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Arene ruthenium dithiolato–carborane complexes for boron neutron capture therapy (BNCT)

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Dedicated to Professor Georg Süss-Fink on the occasion of his 65th birthday

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ABSTRACT

We report the effect of low-energy thermal neutron irradiation on the antiproliferative activities of a highly hydrophobic organometallic arene ruthenium dithiolato–carborane complex [Ru(*p*-cymene) (1,2-dicarba-*closo*-dodecarborane-1,2-dithiolato)] (**1**), and of its formulation in Pluronic® triblock copolymer P123 core–shell micelles (RuMs). Complex **1** was highly active, with and without neutron irradiation, towards human ovarian cancer cells (A2780; IC_{50} 0.14 μM and 0.17 μM, respectively) and cisplatin-resistant human ovarian cancer cells (A2780cisR; IC_{50} 0.05 and 0.13 μM, respectively). Complex **1** was particularly sensitive to neutron irradiation in A2780cisR cells (2.6 × more potent after irradiation compared to non-irradiation). Although less potent, the encapsulated complex **1** as RuMs nanoparticles resulted in higher cellular accumulation (2.5×), and was sensitive to neutron irradiation in A2780 cells (1.4× more potent upon irradiation compared to non-irradiation).

Introduction

Boron neutron capture therapy (BNCT) has raised considerable interest for the treatment of high-grade gliomas and either cutaneous primaries or cerebral metastases of melanoma [1]. This binary method consists of the nuclear reaction of nontoxic and nonradioactive ^{10}B atoms and low-energy thermal neutrons that produces high-energy $^{4}He^{2+}$ α-particles and $^{7}Li^{3+}$ ions. The dissipation of the high kinetic energy of these particles is achieved in a small distance (less than one cell diameter), which allows accurate destruction of the targeted cells [2].

Dicarba-*closo*-dodecarboranes are a class of boron-rich compounds with globular structure and diameter of *ca.* 1 nm (diameter of a rotating phenyl) that possess unusual properties, including high symmetry and remarkable stability [3]. These clusters contain ten boron atoms; they possess a rather low cytotoxicity and are extremely stable in biological media. They are well suited to boron neutron capture therapy [4,5], but also have potential in other fields of drug discovery, molecular imaging, and targeted radionuclide therapy [6]. However, effective delivery of boron agents is still a critical issue which impairs their further clinical development [7]. We have recently discussed how the combination of arene ruthenium(II) complexes and carboranes has unexplored potential in medicine [8]. Such complexes also exhibit unusual chemistry: coordination of the bulky, electron-deficient carborane ligand 1,2-dicarba-*closo*-dodecarborane-1,2-dithiolato to an arene-Ru metal center leads to the isolation of a stable 16-electron complex [Ru(*p*-cymene) (1,2-dicarba-*closo*-dodecarborane-1,2-dithiolato)] (**1**) [9]. However, since this complex is highly hydrophobic, exploration of its biological applications is hampered by the lack of solubility in water [10]. To exploit the chemistry of carborane-containing arene ruthenium complexes in aqueous solution, and to take advantage of their unique properties, we have encapsulated the 16-electron complex **1** in Pluronic® triblock copolymer P123 micelles (Fig. 1). We have recently shown that although entrapment of the 16-

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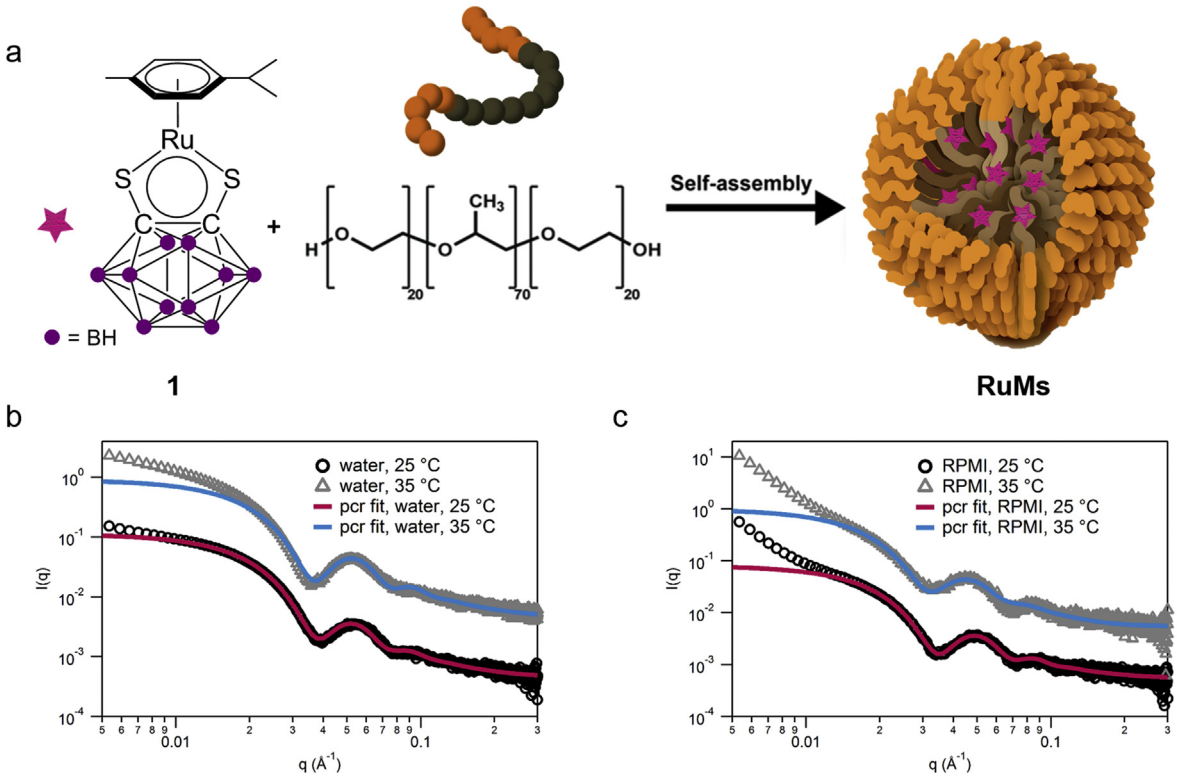

Fig. 1. (a) Self-assembly formation of **RuMs** (purple dots in **1** are B–H vertices). (b) and (c) Small-angle X-ray scattering (SAXS) experimental profiles and fitting with spherical core–shell micelle model of micelles **RuMs** at 25 °C and 35 °C in water and at 25 °C and 35 °C in RPMI, respectively; 5 mg/mL aqueous solutions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

electron complex **1** in Pluronic® micelles (**RuMs**) leads to a reduction in its anticancer potency towards ovarian cancer cells A2780, the micelles exhibit enhanced selectivity towards cancer cells compared to normal cells (up to a factor 8) [11]. This formulation was fully characterised by using a combination of analytical techniques, including synchrotron small-angle X-ray scattering, high-resolution transmission electron microscopy, and light scattering methods [11]. Polymer encapsulation of metal carborane complexes provides the potential for delivering high amounts of boron to cells which is of interest for BNCT [12]. We report here the effect of low-energy thermal neutron irradiation on the antiproliferative activity of both complex **1** and **RuMs** particles in the A2780 ovarian cancer cell line, and in A2780cisR cisplatin-resistant cancer cell line.

Results

Synthesis and characterisation

The organometallic half-sandwich Ru^{II} arene complex [Ru(*p*-cymene) (1,2-dicarba-*closo*-dodecarborane-1,2-dithiolate)] (**1**) was synthesised as reported previously [13]. This complex has a *pseudo*-octahedral structure, with a π-bonded arene occupying 3 coordination sites, a S-bound chelated dithiolato dicarba-*closo*-dodecarborane ligand, and a vacant 6th site (Fig. 1). It is a 16-electron complex and therefore electron-deficient at the metal [14]. Complex **1** is highly hydrophobic and insoluble in water [15]. To achieve dispersion in water [16], we encapsulated complex **1** in the water-soluble amphiphilic triblock copolymer P123 (poly(ethylene glycol)-*block*-poly(propylene glycol)-*block*-poly(ethylene glycol)) (PEO-*b*-PPO-*b*-PEO), according to a previously reported procedure (Fig. 1) [11].

To gain further insight into the structure of **RuMs** in RPMI cell culture medium, and to compare the sizes of the assembly in RPMI *versus* water at ambient temperature and at 35 °C, solutions of **RuMs** were analysed by synchrotron small-angle X-ray scattering (SAXS; Fig. 1). The experimental profiles were fitted using IgorPro software [17] to a core–shell spherical micelle model PolyCoreShellRatio [18] (PCR) according to a previous procedure for similar micelles [19]. Some aggregation was observed for all the samples (high turn at low q values), however the PCR model fitted excellently for all micellar solutions from 0.2 Å^{-1} with very low dispersity parameters (between 0.13 and 0.16, 0 being an ideal mono-disperse system; Table 1).

Cell testing

We studied the time-dependence of the antiproliferative activity of complex **1** and micelles **RuMs** and P123Ms (micelles made of Pluronic® copolymers without complex **1**) in A2780 human ovarian cancer cells (Table 2). Cells were exposed for variable times (1, 4, 16, 24, 48 and 72 h) to complex **1** (dissolved in 5% dimethyl sulfoxide (dmso)/95% saline:RPMI and further diluted in cell culture medium until working concentrations were achieved) or to **RuMs** micelles (dissolved in 100% saline:RPMI, further diluted with cell culture medium to working solutions). After this, drugs were removed and cells were washed and placed in fresh growth medium for a further 72 h as a recovery period. Cell viability was then assessed using the sulforhodamine B (SRB) colorimetric assay. Complex **1** was found to be highly potent towards A2780 cells (Table 2), particularly after 24 h of drug exposure (IC_{50} 170 nM), and it is also 39 × more potent than **RuMs** micelles, which still exhibit good (micromolar) activity towards cancer cells.

Since the optimum time for drug exposure was 24 h, we determined the IC_{50} values of complex **1** and micelles **RuMs** in A2780cisR cells after 24 h of drug exposure. Complex **1** was found

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