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Research paper

Synthesis, characterization and antitumor activity of two new dipyridinium ylide based lanthanide(III) complexes

Andreea Cârâc [a], Rica Boscencu [a], Rodica Mihaela Dinică [b], Joana F. Guerreiro [c], Francisco Silva [c], Fernanda Marques [c], Maria Paula Cabral Campello [c], Călin Moise [d], Oana Brîncoveanu [d], Marius Enăchescu [d], Geta Cârâc [b], Aurel Tăbăcaru [b,*]

[a] Faculty of Pharmacy, "Carol Davila" University of Medicine and Pharmacy, 6 Traian Vuia Street, 020956 Bucharest, Romania
[b] Department of Chemistry, Physics and Environment, Faculty of Sciences and Environment, "Dunarea de Jos" University of Galati, 111 Domneasca Street, 800201 Galati, Romania
[c] Centro de Ciências e Tecnologias Nucleares (C^2TN), Instituto Superior Técnico, Universidade de Lisboa, Portugal
[d] Center for Surface Science and Nanotechnology, Politehnica University of Bucharest (CSSNT-UPB), 313 Splaiul Independentei, 060042 Bucharest, Romania

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ABSTRACT

Two new dinuclear lanthanide(III) complexes, **La-DPY** and **Nd-DPY**, with the stoichiometric formula $[\{Ln(Et_3N)(SO_4)\}_2(\mu\text{-DPY})(\mu_4\text{-}SO_4)]$ (Ln = La, Nd; DPY = ylide form of DPB, Et_3N = triethylamine), were obtained through the reaction of *N*-heterocylic diquaternary salt *N,N'*-diphenacyl-4,4′-dipyridinium dibromide (DPB) and lanthanide(III) sulfate in methanol, in the presence of triethylamine. The obtained complexes were characterized by Fourier transform infrared spectroscopy, proton nuclear magnetic resonance, elemental analysis, UV–vis spectroscopy, thermogravimetric analysis and powder X-ray diffraction. Scanning electron microscopy (SEM) was used to investigate the morphology and particles size of the complexes, confirming that their particles are quite homogenous and uniform. The antitumor activity of the complexes was evaluated in the human cancer cells MCF7 and A2780 and compared to cisplatin, the metal-based drug in cancer therapy. The complexes were found to induce apoptotic cell death and, to a lesser extent, production of ROS, although these are not the unique mechanisms of action. In conclusion, we anticipate that these types of Ln(III) complexes have potential and could be further explored for applications as antitumor agents.

1. Introduction

Lanthanide (Ln) chemistry is currently a very active area of research. Due to their electronic structure these elements have unusual properties that make them suitable for applications in catalysis, organometallic synthesis, electronic and luminescent materials [1–11]. In the biomedical field, lanthanide complexes have received considerable attention, particularly in bioanalysis, imaging and radioimmunotherapy [2]. Recently, some lanthanide complexes have shown photocytotoxic activities in tumoral cells and hence with potential use in photodynamic therapy (PDT) [12–16].

As far as the clinical applications are concerned, lanthanides cannot be administered in the form of simple salts or metal ions, due to their toxic effects, but they can be administered in the form of thermodynamically and kinetically stable complexes. Upon coordination, the ligands play a key role in tuning the properties of the corresponding complexes [17], which is particularly relevant to biological, biochemical and medical applications [18].

Due to their size, lanthanide ions form stable complexes with high geometric flexibility and high coordination number [15–19]. As strong Lewis acids, lanthanide ions coordinate with highly electronegative donor atoms such as nitrogen or oxygen. *N*-donor ligands such as quaternary pyridinium derivatives present a positive charge at the nitrogen atom and therefore are expected to be more suitable for the synthesis of very large complexes of 4f-elements than O-donor ligands [9,20]. Nevertheless, the synthesis of lanthanide complexes with these types of ligands present certain limitations, *i.e.*, if anhydrous conditions are not met, the presence of a nitrogen donor ligand such as 4,4′-bipyridine can favor deprotonation of any coordinated water, with formation of undesired sub-products as hydroxo- or oxo-lanthanide derivatives [20,21].

Heterocyclic ligands present intrinsic versatility to form metal complexes with biomedical importance and, in this direction, a

* Corresponding author.
E-mail address: aurel.tabacaru@ugal.ro (A. Tăbăcaru).

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wide range of complexes with biological activities such as antibacterial, antifungal, antiviral, and antitumor activities have been reported [22–29]. Novel synthetic strategies with the advances made in coordination chemistry strongly influenced the design of prospective lanthanide-based drugs with higher selectivity and improved toxicological and pharmacokinetic profiles [30,31]. In this context, we describe here the synthesis and characterization of the sulfate complexes of La^{3+} and Nd^{3+} with the diquaternary *N,N'*-diphenacyl-4,4′-dipyridinium dibromide (DPB) proligand (Scheme 1). The antitumor activity of these complexes in cancer cell lines was performed and compared with cisplatin, the metal-based drug in clinical use, in order to allow their evaluation as chemotherapeutic agents. The mechanisms involved in cell death were also explored.

2. Experimental section

2.1. Materials and methods

All the chemicals and reagents were purchased from Sigma-Aldrich Co and used without further purification. The *N*-heterocylic diquaternary salt *N,N'*-diphenacyl-4,4′-dipyridinium dibromide (DPB) was prepared by the previously published method [32]. $Ln_2(SO_4)_3$ (Ln = La, Nd) was prepared by dissolving Ln_2O_3 in concentrated H_2SO_4. The solvents were used as supplied or distilled using standard methods.

Elemental analyses (C, H, N, S) were performed in-house with Fisons Instruments 1108 CHNS-O Elemental Analyzer. Before performing the analytical characterization, all samples were dried in vacuo (50 °C, $\sim 10^{-4}$ bar) until a constant weight was reached.

Melting points are uncorrected and were taken on an SMP3 Stuart instrument with a capillary apparatus.

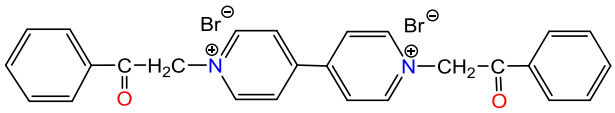
The IR spectra were recorded from 4000 to 450 cm^{-1} with a Spectrum Two IR spectrometer from PerkinElmer. In the following, the IR bands are classified as very weak (vw), weak (w), medium (m), strong (s) and very strong (vs).

The ^1H NMR spectrum of **La-DPY** was acquired at room temperature in dimethyl sulfoxide (DMSO) with a VXR-300 Varian spectrometer operating at 400 MHz, using tetramethylsilane (TMS) as internal standard. In the following, the ^1H NMR signals are classified as singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m). Due to the paramagnetism of Nd(III) ion, the ^1H NMR spectrum of **Nd-DPY** was not acquired.

UV–vis spectra were recorded in the 200–900 nm range for both the ligand and the two complexes at 10^{-5} M in ethanolic solutions using a LAMBDA 950 spectrophotometer from PerkinElmer. For the stability tests of the complexes, the UV–vis spectra, performed in 10^{-5} M DMSO solutions, were collected in a Shimadzu UV-1800 Spectrophotometer (range: 200–800 nm).

Thermogravimetric analyses (TGA) were carried out under a N_2 flow from room temperature to 1200 °C and with a heating rate of 10 °C/min using a PerkinElmer STA 6000 Simultaneous Thermal Analyzer.

Powder X-ray diffraction (PXRD) analyses were carried out in the 5–90° 2θ range on a Rigaku SmartLab X-ray diffractometer using Cu Kα radiation (λ = 0.154060 nm) in the general (Bragg Brentano Focusing) type of measurements, operating at room temperature. The generator was set at 45 kV and 200 mA.



Scheme 1. Structure of *N,N'*-diphenacyl-4,4′-dipyridinium dibromide (DPB).

Scanning electron microscopy (SEM) measurements were carried out using a Hitachi SU 8230 Scanning Electron Microscope equipped with a detector able to achieve the low angle backscattering electrons (LA-BSE), for both morphological and compositional contrast information.

2.2. Synthesis of Ln(III) complexes: General procedure

Complexes **La-DPY** and **Nd-DPY** were synthesized according to the following procedure: in two 50 mL round bottom flasks, 0.277 g of *N,N'*-diphenacyl-4,4′-dipyridinium dibromide (0.5 mmol) were dissolved in 20 mL of methanol under heating at 50–60 °C and stirring until complete dissolution. Lanthanum(III) sulfate (0.283 g, 0.5 mmol) or neodymium(III) sulfate (0.288 g, 0.5 mmol) were then added, and after several minutes of stirring, 1 mL of triethylamine was added. Then, deep violet precipitates started to appear, which were left under reflux and continuous stirring overnight. After cooling, the solid products were filtered off, washed three times with 5 mL of methanol and distilled water, and were finally dried under vacuo for 24 h.

[{La(Et$_3$N)(SO$_4$)}$_2$(μ-DPY)(μ$_4$-SO$_4$)] (***La-DPY***). Violet powder. Yield: 85%. M.p. 200–205 °C dec., weakly soluble in methanol, and dimethylformamide. *Anal.* Calc. for $C_{38}H_{50}La_2N_4O_{14}S_3$ (FW = 1160.83 g mol^{-1}): C, 39.32; H, 4.34; N, 4.83; S, 8.29%. Found: C, 39.15; H, 4.45; N, 4.62; S, 7.98%. IR ν(cm^{-1}): 3100–3000 (vw), 3000–2800 (vw), 1580 (m), 1542 (m), 1487 (vs), 1454 (s), 1420 (vs), 1336 (s), 1281 (w), 1223 (w), 1190 (vs), 1086 (w), 1014 (m), 943 (vw), 889 (s), 845 (w), 818 (s), 785 (w), 694 (vs), 664 (w), 503 (m). ^1H NMR (DMSO d_6, 400 MHz): δ, 1.56q, 3.28 t (30H, (CH_3-CH_2)$_3$N), 5.60 s (2H, $CH_{methine}$), 7.14–7.30 m (10*H*, CH_{phenyl}), 7.60d (*J* = 8.4 Hz, 4H, $CH_{bipyridyl}$), 8.65d (*J* = 8.4 Hz, 4H, $CH_{bipyridyl}$).

[{Nd(Et$_3$N)(SO$_4$)}$_2$(μ-DPY)(μ$_4$-SO$_4$)] (***Nd-DPY***). Violet powder. Yield: 81%. M.p. 200–205 °C dec., weakly soluble in methanol, and dimethylformamide. *Anal.* Calc. for $C_{38}H_{50}Nd_2N_4O_{14}S_3$ (FW = 1171.50 g mol^{-1}): C, 38.96; H, 4.30; N, 4.78; S, 8.21%. Found: C, 38.75; H, 4.48; N, 4.57; S, 7.89%. IR ν(cm^{-1}): 3100–3000 (vw), 3000–2800 (vw), 1580 (m), 1542 (m), 1487 (vs), 1454 (s), 1420 (vs), 1336 (s), 1281 (w), 1223 (w), 1190 (vs), 1086 (w), 1014 (m), 943 (vw), 889 (s), 845 (w), 818 (s), 785 (w), 694 (vs), 664 (w), 503 (m).

2.3. Biological assays

2.3.1. Cellular viability by the MTT assay

Human tumor cell lines, breast MCF7 and ovarian (cisplatin sensitive) A2780, were cultured in RPMI (A2780) or DMEM culture media (Gibco) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics at 37 °C, 5% CO_2 in a humidified atmosphere (Heraeus, Germany). The cells were adherent in monolayers and upon confluence were harvested by digestion with trypsin-EDTA (Gibco). Cell viability was evaluated using the tetrazolium salt MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]), which is reduced by a mitochondrial succinate dehydrogenase in metabolically active cells to insoluble purple formazan crystals [33]. For the assays, cells were seeded in 96-well plates at a density that ensures exponential growth of controls (untreated cells) throughout the experiments. Cells ($2\text{-}3 \times 10^4$ cells/200 μL medium) were allowed to settle overnight followed by the addition of dilution series of the compounds in fresh medium in aliquots of 200 μL per well. Ligand and complexes were first solubilized in DMSO and then in medium, and added to final concentrations from 1 μM to 50 μM. The final concentration of DMSO in cell culture medium did not exceed 1%. After continuous exposure to the compounds for 24 h and 48 h at 37 °C/5% CO_2, the medium was removed, and the cells were incubated with 200 μL of MTT solution in phosphate buffer saline (PBS) (0.5 mg/mL). After 3 h at 37 °C/5%