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Bioactive Azamacrocyclic Zinc(II) Complexes: Synthesis and Characterisation

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Reduction of Me_8[14]diene·2$HClO_4$ (L·2$HClO_4$) afforded three isomeric ligands (L_A, L_B and L_C), among which L_C underwent cyanoethylation with excess acrylonitrile to form the *bis*-cyanoethyl derivative (L_{CX}). Reaction of L_{CX} with zinc(II) chloride yielded the six-coordinate complex [Zn(L_{CX})Cl_2], which subsequently underwent axial ligand substitution with KSCN, KNO_3, $NaNO_2$, KBr and KI to produce a series of octahedral zinc(II) complexes, [Zn(L_{CX})($X)_2$] (X = NCS, NO_3, NO_2, Br or I). All the Zn(II) synthesised complexes were characterized using various analytical and spectroscopic techniques. Their antibacterial activity was evaluated against four selected bacterial strains and most of the complexes exhibited good antibacterial activity against the tested bacteria.

Keywords: Tetraazamacrocyclic ligand, Cyanoethyl derivative, Zinc(II) complexes, Antibacterial studies.

INTRODUCTION

Metal complexes of tetraazamacrocycles have become a focus of significant research due to their diverse applications in coordination [1] and bioinorganic chemistry [2]. The unique structural architecture of tetraazamacrocyclic ligands enables strong coordination with transition metal ions, results to the formation of highly stable metal complexes through the macrocyclic effect [3]. Their well-defined coordination environment often enhances the stability and biological performance of the resulting complexes, making them attractive molecular platforms for medicinal chemistry [2,4]. Owing to these favourable characteristics, tetraazamacrocyclic metal complexes have been widely explored for diverse therapeutic applications, with reports describing their antimicrobial [5,6], anticancer [7,8], antitumor [9] and anti-HIV activities [10]. Due to the strong chelation effect, these complexes have also exhibited their dynamic behaviour in the fields of imaging [11], catalysis [12], sensors [13] and other industrial applications [14].

Zinc(II) macrocyclic complexes have attracted considerable interest in medicinal chemistry and bioinorganic chemistry due to their diverse biological and catalytic properties [15,16]. Their ability to bind and cleave nucleic acids has led to the development of artificial nuclease systems with potential therapeutic applications [17]. These complexes have also been widely investigated for their antimicrobial and anticancer activities [18]. Owing to their structural and functional resemblance to the active sites of zinc-dependent enzymes, they have served as valuable biomimetic models for studying metalloenzymes such as carbonic anhydrase [19].

The synthesis of isomeric ligands (L_A, L_B and L_C) derived from Me_8[14]diene·2$HClO_4$ is available in literature [20]. Recent research has also been carried out on the synthesis of *bis*(cyanoethyl)N-pendant derivative L_{CX} from L_C [5]. However, The X-ray crystallographic structure of L_{CX} has been reported by Chakraborty *et al.* [21] and the ligand has demonstrated excellent structural stability and antibacterial activity in its cadmium(II) and nickel(II) complexes [21,22]. Recently, Soha *et al.* [7] showed that [Zn(L_{CX})($NCS)_2$] effectively inhibited cancer cell proliferation and induced apoptosis in both *in vitro* and *in vivo* models.. Therefore, it was reasonable to use zinc(II) to check the ligand's structural impact and potential uses in medicine. The current study focuses on the synthesis, characterisation and antibacterial investigation of zinc(II) complexes of a tetraazamacrocyclic ligand (L_{CX}).

EXPERIMENTAL

All the chemicals and solvents employed were of analytical reagent grade and no additional purification was required.

Characterisation: Elemental analyses (C, H, N) were performed using a LECO CHNS-932 elemental analyzer. Infrared spectra were recorded on a Shimadzu IR-20 spectrophotometer in the range 4000-400 cm^{-1} using KBr pellets. ^1H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 MHz spectrometer using TMS as internal reference and DMSO-d_6 as solvent. Molar conductance measurements were carried out using a Hanna HI-8820 conductivity bridge at room temperature. Magnetic susceptibilities were measured at room temperature and expressed as effective magnetic moments (μ_{eff}).

Synthesis of ligands: Three isomeric ligands (L_A, L_B and L_C) were previously synthesized [20]. Among them, L_C was used as the precursor for the synthesis of *bis*-cyanoethyl derivative, L_{CX} (**Scheme-I**), according to the reported method [5].

Synthesis of zinc(II) complexes

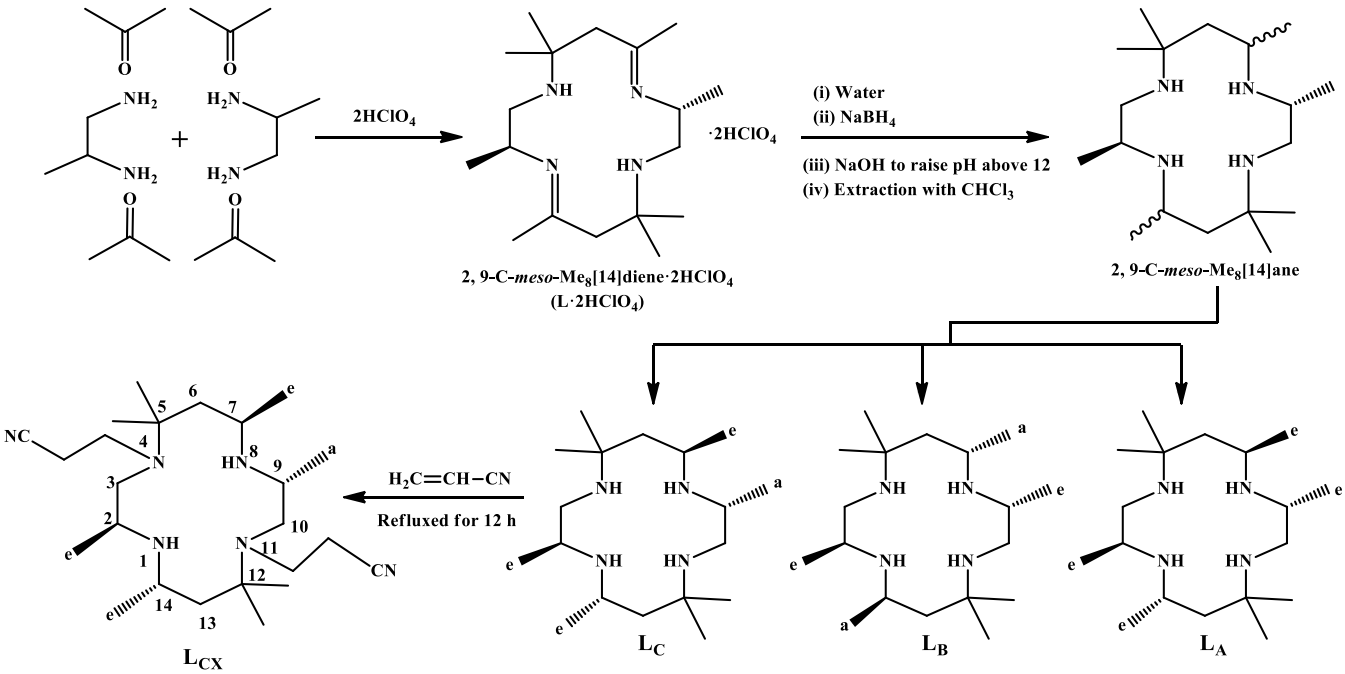
$[Zn(L_{CX})Cl_2]$: This complex was synthesized by the direct reaction of L_{CX} with zinc(II) chloride. In brief, L_{CX} (0.42 g, 1.0 mmol) and $ZnCl_2$ (0.14 g, 1.0 mmol) were separately dissolved in 20 mL of hot methanol and combined while still hot. The resulting solution was heated on a steam bath until its volume was reduced, leading to the formation of an oily product. Upon standing overnight, the oily mass transformed into a sticky solid, which was washed with methanol and filtered. The filtrate was evaporated and the residue was extracted with chloroform to afford $[Zn(L_{CX})Cl_2]$ after solvent evaporation. The product was dried under vacuum over silica gel in a desiccator. The characterization data of this complex have been reported previously [7]. Yield: 72% (554.985 g mol^{-1}); m.p.: 130 ºC; Anal. calcd. (found) %: C, 51.89 (51.85); H, 8.30 (8.25); N, 15.14 (15.18); Molar conductivity, Ω (ohm^{-1} cm^2 mol^{-1}): 28 (in DMSO); 0 (in $CHCl_3$); 224 (in H_2O); Magnetic moment, μ_{eff} (BM): Diamagnetic. IR (KBr, ν_{max}, cm^{-1}): 3244 w (N–H), 2971 m (C–H), 2245 s (C≡N), 1377 m (CH_3), 1173 m (C–C), 608 w (Zn–N); ^1H NMR (DMSO, 400 MHz, δ ppm): For *geminal* dimethyl, 1.14[e] (os, 6H), 1.25[a] (os, 6H); For methyl on chiral carbon, 1.22[e] (od, 6H), 1.34[a] (od, 3H), 1.43[a] (od, 3H); For CH_2, CH and NH, 2.41 (m), 2.75 (m), 3.32 (m), 3.50 (m), 3.79 (m) and 7.25 (m).

Zn(II) complexes synthesised by the axial substitution reactions on $[Zn(L_{CX})Cl_2]$

$[Zn(L_{CX})(NCS)_2]$: $[Zn(L_{CX})Cl_2]$ (0.5549 g, 1.0 mmol) and KSCN (0.20 g, 2.0 mmol) were dissolved separately in 20 mL hot absolute methanol and mixed while hot. The colourless solution thus obtained was heated on a steam bath and completely dried. The residue was extracted with chloroform and filtered and evaporation of the chloroform extract afforded the white complex $[Zn(L_{CX})(NCS)_2]$. The product was stored in a desiccator over silica gel. Yield: 61% (600.24 g mol^{-1}); m.p.: 100 ºC; Anal. calcd. (found) %: C, 51.98 (51.96); H, 7.66 (7.64); N, 18.66 (18.65); IR (KBr, ν_{max}, cm^{-1}): 3206 w (N–H), 2971 w (C–H), 1383 s (CH_3), 1173 m (C–C), 2248 m (C≡N), 600 w (Zn–N), 2083 vs (CN), 835 w (CS), δ(NCS) 478s. Molar conductivity, Ω (ohm^{-1} cm^2 mol^{-1}): 40 (in DMSO); 0 (in $CHCl_3$). Magnetic moment, μ_{eff} (BM): Diamagnetic.

$[Zn(L_{CX})(NO_3)_2]$, $[Zn(L_{CX})(NO_2)_2]$, $[Zn(L_{CX})Br_2]$ and $[Zn(L_{CX})I_2]$: Complexes $[Zn(L_{CX})(NO_3)_2]$, $[Zn(L_{CX})(NO_2)_2]$, $[Zn(L_{CX})Br_2]$ and $[Zn(L_{CX})I_2]$ were synthesised by treating $[Zn(L_{CX})Cl_2]$ with the required potassium or sodium salts (KNO_3, $NaNO_2$, KBr, KI) in methanol as per same procedure mentioned previously.

$[Zn(L_{CX})(NO_3)_2]$: Colour: white; yield: 57% (608.09 g mol^{-1}); m.p.: 100 ºC; Anal. calcd. (found) %: C, 47.36 (47.34); H, 7.56 (7.54); N, 18.42 (18.40); IR (KBr, ν_{max}, cm^{-1}): 3246 w



Scheme-I: Preparation of ligands

(N–H), 2966 w (C–H), 2246 m (C≡N), 1461 s (NO_3), 1383s (CH_3), 1320 s (NO_3), 1175 m (C–C), 595 w (Zn–N); Molar conductivity, Ω (ohm^{-1} cm^2 mol^{-1}): 30 (in DMSO); 0 (in $CHCl_3$); 222 (in H_2O); Magnetic moment, μ_{eff} (BM): Diamagnetic.

[Zn(L_{CX})(NO_2)$_2$]: Colour: white; yield: 64% (576.09 g mol^{-1}): m.p.: 100 ºC; Anal. calcd. (found) %: C, 49.99 (49.97); H, 7.98 (7.97); N, 19.44 (19.42); IR (KBr, ν_{max}, cm^{-1}): 3241 w (N–H), 2975 w (C–H), 2245 s (C≡N), 1462 s asym, 1335 w sym., 1383 vs (CH_3), 1172 m (C–C), 600 w (Zn–N), Molar conductivity, Ω (ohm^{-1} cm^2 mol^{-1}): 29 (in DMSO); 0 (in $CHCl_3$); Magnetic moment, μ_{eff} (BM): Diamagnetic.

[Zn(L_{CX})Br$_2$]: Colour: white, Yield: 49%; (643.89 g mol^{-1}); m.p.: 130 ºC; Anal. calcd. (found) %: C, 44.73 (44.70); H, 7.14 (7.13); N, 13.05 (13.04); IR (KBr, ν_{max}, cm^{-1}): 3250 w (N–H), 2973 w (C–H), 2245 m (C≡N), 1383 s (CH_3), 1174 m (C–C), 595 w (Zn–N); Molar conductivity, Ω (ohm^{-1} cm^2 mol^{-1}): 42 (in DMSO); 0 (in $CHCl_3$); 258 (in H_2O); Magnetic moment, μ_{eff} (BM): Diamagnetic.

[Zn(L_{CX})I_2]: Colour: white; yield: 65%. (737.89 g mol^{-1}): m.p.: 130 ºC; Anal. calcd. (found) %: C, 39.03 (39.02); H, 6.23 (6.21); N, 11.38 (11.35); IR (KBr, ν_{max}, cm^{-1}): 3241 w (N–H), 2970 w (C–H), 2245 m (C≡N), 1383 s (CH_3), 1173 m (C–C), 600 w (Zn–N); Molar conductivity, Ω (ohm^{-1} cm^2 mol^{-1}): 41 (in DMSO); 0 (in $CHCl_3$); 192 (in H_2O); Magnetic moment, μ_{eff} (BM): Diamagnetic.

Antibacterial studies: The antibacterial activities of the synthesised zinc(II) complexes were evaluated against selected bacterial strains using standard procedures reported previously [22]. Experimental conditions, culture media and measurement protocols were identical to those described in earlier publications [21,22].

RESULTS AND DISCUSSION

The characterisation of ligand L_{CX} has been reported previously [5].

The IR spectrum of [Zn(L_{CX})Cl_2] complex displays the characteristic bands for ν(N-H), ν(C-H), ν(CH_3), ν(Zn-N) and ν(C-C) in the expected region. The presence of the cyano (-C≡N) group is indicated by an infrared band at 2245 cm^{-1}. The Zn–Cl stretching vibration could not be assigned since the FT-IR spectrum was recorded only above 400 cm^{-1}. The molar conductivity of [Zn(L_{CX})Cl_2] in DMSO was 28 Ω^{-1} cm^2 mol^{-1}, which is characteristic of a non-electrolyte and shows that both chloride ions are coordinated to the zinc(II) ion within the inner coordination sphere, giving a six-coordinate octahedral complex. In aqueous solution, the molar conductivity increased to Ω^{-1} cm^2 mol^{-1}, corresponding to 1:2 electrolyte behaviour due to the release of the coordinated chloride ions into solution, as represented by the following equilibrium [23]:

$$[Zn(L_{CX})Cl_2] \xrightarrow{H_2O} [Zn(L_{CX})(H_2O)_2]Cl_2$$

The ^{1}H NMR spectrum of the [Zn(L_{CX})Cl_2] exhibits an overlapped signal corresponding to 24H. Nevertheless, the signal's resolution reveals that it is made up of three doublets and two singlets, while the doublets at δ 1.22, 1.34 and 1.43 ppm in the ratio of 2:1:1 correspond to 6H, 3H and 3H, respectively, the singlets at δ 1.14 and 1.25 ppm, each corresponding to 6H, can be attributed to equatorial and axial components of *gem*-dimethyl protons. Two equatorial methyl protons on two chiral carbons can be attributed to the doublet at δ 1.22 ppm, while two axial methyl protons on the other two chiral carbons can be attributed to the other two doublets at δ 1.34 and 1.43 ppm each that correspond to 3H. As reported previously [5], multiplet signals corresponding to the CH_2, CH and NH protons were observed at δ 2.41, 2.75, 3.32, 3.50, 3.79, and 7.25 ppm. The UV visible spectrum of this complex did not give any *d-d* band and it is diamagnetic as expected for d^{10} system. Based on above characterisation, the structure-I (Fig. 1) can be assigned for [Zn(L_{CX})Cl_2].

[Zn(L_{CX})(X)$_2$] (X = NCS, NO_3, NO_2, Br or I): [Zn(L_{CX})Cl_2] underwent axial substitution reactions with KSCN, KNO_3, $NaNO_2$, KBr or KI in 1:2 ratio to obtain [Zn(L_{CX})(X)$_2$]. The IR spectra of these complexes show all characteristic ν(N-H), ν(C-C), ν(C-H), ν(CH_3), ν(Zn-N) and ν(C≡N) bands in the predicted regions. Cl^- ions are substituted by NCS^- groups in [Zn(L_{CX})(NCS)$_2$], as evidenced by the presence of the ν(NCS) band and the ν(C-N) band. The characteristic bands at 2083 cm^{-1} [ν(CN)], 835 cm^{-1} [ν(CS)] and 478 cm^{-1} [δ(NCS)] is attributed due to the N-bonded thiocyanate coordination and confirmed the complex as an isothiocyanato derivative [22]. In the spectrum of [Zn(L_{CX})(NO_3)$_2$], the ν(NO_3) band appeared at 1383 cm^{-1} was split into two bands at 1320 and 1431 cm^{-1}. The separation of 111 cm^{-1} between these bands is the characteristic of unidentate coordination of the nitrate ion [24-26]. However, complex [Zn(L_{CX})(NO_2)$_2$] exhibits the ν_{asym}(NO_2) and ν_{sym} (NO_2) bands at 1462 and 1335 cm^{-1}, respectively [22]. The δ(NO_2) frequency is responsible for the band's appearance at 836 cm^{-1}. The Zn–Br and Zn–I stretching vibrations could not be assigned since the FT-IR spectra were recorded only above 400 cm^{-1}. The molar conductivity was found to be 40 Ω^{-1} cm^2 mol^{-1} in DMSO and 0 Ω^{-1} cm^2 mol^{-1} in $CHCl_3$, which is characteristic of a non-electrolyte and supports coordination of both thiocyanate ions to the Zn(II) centre. These results are consistent with an octahedral geometry. However, a slightly higher conductivity in DMSO may reflect limited solvation or partial dissociation of the complex in this solvent, although further evidence is required to establish any structural change.

$$[Zn(L_{CX})(NCS)_2] \xrightarrow{DMSO} [Zn(L_{CX})(NCS)](NCS)$$

The molar conductivity value of 29-42 ohm^{-1} cm^2 mol^{-1} in DMSO shows the non-electrolytic nature of these complexes as expected for molecular formula assigned [Zn(L_{CX})(X)$_2$] (X = NO_3, NO_2, Br or I). However, the conductance value for [Zn(L_{CX})(X^1)$_2$] (X^1= NO_3, Br or I) 192-258 Ω^{-1} cm^2 mol^{-1} in H_2O corresponding to 1:2 electrolyte can be expressed by the following expression.

$$[Zn(L_{CX})X_2] \xrightarrow{H_2O} [Zn(L_{CX})(H_2O)_2]X_2$$

The magnetochemical measurement of [Zn(L_{CX})(X)$_2$] (X = NCS, NO_3, NO_2, Br or I) is also in good agreement with diamagnetic for d^{10} system. Therefore, based on aforementioned explanation as well as on the fact that axial substitution takes place without change of conformation and configura-

tion of ligand of original complex [21,22,27], the structures II, III, IV, V and VI (Fig. 1) can be assigned for the zinc(II) complexes.

Antibacterial activity: The antibacterial properties of zinc(II) complexes derived from L_{CX}, however, have not been investigated. To address this gap, the antibacterial activity of L_{CX} and its zinc(II) complexes was evaluated against four selected bacterial strains. As summarized in Table-1, the free ligand showed no detectable antibacterial activity, whereas several Zn(II) complexes inhibited bacterial growth, indicating that complex formation enhances the biological activity of the ligand. Among the synthesized complexes, $[Zn(L_{CX})Cl_2]$ (S-01) and $[Zn(L_{CX})(NCS)_2]$ (S-02) exhibited activity against *B. cereus* and *P. aeruginosa*, with S-02 displaying additional activity against *S. aureus*. The dichlorido complex (S-01) exhibit the highest inhibition against *B. cereus*. The dinitrato complex, $[Zn(L_{CX})(NO_3)_2]$ (S-03), was active only against *P. aeruginosa*, whereas the dinitrito complex, $[Zn(L_{CX})(NO_2)_2]$ (S-04), inhibited all tested bacteria except *P. aeruginosa* and showed the highest activity against *S. aureus*. The dibromido (S-05) and diiodido (S-06) complexes exhibited the strongest activity against *P. aeruginosa*, moderate activity against *B. cereus* and *E. coli*, and no activity against *S. aureus*. Dimethyl sulphoxide, zinc(II) salt and ampicillin were included as the negative, metal-ion and positive controls, respectively.

The enhanced antibacterial activity of the zinc(II) complexes relative to the free ligand may be explained by chelation theory [28,29]. Coordination of the ligand to the metal ion can reduce the polarity of the metal centre through charge sharing with the donor atoms and increase the lipophilic

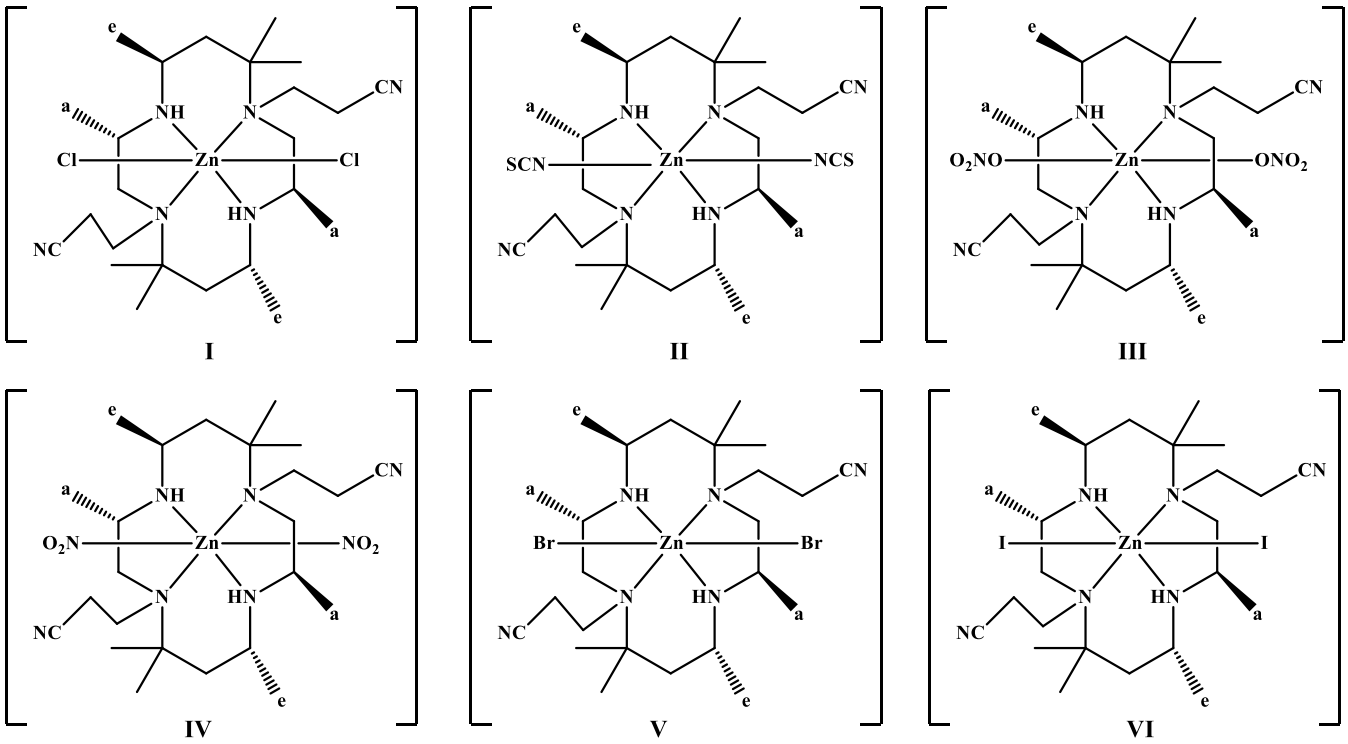


Fig. 1. Structure of zinc(II) complexes

TABLE-1
DIAMETER (mm) OF ZONES OF INHIBITION BY THE LIGANDS AND THEIR ZINC(II) COMPLEXES AGAINST SOME PATHOGENIC BACTERIA

Sample No.	Ligand and its complexes	Zone of inhibition in diameter (mm)			
		Gram-positive bacteria		Gram-negative bacteria	
		S. aureus	*B. cereus*	*E. coli*	*P. aeruginosa*
L	L_{CX}	0	0	0	0
S-01	$[Zn(L_{CX})Cl_2]$	12	15	0	14
S-02	$[Zn(L_{CX})(NCS)_2]$	10	11	0	14.75
S-03	$[Zn(L_{CX})(NO_3)_2]$	0	0	0	15.5
S-04	$[Zn(L_{CX})(NO_2)_2]$	11.5	9	9.5	0
S-05	$[Zn(L_{CX})Br_2]$	0	11	8.5	16.5
S-06	$[Zn(L_{CX})I_2]$	0	13	10	16.5
Negative control	DMSO	0	0	0	0
Positive control	Ampicillin	21	24	23	25
–	$ZnCl_2$	4	2	3	2

character of the complex. High lipophilicity may facilitate diffusion across the bacterial cell membrane, thereby improving antibacterial activity. Since the free ligand was inactive and the isolated complexes are reported to be stable in solution [30], the observed antibacterial activity is more likely associated with the intact zinc(II) complexes than with the individual ligand or metal ion.

Conclusion

This study demonstrates that $ZnCl_2$ readily forms complexes with the N-pendant tetraazamacrocyclic ligand (L_{CX}). Reaction of L_{CX} with zinc(II) chloride afforded the octahedral complex $[Zn(L_{CX})Cl_2]$, which exhibited non-electrolytic behaviour in $CHCl_3$, consistent with a six-coordinate structure. The dichlorido complex underwent axial ligand substitution with NCS^-, NO_3^-, NO_2^-, Br^- and I^- to yield the corresponding octahedral Zn(II) complexes. The conductance values obtained in aqueous solution for some complexes corresponded to those of 1:2 electrolytes confirmed the formation of diaqua complexes. The synthesized zinc(II) complexes exhibited moderate antibacterial activity, whereas the free ligand showed no detectable activity under the experimental conditions.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no data, results or scientific conclusions were generated by AI. All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

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