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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

Fenglong Wang, Xuebin Wang*, Shuye Yang, Jian Liu and Xianzhong Zhang

This paper reports the synthesis of new potential 5-HT_{1A} receptor ligand *N*-[3-[4-(2-methoxyphenyl)piperaziny-1-yl]propyl]cyclam (MPPC) and radiolabelling of it with ^{99m}Tc-nitrido core. The novel neutral complex ^{99m}TcN-MPPC combines 1,4,8,11-tetraazacyclotetradecane (cyclam) ligand as chelate moiety for ^{99m}Tc-nitrido with a 1-(2-methoxyphenyl)piperazine moiety derived from WAY 100635 via a 3-carbon alkyl chain. This provided a reliable and reproducible method for attaching the technetium to the pharmacophore moiety of WAY 100635. ^{99m}TcN-MPPC was prepared by a two-step procedure and the radiochemical purity was found to be greater than 95%. It was hydrophilic and stable for at least 4 h at room temperature. *In vivo* stability study in normal rats showed that no degradation of ^{99m}TcN-MPPC was found in deproteinated blood samples at 2 h post-injection. This effective ^{99m}Tc-labelling strategy for obtaining neutral ^{99m}Tc nitrido complexes would be a useful tool to prepare new SPECT agents to image 5-HT_{1A} receptor with cyclam conjugated ligands.

Keywords: 5-HT_{1A} receptor; technetium-99m; MPPC; ^{99m}TcN-MPPC

Introduction

The 5-HT_{1A} receptor 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. It is therefore of great interest, especially for diagnosis, to develop radioligands of high selectivity and affinity for the receptor to allow brain imaging. Despite the ^{11}C- and ^{18}F-labelled ligands for positron emission tomography (PET) imaging and ^{123}I-labelled ligands for single photon emission computed tomography (SPECT) imaging has 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.[1–5]

Based on the lead structure of *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-*N*-(2-pyridinyl)- cyclohexanecarboxamide (WAY100635), which is used in SPECT imaging and in vitro autoradiographic studies of 5-HT_{1A} receptors, considerable progress has been made in the last few years for the development of technetium-99m complexes.[6–12] There are several different approaches used for attaching the technetium to the pharmacophore moiety of WAY 100635, such as the mixed ligand complex approach (2+1+1, 3+1, 4+1), tetradentate N_2S_2 chelate approach and the tricarbonyl approach.

It is well known that the cyclic polyamines such as 1,4,8,11-tetraazacyclotetradecane (cyclam) can be used as bifunctional chelating agents of rhenium and technetium for labelling bioactive ligands.[13–18] In this study, we introduced the 1-(2-methoxyphenyl) piperazine moiety of WAY 100635 into the structure of the cyclam and obtained a novel neutral complex labelled with ^{99m}Tc(V)-nitrido core. This provided a reliable and reproducible method for attaching the technetium to the pharmacophore moiety of WAY 100635. This effective ^{99m}Tc-labelling strategy would be a favorable method for preparation of neutral ^{99m}Tc-nitrido complexes with cyclam conjugated ligands to image 5-HT_{1A} receptors.

Results and discussion

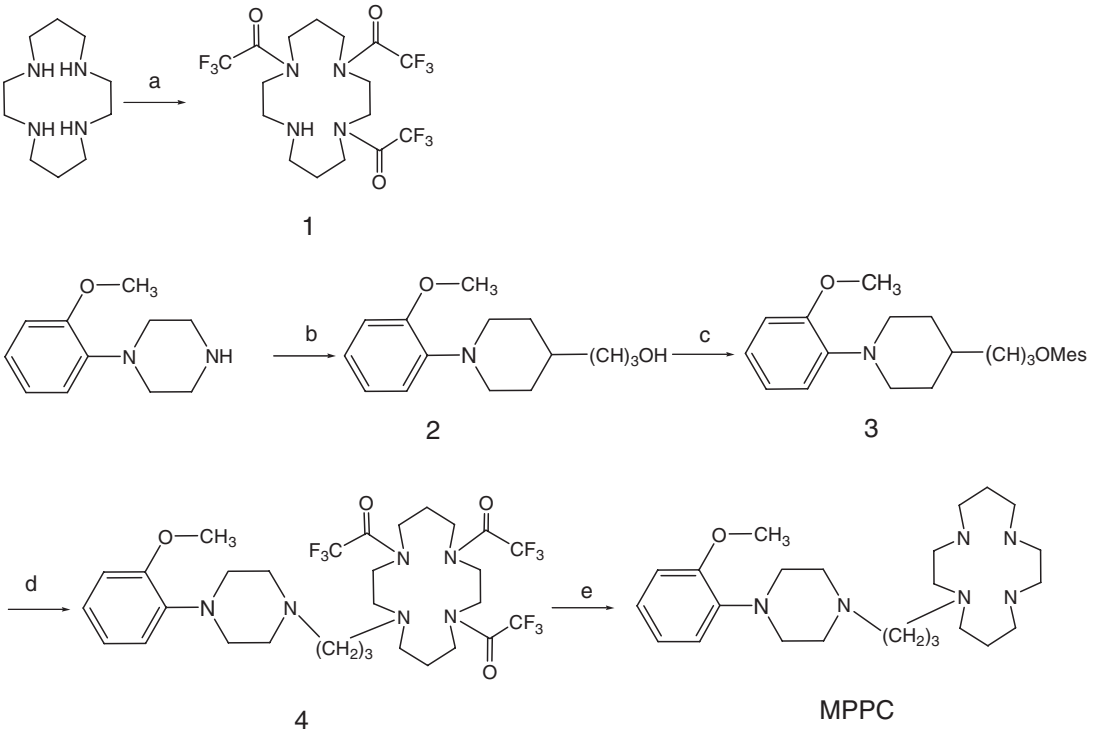
Chemistry

The synthesis of the cyclam chelating unit linked via a 3-carbon alkyl chain to 1-(2-methoxyphenyl) piperazine was accomplished as described in Scheme 1.

The three amine functions of cyclam were protected by an excess of ethyl trifluoroacetate in methanol at room temperature. The preparation of 3-[4-(2-methoxyphenyl)piperaziny-1-yl]propan-1-ol (**2**) was performed by alkylation of 1-(2-methoxyphenyl)piperazine using 3-bromo-1-propanol as alkylating agent. The reaction was carried out in anhydrous CH_3CN in the presence of K_2CO_3. 3-[4-(2-Methoxyphenyl)piperaziny-1-yl]propyl methanesulfonate (**3**) was obtained by mesylation of

Key Laboratory of Radiopharmaceuticals of Ministry of Education, College of Chemistry, Beijing Normal University, Beijing 100875, People's Republic of China

**Correspondence to: X. Wang, Key Laboratory of Radiopharmaceuticals of Ministry of Education, College of Chemistry, Beijing Normal University, Beijing 100875, People's Republic of China.*
E-mail: xbwang@bnu.edu.cn



Scheme 1. Synthesis of N-3-[1-(2-methoxyphenyl)piperidin-4-yl]propylcyclam (MPPC). Reagents: (a) EtOTFA,Et_3N,MeOH; (b) 1-(2-Methoxyphenyl)piperazine, K_2CO_3, CH_3CN; (c) CH_3SO_2Cl, CH_2Cl_2, Et_3N; (d) 1, K_2CO_3, CH_3CN; and (e) NaOH, MeOH.

2 with mesyl chloride and triethylamine in CH_2Cl_2. The synthesis of *N*-[3-[4-(2-methoxyphenyl)piperaziny-1-yl]propyl]Tri-TFA-cyclam (**4**) was performed by alkylation of Tri-TFA cyclam (**1**) using **3** as alkylating agent.

Three protected amine functions of **4** were deprotected in MeOH/NaOH to give *N*-[3-[4-(2-methoxyphenyl)piperaziny-1-yl] propyl]cyclam (MPPC).

Radiochemistry

In order to label MPPC with ^{99m}Tc-nitrido core, the $[^{99m}TcN]^{2+}$ intermediate was first prepared. The final ^{99m}TcN-MPPC complex was obtained in high yield (>95%) by addition of $[^{99m}TcN]^{2+}$ intermediate to MPPC ligand and heating under N_2 at 100°C for 30 min. Quality control of the $[^{99m}TcN]^{2+}$ intermediate and ^{99m}TcN-MPPC was performed by TLC and HPLC. TLC was performed on a polyamide film with saline and acetonitrile as the mobile phase. R_f values for $[^{99m}TcN]^{2+}$ intermediate, ^{99m}TcN-MPPC and other possible components are reported in Table 1. The specific radioactivity of ^{99m}TcN-MPPC was 240 TBq/mmol at end of synthesis.

HPLC studies demonstrated that the reaction resulted in a single complex. The retention time of the ^{99m}TcN-MPPC complex was found to be about 2.20 min, while that of $[^{99m}TcN]^{2+}$ intermediate was 10.50 min. The HPLC chromatograms of $[^{99m}TcN]^{2+}$ intermediate and ^{99m}TcN-MPPC complex are shown in Figure 1. The paper electrophoresis pattern of ^{99m}TcN-MPPC remained at the point of spotting, suggesting that it is a neutral complex.

The partition coefficient (log *P*) of ^{99m}TcN-MPPC was -1.64 ± 0.03 at pH 7.4, which indicated that it is a hydrophilic complex.

In vitro stability of the complex was evaluated by measuring the radiochemical purity (RCP) at different time points after preparation at room temperature (25 °C). The RCP was still over 95% after 4 h, which suggested that ^{99m}TcN-MPPC complex possessed a great stability *in vitro*.

In vivo stability was also tested in normal rats. No degradation of ^{99m}TcN-MPPC was found by HPLC analysis of deproteinated blood samples at different time points post-injection.

Experimental

Materials

The SDH kit was provided by Beijing Shihong Pharmaceutical Center, People's Republic of China. A ^{99}Mo/^{99m}Tc generator was obtained from the China Institute of Atomic Energy (CIAE). All other chemicals were of reagent grade and were used without further purification. The TLC was performed on a polyamide strip and eluted with saline and acetonitrile. Reversed-phase high-performance liquid chromatography experiments were performed on a SHIMADZU SCL-10AVP system with a liquid scintillation analyzer (Packard Bioscience Co., USA). The ODS-C18 column (5 μm, 250 × 4.6 mm, Alltech Associates, Inc., USA) was eluted at a flow rate of 1 mL/min according to the procedure described in the experimental part. NMR spectra were recorded on a Bruker Avance-500 (500 MHz) spectrometers.

Synthesis of Tri-TFA cyclam (1)

Ethyl trifluoroacetate (4.80 mL, 40 mmol) was added dropwise to a mixture of cyclam (2.00 g, 10 mmol) and Et_3N (1.38 mL, 10 mmol) in methanol (20 mL) at room temperature. The addition continued over a period of 5 min. The homogeneous

Table 1. R_f values for some selected complexes

	$[^{99m}TcO_4]^-$	$^{99m}TcO_2 \cdot nH_2O$	$[^{99m}TcN]^{2+}_{int}$	^{99m}TcN-MPPC
Saline	0.1	0.1	0.8–1.0	0.6–0.7
Acetonitrile	0.3–0.5	0.1	0.1	0.1

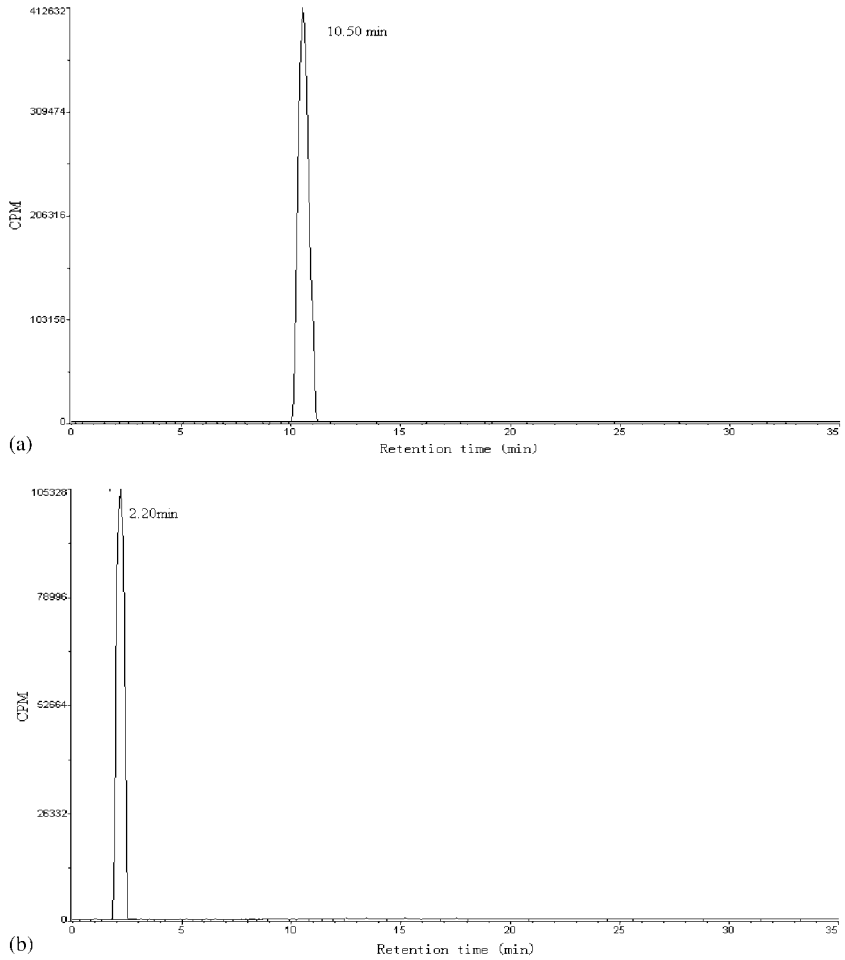


Figure 1. The HPLC chromatograms of $[^{99m}TcN]^{2+}$ intermediate (A) and ^{99m}TcN-MPPC (B).

reaction mixture was cooled with an ice-water bath to control the mild exotherm. Stirring was continued under N_2 for 5 h. Volatiles were removed *in vacuo*. The residue was passed through a small silica gel plug (25 g), eluted with 100% EtOAc. The eluent was concentrated to give the product as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.79–3.51 (m, 12H), 2.95 (m, 2H), 2.72 (m, 2H), 2.18 (m, 2H), 1.86 (m, 2H)

Synthesis of 3-[4-(2-methoxyphenyl)piperaziny-1-yl]propyl methanesulfonate (3)

1-(2-methoxyphenyl)piperazine (0.96 g, 5 mmol) and 3-bromo-1-propanol (1.04 g, 7.5 mmol) were dissolved in anhydrous CH_3CN (20 mL). K_2CO_3 (1.04 g, 7.5 mmol) was added and the mixture was heated under reflux for about 7 h. The solid phase was removed by filtration. The solvent was then evaporated *in vacuo* to obtain **2**, which was used for the next step reaction without further purification.

To the solution of **2** (1.25 g, 5 mmol) and Et_3N (1 mL, 7.5 mmol) in CH_2Cl_2 (50 mL), cooled to 0–5 °C, was added dropwise with stirring a solution of methanesulfonyl chloride (0.87 g, 7.5 mmol) in CH_2Cl_2 (20 mL), and the reaction mixture was then stirred at room temperature overnight. The solution was washed with saturated aqueous ammonium chloride (30 mL) and brine (30 mL) and then dried (Na_2SO_4). The solvent was then evaporated *in vacuo* and the residue was purified by column chromatography on silica gel (MeOH/ CH_2Cl_2, 1:15) to give a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.98–6.89 (m, 4H), 3.73 (s, 3H), 3.45–3.32 (m, 6H), 3.20–3.13(m, 7H), 2.39(m, 2H), 1.42 (m, 2H).

Synthesis of *N*-[3-[4-(2-methoxyphenyl)piperaziny-1-yl]propyl]Tri-TFA-cyclam (4)

To a stirred solution of **1** (0.97 g, 2 mmol) and anhydrous K_2CO_3 (0.42 g, 3 mmol) in anhydrous CH_3CN (20 mL) was added **3** (0.65 g, 2 mmol) and the mixture was heated under reflux for about 9 h. The solid phase was removed by filtration and the solvent was then evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (MeOH/ CH_2Cl_2, 1:16) to give a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.07-6.89 (m, 4H), 3.90 (s, 3H), 3.16–3.04 (m, 12H), 2.95–2.79(m, 16H), 1.82–1.84(m, 6H)

Synthesis of *N*-[3-[4-(2-methoxyphenyl)piperaziny-1-yl]propyl]cyclam (MPPC)

Substrate **4** (0.36 g, 0.5 mmol) was dissolved in methanol (10 mL) and NaOH (0.40 g, 10 mmol) was added at 0°C. The mixture was stirred for 30 min at room temperature. The solid phase was removed by filtration and the solvent was evaporated *in vacuo* to obtain a white solid. Water (20 mL) and chloroform (10 mL) were added. The organic phase was separated and washed with water (3 × 10 mL). The aqueous phases were reextracted with chloroform (15 mL) and the combined organic solutions were dried over Na_2SO_4. Solvent was evaporated *in vacuo* to yield a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.04–6.83 (m, 4H), 3.99(s, 3H), 3.37–2.72 (m, 28H), 1.76–1.81 (m, 6H).

Preparation of the ^{99m}TcN-MPPC

The ^{99m}TcN-MPPC was prepared by a two-step procedure. First, 1 mL saline-containing $Na^{99m}TcO_4$ (about 740 MBq) was added into a SDH kit and kept for 15 min at room temperature to obtain the $[^{99m}TcN]^{2+}$ intermediate. Second, 0.1 mL $[^{99m}TcN]^{2+}$ (about 74 MBq) intermediate was added to the 0.1 mL ethanol containing 1.5 mg MPPC ligand. The pH of the solution was adjusted to 11–12 by addition of 0.9 mL of buffer (composition: 50 mL of 0.15 M Na_2HPO_4+75 mL of 0.1 M NaOH) and the reaction mixture was heated under N_2 at 100 °C for 30 min.

The $[^{99m}TcN]^{2+}$ intermediate and final ^{99m}TcN-MPPC complex were characterized by TLC and HPLC. TLC was performed on a polyamide film with saline and acetonitrile as the mobile phase. HPLC was performed as following: about 5 μL of the ^{99m}TcN-MPPC complex solution was injected into the column, C18 reverse phase column was eluted at flow rate of 1 mL/min and water (solvent A) and acetonitrile (solvent B) were used as the mobile phase in the following gradient (0–20 min, 100–20% B, 20–35 min, 20–100% B).

Determination of the partition coefficient for the complex

The partition coefficient of the complex was determined by measuring the activity that partitioned between the 1-octanol and aqueous phosphate buffer (0.025 mol/L, pH 7.4) under strict equilibrium conditions. 2 mL 1-octanol and 2 mL ^{99m}TcN-MPPC 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 log *P*.

Paper electrophoresis

The sample of the complex was spotted on chromatography paper strips (10 cm × 1 cm), which was 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) at 150 V for 2 h. Then the strips were left to dry, and the distribution of radioactivity on the strip was determined.

In vitro stability study

The stability of ^{99m}TcN-MPPC in the reaction mixture was assayed by measuring the RCP by TLC and HPLC at 1, 2 and 4 h after preparation at room temperature (25 °C).

In vivo stability study

The experiments in rats were carried out in compliance with the national laws relating to the care and experiments on laboratory animals. *In vivo* stability study of the ^{99m}TcN-MPPC in normal male Wistar rats (body mass 250–260 g) was performed by injecting the tracer (0.2 mL, 2.0–3.7 MBq) into the tail vein. Rats were sacrificed at 5, 60 and 120 min post-injection. Blood samples taken were centrifuged for 15 min at 5000 r/min to collect serum. The serum was transferred (1 mL) into a tube and mixed with acetonitrile (1 mL). The resulting mixture was vortexed for 10 s, and centrifuged for 15 min at 5000 r/min. The supernatant liquid was analyzed by HPLC following the same procedure as described above for preparation of the ^{99m}TcN-MPPC.

Conclusions

We used bifunctional chelating agent 1,4,8,11-tetraazacyclotetradecane (cyclam) to attach the technetium-99m to **1**-(2-methoxyphenyl)piperazine, an important pharmacophore for reported 5-HT_{1A} PET and SPECT ligands and obtained MPPC ligand for the first time. This provided a new method for attaching the technetium to the pharmacophore moiety of WAY 100635 and preparing neutral technetium-99m complexes for 5-HT_{1A} receptor imaging. ^{99m}TcN-MPPC complex was prepared by using two-step procedure with high radiochemical purity (> 95%). It was a neutral and hydrophilic complex with high *in vitro* and *in vivo* stability.

In further study, we will characterize and determine the structure of ^{99m}TcN-MPPC using Re model compound. At the same time, the feasibility of this ^{99m}Tc-nitrido complex for 5-HT_{1A} receptor imaging will be evaluated in the future.

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