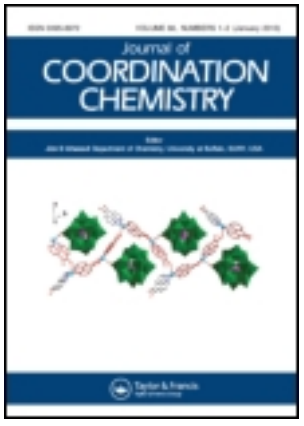

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# Cobalt(II) complexes with thiosemicarbazone as potential antitumor agents: synthesis, crystal structures, DNA interactions, and cytotoxicity

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# Cobalt(II) complexes with thiosemicarbazone as potential antitumor agents: synthesis, crystal structures, DNA interactions, and cytotoxicity

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Two cobalt(II) complexes $[Co(QCT)_2]\cdot Cl\cdot 1.5H_2O$ (**1**) (QCT = quinoline-2-carboxaldehyde thiosemicarbazone) and $[Co(QCMT)(CH_3OH)Cl_2]$ (**2**) (QCMT = quinoline-2-carboxaldehyde $N^4$-methyl-thiosemicarbazone) have been synthesized and structurally characterized. Complex **1** crystallizes in a triclinic system with space group $P\text{–}1$ and complex **2** crystallizes in a monoclinic system with space group $P2(1)/n$. In both complexes the cobalt(II) center is six coordinated with distorted octahedral geometry. The interactions of two complexes with CT-DNA were investigated by electronic absorption spectra, circular dichroism (CD) spectra and fluorescence spectra. Results suggest that the complexes bind to DNA *via* groove binding mode, and complex **2** has stronger binding ability than complex **1**. The *in vitro* cytotoxicity has been tested against the human lung adenocarcinoma cell line A-549, cisplatin-resistant cell line A-549/CDDP, and human breast adenocarcinoma cell line MCF-7. Complex **2** is more cytotoxic than complex **1**, and both of them show higher cytotoxicity than the parent ligands alone. Compared with cisplatin, the two cobalt(II) complexes are more active against A-549/CDDP and MCF-7 cell lines at most experimental concentrations. Notably, although complex 2 is found to be less effective than cisplatin against the parent cell line A-549, it is much more effective than cisplatin against the resistant cell A-549/CDDP.

*Keywords*: Cobalt complexes; Thiosemicarbazone; DNA binding; Cytotoxicity



## 1. Introduction

Metal-based drugs have aroused people's wide concern since the discovery of cisplatin (CDDP) antitumor properties [1–3]. As one of the most widely used compounds in cancer therapy, cisplatin was effective in the treatment of various malignancies including testicular and ovarian carcinomas, osteosarcoma, bladder, head and neck tumors [4, 5]. Despite its great success, cisplatin has quite a few side effects, such as neurotoxicity, nephrotoxicity, myelotoxicity, hematological toxicity and gastrointestinal reactions [6, 7]. Moreover, the treatment efficacy of cisplatin is severely restrained by the problem of both acquired and

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inherent resistance of tumor cells [8]. These limitations have stimulated increasing research on the development of new metal-based anticancer agents with low toxicity and high efficiency. Numerous non-classical platinum-based compounds including monofunctional platinum(II) complexes, polynuclear platinum(II) complexes and platinum(IV) complexes [9–11], and non-platinum compounds [12, 13] have been rationally designed and prepared, and their anticancer activities have been tested.

Transition metal complexes containing metal centers other than platinum as anticancer agents constitute a broad research area of increasing interest [14–16]. Cobalt is an essential element for life and it is less toxic than non-essential metals such as platinum. Vitamin B12, a well known cobalt complex with key biological roles, is very important in the normal functioning of the brain and nervous system, and for the formation of blood. Furthermore, many other cobalt complexes have been found to posses pronounced biological activities, such antiviral and antibacterial activities [17].

It is well known that thiosemicarbazones and their derivatives have displayed wide pharmacological versatility, such as anticancer, antibacterial, antiviral, antifungal, and antimicrobial activities [18–21]. Moreover, these compounds can coordinate to many metal ions, and generate metal complexes that show significant biological activities [22–27]. In this context, we focused our interests on the development of metal complexes of thiosemicarbazone, and investigated their DNA binding ability and cytotoxicity in order to find new potential non-platinum compounds with anticancer activity [28]. We report here the synthesis and crystal structures of two novel cobalt(II) complexes with thiosemicarbazone of quinoline-2-carboxaldehyde. The DNA binding and *in vitro* cytotoxic activities of these cobalt(II) complexes are also presented.



## 2. Experimental

### 2.1. *Chemicals and physical measurements*

Common reagents such as $CoCl_2 \cdot 6H_2O$, thiosemicarbazide, 4-methylthiosemicarbazide, ethanol, methanol, and anhydrous ether are analytical grade and used as received. Disodium salt of calf thymus DNA (CT-DNA), quinoline-2-carboxaldehyde, tris(hydroxy-methyl) aminomethane (Tris), and ethidium bromide (EB) were from Sigma. Infrared spectra were recorded on a Bruker VECTOR22 spectrometer as KBr pellets (4000–500 $cm^{-1}$) and elemental analysis was performed on a Perkin–Elmer 240°C analytical instrument. The electronic absorption spectra were recorded using a UV-3100 spectrometer. The CD spectra were acquired with a JASCO J–810 automatic recording spectropolarimeter. The fluorescence spectra were recorded using an AMINCO Bowman series 2 luminescence spectrometer.

### 2.2. *Synthesis of the compounds*

**2.2.1. Synthesis of the ligands.** Quinoline-2-carboxaldehyde thiosemicarbazone (QCT) was synthesized according to a procedure described previously [28]. Quinoline-2-carboxaldehyde $N^4$-methyl-thiosemicarbazone (QCMT) was obtained following a similar procedure by condensation of 4-methylthiosemicarbazide and quinoline-2-carboxaldehyde (1 : 1 ratio) in ethanol.

**2.2.2. Synthesis of the complexes.** *[Co(QCT)$_2$]·Cl·1.5H$_2$O* (**1**): A methanol solution (5 mL) of $CoCl_2·6H_2O$ (0.5 mM) was added dropwise to methanol solution (10 mL) of QCT (1.0 mmol), and the resulting solution was refluxed for 12 h. Dark red block single crystals suitable for X-ray diffraction were obtained on slow evaporation of the solution of the complex. IR/(KBr, $\nu$/cm$^{-1}$): 3269, 3149 $\nu$(N–H), 1579 $\nu$(C=N), 1126 $\nu$(C=S). Anal. Calcd for $C_{22}H_{22}N_8CoS_2ClO_{1.5}$ (%): C, 46.26; H, 3.80; N, 19.52. Found: C, 46.20; H, 3.75; N, 19.55%.

*[Co(QCMT)(CH$_3$OH)Cl$_2$]* (**2**): A methanol solution (5 mL) of $CoCl_2·6H_2O$ (0.5 mM) was added dropwise to methanol solution (10 mL) of QCMT (1.0 mmol), and the resulting solution was refluxed for 12 h. Dark red block single crystals suitable for X-ray diffraction were obtained on slow evaporation of the solution of the complex. IR/(KBr, $\nu$/cm$^{-1}$): 3184, 2917 $\nu$(N–H), 1527 $\nu$(C=N), 1163 $\nu$(C=S). Anal. Calcd for $C_{13}H_{16}N_4CoSCl_2O$ (%): C, 33.89; H, 2.82; N, 13.17. Found: C, 33.80; H, 2.75; N, 13.20%.

## 2.3. *X-ray crystallography*

Details of the crystal parameters, data collection and refinement are listed in table 1. The structure was determined on a Siemens P4 four-circle diffractometer. Cell constants and an orientation matrix for data collection were obtained by least-squares refinement of diffraction data from 34 reflections in the range $1.84 < \theta < 24.97$. Data were collected at 293 K using monochromated Mo-Kα radiation and the $\omega$–$2\theta$ scan technique with a variable scan speed of 5.0–50.0 min$^{-1}$ in ω and corrected for Lorentz and polarization effects. An

Table 1. Crystal data and structure refinement for [Co(QCT)$_2$]·Cl·1.5H$_2$O (**1**) and [Co(QCMT)(CH$_3$OH)Cl$_2$] (**2**).

| | | |
|---|---|---|
| Chemical formula | $C_{22}H_{22}N_8CoS_2ClO_{1.5}$ **(1)** | $C_{13}H_{16}N_4CoSCl_2O$ **(2)** |
| Formula weight | 580.98 | 406.19 |
| Temperature (K) | 273(2) | 296(2) |
| Crystal system | Triclinic | monoclinic |
| Space group | *P*–1 | *P*2(1)/*n* |
| *a* (Å) | 9.320(2) | 9.187(5) |
| *b* (Å) | 11.325(3) | 15.992(8) |
| *c* (Å) | 13.549(3) | 11.006(5) |
| *α* (°) | 70.776(8) | 90.00 |
| *β* (°) | 81.082(10) | 97.852(10) |
| *γ* (°) | 80.064(9) | 90.00 |
| *V* (Å$^3$) | 1322.6(5) | 1601.8(14) |
| Calculated density (Mg m$^{-3}$) | 1.459 | 1.684 |
| *Z* | 1 | 4 |
| Absorption coefficient (mm$^{-1}$) | 0.941 | 1.540 |
| *F* (000) | 596 | 828 |
| Crystal size (mm) | 0.50 × 0.30 × 0.11 | 0.15 × 0.10 × 0.11 |
| *θ* Range for data collection (°) | 2.08 to 25.00 | 2.26 to 28.38 |
| Limiting indices | $-10 \le h \le 11$<br>$-12 \le k \le 13$<br>$-15 \le l \le 16$ | $-12 \le h \le 10$<br>$-20 \le k \le 20$<br>$-14 \le l \le 11$ |
| Reflection collected/unique | 8579/493 [*R*(int) = 0.1491] | 8678/3970 [*R*(int) = 0.1064] |
| Absorption correction | Semi-empirical | None |
| Refinement method | Full-matrix least-squares on $F^2$ | Full-matrix least-squares on $F^2$ |
| Data/restraints/parameters | 4393/0/325 | 3970/0/213 |
| Goodness-of-fit on $F^2$ | 1.098 | 1.014 |
| Final *R* indices [$I > 2\sigma(I)$] | $R_1 = 0.1253$, $wR_2 = 0.2853$ | $R_1 = 0.0425$, $wR_2 = 0.1159$ |
| *R* indices (all data)[a] | $R_1 = 0.3683$, $wR_2 = 0.4152$ | $R_1 = 0.0471$, $wR_2 = 0.1193$ |
| Largest diff. peak and hole/(e Å$^{-3}$) | 2.847, –1.537 | 0.637, –0.736 |

[a]$R_1 = \Sigma||F_o| - |F_c||/\ \Sigma|\ F_o|$; $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\ \Sigma w(F_o^2)^2]^{1/2}$.

empirical absorption correction was made ($\psi$-scan). The structure was solved by Patterson methods and completed by iterative cycles of least-squares refinement and F-syntheses. Hydrogens were located in their calculated positions and treated as riding on the atoms to which they are attached. All non-hydrogen atoms were refined anisotropically. All calculations were carried out using SHELXTL [29].

### 2.4. *Spectroscopic studies on DNA interaction*

**2.4.1. Electronic absorption spectra.** The concentration of calf thymus DNA (CT-DNA) was determined by recording the UV absorption at 260 nm using the molar absorption coefficient of 6,600 $M^{-1}\,L\,cm^{-1}$[30]. The UV absorbance at 260 and 280 nm of the CT-DNA solution in tris(hydroxy-methyl) aminomethane–HCl buffer gives a ratio of 1.91, indicating that the DNA was sufficiently free of protein [31]. Cobalt(II) complexes were incubated with CT-DNA with a molar ratio of CT-DNA to complex of 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and the concentration of each cobalt (II) complex was $5.0 \times 10^{-5}$ M $L^{-1}$. The intrinsic binding constant $K_b$ for interaction of the studied complexes with CT-DNA was calculated by electronic absorption spectral titration data using the following equation [32]:

$$[\mathrm{DNA}]/(\varepsilon_a - \varepsilon_f) = [\mathrm{DNA}]/(\varepsilon_b - \varepsilon_f) + 1/\mathrm{K_b}(\varepsilon_b - \varepsilon_f),$$

where $\varepsilon_a$, $\varepsilon_f$ and $\varepsilon_b$ correspond to $A_{obsd}$/[complex], the extinction coefficient for the free cobalt (II) complexes and the extinction coefficient for the cobalt (II) complexes in the fully bound form, respectively. The plot of [DNA]/($\varepsilon_a - \varepsilon_f$) *versus* [DNA], $K_b$ is then given by the ratio of the slope to intercept.

**2.4.2. CD spectra.** The CD spectra were recorded at 25 °C with increasing ratio of the complex to CT-DNA. Each sample solution was scanned in the range 220–320 nm with a speed of 10 nm $min^{-1}$. The concentration of CT-DNA was $1.0 \times 10^{-4}$ M $L^{-1}$.

**2.4.3. Fluorescence spectra.** The fluorescence spectra were recorded at room temperature with an excitation wavelength at 530 nm and an emission wavelength of 600 nm. The experiment was carried out by titrating the complex ($5.0 \times 10^{-5}$ M $L^{-1}$) into 3 mL samples containing $1.0 \times 10^{-4}$ M $L^{-1}$ DNA and $1.0 \times 10^{-5}$ M $L^{-1}$ ethidium bromide (EB).

### 2.5. *Cytotoxicity assay*

The *in vitro* cytotoxicity of the two Co(II) complexes against the human lung adenocarcinoma A-549, cisplatin-resistant A-549/CDDP, and human breast adenocarcinoma MCF-7 cell lines by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The growth inhibition rate of treated cells was calculated using the data from three replicate tests by $(OD_{control} - OD_{test})/OD_{control} \times 100\%$, where OD is the optical density. Cisplatin was chosen as a positive reference. The detailed procedure of the assay has been described in our previous work [33].

## 3. Results and discussion

### 3.1. *Crystal structure*

The molecular structure and atom-numbering schemes for $[Co(QCT)_2]\cdot Cl\cdot 1.5H_2O$ (**1**) and $[Co(QCMT)(CH_3OH)Cl_2]$ (**2**) are shown in figures 1 and 2. Selected bond lengths and bond angles are summarized in tables 2 and 3.

In complex **1**, the cobalt(II) ion is coordinated by two QCT ligands in a distorted octahedral geometry through two *cis* quinolinyl nitrogen atoms (N(1) and N(5)) and two *cis* thiolato sulfurs (S(1) and S(2)) in the square plane, and two *trans* imine nitrogen atoms (N(2) and N(6)) in axial positions. chloride ion is a counterion in this complex. Both QCT ligands are tridentate and the ligands show a (*Z*, *E*, *Z*)-configuration for these three donors, respectively. Similar meridional configurations of tridentate NNS ligands around the metal ion center have also been observed in related bischelated complexes of NNS thiosemicarbazones [34]. It is worth noting that the imine nitrogen atom N(3) of one QCT ligand is deprotoned while the other corresponding nitrogen atom N(7) of the other QCT ligand keeps the hydrogen atom. The coexistence of both neutral and anionic form in ligands is also found in some other complexes with thiosemicarbazone [28, 35]. The Co–S [2.204(4) and 2.199(3) Å], $Co–N_{imine}$ [1.891(8) and 1.871(7) Å], and $Co–N_{quinoline}$ (2.010(9) and 2.052(11) Å] bond lengths are similar to those of other six-coordinate distorted octahedral cobalt complexes of tridentate sulfur–nitrogen chelating agents [36–38]. The two $Co–N_{quinoline}$ bond lengths are longer than the $Co–N_{imine}$ bond lengths, indicating a weaker coordination by quinoline N-atoms. The bond angles of N(1)–Co(1)–S(1) (166.6(2)°) and N(5)–Co(1)–S(2) (166.3(3)°) are contracted from the ideal value of 180° for a regular square-planar structure, and the bond angle of N(2)–Co(1)–N(6) (170.8 (4)°) is also contracted from 180°, suggesting a distorted octahedral geometry of this complex. There are hydrogen bonding interactions in the packing of complex **1** (figure S1 (see online supplemental material at http://dx.doi.org/10.1080/00958972.2013.867030)), and the most relevant are given in table 2.

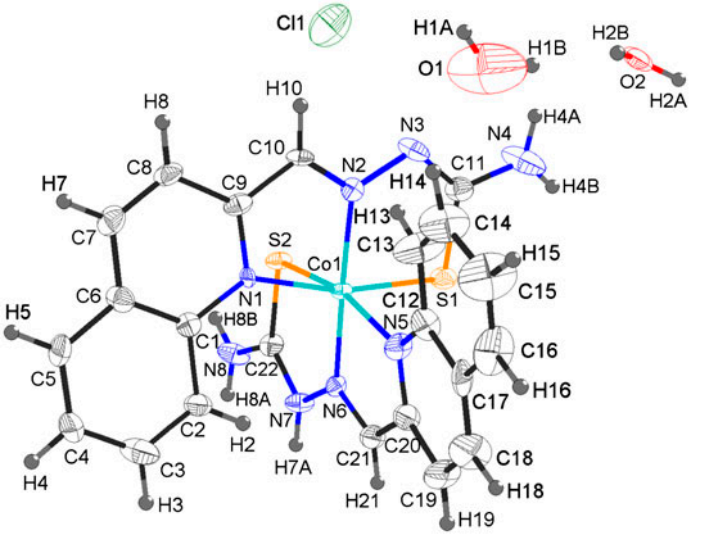


Figure 1. ORTEP diagram for $[Co(QCT)_2]\cdot Cl\cdot 1.5H_2O$ (**1**).

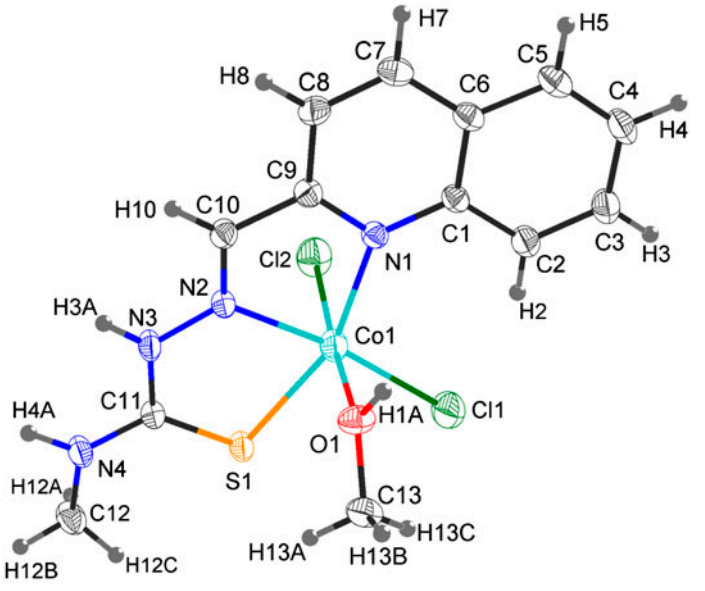


Figure 2. ORTEP diagram for $[Co(QCMT)(CH_3OH)Cl_2]$ (**2**).

Table 2. Selected bond lengths (Å) and angles (°) of $[Co(QCT)_2]\cdot Cl\cdot 1.5H_2O$ (**1**).

| | | | | | |
|---|---|---|---|---|---|
| Co(1)–N(1) | 2.010(9) | Co(1)–N(5) | 2.052(11) | Co(1)–S(1) | 2.204(4) |
| Co(1)–N(2) | 1.891(8) | Co(1)–N(6) | 1.871(7) | Co(1)–S(2) | 2.199(3) |
| C(11)–S(1) | 1.730(11) | C(22)–S(2) | 1.717(9) | | |
| N(6)-Co(1)-N(2) | 170.8(4) | N(1)-Co(1)-N(5) | 94.2(4) | N(6)–Co(1)–S(1) | 87.8(3) |
| N(6)–Co(1)–N(1) | 105.3(4) | N(6)–Co(1)–S(2) | 85.1(3) | N(2)–Co(1)–S(1) | 85.3(3) |
| N(2)–Co(1)–N(1) | 81.4(4) | N(2)–Co(1)–S(2) | 88.9(3) | N(1)–Co(1)–S(1) | 166.6(3) |
| N(6)–Co(1)–N(5) | 81.3(4) | N(1)–Co(1)–S(2) | 87.5(3) | N(5)–Co(1)–S(1) | 90.5(4) |
| N(2)–Co(1)–N(5) | 104.8(4) | N(5)–Co(1)–S(2) | 166.3(3) | S(1)–Co(1)–S(2) | 90.86(13) |
| N(2)–N(3)–C(11) | 112.0(9) | S(1)–C(11)–N(3) | 123.9(11) | S(2)–C(22)–N(7) | 123.0(9) |
| N(6)–N(7)–C(22) | 111.8(8) | S(1)–C(11)–N(4) | 115.2(10) | S(2)–C(22)–N(8) | 118.9(9) |

Hydrogen bonding contacts D–H···A[a]

| | D–H (Å) | H···A(Å) | D···A (Å) | D–H···A (°) |
|---|---|---|---|---|
| N(8)–H(8B)···Cl(1) | 0.86 | 2.47 | 3.314 | 167.6 |
| N(4)–H(4B)···Cl(1) | 0.86 | 2.33 | 3.12 | 153.5 |
| N(4)–H(4A)···O(2) | 0.86 | 2.18 | 2.85 | 134.5 |

[a]i = *x*, *y*, *z*; ii = –*x* + 1, –*y* + 1, –*z*; iii = –*x*, –*y* + 2, –*z*; iv = *x* – 1, *y*, *z*.

In complex **2**, the cobalt(II) ion is also coordinated in a distorted octahedral geometry by one neutral thiosemicarbazone ligand (QCMT). The basal plane is formed by the quinoline and imine nitrogen atoms together with the sulfur atom of the neutral tridentate NNS ligand of QCMT. The fourth basal position is occupied by one chloride ion (Cl(1)). Another chloride ion (Cl(2)) and one oxygen atom (O(1)) from methanol are placed on the axial positions. The Co–S [2.413(6) Å], Co–$N_{imine}$ [2.050(19) Å] and Co–$N_{quinoline}$ [2.199(18) Å] bond lengths of complex **2** are longer than the corresponding bonds of complex **1**. There are hydrogen bonding interactions in the packing of complex **2** (figure S2), and the most relevant are given in table 3.

Table 3. Selected bond lengths (Å) and angles (°) of [Co(QCMT)($CH_3OH$)$Cl_2$] (**2**).

| | | | | | |
|---|---|---|---|---|---|
| Co(1)–N(1) | 2.199(18) | Co(1)–S(1) | 2.413(6) | Co(1)–Cl() | 2.397(6) |
| Co(1)–N(2) | 2.050(19) | Co(1)–O(1) | 2.123(18) | Co(1)–Cl(2) | 2.417(6) |
| N(2)–Co(1)–O(1) | 87.98(8) | N(2)–Co(1)–N(1) | 76.42(7) | O(1)–Co(1)–N(1) | 85.82(7) |
| N(2)–Co(1)–Cl(1) | 172.2(5) | O(1)–Co(1)–Cl(1) | 89.31(6) | N(1)–Co(1)–Cl(1) | 110.68(5) |
| N(2)–Co(1)–S(1) | 81.14(5) | O(1)–Co(1)–S(1) | 92.44(5) | N(1)–Co(1)–S(1) | 157.54(6) |
| Cl(1)–Co(1)–S(1) | 91.67(2) | N(2)–Co(1)–Cl(2) | 90.98(6) | O(1)–Co(1)–Cl(2) | 174.55(5) |
| N(1)–Co(1)–Cl(2) | 88.74(5) | Cl(1)–Co(1)–Cl(2) | 92.38(2) | S(1)–Co(1)–Cl(2) | 92.69(2) |

Hydrogen bonding contacts D–H···A[a]

| | D–H (Å) | H···A(Å) | D···A (Å) | D–H···A (°) |
|---|---|---|---|---|
| N(4)–H(4A)···Cl(2) | 0.956 | 2.314 | 3.181 | 150.53 |
| N(3)–H(3A)···Cl(1) | 0.844 | 2.328 | 3.167 | 172.37 |
| O(1)–H(1A)···Cl(2) | 0.918 | 2.285 | 3.113 | 149.91 |

[a]i = x – 1/2, –y + 1/2, z + 1/2; ii = x – 1/2, –y + 1/2, z + 1/2; iii = x + 1/3, –y + 1/2, z + 1/2.



### 3.2. *Spectroscopic studies on DNA interaction*

It is commonly thought that DNA is the primary pharmacological target of many antitumor compounds, and interactions between metal complexes and DNA is in close relationship with their potential biological and pharmaceutical activities [39–41]. Binding modes to DNA would give insights into the biochemical mechanism of action of the complexes, which have been of interest for development of effective chemotherapeutic agents. Many cobalt(II) complexes that show DNA binding ability and DNA cleavage activity have been reported recently [42–45]. We therefore here in report the results of our investigation on the interactions between the two cobalt(II) complexes and CT-DNA using electronic absorption spectra, CD spectra, and fluorescence spectra.

**3.2.1. Electronic absorption spectra.** It is very common to apply electronic absorption spectroscopy in DNA-binding studies [46]. Electronic absorption spectra of complexes **1** and **2** in the absence and presence of CT-DNA are shown in figure 3. The bands at 298 nm can be assigned to π→π* transitions of the coordinated thiosemicarbazone. Bands around 380 and 400 nm must be due to L→M transfer from sulfur to cobalt. Occurrence of S→M (LMCT) bands is quite common in electronic spectra of metal complexes of thiosemicarbazones

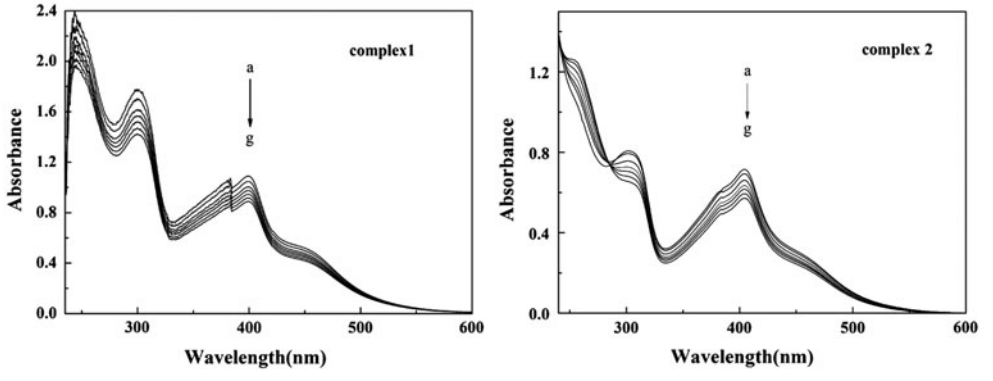


Figure 3. Electronic absorption spectra of complexes **1** and 2 ($5.0 \times 10^{-5}$ M $L^{-1}$) in the absence (a) and presence (b→g) of increasing amounts of CT-DNA at the ratio $r$ = 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2.

[47, 48]. Addition of increasing amounts of CT-DNA results in notable hyprochromicities but no apparent bathochromism. Complex **1** at 298 and 400 nm exhibits hyprochromicity of about 19.7% and 20.0%, respectively. Complex **2** at 298 and 400 nm exhibits hyprochromicity of about 21.8% and 22.5%, respectively. These results suggest that there are interactions between DNA and the cobalt(II) complexes *via* a non-classical intercalation, such as groove binding. Complexes binding with DNA through intercalation usually result in distinct hypochromism and some bathochromism [49, 50]. The binding constants ($K_b$) obtained for complexes **1** and **2** are $8.27 \times 10^3$ $M^{-1}$ and $1.41 \times 10^4$ $M^{-1}$, respectively, which are consistent with hypochromism degree. The results suggest that the binding strength of complex **2** is higher than that of the complex **1**. The $K_b$ values are lower than that observed for classical intercalator EB (order of magnitude of $10^6$ $M^{-1}$) [51], indicating that the two cobalt(II) complexes bind to DNA with less affinity than classical intercalator EB, and it is likely that the cobalt(II) complexes bind to CT-DNA *via* groove binding.

**3.2.2. CD spectra.** CD spectra are useful in monitoring the conformational variations of DNA in solution. The CD spectrum of free helix DNA exhibits a positive band at 275 nm due to base stacking and a negative band at 245 nm due to the helicity of B-type DNA [52]. Figure 4 displays the CD spectra of CT-DNA treated with complexes **1** and **2**. The intensities of both the positive (~275 nm) and negative (~245 nm) bands decrease upon addition of the complexes to DNA. It implies that the cobalt (II) complexes can unwind the DNA helix and lead to the loss of helicity [53, 54]. These changes are indicative of a non-intercalative binding mode and support the groove binding nature, since intercalative binding enhances the intensities of both the bands [55, 56]. The larger decrease in the CD band intensity caused by complex **2** as compared to complex **1** of equal concentration implies that **2** is more effective than **1** in binding to CT-DNA and perturbing the secondary structure of DNA, which is consistent with the results obtained from electronic absorption measurements.

**3.2.3. Fluorescence spectra.** Fluorescence spectra were also used to study the interactions between the two cobalt(II) complexes and DNA by measuring the emission intensity of EB

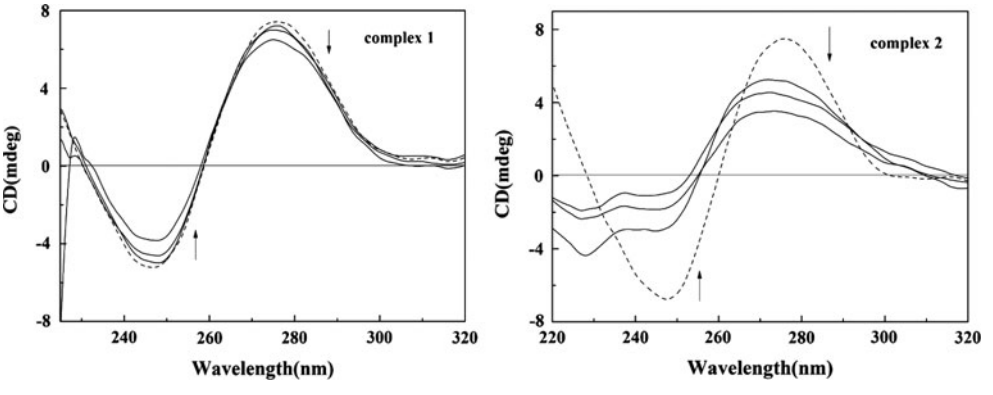


Figure 4. CD spectra of $1.0 \times 10^{-4}$ M $L^{-1}$ CT-DNA in the absence (···) and presence (—) of increasing amounts of complexes **1** and **2** at the ratio $r = 0, 0.2, 0.4, 0.6$.

bound to CT-DNA. EB is weakly fluorescent, but exhibits intense fluorescence when bound to DNA because of its intercalative binding to DNA. However, this enhanced fluorescence could be quenched or partly quenched via formation of strong hydrogen bonding with water, after addition of a second molecule that can replace bound EB or break the secondary structure of DNA [57]. So EB can be used as a probe for determination of DNA structure. Fluorescence titration spectra of complex **2** with CT-DNA are given as an example in figure 5. The emission intensity decreased with the increase of the concentration of complex **2**, which suggests that complex **2** can replace EB in CT-DNA. Besides, the fluorescence intensity decrease follows the order **2** > **1** (insert of figure 5). This behavior suggests that groove binding interactions would occur between the complex and DNA [58, 59], and the electronic absorption spectral and CD spectral analysis also exclude intercalation.

From the discussion above, we may conclude that the cobalt(II) complexes **1** and **2** can interact with DNA by groove binding mode, and the binding strength is in the order **2** > **1**.

### 3.3. *Cytotoxicity assay*

The *in vitro* cytotoxicity of the cobalt(II) complexes was tested against the human lung adenocarcinoma cell line A-549, cisplatin-resistant cell line A-549/CDDP, and human breast adenocarcinoma cell line MCF-7 and compared to cisplatin. The results shown in figure 6 indicate that the inhibitory rates of complex **2** are higher than those of complex **1** on the three cell lines at all concentrations tested (2~50 μM). Interestingly, the two cobalt(II) complexes are more active than cisplatin against A-549/CDDP and MCF-7 cell lines under most

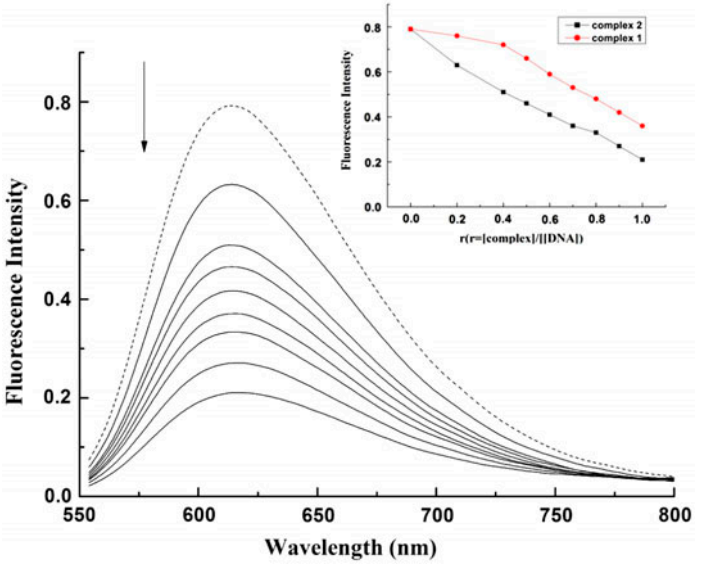


Figure 5. Fluorescence emission spectra (excited at 530 nm) of the ethidium bromide (EB)-CT-DNA system ($1.0 \times 10^{-5}$ M $L^{-1}$) EB, $1.0 \times 10^{-4}$ M DNA) in the absence (dashed line) and presence (solid line) of complex 2 ($2.0 \times 10^{-3}$ M $L^{-1}$, 10 ul per scan). Inset: The fluorescence intensity decrease of the complexes **1** and **2** with the increase of the molar ration of the complex to DNA.

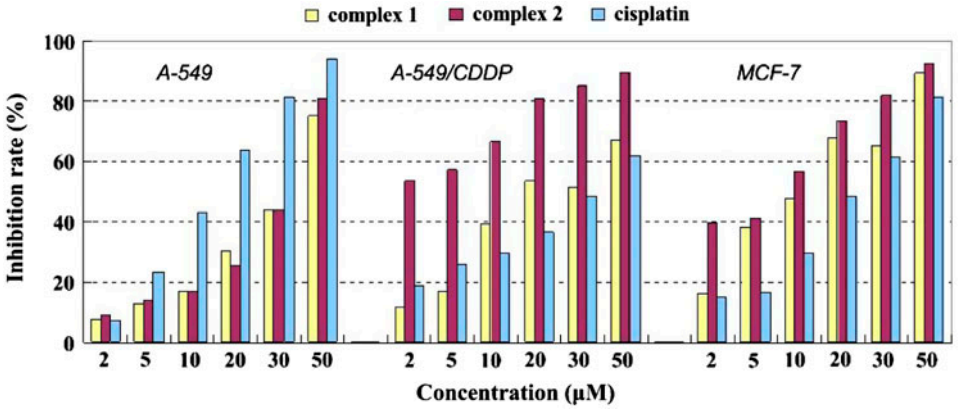


Figure 6. Cytotoxic activity of complexes **1** and **2** against A-549, A-549/CDDP, and MCF-7 cell lines with cisplatin as a positive control.

experimental concentrations. In particular, complex **2** is found to be less active than cisplatin against the parent cell line A-549, but it is found to be much more active than cisplatin against the resistant cell lines A-549/CDDP. The inhibitory rate of complex **2** (53.6%) is about three times higher than that of cisplatin (18.7%) against A-549/CDDP cell line at the concentration of 2 μM. At a compound concentration of 20 μM, the inhibition rate of complex **2** reaches 80.8%, while the inhibition rate of cisplatin is still only 36.5%. The results imply that complex **2** may have potential as a new antitumor agent to be used to overcome the resistance of cisplatin.

Using the same experimental conditions, the *in vitro* cytotoxic effects of the free ligands (QCT and QCMT) and corresponding cobalt(II) complexes (**1** and **2**) were also evaluated against A-549 and A-549/CDDP cell lines. According to the results in figure 7, ligands QCT and QCMT show similar and low growth inhibitory rates against A-549 and A-549/CDDP cell lines. The two cobalt(II) complexes were revealed to be more active than their parent ligands against both cell lines. For example, the inhibitory rates of complex **2** are about two to nine times higher than those of ligand QCMT in the micromolar range (2–50 μM) against

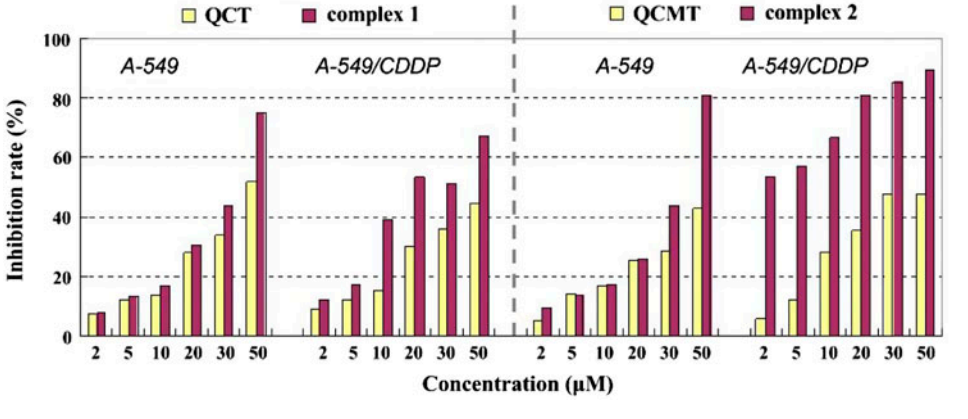


Figure 7. Cytotoxic activity of complexes **1** and **2** and their parent ligands QCT and QCMT against A-549 and A-549/CDDP cell lines.

A-549/CDDP cell lines. This observation is in accord with the phenomenon that the metal complexes usually show higher biological activities than the thiosemicarbazones alone [60–62].

## 4. Conclusion

Two cobalt(II) complexes with thiosemicarbazone of quinoline-2-carboxaldehyde, $[Co(QCT)_2]\cdot Cl\cdot 1.5H_2O$ (**1**) and $[Co(QCMT)(CH_3OH)Cl_2]$ (**2**), have been synthesized and structurally characterized. These complexes bind to CT-DNA through groove binding mode, and complex **2** has stronger binding affinity than complex **1**. Complex **2** exhibits higher growth inhibitory rates than complex **1**, and both of them are more active than the parent ligands alone. It is notable that complex **2** is much more active than cisplatin against the resistant cell lines A-549/CDDP, which suggests that this complex might be a promising antitumor agent to overcome cisplatin resistance.

## Supplementary material

Crystallographic data of $[Co(QCT)_2]\cdot Cl\cdot 1.5H_2O$ (**1**) and $[Co(QCMT)(CH_3OH)Cl_2]$ (**2**) have been deposited at the Cambridge Crystallographic Data Centre with CCDC No. 854024 and 901593. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (Fax: +44-1223-336033; Email: deposit@ccdc. cam.ac.uk or http://www.ccdc.cam.ac.uk).

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