Journal of Organometallic Chemistry 730 (2013) 137–143

Contents lists available at SciVerse ScienceDirect

Journal of Organometallic Chemistry

journal homepage: www.elsevier.com/locate/jorganchem

Synthesis, characterization, electrochemical behavior and *in vitro* protein tyrosine kinase inhibitory activity of the cymene-halogenobenzohydroxamato [Ru(η^6-cymene)(bha)Cl] complexes

Xianmei Shang [a,b], Telma F.S. Silva [a], Luísa M.D.R.S. Martins [a,c], Qingshan Li [d], M. Fátima C. Guedes da Silva [a,e], Maxim L. Kuznetsov [a], Armando J.L. Pombeiro [a,*]

[a] Centro de Química Estrutural, Complexo I, Instituto Superior Técnico, Technical University of Lisbon, Av. Rovisco Pais, 1049-001 Lisbon, Portugal
[b] Tongji School of Pharmacy, Huazhong University of Science and Technology, 13 Hangkong Road, 430030 Wuhan, China
[c] Chemical Engineering Departmental Area, ISEL – Instituto Superior de Engenharia de Lisboa, Rua Conselheiro Emídio Navarro 1, 1959-007 Lisbon, Portugal
[d] School of Pharmaceutical Science, Shanxi Medical University, 86 South Xinjian Road, 030001 Taiyuan, China
[e] Universidade Lusófona de Humanidades e Tecnologias, ULHT Lisbon, Av. do Campo Grande, 376, 1749-024 Lisbon, Portugal

ARTICLE INFO

Article history:
Received 12 October 2012
Received in revised form
9 December 2012
Accepted 10 December 2012

Keywords:
Ruthenium(II) complexes
Synthesis
Protein tyrosine kinase inhibitor
Electrochemistry

ABSTRACT

The ruthenium(II)–cymene complexes [Ru(η^6-cymene)(bha)Cl] with substituted halogenobenzohydroxamato (bha) ligands (substituents = 4-F, 4-Cl, 4-Br, 2,4-F_2, 3,4-F_2, 2,5-F_2, 2,6-F_2) have been synthesized and characterized by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR, cyclic voltammetry and controlled-potential electrolysis, and density functional theory (DFT) studies. The compositions of their frontier molecular orbitals (MOs) were established by DFT calculations, and the oxidation and reduction potentials are shown to follow the orders of the estimated vertical ionization potential and electron affinity, respectively. The electrochemical E_L Lever parameter is estimated for the first time for the various bha ligands, which can thus be ordered according to their electron-donor character. All complexes exhibit very strong protein tyrosine kinase (PTK) inhibitory activity, even much higher than that of genistein, the clinically used PTK inhibitory drug. The complex containing the 2,4-difluorobenzohydroxamato ligand is the most active one, and the dependences of the PTK activity of the complexes and of their redox potentials on the ring substituents are discussed.

1. Introduction

The development of metal-containing drugs with high antitumor activity, low toxicity and a pharmacological outline different from that of the platinum compounds, is a challenge in drug design [1–5]. Ruthenium complexes have shown very promising results in preclinical and clinical studies, and attracted a high attention in cancer research because of their comparative antitumor activity and lower cytotoxicity [6–15]. Ruthenium compounds can have a mechanism of action different from that of Pt drugs and a different spectrum of activity and non-cross-resistance [7,8,16–18].

Protein tyrosine kinases (PTKs) are members of a large family of oncoproteins and proto-oncoproteins, play a major role in mitogenic signal transduction, and are involved in the control of cell proliferation, differentiation and transformation [19]. Continuing activation of PTK is associated with proliferative disorders such as cancer, and PTK inhibitors have been developed as molecular-targeting cancer therapeutic agents. The discovery and development of PTK inhibitors as new cancer therapeutic agents have attracted much attention [20–27]. Many PTK inhibitors with potent activities have already passed or are currently in clinical trials to investigate their applicability as anti-cancer drugs [28].

Ru(II) complexes with cymene ligands possess significant antitumor activity [8b,c,12–15]. Until now, the PTK inhibitory activity of mixed-ligand ruthenium(II) complexes with cymene and halogenobenzohydroxamic acid had not been disclosed, although some ruthenium(II) complexes with cymene and hydroxamic acid have been reported [29]. In the current work, we describe the synthesis, characterization, electrochemical behavior and *in vitro* PTK inhibitory activity of seven new mixed ligand ruthenium(II) complexes with cymene and substituted hydroxamato ligands of general formula [Ru(η^6-cymene)(bha)Cl].

* Corresponding author. Tel.: +351 2184 19237; fax: +351 218464455.
E-mail addresses: pombeiro@ist.utl.pt, pombeiro@popsrv.ist.utl.pt (A.J.L. Pombeiro).

0022-328X/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jorganchem.2012.12.013

2. Experimental section

2.1. Materials

[RuCl(η^6-*p*-cymene)(μ-Cl)]$_2$, methyl 4-fluorobenzoate, methyl 4-chlorobenzoate, methyl 4-bromobenzoate, methyl 2,4-difluorobenzoate, methyl 2,5-difluorobenzoate and methyl 2,6-difluorobenzoate were purchased from Aldrich or Alfa and used as received. 4-fluoro-, 4-chloro-, 4-bromo-, 2,4-difluoro-, 3,4-difluoro-, 2,5-difluoro- and 2,6-difluoro-benzohydroxamic acids were prepared as previously reported [30]. The basic forms of these acids (bha) are denoted by F_4bha, Cl_4bha, Br_4bha, F_{24}bha, F_{34}bha, F_{25}bha and F_{26}bha, respectively. The other reagents were of analytical grade. C and H elemental analyses were performed on a PE-2400-II elemental analyzer. Infrared spectra (4000–400 cm^{-1}) were recorded with a Biorad FTS 3000MX instrument in KBr pellets. The ^{1}H and ^{13}C (Me_4Si internal standard) NMR spectra were recorded on a Bruker Avance II+ 400 MHz (Ultra-Shield Magnet) spectrometer.

2.2. Instrumentation and measurement

The electrochemical experiments were performed on an EG&G PAR 273A potentiostat/galvanostat connected to personal computer through a GPIB interface. Cyclic voltammograms (CV) were obtained in 0.2 M [^{n}Bu$_4$N][BF$_4$]/CH_2Cl_2, at a platinum disc working electrode (d = 1 mm). Controlled-potential electrolyses (CPE) were carried out in electrolyte solutions with the above mentioned composition, in a three-electrode H-type cell. The compartments were separated by a sintered glass frit and equipped with platinum gauze working and counter electrodes. For both CV and CPE experiments, a Luggin capillary connected to a silver wire pseudo-reference electrode was used to control the working electrode potential. The CPE experiments were monitored regularly by cyclic voltammetry, thus assuring no significant potential drift occurred along the electrolyses. Ferrocene was used as an internal standard for the measurement of the oxidation potentials of the complexes; the redox potential values are quoted relative to the SCE by using as internal reference the ferrocene/ferricinium ([Fe(η^5-C_5H_5)$_2$]$^{0/+}$) couple ($E^{ox}_{1/2}$ = 0.525 V *vs.* SCE in CH_2Cl_2) [31].

2.3. Computational details

The full geometry optimization of the complexes has been carried out in Cartesian coordinates at the DFT level of theory using Becke's three-parameter hybrid exchange functional in combination with the gradient-corrected correlation functional of Lee, Yang and Parr (B3LYP) [32] with the help of the Gaussian-03 [33] program package. Symmetry operations were not applied for all structures. A quasi-relativistic Stuttgart pseudopotential described 28 core electrons and the appropriate contracted basis set (8s7p6d)/[6s5p3d] [34] for the ruthenium atom and the 6-31G(d) basis set for other atoms were used. The Hessian matrix was calculated analytically to prove the location of correct minima (no imaginary frequencies were found). Vertical ionization potentials and electron affinities were calculated as differences of the total energies $E_{ox} - E_{neut}$ and $E_{neut} - E_{red}$, where the index "neut" corresponds to a neutral complex, and the indexes "ox" and "red" correspond to oxidized and reduced complexes with unrelaxed geometries.

2.4. General procedure for the synthesis of compounds

The starting complex [RuCl(η^6-*p*-cymene)(μ-Cl)]$_2$ (0.306 g, 0.5 mmol) was added to a mixture of methanol and CH_2Cl_2 (1:1, v/v, 30 mL) with the appropriate halogenobenzohydroxamic acid (1 mmol) and NaOMe (0.054 g, 1 mmol), the resulting clear solution was stirred for 4 h at room temperature, and the color changed from red to orange. The solvent was removed in vacuum, and the residue was dissolved in dichloromethane (10 mL), and the solution was filtered to remove sodium chloride. The orange solution was concentrated (2 mL) and addition of hexane gave an orange precipitate of the complex, which was separated by filtration and dried under vacuum to afford an orange-red solid. The compound is soluble in alcohols, acetone, acetonitrile, dimethyl sulfoxide, and chlorinated solvents.

2.4.1. Synthesis of [Ru(η^6-*p*-cymene)(F_4bha)Cl] (**1**)

Yield: 63%. Anal. Calcd for $C_{17}H_{19}NO_2ClFRu\cdot 1/2H_2O$ (433.87): C, 47.06; H, 4.65; N, 3.23. Found: C, 47.53; H, 4.64; N, 3.22. IR: 3442 (N–H), 3044 (C_{arom}–H), 2959, 2916, 2869, 1606 (C=O), 1488, 1234, 858, 630, 564, 513 (Ru–O) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 7.92–7.72 (H_{arom}, F_4bha), 7.13–7.00 (H_{arom}, F_4bha), 5.37 (d, $^2J_{HH}$ = 8 Hz, 4H, cymene), 2.92 (m, 1H, –C<u>H</u>(CH$_3$)$_2$), 2.28 (s, 3H, –C<u>H</u>$_3$), 1.31 (d, J_{HH} = 7.2 Hz, 6H, (C<u>H</u>$_3$)$_2$CH–) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): 165.3 (C=O), 163.5 (C–F), 129.7, 129.1, 126.2, 115.8, 101.3, 96.8, 81.3, 80.5, 30.9, 30.6, 22.1, 18.9 ppm.

2.4.2. Synthesis of [Ru(η^6-*p*-cymene)(Cl_4bha)Cl] (**2**)

Yield: 75%. Anal. Calcd for $C_{17}H_{19}NO_2Cl_2Ru$ (441.31): C, 46.27; H, 4.34; N, 3.17. Found: C, 46.24; H, 4.44; N, 3.08. IR: 3442 (N–H), 3056 (C_{arom}–H), 2961, 2924, 1846, 1637 (C=O), 1467, 1389, 1091, 878, 567 (Ru–O) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 7.79 (d, J_{HH} = 8.4 Hz, H_{arom}, Cl_4bha), 7.46 (d, J_{HH} = 8.8 Hz, H_{arom}, Cl_4bha), 5.50 (d, J_{HH} = 6 Hz, 4H, H_{arom}, cymene), 5.37 (d, J_{HH} = 6 Hz), 2.95 (m, 1H, –C<u>H</u>(CH$_3$)$_2$), 2.30 (s, 3H, –C<u>H</u>$_3$), 1.31 (d, J_{HH} = 7.2 Hz, 6H, (C<u>H</u>$_3$)$_2$CH–) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): 163.3 (C=O), 159.8 (C–Cl), 140.5, 121.7, 114.7, 102.9, 101.4, 96.5, 81.2, 80.6, 30.8, 30.6, 22.1, 19.0 ppm.

2.4.3. Synthesis of [Ru(η^6-*p*-cymene)(Br_4bha)Cl] (**3**)

Yield: 66%. Anal. Calcd for $C_{17}H_{19}NO_2ClBrRu$ (485.77): C, 42.03; H, 3.94; N, 2.88. Found: C, 42.10; H, 3.72; N, 2.83. IR: 3442 (N–H), 3050 (C_{arom}–H), 2960, 2923, 1831, 1583 (C=O), 1384, 527 (Ru–O) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 7.76 (d, J_{HH} = 8.4 Hz, PBrbha), 7.50 (d, J_{HH} = 8.8 Hz, Br_4bha), 5.73 (d, J_{HH} = 6.0 Hz, 2H, H_{arom}, cymene), 5.50 (d, J_{HH} = 6.0 Hz, 2H), 5.37 (d, $^2J_{HH}$ = 6.0 Hz, 2H), 3.01–2.91 (m, 1H, –C<u>H</u>(CH$_3$)$_2$), 2.40 (s, 3H, –C<u>H</u>$_3$), 1.44 (d, J_{HH} = 7.2 Hz, (C<u>H</u>$_3$)$_2$CH–), 1.31 (d, J_{HH} = 6.8 Hz, (C<u>H</u>$_3$)$_2$C<u>H</u>–) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): 168.4 (C=O), 164.0 (C–Br), 131.3, 130.2, 111.9, 101.3, 96.8, 90.9, 81.3, 80.6, 30.9, 30.7, 22.4, 22.1, 18.9 ppm.

2.4.4. Synthesis of [Ru(η^6-*p*-cymene)(F_{24}bha)Cl] (**4**)

Yield: 58%. Anal. Calcd for $C_{17}H_{18}NO_2ClF_2Ru$ (442.85): C, 46.11; H, 4.10; N, 3.16. Found: C, 46.21; H, 4.42; N, 3.28. IR: 3440 (N–H), 3048 (C_{arom}–H), 2958, 2923, 2866, 1610 (C=O), 1484, 875, 627, 510 (Ru–O) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 9.58 (d, N–H), 8.17–8.09 (m, H_{arom}, F_{24}bha), 7.02–6.80 (m, H_{arom}, F_{24}bha), 5.48 (dd, J_{HH} = 6.0 Hz, 4H, H_{arom}, cymene), 2.91 (m, –C<u>H</u>(CH$_3$)$_2$), 2.33 (s, 3H, –C<u>H</u>$_3$), 1.38 (d, J_{HH} = 6.9 Hz, 6H, (C<u>H</u>$_3$)$_2$C<u>H</u>–) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): 175.6 (C=O), 167.5 (C–F), 160.1 (C–F), 143.7, 139.9, 131.9, 128.7, 126.3, 112.1, 104.3, 101.2, 99.4, 96.7, 81.3, 80.5, 31.2, 30.9, 30.6, 22.1, 18.9, 18.5 ppm.

2.4.5. Synthesis of [Ru(η^6-*p*-cymene)(F_{34}bha)Cl] (**5**)

Yield: 74%. Anal. Calcd for $C_{17}H_{18}NO_2ClF_2Ru$ (442.85): C, 46.11; H, 4.10; N, 3.16. Found: C, 46.02; H, 4.22; N, 3.23. IR: 3436 (N–H), 3061 (C_{arom}–H), 2963, 2925, 1869(vs), 1617, 1600 (C=O), 1521, 1468, 1386, 776, 552 (Ru–O) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 7.77–6.88 (m, 3H, H_{arom}, F_{34}bha), 5.49 (m, 4H, H_{arom}, cymene), 2.34 (s, 3H, –C<u>H</u>$_3$, cymene), 1.28 (m, C<u>H</u>(CH$_3$)$_2$) ppm. ^{13}C NMR (100 MHz,

$CDCl_3$): 165.9 (C=O), 159.3 (C–F), 156.3 (C–F), 137.8, 128.9, 126.3, 118.8, 81.3, 80.5, 30.9, 30.6, 24.1, 22.1, 18.9 ppm.

*2.4.6. Synthesis of [Ru(η^6-p-cymene)(F_{25}bha)Cl](**6**)*

Yield: 71%. Anal. Calcd for $C_{17}H_{18}NO_2ClF_2Ru$ (442.85): C, 46.11; H, 4.10; N, 3.16. Found: C, 45.94; H, 4.26; N, 3.26. IR: 3436 (N–H), 3055 (C_{arom}–H), 2965, 2925, 1602 (C=O), 1577 (strong), 1507, 1203, 1127, 859, 772, 540, 465 (Ru–O) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): 9.73 (d, N–H), 7.85–7.79 (m, H_6, F_{25}bha), 7.12–7.04 (m, H_3, H_4, F_{25}bha), 5.56–5.32 (m, 4H, H_{arom}, cymene), 2.94 (m, 2H, –C<u>H</u>$(CH_3)_2$), 1.39–1.37 (d, J_{HH} = 6.9 Hz, 6H, ($C\underline{H}_3)_2$CH–), 1.31–1.28 (d, J_{HH} = 6.9 Hz, 6H, ($C\underline{H}_3)_2$CH–) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): 169.5 (C=O), 152 (C–F), 148.0 (C–F), 133.4, 128.9, 126.2, 113.9, 81.3, 80.5, 33.6, 30.9, 24.0, 22.1 ppm.

*2.4.7. Synthesis of [Ru(η^6-p-cymene)(F_{26}bha)Cl] (**7**)*

Yield: 50%. Anal. Calcd for $C_{17}H_{18}NO_2ClF_2Ru$ (442.85): C, 46.11; H, 4.10; N, 3.16. Found: C, 46.02; H, 4.24; N, 3.22. IR: 3440 (N–H), 3055 (C_{arom}–H), 2961, 2924, 1626 (C=O), 1466, 1004, 791, 525, 447 (Ru–O) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): 7.62–7.35 (H_{arom}, F_{26}bha), 7.14 (H_{arom}, F_{26}bha), 6.96–6.92 (H_{arom}, F_{26}bha), 5.51, 5.49, 5.37, 5.35 (dd, $^2J_{HH}$ = 6 Hz, 4H, H_{arom}, cymene), 2.93 (m, 1H, –C<u>H</u>$(CH_3)_2$), 2.34 (s, 3H, cymene), 1.31–1.29 (d, J_{HH} = 6.8 Hz, 6H, ($C\underline{H}_3)_2$CH–), 1.27–1.25 (d, J_{HH} = 6.8 Hz, 6H, ($C\underline{H}_3)_2$CH–) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): 168.1 (C=O), 164.1 (C–F), 153.1 (C–F), 135.6, 133.0, 124.9, 103.7, 96.8, 81.5, 80.4, 30.9, 30.6, 22.1 ppm.

2.5. PTK inhibitory activity assay

PTK activity was determined by the ELISA method. The tyrosine kinase was extracted from brain tissue of rat, and microtiter plates were coated using poly-Glu-Tyr (PGT) as substrates. If the tyrosine residues of PGT were phosphorylated by PTKs, they bound to phospho-specific monoclonal antibody that was labeled specifically with HRP. The absorbance was measured to reflect the activity of PTK.

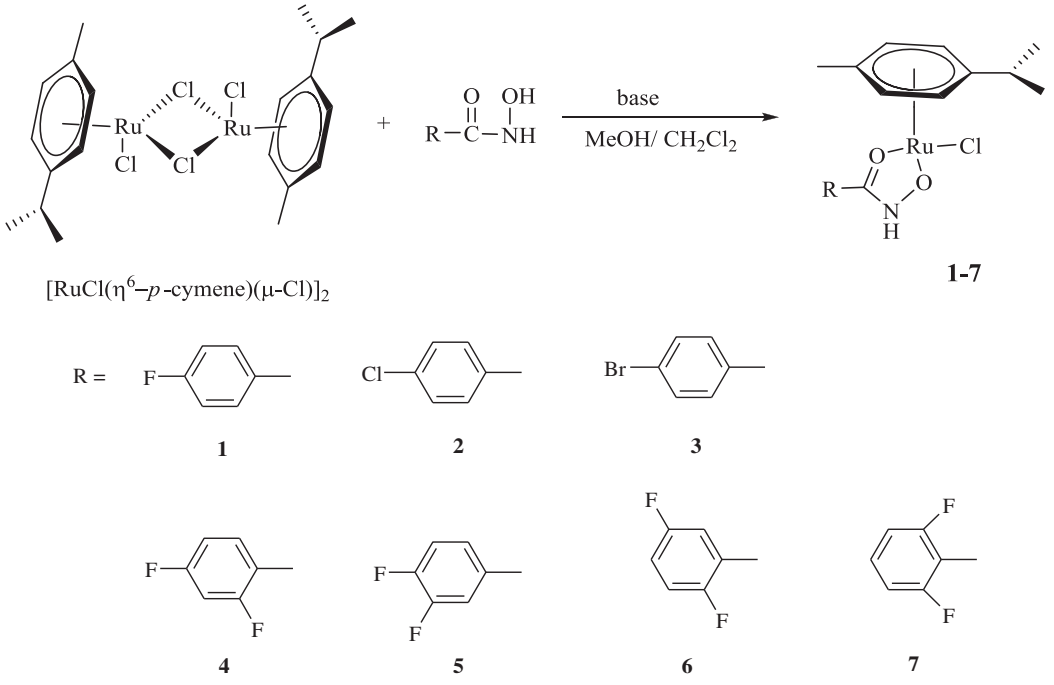
The phosphorylation assays were performed at 37 °C in a final volume of 40 μL tyrosine kinase. The concentrations of PTKs used to construct calibration curves were as follows: 600, 500, 400, 300, 200 and 100 × 10^{-7} U/mL for PTK. A concentration of 500 × 10^{-7}/μL was used for each inhibitor. Phosphorylation reactions were initiated with the addition of 40 mM ATP (10 μL) into each vessel, and the plate was incubated at 37 °C for 30 min. After completion of reaction, liquid was decanted and the vessels were washed four times with Tween-PBS. A volume of 100 μL of blocking solution was added to the vessels and incubated at 37 °C for 30 min. After washing the plate with Tween-PBS, anti-phosphotyrosine (50 μL) was added to the vessels and incubated at 37 °C for 30 min. The reaction liquid was decanted and the remaining solution was removed by rinsing four times with Tween-PBS. One hundred μL of HRP coloring agent was added and incubated at 37 °C for 15 min. The reaction was terminated by addition of 1 N sulfuric acid (100 μL/well). Absorbance was measured at 450 nm in a microplate reader (SpectraMax M5) [22]. All experiments were performed in triplicate.

3. Results and discussion

3.1. Synthesis and characterization

The new ruthenium(II) complexes [Ru(η^6-cymene)(F_4bha)Cl] (**1**), [Ru(η^6-cymene)(Cl_4bha)Cl] (**2**), [Ru(η^6-cymene)(Br_4bha)Cl] (**3**), [Ru(η^6-cymene)(F_{24}bha)Cl] (**4**), [Ru(η^6-cymene)(F_{34}bha)Cl] (**5**), [Ru(η^6-cymene)(F_{25}bha)Cl] (**6**) and [Ru(η^6-cymene)(F_{26}bha)Cl] (**7**) have been prepared by the reaction of [RuCl(η^6-*p*-cymene)(μ-Cl)]$_2$ with the appropriate halogenobenzohydroxamic acid in a mixture of CH_3OH/CH_2Cl_2 (1:1, v/v) under basic conditions in the presence of NaOMe (Scheme 1).

The elemental analysis data of the complexes are in good agreement with the calculated values, and their IR spectra display a sharp band at 1583–1637 cm^{-1}, not present in [RuCl(η^6-*p*-cymene)(μ-Cl)]$_2$, which is assigned to ν_{CO} of the coordinating hydroxamato. Compared to that of the free acid (ν_{CO} for substituted



Scheme 1. Synthesis of the [Ru(η^6-cymene)(bha)Cl] complexes (**1**–**7**).

benzohydroxamic acids are in the 1619–1634 cm^{-1} range), a shift of *ca.* 17–45 cm^{-1} is observed, what is consistent with previous findings [35–38].

In the ^1H NMR spectra of **1**–**7**, the expected resonances are observed for the cymene and the substituted benzohydroxamato. As a result of the coordination of the substituted hydroxamato unit, downfield shifts (0.10–0.20 ppm) of the ligand ring protons are observed relative to the free acid. Similar downfield shifts were also detected for the coordinated *p*-cymene in **1**–**7** as compared to the arene ligand in [RuCl(η^6-*p*-cymene)(μ-Cl)]$_2$. The ^{13}C NMR spectra for **1**–**7** also show the expected resonance signals.

3.2. Biological evaluation

All of the synthesized compounds (**1**–**7**) and the precursor [RuCl(η^6-*p*-cymene)(μ-Cl)]$_2$ were screened for preliminary *in vitro* PTK inhibitory activity by ELISA with genistein as a positive reference compound. As shown in Table 1, all of them exhibit very strong activities with IC_{50} values below 3.11 μM, which are even much higher than that of genistein with an IC_{50} value of 13.65 μM in the same model [39]

Among **1**, **2** and **3** with different *para*-halogen atoms, **1** with the *para*-fluoro substituent showed the best activity with an IC_{50} value of 0.02 μM (the order of activity follows F > Cl > Br), what suggests that the fluorine atom may play an important role for the interaction of the active ruthenium complex with PTK.

For the isomeric difluoro compounds **4**–**7**, the order of activity is $F_{24} > F_{26} > F_{34} > F_{25}$, indicating a higher importance of the *ortho*- and *para*-positions, in comparison with the *meta*-one, for PTK activity. In fact, the most active di-fluoro substituted compounds (**4** and **7**) bear the fluoro substituents only at the *ortho*- and/or *para*-positions, whereas those with a *meta*-F (**5** and **6**) are less active.

3.3. Electrochemistry behavior and theoretical study

The redox properties of **1**–**7** have been investigated by cyclic voltammetry, at a platinum electrode, in a 0.2 M [^{n}Bu$_4$N][BF$_4$]/CH_2Cl_2 solution, at 25 °C. They exhibit a single-electron irreversible anodic process, assigned [40] to the Ru(II/III) oxidation, at the oxidation potential values ($E^{ox}_{p/2}$ in the range of 1.51 – 2.05 V *vs.* SCE) given in Table 2 (Fig. 1 for compound **5** as a typical case).

In the cathodic region, a single-electron irreversible wave is detected, assigned to the Ru(II/I) reduction ($E^{red}_{p/2}$ in the range of −1.51 to −0.51 V *vs.* SCE). The occurrence of a single-electron oxidation (or reduction) has been confirmed by exhaustive controlled potential electrolysis (CPE) at a potential slightly anodic (or cathodic) to that of the corresponding peak potential.

Complex [Ru(η^6-*p*-cymene)(F_{24}bha)Cl] (**4**) is the easiest to oxidize (lowest oxidation potential, $E^{ox}_{p/2}$ = 1.51 V *vs.* SCE) and the most difficult to reduce (lowest reduction potential, $E^{red}_{p/2}$ = −1.51 V *vs.* SCE) (Table 2), and, moreover, is that with the highest PTK inhibitory activity. However, no clear relation, along the series of the complexes, appears to be observed between the redox potential and such a bioactivity.

Table 1
PTK inhibitory activity.[a]

Compounds	IC_{50} (μM)
[RuCl(η^6-*p*-cymene)(μ-Cl)]$_2$	0.18
[Ru(η^6-*p*-cymene)(F_4bha)Cl] (**1**)	0.02
[Ru(η^6-*p*-cymene)(Cl_4bha)Cl] (**2**)	1.52
[Ru(η^6-*p*-cymene)(Br_4bha)Cl] (**3**)	3.11
[Ru(η^6-*p*-cymene)(F_{24}bha)Cl] (**4**)	< 0.02
[Ru(η^6-*p*-cymene)(F_{34}bha)Cl] (**5**)	0.11
[Ru(η^6-*p*-cymene)(F_{25}bha)Cl] (**6**)	0.23
[Ru(η^6-*p*-cymene)(F_{26}bha)Cl] (**7**)	0.02
Genistein	13.65 [39]

[a] The IC_{50} values were determined in triplicate.

Table 2
Cyclic voltammetric data[a] for [Ru(η^6-*p*-cymene)(bha)Cl] halo-substituted complexes **1**–**7** (substituents = 4-F, 4-Cl, 4-Br, 2,4-F_2, 3,4-F_2, 2,5-F_2, 2,6-F_2), and calculated vertical ionization potentials (*I*) and electron affinities (*A*) (eV) of the models **1′**–**7′**.

Complex	Anodic waves		Cathodic waves	
	$E^{ox}_{p/2}$	*I*	$E^{red}_{p/2}$	*A*
[Ru(η^6-*p*-cymene)(F_4bha)Cl] (**1**)	1.60	6.74	−1.33	0.09
[Ru(η^6-*p*-cymene)(Cl_4bha)Cl] (**2**)	1.69	6.77	−0.51	0.25
[Ru(η^6-*p*-cymene)(Br_4bha)Cl] (**3**)	1.67	6.76	−0.55	0.26
[Ru(η^6-*p*-cymene)(F_{24}bha)Cl] (**4**)	1.51	6.70	−1.51	0.11
[Ru(η^6-*p*-cymene)(F_{34}bha)Cl] (**5**)	2.05	6.82	−0.87	0.22
[Ru(η^6-*p*-cymene)(F_{25}bha)Cl] (**6**)	1.70	6.74	−0.82	0.21
[Ru(η^6-*p*-cymene)(F_{26}bha)Cl] (**7**)	1.98	6.65	−0.77	0.13

[a] Potential values (half-peak) in Volt ± 0.02 *vs.* SCE, in a 0.2 M [^{n}Bu$_4$N][BF$_4$]/CH_2Cl_2 solution, at a Pt disc working electrode determined by using the [Fe(η^5-C_5H_5)$_2$]$^{0/+}$ redox couple ($E^{ox}_{p/2}$ = 0.525 V *vs.* SCE) as internal standard at a scan rate of 200 mV s^{-1}.

In order to interpret the experimental redox potentials of the complexes, quantum-chemical calculations of the model compounds [Ru(η^6-C_6H_6)(bha)Cl] (**1′**–**7′**) with a benzene ligand instead of cymene, have been performed at the DFT level of theory (Fig. 2). The calculated structural parameters of bha in **1′** are in good agreement with the experimental data for the Ru(III) complex [Ru(H_2edta)(2-OMe-Pha)] [41] (H_2edta = (HOOC)($^-$OOC)NCH_2CH_2N(COOH)(COO^-); 2-OMe-Pha = 2-methoxyphenylhydroxamate), the only Ru species with hydroxamato ligand for which the X-ray structure is known. Among comparable bonds, the maximum deviation was found for the C=O bond (0.04 Å). The analysis of the composition of frontier MOs of **1′** and the oxidized or reduced species with unrelaxed geometry {**1′**$^+$} or {**1′**$^-$} indicates that, upon oxidation, the electron is removed from the first HOMO of **1′** whereas, upon reduction, the electron goes to the first LUMO.

The main contribution to the HOMOs of **1′**–**7′** comes from the metal and p orbitals of the ONCO fragment of the bha ligand as well as from the Cl^- ligand (Fig. 3). The full geometry optimization of the oxidized complex **1′**$^+$ does not result in a noticeable change of the structure. The Ru–Cl, Ru–O(1), N(2)–O(1), Ru–O(4), and C(3)–C(5) bonds are shortened by 0.020–0.083 Å while the N(2)–C(3) bond is elongated by 0.027 Å upon oxidation. The spin density in **1′**$^+$ is localized mostly on the Ru and O(1) atoms (the contributions are 0.42 and 0.30, respectively). Thus, the oxidation of **1′** affects both the metal atom and the bha ligand.

The LUMOs of complexes **1′**–**7′** are strongly delocalized along the Ru atom and bha and benzene ligands (Fig. 3). The geometry

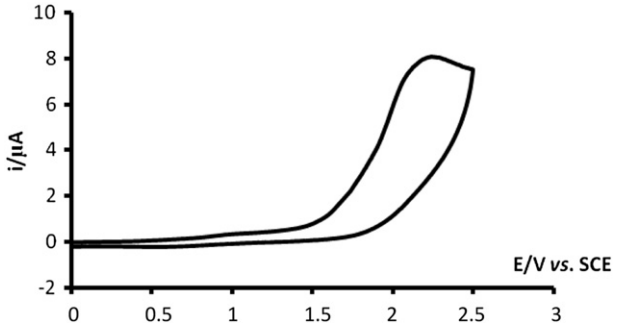


Fig. 1. Cyclic voltammogram (anodic region) of [Ru(η^6-*p*-cymene)(F_{34}bha)Cl] **5** at a Pt disc electrode, in a 0.2 M [^{n}Bu$_4$N][BF$_4$]/CH_2Cl_2 solution (v = 0.2 V s^{-1}).

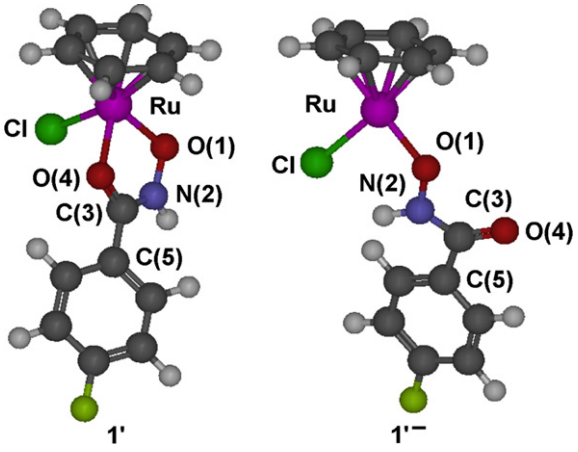


Fig. 2. Equilibrium structures of **1**′ and **1**′⁻.

optimization of the reduced species **1**′⁻ leads to significant structural changes, *i.e.*, a cleavage of the Ru–O(4) bond occurs. As a result, the bha ligand in **1**′⁻ acquired a monodentate coordination mode (Fig. 2). The general structure is stabilized by the intramolecular H-bond between the NH group and the Cl^- ligand. The spin density in **1**′⁻ is localized on the Ru atom with the contribution of 0.93. Thus, despite the delocalized character of the LUMO of **1**′, the reduction of this complex accompanied by the geometry relaxation is metal centered.

The trends of the calculated vertical ionization potentials and electron affinities along the row **1**′–**7**′ are in good agreement with the corresponding trends of the experimental oxidation and reduction potentials, except for complex **7** for which the calculated values are underestimated (Table 2, Fig. 1TS).

3.4. Lever's E_L electrochemical parameter

The introduction of functional groups in the *ortho*-, *meta*- or *para*-positions of the aromatic ring of the bha ligands should influence their electron-donor properties and thus the electrochemical Lever E_L ligand parameter. On the basis of the Lever [42–47] linear relationship (Eq. (1)), by assuming that it is also valid for half-sandwich ruthenium(II) cymene type complexes [48–51], we propose the estimate of the Lever E_L parameter for the various chelating halogenobenzohydroxamatos bha (substituents = 4-F, 4-Cl, 4-Br, 2,4-F_2, 3,4-F_2, 2,5-F_2, 2,6-F_2). However, one should be very cautious with the estimated values since, each of them is based on a single complex and, moreover, the oxidation potential is not the thermodynamic one in view of the irreversibility of the oxidation wave.

$$E = S_M\left(\sum E_L\right) + I_M \quad (\text{V } \textit{vs.} \text{ NHE}) \tag{1}$$

As an example, application of Eq. (1) to **1** ($E^{ox}_{p/2}$ = 1.60 V *vs.* SCE = 1.84 V *vs.* NHE) with the known values of S_M (0.97) and I_M (0.04 V *vs.* NHE) for the $Ru^{II/III}$ redox center [42] and of E_L for Cl^- (−0.24 V *vs.* NHE) [42] and cymene (1.63 V *vs.* NHE) [48], allows to determine the E_L ligand parameter for chelating F_4bha as 0.47 V *vs.* NHE. By following the same calculation methodology, we have determined the E_L parameter for the other bidentate halogenobenzohydroxamato ligands of the present study (Table 3).

The E_L parameter is a measure of the electron-donor character of a ligand, the lower its value the stronger such a character is [42]. For the mono halogenobenzohydroxamato compounds **1**–**3**, the estimated E_L values of their bha ligands follow the order: F–C_6H_4C(O)

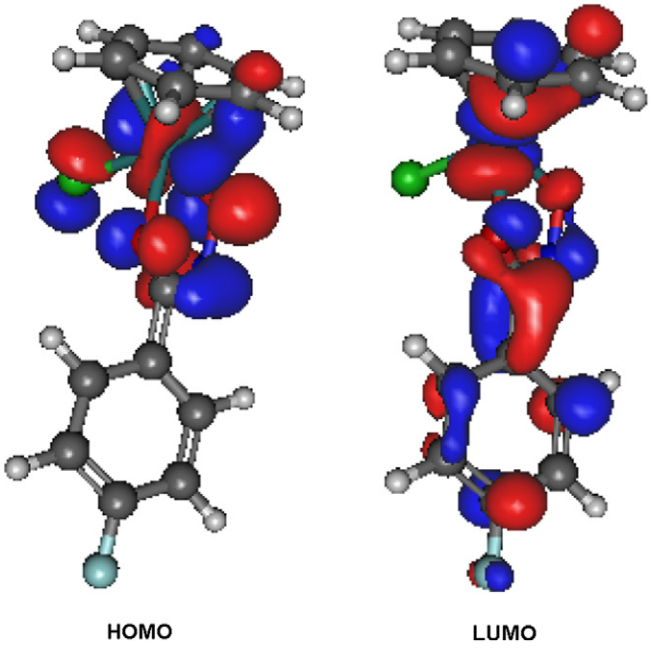
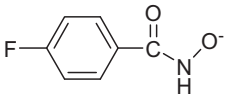
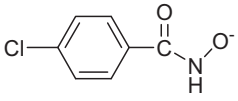
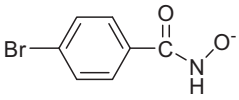
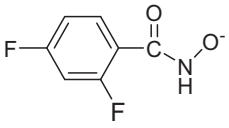
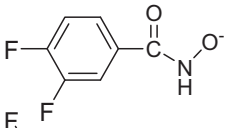
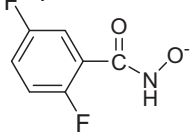
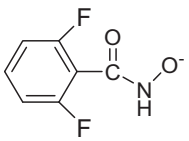
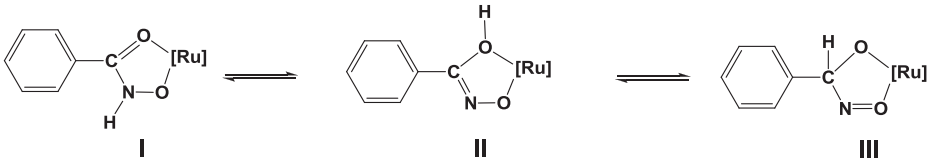


Fig. 3. Plots of the HOMO and LUMO of **1**′.

Table 3
E_L ligand parameter for bidentate substituted halogenobenzohydroxamatos.

Ligand	E_L/V *vs.* NHE
	0.47
	0.56
	0.54
	0.37
	0.93
	0.57
	0.86



Scheme 2. Possible tautomeric equilibria for the bha ligand in **1**–**7**.

NHO^- (F_4bha) < Cl–$C_6H_4C(O)NHO^-$ (Cl_4bha) ≈ Br–$C_6H_4C(O)NHO^-$ (Br_4bha), indicating that the fluoro-benzohydroxamato acts as a stronger electron-donor than the analogous chloro- and bromo-benzohydroxamatos. This can be rationalized on the basis of the Hammett's substituent constant. In fact, the F^- substituent ($\sigma_p = 0.06$) is an overall stronger electron-donor than the other halogens (which have a common σ_p value of 0.23). Correlations of redox potentials with Hammett's and related constants of ligand substituents are well documented [52,53].

The estimated E_L values for the bidentate mono substituted halogeno-benzohydroxamato ligands (*avg.* 0.52 V) indicate an electron-donor ability comparable to that found for the tridentate tris(pyrazol-1-yl)borate (η^3-Tp$^-$; $E_L = 0.52$ V) [48] or the bidentate bis(pyrazol-1-yl)acetic acid (η^2-bpac; $E_L = 0.54$ V) [48], but considerably lower than those found for the bidentate acetylacetonate (acac$^-$; $E_L = -0.16$ V) [43] and fluoro-substituted derivatives, such as 1,1,1-trifluoro-2,4-pentanedionate (tfac$^-$; $E_L = 0.06$ V) [43] or 1,1,1,5,5,5-hexafluoro-2,4-pentanedionate (hfac$^-$; $E_L = 0.34$ V) [43]. The difluoro-benzohydroxamato ligands usually display E_L values lower than that of the mono-fluorinated ligand, thus behaving as weaker electron-donors.

Concerning **4**–**7**, the presence of two fluoro substituents in different positions of the aromatic ring can lead to different redox potentials, but any analysis has to be taken rather cautiously in view, *e.g.*, of the possibility of (i) establishment of H-bonds between F and the NH group, or (ii) ligand tautomerization (Scheme 2), (iii) H-bond between F and the OH group, and (iv) the irreversible character of the oxidation waves.

4. Conclusions

The results indicate that ruthenium(II) complexes with cymene and an halo-substituted benzohydroxamato of the type of this study behave as powerful PTK inhibitors and may constitute a promising source of metal-based antitumor agents. The PTK inhibitory activity is favored by fluoro-substituents at the *ortho* and *para* positions.

The electrochemical study of a series of such compounds has allowed (i) to measure the Ru(II/III) and Ru(II/I) redox potentials, (ii) to compare the effects of the halo-substituents (type, number and position on the halogenobenzohydroxamato ligands), and (iii) to estimate, for the first time, the Lever E_L parameter for these ligands. However, one should be cautious with the estimated values since each of them is based on a single complex and the oxidation potential was not the thermodynamic one in view of the irreversibility of the oxidation wave. It was also assumed that the S_M and I_M values for the octahedral Ru(II/III) redox couple (used in Eq. (1)) are also valid for the half-sandwich cymene complexes of the present study, in accord with our previous proposal [54,55].

Nevertheless, the measured oxidation and reduction potentials of the complexes follow the orders predicted on the basis of their vertical ionization potentials and electron affinities, estimated by DFT calculations which also show the compositions of the frontier MOs and that the oxidation and reduction of the compounds involve the first HOMO and the first LUMO, respectively.

The PTK inhibitory activity does not appear to follow the redox potential of the complexes, and further studies deserve to be undertaken in order to get an insight in the mechanism of action of these complexes which may be helpful for the design of new metal-based anticancer agents.

Acknowledgments

This work has been partially supported by the Foundation for Science and Technology (FCT) (grant no. SFRH/BPD/44773/2008) and its PEst-OE/QUI/UI0100/2011 project, Portugal, the state 'Innovative Drugs Development' key and technology major projects of China (no. 2009ZX09103-104) and the National Natural Science Foundation of China (No: 81102311). T.F.S.S. is grateful to FCT for her PhD (SFRH/BD/48087/2008) fellowship. M.L.K. is grateful to the FCT and IST for a research contract within the Ciência 2007 scientific programme.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.jorganchem.2012.12.013.

References

[1] (a) F. Marchetti, C. Pettinari, R. Pettinari, Coord. Chem. Rev. 249 (2005) 2909;
(b) M. Galanski, M.A. Jakupec, B.K. Keppler, Curr. Med. Chem. 12 (2005) 2075;
(c) L. Ronconi, P.J. Sadler, Coord. Chem. Rev. 251 (2007) 1633.
[2] (a) Y.P. Ho, S.C.F. Au-Yeung, K.K.W. To, Med. Res. Rev. 23 (2003) 633;
(b) M.A. Jakupec, M. Galanski, B.K. Keppler, Rev. Physiol. Biochem. Pharmacol. 146 (2003) 1.
[3] I. Kostova, Recent Pat. Anticancer Drug Discov. 1 (2006) 1.
[4] (a) T. Gianferrara, I. Bratsos, E.A. Alessio, Dalton Trans. (2009) 7588;
(b) C.G. Hartinger, P.J. Dyson, Chem. Soc. Rev. 38 (2009) 391.
[5] (a) G. Jaouen, N. Metzler-Nolte (Eds.), Medicinal Organometallic Chemistry, Topics in Organometallic Chemistry, Springer-Verlag, Berlin, 2010;
(b) G. Jaouen (Ed.), Bioorganometallics, Wiley-VCH Verlag GmbH & Co., Weinheim, 2006.
[6] C. Dittrich, M.E. Scheulen, U. Jaehde, B. Kynast, M. Gneist, H. Richly, S. Schaad, V.B. Arion, B.K. Keppler, Proc. Am. Assoc. Cancer Res. 46 (2005) P472.
[7] (a) C.G. Hartinger, S. Zorbas-Seifried, M.A. Jakupec, B. Kynast, H. Zorbas, B.K. Keppler, J. Inorg. Biochem. 100 (2006) 891;
(b) C.G. Hartinger, M.A. Jakupec, S. Zorbas-Seifried, M. Groessl, A. Egger, W. Berger, H. Zorbas, P.J. Dyson, B.K. Keppler, Chem. Biodivers. 5 (2008) 2140;
(c) I. Berger, M. Hanif, A.A. Nazarov, C.G. Hartinger, R.O. John, M.L. Kuznetsov, M. Groessl, F. Schmitt, O. Zava, F. Biba, V.B. Arion, M. Galanski, M.A. Jakupec, L. Juillerat-Jeanneret, P.J. Dyson, B.K. Keppler, Chem.—Eur. J. 14 (2008) 9046.
[8] (a) H. Chen, J.A. Parkinson, R.E. Morris, P.J. Sadler, J. Am. Chem. Soc. 125 (2003) 173;
(b) P. Nowak-Sliwinska, J.R. van Beijnum, A. Casini, A.A. Nazarov, G. Wagnieres, H. van den Bergh, P.J. Dyson, A.W. Griffioen, J. Med. Chem. 54 (2011) 3895;
(c) F. Giannini, G. Süss-Fink, J. Furrer, Inorg. Chem. 50 (2011) 10552;
(d) F. Caruso, M. Rossi, A. Benson, C. Opazo, D. Freedman, E. Monti, M.B. Gariboldi, J. Shaulky, F. Marchetti, R. Pettinari, C. Pettinari, J. Med. Chem. 55 (2012) 1072;
(e) F. Giannini, J. Furrer, A.-F. Ibao, G. Süss-Fink, M. Baquie, B. Therrien, O. Zava, M. Baquie, P.J. Dyson, P. Stepnicka, J. Biol. Inorg. Chem. 17 (2012) 951;
(f) G. Süss-Fink, Dalton Trans. 39 (2010) 1673.
[9] E. Alessio, G. Mestroni, A. Bergamo, G. Sava, Curr. Top. Med. Chem. 4 (2004) 1525.
[10] (a) S.J. Dougan, P.J. Sadler, Chimia 61 (2007) 704;
(b) A. Habtemariam, M. Melchart, R. Fernandez, S. Parsons, I.D.H. Oswald,

A. Parkin, F.P.A. Fabbiani, J.E. Davidson, A. Dawson, R.E. Aird, D.I. Jodrell, P.J. Sadler, J. Med. Chem. 49 (2006) 6858.
[11] J.M. Rademaker-Lakhai, D. van den Bongard, D. Pluim, J.H. Beijnen, J.H. Schellens, Clin. Cancer Res. 10 (2004) 3717.
[12] M.G. Mendoza-Ferri, C.G. Hartinger, M.A. Mendoza, M. Groessl, A.E. Egger, R.E. Eichinger, J.B. Mangrum, N.P. Farrell, M. Maruszak, P.J. Bednarski, F. Klein, M.A. Jakupec, A.A. Nazarov, K. Severin, B.K. Keppler, J. Med. Chem. 52 (2009) 916.
[13] A.D. Phillips, O. Zava, R. Scopelitti, A.A. Nazarov, P.J. Dyson, Organometallics 29 (2010) 417.
[14] W. Kandioller, C.G. Hartinger, A.A. Nazarov, M.L. Kuznetsov, R.O. John, C. Bartel, M.A. Jakupec, V.B. Arion, B.K. Keppler, Organometallics 28 (2009) 4249.
[15] M.G. Mendoza-Ferri, C.G. Hartinger, R.E. Eichinger, N. Stolyarova, K. Severin, M.A. Jakupec, A.A. Nazarov, B.K. Keppler, Organometallics 27 (2008) 2405.
[16] Y.K. Yan, M. Melchart, A. Habtemariam, P.J. Sadler, Chem. Commun. (2005) 4764.
[17] V. Brabec, O. Nováková, Drug Resist. Updat 9 (2006) 111.
[18] I. Ott, R. Gust, Arch. Pharm. Chem. Life Sci. 340 (2007) 117.
[19] M. Srinivasan, S.G. Trivadi, Clin. Biochem. 37 (2004) 618.
[20] M. Bolla, B. Rostaing-Puissant, S.P. Bottari, M. Chedin, J. Marron-Charriere, M. Colonna, E. Berland, E. Chambaz, Breast Cancer Res. Treat. 39 (1996) 327.
[21] A. Hirabayashi, H. Mukaiyama, H. Kobayashi, H. Shiohara, S. Nakayama, M. Ozawa, E. Tsuji, K. Miyazawa, K. Misawa, H. Ohnota, M. Isaji, Bioorg. Med. Chem. 16 (2008) 9247.
[22] K.J. Jung, E.K. Lee, B.P. Yu, H.Y. Chung, Free Radical Biol. Med. 47 (2009) 983.
[23] L. Shi, X.E. Feng, J.R. Cui, L.H. Fang, G.H. Du, Q.S. Li, Bioorg. Med. Chem. Lett. 20 (2010) 5466.
[24] H. Hori, H. Nagasawa, Y. Uto, K. Ohkura, K.L. Kirk, Y. Uehara, M. Shimamura, Biochim. Biophys. Acta 1697 (2004) 29.
[25] C. Liechti, U. Sequin, G. Bold, P. Furet, T. Meyer, P. Traxler, Eur. J. Med. Chem. 39 (2004) 11.
[26] H.Q. Li, T.T. Zhu, T. Yan, Y. Luo, H.L. Zhu, Eur. J. Med. Chem. 44 (2009) 453.
[27] Z. Kilic, Y.G. Isgor, S. Olgen, Arch. Pharm. Chem. Life Sci. 342 (2009) 333.
[28] S. Olgen, Y.G. Isgor, T. Coban, Arch. Pharm. Chem. Life Sci. 341 (2008) 113.
[29] P. Buglyo, E. Farkas, Dalton Trans. (2009) 8063.
[30] X. Shang, J. Cui, J. Wu, A.J.L. Pombeiro, Q. Li, J. Inorg. Biochem. 102 (2008) 901.
[31] A.J.L. Pombeiro, M.F.C. Guedes da Silva, M.A.N.D.A. Lemos, Coord. Chem. Rev. 219 (2001) 53.
[32] (a) A.D. Becke, J. Chem. Phys. 98 (1993) 5648;
(b) C. Lee, W. Yang, R.G. Parr, Phys. Rev. B 37 (1988) 785.
[33] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery Jr., T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Pople, Gaussian 03, Revision B.05, Gaussian, Inc., Pittsburgh PA, 2003.
[34] D. Andrae, U. Haeussermann, M. Dolg, H. Stoll, H. Preuss, Theor. Chim. Acta 77 (1990) 123.
[35] P. Ghosh, A. Chakravorty, J. Chem. Soc., Dalton Trans. (1985) 361.
[36] D. Griffith, K. Krot, J. Comiskey, K.B. Nolan, C.J. Marmion, Dalton Trans. (2008) 137.
[37] A. Schluter, K. Bieber, W.S. Sheldrick, Inorg. Chim. Acta 340 (2002) 35.
[38] D.A. Brown, W.K. Glass, N.J. Fitzpatrick, T.J. Kemp, W. Errington, G.J. Clarkson, W. Haase, F. Karsten, A.H. Mahdy, Inorg. Chim. Acta 357 (2004) 1411.
[39] X.E. Feng, W.Y. Zhao, S.R. Ban, C.X. Zhao, Q.S. Li, W.H. Lin, Int. J. Mol. Sci. 12 (2011) 6104.
[40] (a) E. Reisner, V.B. Arion, B.K. Keppler, A.J.L. Pombeiro, Inorg. Chim. Acta 361 (2008) 1569;
(b) E. Reisner, V.B. Arion, M.F.C. Guedes da Silva, R. Lichtenecker, A. Eichinger, B.K. Keppler, V.Yu. Kukushkin, A.J.L. Pombeiro, Inorg. Chem. 43 (2004) 7083;
(c) E. Reisner, V.B. Arion, A. Eichinger, N. Kandler, G. Geister, A.J.L. Pombeiro, B.K. Keppler, Inorg. Chem. 44 (2005) 6704.
[41] J. Comiskey, E. Farkas, K.A. Krot-Lacina, R.G. Pritchard, C.A. McAuliffe (the late), K.B. Nolan, Dalton Trans. (2003) 4243.
[42] A.B.P. Lever, Inorg. Chem. 29 (1990) 1271.
[43] A.B.P. Lever, Inorg. Chem. 30 (1991) 1980.
[44] A.B.P. Lever, E.S. Dodsworth, Inorganic Electronic Structure and Spectroscopy, vol. 2, Wiley, New York, 1999, p. 227.
[45] A.B.P. Lever, in: A.B.P. Lever (Ed.), Comprehensive Coordination Chemistry II, vol. 2, Elsevier, Oxford, 2004, p. 251. ch. 2.19.
[46] A.J.L. Pombeiro, J. Organomet. Chem. 690 (2005) 6021.
[47] A.J.L. Pombeiro, Eur. J. Inorg. Chem. 11 (2007) 1473.
[48] M.F.C. Guedes da Silva, A.J.L. Pombeiro, Electrochim. Acta 82 (2012) 478.
[49] C. Pettinari, F. Marchetti, A. Cerquetella, R. Pettinari, M. Monari, T.C.O. Mac Leod, L.M.D.R.S. Martins, A.J.L. Pombeiro, Organometallics 30 (2011) 1616.
[50] F. Marchetti, C. Pettinari, L.M.D.R.S. Martins, M.F.C. Guedes da Silva, T.F.S. Silva, A.J.L. Pombeiro, Organometallics 30 (2011) 6180.
[51] F. Marchetti, C. Pettinari, R. Pettinari, A. Cerquetella, A. Cingolani, E.J. Chan, K. Kozawa, B.W. Skelton, A.H. White, R. Wanke, M.L. Kuznetsov, L.M.D.R.S. Martins, A.J.L. Pombeiro, Inorg. Chem. 46 (2007) 8245.
[52] M.E.N.P.R.A. Silva, A.J.L. Pombeiro, J.J.R. Fraústo da Silva, R. Herrmann, M. Deus, R.E. Bozak, J. Organomet. Chem. 480 (1994) 81.
[53] M.E.N.P.R.A. Silva, A.J.L. Pombeiro, J.J.R. Fraústo da Silva, R. Herrmann, N. Deus, T.J. Castilho, M.F.C. Guedes da Silva, J. Organomet. Chem. 421 (1991) 75.
[54] F. Marchetti, C. Pettinari, A. Cerquetella, A. Cingolani, R. Pettinari, M. Monari, R. Wanke, M.L. Kuznetsov, A.J.L. Pombeiro, Inorg. Chem. 48 (2009) 6096.
[55] S. Bolaño, J. Bravo, J.A. Castro-Fojo, M.M. Rodríguez-Rocha, M.F.C. Guedes da Silva, A.J.L. Pombeiro, L. Gonsalvi, M. Peruzzini, Eur. J. Inorg. Chem. 35 (2007) 5523.