bone uptake than ^{99m}Tc-MDP, both of the bone uptake and the ratios of bone to the non-targets were increased with the time going. The uptakes of ^{99m}Tc-HYNIC-ACPDP in blood, liver, muscle, spleen and lung were very low. These studies demonstrated that ^{99m}Tc-HYNIC-ACPDP is a very promising new bone imaging agent.

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2,3-Diamino propionic acid based chelators for labeling biomolecules with ^{99m}Tc(I)

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The radioactive labeling of targeting biomolecules with cores such as [^{99m}Tc-^{I}(CO)$_3$]$^+$ requires small and hydrophilic complexes in order to not affect the binding properties of the vectors and to get compounds with adequate pharmacokinetic profile. Most recently, we have introduced novel tridentate bifunctional chelators comprising a small 2,3-diaminopropionic acid coordinating unit and a pendant amine and/or carboxylate group for conjugation to relevant biomolecules [1,2]. Such chelators react efficiently with the moiety "M(CO)$_3$" (M=Re, ^{99m}Tc), yielding well-defined neutral complexes of the type *fac*-[M(k^3-L)(CO)$_3$]. Herein, we report on the optimization of the labeling conditions of the new chelators with *fac*-[^{99m}Tc(CO)$_3$]$^+$, as well as on their in vitro stability. We will also present biodistribution studies of the ^{99m}Tc(I)-complexes in mice and discuss their in vivo stability.

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^{99m}Tc-labelled vasopressin peptide as a potential radiopharmaceutical for small-cell lung cancer imaging

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The aim of the paper was to synthesize and investigate the conjugate of the "4+1" mixed-ligand technetium(III) complex with the vasopressin peptide-^{99m}Tc(NS$_3$)(CN-AVP). The overexpression of vasopressin receptor V2 has been found in the case of small-cell lung cancer.
The "4+1" mixed-ligand technetium complex consists of central metal ion Tc(III) coordinated simultaneously by a tetradentate NS$_3$ tripodal chelator *tris*(2-mercaptoethyl)-amine and a monodentate isocyanide ligand, previously coupled with the selected biomolecule. The identity of the ^{99m}Tc-labelled vasopressin peptide was corroborated by investigation of the analogous rhenium compound. The ^{99m}Tc-labelling vasopressin conjugate was formed in two-step synthesis, via the ^{99m}Tc-EDTA intermediate complex, with the final yield of 95%. After 24 h of incubation of the conjugate in the 10 mM solution of histidine or cysteine, the obtained high-performance liquid chromatography chromatograms have shown the existence of one radioactive species, with the retention time characteristic for the complex studied. The log D value of −0.48±0.02 for the ^{99m}Tc-labelled vasopressin peptide was found. This value (higher than the lipophilicity of the free vasopressin peptide equal to −2.15) can be corrected by introducing a hydrophilic group, *R*, at the periphery of the NS$_3$ ligand.

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Ligand exchange mechanism of *fac*-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ complex for ^{99m}Tc-CO-MIBI radiopharmaceuticals

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Introduction: The water-soluble *fac*-[^{99m}Tc(CO)$_3$H$_2$O)$_3$]$^+$ has been an important precursor for a variety of radiopharmaceuticals. We have previously reported that H$_2$O or CO ligands in *fac*-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ complex could be replaced by MIBI under different reaction conditions. In the present study, we report the theoretical studies on ligand exchange mechanism of *fac*-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ complex for ^{99m}Tc-CO-MIBI radiopharmaceuticals.
Methods: We have proposed the mechanism of the water substitution reactions and the carbonyl ligand exchanges by MIBI and investigate them with DFT(B3LYP)/DZVP method using the Gaussian 03 program package. Moreover, we synthesize the corresponding carrier-added ^{99}Tc-CO-MIBI complexes whose structures were confirmed by LC-MS.
Results: The energy barriers for the water substitution for *fac*-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ are less than 20 kcal/mol, while the energy barriers for CO substitution reaction for ^{99m}Tc(CO)$_3$(MIBI)$_3$ are more than 30 kcal/mol without any catalyst. [^{99m}Tc(CO)$_3$(MIBI)$_3$]$^+$ was easily formed at pH 1.0, while [^{99m}Tc(CO)$_x$(MIBI)$_{6-x}$]$^+$ (x=2,1,0) complexes were obtained when pH >10.
Conclusions: The proposed mechanism of ligand exchange with MIBI on *fac*-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ could explain the complex formation on [^{99m}Tc(CO)$_3$(MIBI)$_3$]$^+$ at pH 1.0. The mechanism of [^{99m}Tc(CO)$_x$(MIBI)$_{6-x}$]$^+$ (x=2,1,0) complexes formation with OH$^-$ ion as a catalyst is in progress.

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Evaluation of hydrolyzed impurities in radiochemical analysis and biological distribution of ^{99m}Tc-ECD

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^{99m}Tc-*L,L*-ethylcysteinate dimer (^{99m}Tc-ECD) is used for investigations of cerebral perfusion and the hydrolyzed form (^{99m}TcO-ECD) is an impurity that can be an interference in the quality of the image. The aim of this study was to develop a method by high-performance liquid chromatography (HPLC) for determination of ^{99m}TcO-ECD in ^{99m}Tc-ECD preparation and to study both biodistribution in mice. The HPLC system was LC20AT Prominence model and a Shim-Pack VP-ODS column (250×4.6 mm i.d., 5 μm). ^{99m}Tc-ECD was prepared by adding 1 ml of 0.9% NaCl, 1 ml of phosphate buffer (pH 7.5) and 1 ml of Na^{99m}TcO$_4$. The radioactive concentration was 55.5 MBq ml^{-1}. 20 μL sample volume was injected in a 1.0 ml min^{-1} flow rate. A linear gradient was performed with ethanol and 12.5 mmol L^{-1} phosphate buffer (pH 2.5). ^{99m}Tc-ECD (555 MBq L^{-1}) was intravenously injected in the mice tail vein in a *Swiss* mice (15–20 g weight). After 10 min, the animals were sacrificed and the injected dose (i.d.) in brain was evaluated. The retention time of ^{99m}TcO-ECD and ^{99m}Tc-ECD was 14.65 and 17.38 min, respectively. Biodistribution results showed normal brain uptake of 0.92% i.d. for ^{99m}Tc-ECD. Higher ^{99m}TcO-ECD reduced significantly the brain

uptake in biodistribution in mice and HPLC method showed to be an important tool for the separation and quantification of ^{99m}Tc-ECD and ^{99m}TcO-ECD.

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Structural modification of small technetium complexes for melanoma imaging

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Structural modification studies of the simple $[MO^{v}(AADT)]$-$(CH_2)_n$-NR_2 complex has led to the identification of new *iso*structural derivatives of the complex, termed [MO^{v}(*iso*AADT)], which display improved in vivo tumor targeting and distribution. Three tertiary amine derivatives of the *iso*AADT analogues were synthesized and characterized along with their oxorhenium complexes. The resulting rhenium complexes form a single isomer with the substituents in the *syn* orientation with respect to the Re=O core and display similar pK_a values but reduced lipophilicity compared to the AADT complexes. Radiolabeling with $^{99m}TcO_4^-$ routinely results in >90% radiolabeling yield in a single step. In vitro and in vivo evaluation of these ^{99m}Tc-labeled complexes ^{99m}Tc-L_1–^{99m}Tc-L_3 in mouse melanoma tumor models demonstrates high tumor uptake. Both the *iso*AADT complexes ^{99m}Tc-L_1 and ^{99m}Tc-L_2 display high tumor uptake (7–9%ID/g) at 1 h post injection in the subcutaneous melanoma tumors and an increasing tumor/nontumor ratios over a 2–3-h time period, which is mainly due to increased retention in the target compared to the non-target organs. These new small molecule ^{99m}Tc-complexes have potential utility in early diagnosis of melanoma metastases.

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^{188}Re, ^{99m}Tc and ^{64}Cu bifunctional bisphosphonate complexes for targeting bone metastases

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Palliation of metastatic bone pain using bisphosphonate (BP) radiopharmaceuticals containing beta-emitters, e.g., rhenium-188 is an effective treatment. Despite proven clinical success, current BP preparations used clinically are not radiochemically homogeneous but consist of a mixture of unknown anionic polymers. We aim to improve upon the specificity and properties of current ^{99m}Tc/^{188}Re-BPs using more stable and well-designed agents. Our design separates a chelating group (e.g., dipicolylamine unit (Tc-99m/Re-188 binding) or dithiocarbamate (Re-188/Cu-64 binding) from the targeting group (BP). Here, we describe the efficient synthesis and preclinical evaluation of a series of bifunctional bisphosphonate complexes. These compounds can be radiolabelled using kit-based methodology and, in contrast with the clinically-approved bisphosphonates, form inert, well-characterised homogenous species. In vivo imaging and biodistribution studies demonstrate that some of them accumulate in areas of high-metabolic bone activity better than the well-established agent ^{188}Re-HEDP, while having low soft-tissue uptake. These results demonstrate the high potential of BP-chelator conjugates as well-defined, well-characterised agents for the diagnosis and treatment of bone metastases.

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Development of a new ^{99m}Tc(I) carbonyl complex with selectivity towards hypoxic tissue using the concept of "click chemistry"

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With the aim to develop a potential ^{99m}Tc-radiopharmaceutical for targeting hypoxic tissue we have applied "click chemistry," reaction based on the Cu(I) catalyzed alkyne-azide cycloaddition, in the synthesis of new 5-nitroimidazol derivative suitable for the ^{99m}Tc(I) tricarbonyl complexation. Metronidazole (commercial antiparasitic with recognized biorreductive capacity) was used as starting reagent. The OH-group was transformed into azide-moiety in a single step through Mitsunobu reaction. This intermediate was reacted with D,L-propargylglycine producing the final ligand that contains substituted-triazol, amino and carboxylic groups as chelating units for technetium. ^{99m}Tc-labelling was performed by substitution using fac-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ as precursor. The single species, with radiochemical purity above 90%, was stable for at least 4 h both in reaction milieu and in human plasma, moderately hydrophilic [logP(octanol:buffer pH 7)=−0.44±0.04] and exhibited a plasma protein binding of 13±3%.
Uptake by human tumor cells (HCT-15) "in vitro" under hypoxic conditions was two-fold higher than in oxic conditions. Biodistribution in normal mice showed low blood and liver activity and excretion through the urinary tract (%Dose in urinary system=60% at 4 h post injection). Studies in animals bearing solid tumors, Lewis carcinoma (by induction with 3LL cells), showed a significant tumor-uptake (%Act./g muscle/tumor from 4.24±0.74 at 4 h).
Results are promising and indicate the potentiality of this approach.

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HER2 targeting with ^{99m}Tc-labeled second generation synthetic Affibody molecule

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Affibody molecules based on a non-immunoglobulin scaffold have demonstrated high potential for in vivo molecular imaging of HER2-expressing tumors. Re-engineering of the molecular scaffold has led to a second generation of optimized Affibody molecules, having a surface distinctly different from the parental protein domain from staphylococcal protein A. The new tracer showed further increased melting point, stability and overall hydrophilicity compared to the parental molecule, and was shown to be more amenable for chemical peptide synthesis. Synthetic Affibody molecule with amino acid sequence mercaptoacetyl-ESE- on N-terminus as a chelator provided stable labeling with nearly quantitative yield. Stability of new conjugate was confirmed both in vitro and in vivo. Labeled conjugate targeted specifically HER2 in vitro and in vivo and demonstrated favorable biodistribution profile with low radioactivity accumulation in normal organs except kidneys (60%ID/g in nude mice). In high HER2-expressing tumor model (SKOV-3), radioactivity uptake was 17%ID/g at 4 h post injection that together with rapid blood clearance gave tumor-to-blood ratio of 40. Capacity of new technetium labeled