Chemico-Biological Interactions 234 (2015) 349–359

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

# Ruthenium complexes as inhibitors of the aldo–keto reductases AKR1C1–1C3

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ARTICLE INFO

*Article history:*
Available online 18 November 2014

*Keywords:*
Ruthenium complexes
AKR1C isoenzymes
Inhibition

ABSTRACT

The human aldo–keto reductases (AKRs) from the 1C subfamily are important targets for the development of new drugs. In this study, we have investigated the possible interactions between the recombinant AKR1C enzymes AKR1C1–AKR1C3 and ruthenium(II) complexes; in particular, we were interested in the potential inhibitory actions. Five novel ruthenium complexes (**1a**, **1b**, **2a**, **2b**, **2c**), two precursor ruthenium compounds (**P1**, **P2**), and three ligands (**a**, **b**, **c**) were prepared and included in this study. Two different types of novel ruthenium(II) complexes were synthesized. First, bearing the sulphur macrocycle [9]aneS$_3$, S-bonded dimethylsulphoxide (dmso-S), and an *N,N*-donor ligand, with the general formula of [Ru([9]aneS$_3$)(dmso)(*N,N*-ligand)](PF$_6$)$_2$ (**1a**, **1b**), and second, with the general formula of [($\eta^6$-*p*-cymene)RuCl(*N,N*-ligand)]Cl (**2a**, **2b**, **2c**). All of these synthesized compounds were characterized by high-resolution NMR spectroscopy, X-ray crystallography (compounds **a**, **b**, **c**, **1a**, **1b**) and other standard physicochemical methods. To evaluate the potential inhibitory actions of these compounds on the AKR1C enzymes, we followed enzymatically catalyzed oxidation of the substrate 1-acenaphthenol by NAD$^+$ in the absence and presence of various micromolar concentrations of the individual compounds. Among 10 compounds, one ruthenium complex (**2b**) and two precursor ruthenium compounds (**P1**, **P2**) inhibited all three AKR1C enzymes, and one ruthenium complex (**2a**) inhibited only AKR1C3. Ligands **a**, **b** and **c** revealed no inhibition of the AKR1C enzymes. All four of the active compounds showed multiple binding with the AKR1C enzymes that was characterized by an initial instantaneous inhibition followed by a slow quasi-irreversible step. To the best of our knowledge, this is the first study that has examined interactions between these AKR1C enzymes and ruthenium(II) complexes.



## 1. Introduction

Platinum-based drugs have been in clinical use for cancer treatment for more than 30 years. The landmark discovery was made by Rosenberg in 1965 with *cis*-diamminedichloridoplatinum(II) (cisplatin), which showed anti-tumoral properties [1]. Although cisplatin and its derivatives are by far the most widely and successfully used metal-based anticancer drugs, they suffer from two main disadvantages: the development of platinum resistance; and the occurrence of severe side effects, such as nephrotoxicity, due to non-specific targeting of DNA [2,3]. Therefore the development of metal-based anticancer drugs containing different transition-metal ions is a valid strategy for more specific targeting [4]. Among these, the ruthenium complexes have recently been shown to be promising alternatives, as two compounds have already entered clinical trials: NAMI-A (*trans*-[imH][RuCl$_4$(dmso-*S*)(im)]; im, imidazole) and KP1019 (*trans*-[indH][RuCl$_4$(ind)$_2$]; ind, indazole), [5,6]. These compounds have been shown to be less toxic and less prone to resistance compared to platinum-based drugs [7]. In addition to the classical mechanism of action associated with their binding to DNA, other modes of action have been

*Abbreviations:* [9]aneS$_3$, 1,4,7-trithiacyclononane; AKR1C1–1C3, aldo–keto reductase (AKR) members 1C1 to 1C3; ATR, attenuated total reflection; CHN, carbon, hydrogen, nitrogen; DMAD, dimethyl acetylenedicarboxylate; DMFDMA, *N,N*-dimethylformamide dimethyl acetal; dmso, dimethylsulphoxide as a ligand; DMSO, dimethylsulphoxide as a solvent; ESI–HRMS, electrospray ionization–high-resolution mass spectrometry; $\eta^6$-*p*-cymene, 1-methyl-4-(1-methylethyl)benzene; His, histidine; IR, infrared; MeCN, acetonitrile; MeOH, methanol; NADH, nicotinamide adenine dinucleotide; NAD$^+$, oxidized form of nicotinamide adenine dinucleotide; NMR, nuclear magnetic resonance; r.t., room temperature; PTA, 1,3,5-triaza-7-phosphaadamantane; UV–Vis, ultraviolet–visual spectroscopy.

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http://dx.doi.org/10.1016/j.cbi.2014.11.005
0009-2797/

reported for these compounds, including binding to plasma proteins (e.g., transferrin, albumin) [8,9] and inhibition of enzyme activities (e.g., cathepsin B, thioredoxin reductase, and several protein kinases) [10–15].

Over the past decade, research has shifted towards organometallic half-sandwich Ru(II) compounds with the general formula of $[Ru(\eta^6\text{-arene})(chel)X](PF_6)_n$, where 'chel' is a neutral or monoanionic *N,N*-, *N,O*- or *O,O*- chelating ligand and 'X' is typically a halide ($n$ = 1 or 0, depending on the charge of chel). Several of these have been shown to have promising anticancer activities *in vitro*, and some also *in vivo* [16–19]. Ruthenium(II)-arene compounds bearing the 1,3,5-triaza-7-phosphaadamantane (PTA) ligand were also intensively investigated by Dyson and co-workers [20]. The toluene derivative $[Ru(\eta^6\text{-arene})Cl_2(PTA)]$, termed RAPTA-T, showed antimetastatic properties *in vivo* [21]. It has also been shown that the π-bonded aromatic ligands are not essential for the anticancer activity of ruthenium(II) complexes. As such, the activities of new 'half-sandwich' Ru(II) compounds in which the face-capping sulphur macrocycle 1,4,7-trithiacyclononane ([9]aneS$_3$) replaces the $\eta^6$-arene ligands have been investigated [22–26]. Also coordination complexes based on $d^6$ metal centres and polypyridyl ligand architectures have already attracted attention in a biological context [27,28]. As the exact mechanisms of action of the ruthenium compounds are still not fully understood, different targets for various ruthenium species have been proposed, and their simultaneous actions on multiple targets represent a valid hypothesis [29].

The human members of the aldo–keto reductase (AKR) subfamily 1C are associated with different diseases and they thus represent important targets for the development of new drugs. The AKR1C1–AKR1C3 enzymes have crucial roles in the biosynthesis and inactivation of all classes of steroid hormones, and also in the biosynthesis of neurosteroids and prostaglandins [30,31]. These enzymes regulate the concentrations of active and inactive androgens, estrogens and progestogens in target tissues, and thus ligand occupancy and *trans*-activation of their corresponding nuclear steroid hormone receptors. AKR1C1–AKR1C3 also regulate the levels of neurosteroids that can modulate the activity of the $GABA_A$ receptor and the concentrations of prostaglandins, which can effect various cellular pathways [31]. Aberrant expression and actions of these AKR1C enzymes may lead to an imbalance in the biosynthesis and inactivation of steroid hormones, neurosteroids and prostaglandins, and to the further development of hormonal-dependent cancers of the breast, prostate and endometrium, as well as to diseases such as benign prostatic hyperplasia, endometriosis and neurological disorders [31].

The human AKR1C enzymes metabolize a variety of xenobiotics, including polycyclic aromatic carbohydrates and other pollutants, and several drugs (e.g., doxorubicin, oracin) [32,33]. Recent research has shown that expression of the AKR1Cs, and especially of AKR1C1 and AKR1C3, is related to resistance to a variety of anticancer drugs, including the platinum-based cisplatin and carboplatin [34,35]. Although the mechanisms of resistance to these metallodrugs and the involvement of the AKR1C enzymes have not been defined in detail, the AKR1C enzymes have been reported to regulate the generation of cisplatin-associated reactive oxygen species, and to disrupt apoptotic pathways [34,35]. The increased expression of the AKR1Cs in cisplatin- and carboplatin-resistant cancers thus calls for drugs that can overcome this resistance by either not affecting the AKR1C levels or by decreasing the AKR1C enzymatic activities. To date, no studies have been published that have examined potential direct interactions between the AKR1C enzymes and these metallodrugs.

Although to the best of our knowledge, there are no published data showing induced expression of *AKR1C* genes after treatment with any of the ruthenium complexes, these compounds may be associated with the generation of ROS and concurrent increased *AKR1C* expression. The aim of the present study was thus to evaluate possible interactions between the recombinant AKR1C enzymes AKR1C1–AKR1C3 and ruthenium complexes; in particular, we were interested in their potential inhibitory actions. This study included 10 compounds: five novel ruthenium complexes, two ruthenium precursor compounds, and three ligands. In general, two different types of ruthenium(II) complexes were synthesized with polypyridyl ligands possessing water-solubilizing functional groups (methoxycarbonyl groups). The first type was bearing the sulphur macrocycle [9]aneS$_3$, S-bonded dimethylsulphoxide (dmso-S), and an *N,N*-donor ligand, with the general formula of $[Ru([9]aneS_3)(dmso)(N,N\text{-ligand})](PF_6)_2$. The second type had the general formula of $[(\eta^6\text{-}p\text{-cymene})RuCl(N,N\text{-ligand})]Cl$.

We evaluated the potential inhibitory actions of these ruthenium compounds against the AKR1C enzymes, and we describe here four active compounds: two novel ruthenium complexes (**2a**, **2b**), and two precursor compounds (**P1**, **P2**). To the best of our knowledge, this is the first report that has investigated inhibition of the AKR1C enzymes by ruthenium(II) complexes.

## 2. Materials and methods

### 2.1. Materials

All of the starting materials were purchased from commercial suppliers (Sigma–Aldrich, UK) and used as received. Compounds **P1** and **c** were synthesized as previously described [36,37]. $^1$H and $^{13}$C nuclear magnetic resonance (NMR) spectra of the complexes (**1a**, **1b**, **2a**, **2b**, **2c**) and the new ligands (**a**, **b**) were recorded on a Bruker Avance III spectrometer at 500 MHz and 126 MHz, at room temperature using tetramethylsilane as the internal standard. The coupling constants ($J$) are given in Hz, and the splitting patterns are designated as: s, singlet; d, doublet; dd, double doublet; t, triplet; hept, heptet; and m, multiplet. The proton assignments used for $^1$H NMR characterization are labelled in Scheme 1. The infrared spectra were recorded with a Perkin-Elmer Spectrum 100 Fourier-transform infrared spectrometer, which was equipped with Specac Golden Gate diamond attenuated total reflection (ATR) as a solid sample support. The measurements were made in the range from 4000 cm$^{-1}$ to 600 cm$^{-1}$. The ultraviolet–visual (UV–Vis) spectra were collected on Perkin-Elmer Lambda 750 UV/Vis/near infrared spectrophotometer, in a methanol solution at $2 \times 10^{-5}$ mol/L. The carbon, hydrogen, nitrogen (CHN) elemental analyses were recorded using a Perkin-Elmer 2400 Series II instrument (CHN), and the electrospray ionization–high-resolution mass spectrometry (ESI–HRMS) was carried out on an Agilent 62224 accurate mass spectrometer, using time of flight liquid chromatography/mass spectrometry.

### 2.2. Single crystal X-ray diffraction

X-ray diffraction data were collected on an Oxford Diffraction SuperNova diffractometer, with a Mo microfocus X-ray source (Kα radiation, $\lambda$ = 0.71073 Å) for compounds **a** and **c**, and a Cu microfocus X-ray source (Kα radiation, $\lambda$ = 1.54184 Å) for compounds **b**, **1a** and **1b**, with mirror optics and an Atlas detector at 150(2) K. The structures were solved by direct methods implemented in SIR92 [38], and refined by a full-matrix least-squares procedure based on $F^2$, using SHELXL-97 [39]. All of the non-hydrogen atoms were refined anisotropically. The hydrogen atoms were placed at their calculated positions and treated using the appropriate riding models. The Mercury, ORTEP and Platon programmes were used for the data analysis and figure preparation [40–43].