Synthesis and Physical Evaluation of $^{99m}Tc(CO)_3$-labeled Cysteine-arylpiperazines for a Neuroreceptor(5-HT_{1A}) Imaging

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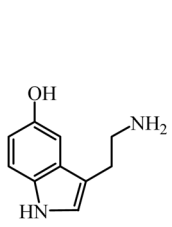
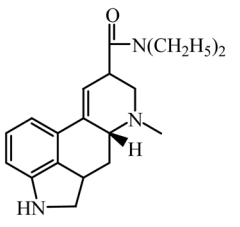
Serotonin (5-HT)[1] is one of the most extensively distributed neurotransmitters which is related to a neuropsychiatric disorder. The structural similarity of 5-HT to lysergic acid diethylamide (LSD) has provided researchers with basic information on the operating mechanism of serotonin in CNS. In line with the development of a histochemical fluorescence technique and a radio labeling method, several classes (5-$HT_{1\sim7}$) of serotonin receptors and their detailed functions have also been discerned. 5-HT_{1A}, one of the serotonin receptor subtypes, is especially implicated in Alzheimer's disease, schizophrenia, anxiety, and depression. Furthermore, the feasibility of visualizing a receptor in the human brain has been studied extensively and demonstrated in the related fields of a single photon emission tomography (SPECT) and a positron emission tomography (PET). Several approaches[2-12] have been pursued for molecular imaging agents by using WAY-100635, a known 5-HT_{1A} receptor antagonist. [^{11}C] WAY-100635[7-9] containing positron emitter with a 20.4 min half life has been established for quantification and visualization of 5-HT_{1A} receptors in the human brain with PET. [^{11}C] DWAY-100635,[10,11] a metabolite of [^{11}C] WAY-100635, has revealed a higher brain uptake than [^{11}C] WAY-100635.

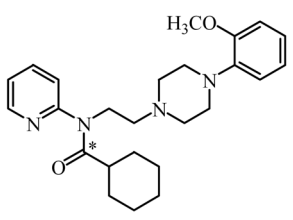
However, the short half life of carbon-11 limits these radio pharmaceuticals from being utilized in a local imaging procedure despite their high specific activity for *in vivo* application. Thus, several studies have been implemented on WAY-100635 derivatives in order to circumvent this issue. M. Karramkam *et al.*[12] studied [^{18}F] fluorinated derivative of WAY-100635, while many efforts[2-6] have been focused on ^{99m}Tc complexes. ^{99m}Tc complexes are generally based on +5 oxidation state, $[^{99m}Tc{=}O]^{3+}$, and they are used for labeling tracers. Recently, complexes[13,14] with $[^{99m}Tc(CO)_3]^+$ have become attractive since they offer air stability, small core size and kinetic inertness. With these merits, we previously described the synthesis of $^{99m}Tc(CO)_3$-labeled histidine-arylpiperazines as potential brain receptor imaging agents for SPECT tracer.[15] In addition to the histidine chelator, we also make an attempt to introduce alternative chelators with different physical properties, small size and easy preparation. Cysteine is one of the most feasible compounds,[16,17] which reveals a smaller tridentate N,O,S ligand with higher affinity to $[^{99m}Tc(CO)_3]^+$ moiety than histidine. Therefore, we suggest herein a synthesizing method for cysteine based arylpiperazines labeled with $[^{99m}Tc(CO)_3]^+$. Also discussed here are the physical properties of the cysteine complex compared to a histidine ligand.



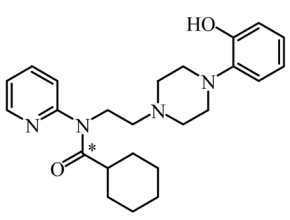
Serotonin



LSD



[^{11}C] WAY-100635



[^{11}C] DWAY-100635

Experimental Section

All the chemicals and reagents used in this study were analytical grade purchased from Aldrich and used without any further purification. All the stages of the reactions were identified with TLC glass sheets pre-coated with silica-gel G-25 UV_{254}, Macherey-Nagel Inc. The NMR spectra were recorded with Varian Gemini 200 (200 MHz ^{1}H, Dongguk Univ.) and Bruker Avance 500 (500 MHz, ^{1}H, KRICT, Daejeon) spectrometers. Mass spectra were measured with the Hewlett Packard HP 1100 series LC/MSD (Chungnam National Univ.). Sodium pertechnetate ([^{99m}Tc] $NaTcO_4$) was obtained from a ^{99}Mo-^{99m}Tc generator (Sam Young Unitech. Co., LTD.). The labeling yield and radiochemical purity were determined by Radio-HPLC as we have previously reported[15] with minor modification on the first flow rate maintained at 1 mL/min (a system equipped with Waters 2695 pump, UV-Detector (Waters 2487), RI-detector (In/US system) and Xterra C-18 column (5 μm, 4.6 × 250 mm)). All radioactivities were measured by using an ionizing chamber (Atomlab 200, Bio-dex).

1-(Chloroalkyl)-4-(4-methoxyphenyl)piperazine (2).

To a mixture of 1-(4-methoxyphenyl)piperazine (1 g, 5.2 mmol) and 1-bromo-chloroalkane (7.8 mmol) in DMF (10 mL), potassium carbonate (1.79 g, 13 mmol) was added and continuously stirred for 6 hrs at 60 °C. The residue was removed by filtration and the solvent was removed under reduced pressure at 40 °C for 2 hrs. The crude product was purified by column chromatography eluting with CH_2Cl_2-MeOH (15 : 1) to obtain (**2a**, **2b**, **2c**) a yellow oil.

1-(2-Chloroethyl)-4-(4-methoxyphenyl)piperazine (2a). Starting from 1-bromo-2-chloroethane (1.12 g, 7.8 mmol), the title compound was prepared by following the same procedure above to give a yellow oil (68% yield).

NMR ($CDCl_3$), δ(ppm) 2.78 (br.t, 4H, piperazine), 2.84 (t, 2H), 3.14 (br.t, 4H, piperazine), 3.67 (t, 2H), 3.89 (s, 3H), 6.97 (m, 4H, Aromatic). (LC/MSD M+1): cald. for 255.12 found 255.1.

1-(3-Chloropropyl)-4-(4-methoxyphenyl)piperazine (2b). Starting from 1-bromo-3-chloropropane (1.23 g, 7.8 mmol), the title compound was prepared by following the same procedure above to give a yellow oil (79% yield).

NMR ($CDCl_3$), δ(ppm) 2.02 (m, 2H), 2.59 (t, 2H), 2.69 (br.t, 4H, piperazine), 3.12 (br.t, 4H, piperazine), 3.66 (t, 2H), 3.89 (s, 3H), 6.97 (m, 4H, Aromatic). (LC/MSD M+1): cald. for 269.13 found 269.1.

1-(4-Chlorobutyl)-4-(4-methoxyphenyl)piperazine (2c). Starting from 1-bromo-4-chlorobutane (1.34 g, 7.8 mmol), the title compound was prepared by following the same procedure above to give a yellow oil (52% yield).

NMR ($CDCl_3$), δ (ppm) 1.87 (m, -CH_2-$\underline{CH_2}$-$\underline{CH_2}$-CH_2-Cl), 3.04 (br.t, 4H, piperazine), 3.61 (t, 2H), 3.68 (br.t, 4H, piperazine), 3.90 (s, 3H), 4.17 (t, 2H), 6.96 (m, 4H, Aromatic). (LC/MSD M+1): cald. for 283.15 found 283.1.

***S*-(1-Alkyl-4-(4-methoxyphenyl)piperazine)-L-cysteine (3).** Under a N_2 condition, L-Cysteine monohydrochloride monohydrate (50 mg, 0.41 mmol) was activated with 0.5 M sodium methoxide (1.65 mL, 0.82 mmol) at −10 °C. After a stirring for 10 min, 1-(Chloroalkyl)-4-(4-methoxyphenyl)-piperazine (**2a**, **2b**, or **2c**) (0.4 mmol) in MeOH (2 mL) was added. The reaction mixture was continuously stirred for 12 hrs at 0 °C. The solvent was removed *in vacuo* at 30 °C. The crude product was purified by a column chromatography eluting with MC : MeOH (2 : 1) to give (**3a**, **3b**, **3c**) a slightly yellow powder.

***S*-(1-Ethyl-4-(4-methoxyphenyl)piperazine)-L-cysteine (3a).** Starting from **2a** (105 mg, 0.41 mmol), the title compound was prepared by following the same procedure above to give **3a** (55% yield).

NMR (D_2O), δ (ppm) 2.81 (t, 2H), 2.82 (br.t, 4H, piperazine), 3.05-2.90 (m, S-$\underline{CH_2}$-CH(NH_2)-), 3.08 (br.t, 4H, piperazine), 3.10 (t, 2H), 3.85 (s, 3H), 3.88 (q, 1H, S-CH_2-$\underline{CH}$(NH_2)-), 7.08 (m, 4H, Aromatic). (LC/MSD M+1): cald. for 340.16 found 340.1.

***S*-(1-Propyl-4-(4-methoxyphenyl)piperazine)-L-cysteine (3b).** Starting from **2b** (111 mg, 0.41 mmol), the title compound was prepared by following the same procedure above to give **3b** (59% yield).

NMR (D_2O), δ(ppm) 1.84 (m, piperazine-CH_2-$\underline{CH_2}$-CH_2-S-), 2.69 (t, 2H), 2.78 (t, 2H), 2.94 (br.t, 4H, piperazine), 3.05 (m, S-$\underline{CH_2}$-CH(NH_2)-), 3.15 (br.t, 4H, piperazine), 3.89 (s, 3H), 4.02 (q, 1H, S-CH_2-$\underline{CH}$(NH_2)-), 7.15 (m, 4H, Aromatic). (LC/MSD M+1): cald. for 354.18 found 354.1.

***S*-(1-Butyl-4-(4-methoxyphenyl)piperazine)-L-cysteine (3c).** Starting from **2c** (117 mg, 0.41 mmol), the title compound was prepared by following the same procedure above to give **3c** (53% yield).

NMR (D_2O), δ(ppm) 1.86 (m, piperazine-CH_2-$\underline{CH_2}$-$\underline{CH_2}$-CH_2-S-), 2.69 (t, 2H), 2.78 (t, 2H), 2.94 (br.t, 4H, piperazine), 3.05-2.90 (m, S-$\underline{CH_2}$-CH(NH_2)-), 3.15 (br.t, 4H, piperazine), 3.87 (s, 3H), 3.90 (q, 1H, S-CH_2-$\underline{CH}$(NH_2)-), 7.15 (m, 4H, Aromatic). (LC/MSD M+1): cald. for 368.19 found 368.2.

Labelling study. A vial containing cysteine based aryl-piperazine (**3a**, **3b**, **3c**, 1 mg in pure water, 1 mL) was sealed and purged with N_2 gas for 2 min. $[^{99m}Tc(H_2O)_3(CO)_3]^+$ prepared using a carbonyl reaction kit was adjusted to pH 7.4 with 0.5 N phosphate buffer and then cooled down in an ice bath. 100 μL (about 5 mCi) of $[^{99m}Tc(H_2O)_3(CO)_3]^+$ was added to the vial containing the cysteine ligand along with an incubation at 75 °C for 30 min. The $^{99m}Tc(CO)_3$ complex was cooled and filtered through a 0.45 μL membrane filter. Radiochemical purity and yield were measured with HPLC (radioactive detector, Xterra C-18 column with solvent gradient system).

Determination of Log P. To an equal volume of *n*-octanol and water (1 mL/1 mL) in a test tube, $^{99m}Tc(CO)_3$ complex ($^{99m}Tc(CO)_3$-cysteine-arylpiperazine (4) or $^{99m}Tc(CO)_3$-Histidine-Arylpiperazine) was added. After vigorously stirring it for 10 min, the mixture was partitioned with a centrifuge at a speed of 6000 rpm for 3 min. Each layer (500 μL) was taken and counted with an ionizing chamber. P and Partition coefficients (Log P) were determined by using the following equation;

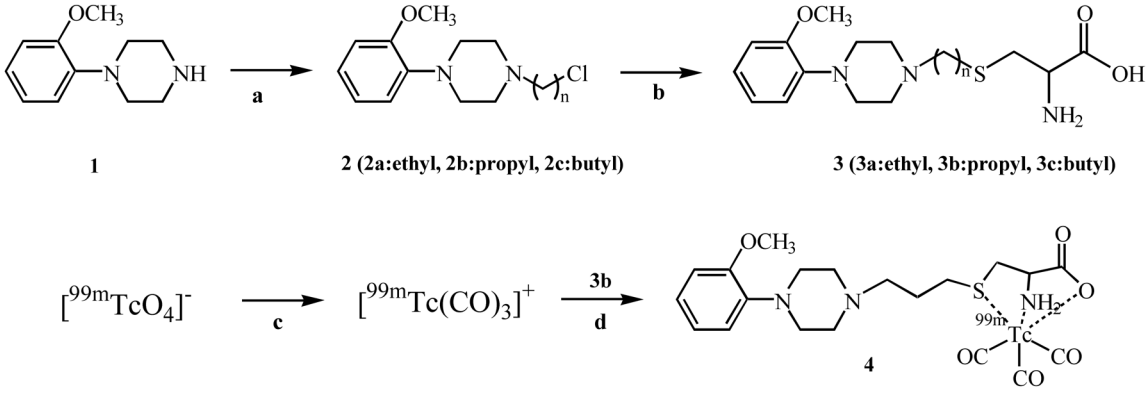
$$P = \frac{\text{Octanol activity}}{\text{Water activity}}, \quad \text{Log P} = \left(\frac{\text{Octanol activity}}{\text{Water activity}}\right)$$

***in-vitro* stability.** The *in vitro* stability of the $^{99m}Tc(CO)_3$ complexes was assessed by incubation at 36 °C at pH 7.0 for 0, 2, 4 and 6 hrs. The stability of each sample was analyzed by a radio HPLC under the same conditions used as we have previously reported.

Electrophoresis. Whatman paper (2 cm × 30 cm) saturated with 0.5 M phosphate buffer (pH 7.4) was placed in electrophoresis containers with 0.5 M phosphate buffer (pH = 7.4). Each 1 μL sample ($^{99m}Tc(CO)_3$-cysteine-arylpiperazine (**4**) and $^{99m}Tc(CO)_3$-histidine-arylpiperazine) was spotted and developed at a constant potential of 220 V for 120 min. After drying the strips, the distribution of the radioactivity on the paper was determined with an ITLC scanner.

Results and Discussion

With our stimulation of our results to develop a $^{99m}Tc(CO)_3$-histidine-arylpiperazine, we made efforts to develop a new chelating system for 5-HT_{1A} receptor imaging. This study is based on 5-HT_{1A} antagonists such as WAY100635,



Scheme 1. Reaction pathways for ^{99}mTc(CO)$_3$-cysteine-arylpiperazines. Reagents and conditions; (a) 1-bromo-chloroalkane/K_2CO_3/DMF/ 60 °C, 6 hrs; (b) L-cysteine/$NaOCH_3$/CH_3OH/−10 °C, 12 hrs; (c) Carbonyl reaction kit ($Na_2B_4O_7$, Na_2BH_3CO, Na_2CO_3, sodium tartrate)/ H_2O, 110 °C, 15 min; (d) 3b/PBS buffer, 75 °C, 30 min.

WAY100135 and BMY 7378, which are mainly composed of a 1-(2-methoxyphenyl) piperazine moiety.

In order to synthesize a smaller ^{99m}Tc-complex in size, we have focused on cysteine as a $[^{99m}Tc(CO)_3]^+$ chelator. Generally, the preparation of a cysteine based ligand has been performed by using protected cysteine, *N*-Boc-cysteine methylester, but we have a chance to know that cysteine undergo directly *S*-alkylation under a strong base condition at the low temperature. Comparative experiments for a protected cysteine and a cysteine free base gave the same *S*-alkylated compounds and the same product after a hydrolysis of the protected cysteine derivatives. The synthesis and labeling process of the target compounds are shown in Scheme 1.

The reaction of arylpiperazine **1** with 1-bromo-chloroalkane provided selective *N*-alkylated compounds, **2a**, **2b** and **2c**, in excess of a 60% yield. Selective *S*-alkylation was achieved through activation of cysteine with 2 eq of 0.5 N sodium methoxide solution under a nitrogen bubbling and a reaction with **2a**, **2b** and **2c** at −10 °C. The yields of **3a**, **3b** and **3c** were 55%, 59% and 53% respectively.

In oder to compare the basic properties of the cysteine and histidine complexes, we representatively used precursors with propyl spacer (**3b** and histidine-C3-arylpiperazine). The $[^{99m}Tc(CO)_3]^+$ precursor was produced over 99% yield by reacting $^{99m}TcO_4^-$ with a carbonyl reaction kit by referring the literature method described by Alberto *et al.*[13,14] $[^{99m}Tc(CO)_3]^+$ complexes (**4**, and $[^{99m}Tc(CO)_3]$-histidine-C3-arylpiperazine) were prepared by simply incubating the precursors and $[^{99m}Tc(CO)_3]^+$ at 75 °C for 30 min. The radiochemical purity and stability of the complexes were checked by RP-HPLC solvent gradient system.[15] **4** and $[^{99m}Tc(CO)_3]$-histidine-C3-arylpiperazine respectively were identified at the retention times of 17.86 min and 16.94 min with high purity (Fig. 1). Their radiochemical stability at different times are shown in Table 1. This data showed that each complex has good stability up to 6 hrs and no decomposition was observed during the times used in this study.

Paper chromatograms of **4** and $[^{99m}Tc(CO)_3]$-histidine-C3-arylpiperazine are shown in Figure 2. After electrophoresis with a 0.5 M phosphate buffer solution (pH 7.4), each complex remained at its spotting site, showing that they can be characterized as charged neutral complexes. The partition coefficients of the cysteine and histidine complexes were −0.19 and 0.71, respectively, which means that cysteine more hydrophilic than histidine (Table 2).

For ideally crossing the blood-brain barrier,[18] pharmaceuticals should be less than 650 dalton in the molecular mass, neutral in electrical charge, 0.5-2.5 value in log P. As a result of several experiments, all of the complexes in this presentation meet the majority of these criteria, but cysteine complex (**4**) showed slightly less lipophilicity comparing to the ideal Log P.

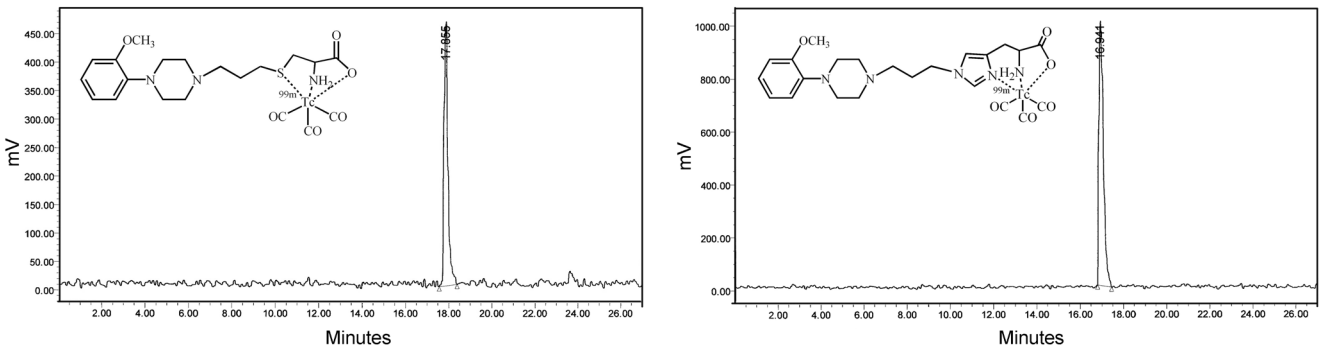


Figure 1. RP-HPLC chromatograms of ^{99m}Tc(CO)$_3$-cysteine-arylpiperazines (left) and ^{99m}Tc(CO)$_3$-histidine-arvlpiperazines (right).

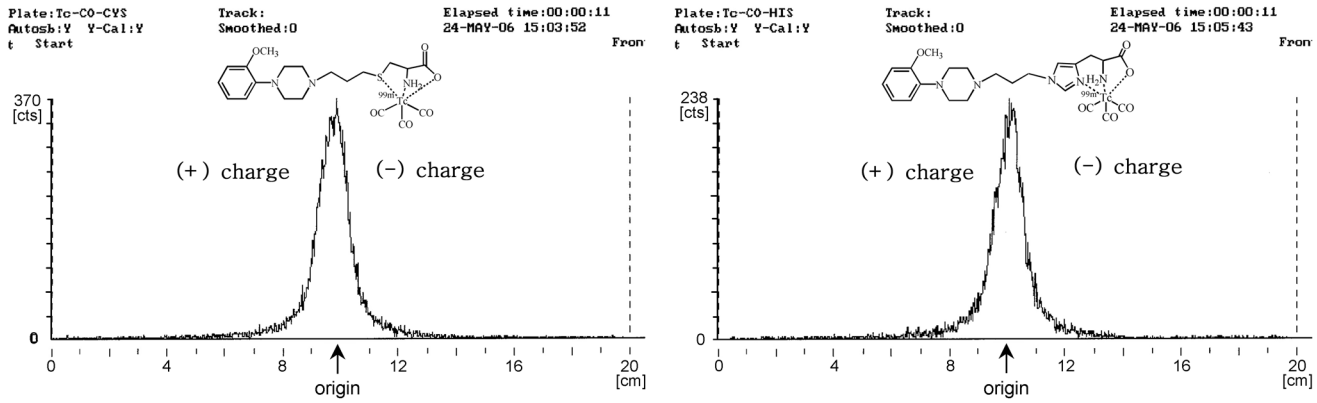


Figure 2. ITLC chromatograms after paper electrophoresis.

Table 1. The stability of cysteine and histidine arylpiperazine complexes

	0 hr	2 hr	4 hr	6 hr
Cysteine complex	>99%	>99%	>99%	>99%
Histidine complex	>99%	>99%	>99%	>99%

Table 2. Lipophilicity tests

	Counts of Water layer	Counts of Octanol layer	Log P
Cysteine complex (2.48 mCi)	0.660 mCi / 0.5 mL	0.423 mCi / 0.5 mL	−0.19
Histidine complex (2.35 mCi)	0.189 mCi / 0.5 mL	0.985 mCi / 0.5 mL	0.71

Conclusion

A cysteine based chelator has been successfully prepared by direct *S*-alkylation, which can be conveniently applied to the preparation of other cysteine based complexes. These results demonstrate that the two complexes used in this study, which containing different chelators have the stability in an *in vitro* condition and sufficiently meet most of the criteria required to pass through BBB, suggesting the possibility of these complexes to be used as neuroreceptor imaging agents.

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