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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: κ*P*, κ^2*N,N* or κ^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**)Cl_2] (**4a**) shows cytotoxicity towards A2780sens and Hct116 cells in the μM range but not in H4IIE cells. The cytotoxicity is decreased upon introduction of a methyl group as [(*p*-cymene)Ru(**1b**)Cl_2] (**4b**) shows only modest toxicities in the cell lines investigated. The κ*P* compound [(*p*-cymene)Ru(**2a**)Cl_2] (**5a**) shows selective toxicity in H4IIE cells after 72 h whereas the κ^2*N,N* compound [(*p*-cymene)Ru(**2a**)Cl]OTf (**5a′**) showed no toxicity in the cell lines investigated which again.

1. Introduction

Since the authorization of cisplatin in 1978, the interest in and development of metal-based drugs prospers consistently. Still, cisplatin, *cis*-[$Pt(NH_3)_2Cl_2$], 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*-[$RuCl_4$(DMSO)(Im)]ImH (NAMI-A, where Im = imidazole) has completed phase I clinical trials [18]. Interestingly, NAMI-A is more active against metastases than against primary tumours [19]. Half-sandwich Ru(II) arene complexes of the type [(η^6-arene)Ru(YZ)(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(η^6-*p*-cymene)Cl_2(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**), 1-methylimidazol-2-yl (**b**)} and [(cym)Ru(κ*P*-**1b**)Cl_2] (**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. ^{1}H and ^{31}P NMR spectra were recorded on a Bruker DRX 200 and Bruker DRX 500 spectrometer. The ^{1}H spectra were calibrated against the residual proton signal of the solvent as an internal reference (methanol-d_4: δ_H = 3.31 ppm; D_2O: δ_H = 4.79 ppm, $CDCl_3$: δ_H = 7.26 ppm) while the $^{31}P\{^1H\}$ NMR spectra were referenced to external 85% H_3PO_4. 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(κ*P*-**1a**)Cl_2] (**4a**)

Ligand **1a** (83 mg, 0.33 mmol) and $[Ru(cym)Cl_2]_2$ (100 mg, 0.16 mmol) were dissolved in dry CH_2Cl_2 (15 mL) and stirred for 24 h. The dark red solution was concentrated to 5 mL and Et_2O 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%). ^{1}H NMR (200 MHz, methanol-d_4): δ = 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). $^{31}P\{^1H\}$-NMR (81 MHz, methanol-d_4): δ = 22 (s). EI-MS (CH_3OH): m/z (%) = 558 (40) $[M]^+$, 523 (28) $[M - Cl]^+$, 486 (100) $[M - 2Cl]^+$, 389 (27) $[M - C_{10}H_{14}]^+$, 352 (45) $[M - C_{10}H_{14} - Cl]^+$. $C_{25}H_{27}Cl_2N_2PRu$ (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(κ*P*-**2a**)Cl_2] (**5a**)

Ligand **2a** (50 mg, 0.21 mmol) and $[Ru(cym)Cl_2]_2$ (63 mg, 0.1 mmol) were dissolved in dry CH_3CN (25 mL) and stirred for 24 h. The dark red solution was concentrated to 5 mL. The red precipitate was filtered off, washed with Et_2O and dried in vacuo. Yield: 27 mg (24%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 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). $^{31}P\{^1H\}$-NMR (81 MHz, $CDCl_3$): δ = −1 (s). ESI-MS (CH_3OH): m/z (%) = 513.4 (43) $[M - Cl]^+$, 477.4 (100) $[M - 2Cl]^+$. $C_{22}H_{25}Cl_2N_4PRu \cdot H_2O$ (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(κ^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 CH_3CN (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 CH_3CN (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 Et_2O was added. The mixture was kept at −18 °C. The yellow precipitate was filtered off, washed with Et_2O and dried in vacuo. Yield: 154 mg (70%). ^{1}H 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). $^{31}P\{^1H\}$-NMR (81 MHz, methanol-d_4): δ = −22 (s). ESI-MS (CH_3OH): m/z (%) = 493.5 (100) $[M + O]^+$, 477.5 (15) $[M]^+$, $C_{23}H_{25}ClF_3N_4O_3PRuS \cdot ½\ H_2O$ (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(κ^2*N,N*-**2b**)Cl]Cl (**5b**)

$[Ru(cym)Cl_2]_2$ (100 mg, 0.16 mmol) and **2b** (118.9 mg, 0.33 mmol) were dissolved in dry CH_3CN (15 mL) and stirred for 24 h. The orange solution was concentrated to 5 ml and Et_2O was added. The resulting solid was filtered off, washed with Et_2O and dried in vacuo. Yield: 158 mg (84%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 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). $^{31}P\{^1H\}$-NMR (81 MHz, $CDCl_3$): δ = −60 (s). ESI-MS (CH_3OH): m/z (%) = 541.4 (76) $[M]^+$, 505.5 (35) $[M - Cl]^+$, 407.3 (100) $[M - C_{10}H_{14}]^+$, 371.4 (37) $[M - C_{10}H_{14} - Cl]^+$. $C_{24}H_{29}Cl_2N_4PRu \cdot 2H_2O$ (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(κ^3*N,N,N*-**3a**)]Cl_2 (**6a**)

$[Ru(cym)Cl_2]_2$ (50 mg, 0.082 mmol) and **3a** (38.2 mg, 0.16 mmol) were dissolved in dry CH_3CN (30 mL) and refluxed for 2 h. The yellow precipitate was collected by filtration, washed with a small amount of CH_3CN and dried in vacuo. Yield: 21 mg (24%). ^{1}H 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). $^{31}P\{^1H\}$-NMR (81 MHz, methanol-d_4): δ = −103 (s). ESI-MS (CH_3OH): m/z (%) = 467.3 (100) $[M]^+$, 234 (58) $[M - \mathbf{3a}]^+$. $C_{19}H_{23}Cl_2N_6PRu \cdot 5/2H_2O$ (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(κ^3*N,N,N*-**3b**)]Cl_2 (**6b**)

$[Ru(cym)Cl_2]_2$ (100 mg, 0.16 mmol) and **3b** (91 mg, 0.33 mmol) were dissolved in dry CH_3CN (25 mL) and refluxed for 1.5 h. The yellow precipitate was collected, washed with a small amount of CH_3CN and dried in vacuo. Yield: 26 mg (18%). ^{1}H 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). $^{31}P\{^1H\}$-NMR (81 MHz, methanol-d_4): δ = −116 (s). ESI-MS (CH_3OH): m/z (%) = 509 (100) $[M - Cl]^+$, 461 (29) $[M - C_4N_2H_6 + Cl]^+$. $C_{22}H_{29}Cl_2N_6PRu$ (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(κ^2*N,N*-en)Cl]Cl (**7**)

$[Ru(cym)Cl_2]_2$ (100 mg, 0.16 mmol) was solved in dry CH_3CN (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 CH_3CN and dried in vacuo. Yield: 99 mg (84%). ^{1}H 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 (CH_3OH): 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-