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Feature article

Chiral anionic binuclear zinc complexes based on diaminocyclohexane ligand and their in vitro antiproliferative studies

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ABSTRACT

Two novel binuclear chiral anionic Zn(II) complexes, $[Zn_2LCl_3]^-$ $(C_2H_5)_3N^+$ 1 and $[Zn_2L(CH_3COO)_3]^-$ $(C_2H_5)_3N^+$ 2 counterbalanced by triethyl ammonium cation, have been synthesized from ligand, 2,2′-((1*E*,1′*E*)-((1R,2R)-cyclohexane-1,2-diylbis(azanylylidene))bis(methanylylidene))dipheno, H_2L. The ligand, H_2L and its complexes are characterized by elemental analyses, IR, ^1H and ^{13}C NMR, ESI-MS, electronic and thermal studies. Complex **1** has been additionally characterized by single crystal X-ray diffraction studies and confirmed a slightly distorted tetragonal pyramid coordination environment in which both zinc atoms are five coordinated by two imine nitrogen atoms, two oxygen atoms and one chloride ion located at the polyhedron apex. Ligand, H_2L and its Zn(II) complexes have been subjected to antiproliferative studies against HCT p53 wild type as well as HCT p53 null cell lines, and the results suggest complexes to be an effective antiproliferative agent against these cancer cell lines in comparison to ligand, H_2L. Furthermore, DNA fragmentation studies revealed that Zn(II) complexes induce significant p53 independent apoptosis in cancer cell lines.

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1. Introduction

The clinical success of cisplatin and related platinum based anticancer drugs encouraged the researchers to discover more selective and less toxic metallopharmaceuticals [1,2]. The important problems associated with the use of cisplatin in curative therapy are the severe toxic side effects viz., nephrotoxicity, neurotoxicity, ototoxicity, low water solubility, instinct, and acquired resistance manifested in various types of cancers [1–3]. To date, a large number of metal-based chemotherapeutic agents have been developed to treat or cure a variety of cancers but most of them demonstrate restricted efficacy due to the problem of delivery and penetration, and low selectivity for the tumor cells causing severe damage to healthy tissues compared with other transition metals [1,2]. Therefore, researchers have diverted their attention towards the synthesis of molecularly-targeted rationally designed metal-based drugs to minimize unwanted side effects as well as to improve tumor selectivity while retaining the desirable therapeutic effectiveness [4]. Among transition metals, zinc is the most prominent trace metal in human body after iron and plays an important role in various biological processes, and act as a major regulatory ion in the metabolism of cells in human body [1–3,5,6]. A search of literature reveals that zinc is cytoprotective and suppresses apoptotic pathways, and has shown a significant efficacy in the prevention of colon and thyroid cancer through induction of cell cycle arrest and apoptosis [1,2,7–10]. In recent years, zinc-containing compounds are regarded as one of the most promising alternatives to cisplatin as anticancer drugs. Magda et al. have synthesized several water-soluble complexes of zinc ionophore 1-hydroxypyridine-2-thione (ZnHPt), and propose them to be a potential new class of anticancer agents [11]. In order to widen the scope of investigations on new biologically active pharmaceuticals of zinc, we hereby design the synthesis of some novel chiral binuclear anionic zinc complexes derived from chiral salen ligand, obtained by the condensation of o-hydroxybenzaldehyde and (*1S,2S*)-(+)-1,2-diaminocyclohexane. Literature reveals that chiral salen-type ligands and their complexes have been studied extensively because of their wide applications in various areas of research viz., medical sciences, non-linear optical materials, asymmetric catalysis and magnetic materials [7,12]. The synthesized and isolated salen ligand, H_2L and its Zn(II) complexes have been characterized by elemental analyses and various spectroscopic studies viz., UV–Vis, IR, NMR, ESI-MS spectrometry and thermal investigation. Complex **1** has been additionally characterized by single crystal X-ray diffraction measurement. Moreover, anti-proliferative effects of the synthesized compounds have been studied on HCT p53 wild type as well as HCT p53 null cell lines. The results revealed that the compounds induce significant apoptosis in p53 independent manner in cancer cells, which is further confirmed by DNA fragmentation.

2. Experimental

The starting materials, (*1S,2S*)-(+)-1,2-diaminocyclohexane, zinc chloride, zinc acetate and o-hydroxybenzaldehyde were purchased from Aldrich. All other reagents and solvents were of high purity and used as purchased without any further purification.

2.1. Physical measurements

C, H, and N elemental analyses were recorded on Elementar Varrio EL analyzer. FT-IR (4000–400 cm^{-1}) spectra were obtained as a KBr pellet using Perkin Elmer 621 spectrophotometer. ^1H and ^{13}C NMR spectra of ligand and its Zn(II) complexes were recorded in $CDCl_3$ using JEOL 400 spectrometer. Mass spectrometry was performed using Agilent technologies ion trap LC/MS 6320 mass spectrometer with electrospray positive ionization mode. Electronic spectra of the complexes were obtained in methanol on Pharmacia LKB-Biochem, UV/Vis spectrophotometer at room temperature. Thermal behavior of the synthesized compounds was studied by using SDTQ-600 (TA Instrument) in helium atmosphere (100 mL min^{-1}) at heating rate of 20 °C/min at temperature 20–800 °C.

2.2. Synthesis of salen ligand: H_2L

The salen ligand was prepared according the methods reported in the literature [13]. A methanolic solution of 2-hydroxybenzaldehyde (2 mmol) was added dropwise to the methanolic solution of (*1S,2S*)-(+)-1,2-diaminocyclohexane (1 mmol). The reaction mixture was stirred for 5 h resulting into a yellow colored solution, which was concentrated to 1 ml followed by the addition of 20 ml of diethyl ether to cause precipitation. The precipitate was removed by filtration, and the filtrate was evaporated to get analytically pure compound.

Yield 78%, Color: Yellow, Mp. 120 °C; Molecular formula $C_{20}H_{22}N_2O_2$; Anal Calc. C, 74.50; H, 6.88; N, 8.69% Found: C, 74.45; H, 6.82; N, 8.65% ^{1}HNMR ($CDCl_3$): δ (ppm) 13.32 (Ar–O**H**), 8.25 (s –C**H**═N), 6.78–7.24 (m Ar–**H**), 3.29 (**H**(a) N–CH–CH–N), 3.32 (H(a) N–CH–CH–N), 195–1.88 (m, –C**H$_2$**–CHN–CHN–C**H$_2$**–), 1.87–1.46 (m, –CH_2–C**H$_2$**–C**H$_2$**–CH_2–), ^{13}CNMR ($CDCl_3$): δ (ppm) 161.0 (–**C**H═N), 164.7 (–**C**–OH), 132.2–116.8 (Ar–**C**), 72.6 (N–**C**H–**C**H–N), 33.1 (–**C**H_2–CHN–CHN–**C**H_2–), 24.2 (–CH_2–**C**H_2–**C**H_2–CH_2–), ESI-MS (M + Na)$^+$ (m/z), 323.2, IR, 2990 cm^{-1} $\nu_{(Ar-OH)}$, 1690 cm^{-1} $\upsilon_{(CH=N)}$, 1185 $\upsilon_{(ArC-O)}$.

2.3. Synthesis of complex, **1** $[Zn_2LCl_3]^-$ $(C_2H_5)_3N^+$

A solution of zinc chloride (2 mmol) in methanol was added dropwise into 10 ml methanol solution of ligand (1 mmol). The resultant reaction mixture was stirred for 5 h followed by addition of triethylamine (1 mmol) with constant stirring. The resulting solution was refluxed for 2 h and then left for evaporation at room temperature. After few days, yellow colored crystals suitable for single crystal X-ray diffraction were separated out.

Yield 75%, Color: Yellow, Mp. 215 °C; Molecular formula $C_{26}H_{36}N_3O_2Cl_3Zn_2$; Anal Calc. C, 47.33; H, 5.49; N, 6.37% Found: C, 47.31; H, 5.45; N, 6.33%, ^{1}HNMR ($CDCl_3$): δ (ppm) 8.45 (s –CH═N), 6.79–7.25 (m Ar–H), 3.42 (**H**(a) N–CH–CH–N), 3.43 (**H**(a) N–CH–CH–N), 197–1.92 (m, –C**H$_2$**–CHN –CHN–C**H$_2$**–), 1.90–1.49 (m, –CH_2–C**H$_2$**–C**H$_2$**–CH_2–), ^{13}CNMR ($CDCl_3$): δ (ppm) 164.6 (–**C**H═N), 167.5(–**C**–OH), 142.8–121.3 (Ar–**C**), 74.4 (N–**C**H–**C**H–N), 35.3 (–**C**H_2–CHN–CHN–**C**H_2–), 25.1 (–CH_2–**C**H_2–**C**H_2–CH_2–), ESI-MS (M + Na)$^+$ (m/z), 659.7, IR, 1620 cm^{-1} $\upsilon_{(CH=N)}$, 1195 $\upsilon_{(ArC-O)}$, 570 cm^{-1} $\upsilon_{(Zn-N)}$.

2.4. Synthesis of complex, **2** $[Zn_2L(CH_3COO)_3]^-$ $(C_2H_5)_3N^+$

A methanol solution of $Zn(OAc)_2\cdot 2H_2O$ (2 mmol) was added dropwise into 10 ml methanol solution of ligand (1 mmol). The resultant reaction mixture was stirred for 5 h followed by addition of triethylamine (1 mmol) with constant stirring. The resulting solution was heated under refluxing conditions for 2 h and then evaporated at