DNA-Binding Study of Nickel(II) and Zinc(II) Complexes with Two Novel Chromenone-Based *Schiff*-Base Ligands

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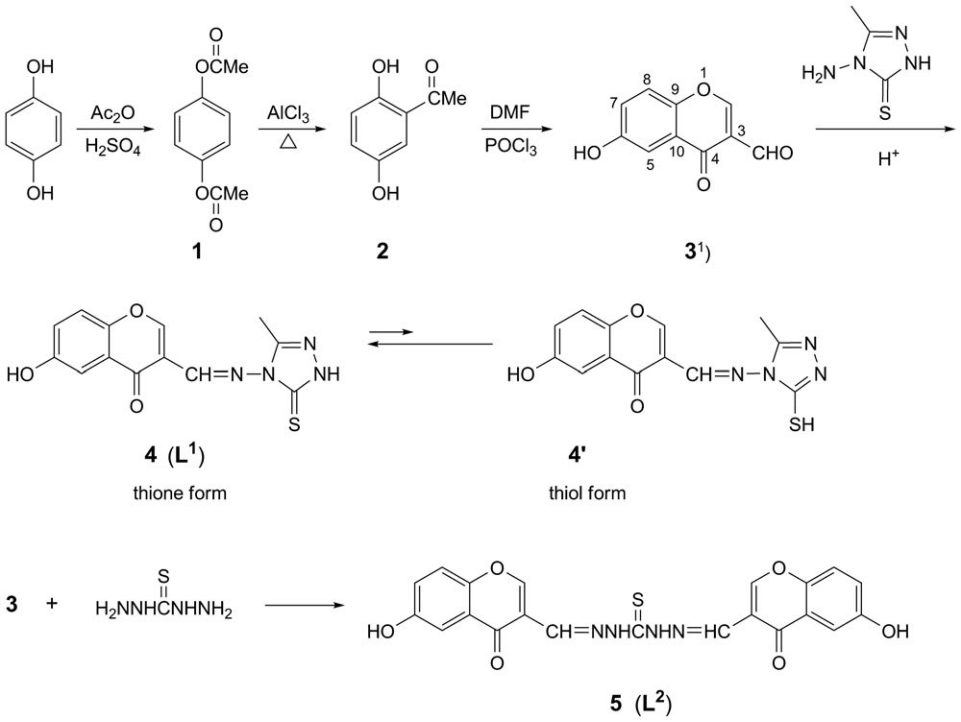
Two new chromenone-based *Schiff*-base ligands, 3-{[(1,5-dihydro-3-methyl-5-thioxo-4*H*-1,2,4-triazol-4-yl)imino]methyl}-6-hydroxy-4*H*-1-benzopyran-4-one (**L**^{1}) and 2,2′-bis[(6-hydroxy-4-oxo-4*H*-1-benzopyran-3-yl)methylene]carbonothioic dihydrazide (**L**^{2}), and their Ni^{II} and Zn^{II} complexes were prepared. All the complexes were characterized by elemental analysis, IR data, and molar conductivity. The binding of these four complexes to calf-thymus DNA was carefully investigated by UV/VIS spectroscopy, fluorescence spectroscopy, viscosity measurements, and CD spectra. The experimental results indicate that the four complexes bind to calf-thymus DNA in an intercalative mode, with the intrinsic binding constants (K) of $3.94 \cdot 10^4$ ([Ni**L**^{1}]), $5.15 \cdot 10^3$ ([Zn**L**^{1}]), $4.12 \cdot 10^4$ ([Ni**L**^{2}]), and $3.75 \cdot 10^4$ M^{-1} ([Zn**L**^{2}]). These data show that the complexes of **L**^{2} can interact more strongly with DNA than complexes of **L**^{1}, and the Ni^{II} complexes have a higher binding constant than Zn^{II} complexes.

Introduction. – Over the past decades, the interactions of small molecules with DNA have attracted a great deal of attention since many anticancer drugs exert their antitumor effects through binding to DNA in one way or another, thereby blocking the replication of DNA and inhibiting the growth of tumor cells [1–3]. In many useful applications of such small molecules, the compounds bind to DNA in an intercalation mode. At present, tremendous interest has been aroused to explore the potential applications of metal complexes as possible therapeutic agents and as nonradioactive probes of nucleic acid structure [4–6]. However, most of these studies are focused on Ru^{II} complexes of 2,2′-bipyridine (bpy), 1,10-phenanthroline (phen), and their modified variants [7–10]. DNA-Binding studies of Ni^{II} and Zn^{II} complexes of *Schiff* bases, which have been intensively used in the medicinal and pharmaceutical field, are rarely reported.

Flavonoids are ubiquitous in nature, especially in plants, and are known to be kinase inhibitors. They also show activity in apoptosis, which is implicated in cancer chemotherapy [11–13]. Chromenones, as one kind of flavonoids, have a structure similar to those of flavonoids. *Balbi* and co-workers [14] and *Ganguly* and co-workers [15][16] have reported a large amount of chromenone derivatives and investigated their biological activities. However, their *Schiff*-base compounds and the corresponding Ni^{II} and Zn^{II} complexes have not been synthesized, and DNA-binding activities of metal complexes have not been studied either. As known, triazole and thiourea derivatives also display a broad range of biological activities, showing potential applications as antitumor, antibacterial, antifungal, and antiviral agents [17][18].

With the endeavor to synthesize effective DNA-binding complexes for biological use, we now report on the successful synthesis of the two new chromenone-based *Schiff*-base ligands **L¹** and **L²** containing a triazole or a thiourea group (see *Scheme* and *Exper. Part*), and of their Ni^{II} and Zn^{II} complexes. The intrinsic binding constants K of the complexes with DNA vary from $5.15 \cdot 10^3$ M^{-1} to $4.12 \cdot 10^4$ M^{-1}, and the quenching constants K_q vary from $2.22 \cdot 10^3$ M^{-1} to $1.30 \cdot 10^5$ M^{-1}. The K and K_q values for these complexes decrease in the order $[Ni\mathbf{L^2}] > [Ni\mathbf{L^1}] > [Zn\mathbf{L^2}] > [Zn\mathbf{L^1}]$. These values are similar to those of some known DNA intercalators, such as $K = 4.8 \cdot 10^4$ M^{-1} for $[Ru(bpy)_2(phi)]Cl_2$ (phi = 9,10-phenanthrenequinonediimine) [7] or $K = 2.1 \cdot 10^4$ M^{-1} for $[Ru(bpy)_2(ddt)]^{2+}$ (ddt = 3-(pyrazin-yl)-5,6-diphenyl-*as*-triazine) [8]. The large K and K_q values are the direct evidence that the complexes have good ability to bind to DNA in the intercalation mode.

Scheme. *Synthesis of the* Schiff-*Base Ligands* **L¹** *and* **L²**



Results and Discussion. – 1. *Characterization of Ni^{II} and Zn^{II} Complexes. Compositions and Properties of the Complexes.* The likely structures of the four complexes are shown in *Fig. 1*. These assumptions are in accord with the results of elemental analysis, IR data, TG/DTA (thermogravimetric/differential thermal analysis), and molar conductivity. The results suggest that $[Ni\mathbf{L^1}]$ is composed of $[Ni\mathbf{L^1} \cdot$

¹) Arbitrary atom numbering; for systematic names, see *Exper. Part.*

$H_2O] \cdot 2\ NO_3 \cdot H_2O$ and $[ZnL^1]$ of $[ZnL^1 \cdot (H_2O)_3] \cdot 2\ NO_3 \cdot 0.5\ H_2O$, whereas the complexes of L^2 are composed of $[ML^2_2] \cdot 2\ NO_3$ ($M = Ni^{II}$ or Zn^{II}). The complexes are air-stable for extended periods and soluble in DMSO, DMF, and MeOH, slightly soluble in EtOH and H_2O, and insoluble in benzene and Et_2O. The molar conductivity value Λ_m is 166 and 172 $S \cdot cm^2 \cdot mol^{-1}$ for $[NiL^1 \cdot H_2O] \cdot 2\ NO_3 \cdot H_2O$ and $[ZnL^1 \cdot (H_2O)_3] \cdot 2\ NO_3 \cdot 0.5\ H_2O$ in MeOH, and 154 and 162 $S \cdot cm^2 \cdot mol^{-1}$ for $[NiL^2_2] \cdot 2\ NO_3$ and $[ZnL^2_2] \cdot 2\ NO_3$ in MeOH; these results show that the four complexes are 2:1 electrolytes [19].

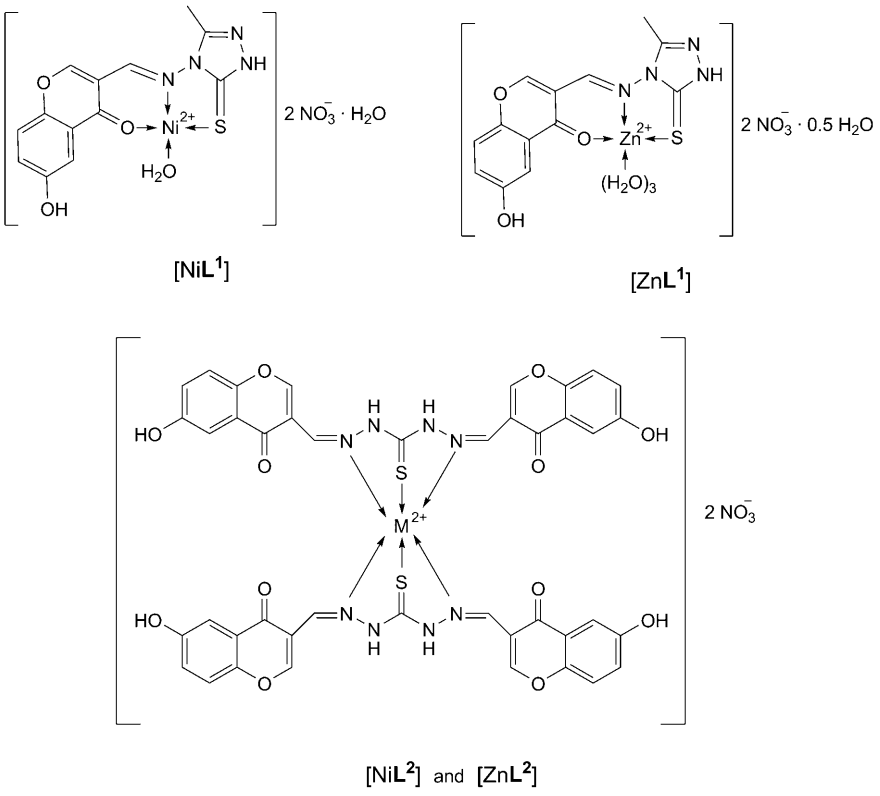


Fig. 1. *Proposed structures of the complexes [Ni**L**¹], [Zn**L**¹], [Ni**L**²], and [Zn**L**²].* For convenience, the short forms [M**L**¹] and [M**L**²] (M = Ni or Zn) are used for these complexes.

IR Spectra. The $\tilde{\nu}$(C=N) vibration of the free ligands **L**¹ and **L**² appear at 1595 and 1590 cm^{-1}, whereas those of [Ni**L**¹] and [Zn**L**¹] are shifted to 1578 and 1562 cm^{-1}, and those of [Ni**L**²] and [Zn**L**²] to 1575 and 1569 cm^{-1}, respectively, indicating that the N-atom of the imino group C=N of the two ligands is bound to a metal ion. The $\tilde{\nu}$(C=O) vibrations of **L**¹ and **L**² are at 1630 and 1633 cm^{-1} and shifted to 1635 and 1636 cm^{-1} in the case of [Ni**L**¹] and [Zn**L**¹]; however, the absorption of **L**² at 1633 cm^{-1} is not shifted in the case of [Ni**L**²] and [Zn**L**²]. This indicates that the O-atom of the C=O group of **L**¹ is involved in a coordination bond with the Ni^{II} or Zn^{II} ion, in contrast to that of **L**². The absorptions at 1257 and 1237 cm^{-1} of **L**¹ and **L**² are assigned to the $\tilde{\nu}$(C=S) stretch

[17], which are shifted to 1233 and 1230 cm^{-1} in the case of [Ni**L**^{1}] and [Zn**L**^{1}], and to 1215 and 1219 cm^{-1} in the case of [Ni**L**^{2}] and [Zn**L**^{2}]. The $\tilde{\nu}$(N–H) vibration of **L**^{1} is at 3191 cm^{-1}, and after coordinating to the metal, the N–H vibration still appears at 3196 in the case of [Ni**L**^{1}] and at 3120 in the case of [Zn**L**^{1}], suggesting that **L**^{1} forms the complexes in its thione form [17][18]. For the four complexes, the bands at 1383 and 1384 cm^{-1} are assigned to the free NO_3^- ion [20].

^{1}H-NMR Spectra. The ^{1}H-NMR spectra ((D$_6$)DMSO, 200 MHz) of **L**^{1} and [Zn**L**^{1}] are assigned as follows: **L**^{1}[1]): δ(H) 2.34 (*s*, Me); 7.28 (*dd*, J = 2.9, 9.0 Hz, H–C(7)); 7.39 (*d*, J = 2.9 Hz, H–C(5)); 7.62 (*d*, J = 9.0 Hz, H–C(8)); 9.05 (*s*, H–C(2)); 10.22 (*s*, CH=N); 10.26 (*s*, exchangeable, OH); 13.73 (*s*, exchangeable, NH). [Zn**L**^{1}][1]): δ(H) 2.34 (*s*, Me); 7.28 (*dd*, J = 2.9, 9.0 Hz, H–C(7)); 7.37 (*d*, J = 2.9 Hz, H–C(5)); 7.62 (*d*, J = 9.0 Hz, H–C(8)); 9.01 (*s*, H–C(2)); 10.18 (*s*, CH=N); 10.26 (*s*, exchangeable, OH); 13.73 (*s*, exchangeable, NH). Thus, the N–H of the 1*H*-1,2,4-triazole moiety of both **L**^{1} and [Zn**L**^{1}] is observed, indicating that both the ligand and the complex exist as in their thione form (see **4** in the *Scheme*) [17][18]. This is consistent with the results obtained from the IR spectra.

UV/VIS Spectra. The study of the electronic spectra of the complexes in the UV region was carried out in buffer solution (containing 1% MeOH). [Ni**L**^{1}] and [Zn**L**^{1}] give rise to two strong bands at λ_{max} 202 and 246 nm, and 204 and 247 nm, respectively. [Ni**L**^{2}] and [Zn**L**^{2}] also exhibit a strong band at 202 nm. The band at 202 or 204 nm is assigned to the $\pi \rightarrow \pi^*$ transition absorbance of the chromenone moiety. The band at 246 or 247 nm is due to the absorbance of the triazole ring. The VIS spectra of [Ni**L**^{1}] and [Ni**L**^{2}] were measured in MeOH solution (concentration $1 \cdot 10^{-3}$ M). [Ni**L**^{1}] shows one absorption band at 525 nm (ε = 248), but [Ni**L**^{2}] exhibits two absorption bands at 603 (ε = 73) and 903 nm (ε = 63). The different d–d transitions of the Ni^{2+} ion suggest a different coordination configuration (*Fig. 1*).

2. *DNA-Binding Interaction. Absorption Titration and Ethidium Bromide (EB) Replacement Experiment.* The interaction of [Ni**L**^{1}], [Zn**L**^{1}], [Ni**L**^{2}], and [Zn**L**^{2}] with DNA were investigated by means of spectrometric titration and viscosity measurements to evaluate their binding affinities and modes [21]. Electronic-absorption spectroscopy is one of the most useful techniques for DNA-binding studies of metal complexes. The UV titration spectra of [Ni**L**^{1}] and [Ni**L**^{2}] are given in *Fig. 2* as examples (those of [Zn**L**^{1}] and [Zn**L**^{2}] are not shown). In the presence of DNA, the absorption band of [Ni**L**^{1}] at 202 nm exhibits hypochromism of *ca.* 27.1% and bathochromism of 16 nm ([Zn**L**^{1}] at 204 nm: *ca.* 11.1% and 3 nm, resp.); the corresponding values of [Ni**L**^{2}] at 202 nm are *ca.* 37.6% and 9 nm (Zn**L**^{2} at 202 nm: 36.5% and *ca.* 8 nm, resp.). These results suggest an association of all four metal complexes with DNA. Hypochromism and bathochromism are both the spectral features of DNA concerning its double-helix structure, hypochromism meaning that the DNA binding mode of the complex is electrostatic or by intercalation which can stabilize the DNA duplex [22]. The remarkable hypochromism (27.1, 11.1, 37.6, and 36.5%) and redshift (16, 3, 9, and 8 nm) for [Ni**L**^{1}], [Zn**L**^{1}], [Ni**L**^{2}], and [Zn**L**^{2}] are direct evidences of the intercalation mode. After binding of the complex to the base pairs of DNA, the π^* orbital of the intercalated ligand can couple with the π orbital of the base pairs, thus, decreasing the $\pi \rightarrow \pi^*$ transition energies, which results in hypochromism and bathochromism.

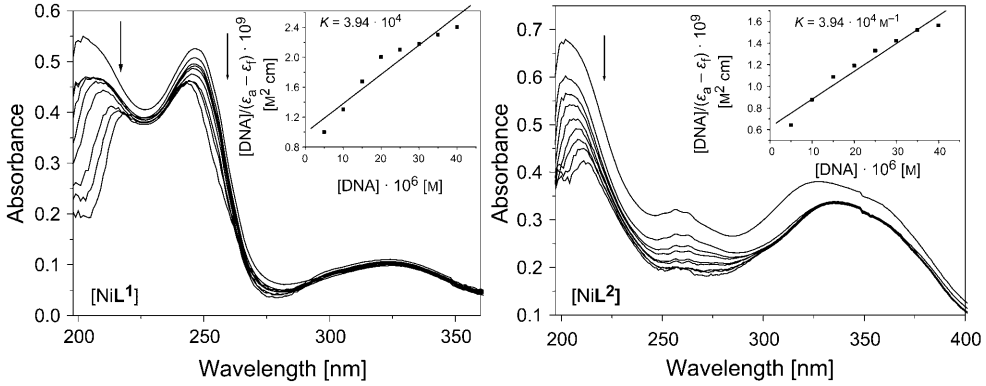


Fig. 2. *UV Spectra of the complexes [Ni***L**^{1}*] and [Ni***L**^{2}*]* (10 μM) *in the presence of increasing amounts of DNA* [DNA] = 0–40 μM. The arrow indicates the absorbance changes upon increasing DNA concentration.

To test the complex-binding to DNA by intercalation, ethidium bromide (EB) was employed, as EB interacts with DNA as a typical indicator of intercalation [23]. *Fig. 3* shows the VIS spectra of EB, EB/DNA, and EB/DNA/[Ni**L**^{1}] as an example. Thus, the maximal absorption of EB at 479 nm (*Fig. 3*,*a*) decreases and simultaneously shifts to 509 nm in the presence of DNA (*Fig. 3*,*b*), which is characteristic of intercalation. A mixture EB/DNA/[Ni**L**^{1}] shows an absorption increase at 509 nm and a redshift (*Fig. 3*,*c*), compared to *Fig. 3*,*b*. These results suggest that a competitive intercalation of [Ni**L**^{1}] and EB in DNA takes place, thus releasing some free EB from the EB/DNA system when [Ni**L**^{1}] is added. The other complexes show a similar behavior, establishing that the four complexes bind to DNA mainly by intercalation.

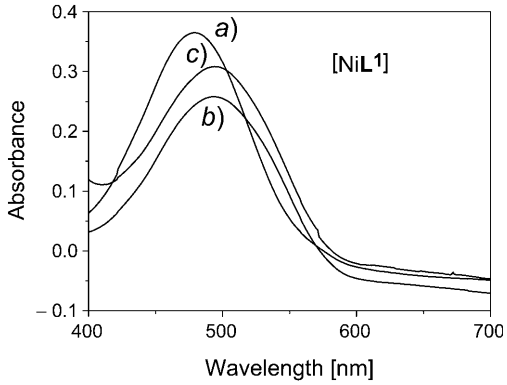


Fig. 3. *VIS Spectra of* a) *EB* ($1 \cdot 10^{-5}$ M), b) *EB* ($1 \cdot 10^{-5}$ M)/*DNA* ($2.5 \cdot 10^{-5}$ M), *and* c) *EB* ($1 \cdot 10^{-5}$ M)/*DNA* ($2.5 \cdot 10^{-5}$ M)/*[Ni***L**^{1}*]* ($2.5 \cdot 10^{-5}$ M)

Spectrofluorimetric Titration and Ethidium Bromide (EB) *Replacement Experiment.* Upon addition of DNA, the emission intensity of [Ni**L**^{1}], [Zn**L**^{1}], [Ni**L**^{2}], and [Zn**L**^{2}] increases by a factor of *ca.* 1.21, 1.11, 1.3, and 1.15 ([DNA]/[complex] = 2). The results of the emission titrations suggest that the four complexes can be protected from

solvent H_2O molecules by the hydrophobic environment inside the DNA helix, since the latter reduces the accessibility of solvent H_2O molecules to the complex, and the complex mobility is restricted at the binding site, leading to a decrease of the vibrational modes of relaxation [24–26]. Hence, the fluorescence emission intensity of the complexes is enhanced after the DNA is added. A spectrofluorimetric titration of [Ni**L**^{1}] is shown in *Fig. 4*.

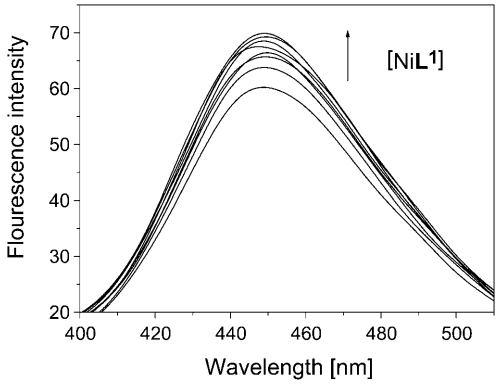


Fig. 4. *Emission-enhancement spectra of [Ni***L**^{1}*]* (10 μM) *in the presence of 0–20* μM *DNA*. The arrow indicates the emission-intensity changes upon increasing DNA concentration.

The DNA-binding modes of the four complexes were further monitored by a fluorescent EB displacement assay [27]. EB is a conjugate planar molecule. Its fluorescence intensity is very weak, but it is greatly increased when EB is specifically intercalated into the base pairs of double-stranded DNA. When EB is expelled by another intercalator, the fluorescence of the DNA–EB complex is quenched [28]. Therefore, a fluorescence-based competition technique can provide indirect evidence for the DNA-binding mode [29][30]. The experimental results show that the fluorescence intensity of the DNA–EB system decreases upon the addition of each of the four complexes. The results also imply that the complexes bind to DNA by intercalation, and they show that the Zn^{II} complexes exhibit a weaker interaction with the DNA–EB system than the Ni^{II} complexes [31]. According to the linear *Stern–Volmer* equation, the quenching constants K_q for [Ni**L**^{2}], [Ni**L**^{1}], [Zn**L**^{2}], and [Zn**L**^{1}] are $1.30 \cdot 10^5$, $2.03 \cdot 10^4$, $7.44 \cdot 10^3$, and $2.22 \cdot 10^3$ M^{-1}, respectively (*Fig. 5*). This order is entirely consistent with the results obtained from the spectrofluorimetric titrations.

Viscosity Measurements. To further clarify the interactions between the four complexes and DNA, viscosity measurements were carried out. Hydrodynamic measurements that are sensitive to length change (*i.e.*, viscosity and sedimentation) are regarded as the least ambiguous and the most critical tests of a binding model in solution in the absence of crystallographic structural data. A classical intercalation model demands that the DNA helix must lengthen as base pairs are separated to accommodate the binding molecule, leading to an increase of DNA viscosity. In contrast, a partial and/or nonclassical intercalation of a molecule could bend (or kink) the DNA helix, reducing its effective length and, concomitantly, its viscosity. The effects of the four complexes on the viscosity of DNA at 25.0° are shown in *Fig. 6*. The

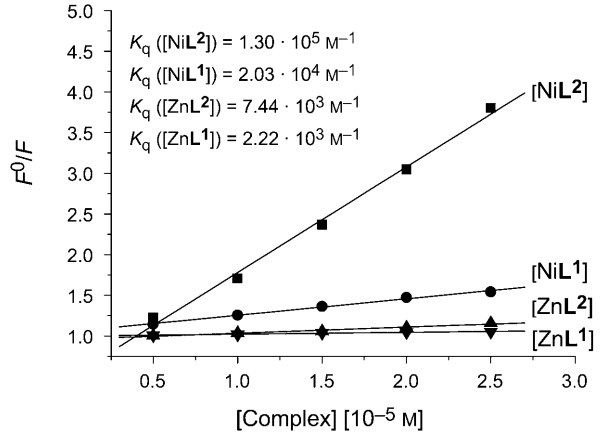


Fig. 5. Stern – Volmer *plot of the fluorescence titration data of the complexes [Ni***L**^{1}*], [Ni***L**^{2}*], [Zn***L**^{1}*], and [Zn***L**^{2}*]*

viscosities of the DNA increase steadily with increasing amounts of each of the four complexes. This behavior is consistent with that of other intercalators, *e.g.*, EB. Thus, the viscosity results clearly show that the four complexes can intercalate between adjacent DNA base pairs, causing an extension in the helix [32].

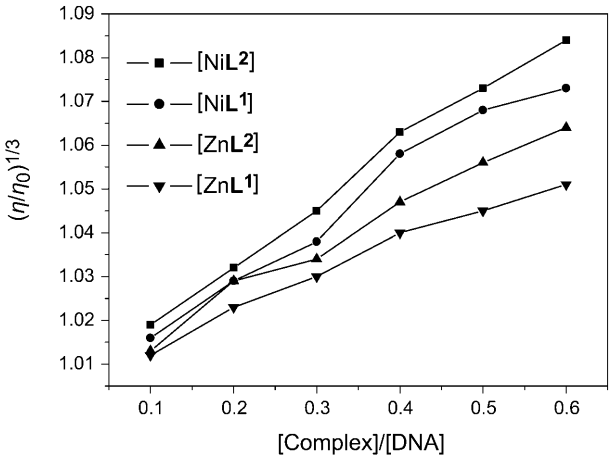


Fig. 6. *Effect of increasing amounts of the complexes on the relative viscosity of calf thymus DNA at 25.0°*

CD Spectra Studies. The foregoing studies suggest that the two Ni^{II} complexes [Ni**L**^{1}] and [Ni**L**^{2}] bind more strongly to DNA than the Zn^{II} complexes. We wanted to find out if this strong interaction can change the conformation of DNA, so the intrinsic CD spectra of calf-thymus DNA were examined. As shown in *Fig. 7*, the CD spectrum of free DNA exhibits a negative band at 241 nm due to the helicity and a positive band at 275 nm due to the base stacking, which are the characteristics of DNA in the right-hand B form [33]. On addition of the complexes to DNA, there is an increase in

ellipticity of the positive band at 275 nm, which is, however, accompanied by a redshift of 4 and 7 nm for [Ni**L**^{1}] and [Ni**L**^{2}], respectively, of the negative band at 241 nm. This result shows that the two Ni^{II} complexes can effectively interact with the DNA base pairs and thus slightly change the DNA helicity of the B form [34][35].

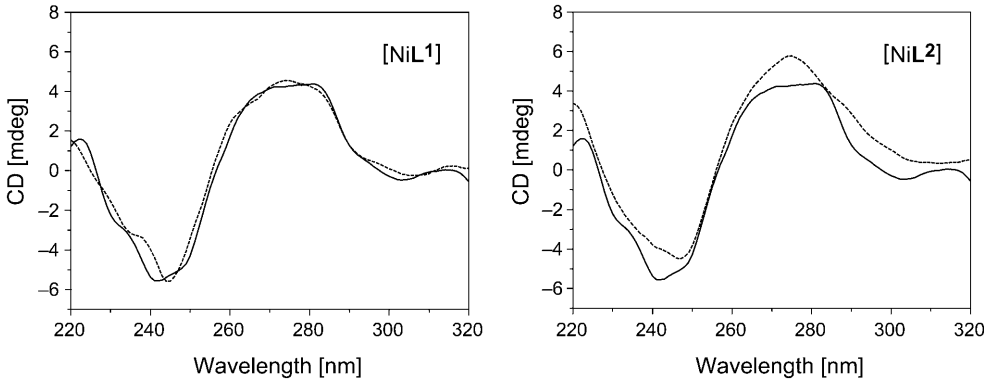


Fig. 7. *CD Spectra of DNA* ($6.0 \cdot 10^{-5}$ M) *in the presence* (– – –) *and absence* (—) *of* Ni^{II} *complex.* [Ni^{II} complex]/[DNA] 0.5.

Conclusion. – Two new chromenone-based *Schiff* bases and their Ni^{II} and Zn^{II} complexes were successfully synthesized. Electronic absorption spectra, fluorescence spectra, and viscosity measurements indicate that the four complexes can bind to calf-thymus DNA, presumably *via* an intercalation mechanism. The DNA-binding constants K and the EB quenching constants K_q were determined. The primary reason that the four complexes can intercalate into DNA may due to the planar structure of the chromenone moiety and the enhancement of rigidity of the complexes. The presented results may contribute to the development of transition-metal complexes with chromenone derivatives of pharmaceutical value, and may also provide some guidance for the development of intercalation compounds.

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Experimental Part

1. *General.* Acetic anhydride, hydroquinone, and transition-metal nitrates were produced in China. Calf-thymus DNA and ethidium bromide (EB) were purchased from *Sigma Chemicals Co.*, USA. All the experiments involving interaction of the complexes with DNA were carried out in doubly dist. H_2O buffer containing 5 mM *Tris* (2-amino-2-(hydroxymethyl)propane-1,3-diol) and 50 mM NaCl, and adjusted to pH 7.1 with aq. HCl soln. A soln. of calf-thymus DNA in the buffer gave a ratio of UV absorbance of *ca.* 1.8 – 1.9 : 1 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein [36]. The DNA concentration per nucleotide was determined by absorption spectroscopy and the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm [37]. CD Spectra: *Olos RSM 1000*. UV/VIS Spectra: *Varian-Cary-100-Conc* spectrophotometer; λ_{max} (ε) in nm. Fluorescence spectra: *Hitachi-RF-4500* spectrofluorophotometer. IR Spectra: *Thermo-Mattson-FT-IR* spectrometer; KBr disks; $\tilde{\nu}$ in cm^{-1}.

^{1}H-NMR Spectra: *Varian-VR-300-MHz* and *Bruker-Avance-DRX-200-MHz* spectrometer; in (D_6)DMSO; δ in ppm, J in Hz. Elemental analyses: *Vario-EL* analyzer.

2. *Ligands.* $\mathbf{L}^1$ *and* $\mathbf{L}^2$ *Hydroquinone Diacetate* (= *Benzene-1,4-diol Diacetate*; **1**). The mixture of hydroquinone (22 g, 0.20 mol), Ac_2O (42.0 g, 0.41 mol), and one drop of 98% H_2SO_4 soln. was stirred for 5 min. The gel-like product was poured into crushed ice, and the mixture left for 4 h. After filtration and washing with H_2O, the solid was recrystallized from 50% EtOH: 20.0 g (52%) of **1**. White crystals. M.p. 120–122°. ([38] 121°).

2,5-Dihydroxyacetophenone (= *1-(2,5-Dihydroxyphenyl)ethanone*; **2**) [39]. The mixture of dry **1** (20.0 g) and dry $AlCl_3$ (50.0 g) was heated to 110–120° for 30 min (drying tube packed with $CaCl_2$ and HCl absorption unit), and then heated at 160–165° for 3 h. After cooling, 36% HCl soln. (10 ml) was added, and then the mixture poured into crushed ice. After filtration and several washings with H_2O, the solid was recrystallized from 95% EtOH: 10.0 g (64%) of **2**. Green needles. M.p. 202–203°.

*6-Hydroxy-4-oxo-4*H-*1-benzopyran-3-carboxaldehyde* (**3**). $POCl_3$ (20 ml) was added slowly and dropwise into the soln. of dried **2** (3.0 g, 19.7 mmol) in dry DMF (40 ml) in an ice-water bath. Then the mixture was stirred at r.t. overnight and then heated at 40° for 1 h. After cooling, the mixture was poured into crushed ice and left for 4 h. Filtration and recrystallization of the solid from DMF/H_2O gave pure **3** (2.4 g, 65%). Brown powder. M.p. 230–231°. IR: 1697 (C=O), 1632 (CH=O). ^{1}H-NMR (300 MHz)[1]): 7.27 (*dd*, J = 2.7, 9.0, H–C(7)); 7.39 (*d*, J = 2.7, H–C(5)); 7.61 (*d*, J = 9.0, H–C(8)); 8.84 (*s*, H–C(2)); 10.1 (CHO); 10.24 (OH, exchangeable).

*3-{[(1,5-Dihydro-3-methyl-5-thioxo-4*H-*1,2,4-triazol-4-yl)imino]methyl}-6-hydroxy-4*H-*1-benzopyran-4-one* (**4**; $\mathbf{L}^1$). A mixture of **3** (0.190 g, 1 mmol) and 4-amino-2,4-dihydro-5-methyl-3*H*-1,2,4-triazol-3-thione (0.130 g, 1 mmol) in MeOH (40 ml) was refluxed for 10 min. Then, AcOH (0.5 ml) was added. The yellow precipitate was washed several times with MeOH and dried *in vacuo*: **4** (= $\mathbf{L}^1$; 80%). M.p. 277–278°. IR: 1630 (C=O), 1595 (C=N), 1257 (C=S), 3191 (NH), 2951 (Me). ^{1}H-NMR (200 MHz)[1]): 2.34 (*s*, Me); 7.28 (*dd*, J = 2.9, 9.0, H–C(7)); 7.39 (*d*, J = 2.9, H–C(5)); 7.62 (*d*, J = 9.0, H–C(8)); 9.05 (*s*, H–C(2)); 10.22 (*s*, CH=N); 10.26 (*s*, exchangeable, OH); 13.73 (*s*, exchangeable, NH).

*2,2′-Bis[(6-hydroxy-4-oxo-4*H-*1-benzopyran-3-yl)methylene]carbonothioic Dihydrazide* (**5**; $\mathbf{L}^2$). A soln. of carbonothioic dihydrazide (0.053 g, 0.5 mmol) in MeOH (50 ml) was added dropwise to the soln. of **3** (0.190 g, 1.0 mmol) in MeOH (30 ml) at 70°. The resulting soln. was refluxed for 4 h, and then the yellow precipitate was washed several times with MeOH: **5** (= $\mathbf{L}^2$; 75%). M.p. 235–237°. IR: 1633 (C=O), 1590 (C=N), 1237 (C=S). ^{1}H-NMR (300 MHz)[1]): 7.26 (*dd*, J = 3.0, 8.7, H–C(7)); 7.32 (*d*, J = 3.0, H–C(5)); 7.48 (*d*, J = 8.7, H–C(8)); 9.23 (*s*, H–C(2)); 10.16 (*s*, CH=N).

3. *Complexes.* 3.1. Ligand $\mathbf{L}^1$ (0.151 g, 0.5 mmol) in MeOH (30 ml) was heated to 70°, then $Ni(NO_3)_2 \cdot 6\,H_2O$ (0.146 g, 0.5 mmol) in MeOH (5 ml) was added. After 30 min stirring, a clear light green soln. was obtained. The clear soln. was concentrated, and then AcOEt was added. The resulting yellow green solid was collected by centrifugation and washed with AcOEt including a small portion of EtOH. The product [Ni$\mathbf{L}^1$] was dried *in vacuo*. [Zn$\mathbf{L}^1$], a yellow solid, was prepared in the same way.

*Aqua{3-{{[1,5-dihydro-3-methyl-5-(thioxo-*κS*)-4*H-*1,2,4-triazol-4-yl]imino-*κN*}methyl}-6-hydroxy-4*H-*1-benzopyran-4-one-*κO^4*}nickel(2+) Nitrate Hydrate (1:2:1)* ([Ni$\mathbf{L}^1$] or [Ni$\mathbf{L}^1 \cdot H_2O$]$\cdot 2\,NO_3 \cdot H_2O$): Yield 65%. Thermal analysis: T 75° (H_2O loss 3.35; calc. 3.46). Λ_m = 166 S·cm^2·mol^{-1} (10^{-3} M in MeOH at 25°). UV: 202 (55100), 246 (52900) ($1 \cdot 10^{-5}$ M in MeOH/buffer soln. 1:99); d–d transition 525 (248) ($1 \cdot 10^{-3}$ M in MeOH). IR: 1635 (C=O), 1578 (C=N), 1233 (C=S), 3196 (NH), 1384 (NO_3). Anal. calc. for $C_{13}H_{14}N_6NiO_{11}S$ (521.04): C 29.97, H 2.71, N 16.13; found: C 30.05, H 3.02, N 16.48.

*Triaqua{3-{{[1,5-dihydro-3-methyl-5-(thioxo-*κS*)-4*H-*1,2,4-triazol-4-yl]imino-*κN*}methyl}-6-hydroxy-4*H-*1-benzopyran-4-one-*κO^4*}zinc(2+) Nitrate Hydrate (2:4:1)* ([Zn$\mathbf{L}^1$] or [Zn$\mathbf{L}^1 \cdot 3(H_2O)$]$\cdot$ $2\,NO_3 \cdot 0.5\,H_2O$): Yield 70%. Thermal analysis: T 72° (H_2O loss 8.47; calc. 8.12). Λ_m = 172 S·cm^2·mol^{-1} (10^{-3} M in MeOH at 25°). UV ($1 \cdot 10^{-5}$ M in MeOH/buffer soln. 1:99): 204 (30900), 247 (23010). IR: 1636 (C=O), 1562 (C=N), 1230 (C=S), 3220 (NH), 1384 (NO_3). Anal. calc. for $C_{13}H_{17}N_6O_{12.5}SZn$ (554.78): C 28.14, H 3.09, N 15.15; found: C 28.41, H 2.92, N 15.84.

3.2. Ligand $\mathbf{L}^2$ (0.135 g, 0.3 mmol) in MeOH (30 ml) was heated to 70°, then $Ni(NO_3)_2 \cdot 6\,H_2O$ (0.146 g, 0.5 mmol) in MeOH (5 ml) was added. After 30 min stirring, a clear light green soln. was obtained. The clear soln. was concentrated, and then AcOEt was added. The resulting green solid was

collected by centrifugation and washed with AcOEt including a small portion of EtOH. The product [Ni**L²**] was dried *in vacuo* [Zn**L²**], a yellow solid, was prepared in the same way.

*Bis{2,2′-bis[(6-hydroxy-4-oxo-4*H*-1-benzopyran-3-yl)methylene]carbonothioic dihydrazide-*κ*N²,*κ*N²′,*κ*S}nickel(2+) Nitrate (1:2)* ([Ni**L²**] or ([Ni**L²**$_2$]·2 NO_3): Yield 75%. $\Lambda_m = 154$ S·cm²·mol^{-1} (10^{-3} M in MeOH at 25°). UV: 202 (68000), 326 (38100) ($1 \cdot 10^{-5}$ M in MeOH/buffer soln. 1:99); d–d transition 603 (73), 903 (63) ($1 \cdot 10^{-3}$ M in MeOH). IR: 1632 (C=O), 1575 (C=N), 1215 (C=S), 1383 (NO_3). Anal. calc. for $C_{42}H_{28}N_{10}NiO_{18}S_2$ (1083.55): C 46.55, H 2.59, N 12.93; found: C 46.74, H 2.52, N 12.68.

*Bis{2,2′-bis[(6-hydroxy-4-oxo-4*H*-1-benzopyran-3-yl)methylene]carbonothioic dihydrazide-*κ*N²,*κ*N²′,*κ*S}zinc(2+) Nitrate (1:2)* ([Zn**L²**] or [Zn**L²**$_2$]·2 NO_3): Yield 70%. $\Lambda_m = 162$ S·cm²·mol^{-1} (10^{-3} M in MeOH at 25°). UV ($1 \cdot 10^{-5}$ M in MeOH/buffer soln. 1:99): 202 (61900), 320 (20100). IR: 1632 (C=O), 1569 (C=N), 1219 (C=S), 1384 (NO_3). ^{1}H-NMR (300 MHz): 7.32 (br., H–C(7)); 7.56 (br., H–C(5)); 7.88 (br., H–C(8)); 9.17 (br., H–C(2)); 10.13 (br., CH=N). Anal. calc. for $C_{42}H_{28}N_{10}O_{18}S_2Zn$ (1090.27): C 46.26, H 2.57, N 12.85; found: C 46.30, H 2.62, N 12.61.

4. *Absorption Titration.* Absorption titration experiments were performed with fixed conc. of the complexes (10 μM) while gradually increasing the conc. of DNA (1 mM) at 25°. While measuring the absorption spectra, an equal amount of DNA was added to both the complex soln. and the reference soln. to eliminate the absorbance of DNA itself. To further compare quantitatively the affinity of the complexes bound to DNA, based on the absorption titrations, the intrinsic binding constants K were determined with *Eqn. 1* [40], where [DNA] is the conc. of DNA in base pairs, ε_a, ε_f, and ε_b correspond to the apparent absorption coefficient A_{obsd}/[complex], the extinction coefficient for the free complex, and the extinction coefficient for the complex in the fully bound form, resp.

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/[K(\varepsilon_b - \varepsilon_f)] \quad (1)$$

5. *Fluorescence Spectra.* Fixed amounts of complexes were titrated with increasing amounts of DNA at 25°. An excitation wavelength of 323 nm for [Ni**L¹**] and [Zn**L¹**], and of 318 nm for [Ni**L²**] and [Zn**L²**] was used, and the fluorescence-emission intensity was monitored at 447 nm for **L¹** complexes and at 451 nm for **L²** complexes.

EB is a common fluorescent probe for DNA structure and has been employed in examinations of the mode and process of metal-complex binding to DNA. A soln. (2 ml) of 10 μM DNA and 0.025 μM EB [41] was titrated by 5–25 μM complex (λ_{ex} 500 nm, λ_{em} 520–650 nm) at 25°. The data were plotted according to the classic *Stern–Volmer Eqn. 2* [42], where F^0 is the emission intensity in the absence of quencher, F is the emission intensity in the presence of quencher, K_q is the quenching constant, and [Q] is the quencher conc. (*Fig. 5*). The shape of the *Stern–Volmer* plots can be used to characterize the quenching as being predominantly dynamic or static. Plots of F^0/F *vs.* [Q] appear to be linear, and K_q depends on temperature.

$$F^0/F = K_q[Q] + 1 \quad (2)$$

6. *Viscosity Measurements.* Viscosity experiments were conducted on an *Ubbelodhe* viscometer, immersed in a thermostatic water bath maintained at 25.0°. Each complex was introduced into a DNA soln. (10 μM) present in the viscometer. Data was presented as $(\eta/\eta_0)^{1/3}$ *vs.* the ratio of the conc. of the complexes and DNA, where η is the viscosity of DNA in the presence of complexes and η_0 is the viscosity of DNA alone [43] (*Fig. 6*).

7. *CD Spectra.* The CD spectra were recorded at r.t. by increasing the complex/DNA ratio ($r = 0.0$, 0.5). Each sample soln. was scanned in the range 220–320 nm. The conc. of DNA was $6.0 \cdot 10^{-5}$ M.

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