Inorganic Chemistry Communications 20 (2012) 238–242

Contents lists available at SciVerse ScienceDirect

Inorganic Chemistry Communications

journal homepage: www.elsevier.com/locate/inoche

Chelating behavior and biocidal efficiency of tryptophan based mixed-ligand complexes

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ARTICLE INFO

Article history:
Received 13 February 2012
Accepted 8 March 2012
Available online 17 March 2012

Keywords:
Tryptophan-based mixed-ligand complexes
DNA binding
DNA cleavage
Microbial property

ABSTRACT

A new family of tryptophan based mixed-ligand complexes has been synthesized and characterized. DNA (Calf thymus) binding properties of the complexes have been explored by UV–vis, viscosity measurements and cyclic voltammetry. All the experimental evidence suggest that the ancillary ligand 2,2′-bipyridine influences the intercalative binding of these complexes to CT DNA. The DNA cleavage efficiencies of these complexes with pBR322 DNA were investigated by gel electrophoresis. The complexes were found to promote the cleavage of pBR322 DNA from the supercoiled form I to the open circular form II in the presence of an oxidizing agent (H_2O_2). Microbial property of these complexes as antibacterial agents has been investigated against Gram-negative and Gram-positive bacteria.

Many of chemotherapeutic agents currently used in cancer therapy are agents which inhibit tumor growth by inhibiting the replication and transcription of DNA. A number of metal chelates are of current interest due to their important applications in nucleic acid chemistry as DNA probes of DNA structure in solutions, reagents for the mediation of strand scission of duplex DNA under physicochemical conditions, and as chemotherapeutic agents and in the genomic research [1]. Complexes bearing amino acid functionality enhance the selective effect of DNA binding as well as for other therapeutic targets, providing a key recognition element of artificial nuclease activity to the molecule [2].

Amino acids play central roles both as building blocks of proteins and as intermediates in metabolism. Tryptophan is an essential amino acid for human nutrition, which is the precursor for the synthesis of serotonin, an important neuromediator associated to mood, stress response, sleep and appetite regulation. Various transition metal complexes derived from 2,2′-bipyridine [3,4] and amino acid derivatives [5] have been reported as good candidates for DNA structural probes, DNA cleavage studies, DNA molecular light switches and new therapeutics.

At present, the interaction of transition metal complexes with DNA has been a pet subject of researchers in the field of bioinorganic chemistry [6–8]. Metal complexes binding with nucleic acid are currently investigated because of their utility as DNA structural probes, DNA foot printing and sequence-specific cleavage agent and potential anticancer drug [9–11]. Recent reports have also shown that amino acid based metal(II) complexes show efficient DNA cleavage activity [12–14]. As pathogenic bacteria continuously evolve resistance to currently use antibacterial agents, the discovery of novel and potent antibacterial agents is the best way to overcome bacterial resistance and develop effective therapies [15].

As part of a research program aim at the design of chemical nucleases, we describe herein the synthesis, structure, anti-microbial and cleavage studies of tryptophan derived Schiff base and its mixed ligand transition metal complexes. However, a survey of the literature reveals that no work has been carried out on the above mentioned mixed ligand complexes of tryptophan derivatives containing 2,2′-bipyridine and various metal ions.

The electron transfer mechanism of the mixed ligand metal complexes is investigated by the aid of cyclic voltammetry. We hope that our results should be of value in designing of new metallonucleases.

The Schiff base ligand and its mixed ligand complexes were prepared by a typical procedure [16–22] (Fig. 1). We got the likely composition of complexes: $[ML(bpy)_2]Cl$ where bpy = 2,2′-bipyridine through elemental analyses, magnetic susceptibility, NMR, UV, IR, Mass spectra and molar conductivity measurements since no single crystals suitable for X-ray determination could be isolated (Fig. 2).

Absorption studies were performed to further ascertain the predicted binding trend of Schiff base complexes with CT DNA. The absorption spectra of $[CuL(bpy)_2]Cl$ in the absence and presence of DNA are given in Fig. 3.

If the binding mode is intercalation, the orbital of intercalated ligand can couple with the orbital of the base pairs, reducing the π–π* transition energy and resulting in bathochromism. If the coupling orbital is partially filled by electrons, it results in decreasing the transition probabilities and resulting in hypochromism [23]. Hypochromism means the metal complexes binding with calf thymus DNA resulting in its breakage and perturbation. The extent of the

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doi:10.1016/j.inoche.2012.03.016

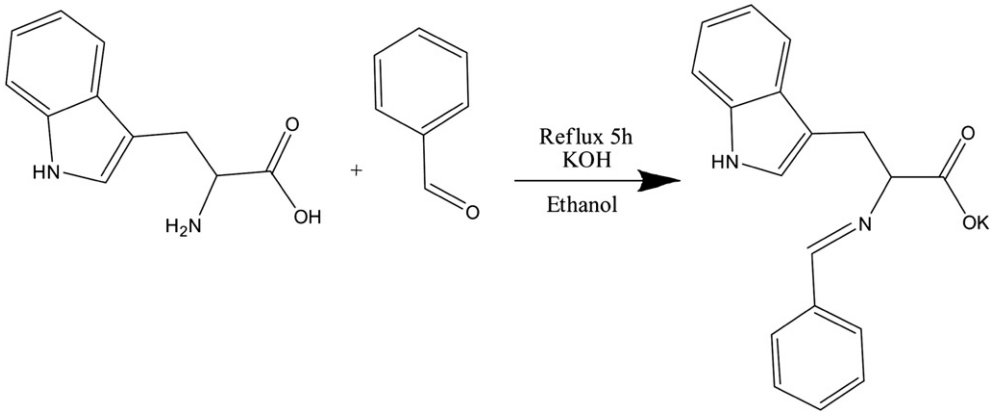

Fig. 1. Synthesis of Schiff base.

hypochromism in the metal-to-ligand charge transfer (MLCT) band is commonly consistent with the strength of intercalative interaction [24]. The absorption spectrum of $[CuL(bpy)_2]Cl$ complex showed an intensive absorption band at 383.6 nm, $[CoL(bpy)_2]Cl$ at 343 nm, $[NiL(bpy)_2]Cl$ complex at 310.8 nm and $[ZnL(bpy)_2]Cl$ at 351.5 nm respectively in 5 mM Tris–HCl and 50 mM NaCl buffer solution (pH 7.2). These are due to intra ligand π–π* transitions. On increasing the concentration of CT DNA resulted in the slight bathochromic shift in the range 3.1–1.3 nm and significant hypochromicity lying in the range 41–24%. Intrinsic binding constants of $3.1\times10^6\ M^{-1}$, $2.2\times10^6\ M^{-1}$, $1.9\times10^6\ M^{-1}$ and $1.3\times10^6\ M^{-1}$ were determined for $[CuL(bpy)_2]Cl$, $[CoL(bpy)_2]Cl$, $[NiL(bpy)_2]Cl$ and $[ZnL(bpy)_2]Cl$ respectively. These values (Table 1) are comparable to the classical DNA intercalators EB (ethidium bromide, $1.4\times10^6\ M^{-1}$ [25]). All the metal complexes showed decrease in absorption intensity (hypochromism) with a slight red shift which is due to the intercalative binding between DNA and metal complexes.

Viscosity measurement is sensitive to the changes in the length of DNA molecule. It is regarded as the least ambiguous and the most critical means studying the binding mode of metal complexes with DNA in solution, and provides stronger arguments for intercalative-binding mode [26,27]. The viscosity measurement was introduced to furthermore support this interaction between the complex and DNA. Intercalation was the effect of increasing DNA viscosity. The significant increase in the viscosity of DNA on addition of complex results due to the intercalation which leads to the separation among the DNA bases to the increase in the effective size in DNA which could be the reason for the increase in the viscosity. Plot of $(\eta/\eta o)^{1/3}$ versus [complex]/[DNA] gives a measure of the viscosity changes (Fig. 4).

A plodding increase in the relative viscosity was observed on addition of the metal complexes to DNA solution suggesting mainly intercalation mode of binding nature of the complexes.

The application of the cyclic voltammetric technique to study the interaction between metal complexes and DNA provides a useful complement to the previously used spectral studies. The cyclic voltammogram of copper complex in buffer pH = 7.2 at 25 °C is given Fig. 5.

The cyclic voltammogram of the $[CuL(bpy)_2]Cl$ in the absence of CT-DNA featured three anodic peaks ($E_{Pa1} = -0.060$ V, $E_{Pa2} = 0.008$ V, $E_{Pa3} = 0.277$ V) and three cathodic peaks ($E_{Pc1} = -0.617$ V, $E_{Pc2} = -0.415$ V, $E_{Pc3} = -0.175$ V). The oxidation of peak E_{Pa1} belongs to Cu(0)/Cu(I) and reduction of Cu(I) occurred, upon scan reversal at −0.617 V. The separation of the anodic and cathodic peak potential is 0.557 V, the ratio of anodic to cathodic peak currents, $ip_a/ip_c = 1.14$, indicating a quasi-reversible redox process. The oxidation of peak E_{Pa2} belongs to Cu(I)/Cu(II) and reduction of Cu(II) occurred, upon scan reversal at −0.415 V. The separation of the anodic and cathodic peak potential is 0.423 V, the ratio of anodic to cathodic peak currents, $ip_a/ip_c = 1.41$, indicating a quasi-reversible redox process. The oxidation

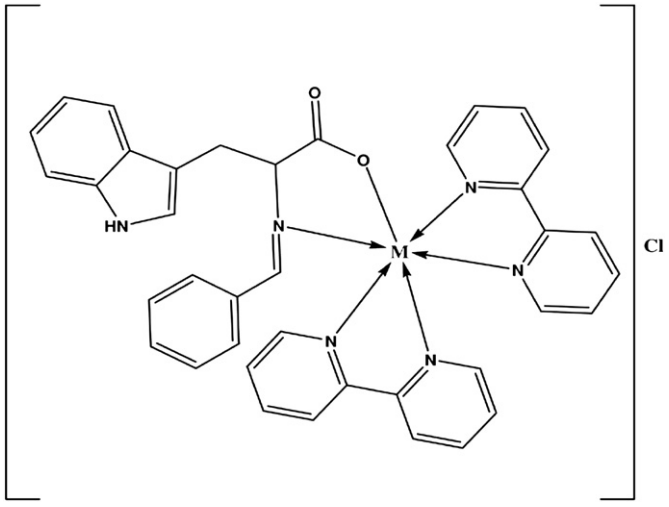

Fig. 2. The proposed structure of the metal complexes, M=Cu(II), Ni(II), Co(II) and Zn(II).

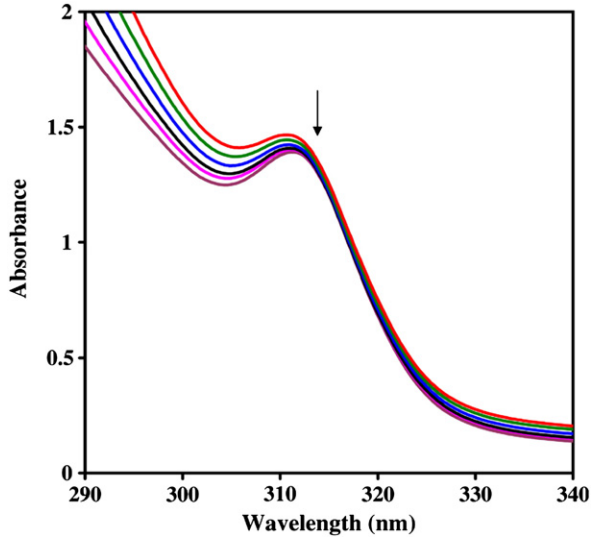

Fig. 3. Absorption spectral changes on addition of CT DNA to the solution of $[NiL(bpy)_2]Cl$ in buffer pH = 7.2 at 25 °C in the presence of increasing amount of DNA. Arrow indicates the changes in absorbance upon increasing the DNA concentration.

Table 1
Electronic absorption spectral properties of Cu(II), Co(II), Ni(II) and Zn(II) complexes.

Complex	λ max		Δλ	H%	$K_b \times 10^6$
	Free	Bound	(nm)		(M^{-1})
$[CuL(bpy)_2]Cl$	383.6	385.2	1.6	41	3.1
$[CoL(bpy)_2]Cl$	343.0	345.5	2.5	29	2.2
$[NiL(bpy)_2]Cl$	310.8	313.1	2.3	32	1.9
$[ZnL(bpy)_2]Cl$	351.5	353.4	1.9	24	1.3

of peak E_{Pa3} belongs to Cu(II)/Cu(III) and reduction of Cu(III) occurred, upon scan reversal at −0.175 V. The separation of the anodic and cathodic peak potential is 0.452 V, the ratio of anodic to cathodic peak currents, $ip_a/ip_c = 1.85$, indicating a quasi-reversible redox process. The formal potential $E_{1/2}$ taken as average of E_{Pa} and E_{Pc} is −0.338 V, −0.204 V and 0.051 V. When CT-DNA is added to a solution of complex both the anodic and cathodic peak current heights of the complex decreased in the same manner of increasing additions of DNA (Fig. 5). Also during DNA addition the anodic peak potential (Epa), cathodic peak potential (Epc), and $E_{1/2}$ (calculated as the average of Epc and Epa) all showed positive shifts. These positive shifts are considered as evidence for intercalation of the complex into the DNA, because this kind of interaction is due to hydrophobic interaction. From the other point of view, if a molecule binds electrostatically to the negatively charged deoxyribose-phosphate backbone of DNA, negative peak potential shifts should be detected. Therefore, the positive shift in the CV peak potentials of complex is indicative of intercalative binding mode of the complex with DNA [28].

The cyclic voltammogram of the $[NiL(bpy)_2]Cl$ in the absence of CT-DNA featured two anodic peaks ($E_{Pa1} = 0.008$ V, $E_{Pa2} = 0.274$ V) and two cathodic peaks ($E_{Pc1} = -0.418$ V, $E_{Pc2} = -0.182$ V). The oxidation of peak E_{Pa1} belongs to Ni(I)/Ni(II) and reduction of Ni(II) occurred, upon scan reversal at −0.418 V. The separation peak potential is 0.426 V, the ratio of $ip_a/ip_c = 1.36$, indicating a quasi-reversible redox process. The oxidation peak E_{Pa2} belongs to Ni(II)/Ni(III) and reduction of Ni(II) occurred, upon scan reversal at −0.182 V. The separation peak potential is 0.456 V, the ratio of $ip_a/ip_c = 1.08$, indicating a quasi-reversible redox process. The formal potential $E_{1/2}$ taken as average of E_{Pa} and E_{Pc} is −0.205 V and 0.046 V.

For Co(III) → Co(II), the redox couple cathodic peak appears at 0.105 in the absence of CT DNA (Epa = 0.357 V, Epc = 0.118 V, ΔEp = 0.239 V and $E_{1/2} = 0.237$ V). The ratio of ipc/ipa is approximately unity. This indicates that the reaction of the complex on the glassy carbon electrode surface is quasi-reversible redox process. The incremental addition of CT DNA to the complex causes

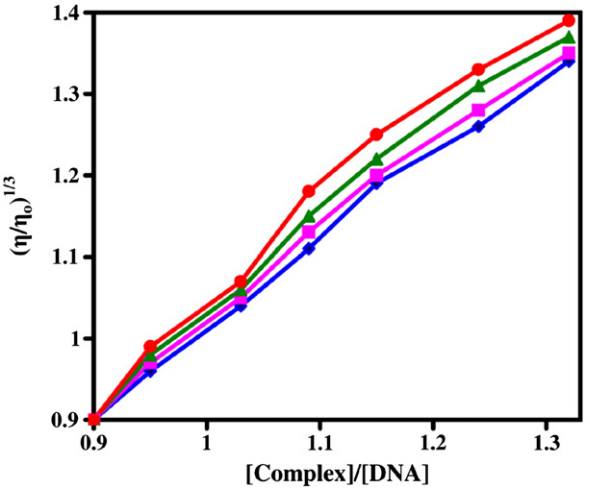

Fig. 4. Effect of increasing amounts of $[CuL(bpy)_2]Cl$ (●), $[CoL(bpy)_2]Cl$ (♦), $[NiL(bpy)_2]Cl$ (▲), $[ZnL(bpy)_2]Cl$ (■) on the viscosity of DNA.

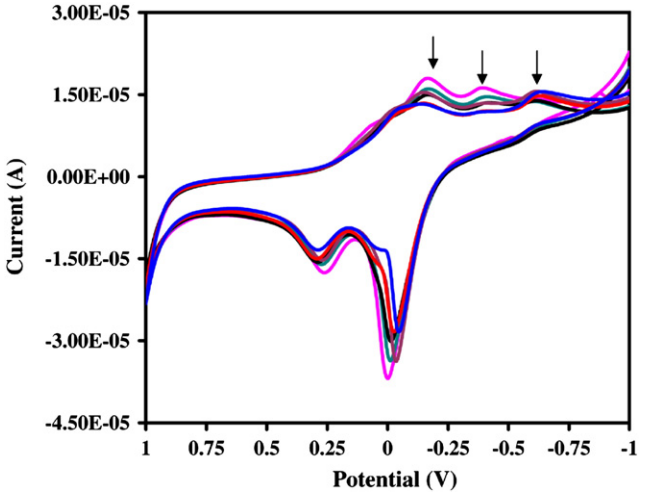

Fig. 5. Cyclic voltammogram of $[CuL(bpy)_2]Cl$ in the presence of increasing amount of DNA in buffer pH = 7.2 at 25 °C. Arrow indicates the changes in voltammetric currents upon increasing the DNA concentration.

a positive shift in formal potential ($E_{1/2}$) indicating that $[CoL(bpy)_2]Cl$ has bonded favorably with DNA *via* intercalation. Finally Zn(II) complex exhibits quasi-reversible transfer process with redox couple [Zn(II) → Zn(0)], cathodic peak appears at −0.091 V in the absence of DNA ($Ep_a = 0.412$ V, $Ep_c = -0.091$ V, ΔEp = 0.503 V and $E_{1/2} = 0.161$ V). The ratio of ip_a/ip_c is 0.90 V. This also indicates the quasi-reversible redox process of the metal complex. The incremental addition of CT DNA to the complex also causes a positive shift in formal potential ($E_{1/2}$) indicating that $[ZnL(bpy)_2]Cl$ stabilizes the duplex (GC pairs) by intercalation. The electrochemical parameters of the Cu(II), Ni(II), Co(II) and Zn(II) complexes are shown in Table 2. From these data, it is understood that all the synthesized complexes interact with DNA through intercalating way.

There has been considerable interest in DNA cleavage reactions that are activated by transition metal complexes [29,30]. Change in the electrophoretic mobility of plasmid DNA on agarose gel is commonly taken as evidence for direct DNA–metal interactions. The delivery of metal ion to the helix, in locally generating oxygen or hydroxide radicals, yields an efficient DNA cleavage reaction. Fig. 6 illustrates the gel electrophoretic separations showing the cleavage of plasmid pBR322 DNA induced by the complexes under aerobic conditions and in presence of H_2O_2, respectively.

When circular pBR322 DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact supercoiled form (Form I). If scission occurs on one strand, the supercoiled form will relax to generate a slower-moving nicked form (Form II). Control experiments using DNA alone (Lane 1) and H_2O_2 + DNA (Lane 2) do not show any apparent cleavage of SC DNA. All the complexes showed pronounced nuclease activity in the presence of an oxidant H_2O_2 (Lanes 4–7), which may be due to the increased production of hydroxyl radicals. The production of hydroxyl free radical is due to the

Table 2
Electrochemical parameters for the interaction of DNA with Cu(II), Co(II), Ni(II) and Zn(II) complexes.

Complex	Redox couple	$E_{1/2}$ (V)		ΔEp(V)		Ip_a/Ip_c
		Free	Bound	Free	Bound	
$[CuL(bpy)_2]Cl$	Cu(III) → Cu(II)	0.051	0.056	0.452	0.468	1.85
	Cu(II) → Cu(I)	−0.204	−0.217	0.423	0.398	1.41
	Cu(I) → Cu(0)	−0.338	−0.332	0.557	0.554	1.14
$[NiL(bpy)_2]Cl$	Ni(III) → Ni(II)	0.046	0.077	0.456	0.467	1.08
	Ni(II) → Ni(I)	−0.205	−0.191	0.426	0.402	1.36
$[CoL(bpy)_2]Cl$	Co(III) → Co(II)	0.237	0.257	0.239	0.245	1.18
$[ZnL(bpy)_2]Cl$	Zn(II) → Zn(0)	0.161	0.178	0.503	0.524	0.90

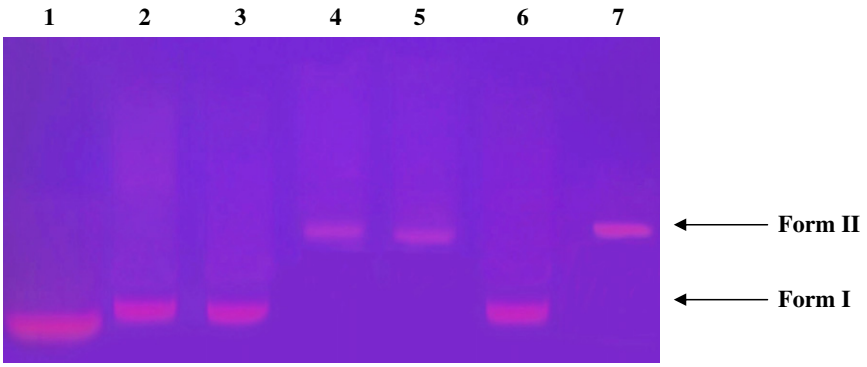

Fig. 6. Changes in the agarose gel electrophoretic pattern of pBR322 DNA induced by H_2O_2 and Cu(II), Ni(II), Co(II) and Zn(II) complexes: Lane 1, DNA alone; Lane 2, DNA + H_2O_2; Lane 3, DNA + [KL] + H_2O_2;Lane 4, DNA + [CuL(bpy)$_2$]Cl complex + H_2O_2; Lane 5, DNA + [NiL(bpy)$_2$]Cl complex + H_2O_2; Lane 6, DNA + [CoL(bpy)$_2$]Cl complex + H_2O_2; and Lane 7, DNA + [ZnL(bpy)$_2$]Cl complex + H_2O_2.

reaction between the metal complex and oxidant. The $OH^{\cdot}$ free radicals participate in the oxidation of the deoxyribose moiety, followed by hydrolytic cleavage of a sugar phosphate back bone.

Currently much attention has been focus to the synthesis of new metal complexes and the evaluation of these agents for antibacterial activity. In the present work, we wish to test the activity of the ligand and its metal(II) complexes against bacteria. Ampicillin is used as a standard drug for comparison. The antibacterial activity of the synthesized azomethines was screened by the agar well diffusion method [31,32] against Gram-negative and Gram-positive bacteria, namely *S. aureus and B. subtilis* (as Gram positive bacteria) and *P. aeruginosa, E. coli* and *S. typhi* (as Gram negative bacteria). The diffusion agar technique was used to evaluate the antibacterial activity of the synthesized mixed-ligand complexes. The biocidal activity data of the investigated compounds are summarized in Table 3.

From this Table, it has been reported that the ligands having oxygen and nitrogen donor atoms upon coordination with metal ion inhibit production of enzyme in the micro-organisms. All the metal complexes have higher inhibitory effect than free ligand. Control experiment using DMF alone does not show any antimicrobial expressions. This higher antimicrobial activity of the metal complexes compared to Schiff bases may be due to the change in structure due to coordination and chelating effect to make metal complexes to act as more powerful antibacterial agents, thus killing the microbe or by inhibiting multiplication of the microbe by blocking their active sites [33]. The chelation reduces the polarity of the ligand due to partial sharing of its negative charge with the metal ion, which increases the lipophilic nature of the complex, favoring transportation of the complexes across the lipid layer of the cell membrane, which enhances antibacterial activities.

In summary, mixed ligand Cu(II), Ni(II), Co(II) and Zn(II) complexes of tryptophan derived Schiff base and 2,2′-bipyridine have been synthesized and characterized. The ligand to metal stoichiometry and the nature of bonding were ascertained on the basis elemental analyses, position of molecular ion peaks in the mass spectra and conductivity data. The interaction of the mixed ligand complexes with CT-DNA has been investigated by using absorption spectra, electrochemical techniques and viscometry. All the experiments reveal that the mixed ligand complexes interact with CT-DNA in the mode of intercalation. The results of agarose gel electrophoresis indicate that the complexes exhibit good cleavage potential of pBR322 DNA in the presence of H_2O_2. Antibacterial activity study shows that the complexes exhibit good biological activity against different pathogenic species. These results should be valuable in understanding the interaction with DNA as well as laying a foundation for the rational design of powerful agents for probing and targeting nucleic acids.

Table 3
Minimum inhibitory concentration values of the synthesized compounds against the growth of five bacteria (mg/mL).

Complex	*S. aureus*	*P. aeruginosa*	*E. coli*	*B. subtilis*	*S. typhi*
[KL]	8	11	6	10	13
[CuL(bpy)$_2$]Cl	18	21	24	25	29
[NiL(bpy)$_2$]Cl	13	16	20	18	23
[CoL(bpy)$_2$]Cl	15	19	17	20	18
[ZnL(bpy)$_2$]Cl	16	13	18	21	20
Ampicillin	12	16	14	16	19
DMF	–	–	–	–	–

Acknowledgments

The authors express their sincere thanks to the College Managing Board, Principal and Head of the Department of Chemistry, VHNSN College, Virudhunagar, India for providing necessary research facilities. NR thanks University Grants Commission (UGC), New Delhi for financial assistance.

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[16] The potassium salt of the Schiff base ligand (KL) was prepared by the following reaction. The potassium salt of tryptophan was prepared by following general procedure. The tryptophan (0.01 mol) dissolved in 1:1 water–ethanol (40 mL) was added to a hot ethanolic solution (30 mL) of KOH and the resulting solution was stirred to obtain a homogeneous solution. Then, to this solution an ethanolic solution of benzaldehyde (0.01 mol) was added drop-wise and the resultant mixture was refluxed for *ca*. 5 h. The pale yellow colored solution was obtained. Then the solution was reduced to one-third on a water bath. The solid complex precipitated was filtered off, washed thoroughly with ethanol and dried in *vacuo.* Yield 75%.

[17] Anal.Calc. for **[$C_{18}H_{14}N_2O_2K$]:** C, 65.8; H, 4.5; N, 8.7; O, 9.9. Found: C, 65.6; H, 4.3; N, 8.5; O, 9.7; FT-IR (KBr disc): 3,252 (NH), 1,667 (HC═N), 1,456 υ_{asy}(COO), 1,375 υ_{sy}(COO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 6.8–7.5 (phenyl multiplet), 8.9 (s, 1H, –CH═N), 2.4 (–CH_2) ppm; ^{13}C NMR ($CDCl_3$): d = 53.90 539 (CH_2), 55.40 (CH), 105.62, 108.15, 111.82, 112.37, 117.95 (aromatic C), 118.74, 121.40, 125.11, 126.33, 128.53, 136.62 (indole C), 167.11 (CH═N), 171.56 (COO–) ppm; λ_{max} in DMSO 42,564 and 36,978 cm^{-1}; MS: *m/z* 330.

[18] The complexes were prepared by mixing the appropriate molar quantity of the above ligand and the metal salts using the following procedure. An ethanolic solution of Schiff base (0.003 mol) was stirred with the ethanolic solution (5 mL) of anhydrous metal(II) chlorides (0.003 mol) for *ca.*1 h. To the above mixture, a methanolic solution (5 mL) of 2,2′-bipyridine (bpy) (0.006 mol) was added in a 1:1:2 molar ratio and the stirring was continued for *ca.*1 h. The solid product obtained was filtered and washed with ethanol. Yield 73–85%. The ligand is soluble in common organic solvents but the complexes are soluble only in $CHCl_3$, DMF and DMSO.

[19] Anal.Calc. for **[$C_{38}H_{30}N_6O_2Cu$]Cl:** C, 68.7; H, 4.8; N, 12.7; O, 4.9; Cu, 9.8%. Found: C, 68.5; H, 4.6; N, 12.6; O, 4.8; Cu, 9.5%; FT-IR (KBr disc): 3,252 (NH), 1,634 (HC═N), 1,444 υ_{asy}(COO), 1,317 υ_{sy}(COO); 513 (M═O), 478 (M═N) cm^{-1}; $\wedge_m \times 10^{-3}(\Omega^{-1}\,mol^{-1}\,cm^2)$ 45.32; μ_{eff} (BM) 1.73; λ_{max} in DMSO 27,243, 35,109 and 13,780 cm^{-1}; MS: *m/z* 663.

[20] Anal.Calc. for **[$C_{38}H_{30}N_6O_2Ni$]Cl:** C, 69.4; H, 4.8; N, 12.9; O, 5.1; Ni, 9.2%. Found: C, 69.0; H, 4.6; N, 12.7; O, 4.9; Ni, 8.9%; FT-IR (KBr disc): 3,252 (NH), 1,615 (HC═N), 1,431 υ_{asy} (COO), 1,315 υ_{sy}(COO); 516 (M═O), 473 (M═N) cm^{-1}; $\wedge_m \times 10^{-3}(\Omega^{-1}\,mol^{-1}\,cm^2)$ 51.20; μ_{eff} (BM) 3.19; λ_{max} in DMSO 14,746, 17,165 and 23,387 cm^{-1}; MS: *m/z* 658.

[21] Anal.Calc. for **[$C_{38}H_{30}N_6O_2Co$]Cl:** C, 69.4; H, 4.9; N, 12.8; O, 5.2; Co, 9.1%. Found: C, 69.1; H, 4.6; N, 12.7; O, 4.9; Co, 8.9%; FT-IR (KBr disc): 3,256 (NH), 1,593 (HC═N), 1,436 υ_{asy} (COO), 1,319 υ_{sy}(COO); 507 (M═O), 481 (M═N) cm^{-1}; $\wedge_m \times 10^{-3}(\Omega^{-1}\,mol^{-1}\,cm^2)$ 55.38; μ_{eff} (BM) 4.85; λ_{max} in DMSO 14,989, 17,511 and 22,649 cm^{-1}; MS: *m/z* 659.

[22] Anal.Calc. for **[$C_{38}H_{30}N_6O_2Zn$]Cl:** C, 68.7; H, 5.0; N, 12.9; O, 5.0; Zn, 9.9%. Found: C, 68.3; H, 4.5; N, 12.6; O, 4.8; Zn, 9.8%; FT-IR (KBr disc): 3,256 (NH), 1,599 (HC═N), 1,444 υ_{asy}(COO); 1,317 υ_{sy}(COO); 503 (M═O), 478 (M═N) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 6.9–7.2 (phenyl multiplet), 7.7–8.2 (phen multiplet), 8.6 (s, 1H, –CH═N) 2.6 (–CH_2) ppm; ^{13}C NMR($CDCl_3$): d = 52.60 (CH), 54.02 (CH2), 105.13, 108.05, 113.69, 112.77, 118.36 (aromatic C), 120.05, 125.27, 127.17, 128.78, 139.34, 147.14 (indole C), 157.25 (CH═N), 165.47(COO–)ppm; $\wedge_m \times 10^{-3}$ $(\Omega^{-1}\,mol^{-1}\,cm^2)$ 57.91; λ_{max} in DMSO 29,489, 33,109 cm^{-1}; MS: *m/z* 665.

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