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Synthesis, characterization and preliminary cytotoxicity evaluation of five Lanthanide(III)–Plumbagin complexes

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ABSTRACT

Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone, H-PLN) was isolated from *Plumbago zeylanica*, the anticancer traditional Chinese medicine (TCM). Five new lanthanide(III) complexes of deprotonated plumbagin: $[Y(PLN)_3(H_2O)_2]$ (**1**), $[La(PLN)_3(H_2O)_2]$ (**2**), $[Sm(PLN)_3(H_2O)_2]\cdot H_2O$ (**3**), $[Gd(PLN)_3(H_2O)_2]$ (**4**), and $[Dy(PLN)_3(H_2O)_2]$ (**5**) were synthesized by the reaction of plumbagin with the corresponding lanthanide salts, in amounts equal to ligand/metal molar ratio of 3:1. The PLN–lanthanide(III) complexes were characterized by different physicochemical methods: elemental analyses, UV–visible, IR and ^1H NMR and ESI-MS (electrospray ionization mass spectrum) as well as TGA (thermogravimetric analysis). The plumbagin and its lanthanide(III) complexes **1**–**5**, were tested for their *in vitro* cytotoxicity against BEL7404 (liver cancer) cell lines by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The five PLN–lanthanide (III) complexes **1**–**5** effectively inhibited BEL7404 cell lines growth with IC_{50} values of 11.0 ± 3.5, 5.1 ± 1.3, 6.1 ± 1.1, 6.4 ± 1.3, and 9.8 ± 1.5 μM, respectively, and exhibited a significantly enhanced cytotoxicity compared to plumbagin and the corresponding lanthanide salts, suggesting a synergistic effect upon plumbagin coordination to the Ln(III) ion. The lanthanide complexes under investigation also exerted dose- and time-dependent cytotoxic activity. $[La(PLN)_3(H_2O)_2]$ (**2**) and plumbagin interact with calf thymus DNA (ct-DNA) mainly *via* intercalation mode, but for $[La(PLN)_3(H_2O)_2]$ (**2**), the electrostatic interaction should not be excluded; the binding affinity of $[La(PLN)_3(H_2O)_2]$ (**2**) to DNA is stronger than that of free plumbagin, which may correlate with the enhanced cytotoxicity of the PLN–lanthanide(III) complexes.

1. Introduction

Since the success of cisplatin and related platinum complexes as anticancer agents, developing other active transition metal anticancer complexes with better efficiency has attracted many bioinorganic chemists' interest and become a central research theme in bioinorganic chemistry [1–5]. Lanthanide complexes have attracted medicinal inorganic chemists' attention, because lanthanides manifest an antitumor activity and may be developed into future anticancer drugs. In the past twenty years, a number of lanthanide complexes have been synthesized and their cytotoxicities evaluated. Some examples are La(III) complexes with 1,10-phenanthroline-2,9-bis-α-amino acid conjugates [6] or 1,10-phenanthroline [7], coumarines [8–10], 3,5-pyrazoledicarboxylic acid [11], Sm(III) and Gd(III) complexes with acenocoumarol [12], cerium(III) and neodymium(III) complexes with 5-aminooritic acid [13] and many other lanthanide complexes [14,15].

In order to develop new metal-based anti-cancer drugs, recently, new strategies have been applied in the designs of antitumor coordination compounds as drugs, such as synthesizing new ligands or metal complexes with different reaction mechanisms. Among them, new coordination compounds based on the traditional Chinese medicines (TCMs) provide a novel approach to potential (pro-)drugs [16–23], which have been recently reviewed by Chen and Liang [24].

It is well known that over long-term folk practice, a large number of TCMs have been screened and used for treating and preventing various chronic conditions, such as cancer, atherosclerosis, aging, diabetes, and other degenerative diseases [25,26]. Plumbagin, 5-hydroxy-2-methyl-1,4-naphthoquinone (H-PLN, Scheme 1) is a potent toxic natural product extracted from *Plumbago zeylanica L.* (*Plumbaginaceae*), which has been used in China and other Asian countries for the treatment of rheumatoid arthritis, dysmenorrhea, injury by bumping, and even cancer [27–29]. Plumbagin is structurally derived from quinones which are a broadly distributed class of

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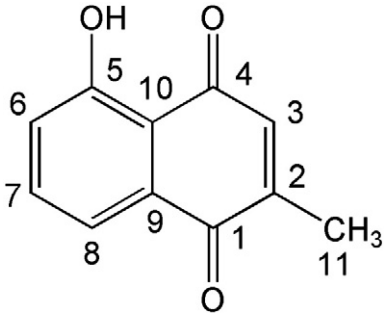

Scheme 1. Plumbagin (H-PLN).

naturally occurring substances with a variety of biological activities, e.g. antibacterial, anticancer, antioxidant and anti-inflammatory. Earlier studies have demonstrated that the metal complexes of many quinones chemotherapeutic drugs such as daunorubicin and adriamycin used today, display less cardiotoxicity than the parent drug but effectively kill p388 leukemia [30]. These findings have stimulated increasing interest in the study of the metal complexes of plumbagin with an antitumor activity. Although the lanthanide plumbaginates and their antibacterial activity have been reported previously [31–35], but firstly, the cytotoxicity of the traditional Chinese medicine (TCM) plumbagin in its copper chemistry has been reported recently. It was observed that these copper(II) plumbaginates exhibited a significantly enhanced cytotoxicity vs. free plumbagin [20].

As a part of our continuing work on the synthesis, characterization and application of metal complexes with plumbagin [20], herein, we report the synthesis, characterization and *in vitro* cytotoxicity against BEL7404 cell lines of five new lanthanide plumbaginates. The binding properties of plumbagin and $[La(PLN)_3(H_2O)_2]$ (**2**) to DNA were investigated by means of UV–visible (UV–vis), fluorescence, circular dichroism (CD) spectroscopy, and agarose gel electrophoresis assay.

2. Experimental section

2.1. Materials

All the metallic salts were purchased from Alfa co. Ltd. The solvents used were analytical grade. All the materials were used as received without further purification unless noted specifically. Tris–HCl–NaCl buffer solution (5 mM Tris, 50 mM NaCl), pH was digitally adjusted to 7.35 by titration with hydrochloric acid with Sartorius professional meter, Tris was prepared using double distilled water. Calf thymus DNA (ct-DNA) was purchased from Sino-American Biotech. co. Ltd, Beijing of China. They were both used without further purification. A Tris-buffer solution of ct-DNA gave ratios of UV absorbance at 260 and 280 nm of ca. 1.8 − 1.9:1, indicating that the DNA was sufficiently free of protein. The DNA concentration per nucleotide in base pairs was determined spectrophotometrically by employing a molar absorptivity (6600 M^{-1} cm^{-1}) at 260 nm. Stock solution were stored at 4 °C and used for no more than 4 days.

Traditional Chinese medicine material, roots of *P. zeylanica* were collected in Guangxi province of China, in September, 2004 and identified by Prof. S. Q. Tang (School of Life Science, Guangxi Normal University). A voucher specimen was deposited at the School of Chemistry & Chemical Engineering, Guangxi Normal University of China.

2.2. Instrumentation

Infrared spectra were obtained on a Perkin-Elmer FT-IR Spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-500 NMR spectrometer using CD_3Cl or DMSO-d_6 as a solvent. Elemental analyses (C, H) were carried out on a Perkin Elmer Series II CHNS/O 2400 elemental analyzer. ESI-MS (electrospray ionization mass spectrum) were recorded on a Bruker HCT Electrospray Ionization Mass Spectrometer. TGA (thermogravimetric analysis) were recorded on Perkin-Elmer Pyris Diamond TG/DTA analyzer. UV–visible (UV–vis) absorption spectra were performed on a Varian Cary100 UV–Visible spectrophotometer. Fluorescence measurements were performed on a Shimadzu RF-5301/PC spectrofluorophotometer. The circular dichroic spectra of DNA were obtained by using a JASCO J-810 automatic recording spectropolarimeter operating at 25 °C. The region between 220 and 320 nm was scanned for each sample. Microscope images were recorded on Nikon TE2000.

2.3. Isolation and structure identification of plumbagin

Dried and powdered roots (15 kg) of *P. zeylamca* were successively and exhaustively extracted with 95% ethanol at 50 °C. The ethanol extracted solution was concentrated under reduced pressure to give a dark brown residue. The residue was suspended in water, and then partitioned successively with n-hexane and EtOAc, respectively, to afford the residues of n-hexane (23 g) and EtOAc (252 g), respectively. The EtOAc soluble-extract (252 g) was purified by a silica gel column chromatography using n-hexane-EtOAc (from 9:1 to 0:1, v/v) as a solvent system to give six fractions (F1–F6). Orange needle-like crystals of plumbagin were obtained from fraction 1 (F1) by recrystallizing in n-hexane-EtOAc (5:1), with a purity of over 98% as determined by HLPC (by peak area normalization). The total yield of plumbagin obtained after extraction and purification by column chromatography was 13.3 g (0.09% basing on the dried and powdered roots (15 Kg) of *P. zeylamca*). IR (ν, cm^{-1}, KBr, s = strong, m = medium, w = weak): 3445 m, 1663 s, 1645 s, 1567 m, 1456 m, 1365 m, 1229 m, and 752w. ^{1}H NMR ($CDCl_3$, 500 M Hz) (δ ppm): δ2.22 (3 H, s(singlet), -CH_3), δ 6.83 (1H, s, H-3), δ 7.28 (1H, d (doublet), J = 8.5 Hz, H-6), δ 7.60 (1H, d, J = 6.2 Hz, H-8), δ 7.66 (1H, d, J = 7.5 Hz, H-7), δ11.99(1H, s, -OH), ^{13}C NMR ($CDCl_3$, 125 M Hz) (δ, ppm): δ 16.5(-CH_3), δ 115.2(C-10), δ119.3(C-8), δ124.2(C-6), δ 132.0 (C-9), δ135.5(C-7), δ 136.1(C-3), δ149.6(C-2), δ161.2(C-5), δ184.8(C-1), δ190.3(C-4).

2.4. Synthesis of $[Y(PLN)_3(H_2O)_2]$ (**1**)

An ethanolic solution (15 mL) of plumbagin (0.564 g, 3 mmol) was added to an aqueous solution (15 mL) of $YCl_3 \cdot 6H_2O$ (0.303 g, 1 mmol), and was adjusted pH to 6.0 with dilute ammonia solution. The reaction mixture was refluxed and stirred with an electromagnetic stirrer for 2 h. At the moment of mixing of the solutions, red brown precipitate was obtained. After cooling to room temperature, the precipitate was filtered, washed three times with water and ethanol, and dried in a desiccators containing P_2O_5 to constant weight. Finally the red brown solid of $[Y(PLN)_3(H_2O)_2]$ (**1**) was obtained. Yield: 0.38 g (56% yield basing on plumbagin); *Anal.* Calc. for $C_{33}H_{25}O_{11}Y$: C, 57.74; H, 3.67. Found: C, 57.65; H, 3.53%. IR (ν, cm^{-1}, KBr): 3433 m, 1642 m, 1604 s, 1424 m, 1254 m, 622w. ESI-MS (in DMSO-H_2O-CH_3OH): *m/z* 668.9 (Calc. 669.4) $[Y(PLN)_3 + H_2O + H]^+$, *m/z* 463.1 (Calc. 463.2) $[Y(PLN)_2]^+$. UV–Vis (DMSO): $\lambda_{max} = 270$, $\lambda_{max} = 424$ nm.

2.5. Synthesis of $[La(PLN)_3(H_2O)_2]$ (**2**)

A purple solid of $[La(PLN)_3(H_2O)_2]$ (**2**) was synthesized by the same method as that employed for $[Y(PLN)_3(H_2O)_2]$ (**1**) using $LaCl_3 \cdot 6H_2O$ to replace $YCl_3 \cdot 6H_2O$ (plumbagin 3 mmol, $LaCl_3 \cdot 6H_2O$ 1 mmol). Yield: 0.40 g (55% yield basing on plumbagin); *Anal.* Calc. for $C_{33}H_{25}LaO_{11}$: C, 53.82; H, 3.42. Found: C, 54.71; H, 3.31%. IR (ν, cm^{-1}, KBr): 3433 m, 1642 m, 1610 s, 1424 m, 1251 m, 614w. ^{1}H NMR(DMSO-d_6, 500 M Hz) (δ ppm): δ2.01 (3 H, s, -CH_3), δ 6.63 (1 H, s, H-3), δ 6.87