Mixed ligand cobalt complexes of lincomycin with diimine ligands: Synthesis, spectroscopic and biological investigation

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Abstract

Four new mixed ligand cobalt complexes with the antibiotic lincomycin and diimine ligands have been synthesized by reacting lincomycin with suitable precursor complexes to obtain $[Cobpy_2Lin]Cl_2.8H_2O$ (**1**), $[Cobpy_2Lin]Cl_3$ (**2**), $[Cophen_2Lin]Cl_3$ (**3**), $[Copd_2Lin]Cl_3$ (**4**) in good yield. These complexes were characterized by their electronic spectra. Antibacterial susceptibility tests of these complexes against five test isolates showed that two of these complexes (**2** and **3**) possess improved antibacterial activity or have activities in the same range with lincomycin, the parent antibiotic. Ethidium bromide fluorescence quenching experiment of complex **3** resulted in slight decrease in the emission intensity of CT DNA-bound ethidium bromide due to partial intercalation of the complex into CT DNA. The partial intercalation of the complex **3** was due to the planar phenanthroline ligand moiety and the weak interaction with DNA was caused by the bulky ancillary lincomycin ligand. This substantiates the weak antibacterial activity of the complex and that of the other complexes as the complexes can only bind to the DNA of the test organisms weakly.

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INTRODUCTION

Lincosamide antibiotics (lincomycin and clindamycin) are widely used in clinical practice for the treatment of diverse infections. Lincomycin, the first lincosamide discovered, was isolated from *Streptomyces linconensis* (Hoeksema *et al*., 1964). At low concentrations, lincomycin inhibits protein synthesis in Gram-positive bacteria without interfering with DNA and RNA synthesis (Josten and Allen *et al*., 1964). Precisely, lincomycin acts on the 50s subunit of the ribosomes of a Gram-positive bacteria but not on the corresponding subunit of a Gram-negative organism (Chang *et al*., 1966). However, resistance to lincomycin by some species of *Staphylococcus* such as *Staphylococcus aureus* BM4611 (Josten and Allen *et al*., 1964) has been reported and the biochemical mechanism of lincosamide inactivation by this species (Leclercq *et al*., 1987; Brisson-Noel *et al*., 1988) has been described.

Complexation of lincosamide antibiotics by some metal ions have been examined. This includes the interactions of palladium with lincomycin (Yi *et al*., 2008) and clindamycin (Yi *et al*., 2009) while the analytical applications of 1:1 palladium(II) complexes of the two compounds obtained in solution have been investigated (Ahou-Attia and El-Anwar *et al*., 2000; Yoshikazu *et al*., 1987). The structure and dynamics of Cu(II) complexation of lincomycin in aqueous solution has also been studied by proton and carbon-13 NMR (Gaggelli *et al*., 2002). The oxidative cleavage activity of this Cu-lincomycin specie was reported to be a fairly efficient promoter of $^{\bullet}OH$ generation (Jezowska-Bojczuk *et al*., 2001). However, the studies of metal complexation by lincomycin including its determination as palladium complex has been centered on species in solution (Egutkin *et al*., 1984). It is, therefore, noteworthy to obtain metal complexes of lincomycin in solid state, to characterize and explore them for different biological applications.

The afore-mentioned uses of lincosamides and their metal complexes, the therapeutic properties of copper, and DNA binding and cleavage abilities of polypyridyl ligands are the motivation behind this research. To this end, heteroleptic transition metal complexes containing antimicrobial lincomycin and

polypyridyl ligands were synthesized and explored for potential biological applications.

MATERIALS AND METHODS

All chemicals used for syntheses were of analytical grade and were used as received. $CoCl_2.6H_2O$, potassium bromide (KBr), ethidium bromide, 2,2′-bipyridine (bpy), 1,10-phenanthroline monohydrate (phen), sodium chloride and sodium hydroxide (NaOH) were obtained from S. D. Fine Chemicals Limited (India). Potassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4) and Calf thymus DNA sodium salt (CT DNA) were obtained from SRL (India). Lincomycin hydrochloride was obtained from Drugfield Pharmaceuticals Plc, Nigeria while $Cobpy_2Cl_2$ (Kumar *et al.* 2011) and, $[Cophen_2Cl_2]Cl$, $[Cobpy_2Cl_2]Cl$ and $[Copd_2Cl_2]Cl$ (Vleck AA 1967; Ghosh *et al.* 2006) were prepared following literature procedures.

Synthesis of the Cobalt complexes

$[Cobpy_2Lin]Cl_2.8H_2O$ (1)

0.225 g (0.50 mmol) of $Cobpy_2Cl_2$ and 0.231 g (0.50 mmol) of lincomycin hydrochloride monohydrate were dissolved in 10 mL of methanol; 0.2 mL triethyl amine was added and the solution was refluxed for 4 hours. The dark red solution was allowed to evaporate at room temperature producing red viscous substance with tiny crystals. The product was re-dissolved in methanol and purified by column chromatography using alumina as the stationary phase and chloroform, acetone and methanol as eluent. Calculated: C, 46.02; H, 6.61; N, 8.47. Found: C, 46.09; H, 6.63; N, 9.22. UV-Vis (H_2O, nm): 388, 391, 509, 741, 975. FT-IR (KBr, ν/cm^{-1}): 3335 br, 3111, 3078, 2958, 2918, 2870, 2847, 2787, 1651 (amide I C=O), 1606, 1558 (amide II C=O), 1521, 1448, 1377, 1311, 1247 (C-O-C), 1159, 1099 (S-CH_3), 1051 (S-CH_3), 964, 906, 775, 731, 709, 542, 520, 491, 424.

$[Cobpy_2Lin]Cl_3$ (2)

0.115 g (0.25 mmol) of lincomycin hydrochloride monohydrate, 0.136 g (0.25 mmol) of $[Cobpy_2Cl_2]Cl$ and 0.1 mL of triethyl amine were refluxed with stirring in 15 mL methanol for 8 hours. The obtained blood red solution was transferred into a beaker and set aside to evaporate slowly at room temperature. The remaining solution was purified by column chromatography using alumina as the stationary phase and chloroform, acetone and methanol as eluent. UV-Vis (H_2O, nm): 388, 391, 509, 741, 975. FT-IR (KBr, ν/cm^{-1}): 3296 br, 3076, 2960, 2918, 2847, 2787, 1647 (amide I C=O), 1606, 1518 (amide II C=O), 1450, 1371, 1311, 1247 (C-O-C), 1159, 1101 (S-CH_3), 1049 (S-CH_3), 964, 906, 775, 731, 655, 615

$[Cophen_2Lin]Cl_3$ (3)

0.116 g (0.25 mmol) of lincomycin hydrochloride monohydrate, 0.1420 g (0.25 mmol) of $[Cophen_2Cl_2]Cl$ and 0.1 mL of triethyl amine were refluxed with stirring in 15 mL methanol for 8 hours. The obtained blood red solution was transferred into a beaker and set aside to evaporate slowly at room temperature. The remaining solution was purified by column chromatography using alumina as the stationary phase and chloroform, acetone and methanol as eluent. Calculated: C, UV-Vis (H_2O, nm): 388, 391, 509, 741, 975. FT-IR (KBr, ν/cm^{-1}): 3350, 3298, 3238, 3057, 2956, 2918, 2847, 2787, 1647 (amide I C=O), 1518 (amide II C=O), 1427, 1383, 1315, 1224 (C-O-C), 1145, 1099 (S-CH_3), 1051 (S-CH_3), 966, 850, 802, 723, 661, 613, 543.

$[Copd_2Lin]Cl_3$ (4)

0.115 g (0.25 mmol) of lincomycin hydrochloride monohydrate, 0.146 g (0.25 mmol) of $[Copd_2Cl_2]Cl$ and 0.1 mL of triethyl amine were refluxed with stirring in 15 mL methanol for 1 hour 30 minutes. The orange precipitated powder was filtered and air dried. FT-IR (KBR, ν/cm^{-1}): 3071, 3013, 1694 (amide I C=O), 1574 (amide II C=O), 1476, 1424, 1307,1258 (C-O-C), 1210, 1195, 1134, 1072 (S-CH_3), 1021 (S-CH_3), 934, 861, 841, 736, 707, 693.

Fluorescence measurements

Competitive binding fluorescence measurements of **3** with ethidium bromide (EtBr)-bound CT DNA solution in phosphate buffer were monitored. The changes in fluorescence intensities at 600 nm (510 nm excitation) of EtBr bound to DNA were recorded with an increasing amount of the complex concentration. Concentrations of DNA and EtBr were 20 μM in all cases.

RESULTS AND DISCUSSION

Characterization of the complexes

Heteroleptic cobalt (II) complexes of lincomycin have been synthesized and isolated in solid state. The isolated complexes were characterized and the coordination mode of lincomycin assigned based on the only possible coordination mode for lincomycin as previously reported (Ahou-Attia and El-Anwar *et*

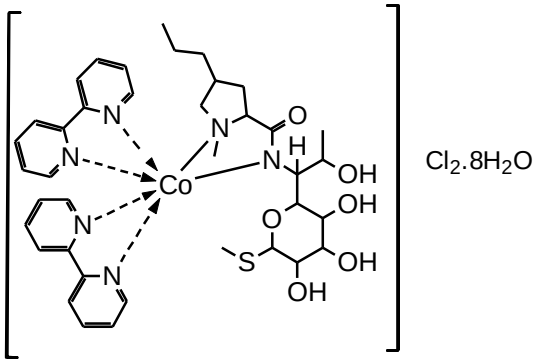
al., 2000; Jezowska-Bojczuk *et al*., 2001; Yi *et al*., 2008; Yi *et al*., 2009;). These heteroleptic cobalt complexes (**1-4**) with lincomycin and polypyridyl ligands are soluble in aqueous and organic solvents such as acetone, ethyl acetate, chloroform and methanol.

The amide I and II bands at 1656 and 1566 cm^{-1} as well as S-CH_3 stretch at 1050-1100 cm^{-1} in lincomycin still appear in the FTIR spectra of all the complexes. This shows that these groups are not involved in coordination to cobalt. However, the weak N-H stretch at 2576 cm^{-1} in lincomycin are absent in the spectra of the complexes indicating the deprotonation and coordination of the amine nitrogen to cobalt.

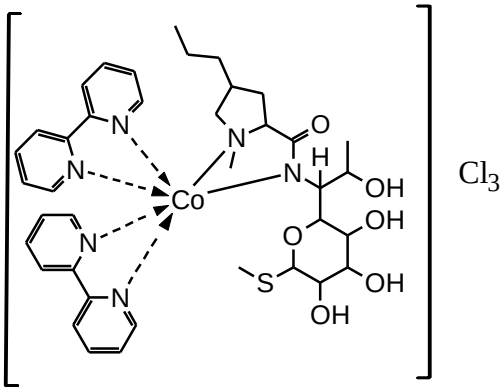
The NMR spectra of the complexes are not well resolved because of the paramagnetic character of Co^{II} complexes which results in broad NMR signals. Nonetheless, the ^{1}H-NMR spectra of the complexes **2-4** were taken in chloroform in which the parent antibiotic (free lincomycin ligand) is insoluble. The spectra of the complexes support complex formation as evidenced from peaks corresponding to both lincomycin and polypyridyl ligands present in the ^{1}H-NMR spectra of the complexes. Peaks in the aliphatic region are due to lincomycin and those in the aromatic region are due to the polypyridyl portions of the complexes. The ^{1}H-NMR spectra of the complexes are available on request and the proposed structures of the complexes are given in Figure 1.

Biological Studies

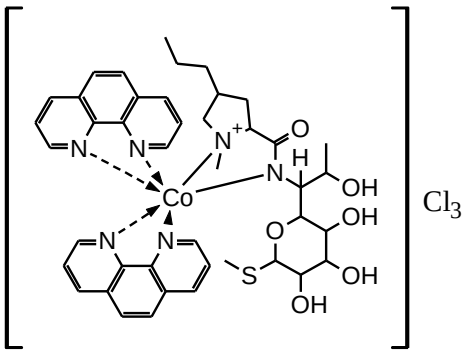
The zones of inhibition of the complexes and its parent antibiotic, lincomycin are given in Table 1. All lincomycin complexes exhibited lower antibacterial activity against *Staphylococcus epidermidis* and S*taphylococcus aureus* than lincomycin itself. Against *Bacillus megaterium*, only **4** had slightly higher antibacterial activity than the parent ligand lincomycin while **3** had antibacterial activity in the same range with the parent ligand lincomycin. Complex **3** also has similar activity against *Bacillus subtillis* with lincomycin while the other complexes exhibited lower activity.



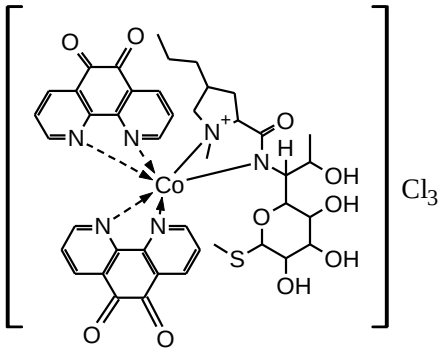
[Cobpy$_2$Lin]Cl$_2$.8H$_2$O (1)



[Cobpy$_2$Lin]Cl$_3$ (2)



[Cophen$_2$Lin]Cl$_3$ (3)



[Copd$_2$Lin]Cl$_3$ (4)

Figure 1: Proposed structures of complexes **1-4**

When complex **3** was added to CT DNA pretreated with EtBr ([DNA]/[EtBr]=1:1) and equilibrated for 20 minutes at room temperature after addition of DNA to the metal complex, the DNA-induced

emission intensity of ethidium bromide (EtBr) decreased slightly (Figure 2). This indicates the weak binding of this complex to DNA and is responsible for the low antimicrobial activity of the complex.
The quenching of EtBr emission is by acceptance of excited state electron from EtBr by the complex since lincomycin is known not to interact with DNA (Josten and Allen *et al.*, 1964).

Table 1: Antibacterial susceptibility tests of lincomycin and its complexes against test bacterial isolates.

AGENTS	ZONE OF INHIBITION (mm)		
	100 µg	200 µg	300 µg
Bacillus subtilis			
1	9	12	14
2	9	11	14
3	10	14	17
4	8	11	16
Lin	12	13	17
Bacillus megaterium			
1	6	7	9
2	6	7	9
3	7	9	11
4	8	10	12
Lin	7	9	11
Staphylococcus epidermidis			
1	10	12	15
2	5	7	10
3	6	10	13
4	3	6	8
Lin	12	15	17
Staphylococcus aureus			
1	6	9	11
2	6	8	11
3	8	10	11
4	7	8	10
Lin	11	13	16
Klebsiella pneumonia			
1	7	9	12
2	3	5	7
3	9	10	12
4	9	10	11
Lin	12	14	17

- Lin = lincomycin

The slight decrease in the emission intensity of CT DNA-bound ethidium bromide shows it is a weak DNA intercalator and explains the weak antibacterial activity of the complexes. This shows that the planar diimine ligand moiety can only interact weakly with the DNA of the test organisms due to the bulky ancillary lincomycin ligand. Similar observations have been observed for other complexes with bulky ancillary ligands (Abosede *et al.*, 2016).

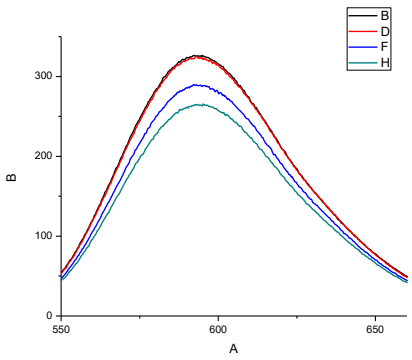


Figure 2: Effect of addition of $Co(Phen)_2Lin$ (3) 0-700 µM in phosphate buffer; [DNA],[EtBr]=20 µM; [DNA]/[Co]=0-35 on the emission intensity of the CT DNA-bound ethidium bromide (20 µM) at different concentrations. Excitation wavelength is 510 nm

CONCLUSION

Mixed ligand lincomycin complexes of lincomycin with diimine ligands were synthesized and characterized. The antibacterial activities of the lincomycin complexes were lower than that of the

parent antibiotic, lincomycin, except for **3** which has comparable activity against *Bacillus sp* and **4** which has higher activity against *Bacillus megaterium*. Ethidium bromide fluorescence quenching experiment of complex **3** showed that it is a weak DNA intercalator and this explains its weak antibacterial activity and that of the other complexes.

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