Inorganic and Radiopharmaceutical Chemistry

Isabel Rego Santos

The Group conducts basic and applied-oriented research in modern Radiopharmaceutical Chemistry, an important topic in Life Sciences. Our main goal is to find innovative radioactive tools for **molecular imaging and/or targeted radiotherapy with interest for Nuclear Medicine**. These are emerging fields that aim to integrate patient-specific and disease-specific molecular information with traditional anatomical imaging readouts and to provide *tailor made* therapy. To achieve such goal the Group has developed and implemented expertise on organic, inorganic and organometallic chemistry of *d*- and *f*-elements, radiochemistry, radiopharmacy, cell and animal studies and biochemistry. This combination, **unique in the country,** is also possible due to the facilities implemented and maintained by the Group, such as laboratories and equipment for the synthesis and characterization of inactive and radioactive compounds, animal facility, laboratories and equipment for animal studies, cell culture and biological evaluation of radioactive compounds.
Our expertise and infrastructures justify the participation in national and international research projects, the support of an international pharmaceutical company and funding from CIMAGO/FLAD.

Research: During 2006**,** we went on with studies on **halogen** and **metal *d*- and *f*-based radioactive tools for biomedical applications**. For imaging we have explored mainly the γ emitters ^{99m}Tc and ^{67}Ga, while for therapy β and Auger emitters have been studied, namely ^{153}Sm, ^{166}Ho, ^{125}I and ^{99m}Tc. In terms of targeting our interest is on cancer, CNS pathologies and myocardial imaging. **The main scientific achievements are reported in the next pages.** The quality of our basic and applied–oriented research allowed the publication in journals of high impact, as well as the filling of patent applications.

Education and Training: At the **graduation level**, the group teaches, in a regular way, Radiopharmacy at the ESTSeL and at the Faculty of Pharmacy/University of Lisbon. Under a collaboration protocol, our facilities are also used every year, during two weeks, by students of the Nuclear Medicine Course, ESTeSL.

At the post-graduate level the **Group** has **organized, teaches and coordinates the Master Course** *Biomedical Inorganic Chemistry: Diagnostic and Therapeutical Applications* (Third Edition) (DR nº 123, 26/05/04, II série). For this Master Course the group established a collaboration protocol with the University of Lisbon (Faculties of Sciences, Pharmacy and Medicine), Hospital Garcia de Orta and Instituto Português de Oncologia/Lisboa. We have also participated in PhD Teaching Programs organized by other Universities.

At the **International level**, the Group participated in the European Radiopharmacy Course, INSTN and has been partner in the EC/COST RTD ACTIONS, and Virtual Radiopharmacy/V Framework Program.

We have also trained several young scientists, funded by FCT grants, namely **BIC**, **MSc**, **PhD** and **Post-Doctoral** researchers. Our expertise has also been provided to some Nuclear Medicine Centers, to the Portuguese Medicines Evaluation Agency and IAEA.

Research Team

Researchers

I. SANTOS, Princ. Researcher, Agregação, Group Leader
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J. G. CORREIA, Princ. Researcher
M. C. OLIVEIRA, Aux. Researcher
C.FERNANDES, Aux. Researcher
L.GANO, Aux. Researcher
F. MARQUES, Aux. Researcher
P. RAPOSINHO, Aux. Researcher

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Collaborators

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J. RUEFF, Full Prof., FCM, UNL
A. S. RODRIGUES, Prof., FCM, UNL
R. ALBERTO, Full Prof. University of Zurich

Tc(I)/Re(I) Tricarbonyl Complexes Combining a Bis-agostic (k^3-H, H, S) Binding Motif with Pendant and Integrated Bioactive Molecules

L. Maria, A. Paulo, R. Alberto,[1] I. Santos,

Objectives

This project aims to introduce small-sized and versatile Tc(I)/Re(I) organometallic building blocks displaying unprecedented coordination environments and suitable for the labelling of bioactive molecules.

Results

Incorporation of a *d*-transition metal into a biomolecule for molecular imaging is quite challenging, since its metabolic fate and biophysical properties must not be affected. For radiopharmaceuticals in particular, in which *e.g.* ^{99m}Tc is combined with a receptor targeting molecule the topology, size and molecular weight of the ligands are of utmost importance. Based on dihydrobis(mercaptoazolyl)borates, we were able to demonstrate that one agostic hydride binds very efficiently to the *fac*-$[M(CO)_3]^+$ (M = Re, ^{99}Tc, ^{99m}Tc) moiety in aqueous media [1]. Coupling of bioactive fragments to this type of ligands led to biocomplexes exhibiting excellent affinity and selectivity for Central Nervous System (CNS) receptors of the 5-HT_{1A} serotonergic type [2]. Still, the two mercaptoimidazolyl groups are relatively bulky and we have introduced a novel class of ligands, trihydro(azolyl)borates (azolyl= mercaptoazolyl and benzothiazolyl) which are the smallest soft scorpionates described so far. We have shown, for the first time, that is possible to stabilize Re and Tc tricarbonyl complexes with the trihydro(azolyl)borates which coordinate through two B-H…M agostic interactions and one sulphur atom of the azolyl ring (Fig. 1) [3,4].

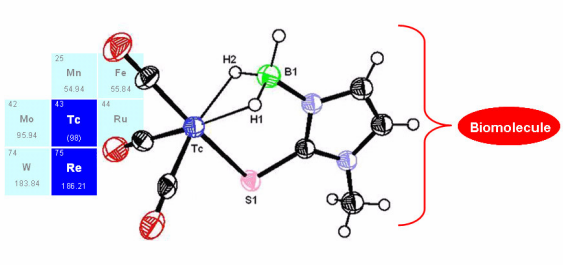


Figure1

The resulting building blocks were already used to incorporate small biomolecules, using the so-called pendant or integrated approaches (Fig. 1). Most interestingly we have also shown that is possible to prepare these complexes at no carrier level (^{99m}Tc), being the final complexes stable in water and also in the presence of high concentration of chloride. Despite this stability, the B-H…M bonds of some of the compounds could be selectively cleaved by unidentate π-acceptors yielding ″2+1″ mixed-ligand complexes in a quantitative way (Fig. 2) which means that these systems can also be useful for labelling biomolecules using the so-called ″2+1″ approach [5].

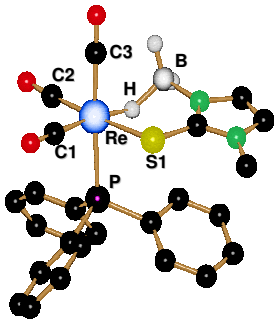


Figure 2

Published work:

3. L. Maria, C. Moura, A. Paulo, I. C. Santos, I. Santos, Synthesis and Structural Studies of Rhenium(I) Tricarbonyl Complexes with Thione Containing Chelators, *J. Organomet. Chem.* 2006, 691, 4773-4778.

4. R. Garcia, L. Gano, L. Maria, A. Paulo, I. Santos e H. Spies, Synthesis and Biological Evaluation of Tricarbonyl Re(I) and Tc(I) Complexes Anchored by Poly(azolyl)borates: Application on the Design of Radiopharmaceuticals for the Targeting of 5-HT_{1A} Receptors, *J. Biol. Inorg. Chem.* 2006, 11, 769-782.

5. L. Maria, A. Paulo, I. C. Santos, I. Santos, P. Kurz, B. Spingler, R. Alberto, Very Small and Soft Scorpionates: Water Stable Technetium Tricarbonyl Complexes Combining a Bis-agostic (k^3-H, H, S) Binding Motif with Pendant and Integrated Bioactive Molecules *J. Am. Chem. Soc.* 2006, 128, 14590-14598.

6. L. Maria, A. Paulo, I. Santos, Tripodal Ligands with the Coordination Motifs κ^2-BH_2 or κ^3-BH_3 Relevant for Biomedical Applications of Organo-metallic Complexes (2006) *European Patent Application* 06075127.8.

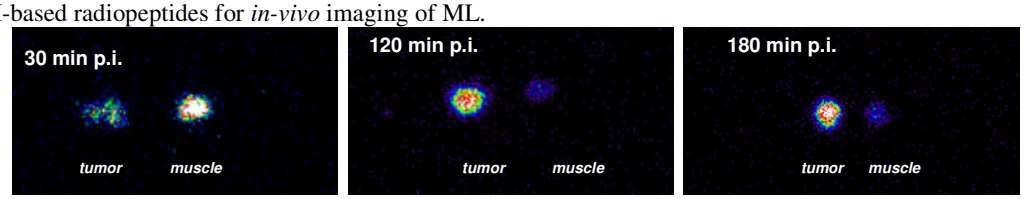
7. L. Maria, A. Paulo, I. Santos, R. Alberto, Rhenium(I) and ^{99m}Technetium(I) Building Blocks Bearing the (κ^3-S,H,H') Coordinating Motif for the labelling of Small Biomolecules, in *Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine 7*. SGEditoriali, Padova, 2006, pag. 127-129.

[1] Institute of Inorganic Chemistry, University of Zürich, Switzerland.

Imaging of Murine Melanoma Using [^{99m}Tc(CO)$_3$-pz-NAPamide]-Conjugate as a Radioactive Probe

P. Raposinho, J. D. G. Correia, I. Santos, M. F. Botelho,[1] C. Santos[1]

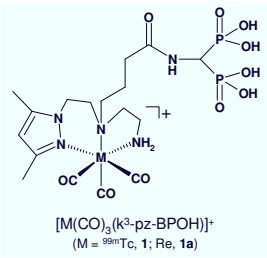
The use of radiolabeled analogs (γ-emitting radionuclides) of the α-melanin-stimulating hormone has emerged as a promising approach for *in-vivo* targeting of the melanocortin-1 receptor (MC1R), which is overexpressed in melanoma (ML). The analog NAPamide, conjugated to the pyrazolyl-containing backbone (pz) through the Lys residue (Ac-Nle-Asp-His-*D*Phe-Arg-Trp-Gly-Lys(pz)-NH_2), was radiolabeled with *fac*-[^{99m}Tc(CO)$_3$]$^+$, and its potential for murine ML imaging was evaluated in ML B16F1-bearing mice. The radiopeptide exhibited a good tumor uptake (4.2 ± 0.9%ID/g, 4h), which is MC1R-mediated as revealed by *in vivo* receptor-blockade with the potent agonist NDP-α-MSH (*ca.* 60% tumor uptake reduction). The highly favorable tumor/muscle ratio (*e.g.* 24 at 4h p.i) observed, and the long residence in the tumor are also key features for the development of new α-MSH-based radiopeptides for *in-vivo* imaging of ML.



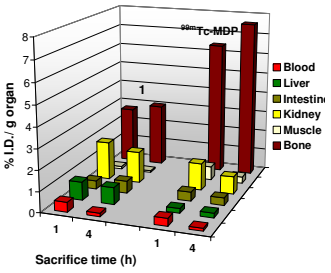
[1] IBILI, University of Coimbra, Portugal.

A Bisphosponate-Containing ^{99m}Tc(I) Tricarbonyl Complex Potentially Useful as Bone-Seeking Agent

E. Palma, B. Oliveira, J. D. G. Correia, L. Gano, I. Santos

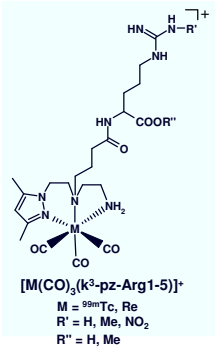


Aiming to develop new bone-seeking radiotracers with improved biological properties, we have prepared new conjugates comprising a pyrazolyl-containing backbone (pz) for metal coordination, and a pendant bisphosphonic acid group. Reaction of this conjugate with *fac*-[^{99m}Tc(CO)$_3$]$^+$ yielded (> 95%) the single radioactive species [^{99m}Tc(CO)$_3$(k^3-pz-BPOH)]$^+$ (**1**), which revealed high stability both *in vitro* and *in vivo*. The corresponding Re surrogate (**1a**) was prepared and used for structural characterization of the radioconjugate by RP-HPLC. Biodistribution studies in mice indicated a fast rate of blood clearance and high rate of total radioactivity excretion, occurring primarily through the renal-urinary pathway. Despite presenting moderate bone uptake (3.04 ± 0.47% ID/g organ, 4 h p.i), significantly lower than that observed for the commercial product ^{99m}Tc-MDP, the high stability of **1** and its adequate *in vivo* pharmacokinetics is encouraging for further studies.



Labeling of *L*-Arginine Derivatives with the Moiety *fac*-[^{99m}Tc(CO)$_3$]$^+$ using a Bifunctional Pyrazolyl-Containing Chelator: Chemistry and Radiochemistry

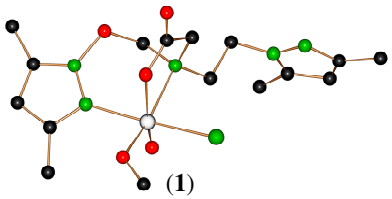
B. Oliveira, J. D. G. Correia, P. Raposinho, I. Santos



The targeting and visualization *in vivo* of Nitric Oxide Synthase (NOS) expression using a radiolabeled substrate/inhibitor of this enzyme (*e.g. L*-Arginine derivatives) is a challenging approach that could provide insight into a wide variety of pathophysiological processes. Taking into account our efforts in the development of new radioactive probes for *in vivo* tumor targeting we have prepared and characterized the new conjugates **pz-Arg1-5**, which contain a bifunctional pyrazolyl-containing ligand (pz) for metal stabilization and a pendant *L*-Arginine derivative (*L*-Arg). We have synthesized and characterized the stable (*in vitro*) radioactive complexes [^{99m}Tc(CO)$_3$(k^3-pz-Arg1-5)]$^+$ (**1-5**). The corresponding Re surrogates were also prepared and fully characterizeed for structural identification/characterization of the organometallic radioactive complexes. Enzymatic studies and biological evaluation of the ^{99m}Tc-complexes are underway.

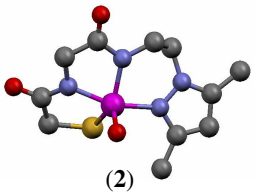
Novel Metal Fragments Anchored by Pyrazolyl-Based Chelators for Labelling Biomolecules with Clinical Relevance

C. Moura, T. Esteves, P. Campello, A. Paulo, I. Santos



(**1**)

This projects searches for novel labelling methodologies based on M(V)/M(III) (M = Re, Tc) metallic fragments anchored by tailor-made chelators of the pyrazolyl type. To achieve this goal, two novel classes of ligands, combining pyrazolyl groups with amino, thiol or carboxylic coordinating functions, were prepared and their coordination capability towards the $[M(O)]^{3+}$ (M = Re, ^{99m}Tc) moiety evaluated. The resulting monoxocomplexes, anchored by κ^3-N_2O (**1**) or κ^4-N_3S (**2**) chelators, were fully characterized, either in the solid state or in solution, and their suitability to enter into the chemistry of related M(III) complexes is under investigation. The synthesis of M(V)/M(III) complexes at the no-carrier added level (^{99m}Tc) is also being studied to assess their interest in the design of target-specific radiopharmaceuticals.

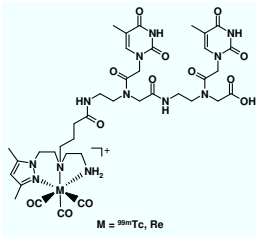


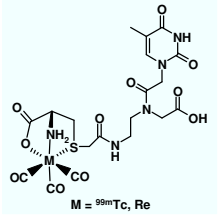
(**2**)

Synthesis, Characterization and Stability Studies of Re(I) and ^{99m}Tc(I) Tricarbonyl Complexes Bearing a PNA Units

C. Xavier, R. Alberto[1], I. Santos

The main goal of this project is to find novel ^{99m}Tc probes for non-invasive imaging of endogeneous gene expression, using the antisense approach. Such approach involves the direct or indirect labeling of DNA/PNA sequences with clinical relevance. Some monomeric and dimeric PNA's, having thymine as nucleobase, have been synthesized, characterized and coupled to two chelators with recognized affinity for the *fac*-$[M(CO)_3]^+$ (M=Re, ^{99m}Tc) moiety. The high specific activity of the ^{99m}Tc complexes, as well as their *in vitro* and *in vivo* stability has shown the utility of these tridentate bifunctional chelators for labeling PNA sequences with clinical relevance. These studies are currently underway.

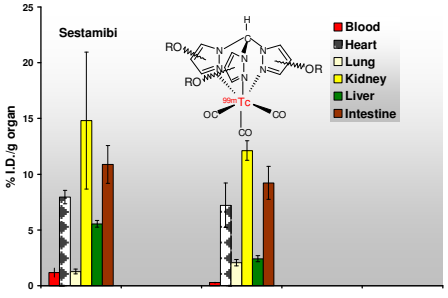




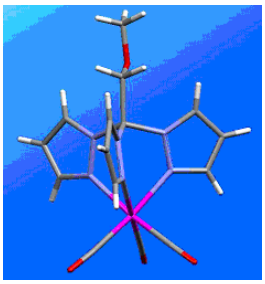
[1] Zurich University, Switzerland

New Myocardial Imaging Agents Based on ^{99m}Technetium(I) Tricarbonyl Complexes

L. Maria, M. Videira, S. Cunha, L. Gano, A. Paulo, I. Santos



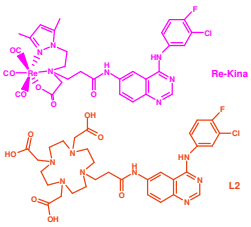
Searching for novel ^{99m}Tc compounds suitable for myocardial perfusion imaging we have studied organometallic complexes anchored by tripodal chelators of the tris(pyrazolyl)methane type. When combined with the *fac*-$[M(CO)_3]^+$ (M = Re, ^{99m}Tc) core, some of these chelators yielded cationic and lipophilic complexes as required for myocardial perfusion imaging. Most relevantly, one of these complexes showed a biodistribution profile in mice similar to Sestamibi®, a radiopharmaceutical in clinical use for myocardial perfusion studies. The best performing complex is being currently evaluated in other animal models, namely by dynamic studies. Chemical modifications of the tripodal ligands are also underway in order to get a better understanding on structure/activity relationship (SAR) and to further improve the biological performance of the complexes



Novel Biomarkers for Molecular Imaging of Epidermal Growth Factor Receptors Positive Tumours.

C. Fernandes, A. Paulo, M. C. Oliveira, R. Garcia, L. Gano, M.P. Campello, A. Bourkoula[1], I. Pirmettis[1], I. Santos

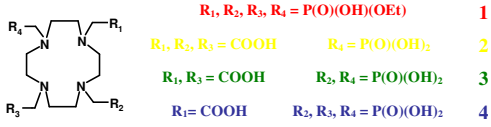
Epidermal growth factor receptors (EGFR) are often over expressed in cancer cells. In our search for the development of novel SPECT/PET radioligands for early detection and staging of EGFR positive tumours new radioactive quinazoline probes labelled with ^{125}I, ^{99m}Tc, ^{111}In and $^{67/68}$Ga are being explored. *In vitro* studies indicate that iodinated quinazoline (**^{127}I-Kina**) inhibits A431 cell growth possessing higher potency than the parent compound (**1**) to inhibit EGFR autophosphorylation. The coordination of **1** to a metal fragment still leads to compounds (**Re-Kina**) which inhibit significantly EGFR autophosphorylation. This, associated to the *in vitro/in vivo* stability suggests the potential of this class of compounds as biomarkers for molecular imaging of EGFR. To extend these studies to other metals ($^{67/68}$Ga, ^{111}In) the novel conjugate **L2** was synthesized and the preparation of gallium and indium complexes are currently under evaluation.



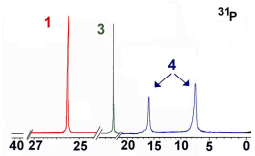
1. *Institute of Radioisotopes -Radiodiagnostic Products, NCSR "Demokritos", Athens, Greece*

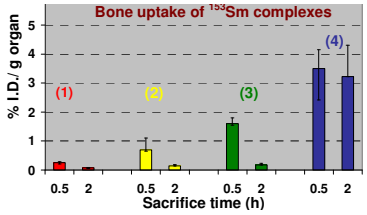
Pharmacokinetics Tuning of Radiolanthanide Complexes by Modification of Functional Groups Anchored on Tetraazamacrocycles

M.P. Campello, F. Marques, L Gano, S. Lacerda, M. Balbina, M. Főrsterová[1], P Hermann[1], I. Lukes[1], I. Santos



New cyclen derivatives with different pendant arms have been synthesized and characterized to evaluate the effect of the pendant arms on the pharmacokinetics of the corresponding ^{153}Sm/^{166}Ho complexes. All complexes have shown fast complexation rate, good *in vitro/in vivo* stability, rapid clearance from most organs and rapid total excretion. The main differences are related with the highest accumulation and the longest residence in bone of complexes with ligand **4**, which highlights the importance of the number and type of phosphonic appended arms. Radiochemical and biological behaviour of complexes has proven their ability for targeted radionuclide therapy.



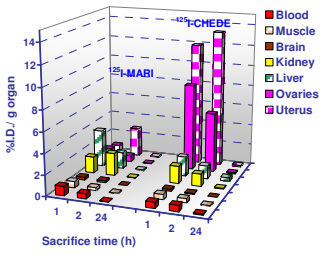


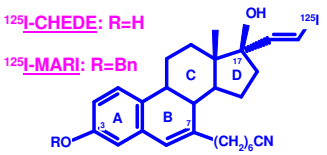
[1] Dpt of Inorganic Chemistry, Univerzita Karlova, Prague, Czech Republic.

Novel Estrogen Receptor Ligands as Potential Probes for Targeted Tumour Imaging and Therapy

C Neto, M C.Oliveira ,L.Gano, F.M. Marques, G. Morais[1], T. Thiemann[1], A C Santos[2], F. Botelho[2], C Oliveira[2]

Following our previous work on new ligands for targeted therapy and/or imaging of breast cancer a set of radioiodinated C7-substituted 17α-iodovinylestra-1,3,5(10),6-tetraene-3,17β-diols are being evaluated to study the effect of a C7-alkyl chain on the biological behaviour of the parent compound. Thus, **^{125}I-CHEDE** was obtained in high radiochemical purity and specific activity. Biodistribution studies in immature female rats have shown high target tissue uptake and selectivity suggesting the potential application of this novel class of compounds for imaging and therapy of ER rich tumours. Specific binding studies are currently underway. The influence of C3-benzyl group (^{125}I-MARI) on the biological behaviour of the compound was also evaluated by comparing the biological profiles. The sharp decrease of target tissue uptake supports the importance of a free phenol group for estrogen receptor binding.





[1] Interdisciplinary Graduate School of Engineering Science, Kyushu University, Japan, [2] CIMAGO, FMUC, Coimbra