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Comparative solution equilibrium and structural studies of half-sandwich ruthenium(II)(η^6-toluene) complexes of picolinate derivatives

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ABSTRACT

Five Ru(II)(η^6-toluene) complexes formed with 2-picolinic acid and its various derivatives have been synthesized and characterized. X-ray structures of four complexes are also reported. Complex formation processes of [Ru(II)(η^6-toluene)$(H_2O)_3$]$^{2+}$ organometallic cation with the metal-free ligands were studied in aqueous solution in the presence of chloride ions by the combined use of ^{1}H NMR spectroscopy, UV–visible spectrophotometry and pH-potentiometry. Solution stability, chloride ion affinity and lipophilicity of the complexes were characterized together with *in vitro* cytotoxic and antiproliferative activity in cancer cell lines being sensitive and resistant to classic chemotherapy and in normal cells as well. Formation of mono complexes such as [Ru(η^6-toluene)(L)(Z)]$^{+/0}$ (L: completely deprotonated ligand; Z = H_2O/Cl^-) with high stability and [Ru(η^6-toluene)(L)(OH)] was found in solution. The pK_a values (8.3–8.7) reflect the formation of low amount of mixed hydroxido species at pH 7.4 at 0.2 M KCl ionic strength. The complexes are fairly hydrophilic and show moderate chloride ion affinity and fast chloride-water exchange processes. The studied complexes exhibit no cytotoxic activity in human cancer cells (IC_{50} > 100 μM), only complexes formed with 2-picolinic acid (**1**) and its 3-methyl derivative (**2**) represented a moderate antiproliferative effect (IC_{50} = 84.8 (**1**), 79.2 μM (**2**)) on a multidrug resistant colon adenocarcinoma cell line revealing considerable multidrug resistant selectivity. Complexes **1** and **2** bind to human serum albumin covalently and relatively slowly with moderate strength at multiple binding sites without ligand cleavage.

1. Introduction

Ruthenium complexes have emerged as attractive alternatives to platinum based compounds such as *cisplatin*, *carboplatin* and *oxaliplatin* which are undoubtedly successful anticancer drugs but have several drawbacks such as serious side-effects and lack of activity against certain types of cancer. Ruthenium compounds have different physico-chemical and pharmacokinetic properties compared to the platinum drugs, and they have different mechanism of action as well, that is the reason why they are the subject of extensive drug discovery efforts [1–3]. Imidazolium *trans*-[tetrachlorido(DMSO)(imidazole)ruthenate (III)] (NAMI-A) was the first Ru(III) complex reached clinical trials [4], while sodium *trans*-[tetrachloridobis(1*H*-indazole)ruthenate(III)] (NKP-1339, IT-139) is one of the most promising investigational non-Pt drugs in current clinical development. NKP-1339 is active against solid malignancies such as non-small cell lung cancer, colorectal carcinoma and the treatment is accompanied by minor side effects [5,6]. While *cisplatin* induces DNA damage *via* adduct formation [7], endoplasmic reticulum stress and reactive oxygen species-related effects were found to be involved in the mechanism of action of NKP-1339 [5,8]. Ru(III) complexes are considered as prodrugs that are activated by reduction and it provides the impetus for the development of various Ru(II) anticancer compounds [5]. It is noteworthy that a novel Ru(II) compound [Ru(4,4′-dimethyl-2,2′-bipyridine)$_2$-(2-(2′,2″:5″,2‴-terthiophene)-imidazo[4,5-f][1,10]phenanthroline)]Cl_2 (TLD-1433) has entered a human clinical trial recently as nontoxic photosensitizing agent [9]. Ru(II) is often stabilized in the +2 oxidation state by the coordination of η^6-arene type ligands and there are two main prototypes of Ru(II)-arene complexes [3]: i) 1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]decane (PTA) containing Ru(II)-arene (RAPTA) compounds such as [Ru(η^6-*p*-cymene)(PTA)Cl_2] (RAPTA-C) possessing significant antimetastatic property and is ready for translation into clinical evaluation [10,11]; ii) the

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bidentate 1,2-ethylenediamine (en) containing Ru(II)-arene (RAED) complexes such as [Ru(η^6-biphenyl)(en)Cl]PF_6 (RM175) that possesses a similar cytotoxic activity to *cisplatin* [12,13]. In most of the half-sandwich organoruthenium(II) compounds a bidentate ligand with an (*O*,*O*), (*O*,*S*), (*O*,*N*), (*N*,*N*) or (*N*,*S*) binding mode is coordinated and a chloride ion acts as the leaving group [3,14–16]. Aquation (replacement of the chlorido ligand by a water molecule) facilitates the reaction with biological macromolecules such as proteins or DNA, therefore the strength of the Ru−Cl bond and the rate of its cleavage have a strong impact on the bioactivity of the Ru(II)-arene complexes [17]. Notably, the chemical and pharmacological properties of the Ru(II)-arene half-sandwich compounds can be fine-tuned by variation of the coordinated ligand, the arene ring and the leaving group [1,3,10]. Although a large number of Ru(II)-arene compounds has been developed and extensively investigated, information about their solution speciation and stability constants is still limited in the literature. Most of the solution equilibrium studies are focused on [Ru(η^6-*p*-cymene)(X,Y)Cl] type complexes [18–24]. For the better understanding of the pharmacokinetic properties and mechanisms of action of these metal complexes, the knowledge of the aqueous chemistry and the most plausible chemical forms in water, especially at physiological pH, is a mandatory prerequisite.

In our previous works we have studied the biological activity of Ru(II)(η^6-*p*-cymene) complexes of various pyridine derivatives [25–28] and moderate-to-low cytotoxicity was found in six tumor cell lines; although the complex of pyridine-2-carboxylic acid (2-picolinic acid, picH) represents an enhanced antiproliferative activity (*e.g.* IC_{50} = 82 μM in HeLa cells, 36 μM in FemX cells [27]) and antimetastatic effect based on wound migration assay [25]. The solution speciation of Ru(II)(η^6-*p*-cymene) picolinate complexes was also studied by some of us revealing the formation of mono-ligand complexes with high stabilities [23]. Notably, the Os(II) congener of the picolinate complex showed very high *in vitro* cytotoxic activity [29].

As the physico-chemical and biological properties can be modified by the exchange of the arene ring, in this work we have prepared and structurally characterized Ru(II)(η^6-toluene) complexes formed with picH and its 3-methyl (3-Me-picH), 5-bromo (5-Br-picH), 4-carboxylic (2,4-dipicH_2) and 5-carboxylic (2,5-dipicH_2) derivatives (Chart 1). In addition to the determination of the solid phase structures of four complexes by X-ray crystallography, solution speciation of these Ru(II)(η^6-toluene) complexes in water was revealed by UV–visible (UV–vis) spectrophotometry, ^{1}H NMR spectroscopy and pH-potentiometry involving studies on their stability and chloride ion affinity. The antiproliferative and cytotoxic effectiveness of these complexes in multidrug resistant/non-resistant human cancer lines was also tested. Interactions between human serum albumin and the complexes showing antiproliferative effect were monitored using fluorometry and ultrafiltration.

2. Experimental

2.1. Chemicals

All solvents were of analytical grade and used without further purification. 2-Picolinic acid (picH), 3-methylpyridine-2-carboxylic acid (3-Me-picH), 5-bromo-2-pyridinecarboxylic acid (5-Br-picH), 2,4-pyridinedicarboxylic acid monohydrate (2,4-dipicH_2·H_2O), 2,5-pyridinedicarboxylic acid (2,5-dipicH_2), $RuCl_3$·$3H_2O$, KCl, HCl, KOH, 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS), 1-methylimidazole (N-MeIm), human serum albumin (HSA, as lyophilized powder with fatty acids, A1653), $NaClO_4$, KH_2PO_4, NaH_2PO_4·$2H_2O$, Na_2HPO_4·$2H_2O$ were purchased from Sigma-Aldrich in *puriss* quality. Doubly distilled Milli-Q water was used for preparation of samples. The purity of the ligands and the exact concentration of their stock solutions were determined by pH-potentiometric titrations and by the computer program Hyperquad2013 [30]. [Ru(η^6-toluene)Cl_2]$_2$ was prepared according to a well-known procedure [31]. A stock solution of [Ru(η^6-toluene)$(Z)_3]^{2+/+/0/-}$, where Z is H_2O or Cl^-, was obtained by dissolving [Ru(η^6-toluene)Cl_2]$_2$ in water and the exact concentration of this stock was determined with pH-potentiometric titrations. The modified phosphate-buffered saline (PBS′) contains 12 mM Na_2HPO_4, 3 mM KH_2PO_4, 1.5 mM KCl and 100.5 mM NaCl; and the concentration of the K^+, Na^+ and Cl^- ions corresponds approximately to that of the human blood serum (c(K^+) = 3.5–5.1 mM, c(Na^+) = 135–145 mM, c(Cl^-) = 96–106 mM [32]). HSA solution was freshly prepared before the experiments and its concentration was estimated from its UV absorption: ε_{280nm}(HSA) = 36,850 M^{-1} cm^{-1} [33]. Stock solution of N-MeIm was prepared on a weight-in-volume basis in PBS′ solution.

2.2. Synthesis of the complex [(η^6-toluene)RuCl(μ-Cl)]$_2$ with different picolinic acids

For the characterization of the prepared complexes ^{1}H and ^{13}C NMR spectroscopy, elemental analysis and electrospray ionization mass spectrometry (ESI-MS, Fig. S1) were used in addition to X-ray crystallography (*vide infra*). NMR spectra were recorded on a Bruker Avance III 500 spectrometer or a Bruker Ultrashield 500 Plus instrument, and DMSO-d_6 was used as solvent. ESI-MS measurements were performed using a Micromass Q-TOF Premier (Waters MS Technologies) mass spectrometer equipped with electrospray ion source. Elemental analysis of all compounds was performed with a Perkin–Elmer 2400 CHN Elemental Analyser (Perkin–Elmer, Waltham, MA) at the Microanalytical Laboratory of the University of Vienna.

2.2.1. Synthesis of the precursor [Ru(η^6-toluene)Cl(μ-Cl)]$_2$

[Ru(η^6-toluene)Cl(μ-Cl)]$_2$ was prepared according the literature procedure used for the analogous [Ru(η^6-benzene)Cl(μ-Cl)]$_2$ [31] by adding 5 mL of 1-methyl-1,4-cyclohexadiene to a solution of 0.5 g $RuCl_3$·$3H_2O$ (1.9 mmol) in 40 mL of absolute ethanol. This mixture was refluxed for 8 h. The reddish brown precipitate formed during the synthesis was filtered off, washed with diethyl ether and left to dry in exsiccator. Yield: 85%, 0.450 g; ^{1}H NMR (500.26 MHz, DMSO-d_6, δ, ppm): 2.12 (3H, s, CH_3), 5.68 (3H, m, C2, C4, C6 toluene), 5.97 (2H, m, C3, C5 toluene); ^{13}C NMR (125.79 MHz MHz, DMSO-d_6) 18.73 (CH_3), 82.22 (C4 toluene), 84.83 (C5, C3 toluene), 89.28 (C6, C2 toluene), 105.82 (C1 toluene).

2.2.2. Synthesis of chlorido[(pyridine-κN-2-carboxylato-κO)(η^6-toluene)ruthenium(II)] (1)

A solution of picH (0.015 g, 0.13 mmol) in 2 mL of 2-propanol was added to a warm solution of [Ru(η^6-toluene)Cl_2]$_2$ (0.030 g, 0.057 mmol) in 25 mL of 2-propanol. The reaction mixture was stirred at room temperature for 7 days and the yellow-range precipitate was formed. Solution was filtered off and product was dried in exsiccator. Yield: 58%, 0.023 g; ^{1}H NMR (500.26 MHz, DMSO-d_6, δ, ppm): 2.15 (3H, s, CH_3), 5.60 (2H, m, C2, C6 toluene), 5.70 (1H, t, C4 toluene), 5.99 (2H, m, C3, C5 toluene), 7.71 (1H, dd, C5 ligand), 7.75 (1H, d, C3,

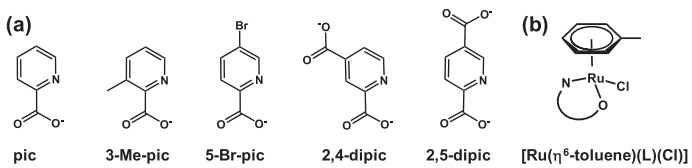

Chart 1. Chemical structures of the ligands in their completely deprotonated forms (a) and the general formula of the prepared [Ru(η^6-toluene)(L)(Cl)] complexes.