Synthesis and *In Vivo* Evaluation of a New Fluorine-18 Labeled Dopamine D2 Radioligand with Benzofuran Benzamide Skeleton

Futoshi Takao,[a] Shigeki Sasaki,[a] Minoru Maeda,*,[a] Toshimitsu Fukumura,[b] Takashi Tahara,[b] Kouji Masuda,[b] and Yuichi Ichiya[b]
[a]*Faculty of Pharmaceutical Sciences and* [b]*Faculty of Medicine, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan*

Summary

N-[(1-Ethyl-2-pyrrolidinyl)methyl]-5-(2-[^{18}F]fluoroethyl)-2,3-dihydrobenzofuran-7-carboxamide ([^{18}F]**5**) was synthesized *via* nucleophilic substitution with K^{18}F/Kryptofix222 complex in 5.4~6.8% radiochemical yields with a specific activity of larger than 5.6 TBq/mmol (150 Ci/mmol) at the end of the 110 minutes synthetic period. Its *in vivo* affinity toward CNS dopamine D2 receptors was investigated using rats in order to evaluate as a radiotracer for the PET (positron emission tomography) study of the dopamine D2 receptors. In biodistribution experiments, [^{18}F]**5** exhibited striatal accumulation, although the whole brain radioactivity was cleared rapidly. The striatal/cerebellar radioactivity ratio, which corresponds to the ratio of a brain D2 receptor-rich to poor region, gradually increased to about 12 at 60 minutes after the injection. The striatal uptake was inhibited with pretreated haloperidol, a dopamine D2 antagonist, indicating that the striatal accumulation was due to the specific binding with D2 receptors. Thus, [^{18}F]**5** appears to be a potential *in vivo* radiotracer for dopamine D2 receptors.

Keywords: [^{18}F]fluoroethylbenzofuran; positron emission tomography; dopamine D2 receptor

Introduction

In vivo imaging of neurotransmitter systems with selective radioligands have been expected to be useful for diagnosis of various diseases of the central nervous system (CNS). Among many radiolabeled dopamine D2 ligands which have been investigated for PET or SPECT studies of CNS dopamine D2 system, substituted benzamides have attracted much attention due to their selective, high affinity and reversible nature of binding to dopamine D2 receptors.[1] IBF(**1**)[2] and eticlopride (**2**) are representatives of such benzamides. We already reported that [^{18}F]fluorinated eticlopride derivative (**3**) with high *in vitro* affinity to D2 receptors (Table 1) showed only moderate *in vivo* selectivity as illustrated by striatal/cerebellar radioactivity ratio of 5.2 in rats at 90 min,[3] which ratio corresponds to

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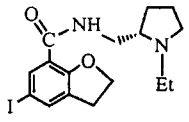
the ratio of a brain D2 receptor-rich to poor region. Furthermore, we synthesized IBF derivatives (**4** and **5**) with *in vitro* affinity toward D2 receptors.[4] In this paper, we wish to report on the synthesis and *in vivo* biodistribution study in rats of [^{18}F]labeled benzofuran derivative (**5**), which exhibited larger striatal/cerebellar ratio in spite of its lower *in vitro* affinity to D2 receptors (Table 1).

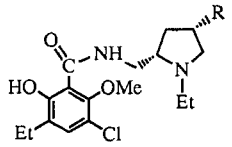
Table 1. In Vitro Receptor Binding[a)]

Compound	IC_{50} (nM)[b)]
IBF (**1**)	0.16[2)]
2	2.9[3a)]
3	1.9[3b)]
4	2.2[4)]
5	36[4)]

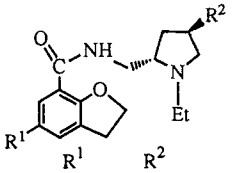
a) Data taken from the literature, in which bovine[3a)] or rat[3b, 4)] striatal tissue homogenates were used in vitro.
b) IC_{50} represents the concentration required to inhibit specific binding of [^{3}H]spiperone by 50%.



IBF (**1**)



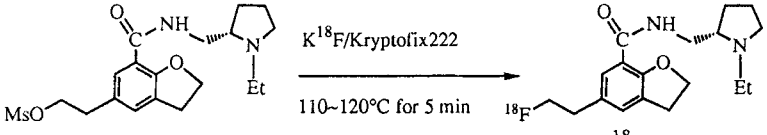
Eticlopride (**2**), R=H
Fluoroeticlopride (**3**), R=F



	R^1	R^2
4:	I	OH
5:	FCH_2CH_2	H

Results and Discussion

Chemistry. We already reported the synthesis of unlabeled ligand (**5**),[4] which was used as a HPLC standard in radiosynthesis. Aqueous [^{18}F]fluoride was used to prepare K^{18}F/Kryptofix222,[5] and [^{18}F]fluorination with the mesylate precursor (Fig. 1) was performed in the absence of the solvent in the analogous manner to the synthesis of [^{18}F]**3**.[3] A single purification by HPLC with a normal phase column provided [^{18}F]**5** in 5.4~6.8 % radiochemical yields (total synthetic time: 110 min, uncorrected for decay) with high chemical and radiochemical purity, which specific activities were estimated to be larger than 5.6 TBq (150 Ci) /mmol. The same [^{18}F]fluorination with either tosylate or mesylate of **4** did not give the corresponding [^{18}F]labeled derivative.



6 [^{18}F]**5**

Fig 1. Radiochemical Synthesis of [^{18}F]**5**

***In Vivo* Tissue Biodistribution in Rats.** A solution of [^{18}F]**5** in saline-5% ethanol was injected into rat tail veins. The animals were anesthetized with ether, and were killed at different times after the injection. Their tissues were dissected, weighed, and analyzed for radioactivity. Brain

regional distributions of radioactivity were investigated using a brain regional-dissection technique. The biodistribution of [^{18}F]**5** is summarized in Table 2.

Initial uptake was cleared in almost all tissues except in the bone and striatum. The bone activity increased gradually to 1.05 %dose/g at 60 min, indicating slow defluorination *in vivo*. Although the radioactivity uptake in the striatum was not very high (0.40 % dose/g at 30 min), the radioactivity was clearly retained, and the striatal/cerebellar radioactivity ratio which corresponds to *in vivo* selectivity between receptor-rich and receptor-poor brain region reached to 12 at 60 min. In the previous study it was reported that [^{18}F]**3** with higher *in vitro* affinity toward D2 receptors (Table 1) exhibited lower ratio of 5.22 at 90 min.[3c] It is interesting, in contrast to this, that [^{18}F]**5** with lower *in vitro* affinity achieved the higher striatal/cerebellar ratio. Cerebellum uptake which is used to estimate nonspecific binding is rapidly cleared with the use of [^{18}F]**5** from 0.20 %dose/g (5 min) to 0.02 %dose/g (60 min), whereas the clearance of [^{18}F]**3** was slower as illustrated by the change from 0.20 %dose/g (30 min) to 0.09 %dose/g (120 min).[3c] Hence, less nonspecific binding of [^{18}F]**5** to cerebellum is attributable to its relatively high *in vivo* selectivity.[6]

Table 2. Tissue Distribution of Radioactivity in Rats of [^{18}F]Fuoroethylbenzofuran(**5**).[a]

Tissue	Biodistribution %dose/g			Inhibition[b] %dose/g after 30 min	
	5 min	30 min	60 min	control	Haloperidol
Brain	0.26±0.02	0.12±0.01	0.05±0.02	0.09±0.01	0.06±0.01
Striatum	0.33±0.01	0.40±0.05	0.24±0.12	0.30±0.01	0.06±0.01
Cerebellum	0.20±0.03	0.07±0.01	0.02±0.01	0.06±0.02	0.05±0.01
Cerebral cortex	0.29±0.02	0.12±0.04	0.02±0.02	0.08±0.01	0.07±0.01
Hippocampus	0.20±0.02	0.10±0.03	0.03±0.02	0.07±0.00	0.06±0.01
Blood	0.22±0.02	0.21±0.01	0.08±0.04	0.15±0.03	0.21±0.02
Bone	0.14±0.05	0.74±0.03	1.05±0.20	0.52±0.09	0.87±0.04
Lung	1.84±0.30	0.36±0.19	0.18±0.07	0.47±0.06	0.58±0.14
Liver	1.15±0.21	0.71±0.11	0.36±0.14	0.51±0.05	0.82±0.06
Kidney	2.52±0.12	0.89±0.06	0.31±0.14	0.65±0.09	0.89±0.02
Heart	0.54±0.01	0.25±0.02	0.12±0.04	0.20±0.01	0.28±0.02
Small intestine	0.84±0.12	0.47±0.10	0.22±0.03	0.39±0.04	0.58±0.13
Striatum/cerebellum	1.65	5.7	12	5.0	1.2
Striatum/cerebral cortex	1.13	3.3	12	3.75	0.86
Brain/blood	1.18	0.57	0.63	0.6	0.29

a) Expressed as %dose/g with the mean ± S.D. of three rats. b) 0.8μmol/kg of haloperidol was injected intravenously 30 min before injected of the radiolabeled ligand.

Uptake inhibition experiment was carried out to confirm that the striatal uptake of [^{18}F]**5** is mediated by specific binding to dopamine D2 receptors with the use of haloperidol as a competitive ligand which is a neuroleptic drug to block CNS dopamine D_2 receptors. A saline solution of haloperidol (0.8 μmol/kg) was injected into rat tail veins, and 30 minutes later a solution of [^{18}F]**5** in saline-5% ethanol was injected, then the tissue distribution were investigated 30 minutes after the last injection (Table 2). The uptake of [^{18}F]**5** in the striatum was decreased to 20% of control, while the uptake in the other region of the brain showed no change. As a result, the striatal/cerebellar ratio was decreased to 1.2. Thus, the uptake inhibition by haloperidol clearly demonstrated the specific binding of [^{18}F]**5** to dopamine D2 receptors.

From the estimated density of dopamine D2 receptors (50 pmol/g)[7], a typical dose in our study (0.11 nmol based on the specific activity of 150 Ci/mmol), and the maximum striatal uptake (0.40 %dose/g at 30 min, Table 2), it could be estimated that fewer than 0.9% of the receptors were bound to [^{18}F]**5**, suggesting that doses of the radioligand used in the present *in vivo* binding experiments were much lower than receptor-saturation doses in the rat striatum.

In conclusion, [^{18}F]fluorinated benzofuran derivative ([^{18}F]**5**) exhibited relatively high striatal/cerebellar radioactivity ratio of about 12 after 60 minutes, and may be a suitable ligand for *in vivo* PET radiotracer study of dopamine D2 receptors.

Experimental

Chemistry. No-carrier-added aqueous [^{18}F]fluoride was prepared in an [^{18}O]H_2O enriched target (8 or 16%) using ^{18}O(p,n)^{18}F nuclear reaction employing a cyclotron at the University Hospital, Kyushu University. Radio-HPLC was carried out with Waters M-5 system, and analyzed both by an absorbance detector at 254 nm (Waters Model 441) and by a radioanalyzer (Aloka, Model RCL550). Radio-TLC was performed on a silica gel plate (Merck Kieselgel 60 F_{254}). Radioactivity was measured either on a radioisotope calibrator (Capintec CRC-30) or on a gamma counter (Packard Auto Gamma-500). Chemical and radiochemical purity of ^{18}F-labeled ligand was determined by HPLC, and the peak of the desired ligand was identified by HPLC co-injection studies. Specific activities were determined by UV spectrometer and a radioisotope calibrator.

(5*S*)-*N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-5-(2-[^{18}F]fluoroethyl)-2,3-dihydrobenzofuran-7-carboxamide ([^{18}F]5) K^{18}F/Kryptofix222 was prepared using Kryptofix222 (3.6 mg) and K_2CO_3 (0.8 mg), and the reagent was dried under vacuum for 15 min. A solution of

(5*S*)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-(2-methanesulfonyloxyethyl)-2,3-dihydrobenzofuran-7-carboxamide (**6**)[4] (1 mg) in acetonitrile (0.06 ml) was added to the above reagent, and the mixture was heated at 110~120°C for 5 min with removing the solvent. The crude residue was dissolved in AcOEt (0.5~0.8 ml) and filtered (Millipore FH 0.5 μ), then purified by normal phase HPLC (t_R=20 min, column: Whatman Partsil 5 PAC 9.4x100 mm; eluent:*n*-hexane:AcOEt:MeOH=400:100: 45; flow rate: 3.0 ml/min) equipped with a guard column (Waters Nova-Pac CN HP) to give [^{18}F]**5** in 5.4~6.8% radiochemical yields (total synthetic time: 110 min, uncorrected for decay). The isolated [^{18}F]**5** was shown to have the identical t_R with the unlabeled **5**, and was chemically and radiochemically pure by radio-HPLC analysis with a reverse phase HPLC column (YMC-Pack ODS-AQ, MeOH:0.004N HCl=9:1; flow rate: 2.0 ml/min) . UV absorbance of the isolated [^{18}F]**5** was less than detectable value, hence the specific activity was estimated to be larger than 5.6 TBq (150 Ci)/mmol. [^{18}F]**5** was dissolved in saline containing 5% ethanol, and used for the *in vivo* experiments.

***In Vivo* Biodistribution.** Male Wistar rats, weighing 200~230 g used for *in vivo* studies were allowed access to food and water *ad libitum*. A saline-5% ethanol solution of [^{18}F]**5** was injected in rat tail veins. The animals were anesthetized with ether, and were killed at indicated times after injection. Organs were first dissected, and brains were regionally dissected to cerebellum, striatum, and cerebral cortex. Tissue radioactivity was measured, corrected for both decay and the tissue weight, and expressed as %dose/g in Table 2. The radioactivity used for each animal experiment ranged from 0.40~0.59 MBq (10.8~16.0 μCi).

Inhibition experiments. A saline solution containing haloperidol at a dose of 0.8 μmol/kg was injected into rat tail veins. Thirty minutes later, a solution of [^{18}F]**5** (0.54~0.59 MBq (14.6~15.8 μCi)) in 0.5 ml saline-5% ethanol solution was injected in rat tail veins. The animals were anesthetized with ether, and killed 30 min after the injection. Tissue radioactivity was determined as described above. Control rats were treated with a saline-5% ethanol solution under identical conditions, and the results are listed in Table 2.

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References

1) Recent examples: P. Damhaut, R. Cantineau, C. Lemaire, A. Plenevaux, L. Christiaens, M. Guillaume, *Appl. Radiat. Isot.*, **43**: 1265 (1992), S. Chumradit, M. P. Kung, H. F. Kung, *J. Labelled Compd. Radiopharm.., (9th Intern. Symp. Radiopharm. Chem. Abs)*, **33**: 223 (1993), Y.-B. Fang, J. Mukheriee, M. Cooper, *ibid*, **33**: 234 (1993), R. H. Mach, P. A. Nowak, R. R. Leudtke, S. T. Elder, J. R. Jackson, P. A. Hoffman, P. H. Evora, P. B. Molinoff, J. G. Scripko, R. L. Ehrenkaufer, *ibid*, **33**: 262 (1993), C. Halldin, T. Högberg, H. Hall, P. Karlsson, C.-E. Hagberg, P. Ström, L. Farde, *ibid*, **33**: 282 (1993), C. Loc'h, M. Guillaume, O. Stulzaft, R. Cantineau, M. Ottaviani, M. Maziere, B. Maziere, **33**: 291 (1993).

2) R. A. Murphy, H. F. Kung, M.-L. Kung, J. Billings, *J. Med. Chem.*, **33**: 171 (1990).

3) a) T. Fukumura, H. Dohmoto, M. Maeda, E. Fukuzawa, and M. Kojima, *Chem. Pharm. Bull.*, **38**: 1740 (1990), b) K. Watanabe, T. Fukumura, S. Sasaki, M. Maeda, and S. Takehara, *ibid*, **39**: 3211 (1991), c) M. Maeda, S. Sasaki, T. Fukumura, E. Fukuzawa, K. Watanabe, M. Kojima, T. Tahara, K. Masuda, and Y. Ichiya, *ibid*, **40**: 1793 (1992).

4) S. Sasaki, F. Takao, K. Watanabe, N. Obana, M. Maeda, T. Fukumura, S. Takehara, *ibid*, **41**: 296 (1993).

5) H. H. Coenen, B. Klatte, A. Knöckel, M. Schüller, and G.Stöchlin , *J. Labelled Compd. Radiopharm.*, **23**: 455 (1986).

6) The following correlation between striatal/cerebellar ratio (str/cer) and the product of the receptor dissociation constant (K_D in n*M*) and the lipophilicity (k_w) was reported for ligands of benzamide class: $\log(str/cer)=2.87-0.82\log(K_D \times k_w)$, R. M. Kessler, M. S. Ansari, T. de Paulis, D. D. Schmidt, J. A. Clanton, H. E. Smith, R. G. Manning, D. Gillespie, M. H. Ebert, *J. Nucl. Med.*, **32**, 1593 (1991).

7) H. Hall, C. Köhler, L. Gawell, *Eur. J. Pharmacol.*, **111**: 191 (1985).