Synthesis, structural investigation and biological application of new mono- and binuclear cobalt (II) mixed-ligand complexes containing 1,10-phenanthroline, acetamide and ethylenediamine

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ABSTRACT

The intensive use of antibiotics has led to an increase of drug resistant bacteria against known antibiotics. To overcome the alarming problem of microbial resistance to antibiotics, the discovery of novel active compounds against new targets is a matter of urgency. Based on this, we report three new mixed ligand complexes of cobalt (II)*viz.* mononuclear ($[Co(phen)_2(Act)(H_2O)]Cl_2.H_2O$ and $[Co(phen)_2(Act)(en)]Cl_2$), and binuclear $[Co_2(phen)_4(Act)_2(en)]Cl_4$ which were synthesized from $CoCl_2.6H_2O$, 1,10-phenanthroline monohydrate, acetamide and ethylenediamine. These complexes were characterized using spectroscopic (ESI-MS, ICP-OES, FT-IR, and UV-Vis), chloride determination as well as melting point and conductance measurement. *Invitro* antibacterial activity was also tested on two Gram positive (*Staphylococcus aureus, Streptococcus pyogenes*) and two Gram negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria using disc diffusion method. The complexes performed well even against the Gram-negative bacteria. The antibacterial result was found comparable with the commercial drug Gentamicin even at lower concentration of complexes. Thus, the synthesized complexes may be considered as potential antibacterial agents after passing cytotoxicity testing.

Keywords: Mixed mono- and binuclear cobalt (II) complex, Bisintercalation, Spectroscopic studies, Antibacterial activity.
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INTRODUCTION

The widespread use and misuse of antibiotics led to a serious public health problem due to bacterial resistance to antibiotics (Golkar *et al.*, 2014; Ventola, 2015; Li and Webster, 2018). According to WHO (2012, 2017), great percentage of hospital-acquired infections are caused by highly resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant enterococci *(*VRE*)* or others (super bacteria: *Escherichia coli, Klebsiella pneumoniae*, etc.). This is expressed in terms of enhanced morbidity and mortality, which makes them survive under the effects of most antibiotics currently in use. Despite the large number of antibiotics and chemotherapeutics available for medical use, parallel emergence of old and new antibiotic resistance created in the last decades revealed substantial medical need for new classes of antimicrobial agents (WHO, 2012a). Treatment of infectious diseases still remains a challenge because of a combination of factors including emerging infectious diseases and the increase in the number of multi-drug resistant microbial pathogens (WHO, 2012b; US Department of Health and Human Services, 2013).

To alleviate this problem, the discovery of novel active compounds with good spectrum of activity and new mechanisms of action against new targets is a matter of urgency (WHO, 2012b; WHO, 2018). From this perspective, the synthesis of new metal complexes and evaluation of their antibacterial activity have been considered by the global community as part of the solution (Saha *et al.*, 2009; Rafique *et al.*, 2010). Based on this, a good number of mononuclear (Ghatole *et al.*, 2012; Pasdar *et al.*, 2015; Atakilt Abebe and Tizazu Hailemariam, 2016; Atakilt Abebe and Getinet Tamiru, 2018), binuclear (Patel *et al.*, 2009; Krishna *et al.*, 2013; Gurumoorthy *et al.*, 2014; Panda *et al.*, 2015;) and metal mixed-ligand complexes have been reported in the literature. In particular, Co (II) containing mixed ligand complexes appeared promising in solving the aforementioned problems (Patel *et al.*, 2009; Chang *et al.*, 2010). In most of these reports, however, Co (II) complexes did not work against Gram-negative bacteria (Shalash and Ali, 2017). This is because their composition prohibited them to penetrate through the cell wall of these bacteria (Gao *et al*., 1999; Podunavac-Kuzmanović *et al*., 2008; Stănilă *et al*., 2011). These phenomena motivated us to look for a solution by synthesis and investigation of biological activities of mononuclear and binuclear mixed-ligand cobalt (II) complexes containing 1,10-phenanthroline (phen), acetamide (act) and ethylenediamine (en) combined. To our knowledge, there is no report with this composition. 1,10-Phenanthroline is rigid planar, hydrophobic, electron-poor, π-acidic, chelating bidentate ligand (Bencini and Lippolis, 2010). These properties enable it to have stacking interaction ability with DNA base pairs that its role in the preparation of medicines mainly targeting the genetic materils is of paramount importance (Chandraleka *et al.,* 2014; Mahalakshmi and Raman, 2016). Acetamide and its derivatives are ligands containing amide moiety acting as an O-, N- donor having a strong ability to form metal complexes and exhibit a wide range of improved biological activities (Kerridge, 1988). Medicinal chemists have carried out considerable research on amide derivatives, Penicillins and cephalosporins, which possess acetamide moiety as the side chain, are widely used antibiotics for the treatment of systemic infections (El-Reash *et al*., 2016; Gull, 2016). Ethylenediamine, its salts and derivatives are frequently found in drugs as well as biologically important molecules (Hast *et al*., 2009). The ethylenediamine portion plays an essential role in the biological activities of the molecules and the structural features of this moiety that confer biological activity are the electron distribution among the atoms and the stereochemistry (Nworie, 2016). Therefore, this study reports the synthesis, structural investigation and antimicrobial activity of new mononuclear and binuclear cobalt (II) mixed-ligand complexes of 1,10-phenanthroline, acetamide and ethylenediamine.

MATERIALS AND METHODS

Chemicals

1,10-phenanthroline monohydrate (BDH Chemical Ltd., Poole, England) (>99%), Acetamide (~99%), Ethylenediamine (>99%), CoCl2.6H2O (>98%), silver nitrate (>99%), acetone (>98%), acetonitrile (>98%), chloroform (>98%), Perchloric acid (70%), methanol (Absolut) (all from Hi Media Laboratories Ltd., India), KBr, dichloromethane, Mueller Hinton agar, and nitric acid (T.V. Industrial Estate, India) were used.

Instruments and Methods

The electronic conductance was measured using 10^{-4} M solution of each complex in distilled water with JENWAY 4200 conductivity meter at room temperature. The electronic spectra were recorded in the 200–800 nm region on cary 60 UV-Vis spectrophotometer. Infrared (FT-IR) spectra were recorded using a Perkin Elmer spectrum BX spectrophotometer in 400-4000 cm^{-1} range in KBr pellets. HR-ESI spectra were conducted on dual ionization ESCi® (ESI/APCi) source options Waters LCT Premier XE (Waters Corp., Manchester, UK) at Academia Sinica, Institute of Chemistry, Taiwan. Cobalt content in each synthesized complex was determined using Perkin Elmer, Optima 7300 VHF Version ICP-OES spectrometer, after digesting each complex in a mixture of concentrated perchloric acid and nitric acid followed by diluting with distilled water. Melting points were determined using SMP30 digital melting point apparatus. Chloride estimations were made thermogravimetrically using the AgCl precipitate obtained from mixture of solution of each complex in distilled water with excess $AgNO_3$ solution (Khopkar, 1998).

Synthesis

New mono- and binuclear cobalt (II) mixed-ligand complexes containing 1,10-phenanthroline, acetamide and ethylenediamine were synthesized using different metal: ligand ratio under optimized reaction condition.

Diaquabis (1,10-phenantroline) cobalt (II) chloride monohydrate $[Co(Phen)_2(H_2O)_2]Cl_2.H_2O$ (1)

A solution of 1,10-phenanthroline monohydrated (1.8399 g, 9.3 mmol) in 30 mL methanol was added drop wise to a magnetically stirred methanolic solution of $CoCl_2.6H_2O$ (1.1043 g, 4.6 mmol) in 100 mL round bottom flask in water bath. The mixture was allowed to stir for 3 h at room temperature and the reaction progress was monitored by TLC. Light-pink homogeneous solution was obtained. The solvent was removed using rotary evaporator. Light-pink powder was collected and washed three times with acetone to remove any unreacted 1,10-phenanthroline. Then, it was recrystallized from methanol.

Yield: (2.4827 g, 98%); FT-IR (KBr pellet, cm^{-1}): 3410 $\nu_{O\text{-}H}$, 1518 $\nu_{C=N}$; UV-Vis (Methanol, nm): 227 $\pi\rightarrow\pi^*$(C=C), 268 $n\rightarrow\pi^*$(C=N), 442, 635, 744 (d-d transition bands); ESI MS (Methanol, m/z):calcd for $[Co(Phen)_2(H_2O)_2\text{-}H^-]$: 454.31$[M\text{-}H]^-$; found: 453.93.

Aquaacetamide ethylene diaminebis (1,10-phenantroline) cobalt (II) chloride monohydrate $[Co(Phen)_2(act)(H_2O)]Cl_2.H_2O$ (2)

To a methanolic solution of $[Co(phen)_2(H_2O)_2]Cl_2.H_2O$ (1.0026 g, 1.8 mmol) in 100 mL round bottom flask and magnetically stirred in water bath, a solution of acetamide (0.1099 g, 1.9 mmol) in 30 mL methanol was added from a dropping funnel. Then, the mixture was refluxed for 1 h at 50-60°C *via* TLC monitoring. The mixture was kept overnight to cool to room temperature. Then, the solvent was removed under vacuum at room temperature and the powder was recrystallized from methanol. Deep-pink powder was collected.

Yield: (0.8291 g, 79%); FT-IR (KBr pellet, cm^{-1}): 3448 (s, br) $\nu_{O\text{-}H}$, 3448 (s, br) $\nu_{N\text{-}H}$, 1545 $\nu_{C=N}$, 1661 $\nu_{C=O}$; UV-Vis (Methanol, nm): 232 $\pi\rightarrow\pi^*$(C=C), 269 $n\rightarrow\pi^*$(C=N), 447, 627, 753 (d-d transition bands).

Acetamidebis (1,10-phenantroline) cobalt (II)-μ-ethylene diamine acetamidebis (1,10-phenantroline) cobalt (II) chloride $[Co_2(phen)_4(Act)_2(en)]Cl_4$ (3)

A solution of ethylene diamine (66.78 μL, 1 mmol) in 30 mL methanol was added from a dropping funnel to a methanol solution of $[Co(Phen)_2(act)(H_2O)]Cl_2.H_2O$ (1.1710 g, 2.0 mmol) being stirred in 100 mL round bottom flask in a water bath at room temperature. The reaction progress was monitored by TLC and the mixture was further refluxed for additional 1 h at 50-60°C resulting in Redish-brown solution. It was left to stand overnight to cool to room temperature. The solvent was removed under vacuum at room temperature and reddish-brown powder was collected, washed with acetone and dried.

Yield: (1.1360 g, 98%); FT-IR (KBr pellet, cm^{-1}): 3436 & 3206$v_{N\text{-}H}$, 1520 $v_{C=N}$, 1648 $v_{C=O}$; UV-Vis (Methanol, nm): 227 π→π*(C=C), 272 n→π*(C=N), 456, 540, 733 (d-d transition bands); ESI MS (Methanol, m/z):calcd for $[Co_2(Phen)_4(Act)_2(en)]^{4+}$:1017.05 [M+]; found: 1016.71

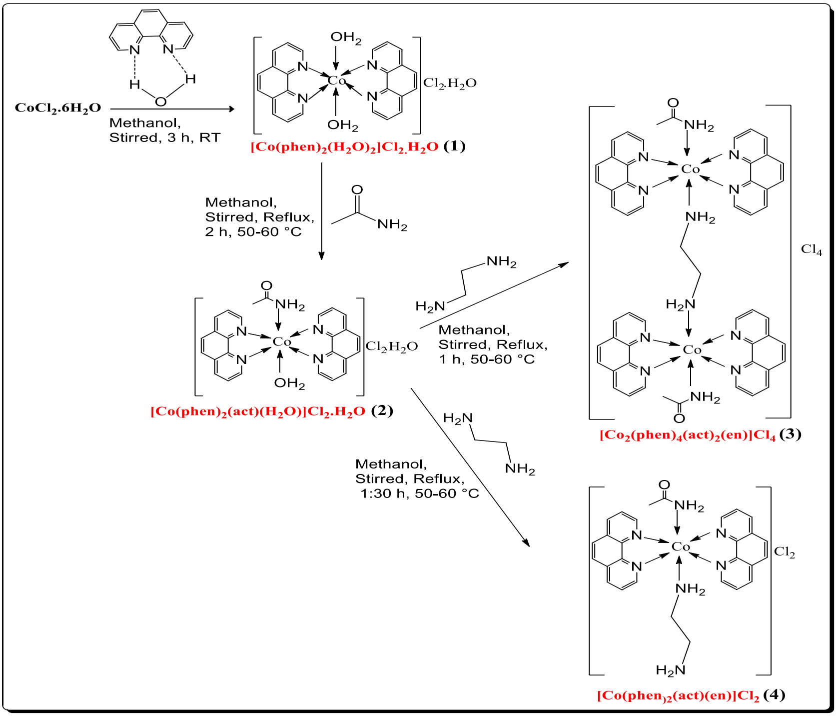
Acetamide ethylene diaminebis (1,10-phenantroline) cobalt (II) chloride $[Co(phen)_2(Act)(en)]Cl_2$ (4)

To a methanolic solution of $[Co(Phen)_2(act)(H_2O)]Cl_2.H_2O$ (0.5855 g, 1.0 mmol) in 100 mL round bottomed flask stirred magnetically in a water bath at room temperature, a solution of ethylene diamine (66.78 µL, 1 mmol) in 20 mL methanol was added from a dropping funnel. The mixture was allowed to stir for 1:30 h and it was further refluxed for 1 h at 50-60°C *via* TLC monitoring. Then it was kept overnight to cool to room temperature. Yellowish precipitate was formed. The precipitate was filtered and washed with acetone and dried).

Yield: (0.5912 g, 97%); FT-IR (KBr pellet, cm^{-1}): 3506 & 3206 $v_{N\text{-}H}$, 1557 $v_{C=N}$, 1649 $v_{C=O}$; UV-Vis (Methanol, nm): 212 π→π*(C=C) , 274 n→π*(C=N), 466, 634, 751 (d-d transition bands); ESI MS (Methanol, m/z): calcd for $[Co(Phen)_2(Act)(en)\text{-}H^-]$: 537.49: found: 537.53.

Antibacterial activity testing

The ligands1,10-phenanthroline (phen), acetamide (act) and ethylenediamine (en) and their metal complexes (**1**), (**2**), (**3**) and (**4**) were evaluated for *in vitro* antibacterial activities against strains of two Gram-positive *Staphylococcus aureus* (*S. aureus* and *Streptococcus pyogenes (S. pyogenes)* and two Gram-negative *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumonia*) bacteria. They were investigated by disc diffusion methods. Muller Hinton agar (MHA) and nutrient Blood agar (BA) were used for culturing the bacterial isolates while diagnostic sensitivity test agar (Oxoid Ltd BASINGSTOK England) was used for sensitivity. The bacterial strains were maintained in the appropriate blood agar base at 4°C. Antibiotic discs gentamicin was used as reference. The minimum inhibitory concentration (MIC) against each bacterium was determined by preparing aqueous solutions of different concentrations of the complexes by serial dilution (100, 200, 300, 400, 500 and 600 mg/L). The experiments were repeated three times to ascertain consistency in results. The antibacterial tests were carried out at Bahir Dar University, Department of Biology, Microbiology Laboratory, Bahir Dar, Ethiopia.



Scheme 1: Synthesis path of the Co (II)-complexes

RESULTS AND DISCUSSION

Physical characterization of Co (II) complexes

The salts (**1**), (**2**), (**3**) and (**4**) were stable in air. They were soluble in water, methanol, ethanol, acetonitrile, and DMSO. The analytical data of the complexes were in agreement with the assigned formulae (Table 1) (Saha *et al.*, 2009).

Molal conductance of the metal complexes. The conductance measurements, recorded for 10^{-4}M solutions of the metal complexes in deionized water, are listed in Table 1. The result confirmed the strong electrolytic nature of the complexes and the cation: anion ratios are in agreement with the proposed structure of both mono- and binuclear Co(II) complexes. Nevertheless, the conductivity of the binuclear complex, $[Co_2(Phen)_4(Act)_2(en)]Cl_4$, was lower than that of corresponding mononuclear complex $[Co(Phen)_2(Act)(en)]Cl_2$. This is a consequence of increase in molar mass, surface area andhydration as a result of increase in the charge of the cation. Hence, the speed of cation mobility decreases due to the decrease in the kinetic energy imparted by the electric field from measurement instrument (Refat *et al.*, 2013).

FT-IR spectroscopy

The infrared spectra, selected characteristic frequencies of the ligands and the complexes are listed in Table 2. The characteristic stretching bands in free ligand 1,10-phenanthroline monohydrate at 1635 cm^{-1} (s), 1584 cm^{-1} (s) and 3437 cm^{-1} (s) due to $\nu_{C=C}$, $\nu_{C=N}$ and $\nu_{O\text{-}H}$ (H_2O), respectively, shifted to 1621 cm^{-1} (s), 1518 cm^{-1} (s) and 3410 cm^{-1} (s, br) in $[Co(Phen)_2(H_2O)_2]Cl_2.H_2O$, indicated coordination of 1,10-phenanthroline. The broadness of the band at 3410 cm^{-1} (s and b) in $[Co(Phen)_2(H_2O)_2]Cl_2.\ H_2O$ is presumably due to increasing in the number and type (coordinated and crystallization) of water molecules (Figure 1a & d).

Table 1. Physical characteristics of Co (II) complexes

Complex /color	**Molecular weight (g/mol)**	**Melting point (ºC)**	**Yield (%)**	**Elemental estimation calculated (found) (%)**		**Molar conductivity ΛM (Scm^2mol^{-1})**
				Co	***Cl***	
$[Co(phen)_2(H_2O)_2]Cl_2.H_2O$ Light-pink	544.41	>228 (decompose)	98	2.8 (2.8)	13.0 (13.0)	243.8
$[Co(phen)_2(act)(H_2O)]Cl_2.H_2O$ Deep-pink	585.47	>314 (decompose)	79	3.2 (3.2)	12.1 (12.0)	357.1
$[Co_2(Phen)_4(Act)_2(en)]Cl_4$ Redish-brown	1159.15	95-97 (melt)	98	4.1 (3.9)	12.3 (12.2)	543.4
$[Co(phen)_2(act)(en)]Cl_2$ Yellow-violet	609.49	256-258 (melt)	97	9.7 (9.4)	11.7 (11.5)	629.8

Table 2. Charcteristic vibration frequence of ligands and complexes

Compound	**Absorption frequencies (cm^{-1})**								
	v_{O-H}	v_{N-H}	v_{C-H}	$v_{C=C}$	$v_{C=N}$	v_{C-N}	$v_{C=O}$	v_{Co-O}	v_{Co-N}
1,10-phenanthroline	3437	-	3029	1635	1589	1328	-	-	-
Acetamide	-	3372 3194	2809	-	-	1390	1672	-	-
Ethylenediamine	-	3424 3337	2925	-	-	1314	-	-	-
$[Co(Phen)_2(H_2O)_2]Cl_{2.}\ H_2O$	3410	-	3040	1621	1518	1341	-	642	510
$[Co(Phen)_2(Act)(H_2O)]Cl_2\ .H_2O$	3448 (s, br)	3448 (s, br)		1621	1545	1403	1661	637	548
$[Co_2(Phen)_4(Act)_2(en)]\ Cl_4$	-	3436 3206		1621	1520	1405	1648	-	580
$[Co(Phen)_2(Act)(en)]\ Cl_2$	-	3506 3206	2964	1621	1557	1410	1649	-	580

The coordination of acetamide to $[Co(Phen)_2(H_2O)_2]Cl_2.H_2O$ to obtain $[Co(Phen)_2(Act)(H_2O)]Cl_2.H_2O$ was confirmed from the appearance of new band at 1672 cm^{-1} (s) characteristic for $\nu_{C=O}$, in addition to the shifts observed on the characteristic absorption frequencies of the former complex. However, the characteristic N-H (NH_2) stretching band in free acetamide disappeared from the complex which indicated that it is coordinated to Co (II) *via* its amine nitrogen (Figure 1e). The coordination of ethylenediamine to $[Co(Phen)_2(Act)(H_2O)]Cl_2.H_2O$ to get $[Co(Phen)_2(act)(en)]Cl_2$ is confirmed by the appearance of new bands at 3512 cm^{-1} (s, b) and 3206 cm^{-1} (s) which are characteristic to stretching frequencies of ν_{N-H} (NH_2). However, the latter bands disappeared in $[Co_2(Phen)_4(act)_2(en)]Cl_4$ which is most probably because the second nitrogen is involved in the coordination to Co (II). The latter is a good confirmation for the formation of the binuclear complex (Atkins,1994; Housecraft and Sharpe, 2005; Tigineh and Liu, 2014).

Electronic absorption spectra

The spectral data of the compounds in methanol are presented in Figure 2 and Table 3. The two absorption bands at in the ultraviolet region, namely 232, 264 nm, in Figure 2a assigned to n→π* (C=N) and π→π*(C=C) transitions for molecular 1,10-phenanthroline appeared shifted towards 227, 268 nm in $[Co(Phen)_2(H_2O)_2]Cl_2.H_2O$. Furthermore, the latter absorption bands appeared towards lower and higher frequencies, confirming the coordination of the other ligands to the metallic ions to give $[Co(Phen)_2(act)(H_2O)]Cl_2$ $.H_2O$, $[Co(Phen)_2(act)(en)]$ Cl_2 and $[Co_2(Phen)_4(act)_2(en)]$ Cl_4.

The visible electronic absorption spectrum of the cobalt (II) complex is dominated by the $^4T_{1g}(F)\rightarrow{}^4T_{1g}(P)$, $^4T_{1g}(F)\rightarrow{}^4A_{2g}(F)$ and $^4T_{1g}(F)\rightarrow{}^4T_{2g}(F)$ transition, which is a typical one for octaedral Co (II) complexes (Iniama and Iorkpiligh, 2013; Bharathi Krishnan, 2016).

MS spectroscopy

The ESI MS spectrum of the complexes recorded dissolving in methanol showed characteristic molecular ion peaks at M/z = 453.93, 537.53, and 1016.71 corresponding to $[Co(Phen)_2(H_2O)_2\text{-}H^-]$, $[Co(Phen)_2(Act)(en)\text{-}H^-]$and $[Co_2(Phen)_4(Act)_2(en)]^{4+}$, respectively (Figure 3a-c). This characterization compounded the evidences found in the former techniques in confirming the achievement of the intended complexes.

Table 3. Electronic spectral data of the salt and complexes

Compounds	**Absorption bands (nm)**	**Assignments**
1,10-phenantroline	232, 264	π–π^*(C=C) and (C=N), n $\rightarrow\pi^*$
Acetamide	261, 280	π–π^*(C=O), n $\rightarrow\pi^*$
Ethylene diamine	289	n $\rightarrow\pi^*$
$CoCl_2.6H_2O$	239, 331, 427	d-d transitions bands
$[Co(Phen)_2(H_2O)_2]Cl_2$. H_2O	227, 268 442 635 744	$\pi\rightarrow\pi^*$(C=C), n$\rightarrow\pi^*$(C=N) $^4T_{1g(F)}\rightarrow{}^4T_{1g(P)}$ $^4T_{1g(F)}\rightarrow{}^4A_{2g(F)}$) $^4T_{1g(F)}\rightarrow{}^4T_{2g(F)}$
$[Co(Phen)_2(act)(H_2O)]Cl_2$ .H_2O	232, 269 447 627 753	$\pi\rightarrow\pi^*$(C=C), n$\rightarrow\pi^*$(C=N) $^4T_{1g(F)}\rightarrow{}^4T_{1g(P)}$ $^4T_{1g(F)}\rightarrow{}^4A_{2g(F)}$) $^4T_{1g(F)}\rightarrow{}^4T_{2g(F)}$
$[Co_2(Phen)_4(act)_2(en)]$ Cl_4	227, 272 456 540 733	$\pi\rightarrow\pi^*$(C=C), n$\rightarrow\pi^*$(C=N) $^4T_{1g(F)}\rightarrow{}^4T_{1g(P)}$ $^4T_{1g(F)}\rightarrow{}^4A_{2g(F)}$) $^4T_{1g(F)}\rightarrow{}^4T_{2g(F)}$
$[Co(Phen)_2(act)(en)]$ Cl_2	212, 274 466 634 751	$\pi\rightarrow\pi^*$(C=C), n$\rightarrow\pi^*$(C=N) $^4T_{1g(F)}\rightarrow{}^4T_{1g(P)}$ $^4T_{1g(F)}\rightarrow{}^4A_{2g(F)}$) $^4T_{1g(F)}\rightarrow{}^4T_{2g(F)}$

Structural investigation

The molar conductivity values of the complexes revealed that they were electrolytic in nature implying that the chloride ions were not in coordination sphere of complexes. The %Cl was determined gravimetrically using aqueous solution of $AgNO_3$ and %Co was obtained *via* ICP-OES after digesting the complexes in strong acid followed by dilution which agreed with the theoretical values of chloride and metal estimation, respectively, and that meant that the proposed formula of complexes was achieved.

The observed shifts in band position and appearance of new non-ligand bands in the complexes infrared spectra confirmed the successful coordination of ligands to metal center in the corresponding complexes. The UV-Visible spectra verified an electronic redistribution and change in band appearance of d-d transition, additionally supported formation of complexes. The three d→d transitions resulting from [$^4T_{1g(F)}\rightarrow{}^4T_{2g(F)}$], [$^4T_{1g(F)}\rightarrow{}^4A_{2g(F)}$] and [$^4T_{1g(F)}\rightarrow{}^4T_{1g\ (P)}$] for all the complexes confirming an octahedral structure as reported in many octahedral cobalt (II) complexes (Iniama and Iorkpiligh, 2013; Bharathi Krishnan, 2016). Furthermore, the ESI-MS results of complexes being in an excellent agreement with the molecular weight of proposed structures of Co (II)-complexes further confirmed the composition and in turn the proposed structures of Co (II)-complexes (**Scheme1)**.

In vitro antimicrobial activity

This experiment showed that the complexes were biologically active against all tested bacteria. The observed increase in antibacterial activity compared to the ligands could be explained on the basis of Overton's concept (Anjaneyulu and Rao, 1986) and Tweedy's chelation theory (Tweedy, 1964).

An important condition for antimicrobial activity of a compound is its ability to pass through the lipid membrane that surrounds the cell. On coordination, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbitals and partial sharing of the positive charge of the metal ion with the donor groups which significantly increases the lipophilicity of the complex. This increased liposolubility enhances the penetration of the complexes into the lipid membrane and interferes with the normal activities of the bacteria (Tweedy, 1964; Anjaneyulu and Rao, 1986; Podunavac-Kuzmanović *et al*., 2008; Warra, 2011; Al-Resayes *et al*., 2016).

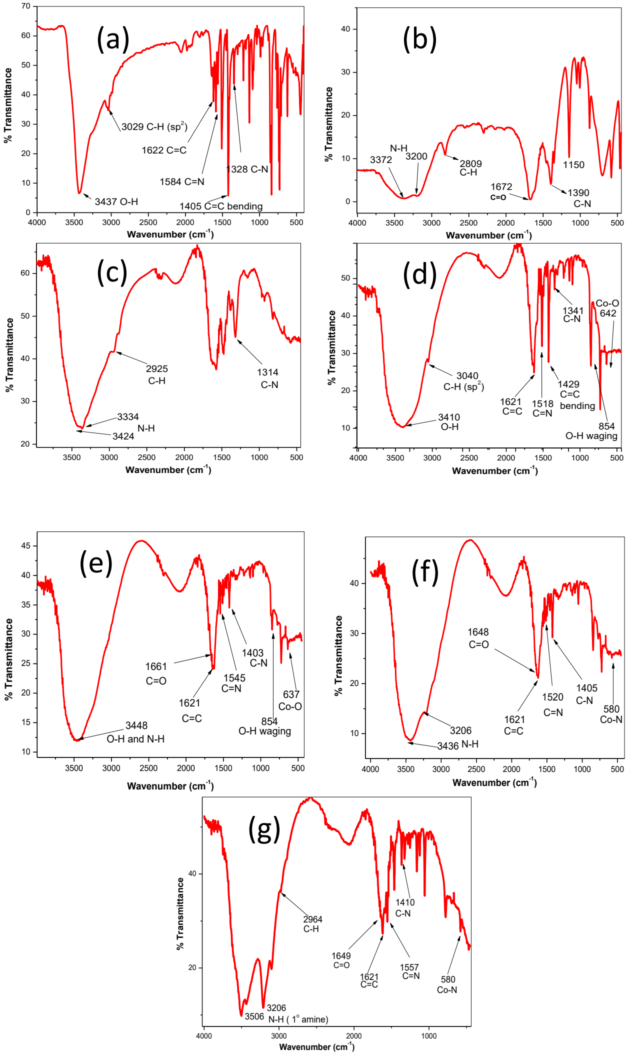


Figure 1. FTIR spectra of (a) 1,10-phenanthroline monohydrate, (b) Acetamide, (c) Ethylenediamine, (d) $[Co(Phen)_2(H_2O)_2]Cl_2.\ H_2O$(1), (e) $[Co(Phen)_2(Act)(H_2O)]Cl_2.H_2O$(2), (f) $[Co_2(Phen)_4(Act)_2(en)]Cl_4$(3), (g) $[Co(Phen)_2(Act)(en)]Cl_2$(4).

Minimum Inhibitory Concentration (MIC) Determination: MIC is the lowest concentration that completely inhibited the growth of microorganisms for 24 hours. The MIC of $[Co(Phen)_2(Act)(H_2O)]Cl_2.H_2O$ was found to be 100 µg/L. Compared to the two newly synthesized mononuclear mixed ligand complexes, the new binuclear mixed ligand complex showed the greatest activity and the result obtained was even better than the antimicrobial activity of commercially available drug Gentamicin (Table 4, Figure 4).

Table 4. Results of the disc diffusion method of the metal salt, ligands and its metal complexes

Compounds in 800 µg/L	**Inhibition zone (mm)**	
	S. aureus (+)	***S. pyog (+)***
$[Co(Phen)_2(H_2O)_2]Cl_2.\ H_2O$	12±0.15	13±0.06
$[Co(Phen)_2(Act)(H_2O)]Cl_2.H_2O$	13±0.10	16±0.20
$[Co_2(Phen)_4(Act)_2(en)]Cl_4$	34±0.06	40±0.06
$[Co(Phen)_2(Act)(en)]Cl_2$	14±0.06	12±0.15
Gentamicin	25±0.10	21±0.10
	E. coli (-)	***K. pne (-)***
$[Co(Phen)_2(H_2O)_2]Cl_2.\ H_2O$	17±0.10	15±0.00
$[Co(Phen)_2(Act)(H_2O)]Cl_2.H_2O$	16±0.06	12±0.00
$[Co_2(Phen)_4(Act)_2(en)]Cl_4$	35±0.21	34±0.06
$[Co(Phen)_2(Act)(en)]Cl_2$	15±0.06	12±0.15
Gentamicin	25±0.12	27±0.00

The high antimicrobial activity of the binuclear mixed ligand complex is probably because of bisintercalation due to the two planar intercalating phenanthroline ring systems covalently linked *via* ethylenediamine. Furthermore, configurational freedom through the bridge presumably enhanced its flexibility and in turn the penetration power of the complex (Feng *et al.*, 2017). All complexes were found water soluble indicating their compatibility with normal human physiological systems and their potential for human medication after *in vivo* cytotoxicity tests.

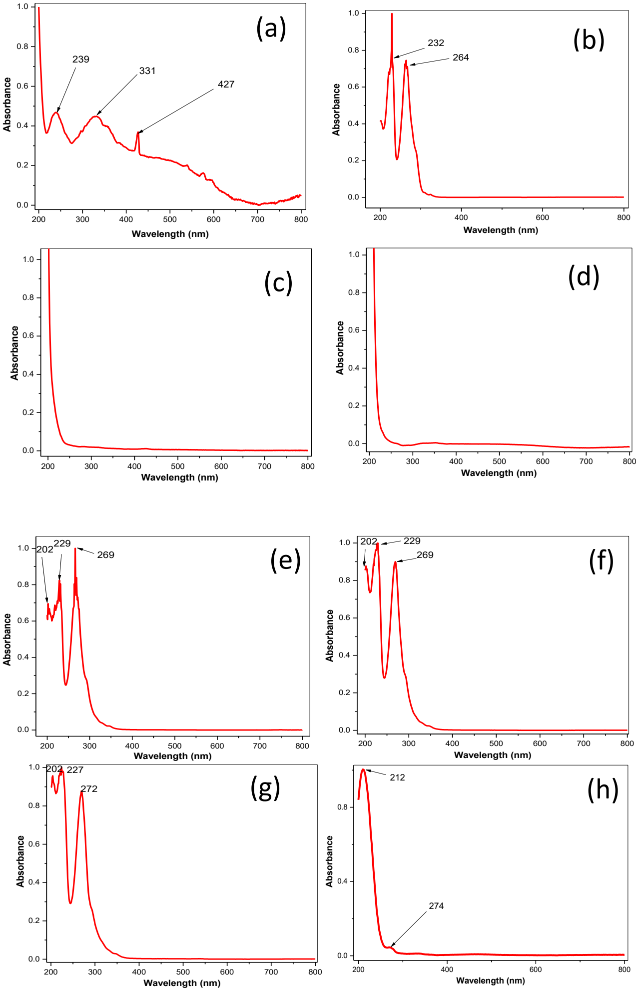


Figure 2. UV-Vis spectra of (a)$CoCl_2.6H_2O$, (b) 1,10-phenanthroline monohydrate, (c) Acetamide, (d) Ethylenediamine, (e) $[Co(Phen)_2(H_2O)_2]Cl_2.\ H_2O$(1), (f) $[Co(Phen)_2(Act)(H_2O)]Cl_2\ .H_2O$(2), (g) $[Co_2(Phen)_4(Act)_2(en)]Cl_4$(3), (h) $[Co(Phen)_2(Act)(en)]\ Cl_2$(4).

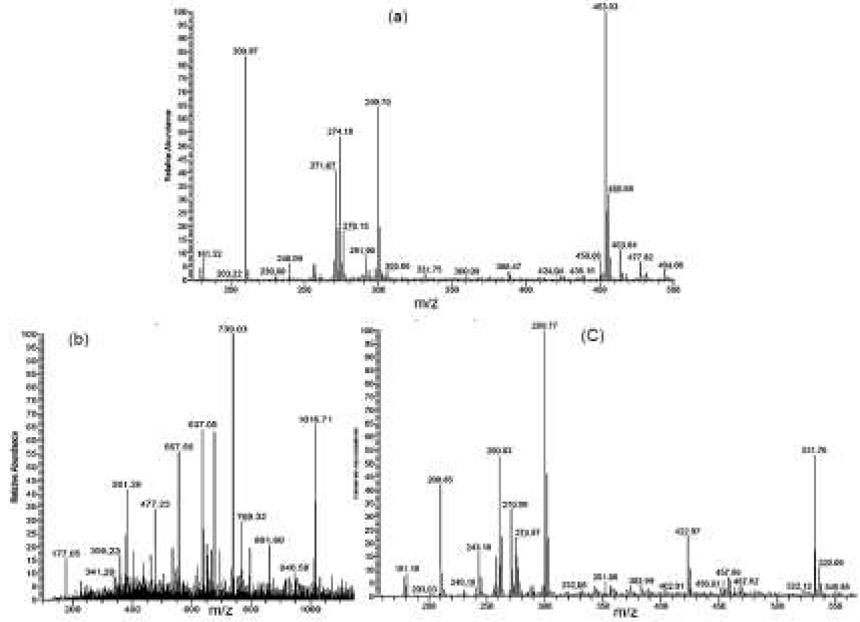


Figure 3. ESI MS spectra of (a) $[Co(Phen)_2(H_2O)_2]Cl_2 . H_2O$(1),(b) $[Co_2(Phen)_4(Act)_2(en)]Cl_4$(3),(c) $[Co(Phen)_2(Act)(en)]Cl_2$(4).

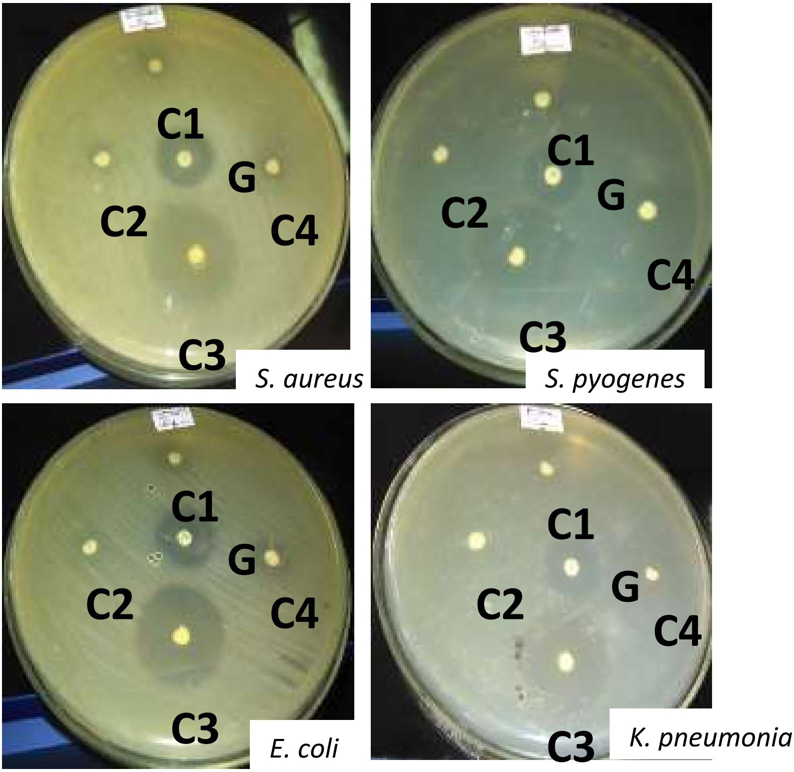


Figure 4. The inhibition observed by the actions of complexes and commercially available antibiotic on Gram-positive bacteria (*S. aureus* and *S. pyogenes*) and Gram-negative bacteria (*E. coli* and *K. pneumoniae*). Where C1, C2, C3 and C4 stand for complex (1), (2), (3) and (4), respectively, and G is for Gentamicin.

CONCLUSION

In this work, two new mononuclear and one binuclear cobalt (II) complexes were synthesized. Based on various physical, chemical and spectroscopic investigations, octahedral geometry has been suggested for all synthesized complexes. *In vitro* antibacterial tests revealed that all the synthesized complexes exhibited antibacterial activity. Most Co (II) complexes are known to be active only against Gram-positive bacteria. However, the synthesized complexes showed awide-range of activities even against the Gram-negative bacteria (*E. coli* and *K. pneumoniae)*. Thus, after *in vivo* cytotoxicity investigation, these complexes could be considered as a potential alternative antibiotic metal-based drugs.

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Competing Interests

There is no conflict of interest among the authors and the funding institution.

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