Radiopharmaceutical Sciences

Isabel Rego Santos

The **Radiopharmaceutical Sciences Group** developed and implemented expertise and facilities to carry on basic/applied oriented research and technology transfer ***on nuclear tools for SPECT and PET molecular imaging and for targeted radiotherapy.*** The group is multidisciplinary with expertise on organic and coordination chemistry, bioconjugation, radiochemistry, animal and cell studies, and molecular biology. Such expertise and facilities enable the RS group to deal with problems of modern Radiopharmaceutical Sciences and to provide education and training at different levels.

The main achievements during 2010:

Research:

1 – Publication of a masterpiece describing a wealth of interesting structural and physicochemical properties of an entire series of lanthanide macrocyclic complexes. Such results helped to interpret kinetic data along the lanthanide series, an important issue for medical applications.

2- Based on the bone-seeking/imaging properties of $^{99m}Tc(CO)_3$-alendronate, a new project between the Group and the Clinical and Translational Oncology Research Unit/IMM/U. Lisbon has started, aiming at the design of multifunctional compounds for the imaging/treatment of bone metastases.

3 - In close cooperation with the Group of Theoretical and Computational Biochemistry/ Faculty of Sciences/U. Porto, molecular docking and molecular dynamic studies were initiated to get insight into the structural parameters responsible for the increased inhibitory effects of Re(I)-complexes towards iNOS

4 – The first ^{99m}Tc-organometallic complex combining specific cell targeting with nuclear internalization has been isolated. This result opens new avenues for the design of Auger therapeutic agents.

5 - Within the framework of a wide cooperation involving our Group, the Cell and Molecular Neuroscience Unit/IMM/U. Lisbon and the ICNAS/U. Coimbra, we have introduced a set of fluorinated azole derivatives aiming at the targeting of amyloid aggregation.

6 - The radiotracer ^{99m}Tc-TMEOP, designed by our Group for myocardial imaging, localizes in the mitochondria, being potentially useful for *in vivo* tumour multidrug resistance (MDR) detection.

Education and Training

1-Graduation:

Radiopharmacy teaching at ESTSeL and at Faculty of Pharmacy/University of Lisbon.

2-Post-graduation:

a) Coordination of the Master Course Biomedical Inorganic Chemistry: Diagnostic and Therapeutical Applications (ITN/UL). Coordination and teaching of Radiochemistry and Biomedical Inorganic Chemistry in the same MSc course.

b) Coordination and teaching of Radiopharmaceutical Chemistry in the Master Course Pharmaceutical and Therapeutical Chemistry/Faculty of Pharmacy/UL.

c) Teaching of Chemical Systems and Reactivity in the 2nd Cycle of Chemistry, Faculty of Sciences/UL

d) Teaching at the Master in Pharmaceutical Sciences, Lusófona University.

e) Teaching at the Master in Human Molecular Biology, Faculty of Sciences/UL

e) Lectures in PhD Teaching Programs organized by Universities/Associated Laboratories, namely ITQB/UNL.

Expertise Provided:

Nuclear Medicine Centers, Portuguese Medicines Evaluation Agency, IAEA, Foreigner Science Foundations (Canada and Uruguay), International Conferences and International Journals.

Publications:

Peer-Review International Journals – 19; Reports - 8; Proceedings – 11; Communications – 24; Invited Lectures and Seminars: 14.

Research Team

Researchers

I. SANTOS, Princ., (Agreg.) Group Leader
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L.GANO, Aux.
F. MARQUES, Aux.
P. RAPOSINHO, Aux.
C.FERNANDES, Aux.
F. MENDES, Aux.
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E. PALMA, Ph.D. student, FCT grant
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T. ESTEVES, Ph.D. student, FCT grant
C. NETO, Ph.D. student, FCT grant
B. OLIVEIRA, Ph.D. student, FCT grant
S. CUNHA, Ph.D. student, FCT grant
F. SILVA, Ph.D. student, FCT grant
M. MORAIS, Ph.D. student, FCT grant
R. GOMES, Undergraduate student
M.ANTUNES, Undergraduate student
A. NEVES, Undergraduate student
H. BATISTA, Undergraduate student

Technical Personnel

RODRIGUES
E. CORREIA

Lanthanide(III) Complexes of *trans*-H_6do2a2p: Structural Studies Along the Series

M. Paula C. Campello, Sara Lacerda, Isabel C. Santos, Giovannia A. Pereira, [1] Carlos F. G. C.Geraldes,[1] Jan Kotek,[2] Petr Hermann,[2] Jakub Vaněk,[3] Přemysl Lubal,[3] Vojtěch Kubíček,[4] Éva Tóth,[4] Isabel Santos

The main goal of this project is the design of Ln–based bone-seeking agents. To get a better insight in the biological behaviour of the radiolanthanide complexes and to improve their pharmacokinetics, complexes of trans-H_6do2a2p- H_6L with Ln(III) ions were investigated at the macroscopic level.

Results

The tetraazamacrocycle 1,4,7,10-tetraazacyclododecane-1,7-bis(acetic acid)-4,10-bis(methylenephosphonic acid), trans-H_6do2a2p-H_6L (Fig.1), reacts with ^{153}Sm/^{166}Ho nitrates yielding very stable radiolanthanide complexes with similar biological profile. Trying to explain these results, the complexation properties of *trans*-H_6do2a2p along the lanthanide series were investigated.

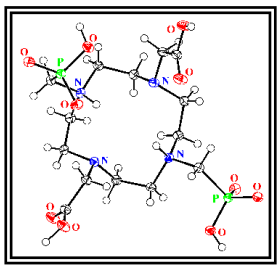
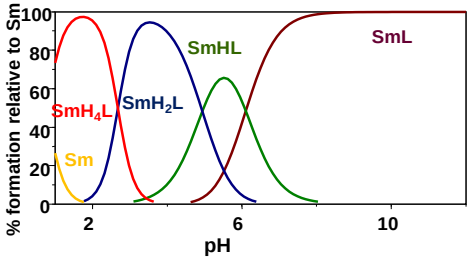


Fig.1. Molecular structure of H_6L.

Potentiometric and ^{1}H/^{31}P NMR studies have shown the formation of very stable and kinetically inert complexes, being the [LnL] species the only one present in solution at pH ≥ 8 (Fig. 2).

Fig. 2. Species Distribution Diagram for Sm-H_6L.



In the solid state the [LnL] complexes (Ln = Ce, Nd, Sm, Eu, Tb, Dy, Er, Yb) are present as twisted square antiprismatic isomers. However, a change from nonacoordinated complexes, with one water molecule in the coordination sphere (Ce→Sm), to anhydrous octacoordinated complexes (Sm→Yb) occurs.
The central ions move more deeply inside the ligand cavity in the Ce–Sm series and then almost do not move further up to Yb. The water coordination has also a strong effect on the opening angle OP-Ln-OP and on the twist angle of the phosphonic/acetic pendant arms (Fig 3).

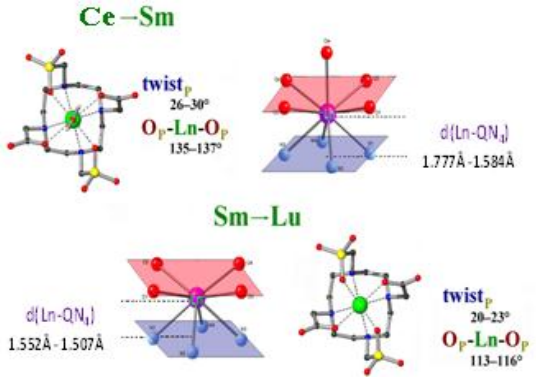


Fig.3 Structural parameters of LnL complexes.

The structures are maintained in solution, as indicated by ^{1}H/^{31}P NMR analysis. The lanthanide induced shifts (LIS) data reflect more the gradual geometrical change of the metal coordination sphere than the change of the hydration number of the complexes which occurs at Sm.
The structural data found for the lanthanide complexes in solution and in the solid state agree with the biological profile found for the [^{153}Sm-do2a2p] and [^{166}Ho-do2a2p] complexes.

Published work:

M. P. C. Campello, S. Lacerda, I. C. Santos, G.A. Pereira,[1] C.F.G.C. Geraldes,[1] J. Kotek,[2] P. Hermann,[2] J. Vaněk,[3] P. Lubal,[3] V. Kubíček,[4] É. Tóth,[5] I. Santos, Lanthanide(III) Complexes of t*rans*-H_6do2a2p in Solution and in the Solid State: Structural Studies Along the Series, *Chem. Eur. J*. **16** (2010) 8446-8465.

M. P. C. Campello, S. Lacerda, I. C. Santos, G.A. Pereira,[1] C.F.G.C. Geraldes,[1] J. Kotek,[2] P. Hermann,[2] J. Vaněk,[3] P. Lubal,[3] V. Kubíček,[4] É. Tóth,[4] I. Santos, Lanthanide(III) Complexes of trans-H_6do2a2p: Synthesis, Structural Studies, Labelling and Biological Evaluation, Cost D38 Action Annual Meeting, 20-22th June, Thessaloniki, Greece.

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[2] Dep. Inorg. Chemistry, Charles University in Prague, Czech Republic
[3] Dep. Chemistry, Masaryk University, Brno Czech Republic
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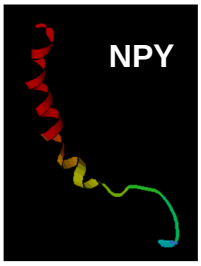
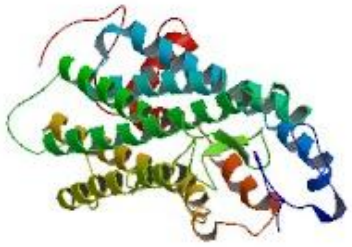
Radiolabeled neuropeptide Y (NPY) analogs for Y1 receptor-targeting in breast cancer

P. Antunes, P. Raposinho, C. Fernandes, I. Santos



The extremely high expression and incidence of neuropeptide Y1 receptors (NPYY1R) in breast cancer make them a promising target for molecular imaging and therapy of this type of tumors. Based on the selective Y1R agonist [Pro^{30}, Nle^{31}, Bpa^{32}, Leu^{34}]NPY(28-36) (NPY1), several short peptides were synthesized and conjugated to DOTA and to a pyrazolyl-based bifunctional chelator (pzNN). DOTA- and pzNN-conjugates were quantitatively labeled with ^{67}Ga and ^{99m}Tc, respectively. These new radiometallated peptides were characterized by comparing their HPLC profiles with the ones obtained for the corresponding *cold* complexes. *In vitro* stability, lipophilicity, and pharmacokinetic profile of the labeled peptides were determined. Binding affinity determination, internalization studies and biological assessment in tumor bearing mice are underway.

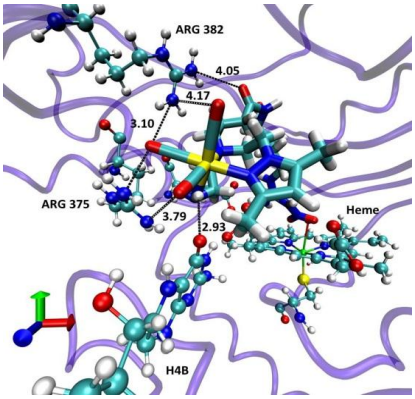


Y1 Receptor model

Insight into the high affinity of Re(I)/^{99m}Tc(I) complexes for the iNOS enzyme

B. L. Oliveira, F. Mendes, P. D. Raposinho, I. Santos, J. D. G. Correia, A. Ferreira, [1] C. Cordeiro,[1] A. P. Freire,[1] I. S. Moreira,[2]P. A. Fernandes,[2] M. J. Ramos[2]

Aiming to find radioactive probes for *in vivo* targeting of Nitric Oxide Synthase (NOS), we have recently introduced a set of Re/^{99m}Tc(CO)$_3$-complexes containing L-arginine derivatives with high *in vitro* (enzymatic assay) and *in vivo* (LPS-induced RAW 264.7 macrophages) affinity for the enzyme. These results prompted us to perform molecular docking and molecular dynamic studies to get an insight into the structural parameters of the Re complexes responsible for their increased inhibitory effect towards iNOS, when compared to the free bioconjugates. Preliminary results showed that the Re(I) complexes really fit into the predicted cavity and interact strongly with the enzyme.



[1] Centro de Química e Bioquímica, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Portugal.
[2] Requimte/Departamento de Química, Faculdade de Ciências da Universidade do Porto, Portugal.

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^{99m}Tc(CO)$_3$-labeled alendronate for bone imaging

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

We have synthesized and characterized the novel complexes *fac*-[M(CO)$_3$(k^3-pz-alendronate)] (M = Re or ^{99m}Tc), and evaluated the biodistribution profile of the ^{99m}Tc(I) complex. This compound is stable at physiological conditions, presents a fast rate of blood clearance, high rate of total radioactivity excretion and high bone uptake.

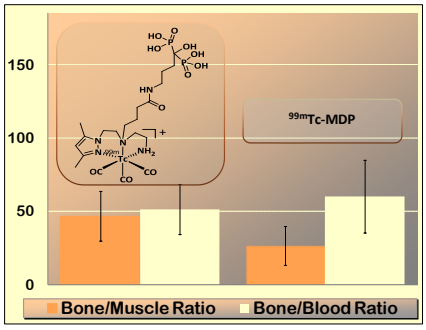


Fig. 1 Target/non target organs ratios.

The target to non target ratios at 4 h p.i. (Fig. 1) were high and comparable to the ones obtained for ^{99m}Tc-MDP, which is the radiopharmaceutical for bone imaging in current clinical use. This biodistribution profile was confirmed by SPECT imaging in Sprague Dawley rats (Fig.2).

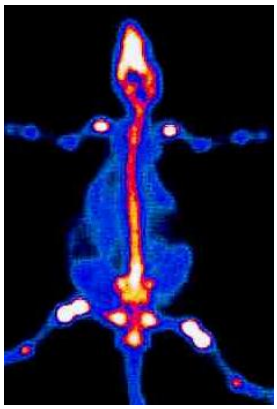


Fig. 2 SPECT imaging in Sprague Dawley rats at 2 h post-injection.

Homodimeric conjugates of a linear α-MSH analog for melanoma imaging

M. Morais, P. D. Raposinho, M. C. Oliveira, J. D. G. Correia, I. Santos

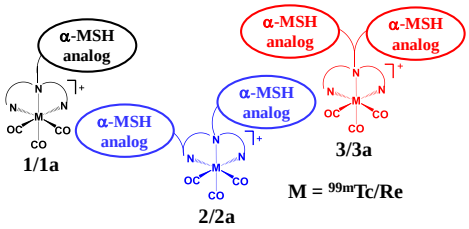


Fig. 1

Aiming to investigate the Melanocortin 1 receptor-targeting properties of $^{99m}Tc(CO)_3$-labeled homodimeric conjugates based on a linear α–MSH analog (NAPamide), we have synthesized the metallated peptides **1/1a**, **2/2a** and **3/3a** (Fig. 1). The rhenium surrogates **1a** and **2a** displayed excellent receptor binding affinities in the subnanomolar range. Cell uptake studies have shown that **3** displayed the highest cellular uptake, when compared to the homodimeric and monomeric derivatives **2** and **1**, respectively (Fig. 2). Complex **3** holds promise as a radioactive probe for melanoma imaging, and is currently under biological evaluation.

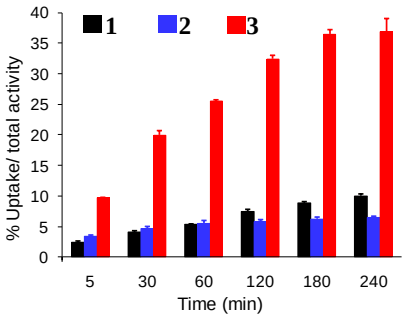
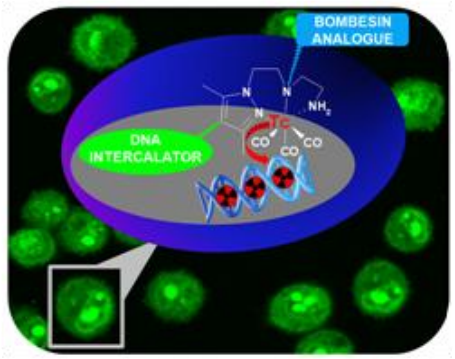


Fig. 2 Uptake in B16F1 melanoma cells (37ºC).

Cell- specific and nuclear targeting with multifunctional $^{99m}Tc(I)$ complexes

T. Esteves, F. Marques, A. Paulo, J. Rino,[1] P. Nanda,[2] C. Smith,[2] I. Santos



To explore the usefulness of ^{99m}Tc as an Auger emitter, we have introduced and biologically evaluated novel multifunctional structures comprising: i) a pyrazolyl-diamine framework (pz*NN) bearing a set of donor atoms to stabilize the $[M(CO)_3]^+$ (M = Re, ^{99m}Tc) core; ii) a DNA intercalating moiety of the acridine orange (AO) type; iii) and a bombesin (BBN) analogue of the type X-BBN[7-14] (where X = SGS, GGG) to provide specificity. Cell uptake studies have shown that the presence of the AO intercalator and metallation did not compromise the capability of the BBN metalloconjugates to accumulate specifically in GRPr-positive PC3 human tumor cells, targeting the nucleus. To the best of our knowledge, these compounds are the first examples of ^{99m}Tc bioconjugates that combine specific cell targeting with nuclear internalization, which are crucial issues for the usefulness of ^{99m}Tc in Auger therapy.

[1] IMM, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal
[2]Research Division, Harry S. Truman Memorial Veterans Hospital, University of Columbia, USA.

PEGylated DOTA-α-MSH analogues for *in vivo* targeting of melanoma

F. Silva, M. P. Campello, M. Baptista, A. Paulo, I. Santos

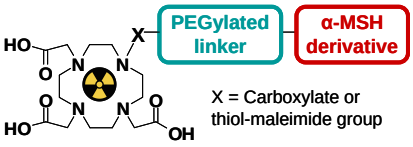


Fig.1 – PEGylated DOTA-NAPamide

In an effort to develop new tools for *in vivo* melanoma imaging, we have synthesized α-MSH derivatives having PEGylated linkers bound to a DOTA chelator (Fig.1). Some of these derivatives were labeled with ^{67}Ga, affording novel radiopeptides, which were characterized by HPLC comparison with the cold Ga congeners and evaluated in B16F1 murine melanoma cells (Fig. 2). Biodistribution studies of these novel radiometallated peptides in tumor-bearing mice are currently underway to assess their ability to target melanoma *in vivo*.

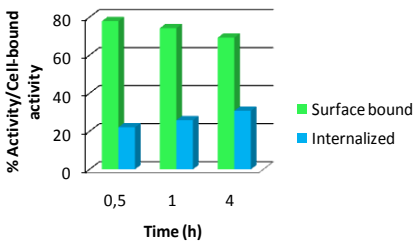


Fig.2 – Cellular uptake of ^{67}Ga-DOTA-PEG2-NAPamide at 37ºC in B16F1 cells.

Radiolabeled benzazole derivatives for *in vivo* imaging of amyloid aggregation

G. Morais, A. Paulo, I. Santos, H. Miranda,[1] T. Outeiro[1]

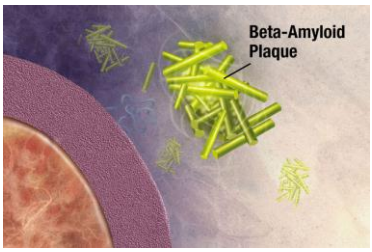


Fig. 1 Schematic drawing of β-amyloid aggregates.

Aiming to prepare compounds exhibiting high affinity and selectivity to amyloid aggregates (Fig. 1), the histopathological feature of neurodegenerative diseases, we have designed and synthesized a number of fluorinated styryl heteroaromatic derivatives (Fig. 2). These compounds were obtained using novel synthetic strategies and fully characterized. Profiting from their intrinsic fluorescence, the *in vitro* affinity of the synthesized compounds towards aggregates of insulin, beta-amyloid ($A\beta_{1-42}$) and α-synuclein was also assessed (Fig. 2). The synthesis and biological evaluation of radiofluorinated congeners are currently underway.

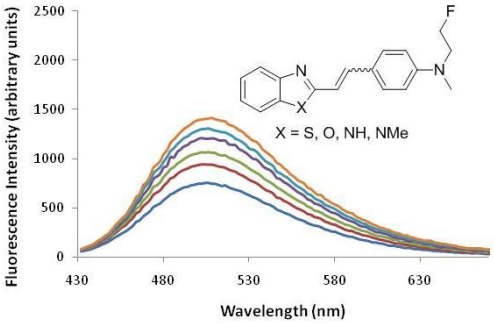


Fig. 2 –Styryl heteroaromatic derivatives and their enhancement of fluorescence upon interaction with $A\beta_{1-42}$ fibers.

[1] IMM, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal

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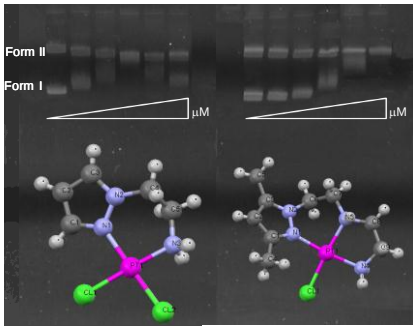
Design of novel anticancer drugs based on Pt(II) complexes with pyrazolyl-containing chelators

S. Gama, F. Mendes, F. Marques, Isabel C. Santos, A. Paulo, I. Santos, E. Gabano,[1] M. Ravera[1]



A series of Pt(II) complexes anchored by bidentate or tridentate pyrazolyl-alkylamine chelators bearing different substituents at the azolyl rings has been prepared to assess their interest as anticancer drugs. The complexes have been fully characterized by classical analytical methods, and in some cases also by X-ray diffraction analysis. Cell uptake, antiproliferative properties and DNA interaction were evaluated. These studies have shown that the complexes were less active than cisplatin on the ovarian carcinoma A2780 cell line. Nevertheless they kept their activity in the cisplatin-resistant A2780cisR cell line and presented a lower resistance factor compared to cisplatin.

[1] DiSAV, Università del Piemonte Orientale "Amedeo Avogadro", Alessandria, Italy

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^{99m}Tc(I) Tricarbonyl complexes for targeting melanotic melanoma

C. Moura, L. Gano, P. Raposinho, A. Paulo, I. C. Santos, I. Santos

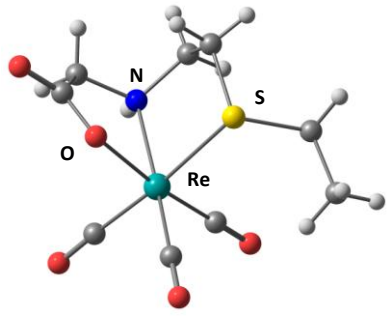

Fig 1

Within our interest on ^{99m}Tc-labeled small-molecules for targeting tumoral tissues, we have pursued with the search of new radioactive probes suitable for the early detection of melanotic melanoma. To achieve this goal we have synthesized and evaluated a series of Re(I)/^{99m}Tc(I) tricarbonyl complexes anchored by (N,N,O) or (S,N,O)-tridentate chelators (Fig. 1) bearing different melanin-avid pharmacophores. In general, the synthesized complexes have shown a moderate to high *in vitro* affinity for melanin, and in some cases were able to target *in vivo* murine melanoma tumors with favorable target/non-target ratios (Fig. 2).

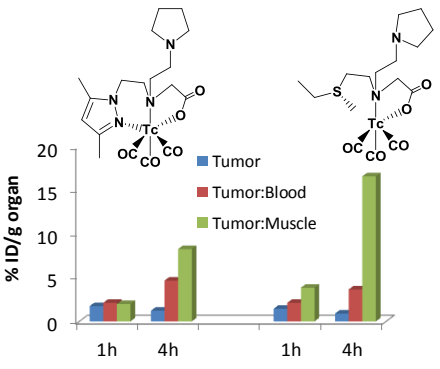

Fig 2

Myocardial localization and excretion mechanism of a novel ^{99m}Tc radiotracer for heart imaging

F. Mendes, L. Gano, C. Fernandes, A. Paulo, I. Santos

We developed a ^{99m}Tc organometallic complex, ^{99m}Tc-TMEOP, which exhibits a high initial and persistent heart uptake associated to rapid blood and liver clearance. More detailed studies in isolated rat hearts were performed for this complex and compared with ^{99m}Tc-sestamibi. Subcellular distribution studies showed that ca. 70% of ^{99m}Tc-TMEOP accumulates in the mitochondria, similarly to ^{99m}Tc-sestamibi (Fig. 1).

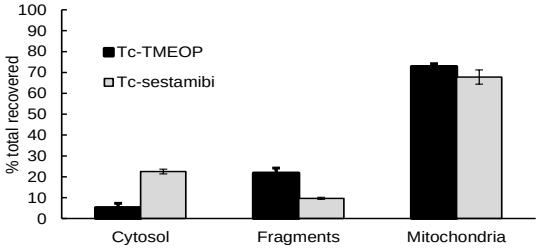

Fig. 1

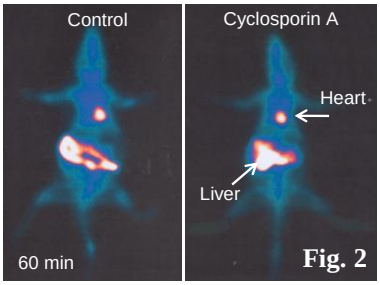

Fig. 2

Biodistribution studies in rats in the presence of cyclosporin A revealed an increase in kidney and liver uptake of ^{99m}Tc-TMEOP, suggesting the involvement of multidrug resistance transporters in the pharmacokinetic profile of this complex (Fig. 2).

Evaluation of novel ^{99m} Tc(I) cationic complexes as probes for multidrug resistance (MDR)

F. Mendes, L. Gano, C. Fernandes, A. Paulo, I. Santos

The cationic radiotracer ^{99m}Tc-TMEOP, originally developed as a myocardial perfusion agent, was evaluated for cancer early detection and non-invasive monitoring of multidrug resistance (MDR) by SPECT. The usefulness of ^{99m}Tc-TMEOP for functional assessment of MDR was studied using nude mice bearing MDR negative and positive tumour xenografts. The biodistribution profile of ^{99m}Tc-TMEOP showed a tumour uptake almost 2 times higher in the MCF7 xenografts compared to the MCF7 PgP tumours (Fig. 1). The *in vivo* MDR phenotype of the tumours was confirmed by detection of protein expression levels (Fig. 2). All together these data indicate that this new complex has potential for *in vivo* tumour MDR detection.

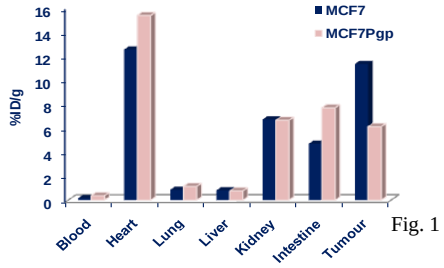

Fig. 1

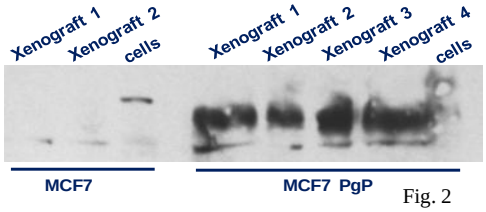

Fig. 2

Novel radiolabeled receptor tyrosine kinase inhibitors for *in vivo* targeting of EGFR

C. Neto, M. C. Oliveira, L. Gano, C. Fernandes, F. Mendes, I. Santos, M. Kuchar[1], T. Kniess[1]

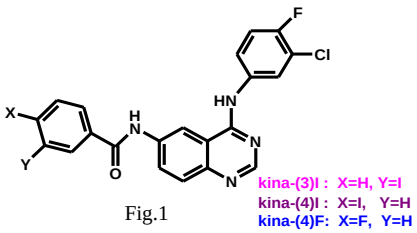

Fig.1

Aiming at the development of new tyrosine kinase inhibitors (TKI) for EGFR tumor imaging, novel anilinoquinazoline derivatives were synthesized and characterized (Fig.1). Western blot analysis showed that all compounds inhibit EGFR autophosphorylation at low micromolar level, being compound kina(4)F the most potent inhibitor (Fig. 2). MTT assay indicates that all compounds are potent inhibitors of A431 cells proliferation (Fig. 3). These data suggest further evaluation of these compounds as SPECT/PET biomarkers for molecular imaging of EGFR positive tumors.

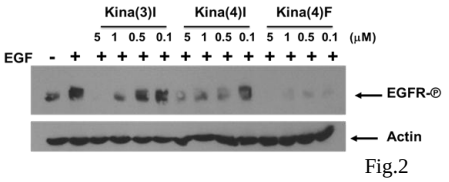

Fig.2

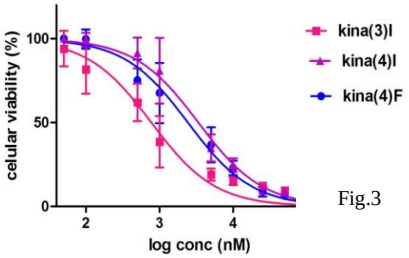

Fig.3

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