Polyhedron 75 (2014) 95–109

Contents lists available at ScienceDirect

Polyhedron

journal homepage: www.elsevier.com/locate/poly

Synthesis, structure, DNA and protein binding studies, and cytotoxic activity of nickel(II) complexes containing 3,3-dialkyl/aryl-1-(2,4-dichlorobenzoyl)thiourea ligands

N. Selvakumaran [a], N.S.P. Bhuvanesh [b], A. Endo [c], R. Karvembu [a,*]

[a] Department of Chemistry, National Institute of Technology, Tiruchirappalli 620015, India
[b] Department of Chemistry, Texas A & M University, College Station, TX 77842, USA
[c] Department of Materials and Life Sciences, Sophia University, 7-1 Kioicho, Chiyoda-ku, Tokyo 102-8554, Japan

ARTICLE INFO

Article history:
Received 3 January 2014
Accepted 10 March 2014
Available online 20 March 2014

Keywords:
Acylthiourea
Ni(II) complex
DNA binding
BSA interaction
Cytotoxicity

ABSTRACT

A new series of nickel(II) complexes of the type $[Ni(L)_2]$ (**1**–**6**) has been synthesized by using the 3,3-dialkyl/aryl-1-(2,4-dichlorobenzoyl)thiourea ligands, and characterized by analytical and spectral (NMR, FT-IR and UV–Vis) techniques. The structures of the ligands (HL1, HL2 and HL3) and complexes (**2** and **5**) have been confirmed by single crystal X-ray diffraction studies. The interaction of the nickel(II) complexes (**2**–**5**) with calf thymus (CT) DNA and bovine serum albumin (BSA) protein was investigated using UV–Vis and fluorescence spectroscopic methods. Absorption and emission spectral studies indicate that complex **2** interacts with CT DNA and BSA protein more strongly than the other complexes (**3**, **4** and **5**). Complexes **2** and **5** exhibited good cytotoxicity against A549 and HT29 cell lines at 50 μM concentration.

1. Introduction

Recently, several highly potent platinum metal-based drugs are used in the cancer treatment, with cisplatin, carboplatin, and oxaliplatin being widely used worldwide. However, several side effects and frequent development of resistance phenomena complicate and hamper the clinical applications [1,2]. A very promising strategy to overcome these obstacles is the use of specific carriers and the change from platinum to other transition metals [3–5]. The transition metal complexes play an important role in nucleic acids chemistry for their diverse applications such as foot printing agents, sequence specific binding, structural probes, and therapeutic agents [6–10]. Nickel is one of the most essential elements for biological systems and is present in the trace quantities [11]. The biological activity of the nickel has been rapidly expanded due to the increasing interest in nickel complexes, which have been shown to act as antiepileptic, anticonvulsant agents or vitamins or have shown antibacterial, antifungal, antimicrobial and anticancer/antiproliferative activities [12–19]. Several reports described the reactivity of DNA with mononuclear nickel(II) complexes [20–23]. Nickel(II) complexes with 2-phenylquinoline-4-carboxylic acid hydrazide ligand showed significant cytotoxicity against MFC cell lines and also bind DNA *via* a groove binding mode; further, these complexes can cleave pBR322 DNA [24]. Nickel(II) complexes of benzoic acid(2-hydroxy-benzylidine)hydrazide ligands bind to DNA base pairs *via* intercalation and π–π stacking interactions; *in vitro* free radical scavenging and cytotoxic potential have also been investigated [25]. Nickel(II) complexes containing N-substituted heterocyclic thiosemicarbazones were found to exhibit excellent DNA/protein binding and antioxidant properties; the binding ability of these complexes varies with the N-terminal substituents [26].

DNA binding ability and cytotoxic activity of thiourea complexes have also been investigated. A new class of hybrid platinum compounds containing acridinylthiourea as intercalating group and its analogs exhibit promising activity toward several tumor cell lines, and show only partial cross resistance with cisplatin [27–29]. Acylthiourea ligands are of considerable interest to inorganic chemists because of their variable coordination behavior and promising biological activities [30–38].

A survey of the literature revealed that there is no benzoyl thiourea ligand reported with two chloro substituents in the benzoyl moiety. Binzet et al. reported the synthesis and characterization of copper(II) and nickel(II) complexes of some 4-bromo-N-(di(alkyl/aryl)carbamothioyl)benzamide derivatives [39]. Arslan et al. prepared copper(II), nickel(II) and cobalt(II) complexes of 4-chloro-N-(di(alkyl/aryl)carbamothioyl)benzamide, and copper(II) and zinc(II)

* Corresponding author. Tel.: +91 431 2503631; fax: +91 431 2500133.
E-mail address: kar@nitt.edu (R. Karvembu).

http://dx.doi.org/10.1016/j.poly.2014.03.010
0277-5387/

complexes with N-pyrrolidine-N′-(2-chlorobenzoyl)thiourea derivatives [40]. Chelating difluoro-substituted acylthiourea ligands were also reported [41]. In our previous reports, we have disclosed the synthesis and cytotoxicity of palladium(II) and nickel(II) complexes containing 3,3-dialkyl/aryl-1-benzoylthiourea ligands [42,43]. We report here the synthesis and characterization of six 3,3-diaryl/alkyl-1-(2,4-dichlorobenzoyl)thiourea ligands and their nickel(II) complexes. We have also established the potential of these complexes in DNA and protein interactions, and cytotoxicity.

2. Experimental

2.1. Materials and methods

All the chemicals were purchased from Sigma Aldrich and used as received. A549 (human lung cancer cell line) and HT29 (human colon adenocarcinoma cell line) were purchased from NCCS, Pune for cell culture experiments. Solvents were purified according to the standard procedures. Elemental analysis was carried out on a Vario EL-III elemental analyzer. Fourier transform infrared (FT–IR) spectra were obtained on a Nicolet–iS5 spectrophotometer as KBr pellets. UV–Vis spectra were recorded using a Shimadzu-2600 spectrophotometer operating in the range of 200–900 nm. Emission spectra were measured on a Jasco V-630 spectrophotometer using 5% DMF in buffer as the solvent. NMR spectra were recorded in $CDCl_3$ by using TMS as an internal standard on a Bruker 400 MHz spectrometer.

2.2. Synthesis of the ligands

A solution of 2,4-dichlorobenzoyl chloride (2.0946 g, 10 mmol) in acetone (60 mL) was added drop wise to a suspension of potassium thiocyanate (0.9718 g, 10 mmol) in anhydrous acetone (60 mL). The reaction mixture was heated under reflux for 45 minutes and then cooled to room temperature. A solution of secondary amine (0.7314–1.9728 g, 10 mmol) in acetone (60 mL) was added and the resulting mixture was stirred for 2 h at 27 °C. Hydrochloric acid (0.1 N, 500 mL) was added and the resulting white solid was filtered off, washed with water and dried *in vacuo*. Single crystals for X-ray diffraction studies were grown at room temperature from acetonitrile solutions of the HL1, HL2 and HL3.

2.2.1. 3,3-Diethyl-1-(2,4-dichlorobenzoyl)thiourea (HL1)

Yield: 82%. White. m.p. 142 °C. *Anal.* Calc. for $C_{12}H_{14}Cl_2N_2OS$ (%): C, 47.22; H, 4.62; N, 9.18; S, 10.51. Found: C, 47.19; H, 4.58; N, 9.15; S, 10.55%. FT-IR (KBr): ν, cm^{-1} 1654 (C⋯O), 1222 (C⋯S), 3237 (N–H). ^1H NMR (400 MHz, $CDCl_3$): δ, ppm 8.45 (s, 1H), 7.61–7.32 (m, 3H), 3.98–3.64 (m, 4H), 1.32–1.31 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ, ppm 177.9 (C⋯S), 162.2 (C⋯O), 138.1, 132.2, 132.1, 131.5, 130.4, 127.8 (C_6H_5), 47.8, 48.1 (CH_2), 13.4, 11.4 (CH_3).

2.2.2. 3,3-Diisobutyl-1-(2,4-dichlorobenzoyl)thiourea (HL2)

Yield: 68%. White. m.p. 153 °C. *Anal.* Calc. for $C_{16}H_{22}Cl_2N_2OS$ (%): C, 61.54; H, 4.23; N, 6.52; S, 7.47. Found: C, 61.51; H, 4.19; N, 6.55; S, 7.45. FT-IR (KBr): ν, cm^{-1} 1701 (C⋯O), 1210 (C⋯S), 3181 (N–H). ^1H NMR (400 MHz, $CDCl_3$): δ, ppm 8.65 (s, 1H), 7.62–7.60 (d, J = 8.4, 1H), 7.46–7.45 (d, J = 1.6 Hz, 1H), 7.36–7.33 (dd, J = 8.4 & 1.6 Hz, 1H), 2.36–2.29 (m, 4H), 2.16–2.09 (m, 2H), 1.02 (d, J = 6.4 Hz, 6H), 0.91–0.93 (d, J = 6.4 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ, ppm 179.1 (C⋯S), 161.3 (C⋯O), 137.9, 132.2, 132.0, 131.2, 130.3, 127.7 (C_6H_5), 61.7 (CH_2–N), 60.7 (CH_2–N), 27.8, 26.0 (CH), 20.1, 20.0 (CH_3).

2.2.3. 3,3-Dibenzyl-1-(2,4-dichlorobenzoyl)thiourea (HL3)

Yield: 76%. Greenish yellow. m.p. 168 °C. *Anal.* Calc. for $C_{22}H_{18}Cl_2N_2OS$ (%): C, 53.18; H, 6.14; N, 7.75; S, 8.87. Found: C, 53.22; H, 6.10; N, 7.69; S, 8.92. FT–IR (KBr): ν, cm^{-1} 1702 (C⋯O), 1243 (C⋯S), 3257 (N–H). ^1H NMR (400 MHz, $CDCl_3$): δ, ppm 8.70 (s, 1H), 7.57 (d, J = 6.8 Hz, 1H), 7.43 (t, J = 1.2 Hz, 1H), 7.36–7.38 (m, 10 H), 7.12 (s, 1H), 5.17 (s, 2H), 4.75 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ, ppm 180.6 (C⋯S), 162.3 (C⋯O), 138.2, 132.2, 131.7, 131.6, 130.5, 129.1, 128.9, 128.4, 127.9, 127.8 (C_6H_5), 56.4, 55.9 (CH_2).

2.2.4. 3,3-Diphenyl-1-(2,4-dichlorobenzoyl)thiourea (HL4)

Yield: 72%. White. m.p. 161 °C. *Anal.* Calc. for $C_{20}H_{14}Cl_2N_2OS$ (%): C, 59.86; H, 3.52; N, 6.98; S, 7.99. Found: C, 59.82; H, 3.55; N, 7.01; S, 7.95%. FT-IR (KBr): ν, cm^{-1} 1693 (C⋯O), 1371 (C⋯S), 3374 (N–H). ^1H NMR (400 MHz, $CDCl_3$): δ, ppm 8.57 (s, 1H), 7.62 (t, J = 1.2 Hz, 1H), 7.51 (t, J = 1.2 Hz, 1H), 7.42–7.48 (m, 10 H), 7.21 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ, ppm 180.6 (C⋯S), 162.3 (C⋯O), 138.2, 132.2, 131.7, 131.6, 130.5, 129.1, 128.9, 128.4, 127.9, 127.8 (C_6H_5).

2.2.5. 3,3-Di-n-butyl-1-(2,4-dichlorobenzoyl)thiourea (HL5)

Yield: 64%. White. m.p. 158 °C. *Anal.* Calc. for $C_{16}H_{22}Cl_2N_2OS$ (%): C, 53.18; H, 6.14; N, 7.75; S, 8.87. Found: C, 53.15; H, 6.09; N, 7.78; S, 8.84%. FT-IR (KBr): ν, cm^{-1} 1677 (C⋯O), 1202 (C⋯S), 3186 (N–H). ^1H NMR (400 MHz, $CDCl_3$): δ, ppm 8.56 (s, 1H), 7.60 (d, J = 6.4 Hz, 1H), 7.45 (s, 1H), 7.35–7.33 (dd, J = 7.6 & 1.2 Hz, 1H), 3.92 (s, 2H), 3.58 (t, J = 6.4 Hz, 2H), 1.76–1.66 (m, 4H), 1.44–1.28 (m, 4H), 0.99–0.91 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ, ppm 178.3 (C⋯S), 161.9 (C⋯O), 137.9, 132.1, 132.0, 131.3, 130.3, 127.7 (C_6H_5), 53.2 (CH_2–N), 30.1, 28.3, 20.1, 20.6 (CH_2), 13.8, 13.7 (CH_3).

2.2.6. 3,3-Diisopropyl-1-(2,4-dichlorobenzoyl)thiourea (HL6)

Yield: 74%. Pale yellow. m.p. 152 °C. *Anal.* Calc. for $C_{14}H_{18}Cl_2N_2OS$ (%): C, 50.45; H, 5.44; N, 8.41; S, 9.62. Found: C, 50.37; H, 5.40; N, 8.35; S, 9.21. FT–IR (KBr): ν, cm^{-1} 1664 (C⋯O), 1213 (C⋯S), 3171 (N–H). ^1H NMR (100 MHz, $CDCl_3$): δ, ppm 8.35 (s, 1H), 7.62 (d, J = 8.4 Hz, 1H), 7.44 (d, J = 1.6 Hz, 1H), 7.33 (dd, J = 8.4 & 2.0 Hz, 1H), 3.42-3.31 (m, 2H), 1.71–1.29 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ, ppm 172.8 (C⋯S), 161.8 (C⋯O), 137.7, 132.4, 132.0, 131.3, 130.3, 127.6 (C_6H_5), 48.2 (CH), 20.0, 19.3 (CH_3).

2.3. Synthesis of nickel(II) complexes

3,3-Dialkyl/aryl-1-(2,4-dichlorobenzoyl)thiourea (HL1–HL6) (0.6080–0.85651 g, 2 mmol) dissolved in ethanol (30 mL) was added to ethanol (30 mL) solution of $NiCl_2{\cdot}6H_2O$ (0.2377 g, 1 mmol). Then, NaOAc (0.1620 g, 4 mmol) dissolved in the minimum amount of water was added. The reaction mixture was stirred at 27 °C for 2 h; a solid product was formed. The product was isolated by filtration, washed with diethyl ether and dried in *vacuo*. Suitable crystals for X-ray diffraction studies were grown at room temperature from the dichloromethane solutions of **2** and **5**.

2.3.1. $[Ni(L1)_2]$ (**1**)

Yield: 65%. Dark brown. m.p. 166 °C. *Anal.* Calc. for $C_{24}H_{26}Cl_4NiN_4O_2S_2$ (%): C, 43.21; H, 3.93; N, 8.40; S, 9.61. Found: C, 43.18; H, 3.95; N, 8.38; S, 9.58%. UV–Vis (5% DMF in buffer): λ_{max}, nm (ε, $dm^3\ mol^{-1}\ cm^{-1}$) 247 (31250), 295 (43200), 371 (14924). FT-IR (KBr): ν, cm^{-1} 1375 (C⋯O), 1205 (C⋯S). ^1H NMR (400 MHz, $CDCl_3$): δ, ppm 7.65–7.16 (m, 6H), 3.73–3.72 (m, 8H), 1.27–1.24 (t, J = 5.6 Hz, 6H), 1.16-1.13 (t, J = 5.6 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ, ppm 172.5 (C⋯S), 171.9 (C⋯O), 135.8, 135.2, 133.5, 132.3, 130.4, 126.5 (C_6H_5), 46.0, 45.5 (CH_2), 13.3, 12.4 (CH_3).