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Mono and binuclear cobalt(II) mixed ligand complexes containing 1,10-phenanthroline and adenine using 1,3-diaminopropane as a spacer: synthesis, characterization, and antibacterial activity investigations

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Abstract

Background: Coordination compounds, in particular cobalt(II) mixed ligand complexes containing 1,10-phenanthroline, have drawn the attention of many investigators as some of them are showing antimicrobial activities.

Result: Herein, we report three novel mixed ligand complexes of cobalt(II) having the formulae $[\text{Co}(\text{L}_1)_2(\text{H}_2\text{O})_2]\text{Cl}_2$, $[\text{Co}(\text{L}_1)_2(\text{L}_2)(\text{H}_2\text{O})]\text{Cl}_2$ and $[\text{Co}_2(\text{L}_1)_4(\text{L}_2)_2(\text{L}_3)]\text{Cl}_4$ (L_1 = 1,10-phenanthroline, L_2 = adenine, L_3 = 1,3-diaminopropane) were synthesized and characterized by elemental analysis, conductivity measurement, infrared, and UV-Vis spectroscopic techniques. Octahedral geometries are proposed to all the complexes. In vitro antibacterial activities of the ligands, salt, and metal complexes were tested on four pathogenic bacteria (*Staphylococcus aureus*, *Salmonella typhus*, *Escherichia coli*, and *Staphylococcus epidermis*) using disc diffusion method.

Conclusions: It is interesting to note that the newly synthesized cobalt(II) complexes are active against gram negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) even though cobalt(II) complexes are well known for their activity against gram positive bacteria.

Keywords: Binuclear, Cobalt(II) complex, 1,10-Phenanthroline, Adenine, 1,3-Diaminopropane, Antibacterial activity

Background

On coordination with suitable ligands, it is possible to impose a set of desired properties on transition metals for specific applications. It is achieved by altering properties such as the stability of different oxidation states, solvophilicity, electrophilic, and nucleophilic properties of the metal ions. It can be done by tuning through variation of the metal and the choice of one of the vast array

of ligands available for complexation [1–3]. While coordinating, the properties of the ligands themselves are also modified. For instance, the pharmacological activities and their crucial role in DNA/RNA base pairing through several hydrogen-bonding patterns of free purines such as adenine can significantly change after complex formation [4–6]. 1,10-Phenanthroline significantly changes its toxicity when coordinated to metals [7, 8]. Based on this, synthesis of different coordination compounds with one or more metal centers by ligand tailoring has become a fascinating research field. Designing such coordination compounds for therapeutic applications has been part of this activity [9–18].

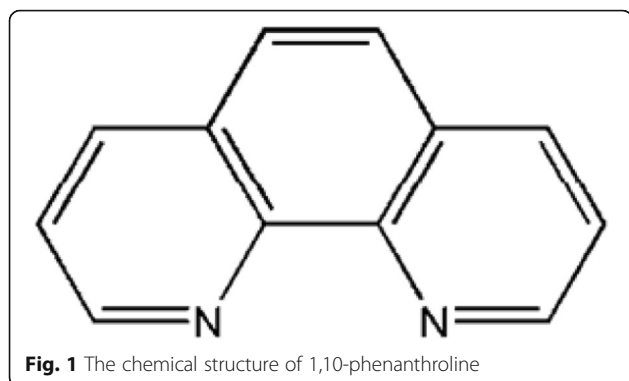
A good number of mononuclear [4, 7, 19, 20] and binuclear [21–25] mixed ligand metal complexes have

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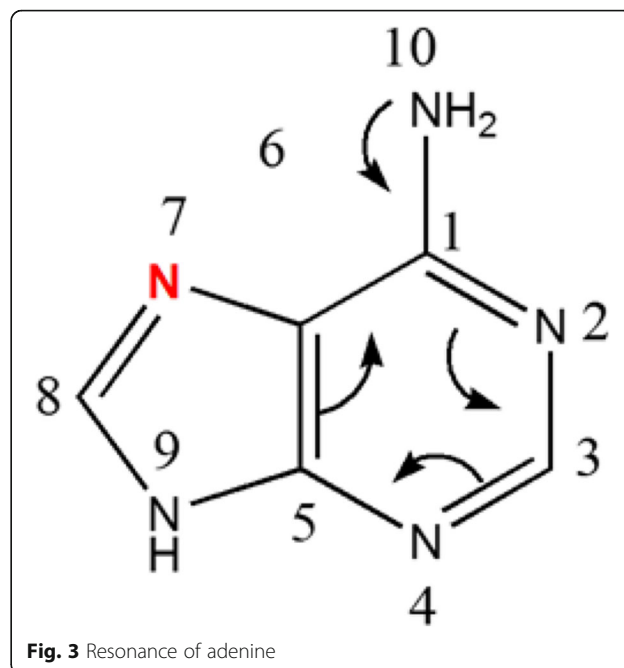
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been reported. In particular, the bioinorganic chemistry of cobalt(II) complexes have drawn the attention of many investigators [26–28]. This is because of the fact that cobalt complexes are showing antibacterial [29] and antifungal [30] activities. However, in most reports, Co(II) complexes are unable to show activities against Gram-negative bacteria [31]. This is probably due to their inability to penetrate the cell wall of these bacteria [32–34], which demands the fine-tuning of Co(II) complexes employing novel ligand functionalities, thereby achieving variable compositions. In this context, two mononuclear and one binuclear Co(II) complexes have been synthesized from 1, 10-phenanthroline, adenine, and 1,3-diaminopropane, and their biological activities were studied and are compared with commercially available antibiotics.

1,10-Phenanthroline (Fig. 1) is rigid planar, chelating bidentate ligand. The stacking interaction of 1,10-phenanthroline with DNA base pairs makes it applicable in medical fields [35, 36].

Adenine (Fig. 2) is a purine nucleotide and can exist in either amino or imino forms. However, the amino tautomer is more stable and therefore predominates under the cellular conditions [37]. Its exocyclic amino group is less likely to coordinate since the lone pair of electrons is largely delocalized into the ring by resonance (Fig. 3). Because it is free from resonance and steric hindrance, the best coordination site for adenine is N₇ of the imidazole ring [38].



Metal complexes of adenine have considerable interest in the design of model complexes involving purines which could mimic interactions of metal ions with DNA [39].

1,3-Diaminopropane is a bidentate ligand (Fig. 4) which is used as a precursor for pharmaceutical, agrochemical, and organic industries [40, 41].

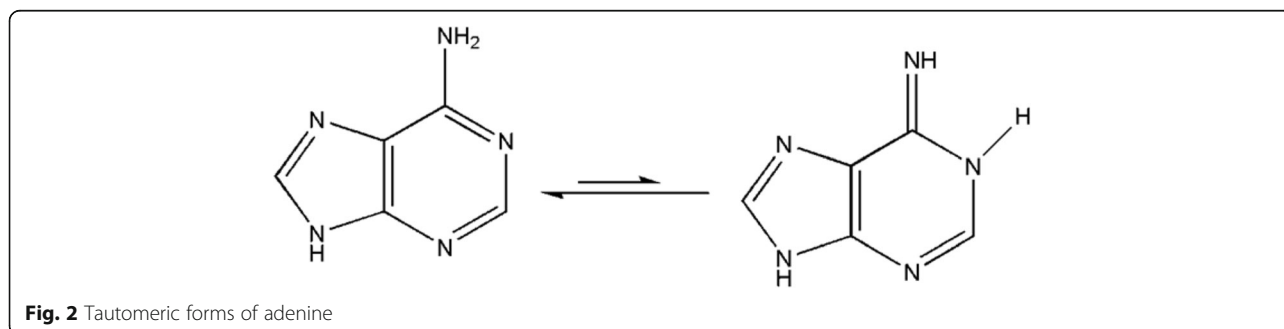
Methods

Materials

The metal salt is CoCl₂·6H₂O (Analar BDH) and the ligands are 1,10-phenanthroline monohydrate (BDH), adenine (99%, ACROS), and 1,3-diaminopropane (ACROS). Muller Hinton Agar and barium chloride (BLULUX Laboratories Ltd, India) were used as such. All other solvents used are of reagent quality.

Physical methods of analysis

All the complexes were analyzed for their metal and halide contents by the conventional methods. The molar conductance was measured using 10⁻³ M solution for





each complex in deionized water with JENWAY 4200 conductivity meter at room temperature. The electronic spectra were recorded in the 50,000–12,500 cm^{-1} region on Sanyo SP65 UV/VIS spectrophotometer. IR spectra were recorded using KBr discs in the 4000–400 cm^{-1} region on AVATAR 330 FT-IR, Thermo Nicolet spectrophotometer. C, H, and N content determination were performed on a Perkin Elmer 2400 elemental analyzer.

Synthesis of complexes

Synthesis of $[\text{Co}(\text{L}_1)_2(\text{H}_2\text{O})_2]\text{Cl}_2$ (1)

A methanolic solution of 1,10-phenanthroline monohydrate (1 g, 5 mmol) was added to a methanolic solution of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (0.6 g, 2.5 mmol) being stirred magnetically in an ice bath. It was further allowed to stir for 1 h at room temperature. It was then refluxed for one more hour and allowed to cool to room temperature. On evaporating the resultant solution, a yellow powder, which was obtained and washed with acetone several times to remove any unreacted 1,10-phenanthroline. It was then re-crystallized from methanol (yield, 1.14 g; 85.84%).

Synthesis of $[\text{Co}(\text{L}_1)_2(\text{L}_2)(\text{H}_2\text{O})]\text{Cl}_2$ (2)

To an aqueous solution of 1 (0.3 g, 0.57 mmol) being stirred magnetically in oil bath at 60 °C, an aqueous solution of adenine (0.077 g, 0.57 mmol) was added. A deep reddish brown homogeneous solution was obtained. The solvent was removed under vacuum. Yellowish orange powder was collected and washed several times with acetonitrile (yield, 0.23 g; 62.9%).

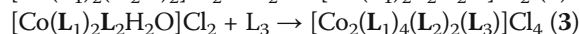
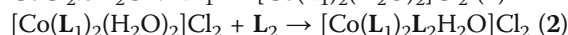
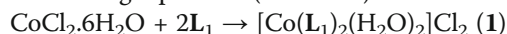
Synthesis of $[\text{Co}_2(\text{L}_1)_4(\text{L}_2)_2(\text{L}_3)]\text{Cl}_4$ (3)

To an aqueous solution of 2 (0.320 g, 0.49 mmol) being stirred magnetically in water bath at 10 °C, an aqueous solution of slightly excess 1,3-diaminopropane (0.022 g, 25 μL) was added. A deep reddish brown homogeneous solution was obtained. The stirring was continued for one more hour. The solvent was removed from the resulting solution under vacuum. A red-brown powder was obtained and was washed with acetone several times (yield, 0.266 g; 73%).

Results

Synthesis and characterization studies

The formation of the complexes can be represented by the following equations (scheme 1):



where L_1 = 1,10-phenanthroline, L_2 = Adenine, and L_3 = 1,3-diaminopropane.

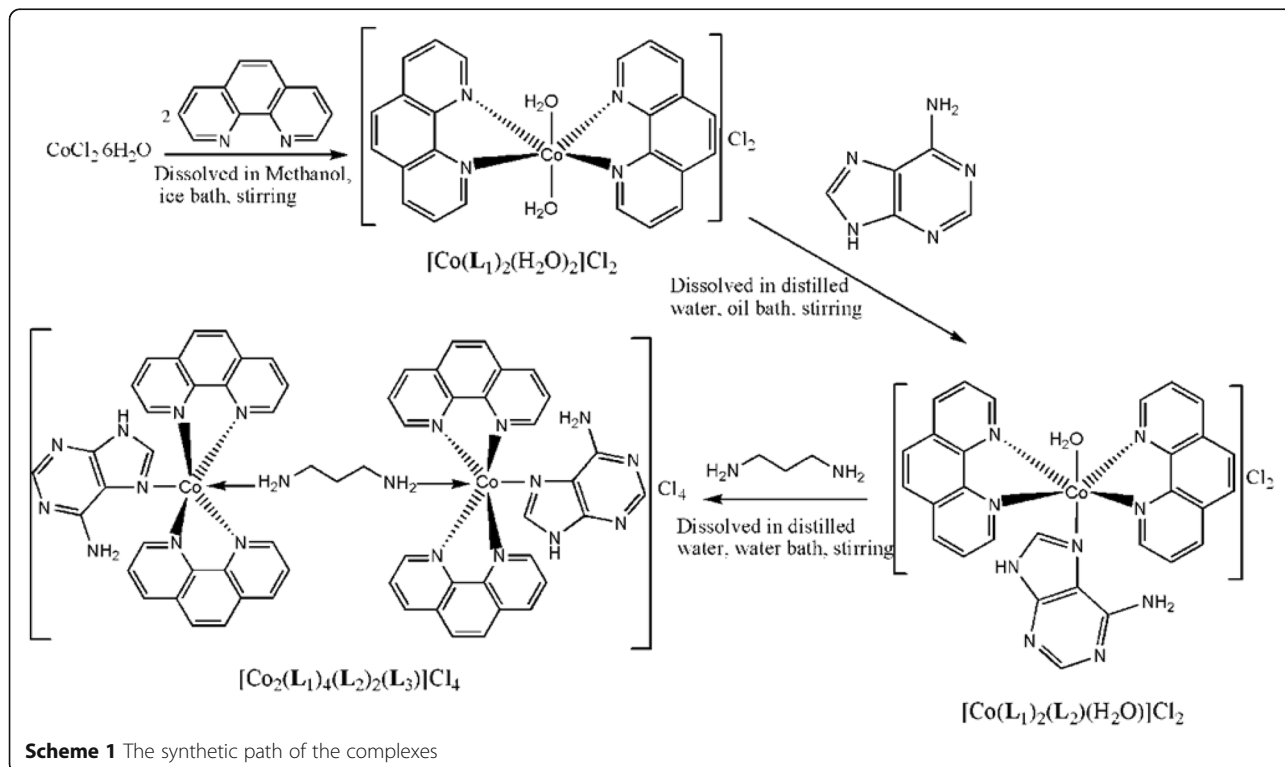


Table 1 Analytical data of the complexes

Complexes	Molar mass (g / mol)	Melting point/°C	Color/appearance	Yield (%)	Elemental analysis, calculated (found) (%)					Λ_M (molar conductivity), $S\ cm^2\ mol^{-1}$
					Co	Cl	C	H	N	
$[Co(L_1)_2(H_2O)_2]Cl_2$	526.29	360	Yellow powder	85.84	11.22, (11.0)	13.50, (13.20)	54.77, (54.71)	3.83, (3.76)	10.65, (10.10)	138.6
$[Co(L_1)_2(L_2)(H_2O)]Cl_2$	643.90	256	Yellow-orange powder	62.90	9.16, (8.94)	11.01, (10.80)	54.01, (53.88)	3.60, (3.33)	19.58, (18.93)	129.4
$[Co_2(L_1)_4(L_2)_2(L_3)]Cl_4$	1,324.83	245	Brownish-red powder	73.00	8.90, (8.84)	10.70, (9.99)	55.30, (54.93)	3.95, (3.66)	21.15, (20.69)	154.2

All the synthesized complexes are soluble in most of the polar solvents including water while insoluble in most of the non-polar solvents and exhibit high melting point.

From elemental analysis and the conductance measurements, the complexes can be formulated as $[Co(L_1)_2(H_2O)_2]Cl_2$ (**1**), $[Co(L_1)_2(L_2)(H_2O)]Cl_2$ (**2**), and $[Co_2(L_1)_4(L_2)_2(L_3)]Cl_4$ (**3**) where L_1 = 1,10-phenanthroline, L_2 = thymine, and L_3 = 1,3-diaminopropane (Table 1).

The important IR spectral band of the ligands and the corresponding complexes are presented in Table 2.

The electronic spectra of the ligands and the corresponding complexes **1**, **2**, and **3** with tentative assignments are presented in Table 3.

It has been observed that complex **3** is having better biological activity than the corresponding precursor complexes **1** and **2** against all the bacterial strains in the present study (Table 4).

The activity indexes of the present study with respect to the commercial antibiotic demonstrated that the complexes are less active. However, it is evident that the MIC of the binuclear complex **3** is better than complexes **1** and **2** (Table 5).

Spectroscopic results

Infrared spectra

The observed shifts in band positions are a clear indication that the ligands are coordinated to the Co(II). The bands at $1620\ cm^{-1}(s)$ and $1585\ cm^{-1}(s)$, characteristic for $\nu_{C=C}$ and $\nu_{C=N}$, stretching vibration respectively in

1,10-phenanthroline monohydrate are shifted to $1628\ cm^{-1}(w)$ and $1522\ cm^{-1}(w)$, respectively, in $[Co(L_1)_2(-H_2O)_2]Cl_2$. The shift in frequency may indicate the change in the bond order in $C=C$ and $C=N$ following the coordination. However, the decrease in their intensity indicates the formation of rigid and symmetric structure. The coordination of adenine to Co(II) in $[Co(L_1)_2(H_2O)_2]Cl_2$ is evident that all its characteristic vibration frequencies appear in $[Co(L_1)_2(L_2)(H_2O)]Cl_2$. The relatively higher electronic concentration in N_7 in the imidazole ring is the most preferable site of coordination [38]. The characteristic vibration frequencies of adenine appeared dominantly. This is probably due to the relative vibration freedom of adenine in the complex compared to the rigid and symmetric configuration of the rest of the complex. The strong and broad band ranging from 3403 to $3043\ cm^{-1}$ is due to the additive combination of the characteristic vibrations of primary amine from adenine and 1,3-diaminopropane which is a strong confirmation of the coordination of 1,3-diaminopropane to $[Co(L_1)_2(-L_2)(H_2O)]Cl_2$. Moreover, the increase in strength and broadness of the band in the frequency range 3403 – $3043\ cm^{-1}$ as well as the appearance of new bands at the vibration frequency $539\ cm^{-1}$ is due to Co–N bond confirms the coordination of 1,3-diaminopropane in the formation of the binuclear complex.

Electronic spectra

Inter-ligand and simple characteristic d-d transitions are exhibited by the complexes. Significant difference in the

Table 2 Characteristic IR absorption (cm^{-1}) of ligands L_1 , L_2 , and L_3 and complexes **1**, **2**, and **3**

Compound	$\nu_{(C=C)}$	$\nu_{(C=N)}$	$\nu_{(C-H)}$	$\nu_{(N-H)}$	$\nu_{(O-H)}$	$\nu_{(C-N)}$
L_1	1581	1610	3043	-	3422	-
L_2	1582	1605	2689	3290	-	1230
L_3	-	-	2800	3350, 2950	-	1100
$[Co(L_1)_2(H_2O)_2]Cl_2$	1587	1626	3048	-	3425	-
$[Co(L_1)_2(L_2)(H_2O)]Cl_2$	1607	1664	3118	Overlapped with OH	3442–3139	1383
$[Co_2(L_1)_4(L_2)_2(L_3)]Cl_4$	1587	1632	3189	3450–3062	-	1387

Table 3 Electronic spectral data of the salt and complexes

Compounds	Absorption bands (cm ⁻¹)	Tentative assignments
L₁	43,103; 37,878	$\pi-\pi^*$; $n \rightarrow \pi^*$
L₂	-	-
L₃	34,602	$n \rightarrow \pi^*$
[Co(L₁) ₂ (H ₂ O) ₂]Cl ₂	44,052; 37,313; 22,624; 15,748; 13,440	$\pi \rightarrow \pi^*$; $n \rightarrow \pi^*$; ${}^4T_{1g(F)} \rightarrow {}^4T_{1g(P)}$; ${}^4T_{1g(F)} \rightarrow {}^4A_{2g(F)}$; $4T_{1g(F)} \rightarrow {}^4T_{2g(F)}$
[Co(L₁) ₂ (L₂)(H ₂ O)]Cl ₂	43,103; 37,174; 22,371; 15,948; 13,280	$\pi \rightarrow \pi^*$; $n \rightarrow \pi^*$; ${}^4T_{1g(F)} \rightarrow {}^4T_{1g(P)}$; ${}^4T_{1g(F)} \rightarrow {}^4A_{2g(F)}$; ${}^4T_{1g(F)} \rightarrow {}^4T_{2g(F)}$
[Co ₂ (L₁) ₄ (L₂) ₂ (L₃)] Cl ₄	44,052; 36,764; 21,929; 18,518; 13,642	$\pi \rightarrow \pi^*$; $n \rightarrow \pi^*$; ${}^4T_{1g(F)} \rightarrow {}^4T_{1g(P)}$; ${}^4T_{1g(F)} \rightarrow {}^4A_{2g(F)}$; ${}^4T_{1g(F)} \rightarrow {}^4T_{2g(F)}$

band number and position of transition absorption between CoCl₂·6H₂O, the ligands, and the complexes is observed. This may be as a consequence of different environments around the metal ion following the coordination [42–44]. The coordination of the ligands to the metal is demonstrated by the shifts in the maximum absorption wave lengths corresponding to ligand centered $\pi \rightarrow \pi^*(C=C)$, $\pi \rightarrow \pi^*(C=N)$, $n \rightarrow \pi^*(C=N)$ as well as metal centered energy states. The absorption band at 37,878 cm⁻¹ of 1,10-phenanthroline due to $n \rightarrow \pi^*(C=N)$ is shifted to 37,313; 37,174; and 36,764 cm⁻¹ in the complexes **1**, **2**, and **3**, respectively [45]. Furthermore, the single band due to the simple d-d transition in the starting salt CoCl₂·6H₂O observed at 23,419 cm⁻¹ is changed to triple absorption bands between 44,062 and 13,642 cm⁻¹ is assigned to ${}^4T_{1g(F)} \rightarrow {}^4T_{1g(P)}$, ${}^4T_{1g(F)} \rightarrow {}^4A_{2g(F)}$, and ${}^4T_{1g(F)} \rightarrow {}^4T_{2g(F)}$ transitions, characteristics of octahedral geometry around Co(II) ion. The equatorial orbitals ($dx^2 - y^2$) of Co(II) are coordinated to the strong field bidentate 1,10-phenanthroline which results strong and shorter bonds compared to those orbitals in the axial position (dz^2) due to Jahn-Teller effect. Consequently, distorted octahedral geometry is achieved. The coordinated 1,10-phenanthroline occupied the base plane with water and adenine occupying the apical position. Based on the above studies, a distorted octahedral geometry is assigned for all the complexes as follows (Fig. 5).

Antibacterial activity

Antibacterial activity is occurring because of the diffusion of the complexes through the lipid membrane of the cells and interacting with the normal activity of the cell. This can be varied by designing the complexes with varying lipophilic properties. The interaction of the complexes with targeted cell could be achieved by tuning the complex in such a way that its components have properties either that bind covalently (irreversible) or interact non-covalently (reversible) or by carrier agents that deliver active ligands in vivo to the biological target [46]. It has been observed that complex **3** showed better biological activities than the precursor complexes **1** and **2** against all the tested strains (Table 4). Based on the proposed structure, it is expected that complex **3** is having configurationally free twisting movement through the bridge, which presumably enhanced its flexibility and in turn enhances the penetration power of the complex into the cytoplasm of the pathogenic cells [47].

Minimum inhibitory concentration (MIC) determination

Minimum inhibitory concentration (MIC) is the lowest concentration that completely inhibited the growth of microorganisms for 24 h. Around 100 µg/mL of complex **3** is sufficient to inhibit the growth of all the tested strains (Fig. 6).

The percentage activity indexes against the reference antibiotic demonstrated that the complexes are less

Table 4 Antibacterial activity of CoCl₂·6H₂O, ligands, metal complexes, and reference antibiotic

Compound	Antimicrobial activity (mean IZ diameter (mm) ± SD)			
	<i>Escherichia coli</i>	<i>Salmonella typhus</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermis</i>
CoCl ₂ ·6H ₂ O	10.50 ± 0.00	11.33 ± 0.58	12.08 ± 1.01	13.92 ± 0.14
L₁	27.33 ± 0.57	35.33 ± 0.28	34.83 ± 0.29	27.28 ± 0.29
L₂	0	0	0	0
L₃	40.00 ± 0.00	50.17 ± 0.29	54.50 ± 0.50	43.92 ± 0.14
[Co ₂ (L₁) ₄ (L₂) ₂ (L₃)]Cl ₄	23.76 ± 0.57	23.92 ± 0.14	23.25 ± 0.66	22.50 ± 1.32
[Co(L₁) ₂ (L₂)(H ₂ O)]Cl ₂	21.33 ± 0.57	20.50 ± 0.00	19.53 ± 0.50	20.17 ± 0.29
[Co(L₁) ₂ (H ₂ O) ₂]Cl ₂	20.67 ± 0.57	19.50 ± 0.50	18.67 ± 0.58	19.25 ± 0.43
Methanol	42.75 ± 0.25	42.67 ± 0.58	44.83 ± 0.29	40.83 ± 0.29
Water	0	0	0	0
Ciprofloxacin	35.25 ± 0.25	40.50 ± 0.50	29.92 ± 0.38	36.08 ± 1.01

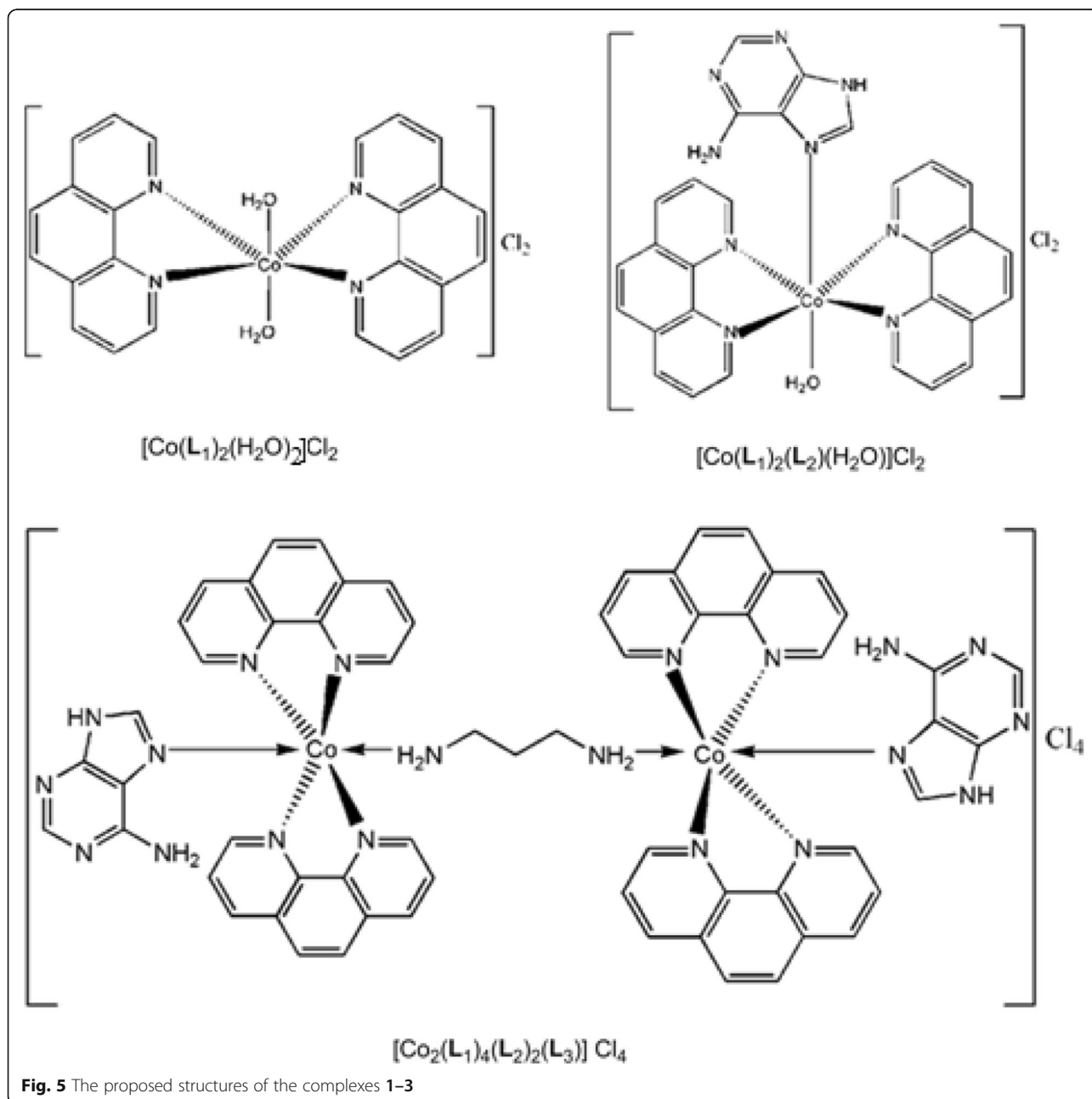
Table 5 The percentage activity index data of the complexes against the tested bacteria compared ciprofloxacin

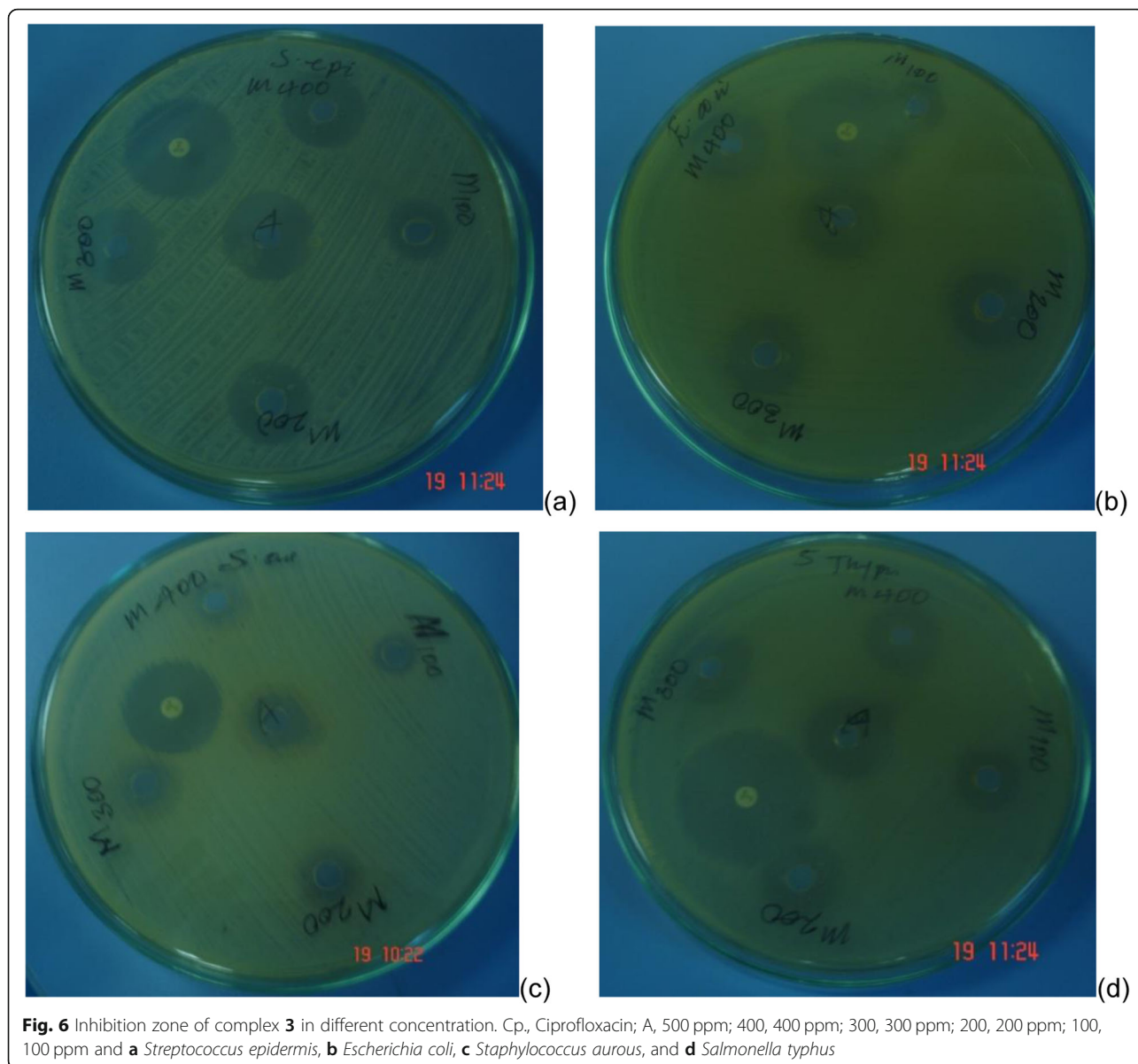
Compound	Microorganism			
	<i>Escherichia coli</i>	<i>Salmonella typhus</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermis</i>
$[\text{Co}_2(\text{L}_1)_4(\text{L}_2)_2(\text{L}_3)]\text{Cl}_4$	- 0.32	- 0.40	- 0.22	- 0.38
$[\text{Co}(\text{L}_1)_2(\text{L}_2)(\text{H}_2\text{O})]\text{Cl}_2$	- 0.39	- 0.49	- 0.35	- 0.44
$[\text{Co}(\text{L}_1)_2(\text{H}_2\text{O})_2]\text{Cl}_2$	- 0.41	- 0.59	- 0.38	- 0.47

active. However, it is evident that the MIC of the binuclear complex **3** is far better than complexes **1** and **2** (Table 5). This is probably because of bisintercalation due to the two planar intercalating 1,10-phenanthroline ring systems covalently linked 1,3-diaminepropane.

Discussion

All the synthesized complexes are soluble in most polar solvents like acetonitrile, DMSO, and water and insoluble in almost all non-polar solvents. From the elemental analysis and conductivity measurement data, it can

**Fig. 5** The proposed structures of the complexes 1–3



be concluded that the complexes can be formulated as $\text{Co}(\text{L}_1)_2(\text{H}_2\text{O})_2\text{Cl}_2$ (**1**), $\text{Co}(\text{L}_1)_2(\text{L}_2)(\text{H}_2\text{O})\text{Cl}_2$ (**2**), and $\text{Co}_2(\text{L}_1)_4(\text{L}_2)_2\text{L}_3\text{Cl}_4$ (**3**). The molar conductance data of the complexes **1**, **2**, and **3** was measured using 10^{-3} M solution for each complex in water and indicates a 1:2 conducting nature for **1** and **2** while a 1:4 conducting nature for **3**. So, the complexes can be formulated as $[\text{Co}(\text{L}_1)_2(\text{H}_2\text{O})_2]\text{Cl}_2$ (**1**), $[\text{Co}(\text{L}_1)_2(\text{L}_2)(\text{H}_2\text{O})]\text{Cl}_2$ (**2**), and $[\text{Co}_2(\text{L}_1)_4(\text{L}_2)_2\text{L}_3]\text{Cl}_4$ [48, 49]. The results obtained from infrared and electronic spectra are in conformity with the above derived formulae.

Based on the analytical and spectroscopic results presented, a distorted octahedral geometry is assigned for all the complexes. The antibacterial activity studies revealed that all the synthesized complexes exhibited

antibacterial activity against even the most drug resistant *Klebsiella pneumoniae* which makes them as potential wide range antibacterial drugs, after in vivo cytotoxicity investigations. The major limitations of these synthesized complexes are that they show minimum inhibition zone than the reference antibiotic ciprofloxacin. However, their antibacterial activity can be improved by tuning the ligand functionality.

Conclusion

Novel mixed ligand Co(II) complexes have been prepared and characterized by various physical, chemical, and spectroscopic techniques. An octahedral geometry has been suggested for all synthesized complexes. Presumably, adenine is coordinated to Co(II) through its

ring nitrogen (N₇). Consequently, the complexes are expected to interact at least by hydrogen bonding with thymine residue of the bacteria genetic material. Furthermore, the synthesis resulted in the alteration of the properties of the starting materials. One advantage of this alteration is to enable the complexes to penetrate the cell wall of the pathogenic cells and consequently, interfere in their normal system to inhibit their growth. This was confirmed from the in vitro antibacterial test results. The result revealed that all the synthesized complexes exhibited antibacterial activity against even the most drug resistant *Klebsiella pneumoniae* which makes them, potentially wide range antibacterial drugs, after in vivo cytotoxicity investigations.

Even though the ligands and the synthesized complexes show antibacterial activity, they are not recommended as antibiotics as compared to the reference ciprofloxacin as the antibacterial activity is less for the complexes than the control. So, more work is needed to be done with respect to the variation of the ligands by causing conformational change to the complexes to achieve a good antibiotic drug by the complexes for therapeutic applications.

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Authors' contributions

The authors YB, MB, and TG participated in conceptualizing, executing, analyzing, and writing the original draft except the authors AA, MT, and WL who were responsible for supervision, editing, and reviewing of this article. Also all authors have read and approved the manuscript.

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Ethics approval and consent to participate

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Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing of interest.

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