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Terpyridyl oxovanadium(IV) complexes for DNA crosslinking and mitochondria-targeted photocytotoxicity

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ABSTRACT

Oxovanadium(IV) complexes $[VO(L^1/L^2)Cl_2]^{n+}$ (**1**,**2**) of (anthracenyl)terpyridine (An-tpy as L^1 in **1**, n = 0) and triphenylphosphonium-appended (anthracenyl)terpyridine (An-tpy-TPP^+ as L^2 in **2**, n = 1) were synthesized, characterized and their DNA crosslinking ability, photocytotoxicity in visible light and cellular localization in cancer cells studied. The bromide derivative of **2**, viz. $[VO(An\text{-}tpy\text{-}TPP)Br_2]Br$ (**3**) is structurally characterized. The structure showed *trans* disposition of two halides in the coordination sphere and the TPP^+ unit is a pendant to the terpyridyl ligand. The DNA melting and comet assay studies on the complexes suggest the formation of DNA crosslinks. Complexes **1** and **2** displayed ~10 fold increase in cytotoxicity on exposure to visible light (400–700 nm) when compared to those in dark in HeLa and MCF-7 cells. FACScan (Fluorescence Associated Cell Sorter Scan) analysis showed cellular apoptosis when treated with the complex in visible light in comparison to their dark controls. Fluorescence microscopic studies using complex **2** revealed its mitochondrial localization within the cancer cells.

1. Introduction

Cisplatin and its analogues, viz. carboplatin and oxaliplatin, are used as chemotherapeutic drugs for a variety of cancers [1–7]. These drugs act as DNA crosslinking agents targeting the nuclear DNA with the loss of two chlorides or the O,O-donor chelating ligand. Such drugs, however, suffer from drawbacks with dose limiting side effects associated with the nuclear excision repair (NER) mechanism and their poor selectivity over normal cells. To augment the efficacy of the drug, Sadler et al. have reported platinum(IV) complexes that could be light activated to generate active platinum(II) species showing activity even against cis-platin resistant cell lines [8–10]. The light activated *trans* diammine platinum(IV) complexes are also reported to show cytotoxic properties [11]. Brabec et al. have shown induction of DNA crosslinks via light activated trans-platin, a molecule which otherwise does not show any apparent activity in the dark [12]. The advantage of inducing DNA crosslinks formation upon light-exposure is based on the facts that (i) cancer cells are generally hypoxic in nature whereas traditional photodynamic therapy (PDT) uses photosensitizers that are dependent on oxygen to kill cancer cells by generation of reactive oxygen species (ROS) and (ii) light activation of the prodrug can be achieved selectively at the cancer site without damaging the unexposed cells thus rendering selectivity [13–18]. Light activated induction of DNA crosslinks dates back to psoralens which require UV-A light for activation [19,20]. Considering the harmful effects of UV-A light, compounds inducing DNA crosslink(s) formation in visible light are viable alternatives to currently available drug candidates [21–25].

We have earlier reported oxovanadium(IV) complexes which induce formation of DNA crosslinks upon visible light exposure [23–25]. The choice of vanadium as the metal is based on its profound biochemistry [26] which includes the following factors: (i) firstly, the similarities between the phosphate and vanadate(V) have a broad impact towards understanding the biochemistry of vanadium [27], (ii) vanadium is a bio-essential metal with V(V)-V(III) species which are biologically relevant and are found in naturally occurring metalloenzymes, (iii) vanadium is found to interact with blood, DNA and RNA binding proteins [28], (iv) decavanadate is known to promote mitochondrial superoxide anion production and it is also known that vanadium selectively accumulates in the mitochondria when decavadate is administered [29,30], (v) vanadium is known to be used for the treatment of diabetes and cancer [31], (vi) vanadium(IV) complexes display a low energy absorption band in the spectral range of 630–800 nm which enables

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such complexes to undergo photo-redox processes to generate reactive oxygen species (ROS), (vii) presence of V(IV)-V(III) redox couple at a significantly negative potential (vs. saturated calomel electrode) reduces the dark cellular toxicity and (viii) the presence of vanadyl (V = O) moiety facilitates dissociation of the *trans* ligand thus enabling facile DNA crosslink formation. Such ligand dissociation process could be facilitated for DNA crosslinks formation upon visible light irradiation with elimination of two labile ligands. In addition to that, the complexes can be suitably designed to specifically deliver them into mitochondria instead of targeting nuclear DNA to circumvent the nuclear excision repair (NER) mechanism. This can be achieved by appending an alkyltriphenylphosphonium cation on the ligand which is known to pass easily through the lipid bilayers by non-carrier-mediated transport and are taken up by the mitochondria of all tissues, in contrast to hydrophilic compounds which rely on the tissue-specific expression of carriers for uptake [32–36]. Several biologically relevant organic compounds as well as metal complexes are known where this strategy has been successfully used to target small molecules specifically into the mitochondria of cells to perform a wide variety of functions [37,38]. Mitochondria being the powerhouse of the cells, they are important for cell's survival. Mitochondria regulate the intrinsic pathway of apoptosis [39,40]. Moreover, they lack the extensive NER machinery to overcome the drug resistance.

Herein, we report the synthesis and anticancer activity of two new dichloro oxovanadium(IV) complexes (**1**, **2**) having (anthracenyl)terpyridine (An-Tpy) based ligands with one bearing a pendant cationic triphenylphosphonium (TPP-An-tpy) moiety in **2** (Fig. 1). Anthracenyl moiety being a fluorophore, the complex having this moiety is suitable for photo-activated chemotherapeutic and cellular imaging study [41–44]. Its emission property can be utilized for cellular imaging via fluorescence microscopy. The triphenylphosphonium moiety with its lipophilic nature is appended to enhance the cellular uptake of the complex and delivering it specifically into the mitochondria of the cells. The complexes were tested for their toxicity against HeLa and MCF-7 cancer cells in both dark and visible light (400–700 nm). Significant results of this work include mitochondria targeting as well as DNA crosslinks induction by complex **2** upon visible light irradiation.

2. Experimental section

2.1. Materials

All the reagents and chemicals were procured from the commercial sources (s.d. Fine Chemicals, India; Sigma-Aldrich, U.S.A.). Solvents were purified by reported procedures [45]. Calf thymus (ct) DNA, agarose (molecular biology grade), Dulbecco's Modified Eagle's medium, propidium iodide and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (U.S.A.). Mitotracker Red (MTR) was purchased from Invitrogen (India). The ligands were prepared by following reported procedures [46,47].

2.2. Instrumentation

The elemental analysis was carried out using a Thermo Finnigan Flash EA 1112 CHN analyzer. The infrared, UV–visible and emission spectra were recorded on Perkin-Elmer Lambda 35, Perkin-Elmer Spectrum one 55 and Perkin-Elmer LS 55 spectrophotometer, respectively. Molar conductivity measurements were made using a Control Dynamics (India) conductivity meter. Magnetic susceptibility of the complexes was measured using Sherwood Scientific magnetic susceptibility balance at 298 K. Cyclic voltammetric measurements were made at 25 °C on a EG & G PAR Model 253 VersaStat potentiostat/galvanostat using a three electrode configuration with a glassy carbon working, platinum wire auxiliary and a saturated calomel reference electrode (SCE). Tetrabutylammonium perchlorate (TBAP, 0.1 M) was used as a supporting electrolyte in dimethylformamide (DMF). ^{1}H NMR spectra for the ligands were recorded at room temperature on a Bruker 400 MHz NMR spectrometer. Electrospray ionization mass spectral measurements were done using Esquire 3000 plus ESI (Bruker Daltonics) Spectrometer. Flow cytometric analysis was performed using Fluorescence Associated Cell Sorter Scan (FACS) Calibur (Becton Dickinson cell analyzer) at FL2 channel (595 nm). Confocal microscopic measurements were done using confocal scanning electron microscope (Leica, TCS SP5 DM6000).

2.3. Synthesis of [VO(An-tpy)Cl_2] (1) and [VO(TPP-An-tpy)Cl_2]Cl (2)

Complexes **1** and **2** were prepared by following a general synthetic procedure in which vanadium(III) chloride (0.16 g, 1.0 mmol) was dissolved in 10 mL methanol and was stirred in air for 30 min during which the solution turned green in color. The green solution was deaerated and then saturated with nitrogen. A methanol solution of the ligand (0.41 g, An-tpy; 0.72 g, TPP-An-tpy (1.0 mmol)) was added to this solution. The reaction mixture was left for stirring at room temperature. The complexes were precipitated after 1 h. The solid was isolated and washed with cold ethanol, tetrahydrofuran, cold acetonitrile, and was finally dried in vacuum over P_4O_{10} [Yield: ~65% for **1** and ~80% for **2**]. The bromo analogue (**3**) of complex **2** was prepared in a similar way by using VBr_3 dissolved in acetonitrile. The reaction

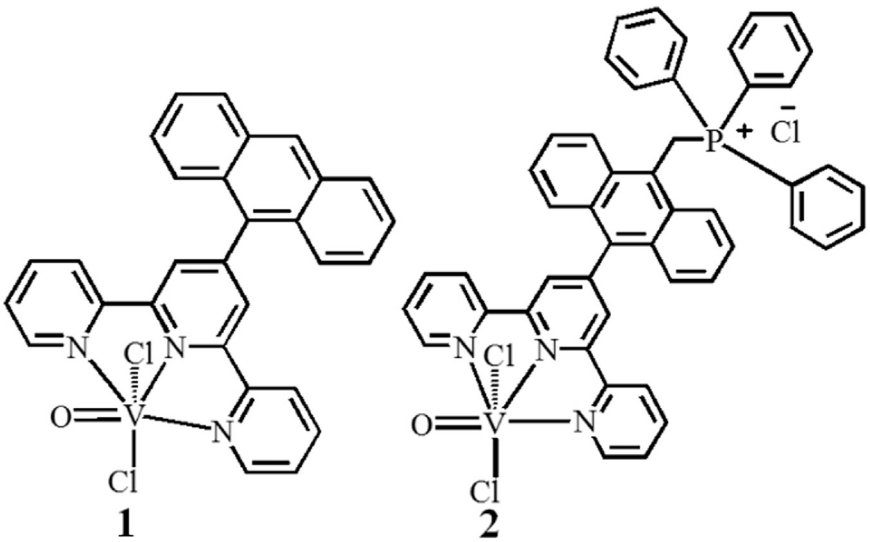

Fig. 1. Schematic drawings of the structures of the oxovanadium (IV) complexes **1** and **2**.