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Original article

Photoactivated DNA cleavage and anticancer activity of pyrenyl-terpyridine lanthanide complexes

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ABSTRACT

Lanthanide(III) complexes [Ln(R-tpy)(acac)$(NO_3)_2$] (Ln = La(III) in **1**, **2**; Gd(III) in **4**, **5**) and [Ln(py−tpy)(sacac)$(NO_3)_2$] (Ln = La(III), **3**; Gd(III), **6**), where R-tpy is 4′-phenyl-2,2′:6′,2″-terpyridine (ph−tpy in **1**, **4**), 4′-(1-pyrenyl)-2,2′:6′,2″-terpyridine (py−tpy in **2**, **3**, **5** and **6**), acac is acetylacetonate and sacac is 4-hydroxy-6-{4-[(β-D-glucopyranoside)oxy]phenyl}hex-3,5-dien-2-onate, were prepared to study their DNA photocleavage activity and photocytotoxicity. Complexes [La(ph-tpy)(acac)(EtOH)$(NO_3)_2$] (**1a**) and [Gd(ph−tpy)(acac)$(NO_3)_2$] (**4**) were characterized by X-ray crystallography. The 1:1 electrolytic complexes bind to calf thymus DNA. The py−tpy complexes cleave pUC19 DNA and exhibit remarkable photocytotoxicity in HeLa cells in UV-A light of 365 nm with apoptotic cell death (IC_{50}: ~40 nM in light, >200 μM in dark). Confocal microscopy using HeLa cells reveal primarily cytosolic localization of the complexes.

1. Introduction

Metal-based photoactivated chemotherapeutic agents are of current interests for their potential utility in the photodynamic therapy (PDT) of cancer [1–4]. PDT has emerged as a new modality of cancer cure due to its non-invasive nature and for its selectivity in which the photo-irradiated cancer cells are selectively damaged leaving the unexposed normal cells intact [5–8]. The currently used PDT agents are primarily porphyrin and phthalocyanine bases. The mode of action of the organic PDT agents is to generate cytotoxic singlet oxygen ($^1O_2$) upon photoactivation involving the $^3(\pi\text{-}\pi^*)$ state of the photosensitizer and molecular oxygen ($^3O_2$) [9–11]. The major drawbacks of such bases include skin sensitivity and hepatotoxicity which leads to jaundice [12,13]. Besides, the potency of an organic PDT agent depends largely on the high quantum yield of singlet oxygen generation which is often difficult to achieve thus making them unsuitable for phototherapeutic applications [11]. These problems could be circumvented using metal-based PDT agents which could be suitably designed utilizing tunable coordination geometries, wide spectral and redox properties thus providing alternate type-I and/or photo-redox pathways to cause cellular photodamage in addition to the generation of singlet oxygen species in a type-II process. The 4*d* and 5*d* metal-based anticancer agents, *viz.*, platinum(IV), ruthenium(II) and rhodium(II) complexes, are reported to display photocytotoxicity in a variety of cancer cells [14–19].

Our work on the 3*d* metal-based complexes has shown that iron(III) and oxovanadium(IV) complexes are remarkably photocytotoxic in various cancer cells in visible light [20–24]. While transition metal-based PDT agents are reported from various research groups, the lanthanide-based PDT agents are virtually

Abbreviations: AO, acridine orange; CCDC, Cambridge Crystallographic Data Centre; ct-DNA, calf thymus DNA; DABCO, 1,4-diazabicyclo[2.2.2]octan; DMEM, Dulbecco's Modified Eagle's Medium; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid; DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate; dppz, dipyrido[3,2-a:2′,3′-c]phenazine; DTPA, diethylenetriaminepentaacetate; EB, ethidium bromide; EDTA, ethylenediaminetetraacetate; FBS, foetal bovine serum; GLUTs, glucose transporters; ISC, intersystem crossing; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MvH, McGhee-von Hippel; NC, nicked circular; PBS, phosphate buffered saline; PDT, photodynamic therapy; PI, propidium iodide; RME, receptor-mediated endocytosis; SC, supercoiled; SOD, superoxide dismutase; TEMP, 2,2,6,6-tetramethyl-4-piperidone.

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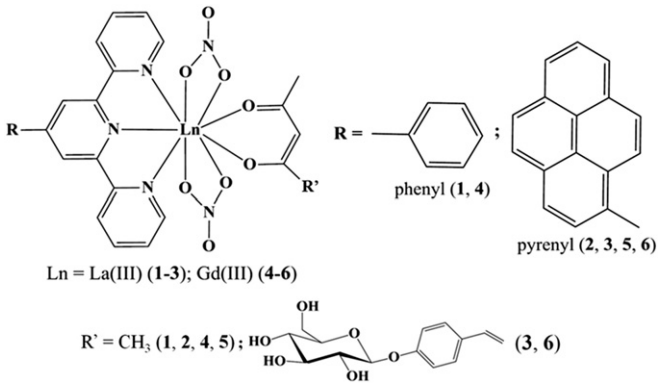
unknown except few complexes containing macrocyclic organic dyes [25–27]. For example, a lutetium(III) texaphyrin (LUTRIN®) complex is an effective PDT agent in near-IR light of 732 nm [25]. The lanthanide complexes are being used as luminescent tags for bio-analytical applications [28–30]. Gadolinium(III) complexes, *viz*., $[Gd(DTPA)(H_2O)]^{2-}$ (Magnevist™) and $[Gd(DOTA)(H_2O)]^{-}$ (Dotarem™), are used as magnetic resonance imaging (MRI) contrast agents [31–33]. The success of the Gd-based MRI agents provides strong impetus to design new lanthanide complexes and explore their photochemotherapeutic potentials. The present work stems from our continued interest to enrich the chemistry of photocytotoxic Ln(III) complexes using their high coordination number and oxophilicity [34,35].

We have chosen terpyridine bases as photoactive organic ligands to achieve high photocytotoxicity in cancer cells. The Ln(III) complexes are expected to be non-toxic in dark owing to their redox stability thus making them suitable for cellular applications in the presence of reducing thiols, *viz*., glutathione. We have recently reported the La(III) and Gd(III) complexes $[Ln(dppz)_2(NO_3)_3]$ and $[Ln(dppz)(acac)_3]$ of dipyridophenazine (dppz) as DNA photocleaving agents showing photocytotoxicity in HeLa cells on irradiation with UV-A light of 365 nm while remaining essentially non-toxic in dark [34,35]. We have now designed and synthesized a series of lanthanide complexes having pyrene-appended terpyridine and acetylacetonate as ligands. The choice of pyrenyl terpyridine (py–tpy) as a ligand is based on the fact that tpy with a pendant pyrenyl moiety could serve as a photosensitizer-cum-DNA binder. The photoactive pyrenyl moiety, also being a fluorophore, could be used for confocal fluorescence microscopy to study the localization of the complex within the cancer cell and for its cytotoxicity on photoactivation. We have also prepared the corresponding phenyl analogues (ph–tpy complexes) as controls to understand the role of the pyrenyl moiety in the cellular damage. The *O*,*O*-donor β-diketonate ligand is chosen for strong complexing nature of the oxophilic lanthanide ions resulting in the formation of a stable complex. We have also used a carbohydrate-appended acetylacetonate ligand to increase the aqueous solubility of the complexes and to augment their cancer cell targeting potential. There is an upregulation of glycolysis and a decrease in the oxidative phosphorylation in cancer cells compared to the normal cells which results in inefficiency in the metabolism of cancer cells. Consequently, the glucose requirements of cancer cells are significantly higher than the normal cells for their uncontrolled growth and proliferation resulting in the overexpression of certain proteins known as GLUTs which form a class of transmembrane proteins mediating the transport of glucose to the cells [36,37]. We have designed the present complexes based on our dual strategy of combining imaging with therapy. In addition, the presence of the nitrate anions in the complexes would improve their aqueous solubility. The use of tridentate terpyridine, as compared to the bidentate phenanthroline bases, has led to higher coordination number of the complexes thus decreasing the possibilty of any hydrolytic DNA damage since lanthanides are known to display significant hydrolytic DNA cleavage activity [38].

Herein, we report the synthesis, characterization, DNA binding, DNA cleavage activity and photocytotoxicity of the lanthanide(III) complexes $[Ln(R\text{-}tpy)(acac)(NO_3)_2]$ (Ln = La(III), **1** and **2**; Gd(III), **4** and **5**) and $[Ln(py–tpy)(sacac)(NO_3)_2]$ (Ln = La(III), **3**; Gd(III), **6**), where R-tpy is an *N*,*N*,*N*-donor ligand, *viz*., 4′-phenyl-2,2′:6′,2″-terpyridine (ph–tpy in **1**, **4**), 4′-(1-pyrenyl)-2,2′:6′,2″-terpyridine (py–tpy in **2**, **3**, **5** and **6**), acac is acetylacetonate and sacac is 4-hydroxy-6-{4-[(β-D-glucopyranoside)oxy]phenyl}hex-3,5-dien-2-onate (Scheme 1). Complexes **1** as $[La(py–tpy)(sacac)(EtOH)(NO_3)_2]$ (**1a**) and **4** were structurally characterized by single crystal X-ray diffraction method.



Scheme 1. Schematic drawing of the La(III) and Gd(III) complexes **1**–**6** and the ligands used.

Significant results of this study include remarkable photocytotoxicity (PDT effect) of the pyrenyl terpyridine complexes in HeLa cells in UV-A light, while being less toxic in the dark. The py–tpy complexes showed cytosolic localization as evidenced from the confocal fluorescence imaging studies.

2. Chemistry

The substituted terpyridine ligand (ph–tpy and py–tpy) was prepared by a reaction of the corresponding aldehyde with 2-acetylpyridine in the presence of NaOH and subsequent condensation of the intermediate product with ammonium acetate in refluxing ethanol [39,40]. The glucose appended β-diketone ligand (Hsacac) was prepared in five synthetic steps. D-Glucose was acylated to get β-D-glucose pentaacetate which was subsequently reacted with Br_2/red P to afford the highly reactive α-acetobromoglucose. The α-acetobromoglucose was reacted with para-hydroxy benzaldehyde in the presence of NaOH using tetrabutylammonium bromide as the phase transfer catalyst to obtain the corresponding β-glycoside which was subsequently reacted with 2,4-pentatedione in presence of boric anhydride using dry DMF as a solvent to get the acetyl protected β-diketone ligand [41]. Finally, the deprotection of the acetyl group by standard method afforded the desired glucose appended β-diketone ligand (Hsacac) in moderate yield. Lanthanide(III) complexes **1**–**6** were prepared by a general method in two synthetic steps. Reaction of a CH_2Cl_2 solution of ph–tpy or py–tpy with an ethanol solution of the corresponding metal nitrate afforded the precursor complex $[Ln(ph–tpy)(NO_3)_3]$ or $[Ln(py–tpy)(NO_3)_3]$ in good yield. The precursor complex upon reaction with Hacac or Hsacac in the presence of triethylamine gave the desired complex in high yield (Scheme 1).

3. Pharmacology

The lanthanide(III) complexes **1**–**6** were evaluated for their DNA binding, DNA cleavage, and cytotoxic activities. Binding studies for the complexes with ct-DNA were done using spectroscopic methods such as electronic absorption titration and DNA melting and hydrodynamic method, *viz*., solution viscosity measurement of complex bound ct-DNA. The DNA cleavage activity of the complexes was studied by determining the ability of each complex to convert the supercoiled (SC) form of DNA to its nicked circular (NC) form. Photocytotoxicity of the complexes was assessed against HeLa cells by MTT assay [42]. The mechanistic aspects of cell death was studied by EB/AO dual staining method using fluorescence microscopy of the HeLa cells in the presence or absence of the