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Research paper

Photoactive platinum(II) complexes of nonsteroidal anti-inflammatory drug naproxen: Interaction with biological targets, antioxidant activity and cytotoxicity

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ABSTRACT

The effect on the therapeutic efficacy of Pt(II) complexes on combining non-steroidal anti-inflammatory drugs (NSAIDs) is an attractive strategy to circumvent chronic inflammation mediated by cancer and metastasis. Two square-planar platinum(II) complexes: [Pt(dach)(nap)Cl] (**1**) and [Pt(dach)(nap)$_2$] (**2**), where dach = (1*R*,2*R*)-dichloro(cyclohexane-1,2-diamine) and NSAID drug naproxen (nap), have been designed for studying their biological activity. The naproxen bound to the Pt(II) centre get released upon photoirradiation with low-power UV-A light as confirmed by the significant enhancement in emission intensities of the complexes. The compounds were evaluated for their photophysical properties, photostability, reactivity with 5′-guanosine monophophosphate (5′-GMP), interactions with CT-DNA and BSA, antioxidant activity and reactive oxygen species mediated photo-induced DNA damage properties. ESI-MS studies demonstrated the formation of bis-adduct with 5′-GMP and the formation of Pt^{II}-DNA crosslinks by gel electrophoretic mobility shift assay and ITC studies. The interaction of the complexes **1** and **2** with the CT-DNA exhibits potential binding affinity (K_b ~ 10^4 M^{-1}, K_{app}~ 10^5 M^{-1}), implying intercalation to CT-DNA through planar naphthyl ring of the complexes. Both the complexes also exhibit strong binding affinity towards BSA (K_{BSA}~ 10^5 M^{-1}). The complexes exhibit efficient DNA damage activity on irradiation at 365 nm via formation of singlet oxygen ($^1O_2$) and hydroxyl radical ($^•$OH) under physiological conditions. Both the complexes were cytotoxic in dark and exhibit significant enhancement of cytotoxicity upon photo-exposure against HeLa and HepG2 cancer cells giving IC_{50} values ranging from 8 to 12 μM for **1** and **2**. The cellular internalization data showed cytosolic and nuclear localization of the complexes in the HeLa cells.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most consumed medicinal drugs commonly used as analgesics, antipyretics and anti-inflammatory drugs and their side-effects were well studied including peptic ulcer bleeding and elevated cardiovascular risks [1–4]. The mode of action of NSAIDs are primarily due to inhibition of prostaglandins synthesis mediated by cyclooxygenase (COX) isoforms, COX-1 and COX-2 [2,3]. NSAIDs are well-known for their anti-inflammatory effects but have received considerable importance in recent years in cancer chemoprevention and management in various aggressive solid tumors [5–8]. Cancer-related inflammation always promotes and aids in proliferation and survival of malignant cells, angiogenesis and metastasis [9–12]. The combination of NSAIDs is an attractive strategy to modulate and enhance the chemotherapeutic potential of existing anticancer drugs. They may have potential clinical applications to circumvent multidrug resistance protein (MRP) mediated multidrug resistance (MDR) and inflammation-induced metastatic tumors. The exact mechanism of antitumorigenesis by NSAIDs although yet non-consensus and may involve both cyclooxygenase (COX)-dependent and independent pathways, play important roles involving a diverse array of transcription factors, cell signaling and cytoskeletal proteins and cell cycle regulators [13,14]. Cyclooxygenases (COXs) known to play an important role and modulate

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tumor growth, progression, metastasis and malignancies, thus are attractive targets in developing next-generation anticancer agents. *Cis*platin, carboplatin and oxaliplatin have been the mainstay of clinical drugs worldwide for the treatment of various solid tumors like testicular, ovarian, colorectal, and non-small cell lung cancers [15–18]. However, the severe unavoidable side effects, high toxicity and multi-drug resistance inherent with these FDA approved Pt(II) drugs are the major obstacles and alternate strategies are needed to overcome these drawbacks. Most of the recent approaches are aiming towards improved and targeted drug delivery using various nanoparticle-based systems or exploring reduction-mediated delivery via Pt(IV) pro-drugs [19,20]. Though a recent survey, deliberately questions the true effectiveness of current nanoparticles in drug delivery and estimated that only 0.7% of the administered dose ends up in a tumor [21]. Thus, developing low molecular weight drugs conjugated to synergistic biologically active ligands with controlled release is an alternative option to modulate the therapeutic efficiencies and MDR associated with present drugs.

The combination of NSAIDs with Pt-drugs is an attractive choice towards solving some of the drawbacks as NSAIDs exhibit anti-tumorigenic properties and reduce cancer-related inflammation [5,9]. Conjugated NSAID ligands also facilitate the intracellular uptake and accumulation in cancer cells due to enhanced lipophilicity of these conjugates. Recently, there are few reports on Pt(IV) prodrugs designed for simultaneous delivery of platins and NSAIDs like aspirin or ibuprofen with reduced side-effects and advantageous anti-inflammatory properties [22–24]. The biological evaluation of different classes of NSAIDs with a variety of metals (Mn, Fe, Co, Ni, Cu, Zn, Pt) are promising towards developing chemo-anti-inflammatory drug candidates [25–28]. In literature, few metal-naproxen complexes with Co(II), Cd(II), Cu(II), Ni(II) are reported [29–35].

Naproxen (nap) is a non-selective COX inhibitor having antipyretic, anti-inflammatory and analgesic properties. Here we have conjugated naproxen ligand to Pt(II) in $[Pt(dach)Cl_2]$ with an intention to utilize synergistic effects of naproxen. This design will lead to a chemo-anti-inflammatory strategy which expected to lower the severe cytotoxicity of platinum drugs with a cytoprotective action from naproxen and reduce dose-dependent side-effects [14–16].

Herein, we report synthesis, photophysical properties, controlled release of naproxen upon photoirradiation with UV-A light (λ = 365 nm), DNA and BSA binding ability, photo-induced DNA cleavage, antioxidant activity and *in vitro* cytotoxicity of two Pt(II) complexes, *viz.* [Pt(dach)(nap)Cl] (**1**) and $[Pt(dach)(nap)_2]$ (**2**), where dach = ((1*R*, 2*R*)-dichloro(cyclohexane-1,2-diamine) and nap = naproxen (Scheme 1). The complexes were tested for their remarkable cytotoxicity against human cervical carcinoma (HeLa) and human liver carcinoma (Hep G2) cells. The complexes also showed around 2-fold enhancement in cytotoxicity upon exposed to UV-A light against HeLa cells.

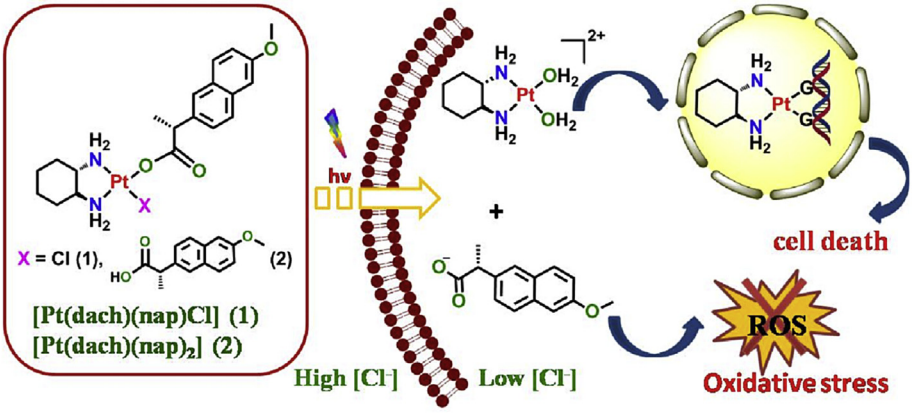
2. Results and discussion

2.1. Synthesis and characterization

[Pt(dach)(nap)Cl] (**1**) and $[Pt(dach)(nap)_2]$ (**2**) were synthesized by reacting dichloro(cyclohexane-1,2-diamine) platinum(II) complex i.e. $[Pt(dach)Cl_2]$ with $AgNO_3$ to precipitate out AgCl and subsequently reacting the *insitu* formed $[Pt(dach)(H_2O)_2]$ with deprotonated naproxen to obtain the desired products (Schemes S1 and SI). The complexes **1** and **2** were synthesized in high yields and were characterized by elemental analysis, ESI-MS, FT-IR, UV–vis spectral techniques. The selected physicochemical data are displayed in Table 1. The ESI-MS of the compounds shows respective molecular ion peaks, $[M+H]^+$ in DMF solution (Figs. S1,S2, SI). The FT-IR data indicate the characteristic binding mode of the carboxylate moiety of naproxen ligand to Pt(II). The two strong bands at ~1600 cm^{-1} and 1378-1384 cm^{-1} ascribed as antisymmetric and symmetric ν(C=O) stretching vibrations of the carboxylate group of the ligand naproxen. The difference (Δ) = [ν_{asym}(C=O) - ν_{sym}(C=O)] = 217-224 cm^{-1} suggesting monodentate mode of binding for the carboxylate of the naproxen to Pt(II) centre [29]. The UV–vis absorption spectra of the compounds in DMF displayed intense ligand-centered broad bands at 274 nm and 319 nm due to π-π^* electronic transitions and 333 nm probably due to the n-π^* transitions of the naphthyl moiety of bound ligand (Fig. 1a). The steady-state emission spectra of the compounds **1** and **2** at room temperature in DMF showed strong emission at λ_{em} = 359 nm on excitation at 333 nm characteristic to naproxen naphthyl moiety (Fig. 1b).

2.2. Stability and photo-release of naproxen

The stability of the complexes **1** and **2** was studied in Tris-HCl buffer (5 mM, pH 7.2) using time-dependent UV–vis spectral changes at ambient condition. They were found to be stable appreciably even after 8 h in solution (Figs. S3 and SI). The speciation of the complexes [Pt(dach)(nap)Cl] (**1**) and $[Pt(dach)(nap)_2]$ (**2**) were studied in DMSO when incubated at 37 °C for 16 h. Platinum(II) complexes shows rapid ligand exchange preferably due to its soft nature towards S-donor DMSO over O-donor naproxen,



Scheme 1. Schematic representation of [Pt(dach)(nap)Cl] (**1**) and $[Pt(dach)(nap)_2]$ (**2**) and proposed possible mechanism of cytotoxicity.