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Research Article

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Syntheses, Characterization and Antibacterial Susceptibility Testing of Transition Metal Complexes of Doxycycline

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Abstract: Binary and ternary cobalt and copper complexes of the pleiotropic antibiotic doxycycline with two polypyridyl ligands were synthesized and characterized by electronic absorption, FT-IR, elemental analysis and electrospray mass spectroscopy. Characterization study indicates that the ligands are bidentate and the complexes are monomeric with octahedral geometry. The antibacterial activities of the complexes—$[CoDox_2(H_2O)_2(CO_3)].5H_2O$(1),$[CoDox_2(H_2O)_2](ClO_4)_3.6H_2O$(2), $[Cophen_2Dox](ClO_4)_3.2H_2O$(3) and $[CubpgDox(H_2O)_2](NO_3)_2.3H_2O$ (4) were tested against *Staphylococcus aureus* and *Klebsiella pneumonia*. These bacteria strains were found to be susceptible to the four complexes but the presence of ancillary polypyridyl ligands did not contribute to the antimicrobial activities of the complexes. Detailed structural analysis of the complexes and their antibiotic activity are presented and discussed.

Keywords: Doxycycline; Metal Complex; Polypyridyl; Copper; Antibacterial

1. INTRODUCTION

Tetracycline is the first semi-synthetic antibiotic of the naturally produced antibiotic—aureomycin (chlorotetracycline) [1-2], the first tetracycline to be discovered by fermentation of *Streptomyces* [3]. Tetracycline was later obtained by fermentation process [4-5] and is the most basic structure common to the other tetracyclines. The successful semisynthesis of tetracycline led to a wide search for superior structurally modified antibiotics and has provided most of the important antibiotic discoveries made since then [6,7]. Among the semisynthetic tetracyclines that are in clinical use are doxycycline, methacycline and minocycline [8]. These antibiotics are among the most frequently prescribed antibiotics for treating bacterial infections for several decades [4] and are highly effective

against a wide spectrum of pathogens [9] though their widespread usage has led to resistant organisms and decreased efficacy against some organisms [10-11]. Thousands [12-15] of novel tetracycline derivatives including penta- [12, 17] and hetero-cyclines [15, 18] have been prepared. Many of these compounds retain or have enhanced antibacterial activity and are active in broad panels of organisms resistant to classical tetracyclines and other antibiotics. Xiao-Yi Xiao and co-workers have also described the design, synthesis, and evaluation of a new generation of tetracycline antibacterial agents (fluorocyclines) which possess potent antibacterial activities against multidrug resistant (MDR) Gram-positive and Gram-negative pathogens [19, 20]. Another successful tetracycline analogue is tigecycline, a 7,9-disubstituted tetracycline derivative and already approved in the US as abroad-spectrum antibiotic [21-22].

These efforts are in response to the emergence of antibiotic-resistant bacterial strains and towards the development of tetracycline analogues with improved potencies and pharmacological properties. However, the number of antibiotics that have been discovered and introduced to clinical use are declining due to rapid resistance of pathogens against these antibiotics, regulatory hurdles and because of economic reasons [14, 22, 23]. This necessitates an urgent need for new classes of antibacterial agents with new modes of action to overcome the virulence of multidrug-resistant pathogens. A possible approach to achieve this is metal complexation of existing drugs [24]. In view of the aforementioned bacterial resistance, the association of activity and toxicity [25] of tetracyclines to metal coordination, the wide application of metal complexes in biology [26] and the use of metal based compounds as chemotherapeutics [27-28], we have synthesized and characterized some metal complexes of doxycycline. The evaluation of their antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumonia* were presented and discussed.

2. EXPERIMENTAL

2.1 Materials

Doxycycline hyclate was a gift from Neimeth International Pharmaceuticals Plc, Nigeria and fresh solutions were used to ensure stability. 1,10-phenanthroline monohydrate was from S. D. Fine Chemicals Ltd., India. All other chemicals and reagents are of analytical grade and used without further purifications.

2.2 Physical Measurements

UV-Vis spectra were recorded on a Jasco UV-Vis spectrophotometer. Infrared spectra were recorded in a range 4,000–400 cm^{-1} on Shimadzu FT-IR-8400 on samples pressed in KBr pellets. Elemental analyses were taken on Elementar Analysen Systeme Vario ® MICRO VI 6.2 GmbH. The electrospray mass spectra were recorded on a THERMO_Finningan_LCQ Advantage max ion trap mass spectrometer. The 10 µl samples (dissolved in methanol) were introduced into the ESI source through Finnigan surveyor autosampler. The mobile phase 90:10 MeOH:H_2O flowed at the rate of 250 µl/min by MS pump. Ion spray voltage was set at 5.3 KV and capillary voltage 34 V. The MS scan run up to 2.5 min and the spectra's print outs are averaged of over 10 scan at peak top in TIC. The ESI-MS were recorded at Central Drug Research Institute (CDRI), Lucknow, India.

2.3 Synthesis of bpg and Precursor Complex, [Cophen$_2$Cl$_2$]Cl

[Cophen$_2$Cl$_2$]Cl [29-30] (phen= 1,10-phenanthroline) and bipyridine glycouril (bpg) [31-33] were prepared by following previously published procedures. The spectroscopic data obtained for the two compounds were in agreement with published data for the two compounds.

2.4 Synthesis of Complexes

[CoDox$_2$(H$_2$O)$_2$]CO$_3$.5H$_2$O (**1**)

To 1.0265 g (2 mM) of doxycycline hyclate in 100 ml of distilled water was added 0.1205 g (1 mM) of $CoCO_3$ and the solution was stirred at 80 ^oC until effervescence stopped and the solution was filtered hot. The orange filterate was kept in freezer for 48 hours and the precipitated orange powder was filtered and dried in vacuum dessicator. Yield: 400 mg (35%). M.p. 107 ^oC. UV-Vis (H_2O): 274, 349; UV-Vis (MeOH): 547,765. Calculated: C, 47.66; H, 5.51; N, 4.94. Found: C, 47.97; H, 5.15; N, 5.24

[CoDox$_2$(H$_2$O)$_2$](ClO$_4$)$_3$.6H$_2$O (**2**)

0.5130 g (1mM) of doxycycline hyclate was dissolved in 10 ml of distilled water. The solution was heated to 80 ^oC and 0.0600 g (0.5 mM) of $CoCO_3$ was added. Heating was continued until effervescence stopped and the solution was filtered hot. To the orange filterate was added aqueous sodium perchlorate. The precipiated powder was filtered and dried in vacuum dessicator. Yield: 314.2 mg (45%). M.p. 168-171. UV-Vis (CH_3CN, nm): 268, 365, 540 sh. UV-Vis (MeOH): 460. Calculated: C, 38.07; H, 4.50; N, 4.04. Found: C, 37.68; H, 3.93; N, 3.71. ESI-MS: (CoDox$_2$(H$_2$O)$_2$+Dox)=1429.1; (Co+Dox)= 502.6; (Co+2Dox)= 947.5

[Cophen$_2$Dox](ClO$_4$)$_3$.2H$_2$O (**3**)

0.2567 g (0.5 mM) of doxycycline and 0.2626 g (0.5 mM) of [Cophen$_2$Cl$_2$]Cl were refluxed in 20 ml of methanol for 1 hour and the resulting solution was filtered. Saturated aqueous $NaClO_4$ was added to the filterate and the precipitated pink product was filtered and dried in vacuum dessicator. Yield: 330 mg (55%). UV-Vis (CH_3CN, nm): 206, 207, 210, 271, 363, 543 sh. Calculated: C, 46.11; H, 3.70; N, 7.01. Found: C, 46.29; H, 3.93; N, 6.77

[CubpgDox(H$_2$O)$_2$](NO$_3$)$_2$.3H$_2$O (**4**)

0.2420 g (1 mM) of $CuNO_3.3H_2O$ and 0.5130 g (1mM) of doxycycline hyclate were stirred at room temperature in 10 ml methanol for 50 minutes after which 0.2940g (1mM) of bipyridine glycouril was added. 10 ml of methanol and 5 ml of water were added and the solution further stirred for 5 hours. The solution was covered with perforated aluminum foil and was allowed to evaporate slowly at ambient conditions. 516.4 mg of green powder was obtained after slow evaporation of the solvent. Yield: 516.4 mg (51%). UV-Vis (H_2O, nm): 273, 316, 327, 356, 625. Calculated: C, 42.38; H, 4.74; N, 13.73. Found: C, 42.53; H, 4.24; N, 11.85. ESI-MS: $[Cu+bpg+Dox+H]^+$= 801.546 (ESI-MS: 800.8); $[CubpgDox]^{2+}$ = 400.773 (ESI-MS: 401.6).

2. 5 Antimicrobial Susceptibility Testing

The *in vitro* antibacterial activities of the ligand and complexes were tested by standard well diffusion method using nutrient agar media as previously described [34]. A sterile swab was used to inoculate the organism on the plate. The plate was left for some time so that the inocula would diffuse into the media. As an alternative to standard cork borer, a sterile needle was used to make 6 mm wells

uniformly on the surface of the plates. Three wells were made in the seeded plates and were labeled well I, II and III. Different concentrations of the test solution (5% aqueous DMSO) were introduced into the wells using sterile syringe**.** The plates were then allowed to stand for 1 hour at room temperature to allow proper diffusion of the test solution to occur. All the plates were incubated at 37 ^{o}C for 24-48 hours before bacteria and zones of inhibition were observed. A zone of clearance round each well signified inhibition and the diameter of such zones were measured in millimeter (mm).

3 RESULTS AND DISCUSSION

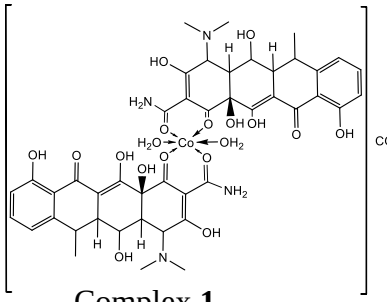
3.1 Synthesis

The synthesis of the new cobalt-doxycycline complex (**1**) was achieved by heating $CoCO_3$ and doxycycline in water and allowing the resulting solution to stand at about -10 ^{o}C for about 50 hours. The addition of excess aqueous sodium perchlorate to the reaction mixture before precipitating complex **1** led to the new Co(III) Doxycycline complex (**2**). Complex **3** was obtained by refluxing $[Cophen_2Cl_2]Cl$ with doxycycline in methanol followed by addition of aqueous sodium perchlorate. Complex **4** was obtained by stirring a stoichiometric amount of copper (II) nitrate trihydrate with doxycycline followed by reaction with bpg. The complexes were obtained in good yield and were stable towards air and moisture.

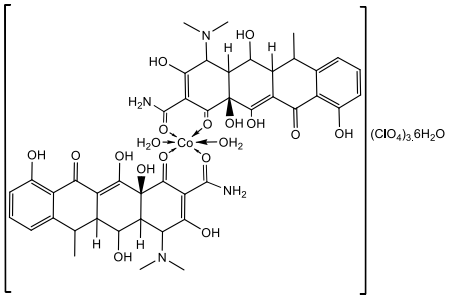
3.2 Characterization

Data obtained from UV-Vis, FT-IR, ESI-MS and elemental analysis were in agreement with the proposed molecular formulae for all the complexes (Scheme **1**).

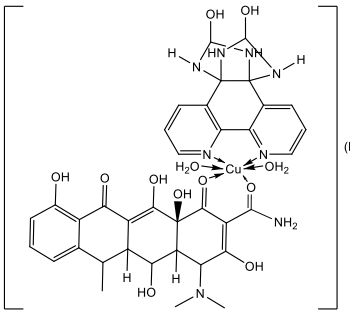
Scheme 1. Proposed structures of the complexes and doxycycline



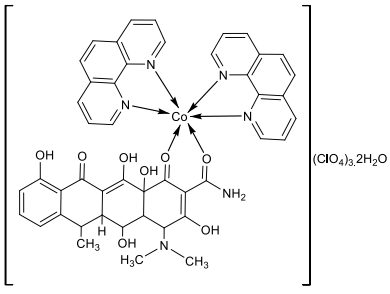
Complex **1**



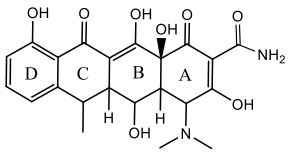
Complex **2**



Complex **3**



Complex **4**



Dox

3.2.1 UV-Vis Spectral Analyses

The UV and visible spectra of doxycycline and the complexes are presented in Fig. **1**. The π-π* transitions of the doxycycline ligands and the diimine ligands in the case of complexes **3** and **4** appeared in the range 210-365. In the visible spectrum of **1**, one band at 547 nm attributed to d–d transition has been found, as expected for distorted octahedral high-spin Co (II) complexes [40]. Two bands which correspond to octahedral geometry of cobalt (III) were seen in the visible region 460-563 nm for complexes **2** and **3** respectively. These bands may be assigned to the transitions $^1A_{1g} \rightarrow {}^1T_{1g}$ (and $^1A_{1g} \rightarrow {}^1T_{2g}$ of Co(III) ion in the pseudo-octahedral symmetry [41]. The electronic spectrum of copper (II) complex **3** showed a broad absorption centred at 625 nm that may be assigned to $^2B_{1g}$ (F)$\rightarrow {}^2A_{2g}$. This broad absorption suggests distorted octahedral geometry as expected from Jahn-Teller effect in hexa-coordinated d^9 metal ion [42]. Dox is the ligand doxycycline with no metal coordinated to it. According to literature [35], the UV-vis absorption spectra of neutral aqueous solutions of free, uncoordinated doxycycline exhibit two main bands centered at 270 and 355 nm. Therefore, the spectrum in the range 400-700 nm was not shown since there is no strong, diagnostic absorption there.

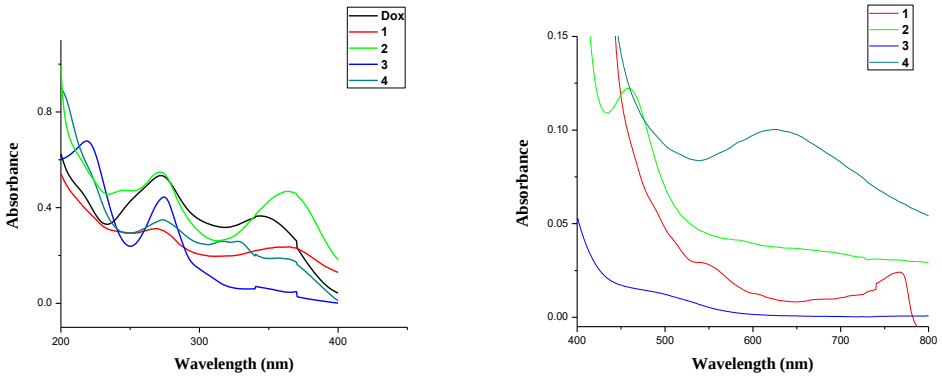


Fig. 1 UV-Vis spectra of doxycycline and complexes **1-4**

3.2.2 Mass Spectral Analyses

The complexes dissociated in the solution phase as evidenced from the ESI mass spectra of **2** and **4** (Figs. 2 and 3). Complex **2** gave peaks at 1429.1, 947.5 and 502.6 corresponding to $[CoDox_2(H_2O)_2+Dox]$, $[CoDox_2]$ and [CoDox] respectively. For complex **4**, there were peaks corresponding to $[M\text{-}2NO_3]^+$, $[M\text{-}2NO_3]^{2+}$ and $[M\text{-}2NO_3+2H_2O]^+$ at 800.8, 401.8 and 837.1 respectively where "M" stands for molecular ion. The mass spectra of other complexes could not be taken because of solubility problems.

3.2.3 Infrared Spectral Analyses

In order to ascertain the coordination sites of doxycycline to the central metal ion we have obtained the infrared spectra of the complexes. The infrared spectra of **1-4** (see supporting information **Fig. 1-4** and Table 1) were compared to that of the ligand and were assigned based on previously published reports [35-39]. For complex **4,** υ(OH) stretching of doxycycline at 3331 cm^{-1} that appeared at 3350 cm^{-1} eliminated the possibility of hydroxyl group participation in coordination. Amide I C=O absorption was absent in complex **4** while amide II absorption (δNH_2 + υC-NH_2) at 1520 cm^{-1} shifted to 1583 cm^{-1}. The C=O absorption of ring C was unchanged and the bands at 1726 cm^{-1} and 1697 cm^{-1} originated from the

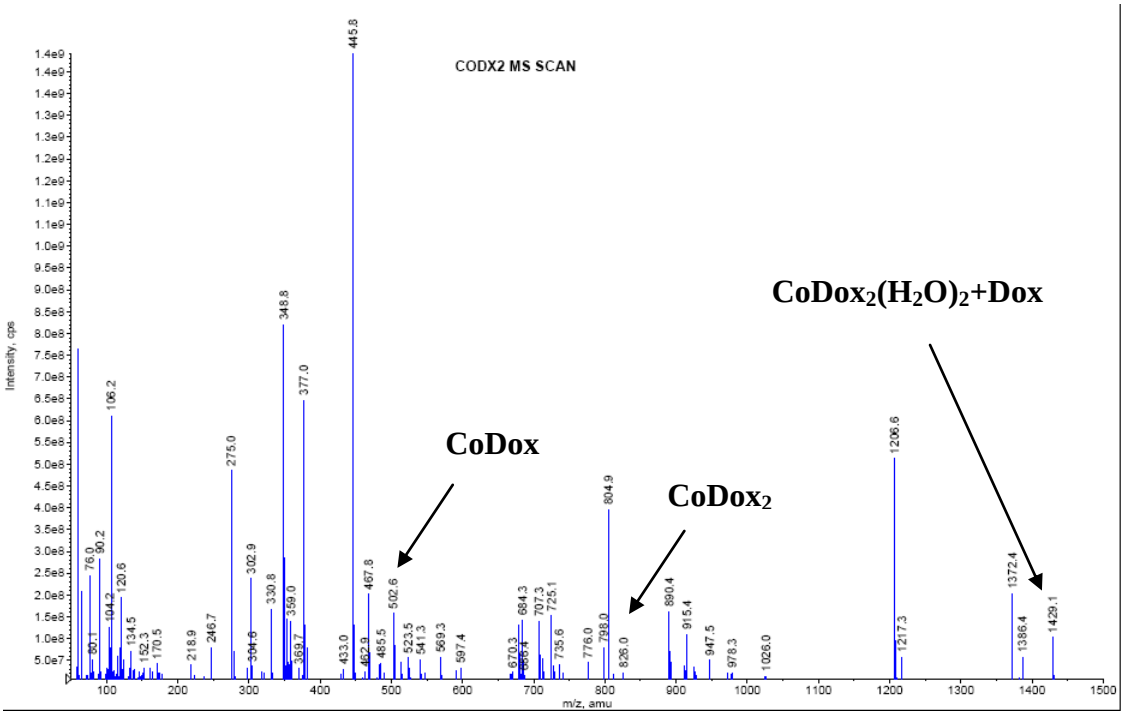


Figure 2. ESI-MS spectrum of complex **2**

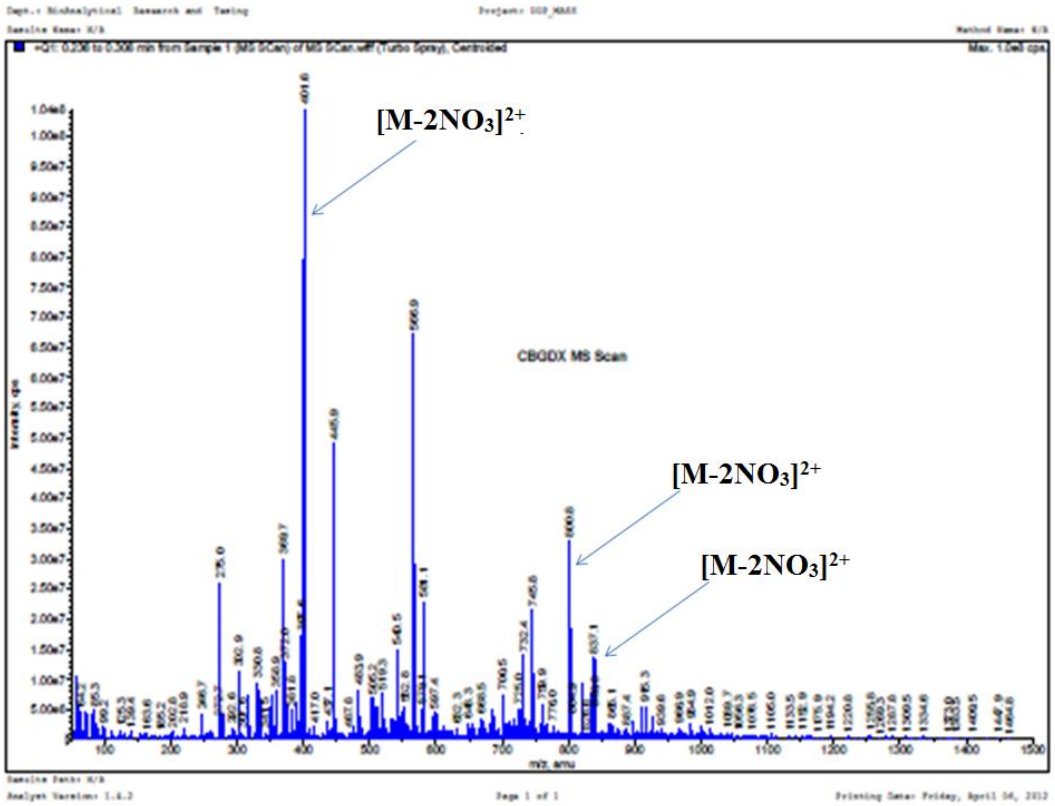


Figure 3. ESI-MS spectrum of complex **4**

C=O absorption of bpg moiety. NH_2 of the amide group was not involved in coordination because δ (NH_2) and υ(NH_2) of doxycyline at 1244 cm^{-1} and 1219 cm^{-1} respectively were essentially unchanged in the complexes. The C=N of bpg moiety appeared at 823 cm^{-1} while NO_3 absorption was seen at 1124 cm^{-1}. The other NO_3 absorption expected at about 1170 cm^{-1} could not be distinguished from doxycycline absorption at 1174 cm^{-1}. ClO_4 absorptions were present in complexes 2 and 3 as identified while CO_3 and NO_3 absorptions were present in complex 1 and 4 respectively (Table1).

Table 1. Assignment of infrared spectra of the complexes

Doxycycline (Dox)	[$CoDox_2(H_2O)_2$]CO_3.$5H_2O$ (1)	[$CoDox_2(H_2O)_2$](ClO_4)$_3$.$6H_2O$ (2)	[$Cophen_2Dox$](ClO_4)$_3$.$2H_2O$ (3)	[$CubpgDox(H_2O)_2$](NO_3)$_2$.$3H_2O$ (4)	Assignment
3452, 3331 3290, 3217	3274,broad	3373 3296, 3182	3435, 3296	3350, 3176	υ-N-H and υ-OH
3010		3057	3078	3097	C-H aromatic
2982, 2883 2839		2879, 2848	2872	2881, 2835	C-H aliphatic
1678, 1616		1726, ca 1610	1662, 1612	1726, 1697, 1614	Amide I C=O, absent in complexes
1520	1577, vs, sh,	1589	1585, 1518	1583	Amide II absorption δNH_2 + υC-NH_2
1458	ca 1500, 1453	1498, 1450	1456, 1427	1454	δ(COH)+ δ(CH_3) of
			1383	1383	BCD Chromophore
1244, 1219	1242, ca 1215	1244, 1217	1240, 1219	1244, 1217	δNH_2 and υC-NH_2
				1124	NO_3
	1080				CO_3
		1089	1095, broad		and ClO_4
	823	823	846	823	C=N of diimine
		507	511		Co-O
	410	445	441		Co-O

a) Infrared spectra of complexes **1**-**3** were similar to that of complex **4**. The new absorptions at 1518, 1427 and 846 cm^{-1} for **3** were ascribed to C=N stretching of phenanthroline while perchlorate absorption appeared at about 1095 cm^{-1} for **2** and **3**. The bands at about 410, 445 and

441 were due to the Co-ligand bonds in **1**, **2** and **3** respectively.Weak Cu–N and Cu-O absorptions were seen in the expected regions.

3.3 Antibacterial Activity

The prevalence of strains *Staphylococcus aureus* resistant to conventional antibiotics had been reported [43]. Antibacterial activities of the complexes were tested as a function of their concentration on *Staphylococcus aureus* and *Klebsiella pneumonia*, pathogens which cause respiratory and urinary tract infections in humans. Data obtained were presented in Figures **3** and **4**. Four concentrations of the complexes were tested i.e. 0.5 mg/ml, 1.0 mg/ml, 1.5 mg/ml and 2.0 mg/ml. The susceptibility of the strains of bacteria towards the complexes were determined by measuring the size of inhibition diameter and compared to that of the parent antibiotic, doxycycline. The activity of complex **3** against *Staphylococcus aureus* was lower than that of the parent ligand, doxycycline, while the others had comparable activity to that of doxycycline especially at high concentration. In the case of *Klebsiella pneumonia*, complexes **1** and **3** had comparable activity to that of doxycycline; complex **2** had higher activity while complex **4** had lower activity at all concentrations used. This kind of observation suggested that the ancillary polypyridyl ligands did not contribute to the antimicrobial activity of the complexes and that the anions played some role in the activity of the complexes. The overall antibacterial activities of these compounds are better that the antibacterial activities of some previously reported compounds [44-46].

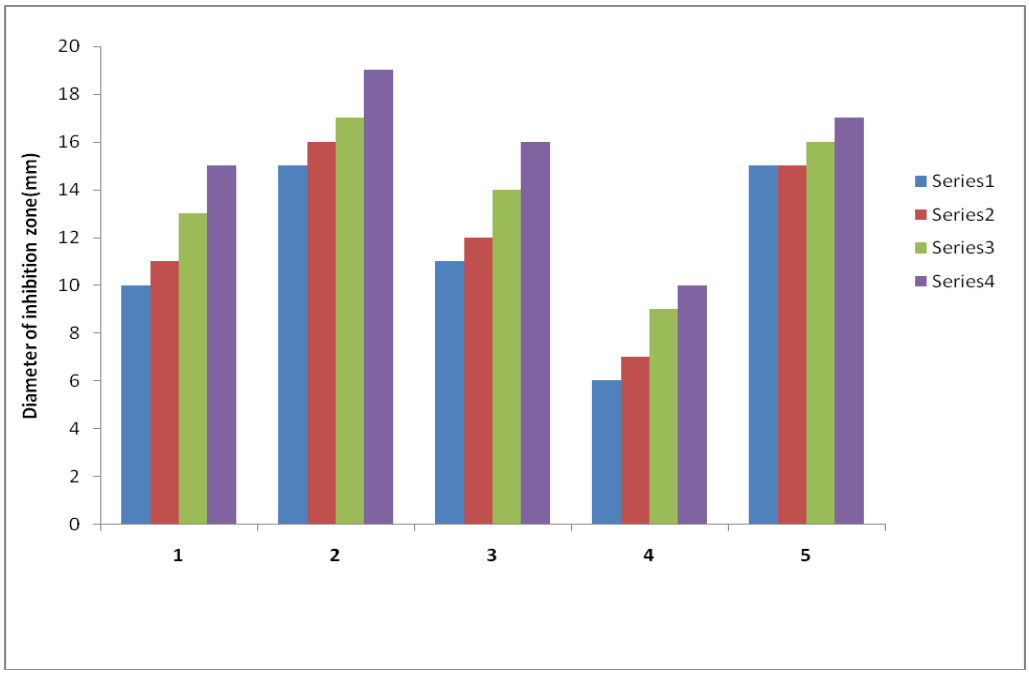


Figure 4. Antibacterial activities of complexes **1**, **2**, **3**, **4** and doxycycline(**5**) on *Klebsiella pneumonia*. Concentrations are 0.5, 1.0, 1.5 and 2.0 mg/ml for series 1, 2, 3 and 4 respectively.

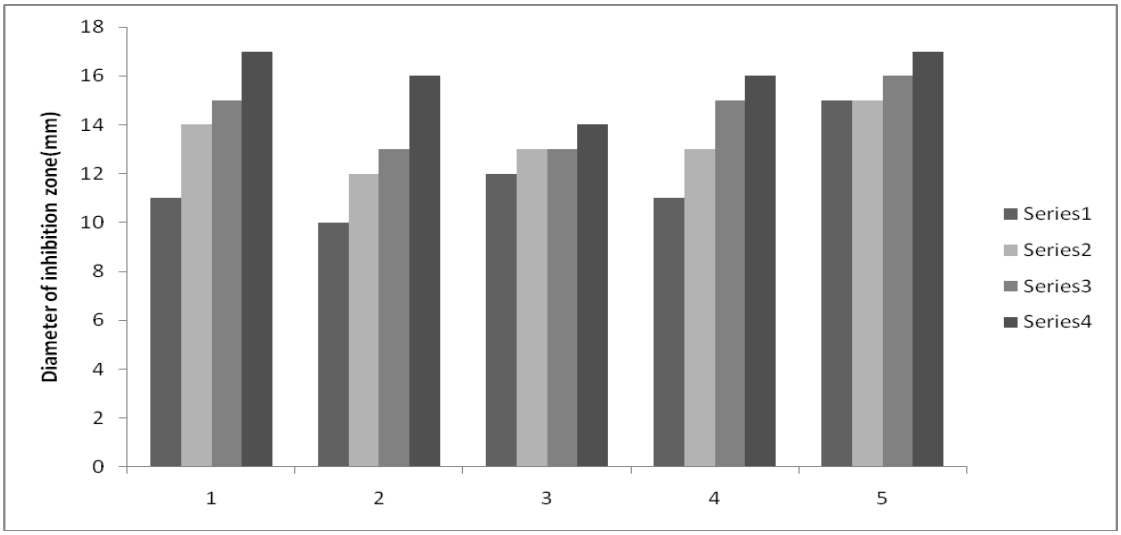


Figure 5. Antibacterial activities of complexes **1**, **2**, **3**, **4** and doxycycline(**5**) on *Staphylococcus aureus*. Concentrations are 0.5, 1.0, 1.5 and 2.0 mg/ml for series 1, 2, 3 and 4 respectively.

4. CONCLUSION

In summary, we present the synthesis of new binary and ternary cobalt (II), cobalt (III) and Cu(II) complexes. The complexes are well characterized by UV-Vis, FT-IR, electrospray mass spectroscopy and elemental analysis. The tentative geometry for the complexes is octahedral. All attempts (i.e. slow evaporation, slow cooling and solvent diffusion techniques) to grow single crystals for X-ray data have failed. The antibacterial activities of these complexes showed that complex **2** had the highest antibacterial activity against *Klebsiella pneumonia*. Complex **2** also had comparable activity with doxycycline against *Staphylococcus aureus*. This makes this compound a valuable addition to the class of potent metal-containing therapeutic agents synthesized by our group [47-50]. Further detailed biological studies on this and other potential therapeutic complexes synthesized by our group are in progress in order to understand the activity of these agents more fully as well as to determine their toxicological profile.

Abbreviations

Phen 1,10-phenanthroline

bpg [4b,5,7,7a-tetrahydro-4b,7a-epiminoethanoimino-6H-imidazo[4,5-f] [1,10] Phenanthroline-6,13-dione]

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