

Inorganic and Radiopharmaceutical Chemistry

Isabel Rego Santos

The **main activity** of the Group is the **design, synthesis and characterization of novel specific radioactive probes with potential interest on Nuclear Medicine diagnostic and/or therapy**. These probes are useful for non-invasive **molecular imaging** of targeted macromolecules and biological processes associated to different pathologies. By using **β^- or Auger emitters**, they might be suitable for **therapy**.

The group is **multidisciplinary**, combining expertise on organic, inorganic and organometallic chemistry of *d*- and *f*-elements, on radiochemistry as well as on animal and cell studies. This combination, **unique in the country**, is possible due to our expertise and 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 facilities, laboratories and equipment for animal studies, cell culture and biological evaluation of radioactive compounds.

The expertise and infrastructures enable basic and applied-oriented research in modern Radiopharmaceutical Chemistry, an important topic in Life Sciences. This justifies our participation in National and International research projects, the support of a Pharmaceutical Company and funding from FLAD. Our know-how on chemistry, radiochemistry and radiopharmacy, and our facilities, have been also used to provide training and education to undergraduate, graduate and post-graduate students.

Research: During 2005, we went on with our studies on **halogen and metal *d*- and *f*-based radiotracers for biomedical applications**. In terms of targeting our

interest is on cancer and CNS pathologies. **The main scientific achievements are reported in the next pages.**

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 protocol, our facilities are also used, every year, during two weeks by the 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* (DR n° 123, 26/05/04, II série). For this Master Course the group established a protocol with the University of Lisbon (Faculties of Sciences, Pharmacy and Medicine), Hospital Garcia da Orta and Instituto Português de Oncologia/Lisboa. We have also participated in a PhD Teaching Program organized by ITQB/UNL. Physicians resident in Nuclear Medicine (*Portaria 555/2003, 11 June*) have also been trained in our laboratories.

At the **International level**, we have participated in the European Radiopharmacy Course, INSTN and have been partners in the EC/COST RTD ACTION, Virtual Radiopharmacy/V Framework Program and in the Coordination Action on Education and Training in Radiation Protection/VI Framework Program.

We have also trained several young scientists, funded by FCT grants, namely **BIC, 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

ISABEL SANTOS, Princ. Agregação, Group Leader
A. PAULO, Aux.
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Novel Estrogen Receptor Ligands as Potential Probes for Targeted Tumour Imaging and Therapy

M. Videira, A. Almeida, M.C. Oliveira, L. Gano, F.M. Marques, I. Santos, T. Thiemann¹, M. Watanabe¹, G.R. Morais¹, F. Botelho², A.C. Santos², C. Oliveira³

Objectives

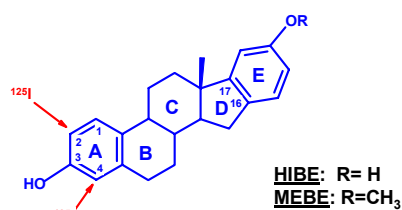
Synthesis of novel radioiodinated (¹²³I/¹²⁵I) estradiol analogues potentially interesting as probes for imaging or for therapy of estrogen receptor (ER) containing tumours. In these radiotracers the presence of the Auger electron emitters (¹²³I/¹²⁵I) might allow a highly selective target radiotherapy, with virtually no damage to the surrounding cells, if the compounds have the ability to achieve DNA causing double strands.

These studies may contribute to the development of new radiopharmaceuticals with potential application in the clinical care of breast and ovarian cancer patients.

Results

In our search for novel ER directed radioligands two families of estradiol derivatives are being explored: (A) areno-annelated estra-1,3,5(10),16-tetraen-3-ol and (B) C7-substituted estra-1,3,5(10),6-tetraen-3,17β-diol.

(A) Two novel areno-annelated estra-1,3,5(10),16-tetraen-3-ol with a hydroxyl (HIBE) or a methoxyl (MEBE) substituent on the E ring have been synthesised and characterized.

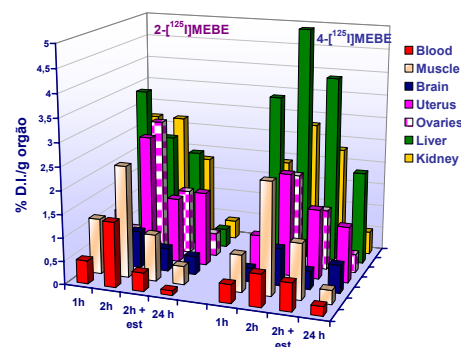


Radioiodination (¹²⁵I) at the *ortho* position of the A ring lead, for both compounds, to the formation of two radioisomers with high specific activity after purification by High Performance Liquid Chromatography (HPLC). All the radioligands studied are stable *in vitro*, more lipophilic than estradiol and have shown low binding to plasma proteins.

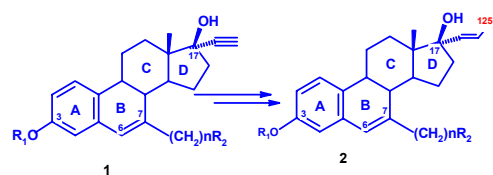
The effect of the site of radioiodination (C₂ vs C₄) on the biological behaviour of the radioiodinated MEBE was evaluated through biodistribution studies in immature female Sprague-Dawley rats. The two isomers, 2-[¹²⁵I]-MEBE and 4-[¹²⁵I]-MEBE, are stable *in vivo* and are mainly excreted through the hepatobiliary pathway. Both localize in the uterus and ovaries *via* a receptor mediated process having the 2-[¹²⁵I]-MEBE isomer higher specific ER binding and uterus selectivity.

Due to the favourable *in vitro/in vivo* stability and biodistribution profile of these radioligands the

evaluation of HIBE radioisomers is currently under investigation.



(B) Several radioiodinated 17α-ethynyl analogues (1) have been synthesised and characterized to be used as precursors in the synthesis of novel radioiodinated C7-substituted estra-1,3,5(10),6-tetraen-3,17β-diols (2). The *trans* isomer of 7-(6'-cyanoheptyl)-3-benziloxy-17α-iodovinylestra-1,3,5(10),6-tetraen-17β-ol and its non-carrier-added [¹²⁵I]iodovinyl analogue (2a) were synthesized, *via* destannylation of the corresponding *trans* tributylstannyl intermediate stereoselectively prepared from (1a). A radioiodinated compound with high specific activity was obtained after HPLC purification and has shown to be very stable *in vitro*. To get insights into the chemical structure/biological activity relationships the radiolabelling and biological evaluation of the other compounds is presently underway.



a: n=6, R₁=Bn, R₂=CN
b: n=4, R₁=H, R₂=CN
c: n=6, R₁=H, R₂=CN
d: n=10, R₁=H, R₂=CN
e: n=8, R₁=H, R₂=CONH₂
f: n=6, R₁=H, R₂=CONH₂

Published or in press work

- G.R. Morais, M.C. Oliveira, T. Thiemann, Synthesis of C7-substituted Estra-1,3,5(10),6-tetraen-3,17β-diols *Lett. Org. Chem.* In press.
- M. Videira, Estrogénios radioiodados para diagnóstico e terapia de tumores da mama Graduation Thesis, FCUL (2005).

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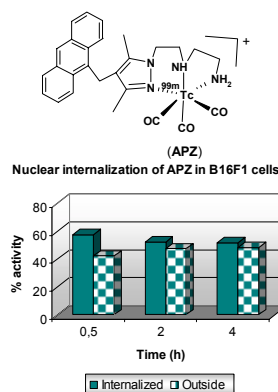
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Technetium-99m Complexes with DNA Binding Ligands for Selective Tumour Radiotherapy

 R. F. Vitor, F. Marques, A. Paulo, P. Raposo, I. Santos, I. Correia¹, J. C. Pessoa¹, A. S. Rodrigues², J. Rueff²

A series of ^{99m}Tc and Re complexes anchored by tridentate pyrazole-diamine chelators functionalized with anthracenyl fragments were prepared and evaluated. At macroscopic level, the interaction of the Re complexes with calf-thymus DNA has been studied by spectrophotometric, fluorescence, circular and linear dichroism (CD/LD) techniques. For the ^{99m}Tc congeners, cellular uptake, nuclear internalization and radiotoxicity studies have been performed in normal V79 and tumour B16F1 murine cells. One of the complexes (APZ) displays an enhanced radiotoxicity in B16F1 cells, which reflects most probably its ability to interact with DNA and its moderate to high cellular uptake and nuclear internalization. Future work involves the functionalization of this promising compound with a tumour seeking peptide, as well as the introduction of other DNA binding fragments, in order to increase the radiotoxicity of the complexes.



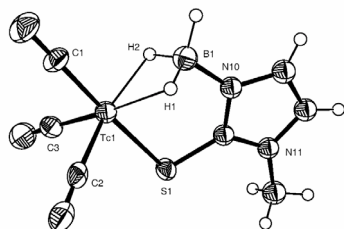
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Tc(I)/Re(I) Tricarbonyl Complexes Anchored by Trihydro(monoazolyl)borates: Novel Building Blocks for Labelling Small Biomolecules with ^{99m}Tc

 L. Maria, R. Garcia, A. Paulo, I. Santos, R. Alberto¹

Following our previous work on poly(mercaptoimidazolyl)borate Tc(I)/Re(I) complexes for radiopharmaceutical applications, we have introduced a novel class of soft scorpionates of the trihydro(mercaptoazolyl)borate type and evaluated their coordination capability towards the *fac*-[M(CO)₃]⁺ (M = Re, ⁹⁹Tc, ^{99m}Tc) moiety. At macroscopic level, X-ray crystallographic analysis of some of the resulting complexes confirmed the presence of the unprecedented donor atom set combining one sulphur atom and two agostic hydrides. Due to their lipophilicity and small-size, trihydro(mercaptoazolyl)borate technetium tricarbonyl complexes are being explored for designing radiopharmaceuticals for the targeting of brain receptors. These building blocks will be explored using the so-called pendant or integrated approaches, as exemplary demonstrated by the synthesis of complexes containing an appended piperazinyl fragment with affinity for serotonergic 5-HT_{1A} receptors or an integrated benzothiazolyl fragment for the recognition of β-amyloid plaques.

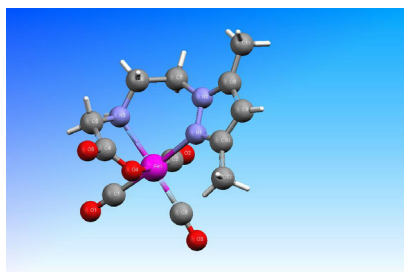


¹ Institute of Inorganic Chemistry, University of Zürich, Switzerland.

Novel Nuclear Imaging Agents for Targeting Epidermal Growth Factor Receptors (EGFR)

 C. Fernandes, C. Oliveira, L. Gano, I. C. Santos¹, I. Santos

The epidermal growth factor receptors (EGFR) belong to the ErbB family of receptor tyrosine kinases (TK) involved in the proliferation of normal and malignant cells. Our main goal is to find ¹²⁵I or ^{99m}Tc probes based on quinazoline derivative inhibitors of tyrosine kinase activity, for early detection and staging of cancers overexpressing EGFR. The bioactive precursor, N-{4-[(3-chloro-4-fluorophenyl)amino]quinazoline-6-yl}-3-bromo propionamide was synthesized and labelled with ¹²⁵I, via halogen exchange, and also coupled to a novel monoanionic asymmetric pyrazolyl containing ligand, [2-(3,5-dimethyl-pyrazol-1-yl)-ethylamino]-acetic acid, for labelling with ^{99m}Tc. The radioactive compounds, obtained with high radiochemical purity, were characterized and their *in vitro* and *in vivo* stability evaluated. The potential of these compounds as SPECT biomarkers for molecular imaging of EGFR positive tumours is currently under investigation. Using these two compounds it will be possible to evaluate the effect of the chelator and/or metal center on the biological activity of the quinazoline pharmacophore.

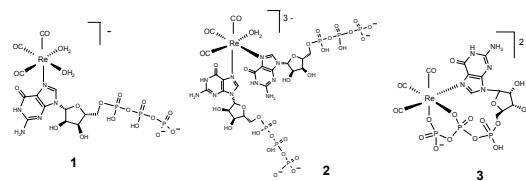


¹ QFES ITN group

Radioactive Probes for in vivo and Non-Invasive Assessment of Gene Expression

 C. Xavier, I. Santos, R. Alberto¹

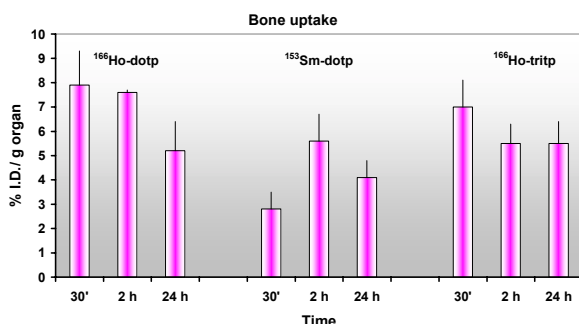
For imaging endogenous gene expression the ribonucleotide coding sequence is the target, and radiolabelled complementary oligonucleotides may be used as a probe. To achieve this goal direct or indirect labeling of DNA sequences has to be explored. To get a better insight into the so-called direct labelling of a DNA sequence, some basic research has been conducted, namely reactions of $\text{fac-}[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ with GMP, GDP or GTP. The organometallic moiety selectively binds to guanine (N7) base, but also to an oxygen atom of the phosphate, leading to a mixture of species of the type **1-3**. Species **3** predominates when the number of phosphates increases. This basic research will continue in order to find novel strategies for direct or indirect labeling of oligonucleotides.



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¹⁵³Sm and ¹⁶⁶Ho Complexes as Potential Therapeutic Bone Agents

M. P. C. Campello, F. Marques, L. Gano, S. Lacerda, I. Santos.

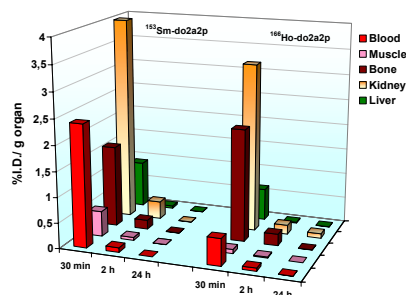
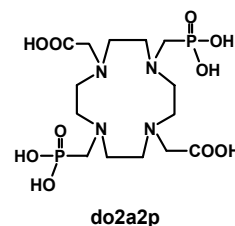


As part of our on going research work on radiolanthanides, ¹⁵³Sm and ¹⁶⁶Ho complexes with macrocyclic ligands containing methylphosphonate and/or methylcarboxylate pendant arms have been studied. Among the evaluated complexes, we found that ¹⁵³Sm and ¹⁶⁶Ho-tritp were prepared with high labeling efficiency, are very stable *in vitro* and *in vivo*, being also significantly taken by the bone. In particular, ¹⁶⁶Ho-tritp has 5-6% I.D./g bone, a high rate of total excretion and a rapid washout from main organs, which led to quite favourable bone/blood and bone/muscle ratios. The values found are comparable to those of ¹⁶⁶Ho-dotp, a compound which is presently in clinical trials. Thus, our studies support the potential interest of ¹⁶⁶Ho-tritp in bone pain palliation.

Biological Evaluation of ¹⁵³Sm and ¹⁶⁶Ho Complexes with a Novel Bis(methylphosphonate) Tetraazamacrocycle

M. P. C. Campello, L. Gano, F. Marques, S. Lacerda, T. Esteves, I. Santos.

To find the effect of the cavity size and/or the nature of the pendant arms on the biological profile of the Ln complexes, a novel bis(methylphosphonate) *do2a2p* was synthesized and characterized by ¹H, ¹³C, ³¹P NMR spectroscopy. With this novel ligand ¹⁵³Sm/¹⁶⁶Ho complexes have been prepared and evaluated. The complexes are obtained in high yield, are anionic, hydrophilic, present a high stability in human serum, up to 48 h, and a moderate

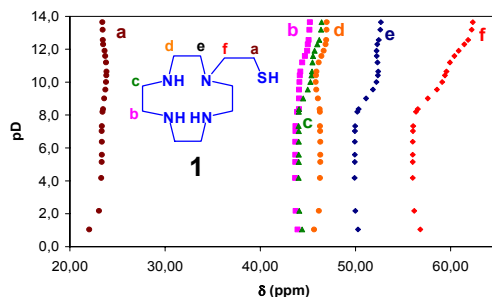


plasmatic protein binding. A relative high *in vitro* hydroxyapatite (HA) adsorption was found for the ¹⁶⁶Ho-do2a2p complex in comparison with ¹⁵³Sm-do2a2p. *In vivo* studies indicated a high stability for both complexes, a fast washout from most organs and a rapid total excretion. A relative high bone uptake was also observed, but the value rapidly decreases over time. These results clearly demonstrate that 12-membered macrocyclic ligands lead to very stable complexes with a biological profile adequate to targeted radiotherapy, being possible to increase and maintain bone targeting by using other bone-seeking groups than methylphosphonates.

Copper Complexes for Diagnostic and/or Therapeutic Applications

 S. Lacerda, M. P. C. Campello, I. Santos, R. Delgado¹

Cu offers an almost unique combination of radionuclides for imaging (^{60-62/64}Cu) and for targeted radionuclide therapy (^{64/67}Cu), being ⁶⁴Cu the most versatile as it can be used for PET imaging and for targeted radiotherapy. Moreover, their physical properties also allow its use in centres remotes from cyclotron. We have been exploring novel chelators to form stable and inert complexes with Cu. A novel macrocycle (**1**) was synthesised, characterised (¹H, ¹³C-NMR, MS) and its protonation and thermodynamic stability constants with Cu²⁺, Zn²⁺ and Cd²⁺ determined by potentiometry and/or by ¹³C-NMR spectroscopy (Figure 1). The values found indicated that K_{ML}[Cu1]>[Cd1]≈[Zn1]. The synthesis of other related ligands is in progress as well as their complexation with Cu, to select the most promising to pursue studies with ⁶⁴Cu.

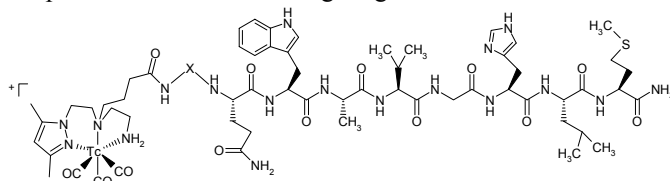


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Pyrazolyl Bombesin Conjugates Labeled with the *fac*-[^{99m}Tc(CO)₃]⁺ Moiety: Biological Behaviour

 S. Alves, J. D. G. Correia, I. Santos, B. Veerendra¹, G. L. Sieckman², T. J. Hoffman², T. Rold4, L. Retzloff,¹ J. McCrate,¹ A. Prasanphanich,¹ Charles J. Smith¹

Following our previous results on the promising biological behaviour of the new tumor-seeking bombesin pyrazolyl conjugate labeled with the *fac*-[^{99m}Tc(CO)₃]⁺ moiety, [^{99m}Tc-Pyrazolyl-G-G-G-Q-W-A-V-G-H-L-M-NH₂], we have introduced a new series of pyrazolyl bombesin conjugates radiolabeled via the tricarbonyl core. These new radiolabeled conjugates are based upon the general structure [^{99m}Tc-Pyrazolyl-X-BBN[7-14][NH₂]] (X = β-Alanine, Serylserylserine, or Glycylglycylglycine). Results of our investigations demonstrate the ability of these new radiolabeled conjugates to specifically target the gastrin releasing peptide receptor subtype 2 which is over-expressed on human, prostate, PC-3 cancerous tissue. Therefore, these studies suggest the tridentate pyrazolyl ligand framework to be an ideal candidate for design and development of low-valent ^{99m}Tc-based diagnostic radiopharmaceuticals based upon bombesin or other targeting vectors.



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² Research Division, Harry S. Truman Memorial Veterans' Hospital, Columbia, Missouri, United States, 65201.

Melanoma targeting with [^{99m}Tc(CO)₃-Pyrazolyl-MSH analogs]-conjugates

P. Raposinho, J. D. G. Correia, S. Alves, I. Santos

Radiolabeled analogs of α-MSH have been investigated for melanoma (ML) imaging due to overexpression of MC-1 receptors in both melanotic and amelanotic ML. Two analogs were conjugated to pyrazolyl (pz1), radiolabeled with the *fac*-[^{99m}Tc(CO)₃]⁺ moiety and their potential for ML diagnosis evaluated *in vitro* (ML B16F1 cells) and *in vivo* (ML-bearing mice). The radiopeptide **1** exhibited a better biological profile than the radiopeptide **2** with a higher degree of cell internalization (about 50% at 3h) and a better cellular retention (only 38% released from the cell after 5h). Consequently, a relatively good and specific tumor uptake (4.2 ± 0.9%ID/g, at 4h, 2.7 ± 0.6%ID/g, at 24h) was observed for the *fac*-[^{99m}Tc(CO)₃-pz1-NAPamide]-conjugate. Other analogs, in particularly cyclic peptides, could be also promising and are under study.

pz1-MSHoct: **pz1**-βAla-Nle-Asp-His-DPhe-Arg-Trp-Lys-NH₂ (1)

pz1-NAPamide: Ac-Nle-Asp-His-DPhe-Arg-Trp-Gly-Lys(**pz1**)-NH₂ (2)

