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Review article

Preparation and biodistribution of 1-((2-methoxyphenyl) piperazine) ferrocenecarboxamide labeled with technetium-99m as a potential brain receptor imaging agent

Nadia Malek Saied [a, *], Najoua Mejri [a], Radhia El Aissi [a, b, c], Eric Benoist [b, c, **], Mouldi Saidi [a]

[a] Radiopharmaceutical Unit, National Centre of Sciences and Nuclear Technology, Sidi Thabet 2020, Tunisia
[b] CNRS, Laboratoire de Synthèse et Physico-Chimie de Molécules d'Intérêt Biologique, SPCMIB, UMR 5068, 118, Route de Narbonne, F-31062 Toulouse Cedex 9, France
[c] Université de Toulouse, UPS, Laboratoire de Synthèse et Physico-Chimie de Molécules d'Intérêt Biologique, SPCMIB, UMR 5068, 118, Route de Narbonne, F-31062 Toulouse Cedex 9, France

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ABSTRACT

The goal of this study is to develop a novel brain receptor imaging agent. This study reports the synthesis, characterization and the biological evaluation of 1-((2-methoxyphenyl) piperazine)ferrocenecarboxamide labeled with technetium-99 m (^{99m}Tc-MP). The ^{99m}Tc-MP was obtained quickly (radiolabelling time < 5 min), in 90% yield. The ^{99m}Tc-complex, characterized by HPLC (20–50% ACN of 0 at 5 min then 50% ACN of 5 at 17 min to finally with 50 at 20% ACN of 17 at 20 min), is stable, neutral and lipophilic enough to cross the blood–brain barrier which was confirmed by octanol/water partition coefficient (LogP = 1.82). *In vivo* biodistribution indicated that this complex had exceptional brain uptake (2.47% ID/g at 5 min and 0.75% ID/g at 60 min). The distribution of the activity at 15 min post-injection in various rat brain regions showed a higher accumulation in the hippocampus area. After blocking with 8-hydroxy-2-(dipropylamino) tetralin, the uptake of hippocampus was decreased significantly from 0.87% ID/g to 0.21% ID/g at 15 min p.i., while the cerebellum had no significant decrease.

The new ^{99m}Tc-cyclopentadienyltricarbonyl technetium complex reported here showed promising biological results, making it an interesting starting point for the development of a new ^{99m}Tc-complex as brain receptor imaging agent.

1. Introduction

Currently, research is concentrated on the development of radiotracers marked by technetium-99 m because it is available as a generator product, has a short half-life of 6 h and emits suitable gamma radiation. The almost ideal properties of ^{99m}Tc for SPECT imaging and its availability still justify the interest in ^{99m}Tc complexes specific for brain receptor imaging. The widespread availability of single-photon-emission tomography (SPECT) facilities in comparison with that of PET facilities, in nowadays, ^{99m}Tc is still the ideal radionuclide in the diagnostic nuclear medicine. To date most Tc compounds assigned as CNS receptor-targeted agents are square-pyramidal complexes of the oxo core $[Tc{=}O]^{3+}$ [1,2]. However, the *in vivo* behavior of such complexes may be influenced by the Tc=O unit offering a free position trans to the oxo ligand for further reaction *in vivo* as observed especially for the '3 + 1' complexes [3,4]. To reduce this *in vivo* reactivity, we consider oxo-free Tc complexes containing the metal in lower oxidation states to be interesting alternatives. In fact, bioorganometallic Tc(I) and Tc(III) complexes described so far show excellent affinities to the target receptor but suffer from insufficient brain uptake [5–7]. It is well known that besides the thermodynamic stability of a complex its kinetic stability or inertness is of equal and sometimes even of greater importance for an application *in vivo*. The most promising organometallic core for the labeling of biomolecules is the technetium-tricarbonyl core [8,9]. The metal center is at the low

* Corresponding author.
** Corresponding author. CNRS, Laboratoire de Synthèse et Physico-Chimie de Molécules d'Intérêt Biologique, SPCMIB, UMR 5068, 118, route de Narbonne, F-31062 Toulouse Cedex 9, France.
E-mail address: nadia.malek@gmail.com (N.M. Saied).

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oxidation state I and the metal tricarbonyl monocationic core $[M(CO)^{3+}]$ has a low-spin d6 electronic configuration which gives to this compound ($M(CO)_3$-core ($M = {}^{99m}Tc$)) the property to be chemically inert. It is very compact, owning an almost spherical shape. If the octahedral coordination sphere is "closed" with an appropriate ligand system, the metal center is efficiently protected against further ligand attack or re-oxidation. Several research teams including ours demonstrated that Tc(I) could be efficiently stabilized by cyclopentadienyl (Cp) derivatives [10,11]. Cp units are particularly attractive ligands since they lead to organometallic half-sandwich complexes of general formula $[\{R\text{-}Cp\}^{99m}Tc(CO)_3]$ (R = organic part or biomolecule), so-called cytectrenes I and II [12,13] Furthermore, the stable piano stool $CpM(CO)_3$ core (Cp = cyclopentadienyl, $M = {}^{99m}Tc$) is one of the most promising moieties for this purpose. Indeed, the formal oxidation state of the metal is +I, and the CO ligands can stabilize low valent metal centers by backbonding. Therefore, the metal is less susceptible to oxidation, as the cyclopentadiene ligand, is small, with low molecular weight, and presents the additional possibility of being coupled to targeting vectors [14].

The application of this kind of '$CpTc(CO)_3$' moiety-based small complexes for brain imaging promising. We anticipated that the substitution of both the ester function and the piperidine derivatives of cytectrene I and II by an amido group and 1-(2-methoxyphenyl) piperazine respectively, should (i) lead, in a few steps, to a new cytectrene with suitable *in vivo* stability and lipophilicity to cross the blood brain barrier (BBB), (ii) allow a better brain targeting, (iii) offer the possibility to develop a multitude of other cytectrenes based on this new model.

Here, we report the synthesis, radiolabeling and biological evaluation of a novel ^{99m}Tc-tricarbonyl complex as a potential brain receptor imaging agent.

2. Materials and methods

2.1. Materials and equipment

All purchased chemicals were of the highest purity commercially available and used without further purification. Analytical grade solvents were used and not further purified unless specified. Reactions were monitored by TLC on *Kieselgel* 60 F_{254} (Merck) on aluminium support under UV light (254 nm). Technetium-99 m as sodium pertechnetate $[Na^{99m}TcO_4]$ was obtained in physiological saline. Chromatographic purification was conducted using "gravity" silica gel obtained from Merck. $Re(CO)_5Cl$ was purchased from Aldrich Chem. Co. $[Re(CO)_3Cl_3][NEt_4]_2$ [15] was prepared as previously reported. Liquid Chromatography (HPLC) analysis was performed on a SCL-10Avp SHIMADZU HPLC system coupled to a UV-Absorbance detector from ICS and a Gabi gamma detector from Raytest. Separations were achieved on a reverse phase C-18 column 250 × 4.6-mm (Shim-pack VP-ODS, SHIMADZU) eluted with a binary gradient system at a flow rate of 1 ml/min. Mobile phase A was water containing 0.1% trifluoroacetic acid, while mobile phase B was acetonitrile (ACN) containing 0.1% trifluoroacetic acid. Gamma camera NaI (GAEBE) brand square head 20 cm/20 cm with photo multiplier low-energy high-resolution, collimator collateral channels, acquisition matrix 256/256.

2.2. Animals

Albino Wistar male rats (Pasteur Institute, Tunisia), with a weight of 250–300 g, were used in all experiments. Animals were housed for one day before the onset of the experiments in our laboratory housing facility. They were carried for in accordance with the principles of the guide to the care and use of experimental animals.

2.3. Synthesis of 1-((2-methoxyphenyl) piperazine) ferrocenecarboxamide 2

2.3.1. Ferrocenoyl chloride 1

Under a nitrogen atmosphere, to a stirred solution of ferrocene carboxylic acid (1.20 g, 5.2 mmol) in freshly distillated dichloromethane (10 ml), was added dropwise oxalyl chloride (4 ml, 46.8 mmol), at 0 °C. The resulting mixture was stirred at ambient temp. for 4 h, then the solvent was removed under reduce pressure. The solution was triturated with hot pentane, then the mixture was filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was crystallized from pentane to give a red crystalline solid (1.25 g, 97%): mp 134 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 4.36 (s, 5H, C_5H_5), 4.66 (s, 2H, C_5H_4), 4.94 (s, 2H, C_5H_4).

2.3.2. 1-((2-methoxyphenyl) piperazine)ferrocenecarboxamide 2

1-(2-methoxyphenyl) piperazine was obtained as active principle from Sigma and stored at 4 °C. The Chlorocarbonyl ferrocene (FeCOCl) was synthesized in our laboratory [16,17], briefly activation of ferrocene carboxylic acid by oxalyl chloride in CH_2CL_2, is stirred in the dark for 3 h under dry N_2. Its structure was confirmed by proton nuclear magnetic resonance and mass spectroscopy. All the other chemicals were used as supplied. A mixture of 1-(2-methoxyphenyl) piperazine (0.422 ml; 2412 mmol) and FeCOCl (500 mg; 2012 mmol) in dry THF (20 ml) and pyridine (0.162 ml; 2012 mmol) is stirred in the dark for 2 h under dry N_2. Removal of volatiles was realized by evaporation under reduced pressure. The resulting crude product was purified by chromatography (silica, then a gradient of Hexane 60%)/AcOEt (40%)) to give the 1-((2-methoxyphenyl) piperazine)ferrocenecarboxamide (82%) as orange powder: mp 134 °C.

^{1}H NMR (DMSO-d_6): δ_H (ppm) = 3.00 (m, 4H, CH_2); 3.82 (s, 7H, 2 × CH_2 + OCH_3); 4.28 (s, 5H, $CH_{Ar\ Cp}$); 4.41 (m, 2H, $CH_{3\text{-}4\ Ar\ Cp}$); 4.60 (m, 2H, $CH_{2\text{-}5\ Ar\ Cp}$); 6.95 (m, 4H, $CH_{Ar\ Ph}$); ^{13}C NMR (DMSO-d_6): δ_C (ppm) = 50.5 (4C, CH_2); 55.3 (1C, OCH_3); 69.2, 69.4, 70.2 (9C, $CH_{Ar\ Cp}$); 78.0 (1C, $C_{Ar\ Cp}$); 111.9, 118.3, 120.8, 122.8 (4C, $CH_{Ar\ Ph}$); 140.8, 152.0 (2C, $C_{Ar\ Ph}$); 168.2 (1C, C=O); MS (CI/NH_3): $[M+H]^+ = 405$; IR (KBr): $\nu_{C=O}$ = 1606 cm^{-1}; $C_{23}H_{19}FeNO$ calcd. C, 65.36; H, 5.98; N, 6.93%; found: C, 64.97; H, 5.97; N, 6.69%.

The purity of the 1-((2-methoxyphenyl) pipérazine)ferrocenecarboxamide was achieved by reverse-phase high performance liquid chromatography (HPLC). The fractions were recorded by an on-line UV-detector measuring the absorbance at 254 nm.

2.4. Synthesis of rhenium complex: Re-MP

A solution of $[Re(CO)_3Cl_3][NEt_4]_2$ (30 mg, 0.047 mmol) and 1-((2-methoxyphenyl) piperazine)ferrocenecarboxamide (30 mg, 0.098 mmol) in DMF (2.5 ml) and a solution of 0.1N HCl (1.8 ml) were combined in a 5 ml glass vial. The mixture was purged with argon and the vial was sealed with a teflon cap. The solution was stirred vigorously during 2 h at 160 °C. After cooling, the cap was removed and the solution poured in a dichloromethane solution (10 ml). After three washings with water (3 × 10 ml), the organic extract was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography on silica gel (eluent: petroleum ether/ethyl acetate: 80/20 then 70/30) to obtain the desired complex Re-MP as an orange powder (7 mg, 29% yield).

IR (KBr): $\nu_{C=O}$ = 1938 and 2027 cm^{-1}.

The purity of the Re-MP was achieved by reverse-phase high performance liquid chromatography (HPLC). The fractions were recorded by an on-line UV-detector measuring the absorbance at 254 nm.

2.5. Synthesis and characterization of the ^{99m}Tc-complex

A 2.5 mg aliquot of 1-((2-methoxyphenyl) piperazine)ferrocenecarboxamide and 5 mg of $Mn(CO)_5Br$ dissolved in DMSO (300 μL) were added to 300 μL of sodium pertechnetate eluate (~300 MBq). The reaction mixture was purged with nitrogen, then heated by microwave irradiation (900 W) for 5 periods of 40 s (a 30 s period was observed between two irradiations). Without optimizing the conditions, the resulting Tc-complex was obtained in 90% yield. After HPLC purification, complex ^{99m}Tc-MP was obtained with high radiochemical purity (>98%).

2.6. ITLC analysis

Labeling yield and stability of the ^{99m}Tc-MP during time, was determined with instant thin layer chromatography (ITLC). In ITLC-SG strips (ITLC-SG; Gelman), samples of the preparation containing labeled compound were applied at approximately 1 cm from the bottom (baseline) of the ITLC strips and were directly placed on an air-tight containers with Hexane/AcOEt (6:4 v/v) as solvent. The development time was 2–3 min, then the strips were mounted on a radio-chromatograph (Minigita) and counting of radioactivity was performed. The strips were then cut in several fractions corresponding to ^{99m}Tc-activity detected picks and the radioactivity was determined in a well gamma counter. Radiolabeled compound migrates with the front line, whereas free pertechnetate remains at the origin. The chromatography was repeated at 30 min, 1 h, 2 h, 4 h and 24 h to check the label stability. Results are expressed as percentage of total ^{99m}Tc-activity.

2.7. Stability of ^{99m}Tc-MP in serum

The *in vitro* stability of the purified complex ^{99m}Tc-MP was evaluated at different time points using the following procedure: in a borosilicated vial, ^{99m}Tc-MP (100 μL) was added to 0.9 ml of fresh rat serum at 37 °C. Aliquots were withdrawn in duplicate during the incubation at different time intervals till 24 h and subjected to chromatography using (ITLC-SG; Gelman) paper and Hexane/AcOEt (6:4 v/v) as solvent. Any increase in the free pertechnetate was considered as the degree of degradation.

2.8. Protein binding assay

The protein binding assay of the purified complex ^{99m}Tc-MP was evaluated at different time points using the following procedure: in a borosilicated vial, ^{99m}Tc-MP (100 μL) was added to 5 ml of fresh rat plasma at 37 °C. Aliquots were withdrawn during the incubation at different time intervals till 24 h and plasma was separated from blood samples by centrifugation (3000 rpm for 4 min) and individual fractions were counted in a well counter.

The plasma aliquots were treated with acetonitrile to precipitate proteins. After centrifugation (2000 rpm for 5 min), the activities of both phases (supernatant and precipitate) were measured separately. The reported value represents the average of triplicate measurements [18].

2.9. Partition coefficient determination

Lipophilicity was studied through the partition coefficient between *n*-octanol and phosphate buffer (0.125 M, pH 7.4). In a centrifuge tube containing 500 μL of each phase, 100 μL of the purified ^{99m}Tc complex solution was added, and the mixture was vortexed one minute at ambient temperature, followed by centrifugation at 5000 rpm for 5 min. 100 μL-aliquots of both buffer and organic layers were count using a well counter. The partition coefficient was calculated using the formula: log P = counts in *n*-octanol/counts in buffer. The reported value represents the average of triplicate measurements.

2.10. Biodistribution of ^{99m}Tc-MP in healthy Wistar rats

The radiotracer ^{99m}Tc-MP (300 μL diluted in saline-EtOH (80/20), 20 MBq) was injected *via* a lateral tail vein. At different intervals after injection, the animals (n = 5) were sacrificed by cervical dislocation. Organs of interest, samples of blood were collected, weighed and counted. Bladder and excreted urine were not weighed. The calculation for blood was based upon measured activity, sample weight and body composition data (considering that blood comprise 7% of body weight). Results were expressed as percent dose per gram of tissue (%ID g^{-1}).

2.11. Regional brain distribution and blocking experiment in rats

Rat (200 g, 5 animals per group) was injected through the tail vein with 0.3 ml (~20 MBq) of ^{99m}Tc-MP complex. The animals were sacrificed at 15 min post-injection time. The brain was rapidly removed, chilled and dissected. Samples from different brain regions (cortex, hippocampus and cerebellum) were collected, weighed and counted. The percentage dose/g of each sample was calculated by comparing sample counts with the counts of injected dose. In order to further confirm that the radiolabeled ^{99m}Tc-MP had specific receptor binding, blocking study was performed by conducting the biodistribution experiment in the presence of 8-Hydroxy-2-(dipropylamino) tetralin (8-OH-DPAT, 5-HT1A agonist, 10 mg/kg body weight) as blocking agent. 10 min after the first injection of blocking agent, ^{99m}Tc-MP complex was injected (~20 MBq, 0.3 ml). Rats were sacrificed at 15 min post-injection time (n = 5). Results were expressed as the percentage of the injected dose per gram tissue (% ID/g). Averages and standard deviations were calculated. The average ratios and standard deviations of % ID/g tissue of the different brain regions to cerebellum were calculated [19].

2.12. Rats gamma camera imaging experiments

Two Albino Wistar male rats (Pasteur Institute, Tunisia), with a weight of 250–300 g were used for the experiments. For each gamma experiment, the rat was initially anesthetized with ketamine. An intravenous perfusion line, filled with saline (0.9% w/v), was used for bolus radioligand (1.60–2.40 mCi) injection. Gamma serial dynamic images were obtained on an Advance gamma camera NaI (Gaebe). Scans were acquired for up to 30 min in 20 frames and were corrected for attenuation and scatter. Gamma images were co-registered to scans and decay-corrected.

2.13. Statistical analysis

Experiments were performed in triplicate unless stated otherwise. Results were reported as mean ± standard deviation. Differences between the data were evaluated with the student t test. The level of significance was set at $P < 0.05$.

3. Results and discussions

3.1. Chemical synthesis of 1-((2-methoxyphenyl) piperazine) ferrocenecarboxamide

The synthesis of 1-((2-methoxyphenyl) piperazine)ferrocenecarboxamide was accomplished using a nucleophilic addition reaction of 1-(2-methoxyphenyl) piperazine on the chlorocarbonyl ferrocene in the presence of THF and Pyridine (Fig. 1).

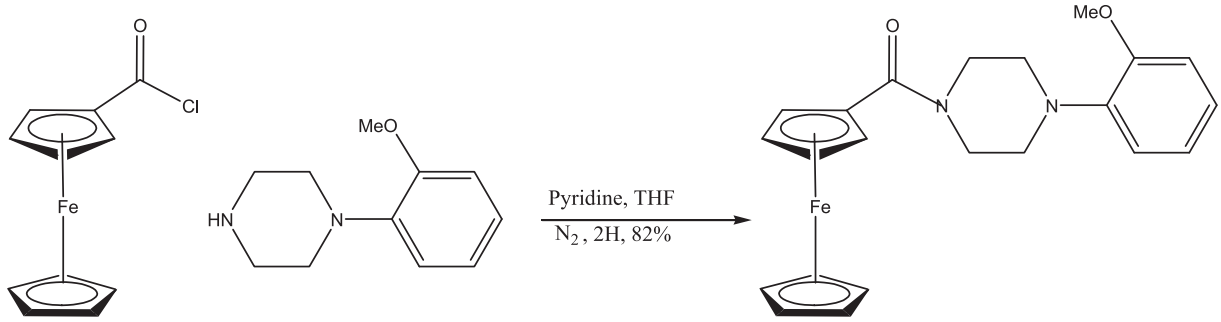

Fig. 1. Structures and reaction scheme for the preparation of 1-((2-methoxyphenyl) piperazine)ferrocenecarboxamide.

The purity of the 1-((2-methoxyphenyl) piperazine)ferrocenecarboxamide was confirmed using C18 reverse-phase HPLC. Only one peak was seen with a retention time of 9.7 min (Fig. 2).

3.2. Radiochemical analysis of ^{99m}Tc-MP

The labeled complex, as shown in the following scheme (Fig. 3). This reaction was accomplished in one pot, reduction, carbonylation and cyclopentadienylation of 99mTcO4− in a rapid and relatively mild manner. This transformation is referred to as a double-ligand transfer (DLT) reaction [20] because two different ligands, Cp and CO, from two different metal atoms, Fe and Mn, were transferred to a third metal atom 99mTc. Exchange mechanism between iron and technetium has been discussed by many authors. High temperature destabilizes the cyclopentadienyl nucleus and promotes the exchange between the central atoms. The destabilization of the complex ferrocene and the release of iron in the reaction medium presents a major advantage in the reduction of pertechnetate (99mTcO4−) passing an oxidation state + VII (very stable complex) to an oxidation state + I. This state oxidation (+I) is stabilized by the bonds of the cyclopentadienyl aromatic structure.

The product was analyzed by HPLC [Shim-pack VP-ODS C18 column; mobile phase: 0–5 min, acetonitrile (20–50%)+0.1% TFA (A) and ultrapure water (80–50%)+0.1% TFA (B); 5–15 min, 50% (A) and 50% (B); 15–17 min, 50–20% (A), 50–80% (B); flow: 1 ml min^{-1}; detector NaI crystal]. Radiochemical purity was confirmed by a single peak at 13 min corresponding to the 99mTc-MP, illustrated in the radiochromatogram (Fig. 4).

Rhenium complex, "cold" metallic analogue to desired ^{99m}Tc-MP complex, was prepared *via* a single-ligand transfer reaction using $[Re(CO)_3Cl_3][NEt_4]_2$, [15] as tricarbonylrhenium(I) entity source. The reaction was carried out by heating a DMF solution of 1-((2-methoxyphenyl) piperazine)ferrocenecarboxamide derivative and the Re(I) precursor, in the presence of a 0.1 N HCl solution, at 160 °C for 2 h. After chromatography purification, rhenium complexes, Re-MP, was obtained in modest yield, to 29%. The chemical identification of the radiotracer was accomplished by HPLC-comparison of its chromatogram with that of the rhenium analogue, as underlined in Fig. 5. Similar retention times were observed (13 min for ^{99m}Tc-MP. 13.7 min for Re-MP), confirming the iso-structurality of both complexes.

3.3. ITLC analysis

ITLC analysis of ^{99m}Tc-MP showed a high labeling efficiency (>95%) and this value was still unchanged up to 24 h. No significant differences were observed between radiochromatographic analysis and counting of the cut ITLC strips. ^{99m}Tc-MP migrated of the mobile phase (Rf = 0.9) and pertechnetate remained at the origin.

3.4. Stability of ^{99m}Tc-MP in serum

The complex showed excellent *in vitro* stability under physiological conditions in rat plasma with >99% of the ^{99m}Tc-MP

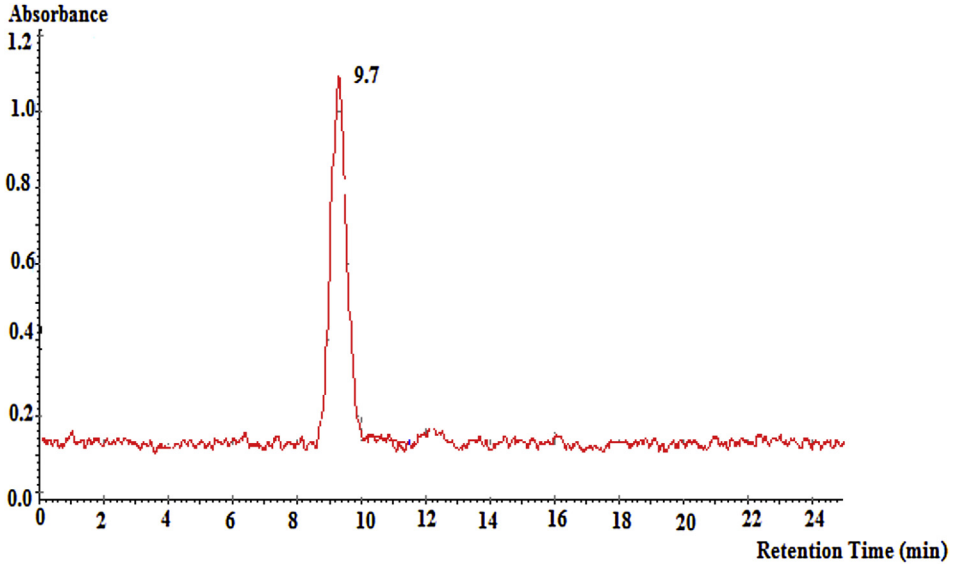

Fig. 2. Chromatogram of the 1-((2-methoxyphenyl) piperazine)ferrocenecarboxamide on C18 column HPLC reverse-phase. Column: C18 Shim pack VP-ODS. Mobile phase: 0–5 min 20–50% Acetonitrile +0.1%TFA (A). Water +0.1% TFA (B) 80–50%. 5–15 min 50% A and 50% B, 15–17 min 50–20% A 50–80 % B. Flow rate: 1 ml/min.

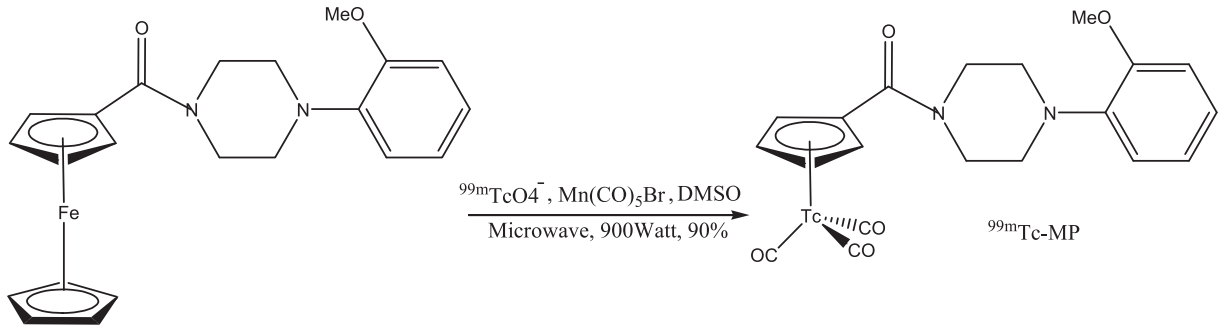

Fig. 3. Synthesis of radiolabeled complex ^{99m}Tc-MP.

compound remaining intact after 24 h. Consequently, no reoxidation to pertechnetate was observed, even after 24 h of incubation, as indicated by ITLC analysis. The data suggested high *in vitro* stability of the complex (Fig. 6).

3.5. Protein binding study

The assays with rat plasma were performed to determine the percentage of protein binding. After incubation of the radiotracer in blood samples, plasma was separated from blood samples by centrifugation and treated with acetonitrile to precipitate the proteins. Two collected fractions (supernatant and protein) were measured by well gamma counter. The ^{99m}Tc-complex activity has been found to be higher in plasma fraction compared to the blood cell one. The protein binding study depicted low binding of the complex with blood protein. Only 10% of the complex was bound to serum proteins after 24 h of incubation (Fig. 7). The major part of the circulating radioactivity corresponds to free complex, which is a favorable feature, in terms of its application in the imaging of 5-HT1A receptor.

3.6. Lipophylicity study

The lipophilicity is an important parameter to predict and interpret the biological activity of a molecule intended to be injected *in vivo*. It is generally accepted that only the lipid pathway provides access to the brain for ^{99m}Tc-complexes [21]. Consequently, the radiocomplexes had to be relatively lipophilic. The lipophilic character of our radiocomplex was assessed by determination of the partition coefficient (P) in physiological conditions (0.05 M Tris HCl buffer, pH 7.4/n-octanol) and was expressed as log P (oct/buffer). A value of 1.82 was found, indicating that ^{99m}Tc-MP is lipophilic. Interestingly, this value is within the range for related radiocomplexes able to cross the blood brain barrier ($logP_{o/w} = 0.5–2.5$) [22,23].

3.7. Biodistribution of ^{99m}Tc –MP in healthy Wistar rats

The *in vivo* behavior of the ^{99m}Tc-MP was evaluated in healthy Wistar rats at different time points, the results were shown in Table 1. Interestingly, the complex exhibits a fast blood clearance (0.53% and 0.27% at 5 and 60 min respectively) combined with a good brain uptake at earlier post-injection time (2.47%ID/g at 5 min). As expected for lipophilic compounds, there is a high and rapid liver uptake that decreases over the time. Excretion of the radioactivity occurred mainly *via* the renal urinary pathway (2.39% and 2.93% at 5 and 60 min respectively) and through the hepatobiliary system as evidenced by the decreasing activity in liver. These data were promising since the complex showed fast blood clearance and excellent brain uptake at 5 min (2.47%ID/g). The retention of the radioactivity in brain is fairly good at 60 min p.i. (0.75%ID/g). The ability of ^{99m}Tc-MP of penetrating the blood brain barrier could be explained by its good properties in terms of charge, small size, lipophilicity and *in vitro*, *in vivo* stability. The brain

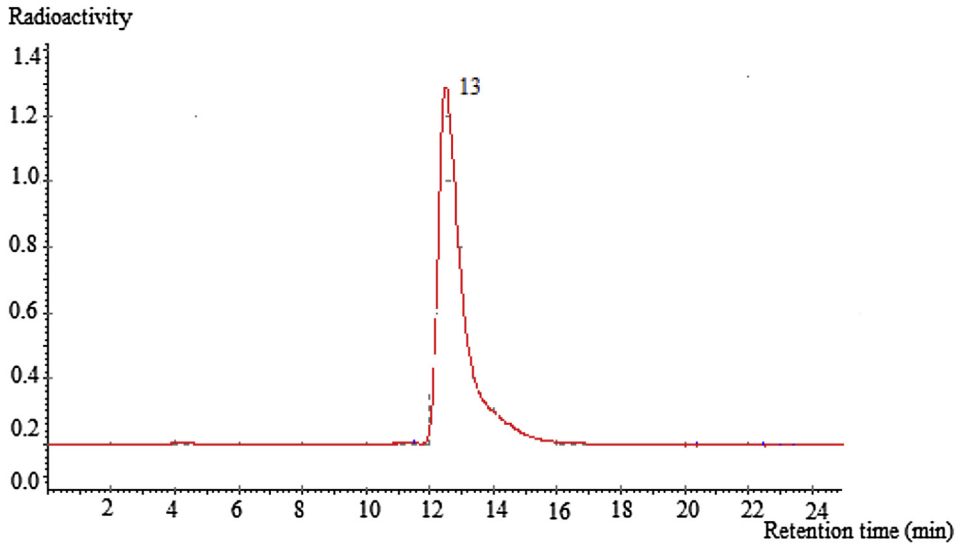

Fig. 4. HPLC radiochromatogram of ^{99m}Tc-MP on C18 column HPLC reverse phase.

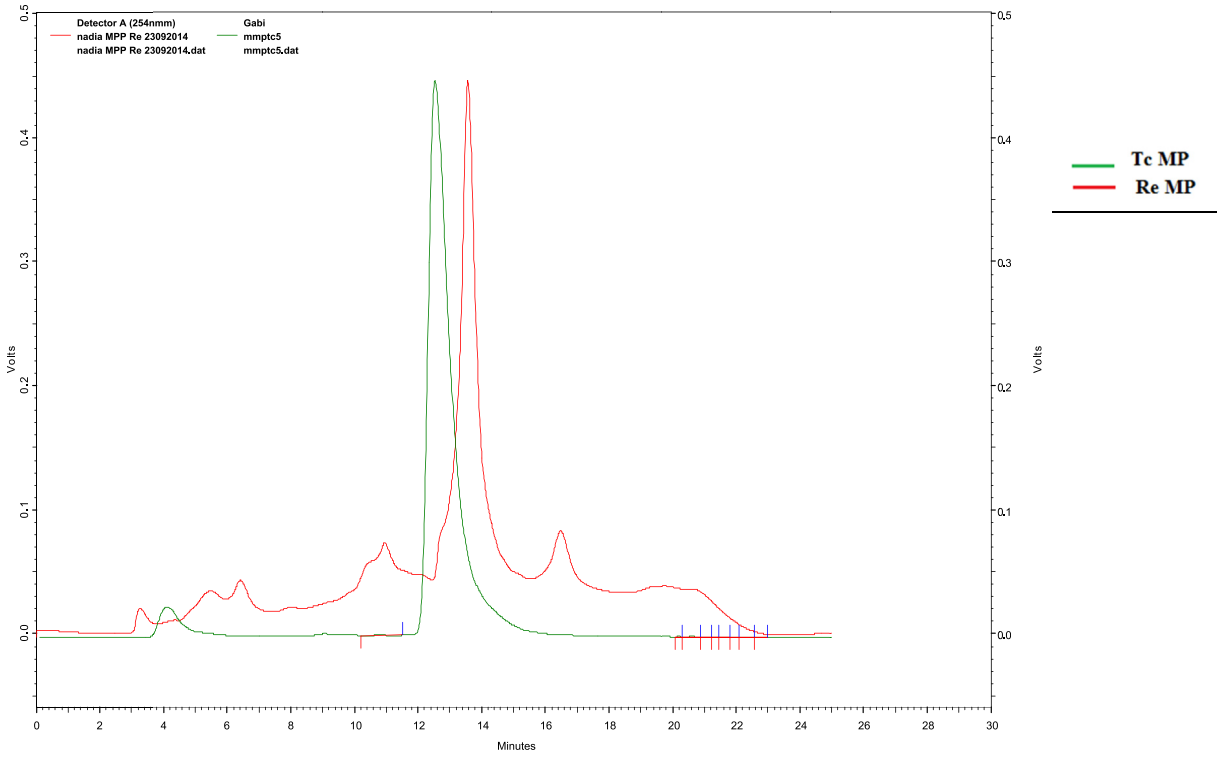

Fig. 5. HPLC comparison of rhenium complex Re-MP and ^{99m}Tc complex ^{99m}Tc-MP.

uptake value of ^{99m}Tc-MP is higher comparable to those complexes DADT (0.6%ID/g), DEEDA (1.63%ID/g), BMPBA (1.77%ID/g) [24–26]. The *in vivo* distribution of many compounds showed a moderate brain uptake, it was clear that more extensive studies in animal models will be needed to correlate the *in vitro* data to predict there *in vivo* behavior in humans.

The biodistribution of the activity in selected regions of the brain was also investigated at 15 min (Fig. 8).

The highest concentration of radioactivity was found in the hippocampus area where the 5-HT$_{1A}$ receptor density was important. For cortex, region where the 5-HT$_{1A}$ receptor density was less important than in the hippocampus, the radioactivity value is lower. Nevertheless, the repartition of the radioactivity in the different regions of the brain seems too homogeneous to establish a clear correlation between the repartition of the 5-HT$_{1A}$ receptors and the brain distribution of the radioactivity. Further studies are ongoing to clarify this point.

3.8. Regional brain distribution and blocking experiment in rats

The distribution of the activity in various rats brain regions was performed in normal rats (Table 2). The biodistribution of the activity in selected regions of the brain was also investigated at 15 min. The highest concentration of radioactivity was found in the hippocampus area where the 5-HT1A receptor density was important. For cortex, region where the 5-HT1A receptor density was less important than in the hippocampus, the radioactivity value is lower. For ^{99m}Tc-MP, the radioactivity concentration of hippocampus (Hipp) at 15 min p.i. was 0.87% ID·g−1. For cerebellum (CB), the uptake was much lower than that of Hipp. The ratio of Hipp/CB was 4.83. For

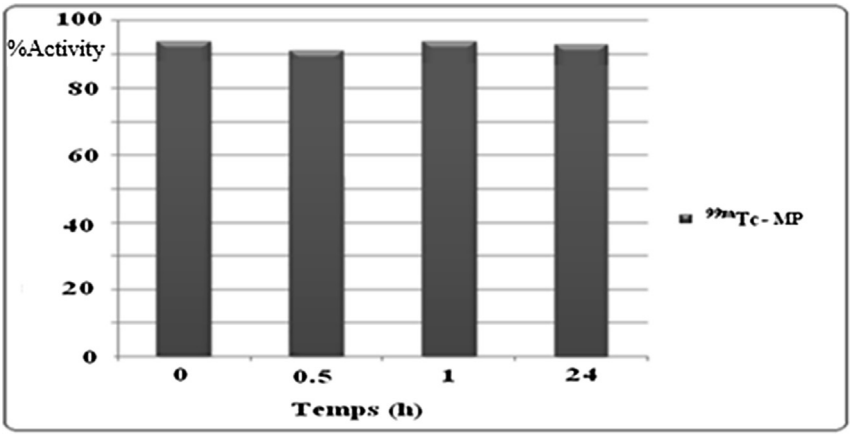

Fig. 6. In vitro stability of ^{99m}Tc-MP in serum.

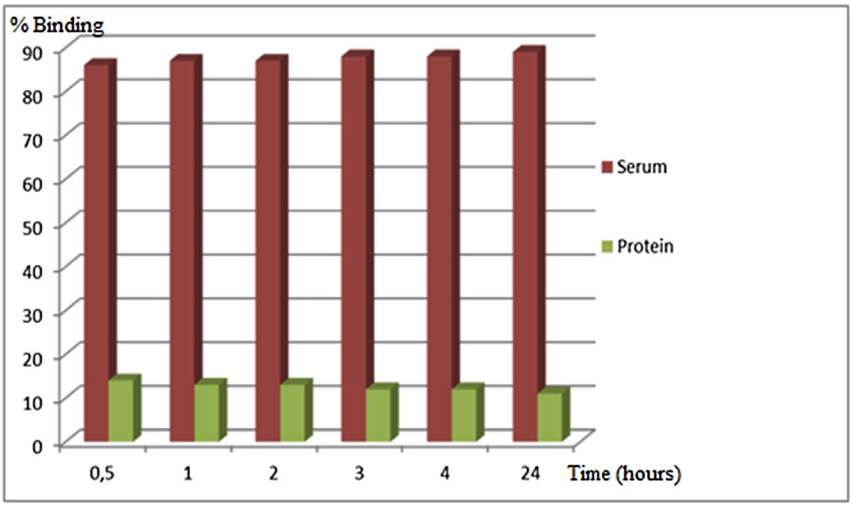

Fig. 7. Protein binding study of ^{99m}Tc- MP.

Table 1
Biodistribution in healthy Wistar rats 5 min, 15 min, 30 min and 60 min after administration of ^{99m}Tc-MP.

Organs	5 min (%ID/g)	15 min (%ID/g)	30 min (%ID/g)	60 min (%ID/g)
Blood	0.53±(0.03)	0.45±(0.04)	0.39±(0.02)	0.27±(0.01)
Heart	0.43±(0.01)	1.03±(0.02)	0.40±(0.05)	0.51±(0.01)
Lungs	5.70±(0.04)	4.11±(0.02)	3.91±(0.04)	2.60±(0.05)
Liver	18.32±(0.04)	16.11±(0.03)	12.65±(0.03)	10.72±(0.02)
Spleen	5.11±(0.02)	3.20±(0.03)	2.41±(0.02)	1.51±(0.05)
Kidney	2.39±(0.03)	2.25±(0.04)	2.82±(0.05)	2.93±(0.02)
Brain	2.47±(0.04)	1.81±(0.01)	0.93±(0.04)	0.75±(0.03)

• Expressed as % injected dose per gram (%ID/g ±SD, n = 5).

Table 2
Regional brain distribution and blocking studies of ^{99m}Tc-MP in normal rats at 15 min p.i. (%ID/g ± SD, n = 5).

	Without blocking	With blocking	P value
Hippocampus	0.87 ± 0.03	0.21 ± 0.03	0.003
Cortex	0.50 ± 0.04	0.35 ± 0.02	0.045
Cerebellum	0.18 ± 0.02	0.17 ± 0.02	0.401
Hippocampus/cerebellum	4.83	1.23	
Cortex/cerebellum	2.77	2.05	

In the blocking and without blocking experiments, the two-tailed paired Student's *t* test was applied to compare the values between before and after blocking. A *P* value of less than 0.05 was considered statistically significant.

cortex, the uptake was 0.5% ID·g−1, in the middle of Hipp (0.87% ID·g−1) and CB (0.18% ID·g−1). The distribution of radioactivity in hippocampus, cerebellum and cortex was correlated very well with the distribution of 5-HT1A receptors in brain. In order to further characterize the *vivo* brain uptake of this ^{99m}Tc labeled complex in rats, a blocking study was carried out to determine the changes of regional brain uptake. After blocking by 8-hydroxy-2-(dipropylamino) tetralin (8-OH-DPAT), the uptake of hippocampus was decreased obviously from 0.87% ID·g−1 to 0.21% ID·g−1 at 15 min p.i. ($P = 0.003$), and the uptake of cortex was also decreased from 0.5% ID·g−1 to 0.35% ID·g−1 at 15 min p.i. ($P = 0.045$), but the cerebellum had no significant decrease ($P = 0.40$) (Table 2). The ratios of Hipp/CB were changed from 4.83 to 1.23 at 15 min p.i. This change is probably due to the competition of 8-OH-DPAT binding to the same 5-HT1A receptor in the brain. Based on the above *in vivo* results, we could conclude that complex ^{99m}Tc-MP had like enough specific binding to the 5-HT1A receptor and hopefully be developed as potential technetium- 99 m receptor imaging agent.

3.9. Rats gamma camera imaging

After injection of ^{99m}Tc-MP into a Wistar rats, there was a rapid and moderate uptake of radioactivity into rat's brain and agree with bio-distribution. The accumulation of radioactivity in the brain is indicative of an efficient blood–brain-barrier passage of labeled compound (Fig. 9).

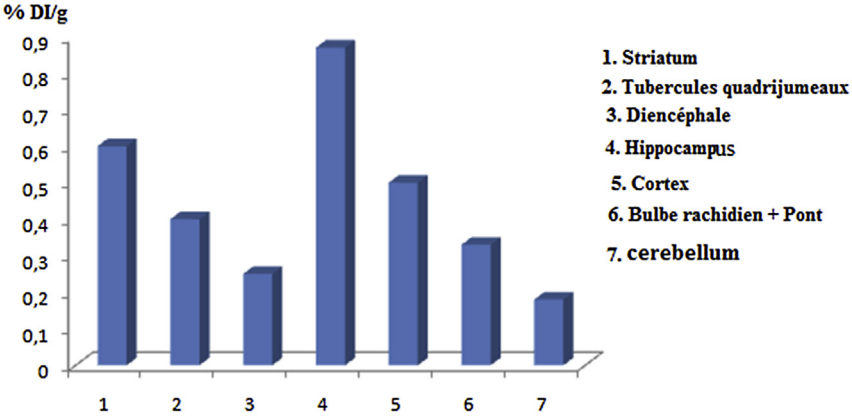

Fig. 8. Regional distribution in rat brain at 15 min p.i.

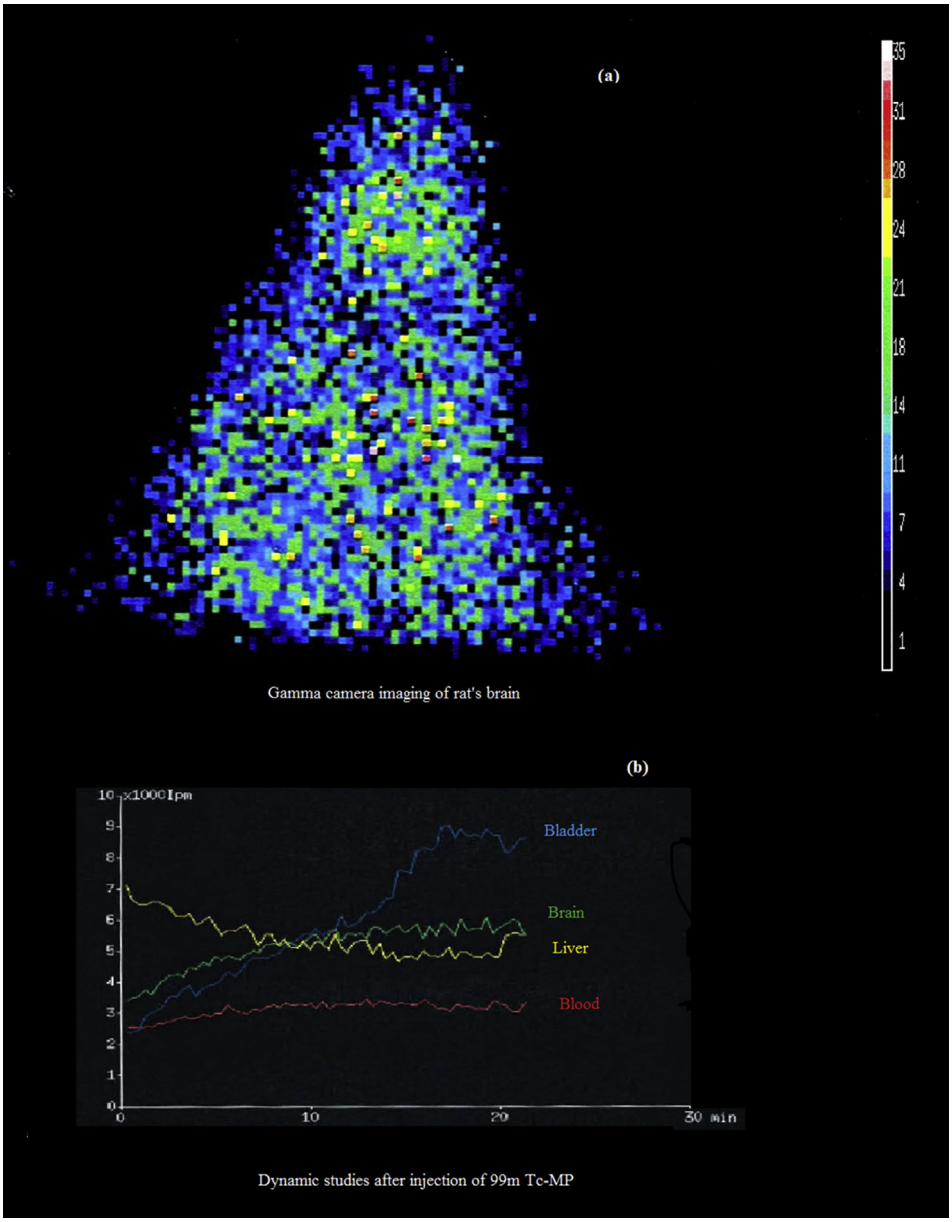

Fig. 9. Gamma camera of rat brain illustrates the distribution of 99mTc-MP accumulation (a) and dynamic studies in the various organs over time (b).

4. Conclusion

We designed and synthesized a novel brain receptor imaging agent ^{99m}Tc-MP with high radiolabeling yield (>90%). According to the results of *in vivo* biodistribution studies, we found that ^{99m}Tc-MP had favorable properties for further study. The preliminary biological studies are interesting. Firstly, the Tc-complex is lipophilic enough to cross the blood brain barrier, as expected (Log P = 1.82). Secondly, biodistribution studies in healthy male rats indicated that this complex presented a very good brain uptake combined with a fairly good retention of radioactivity in brain (0.75% ID/g after 1 h). Then, the distribution of the activity at 15 min post-injection in various rat brain regions showed a higher accumulation in the hippocampus area which is rich in $5HT_{1A}$ receptors.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.05.014.

References

[1] A. Mahmood, J.F. Kronauge, E. Barbarics, E. Freiberg, B.K. Madras, J. Li, A. Davison, A.G. Jones, M. Nicolini, U. Mazzi (Eds.), Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine. SGE Editoriali Padova vol. 5, 1999, p. 393.
[2] I. Heimbold, A. Drews, R. Syhre, M. Kretzschmar, H.J. Pietzsch, B. Johannsen, A novel technetium-99m radioligand for the 5-HT1A receptor derived from desmethyl-WAY-100635 (DWAY), Eur. J. Nucl. Med. 29 (2002) 82–87.
[3] R. Syhre, S. Seifert, H. Spies, A. Gupta, B. Johannsen, Stability versus reactivity of "3_1" mixed ligand complexes in vitro and in vivo, Eur. J. Nucl. Med. 25 (1998) 793–796.

[4] B.A. Nock, T. Maina, D. Yannoukakos, C. Ioannis, M. Papadopoulos, E. Chiotellis, Glutathione mediated metabolism of technetium-99m SNS/S mixed-ligand complexes: a proposed mechanism of brain retention, J. Med. Chem. 42 (1999) 1066–1075.
[5] R. Alberto, R. Schibli, A.P. Schubiger, U. Abram, H.J. Pietzsch, B. Johannsen, First application of fac-[99mTc$(OH_2)_3(CO)_3]^+$ in bio organometallic chemistry: design, structure, and in vitro affinity of a 5-HT1A receptor ligand labeled with 99mTc, J. Am. Chem. Soc. 121 (1999) 6076–6077.
[6] J. Bernard, K. Ortner, B. Spingler, H.-J. Pietzsch, R. Alberto, Aqueous synthesis of derivatized cyclopentadienyl complexes of technetium and rhenium directed toward radiopharmaceutical application, Inorg. Chem. 42 (2003) 1014–1022.
[7] A. Drews, H.J. Pietzsch, R. Syhre, S. Seifert, K. Varnas, H. Hall, C. Halldin, W. Kraus, P. Karlsson, C. Johnsson, H. Spies, B. Johannsen, Synthesis and biological evaluation of technetium(III) mixed-ligand complexes with high affinity for the cerebral 5-HT1A receptor and the alpha1-adrenergic receptor, Nucl. Med. Biol. 29 (2002) 389–398.
[8] R. Alberto, in: G. Jaouen (Ed.), Radiopharmaceuticals, Wiley-VCH, 2005, pp. 97–124.
[9] R. Schibli, A.P. Schubiger, Current use and future potential of organometallic radio- pharmaceuticals, Eur. J. Nucl. Med. Mol. Imaging 29 (2002) 1529.
[10] D. Kuntschke, M. Wenzel, P. Schulze, New [99mTc]-cytectrene amine compounds as specific brain imaging agents, J. Label. Cpd. Radiopharm. 36 (1995) 193.
[11] H. Zeng, H. Zhang, Synthesis and biological evaluation of fatty acids conjugates bearing cyclopentadienyl-donors incorporated $[(99m)Tc/Re(CO)_3]^+$ for myocardical imaging, Eur. J. Med. Chem. 72 (2014) 10.
[12] M. Saidi, K. Kothari, M.R.A. Pillai, A. Hassan, H.D. Sarma, P.R. Chaudhari, T.P. Unnikrishnan, A. Korde, Z. Azzouz, Cyclopentadienyl technetium (99mTc) tricarbonyl piperidine conjugates: biodistribution and imaging studies, J. Label. Compd. Radiopharm. 44 (2001) 603.
[13] M. Kretzschmar, M. Saidi, R. Bergmann, Annual Report 2002, FZR-363, Institute of Bioinorg, Radiopharm. Chem, 2003, p. 53.
[14] R. Alberto, The particular role of radiopharmacy within bioorganometallic chemistry, J. Organomet. Chem. 692 (2007) 1179.
[15] C.L. Ferreira, C.B. Ewart, S.R. Bayly, B.O. Patrick, J. Steele, M.J. Adam, C. Orvig, Glucosamine conjugates of tricarbonylcyclopentadienyl rhenium(I) and technetium(I) cores, Inorg. Chem. 45 (2006) 6979.
[16] S. Arimoto, A.C. Haven, Derivatives of dicyclopentadienyliron, J. Am. Chem. Soc. 77 (1955) 6295.
[17] H.J. Lorkowski, R. Pannier, A. Wende, Über Ferrocenderivate. VIII. Die Darstellung von monomeren und polymeren Ferrocenylenoxadiazolen, J. Prak. Chem. 35 (1967) 149.
[18] E.P. Ephraim, J. Carl, S. Terry, J.W. Michael, A.K. John, Synthesis and biological evaluation of a nonsteroidal bromine-76-labeled androgen receptor ligand 3-[76Br]bromo-hydroxyflutamide, Nucl. Med. Biol. 33 (2006) 705.
[19] Z. Xianzhong, Z. Panwang, L. Jiaojiao, H. Yan, L. Yan, C. Yanling, G. Ting, Y. Wenjiang, W. Xuebin, Preparation and biodistribution of 99mTc-tricarbonyl complex with 4-[(2-methoxyphenyl)piperazin-1-yl]-dithioformate as a potential 5-HT1A receptor imaging agent, Appl. Radiat. Isotopes 65 (2007) 287–292.
[20] M. Wenzel, Tc-99m labeling of cymantrene-analogues with different substituents. A new approach to Tc-99m radiodiagnostics, J. Label. Compd. Radiopharm. 31 (1992) 641–651.
[21] T.H. Belhadj, Y. Coulais, G. Cros, M.H. Darbieu, J.A.M. Tafani, J. Fabre, et al., Technetium labeling of Bi, tri and tetradentate ligands derived from 2-aminocyclopentene-1-dithiocarboxylic acid: characterization and biodistribution of their oxo and nitrido 99mTechnetium complexes, Nucl. Med. Biol. 23 (1996) 353–357.
[22] B. Johannsen, H.J. Pietzsch, Development of technetium-99m-based CNS receptor ligands: have there been any advances? Eur. J. Nucl. Med. 29 (2002) 263–275.
[23] C. Fernandes, J.D.G. Correia, L. Gano, I. Santos, S. Seifert, R. Syhre, R. Bergmann, H. Spies, Dramatic effect of the tridentate ligand on the stability of ^{99m}Tc "3+1" oxo complexes bearing arylpiperazine derivatives, Bioconjugate. Chem. 16 (2005) 660–668.
[24] I. Heimbold, A. Drews, R. Syhre, M. Kretzschmar, H.J. Pietzsch, B. Johannsen, Eur. J. Nucl. Med. 29 (2002) 82–87.
[25] Leon A.S. et al. Techhnical reports series no. 426. IAEA.
[26] Fel L. et al. Techhnical reports series no. 426. IAEA.