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The anti-tumor activity of novel oxovanadium complexes derived from thiosemicarbazones and fluoro-phenanthroline derivatives

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ABSTRACT

Three oxovanadium complexes incorporating thiosemicarbazones and fluoro-phenanthroline derivatives [VO(hntdtsc)(CF_3PIP)] (**1**) (hntdtsc = 2-hydroxyl-1-naphthaldehyde thiosemicarbazone, CF_3PIP = 2-(2-trifluoromethyl phenyl)imidazole[4,5-f][1,10]phenanthroline [VO(hntdtsc)(*m*-CF_3PIP)] (**2**) (*m*-CF_3PIP = 2-(3-trifluoromethyl phenyl)imidazole[4,5-f][1,10]phenanthroline), [VO(hntdtsc)(*p*-CF_3PIP)] (**3**) (*p*-CF_3PIP = 2-(4-trifluoromethyl phenyl)imidazole[4,5-f][1,10]phenanthroline) were newly synthesized and characterized by elemental analysis and spectroscopic techniques. Their interactions with calf-thymus DNA (CT-DNA) and photocleavage properties with plasmid pBR322 DNA were investigated by a host of analytical methods. The results suggest that these three complexes interact with CT-DNA through an non-classical intercalative mode and can efficiently cleavage plasmid pBR322 DNA upon exposure to ultraviolet light. In addition, they all exhibited considerable anti-proliferative activity *in vitro* against human Hela, CaSki, SiHa, ECa9706, ECa109, MDA-MB-231 and MCF-7 tumor cell lines, to have an IC_{50} values for cytotoxicity in the low micromole range 0.31–6.15 μM, which is very close to that of cisplatin (0.52–2.49 μM). Furthermore, their antitumor mechanism has been analyzed by the cell cycle arrest, and apoptosis analysis. The results showed that complexes **2** and **3** caused G0/G1 phase arrest in ECa9706 cells, but differentiatedly induced G0/G1 and S phase arrest in ECa109 cells. And significant apoptosis were observed in both the two tumor cell lines, which indicate these oxovanadium complexes induce proliferative suppression of ECa9706 and ECa109 cells via the induction of apoptosis.

1. Introduction

Since cisplatin has been discovered to be one of most widely prescribed as well as a first and effective treatment for many cancer diagnoses, especially for testicular, pvarian, bladder, lung, and stomach cancers [1–3]. For decades of years, more and more biochemists and pharmacologists have paid their attention to a large diversity of some transition metal complexes bearing various kinds of ligands such as ruthenium(II), nickel(II), cobalt(III), copper(II) and zinc(II) due to their wide range of biological and physiological activities [4–8]. Wherein, vanadium compounds are described for participating in some biogenic vanadium systems such as vanadium-ionophore interaction, vanadate-dependent haloperoxidases, vanadium-nitrogenase, vanadium-thiolate redox interaction, and alkyne-reductase as well as isonitrile-reductase/ligase activities of nitrogenases [9–13]. In recent years, some researchers have been focused on interaction of vanadium compounds with DNA [14–16]. DNA is generally regarded as the primary intracellular target of anticancer agents and hence the interaction between vanadium complexes with DNA can cause DNA damage in cancer cells, blocking the division of cancer cells, and resulting in cell death, which is related to the antitumor activity [17,18]. In this

Abbreviations: CT-DNA, calf thymus DNA; DMF, N, N-dimethylformamide; DMSO, dimethyl sulfoxide; EDTA, N, N′-1,2-ethanediylbis[N-(carboxymethyl)] glycine; ESI-MS, electrospray ionization mass spectra; IC_{50}, 50% inhibition concentrations; MTT, 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide; PBS, phosphate-buffered saline; PI, propidium iodide; Tm, DNA melting temperature where half base pairs are unbound; Tris, tris(hydroxymethyl)aminomethane.

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regard, some oxovanadium complexes containing different types of ligands showing considerable antiproliferative activities against various kinds of cancer cell lines [19,20].

In addition, there are large amounts of reports on transition metal complexes bearing Schiff base ligands exhibiting some fantastic pharmacological effects especially its application as potential clinical therapeutics agents towards cancer [21,22]. On one hand, thiosemicarbazones were usually acted as primary ligands for many metals owing to its general physiological and pharmacological activities including antibacterial, antifungal, antitumor, and antiviral activities [23–25]. On the other hand, some oxovanadium complexes containing thiosemicarbazones were capable to demonstrate both *in vitro* antibacterial and antiproliferative properties [26,27] as well as intense interaction with DNA, thus causing DNA damage in cancer cells, which leading it as new potential DNA-targeted antitumor drugs [28,29]. Moreover, 1,10-phenanthroline derivatives were often served as auxiliary ligands due to their idiosyncratic capacity of coordinating with metal ions by N-alkyl and N-benzyl group as well as a wide variety of flexible applications such as in fields of photovoltaic devices and solar fuels, selective *Typanosoma cruzi* growth inhibitors, the treatment of cancer diseases [30–32], and sequence-selective DNA-binding and cleavage agents for DNA itself [16,27,33].

In our previous work, we have investigated the DNA-binding affinities of three oxovanadium complexes, [VO(hntdtsc)(HPIP)], [VO(hntdtsc)(*m*-HPIP)] and [VO(hntdtsc)(*p*-HPIP)] towards calf-thymus DNA(CT-DNA) as well as their antitumor activities against human cervical cancer Hela, CaSki and SiHa, leukemia ECa9706 and ECa109, esophagus carcinoma MDA-MB-231, and MCF-7 cell lines *in vitro*. They exhibited intense interaction with CT-DNA through a non-classical intercalation mode, and exerted a certain antiproliferative and cytotoxic activities against selected cultured tumor cells [34]. Interestingly, previously we reported several oxovanadium complexes incorporating Schiff base substituted with electro-withdrawing group (–Cl) presented stronger DNA-binding affinities and higher cytotoxic activities than that of substituted with electron-donating group (–$N(Et)_2$) [16]. It occurs to us that the introduction of electro-withdrawing group in ligands probably enhance DNA-binding capacity and antitumor properties.

Thus, in the present work, we have designed and synthesized three oxovanadium complexes incorporating thiosemicarbazones and fluoro-phenanthroline derivatives [VO(hntdtsc)(CF_3PIP)] (**1**) (hntdtsc = 2-hydroxyl-1-naphthaldehyde thiosemicarbazone, CF_3-PIP = 2-(2-trifluoromethyl phenyl)imidazolen[1,10]phenanthroline [VO(hntdtsc)(*m*-CF_3PIP)] (**2**) (*m*-CF_3PIP = 2-(3-trifluoromethyl phenyl)imidazole[4,5-f][1,10]phenanthroline), [VO(hntdtsc)(*p*-CF_3-PIP)] (**3**) (*p*-CF_3PIP = 2-(4-trifluoromethyl phenyl)imidazole[4,5-f] [1,10]phenanthroline) (see Scheme 1), and characterized by elemental analysis, IR, ESI-MS, ^{1}H NMR and ^{13}C NMR. Their interactions with calf-thymus DNA (CT-DNA) were studied by UV–Vis absorption titration, fluorescence spectra, viscosity measurements and thermal denaturation. The photocleavage properties with plasmid pBR322 DNA were investigated and *in vitro* cytotoxicity against human Hela, CaSki, SiHa, ECa9706, ECa109, MDA-MB-231 and MCF-7 tumor cell lines were evaluated by MTT assay. In addition, their antitumor mechanism has been analyzed by cell cycle arrest, apoptosis, and Annexin V-FITC/PI assay.

2. Materials and methods

2.1. Materials

All reagents and solvents used in the synthesis and physical measurements were commercially available and without further purification unless otherwise noted. $VO(acac)_2$ (acac = acetylacetonate) and 1,10-phenanthroline were obtained from Alfa Aesar (Beijing, China). DMSO and $CHCl_3$ were purchased from Shanghai Jingchun Company (Shanghai, China). Cisplatin, CT-DNA and pBR322 DNA were purchased from Aldrich (USA). Ethidium bromide (EB), agarose, thiosemicarbazide, salicylaldehyde and 2-Hydroxy-1-naphthaldehyde were purchased from Sigma (USA). Tris buffer A (Tris = tris(hydroxyl-methyl) aminomethane) containing 5 mM Tris–HCl and 50 mM NaCl (pH = 7.2) was used for absorption titration and viscosity measurements. Tris buffer B containing 50 mM Tris–HCl and 18 mM NaCl (pH = 7.2) was used for the gel electrophoresis experiments. Buffer C containing 1.5 mM Na_2HPO_4, 0.5 mM NaH_2PO_4 and 0.25 mM Na_2H_2EDTA (H_4EDTA = N,N′-ethane-1,2-diylbis[N-(carboxymethyl) glycine]) (pH = 7.0) was used for thermal denaturation. A solution of CT-DNA in buffer A gave a ratio of UV absorbance at 260 and 280 nm of 1.8–1.9:1, indicating that the DNA was sufficiently free of protein [35]. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm [36]. RPMI 1640 medium was purchased from Hyclone (Logan, USA) for the tumor cells culture. Trypsin, fetal calf serum and Annexin V-FITC/PI apoptosis detection Kit were purchased from GIBCO Company (USA). Hoechst 33342 staining solution was purchased from Beyotime Institute of Biotechnology (China). MTT (3-(4,5-dimethylthiazoyl-2-yl)2, 5-diphenyltetrazoliumbromide) and Rhodamine 123 (2-(6-Amino-3-imino-3H-xanthen-9-yl)benzoic acid methyl ester) were purchased from Sigma Company (USA). Double distilled water was used for preparing various buffers, and solutions of compounds were freshly prepared 2 h prior to biochemical evaluation.

2.2. Physical measurements

Microanalysis (C, H, and N) was carried out with a PerkinElmer 240Q elemental analyzer. UV–Visible spectra were recorded on a Shimadzu UV-3101 PC spectrophotometer at room temperature. Fluorescence measurements were performed on a Perkin–Elmer Lambda 55 spectrofluorophotometer. Infrared spectra (IR) were recorded on KBr disks a on a Bomem FTIR model MB102 instrument. Electrospray Ionization Mass Spectra (ESI-MS) were recorded on an LCQ system (Finnigan MAT, USA) using methanol as mobile phase. ^{1}H and ^{13}C NMR spectra were recorded on a Varian-500 spectrometer. All chemical shifts are given relative to tetramethylsilane (TMS). Molar conductivities in DMF (10^{-3} M) solution at room temperature were measured using a DDS-307 digital direct reading conductivity meter.

2.3. Synthesis and characterization

2.3.1. Synthesis of phenanthroline-based ligands: CF_3PIP, m-CF_3PIP and p-CF_3PIP

CF_3PIP was synthesized through a modification of a previously reported procedure [37]. A mixture of 2-(Trifluoromethyl)benzaldehyde (0.69 mL, 5 mmol) and ammonium acetate (7.70 g, 0.1 mol) was added into a stirring solution of 1,10-phenanthroline-5,6-dione (0.99 g, 5 mmol) in 60 mL of glacial acetic acid, and the mixture was continuously stirred at 60 °C for 6 h. Then the deep red solution was cooled to room temperature and diluted with 100 mL distilled water. A grayish yellow precipitate was obtained by neutralization with ammonium hydroxide. Then the mixture was filtered and washed with water for three times. The crude solid power was purified by chromatography over 60–80 mesh SiO_2 using absolute ethanol as eluent. The solvent was removed and the products were collected, and dried in vacuo.