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Cytotoxicity of ruthenium(II) piano-stool complexes with imidazole-based PN ligands

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ABSTRACT

A series of *p*-cymene ruthenium(II) complexes with imidazol-2-yl phosphines as PN ligands was prepared. Depending on the number of imidazolyl substituents in the ligands Ph_{3-n}P(im)_n {**1**-**3**: n = 1-3, im = imidazol-2-yl (**a**), 1-methylimidazol-2-yl (**b**)} different coordination modes were observed: κP , $\kappa^2 N$, N or $\kappa^3 N$, N, N. The complexes were tested for their cytotoxicity in different cancer cell lines. Most of the compounds were found to be non-toxic; The compounds [(*p*-cymene)Ru(**1a**)Cl₂] (**4a**) shows cytotoxicity towards A2780sens and Hct116 cells in the μ M range but not in H4IIE cells. The cytotoxicity is decreased upon introduction of a methyl group as [(*p*-cymene)Ru(**1b**)Cl₂] (**4b**) shows only modest toxicities in the cell lines investigated. The κP compound [(*p*-cymene)Ru(**2a**)Cl₂] (**5a**) shows selective toxicity in H4IIE cells after 72 h whereas the $\kappa^2 N$, N compound [(*p*-cymene)Ru(**2a**)Cl₃OTf (**5a**') showed no toxicity in the cell lines investigated which again.

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1. Introduction

Since the authorization of cisplatin in 1978, the interest in and development of metal-based drugs prospers consistently. Still, cisplatin, *cis*-[Pt(NH₃)₂Cl₂], and its analogues, especially oxaliplatin and carboplatin, are basic chemotherapeutics in combination therapy [1–3]. The therapeutic effect of cisplatin is based on DNA platination which triggers apoptosis [4]. The major draw-back for a successful chemotherapy by platinum-based drugs is acquired resistance towards the applied drug during the course of therapy [5]. In order to circumvent those resistance mechanisms drugs, which address alternative cellular targets have to be developed [6-8].

The most promising metallodrugs besides cisplatin analogues are ruthenium-based drugs [9–13]. Compounds of Ru(II) and Ru(III) are able to overcome cisplatin resistance. Their cytotoxicity and antimetastatic properties are combined with low overall toxicity [9,14–17]. *Trans*-[RuCl₄(DMSO)(Im)]ImH (NAMI-A, where Im = imidazole) has completed phase I clinical trials [18].

¹ X-ray structure analysis.

Interestingly, NAMI-A is more active against metastases than against primary tumours [19]. Half-sandwich Ru(II) arene complexes of the type $[(\eta^6 - \text{arene}) \text{Ru}(\text{YZ})(\text{X})]$, where YZ is a bidentate chelating ligand and X is a good leaving group, show promising in vitro and in vivo anticancer activity [16]. These compounds coordinate to guanine N7 of DNA, which can be complemented by intercalative binding of an extended arene, as well as specific hydrogen-bonding interactions [20,21]. For example, increasing the size of the coordinated arene is accompanied by an increase in activity in human ovarian cancer cell lines [16] and the nature of the chelating ligand YZ and leaving group X seems influence their kinetics and even can change their nucleobase selectivity [22]. The RAPTA family of organometallic Ru(II) compounds contain the water-soluble phosphine ligand phosphaadamantane (pta) or derivatives thereof. Usually these compounds exhibit moderate in vitro activity, and some compounds show no activity in healthy cells up to millimolar concentrations. The pta compounds show little activity against primary tumours in vivo, although they exhibit some capacity to reduce lung metastases derived from a mammary carcinoma xenograft grown in mice [23]. The cytotoxicity of $[Ru(\eta^6$ p-cymene)Cl₂(pta)], in EAC cells is thought to be mediated by mitochondrial and Jun-N (amino)-terminal kinase (JNK) - p53 pathways [24]. For all Ru(II) compounds it is believed that in vivo, analogous to cisplatin, aquation of the chlorido complex is largely suppressed in intracellular fluids (with chloride concentrations are

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about 100 mM), whereas in the cell nucleus with a much lower chloride concentration (ca. 4 mM) the active aqua species forms [25,26]. Although their mechanism of action is still largely unknown, there is some evidence that RAPTA compounds work on molecular targets other than DNA [27–29], implying a biochemical mode of action profoundly different from classical platinum anticancer drugs.

We are currently examining the use of imidazole-based PN ligands in biomedical applications [30–32] as well as in catalysis [33,34]. Here we present coordination chemistry of these PN ligands towards (η^6 -cymene)Ru(II) and basic cytotoxicity studies in different cancer cell lines.

2. Experimental section

The ligands Ph_{3-n}P(im)_n {**1**-**3**: n = 1-3, im = imidazol-2-yl (**a**), 1methylimidazol-2-yl (**b**) and [(cym)Ru(κ P-**1b**)Cl₂] (**4b**) were prepared according published procedures [32,34–37]. All reactions were carried out in Schlenk tubes under an atmosphere of dry nitrogen using anhydrous solvents purified according to standard procedures. All chemicals were purchased from commercial sources and used as received. ¹H and ³¹P NMR spectra were recorded on a Bruker DRX 200 and Bruker DRX 500 spectrometer. The ¹H spectra were calibrated against the residual proton signal of the solvent as an internal reference (methanol- d_4 : $\delta_H = 3.31$ ppm; D₂O: $\delta_H = 4.79$ ppm, CDCl₃: $\delta_H = 7.26$ ppm) while the ³¹P{¹H} NMR spectra were referenced to external 85% H₃PO₄. The MALDI mass spectra were recorded on a Bruker Ultraflex MALDI-TOF mass spectrometer. The elemental composition of the compounds was determined with a PerkinElmer Analysator 2400 at the Institut fur Pharmazeutische und Medizinische Chemie, Heinrich-Heine-Universitat Dusseldorf.

2.1. Synthesis of (cym)Ru complexes

2.1.1. [(cym)Ru(κP-**1a**)Cl₂] (**4a**)

Ligand **1a** (83 mg, 0.33 mmol) and $[Ru(cym)Cl_2]_2$ (100 mg, 0.16 mmol) were dissolved in dry CH₂Cl₂ (15 mL) and stirred for 24 h. The dark red solution was concentrated to 5 mL and Et₂O was added. The precipitate was collected and dissolved in thf, filtered and again precipitated upon addition of *n*-hexane. The red solid was filtered off and dried in vacuo. Yield: 52 mg (28%). ¹H NMR (200 MHz, methanol-*d*₄): $\delta = 0.97$ (d, J = 7.0 Hz, 6H), 1.83 (s, 3H), 2.42 (sept., J = 7.0 Hz, 1H), 5.42 (m, 4H), 7.10 (d, J = 1.2 Hz, 2H), 7.72 (m, 10H). ³¹P{¹H}-NMR (81 MHz, methanol-*d*₄): $\delta = 22$ (s). EI-MS (CH₃OH): m/z (%) = 558 (40) [M]⁺, 523 (28) [M - Cl]⁺, 486 (100) [M - 2Cl]⁺, 389 (27) [M - Cl₀H₁₄]⁺, 352 (45) [M - Cl₀H₁₄ - Cl]⁺. C₂₅H₂₇Cl₂N₂PRu (558.45): calc. C 53.77, H 4.87, N 5.02; found C 53.44, H 4.98, N 4.77.

2.1.2. [(cym)Ru(κP-2a)Cl₂] (5a)

Ligand **2a** (50 mg, 0.21 mmol) and $[Ru(cym)Cl_2]_2$ (63 mg, 0.1 mmol) were dissolved in dry CH₃CN (25 mL) and stirred for 24 h. The dark red solution was concentrated to 5 mL. The red precipitate was filtered off, washed with Et₂O and dried in vacuo. Yield: 27 mg (24%). ¹H NMR (200 MHz, CDCl₃): $\delta = 0.97$ (d, J = 7.0 Hz, 6H), 1.76 (s, 3H), 2.47 (sept., J = 7.0 Hz, 1H), 5.83 (m, 4H), 7.26 (d, J = 1.2 Hz, 4H), 7.39 (m, 5H). ³¹P{¹H}-NMR (81 MHz, CDCl₃): $\delta = -1$ (s). ESI-MS (CH₃OH): m/z (%) = 513.4 (43) [M - Cl]⁺, 477.4 (100) [M - 2Cl]⁺. C₂₂H₂₅Cl₂N₄PRu · H₂O (566.43): calc. C 46.65, H 4.80, N 9.89; found C 47.08, H 5.20, N 9.65.

2.1.3. $[(cym)Ru(\kappa^2 N, N-2a)Cl]OTf(5a')$

 $[Ru(cym)Cl_2]_2$ (101 mg, 0.16 mmol) and AgOTf (85 mg, 0.33 mmol) were dissolved in dry CH₃CN (15 mL) and refluxed for 1 h. Precipitated AgCl was filtered off and the red filtrate was added

to a suspension of **2a** (80 mg, 0.33 mmol) in dry CH₃CN (10 mL). The reaction mixture was refluxed for 1 h and stirred for 24 h at ambient temperature. The resulting yellow solution was concentrated to ca. 3 mL and Et₂O was added. The mixture was kept at –18 °C. The yellow precipitate was filtered off, washed with Et₂O and dried in vacuo. Yield: 154 mg (70%). ¹H NMR (200 MHz, methanol-*d*₄): δ = 1.31 (d, *J* = 6.9 Hz, 6H), 2.04 (s, 3H), 2.91 (sept., *J* = 6.9 Hz, 1H), 5.73 (m, 4H), 7.30 (d, *J* = 1.47 Hz, 2H), 7.46 (d, *J* = 1.5 Hz, 2H), 7.68 (m, 5H). ³¹P{¹H}-NMR (81 MHz, methanol-*d*₄): δ = –22 (s). ESI-MS (CH₃OH): *m/z* (%) = 493.5 (100) [M + O]⁺, 477.5 (15) [M]⁺, C₂₃H₂₅ClF₃N₄O₃PRuS·½ H₂O (671.03): calc. C 41.16, H 3.91, N 8.49; found C 41.15, H 3.42, N 8.43.

2.1.4. $[(cym)Ru(\kappa^2N,N-2b)Cl]Cl$ (5b)

[Ru(cym)Cl₂]₂ (100 mg, 0.16 mmol) and **2b** (118.9 mg, 0.33 mmol) were dissolved in dry CH₃CN (15 mL) and stirred for 24 h. The orange solution was concentrated to 5 ml and Et₂O was added. The resulting solid was filtered off, washed with Et₂O and dried in vacuo. Yield: 158 mg (84%). ¹H NMR (200 MHz, CDCl₃): $\delta = 1.25$ (d, J = 7.0 Hz, 6H), 1.67 (s, 3H), 2.66 (sept., J = 7.0 Hz, 1H), 4.20 (s, 6H), 4.94 (m, 4H), 7.15 (d, J = 1.4 Hz, 2H), 7.56 (m, 5H), 7.83 (d, J = 1.4 Hz, 2H). ³¹P{¹H}-NMR (81 MHz, CDCl₃): $\delta = -60$ (s). ESI-MS (CH₃OH): m/z (%) = 541.4 (76) [M]⁺, 505.5 (35) [M - Cl]⁺, 407.3 (100) [M - C₁₀H₁₄]⁺, 371.4 (37) [M - C₁₀H₁₄ - Cl]⁺. C₂₄H₂₉Cl₂N₄PRu · 2H₂O (612.50): calc. C 47.1, H 5.4, N 9.1; found C 47.1, H 5.3, N 9.2.

2.1.5. $[(cym)Ru(\kappa^3N,N,N-3a)]Cl_2$ (6a)

[Ru(cym)Cl₂]₂ (50 mg, 0.082 mmol) and **3a** (38.2 mg, 0.16 mmol) were dissolved in dry CH₃CN (30 mL) and refluxed for 2 h. The yellow precipitate was collected by filtration, washed with a small amount of CH₃CN and dried in vacuo. Yield: 21 mg (24%). ¹H NMR (200 MHz, methanol-*d*₄): δ = 1.20 (d, *J* = 6.7 Hz, 6H), 2.45 (s, 3H), 3.24 (sept., *J* = 6.7 Hz, 1H), 6.28 (m, 4H), 7.47 (dd, *J* = 1.6 Hz, *J* = 2.94 Hz, 3H), 8.23 (d, *J* = 1.6 Hz, 3H). ³¹P{¹H}-NMR (81 MHz, methanol-*d*₄): δ = -103 (s). ESI-MS (CH₃OH): *m*/*z* (%) = 467.3 (100) [M]⁺, 234 (58) [M – **3a**]⁺. C₁₉H₂₃Cl₂N₆PRu·5/2H₂O (583.38): calc. C 39.1, H 4.8, N 14.4; found C 39.3, H 4.5, N 14.1.

2.1.6. $[(cym)Ru(\kappa^3N,N,N-3b)]Cl_2$ (6b)

[Ru(cym)Cl₂]₂ (100 mg, 0.16 mmol) and **3b** (91 mg, 0.33 mmol) were dissolved in dry CH₃CN (25 mL) and refluxed for 1.5 h. The yellow precipitate was collected, washed with a small amount of CH₃CN and dried in vacuo. Yield: 26 mg (18%). ¹H NMR (200 MHz, methanol-*d*₄): δ = 1.23 (d, *J* = 6.7 Hz, 6H), 2.45 (s, 3H), 3.23 (sept., *J* = 6.7 Hz, 1H), 4.03 (s, 9H), 6.29 (m, 4H), 7.55 (dd, *J* = 1.6 Hz, *J* = 4.0 Hz, 3H), 8.21 (d, *J* = 1.6 Hz, 3H). ³¹P{¹H}-NMR (81 MHz, methanol-*d*₄): δ = -116 (s). ESI-MS (CH₃OH): *m/z* (%) = 509 (100) [M - Cl]⁺, 461 (29) [M - C₄N₂H₆ + Cl]⁺. C₂₂H₂₉Cl₂N₆PRu (580.46): calc. C 45.5, H 5.0, N 14.5; found C 45.2, H 4.9, N 14.1.

2.1.7. $[(cym)Ru(\kappa^2N,N-en)Cl]Cl(7)$

[Ru(cym)Cl₂]₂ (100 mg, 0.16 mmol) was solved in dry CH₃CN (25 mL). A yellow solid precipitated upon addition of an excess of ethylenediamine (en). The reaction mixture was stirred for 20 min to complete the reaction. The solid was filtered off, washed with dry CH₃CN and dried in vacuo. Yield: 99 mg (84%). ¹H NMR (200 MHz, methanol-*d*₄): δ = 1.27 (d, *J* = 7.0 Hz, 6H), 2.43 (s, 3H), 2.70 (m, br, 4H), 2.81 (sept., *J* = 7.0 Hz, 1H), 5.81 (m, 4H). ESI-MS (CH₃OH): *m*/*z* (%) = 295 (100) [M - Cl]⁺, 235 (34) [M - Cl - en]⁺.

2.2. Distribution coefficients (log D)

The *n*-octanol–water distribution coefficients of the compounds were determined using a shake-flask method. PBS buffered bi-

distilled water (100 mL, phosphate buffer, $c(PO_4^{3-}) = 10$ mM, c(NaCl) = 0.15 M, pH adjusted to 7.4 with HCl) and *n*-octanol (100 mL) were shaken together using a laboratory shaker (PerkinElmer), for 72 h to allow saturation of both phases. 1 mg of each compound was mixed in 1 mL of aqueous and organic phase, respectively for 10 min using a laboratory vortexer. The resultant emulsion was centrifuged (3000g, 5 min) to separate the phases. The concentrations of the compounds in the organic and aqueous phases were then determined using UV absorbance spectroscopy (230 nm). log D_{pH} was defined as the logarithm of the ratio of the concentrations of the complex in the organic and aqueous phases log $D = \log[[compound_{(org)}]/[compound_{(aq)}])$, the value reported is the mean of three separate determinations.

2.3. DNA binding studies

The UV/Vis kinetic studies and thermal denaturation temperature T_m determinations for 1:5 complex/DNA mixtures [DNA concentration = M(base pairs)] were performed in a 10 mM phosphate buffer at pH = 7.4. Melting curves were recorded at 2 °C steps for the wavelength 260 nm with an Analytik Jena SPECORD 100 spectrometer and a thermostat. ΔT_m values were calculated by determining the midpoints of melting curves. The experimental ΔT_m values are estimated to be accurate within ± 1 °C. Concentrations of calf-thymus (ct) DNA were determined spectrophotometrically using the molar extinction coefficient $\varepsilon_{260} = 13200 \text{ M}^{-1} \text{ cm}^{-1}$ [38].

2.4. Cell culture

Hct116 human colon carcinoma and H4IIE rat hepatoma cells were grown in Dulbecco's modified Eagle's medium (DMEM, GIBCO; Germany), A2780 human ovarian carcinoma cells were grown in RPMI cell culture medium; all media contained 10% foetal calf serum (PAA Laboratories; Austria), penicillin (100 U/mL) and streptomycin (100 μ g/mL) at 5% CO₂ and 37 °C.

2.5. Determination of cytotoxicity

The effect of the compounds on cell viability was determined using the MTT assay [39]. Cells were plated on 96-multiwell plates (H4IIE, Hct116 cells: 15.000/well, A2780 cells: 35.000/well), allowed to attach for 24 h and then treated with different concentrations of the substances for indicated time points. In all experiments compounds were dissolved in DMSO. The DMSO concentration was equal at all compound concentrations analyzed. The highest DMSO concentration used was 1%; no toxic effect was detected at this concentration. After treatment medium was changed and cells were incubated for 30 min under cell culture conditions with 1 mg/mL MTT. Then the cells were lysed with 100% DMSO. The concentration of reduced MTT as a marker for cell viability was measured photometrically (560 nm) using a Wallace Victor2 1420 multilabel counter (PerkinElmer).

2.6. Statistical analysis

All data were analyzed using one-way analysis of variance, followed by Bonferroni or Dunnet post hoc analysis to determine statistical significance. *P* values < 0.05 were considered statistically significant. The analysis was performed with GraphpadPrism 5.0c.

2.7. Crystallography

Crystals of compounds **5a** and **5a**' \cdot CH₂Cl₂ suitable for X-ray study were selected by means of a polarization microscope and investigated with an STOE Imaging Plate Diffraction System, using

graphite monochromatized Mo K α radiation ($\lambda = 0.71073$ Å). Unit cell parameters were determined by least-squares refinements on the positions of 8000 reflections. Space group type no. 14 was uniquely determined for both compounds. Corrections for Lorentz and polarization effects were applied. The structures were solved by direct methods (SHELXS-86) [40] and subsequent ΔF -syntheses. Approximate positions of all the hydrogen atoms were found in different stages of converging refinements by full-matrix leastsquares calculations on F^2 [40]. Anisotropic displacement parameters were refined for all atoms heavier than hydrogen. With idealized bonds lengths and angles assumed for all the CH, CH₂ and CH₃ groups, the riding model was applied for the corresponding H atoms and their isotropic displacement parameters were constrained to 120%, 120% and 150% of the equivalent isotropic displacement parameters of the parent carbon atoms, respectively. In addition, the H atoms of the CH₃ groups were allowed to rotate around the neighbouring C-C bonds. Crystallographic data of 5b·2CH₃CN·½H₂O were collected at 183(2) K on an Oxford Diffraction Xcalibur system with a Ruby detector using Mo Ka radiation ($\lambda = 0.7107$ Å) that was graphite-monochromated. Suitable crystals were covered with oil (Infineum V8512, formerly known as Paratone N), mounted on top of a glass fibre and immediately transferred to the diffractometer. The program suite CrysAlisPro was used for data collection, multi-scan absorption correction and data reduction [41]. The structure was solved with direct methods using SIR97 [42] and was refined by full-matrix least-squares methods on F² with SHELXL-97 [40]. The structure was checked for higher symmetry with help of the program Platon [43].

3. Results and discussion

3.1. Syntheses and characterization

We prepared the (cym)Ru complexes (cym = p-cymene) by reaction of [{(cym)RuCl₂}₂] and the corresponding ligands **1a,b–3a,b** in acetonitrile (Scheme 1). The corresponding complexes precipitated from solution or were obtained in analytically pure form after addition of Et₂O.

All compounds exhibit sharp singlet resonances in their ³¹P{¹H} NMR spectra (Table 1). The analytical data of complex **4a** are nearly identical to the one of **1b** which has been previously described by Caballero et al. Complexes **6a** and **6b** show one set of signals for the ligands in the ¹H NMR (methanol- d_4), indicating local C_{3v} symmetry. Additionally, their ³¹P{¹H} NMR spectra show the typical coordination shift to about -110 ppm which is indicative for the $\kappa^3 N, N, N$ -coordination mode in complexes of tris(imidazolyl)phosphine ligands [44-46].

The ligands **1a/b** in complexes **4a** and **4b** show the κP coordination mode and the ligands **3a/b** in complexes **6a** and **6b** show the $\kappa^3 N, N, N$ mode. The coordination modes found in the isolated complexes of ligands **2a** and **2b** are more complex. Initially, the reaction of [{(cym)RuCl₂}] with the PN phosphine ligands proceeds via a P coordinated species. The course of the reactions was monitored by ³¹P NMR and even during formation of **6a** and **6b** signals for a transient species, tentatively assigned to κP species (see Fig. ESI1), was observed. All attempts to 'trap' κP -bound ligands **3a/b**, e.g. by reaction of these ligands with [(cym)Ru(en)Cl] (**7**), finally resulted in formation of compounds **6a** and **6b**, respectively.

When ligands **2a** and **2b** were used, κP coordination can compete with the chelating $\kappa^2 N$, N binding mode. Those coordination modes were found in compounds **5a**' and **5b** (both $\kappa^2 N$, N) and **5a** (κP) which were unambiguously assigned by their single crystal structures (see below). As mentioned previously, κP coordination



Scheme 1. Reaction of [{(cym)RuCl₂}₂] in acetonitrile with ligands 1–3 yields compounds 4–6.

leads to a classical coordination shift of the ³¹P resonance towards lower field whereas chelating $\kappa^2 N.N$ (or $\kappa^3 N.N.N$ in **6a.b**) results in a shift towards higher field (Table 1). The reaction of 2b with [{(cym)RuCl₂]₂] yields **5b**, where only $\kappa^2 N, N$ coordination of **2b** is observed. The related ligand 2a can adopt both coordination kP and $\kappa^2 N, N$ modes depending on the chloride concentration in solution. The reaction of stoichiometric amounts of [{(cym)RuCl₂}] and 2a gives a mixture of $[(cym)Ru(\kappa^2 P-2a)Cl_2]$ (5a) and $[(cym)Ru(\kappa^2 N, N-a)Cl_2]$ **2a**)Cl]⁺ and unreacted ligand (Fig. 1). Addition of one equivalent of $Ag(O_3SCF_3)$ to that reaction mixture quantitatively yields **5a**'. This reaction is not reversible as 5a' is persistent even in the presence of high chloride concentration (up to >100 fold) as was shown by ^{31}P NMR spectroscopy. Therefore small electronic changes in the ligand system, like introduction of electron donating methyl groups, can favour one binding mode over the other. This might be of importance as this might alter their cytotoxicity profiles, e.g. in the presence of other coordinating ligands as chloride.

3.2. Solid-state structures

The solid-state structures of compounds **5**a', **5**a and **5**b were determined by single crystal analysis and crystallographic data is summarized in Table 2. Compounds **5**a' and **5**a crystallized in the monoclinic space group *P* 2₁/c. Compound **5**b crystallized as

Table 1 ³¹P{¹H} resonances (δ_C) of the (cym)Ru complexes **4a,b–6a,b** and the corresponding coordination shifts ($\Delta \delta = \delta_C - \delta_L$).

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Compound	$\delta(^{31}P)/ppm$	$\Delta\delta(^{31}P)/ppm$		
4a	22 ^a	+45		
4b	8 ^b	+35		
5a	1 ^b	+46		
5a′	-22 ^a	+23		
5b	-60^{b}	-15		
6a	-103^{a}	-43		
6b	-116^{a}	-44		

^a In methanol-d₄.

^b In CDCl₃.

solvate **5b**·3CH₃CN· $\frac{1}{2}$ H₂O in the monoclinic space group *P* 2₁/n. The ruthenium atom in all structures is in octahedral coordination sphere with the η^6 -cymene ligand occupying one face of the octahedron. In **5a** the other three positions are occupied by two chlorido ligands and κ *P*-bound **2a** (Fig. 2). In **5a**' and **5b** the $\kappa^2 N$,*N*-coordination mode of the PNN ligands **2a** and **2b** is found (Fig. 3 and Fig. 4). The ruthenium atom is coordinated by **2a/b** in the chelating $\kappa^2 N$,*N* mode and a chlorido ligand. The other chloride or CF₃SO₃ acts as counter ion to the complex cations, respectively. The metric parameters found in **5a** and **5a**'/**5b** are within the range found for other compounds [(cym)Ru(PR₃)Cl₂] [31,35,47–49] and complexes [(cym)Ru(N–N)Cl]⁺ with diamino ligands [31,50,51].

In the solid-state structures of **5a** and **5b** the phenyl substituents of the corresponding ligand **2a/b** and the *p*-cymene ligand at the ruthenium atom adopt *cis* (**5b**) and *trans* positions (**5a'**) (Scheme 2). The compounds **5a** and **5a'**, bearing ligand **2a** with NH functionalities, show hydrogen bonding in their solid-state structures.

In the solid state of **5a**' intermolecular hydrogen bonds are formed between N2H1…O1_(triflate) and N4H2…Cl1 thus forming one-dimensional arrays. Also in the solid state of **5a** the intermolecular hydrogen bonds N3H2…Cl2 result in formation of onedimensional arrays. Additionally a weak bifurcated intramolecular hydrogen bond between N1H1 and Cl1 and Cl2 is found.

3.3. Biological studies

Biological studies were performed on compounds **4a**,**b**, **5a**,**b** and **5a**' as well as **6a** and **6b**. The *n*-octanol–water distribution coefficients of compounds **4a**,**b**, **5a**,**b** and **5a**' were determined as log $D_{7,4}$ values using phosphate buffered saline (PBS). The log $D_{7,4}$ value decreases within the series **4a** > **4b** > **5a**' > **5a** > **5b** (Table 3). The introduction of additional imidazolyl groups in the κP complexes increases the water-solubility of the compounds as does the introduction of charge in complexes **5a**/**b**. Surprisingly, the complexes having *N*-methyl groups in the PN ligands are more water soluble than their NH-congeners.

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Table 2

Crystallographic data for compounds **5a**, **5a**' and **5b** · 2CH₃CN · ½H₂O.

Compound	5a	$5a' \cdot CH_2Cl_2$	$\pmb{5b} \cdot 2CH_3CN \cdot \frac{1}{2}H_2O$	
Empirical formula	C ₂₂ H ₂₅ Cl ₂ N ₄ PRu	C24H27Cl3F3N4O3PRuS	C ₂₈ H ₃₆ Cl ₂ N ₆ O _{0.5} PRu	
Formula weight	548.40	746.96	667.57	
Temperature/K	291(2)	291(2)	183(2)	
Wavelength/Å	0.71073	0.71073	0.71073	
Crystal system	Monoclinic	Monoclinic	Monoclinic	
Space group	P 2 ₁ /c	P 21/c	P 2 ₁ /n	
Unit cell dimensions				
a/Å	17.2041(9)	14.985(2)	10.98070(16)	
b/Å	9.7578(8)	7.7408(8)	12.8114(2)	
c/Å	14.1705(7)	26.642(4)	23.9097(4)	
$\alpha / ^{\circ}$	90	90	90	
β/°	103.729(6)	98.273(18)	93.5635(14)	
$\gamma/^{\circ}$	90	90	90	
Volume/Å ³	2310.9(3)	3058.2(7)	3357.07(9)	
Ζ	4	4	4	
Density (calculated)/Mg/m ³	1.576	1.622	1.321	
Absorption coefficient/mm ⁻¹	0.996	0.947	0.701	
F(000)	1112	1504	1372	
Crystal size/mm ³	0.3 imes 0.3 imes 0.3	0.2 imes 0.2 imes 0.2	$0.24\times0.18\times0.11$	
Theta range for data collection	2.42-24.99°	1.91-25.92°	2.63–33.14°	
Index ranges	$-20 \leq h \leq 20$,	$-18 \leq h \leq 18$,	$-16 \le h \le 14$,	
	$-11 \le k \le 11$,	$-9 \leq k \leq 9$,	$-19 \le k \le 13$,	
	$-16 \leq l \leq 16$	$-32 \leq l \leq 32$	$-36 \le l \le 36$	
Reflections collected	29,702	35,580	31,289	
Independent reflections	4013 [R(int) = 0.0438]	5920 [$R(int) = 0.0977$]	12,777 [R(int) = 0.0355]	
Completeness to theta	98.3% to 24.99°	99.4% to 25.92°	99.9% to 33.14°	
Absorption correction	None	None	Semi-empirical from equivalents	
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F ²	Full-matrix least-squares on F^2	
Data/restraints/parameters	4013/0/279	5920/0/364	12,777/7/380	
Goodness-of-fit on F^2	1.045	0.932	0.967	
Final R indices $[I > 2$ sigma $(I)]$	$R_1 = 0.0283, wR_2 = 0.0665$	$R_1 = 0.0373, wR_2 = 0.0603$	$R_1 = 0.0456, wR_2 = 0.1232$	
R indices (all data)	$R_1 = 0.0365, wR_2 = 0.0681$	$R_1 = 0.1079, wR_2 = 0.0653$	$R_1 = 0.0790, wR_2 = 0.1324$	
Largest diff. peak and hole/e $Å^{-3}$	0.626 and -0.323	0.569 and -0.650	1.027 and -0.703	
5 1			-	

A decrease in thermal denaturation temperature of 7 °C in the presence of **4a**, **4b** and **5a**', respectively and of 4 °C in the presence **5b** was recorded for calf-thymus DNA (ctDNA) at a molar ratio of r = 0.2 where [DNA] is given in M(base pairs) (Table 3 and Supporting information). Interestingly, the melting curve in the presence of **5a** shows two stages corresponding to decreases in thermal denaturation temperature of 8 and 28 °C respectively. This might reflect a rearrangement of the coordination mode of the ligand in the complex as discussed before.

The cytotoxicity of the compound was determined towards three different cell lines using the MTT assay method. For comparison, the compound [(cym)Ru(en)Cl]Cl (**7**) was also introduced in the cell line studies (Table 4 and Fig. 5). Cell lines used were Hct116 human colon carcinoma, H4IIE rat hepatoma and A2780 human ovarian carcinoma cells (cisplatin sensitive). The cytotoxicity values for these compounds fall in the range commonly observed for various Ru(arene)-type complexes [31,52,53].



Fig. 1. ³¹P{¹H}-NMR spectrum of the reaction mixture of [{(cym)Ru(L₂)₂] and **2a** in DMSO- d_6 . The ligand **2a** and complexes [(cym)Ru($\kappa^2 P$ -**2a**)Cl₂] (**5a**) and [(cym)Ru($\kappa^2 N$, N-**2a**)Cl]+ with κP and $\kappa^2 N$, N-coordination mode show resonances at -50, 3 and 23 ppm.



Fig. 2. Molecular structure of **5a**. Non-acidic hydrogen atoms are omitted for clarity. The displacement ellipsoids are shown on a 50% level. Selected bond lengths [Å] and angles [°]: Ru1-P1 2.3477(7), Ru1-Cl1 2.4173(7), Ru1-Cl2 2.4165(8), P1-Ru1-Cl1 87.41(3), P1-Ru1-Cl2 86.60(3), Cl1-Ru1-Cl2 87.39(3).

As expected, the complexes coordinatively saturated **6a** and **6b** are not cytotoxic at concentrations up to 100 μ M after 24, 48 and 72 h of incubation, respectively (Table 4). Compounds **5a**' and **5b** containing $\kappa^2 N$, N bonded ligands show no toxicity in the cell lines used. The steric repulsion of the imidazolyl groups in **5b** may hinder coordination to DNA bases. Additionally, the NH groups in **5a**' point away from a possible binding site due to the ligand geometry. In complexes [(arene)Ru(en')X] with ethylenediamine derivatives (en') it has been shown that NH functionalities in the en' ligand are essential for efficient DNA binding [20,54,55].

The compounds [(*p*-cymene)Ru(**1a**)Cl₂] (**4a**) shows cytotoxicity towards A2780sens and Hct116 cells in the μ M range but not in



Fig. 3. Molecular structure of **5**a'·CH₂Cl₂. Uncoordinated counter ion, non-acidic hydrogen atoms and solvent molecule are omitted for clarity. The displacement ellipsoids are shown on a 50% level. Selected bond lengths [Å] and angles [°]: Ru1-N1 2.096(4), Ru1-N3 2.087(3), Ru1-Cl1 2.4006(15), N1-Ru1-N3 84.28(14), N1-Ru1-Cl1 85.21(12), N3-Ru1-Cl1 85.84(11).



Fig. 4. Molecular structure of **5b**·2CH₃CN·½H₂O. Uncoordinated counter ion, hydrogen atoms and solvent molecules are omitted for clarity. The displacement ellipsoids are shown on a 50% level. Selected bond lengths [Å] and angles [°]: Ru1-N1 2.090(2), Ru1-N3 2.097(2), Ru1-Cl1 2.3897(7), N1-Ru1-N3 2.3897(7), N1-Ru1-Cl1 85.21(6) N3-Ru1-Cl1 84.61(6).



Scheme 2. The *trans* and *cis* isomers found in the solid-state structures of 5a' (R = H) and 5b (R = CH₃).

H4IIE cells. The cytotoxicity is decreased upon introduction of a methyl group as [(*p*-cymene)Ru(**1b**)Cl₂] (**4b**) shows only modest toxicities in the cell lines investigated. In general **4a** is more cytotoxic then **4b**. As mentioned above, free NH functions as in [(arene) Ru(en')X] should favour DNA binding. This is not a valid explanation here, as the melting temperatures for **4a** and **4b** are essentially the same.

Although **5a** and **4a** both have the κP binding mode and therefore resemble RAPTA-type [(arene)RuCl₂(pta)] complexes, **5a** is almost non-toxic in the cell lines investigated within 24 of incubation. After 72 and 96 h of incubation a selective toxicity of **5a**

Table 3

Experimental *n*-octanol/water (PBS buffer pH 7.4) distribution coefficients (log *D*_{7.4}) and DNA melting temperatures (ΔT_m) at a ratio of metal complex/DNA (in base pairs) of 1/5. The ΔT_m values are estimated to be accurate within ± 1 °C.

Compound	log D _{7.4}	$T_{\rm m}/^{\circ}{\rm C}$	$\Delta T_{\rm m}/^{\circ}{\rm C}$
4a	1.25 ± 0.03	66	7
4b	1.14 ± 0.05	66	7
5a	0.14 ± 0.03	45, 65	28, 8
5a′	-0.33 ± 0.02	66	7
5b	-0.68 ± 0.01	69	4

Table 4

Cell viability tests after 24 and 72 h of incubation, IC_{50} values given in μM (n.d. not determined).

Cell line	Incubation time	4 a	4b	5a	5a′	5b	6a	6b
A2780sens	24 h	83	>100	>100	>100	>100	n.d.	n.d.
	72 h	38	95	> 100	>100	>100	n.d.	n.d.
Hct116	24 h	66	>100	> 100	>100	>100	> 100	>100
	72 h	58	>100	n.d.	n.d.	n.d.	> 100	>100
H4IIE	24 h	>100	>100	> 100	>100	>100	>100	>100
	72 h	>100	>100	54	>100	>100	>100	>100



Fig. 5. Cytotoxic effects **5a**, **5a**', **5b** and **7** in H4IIE and A2780 cells. H4IIE rat hepatoma and A2780 human ovarian carcinoma cells were incubated with **5a**, **5a**', **5b** or **7** (100 μ M) for 72 h, then MTT reduction as a marker of cell viability was measured (absorbance at 560 nm). Results are expressed as viable cells in percent of control value \pm SD (n = 3, *p < 0.05 vs. corresponding DMSO control).



Fig. 6. Cytotoxic effects of **5a** in H4IIE. H4IIE rat hepatoma cells were incubated with different concentrations of **5a** for 48, 72 and 96 h, then MTT reduction as a marker of cell viability was measured (absorbance at 560 nm). Results are expressed as viable cells in percent of control value \pm SD (n = 3, *p < 0.05 vs. corresponding DMSO control).

towards H4IIE cells is found (Table 4 and Fig. 6). Reason for the decreased toxicity of compound **5a** to **4a** might be the hydrophobicity. The selective toxicity of **5a** in H4IIE cells might be an effect of **5a** in the cell cycle leading to delayed toxic effects. In addition it is well known, that RAPT-type complexes not only act as DNA binders. The poor correlation found here could also point towards a different mechanism, where proteins are favoured targets for the compounds. For corresponding complexes this has been shown by the groups of Messori and Davey [29,56].

4. Conclusion

We prepared a series of ruthenium(II) arene complexes with ambidentate PN ligands. All compounds displaying a chelating $\kappa^2 N, N$ or $\kappa^3 N, N, N$ -coordination mode show no cytotoxicity towards the used Hct116, H4IIE and A2780 cell lines. The most lipophilic complex **4a** show the highest cytotoxicity within the series of compounds, with IC₅₀ values in the micromolar range. Complex **5a**, in which ligand **2a** is bound in the κP mode, shows a selective cytotoxicity towards H4IIE cells after 72 h. In contrast complex **5a**', in which ligand **2a** is bound in the $\kappa^2 N$, N mode, shows no cytotoxic effects in the cell lines investigated.

Appendix A. Supplementary material

CCDC 867104, 867105 and 861637 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Appendix B. Supplementary information

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jorganchem.2012. 06.027.

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