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Organic synthesis and biological evaluation of novel "3 + 1" mixed ligands of technetium-99m Gabapentin as receptor imaging agents

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Abstract This work focuses on the "3 + 1" mixed ligands of ^{99m}Tc labeled Gabapentin as $\alpha 2\delta$ receptor imaging agents in the brain. Gabapentin 1-(aminomethyl)cyclohexanacetic acid as monodentate and two tridentates: tridentate **A**; 3-(2-imino-thiozolidin-4-one)-quinozoline-4-(3H)-one and tridentate **B**; *N*-(4-chlorophenyl)-2-imino-2H-chromene-3-Carbothioamide which were synthesized and characterized by infrared analysis (IR), ^{1}H nuclear magnetic resonance (NMR), and mass spectrum. ^{99m}Tc-complexes were prepared by the "3 + 1" mixed ligand approach. The labeling conditions were optimized and the complexes was extracted by chloroform and purified by high performance liquid chromatography. ^{99m}Tc-complexs were lipophilic and stable for at least 8–12 h at room temperature. The biodistribution of the ^{99m}Tc-complexes was evaluated in mice. The brain uptake was 4.5% and 3.5% ID/g (percentage of the injected dose per gram) at 5 min, and the retention was 1.5% and 1.7% ID/g at 120 min for ^{99m}Tc-complex **A** and ^{99m}Tc-complex **B**, respectively.

Keywords "3 + 1" mixed ligands · Technetium-99m · Gabapentin · Imaging agents

Introduction

The transport and accumulation into the target organ is an important issue for ^{99m}Tc radiopharmaceuticals development. The development of radiopharmaceuticals designed to bind specific receptors, including membrane transport systems, is receiving much interest due to their potential to achieve improved in vivo monitoring of biochemical and physiological functions [1]. Technetium-99m (^{99m}Tc) is the radionuclide of choice for diagnostic imaging with single photon emission computed tomography (SPECT) due to its ideal nuclear properties (Eγ = 140 keV, $T_{1/2}$ = 6 h, no β-emission) and availability from a ^{99}Mo/^{99m}Tc generator [1]. These properties have led to the search of novel ^{99m}Tc-based radiopharmaceuticals incorporating ligands specifically designed to probe protein receptors and transporters [2–4]. A new 3 + 1 mixed-ligand approach has been proposed as a new strategy for developing novel neutral ^{99m}Tc-radiopharmaceuticals containing the Tc = O core [5, 6]. This approach, which consists of a tridentate ligand and a monodentate co-ligand surrounding the Tc = O core, has evolved successfully to generate novel lipophilic potential radiopharmaceuticals with high brain uptake and retention [7–9]. This strategy also offers an easy access to ^{99m}Tc-based probes with affinity and selectivity to protein receptors, in which the receptor ligand is appended either into the tridentate or the monodentate ligand [10–12].

One of the reported receptors ligands is WAY 100635, a potent antagonist of pre- and post-synaptic 5-HT$_{1A}$ receptors, with residue 1-(2-methoxyphenyl) piperazine [13]. Fragment of WAY 100635 was combined with different technetium tetradentate N2S2 chelates, amine-amide dithiols or diamine dithiols [14–17]. The major disadvantage of these compounds is their poor brain uptake in experimental animals which precludes their usefulness as brain receptor imaging agents. This has been attributed mainly to their high molecular size. However, "3 + 1" oxotechnetium mixed ligands complexes of the general type ^{99m}TcO[SN(R)S][S] have been synthesized as 5-HT$_{1A}$

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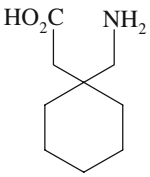
Fig. 1 Chemical structure of Gabapentin



radioligands by introducing the receptor-binding 1-(2-methoxyphenyl)piperazine moiety on the monodentate ligands [18, 19]. These complexes showed in vitro affinity for 5-HT_{1A} in nanomolar range and moderate brain uptake in mice and rats.

Also, Gabapentin (Neurontin®) 1(aminomethyl) cyclohexanacetic acid is anticonvulsant analgesic drug synthesized nearly 40 years ago [20]. The original concept was to increase the lipophilicity of the inhibitory neurotransmitter GABA (γ-aminobutyric acid) by addition of a cyclohexyl substituent, thereby increasing its CNS penetrating properties yet retaining a similar pharmacology. Recently, the [^{3}H] Gabapentin binding protein was subsequently purified from pig brain and shown to be the $\alpha 2\delta$ subunit receptor of the voltage dependent calcium channel complex [21]. So the success in synthesizing "3 + 1" oxotechnetium mixed ligands complexes of Gabapentin (Fig. 1) will provide potential radiopharmaceuticals.

The present work is concerning the technetium-99m labeling of Gabapentin by the "3 + 1" mixed-ligand approach in which it acts as monodentate (L_1) and using each of quinazoline derivative and thioamide derivative as tridentate (L_2). Quinazolines are classes of fused heterocycles that are considerable interest because of their safe biological properties [22]. Also, thioamide derivatives are used for therapeutic purposes such as treatment of tuberculosis, leprosy, and thyrotoxicosis [23, 24]. Theoretically possible complexes produced from this labeling reaction are neutral mixed ligand complex (^{99m}Tc(O)L_1L_2), binuclear complex of the tridentate ligand [(^{99m}Tc(O))$_2$(L_2)$_3$)] and anionic complex of the monodentate ligand [(^{99m}Tc(O)(L_1)$_4$]. Of all these complexes, the neutral mixed ligand complex is the most produced complex specially when the molar ratio of mono- to tridentate is 1:1 [25]. The presumable structure of ^{99m}Tc-complex **A** and ^{99m}Tc-complex **B** are shown in Figs. 2 and 3, respectively.

Experimental

Materials and methods

Gabapentin, 1(aminomethyl)cyclohexanacetic acid, was obtained as a gift from AMOUN PHARMACEUTICAL CO. Cairo, Egypt. ($C_9H_{17}NO_2$), MW = 171.24.

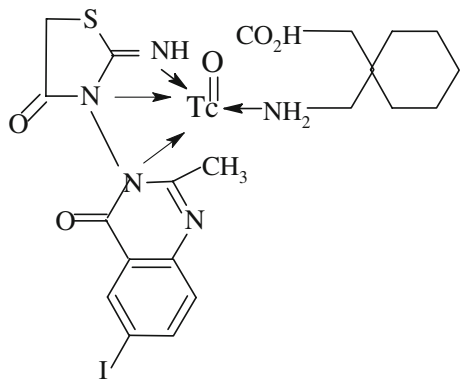


Fig. 2 The presumable structure of ^{99m}Tc-complex **A**

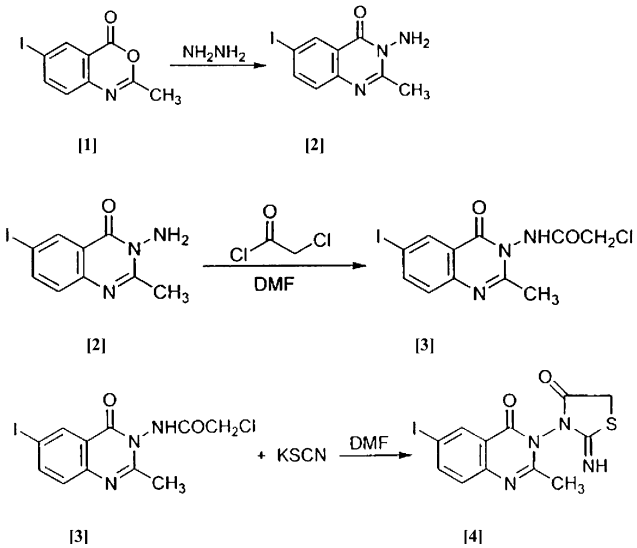
Pertechnetate-99m solution was obtained by elution from the sterile ^{99}Mo/^{99m}Tc generator (Elutic, Brussels, Belgium).

White Albino mice were used for biodistribution studies.

Synthesis and structure confirmation of tridentate ligands

Synthesis of 2-imino-thiazolidin-4-one derivative of 4(3H) quinazoline (A)

Addition of benzoxazine derivative [**1**] to hydrazine hydrate gives 3-amino-quinazoline-4(3H)-one derivative [**2**] which is converted to 3-(*N*-acylamine)-quinazoline-4(3H)-one derivative [**3**] by adding chloroacetyl chloride then potassium thiocyanate is added to give the tridendate 3-(2-imino-thiozolidin-4-one)-quinozoline-4-(3H)-one derivative [**4**]



– One mole of benzoxazine derivative was added to one mole of hydrazine hydrate in methanol under reflux for 1 h then filtration and recrystallization using ethanol was performed to give product [**2**].

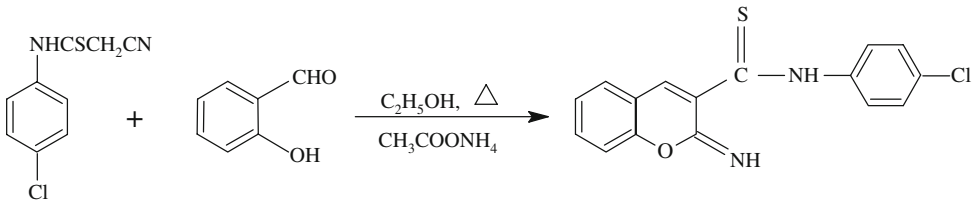
– One mole of product [**2**] was stirred with one mole of chloroacetyl chloride in DMF for 3 h after that the mixture was poured on crushed ice and product [**3**] was obtained by filtration and recrystallization in ethanol.
– One mole of product [**3**] was refluxed in DMF with potassium thiocyanate for 3 h and poured on crushed ice in presence of hydrochloric acid. The tridendate ligand [**4**] was obtained by filtration and recrystallization in acetic acid with melting point 140 °C.

Chemical analyses

– IR spectrum of the tridentate ligand showed absorption bands at 3448 cm^{-1} (NH), 3093 cm^{-1} (CH-arom.), 1743, 1671 cm^{-1} (C=O), 1609 cm^{-1} (C=N),
– ^{1}HNMR spectrum of the tridentate ligand in (DMSO-d6) revealed signals at
– $\delta = 2.36$ (s, 3H, CH_3), 4.05 (s, 2H, CH_2, thiazole), 7.41 (d, 1H, CH-C), 8.07 (d, 1H, CH-b),
– 8.36 (s, 1H, CH-a), 12.61 (br, 1H, NH),
– The mass spectrum of the tridentate ligand showed a molecular ion peak at m/z = 400 (40.7%) and base peak at m/z = 353 (100%). Other significant peaks were observed at m/z: 327 (12.6%), 326 (6.5%), 116 (12.6%) and 75 (43.0%).

Synthesis of *N*-(4-chlorophenyl)-2-imino-2H-chromene-3-carbothioamide (**B**)

The process of synthesis depends on the reaction of *N*-(4-chlorophenyl) cyanothioacetamide with salicylaldehyde in presence of ammonium acetate giving the required *N*-(4-chlorophenyl)-2-imino-2H-chromene-3-carbothioamide.



– One mole of *N*-(4-chlorophenyl) cyanothioacetamide was refluxed for 3 h with one mole of salicylaldehyde in presence of ammonium acetate then recrystallization using ethanol was performed to give the final product with melting point 180 °C.

Chemical analyses

– IR spectrum of thioamide tridentate ligand showed absorption bands at 3306 cm^{-1} (NH), 3000 cm^{-1} (CH-aromatic), 1586 cm^{-1} (C=N).

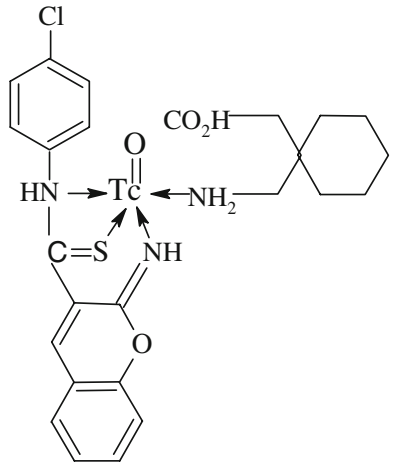


Fig. 3 The presumable structure of ^{99m}Tc-complex **B**

– ^{1}HNMR spectrum of thioamide tridentate ligand in (DMSO-d6) revealed signals at $\delta = 7.22$–8.01 (m, 8H, aromatic H), 8.56 (s, 1H, cromene), 9.8–9.6 (2 s, 2H, 2NH).
– The mass spectrum of thioamide tridentate ligand showed a molecular ion peak at m/z = 314 (52.5%) and base peak at m/z = 313 (M − 1). Also significant peaks were observed in the spectrum at m/z = 281 (49.1%), 246 (15%), 171 (47.0%) and 118 (43.0%).

Synthesis of ^{99m}Tc complexes at tracer level

First, 125 mg of glucoheptonate was dissolved in 50 mL water, then 1 N HCl solution of 50 mg $SnCl_2 \cdot 2H_2O$ was added to the solution and the pH was adjusted to 7.5. After filtration through a 0.22 μm millipore filter, the solution was divided into 50 vials and lyophilized.

The complex was prepared by ligand exchange reaction using ^{99m}Tc(V)O-glucoheptonate as precursor and equimolar quantities of the two ligands. A glucoheptonate kit was reconstituted with ^{99m}Tc-pertechnetate solution (20–50 mCi). To an equal molar (1×10^{-6}–1×10^{-8} mol) mixture of monodentate ligand and tridentate ligand in ethanol solution, 20–50 mCi (740–1850 MBq) of the ^{99m}Tc-glucoheptonate precursor (radiochemical purity >95%) was added. The mixture was agitated in a vortex mixer and left to react in a water bath at 70 °C for 30 min [26]. The reaction time, pH, and temperature of the

reaction system were optimized to achieve a high labeling yield.

The ^{99m}Tc complex was extracted three times with 2 mL of organic solvent (chloroform) and the organic phase was separated. The percent radiochemical yield of the ^{99m}Tc-complex was estimated by determination of the activity in the organic phase related to free ^{99m}Tc-pertechnetate in the aqueous phase.

Radiochemical purity

The radiochemical purity of the organic extract was determined by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC):

TLC analysis

The radiochemical purity of the ^{99m}Tc complexes were determined by thin layer chromatography-silica gel (TLC-SG). (TLC-SG) sheets were marked 2 cm from the base and lined into fragments 1 cm each up to 14 cm using non-pointed pencil. A spot (5 μL) from the organic phase was applied using micropipette, and then the sheet was developed in an ascending manner in a closed jar contains the developing solvent of $CHCl_3$:CH_3OH = 9:1 (v/v) [13]. The sheets after complete development, were removed, dried, and cut into strips, each strip is 1 cm width, then the strip was counted in a well type γ-counter, free $^{99m}TcO^-_4$ moves with the solvent front (R_f = 0.8–1) while ^{99m}Tc-complexes remain at the starting line (R_f = 0–0.1).

HPLC analysis

To perform the HPLC analysis of the labeled compound, monodentate and tridentate cold solutions were injected into the column (RP18—250 × 4 mm, 5 μm, Lischrosorb) build in HPLC Shimadzu model consisting of pumps LC-9A with a Rheohydron injector and UV spectrophotometer detector (SPD-6A) adjusted to the wave length 254 nm. The column was eluted with the isocratic solvent methanol:water ratio (70:30) and the flow rate was adjusted to 1 mL/min. Then 10 μL of the organic phase, containing ^{99m}Tc-complexes, were injected into the column of HPLC and the fractions of 1 mL were collected and counted using NaI(TI) well crystal coupled to SR-7 scaler ratemeter.

Determination of the partition coefficient for ^{99m}Tc complexes

The partition coefficient was determined by mixing the ^{99m} Tc-complexes with equal volumes of 1-octanol and phosphate buffer (0.025 M at pH 7.4) in a centrifuge tube. The mixture was vortexed at room temperature for 1 min and then centrifuged at 5,000 rev./min for 5 min. Subsequently 100 μL samples from the 1-octanol and aqueous layers were pipetted into other test tubes and counted in a gamma counter. The measurement was repeated three times. The partition coefficient value was expressed as log p [13], which was found to be equal to 1.7 ± 0.2 and 1.5 ± 0.1 for ^{99m}Tc complexes **A** and **B**, respectively, showing that the two complexes are good lipophilics and possibly cross the intact blood-brain barrier (BBB).

Biodistribution study of ^{99m}Tc complexes

Twelve normal female Albino mice (weighing 20–25 g) were divided into four groups. Each mouse was injected with ^{99m}Tc-complex (3–5 MBq/100 μL saline solution containing the ^{99m}Tc complex) in the lateral tail vein. Mice were sacrificed at 5, 30, 60, and 120 min post-injection. Blood samples were collected at the time of decapitation. Organs were dissected, weighed, and their radioactivity was measured using a well-type NaI scintillation detector. Samples of fresh blood, bone and muscle were collected in pre-weighed vials and counted. Blood, bone and muscles were assumed to be 7%, 10% and 40%, respectively [27], of the total body weight. Whole brains were removed and frozen for an hour. The organs were weighed and the radioactivity was assessed radiometrically. The results were expressed as % dose/organ and % dose/g. The brain/blood ratio was calculated from the corresponding percentage of the injected dose per gram (% ID/g) values.

Results and discussion

Effect of reaction time

The effect of the reaction time on the radiochemical yield of ^{99m}Tc-complexes were studied as indicated in Fig. 4. The reaction mixture was incubated in a water bath at 70 °C for different time periods, ranging from 5 to 60 min. It is clear from Fig. 4 that at 5 min reaction time the radiochemical yield was low (45.3% for ^{99m}Tc complex **A** and 32.5% for ^{99m}Tc complex **B**) because the time is not sufficient for the transfer of reduced technetium from ^{99m}Tc(V)O-glucoheptonate complex to the monodentate and tridentate ligands, while by increasing the reaction time up to 30 min the radiochemical yield increased to 80% for ^{99m}Tc complex **A** and 75% for ^{99m}Tc complex **B** which are the maximum yields for the two complexes. With further increase in reaction time up to 45 min, no increase in the radiochemical yields were observed.

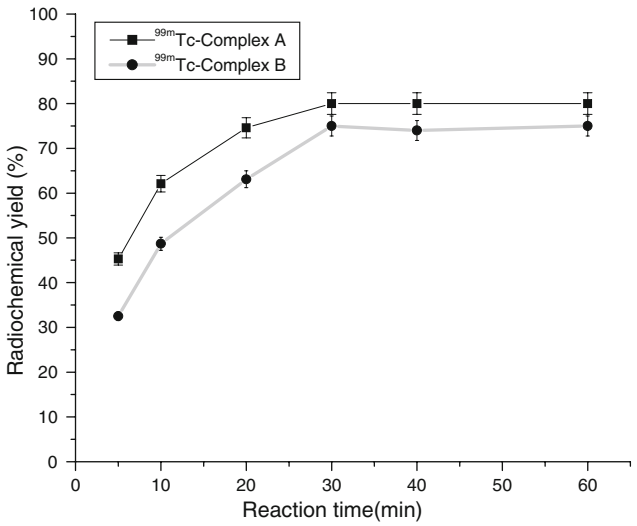


Fig. 4 Radiochemical yield of ^{99m}Tc complexes as a function of the reaction time. Reaction conditions: equimolar mixture of monodentate ligand and tridentate ligand, the ^{99m}Tc-glucoheptonate precursor, at pH 7, the reaction mixture was kept at 70 °C for X min.

Effect of pH of the reaction system

The pH of the reaction medium is a very critical factor; the optimum pH was found to be 7 which gives a yield of 80% for complex **A** and 75% for complex **B**. The data presented in Fig. 5 reflects the results obtained from the preparation of the ^{99m}Tc-complexes at different pH values using buffer systems with a range from pH 2 to pH 11. The results confirmed the influence of pH of the reaction mixture on the radiochemical yield of the ^{99m}Tc-complexes. The percentage of ^{99m}Tc-complexes increased gradually with the increase in pH up to 7 reaching a maximum yield. On increasing the pH of the reaction medium above pH 7, the yield of ^{99m}Tc-complex **A** decreased to 59.1% and 48.2% at pH 9 and 11, respectively. The same behavior was observed for ^{99m}Tc-complex **B** at alkaline medium. This may be attributed to the fact that the reduction at high pH usually does not release all oxygen atoms in the pertechnetate molecule, leading to complexes with a TcO^{3+} core [28].

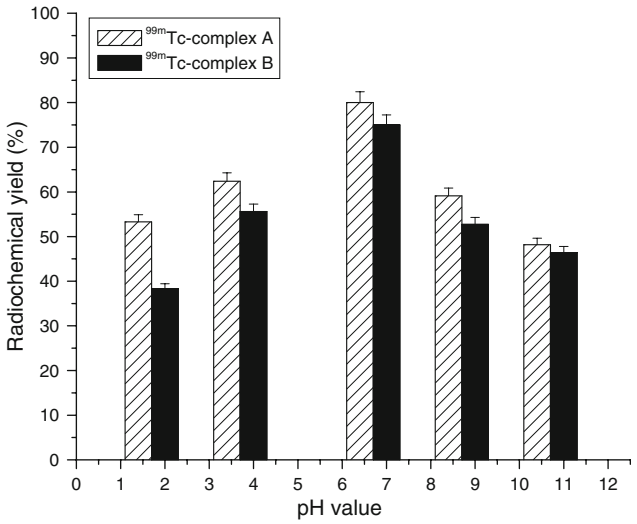


Fig. 5 Radiochemical yield of ^{99m}Tc complexes as a function of pH value. Reaction conditions: equimolar mixture of monodentate ligand and tridentate ligand, the ^{99m}Tc-glucoheptonate precursor, at pH X, the reaction mixture was kept at 70 °C for 30 min.

Effect of temperature of the reaction system

The reaction temperature plays an important role in the labeling process. The reaction mixture was heated at different temperatures ranging from 25 °C to 100 °C for 30 min. The results were presented in Fig. 6. As observed from these data, there is a significant effect of the reaction temperature on the percent labeling yield. When the reaction was performed at room temperature (25 °C) the radiochemical yield was low, but by increasing the reaction temperature to 50 °C the radiochemical yield increased. Maximum radiochemical yields of ^{99m}Tc-complexes were obtained at 70 °C reaction temperature. Also, it is clear from this result that the radiochemical yield decreased by increasing the reaction temperature reaching (57.1% for complex **A** and 52.1% for complex **B**) at 100 °C. This decrease in the radiochemical yield at high temperatures may be due to the decomposition of the ^{99m}Tc-complexes.

HPLC analysis of the ^{99m}Tc-complexes

The retention times of the non labeling monodentate ligand, tridentate ligand **A** and tridentate ligand **B** were 4, 6 and 7 min, respectively. The radiochromatogram for each complex shows two peaks one at fraction No. 2 which

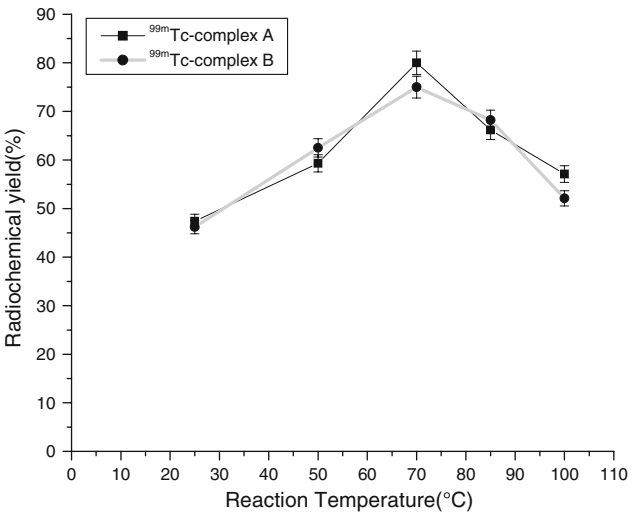


Fig. 6 Radiochemical yield of ^{99m}Tc complexes as a function of reaction temperatures. Reaction conditions: equimolar mixture of monodentate ligand and tridentate ligand, the ^{99m}Tc-glucoheptonate precursor, at pH 7, the reaction mixture was kept at X °C for 30 min.

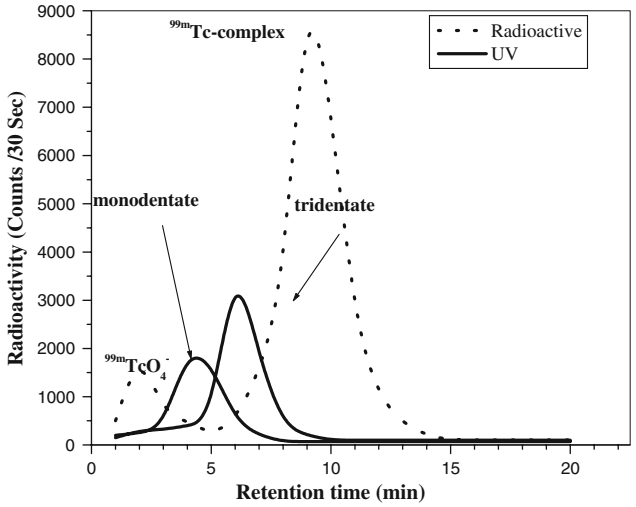


Fig. 7 HPLC radiochromatogram of ^{99m}Tc complex **A** conditions: Solvent, methanol:water 70:30, column: RP18, the flow rate: 1 mL/min. and U.V. wave length: 254 nm.

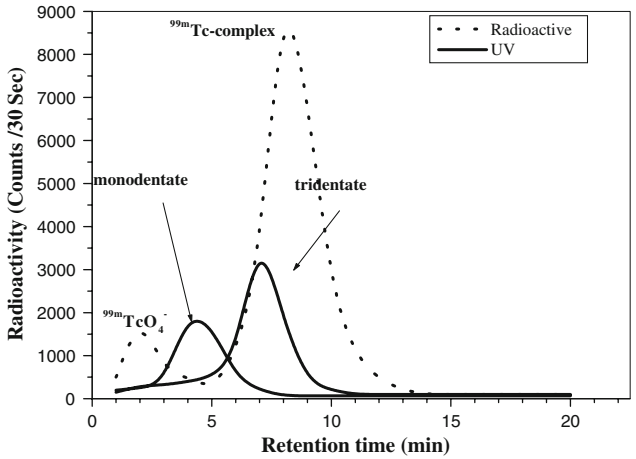


Fig. 8 HPLC radiochromatogram of ^{99m}Tc complex **B** conditions: Solvent, methanol:water 70:30, column: RP18,the flow rate: 1 mL /min. and U.V. wave length: 254 nm.

corresponds to the free pertechnetate, while the second peak appears at fraction No. 9 which corresponds to $^{99\ m}$Tc-complex **A** and fraction No. 8 which corresponds to $^{99\ m}$Tc-complex **B** which is in coincidence with the UV signal. The tracer was separated by HPLC as shown in Figs. 7 and 8. The fractions were collected and sterilized by Millipore filter (0.22 μm) under aseptic conditions.

Stability

The in vitro stability of ^{99m}Tc-complexes were studied and the experiment was carried out for different time periods ranging from 1 to 12 h at room temperature. From the results of this experiment, it can be deduced that the both complexes were stable (radiochemical purity >90%) after being purified by HPLC. ^{99m}Tc-complexes display excellent in vitro stability with no decomposition observed during these time periods at room temperature.

Biodistribution

Two in vivo evaluations of the ^{99m}Tc complexes were performed during this study, the first one was the investigation of blood-brain barrier penetration, and the second was the biodistribution in different organs in normal healthy mice. The studies were carried out on normal mice weighing (20–25 g). The in vivo behavior of the ^{99m}Tc complexes were evaluated in mice at 5, 30, 60, and 120 min post-intravenous injection. Tables 1 and 2 show the results expressed as % ID/total organ in the most relevant organs for complex **A** and complex **B**, respectively. The ^{99m}Tc complexes, after an intravenous injection, were able to cross the blood brain barrier, resulting in a significant initial brain uptake (1.8% for complex **A** and 1.4% for complex **B** at 5 min post-injection) and a good retention (0.6% for complex **A** and 0.7% for complex **B** at 120 min) observed in mice. In addition, the ratio of the brain to the blood, expressed as percentage uptake/g tissues, has increased gradually as time passes, which may be due to the rapid clearance of the tracer from the blood. The ratio (brain/blood) was 0.52 after 120 min post-injection for complex **B**. The washout of activity from the brain was faster during the first 30 min for the complex **A**, compared to the washout from 30 to 60 min. The complexes have high initial blood, muscle and liver uptake as expected for lipophilic compounds. Nevertheless, the blood and the muscle clearance is quite fast. Excretion occurs mainly through the hepatobiliary tract compared with urinary elimination.

Conclusion

In this work our attempt to synthesize ^{99m}Tc-complexes as potential agents for imaging brain receptors has been focused on three basic tasks: synthesis, characterization and evaluation.

The 3 + 1 concept for the preparation of neutral, lipophilic, and small size oxotechnetium complex has been applied in the development of novel diagnostic or therapeutic radiopharmaceuticals. In an effort to develop ^{99m}Tc-based radioligands for brain receptors, the "3 + 1" concept was applied and oxotechnetium complexes of the general formula TcO[NN(R)N][N] and TcO[NS(R)N][N] were successfully synthesized and characterized. In general, the preparation of the 3 + 1 complex requires the simultaneous action of a tridentate ligand, containing the NNN or NSN donor atom sets and a monodentate co-ligand, on a suitable oxotechnetium (V). The complex was prepared by ligand

Table 1 Biodistribution pattern of $^{99\ m}$Tc-complex **A** in normal mice at different times

Organs and body fluids	% ID/organs and body fluid at different times post-injection			
	5	30	60	120
Blood	25.3 ± 1.4	18.6 ± 1.5	12.1 ± 0.9	8.2 ± 0.6
Bone	3.8 ± 0.9	3.2 ± 0.8	2.2 ± 0.7	1.7 ± 0.2
Muscle	4.3 ± 0.5	4.0 ± 0.7	3.1 ± 0.6	2.5 ± 0.5
Brain	1.8 ± 0.1	1.1 ± 0.2	0.8 ± 0.1	0.6 ± 0.1
Lungs	2.1 ± 0.1	1.3 ± 0.2	0.6 ± 0.1	0.3 ± 0.1
Heart	1.6 ± 0.2	1.3 ± 0.1	0.7 ± 0.1	0.4 ± 0.1
Liver	14.2 ± 1.7	14.8 ± 1.4	12.4 ± 1.5	10.4 ± 1.1
Kidneys	3.7 ± 0.8	3.2 ± 1.2	5.0 ± 0.9	3.8 ± 0.7
Spleen	1.1 ± 0.2	1.8 ± 0.4	0.9 ± 0.2	0.7 ± 0.1
Intestine	4.7 ± 0.6	9.3 ± 1.3	18.3 ± 2.4	23.4 ± 1.6
Urine	2.1 ± 0.9	3.8 ± 1.1	6.2 ± 0.8	8.7 ± 1.2
Brain[a]	4.5 ± 0.2	2.7 ± 0.3	2.0 ± 0.1	1.5 ± 0.1
Blood[a]	14.5 ± 0.6	10.6 ± 0.4	6.9 ± 0.3	4.7 ± 0.2
B/Bl, g	0.3	0.25	0.29	0.32

[a] Injected dose/g tissue, *B* brain *Bl* blood

Table 2 Biodistribution pattern of ^{99m} Tc-complex B in normal mice at different times

Organs and body fluids	% ID/organs and body fluid at different times post-injection			
	5	30	60	120
Blood	28.5 ± 1.6	20.8 ± 1.5	12.0 ± 0.8	5.8 ± 0.2
Bone	3.4 ± 0.2	2.3 ± 0.2	1.8 ± 0.1	1.4 ± 0.1
Muscle	4.8 ± 0.3	3.2 ± 0.4	2.1 ± 0.2	1.5 ± 0.1
Brain	1.4 ± 0.2	1.2 ± 0.1	0.9 ± 0.2	0.7 ± 0.1
Lungs	0.5 ± 0.1	1.4 ± 0.2	0.6 ± 0.2	0.4 ± 0.1
Heart	1.4 ± 0.1	1.0 ± 0.1	0.4 ± 0.1	0.2 ± 0.1
Liver	9.4 ± 1.2	13.3 ± 1.3	11.3 ± 1.3	9.6 ± 1.2
Kidneys	3.2 ± 0.2	4.8 ± 0.4	7.0 ± 1.1	5.8 ± 0.9
Spleen	0.2 ± 0.1	0.9 ± 0.1	0.3 ± 0.1	0.1 ± 0.1
Intestine	5.7 ± 0.4	10.3 ± 1.4	17.0 ± 0.9	4.5 ± 2.3 2
Urine	4.2 ± 0.2	6.1 ± 0.8	9.5 ± 1.2	13.5 ± 1.4
Brain[a]	3.5 ± 0.5	3.0 ± 0.2	2.2 ± 0.4	1.7 ± 0.2
Blood[a]	16.3 ± 0.9	11.9 ± 0.6	6.9 ± 0.3	3.3 ± 0.1
B/Bl, g	0.2	0.25	0.32	0.52

[a] Injected dose/g tissue, *B* brain *Bl* blood

exchange reaction using ^{99m}Tc(V)O-glucoheptonate as precursor and equimolar quantities of the two ligands. The mixture was left to react in a water bath at 70 °C for 30 min at pH7. This reaction system achieved high labeling yields (75% and 80%). The ^{99m}Tc-complexes were able to cross the blood-brain barrier and displayed good initial brain uptake in healthy mice (4.5% ID/g and 3.5% ID/g for complex **A** and complex **B** at 5 min post-injection) indicating their suitability for brain receptor imaging.

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