Novel $^{99m}Tc(CO)_3$ Complexes with WAY-100635 Moiety for the Development of 5-HT_{1A} Receptor Imaging Agent

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Received January 24, 2009, Accepted March 25, 2009

The aim of this study is to develop and synthesize 5-HT_{1A} receptor imaging agents with WAY-100635 moiety and $^{99m}Tc(CO)_3$ core. WAY-100635 is commonly known as 5-HT_{1A} antagonist and its labeled compound ([^{11}C] WAY-100635) has been used as effective radioligand for imaging brain 5-HT_{1A} receptors with PET(Positron Emission Tomography). However, there are several restrictions in using a radioisotope of C-11 and requires for more effective radioisotopes and ligands. In order to produce a structure most similar to WAY-100635, WAY-100635 derivatives containing a cysteine chelator were designed and confirmed by using *in silico* (Hyperchem). The novel compounds **(7a, 7b, 7c)** were prepared in five or 7 steps with yields of 16%, 36% and 42%, respectively and radiolabeled with $[^{99m}Tc(CO)_3(H_2O)_3]^+$. The labeling yield was 99% for all the newly synthesized compounds. $[^{99m}Tc(CO)_3]$-WAY-100635 derivatives show a neutral charge which were confirmed by paper electrophoresis.

Key Words: WAY-100635, Central nervous system (CNS), 5-HT_{1A}, ^{99m}Tc

Introduction

Since two brain perfusion agents (ECD (L,L-Ethylcysteinate Dimer) and HMPAO (Hexamethyl Propyleneamine Ocime) were developed,[1-4] significant studies have been made in the past two decades for developing new brain imaging agents. Recently, many efforts are focusing on the development of radiopharmaceuticals specific to cross the blood-brain barrier and to bind to the brain receptor, such as 5-HT_{1A} receptors, which may lead to improved clinical tools for nuclear medicine.[5-19] Several approaches[12-16] with 5-HT_{1A} antagonist ([^{11}C] WAY-100635) have been pursued as PET imaging agents for several neuropsychiatric disorders, such as Alzheimer's disease, schizophrenia, suicidal behavior and depression. However, the rapid metabolism of [^{11}C] WAY-100635 by amide hydrolysis makes it difficult to apply radioligand kinetic models that require a intravenous injection.[16,17] Therefore, structural analogues of [^{11}C] WAY-100635 such as CPC-222, SWAY, and (R,S)-JWAY were developed in order to resist a amide hydrolysis in the human body (Fig. 1).[14,16] Moreover, the short half life of carbon-11($t_{1/2}$ = 20 mins) and the need for a special technique in its preparation set a limit for these radiopharmaceuticals for a localized imaging despite their high specific activity *in vivo*.

The drawbacks that limit the accessibility, and the high cost in the use of [^{11}C] PET isotope lead the focus of our research towards SPECT isotopes. ^{99m}Tc ($t_{1/2}$ = 6 h) is the most feasible isotope for SPECT, which can be produced by ^{99}Mo/^{99m}Tc generator and at relatively low cost.[5-11] ^{99m}Tc complexes are generally based on +5 oxidation state $[^{99m}TcO]^{3+}$ but the accessibility of oxidation state is varied from +Ⅰ to +Ⅶ. Recently, the advent of $[^{99m}Tc(CO)_3]^+$,[20,21] Tc(Ⅰ), has lead to significant attractions since it offers high specific activity complex, small size, and high kinetic stabilities with chelators. With these merits, several approaches to attach chelators to several compounds, for instance aryl piperazine,[5-9] 5-hydroxy tryptamine,[10] and phenyltropane derivatives,[11] have been utilized to visualize the central nervous system(CNS). Unfortunately, the brain uptake of these complexes is limited.

One of the major considerations in designing a novel metal complex is to evaluate the geometric structure of its metal core. However, most of the current studies are using a long spacer in the main molecule body instead of considering a 3D structure. In this study, we have focused on WAY-100635 derivatives modified in the cyclohexyl part to introduce a cysteine-based chelator portion and checked its 3D structure with Hyperchem.

Here, we describe the design and the synthetic method of WAY-100635 derivatives containing a cysteine chelator and also discuss physical properties of ^{99m}Tc-tricarbonyl complexes, potential 5-HT_{1A} receptor imaging agents (Fig. 2).

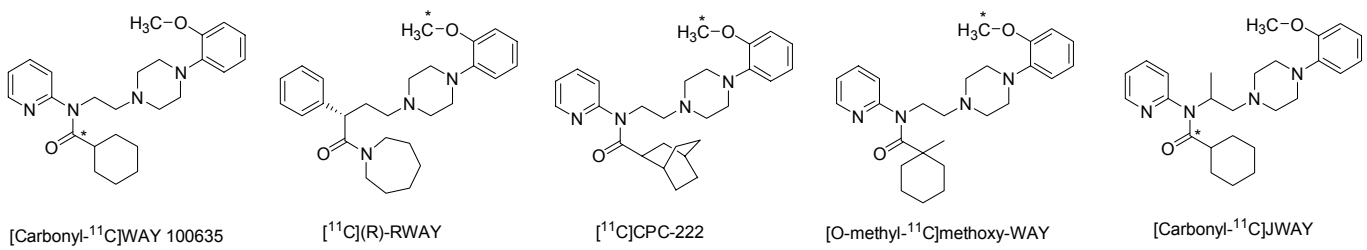


Figure 1. Analogues for using PET tracer with WAY-100635 moiety.

A) WAY-100635 B) 8**b**

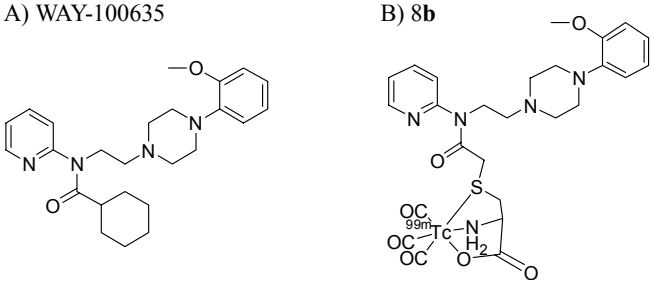


Figure 2. Structures of WAY-100635 and designed WAY derivative.

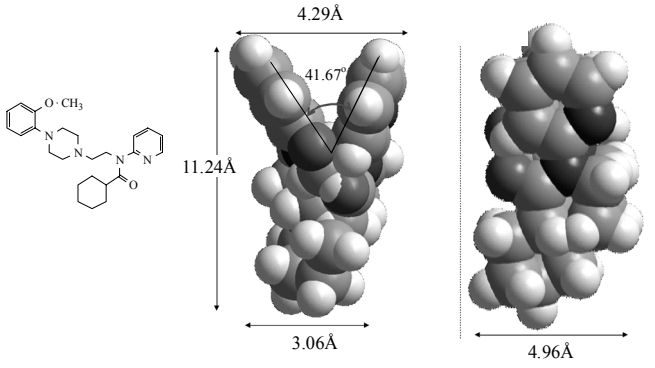
Results and Discussion

In our previous reports,[22,23] we described the introduction of amino acid-based chelating systems (histidine and cysteine) to the key moiety of 5-HT_{1A} receptor imaging agent, arylpiperazine. These compounds were developed from the viewpoint of the structural similarity to many 5-HT_{1A} antagonists (WAY-100635, WAY-100135 and BMY 7378) having an arylpiperazine moiety. Our ongoing project is to conveniently visualize a brain receptor by using easily accessible radioisotopes such as ^{99m}Tc.

As mentioned above, [Carbonyl-^{11}C]-WAY 100635 is now the most commonly used ligand for *in vivo* patient studies, but its metabolic instability caused by an amide hydrolysis, limits its application. To overcome this issue, the cyclohexyl ring has been replaced with a more bulky group such as adamantine (bicyclo[2,2,2]octane), azepane or metylcycloheane to resist an amide hydrolysis (Fig. 1). Our novel compounds (**8a**, **8b**, **8c**) were also designed based on this premiss, replacing the cyclohexyl group with a chelator part as a bulky group. We first checked the structural conformation by comparing **8b** with WAY 100635 by using Hyperchem (Fig. 3). The com putation was performed with minimal set(STO-3G) in *Ab initio* menu.

8b shows a structural similarity to WAY-100635, where the only difference is in the chelator part. The chelator part

A) WAY-100635



B) 8**b**

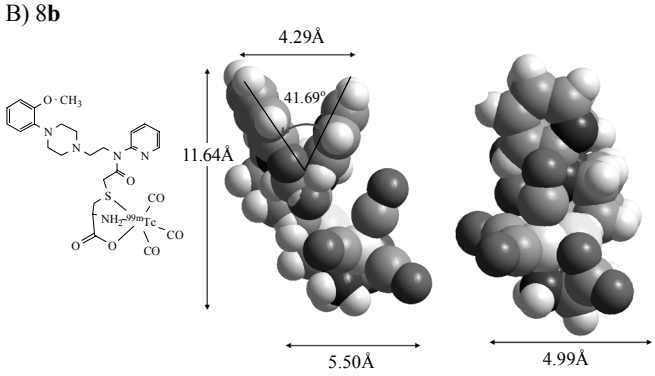
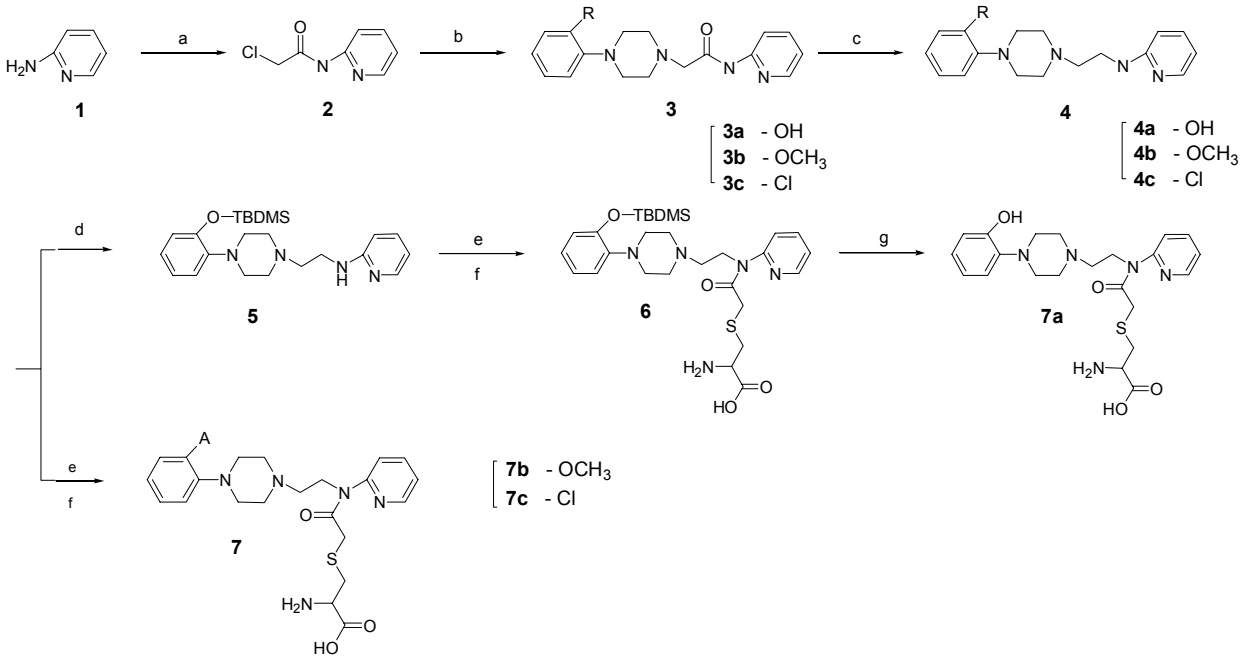
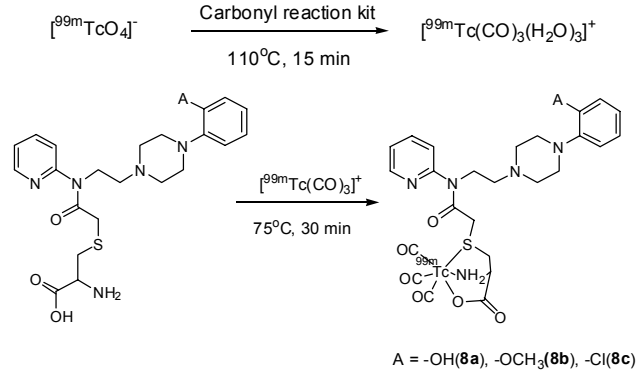


Figure 3. 3D-structural conformation.



Scheme 1. Reaction pathways for WAY-100635 derivatives. Reagent and condition; (a) chloroacetylchloride/TEA/MC/ RT, 12 hrs; b)1-(2-substituted-phenyl)piperazine/K_2CO_3/ DMF/ 50 °C, 12 hrs; c) $LiAlH_4$/THF/ 0 °C, N_2; d) TBDMS-Cl/DBU/ACN/RT, 24 hrs; e) chloroacetylchloride/TEA/THF/0 °C, 10min; f) activated cysteine/THF,MeOH/-18 °C, 2 hrs; g)Et_3N·HF/THF/RT, 6 hrs.



Scheme 2. Preparation of $Tc(CO)_3$-complexes. (**8a**: $^{99m}Tc(CO)_3$-Cys-DWAY, **8b**: $^{99m}Tc(CO)_3$-Cys-WAY, **8c**: $^{99m}Tc(CO)_3$-Cys-ClWAY)

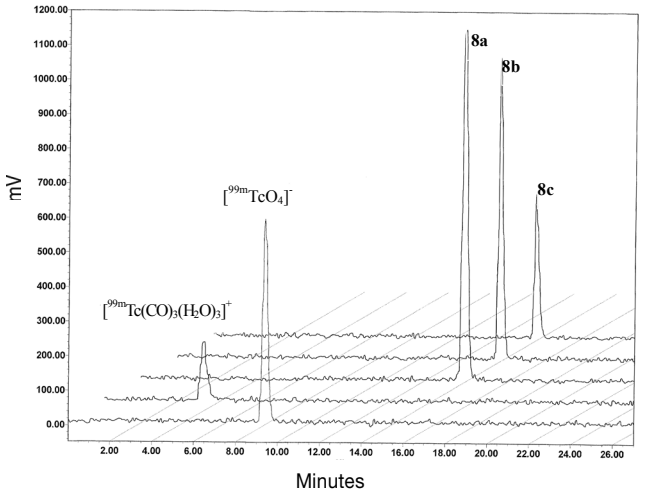


Figure 4. RP-HPLC chromatograms of $[^{99m}TcO_4]^-$, $[^{99m}Tc(CO)_3(H_2O)_3]^+$, and **8** series

containing $Tc(CO)_3$ reveals to be somewhat larger than the cyclohexyl group in the main molecular body of WAY-100635. Despite the addition of the cysteine-based chelator, **8b** (41.69°) did not show a significant change in the steric effect compared to that of WAY-100635 (41.67°) in the main body angle.

The reaction and labeling procedures are shown in Scheme 1 and Scheme 2, respectively. In order to introduce a cysteine chelating system to **5**, we tried several reactions. First, we tried a direct alkylation to its 2nd amine residue but didn't get a product even though we used NaH as a base. Therefore, chloroacetylchloride was chosen as an alternative. After completing the reaction, our desired product, chloroacetyl amide, had formed another side product as time passed. The side product was first checked by TLC and finally identified as cyclized quaternary ammonium, an intra-molecular reaction product reacting with chloroacetylamide and piperazine, which was confirmed by NMR. To settle the problem, one-pot reaction was performed using the synthetic procedure for **6**. The overall yields (**7a**, **7b**, **7c**) were 16%, 36% and 42%, respectively.

Table 1. The stability test of labeled compounds (**8a**, **8b**, **8c**)

	1.5 hrs	3 hrs	4.5 hrs	6 hrs
8a ($[^{99m}Tc(CO)_3]$-DWAY-100635)	> 99%	> 99%	> 99%	> 99%
8b ($[^{99m}Tc(CO)_3]$-WAY-100635)	> 99%	> 99%	> 99%	> 99%
8c ($[^{99m}Tc(CO)_3]$-ClWAY-100635)	> 99%	> 99%	> 99%	> 99%

The labeling procedure has a total of 2 steps including the formation of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ and the introduction of $^{99m}Tc(CO)_3$ to a radioligand. $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was prepared by the procedure reported by Alberto R. *et al.*,[21] with slight modification. The radiochemical purities and a stability of the $^{99m}Tc(CO)_3$-complexes (**8a**, **8b**, **8c**) were checked by RP-HPLC solvent gradient systems. All the complexes revealed high labeling yield (> 99%). As shown in Fig. 4 Chromatogram data of $[^{99m}TcO_4]^-$, $[^{99m}Tc(CO)_3(H_2O)_3]^+$ and $^{99m}Tc(CO)_3$-complexes (**8a**, **8b**, **8c**) were identified at 9.36 min, 4.73 min and about 15 min, respectively.

The *in vitro* stability according to various time points at 37 °C are shown in Table 1. The data show robust complexes (**8a**, **8b**, **8c**) and have good stability over 6 hrs in solution state. To find out the complex's charge, electrophoresis was performed for 120 min at a constant 300 V and each complex remained at spotting position. These results show that all the complexes (**8a**, **8b**, **8c**) have a neutral charge.(Fig. 5)

Over all, our newly synthesized compound from WAY-100635 moiety with a cysteine based chelator will show relatively good characteristics because all the complexes (**8a**, **8b**, **8c)** have less than or almost equal to 650 dalton in molecular weight. Moreover, the newly synthesized complexes show a similar 3D-structure to WAY-100635 with neutral charge and are stable upto 3 days. These charactersitics are ideal for drug development to be able to cross the blood brain barrier.[24]

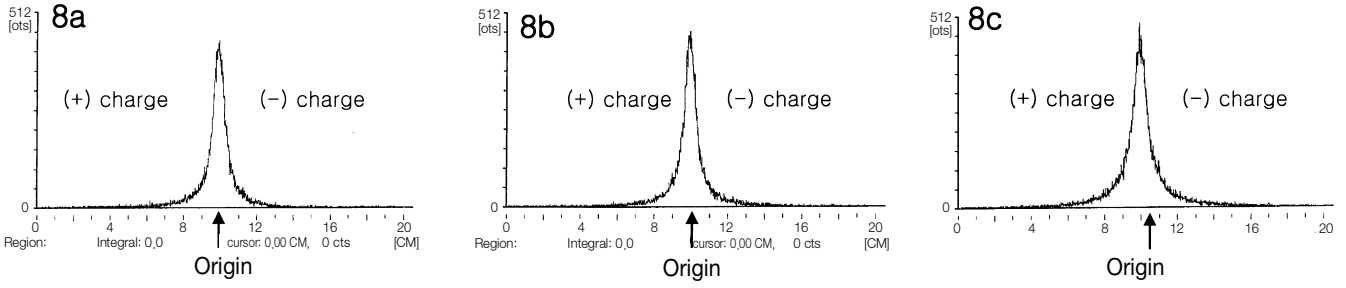


Figure 5. ITLC chromatograms after electrophoresis. ADVANTEC PS-2020 system, Electrolyte (0.1 M phosphate buffer, pH 7.4) Poten- tial (300 V), Current (30 mA) and progressing time (120 min)

Experimental Section

Material and reagents. All chemicals and reagents used were analytical grade purchased from Sigma-Aldrich Co. TLC and column chromatography were performed with glass sheets pre-coated with silica-gel G-25 UV_{254} (Macherey-Nagel Inc.) and silica gel 60 with 70 ~ 200 mesh (MERCK), respectively. 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 checked by HPLC (a system equipped with Waters 2695 pump, UV-Detector (Waters 2487), RI-detector (In/US γ-detector system)). Separations were achieved in a Xterra C-18 column (5 μm, 4.6 × 250 mm) eluted with binary gradient solvent condition of TEAP buffer, pH 2.25 (A) and MeOH (B) at a 1.0 mL/min flow rate. The elution profile was like that, solvent (A) 100% for 3 min, 75% for 6 min, 65%-0% for 11 min, 0% for 2 min and 100% for 3 min. All radioactivities were measured by using an ionizing chamber (Atomlab 200, Bio-dex). All compound synthesized in this paper was named based on ChemBioDraw Ultra 11.0 (ChembridgeSoft) and the 3D structures were identified by Hyperchem 7.0.

2-Chloro-*N*-(pyridin-2-yl)acetamide (2): To a solution of 10 g (106 mmol) of 2-aminopyridine and 14.8 mL (1.0 eq, 106 mmol) of triethylamine in 150 mL of dichloromethane, 10.2 mL (1.2 eq, 127 mmol) of chloroacetylchloride were dropwisely added under N_2 condition at 0 °C. The reaction mixture was stirred for 12 hrs at room temperature. The reaction was stopped by dropwise addition of saturated $NaHCO_3$. The organic layer was extracted twice with dichloromethane. The organic layers were washed once with brine and water, dried over Na_2SO_4, filtered and concentrated. The reaction mixture was chromatographed on silica gel eluting with CH_2Cl_2: MeOH (15:1) to give a white powder (yield : 92%). ^{1}H NMR ($CDCl_3$) δ 4.23(s, 2H), 7.15(t, 1H), 7.80(t, 1H), 8.24(d, 1H), 8.36(d, 1H), 9.0(br s, 1H). (LC/MSD M+1): cald for 171.6 found 170.9.

2-(4-(2-Substituted-phenyl)piperazin-1-yl)-*N*-(pyridin-2-yl)-acetamide (3): To a solution of 2 g (11.7 mmol) of **2** in 20 mL of DMF, 1.2 eq (14.1 mmol) of 1-(2-substituted-phenyl) piperazine dissolved in 40 mL of DMF was added and subsequently added 2.5 eq of K_2CO_3. The reaction mixture was stirred for 12 hrs at 50 °C. The reaction was stopped by addition of pure water and the organic portion was extracted twice with EA.The EA solution was combined together, washed with brine, dried with Na_2SO_4, and concentrated. The yellow sticky oil was chromatographed on a silica gel with EA: hexane (1:1) to get a pale yellow sticky oil form.

2-(4-(2-Hydroxyphenyl)piperazin-1-yl)-*N*-(pyridin-2-yl)-acetamide (3a): The title compound was prepared by following the same procedure as above (**3**) only different in the starting material 2-(piperazin-1-yl)phenol (2.51 g) to obtain a product (yield : 76%, 2.8 g). ^{1}H NMR ($CDCl_3$) δ 2.82(br, 4H), 3.01(br, 4H), 3.25(s, 2H), 6.83(d, 1H), 6.90(d, 1H), 7.04(m, 2H), 7.21(t, 1H), 7.69(t, 1H), 8.22(d, 1H), 8.26(d, 1H), 9.50(br s, 1H). (LC/MSD M+1): cald for 313.4 found 313.0.

2-(4-(2-Methoxyphenyl)piperazin-1-yl)-*N*-(pyridin-2-yl)-acetamide (3b): The title compound was prepared by following the same procedure as above(**3**) only different in the starting material 1-(2-methoxyphenyl)piperazine (2.71g) to obtain a product (yield : 85%, 3.3 g). ^{1}H NMR ($CDCl_3$) δ 2.88(br, 4H), 3.21(br, 4H), 3.28(br, 2H), 3.86(s, 3H), 6.88(d, 1H), 6.93-7.06(m, 4H), 7.71(t, 1H), 8.25(d, 1H), 8.32(d, 1H), 9.65(br s, 1H). (LC/MSD M+1): cald for 327.4 found 327.

2-(4-(2-Chlorophenyl)piperazin-1-yl)-*N*-(pyridin-2-yl)acetamide (3c): The title compound was prepared by following the same procedure as above (**3**) only different in the starting material 1-(2-chlorophenyl)piperazine (2.77g) to obtain a product (yield : 88%, 3.4 g). ^{1}H NMR ($CDCl_3$) δ 2.67(br, 4H), 2.98(br, 4H), 3.18(s, 2H),6.78(t, 1H), 6.84(d, 1H), 6.87(d, 1H), 7.02(t, 1H), 7.16(d, 1H), 7.51(t, 1H), 8.05(d, 1H), 8.11(d, 1H), 9.50(br s, 1H). (LC/MSD M+1): cald for 331.8 found 331.0.

***N*-(2-(4-(2-Substituted-phenyl)piperazin-1-yl)ethyl)pyridin-2-amine (4):** To 7 mmol of proper compound **3** dissolved in 100 mL of dried THF was added 3.8 eq (1.04 g) of $LiAlH_4$ under N_2 condition at 0 °C. The reaction mixture was stirred for 3 hrs at room temperature and then reaction was stopped by dropwise addition of saturated NH_4Cl (10 mL) at 0 °C. The suspension was stirred for 30 more mins and filtered off. The filtrate was extracted twice with 100 mL of CH_2Cl_2. The combined organic layer was washed with brine and pure water, dried with Na_2SO_4 and concentrated. The residue was purified by using silica gel chromatography with MeOH : CH_2Cl_2 (1:10) to give a pale oil form.

2-(4-(2-(Pyridin-2-ylamino)ethyl)piperazin-1-yl)phenol (4a): The title compound was prepared by following the same procedure as above (**4**) only different in the starting material from **3a** (2.2 g) to give a product (yield : 72%, 1.51 g). ^{1}H NMR ($CDCl_3$) δ 2.66(br s, 4H), 2.68(t, 2H), 2.88(t, 4H), 3.36 (quar, 2H), 5.25(br, 1H), 6.38(d, 1H), 6.50(t, 1H), 6.78(t, 1H), 6.87(d, 1H), 7.00(t, 1H), 7.10(d, 1H), 7.36(t, 1H), 8.01(d, 1H). (LC/MSD M+1): cald for 299.4 found 300

***N*-(2-(4-(2-Methoxyphenyl)piperazin-1-yl)ethyl)pyridin-2-amine (4b):** The title compound was prepared by following the same procedure above (**4**) only different in the starting material **3b** (2.3 g) to give a product (yield : 77%, 1.70 g). ^{1}H NMR ($CDCl_3$) δ 2.79(br, 4H), 2.81(br, 2H), 3.16(br, 4H), 3.47 (q, 2H), 3.88(s, 3H), 5.36(br, 1H), 6.47(d, 1H), 6.59(t, 1H), 6.87-7.04(m, 4H), 7.44(t, 1H), 8.10(d, 1H). (LC/MSD M+1): cald for 313.4 found 313.1.

***N*-(2-(4-(2-Chlorophenyl)piperazin-1-yl)ethyl)pyridin-2-amine (4c):** The title compound was prepared by following the same procedure above (**4**) only different in the starting material **3c** (2.4 g) to give a product (yield : 75%, 1.75 g). ^{1}H NMR ($CDCl_3$) δ 2.94(br, 6H), 3.24(br, 4H), 3.59(br d, 2H), 5.76(br, 1H), 6.54(d, 1H), 6.59(t, 1H), 7.00(t, 1H), 7.07(d, 1H), 7.23(t, 1H), 7.41(d, 1H), 7.43(t, 1H), 8.05(d, 1H). (LC/MSD M+1): cald for 317.8 found 317.0.

***N*-(2-(4-(2-(tert-Butyldimethylsilyloxy)phenyl)piperazin-1-yl)ethyl)pyridin-2-amine (5):** To protect the hydroxy group,

0.73g (1.2 eq, 4.8 mmol)of TBDMS-Cl and 0.73 g (1.2 eq, 4.8 mmol) of DBU was added to 1.2g (4 mmol) of **4a** dissolved in 25 mL of CH_3CN. The reaction mixture was stirred for 24 hrs at room temperature and the solvent was removed under a reduced pressure. The residue, a crude compound, was dissolved in small portion of MC and purified with chromatography with a solvent of EA : hexane (8:2) to give a pale sticky oil (yield : 68%, 1.13 g). ^{1}H NMR ($CDCl_3$) δ 0.00(s, 6H), 0.79(s, 9H), 2.60(br s, 4H), 2.72(t, 2H), 2.96(t, 4H), 3.31(br, 2H), 5.38(br, 1H), 6.30(d, 1H), 6.37(t, 1H), 6.58(d, 1H), 6.71(m, 3H), 7.21(t, 1H), 7.86(d, 1H). (LC/MSD M+1): cald for 413.6 found 413.1.

2-Amino-3-(2-((2-(4-(2-(tert-butyldimethylsilyloxy)phenyl)-piperazin-1-yl)ethyl)(pyridin-2-yl)amino)-2-oxoethylthio) propanoic acid (6): 1.1 g (2.7 mmol) of **5** was dissolved in 10 mL of anhydrous THF and then 370 μL (1 eq, 2.7 mmol) of TEA was added. The reaction mixture was cooled down to -18 ^oC using dry-ice/CCl_4 bath. 233 μL (1 eq, 2.7 mmol) of chloroacetylchloride was added dropwisely for 2 min and stirred for 10 min. When white suspension occurred, it was swiftly filtered by using glass wool. The filtrate was cooled down repeatedly to -18 ^oC and then 480 mg (1.5 eq, 4.0 mmol) of cysteine activated by 0.1 M CH_3ONa (4.0 mL, MeOH solution) was quickly added. The final reaction mixture was stirred for 2 hrs at room temperature and reduced its volume by reduced pressure. The purification step was carried out by using column chromatography with the solvent of MeOH : MC (1:4) to give a product as a yellow oil (yield : 55%, 840 mg) ^{1}H NMR ($CDCl_3$) δ 0.00(s, 6H), 0.79(s, 9H), 2.55(br, 6H), 2.79(br m, 4H), 2.78-3.04(dd, 2H), 3.12(s, 2H), 3.54(m, 1H), 3.87(t, 2H), 6.61(d, 1H), 6.67-6.74(m, 3H), 7.23(t, 1H), 7.39(d, 1H), 7.78(t, 1H), 8.36(d, 1H). (LC/MSD M+1): cald for 574.82 found 574.2.

Cysteine based WAY-100635 derivatives. [2-Amino-3-(2-((2-(4-(2-substituted-phenyl)piperazin-1-yl)ethyl)(pyridin-2-yl)-amino)-2-oxoethylthio)propanoic acid] (7): The procedure for preparation of **7** except **7a** was same procedure described in **6** and purified. The purification step was carried out by using prepTLC (20 × 20 cm × 0.25 mm) plates with solvent of MeOH : CH_2Cl_2 (1 : 1)

Cysteine based DWAY-100635. 2-Amino-3-(2-((2-(4-(2-hydroxyphenyl)piperazin-1-yl)-ethyl)(pyridin-2-yl)amino)-2-oxoethylthio)propanoic acid (7a): To remove the TBDMS, 300 mg (0.5 mmol) of **6** was treated with 410 μL (5 eq, 2.5 mmol) of Et_3N·HF in 10 mL of THF and the mixture was stirred at room temperature for 6 hrs. After the reaction was completed, solvent was removed under reduced pressure. Then the crude product was dissolved in 3 mL of MeOH and was purified by using column chromatography with MeOH : CH_2Cl_2 (1 : 1) to obtain a yellow oil (yield: 83%, 200 mg). To get a more purified compound, purification was performed by using prepTLC (20 × 20 cm × 0.25 mm) plates with solvent of MeOH : CH_2Cl_2 (1:1) to obtain a yellow oil. ^{1}H NMR (CD_3OD) δ 2.67(br m, 6H), 2.8-2.95(m, 2H), 2.94(br m, 4H), 3.35(s, 2H), 3.72(m, 1H), 4.06(t, 2H), 6.877.00(m, 4H), 7.43(t, 1H), 7.56(d, 1H), 7.97(t, 1H), 8.53(d, 1H). (LC/MSD M+1): cald for 460.6 found 460.1.

Cysteine based WAY-100635. 2-Amino-3-(2-((2-(4-(2-methoxyphenyl)piperazin-1-yl)-ethyl)(pyridin-2-yl)amino)-2-oxoethylthio)propanoic acid (7b): The title compound(yield : 62 %) was prepared by following the same procedure as **7** only different in the starting material **4b**. ^{1}H NMR (CD_3OD) δ(ppm) 2.68(br s, 4H), 2.80(t, 2H), 2.92(quar, 2H) 2.96(br s, 4H), 3.34(s, 2H), 3.76(m, 1H), 3.84(s, 3H), 4.22(t, 2H), 6.86-6.99(m, 5H), 7.32(t, 1H), 7.74(d, 1H), 7.85(t, 1H). (LC/ MSD M+1): cald for 474.6 found 474.1.

Cysteine based Cl-WAY-100635. 2-Amino-3-(2-((2-(4-(2-chlorophenyl)piperazin-1-yl)ethyl)(pyridin-2-yl)amino)-2-oxoethylthio)propanoic acid (7c): The title compound(yield : 69 %) was prepared by following the same procedure as **7** only different in the starting material **4c.** ^{1}H NMR (CD_3OD) δ 2.80(br, 4H), 3.05(br, 4H), 3.10(br, 2H), 3.21-3.31(m, 2H), 3.36(s, 2H), 3.77(m, 1H), 4.09(m, 2H), 6.64(t, 1H), 7.00-7.50(m, 4H), 7.61(d, 1H), 8.00(t, 1H), 8.57(d, 1H). (LC/MSD M+1): cald for 479.0 found 479.0.

Labeling study (8a, 8b, 8c). A carbonyl reaction kit containing $NaBH_4$ (2.5 mg), Na-Tartrate (8.5 mg), Na_2CO_3 (7 mg) and Na-boranocarbonate(4.5 mg) under N_2 atmosphere was added to 1 mL of [$^{99m}TcO_4^-$] containing ~ 185MBq (5mCi), and heated at 110 ^oC for 15 min. [$^{99m}Tc(CO)_3(H_2O)_3]^+$ was then adjusted to pH 7.4 with a PBS buffer and it was cooled down on ice bath. The product was identified with HPLC [$^{99m}Tc(CO)_3(H_2O)_3]^+$ was added to compound **7** (**7a**, **7b**, **7c**, 1 mg in pure water (1 mL)) followed by incubation at 75 ^oC for 30 min. The resulting complex was characterized by HPLC.

***In vitro* stability test.** The stability test of $^{99m}Tc(CO)_3$-complexes was performed by incubation at 37 ^oC, pH 7.0, and at different time intervals (1.5, 3, 4.5, and 6 hrs).

Electrophoresis. Filter paper (2 cm × 35 cm) presoaked in 0.5 M phosphate buffer (pH 7.4) was placed in an electrophoresis container. Each sample was spotted at the center of paper and then developed for 120 min at constant 300 V. After drying, each paper was checked by ITLC scanner.

Conclusion

In molecular design, the first requirement to be considered, even if we handle the chelating system like radiopharmaceuticals, is to check the 3D-structural relation of molecular body. Our proposed compounds (**8a**, **8b**, **8c**) were also designed and synthesized to maintain the structure prototype of WAY-100635 as possible.

Our novel compounds (**8a**, **8b**, **8c**) show good labeling yield and the data, based on our experiments for physical properties show promising radiolabeled tracers which can be utilized for the imaging of 5-HT_{1A} receptors associated with multiple neurodisorders.

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