Journal of Inorganic Biochemistry 175 (2017) 198–207

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

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

Varying the metal to ethacrynic acid ratio in ruthenium(ii)/osmium(ii)-*p*-cymene conjugates

Emilia Păunescu, Mylène Soudani, Catherine M. Clavel, Paul J. Dyson*

Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland

ARTICLE INFO

Keywords:
Bioorganometallic chemistry
Metal-based drugs
Anticancer drugs
Drug resistance
Ethacrynic acid

ABSTRACT

Following the identification of a ruthenium(II)-arene complex with an ethacrynic acid-modified imidazole ligand, which inhibits glutathione transferase (GST) and is cytotoxic to chemo-resistant cancer cells, a series of structurally related ruthenium(II)- and osmium(II)-*p*-cymene compounds have been prepared. In these complexes the ethacrynic acid is linked to the metals via appropriately modified pyridine ligands. The influence of the metal center and the metal:ethacrynic acid ratio on the cytotoxicity of the compounds was evaluated with the derivatives with one metal center and two ethacrynic acid moieties being the most potent against chemo-resistant A2780cisR cells (human ovarian cancer cells with acquired resistance to cisplatin). Moreover, compared to a complex with an ethacrynic acid-modified imidazole ligand (RAIMID-EA, Figure 2), these complexes display a significant degree of cancer cell specificity.

1. Introduction

The Group 8 metals, iron, ruthenium and osmium, have been extensively explored in the development of metal-based anticancer drugs [1–9]. Ruthenium compounds, in particular, are viewed as promising alternatives to classical platinum-based compounds such as cisplatin, oxaliplatin and carboplatin. The ruthenium(III) complexes imidazolium *trans*-[tetrachlorido(dimethylsulfoxide)(1*H*-imidazole)ruthenate(III)] (NAMI-A) and indazolium *trans*-[tetrachloridobis(1*H*-indazole)ruthenate(III)] (KP1019 – and the sodium salt termed KP1339) (Fig. 1) have been evaluated in clinical trials [10–13]. NAMI-A showed strong efficacy toward solid tumor metastases [14], whereas the indazole complexes KP1019 and KP1339 demonstrated excellent activity in several primary tumor models [11,12,15,16]. In a phase I clinical study of NAMI-A, one patient with pre-treated, progressive non-small cell lung cancer (NSCLC), the disease was stabilized for 21 weeks following treatment with NAMI-A [10] and, in a phase I/II study, in which NAMI-A was combined with gemcitabine for the second- or third-line therapy of metastatic NSCLC patients, partial remission was observed in one patient and stable disease was observed for at least 6–8 weeks in 10 out of 18 patients evaluated [13]. In the case of KP1019 a dose escalation trial was performed in patients with advanced solid tumors without further therapeutic options [11]. The pharmacokinetic analysis suggested that the drug is rapidly bound to plasma proteins and has a long half-life while clearance and the volume of distribution were low and KP1019 was extremely well tolerated with only limited side effects observed. In order to avoid dose limitation by the high infusion volume, the clinical development was recently redirected to a more salt, KP1339, and in the phase I study on KP1339 a total of 34 patients with various solid tumors were treated with only very minor side effects observed. Partial response was observed in one patient with a neuroendocrine tumor (NET) and stable disease in seven patients [12].

Ruthenium(III) complexes are considered as prodrugs that are activated by reduction to more reactive ruthenium(II) species in the tumor environment [17,18]. Organometallic half-sandwich ruthenium (II)-arene complexes, despite being in an 'activated state' tend to be more stable that ruthenium(III) complexes and some show considerable potential as tumor-inhibiting agents. Two prototype ruthenium(II)-arene compounds are [Ru(η^6-*p*-cymene)(PTA)Cl_2] PTA = 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane (RAPTA-C) and [Ru(η^6-biphenyl)Ru(en)Cl]$^+$ (RM175, en = ethylenediamine). The latter complex displays a similar cytotoxicity to that of cisplatin in certain cancer cell lines and was shown to reduce tumor growth in a cisplatin-resistant model [19–23], whereas the RAPTA complexes have modest cytotoxicites, but relevant antimetastatic [24–26], antiangiogenic [27] and antitumor [28] properties *in vivo*. Remarkably, if RAPTA-C is applied with concurrent tumor oxygenation, i.e. during tumor normalization, its ability to reduce tumor growth is greater than that of the highly cytotoxic agent doxorubicin, even at a considerably lower dose [29].

The development of metal-based drugs based on osmium has gained interest in recent years. Osmium offers several distinct features from ruthenium, including slower ligand exchange kinetics, different redox

* Corresponding author.
E-mail address: paul.dyson@epfl.ch (P.J. Dyson).

http://dx.doi.org/10.1016/j.jinorgbio.2017.07.027
Received 2 June 2017; Received in revised form 24 July 2017; Accepted 26 July 2017
Available online 28 July 2017

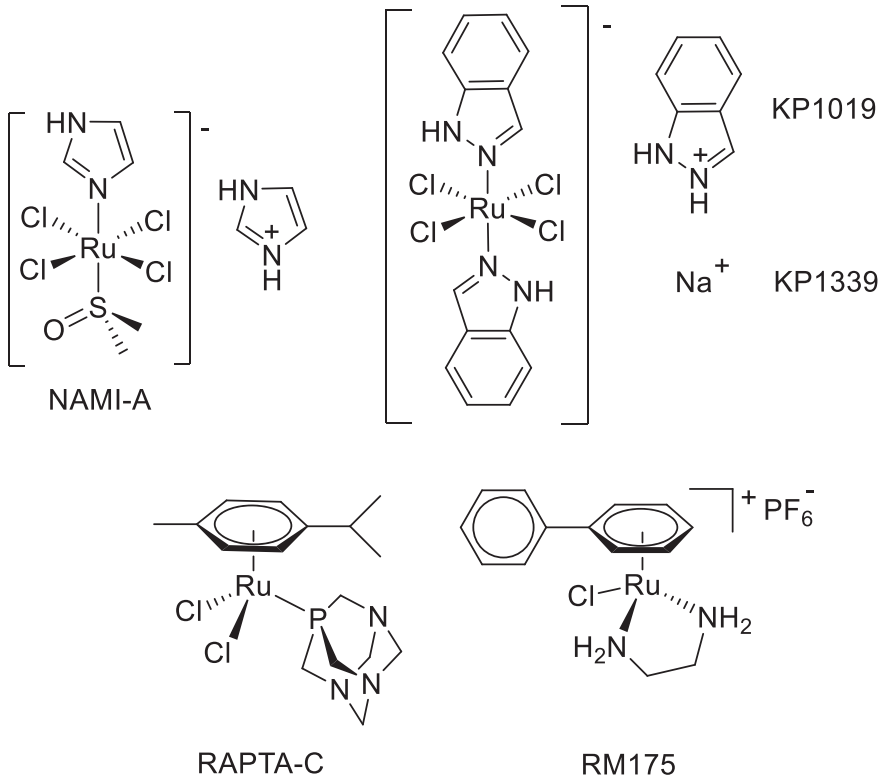

Fig. 1. Structures of the Ru(III) complexes NAMI-A and KP1019/KP1339 and the Ru(II) complexes RAPTA-C and RM175.

properties and markedly stronger spin-orbit coupling [3]. Thus, osmium complexes are considered interesting alternatives to ruthenium-based anticancer agents because of their slower ligand exchange kinetics (and hence stability) under physiological conditions [3].

One way to modify the biological properties of ruthenium(II)- and osmium(II)-arene complexes is to conjugate an organic molecule of known biological function to the organometallic fragment, either via derivatization of the η^6-coordinated arene or via an appropriate mono or bidentate leg ligand (Fig. 2). One of the first examples of this approach involves the conjugation of ethacrynic acid to the ruthenium(II)-*p*-cymene fragment via a modified imidazole ligand [30]. Further examples involve anchoring ethacrynic acid units to ruthenium(II) or osmium(II)-*p*-cymene via monodentate phosphine leg ligands [31,32]. Ethacrynic acid is an inhibitor of glutathione transferases (GSTs) that catalyze the conjugation of glutathione to a variety of exogenous and endogenous electrophiles, being thus involved in the inactivation of various anticancer agents [33,34]. Overexpression of GSTs leads to drug resistance due to enhanced drug detoxification and drug inactivation. The resulting (mercapturate) products are easily eliminated from the organism [35]. As GSTs play an important role in the mechanism of anticancer drug resistance [33,36–38], GST inhibitors and GST-activated prodrugs have been used to sensitize drug-resistant cancer cells [39–41]. Ethacrynic acid, ([2,3-dichloro-4-(2-methylene-1-oxobutyl)phenoxyl] acetic acid, EA-H Fig. 2), inhibits the major classes of GST (α, μ, and π), with the π isozyme being the most active [42]. EA-H has been shown to sensitize cancer cells to the cytotoxic effect of alkylating agents such as melfalan [43–46], carmustine [47], mitomycin C [48], nitrogen mustard [49,50], and chlorambucil [42,50]. EA-H sensitization of tumors to doxorubicin [49,51] and cisplatin [43,52,53] was also observed.

The development of GST-activated organometallic prodrugs represents an interesting approach to overcome resistance. For example, in the case of a platinum(IV) anticancer compound incorporating two EA moieties as axial ligands, binding to GST cleaves the Pt-EA bonds releasing cisplatin while simultaneously inactivating the enzyme [54–56]. Bifunctional RAPTA derivatives have been designed to achieve a similar outcome [57], and conjugation of an EA unit to the arene ring or via an imidazole leg ligand (Fig. 2) [30,54] leads to improved antiproliferative activity [30,54,57–59].

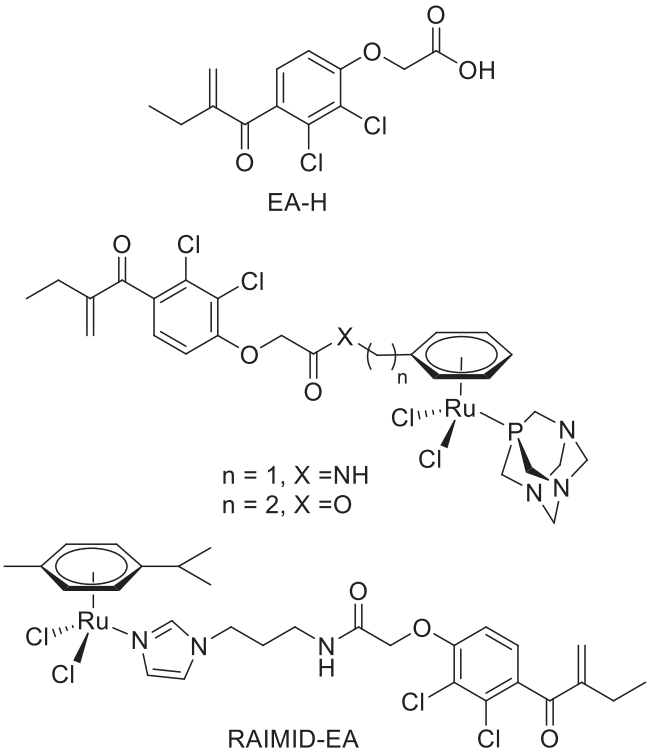

Fig. 2. Structures of ethacrynic acid (EA-H) and of reported Ru(II)-arene ethacrynic acid conjugates that act as both GST inhibitors and cytotoxic agents.

It has also been shown that $[(\eta^6\text{-arene})\text{Ru(en)Cl}]^+$ (arene = *p*-cymene, biphenyl or 9,10-dihydrophenanthrene), are non-selective