Arabian Journal of Chemistry (2016) 9, S743–S751

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

Synthesis, characterization and biological screening of some Schiff base macrocyclic ligand based transition metal complexes as antifungal agents

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Received 28 March 2011; accepted 4 August 2011
Available online 11 August 2011

KEYWORDS

Macrocycle;
Metal complexes;
Antifungal;
Cytotoxicity;
MTT assay

Abstract As part of a continuing search for the synthesis of new antimicrobial agents some macrocyclic tetradentate nitrogen donor (N_4) ligand based transition metal complexes of the type $[M(C_{12}H_{20}O_8N_4)Cl_2]$ where M = Cu(II), Ni(II) and Co(II) were of our interest. All the compounds were synthesized and evaluated *in vitro* for their anticandidal property by performing minimum inhibitory concentration (MIC) along with ergosterol composition assay against *Candida albicans* ATCC 10261, *Candida glabrata* ATCC 90030, and *Candida tropicalis* ATCC 750, respectively. Results obtained indicate that growth and ergosterol content decreased significantly in the presence of the test compounds. All the synthesized compounds under investigation were also tested for toxicity by MTT assay on H9c2 cardiac myoblasts and the results showed that these compounds and the ligand offered remarkable viability in the range of 87–94% at a concentration of 25 μM. Ni(II) complex (KNi) was found to be the most active and least cytotoxic among all the compounds screened.

1. Introduction

Schiff base complexes are considered to be among the most important stereochemical models in main group and transition metal coordination chemistry due to their preparative accessibility and structural variety (Alexander, 1995). Study of the interaction between drugs and transition metals is an important and active research area in bioinorganic chemistry (Albert, 1979; Hughes, 1981; Tao et al., 2003; Chakrabarti

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2011.08.003
1878-5352

et al., 2000). It is well known that the action of many drugs is dependent on the coordination with metal ions (Albert, 1979) or/and the inhibition (Hughes, 1981) on the formation of metalloenzymes. Therefore, metal ions might play a vital role during the biological process of drug utilization in the body. A large number of Schiff bases and their complexes have been studied for their interesting and important properties, e.g. their ability to reversibly bind oxygen (Jones et al., 1979), catalytic activity in the hydrogenation of olefins (Olie and Olive, 1984) and transfer of an amino group (Dugas and Penney, 1981), photochromic properties (Margerum, 1971), and complexing ability toward some toxic metals (Sawodny and Riederer, 1977). The high affinity for the chelation of the Schiff bases toward the transition metal ions is utilized in preparing their solid complexes. Schiff bases are potential anticancer drugs and, when administered as their metal complexes, the anticancer activity of these complexes is enhanced in comparison to the free ligand (Shahabadi et al., 2010). Some biologically active Schiff base ligands, hydrazine-pyrrole-2-carboxaldehyde, hydrazine-furan-2-carboxaldehyde and hydrazine-thiophene-2-carboxaldehyde and their phenyl derivatives and their Co(II), Cu(II) and Ni(II) mixed complexes have been synthesized and characterized (Chohan, 2008). Complexes of Cu(II), Ni(II), Zn(II), Pd(II) and UO_2(II) with a tridentate ONS donor Schiff base; 3-(*o*-mercaptophenyl-iminomethyl) salicylic acid, were synthesized and characterized by Nag et al. (2005). Presently the primary and opportunistic fungal infections continue to increase rapidly because of the increased number of immunocompromised patients. As known, not only biochemical similarity of the human cell and fungi forms a handicap for selective activity, but also the easily gained resistance is the main problem encountered in developing safe and efficient antifungals. Candidiasis, a fungal disease caused by a diploid opportunistic fungal pathogen *Candida* proves to be life threatening mycoses fatal for immuno compromised patients, e.g. AIDS and transplantation surgery (Lott et al., 2005). Risk factors that increase incidence of *Candida* infection include compromised immunity, hormonal imbalances, prolonged use of broad spectrum antibiotics and oral contraceptives, pregnancy, metabolic and nutritional disorders (Odds, 1985; D'Souza and Heitman, 2001). Number of antifungal agents are available for the treatment of *Candidal* infections (Gupta and Thomas, 2003; Carrillo-Munoz et al., 2006) majority of them being polyenes such as Amphotericin B and Nystatin or the azoles, such as Itraconazoles and Fluconazole. Currently, use of standard antifungal therapies is 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 (Chami et al., 2004). Therefore, our studies were conducted to synthesize some macrocyclic tetradentate ligand based transition metal complexes and screen them for their anticandidal activity.

2. Materials and methods

2.1. Chemistry

Solvents and organic reagents were purchased from Sigma Aldrich, Merck (Germany) and Loba Chemie (India), and were used without further purification. Melting points (mp) were performed using a Mel-temp instrument, and the results were uncorrected. Elemental analyses were performed on HeraeusVario EL III analyzer at the Central Drug Research Institute, Lucknow, India. The results were within ±0.4% of the theoretical values. Electronic spectra were recorded on a Shimadzu UV 1601 PC UV–Visible spectrophotometer. IR spectra were recorded on Perkin–Elmer model 1600 FT-IR RX1 spectrophotometer as KBr discs. Far IR spectra were recorded as CsI pellets in the region 650–100 cm^{-1} using a JASCO FT-IR spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE 400 spectrometer using DMSO-d_6 as solvent with TMS as the internal standard. Positive and negative 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. Splitting patterns are designated as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet. Chemical shift values are given in ppm. ESI MS was recorded on a MICROMASS QUATTRO II triple quadrupole mass spectrometer. Reactions were monitored using thin-layer chromatography (TLC) using commercially available precoated plates (Merck Kieselgel 60 F254 silica). Visualization was achieved with UV light at 254 nm or I2 vapor staining.

2.1.1. Synthesis of ligand: 6, 7, 14, 15-tetrahydroxy-1, 4, 9, 12-tetraazacyclohexadecane-5, 8, 13, 16-tetrone (1)

The hot ethanolic solution (25 ml), of tartaric acid (8.30 g, 0.05 mol) and a hot ethanolic solution (25 ml) of ethylenediamine (3.00 g, 0.05 mol) were mixed slowly with constant stirring. This mixture was refluxed at 60–70 °C for 7 h in presence of few drops of concentrated hydrochloric acid. On keeping it overnight at 0 °C, a cream white precipitate was formed, which was filtered, washed with ethanol and dried in vacuo over P_4O_{10} and recrystallized from methanol.

Yield: 75%; mp > 300 °C; IR (KBr) cm^{-1}: 3443 (O–H), 3196 (N–H), 3010 (C–H), 1617 (C═O), 1411 (C–N), 1066, 885, 771; ^1H NMR (300 MHz, δ ppm from TMS in DMSO-d_6, 300 k): δ 11.67–12.56 (4H, Br N–H), δ 4.20–4.35 (s, 4H, C–H), δ 2.94–3.02 (s, 8H, C–H_2); ^{13}C NMR ($CDCl_3$) (δ, ppm): 173.5 (C═O), 38.09 (4CH_2); ESI MS (*m*/*z*) 348 $[M]^+$, 349 $[M+1]^+$; Elem. Anal. Calcd. C 41.39%, H 5.70%, O 36.76%, N 16.09%; found C 41.40%, H 5.72%, O 36.78%, N 16.10%.

2.1.2. Synthesis of cobalt(II) complex

Solution of $CoCl_2 \cdot 6H_2O$ (0.711 g, 3 mmol) dissolved in 20 ml methanol was added dropwise to a methanolic solution (20 ml) of the ligand (1.044 g, 3 mmol) with continuous stirring. The resulting solution was stirred for 7 h at 30 °C 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 recrystallized from methanol.

Yield: 65%; mp > 300 °C; UV–Vis (DMSO) cm^{-1}: 12,748–13,320, 16,885–17,487, 24,722–25,350; IR (KBr) cm^{-1}: 3443 (O–H), 3194 (N–H), 2977 (C–H), 1606 (C═O), 1369 (C–N), 1085, 881, 637, Far IR (CsI, cm^{-1}) 452 (Co–N), 345 (Co–

Cl). ^{1}H NMR (300 MHz, δ ppm from TMS in DMSO-d_6, 300 k): δ 11.70–12.60 (4H, Br, N–H), δ 4.22–4.40 (4H, C–H), δ 2.97–3.08 (8H, C–H_2), ^{13}C NMR ($CDCl_3$) (δ, ppm) 171.2 (C═O), 46.00 ($4CH_2$). ESI MS (m/z) 478 $[M]^+$, 480 $[M+2]^+$. Molar conductance, Λ_m ($\Omega^{-1}cm^{-2}$ mol^{-1}, 10^{-3} DMSO, r.t.): 32. μ_{eff} (r.t., BM): 4.98. Elem. Anal. Calcd. C 30.15%, H 4.10%, O 26.77%, N 11.72%; found C 30.17%, H 4.11%, O 26.78%, N 11.75%.

2.1.3. Synthesis of nickel(II) complex

This compound was also synthesized by the above same procedure except that $NiCl_2{\cdot}6H_2O$ was used instead of $CoCl_2{\cdot}6H_2O$. A light green product was obtained which was recrystallized from methanol.

Yield 62%; mp > 300 °C; UV–Vis (DMSO) cm^{-1}: 11,668, 13,351, 14,662, 19,120, 24,390; IR (KBr) cm^{-1}: 3417(O–H), 3190 (N–H), 2978 (C–H), 1602 (C═O), 1386 (C–N), 1063, 811, 614, Far IR (CsI, cm^{-1}) 468 (Ni–N), 340 (Ni–Cl). ^{1}H NMR (300 MHz, δ ppm from TMS in DMSO-d_6, 300 k): δ 11.71–12.60 (4H, Br, N–H), δ 4.25–4.37 (4H, C–H), δ 2.96–3.10 (8H, C–H_2).^{13}C NMR ($CDCl_3$) (δ, ppm) 176.5 (C═O), 49.50 ($4CH_2$). ESI MS (m/z) 479 $[M]^+$, 481 $[M+2]^+$. Molar conductance, Λ_m ($\Omega^{-1}cm^{-1}$ mol^{-1}, 10^{-3} DMSO, r.t.): 34. μ_{eff} (r.t., BM): 2.97. Elem. Anal Calcd. C 30.16%, H 4.10%, O 26.79%, N 11.72%; found C 30.17%, H 4.12%, O 26.80%, N 11.73%.

2.1.4. Synthesis of copper(II) complex

This compound was also synthesized by the above same procedure except that $CuCl_2{\cdot}2H_2O$ was used instead of $CoCl_2{\cdot}6H_2O$. A Sky blue product was obtained, which was recrystallized from methanol.

Yield: 67%; mp > 300 °C; UV–Vis (DMSO) cm^{-1}: 13,824–14,392, 18,892–19,365, 24,870–25,435; IR (KBr) cm^{-1}: 3419 (O–H), 3189 (N–H), 2976 (C–H), 1600 (C═O), 1390 (C–N), 1075, 888, 644, Far IR (CsI) cm^{-1}: 431 (Cu–N), 335 (Cu–Cl). ^{1}H NMR (300 MHz, δ ppm from TMS in DMSO-d_6, 300 k): δ 11.72–12.61 (4H, N–H), δ 4.23–4.39 (4H, C–H), δ 2.97–3.11 (8H, C–H_2); ^{13}C NMR ($CDCl_3$) (δ, ppm): 174.9 (C═O), 44.80 ($4CH_2$). ESI MS (m/z) 483 M^+, 485 $[M+2]^+$. Molar conductance, Λ_m ($\Omega^{-1}cm^{-1}$ mol^{-1}, 10^{-3} DMSO, r.t.): 29. μ_{eff} (r.t., BM): 1.98; Elem. Anal. Calcd. C 29.86%, H 4.10%, O 26.52%, N 11.60%; found C 29.88%, H 4.12%, O 26.55%, N 11.63%.

2.2. Biological activity

2.2.1. In vitro antifungal activity

2.2.1.1. Growth conditions and media. *Candida albicans* ATCC 10261, *Candida tropicalis* ATCC 750 and *Candida glabrata* ATCC 90030 used in this study were obtained from the Indian Institute of Integrative Medicine, Jammu, India. Stock cultures were maintained on slants of nutrient agar (yeast extract 1%, peptone 2%, D-glucose 2% and agar 2.5%) (HiMedia) at 4 °C. To initiate growth for experimental purposes, one loop full of cells from an agar culture was inoculated into 30 ml of YEPD (yeast extract, peptone, glucose) nutrient medium and incubated at 37 °C for 24 h, 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 (yeast extract, peptone, glucose) medium and grown for 8–10 h, i.e., up to mid-log phase (10^6 cells ml^{-1}). Microbial cultures, freshly grown at 37 °C were appropriately diluted in sterile YEPD broth to obtain the cell suspension at 10^6 CFU ml^{-1}. All inorganic chemicals were of analytical grade and were procured from E. Merck (India).

2.2.1.2. Minimum inhibitory concentration. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of test compounds that causes decrease in absorbance compared with that of the control (no test compound). MIC was determined *in vitro* in triplicate by broth dilution method (NCCLS, 2002) and average of them was considered. Synchronized yeast phase *C. albicans*, *C. tropicalis* and *C. glabrata* cells were used in all experiments.

2.2.1.3. Sterol composition assay. A single *Candida* colony from an overnight Sabouraud Dextrose Agar (Difco) plate culture was used to inoculate 50 ml of Sabouraud Dextrose Broth (Difco) for control and for various concentrations of test compounds. The cultures were incubated for 16 h and harvested by centrifugation at 2700 rpm for 5 min. The net 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 85 °C water bath for 1 h. Following incubation, tubes were allowed to cool. Sterols were then extracted by the 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 the sterol extract was diluted fivefold in 100% ethanol and scanned spectrophotometrically between 240 and 300 nm with a Spectrophotometer (Systronics UV–Visible Spectrophotometer 117). Ergosterol content is calculated as a percentage of the wet weight of the cell as described by Breivik and Owades (1957).

2.2.2. MTT assay

H9c2 rat cardiac myoblasts were cultured and maintained as monolayer in Dulbecco's modified Eagle's medium (DMEM), high glucose, supplemented with 10% fetal bovine serum (heat inactivated), 100 units/ml penicillin, 100 µg/ml streptomycin, and 2.5 µg/ml amphotericin B, at 37 °C in humidified incubator with 5% CO_2 (Gupta et al., 2006). Only viable cells were used in the assay. Exponentially growing cells were plated at 1.2×104 cells per well into 96-well plates and incubated for 48 h before the addition of drugs to achieve the maximum confluency of the cells. Stock solutions were prepared by dissolving the compounds in 20% (v/v) DMSO and further diluted with fresh complete medium to achieve 1 M concentration. Cells were incubated with different concentrations of the standard drug SD and compounds KNi, KCu and ligand (K) for 48 h at 37 °C in 5% CO_2 humidified incubator together with untreated control sample. At appropriate time points, cells were washed in PBS, treated with 50 µl MTT solution (5 mg/ml, tetrazolium salt) and incubated for 45 min at 37 °C. After 45 min of incubation at 37 °C, the cell supernatants were discarded, MTT crystals were dissolved with acid isopropanol and the absorbance measured at 570 nm. All assays were performed in triplicate. Percent viability was defined as the rela-

tive absorbance of treated versus untreated control cells. Plates were analyzed in an ELISA plate reader (Labsystems Multiskan RC, Helsinki, Finland) at 570 nm with a reference wavelength of 655 nm.

3. Results and discussion

Some macrocyclic tetradentate nitrogen donor (N_4) ligand 6,7,14,15-tetrahydroxy-1,4,9,12-tetraazacyclohexadecane-5,8,13,16-tetrone based transition metal complexes of the type $[M(C_{12}H_{20}O_8N_4)Cl_2]$ where M = Cu(II), Ni(II) and Co(II) were isolated in satisfactory yields. The structure of the ligand and its metal complexes were established using various spectroscopic studies. The analytical data of these compounds are in good agreement with their composition. The compounds do not undergo any weight loss up to 255 °C, 245 °C, 250 °C, respectively, which suggest their fair thermal stability. The structure of all complexes was established by comparing spectral data (IR, UV–Vis and ^{1}H NMR) with their respective ligands and was further supported by their ESI MS and thermo gravimetric analysis.

3.1. Electronic spectra

3.1.1. Cobalt(II) complex

The electronic spectrum of the mononuclear cobalt(II) complex exhibits absorption bands in the range 12,748–13,320, 16,885–17,487 and 24,722–25,350 cm^{-1}, which may be assigned to $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)(\nu_1)$, $^4T_{1g} \rightarrow {}^4A_{2g}(\nu_2)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)(\nu_3)$ transitions, respectively, 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 4.98 BM. This value is indicative of an octahedral geometry (Carlin, 1965; Chandra and Gupta, 2004) of these complexes.

3.1.2. Copper(II) complex

The electronic spectrum of the mononuclear copper(II) complex recorded at room temperature, in DMF solution, shows broad band absorption in the range 13,824–14,392, 18,892–19,365 and 24,890–25,435 cm^{-1}, which may be assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$, $(d_{x^2} - y^2 \rightarrow d_{z^2})(\nu_1)$, $^2B_{1g} \rightarrow {}^2B_{2g}$, $(d_{x^2} - y^2 \rightarrow d_{zy})(\nu_2)$, and $^2B_{1g} \rightarrow {}^2E_g$, $(d_{x^2} - y^2 \rightarrow d_{zy}, d_{yz})(\nu_3)$ transition and it is in conformity with octahedral geometry, 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 1.98 BM. corresponding to the presence of one unpaired electron and it supports an octahedral geometry (Chandra, 2009).

3.1.3. Nickel(II) complex

The magnetic moment of the Ni(II) complex at room temperature lies at 2.97 BM. These values are in tune with high spin configuration and show the presence of an octahedral environment around the Ni(II) ion. The electronic spectra of the nickel(II) complex showed transition bands at 11,668, and 13,351, 14,662 cm^{-1} which are assigned to the transition $^3A_{2g} \rightarrow {}^3T_{2g}$, 19,120(shoulder), 24,390 cm^{-1} which are assigned to the transition $^3A_{2g} \rightarrow {}^3T_{1g}(F)$. These bands indicate an octahedral geometry around the nickel(II) ion in its complex.

3.2. IR spectra

3.2.1. Macrocyclic ligand

IR spectrum of the ligand does not exhibit any band corresponding to the free primary amine group. The absence of a broad absorption band in the region 3100–2500 cm^{-1} characteristic for (O–H) group of carboxylic acid and presence of an absorption band in the region 1675–1775 cm^{-1} characteristic for the carbonyl group (C═O) in tartaric acid, indicates that the OH group of tartaric acid was detached from the COOH group to form a bond between carboxyl carbon atom and amino group nitrogen of ethylenediamine, also suggesting complete condensation of the reactants and the elimination of water molecule. This has been confirmed by the appearance of a strong signal at 1411 cm^{-1} which may be attributed to the C–N bond. A sharp medium intensity band observed at 3196 cm^{-1} may be assigned to υ(N–H) of the secondary amine group. The ligand also shows a signal for the C═O at 1617 cm^{-1} and C–H at 3010 cm^{-1} vibrations. The low frequency of C═O group as compared to acetone (1715 cm^{-1}) is attributed to resonance with lone pair of the nitrogen.

3.2.2. Complexes

The molar conductance measurements of the complexes in DMF correspond to nonelectrolytic nature. Thus, the complexes may be formulated as $[M(K)Cl_2]$ where M = Ni(II), Co(II), and Cu(II) and K is $[(C_{12}H_{20}O_8N_4)]$. The shifting in the band of υ(C–N) toward the lower wave number in the metal complexes indicates that the coordination takes place through the nitrogen of the υ(C–N) group, hence implying that the ligand (K) is tetradentate. This indicates the flow of electron density toward 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 482, 470, 452 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. Other vibrating signals seen at 347, 337 and 330 cm^{-1} in the spectra of metal complexes give us proof of the presence of metal–chlorine bond in Co(II), Ni(II) and Cu(II) complex, respectively.

3.3. ^{1}H NMR spectra

The ^{1}H NMR spectrum of the ligand shows a broad signal in the range 11.67–12.56 ppm which is attributed to amide CO–N–H, (4H) and does not show any signal corresponding to the primary amine. A signal appearing in the range 4.20–4.35 ppm has been ascribed to methylene protons OC–N–CH_2, (8H), whereas C–H (4H) protons appear in the range 2.94–3.02 ppm. 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 toward the metal ions and also the macrocyclic nature of the product.

3.4. Electro spray ionization mass spectra (ESI MS)

The electro spray ionization mass spectra (ESI MS) of the ligand and the complexes were studied in DMSO solution. A

positive ion ESI mass spectrum of the ligand confirms the proposed formula by showing a peak at m/z 349 corresponding to the moiety ($C_{12}H_{20}O_8N_4$) atomic mass m/z 348.14. The series of peaks in the range m/z 63.5, 102.9, 134.9, 192.9, 244.7, 306.1, etc., may be assigned to various fragments. These data suggest 2 + 2 condensation of tartaric acid and ethylenediamine. Their intensity gives an idea of the stability of fragments. Similarly positive ion ESI MS of cobalt and copper, negative ion ESI MS nickel complexes shows a peak at m/z 478, 483, 479, 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. In some cases, the molecular ion peak was also associated with the solvent, water molecules and some adduct ions from the mobile phase solution (Yamashita and Fenn, 1984; Mann, 1990).

3.5. Thermo gravimetric analysis (TG/DTA)

3.5.1. Macrocyclic ligand

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 ligand is stable up to 215 °C

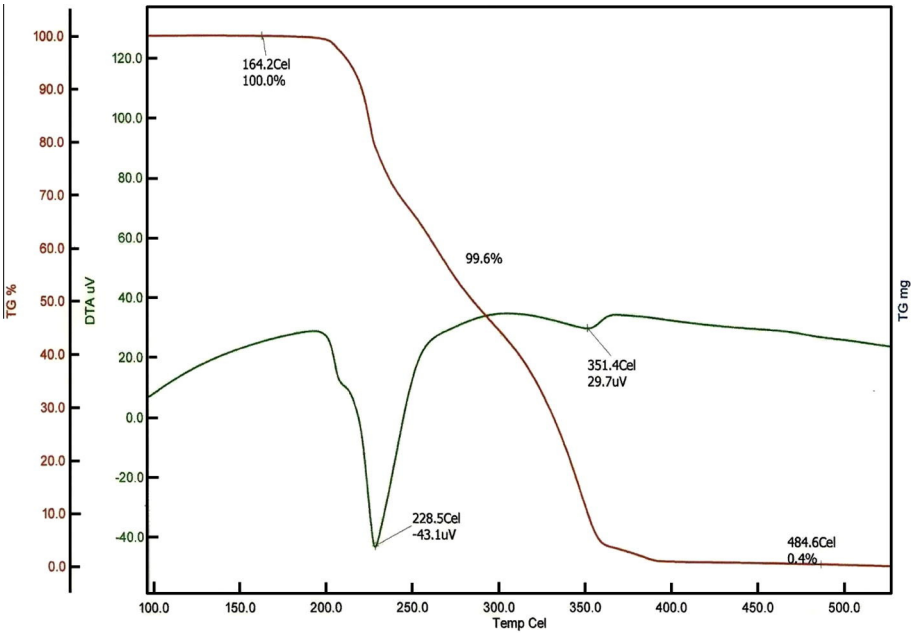


Figure 1 TG/DTA spectrum of macrocyclic ligand (K).

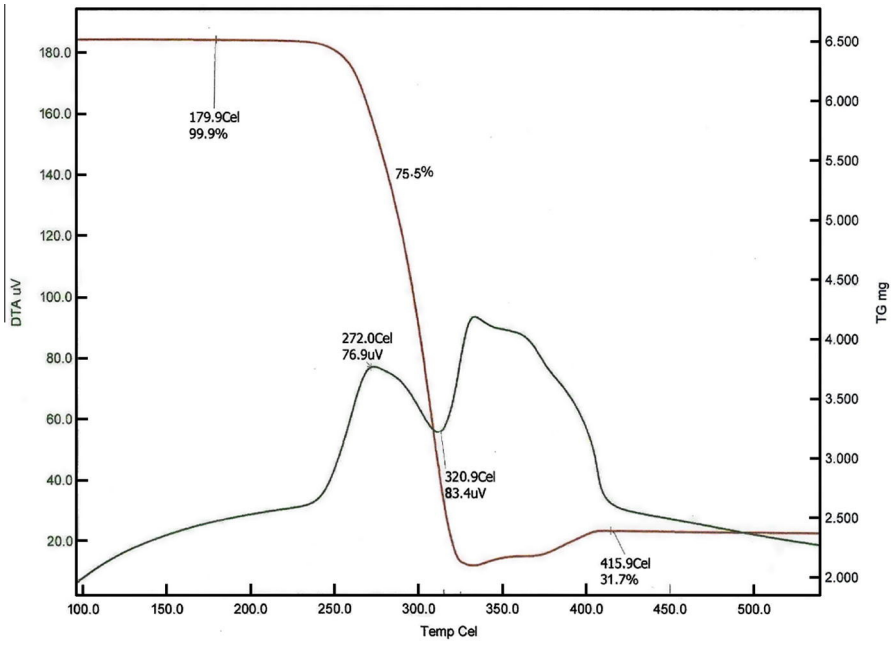


Figure 2 TG/DTA spectrum of Co(II) complex.

Table 1 Minimum inhibitory concentrations (MIC) of bioactive compounds tested against different *Candida spp.*

Bioactive compound	MIC (μg/ml)		
	Candida albicans ATCC 44829	*Candida tropicalis* ATCC 750	*Candida glabrata* ATCC 90030
$[Ni(K)Cl_2]$	80	90	100
$[Cu(K)Cl_2]$	90	110	120
$[Co(K)Cl_2]$	140	150	170
Ligand [K]	190	300	300

and shows a continuous weight loss up to 380 °C, Therefore the whole ligand gets decomposed in a single step (Fig 1). The DTA of the ligand shows two endothermic peaks; one broad endothermic peak at 228 °C with a shoulder at 210 °C, which correspond to the melting and the first inflexion point. The second inflexion on the DTA curve occurs at 351 °C which represents a small weight loss step from 360 °C to 380 °C.

3.5.2. Complexes

The thermal gravimetric (TG) analysis was used as a probe to prove the associated water or solvent molecules to be in coordination sphere or in crystalline form (Emara and Omima, 2007). The thermo gram of copper(II), nickel(II) and cobalt(II) complexes are more stable than the macrocyclic ligand and does not decompose up to 255 °C, 253 °C and 250 °C, respectively (Fig. 2). It shows a major step of decomposition from 255 °C to 330 °C which is detected by DTA at 320 °C, this corresponds to the loss of two phthalic acids and two ethylenediamine moieties (observed weight 75.5%, theoretical weight 72.85%). It is very interesting to note that the complexes gain some 7% weight from 335 °C to 410 °C and do not decompose further. This weight gain of complexes may be attributed to the migration of the metal (M_{layer}) to the new vacant sites produced by the partial reduction of M^{2+} to M^{1+} in case of Copper and M^{4+} to M^{2+} in case of cobalt and nickel, then subsequent oxidation of M^{1+}, M^{2+} in copper, cobalt and nickel complexes, respectively (Gaillot et al., 2005; Cheney et al., 2008).

3.6. Anticandidial activity

3.6.1. Minimum inhibitory concentration (MIC)

Table 1 summarizes the *in vitro* susceptibilities of three *Candida* species. The data are reported as MICs required to inhibit the growth of the tested species. All the *Candida* species were found to be more sensitive to Ni(II) and Cu(II) complexes, compared to Co(II) complex and the ligand.

3.6.2. Sterol assay

Sterol quantitation method is employed to determine ergosterol content of fungal cell membrane. An important mechanism of action by which antifungal drugs inhibit yeast cell growth is through disruption of the normal sterol biosynthetic pathway, leading to a reduction in ergosterol biosynthesis (Kelly et al., 1995). Ergosterol is the major sterol component of the yeast cell membrane and is responsible for maintaining cell integrity and function (Bard et al.,

Table 2 % Ergosterol decrease of control cells and treated samples in different *Candida* species. The data represents mean of three experiments.

Candida albicans ATCC 10261		*Candida tropicalis* ATCC 750		*Candida glabrata* ATCC 90030	
Sample	% Decrease	Sample	% Decrease	Sample	% Decrease
$[Ni(K)Cl_2]$					
Control	0	Control	0	Control	0
20 μg/ml	22	30 μg/ml	20	30 μg/ml	18
40 μg/ml	48	60 μg/ml	39	60 μg/ml	35
80 μg/ml	79	90 μg/ml	70	100 μg/ml	68
$[Cu(K)Cl_2]$					
Control	0	Control	0	Control	0
30 μg/ml	18	30 μg/ml	16	40 μg/ml	16
60 μg/ml	32	60 μg/ml	32	80 μg/ml	30
90 μg/ml	67	110 μg/ml	62	120 μg/ml	60
$[Co(K)Cl_2]$					
Control	0	Control	0	Control	0
50 μg/ml	15	50 μg/ml	13	50 μg/ml	13
100 μg/ml	28	100 μg/ml	25	100 μg/ml	24
140 μg/ml	51	150 μg/ml	47	170 μg/ml	45
Ligand [K]					
Control	0	Control	0	Control	0
50 μg/ml	12	50 μg/ml	10	50 μg/ml	09
100 μg/ml	21	100 μg/ml	19	100 μg/ml	16
190 μg/ml	41	300 μg/ml	39	300 μg/ml	28

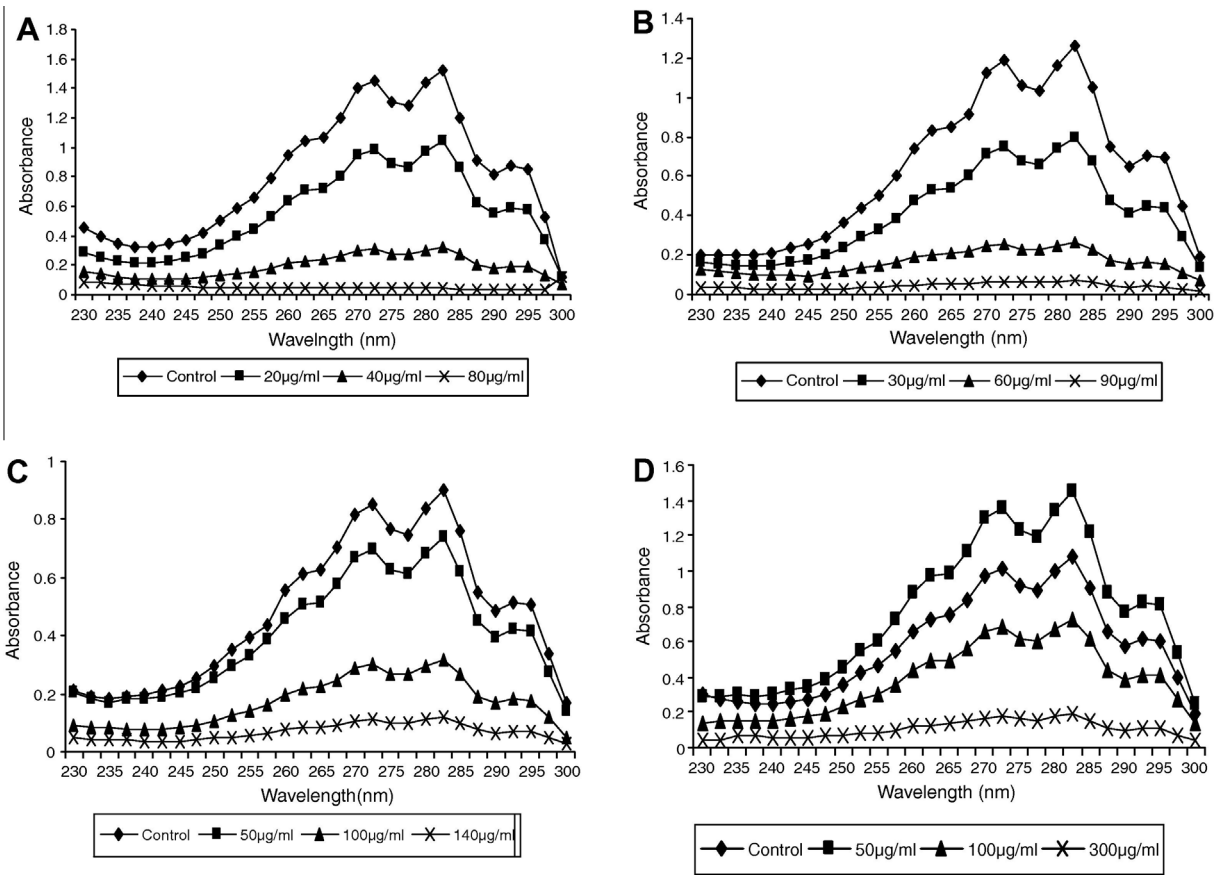


Figure 3 UV spectrophotometric sterol profile of *Candida albicans*. (A) In presence of Ni(II) complex in concentration range of 20–40 µg/ml. (B) Cu(II) complex in concentration range of 30–90 µg/ml. (C) Co(II) complex in concentration range of 50–140 µg/ml. (D) Ligand in concentration range of 50–190 µg/ml. (Data represents mean of three experiments).

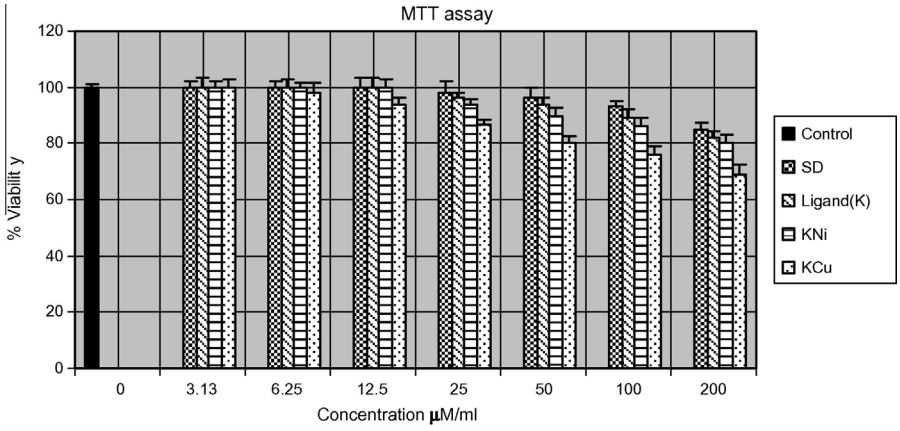
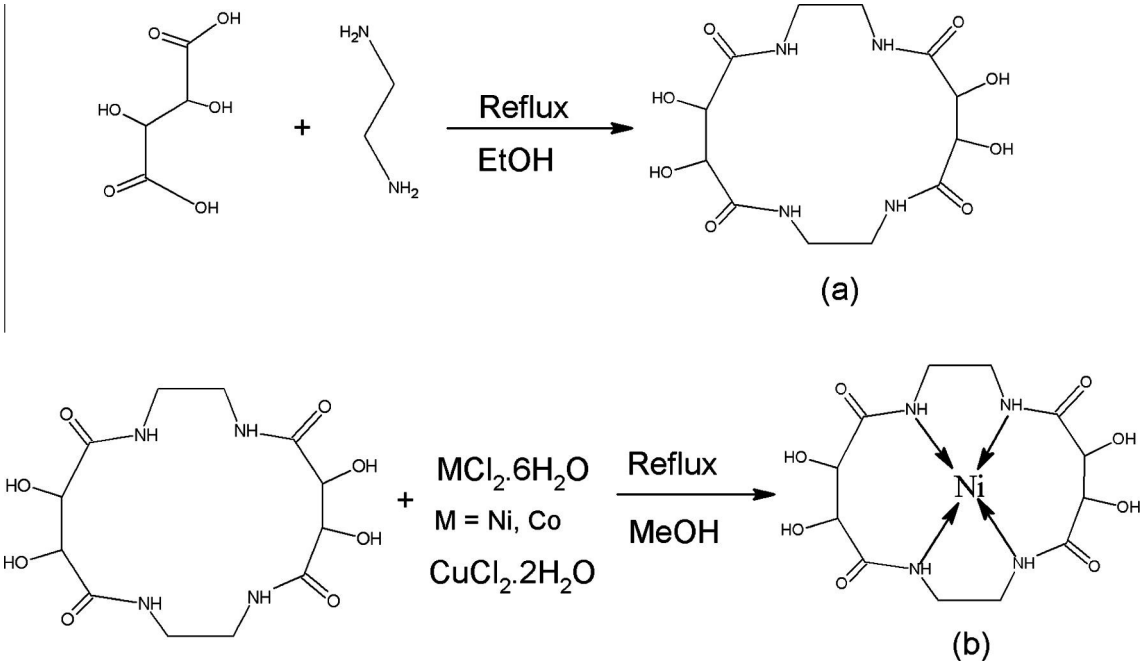


Figure 4 Percentage of viable cells after 48 h pre-treatment of H9c2 myoblasts with SD, ligand (K), KNi and KCu evaluated by MTT assay.

1978; Rodriguez et al., 1985). Therefore, disruption of this pathway by drugs or additional compounds leads to fungistasis. Total sterol content of samples treated with varying concentrations of Ni(II) complex, Co(II) complex, Cu(II) complex and ligand was studied (Table 2). From the results, it is clear that with increase in test compound concentrations, % ergosterol inhibition increased and finally at MIC values, a flat line was observed indicating absence of ergosterol in the sample. Fig. 3(A, B, C, D) shows typical scans of % ergosterol inhibition in *C. albicans* ATCC 10261 in the presence of different test compound concentrations. It was observed that Ni(II) complex and Co(II) complex drastically reduces ergosterol content of cell membrane followed by the Cu(II) complex and the ligand itself.



Scheme 1 (a) Synthesis of ligand and (b) its metal complexes [Cu(II), Co(II), and Ni(II)].

3.7. Toxicity profile

To ensure the toxicity of the active compounds (KNi and KCu) and the ligand (K) they were tested against H9c2 cardiac myoblasts. A subconfluent population of H9c2 cells was treated with increasing concentrations of these compounds and the number of viable cells was measured after 48 h by MTT cell viability assay. The concentration range of all the compounds was 3.13–200 μM. Fig. 4 depicts that all the compounds including the reference drug (Fluconazole) showed a viability of 100% at the concentration range of 3.13 μM. At the concentration of 6.25 μM ligand (K), compound KNi and the reference drug showed 100% viability whereas compounds KCu showed 98% viability. At the concentration of 12.5 μM, ligand (K), compound KNi, and reference drug again showed 100% viability while compound KCu showed 94% viability. At the concentration of 25 μM reference drug, ligand (K), compound KNi, and compound KCu offered a remarkable viability of 98%, 96%, 94% and 87%, respectively. The range of viability, however, got sharply decreased at the concentration of 200 μM. The results indicated that the ligand (K) and the compound (KNi) showed remarkable viability of 94% and 96%, respectively at a concentration of 25 μM. The mean percentage viability sharply decreased with increase in concentration above 50 μM (see Fig. 4; Scheme 1).

4. Conclusion

This research work has achieved the synthesis of a new series of macrocyclic tetradentate nitrogen donor (N_4) ligand 6,7,14,15-tetrahydroxy-1,4,9,12-tetraazacyclohexadecane-5,8,13,16-tetrone based transition metal complexes of the type $[M(C_{12}H_{20}O_8N_4)Cl_2]$ where M = Cu(II), Co(II) and Ni(II) and screened them 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 leads to enormous reduction in ergosterol content followed by Cu(II) complex, Co(II) complex and the ligand, respectively. Toxicity studies performed on H9c2 cardiac myoblasts showed that the compound KNi and the ligand (K) showed viability of 96% and 94%, respectively, at a concentration of 25 μM.

Acknowledgment

The authors would like to thank the University Grants Commission (UGC) for their financial assistance.

References

Albert, A., 1979. The Physico-Chemical Basis of Therapy: Selective Toxicity, sixth ed. Chapman & Hall, London.
Alexander, V., 1995. Chem. Rev. 95, 273.
Bard, M., Lees, N.D., Burrows, L.S., Kleinhans, F.W., 1978. J. Bacteriol. 135, 1146.
Breivik, O.N., Owades, J.L., 1957. J. Agri. Food Chem. 5, 360.
Carlin, R.L., 1965. In: Transition Metal Chemistry, vol. 1. Marcel Dekker, Inc., New York.
Carrillo-Munoz, A.J., Giusiano, G., Ezkurra, P.A., Quindos, G., 2006. Rev. Esp. Quimioter. 19, 130.
Chakrabarti, S., Dasgupta, D., Bhattacharyya, D., 2000. J. Biol. Phys. 26, 203.
Chami, N., Chami, F., Bennis, S., Trouillas, J., Remmal, A., 2004. Braz. J. Infect. Dis. 8, 217.
Chandra, S., 2009. J. Coord. Chem. 62, 1327.
Chandra, S., Gupta, L.K., 2004. Spectrochim. Acta A 60, 2767.

Cheney, M.A., Bhowmik, P.K., Qian, S., Joo, S.W., Hou, W., Okoh, J., J.M., 2008. Nanomaterials 2008, 8.
Chohan, Z.H., 2008. J. Enzyme Inhib. Med. Chem. 23, 120.
D'Souza, C.A., Heitman, J., 2001. J. Clin. Invest. 108, 1577.
Dugas, H., Penney, C., 1981. Bioorganic Chemistry. Springer, New York.
Emara, A.A.A., Omima, M.I.A., 2007. Transition Met. Chem. 32, 889.
Gaillot, A.C., Lanson, B., Drits, V.A., 2005. Chem. Mater. 17, 2959.
Gupta, A.K., Thomas, E., 2003. Dermatol. Clin. 21, 565.
Gupta, M.K., Neelakantan, T.V., Sanghamitra, M., Tyagi, R.K., Dinda, A., Mualik, S., Mukhopadhyay, C.K., Goswami, S.K., 2006. Antioxid. Redox Signal. 8, 1081.
Hughes, M.N. (Ed.), 1981. The Inorganic Chemistry of Biological Processes, second ed. Wiley, New York.
Jones, R.D., Summerville, D.A., Basolo, F., 1979. Chem. Rev. 79, 139.
Kelly, S.L., Lamb, D.C., Corran, A.J., Baldwin, B.C., Kelly, D.E., 1995. Biochem. Biophys. Res. Commun. 207, 910.
Lott, T.J., Fundyga, R.E., Kuykendall, R.J., Arnold, J., 2005. Fungal Genet. Biol. 42, 444.
Mann, M., 1990. Org. Mass. Spectrom. 25, 575.
Margerum, J.D., Mller, L.J., 1971. Photochromism. Interscience Wiley.
Nag, J.K., Pal, S., Sinha, C., 2005. Trans. Metal Chem. 30, 523.
NCCLS, 2002. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard M27-A2. National Committee on Clinical Laboratory Standards, Wayne, PA. p. 30.
Odds, F.C., 1985. Crit. Rev. Microbiol. 12, 45.
Olie, G.H., Olive, S., 1984. The Chemistry of the Catalyzes Hydrogenation of Carbon Monoxide. Springer, Berlin.
Rodriguez, R.J., Low, C., Bottema, C.D.K., Parks, L.W., 1985. Biochem. Biophys. Res. Commun. 112, 47.
Sawodny, W.J., Riederer, M., 1977. Angew. Chem. Int. Ed. Engl. 16, 859.
Shahabadi, N., Kashanian, S., Darabi, F., 2010. Eur. J. Med. Chem. 45, 4239.
Tao, D., Yang, X., Li, Y., Gio, Y., Yang, L., 2003. Chem. Pharm. Bull. 51, 494.
Yamashita, M., Fenn, J.B., 1984. J. Phys. Chem. 88, 4451.