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2-Amino-3-(1-(4-(4-(2-methoxyphenyl) piperazine-1-yl)butyl)-1*H*-1,2,3-triazol-4-yl) propanoic acid: synthesized, ^{99m}Tc-tricarbonyl labeled, and bioevaluated as a potential $5HT_{1A}$ receptor ligand

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To develop a novel $5HT_{1A}$ receptor imaging agent, a new methoxyphenyl piperazine derivative was synthesized and radiolabeled with ^{99m}Tc-tricarbonyl precursor. We used the Cu (I)-catalyzed cycloaddition of azide and terminal alkynes to synthesize 1, 2, 3 triazole as the metal chelating system. This synthesis provided reliable and reproducible method to attach technetium to the methoxyphenyl piperazine moiety. ^{99m}Tc-tricabonyl labeling of ligand was performed at high radiochemical purity (greater than 95%). The radiolabeled compound was stable at least 24 h in room temperature. In vitro stability study in human serum albumin showed more than 90% stability in 37 °C incubation for 6 h. Biodistribution studies in rat have shown brain hippocampus uptake of 0.31 ± 0.02 %ID/g at 5-min post-injection. The favorable in vitro/in vivo stability, lipophilicity, and biodistribution profiles suggest that this radioconjugate is a good candidate for further exploration of its potential clinical application.

Keywords: methoxyphenyl piperazine; ^{99m}Tc-tricarbonyl; click chemistry; $5HT_{1A}$ receptor

Introduction

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) is involved in numerous central nervous system functions and psychiatric disorders.[1,2] The receptors that are activated by 5-HT have been divided into different classes which of these receptors, the $5HT_{1A}$ receptors have been implicated in the pathogenesis of depression, anxiety, schizophrenia, epilepsy, and eating disorders.[3] WAY100635 is a well-known high-affinity $5HT_{1A}$ receptor antagonist.[4,5] Many ^{11}C and ^{18}F radiolabeled derivatives of WAY100635 have been synthesized and evaluated for use in positron emission tomography,[6–9] Whereas its iodinated analogs have been reported for use in single-photon emission computed tomography imaging of $5HT_{1A}$ receptors.[10,11]

Because ^{99m}Tc is the radionuclide of choice in diagnostic nuclear medicine, many efforts have been focused on developing ^{99m}Tc-based radioligands for $5HT_{1A}$ receptors. The successful development of ^{99m}Tc-TRODAT as a radioligand for the dopamine transporter has shown the feasibility of imaging specific transporters in the brain with radiotracers based on ^{99m}Tc.[12,13] Accordingly, many various radioligands have been made by several groups to develop ^{99m}Tc complex for $5HT_{1A}$ receptor imaging,[14–20] but there is no ideal ^{99m}Tc agent for imaging central nervous system receptors.[21] Over the last years, the radiochemistry of ^{99m}Tc-tricarbonyl complexes has been extended to find useful applications in radiochemistry and nuclear medicine. The basis for this kind of radiochemistry is the convenient preparation of the Tc-tricarbonyl precursor $[^{99m}Tc(CO)_3(H_2O)_3]^+$ either in the classic way with CO_{gas} and borohydride reduction or with an IsoLink kit.[33,34] In the resulting precursor, the three water molecules can easily be replaced by ligands that contain suitable donor atoms to form stable complexes.

The click chemistry that is a Cu (I)-catalyzed cycloaddition of azides and terminal alkynes has only recently found applications in the design of ligands for transition metals.[22–30] This catalyzed cycloaddition give high chemical yield under mild reaction conditions even in aqueous media. It is efficient, selective, regiospecific, and devoid of side reactions. Their organometallic ^{99m}Tc $(CO)_3$ complexes proved to be stable in in vivo studies. This reaction allows simultaneous formation of the chelating system and conjugation to a biomolecule in a single high-yielding step. Unexpectedly, few examples of 1,2,3-triazole chelators are reported that were obtained via click chemistry,[26,31] even though triazoles are known to be potent ligand systems for various transition metals.[32]

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The present study demonstrates the application of click chemistry in the preparation of an organometallic ^{99m}Tc complex of a methoxyphenyl piperazine derivative, the pharmacophore moiety of WAY100635. Methoxyphenyl piperazine has been derivatized to 1-(3-azidopropyl)-4-(2-methoxyphenyl) piperazine that undergoes [3 + 2] cycloaddition reaction with propargyl glycine. The 1, 2, 3-triazole derivative thus formed has been radiolabeled with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ core. And the properties of radioligand have been investigated by carrying out suitable in vitro and in vivo studies.

Results and discussion

The 1-(2-methoxyphenyl) piperazine unit of WAY100635 is a moiety that has favorable affinity toward $5HT_{1A}$ receptors and was chosen as the parent molecule for derivatization to an azide in preparation of a clicked 1, 2, 3-triazole. Because of good results in previous studies, propyl spacers were used for linkage of 1-(2-methoxyphenyl) piperazine.[9] The 1-(2-methoxyphenyl) piperazine was reacted with 3-bromo-1-propanol as the alkylation agent to prepare 3-(4-(2-methoxyphenyl) piperazine-1yl)propanol **1**. The reaction was carried out in anhydrous CH_3CN in the presence of K_2CO_3 by refluxing overnight (Figure 1). By treating compound **1** with methanesulfonyl chloride in the anhydrous CH_2Cl_2 in the presence of Et_3N, the methyl 3-(4-(2-methoxyphenyl) piperazine-1-yl) propane-1-sulfonate **2** was obtained. Compound **2** was converted to an azide in the presence of NaN_3 and it was ready to click reaction via azide complex.

All the intermediates and final product have been characterized by infrared (IR), NMR, and ESI-mass spectroscopy (MS). In ^{1}H NMR, a downfield shift has been observed for C-1 proton from δ 3.85–3.83 ppm (corresponding to compound **1)** to δ 4.38–4.35 ppm, indicating the desired mesylation of compound **1**. The SN2 azidation reaction was performed completely. Corresponding to this in IR spectra, the stretching band at 2095 cm^{-1} (characteristic of N_3 functionality) indicated the desired derivatization. In addition, the C-1 proton shift from δ 4.38–4.35 ppm in mesylated compound to δ 3.39–3.37 ppm in azide compound was a further confirmation of azidation reaction.

On the basis of click reaction, a one-pot procedure was chosen for labeling the compound **3** with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor. The (3 + 2) cycloaddition and labeling was performed using compound **3**, propargyl glycine, $Cu(OAc)_2$, sodium ascorbate, and $[^{99m}Tc(CO)_3(H_2O)_3]^+$ that were heated in 100 °C (Figure 1).

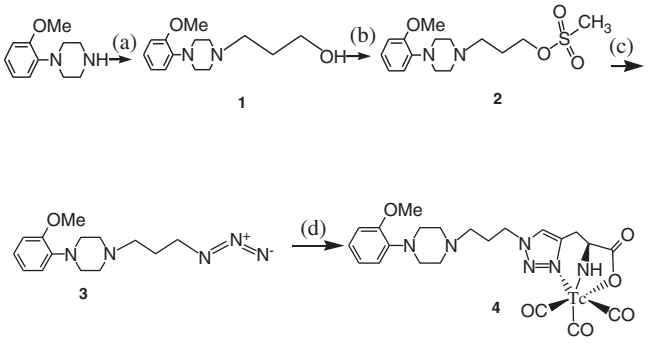

Figure 1. Synthesis of radioligand. Reagents: (a) 3-bromo-1-propanol and K_2CO_3, CH_3CN; (b) CH_3SO_2Cl, Et_3N, and CH_2Cl_2; (c) NaN_3 and dimethylformamide; and (d) propargyl glycine, $[^{99m}Tc(CO)_3(H_2O)_3]^+$, copper acetate, sodium ascorbate, and *t*BuOH/H_2O.

The $^{99m}Tc(CO)_3$-triazole complex (**4**) has been characterized by comparison with the corresponding rhenium complex (**5**), which has been synthesized using $[NEt_4][Re(CO)_3Br_3]$ precursor using conditions similar to ^{99m}Tc labeling. The ultraviolet-HPLC chromatogram retention time of rhenium complex (Figure 2c) was observed to be 18.62 min, which matches well with 18.82 min γ-HPLC chromatogram retention time of $^{99m}Tc(CO)_3$ complex (**4**) (Figure 2b). The complex showed a retention time of 18.82 min that was separated from the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ eluting out at 17.54 min. Because of closeness of their retention time for both compounds, further investigation was performed. Corresponding to this, after a 24-h period, the reaction mixture was re-injected to HPLC, but no further $[^{99m}Tc(CO)_3(H_2O)_3]^+$ peak was observed. This is a further indication that this observed peak belongs to desired labeled ligand. According to the literature, the labeled compound should possess a relatively small size with lipophilic characteristic to cross the blood–brain barrier.[10] The calculated partition coefficient (log $P_{oct/wat}$) for $^{99m}Tc(CO)_3$ complex was 0.34 ± 0.02 that seems not to be lipophilic enough to cross blood–brain barrier; however, biodistribution experiments showed a moderate brain uptake (Table 2).

The radioligand was found to be stable even after a 24-h period. In serum stability studies, nearly 4.36 ± 0.09% of the activity was associated with the precipitate obtained after ethanol

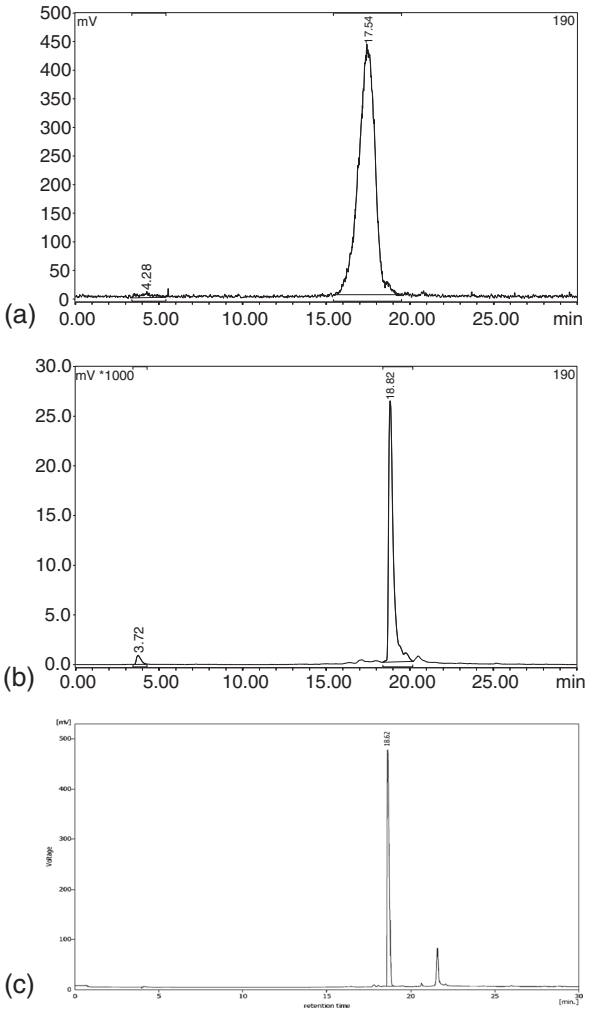

Figure 2. HPLC profile of (a) $[^{99m}Tc(CO)_3(H_2O)_3]^+$, (b) $^{99m}Tc(CO)_3$-triazole complex, and (c) $Re(CO)_3$-triazole complex.

addition, indicating the low binding of complex with serum proteins. The ethanol fraction was characterized by HPLC where a single peak was observed at the same time (18.82 min, >90%) as that of the complex, indicating no decomposition of the complex and therefore its stability under 37 °C incubation.

Biological evaluation of radiolabeled ligand was performed in rat. The results are shown in Table 1. In biodistribution studies, clearance from the blood circulation was fast. The remaining activity in the blood decreased from 2.22 ± 0.35 %ID/g at 2 min to 0.42 ± 0.20 %ID/g at 30 min. Whole-body clearance showed significant accumulation of activity in kidneys and liver 2 min after injection (7.56 ± 1.09 and 6.15 ± 1.04 %ID/g, respectively). Within 30 min, activity decreased in kidney and liver with a moderate clearance (3.51 ± 0.47 and 4.11 ± 0.59 %ID/g, respectively). This favorable clearance is probably due to the balance in lipophilicity for this radioligand and indicates both renal excretion and hepatobiliary metabolism for tracer. The distribution of the activity in different parts of rat brain regions showed mild overall accumulation of radiotracer in the brain (Table 2). The radioactivity concentration of hippocampus and cerebellum at 5-min post-injection was 0.31 ± 0.02 and 0.10 ± 0.01 %ID/g, respectively (Figure 3). The uptake in cortex was 0.25 ± 0.01 %ID/g at 5 min. At 30-min post-injection, the retention of activity in hippocampus was observed (0.26 ± 0.05 %ID/g), whereas the uptake in cerebellum decreased gradually (0.05 ± 0.01 %ID/g). The cortex activity remained almost constant (0.19 ± 0.01 %ID/g). The results showed that distribution of activity in different parts of the brain

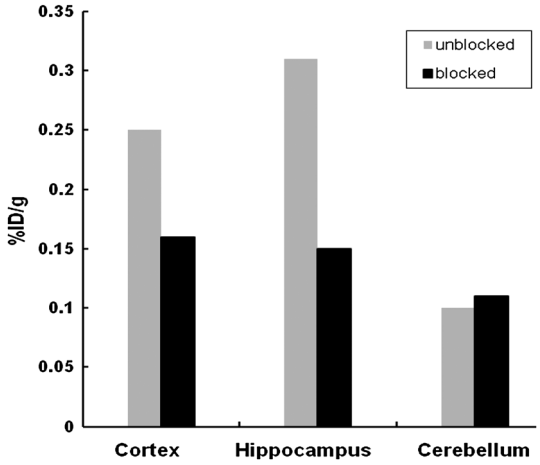

Figure 3. Uptake of radioactivity in regions of the brain in rat 5-min post-injection of ^{99m}Tc $(CO)_3$-triazole complex.

was correlated with the location of $5HT_{1A}$ receptors in the brain. The retention of activity in hippocampus could be caused by specific binding of the radioligand to the $5HT_{1A}$ receptors and tolerable lipophilicity of this complex (becauseof a 3-carbon length spacer and carbonyl itself as a precursor).

In blocking investigation, the uptake in different parts of the brain was decreased, which for hippocampus and cortex was statistically significant ($P < 0.05$) (0.31–0.15 and 0.25–0.16%,

Table 1. Biodistribution of ^{99m}Tc-methoxyphenyl piperazine-triazole (with 3-carbon spacer) in normal rat (%ID/g ± standard deviation, $n = 3$)

	Post-injection time (min)				
Organs	2	5	5 block	15	30
Blood	2.22 ± 0.35	2.02 ± 0.4	1.95 ± 0.42	0.97 ± 0.25	0.42 ± 0.2
Kidney	7.56 ± 1.09	9.34 ± 1.21	9.98 ± 1.45	2.52 ± 0.21	3.51 ± 0.47
Spleen	0.99 ± 0.13	1.15 ± 0.28	1.23 ± 0.18	2.04 ± 0.25	1.62 ± 0.12
Intestine	2.82 ± 0.52	1.76 ± 0.54	1.31 ± 0.08	3.36 ± 0.12	6.73 ± 1.27
Liver	6.15 ± 1.04	6.51 ± 0.99	5.59 ± 0.91	4.47 ± 0.53	4.11 ± 0.59
Lung	1.83 ± 0.39	1.17 ± 0.23	1.43 ± 0.25	0.87 ± 0.07	0.78 ± 0.12
Heart	1.47 ± 0.17	0.92 ± 0.15	0.85 ± 0.37	0.63 ± 0.24	0.58 ± 0.10

Table 2. Biodistribution of ^{99m}Tc-methoxyphenyl piperazine-triazole (with 3-carbon spacer) in different regions of the brain in normal rat (%ID/g ±standard deviation), $n = 3$

Region of brain	2 min	5 min	5 min[a]	15 min	30 min
Cortex	0.22 ± 0.04	0.25 ± 0.01	0.16 ±0.02[b]	0.22 ± 0.06	0.19 ± 0.01
Hippocampus	0.26 ± 0.02	0.31 ± 0.02	0.15 ± 0.04[b]	0.20 ± 0.02	0.26 ± 0.05
Cerebellum	0.13 ± 0.02	0.10 ± 0.01	0.11 ± 0.02	0.09 ± 0.03	0.05 ± 0.01
ROB	0.12 ± 0.01	0.13 ± 0.06	0.09 ± 0.01	0.10 ± 0.02	0.08 ± 0.06
Brain	0.15 ± 0.01	0.11 ± 0.05	0.09 ± 0.03	0.09 ± 0.01	0.08 ± 0.06
Hippocampus/cerebellum	2	3.1	1.36	2.2	5.2
Brain/blood	0.07	0.06	0.05	0.09	0.19

ROB, rest part of the brain except cortex, hippocampus, and cerebellum.
[a]blocked,
[b]Two-tailed *t*-test showed statistically significant difference in 5-min post-injection between cortexes and hippocampus blocked and unblocked in rats brain.

respectively). The clearance of nonspecific uptake from brain and specific affinity to $5HT_{1A}$ receptors in brain could be the main reason for the blocking results. There were no significant changes in activity of other major organs with blocking agent.

To determine the affinity of the $^{99m}Tc(CO)_3$-triazole complex toward binding with $5HT_{1A}$ receptor, the in vitro studies were carried out in rat homogenized hippocampus membrane at 37 °C temperature. The percentage of binding of the complex in membrane receptors was found to be $39 \pm 2.64\%$ when 100 µl (6.6 MBq) of HPLC purified tracer was added. The binding in competition studies in the presence of 0.2 nM of $5HT_{1A}$ full agonist 8-OH-DPAT was $13 \pm 0.89\%$. The results show the non-specific binding in the presence of blocking agent is less than 35% of total binding. On the other hand, the binding for $^{99m}Tc(CO)_3$-triazole complex is receptor mediated and specific.

Experimental

All reagents were obtained from known commercial sources such as Sigma-Aldrich (Munich, Germany) and Fluka (Munich, Germany) and used without further purification. The $^{99m}TcO_4^-$ was eluted from an in-house $^{99}Mo/^{99m}Tc$ column generator using 0.9% saline. IR spectra were recorded as KBr pellets on Perkin Elmer (Shelton, USA) spectrometer. High-resolution fast bombardment MS was performed using an Agilent 1100 (Alto, USA)/Bruker Daltonic (Ion trap) VL (Bremen, Germany) instrument. ^1H NMR spectra were obtained on Bruker 500 MHz instrument using $CDCl_3$ as the solvent.

Monitoring of all reactions was performed with analytical reversed-phase HPLC (RP-HPLC) on a JASCO 880-PU intelligent pump HPLC system (Tokyo, Japan) equipped with a multiwavelength detector and a flow-through Raytest GABI g-detector. CC 250/4.6 Nucleosil 120–5 C-18 column from Teknokroma (Barcelona, Spain) was used for HPLC. The HPLC solvents consisted of 0.1% trifluoroacetic acid/water (solvent A) and acetonitrile (solvent B). The followed gradient program was used in HPLC system: 0 min 95% A, 5 min 95% A, 25 min 0% A, 27 min 0% A, 30 min 95%A, flow = 1 ml/min, $\gamma = 280$ nm.

Quantitative gamma counting was performed on an ORTEC model 4001 M g-system (London, England) well counter.

Synthesis of 3-(4-(2-methoxyphenyl) piperazine-1yl) propanol (1)

A 1-(2-Methoxyphenyl) piperazine (0.96 g, 5 mmol) and 3-bromo-1-propanol (1.04 g, 7.50 mmol) were dissolved in anhydrous CH_3CN (20 ml). K_2CO_3 (1.04 g, mmol) was added, and the mixture was heated under reflux overnight. The solid phase was removed by filtration. The product was purified on a silica-gel column using $MeOH/CH_2Cl_2$ 1 : 15 as the eluting solvent. Yield: 95.2%; white solid; ^1H NMR ($CDCl_3$) δ: 7.03–6.86 (m, 4H), 3.87 (s, 3H), 3.85–3.83 (T, 2H), 3.11 (broad s, 4H), 2.76 (broad s, 4H), 2.73–2.71 (T, 2H), 1.80–1.75 (m, 2H).

Synthesis of methyl 3-(4-(2-methoxyphenyl) piperazine-1-yl) propane-1-sulfonate (2)

To the solution of **1** (625 mg, 2.51 mmol) and Et_3N (419.3 µl, 3.01 mmol) in CH_2Cl_2 (25 ml), cooled to 0–5 °C, was added dropwise with stirring a solution of methane sulfonyl chloride (193.4 µl, 1.26 mmol) for 30 min. The reaction mixture was then stirred at same temperature and was monitored with thin layer chromatography to complete the reaction. The reaction mixture was washed with cold water, cold 10% HCl solution, $NaHCO_3$ (sat.), and NaCl (sat.) and then dried with Na_2SO_4. The solvent was then evaporated in vacuum, and the residue was purified by column chromatography on silica-gel using 1 : 15 $MeOH/CH_2Cl_2$ as eluting solvent. Yield: 95%; slightly yellow oil; ^1H NMR (500 MHz, $CDCl_3$) δ: 7.06–6.85 (m, 4H), 4.38–4.35 (T, 2H), 3.87 (s, 3H), 3.80–3.55 (T, 2H), 3.51 (broad s, 4H), 3.15 (broad s, 4H), 3.05 (s, 3H), 2.32–2.26 (M, 2H).

Synthesis of 1-(3-azidopropyl)-4-(2-methoxyphenyl) piperazine (3)

To a stirred solution of compound **2** (189.1 mg, 0.69 mmol) in 10-ml dry dimethylformamide, sodium azide (138 mg, 2.07 mmol) was added, and the stirring was continued at 100 °C overnight. Water was then added to the reaction mixture and the aqueous phase extracted with diethyl ether (3 × 10 ml). The organic extract was washed with water, brine, and dried over Na_2SO_4. The solvent was removed under vacuum, and the product was purified by silica-gel column using 1% $MeOH/CH_3Cl$ as the eluting solvent. Yield: 87% colorless crystals ^1H NMR (500 MHz, $CDCL_3$) δ: 7.03–6.86 (m, 4H), 3.87 (s, 3H), 3.39–3.37 (t, 2H), 3.11 (broad s, 4H), 2.66 (broad s, 4H), 2.50–2.52 (t, 2H), 1.86–1.80 (m, 2H). IR (KBr, ν cm^{-1}): 2095. ESI-MS *m/z*: 275 [M] (calcd for $C_{14}H_{21}N_5O$: 275.17).

Preparation of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor

The precursor $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was prepared according to the reported procedure.[33] In a closed vial, 4.5 mg Na_2CO_3, 5.5 mg $NaBH_4$, and 20-mg sodium potassium tartrate were added, and the vial was flushed with CO_{gas}, and after that, the 30-mCi activity that was eluted from a commercial $^{99}Mo/^{99m}Tc$ generator was added and heated to 90 °C for 30 min. After cooling the vial to room temperature, pH was neutralized to 7 through adding HCl (1 N). Radiochemical purity of the precursor was checked by RP-HPLC.

Preparation of $^{99m}Tc(CO)_3$-1-(3-azidopropyl)-4-(2-methoxyphenyl) piperazine (4)

Compound **3** (5 mg, 1.6×10^{-2} mmol) was dissolved in *t*BuOH/H_2O, then aqueous solutions of propargyl glycine (100 µl, 0.1 M), copper acetate (20 µl, 0.01 M) and sodium ascorbate (40 µl, 0.01 M) were added. The $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor (100 µl, 10 MCi) was added in the reaction mixture. The reaction mixture was heated at 100 °C for 1 h. The complex prepared was characterized by HPLC.

Preparation of Re $(CO)_3$-1-(3-azidopropyl)-4-(2-methoxy phenyl) piperazine (5)

Compound **3** (5 mg, 18 µmol), $[NEt_4]_2[ReBr_3(CO)_3]$ (13.5 mg, 18 µmol), propargyl glycine (2.03 mg, 18 µmol), copper (II) acetate (101 µg, 0.56 µmol), and sodium ascorbate (112 µg, 0.56 µmol) were dissolved in *t*-butanol/H2O (1 : 1, 2 ml). The reaction mixture was incubated in a sealed vial at 100 °C for 1 h. The $[NEt_4]_2[ReBr_3(CO)_3]$ precursor was prepared from rhenium pentacarbonyl bromide $[Re(CO)_5Br]$, using a published procedure.[35] The compound **5** was characterized by ESI-MS *m/z*: 657 [M] (calcd for $C_{22}H_{26}N_6O_6Re$: 657.15).

Partition coefficient and in vitro stability study

The partition coefficient of the complex was determined by measuring the activity that partitioned between the 1-octanol and water. A 1-ml 1-octanol and 1-ml water and labeled complex (100 µl, 89 µCi) were mixed in a centrifuge tube. The mixture was vortexed at room temperature for 5 min and then centrifuged at 2000 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 = (count per minute in octanol–count per minute in background)/(count per minute in water–count per minute in background). The final partition coefficient value was expressed as log P.

The stability of $^{99m}Tc(CO)_3$ complex was assayed by monitoring the HPLC elution profile and determined the radiochemical purity after incubation at room temperature for 24 h. To determine the in vitro serum stability, 100 µl of radiolabeled complex was incubated with 1-ml human serum at 37 °C for 24 h. A 1-ml ethanol was added to the aforementioned solution. The precipitate was separated by centrifugation. The supernatant was injected in HPLC to determine the stability of the complex.

Biodistribution study

Animal experiments were performed in compliance with the regulations of our institution and with generally accepted guidelines governing such work. A group of three rats (220–280 g) received 20 MBq of high specific activity radiotracer in 0.3 ml of buffered saline via a tail vein. The radiolabeled complex was purified by RP-HPLC and collected in a vial. The solvent was removed under vacuum. The radioligand was diluted by saline for injection. The HPLC chromatogram revealed any decomposition of complex. Groups of three rats per time point with results expressed (mean ± standard deviation) as the percentage injected dose per gram of wet tissue (%ID/g). For blocking studies, rats received a tail vein injection of a solution (50 μl) of 8-OH-DPAT the putative blocker (2 mg/kg) 1 min before administration of the radiotracer and were dissected. The brain was rapidly removed, chilled, and dissected. Samples from different brain regions (cortex, hippocampus, and cerebellum) were collected, weighed, and counted.

Receptor binding assay

Preparation of a membrane receptor

Rats were decapitated, and their brains were rapidly chilled and dissected to obtain the hippocampus on an ice-cold plate. Freshly dissected tissues were homogenized in 40 volumes of ice-cold Tris–HCl buffer (50 mM, pH = 7.5 at room temperature) by Bandelin Sonopuls HD 2070 (Berlin, Germany) for 30 s and centrifuged at 30,000 g for 15 min at 4 °C. Then, the resulting pellets were resuspended in the same buffer, and the process was repeated for three times. Between the washing processes, the resuspended pellets were incubated for 10 min at room temperature to remove the endogenous 5-HT. The final suspended pellets were stored in 500 μl aliquots at −70 °C. The protein content determination was performed according Bradford methods, by using bovine serum albumin as standard.[36]

Specific radioligand binding assay

A 100 μl (10 μCi) of purified radioligand by HPLC was added to 50 μl volume of rat hippocampal homogenates aliquote (which corresponded to 50 μg of protein), and final volume was adjusted to 2 ml by Tris–HCl buffer (50 mM Tris–HCl, 0.1% ascorbic acid, 2 mM $CaCl_2$, pH = 7.5) in centrifuge vials. For another triplicate of samples was added 50 μl (3.3 μg) of $5HT_{1A}$ full agonist 8-OH-DPAT for competition of radioligand. Incubation was carried out for 30 min at 37 °C. After centrifugation at 15,000 g at 4 °C for 10 min. After washing for three times by same buffer, the pellets were counted by gamma counter and subsequently washed twice with 10-mM Tris–HCl containing 150-mM NaCl. Specific radioligand binding was determined by subtraction of total binding and nonspecific binding. The experiments were carried out in triplicate.

Conclusion

In this study, a new methoxyphenyl piperazine derivative was successfully synthesized and radiolabeled in high yield specific activity (23.13 MBq/μmol) by $[^{99m}Tc\ (CO)_3(H_2O)_3]^+$ precursor via click chemistry as a potential $5HT_{1A}$ receptor imaging agent. Preliminary biological evaluation showed moderate brain uptake. Accumulation in the different parts of the brain in rat, followed by excretion via the kidney and liver, were observed for the prepared conjugate. Receptor binding studies indicated specific binding in rat brain membrane. It would be best to modify the complex to increase the lipophilicity and respectively brain uptake.

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Conflict of Interest

The authors did not report any conflict of interest.

References

[1] N. Aznavour, L. Zimmer, *Neuropharmacology* **2007**, *52*(3), 695–707.
[2] C. Gross, X. Zhang, K. Stark, S. Ramboz, R. Oosting, L. Kirby, L. Santarelli, S. Beck, R. Hen, *Nature* **2002**, *416*(6879), 396–400.
[3] W. C. Drevets, E. Frank, J. C. Price, D. J. Kupfer, P. J. Greer, C. Mathis, *Nucl. Med. Biol.* **2000**, *27*(5), 499–507.
[4] A. Fletcher, D. J. Bill, I. A. Cliffe, E. Forster, Y. Reilly, *Br. J. Pharmacol.* **1994**, *112*, 91P.
[5] E. A. Forster, I. A. Cliffe, D. J. Bill, G. M. Dover, D. Jonse, Y. Reilly, A. Fletcher, *Eur. J. Pharmacol.* **1995**, *281*(1), 81–88.
[6] S. Houle, J. N. Dasilva, A. Wilson, *Nucl. Med. Biol.***1996**, *23*, 463–466.
[7] L. Lang, E. Jagoda, B. Schmall, M. Sassaman, Y. Ma, W. Eckelman, *Nucl. Med. Biol.* **2000**, *27*, 457–462.
[8] D. Lebars, C. Lemaire, N. Ginovart, A. Plenevaux, J. Aerts, C. Brihaye, W. Hassoun, V. Leviel, P. Mekhsian, D. Weissmann, J. F. Pujol, A. Luxen, D. Comar, *Nucl. Med. Biol.* **1998**, *25*, 343–350.
[9] S. Marchais, B. Nowicki, H. Wikstrom, L. T. Brennum, C. Halldin, V. W. Pike, *Bioorg. Med. Chem.* **2001**, *9*, 695–702.
[10] M. Vandecapelle, F. De Vos, H. Vermersch, G. De Ley, K. Audenaert, D. Leysen, R. A. Dierckx, G. Slegers, *Nul. Med. Biol.* **2001**, *28*, 639–643.
[11] Z.-P. Zhuang, M.-P. Kung, H. F. Kung, *J. Med. Chem.* **1994**, *37*, 1406–1407.
[12] H. F. Kung, H. J. Kim, M. P. Kung, S. K. Meegalla, K. Plossl, H. K. Lee, *Eur. J. Nucl. Med. Mol. Imaging* **1996**, *23*(11), 1527–1530.
[13] H. F. Kung, *Nucl. Med. Biol.* **2001**, *28*(5), 505–508.
[14] R. Alberto, R. Schibli, A. P. Schubiger, U. Abram, H. J. Pietzsch, B. Johannsen. *J. Am. Chem. Soc.* **1999**, *121*(25), 6076–6077.
[15] A. Drews, H. J. Pietzsch, R. Syhre, S. Seifert, K. Varnas, H. Hall, C. Halldin, P. Karlsson, C. Johnsson, H. Spies, B. Johannsen, *Nucl. Med. Biol.* **2002**, *29*(4), 389–398.
[16] I. Heimbold, A. Drews, M. Kretzschmar, K. Varnas, H. Hall, C. Halldin, R. Syhre, W. Kraus, H. J. Pietzsch, S. Seifert, *Nucl. Med. Boil.* **2002**, *29*(4), 389–398.
[17] I. Heimbold, A. Drews, R. Syhre, M. Kretzschmar, H. Pietzsch, *Eur. J. Nucl. Med.* **2002**, *29*, 82–87.
[18] A. Leon, A. Rey, L. Mallo, I. Pirmettis, M. Papadopoulos, E. Leon, M. Pagano, E. Manta, M. Incerti, C. Raptopoulou, *Nucl. Med. Biol.* **2002**, *29*(2), 217–226.
[19] D. Papagiannopoulou, I. Pirmettis, C. Tsoukalas, L. Nikoladou, G. Drossopoulou, C. Dalla, M. Pelecanou, Z. Papadopoulou-Daifotis, M. Papadopoulos, E. Chiotellis, *Nucl. Med. Biol.* **2002**, *29*(8), 825–832.
[20] A. Chiotellis, C. Tsoukalas, M. Pelecanou, I. Pirmattis, M. Papadopoulos, *Appl. Rad. Iso.* **2012**, *70*, 957–964.
[21] IAEA. Technical Reports Series. No. 426, **2004**.
[22] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2001**, *113*, 2056–2075 Angew. Chem. Int. Ed. **2001**, 40, 2004–2021.
[23] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew, Chem.* **2002**, *114*, 2708–2711; Angew. Chem. Int. Ed. **2002**, 41, 2596–2599.
[24] T. R. Chan, R. Hilgraf, K. B. Sharpless, V. V. Fokin, *J. Am. Chem. Soc.* **2004**, *126*, 2853–2855.
[25] B. Colasson, M. Save, P. Milko, J. Roithova, D. Schroder, O. Reinaud, *Org. Lett.* **2007**, *9*, 4987–4990.
[26] Q. Dai, W. Z. Gao, D. Liu, L. M. Kapes, X. M. Zhang, *J. Org. Chem.* **2006**, *71*, 3928–3934.
[27] R. J. Detz, S. A. Heras, R. De Gelder, P. Van Leeuwen, H. Heiemstra, J. N. H. Reek, J. H. Van Maarseveen, *Org. Lett.* **2006**, *8*, 3227–3230.
[28] R. M. Meudtner, R. Ostermeier, M. Goddard, C. Limberg, S. Hecht, *Eur. J. Inorg. Chem.* **2007**, *13*, 9834–9840.

[29] U. Monkowius, S. Ritter, B. Konig, M. Zabel, H. Yersin, *Eur. J. Inorg. Chem.* **2007**, *13*, 9834–9840.
[30] T. L. Mindt, H. Struthers, L. Brans, T. Anguelov, C. Schweinsberge, V. Maes, D. Tourwe, R. Schibi, *J. Am. Chem. Soc.* **2006**, *128*, 15096–15097.
[31] D. S. Moore, S. D. Robinson, *Adv. Inorg. Chem.* **1988**, *32*, 171–239.
[32] A. J. Link, M. K. S. Vink, D. A. Tirrell, *J. Am. Chem. Soc.* **2004**, *126*, 10598–10602.
[33] R. Alberto, R. Schibli, A. Egli, A. P. Schubiger. *J. Am. Chem. Soc.* **1998**, *120*, 7989–7988.
[34] M. E. Dyszlewski, M. J. Bushman, R. Alberto, J. W. Brodack, H. Knight, J. MacDonald, L. K. Chinen, E. G. Webb, E. Vries, M. Pattipawaej, J. L. Vanderhyden, *J. Labelled Compd. Radiopharm.* **2001**, *44*, S483–S485.
[35] R. Alberto, A. Egli, U. Abram, *et al. Dalton Trans.* **1994**, *19*, 2815–2820.
[36] M. Bradford, *Anal. Biochem.* **1976**, *72*, 248–54.