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Photolabile ruthenium complexes to cage and release a highly cytotoxic anticancer agent☆

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ABSTRACT

CHS-828 (*N*-(6-(4-chlorophenoxy)hexyl)-*N′*-cyano-*N″*-4-pyridyl guanidine) is an anticancer agent with low bioavailability and high systemic toxicity. Here we present an approach to improve the therapeutic profile of the drug using photolabile ruthenium complexes to generate light-activated prodrugs of CHS-828. Both prodrug complexes are stable in the dark but release CHS-828 when irradiated with visible light. The complexes are water-soluble and accumulate in tumour cells in very high concentrations, predominantly in the mitochondria. Both prodrug complexes are significantly less cytotoxic than free CHS-828 in the dark but their toxicity increases up to 10-fold in combination with visible light. The cellular responses to light treatment are consistent with release of the cytotoxic CHS-828 ligand.

1. Introduction

The effectiveness of many anticancer agents can be compromised by factors such as systemic toxicity, low bioavailability, and metabolism. A prodrug approach can potentially circumvent these issues [1], where the drug is delivered in an inert, bioavailable form, then converted to the active form in the tumour region. Among the approaches under investigation for anticancer prodrug design, photocaging is gaining increasing interest as a means of selectively activating a prodrug in the tumour environment. In this strategy, a drug is 'caged' in an inactive form then 'uncaged' by irradiation with light [2]. The use of light as a trigger has the advantage of providing spatial and temporal control over the region of drug release, making this a potentially very selective means of prodrug activation. One key consideration is the irradiation wavelength, with 600–800 nm being the optimum window for maximum tissue penetration with minimum damage [3].

Ruthenium (II) polypyridyl complexes are particularly suited to photocaging as they can form stable complexes in the dark with a range of ligands, then undergo photosubstitution when irradiated with visible light [4]. Etchenique et al. first employed this approach in the photocaging of amine neurochemicals [5], while more recent work has focused on anticancer therapeutics, with pioneering work from Kodanko and Turro demonstrating photocaging of a nitrile-containing cathepsin K inhibitor [6]. We and others have subsequently expanded this approach to include imidazoles [7,8], and purines [9], with two very recent examples from Kodanko et al. focussing on pyridine-based drugs [10,11]. In this study we investigate the application of a photolabile ruthenium complex to cage and release a highly cytotoxic anticancer agent, CHS-828 (*N*-(6-(4-chlorophenoxy)hexyl)-*N′*-cyano-*N″*-4-pyridyl guanidine) (Fig. 1). This pyridine-containing compound is an inhibitor of the enzyme nicotinamide phosphoribosyltransferase (NAMPT) [12], which is overexpressed in a number of cancers [13]. CHS-828 exhibited potent antitumor activity in preclinical tumour models [14,15], and has subsequently completed several Phase I clinical trials against solid tumours [16–18]. However, in each trial the drug was found to induce a number of dose-limiting side effects such as gastrointestinal toxicity and thrombosis, in addition to low bioavailability and large variations in pharmacokinetics. Here we investigate whether incorporation of CHS-828 into two photolabile ruthenium complexes can improve upon these limitations.

2. Experimental

2.1. General procedures

2.1.1. Materials

All other chemicals were obtained from commercial sources and used with further purification.

2.1.2. Instrumentation and methods

^{1}H NMR spectra were collected at 300 K on a Bruker 300 MHz spectrometer using commercially available deuterated solvents.

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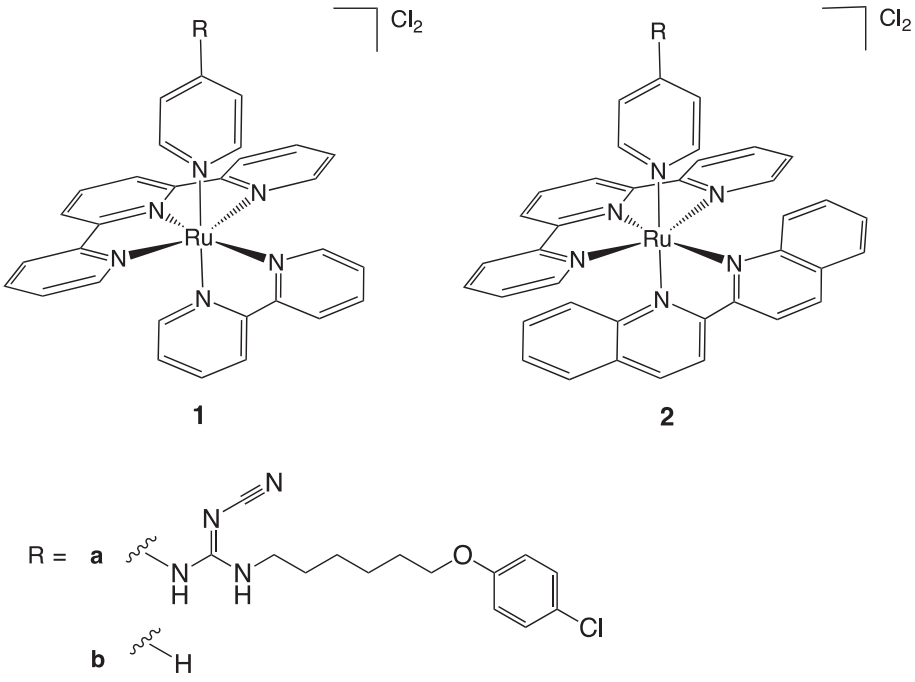

Fig. 1. Chemical structures of ruthenium complexes.

Isotopic impurities were used as internal reference signals. Mass spectrometry was performed using Electro-Spray Ionisation using an amazon SL spectrometer. Elemental analyses (C, H, N) were conducted by the Chemical & MicroAnalytical Services Pty Ltd., Campbell Microanalytical Laboratory, at the University of Otago. ICPMS was conducted at the National Measurements Institute, Pymble, NSW, Australia. UV–visible measurements were performed on a Cary 4E UV–visible spectrometer using a 1 cm × 1 cm quartz cuvette.

Scans were run at room temperature from 300 to 700 nm.

2.2. Synthesis

CHS-828 [12], [Ru(tpy)(bpy)Cl]Cl (tpy = 2,2′;6′,2″-terpyridine), (bpy = 2,2′-bipyridine) [19], [Ru(tpy)(biq)Cl]Cl (biq = (2,2′-biquinoline) [19], $[Ru(tpy)(bpy)(py)]Cl_2$ (py = pyridine) [20], $[Ru(tpy)(biq)(py)]Cl_2$ [20] were prepared according to literature procedures.

All reactions were carried out under nitrogen using standard Schlenk techniques. The synthesis and purification of the final complexes were performed under low ambient light to avoid photodegradation.

$[Ru(tpy)(bpy)(CHS-828)]Cl_2$ (1a)

A solution of [Ru(tpy)(bpy)Cl]Cl (263 mg, 0.5 mmol) in 1:3 water/MeOH (40 mL) was heated at reflux for 30 min in the absence of light. CHS-828 (222 mg, 0.6 mmol) was added and the reaction heated at reflux for further 12 h, then the solvent removed under reduced pressure. The residue was purified by column chromatography on an alumina column (neutral, Brockmann activity 3) with a gradient eluent of dichloromethane:MeOH (10:1 to 2:1). Residual [Ru(tpy)(bpy)Cl]Cl elutes first as a pink band followed by Complex (1a) as an brown band. The fractions were combined, concentrated and precipitated with diethyl ether to give a red/brown powder, which was collected by filtration, washed with diethyl ether (2 × 20 mL) and dried under vacuum. Final yield of Complex (1a) = 288 mg (62%) of red/brown microcrystals.

^{1}H NMR (300 MHz, Methanol-d_4) 9.51 (1H, d, J 5.5), 8.78 (1H, d, J 8.2), 8.66 (2H, d, J 8.1), 8.53 (3H, d, J 8.0), 8.34 (1H, t, J 7.8), 8.26 (1H, t, J 8.0), 8.02 (2H, d, J 7.6), 7.99–7.90 (3H, m), 7.80 (1H, t, J 7.9), 7.60 (2H, d, J 5.5), 7.37 (2H, t, J 6.6), 7.29 (1H, d, J 5.6), 7.21 (2H, d, J 8.6), 7.10 (1H, t, J 6.7), 6.85 (2H, d, J 8.4), 6.65 (2H, d, J 5.7), 3.90 (2H, t, J 6.3), 3.31 (1H, s), 3.12 (2H, t, J 6.9), 1.75–1.63 (2H, m), 1.42 (4H, p, J 7.5), 1.30 (3H, q, J 8.0, 7.5). ESI-MS +: m/z = 431.17 ($[Ru(tpy)(bpy)(CHS-828)])^{2+}$, 861.24 ($[Ru(tpy)(bpy)(CHS-828)]$-H)$^+$. Elemental analysis for $[Ru(tpy)(bpy)(CHS-828)](Cl)_2(H_2O)_2(CH_3OH)$ ($C_{45}H_{49}Cl_3N_{10}O_4Ru$). Calculated: C, 53.98; H, 4.93; N, 13.99. Found: C, 53.69; H, 4.94; N, 13.90.

$[Ru(tpy)(biq)(CHS-828)]Cl_2$ (2a)

A solution of [Ru(tpy)(biq)Cl]Cl (313 mg, 0.5 mmol) in 1:3 water/MeOH (40 mL) was heated at reflux for 30 min in the absence of light. CHS-828 (222 mg, 0.6 mmol) was added and the reaction heated at reflux for further 12 h, then the solvent removed under reduced pressure. The residue was purified by column chromatography on an alumina column (neutral, Brockmann activity 3) with a gradient eluent of dichloromethane:MeOH (10:1 to 2:1). Residual [Ru(tpy)(biq)Cl]Cl elutes first as a bright pink band followed by Complex (2a) as an purple band. The fractions were combined, concentrated and precipitated with diethyl ether to give purple crystalline solid, which was collected by filtration, washed with diethyl ether (2 × 20 mL) and dried under vacuum. Final yield of Complex (2a) = 299 mg (58%) of purple microcrystals.

^{1}H NMR (300 MHz, Methanol-d_4) 9.03 (1H, q, J 8.9), 8.88 (1H, d, J 8.2), 8.77 (1H, d, J 8.5), 8.70 (1H, d, J 8.1), 8.52 (1H, d, J 8.0), 8.41 (2H, t, J 8.6), 8.34 (1H, t, J 8.0), 8.09–7.87 (4H, m), 7.86 (1H, d, J 8.1), 7.72 (1H, d, J 5.6), 7.49 (2H, t, J 7.5), 7.38 (1H, td, J 6.1, 5.7, 3.0), 7.22 (3H, dd, J 8.7, 5.7), 6.87 (2H, d, J 9.0), 6.76 (1H, d, J 8.8), 6.57 (1H, d, J 9.8), 4.00–3.82 (3H, m), 3.15–2.97 (2H, m), 1.70 (2H, t, J 7.3), 1.41 (5H, s), 1.29 (2H, d, J 9.8). ESI-MS +: m/z = 481.93 ($[Ru(tpy)(biq)(CHS-828)])^{2+}$, 961.18 ($[Ru(tpy)(biq)(CHS-828)]$-H)$^+$.

Elemental analysis for $[Ru(tpy)(biq)(CHS-828)](Cl)_2(CH_3OH)_4$ ($C_{56}H_{61}Cl_3N_{10}O_5Ru$). Calculated: C, 57.90; H, 5.29; N, 12.06. Found: C, 58.13; H, 5.25; N, 12.04.

2.3. Spectroscopic studies

Solutions of the ruthenium complexes in water were prepared in a quartz cuvette to give a final concentration of 50 μM. Solutions prepared in methanol or DMSO and diluted with water to give a final composition of 95:1 water/methanol or water/DMSO. The cuvette was irradiated with an LED-EXPO lamp (LuzChem) at 465 ± 10 nm,