6. For the present complexes, the *g* values lie in the range 1.9350–1.9980, with giso (2.0), which are characteristic of an octahedral geometry.[21] The room temperature magnetic moment for the chromium(III) complexes is slightly less than required. The observed magnetic moment values of 3.63–3.78 BM and the electronic spectra of the complexes support the octahedral structure of the complexes.

### 3.5. X-ray powder diffraction study

The possible lattice dynamics of the finely powdered $[CrCl_2(L)(H_2O)_2]$ and $[(CrCl(L)_2(H_2O)]$ type of products have been reported on the basis of X-ray powder diffraction studies.[22] The results show that the compound belongs to an 'orthorhombic' crystal system, having unit cell parameters of $a$ = 9.57, $b$ = 17.21 $c$ = 21.15 and a maximum deviation of $2\theta$ = 0.039–0.045° and $\alpha$ = 90°, $\beta$ = 90°, $\gamma$ = 90°.

On the basis of above studies an octahedral environment around the metal atoms has been proposed (Fig. 2).

**Table 6** ESR spectral data of the chromium(III) complexes

| Compound | $H_0$ | *g* value | Temp./°C | Magnetic moment ($\mu$) |
|---|---|---|---|---|
| $[CrCl_2(L^1)(H_2O)_2]$ | 3371.12 | 1.9879 | 25 | 3.70 |
| $[CrCl(L^1)_2(H_2O)]$ | 3354.07 | 1.9980 | 25 | 3.75 |
| $[CrCl_2(L^2)(H_2O)_2]$ | 3463.28 | 1.9350 | 25 | 3.66 |
| $[CrCl(L^2)_2(H_2O)]$ | 3417.36 | 1.9610 | 25 | 3.74 |

### 3.6. Antimicrobial assay

The ligands and their chromium complexes, synthesized through the conventional as well as the microwave heating method, were evaluated for their antimicrobial activity against two bacteria (*Bacillus subtilis* and *Escherichia coli*) and two fungi (*Candida albicans* and *Aspergillus niger*). The results are summarized in Chart 1 and 2. The results were compared with those of the standard drug streptomycin for bacteria and fluconazole for fungi. All the ligands and their respective Cr(III) complexes were found to be sensitive against all the fungal and bacterial strains tested. The results reveal that there is a considerable increase in the toxicity of the complexes as compared to the free ligands. The biological activity of the ligands exhibited a marked enhancement on the coordination with the metal ions against all the tested bacterial/fungal strains, which shows that metal chelates are more active than the ligands. This may be explained by Tweedy's chelation theory,[23] according to which, chelation reduces the polarity of the central metal atom because of partial sharing of its positive charge with the ligand, due to which the lipophilic character of the metal chelate increases and favours its permeation through the lipid layer of the cell membrane. It has also been proposed that the ultimate action of the compounds is the denaturation of one or more proteins of the cell as a result of which normal cellular processes are impaired,[24] and the deactivation of various cellular enzymes that play vital roles in the different metabolic pathways of these microorganisms.

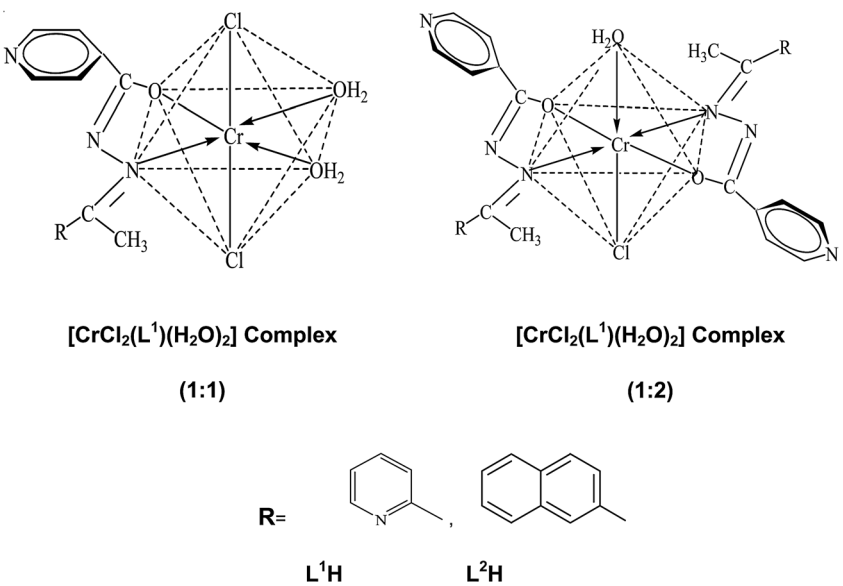


**Fig. 2** Suggested structures for the chromium complexes.

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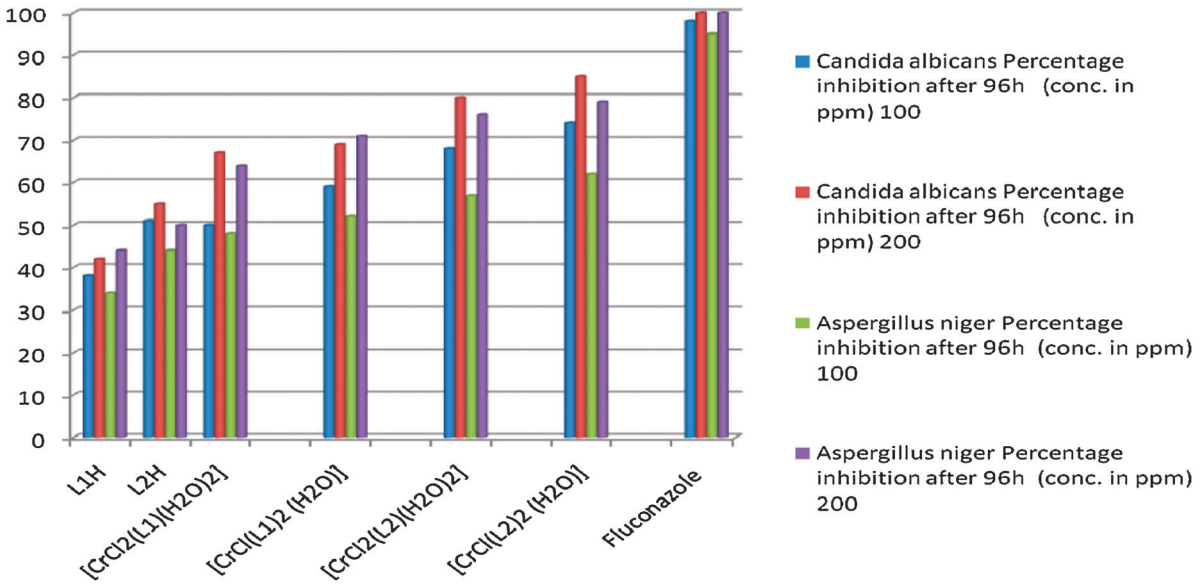


**Chart 1** Antifungal screening data for the ligands ($L^1H$ and $L^2H$) and their chromium(III) complexes.

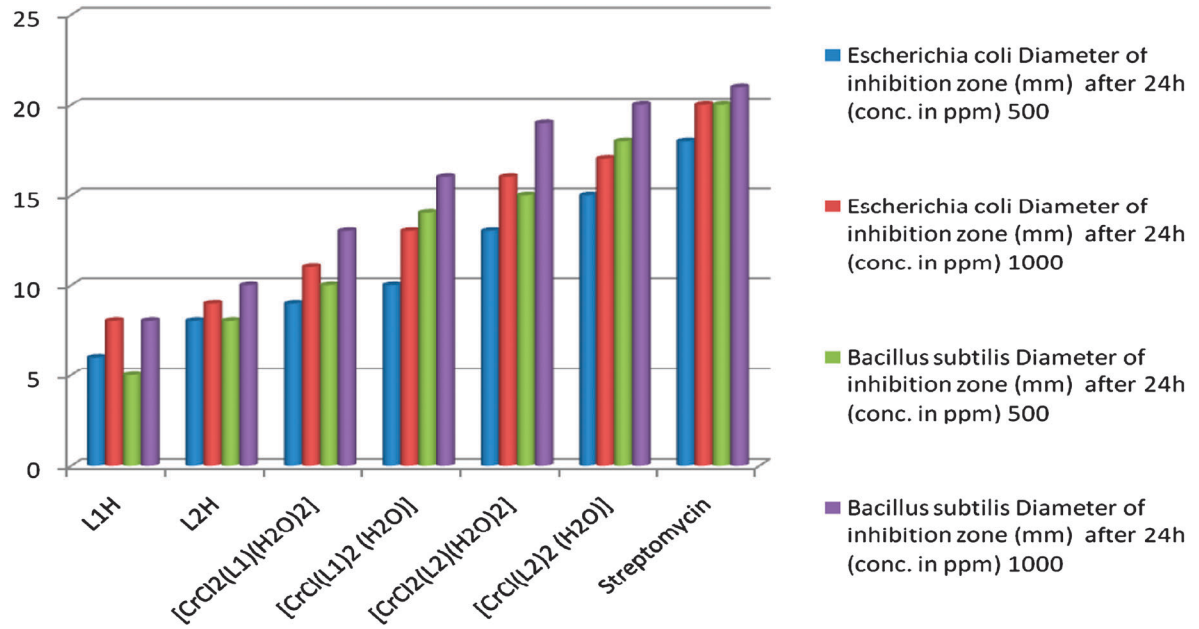


**Chart 2** Antibacterial screening data for the ligands ($L^1H$ and $L^2H$) and their chromium(III) complexes.

**Antifertility test**

*Results.* Treatment with the ligands ($L^1H$ and $L^2H$) and their chromium(III) complexes at the dose level of 20 mg $kg^{-1}$ b.wt, for a period of 60 days, showed the following variation in the different end points.

*Body and organ weights.* No significant change in body weight over the experimental period among the treated groups was noticed. However, a significant reduction in the weight of the testes, epididymis, ventral prostate and seminal vesicle were noticed after treatment with the ligands ($L^1H$ and $L^2H$) and their chromium(III) complexes Table 7.

*Sperm dynamics.* The sperm motility of the spermatozoa and sperm density in testes and cauda epididymis were decreased significantly ($p \leq 0.1$ to 0.001) after treatment with the ligands ($L^1H$ and $L^2H$) and their chromium(III) complexes in all the treated groups Table 8.

*Tissue biochemistry.* Oral administration of the ligands and their metal complexes caused a significant reduction in glycogen and sialic acid, whereas the testicular protein and cholesterol contents of the reproductive sex organs were increased significantly ($p \leq 0.1$ to 0.001) Table 9.

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**Table 7** Effect of ligands and their corresponding metal complexes on the reproductive organs weight of male rats[a]

| Group | Treatment | Body weight (g) | | Organ weight (mg/100 g b. weight) | | | |
|---|---|---|---|---|---|---|---|
| | | Initial | Final | Testes | Epididymis | Seminal vesicle | Ventral prostate |
| A | Control | 220.0 15.8 | 237.0 16.5 | 1100.0 75.8 | 520.0 14.1 | 440.0 15.5 | 390.0 13.2 |
| B | $L^1H$ | 228.0 13.7 | 245.0 17.5 | 950.0 45.0$^b$ | 430.8 15.4$^b$ | 380.0 21.7$^b$ | 305.0 15.5$^b$ |
| C | $L^2H$ | 233.0 14.1 | 251.0 15.6 | 936.0 17.7$^b$ | 410.0 15.3$^c$ | 366.0 20.3$^b$ | 292.0 15.2$^b$ |
| D | $[CrCl_2(L^1)(H_2O)_2]$ | 238.0 13.5 | 247.0 14.7 | 900.0 17.0$^b$ | 350.0 16.3$^c$ | 300.0 14.4$^b$ | 250.0 14.9$^b$ |
| E | $[CrCl(L^1)_2(H_2O)]$ | 225.0 10.7 | 235.0 13.4 | 870.0 15.0$^c$ | 310.0 14.3$^c$ | 310.0 13.2$^b$ | 245.0 13.9$^c$ |
| F | $[CrCl_2(L^2)(H_2O)_2]$ | 220.0 13.1 | 229.0 13.5 | 840.0 14.8$^c$ | 267.0 13.2$^c$ | 288.0 13.8$^c$ | 233.0 16.6$^c$ |
| G | $[CrCl(L^2)_2(H_2O)]$ | 225.0 14.7 | 220.0 13.8 | 670.0 18.6$^c$ | 226.0 11.5$^c$ | 245.0 17.4$^c$ | 210.0 14.8$^c$ |

[a] Mean ± 6 SEM of six animals. Groups B, C, D, E, F, G and H compared with Group A. [b] $p \leq 0.01$. [c] $p \leq 0.001$.

**Table 8** Sperm dynamics and fertility after the administration of ligands and their corresponding metal complexes[a]

| Group | Treatment | Sperm motility % (*Cauda epididymis*) | Sperm density (million/mL) | | Fertility % |
|---|---|---|---|---|---|
| | | | Testes | *Cauda epididymis* | |
| A | Control | 75.1 2.0 | 4.7 0.9 | 54.0 3.9 | 100(+ve) |
| B | $L^1H$ | 60.1 1.7$^c$ | 3.9 0.6$^b$ | 45.0 3.2$^b$ | 75(−ve) |
| C | $L^2H$ | 58.0 1.5$^c$ | 3.7 0.5$^b$ | 47.0 3.2$^b$ | 78(−ve) |
| D | $[CrCl_2(L^1)(H_2O)_2]$ | 45.0 1.5$^c$ | 2.1 0.5$^c$ | 40.0 1.2$^c$ | 80(−ve) |
| E | $[CrCl(L^1)_2(H_2O)]$ | 40.0 1.7$^c$ | 2.0 0.4$^c$ | 38.0 1.1$^c$ | 82(−ve) |
| F | $[CrCl_2(L^2)(H_2O)_2]$ | 37.0 1.6$^b$ | 1.8 0.3$^c$ | 35.0 1.1$^c$ | 85(−ve) |
| G | $[CrCl(L^2)_2(H_2O)]$ | 36.7 1.4$^c$ | 1.5 0.7$^c$ | 34.0 1.2$^c$ | 88(−ve) |

[a] Mean ± 6 SEM of six animals. Groups B, C, D, E, F, G and H compared with Group A. [b] $p \leq 0.01$. [c] $p \leq 0.001$.

*Serum testosterone.* A significant reduction ($p \leq 0.1$ to 0.001) in the serum testosterone level was observed after treatment with the ligands and their chromium(III) complexes Table 9.

## 4. Discussion

The administration of ligands ($L^1H$ and $L^2H$) and their corresponding chromium(III) complexes brought about a marked reduction in the weight of testes and other accessory sex organs. The reduction in testicular weight may be due to the tubule size, alteration of spermatogenesis with marked reduction of gametes production combined with a reduction in the seminiferous diameter in treated animals relative to the control,[25] and inhibition of the steroid biosynthesis of leydig cells.[26] The testes, epididymis, and other accessory sex organs are dependent on androgen for their growth and function. The reduction in the weights of these sex accessory organs may be due to the decreased production of androgen.[27]

The decreased sperm density in the testes and cauda epididymis is an indicator of reduced spermatogenesis, and the reduced sperm motility may be due to the altered enzymatic activity of the oxidative phosphorylation process.[28] Thus, a decrease in sperm motility and density after oral administration of the ligands and their corresponding chromium(III) complexes may be due to an androgen deficiency, which caused the impairment in testicular function by altering the enzymatic activities responsible for spermatogenesis, suggesting thereby an antiandrogenic effect of these compounds. The decrease in male fertility could be explained by the fact that the ligands and their metal complexes acted directly on the testes and influenced the androgen biosynthesis pathway.[29] The ligands and their chromium(III) complexes also induced biochemical changes in the testes and sex accessory organs.

The results demonstrate a marked decrease in testicular glycogen, which may be due to interference during glucose metabolism.[30] Inhibition of glycogen synthesis eventually affects the protein synthesis and thus inhibits spermatogenesis.[31]

Sialic acids are concerned with changing the membrane surface of maturing spermatozoa as well as with the development of their fertilizing capacity.[32] Thus, decreased levels of sialic acid in the testes and sex accessory organs may inhibit the fertilizing capacity of sperms.

The increased testicular cholesterol is attributed to decreased concentration of androgen, which results in impaired spermatogenesis.[33] Similarly, the elevation in the testicular protein contents after treatment with the ligands and their metal

**Table 9** Biochemical changes in the testes of male rats after treatment with the ligands and their corresponding metal complexes[a]

| Group | Treatment | Testicular sialic acid (mg $g^{-1}$) | Testicular protein (mg $g^{-1}$) | Testicular glycogen (mg $g^{-1}$) | Testicular cholesterol (mg $g^{-1}$) | Serum testosterone (mg $mL^{-1}$) |
|---|---|---|---|---|---|---|
| A | Control | 5.08 0.10 | 250.00 9.80 | 2.75 0.10 | 5.38 0.45$^b$ | 2.50 0.50 |
| B | $L^1H$ | 4.30 0.08$^b$ | 295.00 10.50$^b$ | 2.30 0.10$^b$ | 6.80 0.70$^b$ | 2.10 0.30$^b$ |
| C | $L^2H$ | 4.20 0.08$^b$ | 283.00 10.00$^b$ | 2.12 0.10$^b$ | 6,70 0.50$^b$ | 1.75 0.20$^b$ |
| D | $[CrCl_2(L^1)(H_2O)_2]$ | 3.10 0.07$^c$ | 330.00 8.70$^c$ | 1.90 0.09$^b$ | 7.50 0.90$^c$ | 1.70 0.20$^b$ |
| E | $[CrCl(L^1)_2(H_2O)]$ | 3.20 0.30$^c$ | 350.00 7.50$^c$ | 1.80 0.08$^c$ | 7.70 0.80$^c$ | 1.50 0.10$^c$ |
| F | $[CrCl_2(L^2)(H_2O)_2]$ | 2.90 0.10$^c$ | 330.00 10.50$^c$ | 1.60 0.09$^c$ | 7.40 0.80$^c$ | 1.55 0.14$^c$ |
| G | $[CrCl(L^2)_2(H_2O)]$ | 2.77 0.08$^c$ | 345.00 9.50$^c$ | 1.55 0.60$^c$ | 7.20 0.50$^c$ | 1.40 0.09$^c$ |

[a] Mean ± 6 SEM of six animals. Groups B, C, D, E, F, G and H compared with Group A. [b] $p \leq 0.01$. [c] $p \leq 0.001$.

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complexes may be due to the hepatic detoxification activities caused by these compounds, which results in an inhibitory effect on the activities of enzymes involved in the androgen biotransformation.

A marked reduction in testosterone content, in association with a highly reduced circulating level of this hormone, confirmed alterations in the reproductive physiology of the rats. These results suggested that the ligands ($L^1H$ and $L^2H$) and their chromium(III) complexes exert inhibitory effects on testicular function and lead to the infertility in male rats. Further, addition of a metal ion to the ligand enhances the activity.

## 4. Conclusions

Microwave (MW) irradiation is an efficient and environmentally-benign method to accomplish various inorganic syntheses to afford products in higher yields in shorter reaction periods. 1-(2-Pyridyl) ethanone isonicotinoyl hydrazone ($L^1H$) and 1-(2-naphthyl) ethanone isonicotinoyl hydrazone ($L^2H$) behave as monofunctional bidentate ligands with the metal ion under different reaction conditions. An octahedral geometry for the Cr(III) complexes have been proposed. The biological screening data of the ligands and their complexes indicate that the complexes are more potent than the parent ligands.

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