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Synthesis and Biodistribution of (*R*,*S*)-[*O*-Methyl-^{11}C]-1-[3-(5-Methoxy-1,2,3,4-tetrahydro-1-naphtalenyl)propyl]-4-Phenylpiperazine (PNU-157760), A Putative Radioligand for 5-HT$_{1A}$ Receptors

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Racemic 1-[3-(5-methoxy-1,2,3,4-tetrahydro-1-naphtalenyl)propyl]-4-phenylpiperazine (PNU-157760) was labeled with carbon-11 ($t_{1/2}$ = 20.4 min) as a putative radioligand for the noninvasive assessment of 5-HT$_{1A}$ receptors *in vivo* with positron emission tomography (PET). The radiochemical synthesis consisted of O-methylation of desmethyl precursor with [^{11}C]methyl iodide in the presence of potassium *tert*-butoxide in DMF. The desmethyl precursor for the radiosynthesis of [^{11}C]PNU-157760, was prepared by a convenient one-step demethylation of PNU-157760 with boron tribromide. (R,S)-[O-Methyl-^{11}C]-1-[3-(5-methoxy-1,2,3,4-tetrahydro-1-naphtalenyl)propyl]-4-phenylpiperazine with >99% radiochemical purity was obtained in 30 min with a radiochemical yield of 10 ± 5% (EOS, nondecay corrected) and a specific radioactivity of 2.5 ± 1 Ci/μmol. Biodistribution studies in rats showed that [^{11}C]PNU-157760 readily crosses the blood–brain barrier with a maximum of brain uptake at 30 min after injection; however, the low specific-to-nonspecific binding ratio *in vivo* as evidenced by the low hippocampus/cerebellum uptake ratio (1.17 at 60 min postinjection) does not make [^{11}C]PNU-157760 a promising radioligand for serotonin 5-HT$_{1A}$ receptors.

Key Words: [^{11}C]PNU-157760; 5-HT$_{1A}$ antagonist; rats; biodistribution; radiosynthesis; serotonin receptor; PET.

INTRODUCTION

Recent studies of the serotoninergic system implicate the involvement of the 5-HT$_{1A}$ serotonin receptor in several neurological and psychiatric disorders such as depression, anxiety, schizophrenia, and Alzheimer's disease (*1–3*). Development of a radioligand for the *in vivo* measurement of 5-HT$_{1A}$ receptor occupancy by positron emission tomography (PET) (for reviews see (*4*, *5*)) would help to unravel the function of these receptors and their role in various physiological and pathological states.

Among the variety of 5-HT$_{1A}$ agonists and antagonists labeled with PET and SPECT isotopes (*6–11*) only WAY-100635, [*O*-methyl-^{11}C] and [*N*-carbonyl-^{11}C], has proven to be a potential radioligand for delineation of serotonin receptors in

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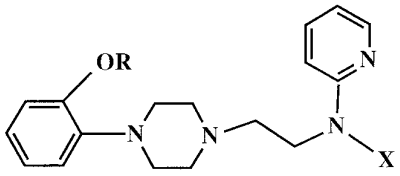

WAY-100635	R=CH3	X=cyclohexylcarbonyl
WAY-100634	R=CH3	X=H
desmethylWAY-100635	R=H	X=cyclohexylcarbonyl
p-[^{18}F]MPPF	R=CH_3	X=p-[^{18}F]fluorobenzoyl
[^{11}C]CPC-222	R=$^{11}CH_3$	X=bicyclooctylcarbonyl
(18)	R=H	X=cyclohexyl[^{11}C-carbonyl]
(19)	R=$^{11}CH_3$	X=H
(9)	R=$^{11}CH_3$	X=substituted phenylcarbonyl
(20)	R=CH_3	X=[^{18}F]fluoroarylcarbonyl
(16, 27)	R=$^{11}CH_3$	X=cycloalkylcarbonyl
(6)	R=CH_3	X=[^{123}I]iodoarylcarbonyl
(9,11,19)	R=[^{18}F]fluoroalkyl	X=cyclohexylcarbonyl

FIG. 1. WAY-100635 and its analogues.

the living human brain (*12*, *13*). Nevertheless, the metabolic pattern of [^{11}C]WAY-100635 complicates quantification of PET imaging data (*14*) and might be responsible for the failure of [*O*-methyl-^{11}C]WAY-100635 to demonstrate changes in 5-HT_{1A} receptor concentrations (*10*). WAY-100635 is a silent high affinity 5-HT_{1A} receptor antagonist (K_d = 0.4 nM, rat hippocampal membrane homogenates) (*15*). This ligand is rapidly metabolized by human liver (*16*), the main metabolic route being decarbonylation to give WAY-100634 (Fig. 1), which in turn is rapidly converted into more polar metabolites, contributing to both specific and nonspecific radioactivity binding (*14*, *17*). Published data do not exclude the possibility of dealkylation on the phenolic moiety, leading to formation of desmethyl-WAY-100635, which was shown to have a performance comparable to that of [carbonyl-^{11}C]WAY-100635 (*18*).

A number of fluoroalkyl, fluoroaryl, and iodoaryl analogues of WAY-100635 have been synthesized in search of a more *in vivo* stable 5-HT_{1A} receptor radioligand (*9*, *11*, *16*, *19–22*). SPECT imaging with the *p*-iodophenyl derivative of WAY-100635, *p*-[^{123}I]MPPI, failed to visualize 5-HT_{1A} receptors in humans, inspite of encouraging results achieved on rats and nonhuman primates (hippocampus/cerebellum radioactivity ratio maximum was 3 at 50 min postinjection in cynomolgus monkey (*7*). The *p*-fluorobenzoyl derivative of WAY-100635, *p*-MPPF (K_d = 1.2 nM in biogenic amine assay (*23*), K_i = 3.3 nM against 8-OH-PIPAT in rat hippocampal

homogenates) was labeled with ^{18}F-fluoride (*24*). This ligand was able to develop a specific signal in the rat and cynomolgus monkey brain, observed hyppocampus/cerebellum ratio at 30 min postinjection being 4 in rats (*21*) and 3 in cynomolgus monkey (*25*), though the degree of specific binding was much greater for [^{11}C]WAY-100635 (hyppocampus/cerebellum ratio was 16 in rats (*15*) and 5.5 in macaca monkey (*26*)). Comparison between ^{11}C-carbonyl WAY-100635 and *p*-[^{18}F]MPPF showed that the brain uptake in rats at 30 min postinjection was 5 times greater for WAY-100635, the blood clearance and the rate of metabolism was fast and similar for both ligands (at 30 min postinjection approximately 40% of radioactivity was found as a parent compound) (*20*). Analysis of arterial plasma in monkey showed that only 20% of radioactivity remained as the parent compound at 30 min postinjection (*25*). The animal biodistribution data reveal that the pharmacological profile of *p*-[^{18}F]MPPF is similar to that of *p*-[^{123}I]MPPI, thus leaving a minor hope for its possible use as a radioligand for studies in humans due to the drastic metabolism difference between species.

It was shown by *in vitro* metabolic assay with human liver cytosol and microsomal preparations that metabolically stable analogues of WAY-100635 pertaining high affinity for the serotonin receptor could be obtained by the substitution of cyclohexyl moiety for the bicycloalkyl group (*16*). Recently images of the 5-HT$_{1A}$ receptor-rich areas in the living human brain were obtained with [*O*-methyl-^{11}C]CPC-222 (*27*). No decarbonylation metabolite, [*O*-methyl-^{11}C]WAY-100634, was found in the plasma of healthy volunteers and a good brain penetration with the peak cortical concentration corresponding to 2% of the injected dose was reported. The degree of nonspecific binding with this tracer was higher than that observed with [carbonyl-^{11}C]WAY-100635 (the ratio of radioactivity in temporal cortex to that of the cerebellum reached a plateau of 3 by 45 min after injection of [*O*-methyl-^{11}C]CPC-222 as compared to 25 at 60 min for [carbonyl-^{11}C]WAY) (*13*, *27*). This disappointing result could be explained partially by the higher lipophilicity of the CPC-222, calculated logP = 5.3, and its lower affinity for the serotonin receptor, IC_{50} = 8.1 nM versus [^{3}H]8-OH-DPAT on rat hippocampal homogenates, as compared to WAY-100635, IC_{50} = 6.0 nM and logP = 4.7 (*16*).

We considered the possibility to label an alternative compound, which would have affinity and selectivity to 5-HT$_{1A}$ receptors comparable to that of WAY-100635, but would not be susceptible to decarbonylation. The compound, PNU-157760 (**1**) (see Fig. 2), was chosen from series of 1-aryl-4-((1-tetralinyl)alkyl)piperazines; it exhibited *in vitro* high affinity and selectivity toward 5-HT$_{1A}$ receptors (racemate, IC_{50} = 0.50 nM, [^{3}H]8-OH-DPAT in rat hippocampus homogenates, ratios receptor IC_{50}/IC_{50} 5-HT$_{1A}$: 5-HT$_2$ = 460, D_2 = 220, α_1 = 86, α_2 = 520) (*21*). The core structure of PNU-157760 is 1-phenylpiperazine to which a 5-methoxytetralinyl ring is attached via a three-membered alkyl chain (*28*). The molecule does not contain the carboxamide-moiety susceptible to metabolic cleavage and could be easily labeled by *O*-methylation of the phenol group of the desmethyl analogue.

In this article we report the preparation of the des-methyl precursor, radiolabeling with [^{11}C]methyl iodide and preliminary biodistribution experiments in rats of the putative 5-HT$_{1A}$ receptor radioligand (*R*,*S*)-[*O*-methyl-^{11}C]-1-[3-(5-methoxy-1,2,3,4-tetrahydro-1-naphtalenyl)propyl]-4-phenylpiperazine (PNU-157760).

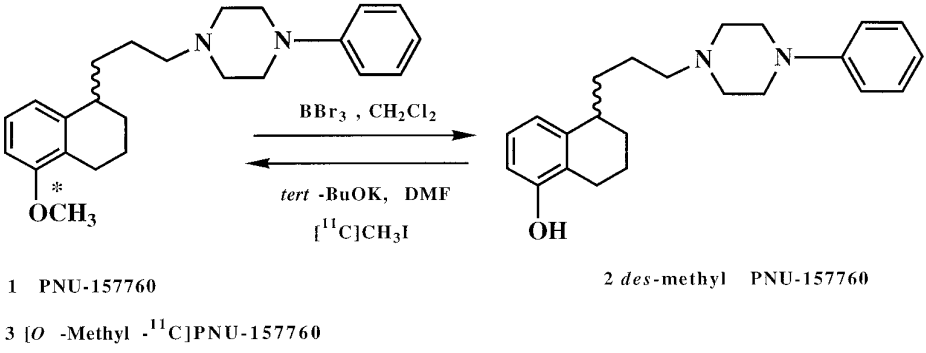

FIG. 2. Synthesis of [*O*-methyl-^{11}C]PNU-157760.

RESULTS AND DISCUSSION

Chemistry

Des-methyl precursor was prepared conveniently from racemic (R,S)-[*O*-methyl-^{11}C]-1-[3-(5-methoxy-1,2,3,4-tetrahydro-1-naphtalenyl)propyl]-4-phenylpiperazine, a free-base of PNU-157760, by *O*-demethylation of phenol ether with boron tribromide as described by McOmie (*29*).

Radiolabeling with [^{11}C]methyl iodide in the presence of strong bases is accompanied by the formation of [^{11}C]methanol. Nucleophiles present in the methylation mixture, the precursor phenolate and hydroxide anion are competing for the [^{11}C]methyl iodide. Thus optimal temperature and substrate/base concentration should be determined in order to maximize incorporation of [^{11}C] into the final product.

In Table 1 the yields of (R,S)-[*O*-methyl-^{11}C]-1-[3-(5-methoxy-1,2,3,4-tetrahydro-1-naphtalenyl)propyl]-4-phenylpiperazine and [^{11}C]methanol are reported in relation to the amount of precursor/base used at different temperatures. The reaction was carried out in 100 μl of DMF and [^{11}C]-incorporation yields were assessed as percentage of the total radioactivity in the reaction mixture by integration of HPLC γ-radioactivity chromatogram.

We observed that partition of the [^{11}C] label between methanol and the product is the subject of both kinetic and thermodynamic control (Table 1). The first three data points reflect the situation when [^{11}C]methyl iodide was trapped in the reaction mixture at -15°C, followed by 1.5 min heating at 100°C. Lowering the temperature preferentially decreased the rate of methylation, thus 15–30% of [^{11}C]methanol was found in the reaction mixture. Methylation reaction was fast at room temperature; no unreacted [^{11}C]methyl iodide was left when precursor concentration was 10 mg/ml and the hydrolysis to [^{11}C]methanol was negligible (5%). Use of the lower substrate amount (0.5 mg) resulted in the decrease of the methylation rate but did not affect the formation of [^{11}C]methanol, thus [^{11}C]methanol/product ratio was higher and 20% of [^{11}C]methyl iodide remained unreacted. Decrease of the base

TABLE 1
Radiolabeling of Des-methyl PNU-157760 with [^{11}C]Methyl Iodide in 100 μl of DMF, 1.5-Min Reaction Time (Percentage of Radioactivity, Mean of Three Experiments, ±SD)

Prec. (mg)	Base (mg)	Temperature (°C)	^{11}C-MeOH (%)	^{11}C-CH$_3$I (%)	^{11}C-PNU (%)
			t-BuOK		
1	1	−15/100	27 ± 8	0	59 ± 1
1	0.7	−15/100	20 ± 5	1 ± 1	58[a]
1	0.1	−15/100	14	15 ± 10	30[a]
1	1	0	14 ± 6	5 ± 4	70 ± 8
1	1	100	5 ± 3	0	82 ± 2
1	1	40	2 ± 1	0	85 ± 4
1	1	26	5 ± 3	0	78 ± 4
0.5	1	26	31 ± 5	20 ± 10	40[a]
			TBAH (μl)[b]		
1	2	26	40	0	45[a]

[a] Mean of two experiments.
[b] Used as a 60% aqueous solution

concentration (0.1 mg) reduced the rate of both competing reactions. We considered also tetrabutylammonium hydroxide (60% solution in water) as an alternative base catalyst. In this case significant hydrolysis to [^{11}C]methanol (40% of [^{11}C]radioactivity) was observed due to the presence of water.

Under the optimized conditions in the presence of potassium *tert*-butoxide only 2 ± 1% of [^{11}C]methanol was formed, thus other alternative bases for the phenolate preparation were not considered. Trapping of [^{11}C]methyl iodide at 40°C in 100 μl of DMF containing 1 mg of precursor and 1 mg of potassium *tert*-butoxide proceeded almost quantitatively, as measured by monitoring the radioactivity of the ascarite/molecular sieves/activated carbon trap placed at the reactor outlet. At the end of [^{11}C]methyl iodide transfer under these conditions 85% of radioactivity was incorporated into [^{11}C]PNU-157760, 13% of activity was randomly distributed in five minor radioactive side-products, which were not identified, but could likely derive from the N-methylation and hydrolysis of the final compound.

The identity of (R,S)-[*O*-methyl-^{11}C]-1-[3-(5-methoxy-1,2,3,4-tetrahydro-1-naphtalenyl]-4-phenylpiperazine was confirmed by HPLC coinjection of the radioactive product with authentic PNU-157760 using four different conditions. The structure of the labeled product was confirmed by the mass spectrum of the product fraction, collected after HPLC purification of a carrier-added synthesis (see Experimental). EI-LC-MS of the methanolic solution showed the molecular ion at m/z 364 as expected for 1-[3-(5-methoxy-1,2,3,4-tetrahydro-1-naphtalenyl)propyl]-4-phenylpiperazine; moreover the relative intensity of the ion at m/z 365 perfectly corresponded to that expected on the basis of natural isotope abundance for the molecular formula $C_{24}H_{32}N_2O$ (27.9% of ion at m/z 364), thus excluding the presence in the collected fraction of N-methylated products with MW 365 Da.

TABLE 2
Tissue Distribution of [^{11}C]PNU-157760 in Rats (Percentage Injected Dose/g of Tissue; Average of Three Rats ± SD)

Organ	10 min	30 min	60 min	90 min
Blood	0.02 ± 0.006	0.04 ± 0.02	0.02 ± 0.003	0.012 ± 0.004
Plasma	0.02 ± 0.01	0.05 ± 0.03	0.03 ± 0.01	0.016 ± 0.003
Heart	0.10 ± 0.04	0.16 ± 0.03	0.06 ± 0.01	0.03 ± 0.003
Lung	0.40 ± 0.20	0.40 ± 0.20	0.16 ± 0.04	0.10 ± 0.01
Liver	0.30 ± 0.07	1.30 ± 0.60	0.48 ± 0.06	0.40 ± 0.02
Adrenal gland	0.40 ± 0.06	2.40 ± 0.70	0.80 ± 0.20	0.90 ± 0.30
Kidney	0.30 ± 0.10	0.70 ± 0.20	0.24 ± 0.04	0.20 ± 0.09
Spleen	0.20 ± 0.04	0.50 ± 0.20	0.23 ± 0.04	0.17 ± 0.05
Intestine	0.10 ± 0.05	0.40 ± 0.20	0.73 ± 0.02	0.16 ± 0.06
Muscle	0.10 ± 0.02	0.20 ± 0.10	0.10 ± 0.07	0.05 ± 0.02
Brain	0.10 ± 0.03	0.30 ± 0.10	0.10 ± 0.02	0.07 ± 0.02

In a typical experiment starting from 600 mCi of [^{11}C]carbon dioxide 100 mCi of [^{11}C]PNU-157760 ready for injection were obtained in 30 min with radiochemical purity greater than 99% and specific radioactivity of 2 Ci/μmol (EOS). No detectable amounts of nonradioactive impurities were found in the final preparation after the HPLC purification on semipreparative column.

Biodistribution Studies

The biodistribution of radioactivity after [^{11}C]PNU injection in rats is reported in Table 2. Concentration of [^{11}C]PNU-157760 in blood was stable during the entire period of observation ranging between 0.012 and 0.04% of injected dose per gram of tissue (% ID/g). The tracer preferentially accumulated in plasma where radioactivity concentration was approximately 1.3 times higher than that in blood.

Organs with higher degree of [^{11}C]PNU-157760 accumulation were intestine, liver, lung, spleen, and kidney. Radioactivity uptake reached a peak at 30 min after tracer injection in liver (1.3 ± 0.6, % ID/g) and at 60 min in intensine (0.73 ± 0.02), suggesting metabolism of the compound to occur mainly in gastrointestinal tract. [^{11}C]PNU-157760 uptake was maximum in adrenal gland (2.4 ± 0.7% ID/g, 30 min postinjection), which is known to be rich in α-adrenergic receptors (*30*). Radioactivity appeared in the gland early (0.4 ± 0.06% ID/g, 10 min postinjection) and was persistent (at 90 min after injection adrenal gland/plasma concentration ratio achieved 56.2). *In vitro* PNU-157760 exhibited a moderate affinity to α_1 adrenergic receptor, IC_{50} = 43 nM, [^{3}H]prazosin (*31*). On the other hand, remarkable high uptakes in the adrenal gland were also found for several fluorine-18-labeled 5-hydroxy-2-aminotetralin derivatives that are potential dopamine agonists (*32*). Adrenal gland is rich in catecholamine and steroid synthesizing enzymes (*33*, *34*). It was shown that 7,12-dimethylbenzanthracene and 7-hydroxymethyl-12-methylbenzanthracene, but not benzopyrene, selectively produce necrosis in the two inner zones of the rat adrenal cortex and are toxic to cultured rat adrenocortical cells.