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Original article

Preparation and characterization of technetium and rhenium tricarbonyl complexes bearing the 4-nitrobenzyl moiety as potential bioreductive diagnostic radiopharmaceuticals. *In vitro* and *in vivo* studies

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Abstract

The synthesis of a ligand containing a nitrobenzyl group as bioreductive pharmacophore and the preparation of the corresponding technetium and rhenium complexes are presented. ^{99m}Tc labelling was performed in high yield (>90%) by ligand substitution using *fac*-$[^{99m}Tc(CO)_3(H_2O)_3]^+$ as precursor. The structure of the technetium complex was established by chromatographic comparison with the analogous rhenium compound which was fully characterized by elemental analysis, spectroscopic methods and X-ray crystallography. Reduction potential of the rhenium complex was in the characteristic range for bioreductive compounds. Biodistribution in normal mice was characterized by fast blood and soft tissue depuration and combined excretion via the hepatobiliary and urinary systems. Tumour uptake was low, probably due to low lipophilicity but tumour/muscle ratios were favourable as a consequence of high excretion.

Keywords: Bioreductive compounds; Technetium; Rhenium; Tricarbonyl complexes; Hypoxia imaging

1. Introduction

Diagnostic radiopharmaceuticals are compounds containing gamma-emitters, the radiation of which readily penetrates the body, thus permitting external detection and measurement. The pattern of biodistribution of radiation allows the physician to make a diagnostic evaluation of both morphology and function [1]. ^{99m}Tc is the radionuclide of choice in nuclear medicine due to its ideal nuclear properties for imaging ($t_{1/2} = 6$ h, $E_\gamma = 140$ keV). In technetium radiopharmaceuticals the metal is bound to a transporting moiety that delivers the radioactivity to a specific site in the body determined by the properties of the transporter [2]. Current research is directed towards the development of radiopharmaceuticals that can potentially serve as ligands for bioactive substrates and can be applied in the *in vivo* evaluation of biochemical functions in a non-invasive way [3–5].

An area of special interest in radiopharmacy is the development of suitable tracers for imaging hypoxic tissue. Oncology would highly benefit from agents that effectively target hypoxic cell populations of solid tumours that are characterized by increased radioresistance and diffusion limitations that hinder the treatment [6,7]. Bioreductive compounds, which are selectively reduced in hypoxic tissue to reactive intermediates that bind to intracellular molecules, have been utilized for the development of potential radiodiagnostic markers of tumour

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hypoxia. 2-Nitroimidazole has been the preferred bioreductive pharmacophore but other functional groups as nitroaromatics and *N*-oxides have also been studied. Attachment of these bioreductive moieties to technetium is achieved through the use of a variety of chelators, namely propylene amine oxime (PnAO), diaminodithiols (DADS) and nitrido complexes [8–12]. However, properties of the synthesized technetium complexes are not yet ideal and new potentially active compounds are still being developed.

With the aim to develop new potential ^{99m}Tc-radiopharmaceuticals for imaging hypoxia with potential application in oncology and cardiology, we have selected the nitrobenzyl functional group as the bioreductive pharmacophore [13], while the *fac*-$[^{99m}Tc(CO)_3]^+$ synthon was chosen as the metal core for the synthesis of the complexes. The widely employed ^{99m}Tc-tricarbonyl core is under intense investigation for the labelling of small biomolecules. Alberto et al. succeeded in carrying out low pressure synthesis of the Tc–carbonyl complexes, which can be used as precursors to obtain a great diversity of potential radiopharmaceuticals. In these complexes CO is tightly bound and stabilizes the low oxidation state of the metal. In aqueous medium, which is the usual medium for the preparation of technetium-99m radiopharmaceuticals, the well characterized aqua ion of technetium $[Tc(OH_2)_3(CO)_3]^+$ is formed. The final step of the synthetic procedure consists of exchanging the weakly bound water molecules with an incoming mono or multidentate ligand attached to a derivatized biomolecule [14,15]. A wide variety of functional groups containing donor atoms are present in these ligands: *N*-heterocycles such as imidazoles, pyridines and pyrazoles, amides, carboxylic acids, thioethers, thiols, etc. According to the properties of the employed ligands, labelled molecules of different charge, lipophilicity and stability might be obtained. This approach has been applied recently for the labelling of peptides, CNS receptor ligands, myocardial imaging agents, DNA intercalators, etc [16–20].

Herein, we describe the synthesis and characterization of the potentially bioreductive ligand 2-[(4-nitrobenzyl)(pyridin-2-ylmethyl)amino]acetic acid. The corresponding ^{99m}Tc and Re complexes were also prepared. The rhenium complex was structurally characterized by elemental analysis, IR, UV–vis and NMR spectra and X-ray crystallography. Electrochemical studies were carried out in order to determine the reduction potential of the nitroaromatic group. The potentiality of the ^{99m}Tc complex as hypoxia imaging agent was evaluated by biodistribution experiments in normal and tumour bearing mice.

2. Chemistry

2.1. Synthesis of 2-[(4-nitrobenzyl)(pyridin-2-ylmethyl)-amino]acetic acid (L)

The ligand 2-[(4-nitrobenzyl)(pyridin-2-ylmethyl)amino]-acetic acid (LH) was designed by combining the 4-nitrobenzyl bioreductive moiety with a pyridinmethylaminoacetic acid derivative, which is an adequate chelator for the synthesis of *fac*-tricarbonyl complexes of technetium and rhenium (Fig. 1).

The ligand LH was synthesized by reacting 4-nitrobenzyl bromide with ethyl 2-[(pyridin-2-ylmethyl)amino]acetate, followed by alkaline hydrolysis as shown in Fig. 2. Characterization was performed by the usual spectroscopic methods (^{1}H NMR, ^{13}C NMR and IR). The overall yield was high (>70%) and all analytical data were consistent with the proposed structure.

2.2. Preparation of the [^{99m}Tc] technetium complex

^{99m}Tc labelling was performed by substitution using *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ as precursor. The tricarbonyl precursor was successfully prepared by two different methods. Method 1 involves the reduction of pertechnetate with sodium borohydride in the presence of CO (g) at atmospheric pressure, while method 2 consists in the addition of pertechnetate to a lyophilized kit formulation containing sodium boranocarbonate, which acts simultaneously as reductant and ''in situ'' source of CO [21]. Both methods produced the desired compound in high yield (>95%) as shown by HPLC analysis. The tricarbonyl precursor had a retention time of approx. 4 min. Comparison with chromatographic profile of the pertechnetate ion that has a retention time of 12 min demonstrated that the formation of the precursor was quantitative. Minor peaks of longer retention times probably corresponding to polymeric species were observed. Radiochemical purity was also checked by paper chromatography. Retardation factors of *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ in butanone and methanol/HCl 95:5 are 0 and 0.9–1, respectively, and allow differentiation of potential radiochemical impurities, namely pertechnetate and reduced hydrolyzed technetium.

Substitution was performed by the addition of ligand LH to an aqueous solution of *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ at neutral pH. HPLC analysis of the labelling mixture revealed the formation of mainly one complex (retention time of 20 min) with a high radiochemical purity (>90%). The radioactivity recovery from the column was monitored by means of an on-line solid scintillation detector coupled to the HPLC system and found to be quantitative. The complex was stable for at least 6 h. The HPLC purified complex is obtained in carrier free form, since the ligand LH has a retention time of approx. 15 min. The partition coefficient of the technetium complex between octanol and phosphate buffer pH 7.4 was measured in order to assess its lipophilicity. A log *P* of 0.02

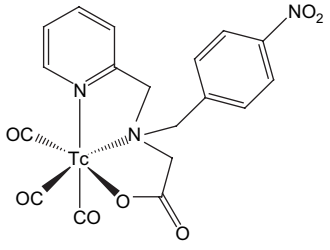

Fig. 1. Proposed structure of ^{99m}Tc complex.

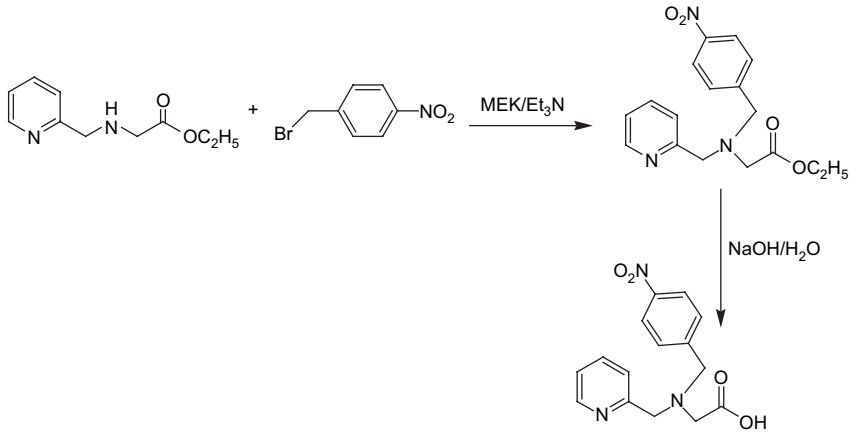

Fig. 2. Synthesis of ligand LH.

was obtained indicating that about half of the activity was extracted by octanol while the other half remained in the aqueous layer.

Corroboration of the structure of the ^{99m}Tc complex was achieved by comparing its HPLC profile with that of the corresponding Re complex upon coinjection [22] (see Section 2.3). Radioactivity and UV detectors showed identical chromatographic profiles, suggesting that the same chemical structure was formed under both chelating conditions.

2.3. Preparation of the rhenium compound

The structural characterization of ^{99m}Tc complexes presents some peculiarities derived from the nuclear properties of the metal. ^{99m}Tc is used *in vivo* in very low concentration ($\leq 10^{-9}$ M). Radioprotection of patients and health care personnel is the cause of this unusually low concentration. As a consequence, the structural characterization of radioactive ^{99m}Tc complexes by the traditional methods (elemental analysis, spectroscopy, X-ray crystallography) is not feasible and usually their chemical evaluation is carried out by using the stable rhenium as a surrogate for technetium [23,24]. Rhenium, as technetium third row congener, exhibits many of the chemical properties of Tc. Furthermore, Re and Tc complexes with the same ligand have essentially identical coordination parameters since the ionic radii of both metals are about the same, due to the lanthanide contraction. In addition, the studies of ^{99m}Tc complexes may be further expanded to the preparation of analogous radioactive rhenium complexes with potential application in radiation therapy.

In our study the rhenium complex was prepared by reacting ligand LH with the tricarbonyl rhenium precursor $[N(CH_3CH_2)_4]_2[Re(CO)_3Br_3]$ [25], as shown in Fig. 3.

HPLC analysis revealed the presence of only one species with a retention time of 20 min and a maximum absorption at 264 nm in agreement with the presence of aromatic systems in the molecule. The rhenium complex was obtained as yellow crystals in high yield (80%). It is very stable in solid state as well as in solution. Elemental analysis performed for C, H and N was consistent with the proposed structure. IR spectra showed the expected symmetric and asymmetric stretching bands of the C≡O bond at 1883 (broad), 1923 and 2022 cm^{-1}, demonstrating the presence of the tricarbonyl core in the molecule. The band at 1664 cm^{-1} can be assigned to the coordinated carboxylate anion of ligand LH and the absorptions at 1523 and 1338 cm^{-1}, which are characteristic of the nitro group, corroborate the presence of the intact bioreductive pharmacophore [26,27]. In agreement with the proposed structure, the NMR spectra revealed the presence of the pyridinyl and nitrobenzyl moieties in the aromatic region, as well as the presence of three pairs of diastereotopic methylene protons in the aliphatic region. X-ray crystallographic studies confirmed the proposed structure. The molecular structure of the rhenium complex is shown in Fig. 4 and selected bond lengths and angles are listed in Table 1. The ligand LH acts as an NNO monoanionic tridentate chelate towards the *fac*-$[Re(CO)_3Br_3]^{2-}$ precursor and replaces the three bromine atoms. The nitro group remains free to interact with the biological system. Bond distances and angles are consistent with the octahedral geometry around the rhenium atom. There are two 5-membered rings in the coordination sphere, defined by the Re–N–C–C–N and the Re–N–C–C–O atoms, which adopt the envelope configuration with C(6) and C(7) lying 0.60 and 0.21 Å, respectively out of the mean plane of the remaining four atoms.

2.4. Electrochemical studies

The redox behaviour of ligand LH and the rhenium complex was studied by cyclic voltammetry in anhydrous DMF. Cathodic region was studied in order to assess the reduction potentials of the ligand and the complex and compare these values with the ones corresponding to enzymes responsible for bioreduction in anaerobic organisms (approx. $E_{1/2}$ −450 mV vs standard hydrogen electrode or −700 mV vs saturated calomel electrode) [28–30]. Fig. 5 shows typical voltammograms for ligand LH and the rhenium complex and Table 2 lists the values of the first and second cathodic peaks. Voltammetric data of the complex indicated that the reduction processes follow a simple pattern that involves two to three reduction waves. All couples

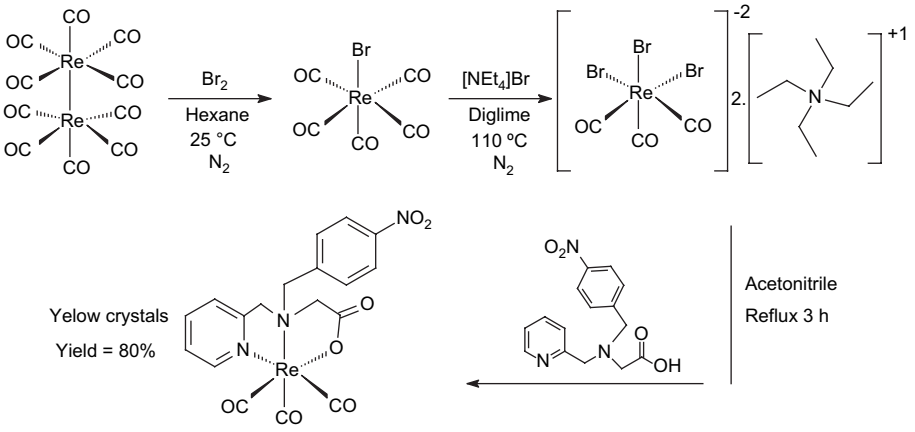

Fig. 3. Preparation of rhenium complex.

correspond to the nitro redox process, while the reduction of the metallic core does not fall within the studied range according to the literature [31,32]. The first cathodic peak could correspond to the nitro-anion radical generation while the second could be attributed to the hydroxylamine reduction [29]. The ligand LH presents the same voltammetric behaviour in the studied region. The reduction of the nitro group in the complex occurs at −805 mV while in the ligand the processes occur approx. 100 mV lower (at −895 mV).

3. Biology

3.1. Biodistribution studies in normal mice

The *in vivo* behaviour of the ^{99m}Tc complex was evaluated by biodistribution studies in normal mice between 0.5 and 24 h post-injection. Fig. 6 shows the results expressed as % dose/organ in the most significant organs and fluids as a function of time. The complex demonstrated low initial blood and lung activity ($1.5 \pm 0.4\%$ and $0.11 \pm 0.4\%$, respectively at 30 min post-injection) and quantitative clearance after 24 h ($0.06 \pm 0.05\%$ and $0.01 \pm 0.01\%$, respectively). Initial liver activity was relatively high ($21.0 \pm 1.1\%$) but was significantly reduced in 2 h ($11.2 \pm 1.4\%$), indicating rapid clearance. Excretion occurs mainly through the hepatobiliary system as demonstrated by high intestinal activity with urinary excretion being also significant (66.0 ± 9.1 and 15.3 ± 2.4, respectively at 2 h post-injection). Stomach and thyroid values were within acceptable levels ($0.1 \pm 0.01\%$ and $0.2 \pm 0.06\%$, respectively, at 30 min), indicating minimal *in vivo* reoxidation.

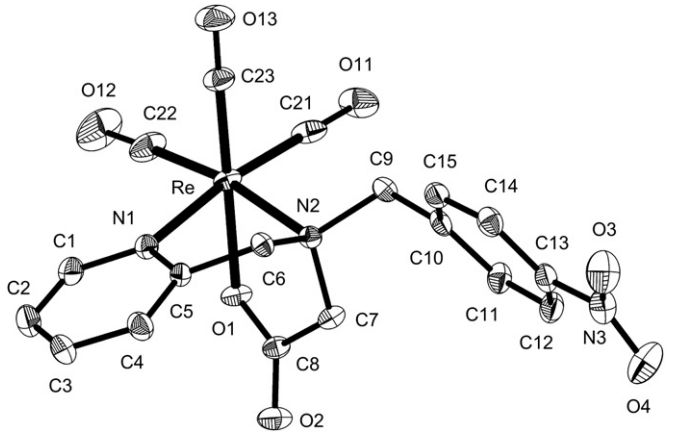

Fig. 4. Labelled ORTEP plot of the rhenium complex showing 30% thermal probability ellipsoids (hydrogen atoms have been omitted for clarity).

3.2. Biodistribution studies in mice bearing induced sarcoma

In order to assess the potentiality of our approach for the design of potential radiopharmaceuticals for nuclear oncology, evaluation in mice bearing induced sarcoma was performed. This animal model was selected because histopathological studies demonstrated a high degree of hypoxia within the tumours.

Tumour uptake (% dose/g) is shown in Table 3. Although uptake was low ($0.19 \pm 0.03\%$ at 0.5 min post-injection) probably due to poor cell penetration caused by low pipophilicity, only minor depuration from the tumor was observed from 0.5 to 12 h suggesting selective retention. Tumour/muscle ratios are favourable from 2 h post-injection due to rapid clearance from blood and muscle (2.2–6.5).

Table 1
Selected bond lengths (Å) and angles (°) for the rhenium complex

Lengths			
Re–C(23)	1.902(5)	Re–O(1)	2.108(3)
Re–C(22)	1.908(6)	Re–N(1)	2.191(4)
Re–C(21)	1.928(6)	Re–N(2)	2.239(4)
Angles			
C(21)–Re–N(1)	172.4(2)	C(23)–Re–C(22)	89.1(3)
C(22)–Re–N(2)	170.8(2)	C(22)–Re–O(1)	93.7(2)
C(23)–Re–O(1)	176.0(2)	O(1)–Re–N(2)	79.4(1)
C(23)–Re–N(2)	97.6(2)	C(23)–Re–N(1)	98.2(2)
C(22)–Re–N(1)	96.9(2)	O(1)–Re–N(1)	78.5(1)
N(1)–Re–N(2)	76.0(1)	C(21)–Re–O(1)	96.8(2)
C(21)–Re–N(2)	97.4(2)	C(23)–Re–C(21)	86.1(2)
C(22)–Re–C(21)	89.3(3)		

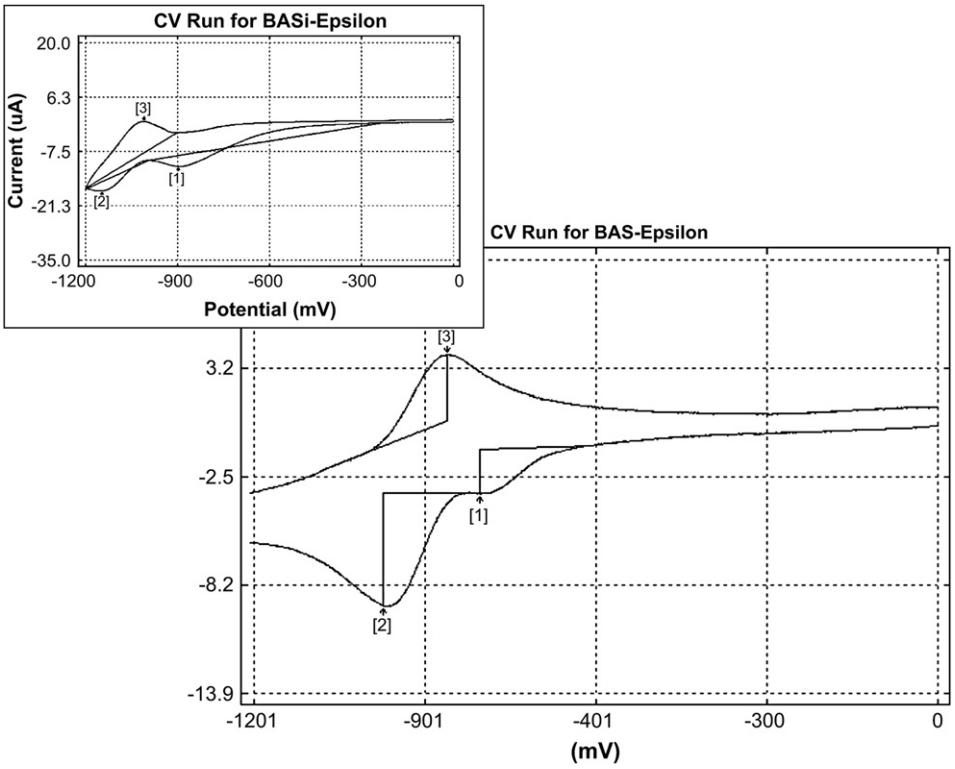

Fig. 5. Voltammogram of rhenium complex and ligand LH (inset).

4. Conclusions

The ligand 2-[(4-nitrobenzyl)(pyridin-2-ylmethyl)amino]-acetic acid was designed combining the 4-nitrobenzyl bioreductive moiety with the chelator (pyridin-2-ylmethyl)-aminiacetate. The ligand synthesized was successfully utilized in the preparation of a new ^{99m}Tc complex with potentiality for hypoxia imaging. ^{99m}Tc complex was obtained with high radiochemical purity (>90%). It is very stable and has medium lipophilicity. The proposed structure was corroborated by means of the comparison with that of the analogous rhenium complex. Reduction potentials of the nitroaromatic group in both the ligand and the rhenium complex were found to be similar to the values of enzymes responsible for the bioreduction in anaerobic organisms. Different types of bioreductive compounds (*N*-oxides, nitrofurans, nitrophenyl mustards, nitroquinolines, etc.) have similar values of reduction potentials [33–36].

Overall biodistribution in normal animals was the expected for medium lipophilicity compounds. Tumour uptake was low, probably due to poor cell penetration. However, the observed tumour/muscle ratios were favourable due to slow clearance from the tumour combined with fast clearance from blood and soft tissues. These ratios are higher than the ones obtained for potentially bioreductive mixed ligand oxotechnetium complexes bearing the same pharmacophore [12], demonstrating the potentiality of our approach. However tumour uptake is insufficient for imaging purposes.

Overall, the technetium-99m complex synthesized may be considered as a starting point for the development of new radiopharmaceuticals for imaging hypoxia. We are currently investigating the application of other pharmacophores and labelling methods in the search for compounds with improved properties.

Table 2
Cyclic voltammetric parameters of ligand LH and the rhenium complex vs saturated Ag/AgCl electrode (scan rate 500 mV s^{-1})

Compound[a]	E_{pc}[b] (mV)	E_{pc}[c] (mV)
L	−895	−1140
fac-[Re(CO)$_3$L]	−805	−974

[a] Concentration 1 mM in DMF.
[b] E_{pc} = potential of the first cathodic peak.
[c] E_{pc} = potential of the second cathodic peak.

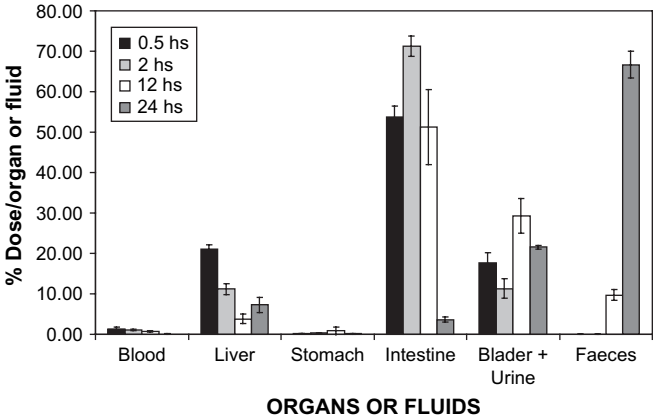

Fig. 6. Biodistribution of ^{99m}Tc complex in normal CD1 mice from 0.5 to 24 h.

Table 3
Tumour uptake as a function of time

% Dose/organ or fluid				
	0.5 h	2 h	12 h	24 h
% Tumour/g (*T*)	0.19 ± 0.03	0.14 ± 0.09	0.13 ± 0.06	0.07 ± 0.01
% Blood/g (*B*)	0.85 ± 0.06	0.74 ± 0.08	0.34 ± 0.02	0.03 ± 0.01
% Muscle/g (*M*)	0.26 ± 0.04	0.04 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
T/M	0.73 ± 0.05	2.2 ± 0.2	6.5 ± 0.9	6.0 ± 1.0
T/B	0.22 ± 0.09	0.18 ± 0.5	0.38 ± 0.07	2.3 ± 0.9

5. Experimental

5.1. General

All laboratory chemicals were reagent grade and were used without further purification. Solvents for chromatographic analysis were HPLC grade. [^{99m}Tc]$NaTcO_4$ was obtained from a commercial generator (Tecnonuclear SA, Argentina). Carbonyl labelling agent Isolink was provided by Mallinckrodt Medical B.V, The Netherlands. IR spectra were recorded as KBr pellets in the range 4000–200 cm^{-1} with a Bomen MB-102 FT-IR spectrophotometer. Elemental analysis was performed on a Carlo Erba EA 1108 analyzer. The NMR spectra were recorded in deuterium chloroform on a Bruker AC 250E spectrometer. Chemical shifts are reported with respect to TMS. HPLC analysis was developed on an LC-10 AS Shimadzu Liquid Chromatography System using a reverse phase column Phenomenex Luna 5 μ, C18 column (4.69 × 300 mm). Elution was performed with a binary gradient system at 1.0 mL/min flow rate using phosphate buffer pH 2.5 with 2% triethylamine as mobile phase A and methanol as mobile phase B; the elution profile was as follows: 0–3 min 100% A; 3–6 min linear gradient to 75% A; 6–9 min linear gradient to 66% A; 9–20 min linear gradient to 0% A; this composition was held for another 10 min. Detection was accomplished either with a photodiode array detector (SPD-M10A, Shimadzu) that recorded UV–vis spectra on flux or with a 3″×3″ NaI (Tl) crystal scintillation detector. Activity measurements were performed either in a Dose Calibrator, Capintec CRC-5R or in a scintillation counter, 3″×3″ NaI (Tl) crystal detector associated to an ORTEC monochannel analyzer.

5.2. Chemistry

5.2.1. Synthesis of 2-[(4-nitrobenzyl)(pyridin-2-ylmethyl)-amino]acetic acid (LH)

Ethyl [(pyridin-2-ylmethyl)amino]acetate (3.00 g, 15.46 mmol) was dissolved in methylethylketone (50 mL) and 4-nitrobenzyl bromide (3.30 g, 15.46 mmol) and triethylamine (1.56 g, 15.46 mmol) were added. The mixture was heated at reflux for 2 h and the solvent was removed under vacuum. The residue was extracted with dichloromethane and concentrated under vacuum. The crude product was purified by silica gel flash column chromatography using 2:1 mixture of ether/petroleum ether to give ethyl [(4-nitrobenzyl)(pyridin-2-ylmethyl)amino]acetate as viscous oil. Yield: 3.80 g (78%). ^{1}H NMR δ_H (ppm, $CDCl_3$): 1.20 (t, 3H, CH_3), 3.32 (s, 2H, CH_2), 3.89 (s, 2H, CH_2), 3.87 (s, 2H, CH_2), 4.09 (q, 2H, $COOCH_2$), 7.10 (m, 1H, PyrH), 7.44 (m, 1H, PyrH), 7.57 (m, 2H, ArH), 7.61 (m, 1H, PyrH), 8.10 (m, 2H, ArH), 8.48 (m, 1H, PyrH). IR (KBr, cm^{-1}): 1735 (s) (C═O ester), 1518 (s), 1345 (s) (NO_2).

Ethyl [(4-nitrobenzyl)(pyridin-2-ylmethyl)amino]acetate (3.08 g, 9.7 mmol) was treated with 0.1 M NaOH (15 mL) for 18 h at room temperature under stirring. The mixture was neutralized and the product crystallized from the reaction mixture. Filtration afforded *2-[(4-nitrobenzyl)(pyridin-2-ylmethyl)amino]acetic acid (LH)*. Yield: 2.6 g (93%). ^{1}H NMR δ_H (ppm, D_2O): 3.38 (s, 2H, CH_2), 3.93 (s, 2H, CH_2), 3.97 (s, 2H, CH_2), 7.18 (m, 1H, PyrH), 7.39 (m, 1H, PyrH), 7.52 (m, 2H, ArH), 7.67 (m, 1H, PyrH), 8.10 (m, 2H, ArH), 8.50 (m, 1H, PyrH). ^{13}C NMR δ_H (ppm, DMSO): 172.12, 158.72, 148.74, 147.51, 146.58, 136.65, 129.48, 122.71, 122.25, 104.37, 58.84, 56.65, 53.95. IR (KBr, cm^{-1}): 1709 (s) (C═O), 1517 (s), 1344 (s) (NO_2), 1217 (s).

5.2.2. ^{99m}Tc labelling

5.2.2.1. Preparation of fac-[^{99m}Tc$(OH_2)_3(CO)_3]^+$ precursor

5.2.2.1.1. Preparation using a kit formulation. ^{99m}Tc-sodium pertechnetate (185–1850 MBq, 1 mL) was added to the Isolink™ kit formulation (Mallinckrodt Medical B.V.) containing sodium tetraborate (2.85 mg), sodium carbonate (7.15 mg), sodium boranocarbonate decahydrate (2.85 mg) and sodium tartrate dihydrate (8.5 mg) and the mixture was incubated at 75 °C for 30 min. After cooling the pH was adjusted to 7 with 1 N HCl. Complex formation was checked by HPLC as described in Section 5.1. Radiochemical purity was also checked by ascending chromatography on Whatman 1 paper/butanone and Whatman 1 paper/methanol/HCl conc. 95:5.

5.2.2.1.2. Preparation using CO (g). The ^{99m}Tc precursor was prepared according to a previously described method [18] as follows. Sodium and potassium tartrate (20 mg), sodium carbonate (4 mg) and sodium borohydride (5.5 mg) were placed in a stoppered vial and flushed with CO (g) for 30 min. ^{99m}Tc-sodium pertechnetate (185–1850 MBq) was added and the mixture incubated at 75 °C for 30 min. After cooling, the pH was adjusted to 7 with 1 N HCl. The complex formation was checked as in Section 5.2.2.1.1.

5.2.2.2. Preparation of technetium-99m complex. A solution of ligand LH, 2-[(4-nitrobenzyl)(pyridin-2-ylmethyl)amino]acetic

acid, in methanol (12.5 mg/mL, 0.1 mL) was added to an aqueous solution of the *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ precursor. After incubation at 50 °C for 30 min an aliquot of the reaction mixture was analyzed by HPLC to verify complex formation.

5.2.3. Preparation of the rhenium complex

A solution of $[NEt_4]_2[Re(CO)_3Br_3]$ (0.077 g, 0.1 mmol) and ligand LH (30 mg, 0.1 mmol) in acetonitrile (20 mL) was heated at reflux for 3 h. The solution was cooled to room temperature. Light yellow plates were obtained upon standing and purified by recrystallization from acetonitrile.

$[Re(CO)_3[(C_5H_4N)CH_2N(CH_2\text{-}p\text{-}NO_2C_6H_4)CH_2COO]$: yellow crystals, yield: 80.0%. Analysis for $C_{18}H_{14}N_3O_7Re$, theoretical: C: 37.89; H, 2.47; N, 7.37. Experimental: C, 37.80; H, 2.35; N, 7.42. IR (KBr, cm^{-1}): 1883 (broad), 1923 and 2022 (C≡O), 1664 (COO−Re), 1523 and 1338 (NO_2). ^1H NMR δ_H (ppm, DMSO-d_6): 8.78 (m, 1H, PyrH), 8.33 (m, 2H, ArH), 8.11 (m, 1H, PyrH), 8.07 (m, 2H, ArH), 7.64 (m, 1H, PyrH), 7.57 (m, 1H, PyrH), 4.91 (d, 1H, CH_2Pyr), 4.90 (d, 1H, CH_2ArH), 4.69 (d, 1H, CH_2ArH), 4.28 (d, 1H, CH_2Pyr), 4.26 (d, 1H, CH_2CO), 2.92 (d, 1H, CH_2CO). ^{13}C NMR δ_C (ppm, DMSO-d_6): 198.40, 197.84, 179.07, 159.90, 152.87, 148.78, 141.41, 140.64, 134.32, 126.77, 124.86, 124.25, 70.95, 68.12, 61.33.

5.2.4. Electrochemical studies

Electrochemical behaviour of the rhenium complex and the ligand (LH) was studied by cyclic voltammetry (Table 2). Anhydrous DMF (spectroscopic grade) was obtained from Aldrich. Tetrabutylammonium perchlorate (0.1 M), used as supporting electrolyte, was obtained from Fluka (electrochemical grade). Cyclic voltammetry was carried out with computer controlled BAS-Epsilon EC equipment. A standard electrochemical three-electrode cell was used with a platinum disc as the working electrode, a platinum wire as the counter electrode and saturated Ag/AgCl as the reference electrode. Cyclic voltammograms were obtained at different scan rates, between 50 and 2000 mV/s in 1 mM DMF solutions of the compounds. Oxygen was removed by purging the solution with extra-pure nitrogen and a continuous stream of this gas was passed over the solutions during the measurements.

5.2.5. X-ray crystal structure determination

A crystal with approximate dimensions $0.50 \times 0.35 \times 0.24$ mm was mounted in air. Diffraction measurements were made on a Crystal Logic Dual Goniometer diffractometer using graphite monochromated Mo radiation. Unit cell dimensions were determined and refined by using the angular settings of 25 automatically centered reflections in the range $11 < 2\theta < 23°$ and they appear in Table 4. Intensity data were recorded using a $\theta-2\theta$ scan to $2\theta_{max} = 50°$, with scan speed 4.0/min and scan range 2.3 plus $\alpha_1\alpha_2$ separation. Three standard reflections monitored every 97 reflections showed less than 3% variation and no decay. Lorentz polarization and psi-scan absorption corrections were applied using Crystal Logic software. The structures were solved by direct methods using SHELXS-86 [37] and refined by full-matrix least squares techniques on F^2 using SHELXL-97 [38]. Further crystallographic details: $2\theta_{max} = 50°$, reflections collected/unique/used 3486/3354 [$R_{int} = 0.0354$]/3354, 318 parameters refined, $[\Delta\rho]_{max}/[\Delta\rho]_{min} = 0.747/-1.089$ e/Å^3, $[\Delta/\sigma]_{max} = 0.003$, $R1/wR2$ (for all data) = 0.0308/0.0651. All hydrogen atoms were located by difference maps and were refined isotropically, all non-H atoms were refined anisotropically.

Table 4
Summary of crystal, intensity collection and refinement data for the rhenium complex

Empirical formula	$C_{18}H_{14}N_3O_7Re$
Formula weight	570.52
Temperature	298
Wavelength	Mo Kα 0.71073
Space group	$P2_1/n$
a (Å)	13.174(5)
b (Å)	10.315(4)
c (Å)	15.210(5)
β (°)	112.09(1)
V (Å^3)	1915.2(12)
Z	4
D_{calcd} (Mg m^{-3})	1.979
Absorption coefficient μ (mm^{-1})	6.392
$F(000)$	1096
Goodness-of-fit on F^2	1.071
R indices	$R1 = 0.0248$,[a] $wR2 = 0.0622$[a]

[a] For 2977 reflections with $I > 2\sigma(I)$.

5.3. Biological studies

All animal studies were approved by the Ethics Committee of the Faculty of Chemistry from Universidad de la República, Uruguay.

5.3.1. Biodistribution studies in normal animals

In vivo evaluation of ^{99m}Tc complexes was performed by biodistribution using normal CD1 mice. Four animals per group (female, 8–10 weeks old and 25–30 g) were injected via a lateral tail vein with the HPLC purified ^{99m}Tc complex reconstituted with saline (0.1 mL, 37–370 kBq [1–10 μCi]). At different intervals after injection (0.5–24 h) the animals were sacrificed by neck dislocation. Whole organs and samples of blood and muscle were collected, weighed and assayed for radioactivity. Total urine volume was collected during the biodistribution period and also removed from bladder after sacrifice. The bladder, urine and intestines were not weighed. Corrections by different sample geometry were applied when necessary. Results were expressed as % dose/organ or fluid.

5.3.2. Biodistribution studies in mice bearing induced sarcoma

A suspension of sarcoma cells CCRF-180II in PBS, containing 2.5×10^6 cells/mL was prepared and 200 μL was injected subcutaneously in the right limb of normal CD1 mice (female, 8–10 weeks old and 25–30 g). After 10–15 days the animals developed palpable tumour nodules and were used for the biodistribution studies according to the procedure described in Section 5.3.1. Results were expressed as % dose/gram for

blood, muscle and tumour. The tumour/muscle ratios were calculated from the corresponding % dose/gram values.

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