Indian Journal of Chemistry
Vol. 57A, March 2018, pp. 409-417

Oxovanadium(IV) complexes of polypyridyl bases as photocytotoxic and DNA crosslinking agents

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Received 29 December 2017; revised and accepted 26 February 2018

Dichlorooxovanadium(IV) complexes of polypyridyl bases (B), viz., $[VO(B)Cl_2]$ (**1** and **2**) of *N,N,N*-donor ligands 2-(2-pyridyl)-1,10-phenanthroline (pyphen in **1**) and 3-(pyridin-2-yl)dipyrido[3,2-a:2',3'-c]phenazine (pydppz in **2**) have been prepared, and their anticancer activity studied. The complexes show good solubility in most polar solvent and are non-electrolytes. They show 1:1 electrolytic property in 20% aqueous DMF, and 1:2 electrolytic character in 20% aqueous DMF upon photo-irradiation with visible light (400–700 nm). Complex **1** shows DNA crosslinking property on dissociation of the V-Cl bond(s) as ascertained from its DNA melting profile and ethidium bromide displacement assay. The complexes **1** and **2** are photocytotoxic towards HeLa (IC_{50}: 16.9 μM and 0.87 μM respectively) and MCF-7 (IC_{50}: 19.5 μM and 1.4 μM respectively) cancer cells, but exhibit less toxicity in normal embryonic fibroblast 3T3 cells with IC_{50} values of ~40 and ~25 μM in visible light (400-700 nm) for complexes **1** and **2** respectively. Complex **2** also displays light induced plasmid DNA cleavage activity. The light-induced cellular damage leads to generation of reactive oxygen species followed by apoptotic cell death.

Keywords: Bioinorganic chemistry, Photocytotoxicity, Cytotoxicity, DNA crosslinks, DNA cleavage, Vanadium, Polypyridyl bases

Cisplatin and its analogues are well known chemotherapeutic agents.[1-4] Cisplatin binds to the N^7 atoms of the guanine bases of nuclear DNA forming DNA crosslinks. These complexes behave as transcription inhibitors resulting in cell death.[5] The rate of dissociation of the Pt-Cl bonds in the intracellular medium is a key factor for the overall efficacy of these drugs. The rapid dissociation of the chlorides of cisplatin in the cellular medium necessitated the development of carboplatin and oxaliplatin as two alternate platinum-based chemotherapeutic drugs with reduced Pt-O bond dissociation rates.[6] The successful use of platinum-based chemotherapeutic drugs has generated interest for other DNA crosslinkers in cancer chemotherapy.[7-9] Although DNA inter- and intra-strand crosslinking (ICL) agents such as mitomycin C, cisplatin and psoralen are found to be potent anticancer drugs, these agents suffer from dose-limiting toxic effects on normal cells and reduced efficacy due to nuclear excision repair (NER) mechanism that operates for nuclear DNA.[10-12] Dichloro(η^6-arene)ruthenium(II) complexes are reported to be less active than cisplatin[13,14] and better activity is reported when used in combination with other drugs. While the cytotoxic effects of titanocene dihalides are comparable to that of cisplatin, they are not preferred for cellular application due to their toxic side effects.[15,16] Copper(II) complexes showing cytotoxic properties are also reported in the literature, but the mechanistic pathways for such redox active complexes are different from that of cisplatin and do not involve formation of any ICLs.[17-19]

The present work stems from our continued interest to design and develop the chemistry of new photoactive six coordinate oxovanadium(IV) complexes as novel DNA crosslinkers having two chloride ligands in *cis* or *trans* disposition to each other mimicking the four coordinate core of *cis*- or *trans*-platin. With this initiative, we have previously reported oxovanadium(IV) complexes showing DNA covalent binding and DNA crosslinking properties.[20-23] *Trans*-dichlorooxovanadium(IV) complex of pyrenylterpyridine (VDC) was earlier developed as

a photo-inducible DNA crosslinking agent.[20] This VDC complex initially forms a mono-adduct with the DNA. It can be photo-activated to generate DNA interstrand crosslinks, eventually leading to cell death. Besides, the dichlorooxovanadium(IV) complexes having pendant photoactive borondipyrromethene (BODIPY) moieties are shown to be highly photocytotoxic due to their mitochondrial localization and mitochondrial DNA crosslinking properties.[21] Oxovanadium(IV) complexes of (anthracenyl)-terpyridine and triphenylphosphonium-appended (anthracenyl)terpyridine ligands are also reported to show DNA crosslinking ability, photocytotoxicity in visible light and cellular localization in the cancer cells.[22] These VO^{2+} complexes showed rapid dissociation of one chloride ligand, while the second chloride ligand becomes labile only on photo-activation. Unlike the cisplatin mechanistic pathway involving rapid hydrolysis of both the Pt-Cl bonds, in these complexes the sequential dissociation of two chloride ligands by light activation makes such complexes unique wherein their activity can be controlled by light. The appendage of a photoactive moiety allows the complexes to show photo-induced cytotoxicity akin to photodynamic therapy (PDT) in which the drug on light-activation generates reactive oxygen species to damage the light exposed cells, while the non-exposed healthy cells remain unaffected.[24-26] We have shown in a recent communication that the ancillary ligand plays an important role in ICL formation and in the cellular organelle localization.[23] While the BODIPY complexes primarily localize in the mitochondria of the cells, appendage of the anthracenyl moiety leads to nuclear localization.[22] Use of polypyridyl bases, *viz.* pyphen (pyridyl-1,10-phenanthroline) and pydppz (3-(pyridine-2-yl)dipyrido[3,2-a:2',3'-c]phenazine) in the dichlorooxovanadium(IV) complexes leads to remarkable photo-induced DNA crosslinking and cytotoxicity giving low IC_{50} values in HeLa and MCF-7 cancer cells.

Herein, we present the calf thymus (ct) DNA binding, plasmid pUC19 DNA cleavage activity of the complexes $[VO(pyphen)Cl_2]$ (**1**) and $[VO(pydppz)Cl_2]$ (**2**) to understand the nature of ROS generation. We have also carried out photocytotoxicity studies using 3T3 embryonic fibroblast normal cells, detection of reactive oxygen species (ROS) via dichlorofluorescein diacetate (DCFDA) assay and cell cycle analysis. This study also includes the evidence for DNA covalent crosslink formation with the dissociation of the chloride ligands from the DNA binding and cleavage studies. In this work DNA crosslink formation in visible light could be directly observed from DNA melting and comet assay data. Further, the photo-cytotoxicity of the complexes in cancer cells, while being inactive in normal cells, thus providing desirable selectivity and cancer cell targeting property, shows the significance of this study.

Materials and Methods

All the reagents and chemicals were purchased from commercial sources (SD Fine Chemicals, India; Aldrich, USA) and solvents were purified by standard procedures.[27] Calf thymus (ct) DNA, ethidium bromide (EB), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2′,7′-dichloro-fluoresceindiacetate (DCFDA), propidium iodide (PI), Dulbecco's modified eagle medium (DMEM), Dulbecco's phosphate buffered saline (DPBS) and fetal bovine serum (FBS) were procured from Sigma, USA. Tris-(hydroxymethyl)aminomethane-HCl (Tris-HCl) buffer solution was prepared using deionized and sonicated triply distilled water. The *N,N,N*-donor polypyridyl bases 2-(2-pyridyl)-1,10-phenanthroline (pyphen) and 3-(pyridin-2-yl)dipyrido[3,2-a:2',3'-c]-phenazine (pydppz) were prepared by following literature procedures.[28,29] The complexes were characterized by: (i) elemental analyses (Thermo Finnigan Flash EA 1112 CHNS analyser), (ii) infrared spectra (Bruker ALPHA FT-IR spectrometer), (iii) electronic spectra (Perkin-Elmer Lambda 650 spectrophotometer), (iv) molar conductivity (Control Dynamics (India) conductivity meter), (v) electrospray ionization mass spectra (ESI-MS) (Bruker Daltonics make Esquire 300 Plus ESI model), and (vi) ^{1}H NMR spectra of the ligands at room temperature (Bruker 400 MHz NMR spectrometer). Flow cytometry (FACS) measurements were made using BD FACS Verse cytometer.

Synthesis of the complexes

Complexes **1** and **2** were prepared by following a general synthetic procedure in which vanadium(III) chloride (1.0 mmol) was dissolved in 10 mL methanol and was stirred in air for 30 min during which the solution turned green. The solution was deaerated and then saturated with nitrogen. A 1.0 mmol methanolic solution of the ligand was added to this solution. The reaction mixture was stirred at room temperature and 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 characterization data for the complexes are available in our preliminary report (vide supporting information data under http://dx.doi.org/10.1002/ejic.201500587).[23]

DNA binding experiments

The fluorescence spectra of the ethidium bromide (EB, 50 μM) intercalated ct-DNA (200 μM) were recorded (λ_{ex} = 546 nm, λ_{em} = 595 nm) in presence of the complexes **1** and **2** (10% DMF-DPBS, pH = 7.2) in the dark and under photo-irradiated conditions (400-700 nm, 10 J cm^{-2}, Luzchem photoreactor). Thiourea, which supresses the formation of crosslinks by reacting with DNA adducts, was used to assess the extent of DNA adduct formation.[30] The solutions were treated with excess of thiourea (10 mM) and the emission spectra were recorded. DNA melting experiments were carried out by monitoring the absorbance of ct-DNA (200 μM) at 260 nm at various temperatures, both in the absence and presence of the complexes (25 μM). Measurements were carried out using a Perkin-Elmer Lambda 650 spectrometer with a temperature controller at an increase rate of 0.5 °C per min of the solution. Ethidium bromide (EB) was used as a control. Viscometric titrations were performed with a Schott Gerate AVS 310 automated viscometer that was maintained at 37 °C in a constant temperature bath. The concentration of ct-DNA was 150 μM in NP (nucleotide pair) and the flow times were measured using an automated timer. Each sample was measured three times and an average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ versus [complex]/ [DNA], where η is the viscosity of DNA in the presence of complex and η_0 is that of DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solutions (t) corrected for that of the buffer alone (t_0); $\eta = (t-t_0)/t_0$. Due corrections were made for the viscosity of DMF solvent present in the solution.

DNA cleavage experiments

The photo-induced plasmid DNA cleavage activity of the complexes **1** and **2** was studied using supercoiled (SC) pUC19 DNA (33.3 μM, 0.2 μg) in a medium of Tris-HCl/NaCl (50 mM, pH 7.2) buffer on irradiation with visible light of wavelength 476 nm (50 mW) using a CW argon-krypton mixed gas ion laser. The nature of ROS generated on photo-irradiation was investigated by studying the mechanistic aspects of the DNA photocleavage in the presence of several quenchers and scavengers like DABCO (1,4-diazabicyclo[2.2.2]octane), L-histidine, NaN_3 and TEMP (2,2,6,6-tetramethylpiperidine) as singlet oxygen quenchers, KI and DMSO as hydroxyl radical scavengers, catalase as a H_2O_2 scavenger, and superoxide dismutase (SOD) as a superoxide radical scavenger.

Cytotoxicity from MTT assay

Cytotoxicity of the complexes using 3T3 embryonic fibroblast normal cells was determined by MTT assay in light and dark by an earlier reported procedure.[31] Approximately 1.0 x 10^4 3T3 embryonic fibroblast normal cells were plated in each well of a 96-well cell culture plate in DMEM cell culture medium supplemented with 10% fetal bovine serum (10% DMEM) and cultured for 12 h at 37 °C in 5% CO_2. Various concentrations of the complexes dissolved in 1% DMSO were added to the cells and incubated for 4 h in dark. The media was subsequently replaced with DPBS and the cells were irradiated with a broad band visible light for 1 h (400–700 nm, 10 J cm^{-2}), using a Luzchem photoreactor (model LZC-1, Ontario, Canada). After photo-irradiation, DPBS was removed and replaced with 10% DMEM, and incubation was continued for a further period of 20 h in dark. After the incubation, 5 mg mL^{-1} of MTT (20 μL) was added to each well and incubated for another 3 h. The culture medium was discarded and 200 μL of DMSO was added to dissolve the formazan crystals. The absorbance was measured at 540 nm using Spectra Max M5 plate reader (Molecular Devices). Cytotoxicity of the complexes was determined as the percentage ratio of the absorbance of the treated cells to the untreated controls. The IC_{50} values were obtained by nonlinear regression analysis (GraphPad Prism 5).

ROS from DCFDA assay

The formation of reactive oxygen species (ROS) was studied using 2',7'-dichlorodihydrofluorescein diacetate (DCFDA) assay. Cell permeable DCFDA dye on oxidation by cellular ROS generates fluorescent DCF having an emission maximum at 528 nm.[32] The percentage of cells generating ROS was determined by flow cytometry analysis. HeLa cells incubated with 1 μM of complex **2** for 4 h were photo-irradiated (400-700 nm) for 1 h in DPBS buffer. The cells were then isolated by trypsinization and single cell suspension of 1×10^6 cells mL^{-1} was prepared. The cells were treated with 10 μM DCFDA

solution in the dark for 15 min at room temperature. The distribution of DCFDA stained HeLa cells was determined by flow cytometry.

Cell cycle analysis

To investigate the effect of the complexes on the cell cycle, 2×10^5 HeLa cells were plated in each well of a 24-wells tissue culture plate in 10% DMEM. After 12 h of incubation at 37 °C in a CO_2 incubator, the cells were treated with DMSO solutions of the complexes **1** (2 μM) and **2** (1 μM) for 4 h in dark. The medium was subsequently replaced with DPBS and the cells were photo-irradiated for 1 h. After light irradiation, DPBS was removed and replaced with 10% DMEM, and incubation was continued in the dark for a further period of either 12 h or 16 h. The cells were then trypsinized and collected into 1.5 mL centrifuge tubes, washed once with DPBS and fixed by adding 1.0 mL of chilled 70% ethanol. The cell suspensions were incubated at −20 °C for 24 h. The fixed cells were then washed twice with 1.0 mL of DPBS by centrifuging at 5000 rpm at 4 °C for 5 min. The supernatants were gently discarded, and the cell pellets were suspended in 200 μL of DPBS containing 50 μg mL^{-1} DNase-free RNase and incubated at 42 °C for 1 h. After digestion of cellular RNA, the cells were stained with 20 μg mL^{-1} propidium iodide (PI) solution for 15 min at 4 °C. Flow cytometric analysis was performed using a FACS Verse Becton Dickinson (BD) cell analyser. Data analysis was performed by using Cyflogic software.

Results and Discussion

Synthesis and general aspects

The complexes were synthesized and characterized using various analytical and spectroscopic methods that are reported in our preliminary communication.[23] The complexes, viz. $[VO(pyphen)Cl_2]$ (**1**) and $[VO(pydppz)Cl_2]$ (**2**), have six coordinate structures in which the polypyridyl bases display *N,N, N*-tridentate chelating mode of coordination. The disposition of the two chlorides could be *cis* or *trans* to each other as the energy difference between the two structures (0.5-0.7 kcal mol^{-1}) is insignificant indicating equal possibility of both the structures.[23] The structure having two chloride ligands in *trans* disposition is of marginally lower energy than that of the *cis* analogue. The complexes showed good solubility in most polar solvents and are non-electrolytes. They displayed 1:1 electrolytic behaviour in 20% aqueous DMF, and 1:2 electrolytic character in 20% aqueous DMF upon photo-irradiation with visible light (400–700 nm). The rapid loss of one chloride on dissolution of the complex in aqueous medium is likely to form the same species from both *cis* and *trans* dichloro species. The *trans* orientation of the chlorides is supported by the IR data showing different V=O stretching frequencies for the pyphen and pydppz complexes. A *cis* disposition of two chloride ligands is likely to result in the V=O bond frequency remaining unchanged as is reported for other *cis*-dichloro oxovanadium(IV) complexes.[20-23]

DNA binding studies

With the knowledge that planar polypyridyl bases can bind to DNA grooves or intercalate within base pairs and that loss of chloride ligand(s) can promote ICL formation, DNA binding data can give important insights into their anticancer properties. The complexes could show dual DNA binding properties. Ethidium bromide (EB) intercalates to calf thymus DNA and being trapped in a hydrophobic environment created by the base pairs, displays fluorescence which is otherwise not present in the aqueous solutions. Addition of the complexes showed a gradual decrease of the fluorescence intensity of intercalated EB due to its displacement (Fig. 1).[33,34] The K_{app} binding constant values for **1** and **2** were 2.5×10^4 and 4.2×10^4 M^{-1}.

To get information about any ICL formation instead of intercalative DNA binding, the stability of the DNA double helix upon binding to **1** and **2** (Fig. 2a) was investigated by thermal denaturation experiments. A small positive shift of the DNA melting temperature (ΔT_m ~1 °C) was observed upon addition of complex **1** to ct-DNA. This low ΔT_m value suggests the primarily crosslinking nature of the complex to ct-DNA in preference to an intercalative mode of binding that normally results in a large positive ΔT_m value (10 °C for intercalative EB).[35] The ΔT_m value for the pydppz complex **2** is marginally higher (ΔT_m ~3 °C) than that of the pyphen complex **1** possibly due to the presence of an extended aromatic ring that may facilitate partial intercalative DNA binding. Viscosity measurements were made to examine the effect of the complexes on the specific relative viscosity of DNA (Fig. 2b). The relative specific viscosity η/η_0 (where η and η_0 are the specific viscosities of DNA in the presence and absence of the complexes, respectively) of DNA gives a measure of the increase of the contour length

associated with the separation of DNA base pairs caused by intercalation and a classical DNA intercalator like ethidium bromide would show a significant increase in the viscosity of the DNA solutions. In contrast, a partially DNA intercalating complex would result in a less pronounced effect on the viscosity.[36] The groove binder Hoechst 33258 was used as a reference compound that showed an insignificant increase in viscosity. The viscosity profile of the pydppz complex **2** is also similar to

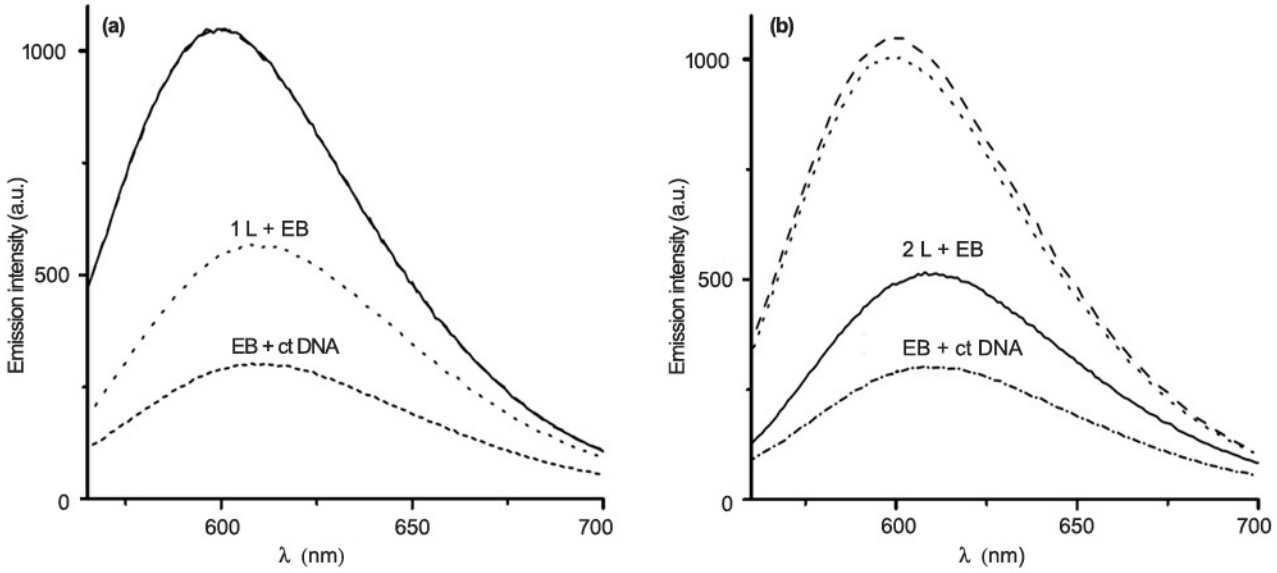

Fig. 1 – Ethidum bromide (EB) displacement assay showing DNA crosslink formation upon photo-exposure of (a) complex **1,** and (b) complex **2,** treated with ethidum bromide (EB, 50 μM) intercalated ct-DNA (200 μM). Emission recorded in dark and light as shown in the figure [λ_{exc} = 546 nm, λ_{em} = 595 nm, photoreactor of 400-700 nm].

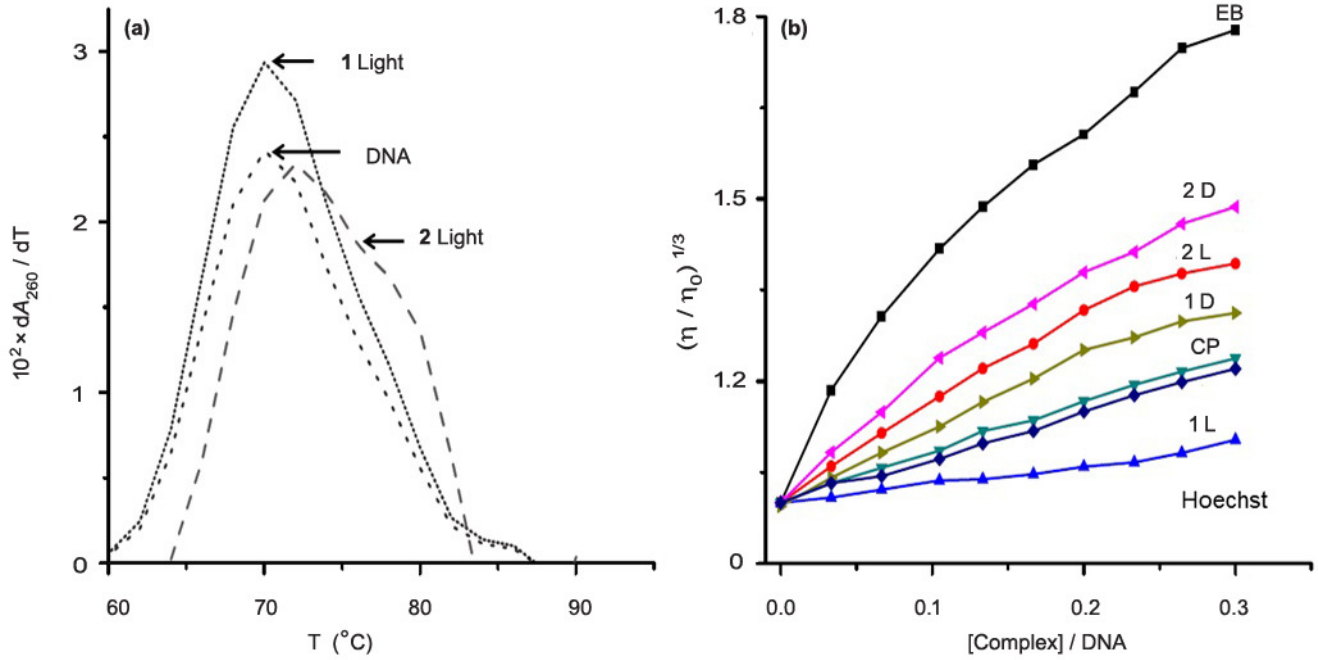

Fig. 2 – (a) Thermal denaturation plots of 200 μM ct-DNA alone and on addition of the complexes **1** and **2** on exposure to light of 400-700 nm (10 J cm^{-2}). (b) Effect of addition of complex **1**, cisplatin (CP) , DNA intercalator ethidium bromide (EB) and groove binder Hoechst dye on the relative viscosity of ct-DNA (200 μM) in DPBS [pH = 7.2].

that of EB, indicating a partial DNA intercalative mode of binding of the pydppz complex in addition to its DNA crosslinking property. Interestingly, the viscosity profile of complex **1** compared well with that of the classical DNA cross-linker, viz. cisplatin.[37]

DNA cleavage and mechanistic aspects

The primary target of binding of these complexes is DNA, hence their DNA cleavage property is of importance. The complexes showed plasmid supercoiled (SC) pUC19 DNA cleavage activity in blue light of 476 nm, forming nicked circular (NC) DNA (Fig. 3). The possibility of involvement of any reactive oxygen species (ROS) was probed by mechanistic studies. Addition of singlet oxygen quenchers like DABCO, TEMP, sodium azide, and L-histidine showed no inhibitory effect on the DNA cleavage activity of complex **2**. The %NC remained above 85% even in the presence of these $^1O_2$ quenchers. Interestingly, hydroxyl radical scavengers like DMSO and KI displayed a significant reduction in the DNA cleavage activity at this wavelength. Catalase as hydrogen peroxide scavenger and SOD as superoxide scavenger showed significant inhibition of the DNA cleavage activity, with the formation of just ~30% NC DNA. The results suggest the involvement of hydroxyl radicals ($HO^•$) as the ROS in the DNA photocleavage reactions in visible light. In the absence of any enhancement of the DNA cleavage activity in the presence of D_2O, the involvement of singlet oxygen ($^1O_2$) is ruled out.[38]

The groove-binding preference of the complexes was studied by using DNA minor groove-binder distamycin-A, which alone showed ~20% cleavage of the SC DNA at 476 nm for an exposure time of 2 h. The DNA cleavage activity of **2** remained unaffected in the presence of distamycin-A, indicating major groove interaction of the pydppz complex. Complex **1** showed ~19% of the NC form under similar conditions. The DNA cleavage activity was also studied under anaerobic medium using argon. Photocleavage of ~12% of SC DNA was observed under such conditions (Fig. 3). The plasmid DNA photocleavage data suggest the formation of hydroxyl radicals as the ROS, binding of the polypyridyl groups at the major groove of DNA, requirement of molecular oxygen for their activity, and the dual possibility of groove binding and ICL formation.

Photocytotoxicity and cellular properties

Photo-induced cytotoxicity of complexes **1** and **2** has been reported earlier in HeLa and MCF-7 cancer cells.[23] The respective IC_{50} values for the complexes **1** and **2** were 16.9 μM and 0.87 μM in HeLa and 19.5 μM and 1.4 μM in MCF-7 cells in light (400-700 nm, 1 h photoexposure, 10 J cm^{-2}, Luzchem photoreactor) and >40 μM in dark for both the cells. An important aspect of anticancer drugs is their selectivity between normal and cancer cells. Reduced toxicity for normal cells enhances the efficacy of the drug. We have studied the photocytotoxicity of the complexes in 3T3 embryonic fibroblast normal cells by MTT assay. The complexes upon prior incubation for 4 h in dark and subsequent photo-exposure to visible light of 400-700 nm (Luzchem photoreactor) for 1 h did not show any significant dose-dependent decrease in cell viability. The IC_{50} value was 26.0 (±1.7) μM for the pydppz complex **2** and 42.0 (±1.4) μM for complex **1** (Fig. 4(a), Table 1). The cells unexposed to light gave an IC_{50} value of >50 μM for both the complexes. The pyphen complex being photo-inactive showed

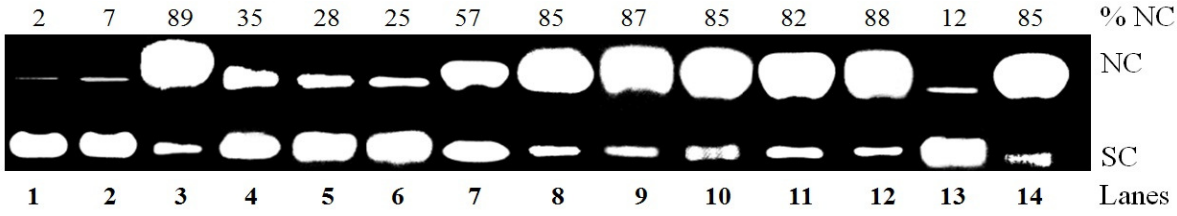

Fig. 3 – Gel diagram showing the mechanistic aspects of DNA photo-cleavage activity of complex **2** (50 μM) on SC pUC19 DNA (0.2 μg, 33.3 μM) in the presence of various singlet oxygen quenchers and radical scavengers under irradiation with blue laser light of 476 nm (50 mW, 120 min exposure, lanes 3-14). [Lane 1, DNA alone; lane 2, DNA + **2** (Dark); lane 3, DNA + **2** (Light); lane 4, DNA+**2** + KI (0.5 mM); lane 5, DNA + **2** + catalase (4 μL) ; lane 6, DNA + **2** + DMSO (4 μL) ; lane 7: DNA+ **2** + SOD (superoxide dismutase, 4 units); lane 8, DNA + **2** + L-histidine (0.5 mM) ; lane 9, DNA+ **2** + DABCO (0.5 mM); lane 10: DNA+ **2** + NaN_3 (0.5 mM); lane 11, DNA+ **2** + TEMP (0.5 mM) ; lane 12, DNA+ **2** + D_2O (16 μL); lane 13, DNA + **2** + Argon; lane 14, DNA + distamycin-A (50 μM) + **2**].

Table 1 — IC_{50} (μM) values of the complexes **1** and **2** in different cell lines

Complex	IC_{50} (μM)					
	HeLa[a]		MCF-7[b]		3T3[c]	
	Light[d]	Dark	Light[d]	Dark	Light[d]	Dark
1	16.9±0.6	>50	19.5±0.3	>50	42.0 ±1.4	>50
2	0.87±0.07	>40	1.4±0.2	>40	26.0 ±1.7	>50

[a]Human cervical cancer cell line. [b]Human breast cancer cell line. [c]Normal embryonic fibroblast cell line.
[d]Photoreactor of 400-700 nm broad band light.

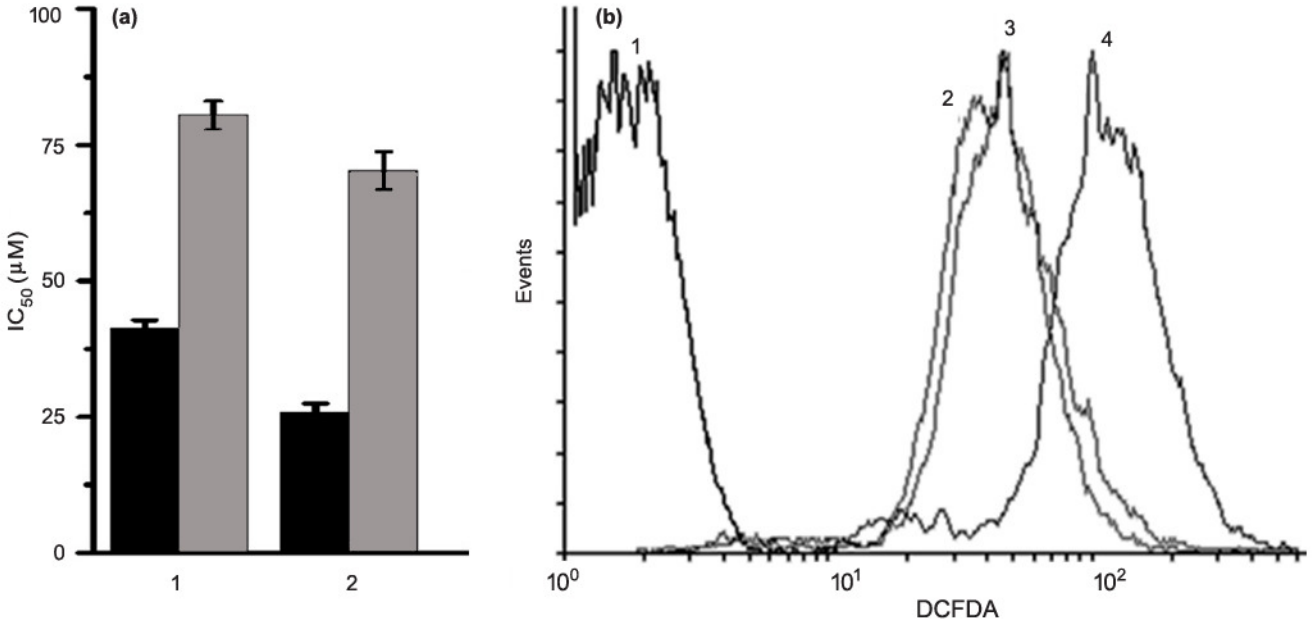

Fig. 4 – (a) Photocytotoxicity of the complexes **1** and **2** in 3T3 normal cells on 4 h incubation in dark followed by exposure to light of 400-700 nm (Luzchem photoreactor of power 10 J cm^{-2}). (b) DCFDA assay for generation of ROS in HeLa cells upon exposure of visible light. [1, Fluorescence of cells alone; 2, fluorescence of cells + DCFDA; 3, fluorescence of cells + DCFDA + **2** in dark; 4, fluorescence of cells + DCFDA + **2** in light].

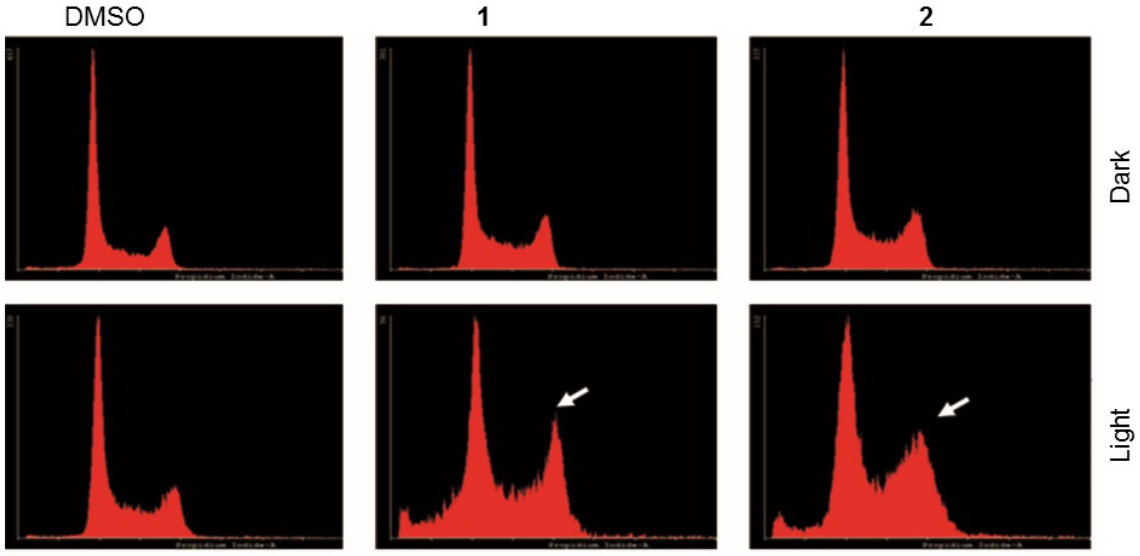

Fig. 5 – Cell cycle profile (sub-G1, G1, S, and G2/M phases) of the HeLa cells treated with complexes **1** (2 μM) and **2** (1 μM) in dark and after irradiation with visible light (400–700 nm, 10 J cm^{-2}).

significantly lower activity than the photoactive pydppz complex. Both the complexes, however, displayed similar ICL properties. The observed lower activity of the complexes in normal cells compared to those in cancer cells may be due to their lower cellular uptake in normal cells. The DNA photocleavage study showed the formation of hydroxyl radicals as the reactive oxygen species (ROS), which is further supported by the DCFDA assay that detects formation of any cellular ROS. Cell permeable DCFDA on oxidation by cellular ROS generates the fluorescent product DCF which has an emission maximum at 525 nm; its formation can be detected by flow cytometry analysis. A substantial positive shift in the fluorescence (indicating ROS generation) was observed for HeLa cells treated with the photoactive pydppz complex **2** upon irradiation with visible light of 400-700 nm when compared to the reaction in the dark as well as for the untreated cells (Fig. 4(b)).

The DNA content of the HeLa cells treated with the complexes **1** and **2** was measured to examine the effect of the complexes on the cell cycle using a red-emitting fluorescent dye, viz. propidium iodide (PI), by flow cytometry (Fig. 5). The experiments were performed under both light and dark conditions. The complexes upon light activation caused irreparable DNA damage which could lead to a G2/M arrest in the cells (12 h) and eventually resulting in their death (16 h). In the dark, the complexes did not induce any apparent change in either G2/M or sub-G1 population of the cells, thus emphasising the role of light activation in cell cycle arrest and cell death.

Conclusions

Dichlorooxovanadium(IV) complexes of polypyridyl bases having one dissociable and one photo-dissociable chloride ligand were designed as vanadium-based DNA crosslinking agents. The incorporation of rigid polypyridyl bases in the complexes facilitates the damage of nuclear DNA in a manner similar to that known for cisplatin as a transcription inhibitor and ICL generator. The ct-DNA binding and plasmid DNA photocleavage studies show that complex **1** with a dichlorooxovanadium(IV) moiety and pyphen ligand, forms DNA crosslinks only upon light activation, whereas complex **2** having a dppz ligand with an additional aromatic moiety displayed a partial intercalative mode of DNA binding along with DNA crosslinks formation. Complex **2** also exhibited excellent DNA cleavage activity upon photo-irradiation *via* the hydroxyl radical pathway, which was established from the pUC19 DNA photo-cleavage mechanistic study and DCFDA assay. To ascertain the possibility of any damage induced by the complexes to the normal healthy cells, MTT assay was performed in normal 3T3 embryonic fibroblast cells; no significant cytotoxicity was observed upon photo-irradiation. The IC_{50} values were significantly higher in normal cells in comparison to the cancerous HeLa and MCF-7 cells.[23] These results are of importance considering the selectivity observed and the importance of DNA inter-strand crosslink formation which is unlikely to get repaired by nuclear excision repair (NER) mechanism that operates for intra-strand DNA crosslinks.

Acknowledgement

We thank the Department of Science and Technology (DST), Government of India, and the Council of Scientific and Industrial Research (CSIR), New Delhi, India, for financial support (SR/S5/MBD-02/2007, EMR/2015/000742 and 01(2559)/12/EMR-II). ARC thanks the DST for J. C. Bose National Fellowship. The FACS facility of Indian Institute of Science is also acknowledged.

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