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INORGANIC CHEMISTRY | RESEARCH ARTICLE

A hexacationic coordination compound from Co (II) and a cationic ligand derived from 4,4′-bipyridine: Synthesis, characterization and investigation for biological application

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Abstract: A hexapositively charged tetrahedral geometry Co(II) complex was synthesized using a new positively charged ligand prepared quaternizing 4,4′-bipyridine by 1-bromobutane. It is characterized by spectrometry (^{1}H NMR, ESI MS, Uv-Vis, ICP OES), carbon, hydrogen and nitrogen (CHN) elemental analysis, halide estimation and conductivity measurement. The complex showed significantly high molar conductivity value in water (640.83 S mol^{-1} cm^2). Its *in vitro* biological activities were tested on one gram-positive (*Staphylococcus aureus*) and one gram-negative (*Klebsiella pneumoniae*) bacteria. It was found active against both bacteria with improved and better activity against the latter.

Subjects: Biochemistry; General Science; Pharmaceutical Science

Keywords: Hexapositively charged complex; cationic ligand; antibacterial acivity

1. Introduction

Anticancer experiments usually target DNA in drug tests (Eriksson, Leijon, Hiort, Norden, & Graeslund, 1994; Lying, Rodger, & Nordén, 1991; Lyng, Rodger, & Nordén, 1992). This is because

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Atakilt Abebe's research group consisted of some staff members of Bahir Dar University and post-graduate (M.Sc.) students. Dr. Atakilt Abebe is a full-time associate professor of Inorganic Chemistry at Bahir Dar University. He received B. Sc. in Chemistry and M.Sc. and Ph.D. in Inorganic Chemistry from Addis Ababa University, Ethiopia. Atakilt Abebe's research group works on the development of metal-based drugs employing heteroaromatic flat molecules as ligands as well as incorporation of phytochemicals with medicinally potent transition metal ions for antibacterial as well as anticancer purposes. Furthermore, the group is working on the synthesis of organic salts aiming for medicinal applications as an alternative to metal-based approaches. In this category, synthesis of new ionic liquids for electrochemistry and as modulators in the properties of materials is also carried out. Atakilt Abebe has authored/coauthored more than 15 peer-reviewed original research articles.

PUBLIC INTEREST STATEMENT

The rapid increase in the number of multidrug-resistant pathogens and cellular-based diseases is fast becoming a global concern. Thus, the discovery of novel active compounds against new targets is a matter of urgency. The conventional approach to address this impulse employing materials originating from wild growing plants and animals has shortened their life span. Apart from studying their attractive chemistry, this problem has initiated the scientific community to consider and investigate transition metal complexes as an alternative solution.

This is the first objective of our group. Furthermore, efficient and environmentally friendly applications of newly prepared materials have been the focus of attention of the global community. Thus, investigation and preparation of materials fulfilling this objective is the second objective of the group.

the drug molecules interfere with tasks of DNA and block cell division which results in the death of problematic cells (Hemmert et al., 2001; Li et al., 1996; Dasari et al., 2014). Therefore, the primary tasks are identification and/or designing of molecules having the ability to interact with DNA (Abdel & Baker, 2017; Abebe, Atlabachew, Liyew, & Ferede, 2018; Ma, Chan, Lee, Kwan, & Leung, 2011; Sheng, Gan, & Huang, 2013). In this regard, molecules with rigid planar or approximately planar structure systems may attract significant consideration (Zimmerman, 1991; Tawani, Amanullah, Mishra, & Kumar, 2016; Yadav et al., 2017; El-Kalyoubi et al., 2017). 4,4′-Bipyridine fulfills these criteria. It has two potential binding nitrogen atoms with a lone pair of electrons which are arranged in a divergent fashion. The pyridine parts of 4,4′-bipyridine freely rotate along a central C–C bond and help in its stacking ability to form the twisted conformation of DNA. 4,4′-Bipyridine is an electron-poor heteroaromatic, π-acidic bidentate ligand. Recognizing its electronic characteristics as well as the proved bridging ligand behavior, several investigations on its coordination compounds with various transition metals for material and device applications are reported (Berben, Faia, Crawford, & Long, 2006; Zhu et al., 2016). In this regard, 4,4′-bipyridine continued to generate significant interest for its potential applications in organic conductors and magnetic materials (Dai et al., 2003; Ferreira, Alcácer, & Morgado, 2011; Mortimer, Dyer, & Reynolds, 2006). However, its aforementioned attractive structure makes it a toxic molecule to be directly employed for biological applications. This is because of its ability to removing essential metal ions from biological systems (Li, Crooks, Wei, & Leon, 2004). It is probably due to this, as far as we are aware, none of the transition metal(II)–4,4′-bipyridine complexes investigated for medicinal applications is known.

In the present work, an attempt for the employment of the convenient features of 4,4′-bipyridine for biological applications is made. This was done by quaternizing one of its nitrogen atoms and then coordinating to cobalt(II) through the unquaternized one. The purpose of quaternization was to remove the bidentate property. Moreover, coordination to the metal ion serves, on the one hand, to avoid the coordination and removal of essential metal ions from the biological system. On the other hand, it tunes the physicochemical properties of the transition metal that the penetration ability into the cellular target for medicinal applications is improved (Lawrence, 2010; Sears, Joyce, & Turro, 2010; Wilkins, 1974).

2. Experimental

2.1 Materials and methods

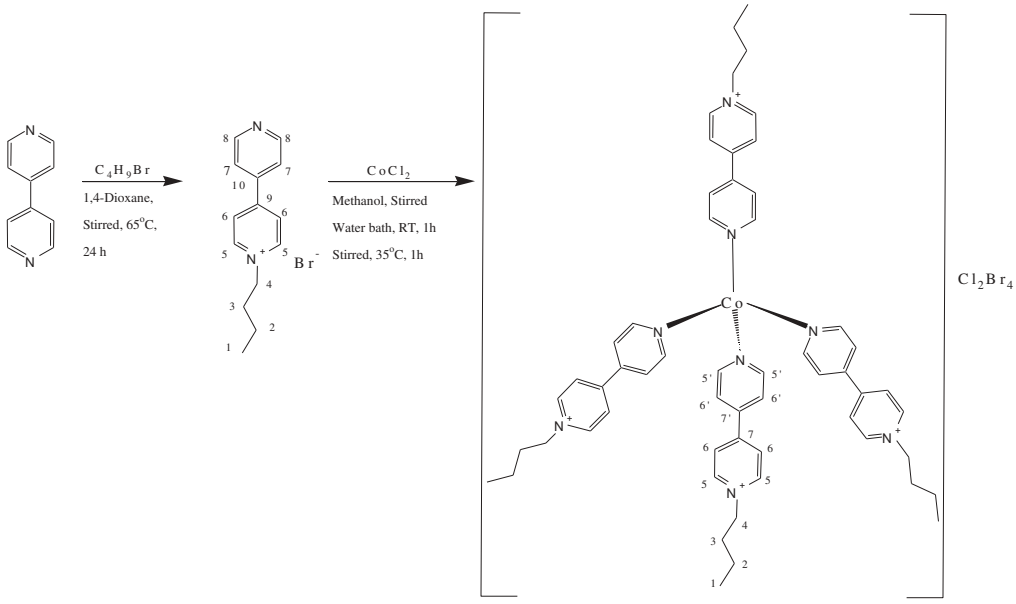
4,4′-Bipyridine (99%), 1-bromobutane, 1,4-dioxane and acetonitrile are obtained from Sigma–Aldrich and are used as received.

^{1}H NMR, using a Bruker AM-270 (270 MHz) and BRUKER 400 MHz spectrometer, was employed to confirm the structures and check the purity of the synthesized ligand salt and complex. ESI MS was used to determine the molecular ion mass of the ligand and complex using Bruker Micro TOF. 5E-CHN2200 elemental analyzer was employed for CHN analysis taking 15 mg sample. Bromide and chloride estimation was conducted taking 10 mg sample dissolved in 20 mL distilled water. Excess $AgNO_3$ solution was added for the formation of silver bromide (AgBr and AgCl) precipitate. Then, the cruddy white precipitate formed was filtered and dried in an oven at 110°C, and the amount of bromide and chloride was calculated from the weight difference. The conductivity of the complex for 6×10^{-4} M solution in deionized water was also investigated using Bante901P portable pH/conductivity/TDS meter at room temperature. Its melting point was measured by Stuart SMP30 melting point apparatus.

2.2. Synthesis of N-butyl-4,4′-bipyridinium bromide, [C_4Bipyr]Br, the ligand

[C_4Bipyr]Br was prepared following a reported procedure (Abebe et al, 2013). To a 4 g (0.0256 mol) of 4,4′-bipyridine dissolved in 30 mL dry 1,4-dioxane in a two-necked 100 mL round-bottomed flask fitted with a condenser, 3.52 g (0.0257 mol, 2.45 mL) of 1-bromobutane dissolved in 10 mL dry 1,4-dioxane was added from a dropping funnel, and the mixture was allowed to stir at 65°C. After 24 h, the reaction was allowed to cool down to room temperature and white precipitates were filtered.

Scheme 1. Synthesis path for the ligand and complex.



2.3. Synthesis of tetrakis-(N-butyl-4,4′-bipyridinium)cobalt(II) tetrabromidedichloride ($[C_4Bipyr)_4Co]Cl_2Br_4$)

To a methanolic solution of $CoCl_2$ (0.0145 g, 0.1168 mmol) being stirred magnetically in a water bath at room temperature, a methanolic solution of N-butyl-4,4′-bipyridinium bromide (0.1304 g, 0.47 mmol) was added from a dropping funnel and stirred for 1 h. The mixture was stirred for 1 h at 35°C. A light pink-colored homogeneous solution was obtained. The methanol was removed in vacuum. The blue powder was collected and washed three times with acetone to remove any excess 1-butyl-N-4,4′-bipyridinium bromide. It was recrystallized from methanol to remove any unreacted $CoCl_2$ (yield: 0.1372 g, 94.68%). The synthesis path is indicated in Scheme 1.

2.3.1. Antibacterial activity testing

The ligand and its metal complex were evaluated for *in vitro* antibacterial activities against strains of one gram-positive (*Staphylococcus aureus*) and one gram-negative (*Klebsiella pneumoniae*) bacteria. The bacterial strains were maintained in the appropriate blood agar base at 4ºC. An antibiotic disc (gentamicin 10 µg) 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. The experiments were repeated three times to obtain consistent results. The antibacterial tests were carried out at Bahir Dar University, Department of Biology, Microbiology Laboratory, Bahir Dar, Ethiopia.

2.3.2. ^1H NMR

The ^1H NMR spectra of the ligand and complex are indicated in Figure 1.

2.3.3. Mass spectra

The electron spray ionization (ESI MS) spectra of the molecular ion peaks for the ligand and complex are presented in Figure 2.

3. Results and discussion

In the synthesis of the ligand, the developed positive charge on the quaternized nitrogen removed the symmetry of 4,4′-bipyridine which is confirmed from the appearance of four and six peaks in ^1H and ^{13}C NMR spectra in the aromatic region of the ligand, respectively (Figure 1(a, b)). This is good evidence that only a monoquaternary N-butyl-4,4′-bipyridinium bromide as a product was obtained. Otherwise, only two and three peaks, respectively, would have appeared in this region.

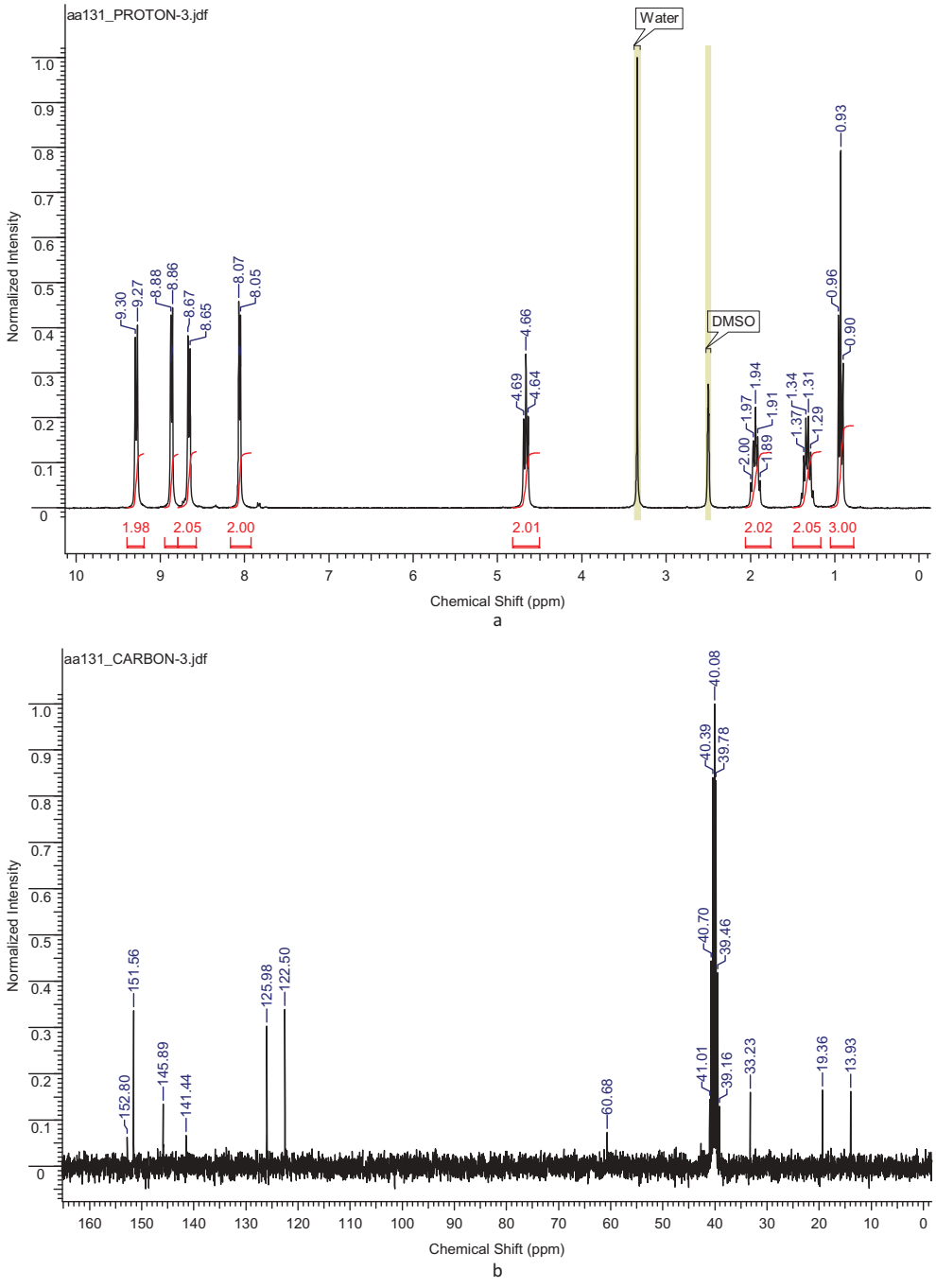
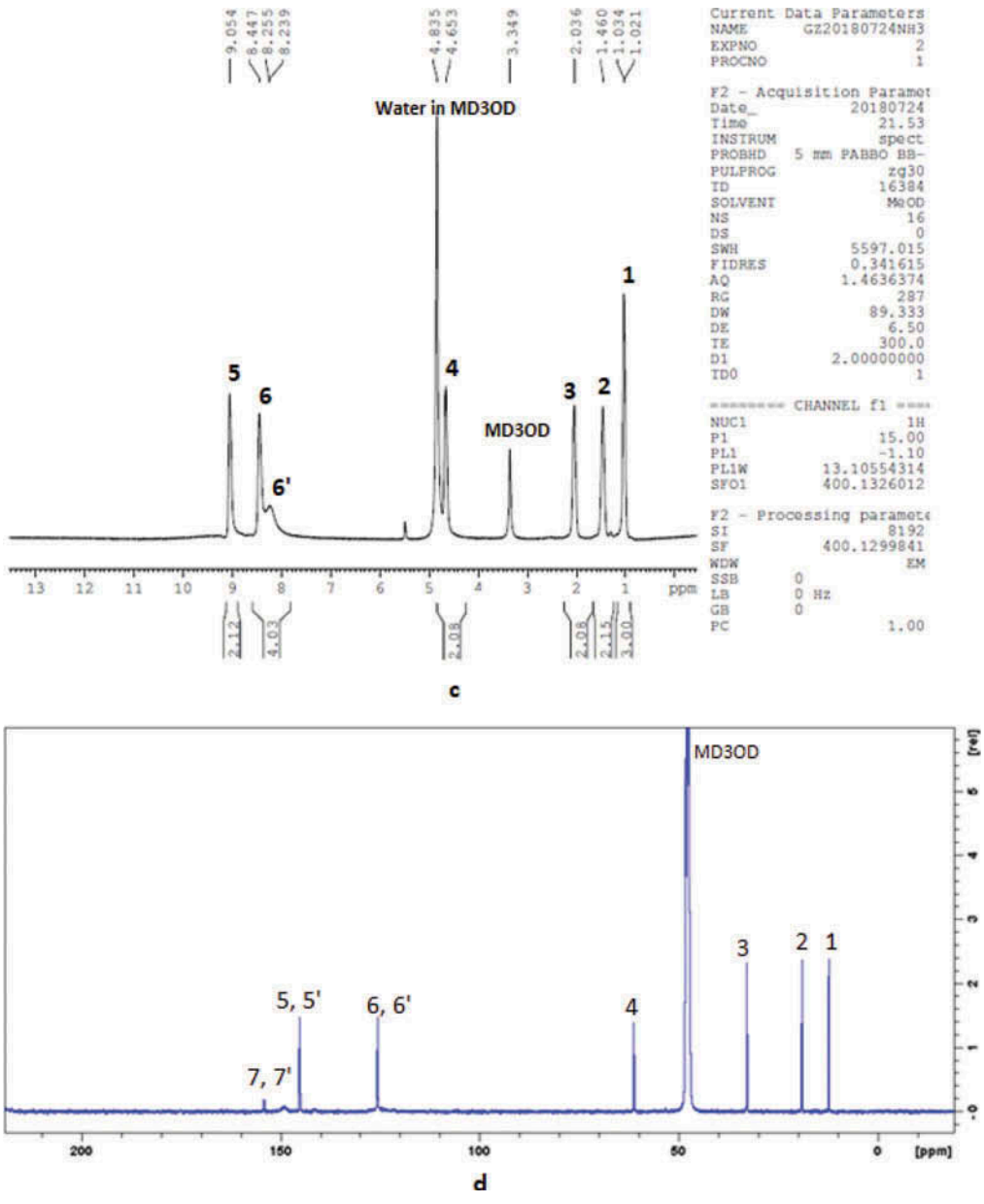


Figure 1. (a) ^{1}H NMR spectrum of $[C_4Bipyr]Br$, (b) ^{13}C NMR spectrum of $[C_4Bipyr]Br$, (c) ^{1}H NMR spectrum of $[(C_4Bipyr)_4Co]Cl_2Br_4$ and (d) ^{13}C NMR spectrum of $[(C_4Bipyr)_4Co]Cl_2Br_4$.

Furthermore, the upfield appearance of an appropriate number of alkyl protons and carbones and the change in the chemical shift of aromatic protons are strong confirmations for the occurrence of quaternization. Moreover, the molecular ion peak (M/z = 213.11) obtained from ESI MS spectra confirmed the acquisition of the intended structure (Figure 2(a)).

Even though the ligand has the same charge as the metal ion, it was found successfully coordinated. This is probably because the coordinating site nitrogen of the ligand is far from its quaternized part. After coordination, the ligand acquired a partial positive charge on the coordinating site nitrogen and consequently became symmetric that the four types of protons and six

Figure 1. (Continued).



types of carbons in the ligand are reduced to two and three, respectively (Scheme 1). This is observed from the change of four and six peaks in the free ligand to two and three peaks in the complex in the aromatic region in ^{1}H and ^{13}C NMR spectra, respectively (Figure 1(a–of d)). The number of protons in the aromatic region was found below the expected probably due to isotropic shift as a consequence of the paramagnetic effect of Co(II) (Figure 1(c)) (Bertini, Messori, Golub, Cohen, & Meyerstein, 1995). As a result of the latter, certain peaks might have appeared out of range (Kruck, Sauer, Enders, Wadepohl, & Gade, 2011). The complex was found stable at room temperature and decomposed in the range 162–164°C without melting. The stability is attributed to the strong field nature of the ligand. It is highly hygroscopic and soluble in methanol, ethanol, acetonitrile and dimethyl sulfoxide (DMSO) which may be due to the large charge (+6) on the cation. CHN elemental analyses and Co(II) and halide estimation experiment results were found in agreement with the assigned structure. Elements found (calculated) are C, 51.22 (51.58); H, 4.98 (5.22); N, 8.20 (8.60); Co, 4.27 (4.53); and X, 29.74 (30.01). The complex

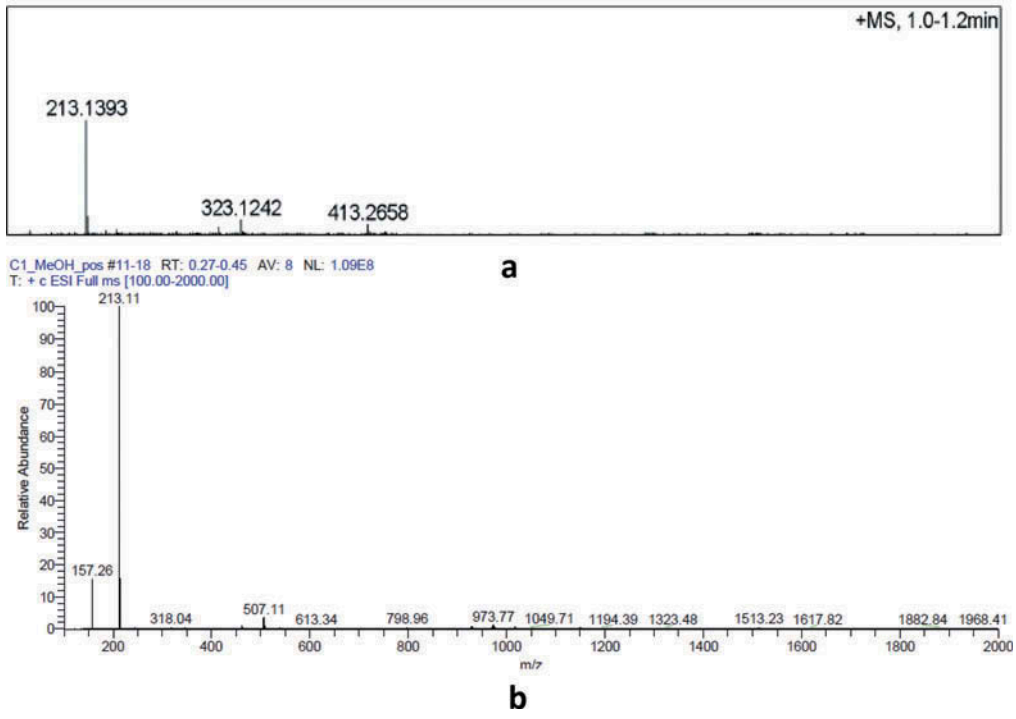


Figure 2. ESI MS spectra of (a) $[C_4Bipyr]^+$ and (b) $[C_4Bipyr)_4Co(CH_3OH)]^{6+}$.

showed significantly high molar conductivity value in water (640.83 S mol^{-1} cm^2) which is expected from 1:6 cation to anion ratio. This is attributed to a large number of the ions and due to the presence of the hydrophobic alkyl chain and positive charge in the cation (Bonchio et al., 2012). The latter reduces the drifting (counter directional) speed due to the interaction with the solvent cavity surrounding the cation (Atkins, 1994).

Furthermore, the ESI MS spectrum of the complex recorded dissolving in methanol showed a characteristic molecular ion peak (M/z = 157.26) which confirmed the coordination of four N-butyl-4,4′-bipyridinium and one methanol molecule to each Co(II) (Figure 2(b)).

The electronic spectra of the ligand and complex in ethanol are presented in Figure 3. There is one absorption band assigned to π→π* transition in the spectrum of the ligand. This transition is also observed in the spectrum of the complex being blue shifted which confirms the coordination of $[C_4Bipyr]^+$ to cobalt(II). The blue shift is due to the increase of the energy gap between π and π* as a consequence of the increase in electron density on π bonding molecular orbital following the coordination. Furthermore, the complex exhibited simple characteristic three d-d transitions assignable to $^4A_2 \rightarrow {}^4T_2$, $^4A_2 \rightarrow {}^4T_1(F)$ and $^4A_2 \rightarrow {}^4T_1(P)$ corresponding to 552, 548 and 546 nm, respectively (inset in Figure 3). A tetrahedral geometry is proposed for the complex since it demonstrated absorption wavelengths in regions of typical Co(II) tetrahedral complexes (Vila et al., 1997).

3.1. Antibacterial activity testing

This test shows that the ligand and the complex are biologically active against the gram-negative (*K. pneumoniae*) and gram-positive (*S. aureus*) strains and were compared with the commercially available gentamicin (Figure 4 and Table 1).

The complex demonstrated improved activity against the gram-negative bacteria (*K. pneumoniae*) compared to the ligand. This may be due to its action on the bacteria by distraction of the cell wall. The MIC of the complex was found to be as low as 50 μg/L.

Figure 3. Uv-Vis spectra of the ligand and complex.

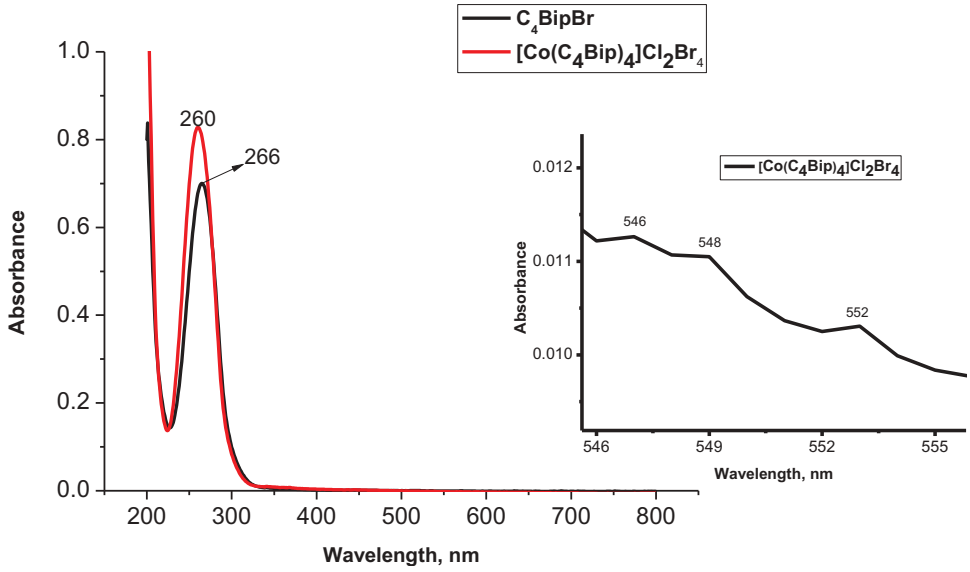


Figure 4. The inhibition observed by the ligand and complex.

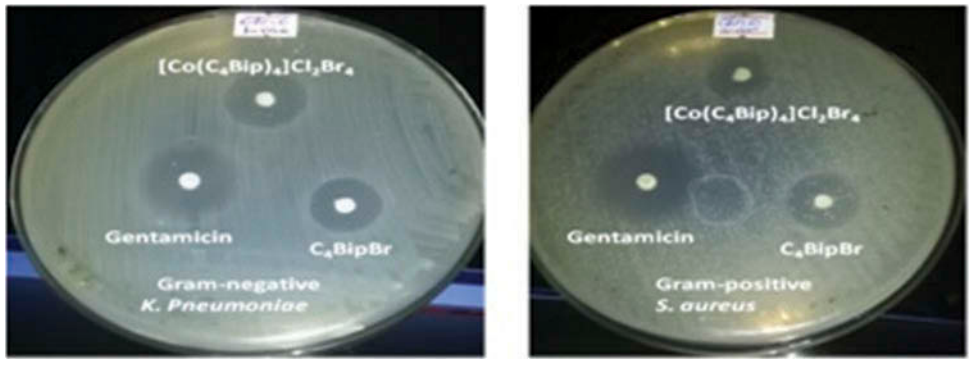


Table 1. Antibacterial studies of the investigated compounds (inhibition zones)

	Inhibition zone (mm)	
Compound	**Gram-negative bacteria *K. pneumoniae***	**Gram-positive bacteria *S. aureus***
C_4BipBr	21.00 ± 0.12	23.00 ± 0.33
$[Co(C_4Bip)_4]Cl_2Br_4$	27.00 ± 0.14	22.00 ± 0.21
Gentamicin	30.00 ± 0.23	30.00 ± 0.14

4. Conclusions

The identity and purity of the synthesized ligand and complex were confirmed from the data obtained using all characterization techniques employed here. The results demonstrated that the cationic ligand successfully coordinated to the metal ion easily probably due to the large space between the charged quaternized part and the coordinating sites of the cationic ligand. The *in vitro* biological activity studies indicated that the complex is biologically active against both the tested bacteria and interestingly demonstrated improved and better activities against the gram-negative drug-resistant *K. pneumonia*.

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Competing Interests
There is no conflict of interests among the authors and the funding institution.

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