Inorganic and Radiopharmaceutical Chemistry

Isabel Rego Santos

The main goal of the Inorganic and Radiopharmaceutical Chemistry Group is the design, synthesis and characterization of novel radioactive probes (specific radiopharmaceuticals) for non-invasive molecular imaging of targeted macromolecules and biological processes associated with different pathologies. The use of some of these probes for therapy, by using β or Auger emitters, produced at the RPI, is also one of our goals.

This group is multidisciplinary, combining expertise on the synthesis and characterization of organic compounds and inorganic and organometallic complexes of *d*- and *f*-elements, as well as on radiochemistry and radiopharmacology. Such a combination, unique in the country, allows the synthesis of compounds at the macroscopic level and their characterization in the solid state (X-ray diffraction analysis) and in solution (multinuclear NMR spectroscopy and HPLC). The expertise and infrastructures, implemented and maintained by the group, also allow the synthesis of the corresponding radioactive compounds (halogen and metal–d and f-radiotracers) and their characterization and biological evaluation, using nuclear techniques and animal models.

The expertise and infrastructures enable basic and applied-oriented research in modern Radiopharmaceutical Chemistry, an important topic in life science. This justifies our participation as a partner in National and International research projects and the support of a Pharmaceutical Company. Our know-how on chemistry, radiochemistry and radiopharmacy and our facilities are used to provide training and education to undergraduate, graduate and post-graduate students.

Due to the Nuclear Medicine needs, background and facilities of the group and due to the available conditions at the RPI, we centered our research on:

I. Halogen-Based Radiotracers: During 2003, estradiol analogues and triphenylethylene derivatives have been isolated characterized and labelled with $^{125/131}$I. The i*n vitro* and *in vivo* behaviour of these compounds is being evaluated.

II. Metal *d*- and *f*- Based Radiotracers: During 2003, we have synthesized and characterized several Re and Tc complexes anchored by heterofunctionalized phosphines or by soft scorpionates functionalised with biomolecules with affinity for the $5HT_{1A}$ receptors. Structure/activity relationship has been established and we concluded that some of our complexes present IC_{50} values (0.172 – 0.710 nM) and specificity which are among the best values reported, so far. The evaluation of the capability of these complexes for crossing the BBB is underway. For CNS imaging, we have also shown that our PNS phosphine allows the synthesis of mixed–ligand complexes of the ″3+1″ type stable *in vitro* and *in vivo*.

For tumor imaging, we have also shown that Re and Tc complexes anchored by pyrazolyl containing ligands are stable *in vitro* and *in vivo* and can be coupled to active peptides, namely somatostatin and minigastrin analogues, leading to stable complexes. The biological evaluation of these *biocomplexes* is underway.

Having in mind β or Auger emitters for targeted anti - tumor therapy, acyclic and cyclic polyamines have been designed and their chemistry studied with Re, Tc, Cu and Ln. Several complexes were synthesized and characterized, and the cytotoxicity and genotoxicity evaluation of some of the compounds was initiated. With Ln, the chemical, radiochemical and biological effect of introducing a pyridine moiety on cyclic polyamines was evaluated, and these studies were also extended to 13- and 14-membered tetraazamacrocycles with different pendant arms.

III. Training: During 2003, young scientists, funded by FCT grants, have been trained in our laboratories, namely BIC, PhD and Post-Doctoral researchers. Radiopharmacy was also taught, in a regular way, in the Technical Nuclear Medicine Course (NMC), at the Escola Superior de Tecnologias da Saúde de Lisboa (ESTeSL) and in the Faculty of Pharmacy of the University of Lisbon. In our laboratories twenty students from the NMC/ESTeSL and two students from the Physics Engineering Graduation Course /IST, Technical University of Lisbon, have been trained during two and one week, respectively. The group has collaborated in a Post-Graduate Teaching Program, organized by ITQB, New University of Lisbon. At the International level we participated in the European Radiopharmacy Course, INSTN, and we are one of the three users participating in the European Community Shared COST RTD ACTION, Virtual Radiopharmacy (VIRAD) within the V Framework Program. We are partner on a Coordination Action on Education and Training in Radiation Protection, approved within the VI Framework Program of the EC. Together with the DQB of the Faculty of Sciences, UL, the Group presented a proposal for a Post-Graduation Course on ″Biomedical Inorganic Chemistry: Imaging and Therapeutical Aplications″, which has been accepted and will be running during 2004/2005. Our expertise has also been provided to some Nuclear Medicine Centers, to the Portuguese Medicines Evaluation Agency, to CRP/AIEA and to Natural Sciences Research Council/Canada.

Inorganic and Radiopharmaceutical Chemistry

Research Team

Researchers

- I. SANTOS, Principal Researcher/Group Leader
- A. PAULO, Auxiliary Researcher
- J.D.G. CORREIA, Auxiliary Researcher
- M.P.C. CAMPELLO, Auxiliary Researcher
- M.C. MELO e SILVA, Auxiliary Researcher
- C. FERNADES, Research Assistant
- L. GANO, Auxiliary Researcher
- F. MARQUES, Auxiliary Researcher
- P. RAPOSINHO, Auxiliary Researcher

Students

- L. MARIA, Post-Doctoral, FCT Grant
- S. ALVES, PhD student, FCT Grant
- R. GARCIA, PhD student, FCT Grant
- R. VITOR, PhD student, FCT Grant
- R. OLIVERA, MSc student, FCT Grant
- E. PALMA, BIC student
- S. LACERDA, BIC student
- C. XAVIER, BIC student

Technical Personnel

- AMADEU RODRIGUES
- ELIZABETE CORREIA

Funding (€)

Research Projects:	95.985,00
Services:	2.469,05
Total:	**98.454,05**

Publications

Books:	1
Journals:	18 (4 in press)
Conf.Communications:	23
Other publications:	4
Patents:	1 (in progress)
THESES:PHD	1

Re and Tc Complexes Anchored by Soft Scorpionates: Development of Radiopharmaceuticals for CNS Receptor Targeting

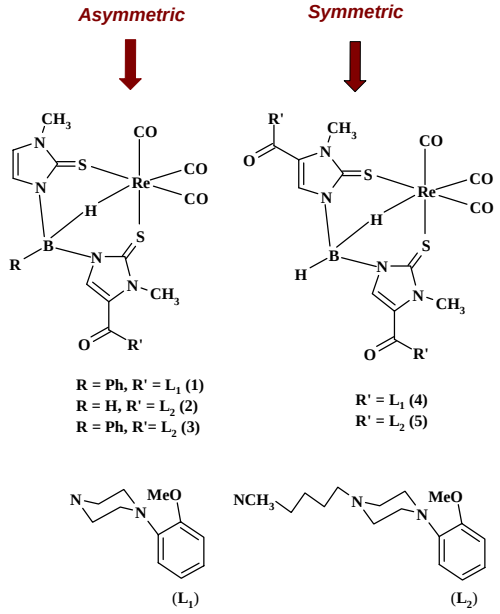
R. Garcia, C. Xavier, L. Maria, A. Paulo, I. Santos, H. Spies,[1]H.-J. Pietzsch, [1] R. Bergmann[1]

Objectives

Development of radiopharmaceuticals for the targeting of CNS receptors, based on Tc and Re carbonyl complexes anchored by soft scorpionates of the bis(mercaptoimidazolyl)borate type.

Results

Bis(mercaptoimidazolyl)borate anchors have been functionalized, in a symmetric or asymmetric fashion, with 1-(2-methoxy)arylpiperazines fragments that are known to recognise selectively the 5-HT_{1A} subclass of serotonergic receptors. The incorporation of the biologically active fragment was done at the 5-position of the imidazole ring, using different spacer lengths between the azole and the pharmacophore (Scheme 1).



Scheme 1 – Functionalized rhenium complexes

Functionalised Re tricarbonyl complexes (**1**-**5**) were synthesised with the symmetric and asymmetric bis(mercaptoimidazolyl)borates, using $(NEt_4)_2[ReBr_3(CO)_3]$ as starting material. The new complexes have been fully characterised, including by X-ray diffraction analysis in the case of **1** and **4**, and their affinities for the 5-H_{T1A} receptors were measured by *in vitro* binding assays.

The increase of the spacer length improved dramatically the affinity for the 5-HT_{1A} receptors (Table 1). Compounds with the longer spacer length (**2**, **3** and **5**) display IC_{50} values (0.172-0.71 nM) which are among the best values reported so far for Re and Tc complexes. The symmetric complex **5** is the one that shows the best affinity (IC_{50} = 0.172 nM), while maintaining an excellent selectivity. Interestingly, this seems to indicate that the the so-called bivalent approach, which relies in the use of two pharmacophores linked through a spacer in a single ligand, can improve the biological properties of this family of complexes. This approach has been explored, in a few instances, by medicinal chemists to design selective CNS receptor subtype ligands but, until now, never applied in the field of radiopharmaceutical chemistry.

Table 1 – Affinities and selectivities of the functionalized rhenium complexes.

Complex	IC_{50} (nM)[a] for 5-HT_{1A}	IC_{50} (nM)[b] for 5-HT_{2A}
1	9290 +/- 212	–
2	0.71 +/- 0.02	44 +/- 0.7
3	0.65 +/- 0.01	579 +/- 5
4	8130 +/- 505	–
5	0.172 +/-	154 +/- 2.6

[a] against OH-DPAT; rat hippocampus homogenate.
[b] against ketanserin; rat cortex homogenate.

The synthesis of the ^{99m}Tc analogues of the best performing complexes (**2**, **3** and **5**) is currently underway. Biodistribution studies in animal models will also be undertaken to assess the capability of the ^{99m}Tc complexes to cross the BBB, while recognising the putative receptors.

Published, accepted or in press work

1. R. Garcia, A. Paulo, A. Domingos, I. Santos, Rhenium(I) tris(carbonyl) Complexes with Soft Scorpionates, *J. Chem. Soc. Dalton Trans*. (2003) 2757-2760.

2. R. Garcia , C. Xavier, A. Paulo, A. Domingos, I. Santos, H. Spies, H.-J. Pietzsch, R. Bergmann, Rhenium(I) Carbonyl Complexes Anchored by Soft Scorpionates – Applications on the Development of. Specific Radiopharmaceuticals, *7th FIGIPS Meeting in Inorganic Chemistry, Lisbon* (2003) oral.

[1]Institute of Bioinorganic & Radiopharmaceutical Chemistry, Germany

Heterofunctionalized Phosphines for Labelling CNS Receptor Ligands with *fac*-[^{99m}Tc(CO)$_3$]$^+$

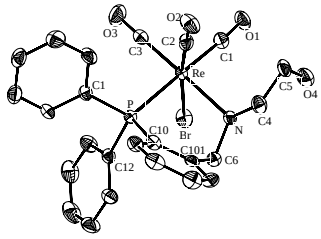
E. Palma, J. D. G. Correia, I. Santos, T. Kniess, H. Spies,[1] R Bergman[1]

Objectives

Development of new phosphine-based chelators for the labelling of Central Nervous System (CNS) receptor-binding ligands with the organometallic moiety *fac*-[^{99m}Tc(CO)$_3$]$^+$.

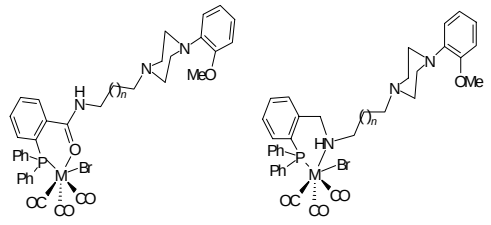
Results

Reduction of the amide in 2-(diphenylphosphanyl)-*N*-(2-hydroxyethyl)benzylamide (**H_2PNO**) yielded the compound 2-(diphenylphosphanyl)-*N*-(2-hydroxy-ethyl)benzylamine (**H_2CH_2PNO**). This ligand reacts with $(NEt_4)_2[ReBr_3(CO)_3]$ leading to compound $[Re(CO)_3(\kappa^2\text{-}H_2CH_2PNO)Br]$, which was fully characterized, including by X-Ray diffraction analysis.



$[Re(CO)_3(\kappa^2\text{-}H_2CH_2PNO)Br]$

In this complex the ligand is bidentate, coordinating to metal centre through the phosphorus and the nitrogen of the secondary amine (κ^2-PN coordination mode). Taking advantage of the



$n = 1$ (L^1); **1** (M = Re), **1a** (M = ^{99m}Tc) $n = 4$ (L^2), **2** (M = Re), **2a** (M = ^{99m}Tc)

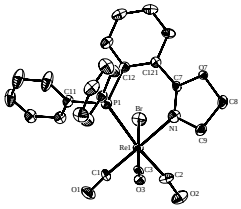
$n = 1$ (L^3); **3** (M = Re), **3a** (M = ^{99m}Tc) $n = 4$ (L^4), **4** (M = Re), **4a** (M = ^{99m}Tc)

coordination properties of the bidentate ligands **H_2PNO** and **H_2CH_2PNO**, the receptor binding ligands **L^1-L^4** were synthesized, and the tricarbonyl complexes **1**-**4** (Re) and **1a**-**4a** (^{99m}Tc) prepared. Complexes **1**-**4** have been used for determination of the receptor binding affinity and selectivity in rat brain homogenates. The IC_{50} values of these compounds for 5-HT_{1A} and 5-HT_{2A} receptors, summarized in the next table, were determined by displacement studies.

[1]Institute of Bioinorganic and Radiopharmaceutical Chemistry, Germany

Complexes	5-HT_{1A}	5-HT_{2A}
1	20 ± 0.1 nM	4680 ± 0.1 nM
2	200 nM	340 nM
3	285 ± 4 nM	490 ± 8 nM
4	1.100 μM	1.190 μM

With the aim of making the coupling to bioactive molecules easier, the compound *N*-(2-bromoethyl)-2-(diphenylphosphanyl)benzamide **HPNBr** was prepared. This new heterofunctionalized phoshine reacted with $(NEt_4)_2[ReBr_3(CO)_3]$ leading to the first example of a Re(I) tricarbonyl complex anchored on a phosphorus–oxazoline ligand, which has been generated during the course of complex formation: $Re(CO)_3(\kappa^2\text{-}PPh_3oxazBr)]$ (**PPh_3oxaz** = 2-(2-diphenyl-phosphinophenyl)oxazoline).



$[Re(CO)_3(\kappa^2\text{-}PPh_3oxazBr)]$

Published, accepted or in press work

1. T. Kniess, J. D. G. Correia, Â. Domingos, E. Palma, I. Santos, Synthesis and Structural Characterization of Novel Re(I) Tricarbonyl Complexes Anchored on a Phosphinoarylbenzylamine and a Phosphinoaryl-oxazoline Generated *in situ*, *Inorg. Chem.* **42** (2003) 6130-6135.

2. E. Palma, J. D. G. Correia, Â. Domingos, I. F. A. Pereira, I. Santos, A. Drews, H. Spies, Phosphine-Based BFCA for Labelling 5-HT_{1A} Receptor Ligands with [^{99m}Tc(CO)$_3$]$^+$, *Annual Congress of the European Asssociation of Nuclear Medicine*, Amsterdão, Holanda (2003) poster.

3. E. Palma, J. D. G. Correia, Â. Domingos, T. Kniess, I. Santos, A Novel Re(I) Tricarbonyl Complex Anchored on a Phosphorus-Oxazoline Ligand Generated in-situ, *7th FIGIPS Meeting in Inorganic Chemistry*, Lisboa, Portugal (2003) poster.

Complexes of d-Transition Elements with Cyclic and Acyclic Polyamines: Radiopharmaceuticals for Selective Tumour Therapy

R. Vitor, A. Paulo, S. Lacerda, P. C. Campello, F. Marques, I. Santos, S. Rodrigues,[1] J. Rueff[1]

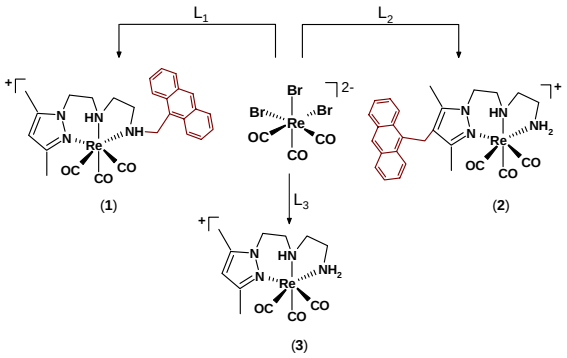
Objectives

Synthesis, characterization and biological evaluation of novel rhenium ($^{186/188}$Re), technetium (^{99m}Tc) and copper ($^{64/67}$Cu) complexes with cyclic and/or acyclic polyamines, having in mind the further development of radiopharmaceuticals (β^- or Auger emitters) for targeted anti-tumour therapy.

Results

1 – Re and Tc Complexes with Acyclic Polyamines

An anthracenyl group, which displays a recognized capacity of intercalating into DNA, has been incorporated into the framework of pyrazolyl-diamine ligands, following two different strategies. The resulting ligands, $\mathbf{L_1}$ and $\mathbf{L_2}$, were used to prepare complexes **1** and **2** (Scheme 1). The novel ligands and the respective complexes were characterized by the common spectroscopic techniques (IR, ^{1}H and ^{13}C NMR). The synthesis of the ^{99m}Tc congeners of **1** and **2** is currently under investigation.

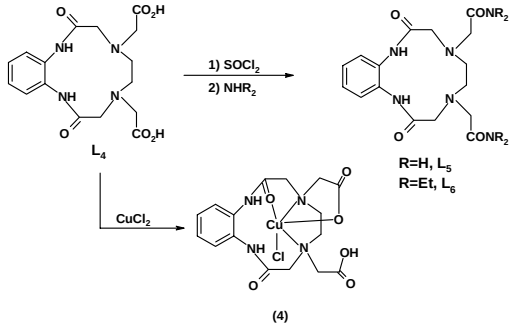


Scheme 1

The introduction of the intercalator moiety in these systems is expected to promote a close proximity with DNA, enhancing the ability of ^{99m}Tc to induce DNA damages. In this approach, it is of crucial importance to assess the influence of the radionuclide, bifunctional ligand and intercalator on the eventual cytotoxic effects of the complexes. Hence, we have evaluated the cytotoxicity and genotoxicity of the prototypical ligand ($\mathbf{L_3}$) and of the parent rhenium complex **3**, in cultured V79 Chinese hamster cells. The methyl-thiazolyl tetrazolium (MTT) cytotoxicity assay demonstrated that these compounds induced just a slightly increase in cell death. Furthermore, genotoxicity assays have shown that **L3** and **3** do not induce sister chromatid exchanges (SCEs) and chromosome aberrations (CA). In the next future, the cytotoxic studies will be extended to $\mathbf{L_1}$ and to $\mathbf{L_2}$ and to the Re complexes **1** and **2,** bearing the anthracenyl moiety, as well as to the ^{99m}Tc analogues.

2 – Cu(II) Complexes with Cyclic Polyamines

In the case of copper, we have focused on macrocyclic ligands, since macrocyclic copper complexes have an enhanced thermodynamic stability and kinetic inertness, if compared with congener complexes containing acyclic chelators. The benzodioxotetraaza-macrocycles $\mathbf{L_4}$-$\mathbf{L_6}$ were synthesized and characterized by the usual analytical techniques (Scheme 2). The coordination chemistry of these ligands with Cu(II) started to be evaluated with $\mathbf{L_4}$. A novel monomeric complex, **4,** has been isolated and characterized, including by X-ray diffraction analysis.



Scheme 2

The synthesis of Cu(II) complexes with ligands $\mathbf{L_5}$ and $\mathbf{L_6}$, as well as with cyclen derivatives containing thiol pendant arms, is underway.

Published, accepted or in press work

1. I. Santos, Catarina Xavier, António Paulo, Ângela Domingos, Synthesis and Structural Studies of Re(V) Complexes Stabilized by a Monoanionic Cyclen Ligand, *Eur. J. Inorg. Chem.* (2003) published on line.

2. P. Antunes, P. M. Campello, R. Delgado, M. G. B. Drew, V. Félix, I. Santos "Metal Complexes of Tetraazacyclophane: Solution and Molecular Modelling Studies, *J. Chem. Soc., Dalton Trans.* (2003) 1852-860.

3. C. Xavier, A. Paulo, J. Ascenso, A. Domingos, I. Santos, M. P. C. Campello, Cationic Re(V) Oxocomplexes Containing an Unprecedented Monoanionic Cyclen Ligand, XXVIII *International Symposium on Macrocyclic Chemistry*, Gdansk, Poland (2003) poster.

[1]Faculdade de Ciências Médicas da Universidade Nova de Lisboa

Bifunctional Pyrazoly-Containing Ligands for Labelling Biological Peptides

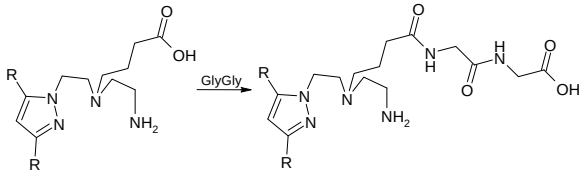
S. Alves, J. D. G. Correia, L. Gano, I. Santos, H. Maecke[1]

Objectives

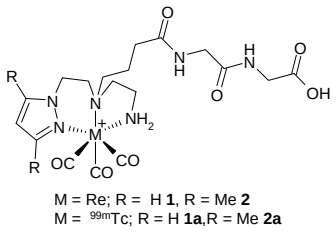
Labelling of bioactive peptides with *fac*-$[^{99m}Tc(CO)_3]^+$ using bifunctional pyrazolyl-containing ligands.

Results

The bifunctional pyrazolyl-containing ligands $\mathbf{L^1}$ and $\mathbf{L^2}$ have been prepared and attached to a small dipeptide (GlyGly), yielding the $\mathbf{L^1}$**-GlyGly** and $\mathbf{L^2}$**-GlyGly** chelators.



The rhenium(I) tricarbonyl model complexes **1** and **2** were obtained by reaction of the appropriate ligand with the organometallic precursor $(NEt_4)_2[ReBr_3(CO)_3]$, under adequate conditions.



The analogue ^{99m}Tc-complexes (**1a**, **2a**) have been obtained in high radiochemical yields (> 90%) and with high specific activities, by reacting the ligands with $[^{99m}Tc(H_2O)_3(CO)_3]^+$. These hydrophilic model complexes ($logP_{o/w}$ = -2,11 ± 0.02, **1a**; -1,12 ± 0.20, **2a**) displayed high stability both *in vitro* and *in vivo*. The biodistribution profile of **1a** and **2a** was examined, being the results shown in Figure 1.

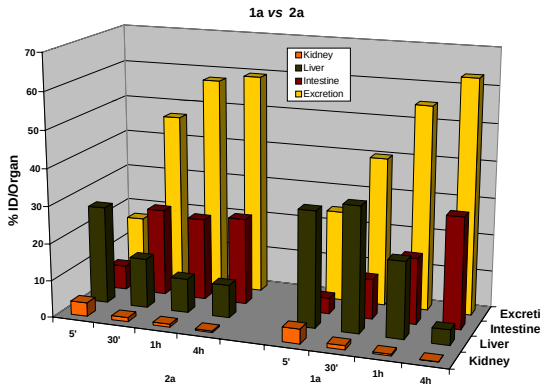


Figure 1: Biodistribution profile of **1a** and **2a**

The overall favourable characteristics of the ^{99m}Tc-complexes incorporating the bifunctional chelates $\mathbf{L^1}$ and $\mathbf{L^2}$, prompted us to couple $\mathbf{L^2}$ to the somatostatine analogue Octreotate (TATE), giving the conjugate $\mathbf{L^2}$-TATE.

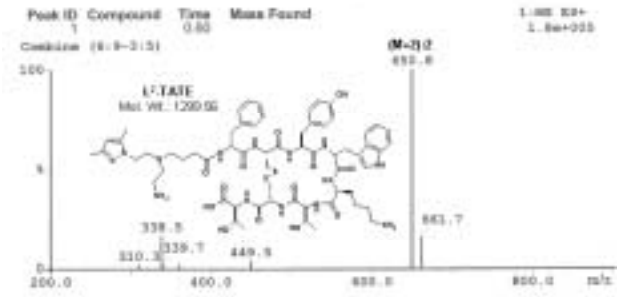
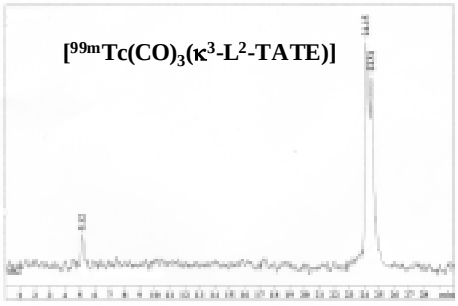


Figure 2: ES/MS of $\mathbf{L^2}$-TATE

The $\mathbf{L^2}$-TATE conjugate was labelled with high radiochemical yield (> 90%). An HPLC chromatogram of the resulting complex is presented.



Biological evaluation of the $[^{99m}Tc(CO)_3(\kappa^3\text{-}L^2\text{-TATE})]^+$ complex is underway.

Published, accepted or in press work

1. S. Alves, J. D. G. Correia, A. Paulo, L. Gano, Â. Domingos, I. Santos, Labelling of Bioactive Peptides with the Unit *fac*-$[^{99m}Tc(CO)_3]^+$, *7th FIGIPS Meeting in Inorganic Chemistry*, Lisboa, Portugal (2003) poster.

2. S. Alves, A. Paulo, J. D. G. Correia, L. Gano, A. Domingos, I. Santos, Complexos Organometálicos de ^{99m}Tc para Marcação de Péptidos Biologicamente Activos, *1º Congresso da Sociedade Portuguesa de Ciências Farmacêuticas*, Lisboa, Portugal (2003) oral.

[1] Institute of Nuclear Medicine, University Hospital Basel, Switzerland.

Mixed Ligand Complexes of Re and Tc with the [PNS/S] Donor Atom Set: Biological Evaluation

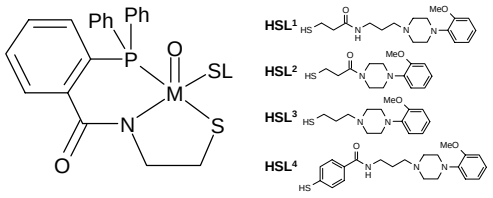
C. Fernandes, L. Gano, J. D. G. Correia, I. Santos, R. Bergman[1], H. Spies[1], S. Seifert[1]

Objectives

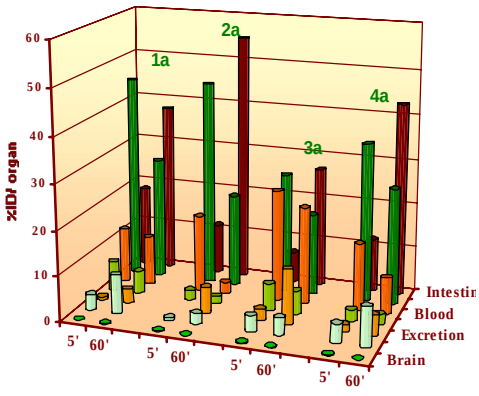
Evaluation of the biological properties (binding affinities, *in vivo*/*in vitro* stabilities and biodistribution in model animals) of "3+1" oxotechnetium(V) complexes with 5-HT_{1A} receptor-binding ligands and silylated groups.

Results

1. Complexes for 5-HT_{1A} Receptors: Mixed-ligand "3+1" oxocomplexes of general formula [MO(κ^3-PNS)(κ^1-SL^{1-4})] (M = Re, **1**–**4**; M = ^{99m}Tc, **1a**–**4a**) have been prepared and fully characterized.

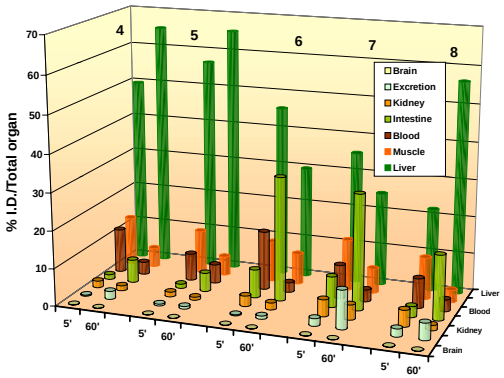


Complex **3** revealed high affinity and selectivity for the 5-HT_{1A} receptors (IC_{50} = 2.35 nM). Besides their high *in vitro* stability, the ^{99m}Tc-complexes exhibit a degree of lipophilicity, which is in the range normally advised for crossing the BBB (log$P_{o/w}$ = 1.70 – 2,01). The biodistribution studies showed low protein-binding and good *in vivo* stability for all complexes, however, poor brain uptake was observed for all the complexes.



2. Complexes with Organosilicon Groups: New oxotechnetium complexes of general formula [^{99m}Tc(O)(PNS)($S(CH_2)_nOSiR_3$)] (**4-6**) were synthesized by direct reduction of [$^{99m}TcO_4$]$^-$ with $SnCl_2$, in the presence of the tridentate heterofunctionalized phosphine **H_2PNS** and of the monodentate silylated thiols [$HS(CH_2)_nOSiR_3$] (n=2, R=Ph (**1**); n=3, R= Ph (**2**); n=3, R= Et (**3**)).

The mixed-ligand Re and Tc complexes of general formula [M(O)(PNS)($S(CH_2)_nOH$)] (n=2: M = ^{99m}Tc, (**7**), M = Re, (**7a**); n = 3: M = ^{99m}Tc, (**8**), M = Re, (**8a**)) were also prepared. Complexes **4-8** were obtained with high radiochemical purity (>95%), and were characterized by comparison of their HPLC profiles with the ones obtained for the corresponding Re compounds. The silylated compounds **4-6** exhibit high *in vitro* stability, and do not exchange with glutathione. The biological behaviour of **4-8** revelead that the silylated complexes are stable in vivo and no significant hydrolysis was observed, as indicated by blood analysis. **4-8** are eliminated essentially through the hepatobiliary tract with low urinary elimination. The silylation increased significantly the lipophilicity of the compounds, but no significant effect was found in their brain uptake or brain retention.



Published, accepted or in press work

1. T. Kniess, C. Fernandes, I. Santos, W. Kraus, H. Spies, Silylated Mixed-Ligand Rhenium Complexes with the [PNS/S] Donor Atom Set, *Inorg. Chim. Acta*, **348** (2003) 237-241.

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Chemical, Radiochemical and Biological Evaluation of Pyridine[14]ane-N_4 Macrocycle Lanthanide Complexes

F. Marques, L. Gano, M.P.C. Campello, J. Costa,[1] Krassimira,[1] R. Delgado,[1] I. Santos

Objectives

To assess the effect of incorporating a pyridine moiety on the basic structure of the [14]ane-N_4 macrocyclic ligand, as well as the effect of the number and type of pendant arms on the chemical, radiochemical and biological behaviour of the radiolanthanide complexes. To evaluate the potential of these complexes for bone pain palliation and/or other therapeutic applications.

Results

The py[14]ane-N_4 macrocyclic ligands evaluated contained methylcarboxylate ($PyAc_3$) and methyl and/or methylphosphonate ($PyMP_2$, PyP_3) pendant arms (fig.1).

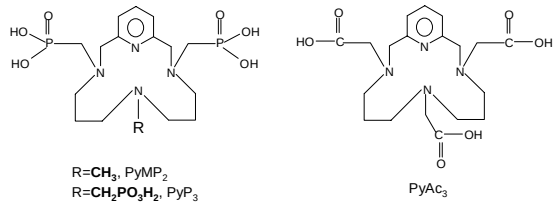

Figure 1- Pyridine macrocyclic ligands

The protonation and stability constants of the pyridine macrocyclic ligands with Ln (Sm, Ho) have been studied by potentiometric and/or ^{1}H NMR spectroscopic techniques. It was found that the overall basicity of the ligands increases in the following order $PyAc_3 < PyMP_2 < PyP_3$, being this trend also observed for the stability constants of the lanthanide complexes, which are very stable.

Pyridine[14]ane-N_4 ligands with methylcarboxylate and/or methylphosphonate pendant arms were labelled with ^{153}Sm and ^{166}Ho. Radiochemical protocols were optimized for chelate concentration and pH in order to achieve >98% chelation efficiency. The stability of the radiocomplexes was evaluated in physiological media and in human serum at 37ºC, using TLC and electrophoresis for control. Lipophilicity was also determined by octanol/ saline coefficient partition. Plasmatic protein binding of the radiolanthanide complexes was assessed by gel filtration.

All the complexes are neutral and hydrophilic at pH=7.4. They are stable at least 3 days in physiological solutions but some transchelation was detected in the presence of human serum. The plasmatic proteins binding was higher than 60%.

Comparative biodistribution studies of both ^{153}Sm and ^{166}Ho complexes in CD-1 mice indicated a similar pattern for all the complexes, which present a very slow rate of total radioactivity excretion and clearance from most of the organs. A very high and fast liver uptake and high hepatic retention of radioactivity was also found. The main differences in the biodistribution are related with the rate of clearance from blood and liver uptake as well as the degree of bone uptake. Accumulation in bone of the radiolanthanide complexes with $PyMP_2$ and PyP_3 is enhanced over the time with the increased number of methylphosphonate groups, likely due to the binding of these groups to hydroxyapatite. TLC analysis of urine and serum collected at sacrifice time demonstrated that radiolanthanide complexes are not stable in vivo leading to different radiochemical species other than free radiolanthanide. The in vivo behaviour of these complexes led us to conclude they are not suitable for therapy. Nevertheless their favourable chemical, radiochemical and in vitro stability may indicate that related ligands can be useful as bifunctional chelators.

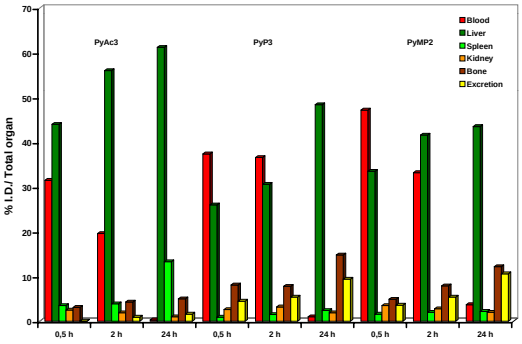

Figure 2 – Biodistribution results of ^{153}Sm-Pyridine radiocomplexes in CD-1 mice

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Lanthanide Complexes with Tetraazamacrocycles Containing Acetate and Methylphosphonate Pendant Arms as Potential Therapeutic Agents

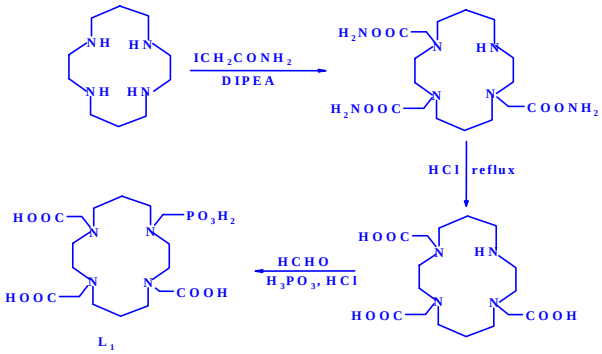
M.P.C. Campello, L. Gano, F. Marques, L. Lima[1], R. Delgado[1], I. Santos

Objectives

To evaluate the interest of radiolanthanide complexes with 13- and 14-membered tetraazamacrocycles as novel therapeutic agents. To study the effect of the cavity size and the nature of the pendant arms on the *in vitro* and *in vivo* behaviour of the ^{153}Sm complexes.

Results

As part of our on-going project on lanthanide complexes with 13- and 14-membered tetraazamacrocycles with methylcarboxylate and methylphosphonate pendant arms, the novel mixed [14]aneN$_4$ macrocyclic ligand **L$_1$** was synthesized and characterized by the usual analytical techniques (scheme 1).



Scheme 1 – Synthesis of **L$_1$**.

Thermodynamic protonation and stability constants of **L$_1$** with several metals were determined by ordinary potentiometric techniques using the Hyperquad program (Fig.1 and 2). It was found that the stability constants with Sm^{3+} and Ho^{3+} are higher than with TETA but lower than with TETP. However, based on pM values, and in contrast with the values obtained with TETA and TETP, the Sm-**L$_1$** is more stable than the corresponding Ho-complex.

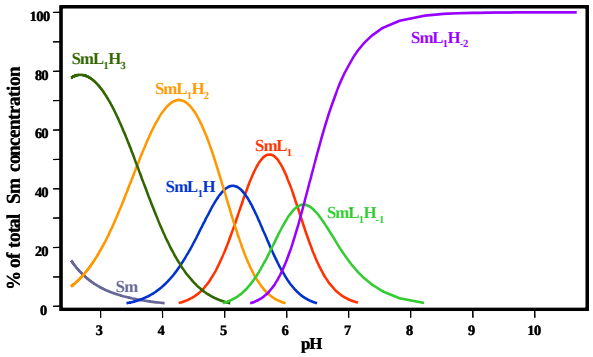


Fig.1 Species distribution for L$_1$ and Sm(III) 2:1

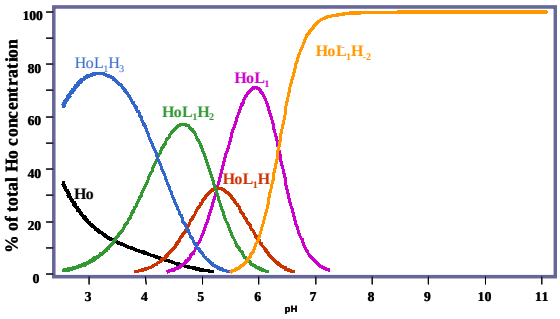


Fig.2 Species distribution for L1 and Ho(III) 2:1

^{153}Sm in the nitrate form was produced from isotopically enriched $^{152}Sm_2O_3$ at RPI. In the sequence of our previous studies, complexes with [14]aneN$_4$ macrocycle ligands were prepared and their radiochemical behaviour and *in vitro* stability determined by ITLC and electrophoresis. The complexes were obtained in relatively low yield (80%), being required a purification step prior to injection. The *in vitro* stability in physiological conditions was highly dependent on the type of pendant arms. The TETA-complex is stable in different physiological solutions but unstable in PBS while TETP-complex is stable in PBS solution but unstable in saline and in physiological acid medium. Taking into account the chemical stability and the pM values of the lanthanide complexes, these findings were unexpected and require further studies. The new mixed macrocycle ligand **L1** show significant structural difference relatively to the congeners TETA and TETP which can be promising for its biological behaviour. Studies of **L$_1$** with ^{153}Sm and ^{166}Ho are underway.

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Synthesis, Radiolabelling and Evaluation of Steroidal and Non-Steroidal Biomolecules as Radioimaging Agents for Human Breast Tumours

M. C. Melo e Silva, L. Gano, F. M. Marques, M.M. Marques[1], G. C. Costa[1], T. Thiemann[2], M. Watanabe[2], J. Wang[2], T. Matsumoto[2],Y. Yamamoto[2], S. Mataka[2]

Objectives

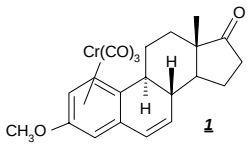
To find ligands for estrogen receptors (ER) to be used as radioimaging agents for the detection of ER^+ human breast tumours.

Results

Novel γ-emitting ER binding radioligands, estradiol analogues (**1**) and triphenylethylene derivatives (**2**) have been synthesized and are being studied.

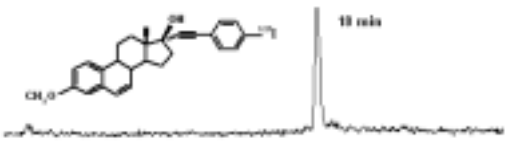
1. Estradiol analogues

Our interest in preparing C-7 substituted estra-1,3,5(10),6-tetraenes lead us to prepare two η^6-estra-1,3,5(10),6-tetraenes (*e. g* **1**). In both cases only one stereoisomer can be isolated in contrast to other estrange-tricarbonylchromium complexes, where complexations are non-selective. X-ray crystal structural analysis discloses that only the more sterically hindered β-facial isomer is formed indicating that the 6,7-olefinic moiety exerts a directive influence on the complexation [1,2].



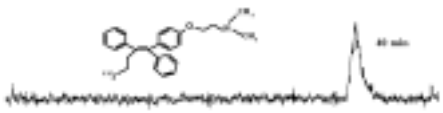
1

Radioiodinated *p*-iodophenylethynyl substituted estra diol analogues have been synthesised by reacting a 17-pyrrolidinylazophenyl substituted intermediate with Na[^{125}I] in the presence of chlorotrimethylsilane (TMSCl) in acetonitrile [3,4].



2. Triphenylethylene derivatives

The radioiodinated triphenylethylene derivative, β-[^{125}I]iodotamoxifen, was similarly obtained by reacting β-bromotamoxifen with Na[^{125}I] in refluxing acetone.



Both radioiodinated compounds were purified and characterised by reversed-phase high performance chromatography (HPLC). The radiochemical purity of the ^{125}I radioiodinated compounds was higher than 95%. These compounds have been prepared with good specific activity.

The potential use of these steroidal and non-steroidal compounds as imaging agents for ER^+ human breast tumors will be assessed by *in vitro* ER binding affinity studies and *in vivo* biodistribution assays in immature female rats.

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