Synthesis, characterization and antimicrobial properties of cobalt(II) and cobalt(III) complexes derived from 1,10-phenanthroline with nitrate and azide co-ligands

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Synthesis, characterization and antimicrobial properties of cobalt(II) and cobalt(III) complexes derived from 1,10-phenanthroline with nitrate and azide co-ligands

Djuikom Sado Yanick Gaëlle¹, Divine Mbom Yufanyi², Rajamony Jagan³ and Moise Ondoh Agwara¹*

Abstract: Two complexes, a cobalt(II) complex [Co(phen)₃(NO₃)₂]·2H₂O (1) and a novel Co(III) complex with mixed ligands [diazido-bis(1,10-phenanthroline-κ² N,N') cobalt(III)]nitrate [Co(phen)₂(N₃)₂]NO₃ (2), have been synthesized and were characterized by physico-chemical and spectroscopic methods. The cobalt(III) complex (2) crystallizes in the orthorhombic crystal system with space group Iba2 with four formula units. The central Co(III) atom is six coordinate with four N atoms of two 1,10-phenanthroline (1,10-phen) molecules and two terminal N atoms from two azide anions giving a distorted octahedral geometry with a CoN₆ chromophore. The molecular structure of the compound is consolidated by face-to-face π–π stacking between the aromatic rings of 1,10-phen ligand. The ligands, metal salt and the complexes were also evaluated for their antimicrobial activities in vitro against eight pathogens (four bacteria and four fungi species).

Subjects: Applied & Industrial Chemistry; Inorganic Chemistry; Materials Chemistry

Keywords: antimicrobial properties; azide; cobalt(III); 1,10-phenanthroline; X-ray crystal structure

PUBLIC INTEREST STATEMENT

The frequent, inappropriate and abusive use of drugs to fight infections caused by microorganisms has led to resistant pathogens which are resistant to many drugs. Efforts made to develop drugs to fight against these resistant pathogens include protection of the efficacy and appropriate use of existing drugs as well as research and development of new drugs. Metal complexes have found application as novel diagnostic and antimicrobial agents. This study presents the synthesis, characterization and structure elucidation of cobalt(II) and cobalt(III) complexes with 1,10-phen, nitrate and azide co-ligands. The complexes were screened for their activities against four pathogenic bacteria and four fungi species. The results indicate that the complexes have activities higher than that of the metal salt but lower than that of 1,10-phen. The most sensitive strains are E. coli, P. aeruginosa, S. typhi, C. albicans ATCC P37037 and C. neoformans. The positive results suggest that the complexes have a broad spectrum of activity.
1. Introduction

The design and synthesis of new metal complexes based on transition metal ions and diimine ligands, such as 1,10-phenanthroline (1,10-phen) and 2,2'-bipyridine, is an interesting field for the development of new functional materials with intriguing structures and potential applications (Amani, Safari, Khavasi, & Mirzaei, 2007). The use of mixed functional ligands in this process can enable the modification of the physical and chemical properties of these metal complexes. Mixed ligand complexes with 1,10-phen is also interesting due to their potential role as models for biological systems such as binding of small molecules to DNA (Jennifer & Muthiah, 2014; Yesilel, Olmez, Yilan, Pasaoglu, & Buyukgungur, 2006). Since the aromatic rings have an extended π-system and can give the ligands various non-covalent π–π interactions which mimic various biological processes, the study of these complexes has gained importance (Jennifer & Muthiah, 2014; Pook, Hentrich, & Gjikaj, 2015). The geometries of the complexes are greatly influenced by factors such as different coordination abilities of the ligands and the counterion. These nitrogen-containing heterocycles are metal-coordinating, electron-deficient aromatic systems which can undergo π–π stacking interactions as π–acceptors (Pook et al., 2015).

The chemistry of transition metal complexes has received much attention in recent years on account of their applications, amongst others, in biological systems as antimicrobial agents (McCann et al., 2000; Mishra, Kaushik, Verma, & Gupta, 2008; Shaaban, Khandar, Dusek, Pajarova, & Mahmoudi, 2013; Yenikaya et al., 2009), DNA studies (Arounaguiri, Easwaran, Ashokkumar, Dattagupta, & Mayya, 2000a, 2000b; Gopinathan, Komathi, & Arumugham, 2014; Kou et al., 2009; Kumar et al., 2008), larvicidal activity (Gopinathan & Arumugham, 2015), and cytotoxicity studies (Deegan, McCann, Devereux, Coyle, & Egan, 2007; Pivetta et al., 2012; Silva et al., 2011). The metal complexes of diimine ligands such as 1,10-phen and bipyridine have received much research interest because of their versatile roles as building blocks for the synthesis of metallo-dendrimers as well as their bioinorganic and biomedicinal applications (Ahmed & Khaled, 2015; Gopinathan & Arumugham, 2015; Molphy, Slator, Chatgilialoglu, & Kellett, 2015; Rajarajeswari, Ganeshpandian, Palaniandavar, Riyasdeen, & Akbarsha, 2014; Wang et al., 2014).

1,10-phen is a heterocyclic bidentate chelating ligand that bonds to metal atoms (ions) using the lone pairs of electrons on the nitrogen atoms resulting in a five-membered ring structure (Bencini & Lippolis, 2010). In this ligand, the σ-donation is complemented by the π-acceptor ability giving the complex formed greater stability (Janiak, 2000; Pook et al., 2015).

The azide anion is a versatile ligand that has been intensively studied because of its ability to coordinate to transition metals with different coordination modes generating a wide variety of fascinating structures (discrete molecules to 3D arrays) and their promising applications in functional materials (Chen, Jiang, Yan, Liang, & Batten, 2009; Lazari et al., 2009). The coordination modes of the azide anion range from monodentate to bridging bi-, tri- and tetra-dentate (Adhikary & Koner, 2010; Goher & Mautner, 1995; Lazari et al., 2009; Ye, Cheng, Li, & Hu, 2004). The most common bridging coordination modes of the azide ligand are μ−1,1 (end-on, EO), μ−1,3 (end-to-end, EE), μ−1,1,3 and others (Batten & Murray, 2003). The azide ion easily coordinates to transition metal ions and due to the π-donor properties of this ligand, its occupied π-orbitals can also overlap with the d-orbitals of the corresponding metal ion.

The nitrate group can function as a bidentate ligand, bridging group, monodentate ligand, and ionic species in various inorganic systems (Biagetti & Haendler, 1966). The functional characteristics probably depend on the nature and number of molecules of other ligands present.
In order to fight against antimicrobial resistance, our research team has recently focused on the synthesis and antimicrobial screening of some transition metal complexes of the ligands hexamethylenetetramine (Tabong et al., 2016), pyridine-2-carboxylic acid (Amah, Ondoh, Yufanyi, & Gaelle, 2015), 2-aminopyridine (Yuoh et al., 2015), mixture of ligands 1,10-phen and 2,2′-bipyridine (Ndosiri et al., 2013; Sado, Agwara, Yufanyi, Nenwa, & Jagan, 2016). The upsurge of resistant pathogens impedes the effective prevention and treatment of an ever-increasing variety of infections caused by bacteria, parasites, viruses and fungi (WHO, 2014). This situation poses a public health and economic burden to countries worldwide (Hawkey & Jones, 2009; Tanwar, Das, Fatima, & Hameed, 2014; WHO, 2014). Several efforts have been made to develop antimicrobial agents to fight against these resistant pathogens amongst which are the protection of the efficacy and appropriate use of existing drugs as well as research and development of new antimicrobial agents that are not affected by the currently known, predicted, or unknown mechanisms of resistance (Spellberg et al., 2008; Weinstein & Fridkin, 2003). Metal complexes of biologically active ligands are a target for the development of new active agents.

In view of the varied applications of cobalt mixed ligand complexes and exploring the good biological properties of cobalt and 1,10-bipy as well as the structure-directing properties of $N_3^-$, we report herein the synthesis and structure elucidation of cobalt(II) and cobalt(III) complexes of 1,10-phen, $N_3^-$ and $NO_3^-$. The effects of the co-ligands on the biological activities of the complexes towards some resistant pathogens, evaluated using in vitro assays, are also presented.

2. Experimental

2.1. Materials and methods
Co(NO$_3$)$_2$·6H$_2$O, sodium azide, 1,10-phen and anhydrous methanol 99.8% were obtained from Sigma–Aldrich. All chemicals (reagent grade) and solvents were used as received.

2.2. Synthesis of the complexes

2.2.1. Synthesis of [Co(phen)$_3$(NO$_3$)$_2$]·2H$_2$O (1)
A 25 mL methanol solution of 1,10-phen (0.60 g, 3.0 mmol) was added drop wise to a 25 mL methanol solution of Co(NO$_3$)$_2$·6H$_2$O (0.29 g, 1.0 mmol). The mixture was refluxed at 90°C for 4 h. The pink precipitate obtained was filtered, washed with methanol and dried in vacuo. Dark brown block-shaped crystals suitable for single crystal X-ray diffraction obtained from the filtrate within two weeks were washed with acetone and dried in vacuo.

2.2.2. Synthesis of [Co(phen)$_2$(N$_3$)$_2$]NO$_3$ (2)
A 25 mL of methanol solution of 1,10-phen (0.39 g, 2.0 mmol) was added drop wise to a 25 mL methanol solution of Co(NO$_3$)$_2$·6H$_2$O (0.29 g, 1.0 mmol). The mixture was refluxed at 90°C for an hour. Sodium azide (0.13 g, 2 mmol) dissolved in 2 mL of water and 8 mL of methanol was added drop wise to the reaction mixture and it was further refluxed for 4 h. The yellow precipitate obtained was filtered, washed with ethanol and dried in vacuo. Dark red block-shaped crystals suitable for single crystal X-ray diffraction obtained from the filtrate within two months were washed with acetone and then dried in vacuo.

2.3. Characterization
The melting point/decomposition temperatures were recorded using a STUART SCIENTIFIC melting point apparatus. The conductivity of the complex was measured in distilled water using a HANNA multimeter type H19811–5, pH/°C/EC/TDS meter at room temperature. The infrared spectrum was recorded using a Bruker ALPHA-P spectrophotometer directly on a small sample of the complex in the range 400–4,000 cm$^{-1}$, while the UV–vis spectrum of an aqueous solution of the complex was recorded using a Bruker HACH DR 3,900 UV–vis spectrophotometer at room temperature. Thermogravimetric measurements were obtained using a Pyris 6 PerkinElmer TGA 4,000 thermal analyser. TGA analysis was conducted between 35 and 930°C under nitrogen atmosphere at a flow rate of 20 mL/min and a temperature ramp of 10°C/min.
2.4. Single crystal X-ray structure determination of [Co(phen)(N$_3$)$_2$]NO$_3$ (2)

Intensity data for the compound was collected using a Bruker AXS Kappa APEX II single crystal CCD Diffractometer, equipped with graphite-monochromated MoK$_\alpha$ radiation ($\lambda = 0.71073$ Å) at room temperature. The selected crystal for the diffraction experiment had a dimension of 0.25 × 0.25 × 0.20 mm$^3$. Accurate unit cell parameters were determined from the reflections of 36 frames measured in three different crystallographic zones by the method of difference vectors. The data collection, data reduction and absorption correction were performed by APEX2, SAINT-plus and SADABS program (Bruker AXS, 2004). The structure was solved by direct methods procedure using SHELXS-97 program (Sheldrick, 2008) and the non-hydrogen atoms were subjected to anisotropic refinement by full-matrix least squares on F$^2$ using SHELXL-97 program (Sheldrick, 2008). The positions of all the hydrogen atoms were identified from difference electron density map and were fixed accordingly. All the aromatic hydrogen atoms were constrained to ride on the corresponding non-hydrogen atoms with a distance of C-H = 0.93 Å and U$_{iso}$(H) = 1.2U$_{eq}$(C). Whereas the hydrogen atoms associated with all the N atoms were restrained to a distance of N-H = 0.88(2) Å.

2.5. Antimicrobial tests

The antimicrobial tests were carried out in the laboratory of Phytobiochemical and Medicinal Plant Study, University of Yaounde I. The tests were done on eight pathogenic micro-organisms, four bacterial strains, Staphylococcus aureus CIP 7625 (Gram-positive) and Pseudomonas aeruginosa CIP 76110, Salmonella typhi and Escherichia coli ATCC 259224 (Gram-negative) and four yeasts, Candida albicans ATCC P37039, C. albicans 194B, Candida glabrata 44B, Cryptococcus neoformans obtained from Centre Pasteur Yaoundé, Cameroon. Reference antibacterial drug gentamycin and antifungal drug nystatin were evaluated for their antibacterial and antifungal activities and their results were compared to those of the free ligands and the complex.

The disc diffusion method, using Muller Hinton Agar, from the protocol described by the National Committee for Clinical Laboratory Standard was used for preliminary screening.

Mueller-Hinton agar was prepared from a commercially available dehydrated base according to the manufacturer’s instructions. Several colonies of each micro-organism was collected and suspended in saline (0.9% NaCl). Then, the turbidity of the test suspension was standardized to match that of a 0.5 McFarland standard (corresponds to approximately 1.5 × 10$^8$ CFU/mL for bacteria or 1 × 10$^7$ to 5 × 10$^7$ cells/mL for yeast). Each compound or reference was accurately weighed and dissolved in the appropriate diluents (DMSO at 10%, Methanol at 10% or distilled water) to yield the required concentration (2 mg/mL for compound or 1 mg/mL for reference drug), using sterile glassware.

Whatman filter paper No. 1 was used to prepare discs approximately 6 mm in diameter, which were packed up with aluminium paper and sterilized by autoclaving. Then, 25 μL of stock solutions of compound or positive control were delivered to each disc, leading to 50 μg of compound or 25 μg of reference drug.

The dried surface of a Müeller-Hinton agar plate was inoculated by flooding over the entire sterile agar surface with 500 μL of inoculum suspensions. The lid was left ajar for 3–5 min to allow for any excess surface moisture to be absorbed before applying the drug impregnated discs. Discs containing the compounds or antimicrobial agents were applied within 15 min of inoculating the MHA plate. Six discs per petri dish were plated. The plates were inverted and placed in an incubator set to 35°C. After 18 h (for bacteria) and 24 h (for yeasts) of incubation, each plate was examined. The disc diameter and the diameter of the zones of complete inhibition (as judged by the unaided eye) were measured. Zones were measured to the nearest whole millimetre, using sliding callipers, which was held on the back of the inverted petri plate. All experiments were carried out in duplicate. The compound was considered active against a microbe if the inhibition zone was 6 mm and above.
2.6. Minimum inhibitory concentration (MIC) of the complexes

The microbroth dilution method was used to determine the MIC of the compounds and the reference antibiotic on a given micro-organism. A polystyrene tray containing 80 wells is filled with small volumes of serial twofold dilutions of the complex and reference antibiotics. The inoculum suspension and standardization is done according to McFarland standard. The bacterial inoculum is then inoculated into the wells and incubated at 37°C overnight. The lowest concentration of antibiotic that completely inhibits visual growth of bacteria (no turbidity) is recorded as MIC.

3. Results and discussion

3.1. Synthesis of the complex

The reaction of Co(NO₃)₂·6H₂O and 1,10-phen with and without azide in methanol yielded two complexes whose physicochemical properties are summarized in Table 1. The complexes which are coloured and air stable were obtained in good yields (>75%). Complex 1, a Co(II) complex, had a sharp melting point (314°C) indicating its purity, while the melting point of complex 2, a Co(III) complex, could not be determined due to the explosive nature of the azide. The molar conductivity values of 68.4 and 120 Ωcm⁻² mol⁻¹ in water, for complexes 1 and 2, respectively, indicate that complex 1 is a non-electrolyte, while complex 2 is a 1:1 electrolyte type.

The chemical reaction leading to the formation of complex 2 is a redox process where Co(II) becomes oxidized to Co(III). The oxidation of Co(II) to Co(III) generates a diamagnetic complex and indicates an octahedral structure for complex 2. Most Co³⁺ complexes are diamagnetic; the mixed ligand under the investigation behaves as a strong field.

3.2. Crystal structure of complex 2

The crystals obtained for complex 1 were not suitable for single crystal X-ray structure determination. Thus, only the crystal structure of complex 2 was determined.

The ORTEP representation of the crystal structure of [Diazido-bis(1,10-phen-c≡N,N')cobalt(III)]nitrate, [Co(C₆H₄N₂)₂(N₃)₂]NO₃ with the atom numbering scheme used in the corresponding tables is shown in Figure 1. The crystal packing diagram for [Co(C₆H₄N₂)₂(N₃)₂]NO₃ seen along the crystallographic c-axes is shown in Figure 2. The crystal data and structure refinement are presented in Table 2, while the selected bond lengths and bond angles are shown in Table 3.

Complex 2 crystallizes in the orthorhombic crystal system with space group Iba₂ with four formula units. The asymmetric unit consists of one molecule of 1,10-phen, one azide anion, one nitrate anion and one Co(III) ion. The molecular structure of complex 2 contains one monomeric cation, [Co(phen)₂(N₃)₂]^⁺ and one nitrate ion, as revealed in Figure 1. Chemically, each Co atom is six coordinate; it is bonded to four N atoms of two phen molecules [Co1—N1 1.955 (2) Å, Co1—N1i 1.955 (2) Å, Co1—N2 1.9356 (17) Å, Co1—N2i 1.9356 (17) Å] and two terminal N atoms from two azide anions [Co1—N3 1.933 (3) Å, Co1—N3i 1.933 (3) Å] giving a slightly distorted octahedral geometry around the Co atom with CoN₂ chromophore. The axial positions are occupied by the N2 and N2’ atoms of two different phen molecules [N2—Co1—N2’ 179.98 (16)°] while the equatorial plane is formed by
the coordinating atoms N1, N1 \[N1—Co1—N1^i 89.01 (13)°\] from phen and N3, N3 \[N3—Co1—N3^i 93.86 (17)°\] from the two azide anions. The Co-N(phen) bond lengths are shorter than Co-N(phen) bonds reported in the literature (Guo, Shi, Si, Duan, & Shi, 2013; Pook et al., 2015). The structure of (2) contains one crystallographically independent Co(III) atom with the angles around Co1 slightly distorted from the ideal 90° and 180° of a perfect octahedron (Richers, Bertke, & Rauchfuss, 2015). The bond angles N3—Co1—N2 89.55 (11)°, N3\[i—Co1—N2 90.44 (10)°\], N3—Co1—N2\[i 90.44 (10)°\] and N3\[i—Co1—N2\[i 89.55 (11)°\] indicate that the Co1—N2 and N3—Co1 bonds are in two different molecular planes perpendicular to each other. The two phen ligands which form five-membered chelate rings with Co are oriented in two different molecular planes (Liang et al., 2003; Meundaeng, Prior, & Rujiwatra, 2013). These bond angles slightly deviate from 90° indicating a distorted square planar arrangement in the equatorial plane. The N5—N4—N3 bond angle of 175.3° (3) indicates that the azide anion is non-linear. The presence of nitrate O and azide N atoms in the molecular structure act as acceptor centres for the formation of C-H ... O and C-H ... N intermolecular interactions. These
Table 2. Crystal data and structure refinement for [Co(C12H8N2)2(N3)2]NO3 (2)

| Crystal data |  |
|--------------|---|
| Chemical formula | C24H16CoN11O3 |
| Mr | 565.41 |
| Crystal system, space group | Orthorhombic, Iba2 |
| Temperature (K) | 296 |
| a, b, c (Å) | 15.5018 (16), 9.8379 (8), 14.7376 (11) |
| V (Å³) | 2247.6 (3) |
| Z | 4 |
| Radiation type | Mo Kα |
| μ (mm⁻¹) | 0.82 |
| Crystal size (mm) | 0.25 × 0.25 × 0.20 |

Data collection

| Diffractometer | Bruker kappa apex2 CCD Diffractometer |
|----------------|-------------------------------------|
| Absorption correction | Multi-scan SADABS |
| T_min, T_max | 0.801, 0.882 |
| No. of measured, independent and observed [I > 2σ(I)] reflections | 18,221, 3,100, 2,655 |
| R(int) | 0.024 |
| (sin θ/λ)max (Å⁻¹) | 0.708 |

Refinement

| R[F² > 2σ(F²)], wR(F²), S | 0.025, 0.070, 1.07 |
| No. of reflections | 3100 |
| No. of parameters | 178 |
| No. of restraints | 1 |
| H-atom treatment | H-atom parameters constrained |
| Δρmax, Δρmin (e Å⁻³) | 0.23, −0.29 |

Absolute structure

| Absolute structure Flack x determined using 1,168 quotients [(I+)(I)]/[(I+)+(I-)] | −0.008 (5) |

Table 3. Selected bond lengths (Å) and angles (°) for complex (2)

| Co1—N3 | 1.933 (3) | N3—Co1—N3i | 93.86 (17) |
| Co1—N3i | 1.933 (3) | N3—Co1—N2 | 89.55 (11) |
| Co1—N2 | 1.9356 (17) | N3—Co1—N2i | 90.44 (12) |
| Co1—N2i | 1.9356 (17) | N3—Co1—N1 | 89.01 (13) |
| Co1—N1 | 1.955 (2) | N3i—Co1—N1i | 88.85 (9) |
| Co1—N1i | 1.955 (2) | N2—Co1—N1 | 83.94 (9) |
| O1—N6 | 1.212 (9) | N3—Co1—N1i | 173.75 (11) |
| O2—N6 | 1.230 (4) | N3—Co1—N1 | 83.94 (9) |
| N4—N3—Co1 | 120.6 (2) | N2—Co1—N1 | 96.07 (9) |
| N5—N4—N3 | 175.3 (3) | N2i—Co1—N1 | 83.95 (9) |
| O1—N6—O2 | 120.8 (3) | N3—Co1—N1i | 173.75 (11) |
| O1—N6—O2i | 120.8 (3) | N3—Co1—N1 | 83.94 (9) |
| O2—N6—O2i | 118.5 (5) | N2—Co1—N1i | 89.01 (13) |
| N2—Co1—N1i | 96.07 (9) | N1—Co1—N1 | 83.94 (9) |
weak, C5-H5 … O2, C11-H11 … O2, C8-H8 … N5, intermolecular interactions, result in the formation of two dimensional supramolecular sheets in the ab plane. The adjacent two-dimensional supramolecular sheets exchange π–π interactions experienced between the 1,10-phen moiety forming an extended three-dimensional network in the solid. The cobalt atoms between the stacked sheets running along the c-direction have an interlayer Co–Co distance of 7.369 Å.

### 3.3. Infrared spectroscopy

The characteristic absorption bands in the IR spectra of the ligands and the complexes (1 and 2) are summarized in Table 4.

In the spectrum of the phen ligand, the absorption bands at 1,586 and 1,492 cm\(^{-1}\) assigned to \(\nu(C=\text{N})\) and \(\nu(C=\text{C})\) stretching vibrations, respectively, are shifted in the complexes to 1,518 and 1,480 cm\(^{-1}\) (1) and 1,578 and 1,512 cm\(^{-1}\) (2), respectively, indicating the participation of the C = N of phen in bonding (Liang et al., 2003; Lu, Zhu, & Yang, 2003). The strong absorption band at 2,103 cm\(^{-1}\) in the spectrum of the azide ligand, assigned to the asymmetric stretching vibration \(\nu_{\text{as}}(\text{N}_3)\) of the azide is shifted to 2,073 cm\(^{-1}\) in complex 2 indicating terminal coordination of the azide to the metal ion (Goher et al., 2004; Liang et al., 2003). The new bands at 497 and 419 cm\(^{-1}\) which were not found in the spectra of the ligands, indicates the presence of Co-N bonding between the metal and the nitrogen atoms of both phen and the azide.

### 3.4. UV–vis spectroscopy

The UV–vis spectra of the complexes were measured in aqueous solution at ambient temperature. The ground term symbol for d\(^6\) Co(III) is 5D and the octahedral high spin spectroscopic ground state is 5T\(_{2g}\). Thus, a single spin-allowed transition \(5E_g \leftarrow 5T_{2g}\) is predicted but this will generally be split because of Jahn-Teller distortion. Most of the six coordinate complexes of cobalt(III) are low spin and have the spectroscopic ground term symbol 1A\(_{1g}\). Two transitions: 1T\(_{1g}\) \leftarrow 1A\(_{1g}\) and 1T\(_{2g}\) \leftarrow 1A\(_{1g}\) are expected for six coordinate low spin Co(III) complexes (Lever, 1984). The ground term symbol of d\(^7\) Co(II) is 4F. In octahedral Co(II) complexes, three transitions \(4T_{2g} \leftarrow 4T_{1g}(\text{F}),\ 4A_{2g} \leftarrow 4T_{1g}(\text{F})\) and \(4T_{1g}(\text{P}) \leftarrow 4T_{1g}(\text{F})\) are expected. The transition to \(^4A_{2g}\) is usually very weak and often appears as a shoulder.

The electronic absorption spectrum of Co(III) (complex 2) shows a single broad band centred at 18,518.5 cm\(^{-1}\) (530 nm). This d–d absorption band in has been assigned to \(^3E_g \leftarrow ^3T_{2g}\) transition (Abdelhak et al., 2014). The electronic spectrum of Co(II) (complex 1) shows a split absorption band centred at 34,482 cm\(^{-1}\) (290 nm) and 31,746 cm\(^{-1}\) (315 nm) probably due to intraligand transitions. A shoulder is observed at 28,571 cm\(^{-1}\) (350 nm) assigned to ligand-to-metal charge transfer transition.

### 3.5. Thermogravimetric analysis of \[\text{Co(phen)}_2(\text{N}_3)_2\text{NO}_3\]

To examine the thermal stability of the complex 2, thermal gravimetric (TG) analysis was carried out. This analysis (Figure 3) shows that the complex is thermally stable up to 250°C and upon further heating, it decomposes in a single step from 300 to 420°C. The major weight loss (~83%) takes place rapidly at 300°C, attributed to the complete decomposition of the sample (calc. 89.6%). A stable mass is reached at 460°C.

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**Table 4. Characteristic IR bands of the ligands and the complexes**

|          | C = N | C-H | C = C | N = N = N | M-N |
|----------|-------|-----|-------|-----------|-----|
| Phen     | 1,586 | 1,638| 1,492 | /         | /   |
| N\(_3\)  | /     | /   | /     | /         | /   |
| \[\text{Co(phen)}_3(\text{NO}_3)_2\]·2H\(_2\)O (1) | 1,518 | 1,625| 1,480 | 2,103     | /   |
| \[\text{Co(phen)}_2(\text{N}_3)_2\text{NO}_3\] (2) | 1,578 | 1,622| 1,512 | 2,073     | 497 |
|          |       |     |       |           | 419 |
Caution! Azide complexes of metals with organic ligands are potentially explosive and should be handled with care.

3.6. Antimicrobial tests

The potency of the metal salt, 1,10-phen, $\text{N}_3^-$ and the complexes together with the reference antibacterial drug (Gentamycin) and antifungal drug (Nystatin) were evaluated against four bacteria and four fungi strains. The diameter of the zone of inhibition (mm) was used to compare the antimicrobial activity of the test compound with that of the reference drug. Results of the antimicrobial activity are presented in Table 5 and histograms shown in Figures 4–6.

The metal salt, 1,10-phen and azide showed considerable antimicrobial activity against the microorganisms. The ligand 1,10-phen was very active against all the fungi and bacteria species tested. The metal salt and azide were more active against bacteria species while 1,10-phen showed higher activity against the fungi. Coordination of the ligands to the metal ion impacted the antimicrobial activity against all fungi and bacteria species tested with inhibition zone diameters in the range 13–38 mm. Complex 1 showed higher activity against the bacteria species while complex 2

| Micro-organisms | Zone of inhibition (mm) |
|------------------|-------------------------|
|                  | $\text{N}_3^-$ | Phen. | $\text{Co(NO}_3)_2\cdot6\text{H}_2\text{O}$ | Complex 1 | Complex 2 | Gentamycin | Nystatin |
| **Bacteria**     |              |       |                                |            |            |            |          |
| E. coli          | 26 ± 1.4     | 24.5 ± 0.7 | 17 ± 0                      | 20.5 ± 0.7 | 20.5 ± 0.7 | 22 ± 0     | 6.0 ± 0   |
| P. aeruginosa    | 37 ± 1.4     | 13.5 ± 0.7 | 17.5 ± 0.7                  | 23.5 ± 0.7 | 16 ± 1.4   | 25 ± 0     | 7.0 ± 0   |
| S. typhi         | 0 ± 0        | 14 ± 0   | 14.5 ± 0.7                  | 17.5 ± 0.7 | 13 ± 1.2   | 30 ± 0     | 6.0 ± 0   |
| S. aureus        | 13.5 ± 0.7   | 17 ± 1.4 | 16 ± 1.2                    | 18.5 ± 1.2 | 16 ± 0     | 30 ± 0     | 6.0 ± 0   |
| **Yeast**        |              |       |                                |            |            |            |          |
| C. albicans ATCC 12C | 35 ± 0 | 30 ± 1.4 | 11.5 ± 0.7                  | 17.5 ± 1.2 | 22 ± 0     | 6.0 ± 0     | 6.0 ± 0   |
| C. albicans ATCC P37037 | 0 ± 0 | 40 ± 0   | 10.5 ± 0.7                  | 16 ± 0     | 36 ± 0     | 7.5 ± 0     | 7.0 ± 0   |
| C. albicans ATCC P37039 | 9 ± 1.2 | 30 ± 1.4 | 13 ± 0                    | 38 ± 0     | 17.5 ± 0.7 | 6.0 ± 0     | 6.0 ± 0   |
| C. neoformans    | 6 ± 0        | 26 ± 0   | 24 ± 1.4                    | 0 ± 0      | 20 ± 0     | 6.5 ± 0     | 9.5 ± 0   |

Figure 3. Thermogravimetric and differential thermogravimetric analyses of complex 2.
Figure 4. Histogram of inhibition zones.

Figure 5. Histogram of inhibition zones against bacteria species.

Figure 6. Histogram of inhibition zones against fungi species.
exhibited higher activities against the fungi species. The complexes were generally more potent towards the fungi species than the reference antifungal (nystatin). The complexes exhibited highest activity against the fungus *C. albicans* ATCC P37039, which can lead to the development of vagina candidiasis. Compared to the reference antibiotic gentamicin, the complexes showed comparable antibacterial activity against the bacteria species *E. coli* and *P. aeruginosa*. These results are comparable to those of the complex \([\text{azido-bis(1,10-phen-κ}^2\text{N,N'}\text{copper(II)})\text{nitrate hydrate, }[\text{Cu(C}_{12}\text{H}_{8}\text{N}_2\text{N}_3]\text{NO}_3\cdot\text{H}_2\text{O} (\text{Sado et al., 2016})].\)

The complexes are also more active than the reference drug nystatin towards the fungi species, with complex 2 being most active. This indicates that reaction of metal ions with the ligands plays an important role in enhancing its antimicrobial activity. This increase in activity could be due to the reduction of the polarity of the metal ion by partial sharing of the positive charge with the ligand’s donor atoms so that there is electron delocalization within the metal complex. This may increase the hydrophobic and lipophilic character of the metal complex, enabling it to permeate the lipid layer of the organism killing them more effectively (Amah et al., 2015; Chohan, Munawar, & Supuran, 2001).

The antimicrobial activities of the complexes were quantified by the determination of their MICs. The MIC indicates the lowest drug concentration at which a visible inhibition growth of the microorganisms is noticed. The values for the different pathogens were determined by using the microbroth dilution test. The MICs values for the complexes are summarized in Table 6 and the histograms on Figure 7.

**Table 6. MIC (mg/mL) of the complexes**

| Species           | MIC (mg/mL)       | Co(NO$_3$)$_2$·6H$_2$O | O-Phen | N$_3$⁻ | Complex 1 | Complex 2 | Gentamycin | Nystatin |
|-------------------|-------------------|------------------------|--------|--------|-----------|-----------|------------|----------|
| **Bacteria**      |                   |                        |        |        |           |           |            |          |
| *E. coli*         | 0.125             | 0.039                  | 0.156  | 1.25   | 0.313     | 0.156     | >2.5       |          |
| *P. aeruginosa*   | 0.625             | 0.039                  | 0.078  | 0.078  | 0.313     | 1.25      | >2.5       | >2.5     |
| *S. typhi*        | 1.25              | 0.039                  | 0.156  | 0.156  | 0.625     | 1.25      | >2.5       | >2.5     |
| *S. aureus*       | 1.25              | 0.078                  | 0.625  | 0.625  | 0.625     | 1.25      | >2.5       | >2.5     |
| **Fungi**         |                   |                        |        |        |           |           |            |          |
| *C. albicans ATCC 12C* | 0.625             | 0.039                  | 1.25   | 0.156  | 1.25      | >2.5      | >2.5       | >2.5     |
| *C. albicans ATCC P37037* | 0.625             | 0.078                  | 0.625  | 0.156  | 0.313     | >2.5      | >2.5       | >2.5     |
| *C. albicans ATCC P37039* | 0.625             | 0.156                  | 1.25   | 0.156  | 1.25      | >2.5      | >2.5       | >2.5     |
| *C. neoformans*   | 1.25              | 0.039                  | 2.5    | 0.078  | 1.25      | >2.5      | >2.5       | >2.5     |

**Figure 7. Histogram of MIC against bacteria species.**
The MIC values indicate that the ligands (1,10-phen and azide) have higher activity against the bacteria species than the fungi, with 1,10-phen being more active than azide. The complexes have activities higher than that of the metal salt but lower than that of 1,10-phen, towards the micro-organisms. The most sensitive strains are the bacteria species *E. coli*, *P. aeruginosa*, *S. typhi* and the fungi *C. albicans* ATCC P37037 and *C. neoformans*. The complexes have a broad spectrum of activity, since they showed high activity towards all the tested strains. The MIC values obtained are lower than those of a copper(II) complex against the same micro-organisms, indicating that the cobalt complexes synthesized are more potent (Yenikaya et al., 2009). Comparison of the MIC values of the complexes with that of the reference drugs indicates that the complexes are potentially more active than the reference drugs. The disparity in the effectiveness of the complexes against the tested pathogens depends either on the impermeability of the cells of the microbes or the difference in the ribosomes of the microbial cells (Yenikaya et al., 2009).

4. Conclusion
A novel mixed ligand Co(III) complex with 1,10-phen and azide co-ligands has been reported. The equatorial and terminal azide ligands coordinate in a non-linear manner to the central metal ion while two 1,10-phen ligands coordinate to the Co(III) ion through four N-atoms, two axial and two equatorial. The Co(III) ion in the complex adopts a slightly distorted octahedral environment comprising four phenanthroline N-atoms and two nitrile N-atoms from the azides. The molecular structure of the compound is consolidated by weak C-H … O and C-H … N intermolecular interactions and face-to face π–π stacking between the aromatic rings of 1,10-phen ligand. The results of the preliminary antimicrobial screening against four pathogenic bacteria and four fungi species indicates that the complexes are very active and could be further screened *in vitro* against a wide range of pathogens. The MIC values indicate that the ligands (1,10-phen and azide) have higher activity against the bacteria species than the fungi, with 1,10-phen being more active than azide. The complexes have activities higher than that of the metal salt but lower than that of 1,10-phen, towards the micro-organisms. The most sensitive strains are the bacteria species *E. coli*, *P. aeruginosa*, *S. typhi* and the fungi *C. albicans* ATCC P37037 and *C. neoformans*. The generally positive results of the antimicrobial screening against four bacteria and four fungi species suggest that complex 1 is more active towards the bacteria, while complex 2 is more active towards the fungi. Both complexes (1 and 2) may represent good candidates as an antibacterial and antifungal agent, respectively. However, additional and profound *in vitro* antimicrobial studies mainly in relation to elucidation of the mechanism of growth inhibition and toxicity of the complexes is ongoing.

Supplementary data
CCDC 1417600 contains the supplementary crystallographic data for the complex. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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