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# Nickel(II) complexes of polyhydroxybenzaldehyde N4-thiosemicarbazones: synthesis, structural characterization and antimicrobial activities

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**Abstract** Nickel(II) complexes with 2,3-dihydroxybenzaldehyde N4-substituted thiosemicarbazone ligands ($H_3L^1$–$H_3L^4$) have been synthesized and characterized with the aim of evaluating the effect of N4 substitution in the thiosemicarbazone moiety on their coordination behavior and biological activities. Two series of nickel(II) complexes with the general formulae $[Ni(H_3L)(H_2L)]ClO_4$ and $[Ni_2(HL)_2]$ were characterized by analytical and spectral techniques. The molecular structure of one of the complexes, namely, $[Ni(H_3L^4)(H_2L^4)]ClO_4$ was established by single crystal X-ray diffraction studies. The crystal structure of this complex revealed that two $H_3L^4$ ligands are coordinated to nickel(II) in different modes; one as a neutral tridentate ONS ligand and the other is as a monoanionic tridentate ($ONS^-$) ligand. The antimicrobial activities of the compounds were tested against 25 bacterial strains via the disc diffusion method, and their minimum inhibitory concentration (MIC) and minimum microbicidal concentration were evaluated using microdilution methods. With a few exceptions, most of the compounds exhibited low-to-moderate inhibitory activities against the tested bacterial strains. However, the complexes $[Ni_2(HL^3)_2]$ (**7**) and $[Ni_2(HL^4)_2]$ (**8**) indicated higher inhibitory activity against *Salmonella enterica* ATCC 9068 (MIC values 15.7 and <15.7 μg/ml, respectively), compared with gentamicin as the positive control (MIC 25 μg/ml). Complex (**7**) also inhibited *Streptococcus pneumoniae* more efficiently (MIC 31.2 μg/ml), compared with gentamicin (MIC > 50 μg/ml). The toxicities of the compounds were tested on brine shrimp (*Artemia salina*), where no meaningful toxicity level was noted for both the free ligands and the complexes. The cytotoxicities of the compounds on cell viability were determined on MCF7, PC3, A375, and H413 cancer cells in terms of $IC_{50}$; complexes $[Ni(H_3L^3)(H_2L^3)]ClO_4$ (**3**), $[Ni_2(HL^3)_2]$ (**7**) and $[Ni_2(HL^4)_2]$ (**8**) exhibited significant cytotoxicity on the tested cell lines.

## Introduction

The synthesis and characterization of coordination compounds with ligands containing nitrogen and sulfur donors have evolved during the last few years as one of the main research areas in coordination chemistry as well as in biochemistry [1, 2]. Among the nitrogen/sulfur compounds, thiosemicarbazones are one of the most studied ligands, not only because of their flexibility to bind metal ions in various modes, but also because of the wide range of applications that they possess [3, 4]. The capability of thiosemicarbazones to coordinate to metals is due to the delocalization of electron density over the NHC(S)NHN system, which is improved by substitution at the N(4) position. Consequently, thiosemicarbazones can coordinate to metal ions as monodentate ligands through the thiocarbonyl sulfur or as bidentate through the thiocarbonyl sulfur and one of the hydrazinic nitrogen atoms, leading to the formation of either a five or a four membered chelate ring (Scheme 1a) [5, 6]. Furthermore, the presence of an additional coordinating group in the carbonylic part opens up many more coordination

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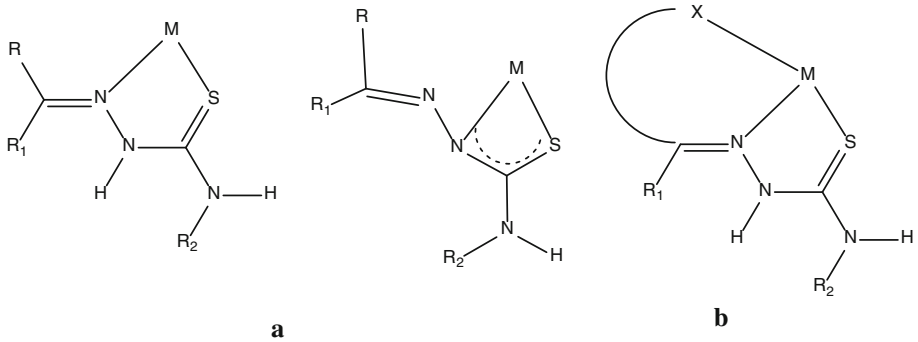
**Scheme 1** Coordination possibilities of thiosemicarbazones

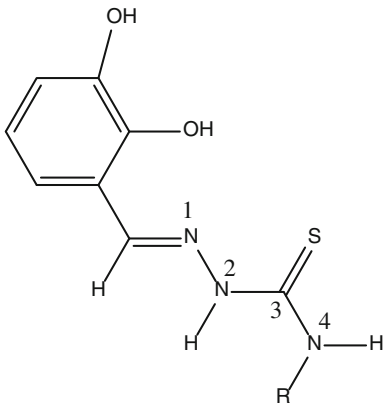



possibilities, increasing the flexibility in coordination to both hard and soft metal ions, and resulting in the formation of complexes having five- and six-membered chelate rings (Scheme 1b) [7]. The biological activities of thiosemicarbazones have gained considerable interest due to their good activities as anticancer [8, 9], antimalarial [10], antiepileptic [11] and antifungal [12] agents. It has been shown that complexation of thiosemicarbazones with metal ions can increase their biological activities. In order to find new compounds with higher activity, variation of the thiosemicarbazone frame has been extensively studied. Relationships between the structures of thiosemicarbazones, their transition metal complexes and their interactions with DNA are evident in a number of cases [13, 14].

A number of thiosemicarbazone metal complexes with ONS-donor ligands, especially salicylaldehyde thiosemicarbazone ligands, have been synthesized and characterized by means of X-ray diffraction and spectroscopic techniques [15, 16]. The ONS-donor set can lead to unusual stereochemical, electrochemical, and electronic properties in many of their metal complexes [17].

The bioinorganic chemistry of nickel has been rapidly expanded since the discovery of nickel in the active site of several metalloenzymes [18]. Although certain nickel compounds are considered to have environmental toxicity and carcinogenic nature, many structurally characterized nickel complexes have shown antibacterial, antifungal, and anticancer/antiproliferative activity. The key target of anticancer metallodrugs is DNA; thus, the interaction of Ni(II) complexes with DNA is mainly dependent on the structure of the ligand and can involve intercalative behavior and/or DNA cleavage ability [19].

Although metal complexes of salicylaldehyde thiosemicarbazone ligands have received considerable attention in view of their chemistry and biological properties, fewer studies have focused on the complexation behavior and biological activities of polyhydroxybenzaldehyde thiosemicarbazone derivatives [20–22]. In this paper, we report the preparation, structural characterization and antimicrobial studies of nickel complexes of 2,3-dihydroxybenzaldehyde N4-substituted thiosemicarbazone.



**Scheme 2** Chemical structure of 2,3-dihydroxybenzaldehyde –N4-substituted thiosemicarbazone. $H_3L^1$; R=H, $H_3L^2$; R=–$CH_3$, $H_3L^3$; R=–$C_6H_5$, $H_3L^4$; R=–$C_2H_5$

## Experimental

### Materials and measurements

2,3-Dihydroxybenzaldehyde, thiosemicarbazide, 4-methyl-3-thiosemicarbazide, 4-ethyl-3-thiosemicarbazide, 4-phenyl-3-thiosemicarbazide, nickel(II) perchlorate hexahydrate, and nickel(II) acetate tetrahydrate were obtained from Sigma-Aldrich. All the solvents used in the reaction were from Merck and used as received.

FT-IR spectra were recorded on a Perkin-Elmer Spectrum RX-1 spectrophotometer as KBr pellets in the frequency range of 4,000–400 $cm^{-1}$. NMR spectra were recorded in deuterated DMSO-$d_6$ on an ECA 400 MHz instrument. Elemental analyses were performed on a Thermo Finnigan Eager 300 CHNS elemental analyzer.

### Synthesis of the ligands

The thiosemicarbazone ligands $H_3L$ (Scheme 2) were prepared following the published procedure [20] by treatment of 2,3-dihydroxybenzaldehyde with N4-substituted thiosemicarbazide in a 1:1 molar ratio, under standard reflux conditions.

Synthesis of complexes $[Ni(H_3L)(H_2L)]ClO_4$ (**1–4**)

To a colorless solution of 2,3-dihydroxybenzaldehyde thiosemicarbazone (1 mmol) ($H_3L^1$; 0.21 g, $H_3L^2$; 0.23 g, $H_3L^3$; 0.29 g, $H_3L^4$; 0.24 g) in ethanol (15 ml) was added an ethanolic solution of $Ni(ClO_4)_2{\cdot}6H_2O$ (0.36 g, 1 mmol). In each case, the dark green solution was stirred for about 4 h under reflux. The clear solution after filtration was left for slow evaporation at room temperature.

Synthesis of complexes $[Ni_2(HL)_2]$ (**5–8**)

2,3-Dihydroxybenzaldehyde thiosemicarbazone (2 mmol) ($H_3L^1$; 0.42 g, $H_3L^2$; 0.46 g, $H_3L^3$; 0.58 g, $H_3L^4$; 0.48 g) was dissolved in 20 ml ethanol. To this, a solution of $Ni(OAc)_2{\cdot}4H_2O$ (0.248 g, 1 mmol) in ethanol (10 ml) was added. The mixture was refluxed for 2 h. The red solid which precipitated out was filtered off and washed thoroughly with water and ethanol followed by ether, dried in air and kept in a desiccator over silica gel.

X-ray crystallography

Green crystals of complex **4** were grown by slow evaporation of the mother liquor and were analyzed by single crystal diffraction. The data were collected at 273 K on a Bruker Apex 2 diffractometer with Mo–Kα radiation ($\lambda = 0.71073$ Å), and the data reduction was performed using Bruker SAINT [23]. The structure was solved using direct methods, which yielded the positions of all non-hydrogen atoms. These were refined first isotropically and then anisotropically. All hydrogen atoms were placed in calculated positions with fixed isotropic thermal parameters and included structure factor calculations in the final stage of full-matrix least-squares refinement. All calculations were performed using the SHELXTL suite of programs [24]. Molecular graphics were drawn using ORTEP [25] and PLATON [26]. Material for publication was prepared using publCIF [27].

In vitro antimicrobial assays

Stock solutions of the test compounds were prepared in 10 % v/v aqueous DMSO solution (Fisher chemicals) at concentrations of 10 mg/ml. Antimicrobial activities of the compounds was tested against 38 microorganisms, including 25 pathogenic bacteria (standard and clinical strains): *Bacillus subtilis*, *Escherichia coli*, *Enterococcus faecalis*, *Listeria innocua*, *L. monocytogenes*, *L. welshimeri*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *S. enteritidis*, *S. typhi*, *S. typhimurium*, *S. dysenteriae*, *S. flexneri*, *S. sonnei*, *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pneumoniae*, *Vibrio alginolyticus*, *V. cholerae*, *V. fischeri*, *V. harveyi*, *V. parahaemolyticus*, *V. vulnificus* and *Yersinia pestis.*

The medium used in the assay for the antibacterial test was Difco yeast extract–peptone–dextrose (YPD) broth and agar medium (BD, NJ, USA). Bacterial initial suspensions were prepared by the direct colony method. The colonies were taken from the plate, suspended in 5 ml of sterile Difco YPD broth and allowed to grow in a rotary shaker (200 rpm) at 37 °C. The bacterial culture was then diluted using sterile 0.85 % saline, and its turbidity was adjusted by comparing with 0.5 McFarland's standard to obtain 108 CFU/ml bacterial suspension [28].

Kirby–Bauer disc–diffusion experiments were carried out as follows. An aliquot of 65 µl of each microorganism was swabbed uniformly across a Difco YPD agar plate using cotton swabs. Then, a sterile filter paper disc (diameter 6 mm) impregnated with 20 µl of the compound solution was placed on the surface of the agar. After treatment, the plate was inverted (to avoid moisture on the agar surface) and incubated for 16–18 h in air at 35 °C. At the end of the incubation period, the plates were examined for the inhibition of bacterial growth by measuring the diameter (in mm) of the inhibition zone surrounding each filter paper disc. In general, larger zones of inhibition indicate a more active compound against the organism [29]. A bacterial strain was considered resistant (*R*) to the drug tested when the zone of inhibition was $\leq$12 mm; intermediate (*I*) when the inhibition zone was between 12 and 15 mm, or susceptible (*S*) if the inhibition zone was $\geq$15 mm [30].

The microdilution plate method with resazurin was applied to determine the minimum inhibitory concentrations (MIC) and minimum microbicidal concentrations (MMC) of the test compounds [31]. The 96-well plates were prepared by dispensing 180 µl of nutrient broth to the first row and 100 µl of the broth to the rest of the plate. A 20 µl aliquot from the stock solution (with a concentration of 10 mg/ml) of the test compound was added into the first row of the plate. Then, twofold serial dilutions were carried out to obtain a range of concentrations from 1,000 to 7.81 µg/ml. A 10 µl aliquot of diluted bacterial suspension was added to each well to give a final concentration of $5 \times 10^5$ CFU/ml. Finally, 10 µl resazurin solution was added to each well inoculated with bacteria, and the plates were incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration of the test compound that prevented the resazurin color change from blue to pink. The experiment was performed in triplicate using gentamicin as positive control. In a solvent control test, the effect of 10 % DMSO was studied on the growth of microorganisms, and no inhibitory activity was observed [32].

The MMC of each compound was evaluated by plating 10 µl of the samples from the wells with no indicator color change, on YPD agar plates, followed by 16-h incubation

at 37 °C. The lowest concentration with no colony was defined as the MMC of the compound against the microorganism [33].

### Brine shrimp lethality assays

Brine shrimp lethality assays (BSLA) were carried out as follows. Sea salt (38 g) was dissolved in distilled water (1 l) to prepare artificial sea water as brine shrimp growth medium. After filtration, brine shrimp (*Artemia salina*) eggs were added to the medium and allowed to hatch by incubation at room temperature in the dark for 48 h. The brine shrimps were then seeded in 6-well plates (10 shrimps and 3 ml of artificial sea water per well) and treated with 6 concentrations of each compound (200, 100, 50, 25, 12.5, 6.25 μg/ml). Control experiments were simultaneously conducted. After 24 h, the number of surviving shrimps was counted and data were analyzed using PROBIT analysis software to determine the median lethal concentration (LC50). The experiments were done in triplicate [34].

### In vitro anticancer activity

Human prostate cancer cell line (PC3) and human malignant melanoma cell line (A375) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Human breast cancer cell line (MCF-7) was acquired from Cell Lines Service (300273; Eppelheim, Germany) and Carcinoma-derived human oral keratinocyte cells (H413) were received from Professor Ian Charles Paterson. MCF-7 and A375 cells were grown in Dulbecco's Modified Eagle Medium (DMEM, Life Technologies, Inc., Rockville, MD, USA) supplemented with 10 % heat-inactivated fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO, USA), 1 % penicillin and streptomycin. PC3 cells were grown in Roswell Park Memorial Institute medium (RPMI) supplemented with 10 % heat-inactivated fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO, USA), 1 % penicillin and streptomycin. H413 cells were grown in Dulbecco's Modified Eagle Medium/Ham's F-12 (DMEM/F12) supplemented with 10 % heat-inactivated fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO, USA), 1 % penicillin and streptomycin. Cells were cultured in tissue culture flasks (Corning, USA) and were kept in an incubator at 37 °C under a humidified atmosphere with 5 % $CO_2$. For experimental purposes, cells in exponential growth phase (approximately 70–80 % confluency) were used.

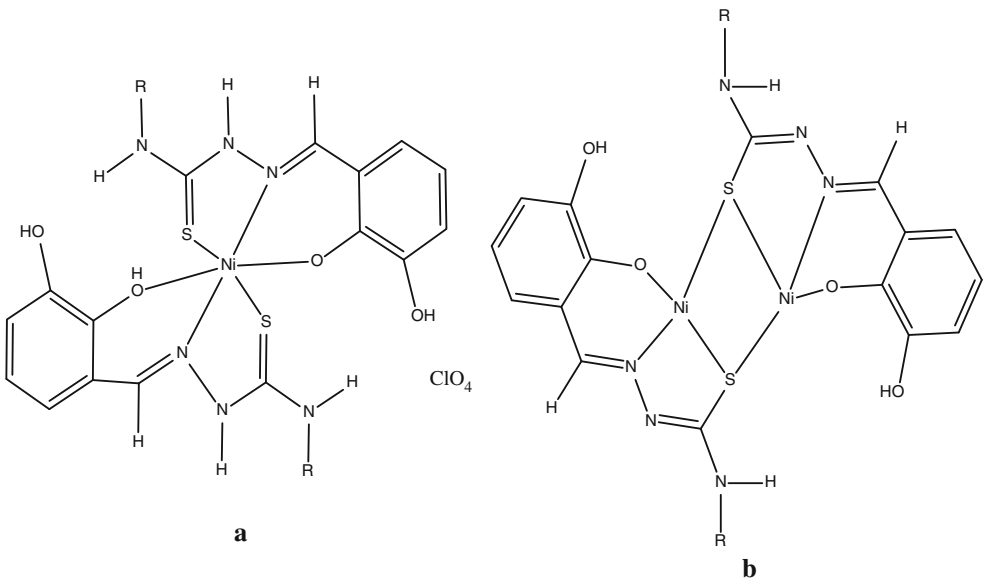
The influence of the ionic solvents was determined by MTT assay [35]. MCF-7, A375, PC3 and H413 cells were treated for 48 h. On the first day, $1.0 \times 10^4$ cells were seeded into a 96-well plate for 24-h incubation assay. The cells were incubated overnight at 37 °C in 5 % $CO_2$. On the next day, the cells were treated with a twofold dilution series of six concentrations of the solvents, and then incubated at 37 °C in 5 % $CO_2$ for 48 h. MTT solution (4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazoliumbromide) was added at 2 mg/ml and after 2 h of incubation at 37 °C in 5 % $CO_2$, DMSO was added to dissolve the formazan crystals. The plates were then read with a Chameleon multitechnology microplate reader (Hidex, Turku, Finland) at 570 nm absorbance. The cell viability percentage after exposure to the solvents for 48 h was calculated by the previously described method [36]. The ratio of the absorbance of treated cells to the absorbance of DMSO-treated control cells was determined as cell viability (%). The concentration of the solvent required to reduce the absorbance of treated cells to 50 % of the DMSO-treated control cells was defined as the $IC_{50}$.

## Results and discussion

### Synthesis and general properties

Four 2,3-dihydroxybenzaldehyde N4-substituted thiosemicarbazones ($H_3L^{1-4}$) have been used in this study, differing in the substituent on nitrogen N4 of the thiosemicarbazone moiety (R = H; $H_3L^1$, $CH_3$; $H_3L^2$, $C_6H_5$; $H_3L^3$ and $C_2H_5$; $H_3L^4$), in order to observe their influence on the biological activities of the complexes. Reaction of these thiosemicarbazone ligands with $Ni(ClO_4)_2{\cdot}6H_2O$ in a molar ratio 1:1 afforded mononuclear complexes **1–4** of the general formula $[Ni(H_3L)(H_2L)]ClO_4$ (Scheme 3a), while reactions of these thiosemicarbazones with $Ni(OAC)_2{\cdot}4H_2O$ in a molar ratio 2:1 (ligand:metal) yielded complexes (**5–8**) of the general formula $[Ni_2(HL)_2]$ (Scheme 3b). The stoichiometries, partial elemental analyses and colors of the complexes are shown in Table 1. The crystals of the nickel(II) complexes (**1–4**), prepared by slow evaporation of the solution remaining after filtration of the reaction mixtures, were green colored and soluble in common organic solvents such as methanol, ethanol, acetone, and acetonitrile. They were stable to air and moisture and could be manipulated in air without noticeable decomposition, although the brightness of the crystals decreased when they were taken out of solution. Complexes (**5–8**), were isolated as brown solids, insoluble in common organic solvents such as methanol, dichloromethane, and acetonitrile, but soluble in DMF and DMSO.

In the mononuclear complexes (**1–4**), the two thiosemicarbazone ligands bound to nickel(II) behave as tridentate ONS chelating ligands, but in different coordination modes as evidenced by the IR spectra and X-ray analysis. One ligand is coordinated in its neutral form through the thione sulfur, azomethine nitrogen and the phenol oxygen, while the other is




**Scheme 3** Proposed structures of the prepared complexes

**Table 1** Stoichiometries, color and partial elemental analysis of nickel(II) thiosemicarbazone complexes

| Complex | Stoichiometries | Yield % | Color | Anal. calc. (found) % | | |
|---|---|---|---|---|---|---|
| | | | | C | H | N |
| $[Ni(H_3L^1)(H_2L^1)]ClO_4{\cdot}2H_2O$ (**1**) | $C_{16}H_{17}N_6O_8S_2ClNi{\cdot}2H_2O$ | 68 | Green | 31.21 (31.30) | 3.44 (3.32) | 13.65 (13.68) |
| $[Ni(H_3L^2)(H_2L^2)]ClO_4{\cdot}2H_2O$ (**2**) | $C_{18}H_{21}N_6O_8S_2ClNi{\cdot}H_2O$ | 72 | Green | 34.55 (34.47) | 3.71 (4.64) | 13.43 (13.49) |
| $[Ni(H_3L^3)(H_2L^3)]ClO_4{\cdot}$(**3**) | $C_{28}H_{25}N_6O_8S_2ClNi$ | 64 | Green | 45.95 (46.01) | 3.44 (3.49) | 11.49 (11.40) |
| $[Ni(H_3L^4)(H_2L^4)]$ $ClO_4{\cdot}H_2O$ (**4**) | $C_{20}H_{25}N_6O_8S_2NiCl{\cdot}H_2O$ | 72 | Green | 36.74 (36.81) | 4.16 (4.05) | 12.86 (12.84) |
| $[Ni_2(HL^1)_2]$ (**5**) | $C_{16}H_{14}N_6O_4S_2Ni_2$ | 62 | Brownish red | 35.86 (35.84) | 2.63 (2.53) | 15.68 (15.66) |
| $[Ni_2(HL^2)_2]{\cdot}H_2O$(**6**) | $C_{16}H_{18}N_6O_4S_2Ni_2{\cdot}H_2O$ | 70 | Brown | 37.15 (37.12) | 3.46 (3.43) | 14.44 (14.38) |
| $[Ni_2(HL^3)_2]$ (**7**) | $C_{16}H_{18}N_6O_4S_2Ni_2$ | 73 | Brown | 48.88 (48.79) | 3.22 (3.20) | 12.21 (12.18) |
| $[Ni_2(HL^4)_2]$ (**8**) | $C_{20}H_{22}N_6O_4S_2Ni_2$ | 76 | Brown | 40.58 (40.64) | 3.75 (3.82) | 14.20 (14.23) |

coordinated in its monodeprotonated form through the thione sulfur, azomethine nitrogen and phenolate oxygen. Magnetic moment measurements indicate the paramagnetic nature of these nickel(II) complexes with room temperature magnetic moments in the range of 2.92–3.08 B.M, consistent with octahedral nickel(II) complexes [37]. The thiosemicarbazone ligand in complexes (**5**–**8**) is bound in its doubly deprotonated form as a tridentate ONS donor in a bridging mode through the thiolate sulfur, azomethine nitrogen and phenolic oxygen. Magnetic moment measurements indicate the diamagnetic nature of these nickel(II) complexes. Due to the poor solubility of these complexes, attempts to grow crystals suitable for X-ray studies were not successful.

## Crystal structure of complex **4**

The crystallographic data and structure refinement parameters obtained from X-ray diffraction analysis for complex **4** are shown in Table 2. Figure 1 shows the molecular structure, and the most important bond lengths and angles are shown in Table 3. This compound crystallizes in the monoclinic space group *P*2(1)/*c* with two molecular weight units per cell. The asymmetric unit of complex **4** (Fig. 2) consists of two distorted octahedral $[Ni(H_3L^4)(H_2L^4)]^+$ cations and two perchlorate counteranions, as well as two water molecules of solvation within the crystal lattice. In each cation, the nickel center is coordinated to two different 2,3-dihydroxybenzaldehyde-N4-ethylthiosemicarbazone ligands, which are placed approximately perpendicular to each other. One neutral ligand is coordinated through the azomethine nitrogen, thione sulfur and phenol oxygen, while the other is monoanionic and coordinated through the azomethine nitrogen, thione sulfur and phenolate oxygen. This arrangement gives rise to an octahedral environment in such a way that four bicyclic chelate systems are formed.

**Table 2** Crystal data and structure refinement parameters for complex **4**

| Parameters | $[Ni(H_3L^4)(H_2L^4)]_2(ClO_4)_2{\cdot}2H_2O$ |
|---|---|
| Empirical formula | $C_{40}\ H_{52}\ Cl_2\ N_{12}\ Ni_2\ O_{18}\ S_4$ |
| Formula weight, *M* | 1,305.50 |
| Temperature, *T* (K) | 273 |
| Wavelength, Mo Ka (Å) | $\lambda = 0.71073$ |
| Crystal system | Monoclinic |
| Space group | *P*2(1)/*c* |
| Unit cell dimensions | |
| *a* (Å) | 12.0076(2) |
| *b* (Å) | 26.8576(4) |
| *c* (Å) | 17.2286(3) |
| α (°) | 90.00 |
| β (°) | 101.3100(10) |
| γ (°) | 90.00 |
| Volume *V* (Å$^3$) | 5,448.24(15) |
| *Z* | 4 |
| Absorption coefficient, μ (cm$^{-1}$) | 1.023 |
| Reflections collected | 48,139 |
| Density (calculated) (mg m$^{-3}$) | 1.592 |
| *F*(0 0 0) | 2,696 |
| Data/parameters/restraint | 11,141/703/0 |
| *S* | 1.009 |
| Independent reflections | 6,858 [*R*(int) = 0.0503] |
| $R[F2 > 2\sigma(F2)]$ | 0.1151 |
| *wR*(*F*2) | 0.1241 |
| Largest difference peak and hole (e Å$^3$) | 0.6618 and 0.7454 |

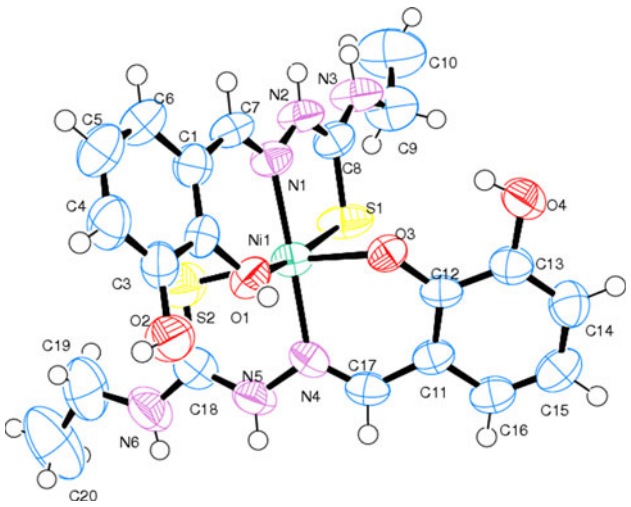



**Fig. 1** Molecular structure of complex $[Ni(H_3L^4)(H_2L^4)]ClO_4{\cdot}2H_2O$, displacement ellipsoids are drawn at 70 % probability level, perchlorate ion and water molecule are omitted for clarity

**Table 3** Selected bond lengths (Å) and bond angles (°) of complex **4**

| Bond lengths | | Bond angels | |
|---|---|---|---|
| Ni1–N4 | 2.015(3) | N4–Ni1–N1 | 178.47(14) |
| Ni1–N1 | 2.025(3) | N4–Ni1–O3 | 88.70(11) |
| Ni2–N7 | 2.032(3) | N1–Ni1–O3 | 92.51(11) |
| Ni2–N10 | 2.031(3) | N4–Ni1–O1 | 92.28(13) |
| Ni1–O3 | 2.054(3) | N1–Ni1–O1 | 86.92(13) |
| Ni1–O1 | 2.108(3) | O3–Ni1–O1 | 84.25(12) |
| Ni2–O7 | 2.048(3) | N4–Ni1–S1 | 96.60(9) |
| Ni2–O5 | 2.114(3) | N1–Ni1–S1 | 84.35(10) |
| Ni1–S2 | 2.3972(1) | O3–Ni1–S1 | 89.14(8) |
| Ni1–S1 | 2.4073(1) | O1–Ni1–S1 | 168.81(10) |
| Ni2–S3 | 2.3958(1) | N4–Ni1–S2 | 83.59(10) |
| Ni2–S4 | 2.4116(1) | N1–Ni1–S2 | 95.09(10) |
| S2–C18 | 1.682(5) | O3–Ni1–S2 | 169.71(8) |
| S1–C8 | 1.691(4) | O1–Ni1–S2 | 89.25(10) |
| S4–C38 | 1.683(4) | S1–Ni1–S2 | 98.48(5) |
| S3–C28 | 1.680(4) | N10–Ni2–N7 | 176.47(13) |
| N7–C27 | 1.282(4) | N10–Ni2–O7 | 87.59(11) |
| N10–C37 | 1.286(5) | N7–Ni2–O7 | 95.28(11) |
| N11–C38 | 1.344(5) | N10–Ni2–O5 | 96.60(11) |
| N4–C17 | 1.274(5) | N7–Ni2–O5 | 85.75(11) |
| N8–C28 | 1.351(5) | O7–Ni2–O5 | 83.99(10) |
| N2–C8 | 1.347(5) | N10–Ni2–S3 | 94.61(9) |
| N1–C7 | 1.283(5) | N7–Ni2–S3 | 83.30(9) |
| N5–C18 | 1.349(5) | O7–Ni2–S3 | 91.31(8) |
| C2–O1 | 1.360(5) | O5–Ni2–S3 | 167.64(8) |
| O2–C3 | 1.375(5) | N10–Ni2–S4 | 82.65(10) |
| O4–C13 | 1.371(4) | N7–Ni2–S4 | 94.85(9) |
| O3–C12 | 1.336(4) | O7–Ni2–S4 | 166.38(8) |
| O5–C22 | 1.358(4) | O5–Ni2–S4 | 87.71(8) |
| O6–C23 | 1.366(5) | S3–Ni2–S4 | 98.90(4) |
| O7–C32 | 1.333(4) | | |
| O8–C33 | 1.365(5) | | |

Because of the different character of the atoms forming the environment around the Ni(II) ion, the coordination octahedron is distorted, where the Ni atoms are in a *trans*-N(1)N(4)-*cis*-O(1)O(3)-*cis*-S(1)S(2) configuration and *trans*-N(7)N(10)-*cis*-O(5)O(7)-*cis*-S(3)S(4) configuration, respectively. The trans angles involving the two imine nitrogens, N(1)–Ni1–N(4) of 178.46(15)° and N(7)–Ni2–N(10) of 176.44(13)°, are <180°. The O(1)–Ni1(1)–O(3) angle is 83.83(11)° and O(5)–Ni2(1)–O(7) angle is 83.78(10)°, while the S(1)–Ni1–S(2) angle of 98.47(5)° and S(3)–Ni2–S(4) of 98.89(5)° are >90°, revealing that the two thiosemicarbazone ligands exert significant steric effects on each other. The bond lengths between each nickel(II) ion and the donor atoms are very unequal,

increasing in the order Ni–N < Ni–O < Ni–S as shown in Table 2. However, the average Ni–N bond distance of 2.025(3) Å is in the normal range for Ni–N bond distances of octahedral nickel(II) complexes with thiosemicarbazones [38]. The coordination modes of the oxygens of the 2,3-dihydroxybenzaldehyde moieties in the two ligands are different; the average value of bond distances O(1)–C(2), 1.360(5) Å, is only 0.015 Å shorter than the average value of bond O(2)–C(3), 1.375(5) Å, while the average value of bond distances O(3)–C(12), 1.336(4) Å, is 0.038 Å shorter than the average value of O(2)–C(3), 1.375(5) Å. Consequently, the Ni–O bond distances are different and lay in the range 2.049–2.120 Å. These values are close to the bond distances observed in other nickel salicylaldehyde complexes. The other values are very similar to those reported for other nickel complexes with ONS-donor thiosemicarbazones [39].

The bicyclic chelate systems Ni(1), S(2), C(18), N(5), N(4), C(17), C(11), C(12), O(3) and Ni(1), S(1), C(8), N(2), N(1), C(7), C(1), C(2), O(1) are considerably deviated from planarity, as evidenced by the maximum deviation of 0.345(2) Å for O(3) and 0.24(3) Å for O(1), respectively, from their respective mean planes.

The packing of the molecules in the unit cell is shown in Fig. 3. The molecules are held together by a system of hydrogen bonds in which both $[Ni(H_3L^4)(H_2L^4)]^+$ cations act as donors through the nitrogens N(2), N(3), N(5), N(6), N(8), N(9), N(11) and N(12) and the oxygens O(2), O(4), O(6), and O(8), and as acceptors through the oxygen atoms O(2), O(3), O(6) and O(7) (Table 4). The two perchlorate anions and water molecules also act as acceptors. The molecular structures of the non-crystallized complexes **1**, **2** and **3** are expected to be similar to that of complex **4**.

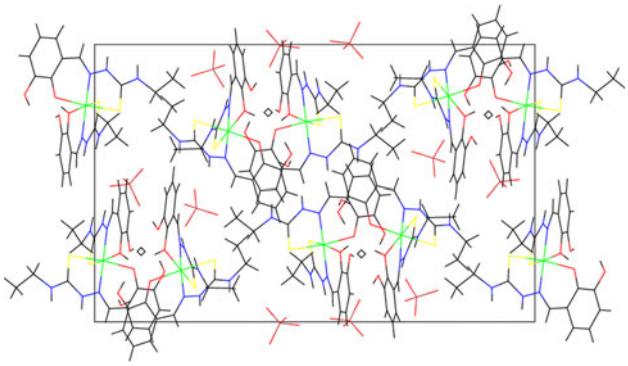


**Fig. 3** Unit cell packing of complex **4** along *a* axis

## Spectroscopic studies

The most characteristic vibrational frequencies and their tentative assignments for the 2,3-dihydroxybenzaldehyde-N4-substituted thiosemicarbazone ligands $H_3L$, and their nickel(II) complexes are listed in Table 5. In the solid state, all the thiosemicarbazones remain in the thione form, as indicated by the bands found at around 1,390–1,340 $cm^{-1}$ attributable to $\nu$(C=S). Furthermore, the asymmetric stretching frequency of the thioamide group is observed in the region of 855–800 $cm^{-1}$. Moreover, the $\nu$(S–H) band that would appear at around 2,500–2,800 $cm^{-1}$ cannot be seen in the spectra of all these ligands, while a sharp band due to the vibration of N2–H is observed at 3,200–3,150 $cm^{-1}$ in the spectra of all the ligands. These results support the existence of the thione tautomer in the solid state [40, 41]. The bands around 3,300–3,420 $cm^{-1}$ are attributed to $\nu$(N4–H), while the bands at the region of 3,500–3,370 $cm^{-1}$ are assigned to OH stretching of the polyhydroxybenzaldehyde thiosemicarbazone. The

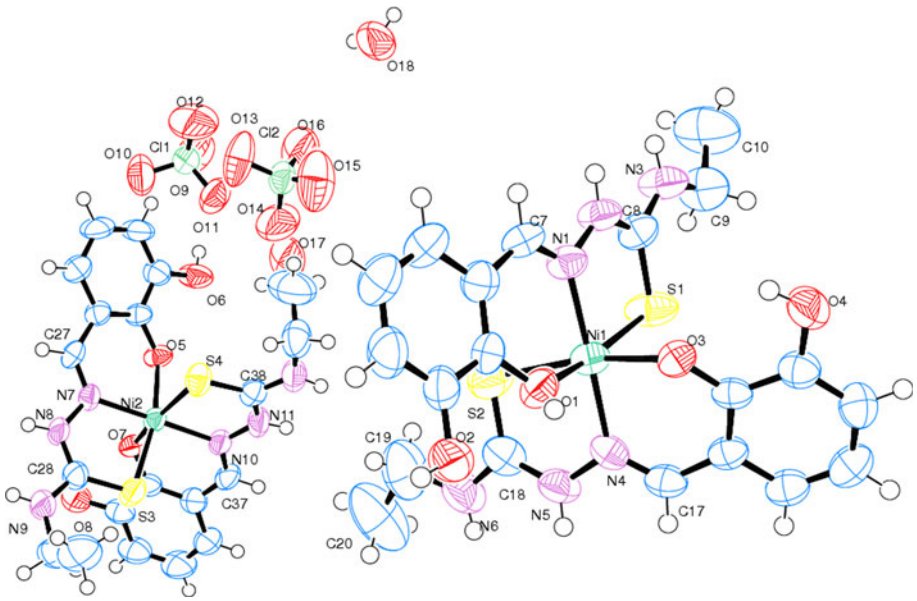



**Fig. 2** Asymmetric unit cell for complex **4**

**Table 4** Hydrogen-bond geometry (Å, °) for complex (**4**)

| Donor–H···acceptor | D–H | H···A | D···A | D–H···A |
|---|---|---|---|---|
| N(2)–H(2A)···O(10) | 0.86 | 2.32 | 3.149(6) | 163 |
| N(2)–H(2A)···O(9) | 0.86 | 2.55 | 3.043(5) | 118 |
| O(2)–H(2B)···O(17) | 0.82 | 1.87 | 2.673(3) | 164 |
| N(3)–H(3A)···O(9) | 0.86 | 2.30 | 3.122(5) | 160 |
| N(3)–H(3A)···O(10) | 0.86 | 2.52 | 3.053(6) | 121 |
| O(4)–H(4A)···O(3) | 0.82 | 2.31 | 2.754(4) | 114 |
| O(4)–H(4A)···O(6) | 0.82 | 1.95 | 2.720(4) | 155 |
| N(5)–H(5A)···O(18) | 0.86 | 2.03 | 2.842(5) | 158 |
| O(6)–H(6A)···O(11) | 0.82 | 2.69 | 2.903(5) | 173 |
| N(6)–H(6B)···O(18) | 0.86 | 2.53 | 3.256(6) | 143 |
| N(8)–H(8A)···O(16) | 0.86 | 2.26 | 3.057(6) | 154 |
| O(8)–H(8B)···O(2) | 0.82 | 1.91 | 2.685(4) | 157 |
| O(8)–H(8B)···O(7) | 0.82 | 2.33 | 2.742(4) | 112 |
| N(9)–H(9A)···O(14) | 0.86 | 2.26 | 3.118(6) | 171 |
| N(11)–H(11A)···O(13) | 0.86 | 2.16 | 2.977(6) | 157 |
| N(12)–H(12A)···O(13) | 0.86 | 2.44 | 3.209(6) | 149 |

azomethine stretching frequency is observed at 1,611–1,542 $cm^{-1}$, while strong to medium bands observed in the range of 1,210–1,060 $cm^{-1}$ are assigned to the hydrazinic (N–N) bonds.

The assignments of the vibrational frequencies of the complexes were made by comparing with those of the free ligands. The IR spectra of the nickel complexes suggest that the coordination of the thiosemicarbazone to nickel(II) occurs through the azomethine nitrogen, phenolate oxygen and thione sulfur. The azomethine $\nu$(C=N) vibration in the range of 1,611–1,542 $cm^{-1}$ in the free ligands shows a positive shift to higher frequency 1,617–1,581 $cm^{-1}$ after complexation, indicating participation of the azomethine group in the coordination to nickel(II), such that the electron density of the azomethine group is reduced due to coordination of nitrogen to the metal atom. However, this band is not a pure vibrational band of C=N stretching, but it is a combination band of [$\nu$(C–N) + $\delta$(NH)] stretching vibrations [42]. Meanwhile, the shift to higher frequency of the $\nu$(N–N) band, observed in the spectra of the complexes, has also been related to the electronic delocalization produced as a consequence of coordination through the azomethine nitrogen atom. Further evidence for the coordination of the azomethine nitrogen is obtained from the presence of a new band 520–580 $cm^{-1}$, assignable to $\nu$(Ni–N), for all the nickel complexes. The interaction of thiosemicarbazone ligands with nickel(II) through sulfur in all of these complexes is confirmed by the shift in the thioamide band to lower energies, together with a decrease in intensity when compared with the corresponding bands of the free ligands. In complexes **1**–**4**, the bands assigned to the C=S stretching at 1,390–1,340 and 855–800 $cm^{-1}$ in the free ligands have shifted to 1,342–1,330 and 780–775 $cm^{-1}$, respectively, while the thioamide stretching in complexes **5**–**8** was observed at 1,330–1,315 $cm^{-1}$ and around 735 $cm^{-1}$. This last band is attributed to $C–S_{bridging}$ stretching. The wavenumber shift of the thioamide band of complexes **5**–**8** (thiol coordination) would be expected to be larger than in the complexes **1**–**4** (thione coordination), due to the lowering of the C–S bond order owing to deprotonation in the case of thiol coordination. The presence of a new band at around 1,525–1,559 $cm^{-1}$ in the IR spectra of complexes **5**–**8** due to the newly formed $\nu$(N=C) supports the above observation. The loss of the O–H proton upon coordination is difficult to assign in polyhydroxybenzaldehyde thiosemicarbazone complexes, due to the presence of more than one OH which can engage in both inter- and intra-molecular hydrogen bonds [21].

**Table 5** IR Spectral assignments for the thiosemicarbazone ligands and their nickel(II) complexes

| Compound | $\nu$(C=N) | $\nu$(C=S)/$\nu$(C–S) | $\nu$(C–O) | $\nu$(N–N) | $\nu$(Ni–O) | $\nu$(Ni–N) |
|---|---|---|---|---|---|---|
| $H_3L^1$ | 1,611 | 1,342, 824 | 1,279 | 1,068 | – | – |
| $H_3L^2$ | 1,543 | 1,389, 853 | 1,290 | 1,043 | – | – |
| $H_3L^3$ | 1,542 | 1,389, 850 | 1,289 | 1,064 | – | – |
| $H_3L^4$ | 1,542 | 1,384, 807 | 1,307 | 1,051 | – | – |
| $[Ni(H_3L^1)(H_2L^1)](ClO_4)_2{\cdot}2H_2O$ (**1**) | 1,617 | 1,335, 779 | 1,270 | 1,087 | 467 | 523 |
| $[Ni(H_3L^2)(H_2L^2)]$ $(ClO_4)_2{\cdot}H_2O$ (**2**) | 1,581 | 1,342, 789 | 1,272 | 1,086 | 474 | 581 |
| $[Ni(H_3L^3)(H_2L^3)](ClO_4)_2$ (**3**) | 1,607 | 1,324, 773 | 1,264 | 1,061 | 455 | 569 |
| $[Ni(H_3L^4)(H_2L^4)](ClO_4)_2{\cdot}2H_2O$ (**4**) | 1,606 | 1,340, 780 | 1,280 | 1,088 | 464 | 569 |
| $[Ni_2(HL^1)_2]$ (**5**) | 1,601 | 1,320, 737 | 1,227 | 1,094 | 486 | 545 |
| $[Ni_2(HL^2)_2]{\cdot}H_2O$ (**6**) | 1,591 | 1,333, 736 | 1,260 | 1,090 | 447 | 521 |
| $[Ni_2(HL^3)_2]$ (**7**) | 1,597 | 1,335, 737 | 1,263 | 1,095 | 424 | 520 |
| $[Ni_2(HL^4)_2]$ (**8**) | 1,595 | 1,316, 734 | 1,260 | 1,089 | 445 | 521 |

However, the coordination of phenolate oxygen is indicated by the lowering in the $\nu$(C–O) band by 10–30 $cm^{-1}$ when compared with the free ligand. These observations are supported by the observation of a Ni–O band at 480–424 $cm^{-1}$.

$^1$H NMR is a helpful tool for identification of the coordination sites in diamagnetic nickel(II) complexes when taken in conjunction with other spectroscopic information. The chemical shifts of the thiosemicarbazone ligands and their diamagnetic nickel complexes $[Ni_2(HL^2)_2]$ (**6**) and $[Ni_2(HL^3)_2]$ (**7**) are presented in Table 6. A singlet observed at 11.41 and 11.78 ppm for the free ligands $H_3L^2$ and $H_3L^3$, respectively, is assigned to the thioamidic proton N(2)H. This signal is absent from the spectra of complexes 6 and 7, showing that coordination of the ligands $H_3L^2$ and $H_3L^3$ to nickel(II) is in the thiolate form [43]. In the $^1$H NMR spectra of both complexes, the azomethine proton signal is observed as two singlets; one is shifted upfield, while the other is shifted downfield when compared with the free ligands. This pattern of shifts is probably due to different withdrawal of electron density from the thiosemicarbazone moiety. The appearance of CH=N as two signals suggests the coordination of two inequivalent azomethine nitrogens in these complexes. Furthermore, two signals are observed at 9.50, 8.90 ppm and 9.21, 9.54 ppm for complexes **6** and **7,** respectively; each integrates as one hydrogen corresponding to the *m*-OH of the thiosemicarbazone moiety.

In the $^1$H NMR spectra of complexes **6** and **7**, the N(4)H appears at 6.30 and 6.38 ppm, respectively, and has shifted upfield compared with the free $H_3L^2$ and $H_3L^3$ ligands, perhaps due to the presence of this proton within the benzene shielding cone. Unfortunately, the $^1$H NMR spectra of complexes $[Ni_2(HL^1)_2]$ (**5**) and $[Ni_2(HL^4)_2]$ (**8**) could not be assigned due to spectral broadening which may be due to the presence of paramagnetic species formed with solvent in the axial positions [44]. The aromatic ring protons are observed as multiplets between 7.59 and 6.61 ppm, which are practically unaffected by metal complexation.

## In vitro antimicrobial assays

The disc diffusion method, developed by Bauer et al., is a well-standardized and highly reproducible qualitative method of antimicrobial susceptibility testing that allows the simultaneous testing of a number of compounds in a relatively easy and inexpensive manner. In this study, the disc diffusion method was applied to test the activity of the compounds against 25 bacterial strains. The diameter of the inhibition zone was correlated with the susceptibility level of each microorganism against the test compound (Table 7). The bacterial strains indicated diverse susceptibilities to these compounds, but a significant percentage of the tested bacterial strains demonstrated high resistance.

In order to gain more quantitative data, microdilution plate analysis was also performed in the present study (Table 8). Overall, most of the compounds demonstrated low-to-moderate antibacterial activities.

The complexes indicated higher inhibitory activities than the corresponding free ligands, and the susceptibility levels of the Gram-negative bacteria to the complexes were higher than those of the Gram-positive bacteria. Complexes (**7**) and (**8**) exhibited significant antimicrobial activity

**Table 6** $^1$H NMR spectral assignments for the thiosemicarbazone ligands and their nickel(II) complexes **6** and **7** in DMSO-$d_6$

| Compound | Chemical shifts, $\delta$ (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | N(2)H | OH | C=N | N(4)H | Aromatic due to Tsc | Aliphatic due to Tsc |
| $H_3L^2$ (**2**) | 11.41(*s*, 1H) | 9.26(*s*, 2H) | 8.38(*s*, 1H) | 8.39(*s*, 1H) | 7.38(*d*, 1H)<br>6.41(*dd*, 1H)<br>6.66(*t*, 1H) | 3.01(*d*, 3H) |
| $[Ni_2(HL^2)_2]\cdot H_2O$ (**6**) | – | 9.50(*s*, 1H)<br>8.90(*s*, 1H) | 8.33(*s*, 1H)<br>8.42(*s*, 1H) | 6.30(*s*, 1H) | 7.33(*d*, 2H)<br>6.75(*d*, 2H)<br>6.61(*t*, 2H) | 2.96(*d*, 3H) |
| $H_3L^3$ (**3**) | 11.78(*s*, 1H) | 9.55(*s*, 1H)<br>9.04(*s*, 1H) | 8.52(*s*, 1H) | 10.03(*s*, 1H) | 7.59(*d*, 1H)<br>7.50(*d*, 2H)<br>7.36(*t*, 2H)<br>7.19(*t*, 1H)<br>6.71(*dd*, 1H)<br>6.69(*t*, 1H) | – |
| $[Ni_2(HL^3)_2]$ (**7**) | – | 9.54(*s*, 1H)<br>9.21(*s*, 1H) | 8.99(*s*, 1H)<br>8.47(*s*, 1H) | 6.38(*s*, 1H) | 7.58–6.63(*m*, 13H) | – |

**Table 7** Inhibitory activity of the compounds against different bacterial species using Kirby–Bauer disc diffusion method

| Tested microorganism | Inhibitory activity of compounds based on the inhibition zones (IZ)[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $H_3L^1$ | $H_3L^2$ | $H_3L^3$ | $H_3L^4$ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| *B. subtilis* | I | I | R | I | I | I | I | I | I | I | I | I |
| *E. faecalis* | I | S | R | S | I | R | R | R | I | I | I | S |
| E. *coli* ATCC 25922 | I | R | R | I | I | I | I | I | I | R | I | I |
| *L. innocua* | R | I | I | I | R | I | I | R | R | I | S | R |
| *L. monocytogenes* | R | I | R | R | I | I | I | I | R | S | S | I |
| *L. welshimeri* | R | R | R | I | I | R | S | I | I | R | S | I |
| *P. mirabilis* | R | R | R | R | R | I | R | I | R | I | I | S |
| *P. aeruginosa* | R | I | R | I | S | S | S | S | I | S | S | I |
| *S. enterica* ATCC 9068 | R | R | R | R | S | S | R | S | S | S | S | S |
| *S. enteritidis* | R | R | R | I | I | S | I | S | I | I | S | S |
| *S. typhi* | R | I | R | I | I | I | I | R | I | R | S | I |
| S. *typhimurium* | R | R | R | R | I | I | I | I | I | R | I | R |
| *S. dysenteriae* | R | R | R | I | I | I | I | I | I | S | I | S |
| *S. flexneri* | I | I | R | I | S | S | I | S | I | I | I | I |
| *S. sonnei* | R | R | R | I | I | I | I | I | I | I | I | S |
| *S. aureus* | I | I | R | I | I | I | I | I | I | I | I | I |
| *S. epidermidis* | I | R | I | I | I | R | S | I | I | I | I | I |
| *S. pneumoniae* | R | R | R | R | I | R | I | I | R | R | S | I |
| *V. alginolyticus* | I | I | R | I | I | I | I | I | I | I | I | I |
| *V. cholerae* | I | I | R | R | I | I | I | I | I | I | I | I |
| *V. fischeri* | S | I | R | S | S | S | I | S | S | S | I | S |
| *V. harveyi* | I | I | R | I | S | S | S | S | S | S | S | S |
| *V. parahaemolyticus* | R | I | I | I | R | R | I | R | I | I | S | I |
| *V. vulnificus* | R | R | R | R | I | I | I | I | I | I | S | S |
| *Yersinia pestis* | I | R | R | R | I | I | I | R | I | I | S | R |

[a] *R* resistant (IZ = 0 mm), *I* intermediate (0 < IZ ≤ 1 mm), *S* susceptible (IZ > 1 mm)

**Table 8** In vitro antimicrobial activity of the compounds using microdilution method

| Tested microorganisms | Minimum inhibitory concentration (MIC)/minimum microbicidal concentration (MMC) (μg/ml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $H_3L^1$ | | $H_3L^2$ | | $H_3L^3$ | | $H_3L^4$ | | 1 | | 2 | |
| | MIC | MMC | MIC | MMC | MIC | MMC | MIC | MMC | MIC | MMC | MIC | MMC |
| *B. subtilis* | 250 | 500 | 500 | 1,000 | 250 | 500 | 1,000 | >1,000 | 250 | 500 | 500 | 500 |
| *E. faecalis* | 250 | 500 | 125 | 250 | 62.5 | 125 | 125 | 250 | 500 | >1,000 | 500 | >1,000 |
| E. *coli* ATCC 25922 | 250 | 500 | 500 | 1,000 | 250 | 500 | 500 | >1,000 | 125 | 250 | 125 | 250 |
| *L. innocua* | 500 | 1,000 | 250 | 500 | 62.5 | 125 | 250 | 250 | 500 | 1,000 | 250 | 500 |
| *L. monocytogenes* | 500 | 1,000 | 250 | 500 | 500 | 1,000 | 500 | >1,000 | 250 | 500 | 250 | 500 |
| *L. welshimeri* | 500 | 1,000 | 1,000 | >1,000 | 125 | 250 | 250 | 500 | 250 | 500 | 1,000 | >1,000 |
| *P. mirabilis* | 250 | 500 | 250 | 500 | 250 | 500 | 125 | 250 | 500 | 1,000 | 250 | 500 |
| *P. aeruginosa* | 250 | 500 | 250 | 500 | 125 | 250 | 250 | 250 | 125 | 250 | 125 | 125 |
| *S. enterica* ATCC 9068 | 500 | 1,000 | 500 | 500 | 250 | 250 | 250 | 500 | 62.5 | 125 | 62.5 | 125 |
| *S. enteritidis* | 250 | 500 | 500 | >1,000 | 125 | 250 | 250 | 500 | 125 | 250 | 62.5 | 125 |
| *S. typhi* | 1,000 | >1,000 | 250 | 500 | 250 | 250 | 250 | 500 | 125 | 250 | 250 | 500 |
| S. *typhimurium* | 500 | 1,000 | 1,000 | >1,000 | 250 | 500 | 1,000 | >1,000 | 250 | 500 | 250 | 500 |
| *S. dysenteriae* | 500 | >1,000 | 500 | 500 | 250 | 500 | 500 | >1,000 | 250 | 500 | 62.5 | 125 |

**Table 8** continued

| Tested microorganisms | Minimum inhibitory concentration (MIC)/minimum microbicidal concentration (MMC) (μg/ml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $H_3L^1$ | | $H_3L^2$ | | $H_3L^3$ | | $H_3L^4$ | | 1 | | 2 | |
| | MIC | MMC | MIC | MMC | MIC | MMC | MIC | MMC | MIC | MMC | MIC | MMC |
| *S. flexneri* | 250 | 500 | 125 | 250 | 250 | 500 | 125 | 250 | 62.5 | 125 | 31.2 | 62.5 |
| *S. sonnei* | 500 | 1,000 | 250 | 500 | 500 | 500 | 500 | >1,000 | 250 | 250 | 125 | 250 |
| *S. aureus* | 250 | 500 | 250 | 250 | 250 | 500 | 125 | 250 | 125 | 250 | 125 | 125 |
| *S. epidermidis* | 250 | 500 | 500 | >1,000 | 62.5 | 125 | 125 | 250 | 250 | 500 | 500 | 500 |
| *S. pneumoniae* | 500 | >1,000 | 1,000 | >1,000 | 125 | 250 | 250 | 500 | 500 | 500 | 1,000 | >1,000 |
| *V. alginolyticus* | 250 | 500 | 250 | 250 | 250 | 500 | 125 | 250 | 125 | 250 | 125 | 250 |
| *V. cholerae* | 500 | 1,000 | 250 | 250 | 125 | 250 | 500 | >1,000 | 250 | 500 | 125 | 250 |
| *V. fischeri* | 62.5 | 125 | 125 | 250 | 250 | 250 | 62.5 | 125 | 31.2 | 125 | 62.5 | 62.5 |
| *V. harveyi* | 125 | 250 | 125 | 125 | 125 | 250 | 250 | 500 | 62.5 | 125 | 62.5 | 125 |
| *V. parahaemolyticus* | 500 | 1,000 | 250 | 500 | 250 | 500 | 250 | 250 | 250 | 500 | 500 | 1,000 |
| *V. vulnificus* | 500 | 1,000 | 1,000 | >1,000 | 250 | 500 | 500 | >1,000 | 250 | 250 | 125 | 250 |
| *Y. pestis* | 125 | 250 | 1,000 | >1,000 | 250 | 500 | 500 | 1,000 | 125 | 250 | 250 | 250 |

| Tested microorganisms | Minimum inhibitory concentration (MIC)/minimum microbicidal concentration (MMC) (μg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3 | | 4 | | 5 | | 6 | |
| | MIC | MMC | MIC | MMC | MIC | MMC | MIC | MMC |
| *B. subtilis* | 250 | 500 | 250 | 250 | 500 | >1,000 | 500 | 500 |
| *E. faecalis* | 250 | 500 | 125 | 500 | 250 | 500 | 500 | 1,000 |
| E. *coli* ATCC 25922 | 250 | 500 | 250 | 500 | 125 | 250 | 250 | 500 |
| *L. innocua* | 125 | 250 | 500 | >1,000 | 500 | >1,000 | 500 | 1,000 |
| *L. monocytogenes* | 250 | 500 | 500 | 1,000 | 1,000 | >1,000 | 125 | 250 |
| *L. welshimeri* | 125 | 250 | 250 | 500 | 500 | 1,000 | 500 | 1,000 |
| *P. mirabilis* | 250 | 500 | 250 | 500 | 1,000 | >1,000 | 250 | 500 |
| *P. aeruginosa* | 62.5 | 125 | 62.5 | 125 | 250 | 500 | 250 | 500 |
| *S. enterica* ATCC 9068 | 62.5 | 62.5 | 31.2 | 62.5 | 62.5 | 62.5 | 62.5 | 125 |
| *S. enteritidis* | 62.5 | 62.5 | 62.5 | 125 | 125 | 250 | 125 | 250 |
| *S. typhi* | 125 | 250 | 250 | 500 | 125 | 250 | 125 | 250 |
| S. *typhimurium* | 125 | 250 | 250 | 500 | 250 | 500 | 500 | 1,000 |
| *S. dysenteriae* | 250 | 250 | 125 | 250 | 125 | 250 | 62.5 | 62.5 |
| *S. flexneri* | 125 | 250 | 62.5 | 62.5 | 250 | 500 | 125 | 250 |
| *S. sonnei* | 250 | 500 | 62.5 | 125 | 250 | 500 | 125 | 250 |
| *S. aureus* | 125 | 250 | 250 | 500 | 250 | 500 | 125 | 250 |
| *S. epidermidis* | 31.2 | 62.5 | 125 | 250 | 250 | 500 | 250 | 250 |
| *S. pneumoniae* | 125 | 250 | 250 | 250 | 250 | 500 | 500 | 1,000 |
| *V. alginolyticus* | 125 | 250 | 250 | 500 | 250 | 500 | 250 | 250 |
| *V. cholerae* | 62.5 | 125 | 250 | 500 | 500 | 500 | 125 | 250 |
| *V. fischeri* | 125 | 250 | 62.5 | 125 | 62.5 | 125 | 31.2 | 62.5 |
| *V. harveyi* | 125 | 250 | 31.2 | 62.5 | 125 | 125 | 62.5 | 125 |
| *V. parahaemolyticus* | 250 | 500 | 500 | >1,000 | 250 | 250 | 125 | 250 |
| *V. vulnificus* | 125 | 125 | 250 | 500 | 125 | 250 | 250 | 500 |
| *Y. pestis* | 250 | 500 | 500 | 1,000 | 125 | 125 | 250 | 500 |

**Table 8** continued

| Tested microorganisms | Minimum inhibitory concentration (MIC)/minimum microbicidal concentration (MMC) (μg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | 7 | | 8 | | Gentamicin | |
| | MIC | MMC | MIC | MMC | MIC | MMC |
| *B. subtilis* | 250 | 500 | 500 | >1,000 | 0.8 | 1.6 |
| *E. faecalis* | 125 | 250 | 125 | 250 | >50 | >50 |
| E. *coli* ATCC 25922 | 250 | 500 | 500 | >1,000 | 1.6 | 3.1 |
| *L. innocua* | 62.5 | 125 | 250 | 500 | 0.8 | 1.6 |
| *L. monocytogenes* | 125 | 250 | 250 | 500 | 1.6 | 3.1 |
| *L. welshimeri* | 62.5 | 125 | 250 | 500 | 1.6 | 3.1 |
| *P. mirabilis* | 250 | 500 | 125 | 250 | 0.8 | 3.1 |
| *P. aeruginosa* | 31.2 | 62.5 | 62.5 | 125 | 3.1 | 6.25 |
| *S. enterica* ATCC 9068 | <15.7 | 15.7 | 15.7 | 31.2 | 25 | 50 |
| *S. enteritidis* | 31.2 | 62.5 | 62.5 | 125 | 3.1 | 6.25 |
| *S. typhi* | 62.5 | 125 | 250 | 500 | 1.6 | 6.25 |
| S. *typhimurium* | 125 | 250 | 500 | >1,000 | 3.1 | 6.25 |
| *S. dysenteriae* | 125 | 250 | 62.5 | 125 | 6.25 | 12.5 |
| *S. flexneri* | 125 | 250 | 250 | 250 | 12.5 | 12.5 |
| *S. sonnei* | 125 | 250 | 31.2 | 62.5 | 6.25 | 12.5 |
| *S. aureus* | 125 | 125 | 62.5 | 125 | 0.8 | 0.8 |
| *S. epidermidis* | 62.5 | 125 | 125 | 250 | 3.1 | 6.25 |
| *S. pneumoniae* | 31.2 | 62.5 | 250 | 250 | >50 | >50 |
| *V. alginolyticus* | 125 | 250 | 125 | 250 | 12.5 | 25 |
| *V. cholerae* | 125 | 250 | 125 | 125 | 6.25 | 12.5 |
| *V. fischeri* | 250 | 500 | 125 | 125 | 6.25 | 12.5 |
| *V. harveyi* | 125 | 125 | 31.2 | 62.5 | 12.5 | 25 |
| *V. parahaemolyticus* | 62.5 | 125 | 125 | 125 | 6.25 | 12.5 |
| *V. vulnificus* | 31.2 | 62.5 | 62.5 | 125 | 12.5 | 25 |
| *Y. pestis* | 62.5 | 125 | 500 | 500 | 1.6 | 3.1 |

against *S. enterica* ATCC 9068. The MIC values of the two compounds (15.7 and <15.7 μg/ml) toward *S. enterica* ATCC 9068 were lower than that of gentamicin, the positive control (25 μg/ml). Complex (**7**) also demonstrated higher inhibitory power against *S. pneumoniae* (MIC 31.2 μg/ml), compared with gentamicin (MIC > 50 μg/ml). Two of the ligands, $H_3L^1$ and $H_3L^4$ indicated significant antibacterial activity against *V. fischeri*, with MIC values <100 (MIC = 62.5 μg/ml), which is, however, higher than gentamicin (MIC = 6.25 μg/ml). The ligand $H_3L^3$ inhibited *E. faecalis*, *L. innocua* and *S. epidermidis* with MIC value of 62.5 μg/ml. However, the inhibitory level of the ligand was comparable with gentamicin only against *E. faecalis* (MIC > 50 μg/ml), and for the other two was much higher than the positive control.

## Brine shrimp lethality assays

The toxicities of the compounds were tested on brine shrimp (*A. salina*), and the results are expressed in $LC_{50}$ values (Table 9). Most of the ligands indicated higher $LC_{50}$ values than their corresponding complexes. In general, none of the compounds indicated high cytotoxicity against

**Table 9** Brine shrimp lethality assay (BSLA)

| Compound | LC50 value (μg/ml) ± SD |
|---|---|
| $H_3L^1$ | 97.79 ± 1.09 |
| $H_3L^2$ | 105.17 ± 1.46 |
| $H_3L^3$ | 9,361.07 ± 0.68 |
| $H_3L^4$ | 77.35 ± 0.93 |
| $[Ni(H_3L^1)(H_2L^1)](ClO_4)_2{\cdot}2H_2O$ (**1**) | 88.32 ± 1.73 |
| $[Ni(H_3L^2)(H_2L^2)]\ (ClO_4)_2{\cdot}H_2O$ (**2**) | 96.38 ± 3.84 |
| $[Ni(H_3L^3)(H_2L^3)](ClO_4)_2$ (**3**) | 63.56 ± 1.27 |
| $[Ni(H_3L^4)(H_2L^4)](ClO_4)_2{\cdot}2H_2O$ (**4**) | 59.29 ± 0.78 |
| $[Ni_2(HL^1)_2]$ (**5**) | 92.41 ± 1.45 |
| $[Ni_2(HL^2)_2]{\cdot}H_2O$ (**6**) | 87.61 ± 1.19 |
| $[Ni_2(HL^3)_2]$ (**7**) | 72.43 ± 1.05 |
| $[Ni_2(HL^4)_2]$ (**8**) | 58.76 ± 0.92 |

**Table 10** Cytotoxic activity of the compounds on different cancer cells in terms of $IC_{50}$

| Compound | $IC_{50}$ value (μg/ml) ± SD | | | |
|---|---|---|---|---|
| | MCF7 | PC3 | A375 | H413 |
| $H_3L^1$ | 50.43 ± 4.77 | 78.22 ± 4.86 | 52.78 ± 3.81 | 43.19 ± 5.12 |
| $H_3L^2$ | 43.75 ± 5.21 | 73.41 ± 6.29 | 57.22 ± 4.29 | 48.97 ± 3.84 |
| $H_3L^3$ | 97.52 ± 6.19 | 109.17 ± 8.45 | 78.31 ± 8.17 | 127.08 ± 13.54 |
| $H_3L^4$ | 26.08 ± 1.86 | 89.43 ± 5.93 | 59.03 ± 6.08 | 53.22 ± 4.26 |
| $[Ni(H_3L^1)(H_2L^1)](ClO_4)_2{\cdot}2H_2O$ (**1**) | 58.36 ± 3.97 | 36.51 ± 2.18 | 37.16 ± 2.53 | 47.21 ± 2.15 |
| $[Ni(H_3L^2)(H_2L^2)]$ $(ClO_4)_2{\cdot}H_2O$ (**2**) | 31.40 ± 2.04 | 45.90 ± 3.92 | 34.28 ± 3.11 | 41.74 ± 3.76 |
| $[Ni(H_3L^3)(H_2L^3)](ClO_4)_2$ (**3**) | 17.43 ± 1.27 | 27.63 ± 0.67 | 24.61 ± 2.98 | 18.08 ± 0.38 |
| $[Ni(H_3L^4)(H_2L^4)](ClO_4)_2{\cdot}2H_2O$ (**4**) | 27.61 ± 0.96 | 52.11 ± 2.87 | 56.73 ± 5.27 | 32.81 ± 2.53 |
| $[Ni_2(HL^1)_2]$ (**5**) | 46.21 ± 5.30 | 51.89 ± 6.02 | 41.83 ± 5.10 | 48.15 ± 5.21 |
| $[Ni_2(HL^2)_2]{\cdot}H_2O$ (**6**) | 39.91 ± 2.49 | 68.55 ± 4.73 | 57.29 ± 7.26 | 51.32 ± 3.70 |
| $[Ni_2(HL^3)_2]$ (**7**) | 14.22 ± 0.72 | 19.27 ± 0.93 | 18.30 ± 2.01 | 14.45 ± 0.62 |
| $[Ni_2(HL^4)_2]$ (**8**) | 27.55 ± 1.61 | 37.43 ± 1.81 | 25.18 ± 1.49 | 26.11 ± 1.05 |

*A. salina,* and the lowest $LC_{50}$ value was obtained for complex (**7**) ($LC_{50}$ = 58.76 ± 0.92 μg/ml).

MTT cell viability assays

The effect of the compounds on cell viability was determined by MTT assay on MCF7, PC3, A375, and H413 cells. Table 10 shows the in vitro anticancer $IC_{50}$ values recorded on the studied cell lines. Complex (**7**) induced the highest cell growth inhibition activity on the tested cell lines; among the rest, complexes (**3**) and (**8**) also exhibited moderately high cytotoxicities against the cancer cells. Other compounds showed low-to-moderate cytotoxicities.

The good antimicrobial and inhibition activity for complex (**7**) could be due to the existence of a phenyl substituent on the thiosemicarbazone moiety, which is more hydrophobic compared with the other substituents. Moreover, the inhibitory effect of complex (**7**) is larger than of complex (**3**) (complex with N4–phenyl substituent). This may be related to the coordinatively unsaturated square planar geometry in complex (**7**), giving the opportunity for easy binding of DNA to the metal [45].

## Conclusion

In this study, reaction of thiosemicarbazones with nickel perchlorate gave mononuclear nickel(II) complexes of the type $[Ni(H_3L)(H_2L)]ClO_4$ in which one thiosemicarbazone acts as uninegative tridentate and the other as neutral tridentate ligand forming bis-octahedral complexes. The reaction with nickel acetate gave nickel(II) complexes of the type $[Ni_2(HL)_2]$ (L = ONS-donor ligand) with the thiosemicarbazone coordinated as a binegative tridentate ligand. The $[Ni_2(HL)_2]$ series are most probably binuclear, based on the spectroscopic results and other studies. Attempts to get good crystals of these complexes were unsuccessful, preventing X-ray diffraction studies. The substituents at nitrogen N4 did not have an effect on the geometry of the nickel(II) complexes, but did influence the biological activity. The results of disc diffusion and microdilution tests indicated various levels of inhibition, conducted by the complexes and their corresponding ligands, against the tested bacterial strains. In general, the antimicrobial activities of the ligands were lower than their corresponding complexes. However, only a few combinations of complexes with the tested bacterial strains gave significant inhibition.

## Supplementary material

CCDC 941774 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via htt:www.ccdc.cam.ac.uk/conts/ retrieving html, or from the Cambridge crystallographic data centre, 12 Union Road, Cambridge CB21E2, UK; fax: (+44) 1223-336033; or e-mail:deposit@ccdc.cam.ac.uk. Supplementary data associated with this article can be found in the online version.

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