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Short communication

Cytotoxic organometallic [Ru(η^6-anethole)(en)(X)]PF_6 (X = Br or I) complexes: Synthesis, characterization and *in vitro* biological evaluation

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ABSTRACT

Two new organometallic compounds with the general formula [Ru(η^6-anethole)(en)(X)]PF_6, where X = Br (5) or I (6) and en = ethylenediamine, were synthesized and fully characterized using standard techniques (NMR, MS and elemental analysis). Displacement of the proton and carbon aromatic chemical shifts of anethole to the upper field in both compounds indicated organometallic Ru(η^6-anethole) bond formation, and the characteristic "piano-stool" structure of these types of compounds was confirmed by the single crystal X-ray diffraction of compound 5. Compounds 5 and 6 exhibited interesting cytotoxicity, especially toward the human colon tumor cell line HT-29, similar to the previously studied compound [Ru(η^6-anethole)(en)(Cl)]PF_6 (4). A slight tendency toward cytotoxicity could be observed by varying the halogen leaving group (IC_{50} value): 6 (10 ± 2 μM) > 5 (18 ± 2 μM) = 4 (18 ± 3 μM). Compound 6 showed 3-fold more biological activity in HT-29 cells than toward the non-tumor colon cell line CCD-841, making it an interesting candidate for further studies and drug development.

Platinum-based compounds such as cisplatin, carboplatin and oxaliplatin are widely recognized chemotherapeutic agents for cancer treatment and compose one of the most relevant research areas of transition metal medicinal chemistry [1]. The deleterious secondary effects (nephro-, neuro- and ototoxicity and myelosuppression) in patients under the regime of these drug types has encouraged a systematic search for new compounds based on different metals [2]. Ruthenium(III) compounds such as NAMI-A (*trans*-[tetrachlorido(1*H*-imidazole)(*S*-dimethylsulfoxide)ruthenate(III)]), KP1019 (indazolium *trans*-[tetrachloridobis(1*H*-indazole) ruthenate(III)]) and NKP1339 (the sodium analog of KP1019) have entered clinical trials so far [3]. The later development of organometallic Ru(II) "piano-stool" structures such as [Ru(η^6-*p*-cymene)(PTA)Cl_2] (RAPTA-C; PTA: 1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]decane) and [Ru(η^6-biphenyl)(en)Cl]PF_6 (RM175; en: ethylenediamine) by Dyson's and Sadler's group, respectively, exhibited promising anticancer behavior [4,5]. These types of compounds have the proper balance between lipophilicity, which is necessary to allow the complex to cross the cell membrane, and hydrophilicity, which is necessary to achieve the appropriate plasma and intracellular concentration [6]. The lipophilicity is modulated mainly by the arene ligand, while the chelate ligands (PTA and en for the compounds mentioned above) and the leaving group (Cl) modulate the reactivity of the complexes, and therefore the cytotoxicity [7].

Our research group has synthesized a series of new organometallic compounds employing phenylpropanoids as arene ligands and varying the *N,N* chelate ligand (2,2′-bipyridine, en and 1,2-diaminobenzene) [8]. Of these, [Ru(η^6-anethole)(en)Cl]PF_6 (**4**) exhibited the most active cytotoxicity toward the tumor cell lines MCF-7 and HT-29, even better than the commercial drug carboplatin. In this study, we synthesized the bromide (**5**) and iodide (**6**) analogs of **4** to assess the cytotoxic properties of the series [Ru(η^6-anethole)(en)(X)]PF_6 (X = Cl, Br or I). Compounds **5** and **6** have been fully characterized (^{1}H and ^{13}C NMR, MS and elemental analysis), and the description of the crystal structural of **5** has also been reported. Anethole, a principal constituent of several essential oils, possesses interesting biological activities itself, such as hepatoprotective [9], anti-inflammatory [10] and antimetastatic [11] activities and induction of apoptosis in human breast cancer cells [12], so its use as a constituent of cytotoxic compounds may lead to compounds with relevant pharmacological activity.

Scheme 1 describes the synthetic procedure followed to isolate the new compounds. The dimeric organometallic compounds [Ru(η^6-anethole)$_2$(X)$_2$]$_2$, where X = Br (**2**) or I (**3**), were obtained easily in 62% and 76% yield, respectively, as insoluble products in aqueous media by reacting the soluble [Ru(η^6-anethole)$_2$Cl$_2$]$_2$ with an excess of a bromide or iodide salt (see Supplementary material). These new dimeric compounds have a similar color as [Ru(η^6-benzene)$_2$(X)$_2$]$_2$, where X = Br or I, and have been previously described by Zelonka and Baird [13]. Compounds **2** and **3** were further reacted with ethylenediamine in MeOH to obtain the desired "piano stool"

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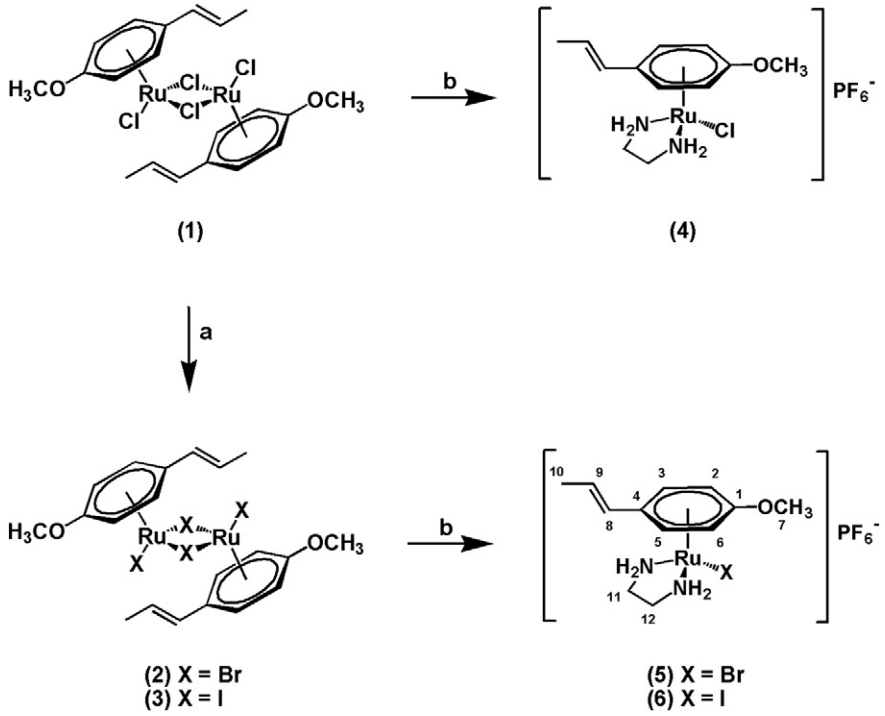

Scheme 1. Synthetic procedure to obtain the organometallic Ru(II) compounds. a) KBr or KI (27 eq.), H_2O, reflux; b) en, MeOH, NH_4PF_6, room temp.

organometallic compound **5** (53% yield) and **6** (34% yield) as a PF_6^- salt. Finally, recrystallization was performed by the slow diffusion of ether into a MeOH solution of **5** and **6**.

The ^1H and ^{13}C NMR chemical shifts of free and coordinated anethole indicated the formation of a Ru(η^6-anethole) bond. Signals associated with H-2/H-6 and H-3/H-5 aromatic protons in free anethole, at δ = 6.86 ppm (d, J = 9 Hz, 2H) and δ 7.29 ppm (d, J = 9 Hz, 2H), respectively, were shifted to a higher field (approximately δ = 5.4 ppm and δ = 6.0 ppm) in the final organometallic compounds **5** and **6** (Fig. 2, Supplementary material) [14]. These proton chemical shifts are in agreement with those previously reported for [Ru(η^6-anethole)(en)Cl]PF_6 (**4**) [8]. The carbon signals associated with the aromatic ring in free anethole at δ = 113.9 ppm (C-2/C-6) and δ 126.8 ppm (C-3/C-5) shifted from approximately 40 to 50 ppm to the upper field in the organometallic compounds. Lower shifts to the upper field (approximately 20 to 40 ppm) were observed for quaternary aromatic carbon (Fig. 3, Supplementary material). Full carbon assignment was established by 2D HSQC/HMBC experiments. Mass spectra revealed fragments associated with the ionic species [Ru(η^6-anethole)(en)Br]$^+$ (m/z = 388.84) and [Ru(η^6-anethole)Cl]$^+$(m/z = 328.76) for **5** and [Ru(η^6-anethole)(en)I]$^+$ (m/z = 436.89) and [Ru(η^6-anethole)I]$^+$ (m/z = 377.16) for **6**, in agreement with theoretical values.

The molecular structure of the [Ru(η^6-anethole)(en)Br]$^+$ complex (**5**) with an atom numbering scheme is shown in Fig. 1, and it reveals the classical "piano-stool" geometry. Selected bond distances and bond angles are shown in Table 1. The Ru—N distances are essentially equivalent, and the N1—Ru—N2 bond angle is comparable to that found in the analogous [(η^6-*p*-cymene)Ru(en)Cl]PF_6 [78.98(10)°] and [Ru(η^6-methylisoeugenol)(en)Cl]PF_6 [78.93(15)°] complexes [5,8]. The torsional N1—C11—C12—N2 angle of the corresponding bridge is 53.8(5)°. The Ru—*Cg1* distance is 1.6810(3) Å (*Cg1* represents the centroid of the C1—C6 ring) and a ring slippage of 0.074 Å. The C7—O1 bond lengths formed by the methoxy ligand (see Table 1) are very close to those reported earlier for [Ru(η^6-methylisoeugenol)(en)Cl]PF_6 [1.336(19) Å] [8]. The substituent group at C4 is tilted out of the mean plane of the arene ring with a torsion angle of C4—C8—C9—C10 of −179.7(4)°. The C9—C8 [1.299(7) Å] bond distance is similar to the average values reported for a Csp^2=Csp^2 bond [15]. All of the other relevant structural parameters (*i.e.*, bond distances and angles) are as expected and are in acceptable agreement with their analogues as reported in the literature. Finally, the structure of the cationic moiety [Ru(η^6-anethole)(en)Br]$^+$ is completed by one PF_6^- anion. The P—F bond lengths ranged from 1.505(5) to 1.573(5) Å [F2—P—F3 = 90.9(4)° and F1—P—F6 = 178.5(3)°]. The octahedral volume is 4.884 Å^3 with an angle variance of 6.04 deg^2 [16]. In the crystal packing, the molecules are linked by hydrogen bonds and P—F···π intermolecular interactions. Accordingly, each [Ru(η^6-anethole)(en)Br]$^+$ cation is connected to two anionic PF_6^- counter ions (see Fig. 5 and Table 4, Supplementary material). The P—F2···*Cg1* and P—F6···*Cg1* distances are 3.352(4) Å and 3.808(5) Å, respectively. Additionally, N—H···F interactions constitute a graph-set descriptor $R_1^2(4)$ motif in the *ac* plane [17]. The supramolecular crystal packing is reinforced by the N2—HN2B···Br1 and N2—H2NB···O1 hydrogen bonds (see Fig. 6 and Table 4, Supplementary material). The H-atoms of the nitrogen link each O and Br atom forming a two-center graph-set $R_1^2(5)$ motif [17]. The combination

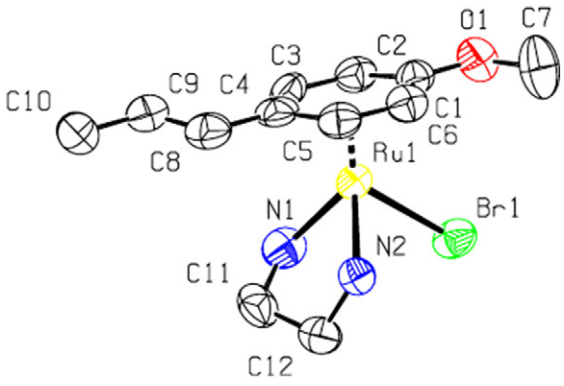

Fig. 1. A view of the asymmetric unit showing the [Ru(η^6-anethole)(en)Br]$^+$ complex. Displacement ellipsoids are drawn at the 50% probability level and H-atoms with counterion PF_6^- have been omitted for clarity.

Table 1
Selected bond lengths (Å) and angles (°).

Ru—N1	2.142(4)
Ru—N2	2.130(4)
Ru—Br	2.5442(7)
C5—C6	1.395(6)
O1—C7	1.435(7)
N1—Ru—N2	78.93(15)
N1—Ru—Br	86.68(14)
N2—Ru—Br	84.62(11)
O2—C1—C6	124.0(4)

of both hydrogen bonds leads to the formation of chains zigzagging along the crystallographic a-axes [100] direction.

The *in vitro* biological activity of organometallic compounds **4** to **6** and carboplatin against the three human tumor cell lines, namely, MCF-7 (breast cancer), PC-3 (prostate cancer) and HT-29 (colon cancer), and one human non-tumor cell line, CCD-841 (CoN, colon epithelial), are shown in Table 2. The results indicated that the synthesized compounds exhibited greater cytotoxic activity toward the colon carcinoma cell line (HT-29) than toward the breast adenocarcinoma (MCF-7), and an activity > 50 μM (not measured) was observed toward the prostate cell line (PC-3). No significant differences were observed in the cytotoxicity of the compounds **4**, **5** and **6** toward the MCF-7 cell line, while a slight increase in activity was exhibited for **6**, in comparison with **4** and **5**, toward the HT-29 cell line. Although the replacement of the chloride leaving group by iodide has been reported, it did not improve the biological activity of [Ru(η^6-arene)(en)Cl]PF_6, where arene = benzene or *p*-cymene [18]. For Ru(II) and Os(II) organometallic iminopyridine and azopyridine complexes, the anticancer activity was considerably improved when iodide instead of chloride was coordinated [19]. The extent of hydrolysis of compounds **4** to **6** after 24 h (Table 2 and Fig. 4, Supplementary material) revealed a trend for the aquation process of the [Ru(η^6-anethole)(en)X]$^+$ (X = Cl, Br or I) complexes, that follow the order Cl > Br > I. In general, the organometallic compounds that undergo hydrolysis process exhibited active cytotoxic behavior, however the more activity showed by the less hydrolyzed iodido analog, specifically toward the tumor colon cell line (HT-29), suggest that different apoptotic pathways (cellular uptake and accumulation) are triggered, in addition to the hydrolysis activation pathway [19]. Additionally, compound **6** showed some degree of selectivity, exhibiting 3-fold greater cytotoxic activity toward the human tumor colon cell line (HT-29) than toward the non-tumor human colon cell line (CCD-841), making it an interesting lead compound for further studies and new compound design.

Two new organometallic compounds were synthesized in this study, namely, [Ru(η^6-anethole)(en)(X)]PF_6, where X = Br (**5**) or I (**6**), as a continuance of the work involving a previously reported chlorido analog (**4**) in order to assess the effect of different leaving groups on the biological activity of the Ru(η^6-anethole)(en) scaffold. Both compounds were characterized by traditional spectroscopic techniques, and X-ray diffraction confirmed the classical "piano-stool" geometry for **5**. The synthesized compounds undergo hydrolysis processes in different extent, and exhibited greater cytotoxic activity toward the human colon cell line (HT-29), showing a slight increasing trend in their biological activity in the order (IC_{50} value): **6** (10 ± 2 μM) > **5** (18 ± 2 μM) = **4** (18 ± **3** μM). Moreover, compound **6** exhibited some selectivity (3-fold) between the human colon tumor (HT-29) and human colon non-tumor (CCD-841) cell lines that make this compound suitable for further drug development studies.

Table 2
Biological activity and extent of hydrolysis of compounds **4**, **5**, and **6** and carboplatin against human breast cancer (MCF-3), human prostate cancer (PC-3), human colon cancer (HT-29) cell lines and a non-tumor human colon epithelial cell line (CCD-841).

Compound	Extent of hydrolysis (%)[a]	IC_{50} (μM)[b]			
		MCF-7	PC-3	HT-29	CCD-841
4 (Cl)	64	21 ± 4	>50	18 ± 3	29 ± 4
5 (Br)	55	22 ± 3	>50	18 ± 2	24 ± 4
6 (I)	24	25 ± 4	>50	10 ± 2	31 ± 4
Carboplatin	–	>50	>50	>50	>50

[a] Extent of hydrolysis of compounds 4–6 after 24 h using 1 mM solutions in 90%D_2O/10%DMSO d_6. Values are means of two independent NMR experiments at room temperature (variability is <3%).

[b] Inhibitory concentrations (50%) in the SRB assay (72 h exposure). Values are means ± standard deviations of three independent experiments.

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Appendix A. Supplementary data

CCDC 1472047 contains the supplementary crystallographic data for compound **5**. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk. Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.inoche.2017.08.024.

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