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Synthesis, characterization and antifungal activity of transition metal complexes derived from a novel macrocyclic compartmental ligand

Rayees Ahmad Sheikh[1], Sheikh Shreaz[2], Luqman Ahmad Khan[2] and Athar Adil Hashmi[1]*

[1]Department of Chemistry, Jamia Millia Islamia (Central University), New Delhi, India
[2]Department of Biosciences, Jamia Millia Islamia (Central University), New Delhi, India

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ABSTRACT

A novel series of macrocyclic tetradentate nitrogen donor (N_4) ligand 5, 8, 13, 16-tetraoxo- 6, 15-dihydroxy-1, 4, 9, 12-tetraazacyclohexa-decane and its transition metal complexes of the type [$M(C_{12}H_{20}O_6N_4)Cl_2$] where M= Cu(II), Ni(II), and Co(II) have been synthesized by template condensation of malic acid and ethylenediamine. The ligand and its complexes have been characterized on the basis of elemental analysis, FTIR, ^{1}H NMR, ESI MS, TG/DTA, UV-Vis spectroscopic techniques, conductivity and magnetic measurements. On the basis of these studies, a six coordinate octahedral geometry around the metal ions in the complexes has been proposed. These metal complexes were also tested for their in vitro antifungal activities against some fungal species to assess their inhibiting potential and the activities shown by these complexes were compared with standard drugs.

Keywords: macrocycle, tetradentate, metal complexes, ergosterol, growth curve.

INTRODUCTION

The application of macrocyclic ligand and their transition metal complexes depends upon the nature of reactants and the corresponding metal ion. The macrocyclic ligands are a growing class of compounds with varying chemistry a wide range of different molecular topologies and sets of donor atoms [1-3]. The chemical properties of macrocyclic complexes can be tuned to force metal ions to adopt unusual coordination geometry. Nature prefers macrocyclic derivatives for many biological fundamental systems such as photosynthesis and transport of oxygen in mammalian and other respiratory systems [4, 5]. *Candida* is an obligate associate of warm-blooded animals, which is a commensal of the skin, gastrointestinal and genitourinary tracts, is responsible for majority of *Candida* bloodstream infections (Candidemia). Clinically, the most

significant member of the genus is *Candida albicans* (47.6%), *Candida tropicalis* (35.4%) followed by *Candida glabrata* and can cause infections (*Candidiasis* or thrush) in humans and other animals, especially in immunocompromised patients such as leukemia, acquired immunodeficiency syndrome or patients who undergo cancer therapy, organ transplantation, severe burn cases, pregnancy are particularly susceptible to opportunistic fungal infections. Numbers of antifungal agents are available for the treatment of *Candidal* infections [6, 7] majority of them being polyenes such as Amphotericin B and Nystatin or the azoles, such as Itraconazoles and Fluconazole. Currently, uses of standard antifungal therapies are scare due to the high toxicity, low efficacy rates, and drug resistance. Recent studies have indicated C. *albicans* resistance to azoles or heptotoxicity and nephrotoxicity linked to polyene use, particularly amphotericin B [8].

In order to avoid these drawbacks, a search for new and more effective compounds is needed for the treatment of this fungal infection. Various new antifungals have demonstrated therapeutic potential. Aza-type macrocyclic ligands show antifertile, antibacterial, antifungal and other biological properties [9, 10]. The macrocyclic systems are of significant interest not only for their pharmacological properties as antibacterial, anticancer, antiviral, antifungal agent [11] but also for their capacity for chemical recognition of anions and metals of biochemical, medical and environmental importance [12-14]. The chemistry of transition metal ion with macrocyclic ligands has become a rapidly growing area of research, because of their importance in biological processes and constitutes the active site in metalloprotiens and enzymes [15-16]. Copper is an important trace element for life processes and several copper containing proteins have been identified [17]. Biochemistry of nickel is well documented. [18] Nickel and its organometal derivatives show good antimicrobial properties [19]. The cobalt is an essential element for life although it does not participate in O_2 metabolism. Some important cobalt-containing metalloproteins are ribonucleotide reductase [20], nitrile hydratase, glucose isomerase [21], where cobalt plays directly or indirectly an important role. Organocobalt complexes have also shown high antibacterial activity against microbes such as *Staphylococcus aureus* and *Enterococcus faecalis* [22].

It has been well established that many drugs have become resistant to microbes, recognizing the antimicrobial properties of macrocyclic transition metal complexes. We therefore have choose to work on the development of new macrocyclic ligand and its metal complexes, which may provide additional options for the treatment of superficial fungal infections, anti microbial in nature even at low doses, and they may help to overcome the limitations of current treatments. Efforts have been made in the last decades to the design and synthesis of macrocycle or macrocyclic complexes and to study their physico-chemical properties [23]. These investigations emphasized the great relevance of these systems in medicinal chemistry. Several synthetic studies are now days available for the preparation of well organized macromolecular systems which exhibit peculiar physico-chemical properties or have well defined pharmacological properties. [11, 24]. So here we report a synthesis and characterization of a macrocyclic tetradentate ligand and its transition metal complexes, which can be used as high potential drug. Anticandidial activities are evaluated by performing: MIC, growth curve studies and ergosterol extraction and estimation assay.

EXPERIMENTAL SECTION

Physical Measurements

Malic acid (Merck Ltd, India), and ethylenediamine (Merck Ltd, India) were purchased and used as received. The solvents were purchased from (Merck India Ltd.). Samples for micro analysis

were dried in vacuum to constant weight. Elemental analyses were performed by a Perkin Elmer 2400 CHNSO Elemental Analyser. IR spectra were recorded as KBr pellets using a Perkin Elmer 1620 FT IR spectrophotometer. Far IR spectra were recorded as CsI pellets in the region 650-100 cm^{-1} using a JASCO FT IR spectrophotometer. ^1H NMR spectra were recorded using a Bruker DPX-300 MHz spectrophotometer operating at room temperature with DMSO d_6 as solvent. The chemical shift (δ) are reported in parts per million (ppm) using tetramethylsilane as internal standard. Positive and negative ESI mass spectra were measured by Bruker (esquire3000_00037) instrument. Thermal analysis (TG/DTA) data were studied under nitrogen atmosphere using a SII Ex Star 6000 TG/DTA 6300 instrument. Magnetic susceptibility measurements were carried out from a microanalysis laboratory by Gouy method at room temperature. Electronic spectra were recorded on a Spectro–UV-Vis Dual Beam 8 auto cell UVS–2700 LABOMED, INC, US spectrophotometer using DMSO as solvent. Melting point was recorded on a Metrex melting point apparatus.

Synthesis of macrocyclic ligand
5, 8, 13, 16-tetraoxo- 6, 15-dihydroxy-1, 4, 9, 12-tetraazacyclohexa-decane
The hot ethanolic solution (30 ml), of malic acid (5 mol.) and a hot ethanolic solution (30 ml) of ethylenediamine (5 mol) were mixed slowly with constant stirring. This mixture was refluxed at 60-70^oC for 8 h in the presence of few drops of concentrated hydrochloric acid. On keeping it overnight at O^0C, a cream white precipitate was formed, which was filtered, washed with ethanol and dried in vacuo over P_4O_{10} and it was recrystallized from methanol (yield 85%), mp>300^oC, IR (KBr, cm^{-1}): 3493(N-H), 2990(C-H), 1645(C=O), 1435(C-N), 1033, 858, 751; ^1H NMR (300 MHz, δ ppm from TMS in DMSO-d_6, 300 k): δ 11.62-12.50 (4H, br N-H), δ 4.18- 4.35(4H, C-H_2), δ 3.99(2H, C-H), δ 2.96(8H, OC-N-C-H_2). ESI MS (m/z) 317 $[M]^+$, 318 $[M+1]^+$. Elem anal calcd C 45.58, H 6.32, O 30.36, N 17.72%; found C 45.60, H 6.35, O 30.39, N 17.75%.

Synthesis of Cobalt (II) complex:
$CoCl_2.6H_2O$ (2 mol) dissolved in 25 mL methanol was added drop wise to a methanolic solution (25 ml) of the ligand (2mol) with continuous stirring. The resulting solution was stirred for 9 hours at 30^oC and the solution was reduced to half of its volume. It was then allowed to stand overnight in a refrigerator. A light pink product separates out, which was isolated by filtration under vacuum. It was washed thoroughly with hexane and dried in vacuo over fused $CaCl_2$. The compound was recovered in solid state. It was recrystallised from methanol Yield 70% and m.p.>300^oC. UV-Vis (DMSO) cm^{-1}, 12,920, 16,260, and 23,450, IR (KBr, cm^{-1}): 3479(N-H), 2978(C-H), 1637(C=O), 1420(C-N), 1043, 881, 748.; Far IR (CsI, cm^{-1}) 465 (Co-N), 346 (Co-Cl). ^1H NMR (300 MHz, δ ppm from TMS in DMSO-d_6, 300 k): δ 11.64-12.54 (4H, br N-H), δ 4.20- 4.38(4H, C-H_2), δ 4.01(2H, C-H), δ 2.98(8H, OC-N-C-H_2). ESI MS (m/z) 447 $[M]^+$, 448 $[M+2]^+$. Molar conductance, Λ_m ($\Omega^{-1}cm^{-1}$ mol^{-1}, 10^{-3} DMSO, r.t.): 30. μeff (r.t., BM): 4.96. Elem. anal. calcd C 32.31, H 4.48, O 21.52, N 12.56%; found C 32.34, H 4.51, O 21.55, N 12.59%;

Synthesis of Nickel (II) complex
The above same procedure was followed except $NiCl_2.6H_2O$ was used instead of $CoCl_2.6H_2O$. A light green product was obtained which was recrystallised from methanol, 65% yield and m.p.>300^oC; UV-Vis (DMSO) cm^{-1}, 11,230, 15,420, and 24,240; IR (KBr, cm^{-1}): 3475(N-H), 2980(C-H), 1632(C=O), 1416(C-N), 1045, 720; Far IR (CsI, cm^{-1}) 445 (Ni-N), 332 (Ni-Cl). ^1H NMR (DMSO d_6, 300K): δ 11.63-12.56(4H, br N-H), δ 4.21- 4.39(4H, C-H_2), δ 4.00(2H, C-H), δ 2.99(8H, OC-N-C-H_2). ESI MS (m/z) 446 $[M]^+$, 417 $[M+2]^+$. Molar conductance, Λ_m ($\Omega^{-1}cm^{-1}$ mol^{-1}, 10^{-3} DMSO, r.t.): 28. μeff (r.t., BM): 2.95. Elem. anal. Calcd: C 32.33, H 4.48, O 21.53, N 12.56%; found C 32.35, H 4.50, O 21.55, N 12.59%;

Synthesis of Copper (II) complex
For the synthesis of copper complex above same procedure was followed except $CuCl_2.6H_2O$ was used instead of $CoCl_2.6H_2O$. A Sky blue product was obtained, which was recrystallised from methanol 67% yield and m.p.>300°C; UV-Vis (DMSO) cm^{-1}, 13,120, 15,340, and 24,480; IR (KBr, cm^{-1}): 3481(N-H), 2974(C-H), 1625(C=O), 1410(C-N), 1073, 882, 749; Far IR (CsI, cm^{-1}) 430 (Cu-N), 329 (Cu-Cl). ^1H NMR (300 MHz, δ ppm from TMS in DMSO-d_6, 300 k): δ 11.65-12.59(4H, br N-H), δ 4.22- 4.40(4H, C-H_2), δ 4.02(2H, C-H), δ 3.01(8H, OC-N-C-H_2). ESI MS (m/z) 421 M^+, 422 $[M+2]^+$. Molar conductance, Λ_m ($\Omega^{-1}cm^{-1}$ mol^{-1}, 10^{-3} DMSO, r.t.): 29. μeff (r.t., BM): 1.96. Elem anal calcd C 31.98, H 4.43, O 21.30, N 12.42%; found C 32.01, H 4.45, O 21.34, N 12.45%;

Antimicrobial Screening

Growth Conditions
All of the fungal species used in this study were obtained from Indian Institute of Integrative Medicines (IIIM) Jammu (India). Stock cultures of *Candida albicans* ATCC 10261, *Candida tropicalis* ATCC 750, *Candida glabrata* ATCC 90030 and *Candida kruesi* ATCC 6258 were maintained on slants of nutrient agar (yeast extract 1%, peptone 2%, D-glucose 2% and agar 2.5%) (HiMedia) at 4ºC. To initiate growth for experimental purposes, one loop full of cells from an agar culture was inoculated into 25ml of respective nutrient media and incubated at 30-37ºC for 24 hr i.e. up to stationary phase (primary culture). The cells from primary culture (10^8 cells ml^{-1}) were re-inoculated into 100 ml fresh YEPD medium and grown for 8-10 h i.e., upto mid-log phase (10^6 cells ml^{-1}). Nystatin was purchased from SIGMA chemicals (USA). Solvents and media used in this study were purchased from Himedia, India.

Determination of MIC
Minimum inhibitory concentration was defined as the lowest concentration of the test molecule that causes inhibition of visible growth of microbial cells. MIC was determined *in vitro* in liquid medium by serial broth dilution method [25]. Nystatin was included as positive control. The MIC values correspond to the lowest concentrations that did not allow for the detection of any visible growth.

Growth studies
For growth studies 10^6 cells (optical density A_{595} = 0.1) of strains were grown aerobically in automated shaker set at 37°C until stationary phase. Growth was followed turbidometrically at 595 nm using Lab Med Spectrophotometer (USA). Required concentrations of test compound were added to culture. The growth rate of fungi and inhibitor were performed in triplicate. Optical density was recorded for each concentration against time (hrs). The growth rate is equivalent to the slope of log (optical density) versus time during the exponential phase.

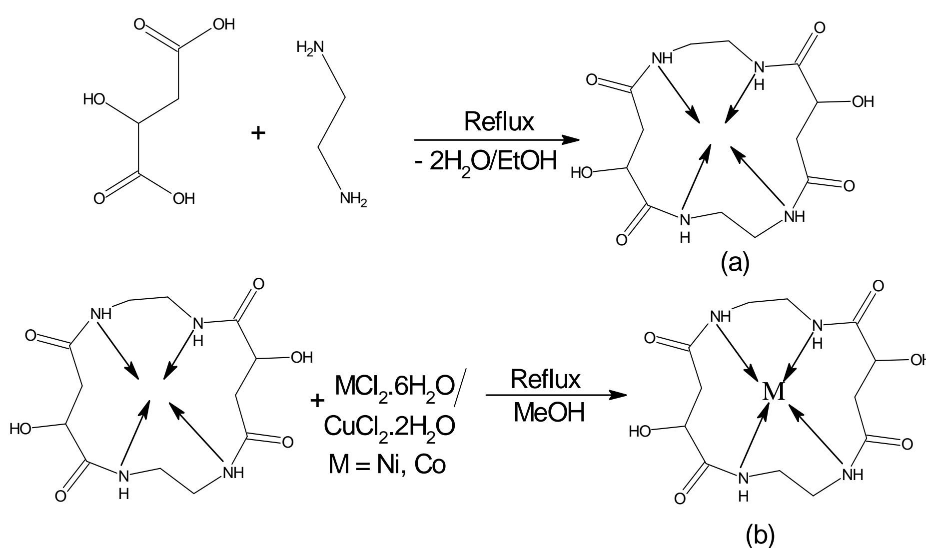
Sterol composition assay
Total intracellular sterols were extracted following the method prescribed by (Breivik & Owades, 1957, [26]. A single *Candida* colony from an overnight Sabouraud Dextrose Agar (Merck Ltd, India) plate culture was used to inoculate 50 ml of Sabouraud Dextrose Broth (Merck Ltd, India) for control and for various concentrations of the test compounds. The cultures were incubated for 16 hrs and harvested by centrifugation at 2,700 rpm for 5 min. The wet weight of the cell pellet was determined. Three milliliters of 25% alcoholic potassium hydroxide solution was added to each pellet and vortex mixed for 1 min. Cell suspensions were transferred to sterile borosilicate glass screw-cap tubes and were incubated in at 85°C water bath for 1 hr. Following incubation, tubes were allowed to cool. Sterols were then extracted by addition of a mixture of 1 ml of sterile distilled water and 3 ml of *n*-heptane followed by vigorous vortex

mixing for 3 min. The heptane layer was transferred to a clean borosilicate glass screw-cap tube and stored at -20°C. Prior to analysis, a 20-µl aliquot of sterol extract was diluted fivefold in 100% ethanol and scanned spectrophotometrically between 240 and 300 nm with a Spectrophotometer (Systronics UV-Visible Spectrophotometer 117) The presence of ergosterol and the late sterol intermediate24 (26) DHE in the extracted sample resulted in a characteristic four-peaked curve. A dose-dependent decrease in the height of the absorbance peaks was evident and corresponded to decreased ergosterol concentration where as flat line indicating absence of sterol in the sample. Ergosterol content is calculated as a percentage of the wet weight of the cell by the following equations: % ergosterol + % 24(28) DHE = $[(A_{281.5}/290) \times F]$/pellet weight, % 24(28) DHE = $[(A_{230}/518) \times F]$/pellet weight and % ergosterol = [% ergosterol + % 24(28) DHE] – % 24(28) DHE, Where F is the factor for dilution in ethanol and 290 and 518 are the E values (in percentages per centimeter) determined for crystalline ergosterol and 24(28) DHE, respectively.

RESULTS AND DISCUSSION

Ligand was prepared by condensing the corresponding acid and the diamine in presence of the few drops hydrochloric acid (scheme 1. a). This ligand was then refluxed at room temperature with the metal chlorides to form their corresponding metal complexes (scheme 1. b).

Scheme 1 (a, b):



The molar conductivity (Λ_m) of the metal complexes measured in 1×10^{-3} mol L^{-1} DMSO at room temperature show low values indicate that they are non electrolyte species [27].

IR Spectra

Ligand and its complexes

In the IR spectrum of ligand absences of a broad absorption band characteristic for hydroxyl group of COOH in malic acid, indicates that the OH group of malic acid was detached from the COOH group to form a bond between carboxyl carbon atom and amino group nitrogen of ethylenediamine, also suggest complete condensation of reactants and elimination of water molecule. This has been confirmed by the appearance of a strong signal at 1435 cm^{-1} which may be attributed to the C-N bond [28]. A sharp medium intensity band observed at 3493 cm^{-1} may be assigned to υ(N–H) of the secondary amino group [29].The ligand also shows a signals for the C=O at 1645 cm^{-1} and C-H at 2990 cm^{-1} vibrations. The low frequency of C=O group as

compared to acetone (1715 cm^{-1}) is attrigrated to resonance with lone pair of the nitrogen. The shifting in the band of υ(C-N) towards the lower wave number in the metal complexes indicating that the coordination takes place through the nitrogen of the υ(C-NH) group, hence implying that the macrocyclic ligand (M) is tetradentate. This indicates the flow of electron density towards the metal atom through the C-N group. This has been finally established through far IR spectra by the appearance of new signals seen at 465, 445, 430 cm^{-1} in the spectra of metal complexes which gives us clear proof for the presence of metal–nitrogen bond in Co(II), Ni(II), and Cu(II) complexes respectively [30, 31]. Other vibrating signals are seen at 346, 333 and 329 cm^{-1} in the spectra of metal complexes give us proof for the presence of metal–chlorine bond in Co(II), Ni(II) and Cu(II) complex respectively [30].

^{1}H NMR Spectra

A broad signal in the range 11.62-12.50 ppm which is attributed to amide CO-N-H, (4H) was shown in the spectrum of ligand [32] and does not show any signal corresponding to primary amine. A signal appearing at 2.96 ppm has been ascribed to methylene protons $OC–N-CH_2$, (8H), while as C-H (2H) protons appear at 3.99 ppm. Another signal appears in the range 4.18-4.35 corresponds to $-CH_2$ (4H). The NMR spectrum of the ligand is consistent with the single species present in the solution, since only one set of signals is observed in the ligand. These proton signals undergo down field shifting in all the metal complexes of the ligand, because of the paramagnetic effect of metal (II) ions and hence support the coordination of the ligand towards the metal ions [33, 34] and also the macrocyclic nature of the product.

Electro spray ionization mass spectra (ESI MS)

ESI MS of the ligand and the complexes were studied in DMSO solution. Fig.1. A negative ion ESI mass spectrum of ligand confirms the proposed formula by showing a peak at m/z 317 corresponding to the macrocyclic moiety [($C_{12}H_{20}O_6N_4$) atomic mass m/z 316.14]. The series of peaks in the range m/z 120.7, 164.6, 254.7, etc, may be assigned to various fragments. These data suggests the 2+2 condensation of malic acid and ethylenediamine. Their intensity gives an idea of stability of fragments. Similarly positive ion ESI-MS of the cobalt, and copper, negative ion ESI-MS nickel complexes shows a peak at m/z 447, 451, 446 respectively which is consistent with the molecular ion fragment and it supports the proposed structure of the complexes. $[M+2]^+$ fragments were observed in all the metal complexes. This may be possible due to presence of isotopic chlorine in low quantities [35]. In some cases, the molecular ion peak was also associated with the solvent, water molecules and some adduct ions from the mobile phase solution [36].

Electronic Spectra

Cobalt (II) complex

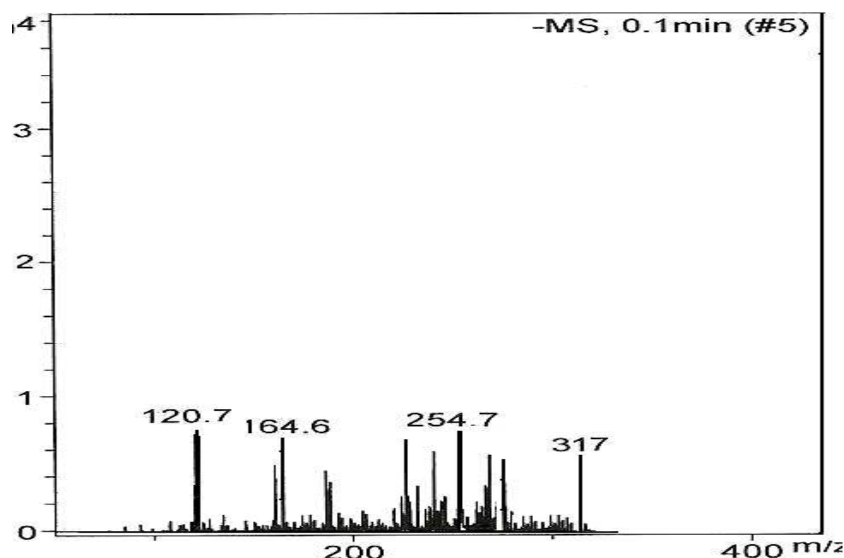
The electronic spectrum of cobalt(II) complex exhibits absorption bands at 12,920, 16,260, and 23,450cm^{-1}, which may be assign to $^4T_{1g}$ (F)→ $^4T_{2g}$ (F)(ν_1), $^4T_{1g}$→ $^4A_{2g}$ (ν_2) and $^4T_{1g}$ (F)→ $^4T_{1g}$ (P)(ν_3) transitions respectively [37]. Suggesting an octahedral geometry around a Cobalt(II) ion, in the complexes under study. Furthermore, the magnetic moment measurements recorded at room temperature lie at 4.96 B.M. This value is indicative of an octahedral geometry [38] of these complexes.

Copper (II) complex

The electronic spectrum of the mononuclear copper(II) complex recorded at room temperature, in DMF solution, shows broad band absorption at 13,120, 15,340, and 24,480cm^{-1}, which may be assign to $^2B_{1g}$→ $^2A_{1g}$, ($d_{x^2-y^2} \rightarrow d_{z^2}$)($\nu_1$), $^2B_{1g}$→ $^2B_{2g}$, ($d_{x^2-y^2} \rightarrow d_{zy}$)($\nu_2$), and $^2B_{1g}$→ ^2E_g, ($d_{x^2-y^2}$ → d_{zy}, d_{yz})(ν_3) transition and it is in conformity with octahedral geometry [38], an indication of

the most probable geometric configuration of the synthesized metal complexes is their magnetic moment values. So, it has been further confirmed by the magnetic moment measurements, room temperature values lie at 1.96 B.M corresponding to the presence of one unpaired electron and it supports an octahedral geometry [39, 40].

Fig. 1



Nickel (II) complex

The electronic spectra of the Ni(II) complexes exhibit three absorption bands at 11,230, 15,420, and 24,240 cm^{-1} these bands may be assign to three spin allowed transition: $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)(\upsilon_1)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)(\upsilon 2)$, and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)(\upsilon 3)$, respectively [39]. This value is indicative to the octahedral geometry. The magnetic moment of the Ni(II) complex at room temperature lie at 2.95 B. M. These values are in tune with high spin configuration and show the presence of an octahedral environment around the Ni(II) ion.

Thermo Gravimetric Analysis (TG/DTA)
Macrocyclic Ligand and its complexes

The thermal analysis (TG/DTA) of the ligand and its metal complexes was recorded under nitrogen atmosphere at the heating rate of 10°C/min. The macrocyclic ligand is stable upto 230°C and shows a continuous weight loss upto 350°C, Therefore the whole macrocyclic ligand gets decomposed in a single step. The DTA of the macrocyclic ligand shows two endothermic peaks; one broad endothermic peak at 248°C with a shoulder at 240°C corresponds to the melting and the first inflexion point. The second inflexion on the DTA curve occurs at 321°C which represents a small weight loss step from 330-350°C.

The thermal gravimetric (TG) analysis was used as a probe to proof the associated water or solvent molecules to be in coordination sphere or in crystalline form [41]. The thermo gram of copper(II), nickel(II) and cobalt(II) complexes are more stable than the macrocyclic ligand and does not decompose upto 280, 273 and 285°C respectively. It shows a major step of decomposition from 290-340°C which is detected by DTA at 330°C, this corresponds to the loss of two malic acids and two ethylenediamine moieties (observed weight 72.5%, theoretical weight 70.88%).

Antimicrobial Activity

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration was defined as the lowest concentration of the ligand and its complexes that causes decrease in absorbance compared with that of the control (no test compound). The MIC of ligand, its metal complexes and standard drug were determined against four fungal species using broth dilution method (BDM). From the results in (Fig. 2) it was observed that, in fungi generally the MIC of MNi<MCo<MCu<M. The yeast species were found to be more sensitive to Ni(II) complex, compared to Co(II), Cu(II) and ligand [M].

Fig. 2: Minimum Inhibitory Concentrations MIC (µg/ml) of ligand and its metal complexes against different fungal species

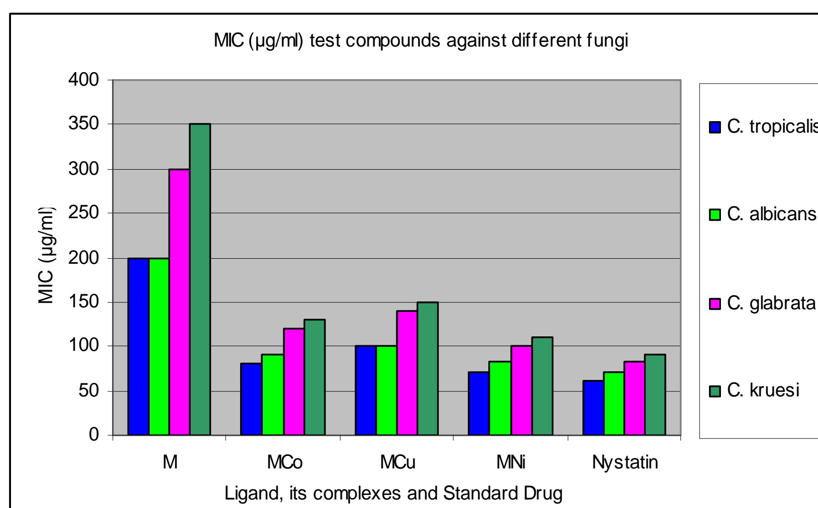
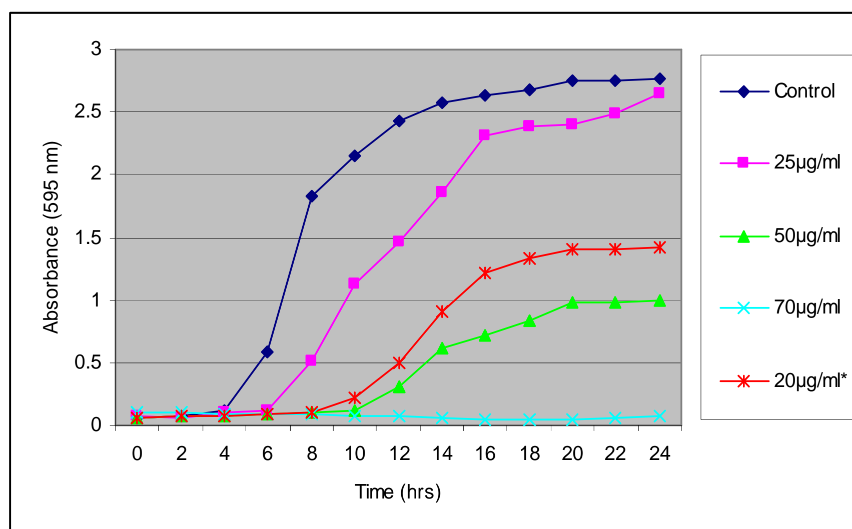


Fig. 3. Effect of Ni(II) complex in concentration range of 25–70µg/ml was studied on *Candida tropicalis* ATCC 750. Growth curve plotted against absorbance at 595 nm and time (hrs) shows complete inhibition of growth at 70µg/ml



** Nystatin (-ve control)*

Growth curve studies

In case of growth curve studies the effect of increasing concentrations of the ligand and its complexes on the growth pattern of different fungal species have been studied. Control cells showed a normal pattern of growth with lag phase of 4 hrs, active exponential phase of 8-10 hrs

before attaining stationary phase. Increase in concentration of test compounds leads to significant decrease in growth. Ni(II) complex when treated against *Candida tropicalis* at concentration of 25µg/ml the growth pattern has changed, the lag phase is extended by 2h, the stationary phase has not reached the same level of cell growth as in case of control and at 50µg/ml the lag phase is further extended by 4h. At concentration of 70µg/ml (MIC level) there is total inhibition of growth showing a flat line (Fig.3). (Nystatin 20µg/ml), showed the lag phase further extended by 4h with respect to control. Significant and pronounced effect is observed for all the synthesized complexes. Ni(II), Co(II) and Cu(II) complex, in concentration dependent manner suppressed growth and delayed exponential phases. At MIC values complete inhibition of growth was observed.

Sterol assay

Total sterol content of samples treated with varying concentrations of ligand [M] and its complexes was studied (Table 1. a, b, c, d). From the results, it is clear that with increase in test compound concentrations, %Ergosterol inhibition increases and finally at MIC value, flat line was observed indicating absence of ergosterol in the sample. High level of the metal complex was able to drastically reduce ergosterol content of cell membrane, although exact mechanism of its action unlike other antifungals or azoles is not understood yet for which sterol quantitation method is a reliable antifungal susceptibility testing method.

Table 1 (a, b, c, d). Ergosterol content of Control cells and treated samples

(a) Ligand (M)

C. tropicalis			***C. albicans***		
Sample	% Ergosterol Content	% Decrease	Sample	% Ergosterol Content	% Decrease
Control	100	0	Control	100	0
50µg/ml	82	18	50µg/ml	85	14
100µg/ml	77	23	100µg/ml	66	20
200µg/ml	59	41	200µg/ml	45	37

C. glabrata			***C. kruesi***		
Sample	% Ergosterol Content	% Decrease	Sample	% Ergosterol Content	% Decrease
Control	100	0	Control	100	0
100µg/ml	71	09	100µg/ml	73	06
200µg/ml	39	18	200µg/ml	43	15
300µg/ml	25	34	300µg/ml	29	31

(b) Ni(II) Complex

C. tropicalis			***C. albicans***		
Sample	% Ergosterol Content	% Decrease	Sample	% Ergosterol Content	% Decrease
Control	100	0	Control	100	0
25µg/ml	85	38	30µg/ml	85	32
50µg/ml	64	65	60µg/ml	66	59
70µg/ml	42	95	82µg/ml	45	86

C. glabrata			***C. kruesi***		
Sample	% Ergosterol Content	% Decrease	Sample	% Ergosterol Content	% Decrease
Control	100	0	Control	100	0
30µg/ml	71	26	40µg/ml	73	22
60µg/ml	39	49	80µg/ml	43	37
100µg/ml	25	79	110µg/ml	29	67

(c) Co(II) Complex

C. tropicalis			*C. albicans*		
Sample	% Ergosterol Content	% Decrease	Sample	% Ergosterol Content	% Decrease
Control	100	0	Control	100	0
25µg/ml	85	27	30µg/ml	85	25
50µg/ml	64	50	60µg/ml	66	46
80µg/ml	42	77	90µg/ml	45	74
C. glabrata			***C. kruesi***		
Sample	% Ergosterol Content	% Decrease	Sample	% Ergosterol Content	% Decrease
Control	100	0	Control	100	0
40µg/ml	71	20	50µg/ml	73	16
80µg/ml	39	40	100µg/ml	43	32
120µg/ml	25	71	130µg/ml	29	63

(d) Cu(II) Complex

C. tropicalis			*C. albicans*		
Sample	% Ergosterol Content	% Decrease	Sample	% Ergosterol Content	% Decrease
Control	100	0	Control	100	0
30µg/ml	85	23	30µg/ml	85	21
60µg/ml	64	40	60µg/ml	66	37
100µg/ml	42	69	100µg/ml	45	67
C. glabrata			***C. kruesi***		
Sample	% Ergosterol Content	% Decrease	Sample	% Ergosterol Content	% Decrease
Control	100	0	Control	100	0
50µg/ml	71	17	50µg/ml	73	12
100µg/ml	39	34	100µg/ml	43	29
140µg/ml	25	61	150µg/ml	29	57

The data represents Mean of three experiments

A whole line of current antifungals target ergosterol biosynthesis pathway or its end product which is unique to fungi. It is important target because it maintains membrane fluidity, asymmetry and integrity. Ergosterol also contributes to proper functioning of membrane bound enzymes. Ni(II) complex has significant effect on ergosterol production (Table 1.b). At MIC value of 70µg/ml, 82µg/ml, 100µg/ml and 110µg/ml reduction of 95%, 86%, 79% and 67% in ergosterol content is seen in case of *C. tropicalis*, *C. albicans*, *C. glabrata* and *C. kruesi* respectively (Fig. 4 a, b, c, d)

Figure 4 (a). UV spectrophotometric sterol profile of *C. tropicalis* ATCC 750 in presence of Ni(II) complex

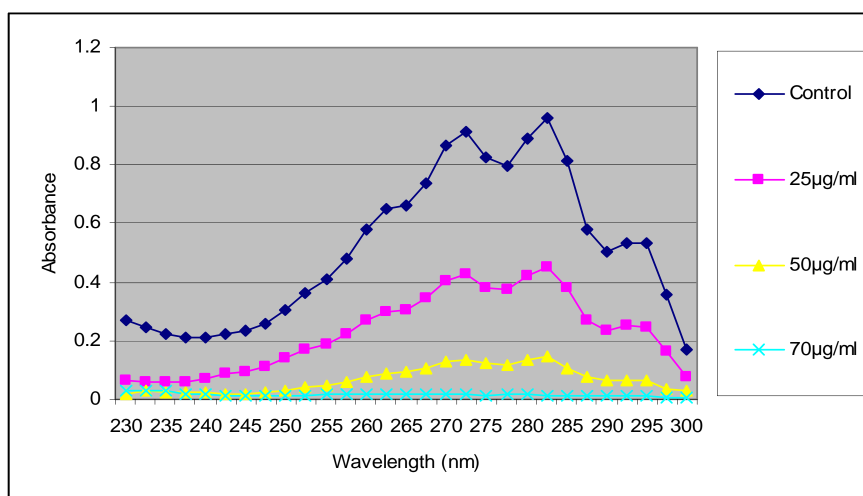


Figure 4 (b). UV spectrophotometric sterol profile of *C. albicans* ATCC 10261 in presence of Ni(II) complex

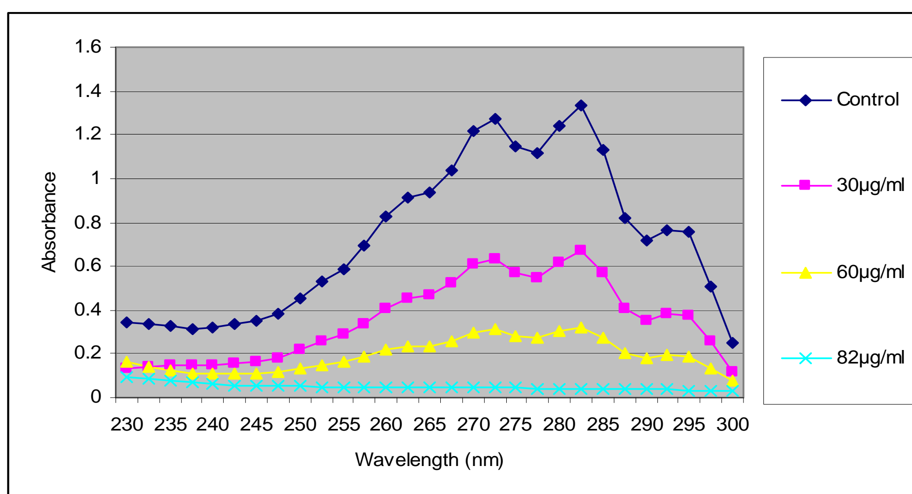


Figure 4 (c). UV spectrophotometric sterol profile of *C. glabrata* ATCC 90030 in presence of Ni(II) complex

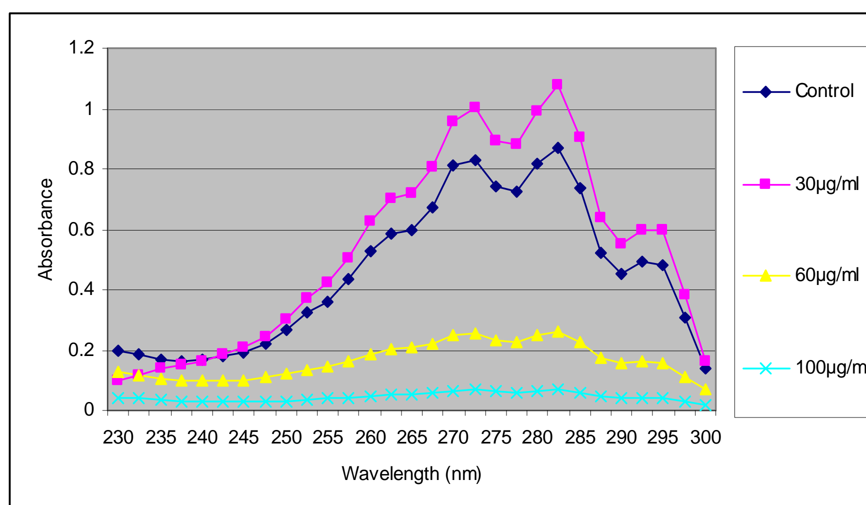
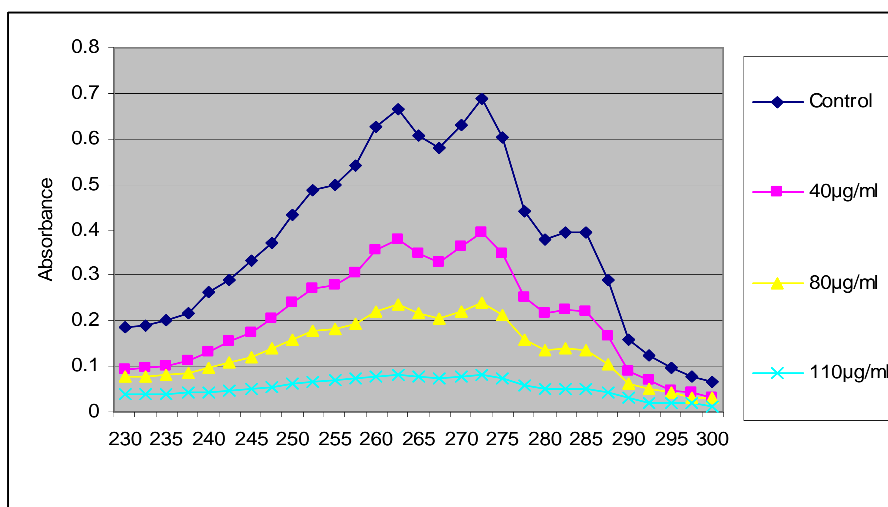


Figure 4 (d). UV spectrophotometric sterol profile of *C. kruesi* ATCC 6258 in presence of Ni(II) complex



CONCLUSION

A new series of macrocyclic tetradentate nitrogen donor (N_4) ligand 5, 8, 13, 16-tetraoxo- 6, 15-dihydroxy-1, 4, 9, 12-tetraazacyclohexa-decane based transition metal complexes of the type $[M(C_{12}H_{20}O_6N_4)Cl_2]$ where M= Cu(II), Co(II) and Ni(II) have been synthesized and screened for their anticandidal property by performing Minimum Inhibitory concentration (MIC) along with ergosterol composition assay against the tested *Candida* species. Antimicrobial results showed that metal complexes have high killing activity compared to the ligand. MIC values decreased almost stoichiometrically with multiplication of structure. A whole line of current antifungals target ergosterol biosynthesis pathway or its end product which is unique to fungi. At respective MIC values Ni(II) complex lead to enormous reduction in ergosterol content followed by Cu(II) complex, Co(II) complex and the ligand respectively.

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