European Journal of Medicinal Chemistry 92 (2015) 332–341

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Photocytotoxic oxovanadium(IV) complexes of ferrocenyl-terpyridine and acetylacetonate derivatives

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ARTICLE INFO

Article history:
Received 1 August 2014
Received in revised form
8 November 2014
Accepted 3 January 2015
Available online 3 January 2015

Keywords:
Medicinal chemistry
Vanadium
Ferrocene
Photocytotoxicity
Cellular imaging
Apoptosis

ABSTRACT

Oxovanadium(IV) complexes [VO(Fc-tpy)(acac)](ClO_4) (**1**), [VO(Fc-tpy)(nap-acac)](ClO_4) (**2**), [VO(Fc-tpy)(py-acac)](ClO_4) (**3**) and [VO(Ph-tpy)(py-acac)](ClO_4) (**4**) of 4′-ferrocenyl-2,2′:6′,2″-terpyridine (Fc-tpy) and 4′-phenyl-2,2′:6′,2″-terpyridine (Ph-tpy) having monoanionic acetylacetonate (acac), naphthylacetylacetonate (nap-acac) or pyrenylacetylacetonate (py-acac) ligand were prepared, characterized and their photocytotoxicity in visible light studied. The ferrocenyl complexes **1**–**3** showed an intense charge transfer band near 585 nm in DMF and displayed Fc^+/Fc and V(IV)/V(III) redox couples near 0.66 V and −0.95 V vs. SCE in DMF-0.1 M TBAP. The complexes as avid binders to calf thymus DNA showed significant photocleavage of plasmid DNA in green light (568 nm) forming •OH radicals. The complexes that are photocytotoxic in HeLa and MCF-7 cancer cells in visible light (400–700 nm) with low dark toxicity remain nontoxic in normal fibroblast 3T3 cells. ICP-MS and fluorescence microscopic studies show significant cellular uptake of the complexes. Photo-irradiation of the complexes causes apoptotic cell death by ROS as evidenced from the DCFDA assay.

1. Introduction

Photodynamic therapy (PDT) which has emerged as a non-invasive treatment modality for various types of cancers using the FDA approved hematoporphyrin drug Photofrin® involves administration of the photosensitizer (PS) to the body, followed by its activation by light at the cancer site with formation of singlet oxygen killing the tumour cells [1–5]. The advantage of PDT over other chemotherapeutic treatments is its selectivity to target only the photo-exposed cancerous cells leaving unexposed normal cells unaffected thus minimizing toxic side effects. Photofrin and its analogues suffer from prolonged skin photosensitivity and hepatotoxicity due to formation of bilirubin on oxidative degradation of the porphyrin core [6,7]. Non-porphyrinic transition metal complexes are shown to be efficient photocytotoxic agents with low dark toxicity [8–15]. The metal complexes could be suitably designed to show novel PDT activity following different reaction pathways, producing hydroxyl radicals or singlet oxygen to cause cellular damage [16,17].

We have recently shown that ferrocenyl conjugates of transition metals significantly enhance their photo-chemotherapeutic potential compared to the one lacking the ferrocenyl unit [18–20]. Ferrocenyl derivatives with their non-toxic, redox active and lipophilic properties have been used to prepare novel antitumor, antimalarial and antifungal agents [21–26]. Mechanistic studies have shown that the anticancer activity of the ferrocenium cation is due to generation of reactive oxygen species (ROS) including •OH radicals and these radicals are responsible for DNA degradation and strand-breakage [27]. We have also shown in a recent report that ferrocene-conjugated oxovanadium(IV) complexes of

Abbreviations: acac, acetylacetonate; ct-DNA, calf thymus DNA; DCFDA, 2′,7′-dichlorofluorescein diacetate; DMEM, Dulbecco's Modified Eagle's Medium; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EB, ethidium bromide; EDTA, ethylenediaminetetraacetate; EPR, electron paramagnetic resonance; FBS, fetal bovine serum; Fc, ferrocenyl; Fc-tpy, 4′-ferrocenyl-2,2′:6′,2″-terpyridine; ICP-MS, inductively coupled plasma mass spectrometry; MLCT, metal-to-ligand charge transfer; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MvH, McGhee-von Hippel; NAC, *N*-acetylcysteine; nap-acac, naphthylacetylacetonate; PBS, phosphate buffered saline; PDT, photodynamic therapy; Ph-tpy, 4′-phenyl-2,2′:6′,2″-terpyridine; PI, propidium iodide; py-acac, pyrenylacetylacetonate; ROS, reactive oxygen species; TBAP, tetrabutylammonium perchlorate.

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http://dx.doi.org/10.1016/j.ejmech.2015.01.003
0223-5234/

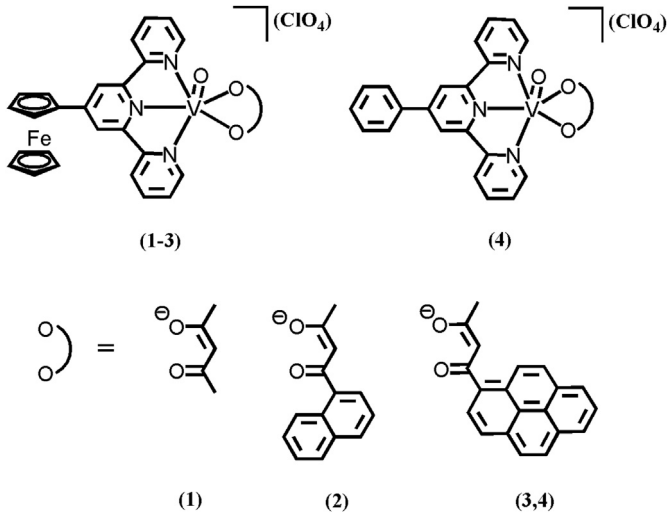
curcuminoids are photocytotoxic in cancer cells [28]. Curcumin has a dione moiety which in its enolized form resembles the metal binding properties of acetylacetonates [29]. The present work stems from our interests to extend this chemistry further by using different acetylacetonate derivatives in a ternary structure in which the oxovanadium(IV) moiety is bound to a tridentate ferrocenylterpyridine ligand and a bidentate acetylacetonate derivative. Curcumin with its poor bioavailability is known to be susceptible to hydrolytic degradation in the physiological medium [30]. In contrast, the acac ligands are expected to be of higher stability in a biological medium. The acac derivatives bearing fluorophoric pendants like naphthyl or pyrenyl moiety could be used to study the cellular localization of the complexes by fluorescence microscopy. The 4′-ferrocenyl-2,2′:6′,2″-terpyridine (Fc-tpy) ligand with its intense charge transfer band is used as a photosensitizer.

Herein, we report the synthesis, characterization, photocytotoxicity and cellular imaging of ferrocenyl-terpyridine (Fc-tpy) oxovanadium(IV) complexes of acetylacetone derivatives, viz. [VO(Fc-tpy)(acac)](ClO_4) (**1**), [VO(Fc-tpy)(nap-acac)](ClO_4) (**2**), [VO(Fc-tpy)(py-acac)](ClO_4) (**3**) and [VO(Ph-tpy)(py-acac)](ClO_4) (**4**), where acac is acetylacetonate, nap-acac is naphthylacetylacetonate and py-acac is pyrenylacetylacetonate (Scheme 1). Complex **4** having the ferrocenyl moiety replaced by a phenyl group was used as a control. Significant results of this study include remarkable photocytotoxicity of the py-acac complexes and cytosolic localization of complex **3** as evidenced from the fluorescence microscopy imaging study. The pyrenyl complex shows similar photocytotoxicity as observed for its reported curcumin analogue [28].

2. Results and discussion

2.1. Chemistry

The terpyridine ligands (Fc-tpy and Ph-tpy) were prepared by reacting corresponding aldehyde with 2-acetylpyridine and NaOH with subsequent condensation of the intermediate species with ammonium acetate in refluxing ethanol [31]. The 1-naphthylacetylacetone (Hnap-acac) and pyrenylacetylacetone (Hpy-acac) were prepared by following a literature procedure [32]. 1-Acetylnaphthalene or 1-acetylpyrene was refluxed with sodium sand in dry ethyl acetate for 4 h and poured into cold water. The aqueous layer was acidified with dilute HCl to precipitate out the product. The metal precursor vanadyl perchlorate was prepared by reacting vanadyl sulphate with calcium perchlorate. Oxovanadium(IV) complexes **1**–**4** were prepared by a general synthetic method in two steps. First, the acetylacetonate derivative after neutralizing with dilute NaOH was reacted with vanadyl perchlorate for 30 min. Corresponding terpyridine ligand in $CHCl_3$–MeOH was then added and stirred for further 30 min. The complex was isolated as its perchlorate salt.



Scheme 1. Schematic drawings of the complexes **1**–**4** and ligands used.

2.2. Pharmacology

Photocytotoxicity of the oxovanadium(IV) complexes **1**–**4** in HeLa and MCF-7 cells was evaluated by MTT assay in dark and visible light (400–700 nm). The cellular uptake of the complexes was evaluated by ICP-MS. The cellular localization studies were carried out by fluorescence microscopy using the blue fluorescence of the naphthyl and pyrenyl groups present in the complexes. The mechanism of cell death on photo-irradiation was assessed by Hoechst staining. The generation of reactive oxygen species (ROS) in the cells on photo-irradiation was evaluated by DCFDA assay. The oxovanadium(IV) complexes were also evaluated for their calf thymus DNA binding strengths and plasmid DNA photocleavage activities. The DNA binding studies were done by spectroscopic methods, viz. electronic absorption titration, DNA melting and viscosity measurements. The DNA cleavage activity of the complexes was studied using plasmid supercoiled (SC) form of DNA. The extent of DNA cleavage forming nicked circular (NC) DNA was quantified by gel electrophoresis.

2.3. Synthesis and general aspects

Complexes [VO(Fc-tpy)(L)](ClO_4) (**1**–**3**) of acetylacetone derivatives (L: acac in **1**; nap-acac in **2**; py-acac in **3**) and the ferrocenyl terpyridine ligand (Fc-tpy) were prepared in good yield. Complex **4** was prepared using Ph-tpy instead of Fc-tpy and used as a control species. Complexes **1**–**4** were characterized from the analytical, mass spectral and other physicochemical data (Table 1). The molar conductance value of ~82 S m^2 M^{-1} of the complexes in DMF at 25 °C suggests their 1:1 electrolytic behaviour. The ESI mass spectra of the complexes in MeCN showed a single peak corresponding to the molecular ion peak (*m/z*) as $[VO(Fc\text{-}tpy)(L)]^+$. The isotopic distribution pattern of the complexes is in accordance with the calculated one. The IR spectra of the complexes in the solid state showed C=O and C=C bands that are shifted to lower wavenumbers compared to the free ligands due to binding to the metal. The complexes showed band for V=O and ClO_4^- within 958–963 cm^{-1} and 1090-1096 cm^{-1}, respectively [33]. The $3d^1$-VO^{2+} complexes are one-electron paramagnetic giving a magnetic moment value of ~1.64 μ_B. The complexes showed broad ^1H NMR spectra due to their paramagnetic nature. The complexes showed eight line pattern in the ESR spectra due to hyperfine coupling of the unpaired electron with the ^{51}V nucleus (I = 7/2), confirming the $3d^1$-VO(IV) oxidation state. Complexes **1**–**3** showed an intense band at ~585 nm which is assignable to the metal to ligand charge transfer transition (MLCT) (Fig. 1) [34]. This band is absent in the spectrum of the phenyl analogue **4** indicating the involvement of the ferrocenyl moiety for this charge transfer transition. Complex **4** showed only a weak d–d transition at 774 nm. The d–d band in **1**–**3** was found to be masked by the MLCT band. The complexes **2**–**4** showed fluorescence property (Fig. 1). Complex **2** having a naphthyl moiety showed emission bands at 406 and 430 nm, while the pyrenyl complexes **3** and **4** showed emission bands at 387 and 409 nm, respectively. Cyclic voltammetry of the redox active complexes in DMF-0.1 M TBAP showed quasi-reversible Fc^+/Fc redox couple near 0.62 V (Fc = ferrocenyl unit) and the V(IV)/V(III)