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# Iron(III) salicylates of dipicolylamine bases showing photo-induced anticancer activity and cytosolic localization

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ABSTRACT

An iron(III) salicylate having a dipicolylamine base (andpa) with a photoactive anthracenyl moiety is prepared, characterized, and studied for its photo-induced anticancer activity and cellular localization in HeLa and MCF-7 cells. Its phenyl analogue is structurally characterized by X-ray crystallography. The complex has a ternary structure in which the dipicolylamine ligand and salicylic acid in dianionic form (sal) display respective tridentate and bidentate mode of coordination in [Fe(sal)(phdpa)Cl] (**1**). Complex [Fe(sal)(andpa)Cl] (**2**) having a pendant anthracenyl moiety shows significant photocytotoxicity in visible light (400–700 nm) giving $IC_{50}$ values of 8.6 ± 0.7 and 3.4 ± 0.9 μM in HeLa and MCF-7 cells, while being essentially nontoxic in the dark ($IC_{50}$ > 100 μM). The complex shows cytosolic localization in the cancer cells. Formation of hydroxyl radicals (·OH) as the reactive oxygen species is evidenced from the pUC19 DNA photocleavage studies.



## 1. Introduction

Iron–bleomycins (Fe–BLMs) as metalloglycopeptides are the naturally occurring anticancer antibiotics [1,2]. Their successful use in cancer therapy has led to the development of the chemistry of synthetic iron complexes as structural models and potent chemotherapeutic agents [3–8]. Iron–bleomycins act as chemical nucleases in which the metal in its high-spin +2 oxidation state activates molecular oxygen to form reactive hydroxyl species that oxidizes the deoxyribose sugar moiety causing damage to DNA [9]. There are only few synthetic models of iron–bleomycins reported in the literature using N-donor multidentate ligand systems stabilizing the metal in its +2 and +3 oxidation states [6–8]. Unlike the natural iron–bleomycins, these model complexes lack any selectivity towards cancer cells over normal cells. The currently available synthetic models are thus likely to damage the healthy cells due to their low $IC_{50}$ values in the dark [6]. A convenient way to circumvent this predicament is to design iron complexes that are nontoxic in the dark under aerobic conditions but gains activity only when exposed to visible light of suitable wavelength. We have earlier shown that iron complexes could be suitably designed to show photo-induced anticancer activity [10–15]. Besides, complexes of other metal ions are also known to show photochemotherapeutic activity [16–25]. Six-coordinate platinum(IV) complexes having two trans-azide ligands are known to generate reactive platinum(II) species on photoactivation showing significant cytotoxicity even in cisplatin resistant cells [16]. Photocytotoxic nitrosyl ruthenium complexes are used to deliver toxic nitric oxide (NO) on exposure to visible light [17]. Dirhodium(II) complexes are reported to show cytotoxicity in visible light [18]. Copper(II) and oxovanadium(IV) complexes having photoactive moieties are known to show excellent PDT effect in visible light [19–22]. Photodynamic therapy (PDT) has evolved as a non-invasive treatment modality of cancer with promising results [23–25]. Porphyrin derivatives with the hematoporphyrin species Photofrin® is currently used as a PDT drug. With the advent of this new methodology, metal-based photochemotherapeutic agents have gained considerable importance as substitutes of the porphyrin-based PDT agents [26,27].

The present work stems from our interest to design new iron complexes which can show significant anticancer activity on photo-irradiation while remaining essentially inactive in the dark. We have chosen iron for being an essential metal for cellular growth including that for cancer cells [28]. The presence of large number iron receptors on cancer cells can be used to successfully transport iron-based anticancer agents into the cell [29]. Besides, there is no report on any major adverse impact of the metal in iron–bleomycins except pulmonary fibrosis [30]. A stable iron

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complex under physiological conditions can overcome the challenges associated with this metal. We have used dipicolylamine base having a pendant photoactive moiety for achieving the desired PDT activity. Salicylic acid in its dianionic form is used for its bio-essential nature and for its affinity to bind iron. Using these ligand systems we have been able to synthesize ternary complexes that show reduced chemical nuclease activity in the presence of reducing cellular thiols. The dipicolylamine base (andpa) having a pendant anthracenyl moiety as a fluorophore is used as a photosensitizer and to study cellular localization of the complex. Herein, we report the synthesis, crystal structure and visible light-induced cytotoxicity of two iron(III) salicylates, viz. [Fe(sal)(phdpa/andpa)Cl] (**1**, **2**), where phdpa is (phenyl)dipicolylamine (in **1**) and andpa is (anthracenyl)dipicolylamine (in **2**) (Fig. 1). Significant results of this study are the observation of remarkable PDT effect of the andpa complex **2** in visible light (400–700 nm) in cervical cancer HeLa and breast cancer MCF-7 cells along with cytosolic localization of the complex from the fluorescence microscopy.

## 2. Experimental

### 2.1. Materials and measurements

The chemicals and reagents were procured from the commercial sources (s.d. Fine Chemicals, India; Aldrich–Sigma, USA; Invitrogen Bio Services, India). They were used without further purification. Supercoiled plasmid pUC19 DNA (CsCl purified) was from Bangalore Genie (India). Tris-(hydroxymethyl)aminomethane–HCl (Tris–HCl) buffer was prepared using deionised and sonicated triple distilled water. Solvents used for this work were purified by standard procedures. Dipicolylamine bases, viz. *N*-benzyl-1-(pyridin-2-yl)-*N*-[(pyridin-2-yl)methyl]-methanamine (phdpa) and 1-(anthracen-9-yl)-*N*,*N*-bis(pyridin-2-ylmethyl)methanamine (andpa) were prepared following literature methods [31,32].

The elemental analyses were done with a Thermo Finnigan FLASH EA 1112 CHNS analyzer. The infrared, absorption and emission spectral measurements were done using Perkin-Elmer make model Lambda 35, Lambda 650 and LS 55 spectrophotometer, respectively, at 25 °C. Molar conductivity measurements were made using a Control Dynamics (India) conductivity meter. Electrochemical studies were done at 25 °C with an EG&G PAR model 253 VersaStat potentiostat/galvanostat with electrochemical analysis software 270 using a three-electrode setup that consists of a glassy carbon working, platinum wire auxiliary and a saturated calomel reference electrode (SCE). Tetrabutylammonium perchlorate (TBAP, 0.1 M) was used as a supporting electrolyte. ESI-MS measurements were made with a Bruker Daltonics make Esquire 300Plus ESI model. Flow cytometric analysis was performed using a FACS Verse (Becton Dickinson (BD)) cell analyzer at FL2 channel (595 nm). Fluorescence microscopy images were obtained from Zeiss LSM5 10 apochromat confocal laser scanning microscope. Magnetic measurements at 298 K were done using Sherwood Scientific, Cambridge (U.K.), magnetic susceptibility balance.

### 2.2. Preparation of [Fe(sal)(phdpa/andpa)Cl] (phdpa, *1*; andpa, *2*)

To a methanol solution of ferric chloride (0.16 g, 1.0 mmol) was added the dipicolylamine base (0.29 g phdpa for **1** and 0.39 g andpa for **2**, 1.0 mmol) dissolved in methanol (20 mL). The solution was stirred for 30 min to get a precipitate of the precursor complex [Fe (phdpa/andpa)$Cl_3$] to which was added drop-wise a solution of salicylic acid ($H_2$sal, 0.14 g, 1.0 mmol) and triethylamine (0.10 g, 1.0 mmol) in 10 mL methanol to obtain 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 vacuum over $P_4O_{10}$.

*[Fe(sal)(phdpa)Cl] (**1**):* Yield: 0.42 g (75%). *Anal.* Calc. for $C_{26}H_{23}ClFeN_3O_3$ (MW: 516.78): C, 60.43; H, 4.49; N, 8.13. Found: C, 60.69; H, 4.27; N, 7.80. ESI-MS in MeOH: *m*/*z* 481.1038 $[M - Cl^-]^+$. IR (in solid phase, $cm^{-1}$): 2940 (m), 2650 (m), 2370 (m), 1600 (s), 1500 (s), 1390 (s), 1280 (m), 1150 (s), 1030 (s), 975 (s), 815 (w), 770 (m), 730 (m), 550 (w), 450 (m) $cm^{-1}$ (s, strong; m, medium; w, weak). UV–Vis (DMF) $\lambda_{max}$, nm (ε, $M^{-1}$ $cm^{-1}$) = 450 (1300), 290 (3360). Molar conductance in DMF at 25 °C: $\Lambda_M$ = 69 S $m^2$ $M^{-1}$. $\mu_{eff}$ = 5.85 $\mu_B$ at 298 K.

*[Fe(sal)(andpa)Cl] (**2**):* Yield: 0.54 g (79%). *Anal.* Calc. for $C_{34}H_{27}ClFeN_3O_3$ (MW: 616.90): C 66.20, H 4.41, N 6.81. Found: C 65.50, H 4.67, N 6.61. ESI-MS in MeOH: *m*/*z* = 580.99 $[M - Cl^-]^+$. IR (in solid phase, $cm^{-1}$): 2950 (br), 2600 (m), 2490 (m), 2350 (s), 1660 (m), 1580 (s), 1450 (s), 1360 (m),1270 (m), 1230 (m), 1150 (m), 1000 (m), 815 (s), 750 (s), 650 (s), 570 (w), 530 (w), 470 (w), 430 (w) $cm^{-1}$ (br, broad). UV–Vis (DMF) $\lambda_{max}$, nm (ε, $M^{-1}$ $cm^{-1}$): 450 (1256), 390 (4450), 370 (4470), 350 (3200), 333 (2440), 300 (5350). Molar conductance in DMF at 25 °C: $\Lambda_M$ = 72 S $m^2$ $M^{-1}$. $\mu_{eff}$ = 5.80 $\mu_B$ at 298 K.

### 2.3. X-ray crystallographic procedure

The crystal structure of [Fe(sal)(phdpa)Cl] $H_2O$ was obtained by X-ray diffraction method. Crystals of **1** as a monohydrate were obtained from a methanol solution of the complex on slow evaporation of the solvent. A suitable crystal was mounted on a glass fiber with epoxy cement and the 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 ($\lambda$ = 0.71073 Å) with increasing $\omega$ (width of 0.3° per frame) at a scan speed of 5 s per frame. Intensity data were collected using $\omega$-2$\theta$ scan mode and corrected for Lorentz-polarization effects and for absorption [33]. The structure solution was done by a combination of Patterson and Fourier techniques and full-matrix least-squares refinement was done using

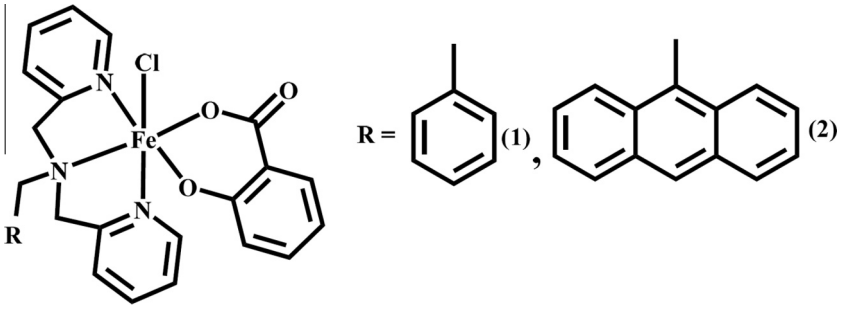


**Fig. 1.** Schematic drawings of the complexes **1** and **2**.