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ORIGINAL PAPER

New microwave assisted radiolabelling method and rat brain biodistribution study of two new ^{99m}Tc-tricarbonyl complexes as potential brain imaging agents

Moez Trabelsi, Abdelkader Mekni, Chandra Solanki, Paul Maltby, Mouldi Saidi

Abstract. Two new cyclopentadienyl piperidine derivatives, namely ferrocene carboxylic acid 1-ethyl-3-hydroxypiperidinyl ester and ferrocene carboxylic acid 4-hydroxypiperidinyl ester, were synthesized. The ligands were then radiolabelled with ^{99m}Tc using two different approaches. The first method consisted of reacting the ligand precursor with $Mn(CO)_5Br$ in pertechnetate $^{99m}TcO_4^-$ in normal saline and dimethyl formamide (DMF) at 150°C for 1 h. The yields were 70% and 90%, respectively. For the second method, the reactions mixtures were placed in a microwave oven for 2 min at 650 watt. The yields were higher than 90% for both ^{99m}Tc complexes. Biodistribution studies showed that tricarbonyl{η^5-[carboxy-3-hydroxy(N-ethyl)piperidine]cyclopentadienyl}technetium(I) had the highest brain uptake. The regional distribution in the brain also demonstrated relatively higher uptake of tricarbonyl{η^5-[carboxy-3-hydroxy(N-ethyl) piperidine]cyclopentadienyl}technetium(I) in the colliculus (1.97% ID/g tissue,) with the colliculus to cerebellum ratio of 1.99. We conclude that the radiolabelling can be achieved by microwave activation, and tricarbonyl{η^5-[carboxy-3-hydroxy(N-ethyl)piperidine]cyclopentadienyl}technetium(I) has the potential for use as central nervous system (CNS) imaging agent.

Key words: technetium-99m • cyclopentadiene technetium carbonyl complexes • microwave synthesis • brain imaging • radiopharmaceuticals

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Introduction

Technetium-99m is the most widely used radionuclide in diagnostic nuclear medicine by virtue of its ready availability, low cost and optimal radiation properties ($t_{½}$ = 6 h, 89% photon yield of 140 keV). Technetium also has a diverse chemistry due to its variable oxidation states ranging from –I to +VII, and coordination numbers up to nine, thus enabling labelling of a wide range of compounds. Moreover, the chemistry of ^{99m}Tc can be conveniently studied using ^{99}Tc which, unlike ^{99m}Tc, can be handled safely in milligram quantities.

Recently, an increased interest has been observed in the development of ^{99m}Tc-radiopharmaceuticals that bind *in vivo* to receptors of the central nervous system (CNS) as they may reflect pathophysiology in several neuropsychiatric disorders [3]. To this end, a series of ^{99m}Tc labelled compounds containing $^{99m}TcO^{3+}$ cation have been reported as potential CNS-Receptor-Imaging agents [1, 2, 5, 6, 8–11]. Efforts towards the development of oxo-free technetium complexes in lower oxidation state of Tc, in particular Tc^{+1} complexes have shown encouraging results with excellent affinity to the target receptors, but have suffered from insufficient brain uptake.

The development of ^{99m}Tc-cyclopentadienyl tricarbonyl piperidine derivatives, in which Tc^{+1} is coordinated to cyclopentadienide ($C_5H_5^-$) and three carbonyl groups, has been reported [4, 15]. These complexes have shown

high uptake in the brain as well as high affinity to the target receptors in rats 20 min after i.v. administration.

In an effort to further understanding the structure/biodistribution relationship of the piperidine derivatives and to achieve better brain retention, two new piperidine derivatives were synthesized, radiolabelled and evaluated by biodistribution studies in the rat brain. A previously published complex was used as Ref. [15]. This complex was radiolabelled according to the method described by Wenzel [18], and showed a high affinity to the entorhinal cortex as well as in regions for α_1 adrenergic receptors, such as thalamus and cortex in rats 20 min after i.v. application. Moreover, in order to improve the reaction conditions for practical and eventual clinical use, the radiochemical labelling was performed using the microwave activation as a new method and compared with the method described by Wenzel.

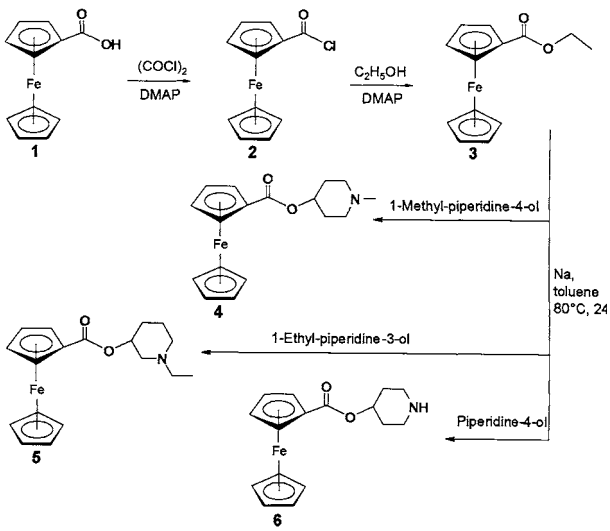

Fig. 1. Synthesis of cyclopentadienyl ligand precursors (**4**, **5** and **6**).

Experimental

Animal experiments

Animal procedures were undertaken in strict accordance with the recommendation of the EEC (86/909/CEE) and of the French National Committee (Decret 87/848) for the Care and Use of Laboratory Animals. For biodistribution experiments, Wistar male rats (200–250 g) were injected through jugular vein with 0.1–0.2 ml of the solution containing 10–15 μCi (0.37–0.55 MBq) of ^{99m}Tc complex, and then were sacrificed by decapitation 20 min after injection (n = 5 rats per time point).

Brain regions (cerebellum, colliculus, diencephalons, hippocampus, striatum, medulla and cortex) identified according to the Atlas by Paxinos and Watson [12] were excised, weighed, and their radioactivity was measured. Radioactivity in each sample was counted in a calibrated NaI(Tl) gamma-counter (CANBERRA, USA) and the results were corrected for the decay of ^{99m}Tc. Uptake was expressed as the percentage of injected dose per gram of tissue (% ID/g tissue).

Synthesis of ligand precursors (Fig. 1)

Synthesis of the derivatizing reagent (2)

The derivatizing agent, ferrocenecarboxylic acid chloride (**2**), was synthesized according to Rolfes and Andersson [13]. At room temperature, a solution of 1.43 ml (2.1 g, 16.6 mmol) of oxalyl chloride in 30 ml of toluene was added to a stirred suspension of 3.0 g (13.0 mmol) of ferrocenecarboxylic acid (**1**) and a catalytic amount of 4-(N,N-dimethylamino)pyridine (DMAP) in 35 ml of toluene. A Dimroth condenser with a bubbler was attached to the flask and the reaction mixture was stirred for 1 h at room temperature during which time there was a colour change from orange to dark red and bubbles of CO_2 were observed. To complete the reaction, the mixture was heated to 80°C, left at that temperature for 2 min and then allowed to cool down. The toluene was evaporated under vacuum and the residue was extracted with 2 × 15 ml and 1 × 8 ml of warm pentane (30°C) which was then removed by aspiration with a Pasteur pipette. The combined extracts were filtered through a glass frit, which was washed with 5 ml of pentane. The filtrate was left for 3 h at room temperature, then put into a refrigerator at 8°C for 1 h and finally left in the freezer at –18°C overnight. The **(2)** precipitated as dark red crystals. After removal of the pentane layer with a Pasteur pipette, the product was dried under vacuum and was used for the synthesis of ethyl ferrocenecarboxylic acid ester (**3**) without further purification. The yield of **(2)** was 2.15 g (67%).

2: MS (m/z (%), 70 eV): 248 (100) $[M]^+$.

Synthesis of ferrocene carboxylic acid ethyl ester (3)

The derivative **(3)** was prepared according to a modification of previously published procedures by Rolfes and Andersson [14]. 1 g (4 mmol) of **(2)** and 1.466 g (12 mmol) DMAP were dissolved in 40 ml of dichloromethane and added to a solution of 3.64 mmol ethanol in 40 ml dichloromethane. The mixture was left to react until the dark red coloration had faded. The DMAP and the excess of **(2)** were removed by filtration on a column (15 cm × 3 cm of aluminium oxide). **(3)** was eluted with 100 ml of dichloromethane. The solvent was evaporated under vacuum. The yield of **(3)** was 87%.

3: MS (m/z (%), 70 eV): 258 (100) $[M]^+$.

Synthesis of ferrocene carboxylic acid 1-methyl-4-hydroxypiperidinyl ester: (4), ferrocene carboxylic acid 1-ethyl-3-hydroxypiperidinyl ester: (5) and ferrocene carboxylic acid 4-hydroxypiperidinyl ester: (6)

A solution of piperidinol (1-methyl-4-hydroxypiperidine, or 1-ethyl-3-hydroxypiperidine, or 4-hydroxypiperidine) (16.5 mmol) in toluene (50 ml) was refluxed utilizing a Dean-Stark trap for 1 h under argon pressure. After cooling to room temperature, the toluene collected in the trap was discarded. Freshly cut sodium metal (0.5 g, 21.7 mmol) was added and the solution refluxed under argon pressure utilizing a Dean Stark trap for 30 min. The solution was cooled slightly and transferred warm under argon pressure to a solution of ferrocene

carboxylic acid ethyl ester **(3)** (994.6 mg, 4.6 mmol) in 50 ml of toluene. The resultant solution was refluxed under argon pressure utilizing a Dean Stark trap for 24 h. The solvent was then evaporated under vacuum. The product was purified by preparative TLC plate (20 × 20 cm, 0.2 mm coating thickness) in ether/diethylamine (95:5). The yellow band (R_f respectively 0.61, 0.82 and 0.95) was carefully scraped out by using a spatula.

A microcolumn (50 × 5 mm) was packed with a small amount of non-absorbing cotton and was used to extract the product from the silica gel using 10 ml of ethanol. The extract was dried under a gentle stream of nitrogen. The residues were crystallized in n-hexane. The yields were about 60%.

4: MS (m/z (%), 70 eV): 327 (100) $[M]^+$.
5: MS (m/z (%), 70 eV): 341 (100) $[M]^+$.
6: MS (m/z (%), 70 eV): 313 (100) $[M]^+$.

Radiochemical studies (Fig. 2)

*Synthesis of tricarbonyl{η⁵-[carboxy-4-hydroxy(N-methyl)piperidine]cyclopentadienyl} technetium(I) (**4a**), tricarbonyl{η⁵-[carboxy-3-hydroxy(N-ethyl) piperidine]cyclopentadienyl}technetium(I) (**5a**), and tricarbonyl{η⁵-[carboxy-4-hydroxypiperidine]cyclopentadienyl}technetium(I) (**6a**)*

A saline solution of $^{99m}TcO_4^-$ was reacted with the ligand precursors **(4)**, **(5)** and **(6)** in the presence of $Mn(CO)_5Br$ to give the respective complexes (**4a**, **5a** and **6a**). 1 mg of the ligand precursor and 1 mg of $Mn(CO)_5Br$ were dissolved in 150 µl of dimethyl formamide (DMF) in a 2 ml sealed vial and 150 µl (about 25 MBq) of saline $^{99m}TcO_4^-$ solution was added.

Two heating methods were used to carry out the radiolabelling:

- Method 1: The vial was heated for 1 h in a bath oil at 150°C.
- Method 2: The vial was placed in a microwave oven for 2 min at 650 watt.

Quality control was performed by TLC on silica gel in ether/diethylamine (95:5).

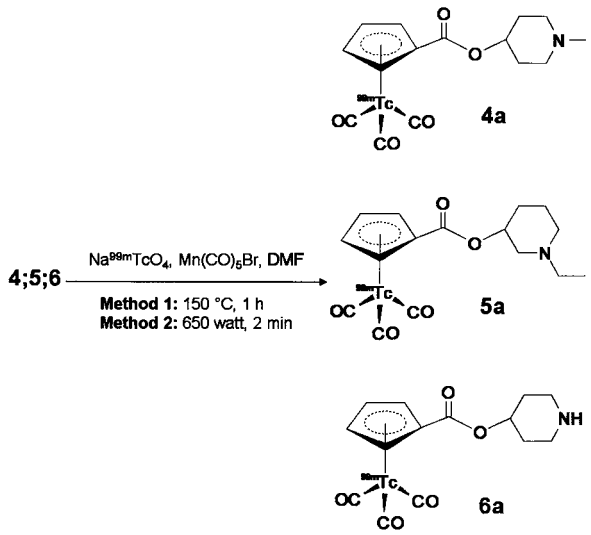

Fig. 2. Synthesis of radiolabelled complexes (**4a**, **5a** and **6a**).

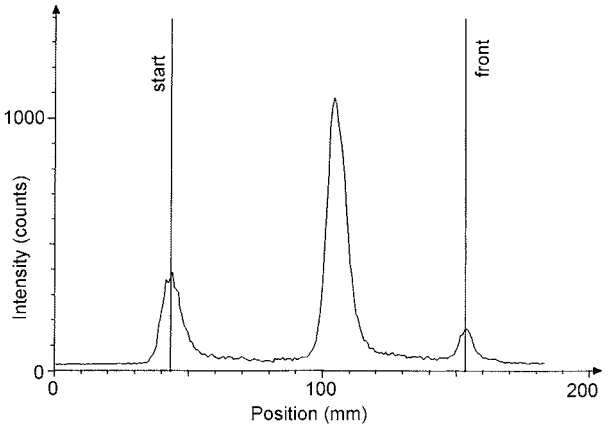

Fig. 3. Typical TLC radiochromatogram of radiolabelling reaction mixture of the complex **5a**.

Purification

The reaction mixtures were purified using TLC by spotting the entire reaction mixture carefully onto a preparative silica gel TLC plate (20 × 20 cm, 0.2 mm coating thickness) marked at 2 cm from one end. The air-dried plate was developed in a mixture of ether/triethylamine (95:5). A well-defined yellow band was observed, which was identified to be a mixture of the product and the starting ligand precursor. The yellow band was carefully scraped out by using a spatula. A microcolumn (50 × 5 mm) was packed with a small amount of non-absorbing cotton and was used to extract the product from the silica gel using 2 ml of ethanol. The extract was dried under a stream of N_2 and then dissolved in 1 ml of saline containing 20% ethanol.

The radiochemical purity of the tracer was estimated by thin-layer chromatography using plastic backed flexible silica gel plates (20 × 5 cm, coating thickness 0.25 mm). A 2 µl aliquot of the reaction mixture was spotted 2 cm from the lower end of the plate. The strips were developed using the ether/triethylamine (95:5) solvent system. After drying, the plates were developed by radiochromatography (miniGITA, Raytest) (Fig. 3).

Lipophilicity studies (P_{hep})

Partition coefficients P_{hep}, of the target complexes in the system n-heptane – 0.9% saline (1:1, v/v.) were determined at room temperature. The saline solution and n-heptane were saturated with each other.

The solutions were pipetted into glass vials, solutions of the complexes were added with a microlitre syringe. The phases were shaken together on a mechanical shaker for 30 min. Radioactivities of the same volumes of the two phases, N_{hep} and N_{sal} (cpm), were measured using a calibrated NaI(Tl) gamma-counter (CANBERRA, USA). The partition coefficient (P_{hep}) values were calculated using the following equation

$$P_{hep} = \frac{N_{hep}}{N_{sal}}$$

Results

Preparation of ^{99m}Tc radiolabelled compounds

The three complexes **4a, 5a** and **6a** were obtained. Using the method of Wenzel [18] by heating at 150°C for 1 h in DMF. The radiochemical yields were 93, 70 and 90%, respectively. The use of microwave activation at 650 watt for only 2 min resulted in the average yield of the radiolabelling of 90%. As shown in the radiochromatogram (Fig. 3), the first band corresponds to the unreacted $^{99m}TcO_4^-$ the second band was identified to be the labelled product and the last one has not been identified.

Lipophilicity studies

For the complexes, the lipophilicity (logarithm partition coefficient, log$P_{hep,}$ in the two-phase system n-heptane/0.9% NaCl solution) was considered a useful parameter in biodistribution. The widespread application of lipophilicity to drug design explains the need for quick procedures to quantify molecular lipophilicity, particularly at the screening level [7, 17]. The results obtained in the experiments are summarized in Table 1.

A general trend was observed in which increased lipophilicity of the radioactive complexes was associated with increased brain uptake. Complex **6a** showed the highest lipophilicity (1.31) and the lowest was observed for the complex **4a**.

*Biodistribution of complex **5a** in rat brain*

Figure 4 shows the brain tissue uptake of radioactivity. Preferential brain accumulation was observed in the colliculus (1.97% ID/g tissue at 20 min p.i.). Cortical accumulation (1.33% ID/g tissue at 20 min p.i.) was much lower than the colliculus accumulation. Lower radioactive concentrations were measured in medulla, hippocampus, diencephalons, striatum and cerebellum (about 1% ID/g tissue at 20 min p.i.).

Table 1. Lipophilicity and % ID of brain uptake 20 min p.i. of the complexes **4a**, **5a** and **6a**. Data are presented as the mean value ± SD, $n = 5$

	log P_{hep}	Brain uptake, % ID
Complex **4a**	0.86 ± 0.09	1.3 ± 0.1
Complex **5a**	0.92 ± 0.10	1.6 ± 0.1
Complex **6a**	1.31 ± 0.07	2.0 ± 0.2

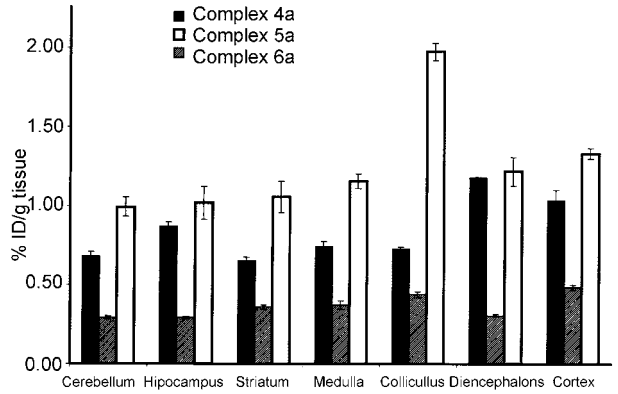

Fig. 4. Comparison of brain tissue radioactive concentration expressed in % ID/g tissue, 20 min after injection of complexes **4a**, **5a** and **6a**. Data are presented as the mean value ± SD in different cerebral regions, $n = 5$ animals per group.

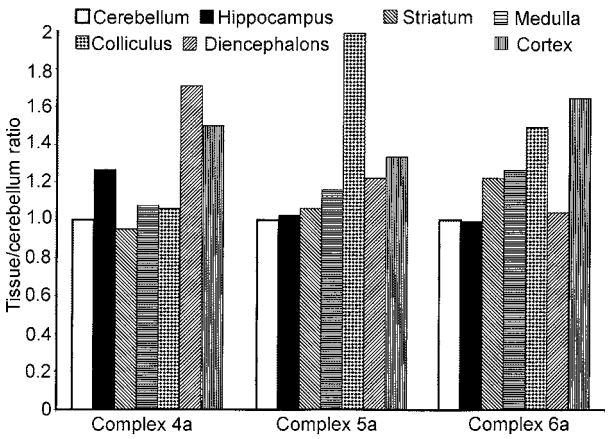

Fig. 5. Comparison of the ratio of tissue/cerebellum uptake of the complexes **4a, 5a** and **6a**, 20 min p.i.

As shown in Fig. 5, the colliculus/cerebellum ratio for **5a** was 1.99 at 20 min p.i. At the same time the cortex/cerebellum ratio was 1.34.

*Biodistribution of complex **6a** in rat brain*

As observed in Fig. 4, the brain uptake of complex **6a** was very low (less than 0.5% ID/g tissue at 20 min p.i.) with no selective uptake observed.

Discussion

The purpose of this study was to improve the radiolabelling reaction for eventual clinical use, and to develop a tricarbonyl technetium-99m based compound with high specificity and fast kinetics for the exploration of brain receptors by SPECT.

As tricarbonyl technetium-99m based compounds are promising radiopharmaceuticals for imaging receptors in the brain, we have developed more compounds in the current study as potential brain receptor binding agents.

The Wenzel method, which suffers from inadequate conditions, mainly the high temperature of 150°C and long reaction time of 1 h, was substituted by a more efficient one. In this study we replaced the oil bath heating by using a microwave oven, consequently the time of the radiolabelling reaction became extremely short (2 min), advantageous for the use of these complexes as imaging agents for clinical purposes.

In order to increase the brain uptake of these tricarbonyl complexes, non-substituted piperidinol was used for the synthesis of complex **6a**. However, the *in vivo* studies in rat showed a lower uptake of complex **6a** as compared to previously published data for the reference compound (complex **4a**) [16]. On the other hand, the complex **5a** has shown the highest uptake (Fig. 4). The highest activity was observed in the cortical region (0.4% ID/g tissue at 20 min). The cortex/

cerebellum ratio was 1.6, approximately the same as the colliculus/cerebellum ratio (1.5) (Fig. 5). This lack of specificity precludes, however, further development of this compound as an imaging agent.

Conclusion

Labelling of cyclopentadienyl piperidine derivatives by the ^{99m}Tc-tricarbonyl moiety could be achieved with a high yield by using the microwave activation method. The complex with ethyl-substituted cyclopentadienyl piperidine can cross easily the blood brain barrier, more easily than that with the non-substituted piperidine, and have more specificity to colliculus tissue. However, more experiments must be achieved to determine precisely the target binding receptors of this complex.

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