

Synthesis, spectroscopic and *in-vitro* antifungal studies of drug based mixed ligand complexes

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(Received: August 18, 2010; Accepted: September 21, 2010)

ABSTRACT

The mixed-ligand complexes of various metal(II) with 5-chloro-7-iodo-8-hydroxyquinoline (Clioquinol) and 5-(methoxymethyl-8-quinolinol) (MMQ) were prepared. The structure of mixed-ligand complexes was investigated using spectral, physicochemical, elemental analyses and thermal studies. Magnetic moment and reflectance spectral studies reveal that an octahedral geometry has been assigned to all the prepared complexes. The ligands, metal salts, complexes, control and standard drug were tested for their *in-vitro* antifungal. The metal complexes exhibit good activity against fungal strains compared with parental compounds and moderate compared with the standard drug (Clioquinol).

Key words: *In-vitro* antifungal activity, mixed-ligand complexes, spectral studies, magnetic moment.

INTRODUCTION

Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline) has antibacterial and antifungal activity and it is widely used in creams and ointments for the treatment of skin diseases¹. Clioquinol is an antifungal drug and antiprotozoal drug. It is neurotoxic in large doses. It is a member of a family of drugs called hydroxyquinolines which inhibit certain enzymes related to DNA replication. The drugs have been found to have activity against both viral and protozoal infections². In spite of successful human trials with CQ for the treatment of (AD), the coordination chemistry of this ligand with copper(II) or zinc(II) and its interaction with β -amyloid in the brain remains unknown. For a better understanding of its mechanism of action, we were incited to study the formation of CQ-metal complexes in a media with an ionic composition similar to the brain extracellular environment³. Thermal analysis, although an old technique, is now proving useful in

the interpretation and determination of different physical parameters such as inorganic and organic thermodynamics and reaction kinetics in different fields of study such as chemistry, polymer science, biology, medicine and pharmaceuticals⁴.

Previously, Kharadi et al. have synthesized a series of 8-hydroxyquinoline derivatives and their transition metal complexes⁵⁻¹⁰. However, no information is available on same derivative but different ligand *in-vitro* antifungal activity. Hence, in this paper, we report *in-vitro* antifungal activity properties of some transition metal and clioquinol complexes.

EXPERIMENTAL

Materials and Instrumentation

All the chemicals and reagents used for the preparation of ligands and complexes were commercial products (E. Merck Ltd, India) and used

without further purification. Cloquinol was purchased from Atul Ltd., Agro Chemical Division, Atul, Valsad (India). Luria broth was purchased from Hi-media Laboratories Pvt. Ltd, India. The organic solvents were purified by standard methods¹¹. Infrared spectra were recorded in a FT-IR Perkin Elmer, USA series-200, Spectrum GX Spectrometer using KBr pellets. C, H, N analyses were carried out using a Perkin Elmer, USA Series-II 2400 analyzer. The metal content of the complexes were determined by the EDTA titration technique¹² after treating them with mixture of HClO₄, H₂SO₄ and HNO₃ (1:1.5:2.5). The magnetic moments were obtained by the Gouy's method using mercury tetrathiocyanatocobaltate(II) as a calibrant ($\chi_g = 16.44 \times 10^{-6}$ c.g.s. units at 20 °C). Diamagnetic corrections were made using Pascal's constant¹³. A simultaneous TGA/DTG had been obtained by a model Perkin Elmer, Pyris-1 Instruments, Perkin Elmer, USA at heating rate of 10°C min⁻¹ under N₂ atmosphere.

Preparation of ligands

Synthesis of 5-chloromethyl-8-quinolinol (CMQ)

5-chloromethyl-8-quinolinol (CMQ) was prepared by chloromethylation of 8-hydroxyquinoline (oxine) according to the method reported in literature¹⁴. The detail of the procedure is given below.

To a mixture of 7.3 gm.(0.05 mole) of 8-hydroxyquinoline (oxine), 8 ml. conc.HCL and 8 ml.(0.05 mole) of 37 % formaldehyde dry HCL gas was passed at room temperature, till the yellow crystals fallout (about 2hours).The precipitates were filtered and air dried yield was 77% and m.p.180°C. (uncorrected)¹⁴.

Synthesis of 5-(methoxymethyl-8-quinolinol) (MMQ)

To a suspension of 2.3 gm. (0.01 mole) of 5-chloromethyl-8-quinolinol (CMQ), methanol (3 times.) and 0.84 gm.(0.01 mole) of sodium bicarbonate (NaHCO₃) added. The mixture was warmed on the steam bath with occasional shaking until most of the alcohol had been evaporated. The pale yellow solid was dissolved in water and made basic with 5 % ammonium hydroxide. The white solid was collected on a filter and dried to give 2.25 gm. The reaction scheme is shown in Fig. 1. Yield, 99%;

m.p. 83 °C. Found (%): C, 69.75, H, 5.72, N, 7.32. C₁₁H₁₁NO₅ (189) requires (%): C, 69.84, H, 5.82. IR (cm⁻¹): 3800-2700(-OH), 1599 and 3026 (aromatic C=C and C-H stretching), 1275-1298 (C-N), 2850, 2940, 1450 (-CH₂-). ¹H NMR: 7.1-7.64 (multiplet, quinoline), 8.51-9.29 (singlet of phenolic-OH), 4.75 (-CH₂-), 3.52 (-OMe).

Synthesis of the mixed-ligand complexes

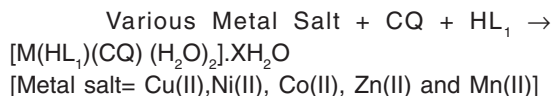
A water solution (100 mL) of various metal salt (10 mmol, 2.41 g) was added to dimethyl formamide solution (100 mL) of ligand (HL₁) (10 mmol, 2.77 g), followed by addition of cloquinol (10 mmol, 3.05 g) in ethanol; the pH was adjusted to 4.5-6.0 with dilute NaOH solution. The resulting solution was refluxed for 7 h and then heated over a steam bath to evaporate up to half of the volume. The reaction mixture was kept overnight at room temperature. The obtained product was washed with ether and dried over vacuum desiccator. The reaction scheme is shown in Fig. 2 whereas physicochemical parameters of complexes data are shown in Table 1.

Antifungal activity studies

In-vitro antifungal activity of the metal salts, control, standard drug, ligands and its complexes were analyzed against various fungal cultures using the Agar-Plate technique¹⁵⁻¹⁶.

RESULTS AND DISCUSSION

The analytical and physical data of the complexes are presented in Table 1. The complexes are colored and stable in air. They are insoluble in water and in most organic solvents but soluble in DMSO. The formation of the complexes is assumed according to the chemical reaction as shown below:



IR spectra

The important infrared spectral bands and their tentative assignments for the synthesized ligands (HL₁) and its complexes were recorded as KBr disks. In the investigated complexes, the bands observed in the region 3418-3437, 1278-1295, 865-875 and 705-710 cm⁻¹ are attributed to -OH

stretching, bending, rocking and wagging vibrations, respectively due to the presence of water molecules. The presence of rocking band indicates the coordination nature of the water molecule.¹⁷ In the cloquinol complexes of divalent metals, the $\nu(\text{C-O})$, appeared at 1120 cm^{-1} region and the position of the band slightly varies with the metal.¹⁸ The $\nu(\text{C-O})$, observed in the free oxine molecule at 1090 cm^{-1} , shifted to higher frequencies in all the mixed ligand complexes giving a strong absorption band at 1110 cm^{-1} . The spectra of the 8-HQ derivatives band at $1275\text{--}1298\text{ cm}^{-1}$ corresponding $\nu(\text{C-N})$, were shifted to lower frequency $1260\text{--}1287\text{ cm}^{-1}$ on complexation. This clearly indicates the coordination of cloquinol in these complexes.

Magnetic moments and electronic spectra

In order to shed some light on the geometrical structure of the complexes, the reflectance spectra of the complexes were recorded in the solid phase at room temperature. The reflectance spectra of the Mn(II) complex shows absorption bands at ~ 14600 , ~ 19720 and $\sim 24400\text{ cm}^{-1}$ assignable to ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{1g}$, ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{2g}$ and ${}^6\text{A}_{1g} \rightarrow {}^4\text{A}_{1g}$, ${}^4\text{E}_g$ transitions, respectively, in an octahedral environment around the Mn(II) ion. The magnetic moment value of the Mn(II) complex is 6.02 B.M. due to a high-spin d^5 -system with an octahedral geometry.^[19] For the Co(II) complex, the reflectance spectra exhibits the bands of medium intensity at ~ 9300 , ~ 18050 and $\sim 18900\text{ cm}^{-1}$, which may reasonably be assigned to ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{F})$, ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}(\text{F})$ and ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{1g}(\text{P})$ transitions, respectively, of an octahedral geometry around the metal ion²⁰ and the magnetic moment value observed is 4.06 B.M. The electronic spectra of the Ni(II) complex exhibits absorption bands at ~ 10200 , ~ 17650 and $\sim 23800\text{ cm}^{-1}$ assignable to ${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{2g}(\text{F})$, ${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{F})$ and ${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{P})$ transitions respectively, in an octahedral geometry. The value of the magnetic moment (2.84 B.M.) may be taken as additional evidence for their octahedral structure.^{21–24} The Cu(II) complex display a broad band at $\sim 15440\text{ cm}^{-1}$ due to the ${}^2\text{E}_g \rightarrow {}^2\text{T}_{2g}$ transition and the magnetic moment value is 1.78 B.M., which is close to spin-only value (1.73 B.M.) expected for an unpaired electron, which offers the possibility of an octahedral geometry.²⁵ The values of the electronic parameters,²⁶ such as the ligand

Table 1: Analytical and physical data of the metal complexes

| Metal complexes | Molecular formula | Mol. wt gm/mole | Yield % | % Metal analysis | | Elemental analysis | | | | | | |
|-------------------------|--|-----------------|---------|------------------|-------|--------------------|------|-------|------|-------|------|-------|
| | | | | Cal. | Found | % C | | % H | | % N | | |
| | | | | | | | Cal. | Found | Cal. | Found | Cal. | Found |
| HL ₁ -Cu(II) | C ₂₂ H ₂₄ N ₃ O ₆ Cu ⁺² | 475.54 | 70 | 13.36 | 13.3 | 55.51 | 5.04 | 55.45 | 4.91 | 5.88 | 5.74 | 5.87 |
| HL ₁ -Ni(II) | C ₂₂ H ₂₄ N ₃ O ₆ Ni ⁺² | 470.69 | 68 | 12.46 | 12.4 | 56.08 | 5.09 | 55.95 | 5.02 | 5.94 | 5.87 | 5.91 |
| HL ₁ -Mn(II) | C ₂₂ H ₂₄ N ₃ O ₆ Mn ⁺² | 466.93 | 72 | 11.76 | 11.7 | 56.53 | 5.13 | 56.44 | 5.05 | 5.99 | 5.91 | 5.89 |
| HL ₁ -Co(II) | C ₂₂ H ₂₄ N ₃ O ₆ Co ⁺² | 470.93 | 70 | 12.51 | 12.5 | 56.05 | 5.09 | 56.00 | 5.04 | 5.94 | 5.89 | 5.80 |
| HL ₁ -Zn(II) | C ₂₂ H ₂₄ N ₃ O ₆ Zn ⁺² | 477.39 | 75 | 13.69 | 13.6 | 55.30 | 5.02 | 55.22 | 4.94 | 5.86 | 5.80 | 5.80 |

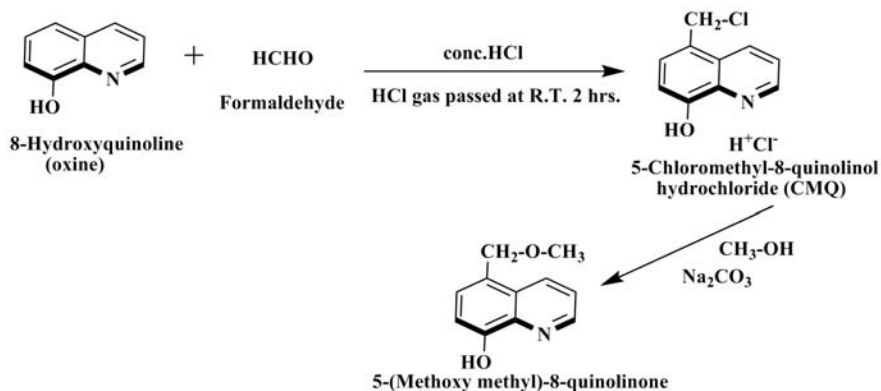


Fig. 1: The suggested structure scheme of ligand

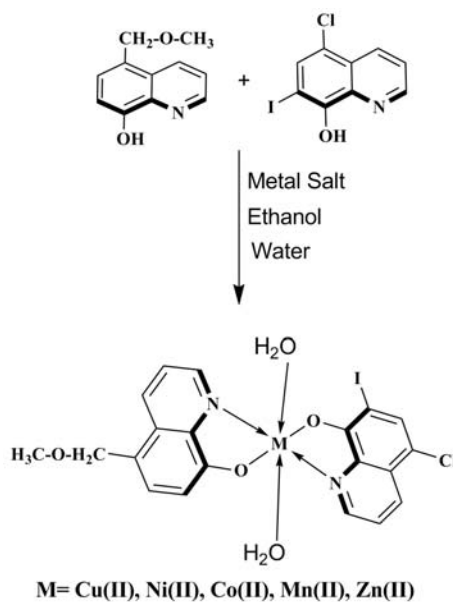


Fig. 2: The suggested structure scheme of ligand complexes

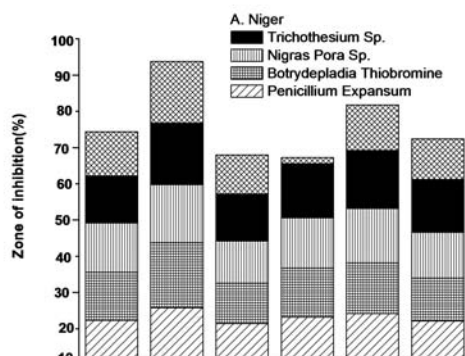


Fig. 3: Comparative analysis for antifungal activity of metal complexes

Table 2: Electronic parameters of the Co(II) and Ni(II) complexes^[a]

| Complexes | Observed band/cm ⁻¹ | | | v_2/v_1 | B | β | β_0 | 10 Dq |
|-----------|--------------------------------|-------|-------|-----------|-----|---------|-----------|-------|
| | v_1 | v_2 | v_3 | | | | | |
| Ni(II)-2 | 10200 | 17650 | 23800 | 1.73 | 723 | 0.70 | 29.7 | 10200 |
| Co(II)-3 | 9300 | 18050 | 18900 | 1.94 | 714 | 0.73 | 26.5 | 10415 |

^[a] The ligand field splitting energy (10 Dq), interelectronic repulsion parameter (B) and covalency factor (nephelauxetic ratio) (β) for the Ni(II) and Co(II) complexes were calculated using the secular equations given by E. König:

For Ni(II) complexe

$$10 Dq = v_1$$

$$10 Dq = \frac{1}{2} [(2v_1 - v_3) + (v_3^2 + v_1v_3 - v_1^2)^{1/2}]$$

$$15 B = (v_2 + v_3) - 3 v_1$$

$$15 B = v_3 - 2 v_1 + 10 Dq$$

For Co(II) complexe

$$\beta = B/B_0 [B_0 (\text{free ion}) = 1030]$$

$$\beta = B/B_0 [B_0 (\text{free ion}) = 971]$$

$$\beta_0 = (1 - \beta).100$$

$$\beta_0 = (1 - \beta).100$$

Table 3: Antifungal activity of compounds.

| Sample | Zone of inhibition at 1000 ppm (%) | | | | |
|-------------------------|------------------------------------|----------------------------------|------------------------|--------------------------|-----------------|
| | <i>Penicillium expansum</i> | <i>Botrydepladia thiobromine</i> | <i>Nigras pora</i> Sp. | <i>Trichothesium</i> Sp. | <i>A. niger</i> |
| HL ₁ | 62 | 66 | 68 | 65 | 61 |
| HL ₁ -Cu(II) | 79 | 90 | 80 | 85 | 85 |
| HL ₁ -Mn(II) | 57 | 56 | 58 | 65 | 54 |
| HL ₁ -Co(II) | 66 | 68 | 69 | 75 | 8 |
| HL ₁ -Zn(II) | 71 | 70 | 75 | 80 | 63 |
| HL ₁ -Ni(II) | 61 | 59 | 63 | 73 | 56 |

field splitting energy (10 Dq), Racah interelectronic repulsion parameter(B), nephelauxetic ratio(β) and ratio v_2/v_1 are summarized in Table 2.

Antifungal activity results

All the synthesized compounds were screened for their bioassay. From comparative analysis as shown in Fig. 3, it is observed that all the complexes exhibited strong activities against fungal microorganisms. In comparison with both the free ligands and metal salt, control (DMSO), clioquinol, the HL₁-Cu(II) complexes were more active against one or more fungal strains, thus introducing novel class of metal-based bactericidal agents. The zone of inhibition was measured around the disc and the results are represented in Table 3. From the graph it is clear that HL₁-Cu(II) is highly active among the complexes of the respective metal.

Abbreviations

CQ= Clioquinol

HL₁ = 5-(methoxymethyl-8-quinolinol)

D. D. Water = Double distilled water

B.M. = Bohr Magneton

ACKNOWLEDGEMENTS

The authors are grateful to Principal, V. P. & R. P. T. P. Science College, Sardar Patel University, Vallabh Vidyanagar-388 120, Gujarat, INDIA, for providing the necessary laboratory facilities. Analytical facilities provided by the Sophisticated Instrumentation Centre for Applied Research & Testing (SICART), Vallabh Vidyanagar, Gujarat, India is gratefully acknowledged.

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