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High nuclearity nickel compounds with three, four or five metal atoms showing antibacterial activity

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Abstract

The effect on DNA and the antibacterial activity of a series of high nuclearity nickel compounds with three, four and five metal atoms were examined. The compounds have a mixed ligand composition with salicylhydroxamic acid and di-2-pyridyl-ketonoxime as chelate agents. In the trinuclear compound $Ni_3(shi)_2(Hpko)_2(py)_2$**(1)**, two metal ions show a square planar geometry while the third one is in an octahedral environment. The compounds with four and five nickel atoms construct metallacrown cores with two distinct connectivities. The tetranuclear vacant metallacrown $[12\text{-}MC_{Ni(II)N(Hshi)2(pko)2}\text{-}4]^{2+}$ shows the connectivity pattern $[-O-Ni-O-N-Ni-N-]_2$, while the pentanuclear $\{[Ni(II)][12\text{-}MC_{Ni(II)N(shi)2(pko)2}\text{-}4]\}^{2+}$ follows the pattern $[-Ni-O-N-]_4$. Two distinct arrangements of the chelates around the ring metal ions were observed; a 6-5-6-5-6-5-6-5 arrangement for the $[12\text{-}MC_{Ni(II)N(Hshi)2(pko)2}\text{-}4]$ core and a 6-6-5-5-6-6-5-5 arrangement for the $[12\text{-}MC_{Ni(II)N(shi)2(pko)2}\text{-}4]$ core. Magnetic variable temperature susceptibility study of the trinuclear compound revealed the presence of one paramagnetic nickel(II) ion with strong crystal field dependence, with $D=5.0(4)$ cm^{-1}, $g_{xy}=2.7(3)$ and $g_z=2.3(3)$. The effect of the synthesized Ni(II) complexes on the integrity and electrophoretic mobility of nucleic acids was examined. Only compounds **2, 3** and **4** altered the mobility of pDNA, forming high molecular weight concatamers at low concentrations or precipitates at higher concentrations. Antibacterial activity screening of the above compounds suggests that nickel compounds **2, 3** and **4** were the most active and can act as potent antibacterial agents.

Keywords: Metallacrown; Nickel; Antibacterial activity

1. Introduction

Metallomacrocycles have gained increasing attention over the past decade due to their potentially unique properties. Metallacrowns, an example of this molecular class, exhibit selective recognition of cations and anions, can display intramolecular magnetic exchange interactions and may be used as building blocks for chiral layered solids [1]. Structurally, metallacrowns[1] resemble crown ethers in their repeating pattern of O–X–X–O, with the oxygen atoms oriented toward the center of a cavity [2–27]. $[9\text{-}MC_{M(ox)N(ligand)}\text{-}3]$ [2], $[12\text{-}MC_{M(ox)N(ligand)}\text{-}4]$ [3–7] and $[15\text{-}MC_{M(ox)N(ligand)}\text{-}5]$ [8–12] metallacrowns with cavity sizes of about 0.35, 0.60 and 0.77 Å, respectively, and with $V^{V}O$, Fe^{III}, Mn^{III}, Ni^{II} and Cu^{II} as metal

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[1]The nomenclature for metallacrowns is as follows: $M'_mA_a[X\text{-}MC_{M(ox)H(Z)}\text{-}Y]$, where X and Y indicate ring size and number of oxygen donor atoms, respectively, MC specifies a metallacrown, M and ox are the ring metal and its oxidation state, H is the identity of the remaining heteroatom bridge, and (Z) is an abbreviation for the organic ligand containing the N/O functionality. There are m captured metals (M′) and a bridging anions (A) bound to the ring oxygens and metals, respectively.

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ions, [12-MC-6] [18], [16-MC-8] [19], [18-MC-6] [13], [18-MC-8] [18], [30-MC-10] [14] and stacking metallacrowns [15] as well as a variety of dimers and fused metallacrowns [6,11,16,17] have been reported. Concerning trinuclear nickel compounds only a few examples have been reported to date [1,20–23], while $[Ni_3(H_2shi)_2(OAc)_2(py)_8](ClO_4)_2$ with a linear arrangement is the only nickel compound with salicylhydroxamato ligands reported [1].

Nickel compounds have been found to inhibit DNA replication and transcription processes in vitro and in vivo and to cause a non-random assault on DNA of cultured mammalian cells, resulting in chromosomal aberrations [24–26]. Nickel compounds are speculated to be carcinogenic [27] both in humans and experimental animals [28,29]. Due to its chemical nature, nickel is expected to form covalent bonds with DNA at several available binding sites, such as nitrogen and oxygen centers of nucleobases as well as phosphate oxygens. Covalent Ni^{2+} coordination to the N7 atom of guanine and adenine has been reported [30] and this covalent interaction can be considered responsible for base depurination, predominantly at the adenine sites, resulting in extensive DNA damage [31]. Recent evidence suggest that DNA repair systems are very sensitive targets for Ni(II), resulting in reduced removal of damaged DNA, which in turn may increase the risk of tumour formation. This is due to the ability of toxic metals to compete with magnesium ions or to displace zinc ions that participate in zinc finger structures of DNA repair enzymes [32,33]. To date only mononuclear Ni(II) complexes showing antibacterial activity have been reported [34–41]. The role of the nuclearity of a metal atom as antibacterial or anticancer factor is not very clear. To date, several high nuclearity complexes with Pt [42,43], Au [44], Ti [45], Sn [46,47] and Bi [48] have been tested as potential anticancer agents [49].

Here we report the synthesis, characterization and the biological study of a trinuclear compound, $Ni_3(shi)_2(Hpko)_2(py)_2$**(1),** the biological study of tetranuclear vacant metallacrowns with mixed ligand composition $[12\text{-}MC_{Ni(II)N(Hshi)2(pko)2}\text{-}4](SCN)_2(dmf)(CH_3OH)$ **(2)** [6] $[12\text{-}MC_{Ni(II)N(Hshi)2(pko)2}\text{-}4](NNN)_2(dmf)(CH_3OH)$ **(3)** [6] $[12\text{-}MC_{Ni(II)N(Hshi)2(pko)2}\text{-}4](OCN)_2(dmf)(CH_3OH)$ **(4)** [6], and a pentanuclear mixed ligand $[Ni(AcO)_2][12\text{-}MC_{Ni(II)N(shi)2(pko)2}\text{-}4](H_2O)$ metallacrown (**5**) [6].

2. Experimental section

2.1. Materials

Chemicals for the synthesis of the compounds were used as purchased. Dimethylformamide (*dmf*) and CH_3OH, distilled from calcium hydride (CaH_2) and magnesium (Mg), respectively, were stored over 3 Å molecular sieves. NH_4SCN, NaOCN, NaN_3 and $NiCl_2\cdot 6H_2O$ were purchased from Aldrich. All chemicals and solvents were of reagent grade. Agarose was purchased from BRL. Tryptone and yeast extract were purchased from Oxoid (Unipath, Hampshire, UK). Molecular weight markers, 1-kb DNA ladder, were from Gibco-BRL. Plasmid pUC19 or pTZ18R was isolated from *E. coli* XL1.

2.2. Culture media: minimal medium salts broth (MMS)

Glucose (1.5%, w/v), 0.5% (w/v) NH_4Cl, 0.5% (w/v) K_2HPO_4, 0.1% (w/v) NaCl, 0.01% (w/v) $MgSO_4\cdot 7H_2O$ and 0.1% (w/v) yeast extract.

2.3. Luria broth medium

Tryptone (1%, w/v), 0.5% (w/v) NaCl and 0.5% (w/v) yeast extract. pH was adjusted to 7.0.

3. Methods

Infrared spectra (200–4000 cm^{-1}) were recorded on a Perkin-Elmer FT-IR 1650 spectrometer with samples prepared as KBr pellets. UV–Vis spectra were recorded on a Shimadzu-160A dual beam and on a Perkin-Elmer Lambda 9 UV–Vis–near-IR spectrophotometer equipped with a Perkin-Elmer 3600 data station. Variable-temperature magnetic susceptibility data were collected with a Quantum Concepts SQUID magnetometer. C, H and N elemental analysis was performed on a Perkin-Elmer 240B elemental analyser. Ni was determined by atomic absorption spectroscopy on a Perkin-Elmer 1100B spectrophotometer. Electric conductance measurements were carried out with a WTW model LF 530 conductivity outfit and a type C cell, which had a cell constant of 0.996. This represents a mean value calibrated at 25 °C with potassium chloride. All temperatures were controlled with an accuracy of ± 0.1 °C using a Haake thermoelectric circulating system. All plastics and glassware used in the experiments with nucleic acids were autoclaved for 30 min at 120 °C and 130 kPa. Heat-resistant solutions were similarly treated, while heat-sensitive reagents were sterilized by filtration.

3.1. Plasmid isolation

Plasmids pUC19 and pTZ18R were isolated from *E. coli* XL1 by alkaline SDS lysis (Strategene). Native DNA was isolated from calf thymus gland using standard procedure. Linear DNA was obtained by incubating the isolated plasmids with the restriction enzyme *Eco*RI. Single-stranded (ss) DNA was prepared by heating double-stranded (ds) DNA at 100 °C for 10 min.

3.2. Agarose gel electrophoresis of nucleic acids

Aliquots of 1–3 μg of each nucleic acid (as indicated in figure legends) were incubated at a constant temperature of

37 °C in the presence of compounds 1–4. Reaction volume was 20 μl. Reaction was terminated by the addition of 5 μl of loading buffer consisting of 0.25% bromophenol blue, 0.25% xylene cyanol FF and 30% glycerol in water. The products, resulting from DNA–compound interactions, were separated by electrophoresis on agarose gels (1%) containing 1 μg/ml ethidium bromide in 40 mM Tris–acetate, pH 7.5, 20 mM sodium acetate and 2 mM Na_2EDTA, at 5 V/cm. The electrophoresis was carried out for about 4 h with a horizontal gel apparatus (Mini-Sub™ DNA Cell, BioRad). Since ethidium bromide forms a fluorescent complex when it binds to DNA, a decrease in fluorescence signifies diminution of the amount of DNA. The gels were visualized in the presence of UV light. All assays were performed in duplicate.

3.3. Antibacterial activity

The antibacterial activity of the compounds was studied against *B. subtilis* (wild type), *B. cereus* (wild type), *S. aureus* (wild type), *E. coli* (XL1), *P. mirabilis* (wild type) and *X. campestris* (ATCC 33013). Screening was performed by determining the Minimal Inhibitory Concentration (MIC). Two different media (LB and MMS) were used. The compounds were dissolved in distilled water with 2-fold serial dilutions from 100 to 12.5 μg ml^{-1}. All cultures were incubated at 37 °C, except *X. campestris*, which was cultivated at 28 °C. Control tests with no active ingredients were also performed.

3.4. Minimal inhibitory concentration (MIC)

Minimal inhibitory concentration (MIC) was determined using 2-fold serial dilutions in liquid media containing 100–12.5 μg/ml of the compound being tested. A preculture of bacteria was grown in LB overnight at the optimal temperature of each species. Two ml of MMS were inoculated with 20 μl of this preculture. This culture was used as a control to examine if the growth of the bacteria tested is normal. In a similar second culture, 20 μl of the bacteria as well as the tested compound at the desired concentration were added. A third sample containing 2 ml MMS supplemented with the same compound concentration was used as a second control to check the effect of the compound on MMS. All samples were in duplicate. We monitored bacterial growth by measuring the turbidity of the culture after 12 and 24 h. If a certain concentration of a compound inhibited bacterial growth, half the concentration of the compound was tested. This procedure was carried on to a concentration that bacteria grow normally. The lowest concentration that inhibited bacterial growth was determined as the M.I.C. value. All equipment and culture media were sterile.

3.5. Preparation of the complexes

All metallacrown compounds were prepared according to procedures reported before [6].

3.6. $Ni_3(shi)_2(Hpko)_2(py)_2\cdot CH_3OH$ (1)

Salicylhydroxamic acid (H_3shi) (0.306 g, 2 mmol) and di-2-pyridyl-ketonoxime (*Hpko*) (0.398 g, 2 mmol) were mixed with NaOH (0.24 g, 6 mmol) in 50 ml of CH_3OH. The resulting solution was added to 100 ml of $NiCl_2\cdot 6H_2O$ (0.716 g, 3 mmol) CH_3OH:py(10:1) solution and was stirred for 2 h. Red/brown crystals of $Ni_3(shi)_2(Hpko)_2(py)_2\cdot CH_3OH$ **(1)** complex suitable for X-ray diffraction studies were obtained by slow evaporation of the mother liquid in a few days. Yield was 70%. Analytical data: ($Fw = 1064.6$) (Found: C, 53.20; H, 3.70; N, 13.10; Ni, 16.55, $Ni_3C_{46}H_{36}N_{10}O_8\cdot CH_3OH$ requires C 53.02; H, 3.78; N, 13.15; Ni, 16.53); IR: ν_{max}/cm^{-1}: (KBr pellet): $\nu(C{=}N)_{pko}$: 1596(vs); $\nu(C{=}N)_{shi}$: 1568(vs); $\nu(C{=}O_{Ph})_{shi}$: 1483(vs); $\nu(N{-}O_H)_{shi}$: 1266(s).

3.7. X-ray crystal structure determination

A red–brown crystal of (**1**) with dimensions of $0.08\times 0.08\times 0.7$ mm^3 was mounted on the top of a glass fiber and data were collected at 173.1 K, with a Bruker (formerly Siemens) SMART Platform CCD diffractometer equipped with an Oxford Cryosystems 'Cryostream' low temperature system. Crystal data and parameters for data collection of compound **1** are presented in Table 1 and selected bond distances and angles in Table 2. A hemisphere of data (1271 frames) was collected. Final cell constants were calculated from a set of 999 strong reflections ($I > 10\sigma(I)$) obtained from the data collection. Four different sets of frames were collected using 0.30° steps in ω. The exposure time was 35 s per frame and the detector-to-crystal distance was ~5 cm. The resolution of the data set was 0.78 Å. The SMART software was used for data acquisition and SAINT for data extraction [50]. The absorption correction was done using SADABS [51] and all refinements were done using the SHELXTL [52] and/

Table 1
Crystal data and structure refinement of $Ni_3(C_7H_4NO_3)_2(C_{11}H_9N_3O)_2(C_5H_5N)_2$**(1)**

Empirical formula	$Ni_3C_{46}H_{36}N_{10}O_8\cdot CH_3OH$
Formula weight	1064.6
Temperature	173.1 K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	P_{bcn}
a	14.7018 (9) Å
b	18.5054 (1) Å
c	15.8609 (1) Å
α	90.00°
β	90.00°
γ	90.00°
Volume	4315.2(17) $Å^3$
Z	4
Density (calculated)	1.633 Mg/m^3
Absorption coefficient	1.368 mm^{-1}
Reflections collected/unique	25700/5262 [R(int) = 0.0819]
Final R indices [2813 $Fo > 4\sigma(Fo)$]	$R1 = 0.0413$, $wR2 = 0.0933$

Table 2
Selected bond distances (Å) and angles (°) of $Ni_3(C_7H_4NO_3)_2(C_{11}H_9N_3O)_2(C_5H_5N)_2$

Bonds (Å)			
$Ni(1)–N(1)_{pyridine}$	2.060(3)	$Ni(2)–O(4)_{phenolato\text{-}shi}$	1.821(3)
$Ni(1)–O(1)_{carbonyl\text{-}shi}$	2.044(2)	$Ni(2)–N(4)_{pyridyl\text{-}pko}$	1.913(3)
$Ni(1)–O(2)_{hydroxamato\text{-}shi}$	2.070(2)	$Ni(2)–N(5)_{ketonoximato\text{-}pko}$	1.891(3)
Ni(1)···Ni(2)	4.509	$Ni(2)–N(2)_{hydroxamato\text{-}shi}$	1.885(3)
Ni(2)···Ni(2)#	3.560		
Angles (°)			
N(1)–Ni(1)–O(2)	172.53(10)	O(4)–Ni(2)–N(2)	91.11(12)
O(1)–Ni(1)–O(1)	160.56(13)	N(5)–Ni(2)–N(4)	81.29(13)
O(4)–Ni(2)–N(4)	89.01(12)	N(2)–Ni(2)–N(4)	177.11(12)
N(2)–Ni(2)–N(5)	98.72(12)	O(4)–Ni(2)–N(5)	169.89(12)

or SHELX97 [53] package of crystallographic programs. The structures were solved with direct methods. All the non-hydrogen atoms were refined anisotropically. The hydrogen atoms were placed in idealized positions and refined using a riding model. The final *R*1, *wR*2, ($I>4\sigma(I)$) and GOF are 0.0413, 0.0933 and 0.931 the maximum and minimum residual peaks in the final difference map are 0.681 and -0.947 e $Å^{-3}$ and the largest shift/esd on the final cycle is 0.007.

4. Results and discussion

4.1. Description of the structure of $Ni_3(shi)_2(Hpko)_2(py)_2$

An ORTEP diagram of the trinuclear $Ni_3(shi)_2(Hpko)_2(py)_2$ complex is shown in Figs. 1 and 2. One Ni(II) ion is octahedral while the other two Ni(II) ions are square planar. The two equivalent square planar Ni(2) atoms are bound to hydroxamato nitrogen, N(2), and phenolato oxygen, O(4), of deprotonated shi^{3-} ligands, and to ketonoximato, N(5), and pyridyl nitrogen, N(4), of two protonated *Hpko* ligands. The octahedral Ni(1) atom is bound to carbonyl, O(1), and hydroxamato, O(2), oxygens of two deprotonated shi^{3-} ligands and to two nitrogens, N(1), N(1)#, of two pyridine ligands. This compound may be considered an acid form having the two *pko* ligands protonated. The protonated hydroxamato groups of Hpko ligands show weak intraligand interaction with O(2), with H(3a)···O(2) separation of 1.615 Å. The tetranuclear [12-$MC_{Ni(II)N(Hshi)2(pko)2}$-4] metallacrown is also in an acid form but there is a significant difference. In the trinuclear compound the acid atom is located at the ketonoximato oxygen, O(3), of the *Hpko* ligands while in the tetranuclear one, it is located at hydroxamato oxygens of the $Hshi^{2-}$ ligands.

The trinuclear nickel atoms array is bending with an dihedral angle Ni(2)–Ni(1)–Ni(2)# of 46.50°. The analogous trinuclear $[Ni_3(H_2shi)_2(OAc)_2(py)_8](ClO_4)_2$ compound with doubly protonated salicylhydroxamato ligands keeps a linear arrangement [1]. The Ni(1)···Ni(2) atom separation is 4.509 Å, similar to those reported for the tetranuclear, pentanuclear and decanuclear [11] metallacrowns. The Ni(2)···Ni(2)# separation is 3.56 Å, closer by 1.2 Å than that reported for the bending tetranuclear metallacrown (4.8 Å). The compound is totally isolated not having any interaction with neighbouring molecules as shown in the packing diagram (Fig. 3a). The nearest separation of Ni(2) to a neighbouring molecule is 4.043(2) Å from C(17)# of a pko ligand.

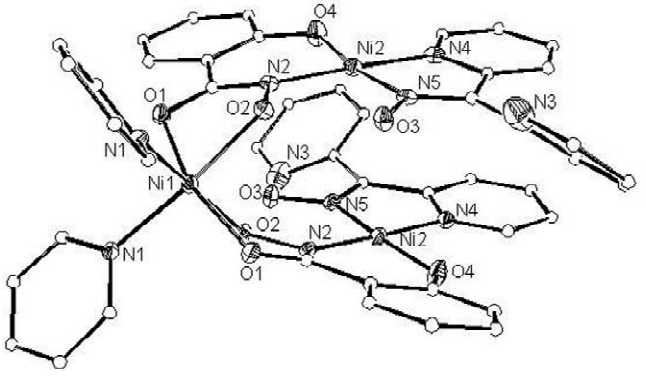


Fig. 1. An ORTEP diagram of $Ni_3(shi)_2(Hpko)_2(py)_2\cdot CH_3OH$ **(1)** with 50% ellipsoids with only the heteroatoms labeled.

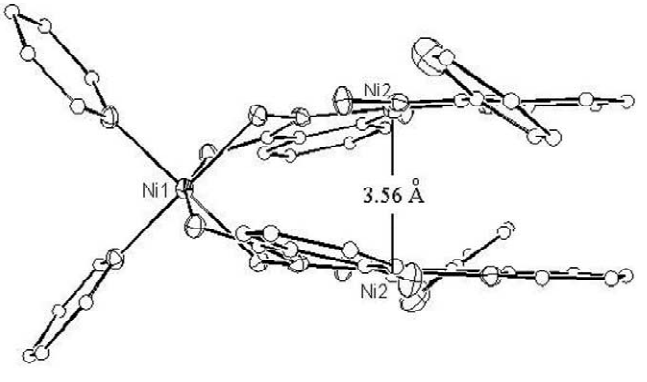


Fig. 2. An ORTEP diagram of $Ni_3(shi)_2(Hpko)_2(py)_2\cdot CH_3OH$ **(1)** showing the bite distance between the two square planar nickel atoms.

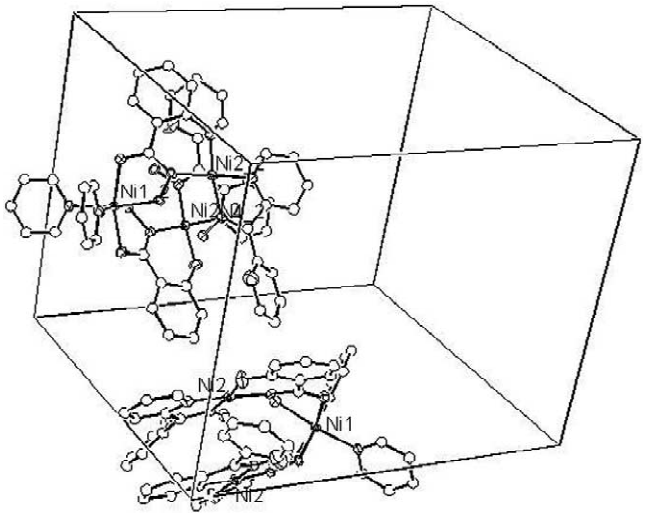
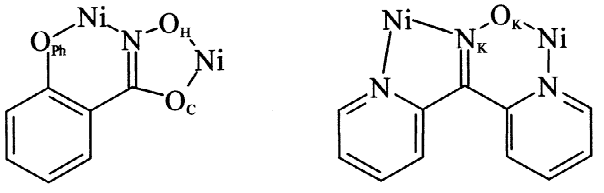


Fig. 3. A packing diagram of **(1)** showing the position of two neighbouring molecules in the cell.



shi *pko*

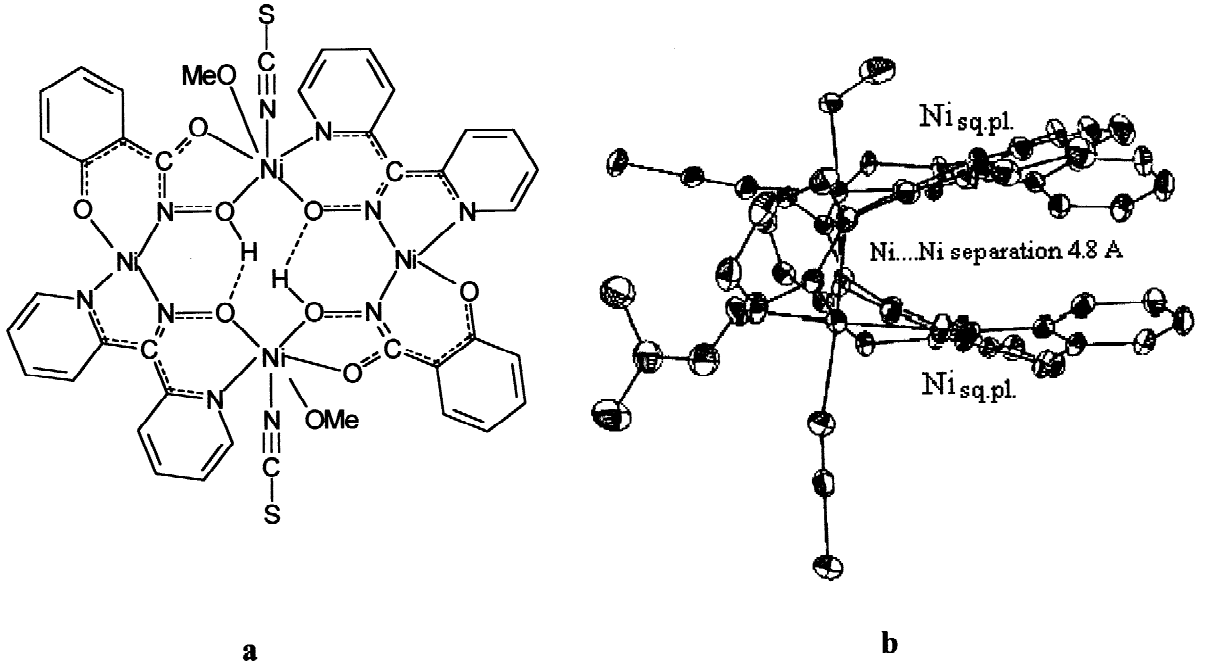
Scheme 1. Drawings showing the binding modes of shi^{3-} and pko^{1-} with metal ions.

4.2. Structural features of the 4 and 5 nuclearity nickel metallacrowns

A series of mixed ligand compounds with unique characteristics was obtained using 2-dipyridyl-ketonoxime in conjunction with salicylhydroxamic acid. *Hpko* is a bifunctional ligand that can bind metals in either five- or six-membered chelate rings (Scheme 1). The ligand can be singly deprotonated when metals are bound. The deprotonated di-2-pyridyl-ketonoxime uses ketonoxime oxygen (O_K) and one pyridine–nitrogen (N) to bind to one nickel and the other pyridine–nitrogen (N) plus ketonoxime nitrogen (N_K) to chelate an adjacent Ni(II). The deprotonated salicylhydroxamic acid acts as a binucleating ligand with the carbonyl and hydroxamate oxygens (O_C and O_H) binding to one nickel and the phenolate oxygen (O_{Ph}) plus the imine nitrogen (N) chelating an adjacent Ni(II).

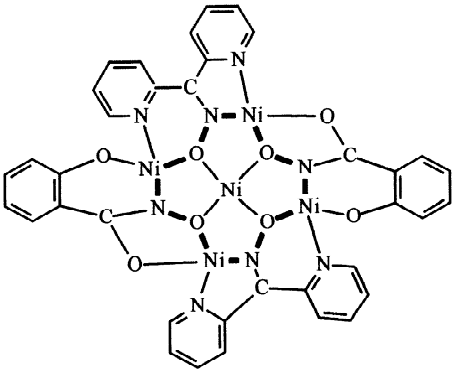
A 2:2 distribution of shi^{3-} and pko^{1-} results in a trinuclear $Ni_3(shi)_2(Hpko)_2(py)_2$ compound **(1)** and a tetranuclear $[12\text{-}MC_{Ni(II)N(Hshi)2(pko)2}\text{-}4]^{2+}$ neutral metallacrown ring **(2–4)** [6]. When Ni(II) is captured in the center, the divalent pentanuclear $\{[Ni(II)][12\text{-}MC_{Ni(II)N(shi)2(pko)2}\text{-}4]\}^{2+}$ complex **(5)** results [6].

The tetranuclear $[12\text{-}MC_{Ni(II)N(Hshi)2(pko)2}\text{-}4]^{2+}$ metallacrown **(2–4)** shows the connectivity pattern $[-O-Ni-O-N-Ni-N-]_2$ (Scheme 2). This metallacrown is in acid form and severely distorted from planarity. Two strong hydrogen bonds stabilize a bent conformation similar to that of the so-called 'molecular tweezer' compounds [54,55]. Two of the nickel atoms are only bound to nitrogen atoms along the metallacrown core in a square planar arrangement,



a **b**

Scheme 2. (a) Drawings showing the connectivity pattern and the arrangement around the nickel ions for the compound $[12\text{-}MC_{Ni(II)N(Hshi)2(pko)2}\text{-}4](SCN)_2(dmf)(CH_3OH)$. (b) An ORTEP diagram of **2** showing the bending form of the metallacrown ring.



Scheme 3. Drawings showing the connectivity pattern and the arrangement around the nickel ions for the metallacrown $Ni(OAc)_2$[12-$MC_{Ni(II)N(shi)2(pko)2}$-4].

while the other two are coordinated only to oxygen atoms along the metallacrown ring in an octahedral environment. The coordination sphere of the octahedral nickel atoms is completed with one thiocyanato for **(2)**, azido for **(3)** or cyanato for **(4)** ligand and a solvent molecule. The pseudohalide ions are bonded to Ni(II) ions through the N atoms. The two square planar Ni(II) ions are positioned 4.8 Å apart for (**2**) [6]. Ligands smaller than this distance can act as substrates for compounds like (**2**), binding between the metal ions.

The divalent pentanuclear $\{[Ni(II)][12\text{-}MC_{Ni(II)N(shi)2(pko)2}\text{-}4]\}^{2+}$ metallacrown, **(5),** is shown in Scheme 3. The two shi^{3-} and two pko^{1-} ligands are arranged in a *trans* configuration to construct a 12-membered metallacrown core with a $[M–N–O]_4$ repeat unit, a Ni(II) encapsulated ion and two acetate anions bridging the encapsulated ion with two ring nickel ions. This results in an overall neutral charge for the molecule. The encapsulated nickel ion is also in an octahedral oxygen environment with four oxygens coming from the shi^{3-} and pko^{1-} ligands and two from the acetate bridging ligands. Two five-membered chelate rings surround the square planar nickel ions and two six-membered chelate rings surround the octahedral nickel ions [6].

4.3. Magnetic susceptibility study

Magnetic susceptibility measurements of (**1**) were carried out at different magnetic fields and in the temperature range 2.0–265 K. Fig. 4 shows the susceptibility data at 0.1 T where the non-Curie behavior of the curve implies strong crystal field dependence. The room temperature value of $\chi_M T$ is 1.46 $cm^3\ mol^{-1}$ K, close to the values expected for one nickel(II) ion, while it decreases to the value of 0.7 $cm^3\ mol^{-1}$ K at 2 K. The solid line represents the best fit according to the Hamiltonian (1) where the *g* parameter and the crystal field anisotropy were taken to be axial.

$$H = D\left[S_z^2 - \frac{1}{3}S(S+1)\right] + E(S_x^2 - S_y^2) + gbH \cdot S \qquad (1)$$

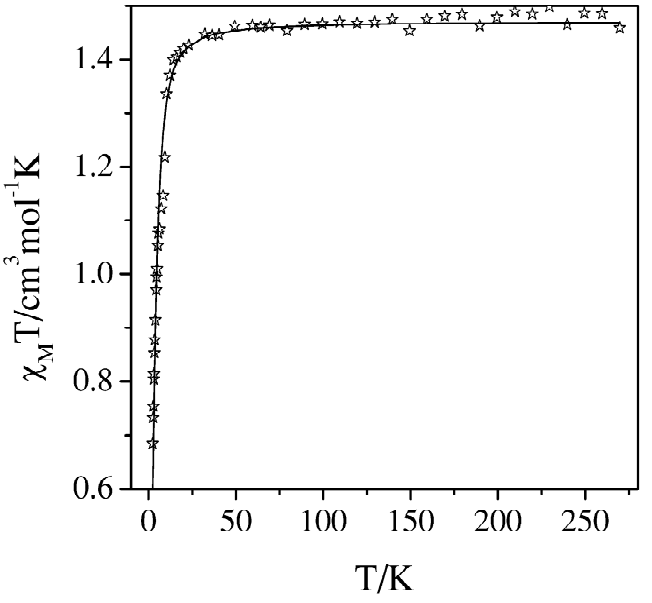


Fig. 4. Temperature dependence of the susceptibility data of compound (**1**), in the form of $\chi_M T$ at 0.1 T. The solid line represents the best fit according to Eq. (1).

The results from the fit are: $D = 5.0(4)$ cm^{-1}, $g_{xy} = 2.7(3)$ and $g_z = 2.3(3)$ indicating the large contribution of the crystal field. In order to verify the above values, magnetization measurements were carried out at different temperatures and in the magnetic field range 0–6.5 T. The 2.5 and 5.0 K curves are shown in Fig. 5. Using the same Hamiltonian formalism the solid lines represent the best fits and the obtained values for the fitting parameters (*D*, *g*) are in the same range as before. In conclusion, compound **(1)** has one paramagnetic nickel(II) ion with strong crystal field dependence.

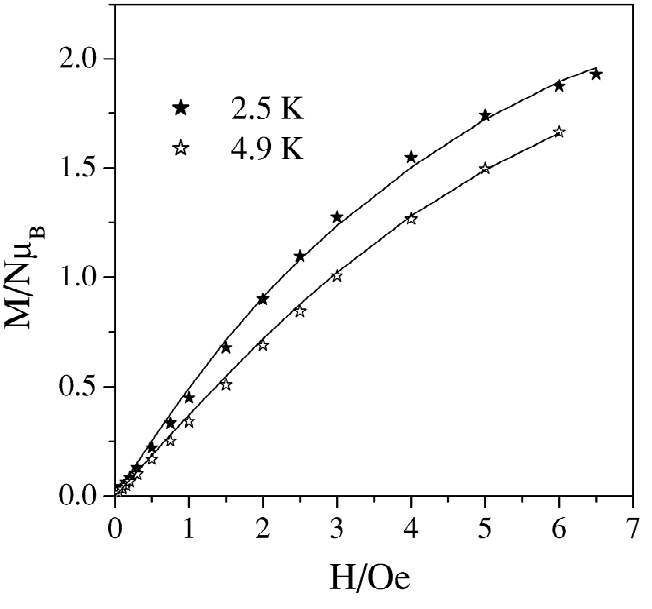


Fig. 5. Magnetization measurements of compound (**1**) at 2.5 and 4.9 K and in the field range 0–6.5 T. The solid lines represent the best fits according to Eq. (1).

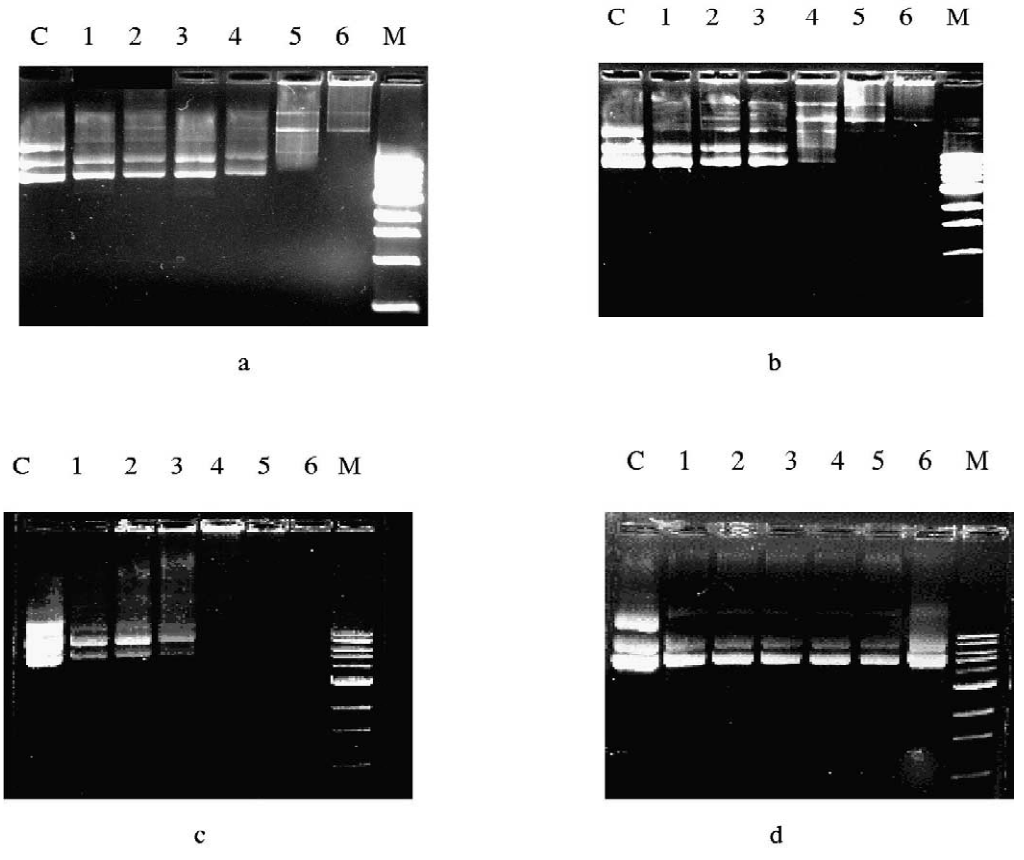


Fig. 6. Agarose (1%) gel electrophoresis patterns of pTZ18R DNA treated with increasing concentrations of compounds **1–4** from Table 3. Two μg of pTZ18R DNA were incubated at 37 °C for 1 h. Lane C: control (untreated pTZ18R DNA). Lanes 1–6: pTZ18R DNA treated with 0.1, 0.3, 0.5, 1, 2 and 5 mM of compound **2** (a), **3** (b), **4** (c) and **1** (d) from Table 3, respectively. Lane M: molecular weight markers, 1 kb DNA ladder: 10.0, 8.0, 6.0, 5.0, 4.0, 3.0, 2.0, 1.5 and 1.0.

4.4. In vitro effects of nickel compounds on DNA

The effect of the synthesized Ni(II) complexes on the integrity and electrophoretic mobility of nucleic acids was examined. Compound **5** at a concentration of 1.2 mM did not affect the electrophoretic mobility of supercoiled or relaxed pDNA. Compounds **2, 3** and **4** altered the mobility of pDNA, forming high molecular weight concatamers at low concentrations or precipitates at higher concentrations (Fig. 6). Compound **1** had a much less pronounced effect on the pDNA electrophoretic mobility at all concentrations tested. pDNA that had been linearized by *Eco*RI and treated with compound **3**, localized, as expected, at the top of the gel at compound concentrations higher than 0.1 mM (Fig. 7). Analogous behavior has been reported for Ni(II) ions interacting with guanine in poly(dG-dC) even at very low concentrations (0.5 mM) [56]. The bridges formation between guanine–Ni(II)–guanine may explain why Ni(II) ions may act either as a precipitant or a renaturing agent for DNA [57]. For the compounds **2, 3** and **4** the bite distance between the two square planar nickel atoms of 4.8 Å (vide infra) may be favor the interaction of Ni(II) to DNA, thus causing the alteration in the mobility of pDNA.

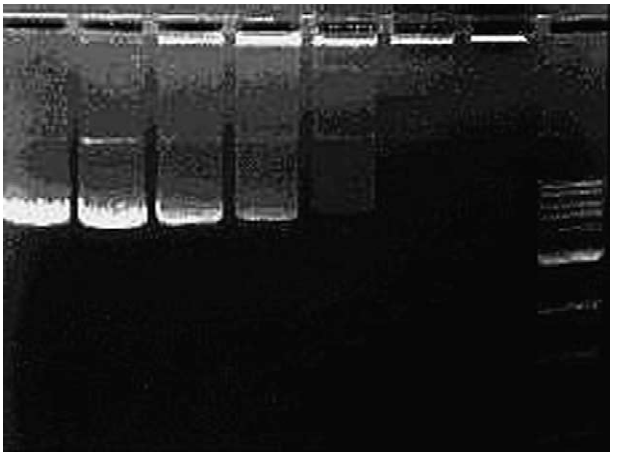


Fig. 7. Agarose (1%) gel electrophoresis pattern of pUC19 DNA linearized by *Eco*RI treatment, incubated with increasing concentrations of compound **3**. Two μg of linearized pUC19 DNA were incubated at 37 °C for 1 h. Lane C: control (untreated linearized pUC19 DNA). Lanes 1–6: linearized pUC19 DNA treated with 0.1, 0.3, 0.5, 1, 2 and 5 mM of compound **3**, respectively. Lane M: molecular weight markers, 1 kb DNA ladder: 10.0, 8.0, 6.0, 5.0, 4.0, 3.0, 2.0, 1.5 and 1.0.

Table 3
MIC (μg/ml) of nickel compounds **1–5**

	Gram-negative bacteria			Gram-positive bacteria		
	X. campestris	*E. coli*	*P. mirabilis*	*B. subtilis*	*B. cereus*	*S. aureus*
1[a]	>100	>100	>100	<50	<100	<100
2	25	25	25	25	<25	25
3	25	25	25	12	<25	25
4	50	25	50	25	25	25
5	50	25–50	100	25	25	25–50
6	>100	>100	>100	>100	>100	>100

[a] **1** $Ni_3(shi)_2(Hpko)_2(py)_2\cdot CH_3OH$ **2** $[12\text{-}MC_{Ni(II)N(Hshi)2(pko)2}\text{-}4](SCN)_2(dmf)(CH_3OH)$**, 3** $[12\text{-}MC_{Ni(II)N(Hshi)2(pko)2}\text{-}4](NNN)_2(dmf)(CH_3OH)$**, 4** $[12\text{-}MC_{Ni(II)N(Hshi)2(pko)2}\text{-}4](OCN)_2(dmf)(CH_3OH)$**, 5** $[Ni(AcO)_2][12\text{-}MC_{Ni(II)N(shi)2(pko)2}\text{-}4](H_2O)$, **6** $Ni(AcO)_2\ 4\ H_2O$.

4.5. Antibacterial study of nickel compounds

Data on antibacterial activity of the tested compounds against *B. subtilis*, *B. cereus*, *S. aureus* (Gram-positive), *X. campestris*, *E. coli* and *P. mirabilis* (Gram-negative), are presented in Table 3. Compounds **2, 3** and **4** were the most active, exhibiting an equal or lower MIC compared to reference compound **6**. The lowest MIC value (12 μg/ml) was observed for compound **3**, with *B. subtilis* being the most sensitive microorganism. The ligands were tested separately on all cultures without causing any growth effect. Until now only mononuclear nickel compounds have been tested as antibacterial agents [34–41]. Therefore, our efforts to correlate nuclearity and antibacterial activity are based only on the present compounds. These results indicate that the tetranuclear metallacrowns **2, 3** and **4** having a bending structure are the most active. All the compounds are not electrolytes in water that exclude side effects of dissociated species. We believe that more polynuclear nickel compounds have to be studied before we reach to a conclusion relating nuclearity and antibacterial activity.

Taking into account the data on the antibacterial activity and the effect on DNA of the tested compounds, we can conclude that antibacterial activity correlate with the compound's ability to cause alterations on DNA.

4.6. Crystallographic data depository

Crystallographic data (without structure factors) for the structure(s) reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-177897. Copies of the data can be obtained free of charge from the CCDC (12 Union Road, Cambridge CB2 1EZ, UK; Tel.: +44-1223-336-408; fax: +44-1223-336-003; e-mail: deposit@ccdc.cam.ac.uk;www:http://ccdc.cam.ac.uk).

6. Notation

H_3shi, salicylhydroxamic acid; *Hpko*, di-2-pyridylketonoxime

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