Synthesis, Characterization and DNA-Binding Properties of the Cu(II) Complex with 7-Methoxychromone-3-carbaldehyde-benzoylhydrazone

Gao-Fei QI, Zheng-Yin YANG,* and Dong-Dong QIN

College of Chemistry and Chemical Engineering and State Key Laboratory of Applied Organic Chemistry, Lanzhou University; Lanzhou 730000, P. R. China.

Received May 27, 2008; accepted October 18, 2008; published online October 22, 2008

A new chromone derivative (7-methoxychromone-3-carbaldehyde-benzoylhydrazone) ligand (HL) and its novel Cu(II) complex have been synthesized and characterized on the basis of elemental analyses, molar conductivities, proton nuclear magnetic resonance, infrared spectra and mass spectra analyses. The general formula of the Cu(II) complex is $[CuL(HB_{2B}O)]Cl\cdot 2H_2O$. In addition, the interaction of the Cu(II) complex and its free ligand with calf-thymus DNA was investigated by electronic absorption spectroscopy, fluorescence spectroscopy and viscosity measurement. Results suggest that both the two compounds can bind with calf-thymus DNA *via* an intercalation mechanism. Furthermore, the Cu(II) complex can bind to DNA more strongly than the free ligand due to the chelating effect of copper(II) ion to the free ligand.

Key words Cu(II) complex; chromone; interaction; calf thymus DNA; intercalation mechanism

The chromone moiety forms the important component of pharmacophores of a number of biologically active molecules of synthetic as well as natural origin and many of them display a remarkable array of biological and pharmacological activities as antimicrobial, anticancer and pesticidal agents.[1,2] Consequently, chromone chemistry continues to draw considerable interest of synthetic organic and medicinal chemists.[3—7] Recently, 3-formylchromone emerged as a valuable synthon for incorporation of the chromone moiety into a number of molecular frameworks has aroused our interest. In previous work from our laboratory, we have presented a detailed investigation of the interactions of 6-substituted chromone-3-carbaldehyde hydrazone Ln(III) complexes[8,9] with calf-thymus DNA (CT-DNA) arised from the fact that a great many hydrazones and their complexes have diverse spectra of biological and pharmaceutical activities, such as anticancer and antioxidative activities.[10—12] However, up to now, the interactions with DNA of 7-substituted chromone-3-carbaldehyde hydrazone and their complexes have not been explored due to the difficulties in synthesis of 7-substituted chromone-3-carbaldehyde. In our first trials, we tried to yield the 7-substituted chromone-3-carbaldehyde starting with 4-methoxy-2-hydroxyacetophenone and *N*,*N*-dimethylformamide (DMF)-$POCl_3$ according to the literature,[13] but get a very low yield (5%). This result has also been reported by the other literature.[14] Later, difluorodioxaborin 1 (Fig. 1) overcome the low yield.[15] Then, we intended to continue the earlier studies of the chromone structure and investigated the binding behaviors of 7-substituted chromone-3-carbaldehyde hydrazone and its complexes with CT-DNA. Considering that copper has an important biological role in all living organisms as an essential trace element and the copper(II) complexes have been used as probes of DNA structure in solution.[16,17] We mainly choose a new chromone derivative (7-methoxychromone-3-carbaldehyde-benzoylhydrazone) ligand (HL) and its a novel Cu(II) complex in this paper as our object of research and investigated the coordination of the chromone derivative ligand to copper(II) and their DNA-binding properties. The interaction of the two compounds with CT-DNA in our studies was investigated using a host of physical methods like spectrometric titrations, ethidium bromide displacement experiments and viscosity measurements.

Experimental

Instrumentation Melting points were determined on a Beijing XT4-100X microscopic melting point apparatus. Elemental analyses (C, H, N) were carried out on an Elemental Vario EL analyzer. The metal contents of the complexes were determined by titration with EDTA (xylenol orange tetrasodium salt used as an indicator and hexamethylidynetetraimine as buffer). IR spectra were obtained in KBr discs on a Therrno Mattson FT-IR spectrophotometer in the 4000—400 cm^{-1} region. ^{1}H-NMR spectra were recorded on a 200 MHz on a Bruker DRX-200 spectrometer in DMSO-d_6 (dimethyl sulfoxide) with TMS (tetramethyl silane) as internal standard. Conductivity measurements were performed in DMF with a DDS-11A conductometer at 25 °C. Electrospray ionization time-of-flight (ESI-TOF) mass spectra were obtained on a Mariner ESI-TOF mass spectrometer. UV–visible spectra were recorded on a Shimadzu UV-240 spectrophotometer. The fluorescence spectra were recorded on a Hitachi RF-4500 spectrofluorophotometer.

Materials and Methods CT-DNA and ethidium bromide (EB) were purchased from Sigma Chemical Co. Saint Louis, MO (U.S.A.). EDTA and $CuCl_2\cdot 2H_2O$ were produced in China. All chemicals used were of analytical grade. All the experiments involving the interaction of the complexes with CT-DNA were carried out in doubly distilled water buffer containing 5 mM Tris [tris(hydroxymethyl)aminomethane] and 50 mM NaCl and adjusted to pH 7.1 with HCl. The solution of CT-DNA in the buffer gave a ratio of UV absorbance of about 1.8—1.9 : 1 at 260 and 280 nm, indicating that the CT-DNA was sufficiently free of protein.[18] The CT-DNA concentration per nucleotide was determined spectrophotometrically by employing an extinction coefficient of 6600 $M^{-1}cm^{-1}$ at 260 nm.[19] The compounds were dissolved in a mixture solvent of 1% CH_3OH and 99% Tris–HCl buffer (5 mM Tris–HCl, 50 mM NaCl, pH 7.1) at the concentration 1.0×10^{-5} M. Absorption titration experiments were performed with fixed concentrations drugs (10 μM) while gradually increasing the concentration of CT-DNA. While measuring the absorption spectra, an equal amount of CT-DNA was added to both the compounds solution and the reference solution to eliminate the absorbance of CT-DNA itself. Viscosity experiments were conducted on an Ubbelodhe viscometer, immersed in a thermostated water-bath maintained to 25 °C. Titrations were performed for the Cu(II) complex and the ligand (0.5—3 μM), and each compound was introduced into a CT-DNA solution (5 μM) present in the viscometer. Data were presented as $(\eta/\eta_0)^{1/3}$ *versus* the ratio of the concentration of the compound and CT-DNA, where η is the viscosity of CT-DNA in the presence of the compound and η_0 is the viscosity of CT-DNA alone. Viscosity values were calculated from the observed flow time of CT-DNA containing solution corrected from the flow time of buffer alone (t_0), $\eta=t-t_0$.[20,21]

To compare the binding affinity of the two compounds bound to DNA,

* To whom correspondence should be addressed. e-mail: yangzy@lzu.edu.cn

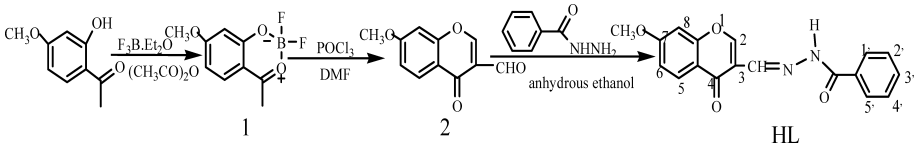

Fig. 1. Scheme of the Synthesis of the Ligand (HL)

fluorescence titration method was used. Fixed amounts of compound were titrated with increasing amounts of DNA, over a range of DNA concentrations from 2.5 to 22.5 μM. An excitation wavelength of 353 nm was used.

Further support for the Cu(II) complex and the ligand binding to DNA *via* intercalation is given through the emission quenching experiment. 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.[22] A 2 ml solution of 10 μM DNA and 0.8 μM EB (at saturating binding levels) was titrated by 10—100 μM the Cu(II) complex and ligand (λ_{ex}=525 nm, λ_{em}=520.0—650.0 nm). According to the classical Stern–Volmer equation[23]:

$$F_0/F = K_q[Q] + 1$$

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 concentration. The shape of Stern–Volmer plots can be used to characterize the quenching as being predominantly dynamic or static. Plots of F_0/F *versus* [Q] appear to be linear and K_q depends on temperature.

Preparations of the Free Ligand and Its Metal Complex. Synthesis of the Ligand The compounds of **1** and **2** (Fig. 1) were prepared according to the literature.[15] Synthesis of the ligand HL was in accordance with the following method: an ethanol solution (20 ml) containing benzoyl hydrazine (1.36 g, 10 mmol) was added dropwise to the compound **2** (2.04 g, 10 mmol) of chloroform solution (10 ml) with stirring. After 10 min, a large amount of light yellow precipitate appeared. Then continuing stirring for 6 h at room temperature, the light yellow precipitate solid was collected by filtration and washed with ethanol three times. Recrystallization from anhydrous ethanol to give the ligand HL, which was dried *in vacuo*. Yield, 88%. mp 176—178 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 11.91 (1H, s, NH), 8.75 (1H, s, CH=N), 8.60 (1H, s, 2-H), 8.02 (1H, d, J=8.9 Hz, H-5), 7.09—7.14 (1H, dd, J=2.4, 8.9 Hz, H-6), 7.21 (1H, d, J=2.4 Hz, 8-H), 7.90—7.93 (2H, d, ph-H(1′ 5′)), 7.48—7.59 (3H, m ph-H(2′ 3′ 4′)), 3.91 (3H, s, CH_3). IR ν_{max} (cm^{-1}) 1670 (C=O of carbonyl), 1639 (CH=N), 1621 (C=O of hydrazonic).

Synthesis of the Cu(II) Complex The ligand (1 mmol, 0.32 g) was dissolved in chloroform (10 ml) and a solution of $CuCl_2 \cdot 2H_2O$ (1 mmol, 0.17 g) in anhydrous ethanol (10 ml) was then added dropwise with stirring. Then the mixture solution was refluxed on an oil-bath at 80 °C for 4 h with stirring. After cooling to room temperature, a large amount of green precipitate appeared. It was separated from the solution by suction filtration, purified by washing several times with ethanol, and dried for 24 h *in vacuo*. [CuL(H_2O)]Cl·2H_2O. Yield: 81%. Analysis: Found (calculated) (%) for $C_{18}H_{19}N_2O_7ClCu$ (%): C, 45.58 (45.58); H, 3.56 (4.04); N, 5.79 (5.91); Cu, 13.45 (13.39). IR ν_{max} (cm^{-1}) 3405 (OH of H_2O), 1646 (C=O of carbonyl), 1622 (CH=N). Λ_m (S cm^2 mol^{-1}): 71.

Results and Discussion

Characterization of the Compounds. Properties of the Compounds and Structure of the Cu(II) Complex The ligand is soluble in chloroform, methanol and ethanol, while the Cu(II) complex is soluble in methanol, slightly soluble in ethanol. The two compounds are soluble in DMF; DMSO; insoluble in water; benzene and diethyl ether. But they are air stable for extended periods. Since the crystal structure of the Cu(II) complex has not been obtained yet, we characterized the complex and determined its possible structure by elemental analyses, molar conductivities, IR and mass (ESI-TOF) date. The likely structure of the Cu(II) complex is shown in Fig. 2.

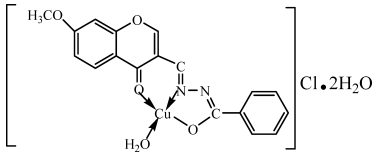

Fig. 2. The Suggested Structure of the Complex

Stability and Molar Conductivity of the Cu(II) Complex in Solution The stability of Cu(II) complex in an aqueous solution has been studied by observing the UV–vis spectra and estimating the molar conductivities at different time intervals for any possible change. The tested Cu(II) complex was prepared in methanol and for experiments freshly diluted in phosphate buffer system (at pH 7.4, 7.8). Then, the UV–vis spectra and molar conductivities were researched at different time intervals. The investigations revealed that the UV–vis spectra have remained unaltered for the solutions and its molar conductance values have no obvious change for very freshly prepared and for over the whole experiment (12 h). It indicates that the Cu(II) complex is quite stable in solution. The molar conductivity of the Cu(II) complex is 71—71.5 (S cm^2 mol^{-1}) in DMF, showing that it is 1 : 1 electrolytes.[24]

IR Spectra The main stretching frequencies of the IR spectra of the ligand and its Cu(II) complex are presented in the experimental section. The ν(C=O) of carbonyl band of the ligand appears at 1670 cm^{-1}, while it becomes at 1646 cm^{-1} in its Cu(II) complex, which makes a shift towards lower frequency by 24 cm^{-1}. It shows that the carbonyl oxygen of the free ligand takes part in the coordination. The Cu(II) complex exhibits band of the ν(OH) vibration at 3405 cm^{-1} which demonstrates that there is crystal water in the complex.[25] The band at 1639 cm^{-1} assigned to the ν(CH=N) stretch for the free ligand was shifted to 1622 cm^{-1} for its Cu(II) complex, indicating that the ligand coordinate to metal ions *via* the azomethine nitrogen.[26] The ν(C=O) of hydrazonic band appears at 1621 cm^{-1} in the free ligand, while the band disapears in its Cu(II) complex. This change indicates that the ν(C=O) of hydrazonic band may lose its original characteristic and form coordinative bond by an enolic format with metal. This point has been further confirmed followed by mass spectrometry.

ESI-TOF Mass Spectra of the Cu(II) Complex In order to further define the structure of Cu(II) complex, ESI-TOF mass spectrometry has been taken. ESI-TOF mass spectra demonstrates clearly the existence of molecular ion peak, and the m/z of 402.8 can be assigned to fragment of [CuL(H_2O)]$^+$. This has further proved that infrared analysis and been in accordance with the other means of characterization.

DNA-Binding Mode and Affinity. Electronic Absorption Titration Electronic absorption spectroscopy is an effective method to examine the binding mode of DNA with

metal complex.[22,27,28] If the binding mode is intercalation, the p* orbital of the intercalated ligand can couple with the p orbital of the base pairs, thus, decreasing the $\pi \rightarrow \pi^*$ transition energy and resulting in the bathochromism. On the other hand, the coupling π orbital is partially filled by electrons, thus, decreasing the transition probabilities and concomitantly resulting in hypochromism.[29] Figure 3 shows the absorption spectra variations of the ligand and its Cu(II) complex in the absence and presence of the CT-DNA (at a constant concentration of the compounds). The electronic spectra of ligand has a hypochromism band at 287 nm and an isobathic point at 332 nm or so, while the Cu(II) complex exhibits hypochromism band at 310 nm and 340 nm or so.

Fluorescence Spectra The ligand and its Cu(II) complex can emit weak luminescence in Tris-buffer with a max wavelength of about 433 nm. The results of the emission titrations for the two compounds with DNA are illustrated in the titration curves (Fig. 4). Upon addition of DNA, the emission intensities at about 433 nm of the two compounds grow to around 1.52 and 1.54 times larger, respectively, than those in the absence of DNA. The results of the emission titrations suggest that both the compounds are protected from solvent water molecules by the hydrophobic environment inside the DNA helix, and that the Cu(II) complex can be protected more efficiently than the ligand. This implies that both the compounds can be inserted between DNA base pairs and that the Cu(II) complex can bind to DNA more strongly than the ligand. In order to further illustrate this point clearly, changes in emission intensities for the ligand and the Cu(II) complex have been plotted against the added DNA concentration per mole compounds at about 433 nm in Fig. 5.

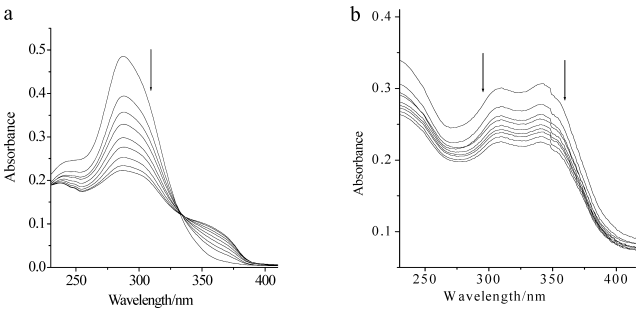

Fig. 3. (a) Electronic Spectra of the Ligand (10 μM) in the Presence of Increasing Amounts of CT-DNA

[CT-DNA]=0—40 μM. Arrow shows the absorbance changes upon increasing CT-DNA concentration.

(b) Electronic Spectra of the Cu(II) Complex (10 μM) in the Presence of Increasing Amounts of CT-DNA

[CT-DNA]=0—40 μM. Arrow shows the absorbance changes upon increasing CT-DNA concentration.

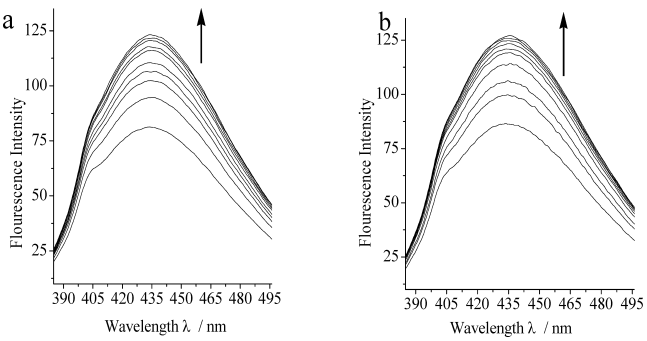

Fig. 4. (a) The Emission Enhancement Spectra of the Ligand (10 μM) in the Presence of 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5 μM CT-DNA

Arrow shows the emission intensity changes upon increasing DNA concentration.

(b) The Emission Enhancement Spectra of the Cu(II) Complex (10 μM) in the Presence of 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5 μM CT-DNA

Arrow shows the emission intensities upon increasing DNA concentration.

Steady-state emission quenching experiments are also used to observe the binding mode of the compounds to DNA. It is well known that EB can intercalate nonspecifically into DNA, which causes it to fluoresce strongly. Competitive binding of other drugs to DNA and EB will result in displacement of bound EB and a decrease in the fluorescence intensity. This fluorescence-based competition technique can provide indirect evidence for the DNA-binding mode. Figure 6 shows the emission spectra of the DNA–EB system with increasing amounts of the ligand and the Cu(II) complex. The emission intensity of the DNA–EB system decreases as the concentration of the two compounds increases, which indicates that two compounds could displace EB from the DNA–EB system. The resulting decrease in fluorescence is caused by EB changing from a hydrophobic environment to an aqueous environment.[30] The quenching plots illustrate that the quenching of EB bound to DNA by the compounds are in good agreement with the linear Stern–Volmer equation. The plots of F_0/F *versus* [Q], K_q is given by the ratio of the slope to the intercept. The K_q values for the ligand and its Cu(II) complex are 1.92×10^4 and $3.05\times10^4\ \mathrm{M}^{-1}$, respectively. The data show that the interaction of the Cu(II) complex with DNA is stronger than that of the ligand, which is consistent with the above fluorescence spectra.

Viscosity Measurements As optical photophysical probes generally provide necessary, but not sufficient, clues to further clarify the interactions between the study complex 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 binding in solution in absence of crystallographic structural data. A classical intercalation model demands that the DNA helix lengthen as base pairs are separated to accommodate the binding ligand, leading to an increase in DNA viscosity. In contrast, a partial, non-classical intercalation of compound could bend (or kink) the DNA helix, reducing its effective length and, concomitantly, its viscosity.[21,31] Viscosity experimental results clearly show that both the compounds can intercalate between adjacent DNA base pairs, causing an extension in the helix, and

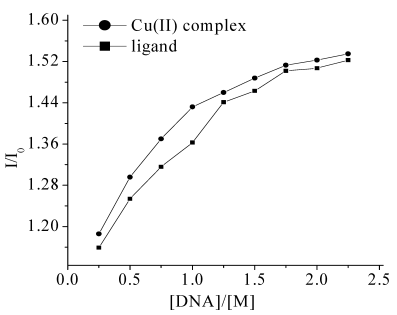

Fig. 5. Changes in Emission Intensities (at About 433 nm) for the Ligand (10 μM) and its Cu(II) Complex (10 μM) in the Presence of Calf Thymus DNA (0—22.5 μM) in Buffer Solutions

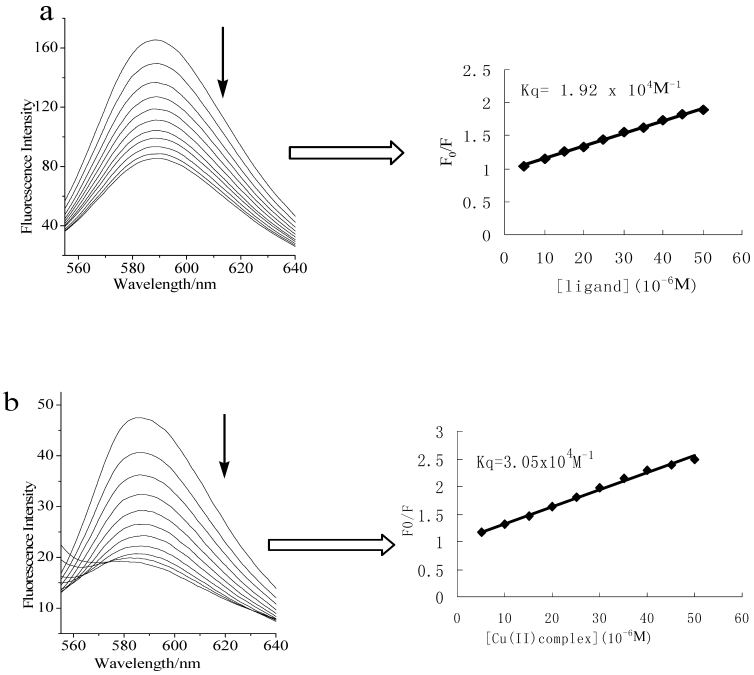

Fig. 6. (a) The Emission Spectra of DNA-EB System (10 μM, 0.8 μM EB), λ_{ex}=525 nm, λ_{em}=520—650 nm, in the Presence of 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 μM Ligand

Arrow shows the emission intensity changes upon increasing ligand concentration. Inset: Stern–Volmer plot of the fluorescence titration data of ligand, K_q=1.92×10^4 M^{-1}. (b) The Emission Spectra of DNA–EB System (10 μM, 0.8 μM EB), λ_{ex}=525 nm, λ_{em}=520—650 nm, in the Presence of 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 μM Cu(II) Complex

Arrow shows the emission intensity changes upon increasing Cu(II) complex concentration. Inset: Stern–Volmer plot of the fluorescence titration data of Cu (II) complex, K_q=3.05×10^4 M^{-1}.

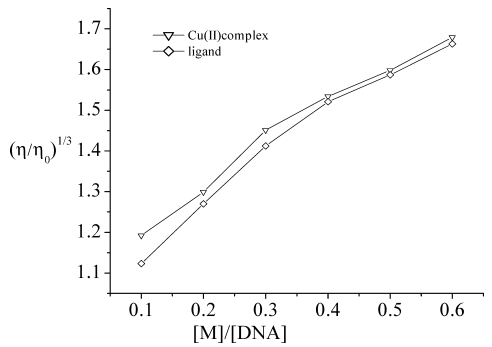

Fig. 7. Effect of Increasing Amounts of the Ligand and the Cu(II) Complex on the Relative Viscosity of CT-DNA at 25 °C

thus increase the viscosity of DNA. The effects of both compounds on the viscosity of DNA are shown in Fig. 7.

Conclusion

Taken together, a new chromone derivative (7-methoxychromone-3-carbaldehyde-benzoylhydrazone) ligand and its novel Cu(II) complex have been prepared and characterized. DNA-binding studies indicate that the Cu(II) complex and its free ligand can interact with calf-thymus DNA by intercalation mechanism. Furthermore, various comparative experiments show that the Cu(II) complex can bind to DNA more strongly than the free ligand, which attribute to chelating effect of the copper(II) ion to the free ligand. Chelating effect (metal ion to free ligand) can enhance the planar functionality of metal complex, so the complex can insert and stack between the base pairs of double helical DNA more easily than the free ligand.[32] The information obtained from the present work would ultimately be helpful to the understanding of the mechanism of metal complexes with nucleic acids, and useful in the development of potential probes of DNA structure and conformation.

Acknowledgments This work is supported by the National Natural Science Foundation of China (20475023) and Gansu NSF (0710RJZA012).

References

1) Wiley P. F., *J. Am. Chem. Soc.*, **74**, 4326—4329 (1952).
2) Koo J., *J. Org. Chem.*, **26**, 635—636 (1961).
3) Drewe J. A., Groundwater P. W., *J. Chem. Soc., Perkin Trans. 1*, **1997**, 601—605 (1997).
4) Tanaka H., Stohlmeyer M. M., Wandless T. J., Taylor L. P., *Tetrahedron Lett.*, **41**, 9735—9739 (2000).
5) Bandyopadhayay C., Sur K. R., Patra R., Sen A., *Tetrahedron*, **56**, 3583—3587 (2000).
6) Majumdar K. C., Bhattacharya T., *Tetrahedron Lett.*, **42**, 4231—4233 (2001).
7) Wang Y., Tan W., Li W. Z., Li Y., *J. Nat. Prod.*, **64**, 196—199 (2001).
8) Wang B. D., Yang Z. Y., Li T. R., *Bioorg. Med. Chem.*, **14**, 6012—6021 (2006).
9) Wang B. D., Yang Z. Y., Crewdson P., Wang D. Q., *J. Inorg. Biochem.*, **101**, 1492— 1504 (2007).
10) Wang L., Zhu Y., Yang Z., Wu J., Wang Q., *Polyhedron*, **10**, 2477—2481 (1991).
11) Yu S., Wang S., Luo Q., Wang L., Peng Z., Gao X., *Polyhedron*, **12**, 1093—1096 (1993).
12) Padhye S., Kauffman G. B., *Coord. Chem. Rev.*, **63**, 127—160 (1985).
13) Nohara A., Umetani T., Sanno Y., *Tetrahedron Lett.*, **22**, 1995—1998 (1973).
14) Nohara A., Umetani T., Sanno Y., *Tetrahedron*, **30**, 3553—3561 (1974).
15) Hogberg T., Vora M., Drake S., Mitscher L. A., Chu D. T. W., *Acta Chem. Scand.*, **B38**, 359—366 (1984).
16) Barton J. K., *Science*, **223**, 727—734 (1986).

17) Hudson B. P., Barton J. K., *J. Am. Chem. Soc.*, **120**, 6877—6888 (1998).
18) Satyanarayana S., Dabrowiak J. C., Chaires J. B., *Biochemistry*, **31**, 9319—9324 (1992).
19) Kumar C. V., Asuncion E. H., *J. Am. Chem. Soc.*, **115**, 8547—8553 (1993).
20) Eriksson M., Leijon M., Hiort C., Norden B., Gradsland A., *Biochemistry*, **33**, 5031—5040 (1994).
21) Xiong Y., He X. F., Zou X. H., Wu J. Z., Chen X. M., Ji L. N., Li R. H., Zhou J. Y., Yu K. B., *Dalton Trans.*, **1**, 19—24 (1999).
22) Barton J. K., Danishefsky A., Golderg J., *J. Am. Chem. Soc.*, **106**, 2172—2176 (1984).
23) Efink M. R., Ghiron C. A., *Anal. Biochem.*, **114**, 199—227 (1981).
24) Geary W. J., *Coord. Chem. Rev.*, **7**, 81—122 (1971).
25) Wang Y., Yang Z. Y., *Transition Met. Chem.*, **30**, 902—906 (2005).
26) Narang K. K., Singh V. P., *Transition Met. Chem.*, **18**, 287—290 (1993).
27) Kelly J. M., Tossi A. B., McConnell D. J., OhUigin C., *Nucleic Acids Res.*, **13**, 6017—6034 (1985).
28) Tysoe S. A., Morgan R. J., Baker A. D., Strekas T. C., *J. Phys. Chem.*, **97**, 1707—1711 (1993).
29) Pyle A. M., Rehmann J. P., Meshoyrer R., Kumar C. V., Turro N. J., Barton J. K., *J. Am. Chem. Soc.*, **111**, 3051—3058 (1989).
30) Zeng Y. B., Yang N., Liu W. S., *J. Inorg. Biochem.*, **97**, 258—264 (2003).
31) Satyanarayana S., Dabrowiak J. C., Chaires J. B., *Biochemistry*, **32**, 2573—2584 (1993).
32) Xu H., Zheng K. C., Lin L. J., Li H., Gao Y., Ji L. N., *J. Inorg. Biochem.*, **98**, 87—97 (2004).