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ScienceDirect
European Journal of Medicinal Chemistry 41 (2006) 1144–1152
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
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Original article

Design and evaluation of "3 + 1" mixed ligand oxorhenium and oxotechnetium complexes bearing a nitroaromatic group with potential application in nuclear medicine oncology

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Received in revised form 25 April 2006; accepted 4 May 2006
Available online 19 June 2006

Abstract

The synthesis and evaluation of a series of oxotechnetium and oxorhenium complexes containing a nitroaromatic moiety as potential radiopharmaceuticals for targeting tumour hypoxia is presented. ^{99m}Tc labelling was performed in high yield (> 85%) and radiochemical purity (> 90%). Their structure was corroborated by means of the rhenium complexes. Reduction potentials were in the range for bioreducible compounds. ^{99m}Tc complexes **III–VI** were selected for "in vivo" experiments in view of the results of cytotoxicity studies. Biodistribution in normal animals was characterized by high initial blood, lung and liver uptake, fast blood and soft tissue depuration and preferential excretion via the hepatobiliary system. Initial tumour uptake was moderate but tumour/muscle ratios for complexes **III** and **IV**, were favourable at all time points. Although the results are encouraging further development is still necessary in order to achieve higher tumour uptake and lower gastrointestinal activity.

Keywords: Bioreducible compounds; Oxotechnetium, Oxorhenium complexes; Hypoxia imaging

1. Introduction

Tumour perfusion and oxygenation status have been recognized as important factors that may cause poor treatment outcome after radio and chemotherapy. Hypoxic cells are very resistant to radiation damage and diffusional limitations also restrict the efficacy of cytotoxic drugs [1,2]. As a result of this situation, Oncology would highly benefit from agents that effectively target the hypoxic cell populations of solid tumours, thus allowing prediction of potential response to therapy [3].

Nuclear medicine techniques, based on the administration of small amounts of radioactive compounds, the so-called radiopharmaceuticals, followed by detection of the radiation escaping from the body, provide a non-invasive and accurate method for "in vivo" imaging highly specific biochemical processes [4]. The design of the adequate radiotracers for each pathological situation is the limiting factor of this method [5,6].

^{99m}Tc is the preferred radionuclide for diagnosis, due to its ideal nuclear properties (γ emission, $t_{1/2}$ = 6 hours, E_γ = 140 keV). About 90% of all diagnostic nuclear medicine procedures are carried out using this isotope. Furthermore, the analogy in chemistry between technetium and rhenium, together with the availability of radionuclides of rhenium with appropriate nuclear properties for therapy (^{186}Re, $t_{1/2}$ = 90.6 h, $E_{\beta max}$ = 1.1 MeV, E_γ = 137 keV; ^{188}Re, $t_{1/2}$ = 17 h, $E_{\beta max}$ = 2.1 MeV, E_γ = 155 keV), contribute to this preference, since the studies of ^{99m}Tc complexes may be further expanded to the preparation of analogous rhenium compounds for therapy. Both technetium and rhenium belong to group 7 of the Periodic Table

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doi:10.1016/j.ejmech.2006.05.006

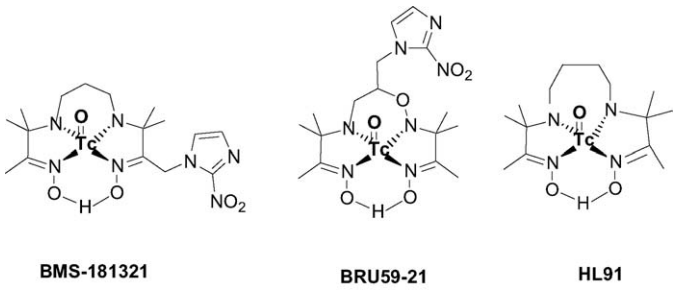


Fig. 1. Proposed ^{99m}Tc bioreductive radiopharmaceuticals.

and as transition metals their chemistry is dominated by the formation of coordination compounds [7,8].

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 hypoxia [9,10]. Different pharmacophores and a variety of approaches for attaching them to the technetium have been proposed over the last years. 2-Nitroimidazole derivatives BMS 181321 and BRU59-21 are the most promising [11,12]. Another potential agent, the HL91 (Fig. 1), which does not contain a bioreducible group has introduced the possibility that the $(TcO)^{+3}$ core itself may have the appropriate redox properties [13]. However, properties of these compounds are not yet ideal and new potentially active compounds are still being developed [14,15].

As part of our project to develop small size, neutral, "3 + 1" mixed ligand oxotechnetium and oxorhenium complexes based on the simultaneous coordination of a tridentate ligand (L) and a monodentate coligand (C) to the metal (M) [16–19], we have synthesised a series of complexes of general formula MOLC (M = Tc or Re) bearing a nitroaromatic derivative as potential radiopharmaceuticals for targeting tumour hypoxia. Four tridentate aminodithiols L (L = R-N($CH_2CH_2SH)_2$) and two monothiols C containing the nitroaromatic moiety (C = *p*-$NO_2C_6H_4CH_2SH$ or *p*-$NO_2C_6H_4SH$) were combined with either ^{99m}Tc or stable Re (Scheme 1).

The rhenium complexes were structurally characterized by IR, UV–vis and NMR spectra and elemental analysis. Electrochemical studies were developed in order to determine the reduction potential of both the nitroaromatic group and the $(MO)^{+3}$ moiety. "In vitro" cytotoxicity in normoxia and hypoxia was also assessed. "In vivo" behaviour, including biodistribution in mice (normal and bearing induced tumours) and γ-camera imaging, was studied using the corresponding ^{99m}Tc-complexes.

2. Chemistry

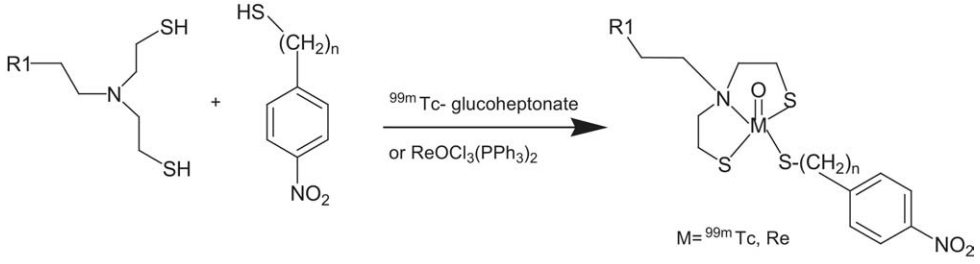
2.1. Preparation of [^{99m}Tc] technetium compounds

^{99m}Tc complexes were prepared by substitution using ^{99m}Tc-glucoheptonate (obtained form a locally produced kit, radiochemical purity > 90%) as precursor and equimolecular amounts (2×10^{-5} mol) of ligand and coligand, as shown in Scheme 1.

According to previous experience and literature data about "3 + 1" mixed ligand technetium complexes bearing similar tridentate ligands, we expect the formation of neutral and lipophilic complexes due to the ionization of the sulphur groups of ligand and coligand [20,21]. Consequently, isolation of the mixed ligand complexes from the reaction mixture was achieved upon extraction by dichloromethane.

The substitution yield, calculated as the percentage of dichloromethane extraction, was above 85% for all compounds. The radiochemical purity of the organic extract was controlled by HPLC. A major peak with a retention time of 13–14 min was obtained for all complexes. The radioactivity of the major peak was above 90%. The radioactivity recovery from the column after injection of complexes was monitored by means of an on-line solid scintillation detector coupled to the HPLC system and found to be quantitative.

Corroboration of the structure of the ^{99m}Tc complexes was achieved by comparing their HPLC profiles with the corresponding to the Re complexes upon coinjection [22] (see Section 2.1.2). Radioactivity and UV detectors showed identical chromatographic profiles, suggesting that the same chemical structure was formed under both chelating conditions.



[^{99m}Tc] complex	Re complex	-R1	n
I	I'	-H	1
II	II'	$-SC_2H_5$	1
III	III'	$-N(C_2H_5)_2$	1
IV	IV'	$-N(C_2H_5)_2$	0
V	V'	$-N(i\text{-}C_3H_7)_2$	1
VI	VI'	$-N(i\text{-}C_3H_7)_2$	0

Scheme 1. Preparation of ^{99m}Tc and Re complexes.

2.2. Preparation of rhenium compounds

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 tracer level (concentration $\leq 10^{-9}$M). Radioprotection of patients and health care personnel is a main concern in nuclear medicine and determines this unusually low concentration. As a consequence, structural elucidation of ^{99m}Tc-complexes by the traditional methods (crystallization followed by spectroscopic characterization) is not possible. An alternative procedure consists in the use of stable rhenium as a surrogate for technetium in the chemical evaluation [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 lantanide contraction. Besides, the studies of ^{99m}Tc complexes may be further expanded to the preparation of analogous rhenium compounds with potential application in radiation therapy.

In our study oxorhenium complexes were prepared by substitution of equimolecular amounts of ligand and coligand (2×10^{-3} mol) on the $[Re(V)O]^{3+}$ precursor trichlorobis(triphenylphosphine)oxorhenium(V) [25], as shown in Scheme 1. Isolation of the mixed ligand complexes from the reaction mixture was achieved upon extraction by dichloromethane followed by crystallization from dichlorometane/methanol mixtures. All complexes were obtained in high yield. They are soluble in acetone, dichloromethane and chloroform, slightly soluble in methanol and ethanol and insoluble in ether or water, indicating their lipophilic nature. They are also stable in solid state as well as in solution. Elemental analysis performed for C, H, N and S for all complexes was consistent with the proposed "3 + 1" structure. IR spectra showed the expected ReO stretching band for monoxocomplexes in the region of 950 cm^{-1} together with intense bands around 1340 and 1500 cm^{-1} arising from the nitro group. The absence of bands associated with the SH groups demonstrates the expected deprotonation of these groups upon complexation. Electronic absorptions were determined from the HPLC eluent using the PDA detector. They are characterized by an intense band in the region of 410–420 nm, probably due to S ———> Re charge transfer transition. Additional absorptions at shorter wavelength correspond to ligand and coligand. ^{1}H NMR was an important tool to complete the structural elucidation. All signals were assigned according to well-supported literature data for similar mixed ligand complexes [26,27]. The results confirm the presence of the ligand and coligand in the complex molecule. The signals of the tridentate ligand fall in the region 1.02–3.95 ppm. The protons corresponding to the methylene groups of the two chelated NCH_2CH_2S moieties are diastereotopic due to the influence of the $(ReO)^{3+}$ core. The ones that are closer (endo) appear downfield (3.4–3.7 ppm) compared to the ones that are remote (exo) in relation to the metal-oxo unit (2.7–3.1 ppm). The chemical shifts of the methylene protons closer to the N of the $NCH_2CH_2R_1$ moiety indicate that the complex has the syn configuration, since they appear in a deshielded environment between 3.80–3.95 ppm. The two doublets at 7.53–7.80 and 8.15–8.22 ppm correspond to the *p*-substituted benzene ring of the coligand. The benzylic protons of complexes **I′**, **II′**, **III′** and **V′** appear at 4.91–4.95 ppm.

Table 1
Cyclic voltammetric parameters in DMF vs. saturated Ag/AgCl electrode (scan rate 500 mV s^{-1})

Reference[a]	E_{pc}[b] (V) [Re(V) reduction]	E_{pc}[c] (V) [NO_2 group reduction]
(*p*-nitrophenyl)methanethiol	–	–1.05
p-nitrothiophenol	–	–0.87
I'	–0.81	–1.09
II'	–0.80	–1.08
III'	–0.81	–1.10
IV'	–0.90	–1.00
V'	–0.85	–1.15
VI'	–0.87	–1.02

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

2.3. Electrochemical studies

The redox behaviour of the rhenium complexes **I′–VI′** and the coligands *p*-nitrothiophenol and (*p*-nitrophenyl)methanethiol was studied by cyclic voltammetry in order to assess if the reduction potentials fall in the appropriate range reported in the literature for bioreductive agents [9,10,28]. The complexes voltammetric data indicate that the reduction processes follow a simple pattern that involves two to three reduction waves. The first couple corresponds to the metal oxido-reduction process [29] being the nitro oxido-reduction process assigned to the second couple. The ligands present only one to two reduction waves in the studied range. Table 1 lists the values of the first and the second voltammetric cathodic peaks for all compounds. The reduction of the monooxo Re (V) core occurred between –0.80 and –0.90 V while the reduction of the nitro group in the complexes lay between –1.00 and –1.15 V. A dependence of the metal reduction on the structure of the coligand was observed, since the reduction potentials in the complexes containing the *p*-nitrothiophenoxide moiety (**IV′** and **VI′**) were more negative than the corresponding potential for the homologous derivatives with the *p*-nitrothiomethylphenoxide moiety (**I′–III′** and **V′**). All the complexes displayed more negative nitro reduction waves than the corresponding free coligands. Fig. 2 shows typical voltammograms for selected coligand and complex.

3. Pharmacology

3.1. Aerobic and hypoxic cytotoxicity

A standard method, using V79 Chinese hamster lung fibroblasts in culture, was used to assess the "in vitro" cytotoxicity of the rhenium complexes, both in oxia and hypoxia [30–33]. Cells were exposed to decreasing concentrations of compound **I′–IV′** (20–1 μM) for 2 hours, either in air or nitrogen atmo-

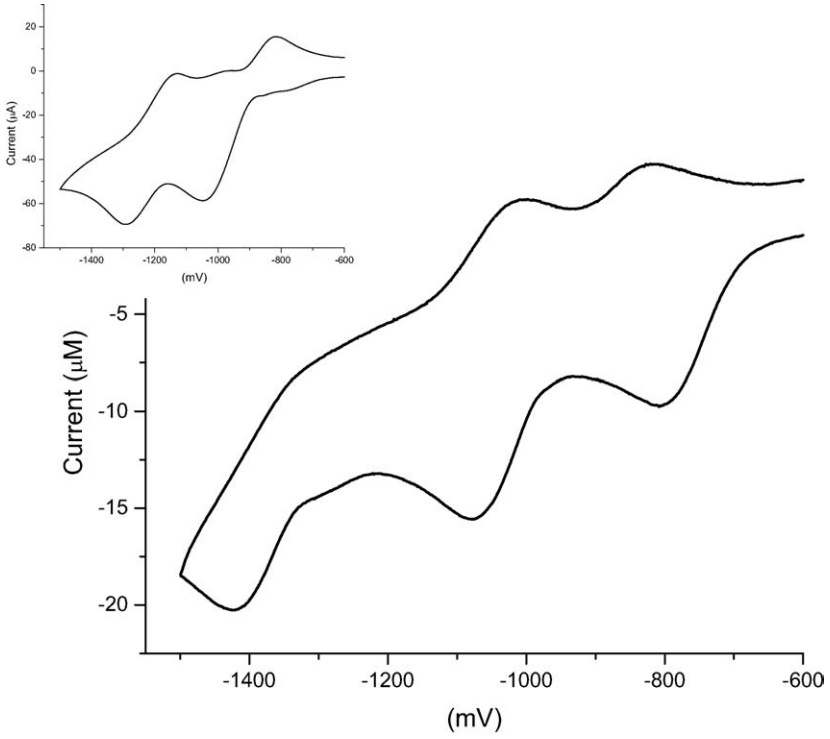


Fig. 2. Voltammogram of complex **I′** and the corresponding coligand *p*-$NO_2C_6H_4CH_2SH$ (inset).

Table 2
Cytotoxicity of complexes **I′–VI′** in V79 cells (Chinese hamster lung fibroblasts)

Complex	Concentration (μM)	% Survival	
		In air	In hypoxia
I′	20	79	100
II′	20	79	96
III′	20	0	0
	5	0	0
	1	80	100
IV′	20	0	0
	5	0	0
	1	100	100

sphere, and then separated from the potentially cytotoxic agents, washed and recultured for 7 days to determine the percentage of cells that have survived. The solvent (DMSO) and the compound 7-chloro-3-[3-(*N,N*-dimethylamine)propylamine]-2-quinoxalinecarbonitrile-1,4-dioxide hydrochloride, which has 100% survival in air and 0% in hypoxia, were used as negative and positive controls, respectively. The results, expressed as percentage of survival relative to the negative control, are shown in Table 2.

Complexes **I′** and **II′** were not significantly cytotoxic either in normoxia or in hypoxia, since the survival was very high (80% and 100%, respectively) already at the highest concentration level (20 μM). Complex **III′** and **IV′**, on the other hand, demonstrated high cytotoxicity both in air and hypoxia. The survival was negligible at 20 and 5 μM. It was necessary to dilute to a very low concentration (1 μM) in order to obtain high recovery of viable cells (80% in air and 100% in hypoxia). However, there is no evidence of selectivity for hypoxia.

These results demonstrate the importance of the structure of the tridentate ligand in the cytotoxicity of the overall complex. The presence of a dialkylamine moiety in the molecule seems to be necessary to achieve this action. On the other hand, small variations of the structure of the nitroaromatic coligand do not seem to affect the "in vitro" cytotoxicity.

Consequently, complexes **I** and **II** were not used for the "in vivo" evaluation. Furthermore, two additional complexes (**V** and **VI**) having the same coligands and a more lipophilic dialkylamine tridentate ligand (diisopropylamine moiety instead of diethylamine moiety) were also analyzed in order to study the effect of an increased lipophilicity in the biological behaviour.

3.2. Biodistribution studies

In order to assess the potentiality of our approach for the design of potential radiopharmaceuticals for nuclear oncology, evaluation in normal and tumour bearing mice was performed.

The results of the biodistribution studies in normal mice of ^{99m}Tc complexes **III–VI** at 0.5, 2 and 24 hours post-injection are shown in Tables 3a, 3b.

All compounds show high initial blood (7.6–11.6% dose), lung (3.0–4.2% dose) and liver uptake (27.0–29.7% dose) at 0.5 h post-injection. This behaviour is similar to previously reported "3 + 1" complexes [17,20]. Blood and lungs clearance was relatively fast (2.7–5.7% injected dose in blood and 0.9–2.3% dose in lungs at 2 h post-injection), while liver activity remained high for a longer period (17.3–23.5% injected dose at 2 h post-injection) The radioactivity was excreted mainly through the hepatobiliary system (43.6–51.3% dose in intes-

Table 3a
Biodistribution of complexes **III** and **VI** in normal CD1 mice

Organ	% Dose/organ					
	Complex III			Complex IV		
	0.5 Hours	2 Hours	24 Hours	0.5 Hours	2 Hours	24 Hours
Blood	7.6 ± 1.6	5.2 ± 2.6	0.34 ± 0.02	9.3 ± 1.5	2.70 ± 0.05	0.65 ± 0.19
Liver	27.2 ± 1.9	17.3 ± 1.2	2.4 ± 0.3	28.7 ± 1.8	23.5 ± 0.1	2.5 ± 0.2
Lungs	3.08 ± 0.01	1.2 ± 0.4	0.15 ± 0.04	3.0 ± 0.6	0.85 ± 0.48	0.60 ± 0.20
Thyroid	0.09 ± 0.03	0.05 ± 0.03	0.03 ± 0.01	0.10 ± 0.05	0.05 ± 0.03	0.05 ± 0.03
Stomach	1.9 ± 0.4	1.2 ± 0.3	0.11 ± 0.03	0.58 ± 0.07	0.36 ± 0.08	0.34 ± 0.08
Intestines + Faeces	26.7 ± 1.6	48.5 ± 3.8	65.0 ± 6.1	24.9 ± 4.2	51.3 ± 1.4	57.0 ± 5.0
Bladder + Urine	5.3 ± 1.2	9.9 ± 1.2	33.2 ± 5.0	10.3 ± 3.1	16.7 ± 3.9	35.4 ± 1.6

Table 3b
Biodistribution of complexes **V** and **VI** in normal CD1 mice

Organ	% Dose/organ					
	Complex V			Complex VI		
	0.5 Hours	2 Hours	24 Hours	0.5 Hours	2 Hours	24 Hours
Blood	11.6 ± 6.3	5.7 ± 0.7	3.2 ± 0.9	10.7 ± 1.8	3.9 ± 0.4	1.7 ± 0.5
Liver	27.0 ± 1.3	21.0 ± 0.2	7.5 ± 0.5	29.7 ± 3.7	17.5 ± 0.5	8.6 ± 1.2
Lungs	4.2 ± 0.7	2.3 ± 0.3	2.0 ± 0.4	3.7 ± 0.5	2.3 ± 0.3	3.6 ± 1.8
Thyroid	0.19 ± 0.04	0.09 ± 0.02	0.08 ± 0.03	0.14 ± 0.03	0.10 ± 0.03	0.06 ± 0.03
Stomach	1.7 ± 0.2	1.1 ± 0.2	0.4 ± 0.1	1.1 ± 0.2	0.6 ± 0.1	0.37 ± 0.09
Intestines + Faeces	24.8 ± 3.9	43.6 ± 2.7	59.8 ± 4.1	24.0 ± 1.0	45.5 ± 1.1	62.7 ± 5.8
Bladder + Urine	7.9 ± 0.1	13.60 ± 1.90	19.9 ± 1.3	9.2 ± 0.8	18.8 ± 2.25	11.6 ± 2.9

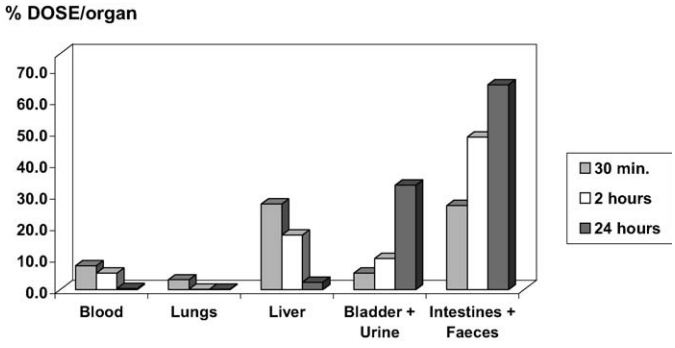


Fig. 3. Biodistribution of complex **III** in normal CD1 mice.

Table 4
Tumour uptake and retention of complexes **III–VI** in CD1 mice bearing induced sarcoma

Complex	Time (hours)	% Dose per gram		
		Tumour (T)	Muscle (M)	(T/M)
III	0.5	1.22 ± 0.17	0.82 ± 0.19	1.5
	2	0.63 ± 0.02	0.21 ± 0.02	3.0
	24	0.23 ± 0.06	0.07 ± 0.02	3.2
IV	0.5	0.88 ± 0.08	0.66 ± 0.15	1.3
	2	0.35 ± 0.02	0.20 ± 0.01	1.8
	24	0.35 ± 0.09	0.13 ± 0.11	2.7
V	0.5	1.36 ± 0.08	1.61 ± 0.16	0.84
	2	0.96 ± 0.2	0.98 ± 0.27	0.98
	24	1.3 ± 0.5	0.6 ± 0.1	2.2
VI	0.5	1.21 ± 0.04	1.27 ± 0.23	0.95
	2	1.22 ± 0.10	0.81 ± 0.06	1.5
	24	1.5 ± 0.1	0.67 ± 0.02	2.2

tines at 2 h post-injection). On the other hand, urinary excretion was lower (9.9–18.8% dose in urine at 2 h post injection). Depuration from blood and soft tissues, after 24 h, was almost complete and excretion after this period above 75%. Stomach and thyroid values were within acceptable levels (approx. 1% and 0.1%, respectively, at 2 h post-injection) demonstrating no "in vivo" reoxidation. Overall "in vivo" behaviour as a function of time for complex **III** is shown in Fig. 3.

Biodistribution results for ^{99m}Tc complexes **III–VI** in mice bearing induced sarcoma as a function of time is shown in Table 4. Initial tumour uptake was moderate (0.9–1.4% per g at 30 min post-injection) but clearance was rather slow (0.4–1.2% per g and 0.23–2.2% per g after 2 and 24 h, respectively).

Tumour to muscle ratio was favourable at all time points for complexes **III** and **IV** and showed a significant increase with time (1.5 and 1.3, 3.0 and 1.75 and 3.2 and 2.7 at 30 min, 2 and 24 h post-injection, respectively) due to soft tissue depuration. Complexes **V** and **VI**, on the other hand, showed higher uptake in tumour at all time points but blood and muscle activity were also significantly higher, probably due to the increased lipophilicity.

3.3. Gamma-camera imaging

In order to corroborate biodistribution results and asses the feasibility of using the studied compounds for nuclear imaging, static gamma-camera images were acquired for complex **III** at 2 and 12 h post-injection.

Images allowed visualisation of the tumour and calculated tumour/soft tissue ratios were coincident with those obtained from biodistribution studies (2 and 3.8 at 2 and 12 h, respec-

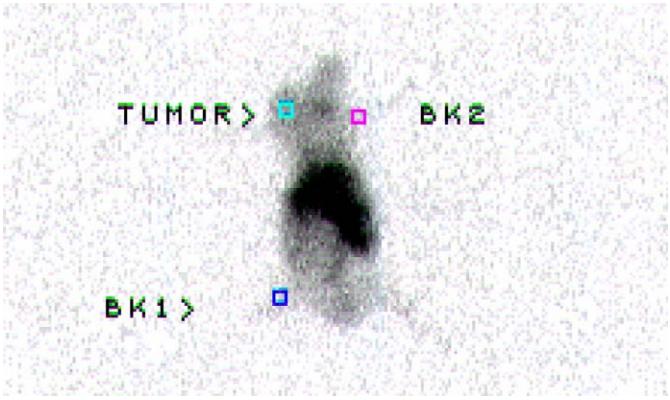


Fig. 4. Scintigraphic image of complex **III** in CD1 mice bearing induced sarcoma at 12 hours post-injection.

tively). However, high gastrointestinal activity due to preferential hepatobiliary excretion is an important drawback for imaging purposes. Scintigraphic image of complex **III** at 12 hours post-injection is shown in Fig. 4.

4. Conclusions

A series of six ^{99m}Tc "3 + 1" mixed ligand complexes formed by simultaneous coordination of four different tridentate aminodithiols and two monodentate thiols bearing nitroaromatic moieties were prepared and evaluated as potential bioreductive radiopharmaceuticals.

Labelling was achieved in high yield (> 85%) and with high radiochemical purity (> 90%) in all cases. The proposed structure was corroborated by means of the analogous rhenium complexes using standard analytical methods (UV–vis, IR, ^{1}H NMR spectroscopy and HPLC and elemental analysis). Presence of ligand and coligand was assessed as well as deprotonation of the three thiol groups upon complexation. As a result of ionization complexes are neutral and lipophilic as demonstrated by their solubility in organic solvents.

Reduction potentials of both the nitroaromatic group and the $(MO)^{+3}$ moiety for all rhenium complexes were determined and found to be in the expected range for potentially bioreductive compounds [28,34–36] (i.e. tirapazamine, $E_{pc} = -0.90$ V vs. SCE [35]). "In vitro" cytotoxicity in oxia and hypoxia was studied for rhenium complexes **I′–IV′** in order to asses the influence of the structure of ligand and coligand in their interaction with cells in culture in the presence and absence of oxygen. Results indicated that the presence of a dialkylamine moiety in the molecule seems to be necessary to achieve this interaction. On the other hand, small variation of the structure of the nitroaromatic coligand does not seem to affect the "in vitro" cytotoxicity. Selectivity of this action in hypoxic conditions could not be assessed.

Taking these results into account, "in vivo" experiments were carried out exclusively using ^{99m}Tc complexes **III–VI**. Overall biodistribution in normal animals was the expected for this type of compounds. Initial tumour uptake was moderate but slow clearance from tumour together with fast depuration for blood and soft tissue leads to favourable tumour to muscle ratios for complexes **III** and **IV,** which also showed a significant increase with time. Complexes **V** and **VI**, on the other hand, showed higher uptake in tumour at all time points but blood and muscle activity were also significantly higher, probably due to the increased lipophilicity. Static gamma-camera images of complex **III** allowed visualization of the tumour and calculated tumour/soft tissue ratios were coincident with those obtained from biodistribution studies. However, high gastrointestinal activity due to preferential hepatobiliary excretion is an important drawback for imaging purposes.

Although these results are encouraging further development is still necessary in order to achieve higher tumour uptake and lower gastrointestinal activity. Furthermore, preparation and evaluation of ^{188}Re-labeled oxorhenium(V) complexes will be the next step since electrochemical properties and biodistribution of analogous technetium and rhenium complexes might be significantly different due to the variation in the oxido-reduction potentials of both metals.

5. Experimental

5.1. General

All laboratory chemicals were reagent grade and used without further purification. Solvents used for chromatographic analysis were HPLC grade. The tridentate ligands were synthesized according to published procedures [37,38]. The monodentate thiols used as coligands were purchased from Fluka. [^{99m}Tc]$NaTcO_4$ was obtained from Elumatic III generator, Cis Bio-international. IR spectra were recorded as KBr pellets in the range 4000–200 cm^{-1} Bomen MB-102 FT-IR spectrophotometer. Elemental analysis was performed on a Carlo Erba EA 1108 analyzer. The NMR spectra were recorded in deuteriochloroform on a Bruker AC 250E spectrometer. Chemical shifts are reported with respect to TMS. HPLC analysis was developed a reverse phase μ Bondapack C18 column (3.9 × 300 mm), eluted with a binary gradient system at a 1.0 ml min^{-1} flow rate. Mobile phase A is phosphate buffer pH 7.4 with 2% triethylamine while mobile phase B is methanol. The elution profile is: 0 min 0% B followed by a linear gradient to 85% B in 7 min; this composition was held for another 15 min. Detection was either accomplished with a photodiode array detector (SPD-M10A, Shimadzu) that recorded UV–vis spectra on flux or a 3″ × 3″ NaI (Tl) crystal scintillation detector. Activity measurements were performed either in a Dose Calibrator, Carpintec CRC-5R or in a scintillation counter, 3″ × 3″ NaI (Tl) crystal detector associated to an ORTEC monochannel analyser.

5.2. Chemistry

5.2.1. Preparation of [^{99m} Tc] technetium compounds

Preparation of the ^{99m}Tc complexes **I-VI** was accomplished by using ^{99m}Tc-glucoheptonate as precursor [18]. A vial containing a lyophilized mixture of 200 mg calcium glucoheptonate and 0.2 mg $SnCl_2.2H_2O$ was reconstituted with 5 ml water

and 0.5 ml of this solution was mixed with [^{99m}Tc] $NaTcO_4$ (0.5–1 ml with an activity of 5–50 mCi (185–1850 MBq). Substitution was performed at coligand/ligand molar ratio of 1 for all complexes, by adding the precursor (with radiochemical purity > 95%) to a centrifuge tube containing the ligand (0.02 mmol) and the coligand (0.02 mmol). The mixture was stirred in a vortex mixer and left to react at room temperature for 10 min. The lipophilic species were extracted with CH_2Cl_2 and the organic layer dried with $MgSO_4$, filtered and analyzed by HPLC.

5.2.2. Structural studies

Rhenium complexes **I′–VI′** were prepared by substitution by mixing the precursor trichlorobis(triphenylphosphine)rhenium(V) oxide and equimolecular amounts of ligand and coligand.

The typical synthetic procedure is given for complex **III**: To a stirred suspension of trichlorobis(triphenylphosphine)oxorhenium(V) (166 mg, 0.2 mmol) in methanol (10 ml) CH_3COONa (1 N in methanol, 2 ml, 2 mmol) was added. A mixture of *N,N*-diethyl-*N′*-(2-mercaptoethyl)ethylenediamine (47 mg, 0.2 mmol) and (*p*-nitrophenyl) methanethiol (33.8 mg, 0.2 mmol) was added under stirring. The solution was heated at reflux until the green-yellow colour of the precursor turned to green. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2 (30 ml) and washed with water. The organic layer was separated from the mixture and dried over $MgSO_4$. The volume of the solution was reduced to 5 ml and then 5 ml of methanol was added.

TLC showed the formation of one complex in all cases. Slow evaporation of the solvents at room temperature afforded the products of the reaction as solids.

5.2.2.1. ***Complex I′***. ReO[EtN($CH_2CH_2S)_2$][*p*-$NO_2C_6H_4CH_2S$], green crystals. Yield: 48%. Analysis for $C_{13}H_{19}N_2O_3S_3Re$ Theoretical: C, 29.21; H, 3.59; N, 5.24. Experimental: C, 29.75; H, 3.43; N, 5.36. IR: (cm^{-1}, KBr): 953 (ReO), 1516, 1342 (NO_2). ^{1}H NMR (250 MHz, $CDCl_3$) δ: 1.35 (t, 3H, $J = 7.5$ Hz, NCH_2CH_3*), 2.65,3.38 (m, 4H, SCH_2*CH_2N), 2,99, 3.62 (m, 4H, SCH_2CH_2*N), 3.88 (q, 2H, $J = 7.5$ Hz, NCH_2*CH_3), 4.92 (s, 2H, SCH_2*-φ), 7.53 (d, 2H, $J = 10$ Hz, aromatic H), 8.15 (d, 2H, $J = 10$ Hz, aromatic H).

5.2.2.2. ***Complex II′***. ReO[$CH_3CH_2SCH_2CH_2N(CH_2CH_2S)_2$] [*p*-$NO_2C_6H_4CH_2S$], green crystals. Yield: 53%. Analysis for $C_{15}H_{23}N_2O_3S_4Re$, Theoretical: C, 30.30; H, 3.90; N, 4.71. Experimental: C, 30.93; H, 3.80; N, 5.57. IR: (cm^{-1}, KBr): 950 (ReO), 1513, 1341 (NO_2). ^{1}H NMR (250 MHz, $CDCl_3$) δ: 1.32 (t, 3H, $J = 7.5$ Hz, SCH_2CH_3*), 2.60 (q, 2H, $J = 7.5$ Hz, SCH_2*CH_3), 2.70 (t, 2H, $J = 7.5$ Hz, NCH_2CH_2 *S), 2.90,3.35 (m, 4H, $ReSCH_2$*CH_2N), 3.02, 3.65 (m, 4H, $ReSCH_2CH_2$*N), 3.95 (t, 2H, $J = 7.5$ Hz, NCH_2*CH_2S), 4.95 (s, 2H, SCH_2*-φ), 7.55 (d, 2H, $J = 10$ Hz, aromatic H), 8.20 (d, 2H, $J = 10$ Hz, aromatic H).

5.2.2.3. ***Complex III′***. ReO[$(Et)_2NCH_2CH_2N(CH_2CH_2S)_2$][*p*-$NO_2C_6H_4CH_2S$], green crystals. Yield: 69 %. Analysis for $C_{17}H_{28}N_3O_3S_3Re$, Theoretical: C, 33.71; H, 4.66; N, 7.05. Experimental: C, 33.92; H, 4.73; N, 7.05. IR: (cm^{-1}, KBr): 943 (ReO), 1511, 1341 (NO_2). ^{1}H NMR (250 MHz, $CDCl_3$) δ: 1.05 (t, 6H, $J = 7.5$ Hz, NCH_2CH_3*), 2.57 (q, 4H, $J = 7.5$ Hz, NCH_2*CH_3), 2.85 (t, 2H, $J = 7.5$ Hz, NCH_2CH_2 *N), 3.00,3.48 (m, 4H, SCH_2*CH_2N), 2.75, 3.62 (m, 4H, SCH_2CH_2*N), 3.85 (t, 2H, $J = 7.5$ Hz, NCH_2*CH_2N), 4.91 (s, 2H, SCH_2*-φ), 7.55 (d, 2H, $J = 10$ Hz, aromatic H), 8.15 (d, 2H, $J = 10$ Hz, aromatic H).

5.2.2.4. ***Complex IV′*** *[39]*. ReO[$(Et)_2NCH_2CH_2N(CH_2CH_2S)_2$][*p*-$NO_2C_6H_4S$], green crystals. Yield: 40.0 %. Analysis for $C_{16}H_{26}N_3O_3S_3Re$, Theoretical: C: 32.53; H, 4.44; N, 7.11. Experimental: C, 32.94; H, 4.62; N, 7.01 IR:(cm^{-1}, KBr): 960 (ReO) 1514, 1338 (NO_2), ^{1}H NMR (250 MHz, $CDCl_3$) δ: 1.11 (t, 6H, $J = 7.5$ Hz, NCH_2CH_3*), 2.58 (q, 4H, $J = 7.5$ Hz, NCH_2*CH_3), 2.64 (t, 2H, $J = 7.5$ Hz, NCH_2CH_2 *N), 2.88,3.48 (m, 4H, SCH_2*CH_2N), 2.93, 3.60 (m, 4H, SCH_2CH_2*N), 3.94 (t, 2H, $J = 7.5$ Hz, NCH_2*CH_2N), 7.80 (d, 2H, $J = 10$ Hz, aromatic H), 8.22 (d, 2H, $J = 10$ Hz, aromatic H).

5.2.2.5. ***Complex V′***. ReO[$(ipr)_2NCH_2CH_2N(CH_2CH_2S)_2$] [*p*-$NO_2C_6H_4CH_2S$], green crystals . Yield: 41.5%. Analysis for $C_{19}H_{32}N_3O_3S_3Re$, Theoretical: C, 36.06; H, 5.10; N, 6.64. Experimental: C, 36.20; H, 5.25; N, 6.57 IR:(cm^{-1}, KBr): 947 (ReO) 1514, 1341 (NO_2), ^{1}H NMR (250 MHz, $CDCl_3$) δ: 1.04 (d, 12H, $J = 7.5$ Hz, $NCH(CH_3)_2$*), 2.18 (m, 2H, NCH*$(CH_3)_2$), 3.01 (t, 2H, $J = 7.5$ Hz, NCH_2CH_2*N), 2.73,3.46 (m, 4H, SCH_2*CH_2N), 3.05, 3.65 (m, 4H, SCH_2CH_2*N), 3.80 (t, 2H, $J = 7.5$ Hz, NCH_2*CH_2N), 4.94 (s, 2H, SCH_2*-φ), 7.80 (d, 2H, $J = 10$ Hz, aromatic H), 8.22 (d, 2H, $J = 10$ Hz, aromatic H).

5.2.2.6. ***Complex VI′***. ReO[$(ipr)_2NCH_2CH_2N(CH_2CH_2S)_2$][*p*-$NO_2C_6H_4S$], green crystals. Yield: 38.3%. Analysis for $C_{18}H_{30}N_3O_3S_3Re$, Theoretical: C, 34.94; H, 4.89; N, 6.79. Experimental: C, 35.40; H, 5.27; N, 6.75, IR:(cm^{-1}, KBr) :950 (ReO) 1514, 1341 (NO_2), ^{1}H NMR (250 MHz, $CDCl_3$) δ: 1.02 (d, 12H, $J = 7.5$ Hz, $NCH(CH_3)_2$*), 2.18 (m, 2H, NCH*$(CH_3)_2$), 2.97 (t, 2H, $J = 7.5$ Hz, NCH_2CH_2*N), 2.67,3.46 (m, 4H, SCH_2*CH_2N), 3.11, 3.66 (m, 4H, SCH_2CH_2*N), 3.85 (t, 2H, $J = 7.5$ Hz, NCH_2*CH_2N), 7.79 (d, 2H, $J = 10$ Hz, aromatic H), 8.21 (d, 2H, $J = 10$ Hz, aromatic H).

5.2.3. Electrochemical studies

Electrochemical behaviour of the six rhenium complexes and the two coligands was studied by cyclic voltammetry. Anhydrous DMF (spectroscopic grade) was obtained from Aldrich. Tetrabutylammonium perchlorate (0.1 mol ml^{-1}), 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 performed at different scan rates, between 0.05 and 2.0 V s^{-1} 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.

5.3. Biological studies

5.3.1. Aerobic and hypoxic cytotoxicity

V79 cells (Chinese hamster lung fibroblasts) were obtained from ECACC (European Collection of Animal Cell Cultures) and maintained in logarithmic growth as subconfluent monolayers by trypsinization and subculture to $1–2 \times 10^4$ cells ml^{-1} twice weekly. The growth medium was EMEM (Eagle's minimal essential medium), containing 10% (v/v) foetal bovine serum (FBS) and penicillin/streptomycin at 100 UI/100 μg ml^{-1}.

Monolayers of V79 cells in exponential growth were trypsinized, and suspension cultures were set up in 50 ml glass flasks: 2×10^4 cells ml^{-1} in 30 ml of EMEM, containing 10% (v/v) FBS and HEPES (10 mM). Flasks were stirred in a water bath at 37 °C, where they were gassed with humidified air or pure nitrogen.

Compounds **I′** to **IV′** stock solutions 150-fold more concentrated were prepared in pure DMSO. Thirty minutes after the start of gassing stock solution was diluted to final concentration 20–0.1 μM and 0.2 ml were added to each flask, two flasks per dose. In every assay there was one flask with 0.2 ml of DMSO as negative control and one flask with 7-chloro-3-[3-(*N,N*-dimethylamine)propylamine]-2-quinoxalinecarbonitrile-1,4-dioxide hydrochloride (positive control).

After 2 h exposure to the compounds, cells were centrifuged and resuspended in plating medium (EMEM plus 15% (v/v) FBS and penicillin/streptomycin). Cell numbers were determined with a haemocytometer and $10^2–10^3$ cells were plated in 6-well plates to give a final volume of 2 ml per 30 mm of well. Plates were incubated at 37 °C in 5% CO_2 for 7 days and then stained with aqueous crystal violet. Colonies with more than 64 wells were counted. The plating efficiency (PE) was calculated by dividing the number of colonies by the number of cells seeded. The percent of control cell survival for the compound-treated cultures was calculated as PE (treated)/PE (control) × 100.

5.3.3. Biodistribution studies

Animal studies were approved by the Ethics Committee of the Faculty of Chemistry from Uruguay. In vivo evaluation of ^{99m}Tc complexes was performed by biodistribution using animals bearing induced tumours.

A suspension of sarcoma cells CCRF-180II in PBS, containing 2.5×10^6 cells ml^{-1} was prepared and 200 μl were injected subcutaneously in the right limb of normal CD1 mice (female, 8–10 weeks old 25–30 g). Ten to 15 days later the animals developed palpable tumour nodules and were used for biodistribution.

Four animals per group were injected via a lateral tail vein with the HPLC purified ^{99m}Tc complex reconstituted with 30% methanol (0.1 ml, 37–370 KBq [1–10 μCi]). At different intervals after injection (1–60 min) 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 per organ and % Dose per gram. The tumour/muscle ratios were calculated from the corresponding percent dose per gram values.

5.3.4. Gamma-camera imaging

Imaging was performed using a rectangular field (21.2 × 15.7 inches) gamma/camera (Sophy Camera DSX) equipped with a low energy, high resolution, parallel hole collimator. Static images from mice bearing induced sarcoma were acquired at 2 and 12 hours after intravenous injection of HPLC purified complex **III** reconstituted with 25:75 methanol/saline (0.1 ml, 10–15 MBq). A 256 × 256 matrix with a 2.66 zoom factor was employed and images were digitally stored for further analysis. Identical regions of interest were placed on the limb containing the tumour and the contralateral one and activity ratios were calculated using the activity/pixel.

Acknowledgements

Comisión Honoraria de Lucha contra el Cáncer, Pedeciba-Química, Professor S. Cáceres and J. Batistoni, Lab. de Biotecnología, Polo Tecnológico, Fac. de Química, Centro de Medicina Nuclear, Fac. de Medicina, Universidad de la República, Uruguay.

References

[1] R. Yyer, E. Engelhardt, C. Stobbe, R. Schneider, J. Chapman, Int. J. Radiat. Oncol. Biol. Phys. 42 (1998) 741–745.
[2] J.E. Moulder, S. Rockwell, Cancer Metastasis Rev. 5 (1987) 313–341.
[3] G.J. Cook, Br. J. Radiol. 2 (2003) S152–S158.
[4] R. Kowalsky, S. Fallen, in: Radiopharmaceuticals in Nuclear Pharmacy and Nuclear Medicine Second Edition, American Pharmacists Association, Washington DC, 2004, pp. 1–15.
[5] J. Dilworth, S. Parrot, Chem. Soc. Rev. 27 (1998) 43–55.
[6] S. Jurisson, J. Lyndon, Chem. Rev. 99 (1999) 2205–2218.
[7] S. Jurisson, D. Berning, W. Jia, D. Ma, Chem. Rev. 93 (1993) 1137–1156.
[8] W. Volkert, T. Hoffman, Chem. Rev. 99 (1999) 2269–2292.
[9] H. Cerecetto, M. González, Mini Rev. Med. Chem. 1 (2001) 219–231.
[10] W.A. Denny, W.R. Wilson, M.P. Hay, Br. J. Cancer 74 (1996) S32–S38.
[11] K.E. Linder, Y.W. Chan, J.E. Cyr, M.F. Malley, D.P. Nowotnik, A.D. Nunn, J. Med. Chem. 37 (1994) 9–17.
[12] T. Melo, J. Duncan, J.R. Ballinger, A.M. Rauth, J. Nucl. Med. 41 (2000) 169–176.
[13] G.J. Cook, S. Houston, S.F. Barrington, I. Fogelman, J. Nucl. Med. 39 (1998) 99–103.

[14] T. Chu, S. Hu, B. Wei, Y. Wang, X. Liu, X. Wang, Bioorg. Med. Chem. Lett. 14 (2004) 747–749.
[15] M.B. Mallia, A. Mathur, S. Subramanian, S. Banerjee, H.D. Sarma, M. Venkatesh, Bioorg. Med. Chem. Lett. 15 (2005) 3398–3401.
[16] A. Rey, I. Pirmettis, M. Pelecanou, M. Papadopoulos, C.P. Raptopoulou, L. Mallo, C.I. Stassinopoulou, A. Terzis, E. Chiotellis, A. León, Inorg. Chem. 39 (19) (2000) 4211–4218.
[17] A. León, A. Rey, L. Mallo, M. Papadopoulos, I. Pirmettis, E. León, M. Pagano, E. Manta, M. Incerti, C. Raptopoulou, A. Terzis, E. Chiotellis, Nucl. Med. Biol. 29 (2002) 217–226.
[18] A. Rey, I. Pirmettis, M. Papadopoulos, J. Giglio, E. León, F. Schreiber, A. Paolino, M. Fernández, R. Fernández, E. Manta, J. Chabalogoity, A. León, World J. Nuc. Med. 4 (2005) 111–119.
[19] H. Cerecetto, M. González, S. Onetto, M. Risso, A. Rey, J. Giglio, E. León, A. León, P. Pilatti, M. Fernández, Arch. Pharm. (Weinheim) 339 (2006) 59–66.
[20] A. Rey, M. Papadopoulos, L. Mallo, I. Pirmettis, E. León, C. Raptopoulou, E. Manta, E. Chiotellis, A. León, J. Lab. Cpd. Radiopharm. 43 (2000) 347–358.
[21] A. Rey, M. Papadopoulos, E. León, L. Mallo, I. Pirmettis, E. Manta, C. Raptopoulou, E. Chiotellis, A. Leon, Appl. Radiat. Isot. 53 (2001) 429–434.
[22] M. Papadopoulos, I. Pirmettis, C. Raptopoulou, E. Chiotellis, M. Friebe, R. Berger, H. Spies, B. Johannsen, Appl. Radiat. Isot. 49 (1998) 961–966.
[23] E. Deutsch, K. Libson, J.-L. Vanderheyden, Technetium, Rhenium and other Metals in Chemistry and Nuclear Medicine 3, in: M. Nicolini, G. Bandolli, U. Mazzi (Eds.), Cortina Int. Verona, 1990, pp. 13–22.
[24] L. Marzilli, M. Banaszcyk, L. Hansen, Z. Kuklenyik, R. Cini, A. Taylor, Inorg. Chem. 33 (1994) 4850–4860.
[25] J. Chatt, G. Rowe, J. Chem. Soc. (1962) 4019–4033.
[26] I. Pirmettis, M. Papadopoulos, S. Mastrostamatis, C. Raptopoulou, A. Terzis, E. Chiotellis, Inorg. Chem. 35 (1996) 1685–1691.
[27] M. Papadopoulos, M. Pelecanou, I. Pirmettis, D. Spyriounis, C. Raptopoulou, A. Terzis, C. Stassinopoulou, E. Chiotellis, Inorg. Chem. 35 (1996) 4478–4483.
[28] P. Wardman, Curr. Med. Chem. 8 (2001) 739–761.
[29] M.F. Cerdá, G. Obal, E. Méndez, A.M. Castro Luna, M.E. Martins, C. Kremer, C.F. Zinola, J. Colloid Int. Sci. 236 (2001) 104–107.
[30] A. Monge, F.J. Martínez-Crespo, A. López de Ceráin, J.A. Palop, S. Narro, V. Senador, A. Marín, Y. Sáinz, M. González, E. Hamilton, A.J. Barker, J. Med. Chem. 38 (1995) 4488–4494.
[31] M.A. Ortega, M.J. Morancho, F.J. Martínez-Crespo, Y. Sáinz, M.E. Montoya, A. López de Ceráin, A. Monge, Eur. J. Med. Chem. 35 (2000) 21–30.
[32] M.H. Torre, D. Gambino, J. Araujo, H. Cerecetto, M. González, M.L. Lavaggi, A. Azqueta, A. López de Cerain, A. Monge-Vega, U. Abram, A.J. da Costa-Filho, Eur. J. Med. Chem. 40 (2005) 473–483.
[33] H. Cerecetto, M. González, M.L. Lavaggi, A. Azqueta, A. López de Cerain, A. Monge, J. Med. Chem. 48 (2005) 21–24.
[34] W. Wilson, R.F. Anderson, W.A. Denny, J. Med. Chem. 32 (1989) 23–30.
[35] A. Monge, J.A. Palop, A. López de Ceráin, V. Senador, F.J. Martínez-Crespo, Y. Sáinz, S. Narro, E. García, C. de Miguel, M. González, E. Hamilton, A.J. Barker, E.D. Clarke, D.T. Greenhow, J. Med. Chem. 38 (1995) 4488–4494.
[36] H. Cerecetto, M. González, M.L. Lavaggi, C. Rigol, C. Olea-Azar, A. Azqueta, A. López de Cerain, A. Monge, A.M. Bruno, Med. Chem. (2006) (in press).
[37] J. Corbin, K. Miller, N. Pariyadath, S. Wherland, L. Bruce, E. Stiefel, Inorg. Chim. Acta 90 (1984) 41–51.
[38] J. Harley-Mason, J. Chem. Soc. (1947) 320–322.
[39] M. Papadopoulos, I. Pirmettis, C. Raptopoulou, E. Chiotellis, M. Friebe, R. Berger, H. Spies, B. Johannsen, Appl. Radiat. Isot. 49 (1998) 961–966.