Synthesis, characterization and evaluation of antimicrobial potential of zinc(II) complexes of nitro-substituted hydroxamic acid chelators

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ABSTRACT
The nitro-substituted hydroxamic acid chelators potassium 4-nitrobenzohydroxamate (4-(NO2)C6H4CONHOK) (KHL1) and 3,5-dinitrosalicylhydroxamate (3,5-(NO2)2(OH)C6H2CONHOK) (KHL2) on reaction with anhydrous zinc(II) chloride (ZnCl2) in 1:2 metal:ligand ratio in MeOH + THF afforded new zinc(II) complexes, [Zn(4-(NO2)C6H4CONHO)2] (1) and [Zn(3,5-(NO2)2(OH)C6H2CONHO)2] (2).

The structural characterization of 1 and 2 has been accomplished by physicochemical studies; elemental analyses, molar conductivity, cyclic voltammetry and spectroscopic techniques in the solid state (IR) and in solution (1H NMR and UV-visible). The stoichiometric composition of 1 and 2 has been authenticated by mole ratio method and the stability constants were determined. IR spectral studies suggested the bidentate nature of ligands involving bonding through carbonyl and hydroxamic oxygen atoms (O,O'-coordination). The mass spectral data of 1 and 2 have indicated these to be mononuclear. In order to infer the biological relevance of newly synthesized complexes, the in vitro antimicrobial activity assay against pathogenic gram-ve bacteria, viz. Salmonella typhi and Escherichia coli; gram+ve bacteria Bacillus cereus and Staphylococcus aureus and fungi Rhizoctonia solani and Fusarium sambucinum by MIC method has shown appreciable antimicrobial potential. The results have been compared with standard Tetracycline and Nystatin drugs.

ARTICLE HISTORY
Received 8 April 2022
Accepted 23 July 2022

KEYWORDS
Zinc(II) complexes; 4-nitrobenzohydroxamate; 3,5-dinitrosalicylhydroxamate; spectral studies; antibacterial activity; antifungal activity
1. Introduction

The coordination chemistry of zinc has drawn considerable attention over the years to form complexes with sulfur-, nitrogen-, and oxygen-containing ligands displaying versatility in structures, bonding modes and coordination number [1]. Zinc is not only an essential trace element for humans, animals, plants and microorganisms, but also the only metal found in representatives of all six International Union of Crystallography classes of enzymes, namely oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases [2]. Zinc is an efficient Lewis acid and useful catalytic agent in hydroxylation and other enzymatic reactions. The catalytic product of the zinc(II)-containing tumor necrosis factor α (TNF-α) converting enzyme (TACE) has been reported to be implicated in diseases, viz. rheumatoid arthritis, Crohn’s disease and multiple sclerosis [3]. The role of zinc in a wide range of cellular processes, such as cell proliferation, reproduction, immune function and defense against free radicals, has been well established [4]. Owing to the pronounced biological activities and as a constituent of proteins and enzymes, zinc is known to be indispensable to growth and development and transmission to genetic messages. The dominance of tetrahedral metal binding sites in metallo-proteins demonstrates its preference for a tetrahedral geometry. Numerous zinc complexes have been reported to possess efficient antimicrobial, anti-convulsant, anti-inflammatory and antitumor activities [5–8].

Hydroxamic acids show wide spectrum of biological activities and generally have low toxicities [9, 10]. Hydroxamic acids containing the –C(O)NHOH functionality constitute an important family of organic bio-ligands. These have been the subject of enormous research interest because of their broad spectrum of biological activities in
medicine as analgesic, anti-inflammatory, collagenase inhibitors, anti-infective, antibiotics, anticancer agents, etc. Cinnamohydroxamic acid and its derivatives have been used for the treatment of symptoms of asthma and other obstructive airway diseases which inhibit 5-lipoxygenase. Hydroxamic acid analogues have been reported to inhibit DNA synthesis by inactivating the enzyme ribonucleotide reductase (RNR). Naturally occurring hydroxamic acid 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) is a powerful antibiotic present in maize [11]. The antiradical and antioxidant properties of hydroxamic acids have been reported [12]. Hydroxamic acids play important roles in many chemical, biochemical, pharmaceutical, analytical and industrial fields [13, 14]. The biological activities of hydroxamic acids are due to their diverse complexation behavior towards transition metal ions [15], which has been studied both in solution as well as in solid state. Hydroxamic acids usually act as bidentate (O,O’)-chelates through the carbonyl and hydroxamic oxygen atoms forming five-membered rings in which the ligand is either singly deprotonated (hydroxamato) or doubly deprotonated [R(O-)C¼NO-] (hydroximato) [16–18]. The experimental and theoretical studies have established that hydroxamic form is the dominant one in free acids or metal hydroxamates. Besides the O,O’-bonding mode, in a few cases N,O- and N,N’-coordination involving the hydroxamate nitrogen and α-amino nitrogen (α-aminohydroxamic acids) has been described [19–21]. The affinity between hydroxamic acids and transition metal ions is quite apparent in the X-ray crystal structures of Ni(II)-containing urease [22] or Zn(II)-containing metalloproteins such as carbonic anhydrase [23], or matrix metalloproteases (MMPs), where hydroxamic acids are bound to the Ni(II) or Zn(II) active sites [24–26]. The intensely colored solutions of metal hydroxamates have been widely used in analytical applications. Hydroxamic acids and their derivatives can be used as pharmacological agents and with electron-withdrawing substituents like –NO₂, –Cl, etc. (–NO₂ in present work) upon complexation with metals show enhanced biological activity [27]. Metal hydroxamates also find use as models for understanding the role of the hydroxamate group in biological systems [28]. With the backdrop of our research interest on the synthesis of vanadium(IV) hydroxamates [29–32] and organotin(IV) hydroxamates [33–35] and in view of the biological importance of zinc complexes, the present work aims at the synthesis of new zinc(II)

**Figure 1.** Structure of ligands. Potassium 4-nitrobenzohydroxamate (KHL¹). Potassium 4-nitrobenzohydroxamate (KHL²).
hydroxamates and evaluation of their antimicrobial potential in search of better anti-
microbial candidates.

2. Experimental

2.1. Materials and instrumentation

Reagent-grade solvents were dried and distilled prior to use. The purity of zinc(II) 
chloride (Merck) was checked by its melting point (290 °C). The ligands potassium 4-
nitro-benzohydroxamate and 3,5-dinitrosalicylhydroxamate (Figure 1a and b) were syn-
thesized by a reported method [36]. Carbon, hydrogen and nitrogen analyses were 
obtained on a Carlo-Erba 1106 Elemental Analyzer. Zinc was estimated by titrating the 
complexes with EDTA using Erichrome Black-T (EBT) as indicator [37]. The molar con-
ductance (10⁻³ M solution in methanol) of complexes was measured at 18 ± 1 °C using 
an Elico Conductivity Bridge type CM-82T; Bench conductivity/TDS meter (cell constant 
K = 1.0). IR spectra were recorded as KBr pellets on a Perkin Elmer Spectrum RX FTIR 
Spectrophotometer. ¹H NMR spectra were recorded on a BRUKER AVANCE II 400 
Spectrometer using TMS as an internal standard and DMSO (deuterated) as solvent. 
Electrospray ionization mass spectra (ESI-MS) were recorded on a Waters QTOF-
MICROMASS mass spectrometer. Cyclic voltammometric measurements were carried 
out on an Autolab Potentiostat 128 N electrochemical analyzer in methanol in single com-
partmental cell of volume 10–15 mL containing a three-electrode system comprising of 
a Pt–disk working electrode, Pt–wire as auxiliary electrode and Ag/AgCl electrode as 
reference electrode. The supporting electrolyte was 10⁻² M KCl in 10⁻⁴ M methanol. 
UV-visible spectra of ligands and complexes were recorded in methanol on a LabIndia 
UV-3000+ UV-visible Spectrophotometer using standard quartz cells of path length 
10 mm at conc. 5 × 10⁻⁵ M at room temperature. The overall stability constant (β₂) of 
complexes were determined using Bjerrum’s method using the equation:

$$\beta_2 = \frac{[ML_2]}{[M][L]^2}$$

The stoichiometry of the complexes were determined by mole-ratio method spec-
trophotometrically (Figure S1). The absorbance is plotted against the mole ratio of 
reactants and stability constant of complexes were calculated. The structures of 
ligands and complexes were drawn in Chemcraft_b536b.

2.2. Synthesis of [Zn(4-NO₂C₆H₄CONHO)₂] (1)

A solution of zinc(II) chloride (1 g, 0.0073 mol) in THF (10 mL) was added to a solution 
of potassium 4-nitrobenzohydroxamate (KHL¹) (2.66 g, 0.0146 mol) in methanol (20 mL). 
The reaction mixture was initially stirred and then refluxed for 6–8 h to ensure comple-
tion of the reaction. The white solid formed during the course of the reaction was 
removed by filtration and identified as KCl. The filtrate was dried under vacuum and 
re-crystallized from 1:1 mixture of methanol and diethylether. A lemon-yellowish solid 
was obtained. C₁₄H₁₀O₈N₄Zn/427 (1). Yield = 2.58 g (82%). M.pt: 220 °C. Calcd. (%): C, 
39.34; H, 2.34; N, 13.11; Zn, 15.31. Found (%): C, 39.25; H, 2.34; N, 13.07; Zn, 15.27. β
(stability constant) = $5.63 \times 10^3$; $\Lambda_m$ (MeOH): 1.65 S·cm$^2$ mol$^{-1}$. IR (KBr matrix, cm$^{-1}$): 3389 (N-H), 1618 (C=O), 1338 (C-N), 970 (N-O) and 470-450 (Zn-O). $^1$H NMR (DMSO-d$_6$), δ(ppm): 8.23 (N-H), 7.95-8.25 (Aromatic protons).

2.3. Synthesis of [Zn((3,5-NO$_2$)$_2$-OHC$_6$H$_2$CONHO)$_2$] (2)

A solution of zinc(II) chloride (0.73 g, 0.0053 mol) in THF (10 mL) was added to a solution of potassium 3,5-dinitrosalicylhydroxamate (KHL$^2$) (3 g, 0.0107 mol) in methanol (20 mL). The reaction mixture was stirred and then refluxed for 6-8 h to ensure completion of the reaction whereupon a white solid appeared. It was filtered off. The excess solvent from the filtrate was removed by distillation. The concentrate was treated with petroleum ether and dried under vacuum repeatedly. An orange solid was obtained. It was re-crystallized from 1:1 mixture of methanol and diethylether. C$_{14}$H$_8$O$_{14}$N$_6$Zn/549 (2). Yield = 2.36 g (78%). M.pt: 150 °C. Calcd. (%): C, 30.60; H, 1.45; N, 15.30; Zn, 11.90. Found (%): C, 30.55; H, 1.45; N, 15.27; Zn, 11.88. β (stability constant) = $7.41 \times 10^3$; $\Lambda_m$ (MeOH): 1.56 S·cm$^2$ mol$^{-1}$. IR (KBr matrix, cm$^{-1}$): 3170 (N-H), 1683 (C=O), 1332 (C-N), 934 (N-O) and 470-450 (Zn-O). $^1$H NMR (DMSO-d$_6$), δ(ppm): 8.69 (N-H), 8.39-8.55 (Aromatic protons).

2.4. Antimicrobial activity assay by two-fold serial dilution method

The *in vitro* antibacterial and antifungal activities of zinc(II) hydroxamates were assayed by minimal inhibitory concentration (MIC) method in a 96-well micro-titre plate (tissue culture grade) by two-fold serial dilution method using oxidation reduction colorimetric indicator dye Resazurin, for the determination of drug-resistance and MIC of antimicrobial agents against different microorganisms. The dye, blue in its oxidized state, turns pink when reduced by viable cells, indicating the growth of bacteria and fungi. The lowest concentration of test complex that prevented this color change was considered as MIC according to CLSI M07-A9. A stock solution of test ligands and complexes was prepared in DMSO (500 µg/mL) for two-fold serial dilution. For MIC assay of each test complex, a row of twelve wells was used out of which the last two wells were taken as control (no test complex added). The 100 µL of the Muller-Hinton broth was placed in each of the ten wells except the first well which contained 200 µL of broth and 500 µg/mL of the test complex. From the first well (containing test complex), 100 µL broth was withdrawn with a sterile tip, and same was added to the 100 µL of the broth in the 2nd well; contents were mixed four times. In this way a range of two-fold serial dilution were prepared (500–7.81 µg/mL) by performing two-fold serial dilution. Resazurin prepared as 0.02% weight/volume in distilled water sterilized by filtration and stored at 4 °C for one week was added to each well. The broth in each of the wells was inoculated with 20 µL of the bacterial/fungal culture and the contents were mixed by ten clockwise and ten anticlockwise rotations on a flat surface. Thereafter, the plate was incubated at 35 °C for 24 h for bacteria and at 28 °C for 72 h for fungi. To evaluate the role of solvent in biological screening if any, separate studies were carried out with DMSO which did not show any activity. The results were
compared with standard antibacterial drug tetracycline and antifungal drug Nystatin for antimicrobial studies. All the experiments were carried out in triplicate.

3. Results and discussion

The reactions of zinc(II) chloride with 4-NO₂C₆H₄CONHOK and 3,5-(NO₂)₂(OH)C₆H₂CONHOK in predetermined 1:2 molar ratio (metal: ligand) in anhydrous methanol and THF under reflux afforded [Zn(4-(NO₂)C₆H₄CONHO)₂] (1) and [Zn((3,5-NO₂)₂(OH)C₆H₂CONHO)₂] (2) in quantitative yields in conformity with their elemental analyses represented by the following equations (Scheme 1):

The complexes are soluble in organic solvents, viz. methanol, acetonitrile and dimethylsulphoxide. The molar conductance values of [Zn(4-(NO₂)C₆H₄CONHO)₂] (1) and [Zn((3,5-NO₂)₂(OH)C₆H₂CONHO)₂] (2) in methanol are of magnitude 1.65 and 1.56 S·cm²·mol⁻¹, respectively, and have indicated their non-electrolytic nature [38]. The stability constants (β) of 1 and 2 have been calculated using Bjerrum’s method (overall stability constant β₂) and found to be 5.63 × 10³ and 7.41 × 10³, respectively, stability being attributed to the formation of five-membered chelate complexes (chelation). All attempts to grow X-ray quality crystals of 1 and 2 were unsuccessful.

3.1. IR spectra

A comparison of IR spectra of 1 and 2 with those of free ligands KHL¹ and KHL² scanned in region 4000-400 cm⁻¹ has supported their formation. KHL¹ exhibited characteristic bands at 3117, 1719, 1339 and 935 cm⁻¹ attributed to ν(N-H), ν(C=O), ν(C-N) and ν(N-O), respectively (Figure S2). Complex 1 displayed bands at 3389, 1618, 1338 and 970 cm⁻¹ due to respective modes (Figure S3). Free KHL² exhibited bands at 3522, 3148, 1703, 1334 and 937 cm⁻¹ attributed to ν(O-H), ν(N-H), ν(C=O), ν(C-N) and ν(N-O), respectively (Figure S4). Complex 2 exhibited bands at 3493, 3170, 1683, 1332 and 934 cm⁻¹, respectively (Figure S5). The retention of bands due to ν(N-H) mode in complexes suggested the exclusion of coordination through nitrogen. The moderate-to-appreciable shifts in ν(C=O) to lower wavenumbers are indicative of lengthening and weakening of carbonyl bond. The shifts of ν(C-N) and ν(N-O) to higher wavenumbers may be ascribed to strengthening of N-O bond and that electron redistribution due to resonance within the chelate ring around zinc results in more double-bond character for C-N bond. The bidentate nature of the ligand involving bonding through carbonyl and hydroxamic oxygen atoms [O,O'-coordination] has

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**Scheme 1.** Synthesis of the complexes.
thus been suggested. The appearance of new sharp bands at 465 and 463 cm$^{-1}$ (not present in free ligand) assigned to $\nu$(Zn-O) further substantiated their formation [39, 40].

### 3.2. $^1$H NMR spectra

A comparison of $^1$H NMR spectra of zinc(II) hydroxamates with those of free ligands further established their formation. Free potassium 4-nitrobenzohydroxamate (KHL$^1$) is known to exhibit signals due to aromatic protons at 8.11–8.44 ppm and $-\text{NH}$ at 8.0 ppm. Complex 1 exhibited resonances at 7.95–8.25 ppm due to aromatic protons and at 8.23 ppm due to $-\text{NH}$ resonance (Figure S6). Free KHL$^2$ showed signals at $\delta$ 10.13 ppm, 8.69 ppm and 8.36–8.53 ppm due to $-\text{OH}$, $-\text{NH}$ and aromatic protons, respectively [33]. Complex 2 exhibited the respective signals at 12.48, 8.69 and 8.39–8.55 ppm, respectively (Figure S7).

### 3.3. UV-visible spectra

UV-visible spectra of free ligands and complexes were recorded in methanol. KHL$^1$ showed one absorption band at 255 nm ($\varepsilon = 25,720 \text{M}^{-1}\text{cm}^{-1}$) and a low intense band at 343 nm ($\varepsilon = 13,920 \text{M}^{-1}\text{cm}^{-1}$) whereas 1 displayed one band with higher intensity (bathochromic shift) than ligand at 270 nm ($\varepsilon = 32,580 \text{M}^{-1}\text{cm}^{-1}$). KHL$^2$ exhibited absorption maxima at 255 nm ($\varepsilon = 25,700 \text{M}^{-1}\text{cm}^{-1}$) while 2 at 225 nm ($\varepsilon = 26,260 \text{M}^{-1}\text{cm}^{-1}$) and a bathochromic shift at 343 nm ($\varepsilon = 27,600 \text{M}^{-1}\text{cm}^{-1}$). These transitions can be attributed to $n\rightarrow\pi^*$ transition (at higher wavelength) and $\pi\rightarrow\pi^*$ transition (shorter wavelength) showing red shift both in 1 and 2, indicative of complexation (Figures S8 and S9).

### 3.4. Mass spectra

Five stable isotopes of zinc occur in nature, with $^{64}$Zn being the most abundant isotope (49.17% natural abundance) [41] and the other isotopes are $^{66}$Zn (27.73%), $^{67}$Zn (4.04%), $^{68}$Zn (18.45%), and $^{70}$Zn (0.61%). The mass spectrum of $[\text{Zn}(4-\text{NO}_2-C_6\text{H}_4\text{CONHO})_2](1)$ (mass $= 427$) (Figure S10) showed less intense molecular ion peak at m/z = 427 (3.34%). The fragment ions at m/z (%) 300 (3.34), 241 (3.53), 181 (100), 160 (3.91), 137 (8.45) and 58 (31.10) corresponded to $[\text{ZnL}^+ + \text{Na}^+ + \text{K}^+]$, $[\text{ZnL} - 5\text{H}^+]$, $[\text{L}]$, $[\text{L} - \text{NO}_2 + \text{Na}^+ + 2\text{H}^+]$, $[\text{C}_6\text{H}_4\text{CONHO} + 2\text{H}^+]$ and $[\text{CONO}]$, respectively (where $\text{L} = 4-\text{NO}_2-C_6\text{H}_4\text{CONHO}$). The mass spectrum of $[\text{Zn}(3,5-(\text{NO}_2)C_6\text{H}_2(\text{OH})\text{CONHO})_2](2)$ (mass $= 549$) (Figure S11) also showed low intense molecular ion peak at m/z = 549 (3.12%). The fragment ions at m/z (%) 521 (6.26), 505 (10.95), 242 (34.59), 224 (8.71), 183 (14.74), 178 (2.36), 137 (1.68) and 80 (1.11) corresponding to $[\text{Zn}(3,5-(\text{NO}_2)\text{OHC}_6\text{H}_2\text{CONHO})_2 - \text{CO}]$, $[\text{M} - \text{NO}_2 + 2\text{H}^+]$, $[\text{L}^+]$, $[\text{L}^+ - \text{H}^+]$, $[\text{L}^+ - \text{OH} - \text{H}^+]$, $[\text{L}^+ - \text{OH} - \text{CONH} + \text{H}^+]$, $[\text{L} - \text{OH} - \text{NO}_2]$, $[\text{C}_6\text{H}_2(\text{NO}_2)\text{CONHO} - \text{NO}_2 + 5\text{H}^+]$ and $[\text{C}_6\text{H}_2\text{CONHO} - \text{CONHO} + 2\text{H}^+]$, respectively, where $\text{M} = [\text{Zn}(3,5-(\text{NO}_2)\text{C}_6\text{H}_2(\text{OH})\text{CONHO})_2]$ and $\text{L} = 3,5-(\text{NO}_2)\text{C}_6\text{H}_2(\text{OH})\text{CONHO}$ supported its formation. The fragment ions corresponding to $[\text{L}]^+$ appeared as the base peak in both the complexes. Schemes 2 and 3 depict the mass fragmentation pattern and Table S1 presents mass spectral data of 1 and 2, respectively.
3.5. Cyclic voltammetry

The electrochemical behavior of free ligands and complexes 1 and 2 studied by cyclic voltammetric technique on the forward scan initiated in positive direction from −2.0 to +2.0 V in methanol has shown these to be electrochemically active. KHL1 displayed a well-defined reductive wave and a feeble oxidative wave at +0.683 V and −0.756 V, respectively (Figure S12). Complex 1 showed a reductive peak at +0.620 V and a feeble oxidative peak at −0.620 V (Figure S13) attributed to the reduction of nitro group (−RNO₂) into hydroxylamine (−RNHOH) and the oxidation of −NHOH group to nitroso (−RNO) group as:

\[
\text{RNO}_2 + 4\text{H}^+ + 4\text{e}^- \rightarrow \text{RNHOH} + \text{H}_2\text{O}
\]

\[
\text{RNHOH} \rightarrow \text{R} - \text{NO} + 2\text{H}^+ + 2\text{H}^+ + 2\text{e}^-
\]
KHL$^2$ displayed two reductive waves at $-0.513$ and $+0.839$ V and a feeble oxidative peak at $-0.664$ V (Figure S14) [33]. Complex 2 showed a reductive peak at $+0.84$ V (Figure S15). The CV data of ligands and respective zinc complexes is depicted in Table 1. It is quite noteworthy that perhaps in the presence of $–\text{NO}_2$ substituents at ligands, no change has occurred in metal oxidation state ($\text{Zn}^{2+}/\text{Zn}^0$) as reported earlier [42] and redox process may be attributed to the ligand.

### 3.6. Proposed geometry and stability of complexes

Based upon physicochemical and spectral studies, four-coordinate distorted tetrahedral geometry around zinc in 1 and 2 may tentatively be proposed (Figures 2 and 3). The high overall stability constant and low molar conductance values (behaving as non-electrolyte) support the integrity and stability of complexes in solution. Furthermore,
no change in color was observed upon dissolving complexes in the solvents. The spectral studies in solution phase (\(^1\)H NMR spectra) and solid phase (IR spectra) were compared to study the structure of complexes. The bands due to –NH, –OH and aromatic stretch in IR spectra and –NH, –OH and aromatic signals observed in NMR spectra have shown that the structure of complexes are similar in solid as well as in solution as a whole entity.

### 3.7. Antimicrobial activity

The zinc(II) complexes with a variety of ligands, viz. 5-bromo 2((2(dimethylamino)ethylamino)methyl)phenol [43], aromatic hydroxamates [44], 3-methylpyridine (3-Mepy), 4-methylpyridine (4-Mepy) and N-methylimidazole (N-MeIm) [45], Schiff bases like 3-(((4-chlorophenyl)imino)methyl)benzene-1,2-diol [46], have been reported to exhibit promising antimicrobial activities. The biological potential of metal complexes is generally known to depend upon the nature of the metal [47], the ligand (the easily dissociative ligands facilitate the transportation of metal ion to the site of action across the cell.
membrane) and the nuclearity of complexes. Also the metal complexes derived from ligands with electron-withdrawing substituents (-NO₂, -Cl) exhibit higher biological activity than those having electron donating substituents (-NH₂, -OH). Hence, the in vitro antimicrobial activity of the ligands and 1 and 2 has been evaluated against selected Gram-positive bacteria Bacillus cereus and Staphylococcus aureus, Gram-negative bacteria Salmonella typhi and Escherichia coli and pathogenic fungi Rhizoctonia solani and Fusarium sambucinum at different concentrations in DMSO employing the standard MIC method as recommended by National Committee for Clinical Laboratory Standards (NCCLS). The commercial antibiotics tetracycline and nystatin have been used as standards for the comparison of results.

3.7.1. Antibacterial activity

KHL¹ inhibited gram +ve bacteria B. cereus and S. aureus at MIC 125 μg/mL. The gram – ve bacteria S. typhi and E. coli were inhibited at MIC = 15.63 and 62.5 μg/mL, respectively. Complex 1 inhibited B. cereus and S. aureus at MIC = 1.95 and 3.90 μg/mL, respectively. S. typhi and E. coli were inhibited at MIC = 3.90 and 31.25 μg/mL, respectively. KHL² inhibited B. cereus and S. aureus at MIC = 7.80 and 125 μg/mL, respectively. S. typhi was inhibited at MIC = 31.25 μg/mL and E. coli at 62.5 μg/mL. Complex 2 showed pronounced activity against all the bacteria in the 1.95–15.63 μg/mL range. It showed minimum inhibitory concentration for B. cereus and S. typhi at 1.95 μg/mL while S. aureus and E. coli were inhibited at 15.63 and 3.90 μg/mL, each showing enhanced activity over the ligands, proving it to be a much efficient inhibitor against the bacteria. The reference drug tetracycline inhibited test bacteria at MIC range of 3.90–15.63 μg/mL. The results indicated that 1 is quite effective against B. cereus, whereas 2 showed promising activity against B. cereus and S. typhi (Figure S16).

Table 2 presents the antibacterial data of ligands and their respective complexes.

Table 2. Antibacterial activity data by MIC method in μg mL⁻¹ (± standard deviation).

| Ligand/complex | Gram +ve B. cereus | Gram +ve S. aureus | Gram –ve S. typhi | Gram –ve E. coli |
|----------------|-------------------|-------------------|------------------|-----------------|
| KHL¹ 1        | 125 ± 0.5         | 125 ± 0.4         | 15.63 ± 0.4      | 62.5 ± 0.2      |
| 1             | 1.95 ± 0.02       | 3.90 ± 0.5        | 3.90 ± 0.05      | 31.25 ± 0.3     |
| KHL² 2        | 7.80 ± 0.5        | 125 ± 0.5         | 31.25 ± 0.4      | 62.5 ± 0.05     |
| 2             | 1.95 ± 0.45       | 15.63 ± 0.3       | 1.95 ± 0.02      | 3.90 ± 0.5      |
| Tetracycline  | 3.90              | 15.63             | 15.63            | 15.63           |

3.7.2. Antifungal activity

The antifungal activity of the ligands and the newly synthesized complexes has been assayed against Rhizoctonia solani and Fusarium sambucinum and compared with standard antibiotic nystatin. Free KHL¹ showed inhibitory effect against R. solani and F. sambucinum at MIC = 62.5 and 125 μg/mL, respectively. Complex 1 inhibited R. solani and F. sambucinum at MIC = 31.25 and 62.5 μg/mL, respectively. KHL² showed efficient inhibitory activity against R. solani and F. sambucinum at 125 and 62.5 μg/mL, respectively. Complex 2 exhibited pronounced activity against R. solani and F. sambucinum at MIC 15.63 and 31.25 μg/mL, respectively, and is quite effective against both fungi.
Table 3. Antifungal activity data by MIC method in μg mL⁻¹ (± standard deviation).

| Ligand/complex | R. solani | F. sambucinum |
|---------------|-----------|---------------|
| KHL¹          | 62.5 ± 0.45 | 125 ± 0.3     |
| 1             | 31.25 ± 0.02 | 62.5 ± 0.4    |
| KHL²          | 125 ± 0.5   | 62.5 ± 0.03   |
| 2             | 15.63 ± 0.03 | 31.25 ± 0.05  |
| Nystatin      | 3.90       | 3.90          |

(Figure S17). Antifungal data of ligands and complexes 1 and 2 are presented in Table 3.

The microbial growth inhibition may be due to bacteriostatic effect of complexes. The possible target of action might be cytoplasmic membrane. Interaction of complexes alters the membrane fluidity [48] and due to expansive K⁺ leakage, the organism perishes. The improved antimicrobial activity of complexes relative to the free ligands may be attributed to the chelation theory [49] inhibiting cellular respiration and ATP synthesis whereby the polarity of the central metal ion gets reduced on complexation because of partial sharing of positive charge with the donor atoms of ligand and possible π-electron delocalization over the whole chelate ring [50], thus increasing the lipophilicity of the metal ion favoring permeation of complex through the cell membrane [51]. The observed antimicrobial activity of complexes can be attributed to the biological significance associated with the zinc(II) ion and the nitro-substituted hydroxamate ligands.

The effectiveness of the antimicrobial activity of complexes studied herein against pathogens in comparison with other zinc(II) complexes can only be made in case experimental assay under similar experimental conditions using same methodology might have employed.

4. Conclusion

New zinc(II) complexes, [Zn(4-NO₂-C₆H₄CONHO)₂] (1) and [Zn(3,5-(NO₂)₂(OH)C₆H₂CONHO)₂] (2), have been synthesized from biologically important 4-nitrobenzohydroxamate and 3,5-dinitrosalicylhydroxamate ligands and characterized by physicochemical and IR, ¹H NMR, UV-visible and mass spectral techniques. The bonding through hydroxamic and carbonyl oxygens (O,O’-coordination) has been indicated by IR spectra. The mononuclear four-coordinate distorted tetrahedral geometry around zinc has tentatively been proposed from physicochemical and spectral studies. The complexes have depicted ligand-centered electrochemical behavior RNHOH/RNO couple due to nitro substituent. Complexes 1 and 2 have shown promising antimicrobial activity assayed by MIC method against the test microorganisms.

Acknowledgements

Authors thank Sophisticated Analytical Instrument Facility (SAIF) Panjab University Chandigarh for recording IR, ¹H NMR and mass spectra; University Grants Commission (UGC) New Delhi for providing cyclic voltammetric facility to the Department under University Grants Commission-Special Assistance Programme-II (UGC-SAP-II); Central Science Workshop, Department of Physics and Department of Biotechnology, Himachal Pradesh University Shimla for laboratory facilities for UV-Visible spectra and antimicrobial assay.
Disclosure statement

No potential conflict of interest was reported by the authors.

Data availability statement

The data that support this study are available in the article and accompanying online Supplementary Material.

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