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Synthesis, characterization and *in vitro* biological evaluation of [Ru(η^6-arene)(*N*,*N*)Cl]PF_6 compounds using the natural products arenes methylisoeugenol and anethole

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ABSTRACT

Five new organometallic Ru(II) compounds (VI–X) with the general formula [Ru(η^6-arene)(*N*,*N*)Cl]PF_6, where arene-*N*,*N* correspond to methylisoeugenol-bipyridine (VI); anethole-bipyridine (VII); methylisoeugenol-ethylenediamine (VIII); anethole-ethylenediamine (IX) and methylisoeugenol-1,2-diaminobenzene (X), have been synthesized, fully characterized and biologically evaluated *in vitro*. The reaction conditions based on the reduction of $[Ru(1,5\text{-}COD)Cl_2]_n$ *in situ* with methyleugenol and estragole, which are natural ligands, induced an alkene isomerization on the allylic substituent of coordinated arenes. The Ru(II)-arene bond formation and isomerization of the C=C bond on the allyl substituent was confirmed using ^{1}H NMR spectroscopy; this result was validated for compound VIII by X-ray diffraction. An XRD analysis revealed the presence of both enantiomers of the complex in the single-crystal. Compounds IX and X exhibited a better cytotoxic activity *in vitro* than carboplatin, which is a commercial drug, against three human tumor cell lines (MCF-7, PC-3 and HT-29).

Introduction

Since 1978, cisplatin has been broadly employed to treat different types of cancer. The use of cisplatin, however, has become limited due to its deleterious secondary effects (nephro-, neuro- and ototoxicity) [1]. New compounds have been developed using different design strategies that aimed either to diminish the secondary toxicity and/or to have activity against cell lines resistant to cisplatin. Ruthenium-based compounds have been studied for this purpose in recent years [2]. Ruthenium can mimic iron when binding certain biological molecules, particularly transferrin, and Ru(II) and Ru(III) complexes can display ligand exchange kinetics similar to Pt(II). Because these attributes are combined with a lower toxicity, ruthenium compounds are suitable for medical applications [3]. Currently, NAMI-A and KP1019, which are ruthenium compounds, are in phase II clinical trials. The mechanism of action for these Ru(III) compounds remains unknown; however, new evidence has indicated that the cytotoxic activity of these complexes is related to the reductive capabilities of the Ru(III) to Ru(II) in the complex [4]. After a chemical reduction, NAMI-A is more active against metastatic growth than the non-reduced complex [5]. For some KP1019 derivatives and a structurally related series of indazole-based Ru(III) compounds, the antiproliferative activity against colon carcinoma (SW-480) was correlated to the increased reductive potential of the corresponding complexes [6]. In addition, the low oxygen concentration levels (hypoxia) of tumor cells promote a more reductive environment than in normal tissue, favoring the active reduced form [7].

"Piano-stool" organometallic Ru(II) type compounds, such as [Ru(η^6-arene)(PTA)Cl_2] (PTA: 1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]decane) and [Ru(η^6-arene)(en)Cl]PF_6 (en: ethylenediamine), have also been evaluated [8,9]. These complexes undergo an intracellular hydrolysis of the metal-chloride bond(s), similar to cisplatin, to generate the aquo-metabolite that binds to DNA or proteins, forming mono- or bifunctional adducts [10]. However, the hydrolysis of these metal-based complexes is not essential during reactions with biomolecules. In [Ru(η^6-*p*-cymene)(PTA)(oxalate)] and [Ru(η^6-*p*-cymene)(PTA)(1,1-cyclobutanedicarboxylate)], a considerable decrease in the rate of hydrolysis was detected, and the cytotoxicity of these compounds against several tumor cells

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remained unchanged [8]. The structure–activity relationships of [Ru(η^6-arene)(XY)Cl]Z complexes where XY is an *N*,*N*-, *N*,*O*- or *O*,*O*-chelating ligand and Z is usually PF_6 revealed that polar substituents in the coordinated aromatic ring tended to decrease the cytotoxic activity of the complex. Incorporating *N*,*N*-chelating ligands, such as ethylenediamine and 1,2-diaminobenzene, increase considerably the cytotoxicity of the complexes [11].

Increasing evidence suggests that combination therapy involving natural products and synthetic drugs can enhance the effectiveness of the treatment through a synergic process [12–15]. This work aimed to synthesize new drugs with ruthenium-bound natural products, improving their pharmacological effects. Toward this purpose, phenylpropanoids, such as methyleugenol (I), estragole (II) and saphrole (III) were used to obtain compounds with the general formula of [Ru(η^6-arene)(*N*,*N*)Cl]PF_6 (*N*,*N*: ethylenediamine, bipyridine and 1,2-diaminobenzene). These types of natural products possess interesting antioxidant, anti-inflammatory and antimicrobial activities [16,17]. A series composed of five organometallic compounds (VI–X) was synthesized, fully characterized and evaluated for cytotoxicity *in vitro*. Moreover, the single-crystal X-ray diffraction data for compound VIII is reported. Previously, an *in vitro* cell viability assay involving boldiplatin, which is a platinum(II) compound synthesized by our research group with a naturally occurred ligand and recognized antioxidant activity, showed that this compound was more active against tumor cell lines than non-tumor cell lines [18]. This difference in activity, which is not observed for the commercial drug oxaliplatin, motivated the synthesis of the above mentioned complexes.

Experimental section

General considerations

The ^{1}H NMR experiments were performed using an Avance 400 Digital Bruker NMR spectrometer operating at 400.13 MHz for ^{1}H. The chemical shifts (δ) are given in ppm, and the coupling constant (*J*) in Hz. The chemical shifts are reported relative to the proton signal of incompletely deuterated DMSO-d_6 (δ 2.49). The mass spectra were recorded on a Bruker MALDI-TOF Microflex spectrometer using the flexControl 3.0 software. α-Cyano-4-hydroxycinnamic acid was employed as a matrix and the spectra were obtained in positive mode. The elemental analyses were performed on a Flash EA™ 1112. The $RuCl_3 \cdot xH_2O$, 1,5-cyclooctadiene (1,5-COD), bipyridine (bipy), ethylenediamine (en), 1,2-diaminobenzene (dab) and 4-allylanisole (estragole) were purchased from Aldrich. The eugenol was extracted through hydrodistillation from cloves (*Eugenia caryophyllata*) and methylated according to an established method [19]. The [Ru(1,5-COD)Cl_2]$_n$ was prepared according to a published procedure [20]. The methylisoeugenol and anethole were obtained after isomerizing methyleugenol and estragole, respectively [21], with a catalyst [{RuCl(μ-Cl)(η^3:η^3-$C_{10}H_{16}$)}$_2$] ($C_{10}H_{16}$: 2,7-dimethylocta-2,6-diene-1,8-diyl) prepared according to an established procedure [22]. The anhydrous solvents were dried and freshly distilled as follows: ethanol (Mg/I_2), THF and diethyl ether (Na/benzophenone), acetonitrile (CaH_2) and hexane (Na). All of the other reagents were obtained from commercial suppliers and were used without further purification.

X-ray crystallography

All of the H-atoms were positioned geometrically and treated as riding atoms with N–H distances of 0.90 Å and C–H distances from 0.98 to 0.93 Å. The isotopic displacement parameters were calculated as follows: $U_{iso}(H) = 1.2U_{eq}(N)$ and $U_{iso}(H) = k\ U_{eq}(C)$, where $k = 1.5$ and 1.2. The crystallographic data and refinement parameters for VIII are summarized in Table 1. The data were collected with a Bruker *SMART* (BRUKER 1996); the cell refinement was performed with Bruker SAINTPLUS V6.02 (BRUKER 1997); the data were reduced with Bruker *SHELXTL* V6.10 (BRUKER 2000); the *SHELXS97* program (Sheldrick, 1990) was used to solve and refine the structure (Sheldrick, 1997) [23,24]. The molecular graphics were produced using *DIAMOND* (Brandenburg, 1999); the software used to prepare material for publication was *PLATON* (Spek, 2003) [25,26].

Synthesis

General procedure for synthesizing the [Ru(η^6-arene)$_2Cl_2$]$_2$ compounds (IV and V) [11]

To a dry, 100 ml round-bottom flask connected to a dry N_2 inlet was added 1.04 g of [Ru(1,5-COD)Cl_2]$_n$ (3.69 mmol of Ru), 14.4 mmol of methyleugenol (I) or estragole (II) and 9.43 g of zinc (144 mmol). Freshly distilled anhydrous THF (25 ml) was added and the reaction mixture was refluxed for 24 h. The solvent was removed under vacuum, and the oily residue was washed with dry hexane (3 × 30 ml). The organic extracts were passed through a glass filter and combined. The organic solvent was removed under vacuum, and the oily product was redissolved in 5 ml of dry acetonitrile. Fifteen ml of a 1 M solution of HCl in ether was added, and the mixture was stirred at room temperature overnight. The solvents were removed under vacuum and the deep red solid was washed with 30 ml of diethyl ether for 1 h. The solid was filtrated and dried under vacuum. Finally, 229 mg of [Ru(η^6-methylisoeugenol)$_2Cl_2$]$_2$ (IV, yield: 18%) and 263 mg of [Ru(η^6-anethole)$_2Cl_2$]$_2$ (V, yield: 22%) were obtained.

Synthesis of [Ru(η^6-methylisoeugenol)(bipy)Cl]PF_6 (VI)

In a 100 ml round-bottom flask, 100 mg of [Ru(η^6-methylisoeugenol)$_2Cl_2$]$_2$ (0.14 mmol) was dissolved in 15 ml of MeOH. A solution of 50 mg of bipyridine (0.32 mmol) in 5 ml of MeOH was added dropwise, and the mixture was stirred at room temperature

Table 1
Crystallographic data and structural refinement parameters for compound VIII.

Empirical formula	$C_{13}H_{22}N_2O_2RuCl,PF_6$
Crystal system	Triclinic
Space group	P-1 (N°2)
Unit cell dimensions	
a (Å)	9.2862(7)
b (Å)	9.5321(7)
c (Å)	11.6940(9)
α (°)	110.247(1)
β (°)	98.322(1)
γ (°)	91.148(1)
Volume (Å^3)	958.19(13)
Z	2
μ (mm^{-1})	1.107
Temperature	293 K
Wavelength ()	0.71073
Crystal size (mm)	0.10 × 0.14 × 0.20
F(000)	520
Theta min–max range collected (°)	1.9–29.0
Measured reflections	9095 (R_{int} = 0.0252)
Independent reflections	4387
Reflections with $I > 2\sigma(I)$	3776
Final *R* indices [($F^2 > 2\sigma(F^2)$)]	*R*1 = 0.0321, *wR*2 = 0.0772
R indices (all data)	*R*1 = 0.0398, *wR*2 = 0.0817
Goodness-of-fit on F^2	1.045
Parameters	475
$\Delta\rho_{max}$, $\Delta\rho_{mim}$ (e^{-3})	0.68/−0.40

for 2 h. To the mixture was added 79 mg of NH_4PF_6 (0.45 mmol), and the mixture was stirred for 15 min. A yellow solid appeared, and the reaction mixture was left at −18 °C overnight. The precipitate was filtrated, washed with 2 ml of cold MeOH and small subsequently portions of diethyl ether and dried under vacuum. Finally, 119 mg of yellow product was obtained (yield: 69%). ^{1}H NMR (DMSO-d_6) δ 1.77 (d, J = 7 Hz, 3H, CH_3), 3.74 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 5.91 (d, J = 6 Hz, 1H, Ar—H), 6.02 (d, J = 16 Hz, 1H, *trans* HC═C), 6.16 (s, 1H, Ar—H), 6.19 (d, J = 6 Hz, 1H, Ar—H), 6.69 (dd, J = 16, 7 Hz, 1H, C═C*H*—CH_3), 7.80 (m, 2H, H-5), 8.25 (dd, J = 8, 8 Hz, 2H, H-4), 8.61 (d, J = 7 Hz, 2H, H-3), 9.20 (d, J = 5 Hz, 1H, H-6), 9.26 (d, J = 5 Hz, 1H, H-6); EM m/z 470.82 ($C_{21}H_{22}N_2O_2RuCl^+$); Anal. Calc. for $C_{21}H_{22}N_2O_2RuClPF_6$: C, 40.95%; H, 3.60%; N, 4.55%. Found: C, 41.19%; H, 3.42%; N, 4.54%.

Synthesis of [Ru(η^6-anethole)(bipy)Cl]PF_6 (VII)

In a 100 ml round-bottom flask, 93 mg of [Ru(η^6-anethole)$_2Cl_2$]$_2$ (0.14 mmol) was dissolved in 20 ml of MeOH. A solution of 50 mg of bipyridine (0.32 mmol) in 5 ml of MeOH was added dropwise, and the mixture was stirred at room temperature for 3 h. The solvent was removed under reduced pressure until approximately 10 ml of solution remained before 79 mg of NH_4PF_6 (0.45 mmol) was added. The mixture was stirred for 1 h. A yellow solid appeared, and the reaction mixture was left at −18 °C for 2 days. The precipitate was filtrated, washed with small portions of diethyl ether and dried under vacuum. Finally, 99 mg of yellow product was obtained (yield: 60%). ^{1}H NMR (DMSO-d_6) δ 1.65 (d, J = 6 Hz, 3H, CH_3), 3.77 (s, 3H, OCH_3), 5.88 (d, J = 6 Hz, 2H, Ar—H), 5.97 (d, J = 16 Hz, 1H, *trans* HC═C), 6.36 (dd, J = 16, 7 Hz, 1H, C═C*H*—CH_3), 6.51 (d, J = 6 Hz, 2H, Ar—H), 7.80 (m, 2H, H-5), 8.27 (dd, J = 8, 8 Hz, 2H, H-4), 8.62 (d, J = 8 Hz, 2H, H-3), 9.43 (d, J = 5 Hz, 2H, H-6); EM m/z 440.79 ($C_{20}H_{20}N_2ORuCl^+$); Anal. Calc. for $C_{20}H_{20}N_2ORuClPF_6$: C, 40.99%; H, 3.44%; N, 4.78%. Found: C, 41.33%; H, 3.28%; N, 4.62%.

Synthesis of [Ru(η^6-methylisoeugenol)(en)Cl]PF_6 (VIII)

In a 100 ml round-bottom flask, 100 mg of [Ru(η^6-methylisoeugenol)$_2Cl_2$]$_2$ (0.14 mmol) was dissolved in 10 ml of MeOH. To the solution was added 30 μl of ethylenediamine (0.45 mmol), and the mixture was stirred at room temperature for 3 h. The reaction mixture was filtrated and the filtrate was concentrated under vacuum until 5 ml of solution remained. Afterward, 120 mg of NH_4PF_6 (0.74 mmol) was added, and the mixture was shaken for 1 h. The reaction mixture was left at −18 °C for 2 days. The precipitate was filtrated, washed with small portions of diethyl ether and dried under vacuum. Finally, 82 mg of yellow product was obtained (yield: 56%). ^{1}H NMR (DMSO-d_6) δ 1.82 (d, J = 6 Hz, 3H, CH_3), 2.18 (m, 2H, CH_2), 2.38 (m, 2H, CH_2), 3.76 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 4.28 (m, 2H, NH_2), 5.40 (d, J = 6 Hz, 1H, Ar—H), 5.64 (d, J = 6 Hz, 1H, Ar—H), 5.78 (s, 1H, Ar—H), 6.07 (d, J = 15 Hz, 1H, *trans* HC═C), 6.45 (dd, J = 15, 7 Hz, 1H, C═C*H*—CH_3); EM m/z 374.77 ($C_{13}H_{22}N_2O_2RuCl^+$); Anal. Calc. for $C_{13}H_{22}N_2O_2RuClPF_6$: C, 30.03%; H, 4.26%; N, 5.39%. Found: C, 30.01%; H, 4.30%; N, 5.52%.

Synthesis of [Ru(η^6-anethole)(en)Cl]PF_6 (IX)

In a 100 ml round-bottom flask, 93 mg of [Ru(η^6-anethole)$_2Cl_2$]$_2$ (0.14 mmol) was dissolved in 20 ml of MeOH. To the solution was added 31 μl of ethylenediamine (0.45 mmol), and the mixture was stirred at room temperature for 3 h. The reaction mixture was filtrated, and the filtrate was concentrated under vacuum until 5 ml of solution remained. Afterward, 120 mg of NH_4PF_6 (0.74 mmol) was added, and the mixture was shaken for 20 min. An orange solid appeared, and the mixture was left at −18 °C for 2 days. The precipitate was filtrated, washed with small portions of diethyl ether and dried under vacuum. Finally, 52 mg of product was obtained (yield: 38%). ^{1}H NMR (DMSO-d_6) δ 1.77 (d, J = 6 Hz, 3H, CH_3), 2.13 (m, 2H, CH_2), 2.30 (m, 2H, CH_2), 3.82 (s, 3H, OCH_3), 4.20 (m, 2H, NH_2), 5.36 (d, J = 6 Hz, 2H, Ar—H), 6.04 (d, J = 6 Hz, 2H, Ar—H), 6.08 (d, J = 16 Hz, 1H, *trans* HC═C), 6.30 (dd, J = 16, 7 Hz, 1H, C═C*H*—CH_3); EM m/z 344.72 ($C_{12}H_{20}N_2ORuCl^+$); Anal. Calc. for $C_{12}H_{20}N_2ORuClPF_6$: C, 29.41%; H, 4.11%; N, 5.72%. Found: C, 29.37%; H, 4.02%; N, 5.96%.

Synthesis of [Ru(η^6-methylisoeugenol)(dab)Cl]PF_6 (X)

In a 100 ml round-bottom flask, 99 mg of [Ru(η^6-methylisoeugenol)$_2Cl_2$]$_2$ (0.14 mmol) was dissolved in 20 ml of MeOH and 4 ml of water. The mixture was refluxed for 1 h and cooled to room temperature before 32 mg of 1,2-diaminobenzene (0.29 mmol) in 4 ml MeOH was added dropwise. The reaction mixture was refluxed for 15 min and cooled to room temperature before 74 mg of NH_4PF_6 (0.44 mmol) was added. The mixture was stirred for 20 min, the solution was concentrated under reduced pressure until approximately 7 ml remained, and the mixture was left al −18 °C overnight. The solid was collected by filtration, washed with small portions of diethyl ether and dried under vacuum. Finally, 82 mg of product was obtained (yield: 52%). ^{1}H NMR (DMSO-d_6) δ 1.80 (d, J = 6 Hz, 3H, CH_3), 3.78 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 5.50 (d, J = 6 Hz, 1H, Ar—H), 5.73 (d, J = 6 Hz, 1H, Ar—H), 5.87 (s, 1H, Ar—H), 6.11 (d, J = 16 Hz, 1H, *trans* HC═C), 6.44 (m, 1H, C═C*H*—CH_3), 6.51 (d, J = 13 Hz, 2H, NH_2), 7.14 (m, 2H, Ar—H), 7.23 (m, 2H, Ar—H), 7.65 (d, J = 13 Hz, 1H, NH); EM m/z 422.75 ($C_{17}H_{22}N_2O_2RuCl^+$); Anal. Calc. for $C_{17}H_{22}N_2O_2RuClPF_6$: C, 35.95%; H, 3.90%; N, 4.93%. Found: C, 36.15%; H, 4.10%; N, 4.84%.

Determination of the octanol/water partition coefficient

The log P values were determined using the shaken flask method [27] with previously described modifications [28]. Octanol was presaturated with water (containing 0.2 M HCl), and the aqueous phase was saturated with octanol. The aqueous samples (5 ml) were vortexed with octanol (5 ml) for 2 h. The mixtures were centrifuged for 10 min at 3000 rpm to separate both phases. The ruthenium compounds were quantified in both phases with a Unicam UV/Vis Spectrometer (UV4).

Cell lines

The experimental cell cultures were obtained from the American Type Culture Collection (Rockville, MD, USA). The HT-29 colon cancer cell line, PC-3 prostate cancer cell line, MCF-7 breast adenocarcinoma cell line and CCD-841 CoN human colon epithelial cell line were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% FCS, 100 U/ml penicillin, 100 μg/mL streptomycin and 1 mM glutamine. The cells were seeded into 96 well microtiter plates in 100 μl volumes at a plating density of 5×10^3 cells/well. After 24 h of incubation at 37 °C under a humidified 5% CO_2 atmosphere to allow cell attachment, the cells were treated with different concentrations of the drugs (compounds VI—XII and carboplatin) and incubated for 72 h under the same conditions. Stock solutions of compounds were prepared in ethanol and the final concentration of this solvent was maintained at 1%. The control cultures received only 1% ethanol.

Cell viability: in vitro growth inhibition assay

The sulforhodamine B assay was used according to the method of Skehan et al. [29,30]. Briefly, the cells were seeded at 3×10^3 cells per well in a 96-flat-bottomed, 200 μl well microplate. The cells were incubated at 37 °C in a humidified 5% CO_2/95% air mixture and treated with the compounds (VI—XII and carboplatin) at different concentrations for 72 h. Afterward, the cells were fixed with 50%

trichloroacetic acid at 4 °C. After washing with water, the cells were stained with 0.1% sulforhodamine B (Sigma–Aldrich, St. Louis, MO, USA), dissolved in 1% acetic acid (50 μl/well) for 30 min, and subsequently washed with 1% acetic acid to remove any unbound stain. The protein-bound stain was solubilized with 100 μl of 10 mM unbuffered Tris base, and the cell density was determined using a spectrophotometric plate reader (wavelength 540 nm). The values are reported as the means ± SD of three independent experiments. The GraphPad software (GraphPad Software, San Diego, CA, USA) was used to calculate the IC_{50} values.

Results and discussion

Synthesis and characterization of the compounds VI–X

The first procedure used to obtain this type of complex was based on the arene ligand exchange reaction in complex [Ru(η^6-naphthalene)(η^4-1,5-cyclooctadiene)] [31]. Reacting this Ru^0 complex with methyleugenol (I) to promote naphthalene displacement with the subsequent hydrochloric acid treatment did not produce the desired [Ru(η^6-methyleugenol)$_2Cl_2]_2$ dimeric complex in useful yields. In contrast, reducing the $[Ru(1,5\text{-}COD)Cl_2]_n$ *in situ* with zinc dust and in the presence of the natural ligands I and II produced dimeric complexes IV and V, which had a characteristic deep red color and were isolated in 18% and 22% yields, respectively (Scheme 1) [11]. For the structurally related natural product saphrole (III), no stable [Ru(η^6-arene)$_2Cl_2]_2$ complex could be isolated. The [Ru(η^6-arene)$_2Cl_2]_2$ complexes (IV and V) were treated with three different *N,N*-chelating ligands (bipyridine (bipy), ethylenediamine (en) and 1,2-diaminobenzene (dab)) to obtain five well-characterized complexes with the following general formula: [Ru(η^6-arene)(*N,N*-chelating)Cl]PF_6. The complexes were crystalized either by slow evaporation in MeOH or a slow diffusion of ether into an MeOH solution. A crystal suitable for X-ray diffraction was obtained for compound VIII.

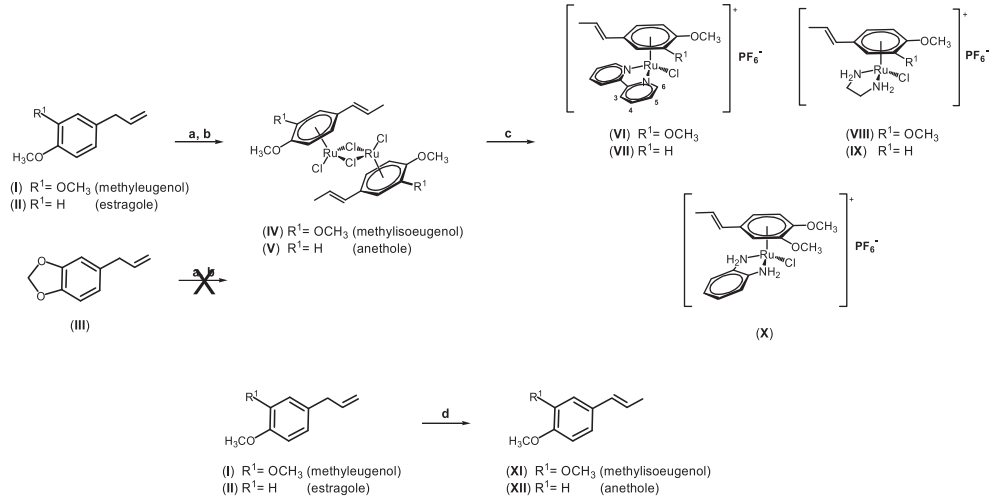
The ^{1}H NMR spectra of compounds VI to X exhibit the expected upfield shifts of the aromatic protons from 7.1 to 7.3 ppm in the natural precursors, to 5.5–6.3 ppm in the coordinated structure, confirming the existence of the organometallic Ru(II)-arene bond [32]. In addition, the ^{1}H NMR spectra show that the pattern of chemical shifts associated with the allyl moiety of the natural products differ in the final Ru(II) complexes, indicating that an isomerization process occurs upon coordination. The signals at δ 3.75 (d, 2H, $J = 7$ Hz, $CH_2CH{=}CH_2$), δ 5.40–5.55 (m, 2H, $CH_2CH{=}CH_2$) and δ 6.40 (dddd, $J = 17, 10, 7, 7$ Hz, 1H, $CH_2CH{=}CH_2$) of the original methyleugenol (I) change to δ 1.77 (d, $J = 7$ Hz, 3H, $CH{=}CHCH_3$), δ 6.02 (d, $J = 16$ Hz, 1H, $CH{=}CHCH_3$) and δ 6.69 (dd, $J = 16$, 7 Hz, 1H, $CH{=}CHCH_3$) in compound VI. The value of the coupling constants of the alkene protons ($J = 16$ Hz) indicated that only the *trans* isomer of methylisoeugenol and anethole were present in the final organometallic compounds.

To confirm that the isomerization occurs on both precursors after coordination, the methyleugenol (I) and estragole (II) were isomerized using a catalyst [{RuCl(μ-Cl)(η^3:η^3-$C_{10}H_{16}$)}$_2$]. The methylisoeugenol (XI) and anethole (XII) were obtained in good yields from I and II, respectively (Scheme 1); however longer reaction times were required (approximately 90 min for I and 150 min for II) compared to the 10 min reported for compound II [21]. The ^{1}H NMR spectra of methyleugenol (I), methylisoeugenol (XI), compound VI, estragole (II), anethole (XII) and compound VII clearly demonstrated that isomerization occurs in the final organometallic compounds (See Supplementary information).

The lack of experimental evidence (Ru^0 intermediate were not isolated) precludes the proposal of a mechanism for the isomerization of the ligands during coordination. However, reported Ru complexes, including $RuCl_2(PPh_3)_3$, $RuCl_3(AsPh_3)_3$, ruthenium carbene catalyst (named Grubbs second-generation catalyst) and the bis(allyl)-ruthenium(IV) dimer [{RuCl(μ-Cl)(η^3:η^3-$C_{10}H_{16}$)}$_2$], employed in this work can perform this type of isomerization on eugenol and/or estragole [33–36]. The isomerizations promoted by the polymeric [Ru(η^4-1,5-COD)$Cl_2]_n$ complex (our starting material) have not been reported in the literature.

X-ray diffraction of [Ru(η^6-methylisoeugenol)(en)Cl]PF_6 (VIII)

The molecular structure with atom-numbering scheme is shown in Fig. 1. The X-ray diffraction analysis of VIII reveals the



a) [Ru(η^4-1,5-COD)$Cl_2]_n$, Zn dust, anh. THF, reflux, 24 h.; b) HCl 1 M in ether, anh. CH_3CN, room temp., 16 h.; c) N,N ligands (bipy, en or dab), NH_4PF_6, MeOH, room temp.; d) 1 mol% [{RuCl(μ-Cl)(η^3:η^3-$C_{10}H_{16}$)}$_2$], H_2O-MeOH 1:1 v/v ratio, 80°C.

Scheme 1. General synthetic procedures for compounds VI to XII.

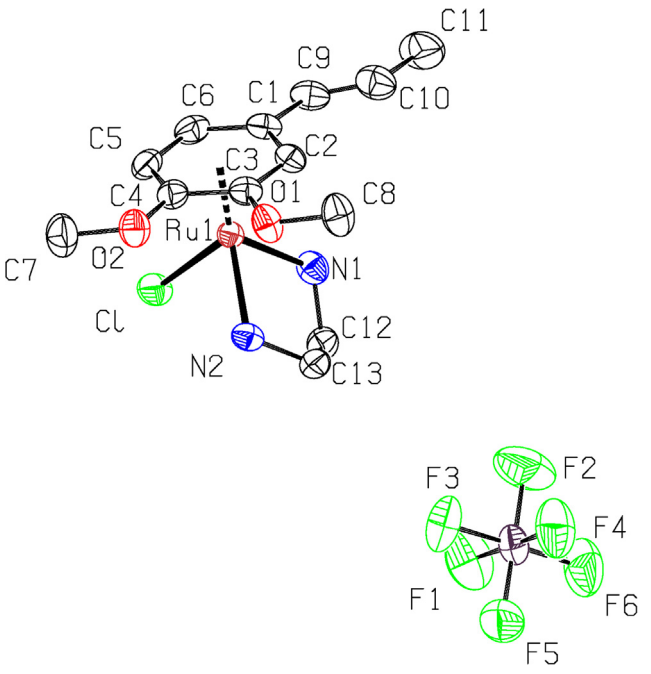


Fig. 1. A view of the asymmetric unit showing the Ru(II) complex and PF_6^- anion. The displacement ellipsoids are drawn at the 50% probability level. H-atoms have been omitted for clarity.

presence of both enantiomers in the same single-crystal. The co-crystallization of isomers is an important phenomenon for optical resolution, as described in the literature. Brunner H. et al. suggested that the stability of the inverted diastereomeric piano-stool complex is attributed to molecular recognition [37]. The opposing absolute configuration of both enantiomers in ruthenium complex VIII compound (in this work) is shown in Fig. 2. The enantiomers form an inverted piano-stool arrangement in a 1:1 ratio.

All bond distances and angles in VIII are as expected (Table 2) and are in acceptable agreement with the described analogs [37–40]. The N–Ru–N bond angles in both enantiomers are comparable to those in [(η^6-*p*-cymene)Ru(en)Cl][PF_6] [78.98(10)°]. The torsional N1–C13–C12–N2 angle of the corresponding bridge is 54.0(3)°. The Ru–Cl bond distance in VIII [2.4149(9) Å], [(η^6-*p*-cymene)$RuCl_2$] and [(η^6-*p*-cymene)Ru(en)Cl][PF_6] [2.39–2.45 Å] are similar [38,39]. The Ru–arene (Ru···*Cg*) bond length (see Table 2) matches that in previous reports. *Cg* is the centroid of the C1–C6 ring. The Ru–arene bond length in [(η^6-*p*-cymene)Ru(O–N)Cl] ranged from 1.655 to 1.689 Å [40]. The structure shows bond lengths in C1–C2, C2–C3 and C3–C4, C4–C5 and C5–C6 in VIII ranged from 1.403 Å to 1.437 Å.

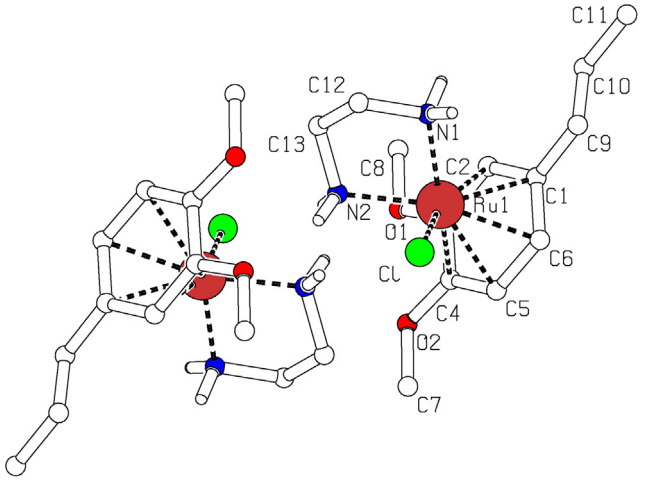


Fig. 2. A view of the two enantiomers. The H-atoms and PF_6^- anions have been omitted for clarity.

Table 2
Selected bond lengths (Å) and angles (°) in Compound VIII.

Ru–centroid	1.6817(2)
Ru–N1	2.132(2)
Ru–N2	2.137(2)
Ru–Cl	2.4148(9)
C4–C5	1.403(4)
C3–O1	1.355(4)
N1–Ru–N2	78.79(10)
N1–Ru–Cl	85.64(8)
N2–Ru–Cl	84.82(8)
O2–C4–C5	125.6(3)

The torsional C2–C1–C9–C10 angle of the corresponding alkyl substituent group (at C1: C9–C10–C11) is −12.7(5)°. The alkyl group is essentially flat relative to the mean plane of the coordinated arene with a C9=C10 bond distance of 1.309(5) Å [C9–C10–C11 = 125.0(3)°]. The P–F bond lengths in PF_6^- anion ranged from 1.565(3) to 1.606 (2) Å [F1–P–F5 = 90.68(16)° and F2–P–F5 = 178.00(18)°].

In the crystal packing of VIII, the Ru(II) complex and PF_6^- anions are linked *via* N–H···F [2.24–2.55 Å] hydrogen bonds and C–H···F [2.46–2.47 Å] intermolecular contacts. The network is reinforced by the N2–H2A···O1 [2.300 Å] and N2–H2B···Cl [2.660 Å] hydrogen bonds (see Fig. 5, Supplementary information). The H atoms of the Nitrogen link each Cl atom forming a four-center graph-set $R_2^2(4)$ motif [41]. The Ru···Ru distance of the two enantiomers in the inverted piano-stool arrangement is 7.056 Å (see Fig. 2). The supramolecular structure is additionally stabilized by π–π interactions with *Cg*···*Cg*′ distance of 3.6489(18) Å.

Octanol/water partition coefficient

The UV–Vis spectra for compounds VI to X were recorded in water and aqueous acidic media (0.2 M HCl) between 190 and 800 nm. All of the compounds were more stable in the aqueous acidic media compared to water during the analysis (2 h). For each compound, two acidic aqueous solutions with different concentrations were prepared (between 0.13 and 1.05 mM) and shaken vigorously with octanol (presaturated with the acidic aqueous solution). The equilibrium concentrations of the compounds were measured in both phases at the following wavelengths for VI, VII, VIII, IX and X, respectively: the organic phase was assessed at 292 nm, 296 nm, 220 nm, 260 nm, and 260 nm; the aqueous phase was assessed at 412 nm, 344 nm, 312 nm, 300 nm, and 312 nm. Table 3 summarizes the log *P* (log [ ]$_{organic}$/[ ]$_{aqueous}$) values for all of the compounds.

In vitro cell viability assay

In vitro biological activity of organometallic compounds VI to X, methylisoeugenol (XI), anethole (XII) and carboplatin against the three human tumor cell lines MCF-7 (breast cancer), PC-3 (prostate cancer) and HT-29 (colon cancer) and one human non-tumor cell line CCD-841 (colon epithelial) are shown in Table 3. The results indicated that the free ligands (XI and XII) exhibited no measurable activity (>100 μM) against any of the studied human cell lines.

Table 3
Comparison of the biological activity of compounds VI to XII and carboplatin against human breast cancer (MCF-3), human prostate cancer (PC-3), human colon cancer (HT-29) and human colon epithelial cells (CCD-841, a non-tumor cell line).

Compound	Log *P*	IC_{50} (μM)[a]			
		MCF-7	PC-3	HT-29	CCD-841
VI	−1.38	>100	>100	>100	>100
VII	−0.83	>100	>100	>100	>100
VIII	−1.30	>100	>100	>100	>100
IX	−0.99	40 ± 4	58 ± 8	18 ± 3	79 ± 10
X	−0.93	73 ± 9	28 ± 4	>100	>100
XI	–	>100	>100	>100	>100
XII	–	>100	>100	>100	>100
Carboplatin	–	78 ± 7	87 ± 12	>100	>100

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

Furthermore, both complexes containing bipy [Ru(η^6-arene)(bipy)Cl]PF_6 where the arene is methylisoeugenol for VI and anethole for VII did not exhibit any measurable activity against the studied cell lines. These results agree with previous reports on [(η^6-arene)Ru(bipy)Cl]$^+$ complexes [11]. The lack of activity displayed by [Ru(η^6-methylisoeugenol)(en)Cl]PF_6 (VIII) was unexpected. [Ru(η^6-anethole)(en)Cl]PF_6 (IX) and [Ru(η^6-methylisoeugenol)(dab)Cl]PF_6 (X) are active compounds that are even better than carboplatin. The lack of activity observed for [Ru(η^6-methylisoeugenol)(en)Cl]PF_6 (VIII) can be correlated with the lower lipophilicity of VIII (see Table 3) due to the additional methoxy substituent on the coordinated aromatic ring compared to IX. The presence of an additional polar group on the coordinated arene (methylisoeugenol vs anethole) tends to decrease the lipophilicity of the organometallic compounds (VI vs VII and VIII vs IX), disfavoring its biological activity. The literature suggests that the presence of a polar substituent on the coordinated arene tends to lower the cytotoxicity of the organometallic complex [11]. When using ethylenediamine (en) or 1,2-diaminobenzene (dab) as the *N*,*N*-chelating ligand, however, organometallic compounds with similar biological activities were generated [11]. Therefore, the higher activity of [Ru(η^6-methylisoeugenol)(dab)Cl]PF_6 (X) versus [Ru(η^6-methylisoeugenol)(en)Cl]PF_6 (VIII) was attributed to the enhanced lipophilicity of the former compound.

Compound IX exhibited the highest biological activity against the human tumor cells under study; its cytotoxicity was approximately 20 times greater than the value reported for anethole [42]. The coordination of the Ru(en)Cl^+ moiety to this biologically active natural product generated compound IX, which exhibited an improved cytotoxic activity. Similarly, coordinating methylisoeugenol to the Ru(dab)Cl^+ moiety produced compound X, which exhibited an increased biological activity compared to the natural product alone. Whether the coordination of these ligands actually improved the cytotoxic activity of the organometallic compound should be established in future works. Previous studies have established that combining anethole with platinum based drugs such as cisplatin and oxaliplatin synergistically increases the *in vitro* cytotoxicity against a human ovarian tumor cell line [15].

Conclusions

The *in situ* reduction of the polymeric [Ru(1,5-COD)Cl_2]$_n$ complex with methyleugenol and estragole generated two new dimeric complexes: [Ru(η^6-methylisoeugenol)$_2Cl_2$]$_2$ (IV) and [Ru(η^6-anethole)$_2Cl_2$]$_2$ (V). Treating these complexes with *N*,*N*-chelating ligands (bipyridine, ethylendiamine and 1,2-diaminobenzene) generates five new well-characterized “piano-stool” compounds: VI–X. The Ru(II)-arene organometallic bond and the alkene isomerization on the allylic chain of the coordinated arene was verified by ^{1}H NMR spectroscopy. The single-crystal X-ray diffraction data for complex VIII confirmed these features. The *in vitro* cytotoxicity measurements showed that [Ru(η^6-anethole) (en)Cl]PF_6 (IX) and [Ru(η^6-methylisoeugenol)(dab)Cl]PF_6 (X) were as cytotoxic as carboplatin, which is a commercial drug, against the three human tumor cell lines studied. Compounds IX and X should be tested against other human tumor cells, particularly cisplatin-resistant cells, in a future study.

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Appendix A. Supplementary material

CCDC 955623 contains 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 material

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jorganchem.2014.09.005.

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