SCIENCE CHINA
Chemistry

• ARTICLES •

July 2011 Vol.54 No.7: 1148–1154
doi: 10.1007/s11426-011-4271-5

Synthesis of several MPP derivatives for ^{99m}Tc-labelling and evaluated as potential 5-HT$_{1A}$ receptor imaging agents

YANG WenJiang, LIN Yan, ZHANG XianZhong*, ZHANG JunBo & WANG XueBin

Key Laboratory of Radiopharmaceuticals (Beijing Normal University), Ministry of Education; College of Chemistry, Beijing Normal University, Beijing 100875, China

Received December 17, 2010; accepted March 4, 2011

The (2-methoxyphenyl) piperazine (MPP) was selected as the functional group and conjugated to dithiocarbamate through different spacers. A series of new MPP derivatives (MPPnDTC, n = 2–6) were synthesized and radiolabelled with ^{99m}Tc-nitrido core or ^{99m}Tc-tricarboxyl core as potential 5-HT$_{1A}$ receptor imaging agents. All the six ^{99m}Tc-labelled complexes were lipophilic and neutral. Biodistribution results showed that those radiotracers had moderate initial brain and hippocampus uptake. There have no significant relation was observed between the biological properties of these tracers with the length of its carbon chain. The radioactivity concentrations of hippocampus of ^{99m}TcN-MPP2DTC, ^{99m}TcN-MPP3DTC, ^{99m}TcN-MPP4DTC, ^{99m}TcN- MPP5DTC, ^{99m}TcN-MPP6DTC and ^{99m}Tc(CO)$_3$-MPP3DTC at 2 min post-injection time (p.i.) were 0.43, 1.15, 0.99, 1.04, 1.13 and 0.83 %ID/g, respectively.

MPP, ^{99m}Tc-nitrido core, ^{99m}Tc-tricarboxyl core, 5-HT$_{1A}$ receptor

1 Introduction

5-HT$_{1A}$ receptor is a member of the serotonin (5-HT) superfamily of receptors. It has been studied extensively due to its role in a number of neuropsychiatric disorders such as schizophrenia, Alzheimer's disease, depression, hallucinogenic behavior, motion sickness, eating disorders and anxiety [1]. Over the past decade, a lot of effort has been put into the development of a radiotracer to image the 5-HT$_{1A}$ receptors *in vivo* by using positron emission tomography (PET) and single photon emission computed tomography (SPECT). Despite the ^{11}C- and ^{18}F- and ^{123}I-labelled ligands for imaging have played a vital role in studying the location and density of 5-HT$_{1A}$ receptor, technetium-99m-labelled ligands would be more desirable for the excellent properties of technetium-99m and its ready availability.

The (2-methoxyphenyl)piperazine (MPP) moiety, residue of WAY 100635, which is known to have high affinity to the 5-HT$_{1A}$ receptor, was selected as the prototype structure for the design of potential radiotracers [2, 3]. Many ^{99m}Tc labeled complexes carrying MPP moiety with high affinity for the 5-HT$_{1A}$ receptor have been reported [4–8]. Unfortunately, most of those agents confront with some problems such as low initial brain accumulation and high non-specific uptake. In our previous studies, MPP analogues were labeled with different technetium-99m cores [9, 10] and fluorine-18 [11]. We had successfully synthesized a ligand containing dithiocarbamate moiety for incorporating ^{99m}Tc-tricarboxyl core. ^{99m}Tc(CO)$_3$-MPPDTF showed moderate brain uptake with good retention [12]. The specific binding of this radiotracer to 5-HT$_{1A}$ receptor was also confirmed partially by blocking experiment in mice.

^{99m}Tc-nitrido core ([^{99m}TcN]$^{2+}$) exhibits a very high chemical stability towards oxidation-reduction reactions and pH variations. The ^{99m}Tc-nitrido core has been found to complex well with ligands containing sulfur atoms, as in dithiocarbamates [13, 14]. The [^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ intermediate is readily replaced by incoming tridentate ligand or one bidentate and one monodentate ligand or three mono-

*Corresponding author (email: zhangxzh@bnu.edu.cn)

dentate ligands [15]. The dithiocarbamate ligands which readily form stable complexes with the tricarbonyl or nitrido precursor have been functionalized for the development of site-specific radiopharmaceuticals using varied biological molecules. Considering the length of spacer between MPP and chelation group may affect the properties of labelled complexes, a series of MPP derivatives containing dithiocarbamate ligands with different carbon linkage length were synthesized. Here we report the radiolabeling and preliminary biological evaluation of ^{99m}Tc-nitrido and ^{99m}Tc-tricarboxyl complexes containing 1-(2-methoxyphenyl) piperazine derivatives as potential 5-HT_{1A} receptor imaging agents.

2 Experimental

2.1 Materials

The DTCZ kit for ^{99m}Tc-nitrido core preparation was provided by Beijing Shihong Pharmaceutical Center, China. (2-Methoxyphenyl) piperazine (MPP, 98+%, Geel, Belgium) was purchased from Acros Organics Co Ltd. All other reagents were of reagent grade and commercially available. Technetium-99m as sodium pertechnetate ($Na^{99m}TcO_4$) was obtained from commercial ^{99}Mo/^{99m}Tc generator system (HTA Co., Ltd. China). High-pressure liquid chromatography (HPLC) experiments were performed on a ALLTECH system with Alltech HPLC pump Model 626 system and Alltech Alltima C-18 reversed phase column (4.6 mm × 250 mm) (Alltech Associates Inc., Deerfield, Illinois, USA). The absorbance was monitored at 220 nm. Reversed-phase extraction Sep-Pak C_{18} Plus cartridges (Waters) were activated with methanol and water before use. Animal experiments were carried out in Kunming mice (average weight about 20 g), obtained from the Animal Center of Peking University. All biodistribution studies were carried out in compliance with the national laws related to the conduct of animal experimentation.

2.2 Preparation of MPPnDTC

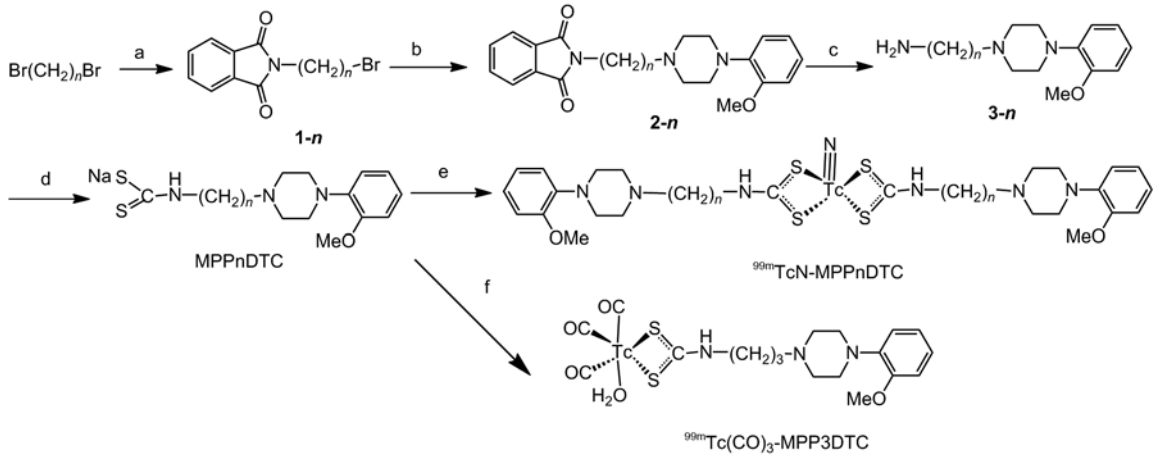
Five ligands MPP2DTC (*N*-2-(4-(2-methoxyphenyl) piperazin-1-yl)ethyl-dithiocarbamate), MPP3DTC (*N*-3-(4-(2-methoxyphenyl)piperarin-1-yl)propyl-dithiocarbamate), MPP4DTC (*N*-4-(4-(2-methoxyphenyl)piperazin-1-yl) butyl-dithiocarbamate), MPP5DTC (*N*-5-(4-(2-methoxyphenyl)piperazin-1-yl) pentyl-dithiocarbamate) and MPP6DTC (*N*-6-(4-(2-methoxyphenyl) piperazin-1-yl) hexyl-dithiocarbamate) with different carbon chain length were synthesized. The synthesis routes were shown in Scheme 1.

The synthesis procedure of MPP3DTC, which is also representative for other four compounds, is as followed:

Two gram (10.8 mmol) of potassium phthalimide and 6.5 g (32.4 mmol) of dibromopropane were refluxed in 40 mL acetonitrile for 6 h. The white solid was removed by filtration and the solvent was then evaporated *in vacuo*. The remaining yellow oil was dissolved in 10 mL of methanol. **1-3** Crystallized as a white powder at −18 °C overnight, yield 53%.

A mixture of 2-(methoxyphenyl) piperazine (1.28 g, 5.71 mmol) and 3.15 g of K_2CO_3 in 20 mL of acetonitrile was refluxed for 30 min. Then the **1-3** (1.53 g, 5.71 mmol, dissolved in acetonitrile) was added dropwise, and refluxed for further 4 h. The solid phase was removed by filtration and the solvent was then evaporated *in vacuo*. The remaining yellow solid was crystallized from methanol to give **2-3** as white crystals, yield 59%. ^{1}H NMR($CDCl_3$): 7.865–7.881(m, 2H, *phenyl*-C-CO), 7.726–7.742(m, 2H, *phenyl*-CH-C-CO), 6.856–7.027(m, 4H, *phenyl*-OCH_3), 3.863(s, 3H, *OCH_3*), 3.815–3.842(t, 2H, CO-N-*CH_2*), 2.641, 3.001(m, 8H, $CH_2N(CH_2CH_2)_2N$), 1.978(m, 2H, *CH_2* $CH_2N(CH_2CH_2)_2N$), 1.618(m, 2H, *CH_2*$N(CH_2CH_2)_2N$).

Compound **2-3** (1.28 g, 3.39 mmol) was dissolved in 10 mL



Scheme 1 The synthetic route of MPPnDTC and radiolabeling with $[^{99m}TcN]^{2+}$ (n = 2–6) and $[^{99m}Tc(CO_3)(H_2O)_3]^+$ ($n = 3$). Reagents: (a) potassium phthalimide, CH_3CN; (b) 2-(methoxyphenyl) piperazine, K_2CO_3, CH_3CN; (c) hydrazine hydrate, CH_3OH; (d) CS_2, NaOH; (e) $[^{99m}TcN]^{2+}$ intermediate; (f) MPP3DTC, $[^{99m}Tc(CO)_3(H_2O)_3]^+$ intermediate.

of methanol and refluxed under stirring. Then 0.2 g hydrazine hydrate was added, and the mixture was refluxed for 3 h. After addition of conc. HCl to adjust the pH to 6, a white solid of phthalic acid hydrazide was precipitated. After filtration, the pH of liquid phase was adjusted to about 12 with 3 mol/L NaOH. The solvent was then evaporated in *vacuo*, and 5 mL water was added. Extracted three times with methylene dichloride (3 mL for each extraction), and the combined organic phases were extracted three times with water (3 mL for each extraction). The organic phases were dried over anhydrous Na_2SO_4. Removal of the solvent gave **3-3** as colorless oil, yield 71%. ^{1}H NMR($CDCl_3$): 6.876–7.076(m, 4H, *phenyl*-OCH_3), 3.889(s, 3H, OCH_3), 3.126, 2.694(m, 8H, N(CH_2CH_2)$_2$N), 2.822–2.849(t, 2H, NH_2-CH_2), 2.505–2.534(t, 2H, $NH_2CH_2CH_2$), 1.891(s, 2H, NH_2CH_2), 1.703–1.759(m, 2H, NH_2CH_2 CH_2CH_2).

Sodium hydroxide (2.41 mmol) was dissolved in 10 mL water and cooled in an ice-salt bath. Then 0.6 g **3-3** (2.41 mmol) was added with stirring, after that 0.2 g carbon disulfide (2.65 mmol) was added immediately. The mixture was stirred for 2 h in an ice-salt bath. Most of the solvent was removed under reduced pressure, and the precipitate collected by filtration. The white crude product was recrystallized from ethanol to give the compound MPP3DTC, yield 78%. ($NaC_{15}N_3H_{22}OS_2\cdot2.5H_2O$) ESI-MS: *m/z* 324.2 (M+, calcd *m/z* 347). Calcd.: C 45.92% H 6.89% N 10.71%. Found: C 45.90% H 6.38% N 10.56%. ^{1}HNMR (500 MHz, D_2O,): δ 7.08–6.91 (m, 4H, CH_3O-*phenyl*-N), 3.76(s, 3H, OCH_3), 3.49–3.46(t, 2H, CS_2NHCH_2), 2.90–2.45(m, 8H, N(CH_2-CH_2)$_2$N), 2.43–2.40(m, 2H, CH_2-piperazine), 1.79–1.73(m, 2H, $CH_2CH_2CH_2$). IR (KBr) (cm^{-1}): 943.8 (C=S)

Other products were characterized by:

^{1}HNMR (500 MHz, D_2O, δ, ppm): MPP2DTC: 6.91–7.09 (m, 4H, CH_3O-*phenyl*-N), 3.76(s, 3H, OCH_3), 3.67–3.70(t, 2H, CS_2NHCH_2), 2.90–2.45(m, 8H, N(CH_2CH_2)$_2$N), 2.43–2.40(m, 2H, CH_2-piperazine).

MPP4DTC: 7.08–6.91 (m, 4H, CH_3O-*phenyl*-N), 3.76(s, 3H, OCH_3), 3.47–3.44(t, 2H, $CS_2NHCH_2CH_2$), 2.96–2.40(m, 8H, N(CH_2CH_2)$_2$N), 2.43–2.40(m, 2H, CH_2-piperazine), 1.56–1.46(m, 4H, $CH_2CH_2CH_2$-piperazine).

MPP5DTC: 7.057–6.910 (m, 4H, CH_3O-*phenyl*-N), 3.749 (s, 3H, -OCH_3), 3.409–3.436(t, 2H, $CS_2NHCH_2CH_2$), 2.96–2.40(m, 8H, N(CH_2CH_2)$_2$N), 2.351–2.382(t, 2H, CH_2-piperazine), 1.524–1.554(m, 2H, $CH_2CH_2CH_2CH_2$-piperazine), 1.453–1.467(m, 2H, $CH_2CH_2CH_2$-piperazine), 1.249–1.278 (m, 2H, CH_2CH_2-piperazine).

MPP6DTC: 7.057–6.910 (m, 4H, CH_3O-*phenyl*-N), 3.749 (s, 3H, -OCH_3), 3.409–3.436(t, 2H, $CS_2NHCH_2CH_2$), 2.96–2.40(m, 8H, N(CH_2CH_2)$_2$N), 2.351–2.382(t, 2H, CH_2-piperazine), 1.524–1.554(m, 2H, $CH_2CH_2CH_2CH_2$-piperazine), 1.453–1.467(m, 2H, $CH_2CH_2CH_2$-piperazine), 1.249–1.278 (m, 2H, CH_2CH_2-piperazine).

ESI-MS and elemental analyses: MPP2DTC: ($NaC_{14}N_3H_{20}OS_2\cdot4H_2O$) *m/z* 310.1 (M+, calcd *m/z* 333). Calcd: C 41.48% H 6.91% N 10.37%. Found: C 41.77% H 7.44% N 10.36%.

MPP4DTC: ($NaC_{16}N_3H_{24}OS_2\cdot0.5H_2O$) *m/z* = 338 (M+, calcd *m/z* 361). Calcd: C 51.89% H 6.76% N 11.35%. Found: C 51.55% H 6.79% N 11.15%.

IR (KBr) (cm^{-1}): 936–958 (C=S), for MPP2DTC to MPP6DTC.

2.3 Preparation of ^{99m}TcN-MPPnDTC

The ^{99m}TcN-MPPnDTC was prepared by a two-step procedure. 1 mL saline-containing $Na^{99m}TcO_4$ (about 185 MBq) was added into a DTCZ kit and heating under N_2 at 100 °C for 15 min to obtain the $[^{99m}TcN]^{2+}$ intermediate. Then 0.2 mL $[^{99m}TcN]^{2+}$ (about 37 MBq) intermediate was added to the MPPnDTC (2 mg ligand dissolved in 0.2 mL water) and incubated for 20 min at room temperature.

The $[^{99m}TcN]^{2+}$ intermediate and final ^{99m}TcN-MPPnDTC complex were characterized by both TLC and HPLC. TLC was performed on a polyamide film with saline and methanol/methylene dichloride (*v*/*v* = 9:1) as the mobile phase. RP-HPLC was performed on a Alltech system with Kromasil C-18 column (4.6 × 250 mm, 5 μm). The flow rate was 1 mL/min with water (solvent A) and methanol (solvent B) were used as the mobile phase in the following gradient (0–10 min, B conc. 60%–100%; 10–30 min, B conc. 100%).

^{99m}TcN-MPPnDTC was purified with semi-preparative column Venusil MP-C18 (10 × 250 mm, 5 μm). The flow rate was set at 3 mL/min with above-described gradient.

2.4 Preparation of ^{99m}Tc(CO)$_3$-MPP3DTC

The ^{99m}Tc(CO)$_3$-MPP3DTC was prepared by a two-step procedure.

$[^{99m}Tc(CO)_3(H_2O)_3]^+$ was prepared by adding 1 mL of $^{99m}TcO_4^-$ eluted from a commercial generator (37 MBq) to a 10 mL vial containing potassium boranocarbonate (3 mg), sodium potassium tartrate tetrahydrate (6.7 mg), and potassium tetraborate pentahydrate (5.2 mg). Then the solution was heated for 15 min in boiling water under N_2. After cooling down to room temperature, the sample was analyzed on TLC and HPLC.

The pH value of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ intermediate was adjusted to 7. Then 2 mg MPP3DTC was added to the above solution. The final complex ^{99m}Tc(CO)$_3$-MPP3DTC was obtained after 30 min incubation at room temperature.

The $[^{99m}Tc(CO)_3(H_2O)_3]^+$ intermediate and final ^{99m}Tc(CO)$_3$-MPP3DTC complex were characterized by both TLC and HPLC, and purified with semi-preparative HPLC.

2.5 Octanol/water partition coefficient

The partition coefficient of the complex was determined by measuring the activity that partitioned between the *n*-octanol and aqueous phosphate buffer (0.025 mol/L, pH 7.4) under

strict equilibrium conditions. 2 mL *n*-octanol and 2 mL radiotracer (^{99m}TcN-MPPnDTC or ^{99m}Tc(CO)$_3$-MPP3DTC) phosphate buffer were mixed in a centrifuge tube. The mixture was vortexed at room temperature for 5 min and then centrifuged at 5000 r/min for 5 min. The counts in 0.1 mL samples of both organic and inorganic layers were determined by a well type gamma counter. The measurement was repeated three times. The partition coefficient (P) was calculated using the following equation: P = (cpm in octanol-cpm in background)/(cpm in buffer–cpm in background). The final partition coefficient value was expressed as logP.

2.6 Paper electrophoresis

The sample of ^{99m}TcN-MPPnDTC and ^{99m}Tc(CO)$_3$-MPP3DTC was spotted on chromatography paper strips (10 cm × 1 cm) which were pretreated with phosphate buffer (0.025 mol/L, pH 7.4). The analyses were carried out using phosphate buffer (0.025 mol/L, pH 7.4) as electrolyte and the voltage was set at 150 V for 2.5 h. Then the strips were left to dry, and the distribution of radioactivity on the strip was determined.

2.7 *In vitro* stability

Both ^{99m}TcN-MPPnDTC and ^{99m}Tc(CO)$_3$-MPP3DTC were incubated at room temperature (25 °C) for 4 h. The radiochemical purity (RCP) was evaluated by TLC chromatography at every single hour and by HPLC analysis at end of 4 h.

2.8 Biodistribution studies

In vivo biodistribution study of ^{99m}TcN-MPPnDTC was carried out in normal Kunming mice. About 0.74 MBq ^{99m}TcN-MPP*n*DTC in 100 μL solution was injected through the tail vein. Mice (n = 3) were sacrificed after 2 min p.i., and the brain was rapidly removed, chilled and dissected. Samples from different brain regions (cortex, hippocampus and cerebellum) were collected, wet weighed and counted in a γ-counter. The percentage of injected dose per gram (%ID/g) for each sample was calculated by comparing its activity with appropriate standard of injected dose (ID), the values are expressed as mean ± SD. The ^{99m}Tc(CO)$_3$-MPP3DTC was evaluated with similar process. About 0.555 MBq ^{99m}Tc(CO)$_3$-MPP3DTC in 100 μL solution was injected through the tail vein. Mice (n = 5) were sacrificed after 2, 60 and 120 min p.i., and samples from different brain regions were collected. The percentage of injected dose per gram (%ID/g) was calculated.

3 Results and discussion

3.1 Chemistry and radiochemistry

The MPP ((2-methoxyphenyl)piperazine) moiety, residue of WAY 100635, which is known to have high affinity to the 5-HT$_{1A}$ receptor, was introduced into the ligand design. The dithiocarbamate ligands containing the MPP moiety could be labelled with [^{99m}TcN]$^{2+}$ core to obtain a series of neutral complexes. Considering the space between the chelate unit and MPP may affect the properties of labelled complexes, five ligands with different carbon chain length were synthesized as described in Scheme 1. The ligands MPPnDTC (n = 2–6) were synthesized in the yield around 10% and characterized by ^{1}H NMR and elemental analysis.

In order to label MPPnDTC with ^{99m}Tc-nitrido core, the [^{99m}TcN]$^{2+}$ intermediate was first prepared through DTCZ kit. The final ^{99m}TcN-MPPnDTC complexes were obtained in high yield (> 90%) by addition of [^{99m}TcN]$^{2+}$ intermediate to MPPnDTC ligand and incubated for 20 min at room temperature. Quality control of the [^{99m}TcN]$^{2+}$ intermediate and ^{99m}TcN-MPPnDTC was performed by TLC and HPLC. TLC was performed on a polyamide film, R_f value for [^{99m}TcN]$^{2+}$ intermediate was 0.8–1.0 and the final complexes ^{99m}TcN-MPPnDTC were 0–0.1 with saline as the mobile phase, when use methanol/methylene dichloride (*v*/*v* = 9:1) as the mobile phase, the R_f value for [^{99m}TcN]$^{2+}$ intermediate was 0–0.1 and the final complexes ^{99m}TcN-MPPnDTC were 0.9–1.0. HPLC analysis showed the similar results as TLC (Figure 1). The retention time of ^{99m}TcN-MPP2DTC, ^{99m}TcN-MPP3DTC, ^{99m}TcN-MPP4DTC, ^{99m}TcN-MPP5DTC and ^{99m}TcN-MPP6DTC was 9.1, 14.9, 15.7, 16.3 and 17.7 min, respectively.

For comparison, MPP3DTC was labeled with ^{99m}Tc-tricarboxyl core, the [^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ intermediate was first prepared according to the procedure of Alberto *et al.* [16]. The final ^{99m}Tc(CO)$_3$-MPP3DTC complex was obtained in high yield (> 80%) by addition of [^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ intermediate to MPP3DTC ligand and incubated for 30 min at room temperature. The R_f value for [^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ intermediate was 0–0.1 and the final complex ^{99m}Tc(CO)$_3$-MPP3DTC was 0.9–1.0 with acetonitrile as the mobile phase. The retention time of [^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ intermediate and ^{99m}Tc(CO)$_3$-MPP3DTC were 8.9 and 13.5 min (Figure 1) in HPLC analysis, respectively.

All the complexes were purified with HPLC for further properties study and biodistribution experiment.

3.2 Physicochemical properties

The physicochemical properties of these ^{99m}Tc-labelled complexes were investigated by electrophoresis and *n*-octanol/water partition coefficient experiments. In paper electrophoresis, more than 90% of this ^{99m}Tc labelled complex did not move after developed at 150 V for 3.5 h, indicating neutrality of all of the complexes. The logP of ^{99m}TcN-MPP2DTC, ^{99m}TcN-MPP3DTC, ^{99m}TcN-MPP4DTC, ^{99m}TcN-MPP5DTC and ^{99m}TcN-MPP6DTC were 2.06, 2.14, 2.10, 2.56 and 2.44 respectively. There is no significant difference of *n*-octanol/water partition coefficient P when carbon

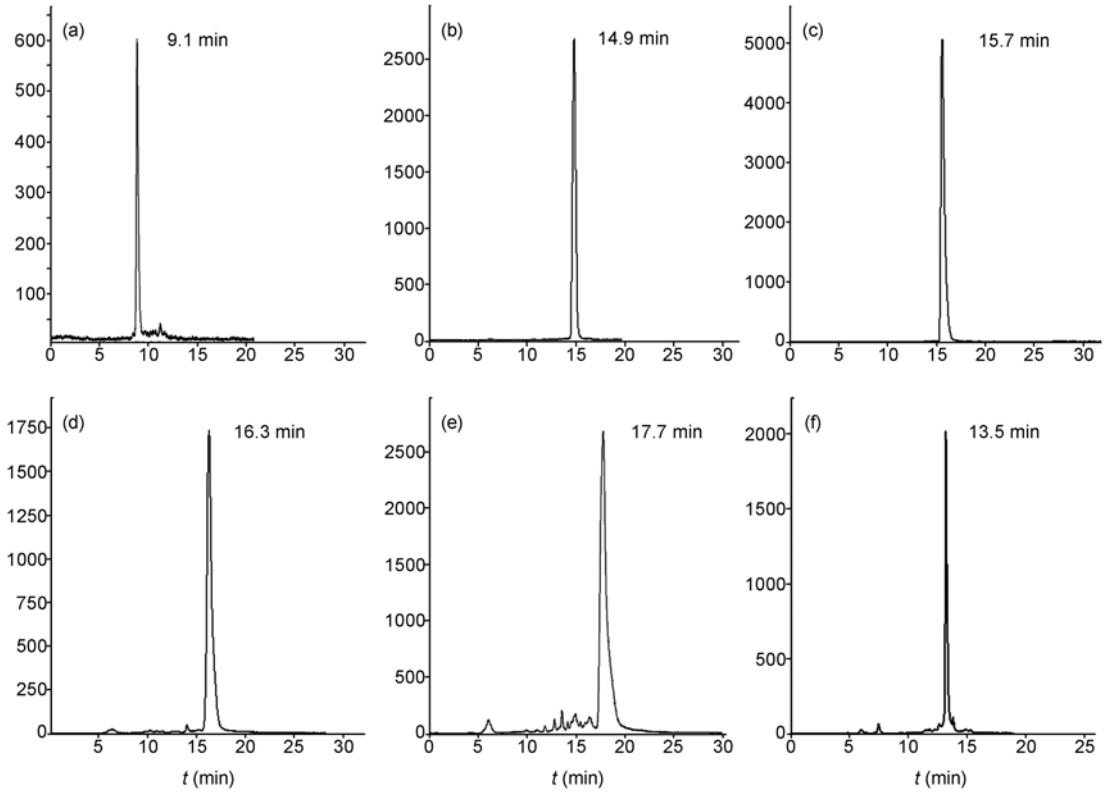


Figure 1 The HPLC chromatograms of ^{99m}Tc labelled MPP derivatives. The retention time of ^{99m}TcN-MPP2DTC (a), ^{99m}TcN-MPP3DTC (b), ^{99m}TcN-MPP4DTC (c), ^{99m}TcN-MPP5DTC (d), ^{99m}TcN-MPP6DTC (e) and ^{99m}Tc(CO)$_3$-MPP3DTC (f) was 9.1, 14.9, 15.7, 16.3, 17.7 and 13.5 min, respectively.

chain length was 2, 3 and 4(P value > 0.05, two tail t test performed by GraphPad InStat). The lipophilic increased significantly when carbon chain length increase to 5 and 6. The logP of ^{99m}Tc(CO)$_3$-MPP3DTC was 1.23, suggesting that the lipophilicity of this compound was lower than that of corresponding ^{99m}Tc-nitrido complex obviously.

In vitro stability of the complexes was evaluated by measuring the radiochemical purity (RCP) at different time points after preparation and purification with HPLC at room temperature (25 °C). The RCP were still over 95% after 4 h, which suggested that all of the complexes were stable in vitro at least for 6 h at room temperature.

3.3 Biodistribution

To evaluate *in vivo* distribution characteristics of ^{99m}TcN-MPPnDTC (n = 2–6) and ^{99m}Tc(CO)$_3$-MPP3DTC, the distribution of the activity in various mice brain regions were performed using normal Kunming mice. The results were shown in Table 1. *In vivo* biodistribution data showed these complexes had moderate initial brain uptake. For ^{99m}TcN-MPP2DTC, ^{99m}TcN-MPP3DTC, ^{99m}TcN-MPP4DTC, ^{99m}TcN-MPP5DTC and ^{99m}TcN-MPP6DTC, the radioactivity concentration of hippocampus (Hipp) at 2 min p.i. were 0.43, 1.15, 0.99, 1.04 and 1.13 %ID/g respectively. The uptake of

Table 1 The biodistribution of ^{99m}Tc-nitrido core labeled MPP derivatives in normal mice at 2 min after injection

	^{99m}TcN-MPP2DTC	^{99m}TcN-MPP3DTC	^{99m}TcN-MPP4DTC	^{99m}TcN-MPP5DTC	^{99m}TcN-MPP6DTC
Hippocampus	0.43±0.09	1.15±0.16	0.99±0.30	1.04±0.13	1.13±0.01
Cerebellum	0.69±0.06	1.65±0.46	1.49±0.56	1.27±0.22	1.50±0.11
ROB[a)]	0.39±0.05	0.82±0.11	0.94±0.25	0.75±0.12	0.87±0.29
Brain[b)]	0.45±0.05	0.99±0.16	1.04±0.30	0.86±0.12	1.00±0.23
Blood	8.94±1.75	31.48±6.11	44.43±9.43	34.44±3.29	32.43±8.24
Hipp/CB	0.62	0.70	0.66	0.82	0.75
Brain/ Blood	0.050	0.031	0.023	0.025	0.031

Expressed as % injected dose per gram (%ID/g). Each value represents the mean ± SD of five animals. a) ROB refers to the rest part of brain except hippocampus and cerebellum. b)Brain includes whole organization of cerebrum and cerebellum.

hippocampus has no significant difference ($P > 0.05$) except ^{99m}TcN-MPP2DTC. Unfortunately, each complex has relative high cerebellum (CB) uptake. Due to the non-specific binding in cerebellum, the Hipp/CB ratio was relatively low compared to other reported ^{99m}Tc labeled complexes carrying MPP moiety which was about 0.7 to 1.6 [4, 7]. The biodistribution results of ^{99m}Tc(CO)$_3$-MPP3DTC were shown in Table 2. The ^{99m}Tc(CO)$_3$-MPP3DTC had lower initial brain and hippocampus uptake compared with ^{99m}TcN-MPP3DTC. The radioactivity concentration of Hipp at 2 min p.i. was 0.83 ± 0.13 %ID/g. The uptake in cerebellum was relatively high, about 1.13 ± 0.16 %ID/g at 2 min p.i. Whole-brain uptake gradually decreased over time. The uptake in both hippocampus and cerebellum decreased gradually. The radioactivity was fast cleared from blood, while increased in liver and kidney. That indicated the tracer was metabolized by both hepatobiliary and renal excretion. Compared to ^{99m}Tc(CO)$_3$-MPPDTF [12], the ^{99m}Tc(CO)$_3$-MPP3DTC showed higher initial brain uptake and fast clearance. At the same time the radioactivity concentration in the blood was increased, while in the liver and other non-target organs were decreased. Despite the molecular weight and lipophilicity of all the radiotracers with ^{99m}Tc-nitrido core and ^{99m}Tc-tricarboxyl core were appropriate for penetrating the blood brain barrier, relatively low initial brain uptakes were found from in vivo distribution results. We need to further modify those complexes to increase the capacity for crossing the BBB.

Although the physical properties such as logP of MPPnDTC can be regulated by changing the length of carbon spacer, biological properties of the series tracers did not showed significant difference. So simply changing the length of carbon chain may not be able to obtain better results. In future research, PEG chain or triazole linker (based on click chemistry) could be considered to increase the spacer length and modify the biodistribution properties.

Table 2 The biodistribution of ^{99m}Tc(CO)$_3$-MPP3DTC in normal mice

	Post-injection time (min)		
	2	60	120
Hippocampus	0.83±0.13	0.34±0.07	0.22±0.02
Cerebellum	1.13±0.16	0.49±0.10	0.33±0.07
ROB [a)]	0.67±0.17	0.30±0.05	0.22±0.05
Brain [b)]	0.75±0.14	0.34±0.06	0.24±0.05
Blood	30.48±9.33	12.10±1.75	8.03±2.10
Heart	6.09±1.83	5.44±1.05	5.27±1.33
Liver	21.75±6.02	34.49±8.30	36.64±5.29
Lung	23.87±7.64	14.03±3.03	13.51±3.76
Kidney	8.42±2.73	11.48±2.57	11.64±2.84
Spleen	13.17±7.24	25.38±3.89	18.04±4.96
Hipp/CB	0.74	0.69	0.69
Brain/Blood	0.025	0.028	0.03

Expressed as % injected dose per gram (%ID/g). Each value represents the mean ± SD of five animals. a) ROB refers to the rest part of brain except hippocampus and cerebellum. b) Brain includes whole organization of cerebrum and cerebellum.

4 Conclusions

Five MPP derivatives MPP2DTC, MPP3DTC, MPP4DTC, MPP5DTC and MPP6DTC with different carbon chain length were successfully synthesized as potential 5-HT$_{1A}$ receptor ligands. Those ligands were labeled with $[^{99m}TcN]^{2+}$ with high yield by using two-step procedure. MPP3DTC was labeled with $[^{99m}Tc(CO)_3(H_2O)_3]^+$. All of these complexes were neutral and lipophilic with high in vitro stability. Preliminary biological experiments in mice showed these complexes had a certain initial brain uptake. Furthermore, we will take in vitro receptor binding assays to determine binding affinity of those radiotracers to 5-HT$_{1A}$ receptor. And modify those complexes for improvement to obtain technetium-99m based 5-HT$_{1A}$ brain receptor imaging agents.

This work was supported by the National Natural Science Foundation of China (20401004) and the Fundamental Research Funds for the Central Universities of China. The authors wish to acknowledge the support of Beijing Shihong Pharmaceutical Center for the donation of DTCZ kit.

1 Gross C, Zhuang XX, Stark K, Ramboz S, Oosting R, Kirby L, Santarelli L, Beck S, Hen R. Serotonin(1A) receptor acts during development to establish normal anxiety-like behaviour in the adult. *Nature*, 2002, 416: 396–400
2 Parsey RV, Belanger MJ, Sullivan GM, Simpson NR, Stabin MG, Van Heertum R, Mann JJ. Biodistribution and radiation dosimetry of C-11-WAY100,635 in humans. *J Nuclear Med*, 2005, 46(4): 614–619
3 Pike VW, Halldin C, Wikstrom H, Marchais S, McCarron JA, Sandell J, Nowicki B, Swahn C-G, Osman S, Hume SP. Radioligands for the study of brain 5-HT$_{1A}$ receptors *in vivo* – Development of some new analogues of WAY. *Nuclear Med Biology*, 2000, 27(5): 449–455
4 Papagiannopoulou D, Pirmettis I, Tsoukalas C, Nikoladou L, Drossopoulou G, Dalla C, Pelecanou M, Papadopoulou-Daifotis Z, Papadopoulos M, Chiotellis E. Oxotechnetium (TcO)-Tc-99m[SN(R)S][S] complexes as potential 5-HT$_{1A}$ receptor imaging agents. *Nuclear Medicine and Biology*, 2002, 29(8): 825–32
5 Heimbold I, Drews A, Syhre R, Kretzschmar M, Pietzsch HJ, Johannsen B. A novel technetium-99m radioligand for the 5-HT$_{1A}$ receptor derived from desmethyl-WAY-100635 (DWAY). *Eur J Nuclear Med*, 2002, 29(1): 82–87
6 Heimbold I, Drews A, Kretzschmar M, Varnas K, Hall H, Halldin C, Syhre R, Kraus W, Pietzsch HJ, Seifert S. Synthesis, biological and autoradiographic evaluation of a novel Tc-99m radioligand derived from WAY 100635 with high affinity for the 5-HT$_{1A}$ receptor and the alpha1-adrenergic receptor. *Nuclear Med Biolog*, 2002, 29(4): 375–387
7 Leon A, Rey A, Mallo L, Pirmettis I, Papadopoulos M, Leon E, Pagano M, Manta E, Incerti M, Raptopoulou C. Novel mixed ligand technetium complexes as 5-HT$_{1A}$ receptor imaging agents. *Nuclear Med Biolog*, 2002, 29(2): 217–226
8 Bolzati C, Mahmood A, Malago E, Uccelli L, Boschi A, Jones AG, Refosco F, Duatti A, Tisato F. The [Tc-99m(N)(PNP)]$^{2+}$ metal fragment: A technetium-nitrido synthon for use with biologically

active molecules the *N*-(2-methoxyphenyl)piperazyl-cysteine analogues as examples. *Bioconjugate Chem*, 2003, 14(6): 1231–1242
9 Fan WW, Lin Y, Zhang XZ, Pang Y, Ma C, Tang ZG, Zhang JB, Wang XB. Synthesis and biological evaluation of Tc-99m-HEDTA/HYNIC-MPP4 complex for 5-HT_{1A} receptor imaging. *Sci China Ser B: Chem*, 2009, 52(5): 590–598
10 Wang F, Wang X, Yang S, Liu J, Zhang X. *N*-[3-[4-(2- Methoxyphenyl) piperaziny-1-yl]propyl]cyclam: Synthesized as a potential 5-HT_{1A} receptor ligand and labelled with ^{99m}Tc-nitrido core. *J Label Comp Radiopharmac*, 2008, 51(10): 347–351
11 Mou T, Yang W, Peng C, Zhang X, Ma Y. [^{18}F]-Labeled 2-methoxyphenylpiperazine derivative as a potential brain positron emission tomography imaging agent. *Appl Radia Isotop*, 2009, 67(11): 2013–2018
12 Zhang XZ, Zhou PW, Liu JJ, Huang Y, Lin Y, Chen YL, Gu T, Yang WJ, Wang XB. Preparation and biodistribution of Tc-99-tricarbonyl complex with 4-[(2-methoxyphenyl)piperazin-1-yl]-dithioformate as a potential 5-HT_{1A} receptor imaging agent. *Appli Radia Isotop*, 2007, 65(3): 287–292
13 Zhang J, Ren J, Lin X, Wang X. Synthesis and biological evaluation of a novel ^{99m}Tc nitrido radiopharmaceutical with deoxyglucose dithiocarbamate, showing tumor uptake. *Bioorg Med Chem Lett*, 2009, 19(10): 2752–2754
14 Zhang J, Lin Y, Sheng X, Wang X. Synthesis of a novel ^{99m}Tc nitrido radiopharmaceutical with isopropyl xanthate, showing brain uptake. *Appl Radiat Isot*, 2009, 67(1): 79–82
15 Alberto R, Schibli R, Schubiger AP, Abram U, Pietzsch HJ, Johannsen B. First application of fac-[Tc-99m$(OH_2)_3(CO)_3]^+$ in bioorganometallic chemistry: Design, structure, and in vitro affinity of a 5-HT_{1A} receptor ligand labeled with Tc-99m. *J Am Chem Soc*, 1999, 121(25): 6076–6077
16 Alberto R, Schibli R, Egli A, Schubiger AP, Abram U, Kaden TA. A novel organometallic aqua complex of technetium for the labeling of biomolecules: Synthesis of [Tc-99m$(OH_2)_3(CO)_3]^+$ from [$^{99m}TcO_4]^-$ in aqueous solution and its reaction with a bifunctional ligand. *J Am Chem Soc*, 1998, 120(31): 7987–7988