Radiopharmaceutical Sciences

Isabel Rego Santos

Apart from the continuing improvement in equipment and technology, the success story of **Nuclear Medicine** is strongly dependent on the availability of powerful nuclear probes for molecular imaging and/or targeted therapy. The **Radiopharmaceutical Sciences Group** does **basic/applied-oriented research and technology transfer** on nuclear tools for ***molecular imaging* and/or *targeted radiotherapy*.** This is a multidisciplinary task based on innovative organic and coordination chemistry, bioconjugation, radiochemistry, animal and cell studies and cellular and molecular biology knowledge afforded by modern genomics/proteomic research. We have implemented and developed expertise on these fields and facilities to carry on such activities. Such combination, **unique in the country,** was reinforced this year with the acquisition of a Microwave-Peptide Synthesizer and justifies our participation in national and international research projects, the support of an international pharmaceutical company, and the involvement in education and training.

Our main achievements are described in this report. However**,** we would like to emphasize the following:

Research:

1 - The **new myocardium imaging,** designed and synthesized by the Group, passed pre-clinical, toxicity and dosimetry tests and is being developed in **GMP** conditions by a **Pharmaceutical Company**, **for human evaluation**. **A significant achievement** for **ITN** and **Portuguese Science and Technology.**

2 - A **new methodology** for preparing *in situ* metal complexes with hybrid poly(azolyl)borates has been implemented, allowing a **fast *in vitro* screening** of the biological properties of the complexes.

3-Biological studies with new **^{99m}Tc-complexes** have **confirmed *in vitro*** DNA damage and apoptotic cell death by **Auger electrons**.

4-Complexes of ***d*- and *f*- elements with tetraazamacrocycles** bearing clinically relevant pendant arms **have been synthesized**, **characterized in the solid state and in solution and biologically evaluated.** This was a significant improvement at the macroscopic level to correlate with the corresponding radioactive complexes.

Education and Training:

1-Graduation, the Group teaches Radiopharmacy at ESTSeL and Faculty of Pharmacy/University of Lisbon. Under a protocol, students of the Nuclear Medicine Course/ESTeSL are trained in our group (two weeks/year).

2-Post-graduation, organizes and coordinates the Master Course *Biomedical Inorganic Chemistry: Diagnostic and Therapeutical Applications.* During 2008, coordinated the Discipline Radiopharmaceutical Chemistry in the *Master Course Pharmaceutical and Therapeutical Chemistry*, Faculty of Pharmacy/UL, and the Discipline Chemical Systems and Reactivity in the 2nd Cycle in Chemistry and lectured in PhD Teaching Programs organized by Universities/Associated Laboratories.

3-International level, teaching in the European Radiopharmacy Course, INSTN.

4-Young scientists: Eighteen are trained in the group, playing a major role in our projects.

Expertise Provided: Nuclear Medicine Centers, Portuguese Medicines Evaluation Agency, IAEA and Foreigner Science Foundations.

Financial support: Mallinckrodt Medical B.V. (a Covidien Company), FCT, CIMAGO/FLAD, BILATERAL and EC/COST ACTIONS.

Publications: Peer-Review International Journals – 12; Patents - 1; Proceedings./Reports- 5; Communications - 24; Thesis: MSc – 5. **Invited Lectures and Seminars**: 8.

Research Team

Researchers

I. SANTOS, Princ., Group Leader
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M. C. OLIVEIRA, Aux.
L.GANO, Aux.
F. MARQUES, Aux.
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F. MENDES, Pos Doctoral, FCT grant, Aux contract since Sept.
C.FERNANDES, Ass..

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S. GAMA, Pos-Doctoral, FCT grant
P. ANTUNES, Pos-Doctoral, FCT grant
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E. PALMA, Ph.D. student, FCT grant
C. MOURA, Ph.D. student, FCT grant
T. ESTEVES, Ph.D. student, FCT grant
C. NETO, Ph.D. student, FCT grant
B. OLIVEIRA, Ph.D. student, FCT grant
M. BALBINA, M.Sc. student
S. CUNHA, M.Sc. student
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F. SILVA, M.Sc. student
F. FIGUEIRA, M.Sc. student
M. MORAIS, M.Sc. student
G.CLEMENTE, M.Sc. student
M. VIDEIRA, BIC grantee, POCI

Technical Personnel

A. RODRIGUES
E. CORREIA

A New Myocardial Perfusion Imaging Agent: Evaluation in Rats and Comparison with ^{99m}Tc-Sestamibi and ^{99m}Tc-Tetrofosmin

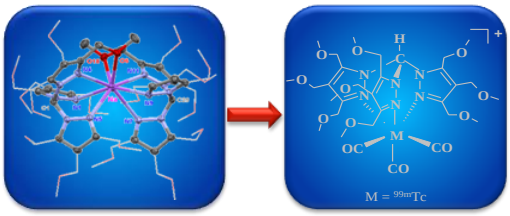
A. Paulo, V. Caveliers[1], C. Fernandes, H. Knight[2], M. Bauwens[1], T. Lahoutte[1], I. Santos

Objective

The main goal of this project is to find good performing nuclear myocardial probes to detect perfusion abnormalities in asymptomatic coronary artery disease (CAD) patients, avoid myocardial infarction, and apply therapeutic regimes, before irreversible myocardial damage occurs. Such probes will improve Nuclear Cardiology, an important and noninvasive tool for the clinical evaluation of patients with known or suspected CAD.

Results

The new myocardial imaging agent ^{99m}Tc-TMEOP (**1**), where TMEOP is a neutral and tridentate nitrogen donor chelator, has been quantitatively synthesized (Scheme 1).



Scheme 1: Synthesis of **1**

The chemical identity of **1** was established by comparing its HPLC chromatogram with the UV-vis trace of the Re congener (**1a**) (Figure 1).

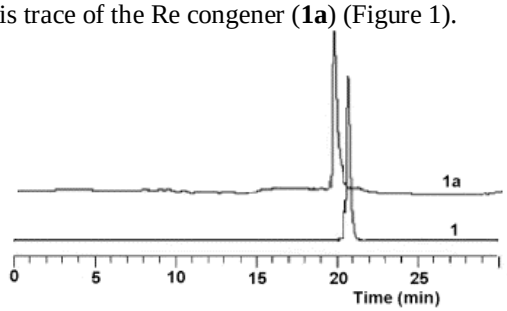


Figure 1. HPLC chromatograms: **1** (γ -detection), **1a** (UV-vis detection)

Complex **1** is stable both *in vitro* and *in vivo*. Dynamic imaging studies have shown that the cardiac uptake of **1** is comparable to ^{99m}Tc-MIBI (**2**) and ^{99m}Tc-TETRO (**3**), but presents a significantly faster clearance from the liver (Figures 2 and 3).

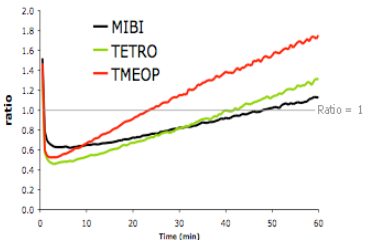


Figure 2. Heart-to-liver ratio of ^{99m}Tc-TMEOP (**1**), ^{99m}Tc-MIBI (**2**) and ^{99m}Tc-TETRO (**3**).

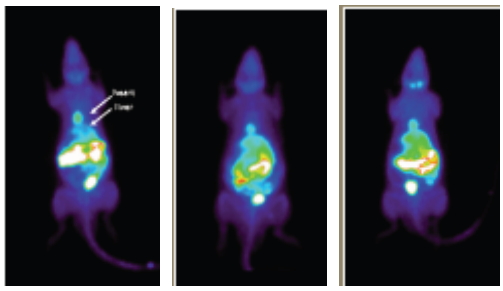


Figure 3. Rat whole body images show diminished liver activity for ^{99m}Tc-TMEOP (**1**) and a better delineation of the heart

High quality SPECT images could be obtained with the new tracer **1** (Figure 4).

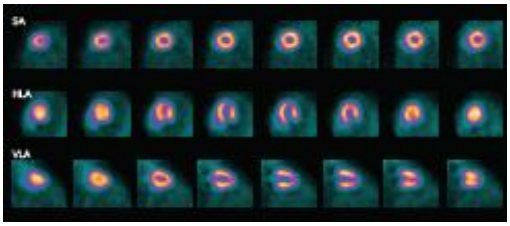


Figure 4. Short-axis (SA), horizontal long-axis (HLA) and vertical long-axis (VLA) slices of a pinhole myocardial perfusion study in Wistar rats, using ^{99m}Tc-TMEOP (**1**).

The high heart uptake and fast liver clearance of **1** should result in less artifacts and improved diagnostic accuracy for detecting CAD, making **1** a very promising compound for further evaluation in human studies.

Published work:

I. Santos, A. Paulo, Tricarbonyl Complexes with Tridentate Chelators for Myocardium Imaging, WO 2008/061792 A2.

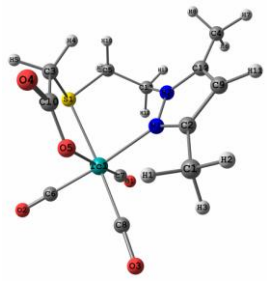
L. Maria, C.Fernandes, R. Garcia , L. Gano, A. Paulo, I.C. Santos, I. Santos, Tris(pyrazolyl)methane ^{99m}Tc Tricarbonyl Complexes for Myocardium Imaging, *Dalton Trans*. DOI: 10.1039/b817451b. **Hot Article**, www.rsc.org/Publishing/Journals/dt/HotArticles.asp.

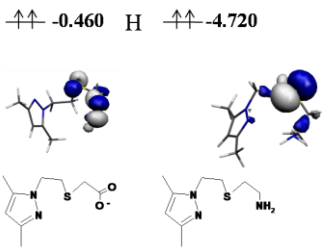
A. Paulo, V. Caveliers, C. Fernandes, H. Knight, M. Bauwens, T. Lahoutte, I. Santos, A New Myocardial Perfusion Imaging Agent: Evaluation in Rats and Comparison with ^{99m}Tc-Sestamibi and ^{99m}Tc-Tetrofosmin, 2008 World Molecular Imaging Congress, Nice (France), September 2008.

[1]Dep. of Nuclear Medicine, Univ. Hospital Brussels (UZ Brussel),
[2]Mallinckrodt Medical B.V., Petten, The Netherlands

Rhenium and Technetium Complexes Anchored by Tridentate Pyrazolyl-Based Chelators for the Labelling of Clinically Relevant Biomolecules

C. Moura, C. Fernandes, A. Paulo, I. Santos, M. J. Calhorda[1]



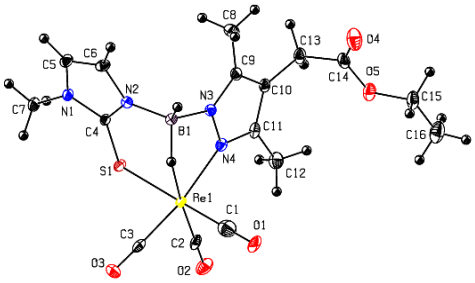


Searching for building blocks suitable for the labelling of biomolecules with the *fac*-$[^{99m}Tc(CO)_3]^+$ and $[^{99m}Tc{=}O]^{3+}$ cores, our group has evaluated novel families of tridentate pyrazolyl-based chelators having different donor atom sets. Within the organometallic approach, *in vitro* and *in vivo* studies demonstrated that the charge of the chelator and/or donor atom set (*e.g.* N,N,O *vs* N,O,S) strongly influence the behaviour of the complexes, particularly in terms of their *in vitro* stability. To have a more rationale insight into such behaviour, DFT calculations were performed. These studies suggested that the differences are not controlled by thermodynamic factors; theoretical studies to evaluate kinetic aspects are underway.

[1] DQB, Faculdade de Ciências da Univ. de Lisboa, Portugal

Rhenium(I) Tricarbonyl Complexes with Hybrid Poly(azolyl)borates: A Novel Class of Compounds Potentially Useful for Radiopharmaceutical Research

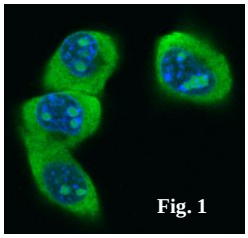
M. Videira, P. Antunes, A. Paulo, I. Santos



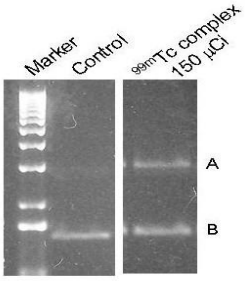
Re(I) tricarbonyl complexes anchored by hybrid poly(azolyl)borates were formed by breaking the Re..H-B bond of the unprecedented *fac*-$[Re\{\kappa^3\text{-}H(\mu\text{-}H)_2B(tim^{Me})\}(CO)_3]$ with azoles (e.g. 2-mercaptomidazoles or pyrazoles). X-ray diffraction analysis of the complexes confirmed the presence of heteroscorpionates displaying (k^3-H, S, S'), (k^3-H, S, N) or (k^3-S, N, N) binding motives. This approach represents a great advantage, since it affords more straightforwardly poly(azolyl)borate Re(I) tricarbonyl complexes, which allows a faster *in vitro* screening of their biological and/or pharmacological properties. Such improvement avoids the independent synthesis of asymmetric scorpionates bearing bioactive vectors, a time consuming and challenging task.

Organometallic Complexes for Targeted Radiotherapy

T. Esteves, S. Gama, F. Mendes, F. Marques, A. Paulo, I. Santos, S. Casimiro,[1] *L. Costa*[1]



Aiming to demonstrate that ^{99m}Tc compounds may hold potential for targeted radiotherapy, we evaluated *in vitro* and *in vivo* a series of Re(I) and ^{99m}Tc(I) tricarbonyl complexes with pyrazole-diamine chelators bearing DNA-binding groups. Fluorescence microscopy studies have shown that the Re complexes can target the nucleus of murine B16F1 melanoma cells (Fig. 1). Some of the ^{99m}Tc congeners induce important damage to plasmid DNA (Fig. 2) and significant cell death through apoptotic pathways, as shown by the PARP assay (Fig. 3). So far, the results obtained indicate that the DNA damage and cell death are induced by the Auger electrons emitted by ^{99m}Tc, although being strongly influenced by the nature of the DNA-binding group.



A- Open circular form
B- Supercoiled circular form

Fig. 2

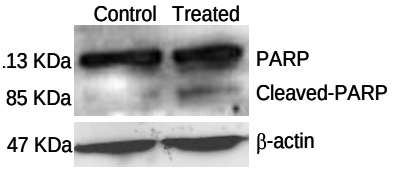


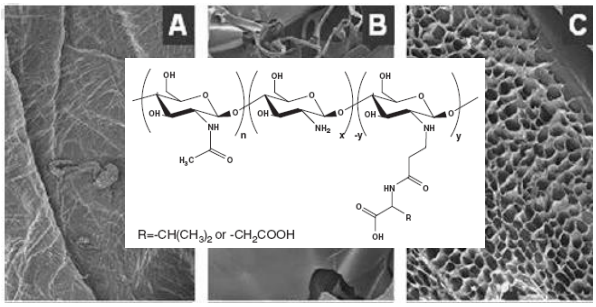
Fig. 3

[1] Inst. de Medicina Molecular, Lisboa, Portugal

Functionalized Natural Polymers (chitosan and dextran) for Delivery of Radioactivity *in vivo*

F. Marques, L. Gano, M. Morais, J. D. G. Correia, M. K. S. Batista,[1,2] C. A. R. Gomes,[2] P. Gomes[1] I. Santos,

Aiming to develop new polymeric systems for the delivery of radioactivity *in vivo* for both internal radiation therapy and radiodiagnosis, we have prepared in high yields (> 95%) ^{153}Sm/^{166}Ho and ^{99m}Tc complexes with amino acid-chitosan and dextran-amine-pyrazolyl-mannose polymers, respectively. These radioactive polymers presented high water solubility and stability, which are essential features for radiopharmaceutical applications. The favourable radiochemical and biological behaviour found, highlights the interest of exploring new chemical modifications of natural polymers in order to improve the biokinetics and specificity of the resulting radioactive complexes.

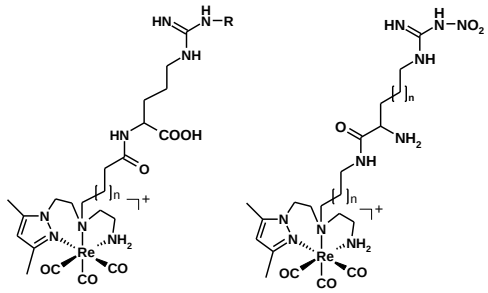


[1] CIQUP, DQ, Fac. Ciências da Universidade do Porto, Portugal
2 LAQUIPAI, DQ, Fac. Ciências da Universidade do Porto, Portugal.

New Re(I)/^{99m}Tc(I) Complexes Containing Pendant L-Arg Derivatives with Enhanced Inhibitory Potency toward iNOS

B. L. Oliveira, F. Figueira, J. D. G. Correia, P. D. Raposinho, C. Cordeiro,[1] A. P. Freire[1] I. Santos

Conjugates containing a pyrazolyl-diamine chelating unit and pendant L-arginine analogues (substrates or inhibitors of NOS) were synthesized to design radioactive compounds for probing inducible nitric oxide synthase (iNOS) levels *in vivo*. Those conjugates allowed the preparation of complexes of the type *fac*-[M(CO)$_3$(k^3-L)]$^+$ (M = Re, ^{99m}Tc). The enzymatic studies revealed that the affinity of the inhibitor-containing conjugates to iNOS seems to be less affected upon metallation than the substrate-containing conjugates. The complexes bearing N$^\omega$-Me- (**1**) or N$^\omega$-NO$_2$- (**2** - **5**) guanidine-substituted analogues of L-arginine present considerable inhibitory action, being the first examples of organometallic complexes able to inhibit the iNOS. The inhibitory potency of complex **3** is identical to that presented by the strong inhibitor N$^\omega$-NO$_2$-L-arginine (K_i = 3 μM).



1 (n = 1, R = Me): K_i = 36 μM
2 (n = 1, R = NO$_2$): K_i = 84 μM
3 (n = 4, R = NO$_2$): K_i = 5 μM
4 (n = 1): K_i = 103 μM
5 (n = 4): K_i = 118 μM

[1] DQB, Faculdade de Ciências da Univ. de Lisboa, Portugal.

^{99m}Tc(CO)$_3$-labeled Bisfosfonates for Bone Imaging

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

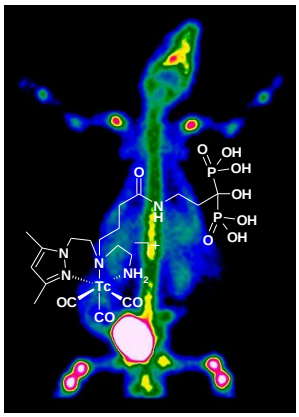


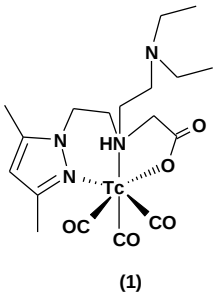
Fig. 1.

Radionuclide bone imaging is useful for evaluation of many pathologic conditions, namely bone metastasis. The radioactive complex *fac*-[^{99m}Tc(CO)$_3$(k^3-**pz-pamidronate**)]$^+$ presents improved radiochemical and biological properties as bone-seeking radiotracer. Besides a fast rate of blood clearance and high rate of total radioactivity excretion, the compound presents significant bone uptake (4.7±0.9 % ID/g organ, 4 h p.i.) and favorable bone/blood (40, 4 h p.i.) and bone/muscle (50, 4 h p.i.) ratios.

Figure 1 shows a SPECT imaging obtained when a Sprague Dawley rat model was injected with *fac*-[^{99m}Tc(CO)$_3$(k^3-**pz-pamidronate**)]$^+$. The image has confirmed the satisfactory biodistribution profile.

Melanoma Targeting with $^{99m}Tc(CO)_3$-labeled Benzamides and α-MSH analogs.

C. Moura, P. Raposinho, A. Paulo , J. D. G. Correia, L. Gano, I. Santos



(1)

Cationic and neutral ^{99m}Tc (I) tricarbonyl complexes, anchored by pyrazolyl-containing chelators with (N_3) or (N_2O) donor atom sets were studied to target MC1 receptors or melanin in melanoma. The targeting of MC1R´s was based on complexes bearing a lactam-based cyclic peptidic antagonist of MC1R, while the targeting of melanin was based on complexes bearing (dialkylaminoalkyl)benzamide pharmacophores or their fragments (**1**). Cellular uptake in B16F1 melanoma cells and tumor uptake in melanoma-bearing mice were evaluated for all compounds. Some of the complexes have shown a moderate cellular and tumor uptake (Fig. 1). Currently, related complexes bearing the same or different pharmacophores conjugated to different positions of the bifunctional chelators are being evaluated to improve their biological performance.

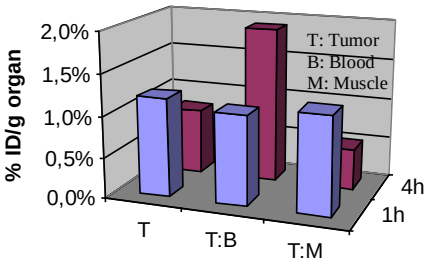
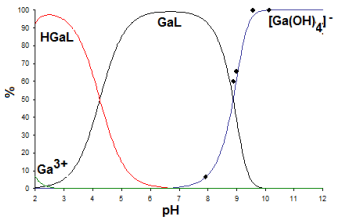


Fig. 1

Ga(III)/ In(III) Complexes for Molecular Imaging and/or Chemoterapeutic Applications

R. Garcia, F. Silva, P. Fousková[1], L. Gano, F. Marques, A. Paulo, E. Tóth[1], I. Santos



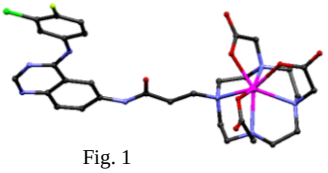
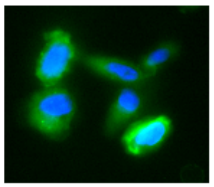


Fig. 1

Ga(III)/In(III) complexes anchored by tetraazamacrocycles bearing quinazoline moieties (Fig.1) were evaluated as radioactive probes for *in vivo* molecular imaging of EGFR´s. The Ga(III) complex (Fig. 1) exhibits a remarkable high thermodynamic stability constant and the congener with ^{67}Ga was prepared in high yield/high radiochemical purity.

Other Ga(III) complexes anchored by pyrazolyl-based chelators were explored as cytotoxic metallopharmaceuticals.

Studies with human PC-3 prostate cancer cells have shown that one of the complexes presents significant cytotoxicity, inducing apoptosis (Fig. 2). The potential of these complexes for the design of imaging or antitumor drugs is currently being explored.



(a)

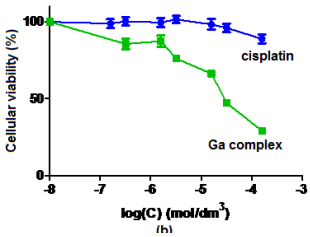


Fig.2

[1] *Centre de Biophysique Moléculaire CNRS, Rue Charles Sadron, 45071 Orléans Cedex 2, France*

Peptide Nucleic Acids Labelled with the *fac*-$[^{99m}Tc(CO)_3]^+$ Moiety for Monitoring Endogenous Gene Expression

C. Xavier, C. Giannini[1], S. Dall'Angelo[1], L.Gano, S. Maiorana[1], R. Alberto[2], I. Santos

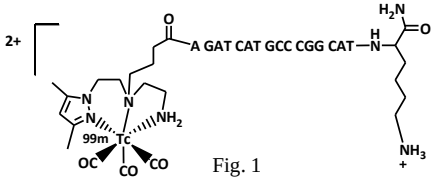


Fig. 1

The main goal of this research project is to find novel ^{99m}Tc probes for non-invasive imaging of endogenous gene expression, using the antisense approach. Taking into account the advantages of Peptide Nucleic Acids (PNAs), we have synthesized a 16-mer PNA antisense sequence (*N*- A GAT CAT GCC CGG CAT-*C*), complementary to a region of *N-MYC* mRNA, which is overexpressed in peripheral and central nervous system tumours. This sequence was labeled with ^{99m}Tc(I) tricarbonyl, using a bifunctional approach (Fig. 1). Studies with cells expressing *N-MYC* mRNA (SH-SY5Y cell line) have shown a relatively high cellular internalization and retention of the ^{99m}Tc complex (Fig. 2). Further *in vitro* and *in vivo* studies are underway.

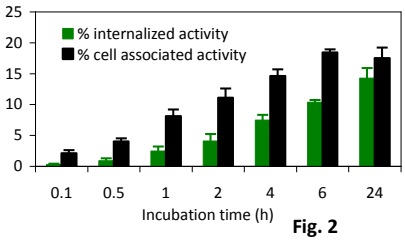


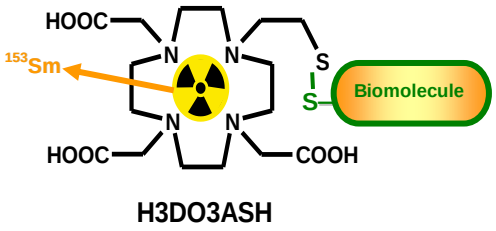
Fig. 2

[1] Dep.of Organic and Industrial Chemistry, Univ. of Milan, Italy
[2] Inst. of Inorganic Chemistry, Univ. of Zurich, Switzerland

Tetraazamacrocycle Bearing Mixed Thiolate/Carboxylate Pendant Arms for Labeling Biomolecules with Radiolanthanides

S. Lacerda, M. P. C. Campello, V. Kubíček[1], P. Fousková[1], E. Toth[1], I. Santos

Following our previous work on the synthesis of bifunctional chelators for labelling biomolecules, we have studied ^{153}Sm and ^{166}Ho radiocomplexes of do3aSH. The pharmacokinetics of these complexes prompted further studies towards the evaluation of the involvement of the thiolate group in the coordination sphere of the lanthanide ion. Protonation and stability contants of do3aSH with Ce^{3+}, Sm^{3+} and Ho^{3+} have been determined by potentiometry and UV-Vis spectroscopy. These results together with relaxivity measurements on the Gd^{3+}-DO3ASH complex, suggest that the thiolate group, even in its deprotonated form, does not coordinate to the metal. The *in vivo* stability and favourable biological profile of ^{153}Sm-DO3ASH, together with the presence of a free thiol group available, make this novel tetraazamacrocycle suitable for functionalization with clinically relevant biomolecules *via* the thiolate pendant arm, which is currently in progress.

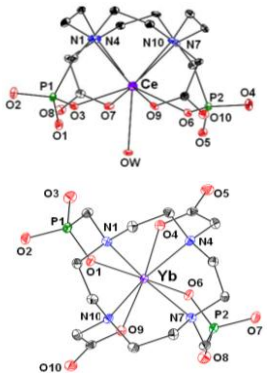


H3DO3ASH

[1] Centre de Biophysique Moléculaire, CNRS, Orléans Cedex 2, France

Structural Studies of Lanthanides Complexes with *trans*-H6DO2A2P.

M. Paula C. Campello, S. Lacerda, I. C. Santos, C. Geraldes[1], P. Hermann[2], J. Kotek[2], P. Lubal[3] I. Santos



Following our previous studies on ^{153}Sm/^{166}Ho-*trans*-do2a2p, we have synthesized and characterized at the macroscopic level complexes of *trans*-H_6DO2A2P with the lanthanide series. The main goal was to evaluate how the structure in the solid state and in solution changes along the series, and also to evaluate the mechanism of formation and dissociation of the complexes. We have found that the stability constants of *trans*-DO2A2P with Ln(III) ions increases along the series, while the kinetic inertness decreases. Reactions of *trans*-do2a2p with $LnCl_3$ yielded mono crystals suitable for X-ray crystallography. The complexes formed are of the type [Ln(*trans*-do2a2p)$(H_2O)_x$] (Ln=Ce, Nd, Sm, x=1; Ln=Sm, Eu, Tb, Dy, Tm Yb, x=0), in which the *trans*-H_6DO2A2P chelator behaves as octadentated. ^{1}H NMR paramagnetic shifts calculations agree with the X-ray data, revealing a twisted square antiprismatic structure for all the complexes (TSA, m' for x = 0; CTSA, m for x = 1).

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Estradiols for Molecular Recognition of ER(+) Breast Tumour: Structure/Activity studies

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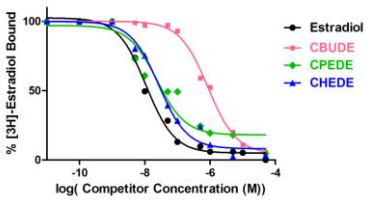
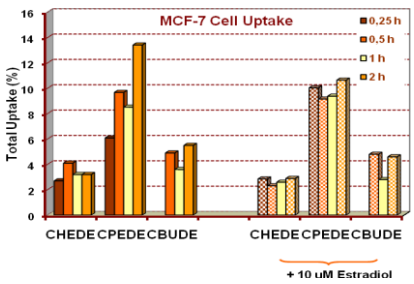


Seeking for new probes for targeting the estrogen receptor (ER), a series of radioiodinated $\Delta^{6,7}$–estradiol derivatives, with C7 ω-cyanoalkyl substituents of different chain length were evaluated, to relate biological behaviour/chemical structure. ER binding data indicate that, compared to estradiol, the addition of a C7 ω-cyanoalkyl chain decreases the binding affinity, but the values increase by lengthening the C7-chain. The trend found was: butyl < pentyl < hexyl (CBUDE; CPEDE, CHEDE). Cell uptake in MCF-7 cells was observed for the three radioligands. However, the cellular uptake seems to be a non ER-mediated process, as the cellular uptake does not change in the presence of estradiol.



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