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Cu(II), Ni(II), Zn(II) and Fe(III) complexes containing a N_2O_2 donor ligand: Synthesis, characterization, DNA cleavage studies and crystal structure of [Cu(HL)Cl]

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ABSTRACT

A polydentate ligand, **H_2L** "[1-(5-isopropyl-2-methyl phenoxy)-3-(N-2-hydroxy benzyl-N-((pyridine-2-yl)amino) propan-2-ol]", containing a N_2O_2 donor moiety was synthesized by refluxing 2-((5-isopropyl-2-methylphenoxy)methyl)oxirane and HBPA (N-(2-hydroxybenzyl)-N-(2-pyridylmethyl)amine). This synthesized ligand was used for the preparation of complexes with different metal ions, viz. [Cu(HL)Cl] (**1**), [Ni(HL)Cl] (**2**), [Zn(HL)Cl] (**3**) and [Fe(HL)Cl_2] (**4**). The ligand and metal complexes were characterized by ^{1}H NMR, mass, ESI-MS, elemental analysis, IR, UV–Vis and electron paramagnetic resonance (EPR) spectroscopy. The crystal structure for one of the complexes, [Cu(HL)Cl], was solved from the X-ray crystallography data. The structure of the complex, based on the trigonality index tau, suggests an intermediate geometry between square pyramidal (sp) and trigonal bipyramidal (tb). Both the ligand and the metal complexes show oxidative cleavage of plasmid DNA (pBR322) without addition of any exogenous agent, even at a concentration of 5 μM. The binding constants for these compounds were found to be in the range 5.33–0.065 $\times$ 10^5 M^{-1}.

1. Introduction

Earlier, transition metal complexes were thought to be an odd choice for studying DNA interactions, however over the last few years they have become attractive agents for nuclease activity as they possess different structures and reactivities. With few exceptions, biological transition metals are confined to coordination sites in proteins or cofactors, and are not in discrete, freestanding coordination complexes [1,2].

Molecules possessing the ability to bind and cleave double stranded DNA under physiological conditions are explored as diagnostic agents in medicinal applications and for the development of genomic research [3–24]. Generally DNA cleavage follows an oxidative or hydrolytic cleavage pathway. The hydrolytic pathway proceeds through hydrolysis of the phosphodiester bond, leading to fragmentation of DNA, and such pathways are mediated by enzymatic processes, while the oxidative process involves the oxidation of nucleobases and/or H abstraction from the sugar moiety [25].

Metal-based synthetic nucleases are of interests for their varied applications in nucleic acid chemistry, including the design of DNA and RNA specific agents capable of controlled cleavage. They are important because of their potential use as chemotherapeutic drugs, gene regulators and molecular biological tools [26,27]. Although redox active transition metal complexes in the presence of oxidants have been extensively used for DNA cleavage reactions, the complexes [Fe (EDTA)]$^{2-}$ [28] (EDTA = ethylene diaminetetraacetic acid), [Cu(phen)$_2$]$^+$ [29] (phen = 1,10-phenanthroline), [Fe-BLM] [30] (BLM = bleomycin), metalloporphyrins [31], Ni-azamacrocycles [32], [Mn(salen)]$^{3+}$ [33] (salen = N,N′-ethylenebis(salicylaldeneaminato), [Cu-desferal] [34], [Co-cyclam] [35], [Rh(phen)$_2$(phi)]$^{3+}$ and [Rh(en)$_2$(phi)]$^{3+}$ [36] (en = N,N′-ethylenediamine; phi = 9,10-phenanthrenequinone diimine), possessing diverse structures and nucleotidal reactivity, have also been reported as prospective candidates. However, the initiation of cleavage in most cases requires exogenous agents such as H_2O_2, mercaptopropionic acid, dithiothreitol or light. This fact limits their in vitro applications; hence DNA cleaving agents functioning without any activation are desirable. Lamour et al. [37], Sissi et al. [38] and Tonde et al. [39] have reported polyhydroxy copper based systems that trigger self activating nuclease activity.

Considering the very sensitive nature of DNA towards oxidative cleavage, efforts have been directed towards the development of molecules capable of cleaving DNA with an oxidative mechanism [40]. Numerous efficient cleaving agents such as reactive oxygen species (ROS) or free radicals capable of inducing an oxidative pathway have been developed over the course of time. The antitumor, antibiotic, drug leinamycin and its analogs have exhibited the crucial role of "Chemical Nucleases" through reduction of molecular oxygen to form reactive hydroxyl species [41,42].

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Thus, in the present work, we report the DNA cleavage activity of novel asymmetric polydentate ligand and transition metal complexes prepared with this ligand.

2. Materials and methods

All reagents and chemicals were purchased from commercial sources and were used without further purification. N-(2-hydroxybenzyl)-N-(2-pyridylmethyl)amine (HBPA) was synthesized by the reported method [43]. Plasmid DNA (pBR322) and calf thymus (Genei, Bangalore, India), superoxide dismutase (SOD), (Sigma, stored at −20 °C), agarose and boric acid (Molecular Biology grade, Sisco Research Laboratories, India), tris(hydroxymethyl)aminomethane (TRIS, AR Grade) and ethylene diaminetetraacetic acid (EDTA, AR Grade, Sisco Research Laboratories, India) were used as received. Ultrapure MilliQ water was used for all the experiments.

3. Experimental

The synthesis of the ligand involves various steps as shown in Scheme 1.

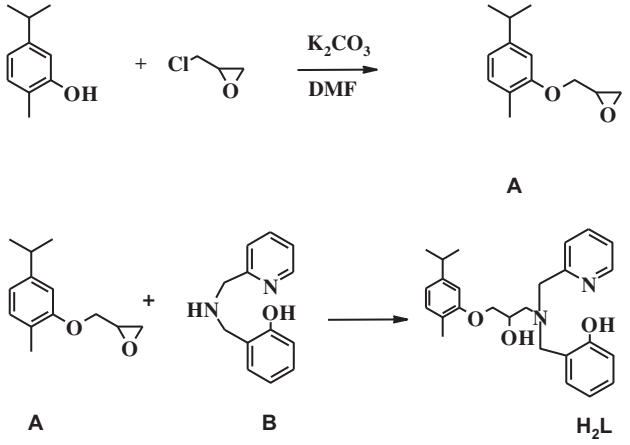
3.1. Synthesis of 2-((5-isopropyl-2-methylphenoxy)methyl)oxirane (*A*)

2-((5-Isopropyl-2-methylphenoxy)methyl)oxirane was synthesized by the reaction of 5-isopropyl-2-methyl phenol (6.6 mmol, 1.00 g) with epichlorohydrin in excess (13.2 mmol, 1.84 g) in the presence of K_2CO_3 (13.3 mmol, 1.83 g) in 20 ml DMF (N,N-dimethylformamide) at 70 °C, stirring for 8 h. The resulting product was dissolved in water and extracted with three 20 ml portions of hexane. The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to give a brown oil.

Yield: 80%; B.P.: 120 °C; ^{1}H NMR (ppm, $CDCl_3$): 1.23 (d, 6H, $2CH_3$), 2.20 (s, 3H, Ar-CH_3), 2.85 (m, 1H, oxirane-CH), 3.38 (m, 1H, Ar-CH), 3.95 (d, 2H, oxirane-CH_2), 4.21 (d, 1H, –CH_2), 6.65 (s, 1H, ArH), 6.8 (d, 1H, ArH), 7.1 (d, 1H, ArH). IR/cm^{-1}: ν(C–O) 1255; ν(C=C) 1416, 1511, 1612 (Supplementary Figs. S3 and S6).

3.2. Synthesis of N-(2-hydroxybenzyl)-N-(2-pyridylmethyl)amine (HBPA) (*B*)

N-(2-hydroxybenzyl)-N-(2-pyridylmethyl)amine (HBPA) was synthesized according to the previously reported procedure [43].



Scheme 1. Schematic representation of the synthesis of the ligand **H_2L**.

3.3. Synthesis of 1-(5-isopropyl-2-methyl phenoxy)-3-(N-2-hydroxy benzyl-N-((pyridine-2-yl)amino)propan-2-ol (*H_2L*)

This compound was synthesized by reacting compound **A** (5.8 mmol, 1.20 g) with **B** (5.8 mmol, 1.23 g) in methanol under reflux at 70 °C for 8 h. The reaction mixture was cooled, filtered and the precipitated product was washed with cold methanol to remove the impurities.

Yield: 66%, M.P.: 134 °C; ^{1}H NMR (ppm, $CDCl_3$): 1.20 (d, 6H, 2-CH_3), 2.08 (s, 3H, –CH_3), 2.84 (d, 2H, –CH_2), 3.10 (m, 1H, –CH), 3.90 (m, 4H, 2-CH_2), 4.10 (d, 2H, –CH_2), 4.25 (m, 1H, –CH), 4.58 (bs, 1H, Ar-OH), 6.7–7.7 (Ar-H). *Anal.* Calc. for $C_{26}H_{32}O_3N_2$: C, 74.44; H, 7.44; N, 7.05. Found: C, 74.54; H, 7.37; N, 6.67%. IR (cm^{-1}): ν(C=C) 1586, ν(C–O–C) 1151. MS (*m*/*z*): 421 [M]$^+$ (Supplementary Figs. S3, S1, S6, and S4).

3.4. Synthesis of the complexes

3.4.1. Synthesis of [Cu(HL)Cl] (*1*)

A 10 ml methanolic solution of **H_2L** (2 mmol, 0.80 g) and a methanolic solution of $CuCl_2{\cdot}2H_2O$ (2 mmol, 0.32 g) were mixed and refluxed for 5–8 h. Triethylamine was added in a catalytic amount for acceleration of the reaction. After cooling the solution to room temperature, a stable green microcrystalline precipitate was formed, which was filtered off and washed with water, ethanol and then ether. Single crystals, suitable for X-ray crystallography, were obtained from methanol/DCM. Yield: 60%; *Anal.* Calc. for Cu-$C_{26}H_{31}O_3N_2Cl$: C, 60.22; H, 6.02; N, 5.40. Found: C, 58.91; H, 5.92; N, 5.42%. ESI-MS *m*/*z*, ion 482.2 [M]$^+$ (Supplementary Figs. S1 and S4).

3.4.2. Synthesis of [Ni(HL)Cl] (*2*), [Zn(HL)Cl] (*3*) and [Fe(HL)Cl_2] (*4*)

Complexes **2**, **3** and **4** were prepared by a similar procedure as for complex **1**, using $NiCl_2{\cdot}6H_2O$, $ZnCl_2$ and $FeCl_3$ instead of $CuCl_2{\cdot}2H_2O$. Complex **2**, (light green): yield, 70%; *Anal.* Calc. for Ni-$C_{26}H_{31}O_3N_2Cl$: C, 60.79; H, 6.07; N, 5.45. Found: C, 60.40; H, 5.99; N, 6.79%. ESI-MS *m*/*z*, ion 477.2 [M]$^+$. Complex **3**, (white): yield, 62%. *Anal.* Calc. for $ZnC_{26}H_{31}O_3N_2Cl$: C, 60.00; H, 6.00; N, 5.38. Found: C, 58.62; H, 5.94; N, 6.56%. ESI-MS *m*/*z*, ion 483.2 [M]$^+$. Complex **4**, (blue): yield: 78%. *Anal.* Calc. for $FeC_{26}H_{31}O_3N_2Cl_2$: C, 57.13; H, 5.53; N, 5.12. Found: C, 58.67; H, 6.01; N, 6.73%. ESI-MS *m*/*z*, ion 510.2 [M]$^+$ (Supplementary Figs. S1 and S4).

3.5. Physical measurements

Elemental analyses (C, H and N) were recorded on a Thermo-Finnigan elemental analyzer. ^{1}H NMR spectra were recorded on a Varian-300 spectrometer. All chemical shifts are relative to tetramethylsilane (TMS). UV–Vis spectra were recorded on a Chemito spectrophotometer (Supplementary Fig. S2). The *X*-band frozen glass EPR spectra of the Cu complex in dimethyl formamide solutions were recorded on a Varian E-109 spectrometer using tetracyanoethylene (TCNE) as a standard.

3.6. X-Ray crystallography

A green crystal of **1** having the size 0.27 × 0.12 × 0.09 mm was obtained by slow evaporation of the reaction mixture and it was mounted on the tip of a glass fiber. Intensity data were collected on a Bruker SMART APEX diffractometer equipped with a CCD area detector using Mo Kα (λ = 0.71073 Å) radiation at 293 K. The data integration and reduction were processed with SAINT [44] software. An empirical absorption correction was applied with SADABS [45]. The structure was solved by direct methods using SHELXS [46] and refined on F^2 by the full-matrix least-squares technique using the