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Synthesis and the characterization of Schiff-base copper complexes: Reactivity with DNA, 4-NPP and BNPP

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ABSTRACT

Two new copper Schiff-base complexes have been synthesized and characterized by use of spectroscopic techniques. The Schiff-base ligands, (*E*)-*N*-((1-methyl-1*H*-imidazol-2-yl)methylene)quinolin-8-amine, MICQ and (*E*)-1-((quinolin-8-ylimino)methyl)naphthalen-2-ol, TL1 were obtained from the reaction of 8-aminoquinoline with 1-methyl-2-imidazolecarboxaldehyde and 2-hydroxy-1-napthaldehyde respectively. The reaction of MICQ with copper(II) chloride produced complex **1**, [Cu(MICQ)Cl](PF_6), whereas the reaction of TL1 with copper(II) acetate resulted in complex **2**, Cu(TL1)(OAc)·CH_3OH. The single crystal X-ray structure determination of both complexes show distorted square planar geometries around the copper center. The reactivity of the complexes with calf thymus DNA, CT-DNA and plasmid DNA have been studied using ethidium bromide displacement fluorescence emission, electronic absorption spectroscopy and agarose gel electrophoresis analysis. From the fluorescence emission studies K_{sv} values of $3.70 \times 10^3\ M^{-1}$ and $7.82 \times 10^3\ M^{-1}$ were obtained for complexes **1** and **2**, respectively. The absorption titration resulted in K_b values of $1.52 \times 10^5\ M^{-1}$ for complex **1** and $5.00 \times 10^5\ M^{-1}$ for complex **2**. The results indicate that both complexes significantly interact with CT-DNA and also show cleavage of supercoiled DNA. In addition, complex **2** was found to hydrolyze the DNA model compounds bis(4-nitrophenyl) phosphate, BNPP and 4-nitrophenyl phosphate, 4-NPP.

1. Introduction

The interaction of transition-metal complexes [1–5] with DNA continues to attract interest in the search for anticancer chemotherapeutic agents due to the success of cisplatin [6]. Cisplatin is currently used in the treatment of a number of malignancies including testicular and ovarian cancer. Despite its worldwide use and success in such treatment, the drug has been found to have serious side effects such as liver toxicity, nausea and vomiting and development of drug resistance. These shortfalls have stimulated researchers to develop new and effective alternatives to cisplatin. Copper complexes are in the forefront of that search. The biochemical activity of copper in many biological systems makes it a good choice for use as chemotherapeutic drug [7,8]. During the past few decades, several copper complexes have been synthesized and studied for their interaction with DNA [9–25]. Metal complex–DNA interactions can be classified as intercalation, electrostatic binding, covalent binding and hydrolytic cleavage. Several copper complexes have been found to exhibit these modes of interaction. Furthermore, a number of the copper complexes have been found to exhibit cytotoxicity levels that are comparable to or greater than that of cisplatin [26–39].

Among the various modes of interactions of metal complexes with DNA, the hydrolysis of phosphate ester bonds in DNA serves as one important reaction for DNA degradation [40,41]. The DNA backbone consists of phosphate ester bonds, which impart high stability to the DNA framework. The half-life of the phosphodiester bond in DNA is estimated to be 130000 years. Hence the hydrolysis of the phosphodiester bonds in DNA is kinetically a slow process under normal physiological conditions. The stability of the phosphodiester bonds in DNA is considered to be nature's mechanism to safeguard the stability of the genetic make-up of life forms. In many biological systems, metalloenzymes catalyze the hydrolysis of the very stable phosphodiester bonds [42,43]. The phosphohydrolases generally contain Zn, Mg, Fe, Mn or Ni. Several studies have been conducted with Cu(II) complexes for the hydrolysis of phosphodiester bonds [44–49]. The hydrolysis of phosphodiester bonds and phosphate monoester bonds in model compounds such as bis(4-nitrophenyl) phosphate (BNPP) and 4-nitrophenyl phosphate (4-NPP) respectively constitute important reactions for understanding the reactivity of metalloenzymes and metal complexes with DNA. Metal complexes imitating hydroxylases could serve as possible chemotherapeutic anti cancer agents. The devel-

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opment of metal complexes that could function as metalloenzymes has therefore been the focus of several researchers [46–49,50–53].

The type of ligands attached to the metal center generally influences the reactivity of a metal-based chemotherapeutic drug. Varying the ligands could tune the reactivity of the complex towards DNA. Planar ligands with pi systems have been noted to be good intercalators [54–58]. The planarity of such ligands contributes to the intercalating mode of binding of the complex to DNA. Copper complexes have been shown to interact non-covalently with DNA if they contain planar aromatic ring ligands capable of inserting between the DNA base pairs. A significant example is the work done by Sigman and co-workers [59] that led to the discovery that the copper complex $[Cu(phen)_2]^+$ (phen = 1, 10-phenathroline) had the ability to intercalate DNA and cause cleavage. Several other phen and related ligands have been coordinated to copper and studied. A significant number of the complexes have been found to exhibit reactivity similar to nucleases [60–64]. Whereas the phen and related ligands have seen more frequent applications, other planar pi systems like quinoline have attracted less attention [65,66]. We are exploring various Schiff-base ligands containing the quinoline moiety to synthesize new copper complexes and study their DNA binding and cleavage capabilities. In this study we report the synthesis of two copper complexes containing the Schiff-base ligands derived from 8-aminoquinoline. The structures of the complexes have been determined by single crystal X-ray diffraction. The reactivity of the complexes towards calf thymus-DNA (CT-DNA) and plasmid DNA has been evaluated. In addition, kinetics experiments with BNPP and 4-NPP indicate that the complexes do hydrolyze the model compounds at reasonable rates.

2. Experimental section

2.1. Reagents and materials

All chemicals were purchased commercially and used as received. Copper(II) chloride, Copper(II) acetate, 8-aminoquinoline, 1-methyl-2-imidazolecarboxaldehyde, 2-hydroxy-1-napthaldehyde, ammonium hexafluorophosphate, ethidium bromide (EB), bis(4-nitrophenyl) phosphate (BNPP) sodium salt, 4-nitrophenyl phosphate disodium salt (4-NPP), calf-thymus DNA (CT-DNA), toluene, $NaH_2PO_4{\cdot}H_2O$ and NaCl were purchased from Sigma Aldrich. Dichloromethane and methanol were purchased from Pharmco-Aaper. Plasmid DNA pBR322 DNA was purchased from Thermo Scientific. The reagents were used without further purification.

2.2. Methods and instrumentation

^1H NMR and ^{13}C NMR were recorded on a Varian 400 MHz spectrometer. A Perkin Elmer Spectrum 100 FT-IR spectrometer was used for the IR spectra. UV–Vis spectra as well as the reactivity studies and absorption titration studies were recorded on the Shimadzu UV-1700 UV–Vis spectrophotometer. Ethidium bromide competitive binding studies were performed using the Jasco-FP 750 spectrofluorimeter. Cyclic voltammetry measurements were performed using a BAS CV-50W voltammetric analyzer. A three-electrode arrangement made up of a glassy carbon working electrode, a platinum wire auxiliary electrode and a Ag/AgCl reference electrode was used. The glassy carbon electrode was polished using alumina before each use. The cyclic voltammograms were recorded in methanol or dichloromethane with tetrabutylammonium hexafluorophosphate, (TBAHP), (0.10 M) as the supporting electrolyte. The concentrations of the complexes were 5.0 mM. Initial scans of the supporting electrolyte were made for the background check. The solutions were purged with N_2(g) for 2–3 min prior to each scan. Scan rate for each measurement was 100 mV/s. Plasmid DNA cleavage analyses were performed by gel electrophoresis using a Gel Doc-IT imaging System equipped with a Hamamatsu camera. Elemental analyses for C, H and N were performed by Galbraith Laboratories, Inc. Knoxville, TN. The X-ray crystal structure determinations and ESI-MS were performed by Dr. Fook S. Tham and Mr. Ron New respectively, of the Department of Chemistry, University of California, Riverside, CA.

2.3. Synthesis of ligands

2.3.1. (E)-N-((1-Methyl-1H-imidazol-2-yl)methylene)quinolin-8-amine, MICQ

This ligand was prepared by the reaction of 1-methyl-2-imidazolecarboxaldehyde (385 mg, 3.50 mmol) and 8-aminoquinoline (500 mg, 3.50 mmol) in 30 mL toluene. The mixture was refluxed overnight. The solution was then rotary evaporated to dryness forming a brown oily product. The oily substance was purified by column chromatography. The alumina column was first eluted with dichloromethane yielding a yellow fraction. The column was then eluted with a 1:1 v/v methanol/dichloromethane solution that resulted in a brown fraction. This fraction was then covered loosely with aluminum foil and left in the fume hood for the solvent to evaporate. A brownish-yellow oil was left. This product was then dissolved in 15 mL of CH_2Cl_2 and the solution was added drop-wise into 100 mL of hexane. A light brownish-yellow precipitate was collected by vacuum filtration. Yield: 308 mg (37.3%). Elemental analysis: Found (Calc.) for $C_{14}H_{12}N_4$, C, 72.31 (71.17); H, 5.37 (5.12); N, 22.01 (23.71). FTIR data (neat, v/cm^{-1}): 3164 (w), 3145 (w), 3036 (w), 1610 (s), 1573 (m), 1504 (s), 1472 (s), 1377 (s), 1334 (s), 1280 (m), 1108 (s), 1082 (m), 1040 (w), 816 (s), 780 (s), 748 (s). ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 4.0 (3H, s), 6.92 (1H, d, J = 7.4 Hz), 7.08 (1H, s), 7.15 (1H, d, J = 8.2 Hz), 7.25 (1H, s), 7.32 (1H, t, J = 7.6 Hz), 7.35 (1H, t, J = 4.1 Hz), 8.05 (1H, dd, J = 8.4, 1.8 Hz), 8.75 (1H, dd, J = 4.3, 1.6), 9.81 (1H, s). ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) 35.9, 110.0, 118.0, 121.5, 126.8, 127.3, 129.1, 131.0, 135.0, 135.6, 138.0, 147.4, 148.1, 182.0.

2.3.2. (E)-1-((Quinolin-8-ylimino)methyl)naphthalen-2-ol, (TL1)

2-Hydroxy-1-napthaldehyde (978 mg, 5.68 mmol) was added to a solution of 8-aminoquinoline (814 mg, 5.65 mmol) in methanol (30 mL). The mixture was refluxed for 4 h. After cooling, a yellow-orange plate like precipitate formed. The product was isolated by vacuum filtration and re-crystallized from methanol. Yield: 1.45 g (80.9%). Elemental analysis: Found (Calc.) for $C_{20}H_{14}N_2O$: C, 80.29 (80.51); H, 4.88 (4.73); N, 9.37 (9.39), FTIR data (neat, v/cm^{-1}): 3058 (w), 3040 (w), 1623 (s), 1609 (s), 1590 (s), 1533 (s), 1488 (m), 1472 (m), 1354 (s), 1299 (s), 1205(s), 1081 (m), 956 (m), 787 (s), 744 (s), 735 (s). ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 6.90 (1H, d, J = 9.6 Hz), 7.26 (1H, t, J = 8.0 Hz), 7.46 (1H, t, J = 8.4 Hz, 7.2 Hz), 7.47 (1H, d, J = 8.8 Hz), 7.51 (1H, t, J = 4.4 Hz), 7.57 (1H, d, J = 8.0), 7.63 (1H, d, J = 12.0 Hz), 7.66 (1H, t, J = 10.0 Hz, 9.6 Hz), 7.75 (1H, dd, J = 7.4 Hz, 1.4 Hz), 7.99 (1H, d, J = 7.6 Hz), 8.18 (1H, dd, J = 8.0 Hz, 1.6 Hz), 9.07 (1H, dd, J = 4.4 Hz, 1.6 Hz), 9.26 (1H, s) 9.28 (1H, s, absent upon D_2O addition), ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) 108.76, 113.06, 118.33, 122.30, 123.62, 124.28, 126.52, 126.63, 126.72, 128.34, 128.94, 129.49, 134.19, 135.90, 137.50, 139.39, 139.90, 146.10, 150.25, 181.83.

2.4. Synthesis of [Cu(MICQ)Cl](PF$_6$) (**1**)

The ligand MICQ, (200 mg, 0.856 mmol) was dissolved in 25 mL of methanol. Copper(II) chloride (144 mg, 0.856 mmol) was dissolved in 25 mL methanol in a different flask. The copper chloride solution was then added drop-wise to the ligand solution. The mixture immediately turned a dark green color. The mixture