Highly Efficient Synthesis and Imaging Studies of an Arylpiperazine Derivative as a 5-HT_{1A} Receptor Imaging Agent

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A novel and straightforward microwave synthesis of a new arylpiperazine derivative **3** which has an N_2S_2 moiety has been developed and radiolabeled with an optimum radionuclide of technetium in the presence of a borohydride exchange resin (BER) as a reducing agent. According to the present study this new radiolabeled compound **4** could have the potential for a diagnostic application as an imaging agent of a serotonin neuroreceptor.

A neurotransmitter system includes the cholinergic nervous system which releases acetylcholine and the adrenergic nervous system which releases noradrenaline. Acetylcholine and noradrenaline are released by a stimulation in the central and peripheral nervous systems. Additionally, many important neurotransmitters such as dopamine, serotonin, and inhibitory GABA (γ-aminobutyric acid) exist in the central nervous system. Among them, the serotonergic system in the brain, specifically, various receptor subtypes of it, is an important neurotransmitter that controls emotion-related actions including worry, anxiety, and physiological functions. Thus, serotonergic nervous system is closely related to mental illnesses such as anxiety, manic-depression, and melanocholia.[1] Serotonin receptors activated by 5-HT,[2] which is one of the serotonin receptor subtypes, have been divided into at least seven classes (5-HT_{1-7}), and each class has been further subdivided into different subtypes (A, B, ...).

So far, from extensive research studies on agonists and antagonists for 5-HT_{1A} receptors, WAY100635 (Figure 1), which is an arylpiperazine compound, is known as a typical antagonist. It was identified that WAY100635 is a crucial ligand for imaging 5-HT_{1A} receptor.[3]

When WAY100635 derivatives were synthesized by a conventional heat treatment, a large quantity of solvent is required, the reaction time is fairly long and the yield is low.[4] Overcoming these inefficencies, the radioligand of WAY100635 derivatives were synthesized by a microwave irradiation (MWI) that induces a direct coupling between the reaction molecules and shows a thermal conductivity leading to a rapid rise in the reaction temperature. MWI has outstanding advantages of a shorter reaction time, higher yield, easier work-up and cleaner reaction due to less side reactions over a conventional heating in organic synthesis.[5–8]

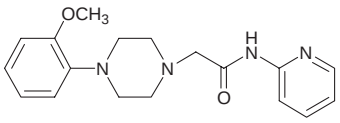
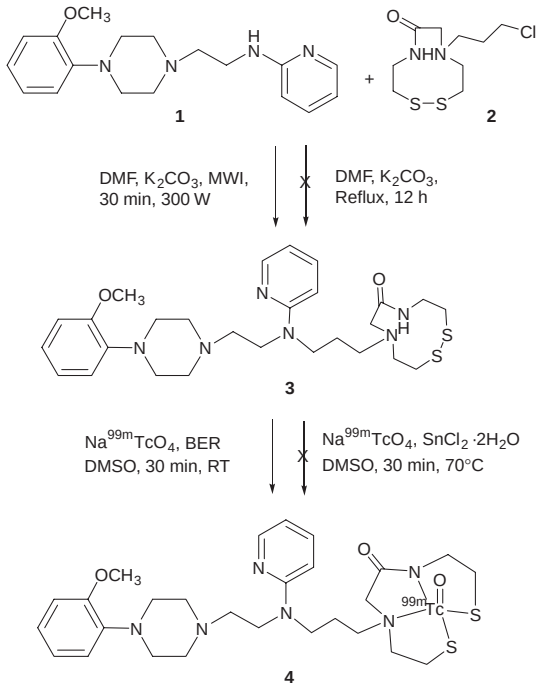


Figure 1. WAY100635 as an antagonist for 5-HT_{1A} receptors.

The labeling of a derivative of WAY100635 with ^{99m}Tc was, at first, achieved by conjugating the WAY100635 derivative, 1-(2-methoxyphenyl)-4-(2-(2-pyridylamino)ethyl) piperazine (**1**) and 8-(3-chloropropyl)-[1,2,5,8]dithiadiazecan-6-one (**2**), which has a chloropropyl spacer, through MWI (Scheme 1). Experimentally, a solution of **1** (0.62 g, 0.2 mmol) and **2** (0.52 g, 0.2 mmol) in DMF (10 mL) was added into a 50-mL round bottom flask containing an excess of K_2CO_3. The mixture was heated under reflux for 12 h. The progress of the reaction was monitored by TLC analyses. Although the compounds **1** and **2** were successfully prepared (yield 75 and 61%, respectively) by a literature method,[6] the coupling of 1 and 2 was unsuccessful. However, the conjugation of **1** and **2** was successfully achieved by the new method using a microwave irradiation with a yield of 20%. A solution of **1** (0.62 g, 0.2 mmol) and **2** (0.52 g, 0.2 mmol) in DMF (10 mL) was added into a 50-mL glass vessel containing an excess of K_2CO_3. The reaction vessel was capped with a TFM teflon cover and placed in a rotor in a microwave reactor. The mixture was irradiated for 30 min at 130 °C in a 300 W and cooled to room temperature. The reaction mixture was evaporated, dissolved in water, and extracted with dichloromethane (100 mL × 2). The organic layer was washed succes-



Scheme 1. Preparation of the ^{99m}Tc-labeled arylpiperazine derivative.

sively with water and brine, and dried over an anhydrous sodium sulfate. Evaporation of the solvent gave the crude product, which was purified by column chromatography, yielding 0.07 g (20%) of the desired product as an oily form. TLC (ethylacetate:*n*-hexane:MeOH = 1:1:2, v/v): **1**, $R_f = 0.561$, **2**, $R_f = 0.929$, **3**, $R_f = 0.789$ (Scheme 1). The complexation of ^{99m}Tc with the prepared chelating ligand **3** was followed by using MWI as shown in Scheme 1.

Interestingly, depending on the reducing agent, the labeling yield was clearly different. In the case of using a conventional reducing agent such as tin(II) chloride, we observed no technetium-labeled complex. Experimentally, an aqueous solution of sodium pertechnetate, $Na^{99m}TcO_4$ (185 MBq) was injected into a vial containing a solution of **3** (0.07 g, 0.13 mmol) in DMSO (2 mL) and tin(II) chloride dihydrate (0.05 mg) in 5 mmol HCl (0.1 mL). After a stirring for 30 min, no technetium-labeled complex was obtained. Further the mixture was then heated in boiling water for 30 min and cooled to room temperature, no technetium-labeled complex was obtained. Alternatively, by using a tetrahydroborate exchange resin (BER), which was newly introduced by us, was very successful in the labeling.[9–11] To a vial containing 5 mg of BER, 0.1 mL of $Na^{99m}TcO_4$ (185 MBq), and a solution of **3** (0.07 g, 0.13 mmol) in DMSO (2 mL) were added at a time. After stirring the mixture at room temperature for 30 min under N_2, it was filtrated by a membrane filter (0.22 μm) before the instrumented analyses. It should be noted that the reduction of pertechnetate as well as the disulfide bond S–S in ligand **3** was occurred during the labeling of ^{99m}Tc. To determine the labeling yield of the ^{99m}Tc-complex **4**, ITLC-SG (silica gel) was performed by using MEK and saline as a development solvent. The ITLC-SG of **4** using MEK gave no peak at the solvent front where $^{99m}TcO_4^-$ would be expected. Some reduced $^{99m}TcO_2$ fragments were observed at the origin after an elution of **4** with saline. These results indicate that ^{99m}Tc-8-[3-({2-[4-(2-methoxyphenyl)piperazine-1-yl]-ethyl}pyridine-2-yl-amino)propyl]-[1,2,5,8]dithiadiazecan-6-one (**4**) with a 99% labeling efficiency was formed with the use of BER.

Radiochemical purity of **4** was determined by HPLC involving a C_{18} reverse-phase column as a stationary phase and water/acetonitrile as a mobile phase, while maintaining a flow rate of 1 mL/min. The HPLC chromatograms of $^{99m}TcO_4^-$, ^{99m}Tc-8-(3-chloropropyl)-[1,2,5,8]dithiadiazecan-6-one (the complexation of ^{99m}Tc and **2**) and **4** showed that the retention times of these species are 3.0, 3.5, and 29.0 min, respectively. The radiolabeling yield of **4** in the reaction mixture was found to be 95%. **4** was stable (>90%) under an hydrolysis condition for approximately 4 h.

Additionally, a filtrated preparation was applied to a paper (20 × 200 mm) for electrophoresis that was eluted for 45 min at a 400 V (20 V/cm) with a 0.1 M phosphate buffer (pH 7.4). The paper electrophoresis investigations in an aqueous solution confirmed the neutral charge of **4**.

Imaging studies were carried on 6 week-old male New Zealand White rabbits. Rabbit was anesthetized with ketamine and xylazine. Rabbit was placed in a posterior posture. The

Figure 2. Image scan of a rabbit after i.v. injection of the ^{99m}Tc-complex **4**.

Diacam gamma camera (Simens, Germany) with a low-energy collimetor was positioned. Energy gate and window width were set to 140 keV and 10%, respectively. Rabbits were injected with 3.7 MBq of **4** per head through the left ear vein. The static image of **4** in the male New Zealand White rabbit at 5 and 180 min post injection is shown in Figure 2. At 5 min post injection image, high activity was found in the liver and a trace amount of **4** seemed to have remained in the brain (Figure 2).

In summary, ^{99m}Tc-8-[3-({2-[4-(2-methoxyphenyl)piperazine-1-yl]ethyl}pyridin-2-yl-amino)propyl]-[1,2,5,8]dithiadiazecan-6-one (**4**) was prepared by the reaction of radioactive ^{99m}Tc with **3** that is a WAY100635 derivative with a labeling yield of 99%. In addition, the facile synthesis of the arylpiperazine derived ligand **3** containing the N_2S_2 binding site for the metals was achieved by a new MWI method with a high yield. The chelating ligand **3** has a high affinity for 5-HT_{1A} receptor antagonist thus it is suitable for being developed as a radiopharmaceutical agent especially as an imaging agent for a neutrotransmitter receptor.

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