SPECT and PET Radioactive Probes Based on Labelled Mercaptoimidazole Derivatives for Brain Receptor Imaging

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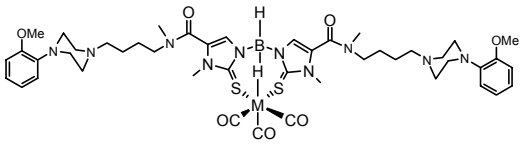
Objectives

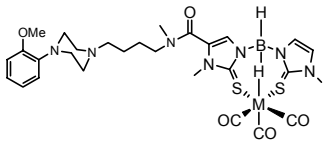
Development of novel radioactive probes for mapping brain 5-HT_{1A} serotonergic receptors by SPECT (^{99m}Tc) or PET (^{11}C) imaging.

Results

Previously, we have found that the rhenium complexes **1** and **2**, anchored by bis(mercaptoimizadolyl)borates and bearing a fragment of WAY-100635, display excellent sub-nanomolar affinities for 5-HT_{1A} receptors. Hence, we proceeded with the synthesis of the ^{99m}Tc-congeners **1a** and **2a**, which were obtained in high yield and high radiochemical purity starting from *fac*-$[^{99m}Tc(CO)_3(H_2O)_3]^+$ (Scheme 1).



M = Re (**1**), ^{99m}Tc (**1a**)



M = Re (**2**), ^{99m}Tc (**2a**)

Scheme 1. Re and ^{99m}Tc complexes

Biodistribution studies in mice demonstrated that **1a** and **2a** are able to cross the BBB. However, both complexes undergo a fast washout (Fig. 1)

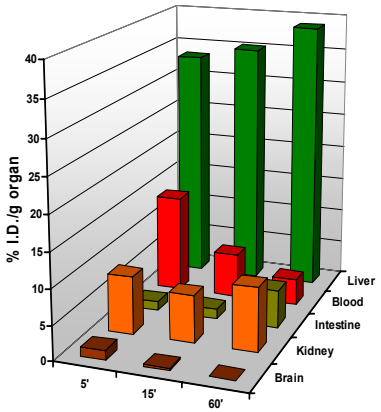
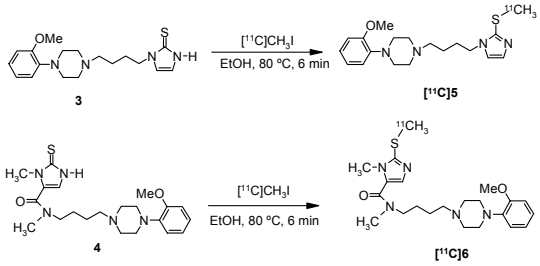


Fig. 1. Biodistribution data for **1a**

The excellent *in vitro* binding properties of complexes **1** and **2**, led us to expect that the corresponding mercaptoimidazoles would also retain, by themselves, good affinity and selectivity for 5-HT_{1A} receptors. Thus, we decided to evaluate the usefulness of ^{11}C-labelled mercaptoimidazoles as radioligands for mapping brain 5-HT_{1A} receptors by means of PET. The novel radioligands [^{11}C]**5** and [^{11}C]**6** were synthesized in radiochemical yields of 20-30% and with a radiochemical purity exceeding 99% (Scheme 2).



Scheme 2. ^{11}C labelled mercaptoimidazoles

Both compounds exhibit excellent subnanomolar affinities (**5**, IC_{50} = 0.576 ± 0.008 nM; **6**, IC_{50} = 0.86 ± 0.02 nM) for the 5-HT_{1A} receptor while displaying a high selectivity towards the 5-HT_{2A} subtype of receptors (IC_{50} > 480 nM). Preliminary biodistribution studies in rats showed an initial brain uptake of 1.14±0.11 %ID/g and 0.37±0.04 %ID/g after 5 min, which decreased to 0.18±0.04 %ID/g and 0.16±0.01 %ID/g after 60 min for compounds [^{11}C]**5** and [^{11}C]**6**, respectively. Due to their washout from the brain, compounds [^{11}C]**5** and [^{11}C]**6** seem not to be good candidates as radioligands for imaging 5-HT_{1A} receptors by PET. Surprisingly, [^{11}C]**6** showed a remarkable and prolonged uptake in the adrenals, promoting further studies to clarify this phenomenon.

Published, accepted or in press work

1. R. Garcia, C. Xavier, A. Paulo, I. Santos, T. Kniess, R. Bergmann, F. Wüst, Synthesis and Biological Evaluation of S-[11C]methylated mercaptoimidazole piperazinyl derivatives as potential radioligands for imaging 5-HT_{1A} receptors by Positron Emission Tomography (PET), *J. Labelled Cpd Radiopharm.* (2004), in press.

[1]Institute of Bioinorganic & Radiopharmaceutical Chemistry, Germany

Novel Bifunctional Pyrazole-Diamine and Pyrazole-Dithioether Chelators for the Labeling of Biomolecules with *fac*-$[M(CO)_3]^+$ (M = Re, ^{99m}Tc)

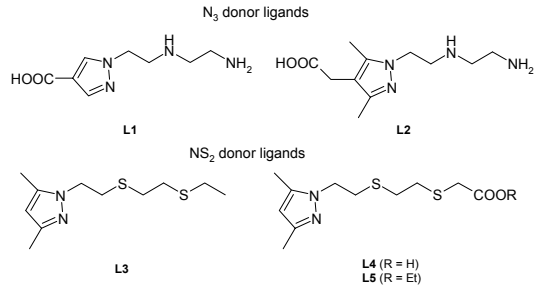
R. F. Vitor, S. Alves, J. D. G. Correia, A. Paulo, I. Santos

Objectives

Design and synthesis of novel asymmetric pyrazolyl tridentate ligands for the labelling of biologically active molecules with the *fac*-$[M(CO)_3]^+$ moieties (M = Re, ^{99m}Tc).

Results

Following our previous work, we synthesized novel asymmetric pyrazolyl-containing ligands with N_3 and NS_2 donor atom sets and with a carboxylate group for direct coupling to biomolecules. Compounds **L1** - **L5** have been prepared by multi-step synthetic procedures, and were characterized by the usual techniques in organic chemistry.



Reactions of the starting materials $[Re(CO)_5Br]$ and/or $(NEt_4)_2[Re(CO)_3Br_3]$ with **L1**-**L5** led to the formation of cationic tricarbonyl complexes of the type *fac*-$[Re(CO)_3(k^3\text{-L})]^+$ (**1**-**5**), in which the pyrazolyl based anchors act as tridentate ligands, as previously reported for related non-functionalised parent complexes. The complexes have been fully characterized by elemental analysis, IR and NMR spectroscopy. Complex **4** was also characterized by X-ray diffraction analysis (Fig. 1).

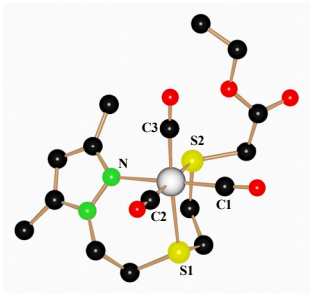


Fig. 1. ORTEP view of complex **4**

The possibility of preparing at non-carrier added level (^{99m}Tc) representative examples of the complexes with the pyrazole-diamine (**L1**) and pyrazole dithioether ligands (**L3** and **L4**) has been evaluated. The corresponding radioactive complexes *fac*-$[^{99m}Tc(CO)_3(k^3\text{-L})]^+$ (**1a**, L = **L1**; **3a**, **L** = **L3**,) have been obtained in yields higher than 90%, by reacting the *fac*-$[^{99m}Tc(H_2O)_3(CO_3)]^+$ precursor with the corresponding ligand, under optimized conditions. The labelling of **L4** yields the complex *fac*-$[^{99m}Tc(CO)_3(k^3\text{-L4})]^+$ (**4a**), partially mixed with *fac* -$[^{99m}Tc(CO)^3(k^3\text{-L5})]^+$ (**5a**), due to the hydrolysis of the ester group in **L4**. The identity of the ^{99m}Tc complexes has been established by HPLC (Figure 2).

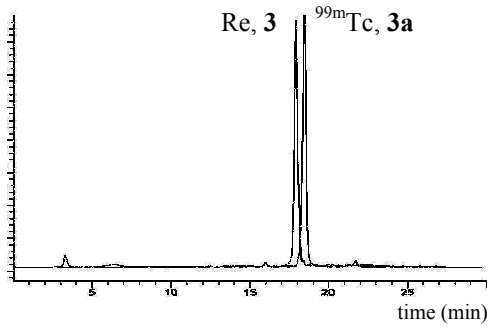


Fig. 2. HPLC trace of complex *fac*-$[Re(CO)_3L3]^+$ (**3**) (254 nm) and γ-trace of the ^{99m}Tc congener **3a**.

The organometallic ^{99m}Tc-complexes **1a** and **3a** were challenged in PBS buffer and in the presence of a large excess of histidine or cysteine, which display great affinity for the *fac*-$[^{99m}Tc(CO)_3]^+$ moiety. In both cases, only a minor decomposition of the complexes could be observed, even for the less stable pyrazole-dithioether **3a**. These findings indicate that pyrazole-diamine and pyrazole-dithioether chelators provide a high kinetic inertness and/or stability to organometallic complexes with the *fac*-$[^{99m}Tc(CO)_3]^+$ moiety, being promising for labeling biomolecules.

Published, accepted or in press work

1. R. F. Vitor, S. Alves, J. D. G. Correia, A. Paulo, I. Santos, Rhenium(I) and technetium(I) tricarbonyl complexes anchored by bifunctional pyrazole-diamine and pyrazole-dithioether chelators *J. Organomet. Chem.* **689** (2004) 4764-4774.

2. R. F. Vitor, A. Paulo, I. Santos Complexos Organometálicos de Rénio(I) com Ligandos bifuncionais do Tipo Pirazolo-Ditioéter, *XIX Encontro Nacional da Sociedade Portuguesa Química,* Coimbra, April 2004, poster.

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

C. Fernandes, I. Pirmettis,[1] I. Santos

Objectives

The epidermal growth factor receptor (EGFR) is known to be overexpressed and/or disregulated in several solid tumour types. Targeting EGFR receptors can be done either with labelled monoclonal antibodies and EGF analogues, which act in the extracellular domain of the receptor, or with small molecules such as quinazoline derivatives, which exhibit high affinity for the EGFR-associated tyrosine kinase. Our main goal is the development of novel radioactive probes based on quinazoline derivatives, for early detection and staging of cancers overexpressing EGFR.

Results

Gefitinib and Tarceva are two tyrosine kinase inhibitors in clinical trials at the most advanced stage (Figure 1).

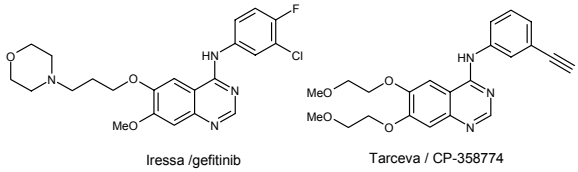


Fig. 1. Chemical structures of gefitinib and tarceva.

The activity of these compounds was considered to be related with the presence of electron–withdrawing groups in the 3' and/or 4' positions of the aromatic rings and also to the fact that they have no substituents in the 5 and 8 positions. Taking this into account, we have chosen the 4-anilinoquinazoline derivatives **4** and **6** to be labelled with ^{99m}Tc (Figure 2).

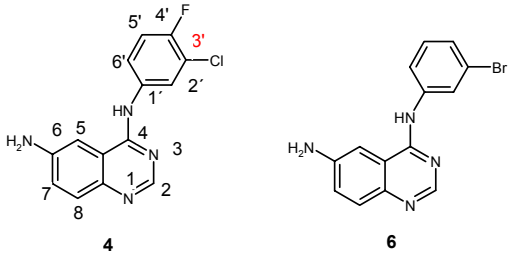
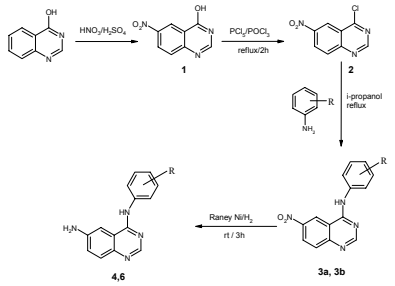


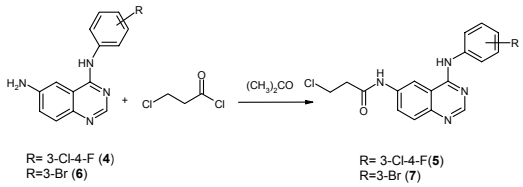
Fig. 2. Quinazoline derivatives to be coupled to ^{99m}Tc.

The presence of the NH_2 group in the 6 position would be expected to increase the potency of the compounds as inhibitors of tyrosine kinase activity, and also could allow their coupling to bifunctional chelators, through an amide bond. Compounds **4** and **6** were synthesised as depicted in Scheme 1.



Scheme 1. Synthesis of the derivatives **4** and **6**.

Nitration of 4-hydroxyquinazoline was achieved by the usual procedure, using nitric acid in the presence of concentrated sulphuric acid. The procedure described in the literature for the synthesis of 4-chloro-6-nitroquinazoline involved the treatment of the 6-nitroquinazoline with PCl_5 at 160 ºC. However, in our hands, this procedure didn't work and it was necessary to add $POCl_3$ to the reaction mixture to obtain the chlorinated compound **2**. Compounds **3a**/**b** were obtained by refluxing **2** with the corresponding halogenated aniline, in isopropanol. Compounds **4** and **6** were obtained by reduction of the nitro group of **3a/b,** using hydrogen (50 psi) and Raney/Ni as catalyst. To increase the possibility of coupling the quinazoline derivatives to bifunctional chelators, compounds **5** and **7** were also synthesized as indicated in Scheme 2.



Scheme 2. Synthesis of **5** and **6**.

The coupling of **4** - **7** to different bifunctional chelators can be performed either through amide bonds or by alkylation of primary or secondary amines. These studies are underway.

Published, accepted or in press work

1. I. Santos, Synthesis and Characterization of Quinazoline Derivatives, *2st RCM on Development of ^{99m}Tc-Based Small Biomolecules Using Novel ^{99m}Tc Cores/ IAEA*, Viena, Austria, November 2004, oral.

[1] NCSR, Demokritos Center, Greece

Metallation Studies and Biological Evaluation of a Tumour-Seeking Peptide

S. Alves, J. D. G. Correia, L. Gano, C. J. Smith[1], I. Santos

Objectives

Peptides containing the amino acid sequence Arg-Gly-Asp (RGD) have been used extensively to target integrin receptors upregulated on tumor cells and neovasculature. The expression patterns of integrin receptors form the basis of attempts to image angiogenesis and tumor formation *in vivo* using RGD-based peptide targeting vectors. Different categories of RGD peptides have been explored, but, due to several reasons, head-to-tail cyclized RGD peptides represent the most promising class for designing imaging agents. Our main goal was the design and synthesis of a novel radioactive probe for mapping angiogenesis, based on a new cyclic-RGD analogue.

Results

As previously reported, our group has introduced novel bifunctional pyrazolyl containing ligands (S. Alves et al., *J. Chem. Soc., Dalton Trans.*, **24** (2002) 4714-4719) adequate for labeling biologically active peptides. Using one of these ligands the new cyclic RGD analogue Cyclo[Arg-Gly-Asp-D-Tyr-Lys(pz1)] (RGD-pz1) has been synthesized by standard solid phase synthetic methods (Figure 1)

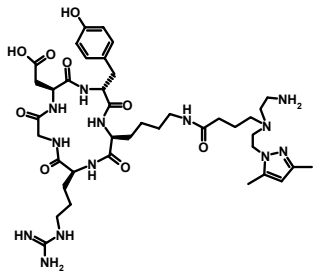


Fig. 1. Cyclo[Arg-Gly-Asp-D-Tyr-Lys(pz1)]

After purification by preparative reversed phase HPLC techniques the structure of the new conjugate was confirmed by MALDI-MS (Figure 2).

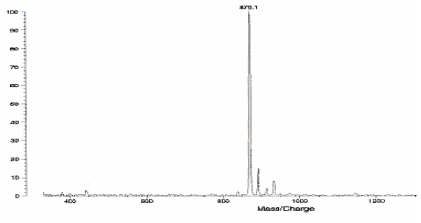


Fig. 2. MALDI-MS of RGD-pz1 conjugate

Metallation of this conjugate with technetium tricarbonyl led to the complex [^{99m}Tc(CO)$_3$-Cyclo[Arg-Gly-Asp-D-Tyr-Lys(pz1)] (**1**) in high yield (>90%) and with high specific activity.

Radiochemical yields of **1** were monitored by RP-HPLC (Figure 3).

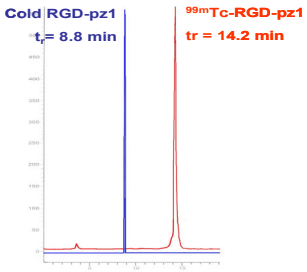


Fig. 3. HPLC profiles of the RGD-pz1 conjugate (UV-detection) and of **1** (γ-detection).

The new conjugate **1** showed remarkable stability *in vitro* in human serum (4 h post-incubation more than 98% of **1** is present). Biodistribution studies in normal CF-1 mice confirmed that this hydrophilic conjugate (**1**) is effectively cleared from the bloodstream 30 min p.i. and more than 60% of the injected dose was excreted at 4h p.i., via the renal-urinary pathway. (Figure 4).

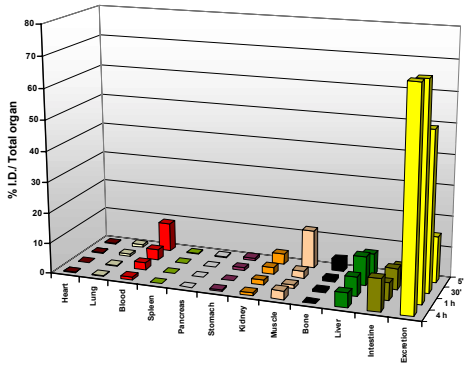


Fig. 4. Biodistribution histogram of **1** in normal CF-1 mice.

To evaluate the *in vivo* stability of **1**, urine, serum, and liver samples were analyzed by HPLC 1 h p.i. For all the samples more than 98% of **1** was found, confirming the remarkable stability of **1**. Evaluation of the specific binding of **1** to receptors expressed on cell lines and *in vivo* pharmacokinetic in tumor-bearing mouse models are underway.

Published, accepted or in press work

1. I. Santos, J. C. Smith, Tumour–Seeking Peptide Conjugation and Metallation Studies, *2st RCM on Development of ^{99m}Tc-Based Small Biomolecules Using Novel ^{99m}Tc Cores/ IAEA*, Viena, Austria, November 2004, oral.

[1] Missouri University

Rhenium Tricarbonyl Complexes Anchored by 5-HT_{1A} Receptor-Binding Ligands: Structure-Activity Relationships

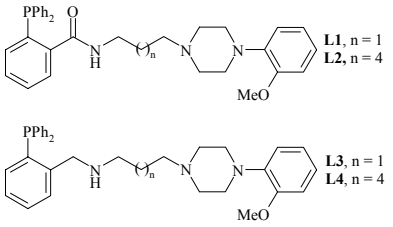
E. Palma, J. D. G. Correia, I. Santos

Objectives

Evaluation of the influence of the spacer length between the (2-methoxyphenyl)piperazine pharmacophore and the phosphinoarylbenzylamide and phosphinoarylbenzylamine chelator groups in complexes of the type $[Re(CO)_3Br(\kappa^2\text{-}L)]$ on the affinity/specificity toward 5-HT_{1A} receptors.

Results

The (2-methoxyphenyl)piperazine pharmacophore, a part of the WAY 100635 structure, has been functionalized with phosphinoarylbenzylamide or phosphinoarylbenzylamine chelator groups using propylene or hexylene alkyl chains as linkers (**L^1** - **L^4**).



The resulting phosphines have been used to synthesize complexes of the type $[Re(CO)_3Br(\kappa^2\text{-}L)]$ (**1**, L = **L^1**, **2**, L = **L^2**; **3**, L = **L^3**; **4**, L = **L^4**), which have been fully characterized, including by X-ray crystallographic analysis (Figures 1 and 2).

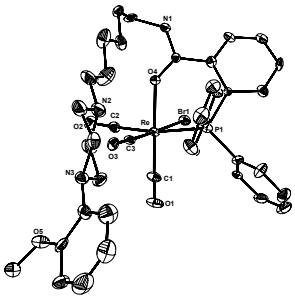


Fig. 1. ORTEP view of *fac*-$[Re(CO)_3Br(\kappa^2\text{-}L^2)]$ (**2**)

The complexes **1** - **4** have been used for determination of the receptor-binding affinity and selectivity in rat brain homogenates. The IC_{50} values of these compounds for 5-HT_{1A}/5-HT_{2A} receptors (**1**, 5-HT_{1A}: 20±0.1nM, 5-HT_{2A}: 4680±0.1nM; **2**, 5-HT_{1A}: 200± 4 nM, 5-HT_{2A}: 340±9nM; **3**, 5-HT_{1A}: 285±4nM, 5-HT_{2A}: 490 ± 8 nM; **4**, 5-HT_{1A}: 1100±4nM, 5-HT_{2A}: 1190±0.5nM) revealed that complex **1** is the only one which presents a relatively interesting affinity and specificity toward the $5HT_{1A}$ receptors for further biological evaluation.

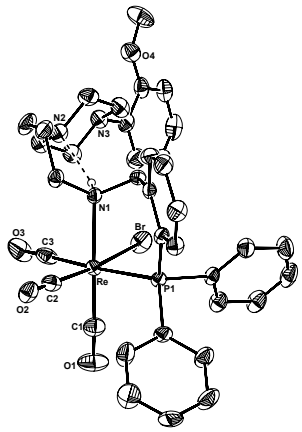


Fig. 2. ORTEP view of *fac*-$[Re(CO)_3Br(\kappa^2\text{-}L^3)]$ (**3**)

For all the other complexes the affinity and specificity towards $5HT_{1A}$ decreased significantly. The fit of the molecule into the binding pocket of the receptors was significantly affected when an amide group in the chelate unit (**1**) was replaced by a secondary amine (**3**). This effect is even more pronounced when the alkyl chain length increases (**1** versus **2**, and **3** versus **4**). The results obtained for **1** could be ascribed to a certain rigidity imposed by the presence of the amide group, which decreases when the coordination to the metal is done through a secondary amine. On contrary, increasing the alkyl chain length a higher mobility of the pendant arm is observed with a folding towards the metal centre. In the case of **3** this folder seems to be favoured due to an intramolecular hydrogen bridge between the secondary amine of the piperazine (Fig. 2). Whether this type of interactions are maintained in solution is difficult to say, although the mobility of the pendant arm seems to exist in solution as indicated by the ^{1}H NMR spectra of **3**.

Published, accepted or in press work

1. E. Palma, J. D. G. Correia, Â. Domingos, I. Santos, R. Alberto, H. Spies, Rhenium and technetium tricarbonyl complexes anchored by 5-HT_{1A} receptor-binding ligands containing P,O/N donor atom sets, *J. Organomet. Chem.* **689** (2004) 4811-4819.

2. E. Palma, J. D. G. Correia, I. F. A. Pereira, I. Santos, H. Spies, A. Drews, R. Alberto, Complexos do tipo *fac*-$[MX(CO)_3(k^2\text{-}PO/N)]$ (M = Re, ^{99m}Tc) funcionalizados com um fragmento arilpiperazina, XIX Encontro Nacional da Sociedade Portuguesa de Química, Coimbra, Portugal (2004) poster.

Rhenium and Technetium Metallointercalators for Selective Tumour Therapy

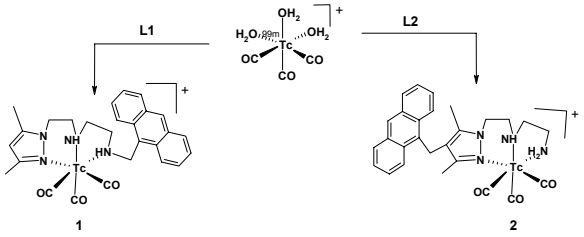
R. F. Vitor, A. Paulo, I. Santos

Objectives

Synthesis, characterization and biological evaluation of rhenium and technetium tricarbonyl complexes with ability to intercalate into DNA, aiming their further application in the design of novel radiotherapeutic (^{99m}Tc/$^{186/188}$Re) or cytotoxic (Re) metallopharmaceuticals.

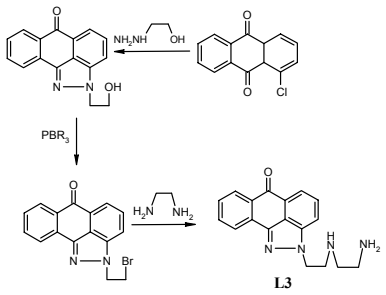
Results

Following our previous work, we have prepared novel ^{99m}Tc(I) tricarbonyl complexes (**1** and **2**) stabilised with pyrazole-diamine ligands (**L1** and **L2**), bearing an anthracenyl group that is a well known DNA intercalator (Scheme 1). Profiting from the versatiliy of the anchor ligands, the intercalating fragment has been introduced at the 4-position of the azole ring (**L1**, **1**) or at terminal amino group (**L2**, **2**)



Scheme 1. Synthesis of ^{99m}Tc complexes bearing an anthracenyl fragment

Complexes **1** and **2** were obtained in almost quantitative yield, at relatively low ligand concentrations (10^{-4} M). Their identity was confirmed by HPLC comparison with the rhenium congeners, which were fully characterized by the common techniques in inorganic chemistry. Evaluation of the *in vitro* stability of **1a** and **2a** demonstrated that both complexes are highly stable under physiologic conditions (PBS, pH=7.4, 37 ºC). Moreover, only negligible *trans*-chelation processes were observed for **1a** and **2a** in the presence of large excess of histidine or cysteine, biological substrates with a well recognized affinity for the *fac*-[^{99m}Tc(CO)$_3$]$^+$ moiety. The interaction of calf-thymus DNA with **L1**-**L2** and with the corresponding rhenium complexes is currently under investigation, using spectrophotometric and circular dichroism (CD) techniques. In the next future, the evaluation of the cytotoxicity and/or radiotoxicity of these compounds and of the ^{99m}Tc complexes **1** and **2** will be also done, using adequate cell lines and the methyl-thiazolyl tetrazolium (MTT) cytotoxicity assay. Our studies were further extended to an anthrapyrazolediamine ligand (**L3**), which was synthesized as depicted in Scheme 2. Anthrapyrazole derivatives are molecules that contain an anthracenyl group fused with the azole ring, which act as DNA-intercalators and have also shown antitumour properties. We expected that **L3** would behave as a tridentate ligand towards the *fac*-[M(CO)$_3$]$^+$ moiety (M = Re, ^{99m}Tc), like the related **L1** and **L2**.



Scheme 2. Synthesis of **L3**

Reactions of **L3** with *fac*-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ proceed smoothly, yielding a stable ^{99m}Tc complex (Fig. 2).

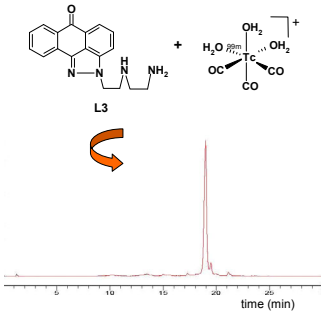


Fig. 1. HPLC profile of [^{99m}Tc- **L3**].

Until now, we were unable to assign a structure for [**^{99m}Tc-L3**], since the corresponding Re surrogate was not yet fully characterized. Reactions of [Re(CO)$_5$Br] or (NEt$_4$)$_2$[Re(CO)$_3$Br$_3$] with **L3** led to the formation of rather insoluble and fluxional complexes, being still necessary to assess the molecular structure of these compounds.

Published, accepted or in press work

1. R. F. Vitor, A. Paulo, I. Santos, R. Alberto, Organometallic Complexes with Anthrapyrazoles: Searching for Novel Metallointercalators, Second International Symposium on Bioorganometallic Chemistry, Zurich, July 2004.

^{153}Sm and ^{166}Ho Complexes with [14]aneN4 Macrocycles: Synthesis and Biological Evaluation

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

Objectives

To find novel Ln macrocycle building blocks suitable for bone pain palliation therapy and/or for labelling biologically active molecules.

Results

Following our previous studies with tetraaza macrocycles containing pyridine and methylcarboxylate or methylphosphonate pendant arms [1], we have explored the stability and biological behaviour of ^{153}Sm and ^{166}Ho complexes with TETA and TET3AP (Figure 1).

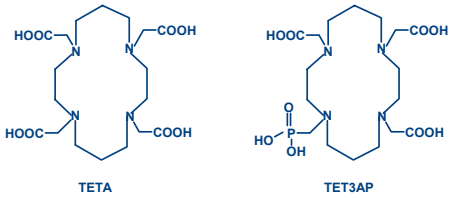


Fig. 1. TETA and TET3AP ligands

After optimizing the experimental conditions the complexes [^{153}SmTET3AP] (**1**), [^{166}Ho-TET3AP] (**2**), [^{153}SmTETA] (**3**), and [^{166}HoTETA] (**4**) have been obtained in high radiochemichal yield (>98%). At physiological pH, complexes **1**-**4** are all negatively charged, as indicated by electrophoresis in tris-HCl buffer 0.1M, pH 7.4. All the complexes are stable, up to 5 days, in physiological solutions such as saline, 0,1M glycine-HCl solution and in human serum. However, they are unstable in PBS, especially the radiolanthanide complexes with TET3AP (**1**, **2**). Biodistribution studies performed in CD-1 mice indicated that the in vivo behaviour was highly dependent on the type of the pendant arms and also on the radiolanthanide. In general, ^{166}Ho complexes (**2**, **4**) present an higher whole body radioactivity excretion than the corresponding ^{153}Sm complexes (**1**, **3**). ^{166}Ho-TETA presents a rapid tissue cleareance with more than 80% of the activity excreted after 2h p.i. and no significant liver, lung, spleen and bone uptake. However ^{166}Ho-TET3AP has a significant higher hepatic retention and increased radioactivity in blood stream with no more than 60% of the activity excreted up to 24 h (data not shown). TLC analysis of urine and serum colleted at sacrificed time demonstrated that ^{166}Ho-TET3AP (**2**) is quite unstable forming in vivo radiochemical impurities, which are very slowly excreted in urine.

Complex **3** is also unstable *in vivo* as indicated by the presence of radiochemical impurities in urine and blood serum. This compound is also slowly excreted and has a high hepatic uptake. Some bone uptake was observed for complexes [^{153}SmTET3AP] (**1**) and [^{166}Ho-TET3AP] (**2**), especially for **1**, certainly due to the presence of the methanephosphonate group.

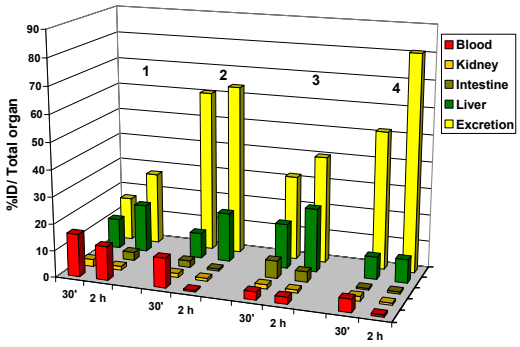


Fig. 2. Biodistribution of ^{153}Sm/^{166}Ho complexes with TET3AP and TETA.

The *in vitro* stability and favourable *in vivo* behaviour of **4** makes it attractive for conjugation to biomolecules and to be explored in the development of therapeutic agents.

Published, accepted or in press work

1. F. Marques, K. P. Guerra, L. Gano, J. Costa, M. P. Campello, L. M. P. Lima, R. Delgado, I. Santos, . ^{153}Sm and ^{166}Ho complexes with Tetraazamacrocycles containing pyridine and Methylcarboxylate or Methylphosphonate Pendant Arms, *J. Biological Inorganic Chemistry* **9** (2004) 859-872.

2. F. Marques, A. Paulo, M. P, Campello, S. Lacerda, R. F. Vitor, L. Gano, R. Delgado, I. Santos, Radiopharmaceuticals for Targeted Radiotherapy, *Radiation Protection Dosimetry in press*.

3. M. P. Campello, K. Guerra, F. Marques, L. Gano, S. Lacerda, R. Delgado, I. Santos, In vitro and in vivo Evaluation of ^{153}Sm and ^{166}Ho Complexes with Tetraazamacrocycles - Effect of the Incorporation of a Pyridine Head Unit, *COST Action D18*, *Athens,* (2004), oral.

4. I. Santos, F. Marques, L. Gano, M. P. C. Campello, S. Lacerda, L. Lima, R. Delgado, Solution and Radiochemical Behaviour of Tetraazamacrocycles with Different Pendant Arms, *COST Action D18, Coruna, Spain,* (2004), oral.

[1]ITQB

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

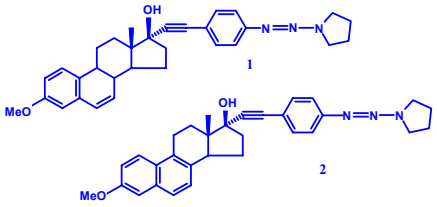
M. C. Oliveira, J. Wang[1], M. Watanabe[1], T. Thiemann[1]

Objectives

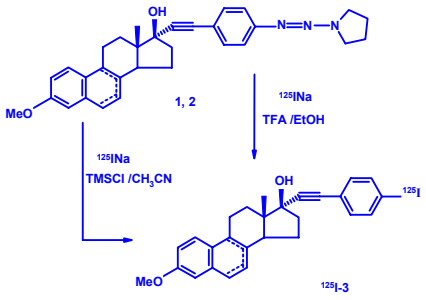
The search for novel selective γ-emitting estrogens that can serve as probes for nuclear imaging of estradiol-sensitive tumours is the main goal of this work.

Results

Estrogen receptor (ER) binding ligands play a crucial role in the management of breast cancer as chemotherapeutic or radioimaging agents. Following our previous work (M. C. Melo e Silva et al. *Zeitschr. F. Naturforschg*, **Sect. B, 58** (2003) 799-804), we have been synthesizing, according with the Sonogashira coupling, 17α-{4-[(pyrrolidin-1-yl)-azo]phenylethynyl}estra-3,17β diols of type **1** and **2**, to be used as precursors of novel ER binding radioligands.



Since the pyrrolidinylazo group in **1** and **2** can be exchanged effectively to an iodo function, the radioiodination of these triazene compounds has been studied. Different reaction conditions have been explored (Scheme 1).



Scheme 1. Synthesis of Iodinated compounds

The best yields were obtained in TMSCl/CH_3CN (Figure 1). Using these mild conditions, the radioiodinated compound ^{125}I-**3** has been prepared, purified and characterized by HPLC. The radiochemical purity of ^{125}I-**3** was higher than 95%, and its specific activity is about 17.4 mCi/μg.

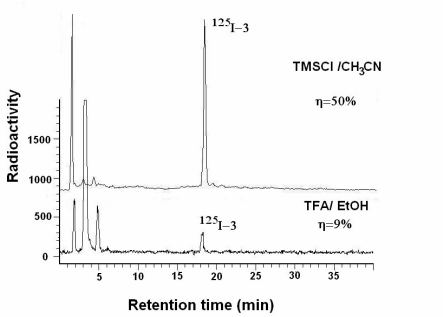


Fig. 1. HPLC of two reactions performed in different conditions.

The stability of ^{125}I-**3** was studied in CH_3CN and in EtOH/PBS (9:1). As shown in Figure 2, the compound ^{125}I-**3** presents a high stability.

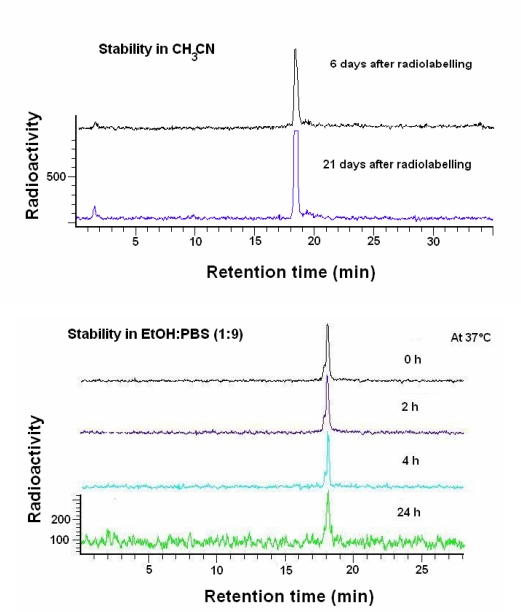


Fig. 2. Stability of ^{125}I-**3**

The possibility of easily attaching an iodo substituent (^{125}I/^{123}I) at the end of the phenyl spacer opens a route to novel pharmaceuticals. *In vitro* ER binding affinity studies and *in vivo* biodistribution assays in immature female rats are currently underway.

Published, accepted or in press work

1. M. C. Oliveira, Síntese e Marcação de Biomoléculas para Detecção do Cancro da Mama, ITN, 2004, oral.

[1]Kyushu University, Fukuoka, Japan