

Synthesis, characterization, antitumor activity of nickel(II) and cobalt(II) complexes of 2,2'-diamino-4,4'-bithiazole and their interaction with dGMP

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Abstract New complexes, of bis(2,2'-diamino-4,4'-bithiazole)sulfate nickel(II) and bis(2,2'-diamino-4,4'-bithiazole)sulfate cobalt(II), have been prepared. The complexes were characterized by infrared and UV-Vis spectroscopy, ^1H NMR, elemental analyses and molar conductivity. The effect of these complexes on the DNA synthesis of sarcoma 180 cells has been studied by the technique of isotopic liquid scintillation. The results indicated that complexes show ability to inhibit DNA synthesis of the tumor cells. In order to provide a molecular basis for understanding the biological effects, the probe, $[\text{trans-en}_2\text{Os}(\eta^2\text{-H}_2)](\text{CF}_3\text{SO}_3)_2$ (en, ethylenediamine) as a monitor was first used to explore interaction of the complexes with 2'-deoxyguanosine-5'-monophosphate (dGMP).

Keywords Synthesis, diaminobithiazole, Ni complex, Co complex

Introduction

The bithiazole moiety, one section of bleomycin, was shown to be responsible for binding of BLM to DNA.¹ During the past decade there has been an explosion in the research effort directed toward the design and synthesis of model compounds that can specifically recognize and cleave DNA. It was, however, indicated that many of these bithiazole derivatives inhibit breakage of DNA by BLM.² The bithiazole derivatives examined only promote helix unwinding and overwinding (positive supercoiling). Recently Hideaki Sasaki found that the bithiazole derivative, 2,2'-bis(2-amino-ethyl)-4,4'-bithiazole, presented Co(II)-activated DNA cleaving activity.³

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Our interest is in designing the coordination compounds of bithiazole derivatives and exploring their biological activity. We report here the synthesis, characterization and antitumor activity of bis(2,2'-diamino-4,4'-bithiazole)sulfate nickel(II) and bis(2,2'-diamino-4,4'-bithiazole) sulfate cobalt(II) and their interaction with dGMP.

Experimental

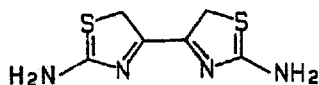
Materials and methods

³H-labelled thymidine was purchased from Institute of Atomic Energy of China. Sarcoma 180 cells were obtained from the Institute of Cancer Research of Shanxi. dGMP was from Sigma Chemical Co. The probe, [*trans-en*₂Os(η^2 -H₂)](CF₃SO₃)₂, was kindly provided by Dr. Zaiwei Li and Prof. Henry Taube (Department of Chemistry, Stanford University). The other chemicals were of analytical grade.

Infrared spectra were recorded in KBr pellets using a Shimadzu IR-435 spectrophotometer in the range of 4000—400 cm⁻¹. ¹H NMR spectra were recorded on a FX-60Q and a Bruker AM-500MHz FT-NMR spectrometers. The electronic absorption spectra were recorded on a Shimadzu UV-Vis 365 recording spectrophotometer. Elemental analyses of the complexes were performed by the laboratory in Institute of Shanxi Coal and Chemistry, Chinese Academy of Sciences. Molar conductance was measured by DDS-12A conductivity meter made in Xiaoshan Scientific Equipment Factory. Radioactivity was determined on PACKARD-Tir-Carb 2200CA liquid scintillation detector.

Synthesis of complexes

The ligand, 2,2'-diamino-4,4'-bithiazole, was prepared according to the literature.⁴ The chemical structure of the ligand is shown below:



Since the ligand is easily oxidizable in air, the following procedures were carried out under nitrogen.

The cobaltous sulfate (CoSO₄·7H₂O) or nickelous sulfate (NiSO₄·6H₂O) (0.5 mmol) was dissolved in the oxygen-free anhydrous methanol (*ca.* 20 mL) in a Schlenk vessel (Solution A). The ligand (1 mmol) was dissolved in 80 mL boiling anhydrous methanol (Solution B). Then solution B was filtered and added to solution A. After standing for 2—5 days at room temperature, dark red crystals (cobaltous complex) and blue crystals (nickelous complex) were obtained, filtered and washed with methanol and dried

in vacuo.

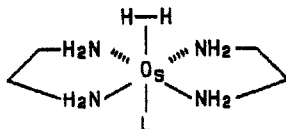
Biological activity

DNA syntheses in cell were assessed by monitoring cellular incorporation of ^3H -labelled thymidine. Sarcoma 180 cells were diluted with PRMI 1640 medium to 1.4×10^7 cells/mL cell suspension. Three concentrations of complexes 5×10^{-6} , 5×10^{-5} , 5×10^{-4} mol/L were selected respectively. Then 100 μL of complex solution and 100 μL of cell suspension were transferred into each well of 96-well plate. The plates were incubated at 37°C under CO_2 for 3 h, and another 1.5 h after addition ^3H -thymidine (15.0 $\mu\text{Ci/nmol}$). The cells were washed with salt water and then filtered through Millipore AP filters. The filters were dried under vacuum at 80°C , and associated radioactivity was determined after addition of scintillation mixture. Every sample were triplicated in parallel and the average value was taken. The rates of incorporation were calculated by the value of CPM, and indicated by $(T/C)\%$ (CPM of complex/CPM of complex free). The rates of inhibition were showed by $(1-T/C)\%$.

Interaction of the title complexes with dGMP

An aqueous solution (10 cm^3) of NiL_2SO_4 (0.01 mol) was added dropwise to aqueous solution (10 cm^3) of dGMP (0.01 mol) with constant stirring ($\text{pH}=7$). A pale green powders were obtained, filtered and washed with distilled H_2O and dried *in vacuo*. Elemental analysis indicated that the new compound was contaminated with a trace of residue. However, all of its IR spectral bands could be assigned according to the relevant literature.⁵

In order to explore interaction of the complexes with dGMP in aqueous solution, we used the probe, $[\text{trans-en}_2\text{Os}(\eta^2\text{-H}_2)]^{2+}$,⁶ depicted below, as a monitor. Since Os(II) complexes are oxidized to Os(III), H_2 is lost, therefore, the procedure was carried out under nitrogen. The probe, NiL_2SO_4 and dGMP were dissolved in D_2O respectively, and the solutions were then transferred to NMR-tube, each solute at 0.010 mol/L. The tube was sealed up and ^1H NMR was recorded after 10 min.



Results and discussion

Characterization of complexes

The interaction of Co(II) and Ni(II) with 2,2'-diamino-4,4'-bithiazole(L) results in the formation of the complexes with the formula CoL_2SO_4 and NiL_2SO_4 respectively, which correspond to analytical data presented in Table 1.

According to analytical data of the complexes, the stoichiometry for metal and ligand is in the ratio 1:2, which differs from previous 2,2'-bithiazole complexes in the ratio 1:3.^{7,8} When the metallic salt and ligand were added in the molar ratio 1:3 during synthesis, the complexes with the ratio 1:2 were still obtained. The molar conductance values of complexes (0.001 mol/L) in DMSO are 9.8 and 10.6 $\text{ohm}^{-1}\cdot\text{cm}^2\cdot\text{mol}^{-1}$ respectively, indicating the nonelectrolytic nature.⁹ These complexes are soluble in water, markedly different from the ligand, which is not soluble in water.

Table 1 Analytical and conductance data for the complexes

Compound	Found (Calcd.)(%)				Molar conductance ^a
	C	H	N	M	
NiL ₂ SO ₄	26.07 (26.14)	2.63 (2.19)	19.91 (20.33)	10.33 (10.65)	9.8
CoL ₂ SO ₄	25.88 (26.13)	2.62 (2.19)	20.14 (20.32)	10.45 (10.68)	10.6

^aMolar conductance of 10^{-3} mol/L solution ($\text{ohm}^{-1}\cdot\text{cm}^2\cdot\text{mol}^{-1}$).

¹H NMR spectroscopy

Proton magnetic resonance indicates the presence of coordinated ligands in complexes as shown by their corresponding proton resonances (Table 2) using the tetramethylsilane as internal standard in DMSO-*d*₆ solution. The change of chemical shifts suggests that coordination has taken place. ¹H NMR of Co(II) complex could not be observed due to its paramagnetism.

Table 2 Proton NMR chemical shifts of free ligand and its complex

Compound	Chemical shifts (ppm)		Peak area ratio (-NH ₂ :ring H)
Ligand	7.02(-NH ₂)	6.62(ring)	2:1
NiL ₂ SO ₄	6.96(-NH ₂)	6.60(ring)	2:1

IR spectroscopy

The infrared spectral data and their assignments were summarized in Table 3. IR spectral data of complexes including bithiazole have never been recorded. Here, the significant change observed was that the band at 1298 cm^{-1} in the spectrum of the ligand was blue-shifted to 1344 cm^{-1} in the complexes. The band at 1298 cm^{-1} in the spectrum of ligand might be due to the skeletal vibration, which was caused by imino-interchange isomer¹⁰ of 2-amino-thiazole. Due to coordination, imino-interchange isomer no longer existed, and skeletal vibration of bithiazole appeared at 1344 cm^{-1} . This suggested that the coordination has taken place through ring nitrogen atom of bithiazole.

The band at 1116, 1054, 990, and 952 cm^{-1} in the spectra of the complexes, which were assigned to ν_{3a} , ν_{3b} , ν_{3c} and ν_1 of SO_4^{2-} , suggested that the SO_4^{2-} is a bidentate ligand¹¹ via oxygen atoms.

Table 3 IR frequencies of ligand and its complexes

Ligand	Compound (cm^{-1})			Assignments
	SO_4^{2-}	NiL_2SO_4	CoL_2SO_4	
3447m		3438m	3424m	$\nu_{\text{N-H}}$
3285m		3272m	3192m	$\nu_{\text{N-H}}$
3127m		3099m	3103m	$\nu=\text{C-H}$
1598s		1609s	1617s	$\delta_{\text{N-H}}$
1528s		1539s	1543s	ν_{ring}
		1515s	1515s	ν_{ring}
1461m				ν_{ring}
1390w				ν_{ring}
1298s		1344s	1357s	ν_{skeletal}
1241m		1273m	1277m	ν_{skeletal}
1034s				ν_{skeletal}
		1116s	1110s	$\nu_{3a}(\text{SO}_4^{2-})^a$
		1054s	1086s	$\nu_{3b}(\text{SO}_4^{2-})^a$
		990m	994m	$\nu_{3c}(\text{SO}_4^{2-})^a$
	1080s ^a			$\nu_3(\text{SO}_4^{2-})^a$
	970m ^a	952m	945m	$\nu_1(\text{SO}_4^{2-})^a$
776m		785m	791m	$\delta_{\text{N-H}}$
704m		718m	736m	$\delta_{\text{N-H}}$

^a Data and assignments were based on Ref. 11.

UV-Vis spectroscopy

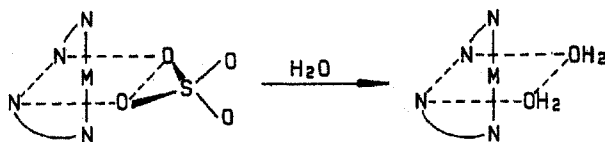
Electronic absorption spectra of the complexes were studied in aqueous solution and data were listed in Table 4.

Table 4 Electronic spectral data and assignments of transition

Compound	Max (nm)	Frequencies (cm^{-1})	Transition
NiL_2SO_4	610	16393	${}^3A_{2g} \rightarrow {}^3T_{1g}(\text{F})$
	1000	10000	${}^3A_{2g} \rightarrow {}^3T_{2g}(\text{F})$
CoL_2SO_4	500	20000	${}^4T_{1g}(\text{F}) \rightarrow {}^4T_{1g}(\text{P})$

The band at 20000 cm^{-1} is assigned to ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ in the CoL_2SO_4 . The bands at 16393 cm^{-1} and 10000 cm^{-1} are attributed to ${}^3A_{2g} \rightarrow {}^3T_{1g}(F)$ and ${}^3A_{2g} \rightarrow {}^3T_{2g}(F)$ in the NiL_2SO_4 . The position of the band suggested an octahedral geometry of the complex, though the third band of Ni(II) complex, ${}^3A_{2g} \rightarrow {}^3T_{1g}(P)$, has not been observed due to the strong absorption of ligand in UV range. It might be explained that the two coordinated H_2O molecules have substituted SO_4^{2-} and the species might be $[\text{NiL}_2(\text{H}_2\text{O})_2]^{2+}$ in the water. Compared with $[\text{Ni}(\text{H}_2\text{O})_6]^{2+}$, the bands of $[\text{NiL}_2(\text{H}_2\text{O})_2]^{2+}$ blue-shifted, indicating that 2,2'-diamino-4,4'-bithiazole is a stronger ligand than H_2O .¹²

On the basis of above evidence and analyses, it has been concluded that the structure of complexes are proposed as follows.



Biological activity

${}^3\text{H}$ -labelled thymidine is indispensable to DNA synthesis. S_{180} cells were incubated with ${}^3\text{H}$ -labelled thymidine and certain concentration complexes. The effects of complexes on the DNA biosynthesis can be known by the incorporation rate of thymidine into cells.¹³ The results of scintillation determination are shown in Table 5. It indicated that the incorporation ${}^3\text{H}$ -TdR is inhibited by these complexes.

Table 5 Effect of complexes on the incorporation of ${}^3\text{H}$ -TdR into S_{180} cell expressed as $(1-T/C)\%$

Compound	Concentration (mol/L)		
	5×10^{-6}	5×10^{-5}	5×10^{-4}
NiL_2SO_4	23.14	52.42	92.50
CoL_2SO_4	54.15	69.98	93.01

Interaction of complexes with dGMP

The molecular hydrogen complex of osmium, $[\text{trans-en}_2\text{Os}(\eta^2\text{-H}_2)]^{2+}$, **1**, is a new ${}^1\text{H}$ NMR recognition probe. When a salt of **1** with CF_3SO_3^- as counter ion is dissolved in water the variable ligand L is water. When a new ligand is added to the solution of **1**, water is partly replaced. For each composition, a characteristic value of δ for the

dihydrogen unit is registered in the ^1H NMR spectrum.

Interaction between the probe and dGMP

dGMP is added to the solution of **1** in D_2O , and each solute is at $0.010 \text{ mol}\cdot\text{L}^{-1}$. ^1H NMR spectra are recorded after 10 min and after 24 h (Fig. 1). As previously illustrated,⁶ the peaks at $\delta=-13.45$, $\delta=-13.83$, -13.86 and $\delta=-9.70$ ppm indicate that the probe is attached to oxygens of water, phosphate and 7-N of dGMP, respectively. The peaks at $\delta=7.96$ and $\delta=6.09$ ppm are assigned to 8-H and 1'-H of free-dGMP and the peaks at $\delta=8.65$ and $\delta=6.32$ ppm are assigned to 8-H and 1'-H of coordinated-dGMP, indicating partly binding of the probe at 7-N of dGMP. After 24 h the peaks at $\delta=7.96$ and $\delta=6.09$ ppm disappeared, and the peak at $\delta=-9.70$ ppm grows up, suggesting completely binding of the probe at 7-N.

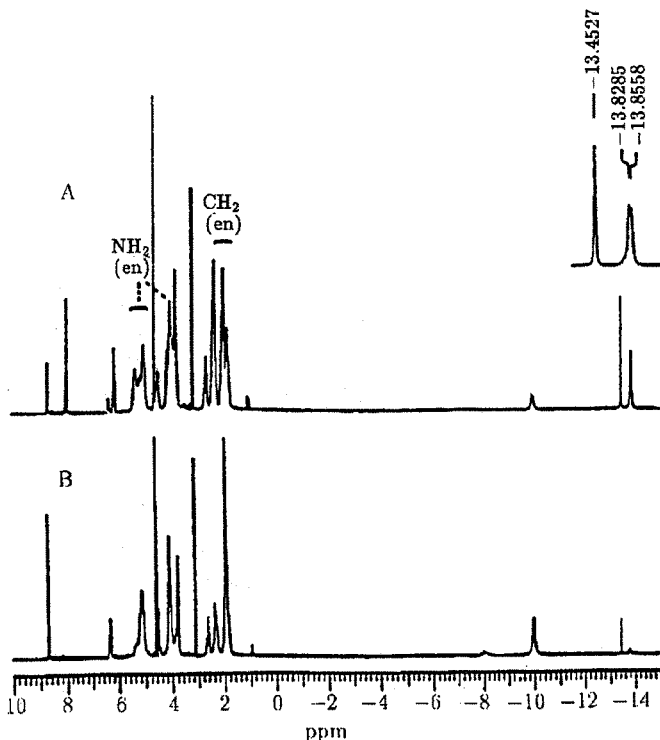


Fig. 1 Proton NMR spectra (200 MHz) of the probe with dGMP in D_2O (both 0.010 mol/L , $\text{pD}=7$). (A) After 10 min. (B) After 24 h.

Interaction between NiL_2SO_4 and dGMP

When the probe is added to a mixing solution (Fig. 2) of NiL_2SO_4 and dGMP in D_2O ($\text{pD}=7.0$) and each solute is at 0.010 mol/L , a new peak at $\delta=-14.02$ ppm

appears after 10 min, indicating that the probe is attached to the oxygen of SO_4^{2-} .¹⁴ The peaks at $\delta=8.86$ and $\delta=6.54$ ppm are assigned to 8-H and 1'-H of coordinated dGMP. However, the original peaks corresponded to 8-H and 1'-H of free-dGMP have not been observed, indicating completely binding of 7-N. Because completely binding of the probe at 7-N is impossible after 10 min as seen in the probe-dGMP binary system we suggested that the part of dGMP might bind at Ni via 7-N. The tumor-inhibiting properties of the complexes might result from the combination of complex to 7-N of dGMP.

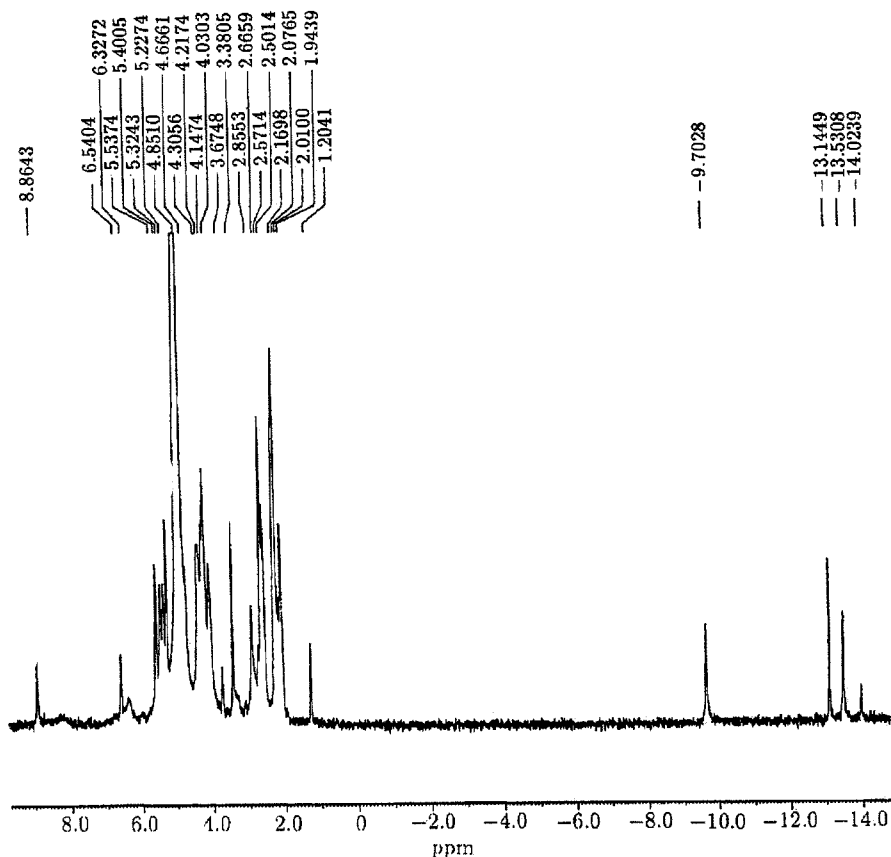


Fig. 2 Proton NMR spectra (500 MHz) of the probe with dGMP and NiL_2SO_4 in D_2O (each one 0.010 mol/L, $\text{pD}=7$) After 10 min.

The IR data (Table 6) of an adduct formed by NiL_2SO_4 with dGMP show that the absorption at 1182 cm^{-1} in free dGMP, corresponding mainly to the C(8)-N(7) stretching vibration, splits into two components at 1202 and 1158 cm^{-1} , suggesting binding between NiL_2SO_4 and 7-N of dGMP.¹⁵ This also supports the behaviour of NiL_2SO_4 with dGMP in aqueous solution.

Table 6 Comparison of the principal IR bands (cm^{-1}) of free dGMP with those of compound formed between NiL_2SO_4 and dGMP

dGMP	NiL_2SO_4	Adduct of NiL_2SO_4 -dGMP	Possible assignment
1696(s)		1667(sh)	$\nu_{\text{C}(6)=\text{O}} > \nu_{\text{C}(6)=\text{C}(5)}$
1605(m)			$\nu_{\text{C}(4)-\text{N}(3)} > \nu_{\text{C}(4)-\text{C}(5)} > \nu_{\text{C}(5)-\text{N}(7)}$
	1609(s)	1613(s)	$\delta_{\text{N}-\text{H}}(2,2'-\text{diamino-4,4'-bithiazole})$
1536(m)			$\nu_{\text{C}(4)-\text{N}(9)} > \nu_{\text{C}(6)=\text{O}} > \nu_{\text{C}(2)-\text{N}(1)}$
	1539(s)	1540(s)	$\nu_{\text{ring}}(2,2'-\text{diamino-4,4'-bithiazole})$
	1515(s)	1517(s)	$\nu_{\text{ring}}(2,2'-\text{diamino-4,4'-bithiazole})$
1482(m)		1487(w)	$\delta_{\text{C}(8)-\text{H}} > \nu_{\text{C}(8)-\text{N}(7)}$
1414(m)		1412(w)	$\delta_{\text{CH}} + \delta_{\text{CH}_2}$
1372(m)		1357(s)	$\nu_{\text{pyrimidine ring}}$
	1344(s)		$\nu_{\text{skeletal}}(2,2'-\text{diamino-4,4'-bithiazole})$
	1273(m)	1275(m)	$\nu_{\text{skeletal}}(2,2'-\text{diamino-4,4'-bithiazole})$
1182(m)		1202(w)	$\nu_{\text{C}(8)-\text{N}(7)} > \nu_{\text{N}(9)-\text{sugar}} > \nu_{\text{C}(4)-\text{N}(3)}$
		1158(w)	
1077(bs)		1084(bs)	$\nu(\text{PO}_3^{2-})$
976(s)		980(s)	$\nu(\text{PO}_3^{2-})$

^a w, weak; m, medium; s, strong; bs, broad and strong; sh, shoulder; ν , stretching; δ , bending.

Efforts are also being made to study the detailed biochemical mechanism to understand these findings.

Acknowledgment

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