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Synthesis, Characterization and antimicrobial activity of macrocyclic metal complexes of Ni(II) and Cd(II)

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Abstract

New macrocyclic complexes derived from the template condensation reaction of bis(benzil)4-chloro-1,2-phenylenediamine(ML^1) and bis(benzi1)4-fluoro-1, 2-phenylenediamine (ML^2) respectively with different diamines in the presence of transition metal chlorides. The complexes were characterized by elemental analysis and spectral techniques i.e. IR, UV-visible, ^{1}H-NMR and mass. Spectral studies show that the geometry around the metal center is octahedral. The Schiff base ligands and their metal complexes were tested against pathogenic bacteria and fungi. All the complexes exhibit antibacterial and antifungal activities against various microorganisms.

Keywords: Template condensation, macrocyclic complexes, tetra-coordinated, spectroscopic studies, antimicrobial activity.

Introduction

The coordination chemistry of macrocyclic ligands and their metal complexes is a fascinating field for inorganic chemists[1]. Macrocyclic ligands has an increased interest due to their features such as the number, nature and arrangement of donor atoms, as well as ligand conjugation, substitution and flexibility. Macrocyclic compounds are interesting ligand-systems due to their good hosting nature for cations, anions and neutral guests[2]. In host-guest chemistry, many biological studies, and phase transfer catalysis of macrocyclic compounds is very useful[3]. The study of macrocyclic complexes is an emerging class of research because macrocyclic complexes possess more selectivity and thermodynamic stability than open chain analogue[4].

Transition metal macrocyclic complexes have unusual attention due to their active part in metalloenzymes and proximity to natural enzymes and proteins. Macrocyclic complexes are considered to mimic the synthetic models of metalloporphyrins and metallocorrins due to their basic structural properties[5]. There is a huge interest in macrocyclic complex synthesisis due to their application in the field of applied science[6]. Macrocyclic transition metal complexes are widely used due to theirbiological activities such asantidiabetic[7], antitumor[8-10], antiviral, antiproliferative, anticarcinogenic[11-13], antifertile[14], herbicidal[15] and antimicrobial[16-23]. Nitrogen containing macrocyclic complexes of transition metals have high stability and have been used widely due to their analytical, industrial and medical applications[24,25].

Due to the increasing interest towards the macrocyclic compounds, the synthesis of some new macrocyclic complexes of Ni^{+2} and Cd^{+2} are reported in this paper.

Materials and methods

All the chemicals were bought from Sigma-Aldrich. All the solvents used were dried by appropriate methods.The nitrogen and chlorine estimation were done by the "Kjeldahl's and Volhard's method, respectively[26]. Metals were estimated gravimetrically[27]. Rast Camphor method was used to determine the molecular weight of macrocyclic metal complexes. FT-IR spectra were taken as KBr discs in the range 4000-400 cm^{-1} on a Shimadzu FTIR-550 spectrophotometer.

Electronic absorption spectra were recorded on an Ultraviolet visible spectrophotometer 752/752N. ^{1}H NMR spectra were recorded on a JEOL-DELTA2-NMR 400MHz spectrometer in $CDCl_3$ using TMS as the internal standard.

Synthesis of ligands (ML^1 and ML^2): The ligands (ML^1 and ML^2) were prepared by dissolving benzil (20mmol, 4.20g) in 40mL of ethanol then diamine i.e. 4-chloro-1,2-phenylenediamine (10mmol, 1.42g) or 4-fluoro-1,2-phenylenediamine (10mmol, 1.26g) was added in 2:1 molar ratio.

The reaction mixture was refluxed for 4-6 h on a ratio head and concentrated to half of the volume. The solution was cooled and the excess solvent was removed by slow evaporation by keeping it in a desiccator overnight. The obtained coloured crystalline products were recrystallized in the same solvent and dried in vacuo[28].

The synthetic route for the ligands is shown in Figure-1 and the analysis and physical properties of these ligands are given in Table-1.

Synthesis of macrocyclic complexes: The macrocyclic complexes were synthesized by the template condensation of ligands bis(benzil)4-chloro-1,2-phenylenediamine (ML^1) or bis (benzil)4-fluoro-1,2-phenylenediamine (ML^2) with 4-chloro-1,2- phenylenediamine and 4-fluoro-1,2-phenylenediamine in the presence of "$NiCl_2.6H_2O$ and $CdCl_2.4H_2O$.

Synthesis of $[M(C_{40}H_{26}N_4Cl_3F)]$ complexes: A weighed amount of ligand ML^1 (10mmol, 5.27g) was taken into a 100 mL round bottom flask and dissolved in methanol. The solution of ligand was mixed with the methanolic solution of 4- fluoro 1,2-phenylenediamine (10mmol, 1.26g) and metal chloride (10 mmol) in 1:1:1 molar ratio. After addition of all the reagents, the contents were boiled under reflux for about 7–8h. The reaction mixture was concentrated to half of its volume and kept in a desiccator at room temperature. The complexes obtained as coloured solids, were washed with methanol and dried under vacuo[28].

Synthesis of $[M(C_{40}H_{26}N_4Cl_2F_2)]$ and $[M(C_{40}H_{26}N_4Cl_4)]$ complexes: To obtain these type of macrocyclic complexes the methanolic solution of ligand ML^1 ((10mmol, 5.27g) or ML^2 (10mmol, 5.10g) was mixed with the 4-chloro-1,2-phenylenediamine and 4-fluoro 1,2-phenylenediamine respectively in the presence of MCl_2 (10mmol) in 1:1:1 molar ratio. After addition was completed, the reaction mixture was refluxed for 7–8h on a ratio head. The mixture was concentrated to half by removing the excess solvent. It was then kept in a desiccator at room temperature for 24 h. The solid coloured complexes were obtained and washed with methanol and dried under vacuo[28]. The template synthesis of themacrocyclic complexes is shown in Figure-2.

All the complexes were recrystallized from 1:1 molar solution of methanol and benzene. The purity of the complexes was checked by thin layer chromatography (TLC). The analysis and physical properties of these complexes are given in Table-1.

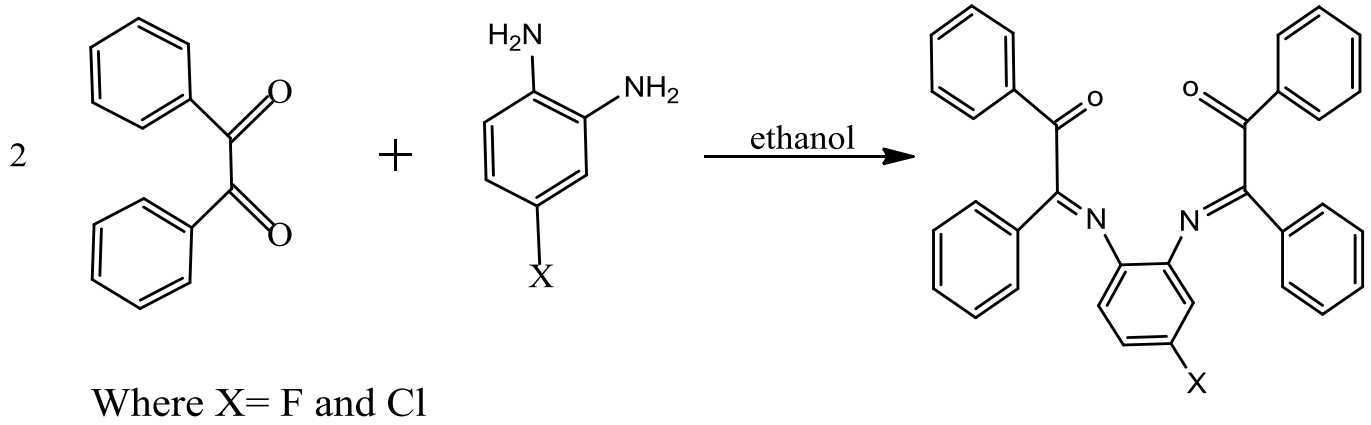

Figure-1: The Synthetic route of ligands.

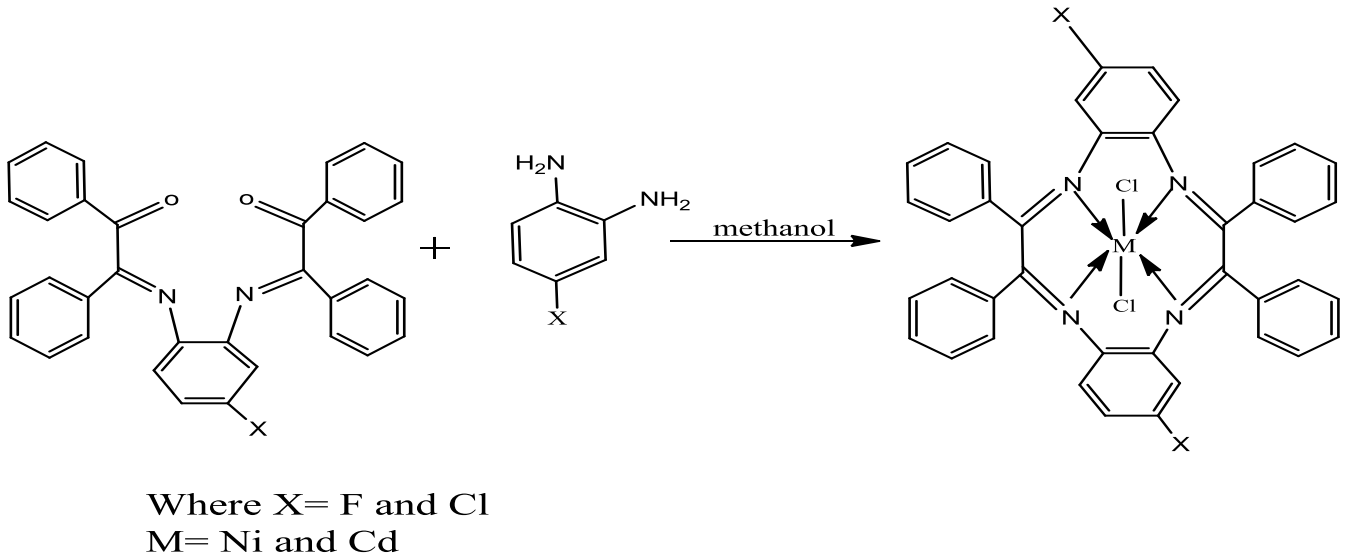

Figure-2: Synthetic route for preparation of the macrocyclic metal complexes.

Table-1: The analysis and physical properties of the ligands and their metal complexes.

Compounds	M.W.	Colour	M.P. (0°C)	Yield	Elemental analysis found (calculated) %				
					C	H	N	Cl	M
$C_{34}H_{23}N_2O_2Cl$ (ML^1)	524.13(527.02)	black	85	70%	77.15 (77.48)	4.24 (4.39)	5.19 (5.31)	6.02 (6.73)	-
$C_{34}H_{23}N_2O_2F$ (ML^2)	508.17(510.56)	Light brown	74	75%	79.03 (79.98)	4.49 (4.54)	5.32 (5.48)	-	-
$[Ni(C_{40}H_{26}N_4Cl_4)]$	761.70 (763.15)	Brown	140	67%	62.58 (62.95)	3.20 (3.43)	7.16 (7.34)	18.31 (18.58)	7.52 (7.69)
$[Ni(C_{40}H_{26}N_4F_2Cl_2)]$	727.42 (730.25)	Black	132	71%	65.52 (65.78)	3.36 (3.58)	7.48 (7.67)	9.51 (9.70)	7.89 (8.03)
$[Ni(C_{40}H_{26}N_4Cl_3F)]$	742.87 (746.71)	Dark Brown	150	65%	64.14 (64.33)	3.31 (3.50)	7.37 (7.50)	14.12 (14.24)	7.59 (7.86)
$[Cd(C_{40}H_{26}N_4Cl_4)]$	814.69 (816.88)	Dark Brown	148	75%	58.70 (58.81)	3.12 (3.20)	6.78 (6.85)	17.30 (17.36)	17.69 (13.76)
$[Cd(C_{40}H_{26}N_4F_2Cl_2)]$	781.67 (783.97)	Black	145	62%	61.17 (61.28)	3.26 (3.34)	7.10 (7.14)	8.97 (9.04)	14.20 (14.33)
$[Cd(C_{40}H_{26}N_4Cl_3F)]$	798.30 (800.42)	Dark brown	162	70%	59.77 (60.02)	3.18 (3.27)	6.90 (6.99)	13.11 (13.28)	13.92 (14.04)

Biological assay: ***Test microorganism:*** All the compounds were evaluated for their antimicrobial properties. The results obtained were compared with the standard drugs Streptomycin for bacteria and Itraconazole for fungi. The microorganisms used were *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*, *Aspergillus fumigatus* and *Candida albicans*.

In vitro antibacterial activity: The test compounds were dissolved in DMSO and the concentration of stock solution was made 5mg/mL. The 50µL of test compound was used in each well. Agar plates were prepared for the antibacterial activity. Mueller-Hinton agar medium and sabouraud dextrose agar medium is the only susceptibility test medium that has been validated by CLSI for screening the antimicrobial activity by well diffusion susceptibility testing. Fresh cultures of test isolates of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* were inoculated in peptone water and kept for incubation for 30 minutes at 37°C. The bacterial suspensions were compared to 0.5Mc Farland turbidity standard. Bacterial cultures were swabbed onto the Mueller hinton agar surface. 50µL from different dilutions prepared from stock was loaded into the respective wells. The *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* plates were kept for incubation at 37°C for 24-48h and results were observed. The antibacterial activity of common standard antibiotic streptomycin was also recorded using the same procedure. The medium with DMSO as solvent was used as a negative control and streptomycin was used as positive control. The experiments were performed three times.

In vitro antifungal activity: Different concentrations of the test compounds under study were prepared from the stock solution (5mg/mL and 2.5mg/mL) and out of which 50µL was used in each well. Saboraud dextrose agar medium is used for screening the antifungal activity by well diffusion susceptibility testing. *Aspergillus fumigatus* and *Candida albicans* were inoculated in normal saline (0.9%) and kept for 48h at 28°C. The fungi were swabbed on to the Saboraud dextrose agar surface. 50µL from different dilutions prepared from stock (5mg/mL and 2.5mg/mL of the compound) was loaded into the respective wells. The antifungal plates were kept for incubation at 28°C for 7 days. The fungal activity of each compound was compared with Itraconazole (5mg/mL (w/v)) as standard drug. The medium with DMSO as solvent was used as a negative control whereas media with Itraconazole was used as positive control. The experiments were performed in triplicates.

Results and discussion

The complexes are colorful solid, monomeric and dissolvable in DMSO, DMF, acetonitrile and chloroform.

IR Spectra: The structure and bonding pattern of ligands and macrocyclic complexes were proposed by recording IR spectra. A pair of band near at 3380 and 3250cm^{-1} due to $\nu_{as}(NH_2)$ and $\nu_s(NH_2)$ was present in the IR spectra of diamines and a band in the range 1670–1680 cm^{-1} corresponding to ν(C=O) was present in the spectra of ligands. The vanishing of these bands and appearance of a new absorption band near 1610–1625cm^{-1} confirms that the carbonyl group of benzil and -NH_2 group of diamines were condensed and results in the formation of a macrocyclic framework. The lower value of $\nu_{(C=N)}$ band is due to the ligand and metal ion coordination and this can be described as a drift of electron density from azomethine nitrogen to the metal atom[29,30] indicates that coordination takes place via nitrogen of C=N groups. Medium intensity band near 1450-1575cm^{-1} may be assigned to stretching vibrations of (C=C) aromatic. The $\nu_{(C-H)}$ symmetric and asymmetric stretching vibrations near 1450-1495cm^{-1} and 1365-1400 cm^{-1} respectively,

might be due to aromatic rings of the complex. In the range of 420-445cm^{-1}, a medium intensity band assigned to $\nu_{(M-N)}$ which indicates the coordination of nitrogen. The IR spectral data of ligands and their metal complexes are shown in Table-2.

^{1}H NMR Spectra: The ^{1}HNMR of the ligands and their Cd(II) macrocyclic complexes were recorded to elucidate the structure of ligands and metal complexes. Multiplets were observed in the region 6.95-7.81ppm, which may be due to the protons of aromatic ring. Other signals were not observed and that confirms the formation of macrocyclic metal compounds by template condensation. The ^{1}H NMR data of ligands and Cd(II) complexes is shown in Table-2.

Electronic studies: The electronic studies of Ni(II) complexes shows that three bands in the region 8500-21700cm^{-1} attributed to three spinal lowed d-d transitions viz. $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$, respectively. The spectra are consistent with the octahedral nature of these complexes. The magnetic moment value for Ni(II) is observed 3.14 B.M. further endorse the electronic spectral findings.

Mass Spectra: The mass spectra of $[Ni(C_{40}H_{26}N_4Cl_4)]$ complex shows the molecular ion peak at m/z 762.25amu (Figure-3). This confirms the proposed the macrocyclic framework with molecular formula $[M(C_{40}H_{26}N_4Cl_2X_2)]$ and confirms the monomeric form of the complex. Peaks at m/z 703.47, 649.11, 463.09, 454.10amu corresponds to $[C_{40}H_{26}N_4Cl_4]$, $[C_{34}H_{21}N_4Cl_4Ni]$, $[C_{22}H_{14}N_4Cl_2Ni]$, $[C_{16}H_6N_4Cl_4Ni]$ fragments respectively and other peaks may be due to different fragments.

Antimicrobial Studies: Antimicrobial activity of the synthesized complexes was studied against some microorganism *E. coli, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis, Aspergillus fumigatus and Candida albicans"*. The results of antimicrobial activity were summarized in Table-3. All the complexes were found to be active against all the bacterial and fungal strains. Generally, the macrocyclic compounds are more active than the open chain derivatives. Macrocyclic metal complexes shows high percent inhibition against bacterial and fungal strains as compared to ligands. It may be because of chelation effect in which the polarities of the ligand and the metal atom are decreased by equal charge distribution over the complete chelate ring. This increases the lipophilic character of the complexes and favors its permeation through the lipoid layer of the bacterial membranes. Ni(II) complexes were found more active than Cd(II) complexes. Concentration also plays an important role, on increasing concentration the percent growth inhibition also increases. The comparative results of antimicrobial studies are shown in Figure-4 and 5.

Table-2: IR and ^{1}HNMR data of ligands and their metal complexes.

Compound	IR spectral data(cm^{-1})			^{1}H NMR spectral data (δ ppm) Aromatic protons(m)
	ν(C=O)	*ν*(C=N)	*ν(M ←N)*	
$C_{34}H_{23}N_2O_2Cl$	1680	1610	-	7.69-8.18
$C_{34}H_{23}N_2O_2F$	1670	1620	-	7.79-8.13
$[Ni(C_{40}H_{26}N_4Cl_4)]$	-	1598	435	-
$[Ni(C_{40}H_{26}N_4F_2Cl_2)]$	-	1600	422	-
$[Ni(C_{40}H_{26}N_4Cl_3F)]$	-	1610	430	-
$[Cd(C_{40}H_{26}N_4Cl_4)]$	-	1600	445	7.41-7.81
$[Cd(C_{40}H_{26}N_4F_2Cl_2)]$	-	1615	428	7.24-7.75
$[Cd(C_{40}H_{26}N_4Cl_3F)]$	-	1622	440	6.97- 7.60

*m= multiplet.

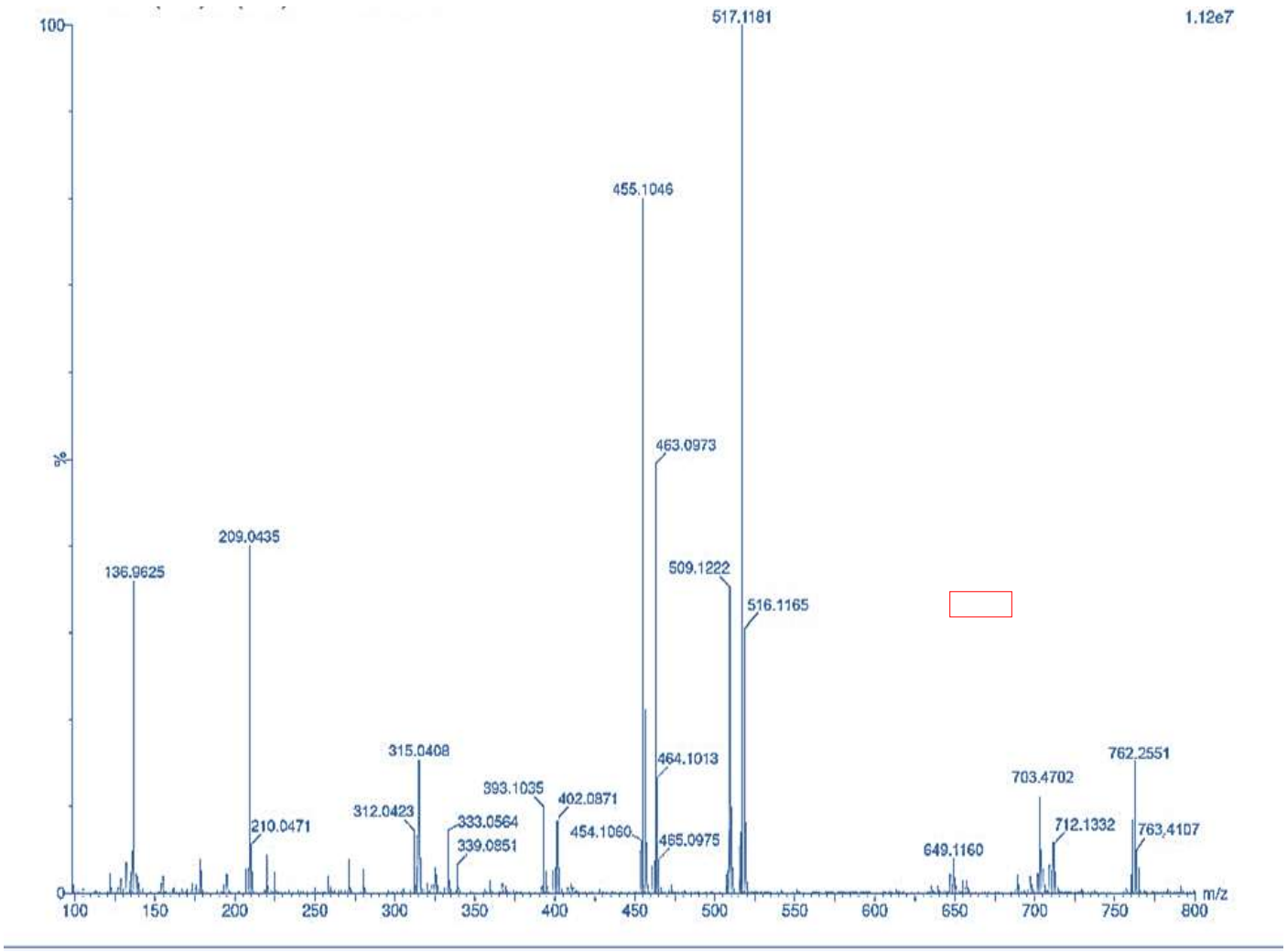

Figure-3: Mass spectra of [Ni($C_{40}H_{26}N_4Cl_4$)] complex.

Table-3: Antimicrobial activity of ligands and their complexes.

Compounds	Diameter of inhibition zone (mm)					
	B. subtilis	S. aureus	P. aeruginosa	E. coli	C. albicans	A. fumigates
$C_{34}H_{23}N_2O_2C_1$	7 ± 0.06	7.2 ± 0.05	8 ± 0.11	6.8 ± 0.05	7 ± 0.08	7.4 ± 0.01
$C_{34}H_{23}N_2O_2F$	6 ± 0.05	7 ± 0.08	7.6 ± 0.09	6 ± 0.05	6.4 ± 0.08	7.0 ± 0.03
[Ni($C_{40}H_{20}N_4Cl_4$)]	13.0 ± 0.06	12.6 ± 0.01	16.0 ± 0.04	14.6 ± 0.02	10.2 ± 0.07	9.0 ± 0.02
[Ni($C_{40}H_{26}N_4F_2C1_2$)]	11.8 ± 0.13	13.0 ± 0.09	15.8 ± 0.03	15.5 ± 0.01	11.6 ± 0.04	8.7 ± 0.04
[C41($C_{40}H_{26}N_4F_2C1_2$)]	9 ± 0.02	10.1 ± 0.01	13.2 ± 0.09	10 ± 0.13	8.0 ± 0.11	7.5 ± 0.01
[Cd($C_{40}H_{26}N_4C1_3F$)]	7.2 ± 0.05	10.6 ± 0.02	12 ± 0.03	12.4 ± 0.06	6.7 ± 0.09	7 ± 0.10
Itraconazole	-	-	-	-	14 ± 0.02	13 ± 0.07
Streptomycin	16 0.01	18 0.11	28 0.02	25 0.18	-	-

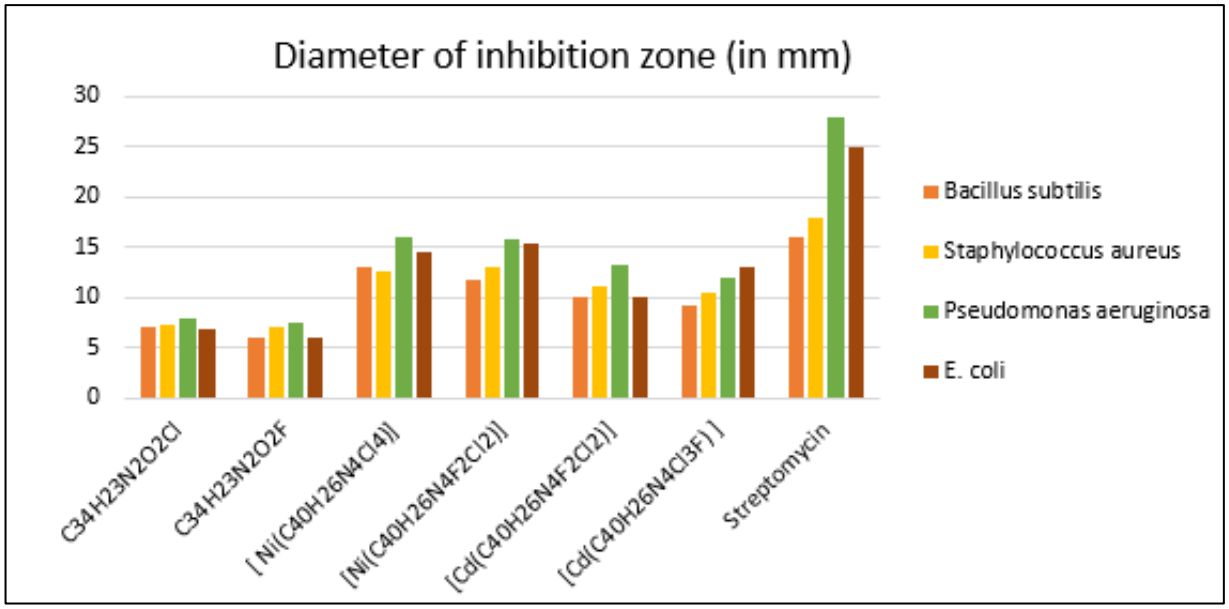

Figure-4: Antibacterial activity of ligands and their metal complexes.

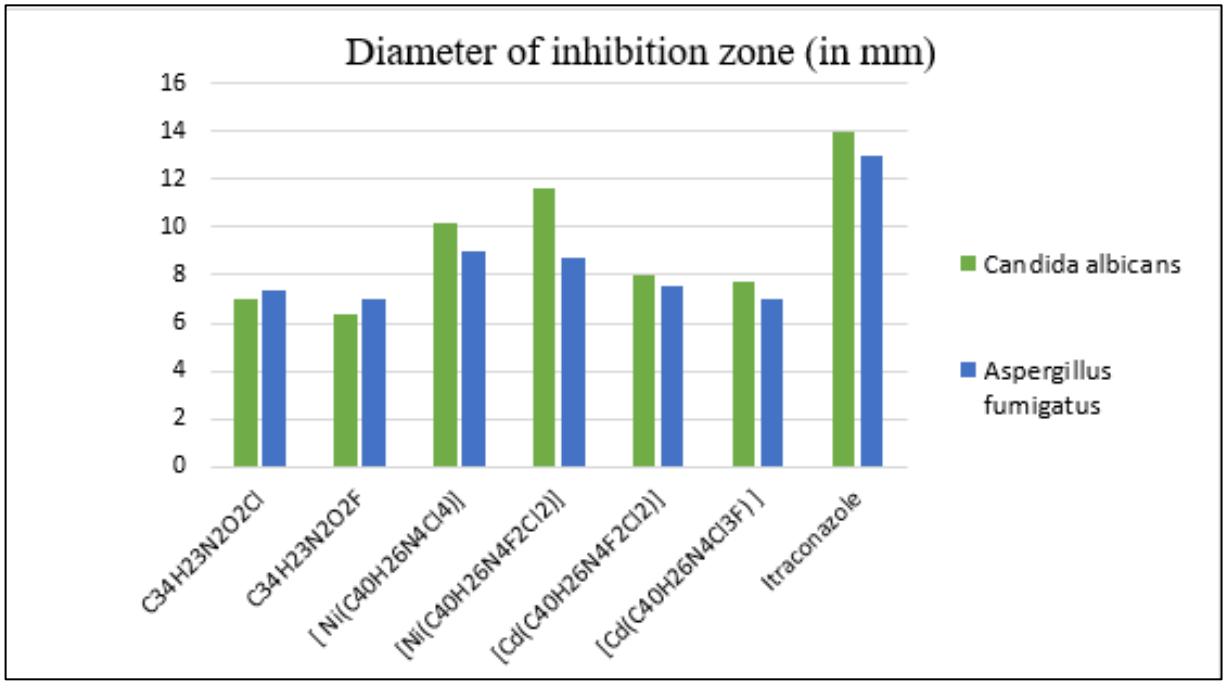

Figure-5: Antifungal activity of ligands and their metal complexes.

Conclusion

The present study shows the formation of macrocyclic metal complexes with Ni(II) and Cd(II) via template condensation. On the basis of different spectral and elemental data, an octahedral geometry is suggested for all the complexes. The results of mass spectra also revealed the formation of monomeric macrocyclic metal complexes. Antimicrobial activity of complexes shows that the macrocyclic complexes are very effective on tested microorganisms. Besides from chelation effect many other factors such as solubility, dipole moment, and conductivity influenced by metal ion also a possible reasons for remarkable antimicrobial activities of these complexes.

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