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Synthesis, characterization and antibacterial activity of cobalt(III) complexes with pyridine-amide ligands

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Abstract

The ligands 2-(N-(X-pyridyl)carbamoyl)pyridine (X = 2, 3 or 4 for HL¹-HL³, respectively) and 2,6-bis(N-(Y-pyridyl)carbamoyl)pyridine $(Y = 2, 3 \text{ or } 4 \text{ for } H_2L^4 - H_2L^6, \text{ respectively})$ in their mono- and di-deprotonated forms have been used to synthesize kinetically stable cobalt(III) compounds $[Co(L^{1-3})_3]$ (1–3) and Na $[Co(L^{4-6})_2]$ (4–6), respectively. The Co(III) ion is in octahedral environment and is surrounded by three bidentate ligands in complexes 1-3 and two tridentate ligands in complexes 4-6. Ligands coordinate the cobalt center via amidic-N and pyridine-N centers forming a 5-membered chelate ring. Complexes 1-6 have thoroughly been characterized by the various spectroscopic analyses (¹H NMR, ¹³C NMR, UV-vis, IR, mass), elemental analysis, and conductivity measurement. All complexes have been assayed for in vitro antimicrobial activity against clinically isolated resistant strains of Pseudomonas, Proteus, Escherichia coli and standard strains of Pseudomonas aeruginosa (MTCC 1688), Shigella flexneri (MTCC 1457), Klebsiella planticola (MTCC 2272). All cobalt compounds show mild to moderate activity. However, complexes $[Co(L^1)_3]$ (1) and Na $[Co(L^4)_3]$ (4) were found to have potent activity against standard and pathogenic resistant bacteria used in the study. Their MIC ranged from 2.7 to 187 µg/ml. In vitro toxicity tests demonstrated that all complexes were less cytotoxic than that of gentamycin on HEK cell lines and the results reveal that these complexes can act as potent antimicrobial agents. © 2007 Elsevier Masson SAS. All rights reserved.

Keywords: Cobalt complexes; Pyridine-amide ligands; Antibacterial activity; Cytotoxic activity; MTT assay

1. Introduction

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Emergence of resistance in bacterial strains has become as one of the prime concerns of the 21st century. There are serious concerns that untreatable pathogens may develop at an alarming rate in the near future. Strategies to address this challenge include the design of improved versions of antibacterial classes already in clinical use and the use of drug combinations. The application of these strategies can be quite successful, but a high risk of rapid resistance development remains [1-3]. Thus, an urgent need for new potent classes of antibiotics with novel modes of action persists. Resistance in bacteria can result from the modification of an antibacterial target or from functional bypassing of that target, or it can be contingent on impermeability, efflux or enzymatic inactivation of the drug [4]. In light of these problems, the search for new antibacterial agents has gained immense popularity in the field of drug development.

In this race of synthesizing new drugs, cobalt complexes have attracted a great deal of attention amongst the scientific community due to their therapeutic uses as tumor imaging agent [5], antitumor [6], transport protein transferrin (Tf) [5,6], antimycobacterial [7], antiischaemic [8], antiviral [9,10], antiparasitic [11], antithrombolytic [11], enzymatic therapeutics [11], anti-inflammatory activities [12] and as metabolic modifier [13]. Herein we describe the synthesis and characterization of the novel Co(III) complexes as the potential candidates for antimicrobial activity against standard as well as clinically isolated resistant bacterial strains with diminished cytotoxicity on the HEK cell line.

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Scheme 1. Synthesis of complexes 1-3.

We have used few pyridine—amide-based bidentate and tridentate ligands in their deprotonated forms to synthesize their cobalt(III) complexes (Schemes 1 and 2). The bidentate ligands, HL^1-HL^3 , form the $[Co(L^{1-3})_3]$ complexes whereas the tridentate ligands, $H_2L^4-H_2L^6$, generate the Na $[Co(L^{4-6})_2]$ complexes. The ligands coordinate *via* N_{amide} and N_{pyridine} donors to the Co(III) ion in an octahedral arrangement. This coordination mode of the ligands, however, leaves one and/or two pyridine rings per ligand un-coordinated that we believe have the ability to interact with additional metal ions [14]. In addition, the central core (cobalt ion) is surrounded by the hydrophobic ligands, which form an outer lipophilic sheath. According to our design, such an arrangement may facilitate the diffusion through bio-membranes, thus enhancing the antibacterial effectiveness of the complexes. It is to be noted that similar pyridine—amide-based ligands have also been used for synthesizing interesting coordination complexes with novel structural features [15–18].

2. Results and discussion

2.1. Ligand design and synthesis

The bidentate ligands, HL¹-HL³, and tridentate ligands, $H_2L^4-H_2L^6$, have been chosen due to their simple and high yield method of preparation and their ability to coordinate metal ions [19]. The ligands HL^1-HL^3 and $H_2L^4-H_2L^6$ in their mono- or di-deprotonated form are expected to coordinate transition metal ion *via* anionic amide-N and pyridine-N forming a 5-membered chelate ring. This results in the formation of tris-ligated metal complex with $[L^{1-3}]^{-1}$ where it functions as a bidentate ligand, while a bis-ligated complex would result from the tridentate analog, $[L^{4-6}]^{2-}$. The interesting part is that one pyridine group per ligand of HL¹-HL³ and two pyridine groups per ligand of H₂L⁴-H₂L⁶ remain uncoordinated due to the geometrical reasons. This arrangement may orient such un-coordinated pyridine rings to interact with the cell membrane and/or crucial cations required to maintain the function of the bacterium cell [14].



Scheme 2. Synthesis of complexes 4-6.

The bidentate ligands, HL^1-HL^3 , and tridentate ligands, $H_2L^4-H_2L^6$, have been synthesized by the coupling of 2-pyridinecarboxylic acid (for HL^1-HL^3) or 2, 6-pyridinedicarboxylic acid (for $H_2L^4-H_2L^6$) and respective amine (2-aminopyridine, 3-aminopyridine or 4-aminopyridine) in the presence of P(OPh)₃ as the coupling reagent. All ligands give satisfactory microanalytical results and show spectroscopic features as reported in the literature [19].

2.2. Synthesis and characterization of cobalt(III) complexes

Complexes 1-6 were synthesized by the reaction of the respective deprotonated ligand, $[L^n]^{-/2-}$ (n = 1-6) with $Co(ClO_4)_2 \cdot 6H_2O$ salt in DMF. The *in situ* generated Co(II) complex was then oxidized to the final Co(III) complex using molecular oxygen (Schemes 1 and 2). The red to brown complexes, 1-3, and yellow-green to deep green complexes, 4-6, were isolated in 60-70% recrystallized yield. Complexes 1-3are non-electrolyte whereas 4-6 behaved as 1:1 electrolytes in organic solvents [20]. The FTIR spectra of complexes 1-6 do not show the N-H stretch and thus clearly indicate the deprotonated nature of the amide group. Moreover, a red shift was observed for the C=O_{amide} stretch of the Co-complexes compared to the free ligand [21]. These features strongly suggest the involvement of the anionic N_{amide} in the coordination. For complexes 1-3, C=O_{amide} stretches were observed at three distinct positions indicating the asymmetry of the three ligands coordinated to the Co(III) ion. For example, three C=O_{amide} stretching frequencies were observed at 1625, 1600, and 1580 cm^{-1} for complex **1**. This asymmetry is most likely the result of the meridional arrangement of the three ligands around the cobalt center. It is well known from the literature that the tris-chelate-Co³⁺-complexes containing bidentate ligands can adopt both facial (fac) and meridional (mer) geometries. Moreover, both these geometrical isomers will have Δ - and Λ - optical isomers [22,23]. In the mass spectra, the molecular ion peak was clearly observed for the $\{[Co(L^{1-3})_3] + H^+\}$ and $\{Na[Co(L^{4-6})_2] + H^+\}$ species for complexes 1-3 and 4-6, respectively, thus asserting the integrity of the complexes in solution. All cobalt compounds also gave satisfactory microanalytical results and authenticated the purity of the bulk samples.

2.3. NMR studies

The diamagnetic nature of all Co³⁺-complexes has helped us in characterizing them by their ¹H (Tables 1 and 2; Figs. S1 and S2) and ¹³C NMR spectra (Tables S1 and S2; Figs. S3 and S4). The NMR spectra of the tris-ligand complexes, 1-3, are quite interesting and deserve special mention. The *mer* arrangement of the three deprotonated bidentate ligands around the cobalt(III) center has caused the asymmetry of all three coordinated ligands. The effect of this asymmetry is clearly visible in the form of 3-fold signals in the proton as well as in the carbon NMR spectra of complexes, 1-3. For example, the deprotonated ligand, $[L^1]^-$, $[L^2]^-$, or $[L^3]^-$, is expected to give eight proton signals due to two chemically different pyridine rings. Cobalt complexes, 1-3, however, show 24 signals due to three asymmetric ligands having eight chemically different protons. Similarly, the ¹³C NMR spectrum of the complex **1** clearly shows 33 signals for three asymmetric ligands each having 11 unique carbon centers (Fig. S3). For example, three different signals were observed at 168.88, 168.99 and 169.92 ppm for the C=O quaternary carbon centers in the case of complex 1. Similarly, the pyridine quaternary carbons are seen at six different positions in the ¹³C NMR spectrum. The 2D $\{^{1}H-^{13}C\}$ correlation spectrum was used for the correct assignment of the various signals for complex 1 (Fig. S5). For instance, H_2' and H_6 pyridine protons (observed as doublets at 7.32, 7.38, 7.38, 8.16, 9.13 and 9.47 ppm) were found to be correlated with the six carbon signals (C_2 ' and C_6) found between 147 and 152 ppm for complex 1.

The ¹H and ¹³C NMR spectra of the complexes **4**–**6** are quite simple as anticipated for symmetrical bis-ligated cobalt(III) complex. All individual protons are well separated and observable in the spectra compared to the free ligand. Moreover, expected coupling between the protons is also clearly visible. For example, the proton H₁₀ is clearly seen as a *triplet* at ~8.0 ppm, while the protons H₉ and H₉' appear as *doublet* at ~7.0 ppm, for complex **4**. The *N*-pyridine protons were observed between 6.70 and 7.65 ppm. In line with the proton NMR spectrum, ¹³C NMR spectra of complexes

Table 1 ¹H NMR data for complexes $1-3^{a}$

Proton	$[Co(L^1)]$ (1)	$[Co(L^2)_3]$ (2)	[Co(L ³) ₃] (3) 8.30, 8.27, 8.05 (d, 3H)				
2	_	8.32, 8.19, 7.98 (s, 3H)					
2'	9.47 (<i>J</i> = 5.2), 9.13 (<i>J</i> = 5.2), 8.16 (d, 3H)	9.00 $(J = 4.6)$, 8.77 $(J = 5.0)$, 8.36 (d, 3H)	8.97 $(J = 5.3)$, 8.79 $(J = 5.4)$, 8.34 (d, 3H)				
3	7.12 $(J = 5.2)$, 5.78 $(J = 7.8)$, 5.60 (d, 3H, $J = 7.9$)	_	6.94 (J = 5.2), 6.53, 6.21 (d, 3H, J = 5.2)				
3'	7.30, 7.29, 7.29 (m, 3H)	7.57, 7.54, 7.34 (m, 3H)	7.60, 7.53, 7.50 (m, 3H)				
4	7.26, 7.26, 7.00 (m, 3H)	7.29, 7.23, 6.98 (d, 3H)	_				
4'	8.12, 7.71, 7.69 (m, 3H)	7.91, 7.60, 7.58 (m, 3H)	7.95, 7.92, 7.91 (m, 3H)				
5	6.97, 6.67, 6.56 (m, 3H)	6.80, 6.77, 6.49 (m, 3H)	6.94 (J = 5.2), 6.53, 6.21 (d, 3H, J = 5.2)				
5'	7.91, 7.74, 7.54 (d, 3H)	8.05, 8.02 (<i>J</i> = 7.6), 7.94 (d, 3H, <i>J</i> = 7.6)	7.90, 7.75, 7.68 (d, 3H)				
6	7.38 $(J = 5.9)$, 7.38 $(J = 5.9)$, 7.32 (d, 3H)	7.92, 7.77, 7.74 (d, 3H)	8.30, 8.27, 8.05 (d, 3H)				
6'		=	_				

⁴ Spectra recorded in DMSO-*d*₆. *J* value is in hertz.

Table 2 ¹H NMR data for complexes **4**–**6**^a

Proton	$Na[Co(L^4)_2]$ (4)	$Na[Co(L^5)_2]$ (5)	$Na[Co(L^6)_2]$ (6)
2	7.65 (d, 4H, $J = 3.8$)	7.84 (d, 4H, $J = 1.6$)	8.10 (d, 4H, J = 5.5)
2'	7.65 (d, 4H, $J = 3.8$)	7.84 (d, 4H, $J = 1.6$)	8.10 (d, 4H, $J = 5.5$)
3	6.70 (m, 4H)	6.77 (m, 4H)	6.72 (d, 4H, $J = 5.6$)
3'	6.70 (m, 4H)	6.77 (m, 4H)	6.72 (d, 4H, $J = 5.6$)
4	7.28 (m, 4H)	6.72 (m, 4H)	—
4′	7.28 (m, 4H)	6.72 (m, 4H)	_
5	7.55 (d, 4H, <i>J</i> = 7.7)	_	6.72 (d, 4H, $J = 5.6$)
5′	7.55 (d, 4H, $J = 7.7$)	_	6.72 (d, 4H, $J = 5.6$)
6	_	7.63 (d, 4H, $J = 1.7$)	8.08 (d, 4H)
6′	—	7.63 (d, 4H, <i>J</i> = 1.7)	8.08 (d, 4H)
9	7.02 (d, 4H, $J = 7.9$)	7.44 (d, 4H, $J = 7.7$)	7.82 (d, 4H, $J = 7.7$)
9′	7.02 (d, 4H, $J = 7.9$)	7.44 (d, 4H, $J = 7.7$)	7.82 (d, 4H, $J = 7.7$)
10	8.00 (t, 2H)	7.80 (t, 4H)	8.05 (t, 2H)

^a Spectra recorded in DMSO- d_6 . J value is in hertz.

4–6 were also simple and all carbon centers were located easily (Fig. S4).

2.4. Absorption spectra

Absorption spectroscopy of the Co(III) complexes of the present ligands is the easiest way to characterize them. Complexes **1–3** are red-brown in colour and display the λ_{max} in the range of 510–540 nm (Fig. S6). Complexes **4–6** are deep green to yellow-green in colour and show the λ_{max} at ~650 nm (Fig. S7). The difference in the colour as well as in the position of the λ_{max} for the Co(III) complexes of bidentate ligands (HL¹–HL³) versus the tridentate ligands (H2⁴–H₂L⁶) is clearly due to the ligand field effect. The high energy features observed between 350 and 500 nm and those below 350 nm could tentatively be assigned as metal to ligand charge-transfer and intra-ligand transitions, respectively.

2.5. Antibacterial studies

In the current study, the synthesized and well characterized Co(III) complexes have been tested against pathogenic clinically isolated resistant strains of *Pseudomonas*, *Proteus*, *Escherichia coli* and standard strains of *Pseudomonas aeruginosa* (MTCC 1688), *Shigella flexneri* (MTCC 1457), *E. coli* (BL21), and *Klebsiella planticola* (MTCC 2272) using

Table 3 Antibacterial studies on the cobalt complexes

the microbroth dilution method (Table 3). Gentamycin was used as a reference drug for the bacteria. Complexes $[Co(L^1)_3]$ (1) and Na $[Co(L^4)_2]$ (4) showed strong activity against resistant strains of *Pseudomonas*, *E. coli* and standard strains of *Shigella*, *Klebsiella* (MIC ranged from 2.7 to 23.43 µg/ml on different strains). Similarly, complex Na $[Co(L^5)_2]$ (5) was found to be effective against *Pseudomonas* (MIC is 5.5 µg/ml). Other compounds appeared to have broad spectrum, as they showed mild to moderate activity towards most of the strains. The effectiveness of the complexes 1 and 4 may be due to the position of the un-coordinated pyridine rings that form a cleft suitable to interact strongly with the cations [14].

2.6. Cytotoxicity studies

In the present study, the cytotoxic activity of the metal complexes has also been determined. The study was used to test the growth inhibition by MTT assay. Data are expressed in terms of % viability which is directly proportional to the metabolic active cell number. Cobalt(III) complexes are less cytotoxic than that of gentamycin (Fig. 1). The cytotoxic activity results suggest that these cobalt complexes are less cytotoxic at moderate concentrations. The importance of such work lies in the possibility that the new complexes might be more efficacious drugs against bacterial infections. For this purpose, a thorough investigation regarding the structure—activity of the complexes and their stability is required in order to understand the variation in their biological effects, which could be helpful in designing more potent antibacterial agents for the therapeutic use.

3. Conclusion

We have shown the preparation of novel cobalt(III) complexes of pyridine—amide-based bidentate and tridentate ligands. Co(III) complexes have thoroughly been characterized by various physical and chemical techniques. The NMR studies authenticate the octahedral arrangement of the ligands around the Co(III) ion. The *meridional* arrangement of the bidentate ligands in the former cobalt complexes, 1-3, has caused the asymmetry which is clearly reflected in their

No.	Complex	MIC (µg/ml)					
		Standard bacterial strains			Resistant bacterial strains		
		Shigella flexneri	Klebsiella Planticola	Pseudomonas aeruginosa	Escherichia coli	Proteus vulgaris	Pseudomonas aeruginosa
1	$[Co(L^1)_3]$	23.43	2.7	187	5.5	187	5.5
2	$[Co(L^2)_3]$	23.43	187	375	187	375	187
3	$[Co(L^3)_3]$	46.87	187	375	187	375	187
4	$Na[Co(L^4)_2]$	23.43	2.7	187	5.5	187	5.5
5	$Na[Co(L^5)_2]$	a	375	375	375	375	5.5
6	$Na[Co(L^6)_2]$	23.43	187	375	187	375	187
Gentamycin		1.46	1.46	1.46	11	187	5.5

^a No activity.



Fig. 1. Cytotoxicity of complexes using MTT assay on HEK cells. Cells are grown in the presence of drug at various concentrations up to 48 h and metabolic activity of these cells was measured using MTT. Results are shown as the % viability. G stands for gentamycin.

NMR spectra. The antibacterial study of the complexes shows significant activity. Their MIC ranged from 2.7 to 375 μ g/ml. On dilution activity decreases, which shows that the complexes are an effective inhibitor at moderate concentrations. Complexes **1** and **4** in particular, showed strong activity against the resistant strains of *Pseudomonas*, *E. coli* and standard strains of *Shigella*, *Klebsiella* (MIC ranged from 2.7 to 23.43 μ g/ml on different strains). All complexes were also tested for their cytotoxic activity on the HEK cell lines and show less cytotoxicity at all tested concentrations when compared with gentamycin. Future studies will be directed to understand the effect of the un-coordinated nature of the pyridine ring on the antimicrobial activity and to evaluate the mode of action of these complexes.

4. Experimental

4.1. Materials and methods

All reagents were obtained from the commercial sources and used as received. FCS, fetal calf serum; PBS, phosphate-buffered saline; and DMEM were obtained from Hyclone, USA, whereas DMSO cell culture tested and MTT were purchased from SRL. N,N'-Dimethyl formamide (DMF) was dried and distilled from 4 Å molecular sieves and was stored over sieves. Acetonitrile (MeCN) was dried by distillation from anhydrous CaH₂. Diethyl ether was dried by refluxing over sodium metal under inert atmosphere. Tetrahydrofuran (THF) was dried by refluxing and distilling from sodium metal and benzophenone. Ethyl alcohol (C₂H₅OH) and methyl alcohol (CH₃OH) were distilled from magnesium ethoxide and magnesium methoxide, respectively. Chloroform $(CHCl_3)$ and dichloromethane (CH_2Cl_2) were purified by washing with 5% sodium carbonate solution followed by water and finally dried over anhydrous CaCl₂, before a final reflux and distillation. Dimethyl sulfoxide (DMSO) was distilled from 4 Å molecular sieves and was stored over sieves. The ligands, 2-(*N*-(2-pyridyl)carbamoyl)pyridine (HL¹), 2-(*N*-(3-pyridyl)carbamoyl)pyridine (HL²), 2-(*N*-(4-pyridyl) carbamoyl)pyridine (HL³), 2,6-bis(*N*-(2-pyridyl)carbamoyl)pyridine (H₂L⁴), 2,6-bis(*N*-(3-pyridyl)carbamoyl)pyridine (H₂L⁵), and 2,6-bis(*N*-(4-pyridyl)carbamoyl)pyridine (H₂L⁶), were synthesized following the procedure for HL¹ reported in the literature [19].

4.2. Physical measurements

The conductivity measurements were done in organic solvents or water using the digital conductivity bridge from Popular Traders, India (model number: PT-825). Elemental analysis data were obtained from Elementar Analysensysteme GmbH Vario EL-III instrument. NMR measurements were done using an Avance Bruker (300 MHz) instrument. Infrared spectra (either as KBr pellet or as a mull in mineral oil) were recorded using Perkin–Elmer FTIR 2000 spectrometer. Absorption spectra were recorded using Perkin–Elmer *Lambda-25* spectrophotometer. Mass spectra were obtained from a Jeol SX102/DA-6000 instrument.

4.3. Syntheses

Caution! Metal perchlorate salts are potentially explosive and should be used in small quantity with great care.

4.3.1. General synthetic procedure for the cobalt(III) complexes

The respective ligand was dissolved in dinitrogen flushed DMF and treated with necessary amount of solid NaH. The resulting mixture was then stirred for 30 min at room temperature. Solid $[Co(H_2O)_6](ClO_4)_2$ was added to the aforementioned

mixture. After 30 min of stirring, dry O_2 was purged to the solution for 2 min. The solution was finally stirred for 1 h. The reaction mixture was filtered followed by the removal of the solvent under reduced pressure. The crude product was isolated after washing with diethyl ether. The crude product thus obtained was dissolved in DMF and subjected to vapour diffusion of diethyl ether. This results in the highly crystal-line product within a day. The recrystallized product was filtered and dried under vacuum.

4.3.1.1. $[Co(L^{1})_{3}]$ (1). Yield: 65%. Anal. Calcd for C₃₆H₃₃N₁₀O₅Co (including one DMF and H₂O): C, 58.06; H, 4.43; N, 18.81. Found: C, 58.36; H, 4.34; N, 18.64. IR (KBr, ν , selected peaks): 1625, 1600, 1580 (C=O) cm⁻¹. $\lambda_{\text{max/nm}}$ (CH₃CN, ε , dm³ mol⁻¹ cm⁻¹): 514 (200), 377 (sh, 3900), 300 (19,000); $\lambda_{\text{max/nm}}$ (DMF, ε , dm³ mol⁻¹ cm⁻¹): 514 (270), 375 (sh, 4200), 300 (24,000), 260 (sh, 36,000). MS (CH₃CN) m/z (%): 654.43 (40) [Co(L¹)₃ + H⁺]. Molecular conductivity ($\sim 1 \text{ mM}$, CH₃CN, 25 °C): $\Lambda = 8 \text{ S cm}^2 \text{ mol}^{-1}$ (the range for 1:1 electrolyte in CH₃CN is 120–160). δ_H (300 MHz, DMSO-d₆, 25 °C, TMS): 9.47, 9.13, 8.16 (d, 3H, H_2' protons for three asymmetric ligands), 7.12, 5.78, 5.60, 7.30, 7.29, 7.29 (d/m, 6H, H₃/H₃' protons for three asymmetric ligands), 7.26, 7.26, 7.05, 8.12, 7.71, 7.69 (m/m, 6H, H_4/H_4' protons for three asymmetric ligands), 6.97, 6.67, 6.56, 7.91, 7.74, 7.74 (m/d, 6H, H₅/H₅' protons for three asymmetric ligands), 7.38, 7.38, 7.32 (d, 3H, H₆ protons for three asymmetric ligands); $\delta_{\rm C}$ (300 MHz, DMSO- d_6 , 25 °C, TMS): 157.02, 156.45, 155.50, 151.07, 150.94, 150.24 (C_2/C_2) for three asymmetric ligands), 119.97, 119.08, 118.10, 128.65 (two signals are merged), 127.08 (C_3/C_3) for three asymmetric ligands), 139.85 (two signals are merged), 141.25, 137.40, 136.48, 135.39 (C₄/C₄' for three asymmetric ligands), 125.39, 124.48, 123.93, 123.50, 120.67, 122.50 (C_5/C_5' for three asymmetric ligands), 148.87, 147.95, 147.54, 159.67, 158.24, 160.31 (C₆/C₆' for three asymmetric ligands), 169.92, 168.88, 168.99 (C_7/C_7' for three asymmetric ligands).

4.3.1.2. $[Co(L^2)_3]$ (2). Yield: 50%. Anal. Calcd for C₃₆H₃₃N₁₀O₅Co (including one DMF and H₂O): C, 58.06; H, 4.43; N, 18.81. Found: C, 58.26; H, 4.32; N, 18.72. IR (KBr, ν , selected peaks): 1622, 1596, 1567 (C=O) cm⁻¹. $\lambda_{\text{max/nm}}$ (DMF, ε , dm³ mol⁻¹ cm⁻¹): 540 (300), 380 (sh, 7050), 345 (sh, 15,600), 330 (sh, 22,700), 300 (34,000). MS (CH₃OH) m/z (%): 654.18 (60) [Co(L²)₃ + H⁺]. Molecular conductivity (~1 mM, DMF, 25 °C): $\Lambda = 7 \text{ S cm}^2 \text{ mol}^{-1}$. δ_H (300 MHz, DMSO-d₆, 25 °C, TMS): 8.32, 8.19, 7.98, 9.00, 8.77, 8.36 (s/d, 6H, H₂/H₂' protons for three asymmetric ligands), 7.57, 7.54, 7.34 (m, 3H, H₃' protons for three asymmetric ligands), 7.29, 7.23, 6.98, 7.91, 7.60, 7.58 (d/m, 6H, H_4/H_4' protons for three asymmetric ligands), 6.81, 6.77, $6.49, 8.05, 8.02, 7.94 \text{ (m/d, 6H, H}_5/\text{H}_5' \text{ protons for three asym-}$ metric ligands), 7.92, 7.77, 7.74 (d, 3H, H₆ protons for three asymmetric ligands); $\delta_{\rm C}$ (300 MHz, DMSO- d_6 , 25 °C, TMS): 148.35, 145.74, 144.90, 150.03, 149.80, 149.38 (C₂/C₂' for three asymmetric ligands), 124.50, 123.53 (two signals are

merged), 126.35, 125.53, 125.04 (C_3/C_3' for three asymmetric ligands), 128.93 (two signals are merged), 129.62, 134.60 (three signals are merged) (C_4/C_4' for three asymmetric ligands), 122.17 (three signals are merged), 122.92 (C_5/C_5' for three asymmetric ligands), 143.45, 141.67, 140.73, 155.78, 154.88, 154.73 (C_6/C_6' for three asymmetric ligands), 169.91, 169.70, 168.95 (C_7/C_7' for three asymmetric ligands).

4.3.1.3. $[Co(L^3)_3]$ (3). Yield: 62%. Anal. Calcd for C₃₆H₃₃N₁₀O₅Co (including one DMF and H₂O): C, 58.06; H, 4.43; N, 18.81. Found: C, 58.26; H, 4.32; N, 18.72. IR (KBr, ν , selected peaks): 1625, 1599, 1582 (C=O) cm⁻¹. $\lambda_{\text{max/nm}}$ (DMF, ε , dm³ mol⁻¹ cm⁻¹): 534 (210), 412 (sh, 2500), 300 (sh, 18,500). MS (CH₃OH) m/z (%): 654.31 (57) $[Co(L^3)_3 + H^+]$. Molecular conductivity (~1 mM, DMF, 25 °C): $\Lambda = 5 \text{ S cm}^2 \text{ mol}^{-1}$. δ_{H} (300 MHz, DMSO- d_6 , 25 °C, TMS): 8.30, 8.27, 8.05 (d, 6H, H₂/H₆ protons for three asymmetric ligands), 8.97, 8.79, 8.34 (d, 3H, H₂' protons for three asymmetric ligands), 6.94, 6.53, 6.21 (d, 6H, H₃/H₅ protons for three asymmetric ligands), 7.60, 7.53, 7.50 (m, 3H, H_3 ' protons for three asymmetric ligands), 7.95, 7.92, 7.91 (m, 3H, H_4' protons for three asymmetric ligands), 7.90, 7.75, 7.68 (d, 3H, H_5' protons for three asymmetric ligands); δ_C (300 MHz, DMSO-d₆, 25 °C, TMS): 129.83 (three signals are merged), 154.60, 152.59, 152.29 (C₂/C₂' for three asymmetric ligands), 122.99 (three signals are merged), 126.60 (two signals are merged), 125.66 (C_3/C_3' for three asymmetric ligands), 150.02, 149.53 (two signals are merged), 141.81 (two signals are merged), 141.09 (C_4/C_4' for three asymmetric ligands), 122.99 (three signals are merged), 125.23 (two signals are merged), 123.44 (C_5/C_5' for three asymmetric ligands), 129.83 (three signals are merged), 155.79 (two signals are merged), 155.06 (C_6/C_6' for three asymmetric ligands), 168.85 (two signals are merged), 168.44 (C_7/C_7 ' for three asymmetric ligands).

4.3.1.4. $Na[Co(L^4)_2]$ (4). Yield: 52%. Anal. Calcd for $C_{34}H_{24}N_{10}O_5CoNa$ (including one H_2O): C, 55.59; H, 3.29; N, 19.07. Found: C, 55.38; H, 2.97; N, 18.78. IR (KBr, ν , selected peaks): 1593, 1578, 1560 (C=O) cm⁻¹. $\lambda_{max/nm}$ (DMF, ε , dm³ mol⁻¹ cm⁻¹): 650 (290), 488 (sh, 1980), 470 (sh, 3040). MS (CH₃OH) *m/z* (%): 716.89 (100) [Na[Co(L⁴)₂] + H⁺], 694.93 (20) [[Co(L⁴)₂] + H⁺]. Molecular conductivity (~1 mM, DMF, 25 °C): Λ = 55 S cm² mol⁻¹ (the range for 1:1 electrolyte in DMF is 60–90). $\delta_{\rm H}$ (300 MHz, DMSO-*d*₆, 25 °C, TMS): 7.65 (d, 4H, *J* = 3.87 Hz, H₂/H₂'), 6.70 (m, 4H, H₃/H₃'), 7.28 (m, 4H, H₄/H₄'), 7.55 (d, 4H, *J* = 7.71 Hz, H₅/H₅'), 7.02 (d, 4H, *J* = 7.98 Hz, H₉/H₉'), 8.0 (t, 2H, H₁₀); $\delta_{\rm C}$ (300 MHz, DMSO-*d*₆, 25 °C, TMS): 146.16 (C₂), 121.94 (C₃), 122.07 (C₄), 116.98 (C₅), 156.94 (C₆), 168.05 (C₇), 160.20 (C₈), 136.11 (C₉), 138.88 (C₁₀).

4.3.1.5. $Na[Co(L^5)_2]$ (5). Yield: 72%. Anal. Calcd for $C_{37}H_{33}N_{11}O_7CoNa$ (including one DMF and two H₂O): C, 53.81; H, 3.99; N, 18.66. Found: C, 53.22; H, 3.90; N, 18.41. IR (KBr, ν , selected peaks): 1652, 1593 (C=O) cm⁻¹. $\lambda_{max/nm}$ (DMF, ε , dm³ mol⁻¹ cm⁻¹): 650 (70),

480 (sh, 910), 385 (sh, 4300), 325 (sh, 13,300). MS (CH₃OH) *m*/*z* (%): 716.97 (50) [Na[Co(L⁵)₂] + H⁺]. Molecular conductivity (~1 mM, DMF, 25 °C): $\Lambda = 60 \text{ S cm}^2 \text{ mol}^{-1}$. δ_{H} (300 MHz, DMSO-*d*₆, 25 °C, TMS): 7.84 (d, 4H, *J* = 1.55 Hz, H₂/H₂'), 6.77 (d, 4H, H₃/H₃'), 6.72 (m, 4H, H₄/ H₄'), 7.63 (d, 4H, *J* = 1.74 Hz, H₆/H₆'), 7.44 (d, 4H, *J* = 7.73 Hz, H₉/H₉'), 7.80 (m, 2H, H₁₀); δ_{C} (300 MHz, DMSO-*d*₆, 25 °C, TMS): 144.63 (C₂), 123.05 (C₃), 140.38 (C₄), 147.02 (C₅), 141.74 (C₆), 166.97 (C₇), 155.29 (C₈), 124.10 (C₉), 133.04 (C₁₀).

4.3.1.6. $Na[Co(L^6)_2]$ (6). Yield: 70%. Anal. Calcd for $C_{37}H_{33}N_{11}O_7CoNa$ (including one DMF and two H_2O): C, 53.81; H, 3.99; N, 18.66. Found: C, 53.74; H, 4.38; N, 18.53. IR (KBr, ν , selected peaks): 1670, 1626, 1599 (C=O) cm⁻¹. $\lambda_{max/nm}$ (DMF, ε , dm³ mol⁻¹ cm⁻¹): 655 (70), 478 (sh, 890), 380 (sh, 4330), 325 (sh, 12,000). MS (CH₃OH) *m*/*z* (%): 716.87 (30) [Na[Co(L²)₂] + H⁺]. Molecular conductivity (~1 mM, DMF, 25 °C): Λ = 55 S cm² mol⁻¹. $\delta_{\rm H}$ (300 MHz, DMSO-*d*₆, 25 °C, TMS): 8.10 (d, 4H, J = 5.46 Hz, H₂/H₂'), 6.72 (d, 4H, J = 5.63 Hz, H₃'/H₅), 6.72 (d, 4H, J = 5.63 Hz, H₃/H₅'), 8.08 (d, 4H, H₆/H₆'), 7.82 (d, 4H, J = 7.7 Hz, H₉/H₉'), 8.05 (m, 2H, H₁₀); $\delta_{\rm C}$ (300 MHz, DMSO-*d*₆, 25 °C, TMS): 153.32 (C₂), 121.62 (C₃), 155.44 (C₄), 124.68 (C₅), 153.32 (C₆), 166.36 (C₇), 162.40 (C₈), 140.83 (C₉), 149.54 (C₁₀).

4.4. In vitro antibacterial activity

All complexes have been screened in vitro against clinically isolated resistant strains of *Pseudomonas*, *Proteus*, *E. coli* and standard strains of *P. aeruginosa* (MTCC 1688), *S. flexneri* (MTCC 1457), *E. coli* (BL21), and *Klebsiella planticola* (MTCC 2272). Various methods [24–27] are available for the evaluation of the antibacterial activity of different types of drugs. However, the most widely used method [27], which consists of determining the antibacterial activity of the drug is to add it in known concentrations to the cultures of the test organisms, was employed.

4.4.1. Microbroth dilution assay

Different concentrations of the test compounds in 200 µl of culture medium were prepared in 96-well flat-bottomed microculture plates (Nunc, Nunclon) by double dilution method. The wells were prepared in triplicate for each concentration. Each well was inoculated with 10.0 µl of bacterial suspension containing 10^6 cells/ml. The plates were incubated at 37 °C for 16 h and the OD was measured at A_{600} nm of the suspension to assess the inhibition of cell growth due to treatment with compounds. All the tests were repeated up to three times.

4.5. In vitro cell growth inhibition assay

Cells were seeded in 96-well plates at a concentration of $2-4 \times 10^3$ cells/well in 200 µl of complete media and incubated for 24 h at 37 °C in 5% CO₂ atmosphere to allow for

cell adhesion. Stock solutions (6 mg/ml) of the compounds made in water were filter-sterilized and then diluted to 3 mg/ml in incomplete media. The 3 mg/ml solutions were further diluted up to 0.73 µg/ml for treatment against HEK cell lines to give final concentrations range of 1500-0.73 µg/ml. All assays were performed in two independent sets of quadruplicate tests. Control group containing no drug was run in each assay. Following 48 h of exposure of cells to drug, each well was carefully rinsed with 200 µl PBS buffer. Cytotoxicity was assessed using MTT (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide). MTT solutions of 20 μ l (5 mg/ml dd H₂O) along with 200 µl of fresh, complete media were added to each well and plates were incubated for 4 h. Following incubation, the medium was removed and the purple formazan precipitate in each well was sterilized in 200 µl DMSO. Absorbance was measured using Techan microplate reader (molecular device) at 570 nm and the results are expressed as % viability which is directly proportional to metabolic active cell number. Percentage (%) viability was calculated as:

% Viability = OD in sample well/OD in control well \times 100.

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Appendix. Supplementary information

Supplementary materials (seven figures and two tables) include ¹H and ¹³C NMR spectra, absorption spectra and ¹³C spectral data for complexes 1-6. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2007.08.015 or from the authors.

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