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Synthesis, Characterization, and Binding to the Translocator Protein (18 kDa, TSPO) of a New Rhenium Complex as a Model of Radiopharmaceutical Agents

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Abstract. A new tridentate 2-phenyl-imidazopyridin-dipropylacetamide ligand (CB239-H) with high (nanomolar) affinity for the TSPO protein was synthesized and its coordination compound with rhenium tricarbonyl, *fac*-[Re(CO)$_3$(CB239-*N*,*N*,*O*)] was investigated. The procedure established for the synthesis of the $^{187/185}$Re complex can be also used for the synthesis of ^{99m}Tc and $^{188/186}$Re analogues, which find application in SPECT diagnosis and in therapy. Because of the tridentate coordination of CB239-H and the kinetic inertness of the carbonyl ligands, the new complex was expected to exhibit low reactivity towards plasma proteins and hence greater resistance to deactivation. Being TSPO overexpressed in numerous types of cancers and in activated microglial cells occurring in inflammatory neurodegenerative diseases, TSPO ligands can be exploited as carriers for receptor-mediated drug targeting and hence can be used in diagnosis as well as in therapy. Very suprisingly, *fac*-[Re(CO)$_3$(CB239-*N*,*N*,*O*)] resulted to be not very stable in diluted human serum but maintained a good affinity towards TSPO.

Introduction

The 18 kDa translocator protein (TSPO, previously known as peripheral-type benzodiazepine receptor or PBR)[1] spans the mitochondrial membrane and has become an attractive target for therapeutic and imaging purposes.[2] TSPO is associated with a number of biological processes including cell proliferation, apoptosis, steroidogenesis, and immunomodulation.[3] Overexpression of TSPO has been found in numerous types of cancers including brain, breast, colon, prostate, and ovarian cancers, as well as in astrocytomas and in hepatocellular and endometrial carcinomas.[4–6] Furthermore, TSPO-specific ligands, such as 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide (PK11195),[7] 7-chloro-5-(4-chlorophenyl)-1-methyl-3H-1,4-benzodiazepin-2-one (Ro-5–4864),[8] and [2-(4-chlorophenyl)-8-amino-imidazo[1,2-*a*]-pyridin-3-yl]-*N*,*N*-di-*n*-propylacetamide (CB86)[9] induce apoptosis and cell cycle arrest in cancer cells (Figure 1).

For these reasons, TSPO ligands have been widely investigated for diagnostic purposes and explored as carriers for receptor-mediated drug delivery. Moreover, some of us have already prepared platinum complexes containing potent and selective TSPO ligands,[10,11] such as CB86 and 2-[6,8-dichloro-2-(1,3-thiazol-2-yl)*H*-imidazo[1,2-*a*]pyridin-3-yl]-*N*,*N*-di-*n*-propylacetamide (TZ6).[12–14] The platinum complexes *cis*-[PtCl$_2$(TZ6-*N*,*N*)] and *cis*-[Pt*X*$_2$(NH$_3$)(CB86-*N*)] (*X* = Cl, I) (Figure 2) have demonstrated to keep the high affinity (nano-

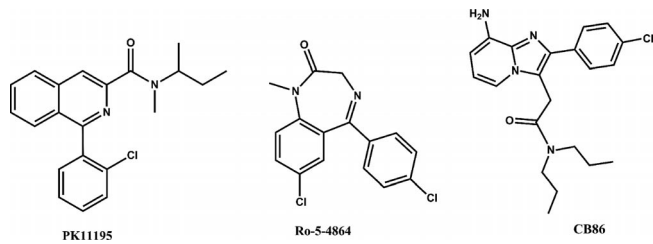

Figure 1. Structure of PK11195, Ro-5–4864, and CB86.

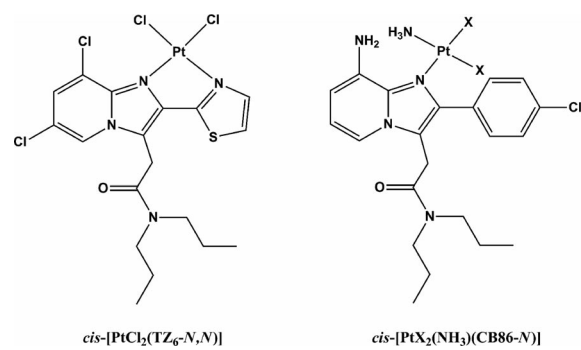

Figure 2. Structure of *cis*-[PtCl$_2$(TZ6-*N*,*N*)] and *cis*-[Pt*X*$_2$(NH$_3$)-(CB86-*N*)] (*X* = Cl, I).

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molar concentration) and selectivity for TSPO characteristic of the free ligands.

TSPO expression is also increased in activated microglial cells occurring in inflammatory neurodegenerative diseases such as Alzheimer, Parkinson, Huntington, and multiple sclerosis.[15] Therefore, TSPO-targeted metal complexes[16] could be also explored for imaging purposes especially for the in vivo early diagnosis of neurodegenerative diseases.

To this end, we have recently prepared a $^{187/185}$Re complex containing the bidentate TSPO ligand TZ6. This latter complex, *fac*-[ReBr(CO)$_3$(TZ6-*N*,*N*)], has been found to be endowed with high affinity for TSPO.[17]

Pharmacokinetic experiments concerning complexes containing the *M*(CO)$_3$ core (*M* = Tc or Re) have addressed the question of minimal denticity of the chelate ligand for optimal clearance and stability in vivo.[18] It has been observed that ^{99m}Tc-tricarbonyl complexes containing a tridentate chelating ligand have good stability in human plasma and in the presence of excess cysteine, histidine, or glutathione.[19] In contrast, complexes with bidentate ligands can be deactivated by plasma proteins both in vitro and in vivo, a phenomenon also observed in the case of platinum-based anticancer drugs.[20] Therefore, tricarbonyl complexes with bidentate ligands are significantly retained in the blood and in the organs of excretion, such as liver and kidneys. Independently from their overall charge and lipophilicity, that appears to play only a limited role, the reason for such a behavior of tricarbonyl complexes with bidentate ligands can be identified in the presence of a coordination site occupied by a substitutionally labile aqua or chlorido ligand.[21,22] Complexation of plasma proteins via this coordination site can explain the prolonged retention in the blood pool and, consequently, in all organs and tissues. Therefore, it is desirable to functionalize biomolecules with a tridentate chelate as this will result, hopefully, in a better pharmacokinetic behavior.

In this work we have used a new tridentate ligand with high selectivity and nanomolar affinity for TSPO, namely 2-(2-(4-chlorophenyl)-3-(2-(dipropylamino)-2-oxoethyl)imidazo[1,2-*a*]pyridin-8-ylamino)acetic acid (CB239-H, Figure 3), for the synthesis of the rhenium complex *fac*-[$^{187/185}$Re(CO)$_3$(CB239-*N*,*N*,*O*)].

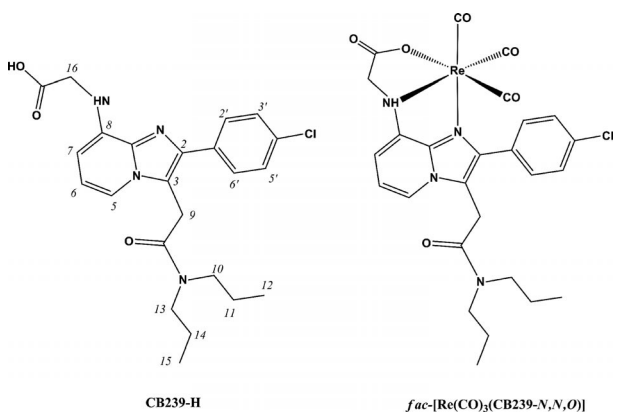

Figure 3. Sketch of CB239-H and *fac*-[Re(CO)$_3$(CB239-*N*,*N*,*O*)].

Besides the synthesis and complete characterization of the ligand and corresponding $^{187/185}$Re(CO)$_3$ complex, in vitro studies were performed to assess their affinity toward the mitochondrial TSPO. In addition, the inertness of the complex in diluted human serum was evaluated. The results are reported in this paper.

Experimental Section

Chemicals: Commercial reagent grade chemicals, including [ReBr(CO)$_5$], and solvents were purchased from Sigma-Aldrich (Milan, Italy) and used without further purification.

HAM'S F12, PBS, trypsin-EDTA, penicillin (10,000 U·mL^{-1}), streptomycin (10 mg·mL^{-1}), L-glutamine solution (100×), and foetal bovine serum (FBS) were purchased from Euroclone (Italy). Disposable culture flasks and Petri dishes were from Corning, Glassworks (Corning, N.Y., USA). The radioligand [^{3}H]-PK11195 (85.7 Ci·mmol^{-1}) was purchased from Perkin-Elmer Life Sciences, PK11195 was purchased from Sigma-Aldrich (Milan, Italy).

Instrumental Measurements: Mass spectrometry: electrospray ionization mass spectrometry (ESI-MS) was performed with an electrospray interface and an ion trap mass spectrometer (1100 Series LC/MSD Trap system Agilent, Palo Alto, CA). ^{1}H 1D and 2D COSY, NOESY, and [^{1}H-^{15}N]-HSQC (natural abundance ^{15}*N*) spectra were recorded with Bruker Avance DPX 300 MHz and Avance II 600 MHz instruments. Standard Bruker pulse sequences were used for the NMR experiments using gradient selected versions when necessary. Chemical shifts are given in ppm. ^{1}H chemical shifts were referenced by using the residual protic peak of the solvent as internal reference (δ = 3.31 ppm for [D$_4$]methanol, 2.50 ppm for [D$_6$]dimethylsulfoxide, 7.24 ppm for [D]chloroform), ^{15}N chemical shifts were referenced to external ^{15}NH$_4$Cl (1 M in HCl 1 M). Elemental analyses were carried out with an Eurovector EA 3000 CHN.

Synthesis of Ethyl 2-(2-(4-Chlorophenyl)-3-(2-(dipropylamino)-2-oxoethyl)imidazo[1,2-a]pyridin-8-ylamino)acetate (CB238): To a stirred solution of CB86 (0.30 g, 0.78 mmol) in anhydrous THF were added, in the order, ethyl bromoacetate (860 μL, 7.8 mmol) and K_2CO_3 (0.54 g, 3.9 mmol). Stirring was continued for additional 24 h at 40 °C, then the solvent was evaporated under reduced pressure and the solid residue was dissolved in 10 % $NaHCO_3$ (25 mL), extracted with ethyl acetate (3 × 20 mL), and dried (Na_2SO_4). Evaporation of the solvent gave a residue, which was purified by silica gel chromatography [light petroleum ether/ethyl acetate 6/4 (v/v) as eluant]. Obtained 0.27 g (73.6 % yield). **IR** (KBr): $\tilde{\nu}$ = 3400, 1745, 1643 cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ = 0.74 (t, 3 H, *J* = 7.4 Hz, CH_3), 0.84 (t, 3 H, *J* = 7.4 Hz, CH_3), 1.28 (t, 3 H, *J* = 7.1 Hz, CH_3), 1.4–1.6 (m, 4 H, CH_2), 3.11 (t, 2 H, *J* = 7.7 Hz, CH_2NCO), 3.27 (t, *J* = 7.7 Hz, 2 H, CH_2NCO), 4.05 (s, 2 H, CH_2CON), 4.0–4.1 (m, 2 H, CH_2COO), 4.23 (q, *J* = 7.1 Hz, 2 H, CH_2), 6.15 (d, *J* = 7.4 Hz, 1 H, Ar), 6.5 (br., 1 H, NH), 6.77 (t, *J* = 7.1 Hz, 1 H, Ar), 7.44 (d, *J* = 8.2 Hz, 2 H, Ar), 7.6–7.7 (m, 3 H, Ar) ppm. **ESI-MS**: calculated for [CB238 + Na]$^+$ = 493. Found: *m*/*z* (% relative to the base peak) = 493.1 (100) [M + Na]$^+$.

Synthesis of 2-(2-(4-Chlorophenyl)-3-(2-(dipropylamino)-2-oxoethyl)imidazo[1,2-a]pyridin-8-ylamino)acetic Acid (CB239-H): To a solution of CB238 (0.20 g, 0.42 mmol) in EtOH (5 mL), NaOH (1 M, 5 mL) was added dropwise. The mixture was stirred at room temperature for 2 h. Afterwards the solvent was evaporated under reduced pressure and the residue was dissolved in 20 mL of water and extracted with ethyl acetate (3 × 20 mL). The cooled water phase was acidified

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with 0.1 M HCl and the resulting precipitate of the pure acid was collected by filtration and dried in a vacuum. Obtained 158 mg (85.1% yield). **IR** (KBr): $\tilde{\nu}$ = 3398, 1639, 1566 cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ = 0.77 (t, 3 H, J = 7.4 Hz, CH_3), 0.85 (t, 3 H, J = 7.4 Hz, CH_3), 1.4–1.6 (m, 4 H, CH_2), 3.11 (t, 2 H, J = 7.7 Hz, CH_2NCO), 3.28 (t, J = 7.7 Hz, 2 H, CH_2NCO), 3.93 (s, 2 H, CH_2CO), 3.98 (s, 2 H, CH_2CON), 6.25 (d, J = 7.4 Hz, 1 H, Ar), 6.86 (t, J = 7.1 Hz, 1 H, Ar), 7.41 (d, J = 8.2 Hz, 2 H, Ar), 7.53 (d, J = 6.6 Hz, 1 H, Ar), 7.67 (d, J = 8.2 Hz, 2 H, Ar) ppm. **ESI-MS**: calculated for $[CB239]^-$ = 441. Found: *m/z* (% relative to the base peak) = 440.9 (100) $[M - H]^-$.

Synthesis of *fac*-[ReBr(CO)$_3$(OH$_2$)$_2$]: This compound was obtained by applying the procedure reported for the synthesis of the compound *fac*-[Re(H$_2$O)$_3$(CO)$_3$]Br.[23] [ReBr(CO)$_5$] (900 mg, 2.21 mmol) was suspended in water (40 mL) in a 250 mL round-bottom flask and heated to reflux for 24 h under gentle magnetic stirring. Periodic rinsing of the reflux condenser allowed unreacted [ReBr(CO)$_5$] deposited on the condenser to be brought back into the reaction solution. The crude mixture was cooled to room temperature and filtered through celite to remove small amounts of impurities. The colorless solution was dried under reduced pressure to give a white powder of the desired compound. Obtained 656 mg (76.9% yield). [ReBr(CO)$_3$(OH$_2$)$_2$] ($C_3H_4O_5BrRe$): calcd. C 9.33; H 1.04%; found: C 9.42; H 1.10%. **ESI-MS**: calculated for $[Re(CO)_3(OH_2)_3]^+$ ($C_3H_6O_6Re$) = 325; found: *m/z* (% relative to the base peak) = 324.9 (100).

Synthesis of *fac*-[Re(CO)$_3$(CB239-*N*,*N*,*O*)]: CB239-H (31.0 mg, 0.07 mmol) was dissolved in methanol (8 mL) and the solution was treated with *fac*-[ReBr(CO)$_3$(OH$_2$)$_2$] (29.9 mg, 0.07 mmol) dissolved in methanol (3 mL). The resulting solution was kept under magnetic stirring for 1 h at room temperature and treated with a solution of KOH in methanol (0.07 mmol in 300 µL). The reaction mixture was kept whilst stirring at room temperature for 1 h and filtered in order to separate the precipitated white solid (KBr). The filtrate was concentrated to a final volume of ca. 3 mL and addition of ca. 10 mL of water caused the precipitation of a brown solid. The crude product was washed with water, dried in a vacuum, and purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH, 4:1 v/v, as eluant). Obtained 25.0 mg (50.2% yield). [Re(CO)$_3$(CB239-*N*,*N*,*O*)]·0.75CH_2Cl_2 ($C_{26}H_{29}ClN_4O_6Re$·0.75CH_2Cl_2): calcd. C 41.40; H 3.54; N 7.22%; found: C 41.93; H 3.76; N 6.66%. **ESI-MS**: calculated for [[Re(CO)$_3$(CB239-*N*,*N*,*O*)] + Na]$^+$ ($C_{26}H_{26}ClN_4NaO_6Re$) = 735.1; found: *m/z* (% relative to the base peak) = 734.9 (100) $[M + Na]^+$.

Stability Studies: High performance liquid chromatography (HPLC) was used to evaluate the inertness of the complex. HPLC was performed with a Waters 1515 instrument equipped with an isocratic HPLC pump and a Dual λ Absorbance Detector Waters 2487. For the HPLC analysis, a reverse-phase column (Phenomenex® Inertsil5, C8, 5 µM, 3.20 × 150 mm) was used. The mobile phase consisted of water/acetonitrile (1/1, v/v) containing 0.1% of trifluoroacetic acid and the flow rate was 1 mL·min^{-1}. The HPLC analysis was performed at ambient temperature using a double detection wavelength (λ_1 = 256 nm and λ_2 = 270 nm). The standard injection volume was 20 µL. A calibration curve of 1.25–50 µM was constructed from linear plots of peak area versus concentration. The calibration curves were used as a quantitative reference for the following experiments.

Stability experiments were carried out in diluted (50%) human serum (Sigma-Aldrich cod. H4522)/phosphate buffer (0.02 M, pH 7.4, isotonized with NaCl). At determined intervals of time, aliquots of 50 µL were withdrawn from the mother solution and added to 250 µL of cold acetonitrile in order to precipitate the serum proteins. After centrifugation for 5 min at 13000 rpm, the clean supernatant was filtered and then analyzed by HPLC. All experiments were performed in triplicate.

Cell Cultures: Rat C6 glioma cells, from Interlab Cell Line Collection (ICLC) (Genova, Italy), were grown in HAM'S F12 with 10% heat-inactivated FBS, 100 U·mL^{-1} penicillin, 100 µg·mL^{-1} streptomycin, and 2 mM L-glutamine in a 5% CO_2 humidified atmosphere at 37 °C.

Membrane Preparation: Membranes from the tumour cell line were prepared as described by *Veenman* et al.[6] with minor modifications. Briefly, rat C6 glioma cells were cultured to 80% confluence, the medium removed and the cells scraped in PBS (pH = 7.2). After detaching, the cells suspended in PBS were homogenized with a Brinkman Polytron (setting 5 for 3 × 15 s). The homogenate was centrifuged at 37,000 g for 30 min at 4 °C and the supernatant was discarded. The final pellet was resuspended in ice-cold 10 mM PBS (pH 7.2) and stored at –80 °C until use.

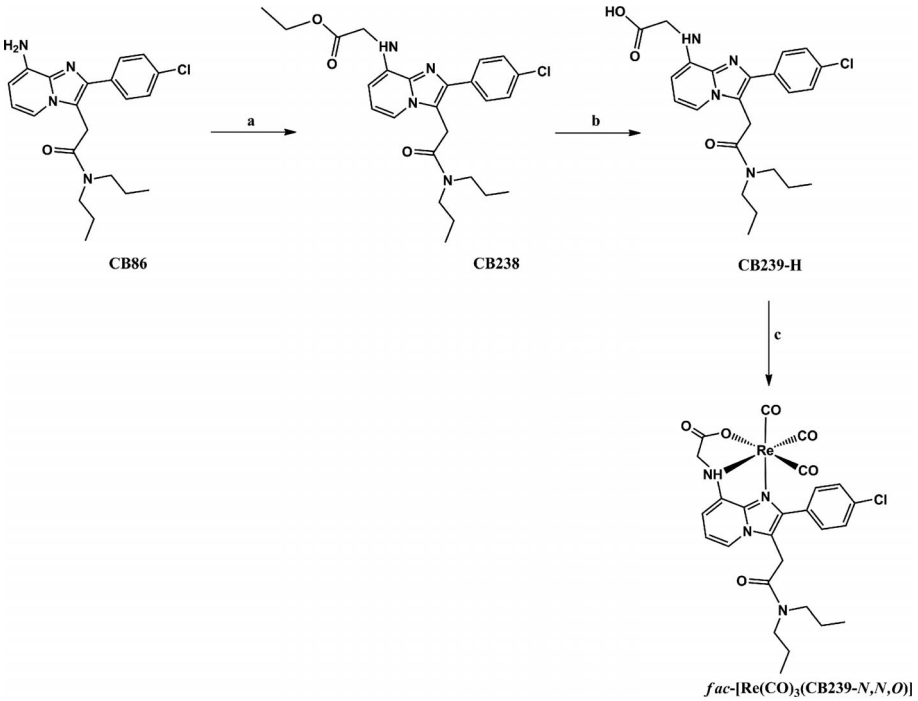
Radioligand Binding Assay at TSPO: Binding of [^{3}H]-PK11195 at TSPO was performed according to *Denora* et al. with minor modifications.[14] In 0.5 mL of incubation buffer (PBS, pH 7.2) were suspended 100 µg of C6 membranes, 0.7 nM [^{3}H]-PK11195, and the compound under investigation or the reference compound (six to nine different concentrations). The samples were incubated for 90 min at 25 °C. Subsequently the incubation was stopped by rapid filtration through Whatman GF/C glass microfiber filters (pre-soaked in 0.3% polyethylenimine for 20 min) and the filters washed with 3 × 1 mL of ice-cold buffer (PBS, pH 7.2). Nonspecific binding was determined in the presence of 10 µM PK11195. Approximately 90% of specific binding was determined under these conditions.

Results and Discussion

Technetium and rhenium have very similar chemistry that enables the same experimental conditions to be applied to the preparation of radiopharmaceuticals for diagnostic (^{99m}Tc) and therapeutic applications ($^{186/188}$Re) in nuclear medicine.[24] Therefore, nonradioactive rhenium complexes are often used as model compounds for the ^{99m}Tc congeners.[25–30] Technetium and rhenium offer also the possibility of exploring a large variety of oxidation states in relation to different types of ligands and bifunctional and trifunctional chelators.[31–34] Following the introduction by *Alberto* and co-workers of a convenient and fully aqueous kit preparation method for the organometallic precursors *fac*-[M(OH$_2$)$_3$(CO)$_3$]$^+$ (M = Tc, Re),[35,36] the so-called tricarbonyl approach has gained considerable attention.

Within the tricarbonyl approach, it is possible to coordinate to the metal core uni-, bi-, and tridentate ligands, the last chelator usually providing complexes with a higher chemical robustness and enhanced in vivo stability. For this reason, tridentate ligands are considered the most appropriate for stabilizing the *fac*-[M(CO)$_3$]$^+$ (M = Tc, Re) core in biological media.

We have recently prepared a $^{187/185}$Re complex containing a bidentate ligand, namely *fac*-[ReBr(CO)$_3$(TZ6-*N*,*N*)], endowed with high affinity for the TSPO.[17] Preliminary stability studies showed that *fac*-[ReBr(CO)$_3$(TZ6-*N*,*N*)] is completely degraded in diluted human serum (50%) in about 3.6 h time. In the presented work, we have extended the investigation to a new TSPO ligand, also having high affinity (nanomolar) and



Scheme 1. (a) THF anhydrous at 40 °C, ethyl bromoacetate, K_2CO_3; (b) EtOH and 1 M aqueous NaOH at 40 °C; (c) MeOH, *fac*-[$ReBr(CO)_3(OH_2)_2$], and KOH; room temperature.

selectivity for the protein, but which can act as tridentate chelator towards rhenium forming the complex *fac*-[$^{187/185}Re(CO)_3$(CB239-*N*,*N*,*O*)], which has all coordination sites occupied and therefore should be endowed with higher inertness in biological medium.

Synthesis and Characterization of the TSPO-ligand CB239-H

The synthesis of CB239-H was achieved by the procedure shown in Scheme 1. In particular, compound CB239-H was obtained by hydrolysis of the corresponding ester, CB238, in aqueous NaOH/ethanol (1:1 v:v) solution. In turn, CB238 was synthesized by reacting the TSPO ligand CB86[12] with ethyl bromoacetate in anhydrous THF. CB239-H was fully characterized by elemental analysis, ESI-MS, IR, and ^{1}H NMR spectroscopy as reported in the Experimental Section.

Synthesis and Characterization of Rhenium Compounds

Fac-[$ReBr(CO)_3(OH_2)_2$] was obtained by applying the procedure previously reported for the synthesis of *fac*-[$Re(H_2O)_3(CO)_3$]Br.[23] Differently from *Zubieta* et al.,[23] who isolated the light green complex *fac*-[$Re(CO)_3(OH_2)_3$]Br by concentration of the mother solution, we obtained the neutral white complex *fac*-[$ReBr(CO)_3(OH_2)_2$] by complete evaporation of the mother liquor. The neutral or slightly acidic pH of our solution prevented the formation of dimeric and oligomeric hydroxo-bridged rhenium compounds, which have been reported to form predominantly in basic conditions.[37,38] Moreover, the ESI-MS spectra did not show any peak having patterns assignable to Re_2 or Re_3 clusters.

The synthesis of *fac*-[$Re(CO)_3$(CB239-*N*,*N*,*O*)] (Scheme 1) was carried out in methanol by reaction of CB239-H with 1.1 equivalents of *fac*-[$ReBr(CO)_3(OH_2)_2$]. Treatment with KOH allowed the deprotonation of the carboxylic group in position 8 of the imidazopyridine ring and its coordination to rhenium. The final, pure, *fac*-[$Re(CO)_3$(CB239-*N*,*N*,*O*)] complex was obtained in good yield by column chromatography on silica gel.

The complex *fac*-[$Re(CO)_3$(CB239-*N*,*N*,*O*)] was characterized by elemental analysis, ESI-MS, ^{1}H 1D NMR and 2D NOESY, COSY and [^{1}H-^{15}N]-HSQC experiments. The ESI-MS analysis revealed the presence of a peak at *m*/*z* (% relative to the base peak) = 734.9 (100) corresponding to the species [[$Re(CO)_3$(CB239-*N*,*N*,*O*)] + Na]$^+$. The experimental isotopic pattern was coincident with the theoretical one.

The ^{1}H NMR spectrum (Figure 4) shows, in the range of 4.5–3.8 ppm, the presence of two overlapping AB systems, one each for the methylene groups 9 and 16 (see Figure 3 for numbering of protons). Because of the asymmetry of the central rhenium core, the two protons of each methylenic group become diastereotopic and generate an AB spin system.

The most deshielded AB system (centered at δ = 4.18 ppm) was assigned to methylene 9 because of its spatial correlations with the singlet at δ = 7.59 ppm (4 H) (Figure 5, cross-peak **A**), belonging to the protons of the 4-Cl-phenyl group (accidentally equivalent), and with the doublet at δ = 8.32 ppm (Figure 5, NOESY cross-peak **B**) assigned to proton 5 of the imidazopyridine ring. The characterization of the imidazopyri-

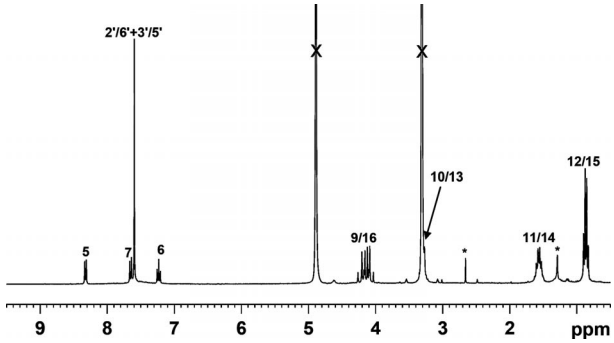


Figure 4. ^{1}H NMR (300 MHz) spectrum of *fac*-[Re(CO)$_3$(CB239-*N*,*N*,*O*)] in [D$_4$]methanol. Solvent peaks are overlayed by a cross. * indicates impurities of the solvent.

dine ring continued with the assignment of the triplet at δ = 7.23 ppm (1 H) to proton 6 (Figure 6, cross-peak **C** with proton 5) and of the doublet at δ = 7.65 ppm (1 H) to proton 7 (Figure 6, COSY cross-peak **D** with proton 6). Finally, proton 7 shows a NOESY cross-peak (**E** in Figure 5) with the most shielded of the above mentioned AB systems (δ = 4.09 ppm), which was assigned to methylene 16.

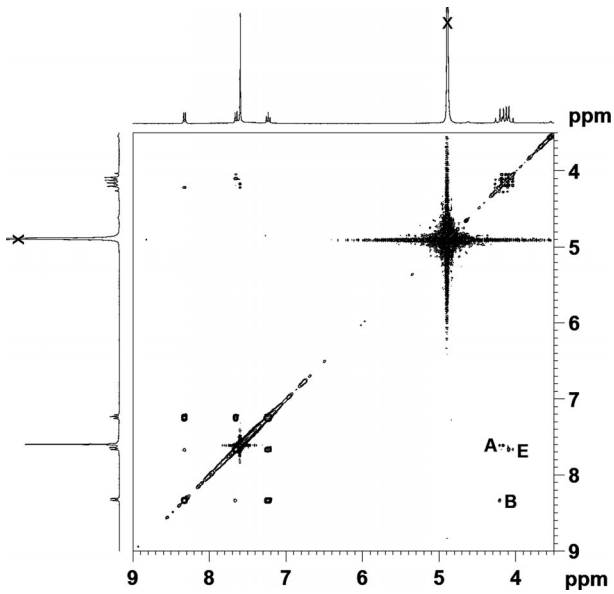


Figure 5. Portion of the 2D NOESY (^{1}H, 300 MHz) spectrum of *fac*-[Re(CO)$_3$(CB239-*N*,*N*,*O*)] in [D$_4$]methanol. Solvent peaks are overlayed by a cross.

The characterization of the dipropylacetamidic chain started from the assignment of the overlapping sextets falling in the range of 1.70–1.40 ppm to the methylenic protons 11 and 14. These sextets show COSY cross-peaks (Figure 6, **F**) with two signals, overlapping with the signal of the solvent, assigned to methylenes 10 and 13, and a COSY cross-peak (Figure 6, **G**) with the two overlapping triplets, falling in the range of 0.94–0.80 ppm, assigned to the methyl groups 12 and 15.

In order to check if the aminic nitrogen in position 8 of the imidazopyridine ring loses its proton after coordination to rhenium, a 2D [^{1}H-^{15}N]-HSQC spectrum in [D$_6$]DMSO (Figure 7) was recorded. The spectrum shows the presence of a

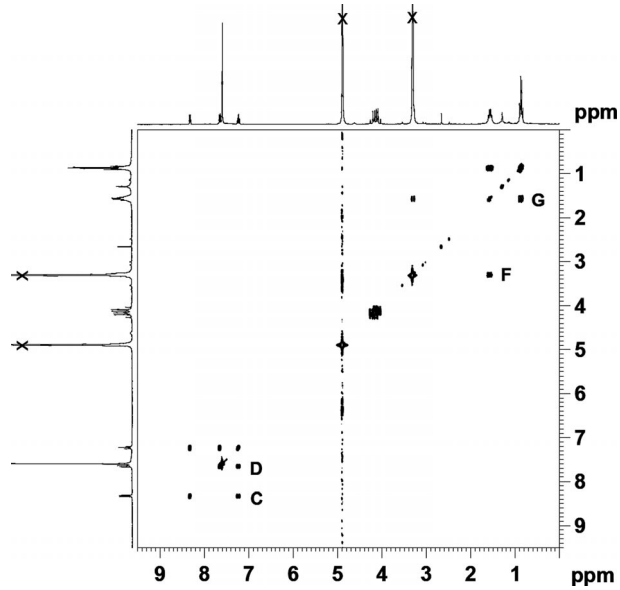


Figure 6. COSY (^{1}H, 300 MHz) spectrum of *fac*-[Re(CO)$_3$(CB239-*N*,*N*,*O*)] in [D$_4$]methanol. Solvent peaks are overlayed by a cross.

cross-peak falling at 8.99/28.2 ppm (^{1}H/^{15}N) consistent with the presence of an aminic proton that was not detectable in [D$_4$]methanol as a result of the exchange with the deuterium of the solvent.

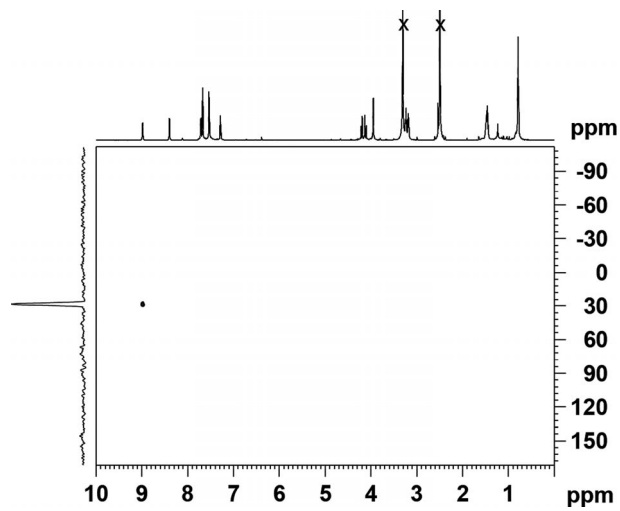


Figure 7. [^{1}H-^{15}N]-HSQC (600 MHz) spectrum of *fac*-[Re(CO)$_3$(CB239-*N*,*N*,*O*)] in [D$_6$]DMSO. Solvent peaks are overlayed by a cross.

Stability Studies

Compound *fac*-[Re(CO)$_3$(CB239-*N*,*N*,*O*)] was challenged in diluted (50 %) human serum/phosphate buffer (0.02 M, pH 7.4, isotonized with NaCl) at 37 °C to determine its inertness.

An almost immediate reaction of the rhenium complex with the serum proteins was observed. This result was quite unexpected and does not comply with the general trend that complexes, in which the *M*(CO)$_3$ core (*M* = ^{99m}Tc or $^{186/188}$Re) is

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bound to a tridentate chelating system have good stability in human plasma.[18] We are currently performing other stability studies in order to unravel the cause of such an unexpected reactivity and, consequently, make the required changes in the metal-coordinating groups.

Radioligand Binding Assays

The affinity of *fac*-[Re(CO)$_3$(CB239-*N*,*N*,*O*)] was evaluated by measuring its ability to displace the reference compound [^{3}H]-PK 11195 from binding to TSPOs in membrane of C6 glioma cells, a tumour cell line, which is well known to over-express TSPO.[5]

The affinity (Table 1) of the free ligand CB239-H and that of *fac*-[Re(CO)$_3$(CB239-*N*,*N*,*O*)] for the TSPO protein is quite good, namely 2.43 and 190 nM, respectively. While the affinity of CB239-H is comparable to that of the reference compound PK11195, that of *fac*-[Re(CO)$_3$(CB239-*N*,*N*,*O*)] is slightly lower but still good for biological applications.

Table 1. Affinities (Ki /nM) of *fac*-[Re(CO)$_3$(CB239-*N*,*N*,*O*)] for TSPO from rat cerebral cortex. Corresponding values for PK11195 and CB239-H are also reported for comparison.

Compound	Ki /nM + SEM
PK11195	1.05 ± 0.05
CB239-H	2.43 ± 0.06
fac-[Re(CO)$_3$(CB239-*N*,*N*,*O*)]	190 ± 43

Conclusions

In this work, a new 2-phenyl-imidazopyridin-dipropylacetamide ligand (CB239-H), with high affinity for the TSPO protein, was synthesized and the corresponding $^{187/185}$Re complex *fac*-[Re(CO)$_3$(CB239-*N*,*N*,*O*)] prepared. NMR techniques (^{1}H 1D and 2D COSY, NOESY, and [^{1}H-^{15}N]-HSQC experiments) were used to fully characterize both the free ligand and the new rhenium complex. The ligand appears to be tri-coordinated to the metal by the imidazopyridine nitrogen, the secondary amine nitrogen, and the carboxylate group.

The affinity of the new compounds for TSPO was evaluated in vitro on membrane extracts from C6 rat glioma cells. *Fac*-[Re(CO)$_3$(CB239-*N*,*N*,*O*)] and CB239-H have demonstrated to be endowed with good affinity for the target protein, namely 190 and 2.43 nM, respectively.

Even though the chemical robustness of *fac*-[Re(CO)$_3$(CB239-*N*,*N*,*O*)] in diluted human serum was lower than expected for a Re(CO)$_3$ core bound to a tridentate ligand, we are confident that some changes in the metal-coordinating groups, such as substitution of the acetyl group with a better donor, can overcome this problem.

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