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# Synthesis, radiolabeling and animal studies of [$^{131}$I]MPPI: A 5-HT$_{1A}$ imaging agent

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**Abstract** The synthesis and biological evaluation of serotonin (5-HT$_{1A}$) imaging agent [$^{131}$I]-4-iodo-*N*-{2-[4-(2-methoxyphenyl)-piperazin-1-yl]-ethyl}-*N*-pridin-2-yl-benzamide ([$^{131}$I]MPPI ) are reported. The chemical structure of aimed compound and intermediates were confirmed by IR, $^{1}$HNMR, and MS. Radiochemical purity was above 99% determined by TLC. Biodistribution of [$^{131}$I]MPPI in rats displayed high uptake in hippocampus and low uptake in cerebellum. The ratio of the uptake of [$^{131}$I]MPPI in hippocampus to that in cerebellum was 2.90 at 30 min post injection. The radioactivity in thyroid was 0.069 and 0.128% ID/g organ at 5 min and 120 min, respectively, and it was increased with time, which suggests that *in vivo* deiodination may be the major route of metabolism. *Ex vivo* autoradiography of brain section displayed significant decrease of radioactivity in hippocampus when pretreated with 8-OH-DPAT, a selective 5HT$_{1A}$ agonist, compared with control. These findings strongly suggested that $^{131}$I-MPPI could be used as an *in vivo* marker for studies of pharmacology of the 5-HT$_{1A}$ receptor system in animals.

**Keywords** 5-HT$_{1A}$, [$^{131}$I]MPPI, Synthesis, Biological evaluation, Imaging agent, Biodistribution

**CLC number** R817

## 1 Introduction

In the last two decades, considerable progress has been made in the understanding of the central nervous system (CNS) serotonin system. It is an important neurotransmission network that regulates various physiological functions and behavior, including anxiety and affective states.[1-3] The family of receptors activated by the neurotransmitter serotonin has been divided into at least seven classes (5-HT$_{1-7}$), some of them further subdivided into different subtypes[4, 5]. Over the past few years, special attention has been paid to 5-HT$_{1A}$, which is certainly the most well-characterized subtype due to the availability of the *R* enantiomer of 8-OH-DPAT, a high selective 5HT$_{1A}$ agonist. 5HT$_{1A}$ receptor is involved in various physiological processes such as the regulation of mood, sleep, and sexual disorder as well as in psychiatric disorders such as anxiety and depression [6, 7]. To develop antagonist for *in vivo* evaluation of 5-HT$_{1A}$ receptors, a series of halogen substituted benzamido derivatives of 4-(2'-methoxyphenyl)-1-[2'(*N*-2''-pyridinylhalobenzamido) ethyl] piperazine were prepared.[8] Of these, the iodinated derivative (the *p*-iodobenzamido compound, [$^{125}$I]-*p*-MPPI) displayed high affinity and selectivity for 5-HT$_{1A}$ receptors. De-

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spite promising *in vivo* binding properties both in rats and baboons, *in vivo* application of $^{123}$I-MPPI in human subjects is hampered by negligible ability to penetrate blood-brain-barrier (BBB). However, MPPI still has potential applications in drug screening in animal models and as an *in vivo* marker for studies of pharmacology of the 5-$HT_{1A}$ receptor system in animals.

Herein, we report the synthesis, radiolabelling and preliminary evaluation of $^{131}$I-MPPI as a 5-$HT_{1A}$ imaging agent.

## 2 Materials and methods

### 2.1 General materials

All reagents were purchased from commercial suppliers and used without further purification unless otherwise stated. When reactions were worked up by extraction with dichoromethane ($CH_2Cl_2$), ethyl acetate (EtOAc) or ethyl ether ($Et_2O$), organic solutions were dried with anhydrous $Na_2SO_4$ and concentrated with a rotary evaporator under reduced pressure.

Melting points were determined on Yanadimoto apparatus and uncorrected. Column chromatography was performed using silica gel, 100—200 mesh. $^1$HNMR spectra were recorded on an AM spectrometer at 400 MHz, with $CDCl_3$-*d* as solvent and tetramethylsilane (TMS) as the internal standard (0 ppm). Mass spectra were run on Varian MAT 2.2 spectrometer.

Rats (Sprague-Dawley, 200–250 g) were purchased from the Center of Experimental Animals of East China. They were allowed free access to food and water in the biodistribution study.

### 2.2 Synthesis of *p*-MPPI

The synthetic route is shown in Fig. 1.

**2.2.1** 2-chloro-*N*-pyridin-2-yl-acetamide (**3**)

To a solution of pyridin-2-ylamine (4.7 g, 0.05 mol) and triethylamine in $CH_2Cl_2$ (50 mL) chloro-acetyl chloride (4.7 mL) was added at low temperature (-78°C) under nitrogen. The mixture was stirred at 0°C for another 1 h, cold water (100 mL) was added and the organic layer was separated. The aqueous layer was adjusted to pH 10 with 50% sodium hydroxide followed by extraction with $CH_2Cl_2$ (50 mL × 4). The combined organic layers were washed with $H_2O$, dried and concentrated under reduced pressure to give **3** (7.5g, 87%) as a gray solid, mp: 118~120°C. IR (KBr, $cm^{-1}$): 3226, 1685, 1581, 1542; $^1$HNMR: δ 4.20 (s, 2H), 7.10 (t, 1H), 7.73 (t, 1H), 8.19 (d, 1H), 8.32 (m, 1H), 8.95 (s, 1H); MS: 170 ($M^+$), 135 (M-Cl), 78 (M-$ClCH_2CONH$).

**2.2.2** 2-[4-(2-methoxy-phenyl)-piperazin-1-yl]-*N*-pyridin-2-yl-acetamide (**4**)

A mixture of product **3** (1 g, 5.86 mmol), 1-(2'-methoxy-)piperazine (1.3 g, 6.77 mmol) and $K_2CO_3$ (0.5 g, 3.6 mmol) in 50 mL DMF was stirred at room temperature. Twenty hours later, the reaction was quenched with water (200mL). The mixture was

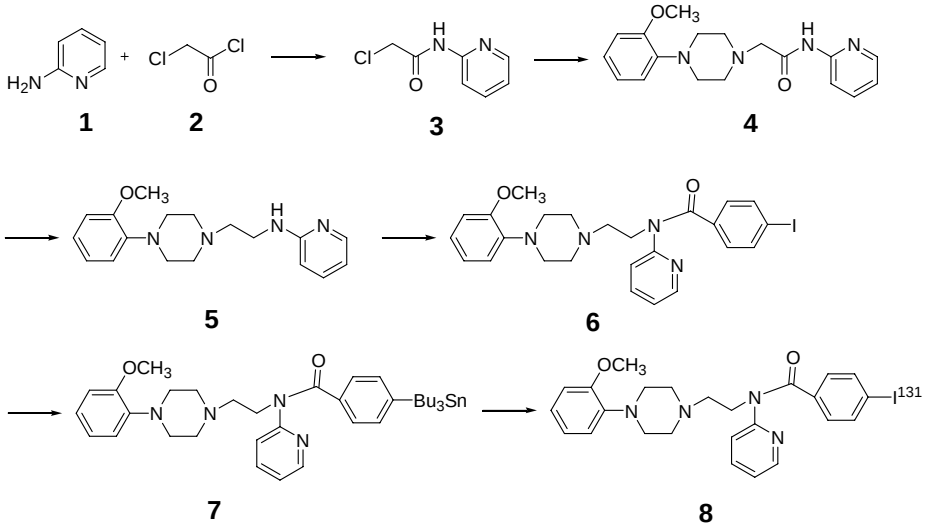



**Fig. 1** Synthesis of MPPI.

adjusted to pH 10 with 50% sodium hydroxide and the so-obtained precipitate was collected to give the product **4** (1.2 g, 63%) as a white solid, mp: 83~85°C. IR (KBr, cm$^{-1}$): 3336, 3304, 2830, 1696; $^1$HNMR: δ1.85 (s, 1 H), 2.85 (t, 4 H), 3.20 (t, 6 H), 3.85 (s, 3 H), 6.80~7.10 (m, 4 H), 7.75 (m, 1 H), 8.30 (m, 1 H), 9.65 (s, 1 H); MS:326 ($M^+$), 190 ((M-pyridinyl-NHCOCH$_2$), 205 (M-pyridinyl-NHCO).

**2.2.3** {2-[4-(2-methoxy-phenyl)-piperazin-1-yl]-ethyl}-pyridin-2-yl-amine (**5**)

A solution of the product **4** (1 g) in THF (20 mL) was added slowly and dropwise to a slurry of LAH (1 g, 3 mmol) in THF (20 mL) at room temperature under stirring. The mixture was then refluxed overnight and then cooled to room temperature. Water (6.0 mL) and NaOH (1 mol/L, 1 mL) were added successively and the mixture was stirred at room temperature for another 10 min and filtered. The filtrate was dried and evaporated under reduced pressure to give the product **5** (0.5 g, 52%) as a white solid, mp: 68~70°C. IR (KBr, cm$^{-1}$):3267, 2830, 1622; $^1$HNMR: δ2.25（s, 2 H）, 2.75（t, 6 H）, 3.10（s, 4 H）, 3.40（t, 2 H）, 3.85（s, 3 H）, 5.25 (s, 1 H), 6.41 (d, 1 H), 6.55 (m, 1 H), 6.85~7.05 (m, 3 H), 7.42 (m, 1 H), 8.35 (m, 1 H); MS: 312 ($M^+$), 205 (M-pyridinyl -NHC H$_2$).

**2.2.4** 4-iodo-*N*-{2-[4-(2-methoxy-phenyl)-piperazin-1-yl]-ethyl}-*N*-pyridin-2-yl-benzamide (**6**)

To a solution of compound **5** (0.5 g, 1.6 mmol) and $Et_3N$ in $CH_2Cl_2$ was added a solution of 4-iodobenzoyl chloride (0.56 g, 2.1 mmol) in $CH_2Cl_2$ dropwise at 0°C in an ice bath. The mixture was stirred at room temperature for 1 h. Water was added and the mixture was extracted with $CH_2Cl_2$. The combined organic layers were dried, evaporated to give the crude product. The crude product was recrystallized from acetone to give the pure compound **6** (0.7g, 81%) as white crystal, mp: 76~78°C. IR (KBr, cm$^{-1}$): 2941, 2816, 1650, 1587, 1502; $^1$HNMR: δ2.60 （s, 4 H）, 2.78 (t, 2 H), 2.92 (s, 4 H), 3.84 (s, 3 H), 4.25 (t, 2 H), 6.70~7.05 (m, 8 H), 7.43 (m, 1 H), 7.56 (d, 2 H), 8.41 (m, 1 H); MS: 543 (M+1), 565 (M+Na), 417 (M-I), 350 (M-$C_{11}H_{15}N_2O$), 313 (M-$C_7H_4OI$) .

**2.2.5** 4-tributylstannyl-*N*-{2-[4-(2-methoxy-phenyl)-piperazin-1-yl]-ethyl}-*N*-pyridin-2-yl-benzamide (**7**)

To a solution of compound **6** (150 mg, 0.28 mmoL) in triethylamine (3.0 mL), bis (tributyltin) (1 mL) and tetrakis (triphenylphosphine) palladium (10 mg) were added. The mixture was heated at 100°C in a sealed vial for 48 h. The solvent was removed under reduced pressure and the residue was purified successively by column chromatography on silica gel to give compound **7** (150 mg, 77%) as a colorless oil. IR (KBr, cm$^{-1}$): 2953, 2923, 2848, 1649, 1586, 1499, $^1$HNMR: δ0.88-1.90 (m, 27 H), 2.65 (s, 4 H), 2.80 (s, 2 H), 3.00 (s, 4 H), 3.82 (s, 3 H), 4.36 (m, 2 H), 6.70~7.41 (m, 11 H), 8.42 (m, 1 H); MS: 705 ($M^+$), 513 (M-$C_{11}H_{15}N_2O$), 417 (M-$Bu_3Sn$).

**2.2.6** Preparation of [$^{131}$I]MPPI (**8**)

The tributyltin precursor **7** (50 μg in 50 μL of ethanol), [$^{131}$I] sodium iodide (~37 MBq), and 1 mol/L HCl (100 μL) were placed in a sealed vial. To this mixture, 100 μL of hydrogen peroxide (3% solution in water, W/V) was added via a syringe at room temperature. The iodination reaction was terminated after 10 min by an addition of saturated sodium bisulfite. The mixture was then extracted with ethyl acetate (3×1 mL) after neutralization with saturated $NaHCO_3$ solution. The combined organic layers were evaporated to dryness, and the remaining residue was stored in a refrigerator for future use or dissolved in ethanol for the analysis of radiolabeling yield and radiochemical purity by TLC.

**2.2.7** Biodistribution in rats

[$^{131}$I] MPPI (0.2 mL, 0.37 MBq, 10% ethanol in saline) was injected through tail veins into rats (divided into 7 groups, 3 in each group). The rats were sacrificed under anesthesia at regular intervals (5, 10, 20, 30, 45, 60, and 120 min) post injection. The organs of interest (brain, heart, liver, spleen, lung, kidney, thyroid, etc.) were dissected and the brain was further separated (cortex, striatum, hippocampus, hypothalamus, and cerebellum), weighed, and the radioactivity was counted in a Packard gamma automatic counter (Model 5000). The uptake of each organ was calculated and expressed as percentage of injection dose per gram.

**2.2.8** *Ex vivo* autoradiography in rat's brain

8-OH-DPAT (0.1 mL, 2 mg/kg, 5 min prior)[9] and saline (0.1 mL, 5 min prior) were injected through tail veins into rats (divided into 2 groups, 2 in each group),

followed by injection of $^{131}$I-MPPI (0.2 ml, 0.37 MBq), respectively. At 30 min post injection of $^{131}$I-MPPI, the rats were sacrificed under anesthesia (diethyl ether). Brains were then rapidly removed and kept frozen at -17°C before sectioning. After being embedded in O.C.T. medium, the brains were cut by a cryostat at consecutive 20 μm coronal sections and thaw-mounted on superfrost/plus microscope slides. The slides containing the brain sections were dried at room temperature and exposed on GS-250 Phosphor Imaging Screen-BI. After a 2-h exposure, the slides were then imaged in GS-250 Molecular Imager and the optical densities were determined with an image analysis system.

## 3 Results and discussion

The synthesis of *p*-MPPI was according to Ref. [8] with some modifications. Briefly, starting from commercially available compound **1** and compound **2** in the presence of triethylamine in $CH_2Cl_2$, reaction under low temperature (-78°C) gave product **3**. Product **3** with high yield (87%) and high purity can be obtained when we conduct the reaction at -78°C rather than at the temperature of 0°C as indicated in the literature. Efforts were made at this step according to the literature; unfortunately the yield was only 5% after purification on the flash chromatography. Coupling compound **3** with 1-(2-methoxy-phenyl)-piperazine overnight can successfully afford compound **4**. Further reduction of the keto-group with LAH furnished compound **5**, which was presented as white solid rather than thick oil as described in the literature. Compound **5** was treated with 4-iodobenzoyl chloride in $CH_2Cl_2$ to generate the cold standard compound **6**, which was then stirred with $(Ph_3P)_4Pd$ and bis(tributyltin) in triethylamine to give product **7**. The overall yield was about 20% and the chemical structures of the aimed compound and all intermediates were confirmed by IR, $^1$HNMR, and MS. Compound **7** also served as a starting material for the radiolabeling reaction. When treated with radioactive [$^{131}$I] sodium iodide in the presence of oxidative agent ($H_2O_2$), the radiolabeling yield of $^{131}$I-MPPI was 90% and the radiochemical purity of $^{131}$I-MPPI was about 99% after being extracted by ethyl acetate. $R_f$ value of $^{131}I^-$ and $^{131}$I-MPPI were 0~0.1 and 0.9~1.0, respectively, as determined by TLC. The radiochemical purity of the product was found to be stable for 2 months when kept in the freezer (>95% pure, analyzed by TLC).

Biodistribution studies (Table 1) in rats showed that in the initial uptakes of [$^{131}$I]-MPPI in liver, lung, and kidney were (1.434±0.039), (1.172±0.345), and (1.805±0.292)%ID/g organ (at 5 min post injection), respectively. The uptake of thyroid at 5 min and 120 min were 0.069 and 0.128%ID/g organ, respectively, and it was increased with time, suggesting that *in vivo* de-iodination may be the major route of metabolism.

**Table 1** Biodistribution of $^{131}$I-MPPI in rats (ID%/g organ, $n=3$)

| Organ | Time / min | | | | | |
|---|---|---|---|---|---|---|
| | 5 | 10 | 20 | 30 | 60 | 120 |
| Heart | 0.777±0.076 | 0.486±0.057 | 0.277±0.051 | 0.190±0.054 | 0.142±0.021 | 0.077±0.005 |
| Liver | 1.434±0.039 | 1.471±0.081 | 1.205±0.287 | 0.952±0.041 | 0.893±0.105 | 0.585±0.088 |
| Spleen | 0.434±0.080 | 0.581±0.071 | 0.318±0.034 | 0.198±0.061 | 0.116±0.027 | 0.102±0.018 |
| Lung | 1.172±0.345 | 0.870±0.177 | 0.574±0.122 | 0.571±0.198 | 0.286±0.015 | 0.146±0.018 |
| Kidney | 1.805±0.292 | 1.414±0.255 | 0.789±0.137 | 0.420±0.113 | 0.428±0.080 | 0.239±0.025 |
| Thyroid | 0.069±0.020 | 0.100±0.007 | 0.073±0.010 | 0.065±0.024 | 0.096±0.035 | 0.128±0.026 |
| Blood | 0.806±0.061 | 0.461±0.039 | 0.385±0.149 | 0.368±0.046 | 0.276±0.048 | 0.127±0.037 |

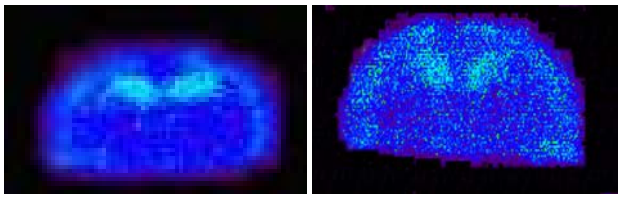
The uptakes of $^{131}$I-MPPI in different brain regions (CB, cerebellum; MD, medulla; FL, frontal lobe; OPL, occipital lobe; ST, striatum; HP, hippocampus; HY, hypothalamus) in rats are shown in Table 2. The radioactivity in HP, a region which is known to be rich in $5HT_{1A}$ receptors, reached (0.226±0.026) and (0.092±0.013)%ID/g at 30 and 60 min post injection, while as shown in Table 2, the activity in CB were (0.079±0.014) and (0.043±0.004)%ID/g at the same time points. A high HP/CB ratio of 2.9 could be achieved at 30 min post injection, which is consistent with Ref. [8].

To further characterize the nature of the HP uptake, the time point (30 min) was chosen for *ex vivo* autoradiography study based on the results got from biodistribution in rats' brains. Blocking study was then performed in rats pretreated with 8-OH- DPAT (2 mg/kg, iv) at 5 min prior to the injection of the radiotracer $^{131}$I-MPPI. As shown in Fig. 2, the specific uptake in hippocampus region of the brain (CA1 and CA3) displayed a marked decrease with very low nonspecific binding. The radioactivity in HP when assayed by optical densities was significantly diminished from (13.98 ± 0.87) to (1.96 ± 0.46) after pretreatment with the agonist as compared with the control. As expected, the tremendous decrease is most likely due to the fact that MPPI shares the same 5HT$_{1A}$ receptor-binding site as 8-OH-DPAT in the brain, therefore MPPI is a potentially useful radiotracer for *in vivo* imaging of the 5-HT$_{1A}$ receptor in animals.

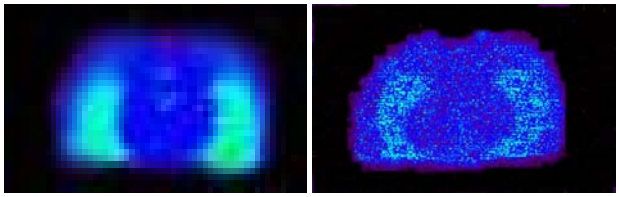
**Table 2** Regional brain distribution of [$^{131}$I]-MPPI (%ID/g, $n = 3$)

| | Time / min | | | | | |
|---|---|---|---|---|---|---|
| | 5 | 10 | 20 | 30 | 60 | 120 |
| CB | 0.430 ± 0.051 | 0.248 ± 0.041 | 0.128 ± 0.010 | 0.079 ± 0.014 | 0.043 ± 0.004 | 0.023 ± 0.000 |
| MD | 0.449 ± 0.046 | 0.308 ± 0.035 | 0.167 ± 0.011 | 0.100 ± 0.013 | 0.050 ± 0.009 | 0.025 ± 0.000 |
| FL | 0.684 ± 0.113 | 0.447 ± 0.081 | 0.227 ± 0.016 | 0.127 ± 0.023 | 0.062 ± 0.011 | 0.035 ± 0.006 |
| OPL | 0.678 ± 0.068 | 0.382 ± 0.07 | 0.206 ± 0.018 | 0.128 ± 0.020 | 0.067 ± 0.016 | 0.046 ± 0.006 |
| ST | 0.601 ± 0.076 | 0.354 ± 0.060 | 0.180 ± 0.010 | 0.113 ± 0.004 | 0.069 ± 0.011 | 0.054 ± 0.012 |
| HP | 0.598 ± 0.029 | 0.480 ± 0.089 | 0.285 ± 0.026 | 0.226 ± 0.026 | 0.092 ± 0.013 | 0.030 ± 0.002 |
| HY | 0.612 ± 0.053 | 0.302 ± 0.142 | 0.192 ± 0.013 | 0.127 ± 0.012 | 0.085 ± 0.011 | 0.059 ± 0.006 |
| Brain* | 0.893 ± 0.168 | 0.513 ± 0.103 | 0.272 ± 0.010 | 0.170 ± 0.046 | 0.073 ± 0.016 | 0.036 ± 0.003 |
| HP/CB | 1.39 | 1.95 | 2.23 | 2.90 | 2.22 | 2.41 |
| ST/CB | 1.40 | 1.43 | 1.41 | 1.46 | 1.58 | 1.35 |

*: ID%/g organ



(CA1)



(CA3)

**Fig. 2** Autoradiography in rats (left: control, right: block).

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