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Iron(III) benzhydroxamates of dipicolylamines for photocytotoxicity in red light and cellular imaging

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ABSTRACT

Benzhydroxamate (BHA) iron(III) complexes [Fe(BHA)(L)Cl]Cl (**1**, **2**), where L is (phenyl)dipicolylamine (phdpa in **1**) and (pyrenyl)dipicolylamine (pydpa in **2**), were prepared and their photocytotoxicity in visible (400–700 nm) and red (600–720 nm) light was studied. Complex **1** was structurally characterized by X-ray crystallography. The complexes have high-spin iron(III) centers. Complex **2**, with a pyrenyl fluorophore, was used for cellular imaging, showing both mitochondrial and nuclear localization in the fluorescence microscopic study. The complex exhibited photocytotoxicity in red light in HeLa cancer cells, giving IC_{50} value of 24.4(±0.4) μM, but remained essentially non-toxic in the dark. The involvement of reactive oxygen species and an apoptotic nature of cell death were observed from the cellular studies.

1. Introduction

Photodynamic therapy (PDT) is a non-invasive method for the treatment of cancer in which an administered drug, on photoactivation at the cancer site, generates a reactive oxygen species (ROS) which selectively damages only the photo-exposed cancer cells, leaving the unexposed healthy cells unaffected [1–7]. Macrocyclic organic dyes based on porphyrin, phthalocyanine and related compounds have been extensively studied as potential PDT agents with Photofrin® having FDA approval as a PDT drug. These compounds generate singlet oxygen ($^1O_2$) as the ROS via a type-II energy transfer pathway. The efficacy of such organic dyes thus largely depends upon their ability to generate $^1O_2$ in the cancer cells [8]. The drawbacks associated with Photofrin® are prolonged skin sensitivity and hepatotoxicity due to formation of bilirubin as a breakdown product of the drug, thus limiting its therapeutic potential [9,10]. An alternate and useful approach to circumvent these predicaments could be designing and synthesizing redox active metal-based PDT agents that are capable of showing similar therapeutic effects in red light through type-I and/or photoredox pathways generating ROS [11]. Biocompatible 3d-metal complexes, with their versatile coordination geometries, varied spectral and redox properties, could be suitably designed to achieve the basic requirements of PDT. While several metal complexes have been studied earlier for their PDT activity, the potential of iron complexes in PDT remains virtually unexplored [12–20]. The present work stems from our interest to develop the chemistry of iron-based PDT agents. There are a few notable recent developments in the chemistry of metal-based photocytotoxic agents. Sadler and co-workers have reported a six-coordinate platinum(IV) complex as a photo-activated metallo-drug with two *trans*-azide ligands, which is stable in the dark, but generates a *trans*-(diguanidine)-platinum(II) adduct on photoactivation, showing cytotoxicity [12,13]. Photocytotoxic nitrosyl ruthenium complexes are reported for site-specific delivery of nitric oxide (NO) on exposure to visible light [14]. Dirhodium(II) complexes have been shown to cause oxidative DNA damage in visible light through both oxygen-dependent and independent pathways [15]. We have reported the photocytotoxicity of copper(II) and oxovanadium(IV) complexes in visible light [16–19]. Although oxovanadium(IV) complexes show low dark cytotoxicity, the low molar extinction coefficient (ε) values of their d–d band near 700 nm make them less effective as near-IR light photosensitizers. Copper(II) complexes are generally unsuitable as PDT agents due to their high dark cytotoxicity, resulting from the reduction of the metal by glutathione and other cellular thiols generating radical species [21,22].

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We have now designed some new iron(III) complexes having the naturally occurring chelating biocompatible hydroxamate ligand and dipicolylamine with a pendant planar pyrenyl moiety as a fluorophore. Iron is an essential bio-element and its complexes, showing significant PDT activity in visible light, could have better therapeutic utility [20]. Herein, we report the synthesis, structure and visible light-induced cytotoxicity of two benzhydroxamate (BHA) iron(III) complexes, *viz.* [Fe(BHA)(L)Cl]Cl (**1**, **2**) where L is (phenyl)dipicolylamine (phdpa in **1**) and (pyrenyl)dipicolylamine (pydpa in **2**) (Fig. 1). A significant result of this study is the observation of mitochondrial localization of the pyrenyl complex **2** from fluorescence microscopy. Photofrin® is also known to localize in the mitochondria for drug action. With the intrinsic pathway of apoptosis largely depending on the mitochondria, targeting this cellular organelle with a suitably designed compound could result in desirable photo-induced cytotoxicity [23–25]. Mitochondria targeting anticancer agents are likely to overcome the resistance mechanism in the conventional chemotherapeutic drug action, thus increasing the potential of the PDT drug. Complex **2** also showed a remarkable photocytotoxic effect in HeLa cancer cells in visible light of 400–700 nm and red light of 600–720 nm, while remaining essentially non-toxic in the dark.

2. Experimental

2.1. Materials and methods

All reagents and chemicals were purchased from commercial sources (S.D. Fine Chemicals, India; Aldrich-Sigma, USA; Invitrogen Bio Services, India) and were used without further purification. Supercoiled pUC19 DNA (CsCl purified) was procured from Bangalore Genie (India). Calf thymus (ct) DNA, agarose (molecular biology grade), distamycin, methyl green, catalase, SOD and ethidium bromide were obtained from Sigma (USA). Tris–(hydroxymethyl)aminomethane-HCl (Tris–HCl) buffer was prepared using deionised and sonicated triple distilled water. The solvents used for the synthesis, electrochemical and spectral measurements were purified by conventional procedures. The dipicolylamine derivatives, viz. N-benzyl-1-(pyridin-2-yl)-N-[(pyridin-2-yl)methyl]-methanamine (phdpa) and N-[(pyren-1-yl)methyl]-1-(pyridin-2-yl)-N-[(pyridin-2-yl)methyl]-methanamine (pydpa) were prepared following literature methods [26,27].

The elemental analyses were performed with a Thermo Finnigan FLASH EA 1112 CHNS analyzer. The infrared, absorption and emission spectra were recorded with Perkin-Elmer Lambda 35, Perkin–Elmer Lambda 650 and Perkin-Elmer LS 55 spectrophotometers, respectively, at 25 °C. Molar conductivity measurements were done with a Control Dynamics (India) conductivity meter. Electrochemical measurements were made at 25 °C with an EG&G PAR model 253 VersaStat potentiostat/galvanostat with electrochemical analysis software 270, using a three-electrode setup consisting of a glassy carbon working, platinum wire auxiliary and a saturated calomel reference electrode (SCE).

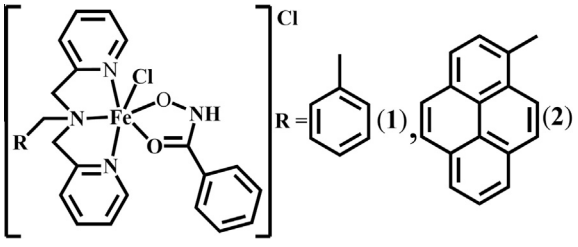

Fig. 1. Schematic drawings of the complexes **1** and **2**.

Tetrabutylammonium perchlorate (TBAP, 0.1 M) was used as a supporting electrolyte for the electrochemical measurements. ESI-MS measurements were carried out with a Bruker Daltonics make Esquire 300Plus ESI model. Flow cytometric analysis was performed with a FACS Calibur (Becton Dickinson (BD)) cell analyzer at the FL2 channel (595 nm). Fluorescence microscopy images were recorded with Olympus IX 81 microscope. Cellular uptake measurements were performed with an inductively coupled plasma optical emission spectrometer, Perkin–Elmer ICP-OES (Model Optima 2000 DV).

2.2. Preparation of [Fe(BHA)(L)Cl]Cl (L = phdpa, *1*; pydpa, *2*)

To a methanolic solution of ferric chloride (0.16 g, 1.0 mmol) was added the dipicolylamine base (L, 0.29 g for **1** and 0.41 g for **2**, 1.0 mmol) dissolved in methanol, and the solution was stirred for 30 min to get a precipitate of the precursor complex [Fe(L)Cl_3], which was filtered and air dried [Yield: for **1**, 0.39 g (~85%); for **2**, 0.52 g (~90%)]. To the suspension of the precursor complex (L, 0.45 g for **1** and 0.57 g for **2**, 1.0 mmol) was added dropwise a solution of benzhydroxamic acid (0.14 g, 1.0 mmol) and triethylamine (0.10 g, 1.0 mmol), also in methanol, to give a deep purple colored solution, which on slow evaporation of the solvent gave a solid that was isolated, washed with diethyl ether and finally dried in a vacuum over P_4O_{10}.

*[Fe(BHA)(phdpa)Cl]Cl·H_2O (**1**·H_2O):* Yield: 0.42 g (~75%). *Anal.* Calc. for $C_{26}H_{27}Cl_2FeN_4O_3$ (MW: 570.27): C, 54.76; H, 4.77; N, 9.82. Found: C, 54.49; H, 5.07; N, 9.53%. ESI-MS in MeOH, *m/z*: 512.17 $[M-2Cl^- + MeO^-]^+$, 480.52 $[M-2Cl^- - H^+]^+$. IR (solid phase, cm^{-1}): 3397 (br), 2944 (m), 2600 (m), 2490 (m), 1604 (s), 1480 (s), 1438 (s), 1345 (m), 1150 (s), 1026 (s), 913 (s), 810 (s), 760 (s), 706 (s), 540 (w), 479 (w) (br, broad; s, strong; m, medium; w, weak). UV–Vis (DMF) λ_{max}, nm (ε, $M^{-1}\,cm^{-1}$): 454 (1420), 268 (6380). Molar conductance in DMF at 25 °C: Λ_M = 67 S m^2 M^{-1}. μ_{eff} = 5.85 μ_B at 298 K.

*[Fe(BHA)(pydpa)Cl]Cl·H_2O (**2**·H_2O):* Yield: 0.54 g (~79%). *Anal.* Calc. for $C_{36}H_{31}Cl_2FeN_4O_3$ (MW: 694.41): C, 62.27; H, 4.50; N, 8.07. Found: C, 62.50; H, 4.76; N, 8.21%. ESI-MS in MeOH, *m/z*: 636.27 $[M-2Cl^- + MeO^-]^+$, 604.47 $[M-2Cl^- - H^+]^+$, 640.07 $[M-Cl^-]^+$. IR (solid phase, cm^{-1}): 3377 (br), 2933 (m), 2666 (m), 1603 (s), 1490 (s), 1448 (s), 1345 (m), 1140 (m), 1070 (m), 1030 (m), 913 (m), 850 (s), 768 (s), 685 (s), 553 (w), 490 (w). UV–Vis (DMF) λ_{max}, nm (ε, $M^{-1}\,cm^{-1}$): 466 (1530), 345 (22,100), 328 (15,800), 315 (8340), 277 (24,190), 267 (18,480). Molar conductance in DMF at 25 °C: Λ_M = 72 S m^2 M^{-1}. μ_{eff} = 5.88 μ_B at 298 K.

2.3. X-ray crystallographic procedure

The crystal structure of [Fe(BHA)(phdpa)Cl]Cl·H_2O (**1**·H_2O) was obtained by the single crystal X-ray diffraction method. Crystals of the composition **1**·H_2O were isolated from a methanol solution of the complex on slow evaporation. Crystal mounting was done on a glass fibre with epoxy cement. All geometric and intensity data were collected at room temperature using an automated Bruker SMART APEX CCD diffractometer equipped with a fine-focus 1.75 kW sealed-tube Mo Kα X-ray source (λ = 0.71073 Å) with increasing ω (width of 0.3° per frame) at a scan speed of 5 s per frame. Intensity data, collected using the ω–2θ scan mode, were corrected for Lorentz-polarization effects and for absorption [28]. The structure solution was done by a combination of Patterson and Fourier techniques and refined by full-matrix least-squares methods using the SHELX system of programs [29]. Hydrogen atoms of the complex were placed in their calculated positions and refined using a riding model. The non-hydrogen atoms were refined anisotropically. A molecular view was obtained using ORTEP [30].