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[^{18}F]FMDAA1106 and [^{18}F]FEDAA1106: Two Positron-Emitter Labeled Ligands for Peripheral Benzodiazepine Receptor (PBR)

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Abstract—We synthesized and evaluated *N*-(5-fluoro-2-phenoxyphenyl)-*N*-(2-[^{18}F]fluoromethyl-5-methoxybenzyl)acetamide ([^{18}F]-FMDAA1106) and *N*-(5-fluoro-2-phenoxyphenyl)-*N*-(2-[^{18}F]fluoroethyl-5-methoxybenzyl)acetamide ([^{18}F]FEDAA1106) as two potent radioligands for peripheral benzodiazepine receptors (PBR). [^{18}F]FMDAA1106 and [^{18}F]FEDAA1106 were respectively synthesized by fluoroalkylation of the desmethyl precursor DAA1123 with [^{18}F]FCH_2I and [^{18}F]FCH_2CH_2Br. Ex vivo autoradiograms of [^{18}F]FMDAA1106 and [^{18}F]FEDAA1106 binding sites in the rat brains revealed that a high radioactivity was present in the olfactory bulb, the highest PBR density region in the brain.

Benzodiazepine receptors are divided into two types: central and peripheral benzodiazepine receptors. Although peripheral benzodiazepine receptor (PBR) was initially identified in the peripheral system, it became clear that its density in the brain regions can equal or exceed the density of central benzodiazepine receptor (CBR) in the corresponding regions.[1–3] Recent studies have shown that PBR might mediate physiological responses in the central nervous system and may be involved in certain pathophysiological events, such as anxiety, by stimulating the production of neuroactive steroids in glial cells in the brain.[4,5] Therefore, the PBR ligands may become effective anxiolytics without the side effects sometimes seen with classical benzodiazepines.[3,5] However, in contrast to the clarification of CBR at the molecular level, the pharmacological characterization of PBR in primate brain has not been fully elucidated.[3,4] Moreover, the precise physiological significance of events mediated through PBR in the brain and the therapeutic potential of PBR antagonists in the pathology and/or etiology of central nervous system disorders are still subjects of controversy.[3–5] As a result there has been great interest in developing radioligands that could be used to visualize the distribution of PBR in a living human brain using positron emission tomography (PET).[6,7]

Recently, *N*-(2,5-dimethoxybenzyl)-*N*-(5-fluoro-2-phenoxyphenyl)acetamide (DAA1106) (Scheme 1) has been reported as a potent and selective ligand for PBR.[8,9] DAA1106 displayed a high affinity for PBR in mitochondrial fractions of rat ($K_i = 0.043$ nM) and monkey ($K_i = 0.188$ nM) brains.[8] Moreover, DAA1106 showed weak affinities ($IC_{50} = 10{,}000$ nM) for melanin, $Kappa_1$ and $GABA_A$ receptors, and negligible affinities ($IC_{50} > 10{,}000$ nM) for 54 others including receptors, ion channels, uptake/transporter and second messenger.[9] To develop a PET tracer that would provide selective imaging of PBR in vivo, and to elucidate the pharmacological role of PBR in the brain, we have labeled DAA1106 by reacting the corresponding desmethyl precursor *N*-(5-fluoro-2-phenoxyphenyl)-*N*-(2-hydroxy-5-methoxybenzyl)acetamide (DAA1123) with [^{11}C]CH_3I[10] in an excellent radiochemical yield as shown in Scheme 1. Moreover, we have determined a high specific binding of [^{11}C]DAA1106 to PBR in the mouse brain. Now, [^{11}C]DAA1106 is being used for investigating PBR in the human brain.

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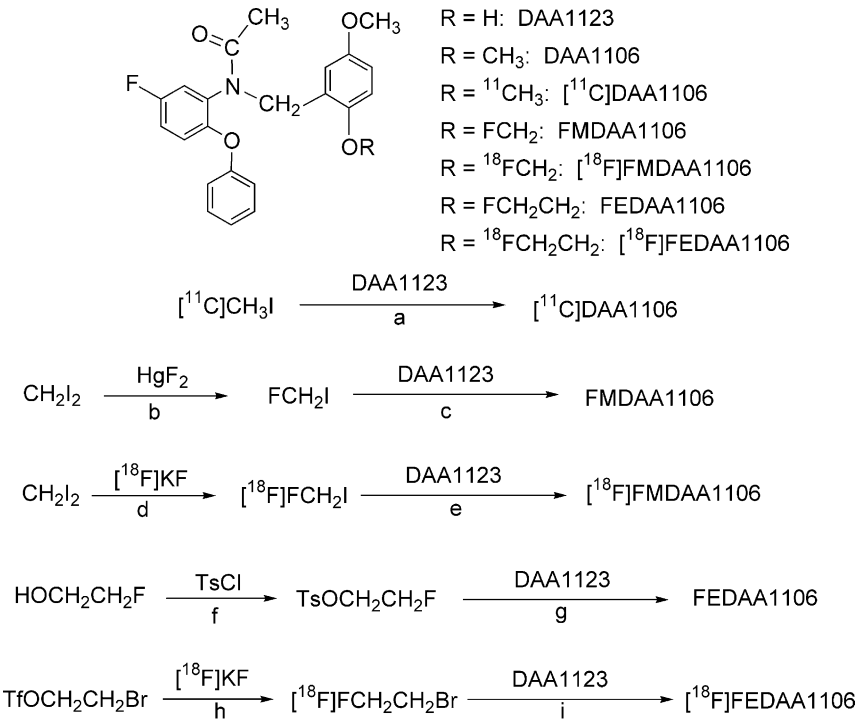

Scheme 1. Structures, chemical synthesis and radiosynthesis: (a) NaH, DMF, 30 °C, 5 min, 81%; (b) 60 °C, 80–100 mm Hg, 10 h, 15%; (c) NaH, DMF, 0 °C, 1 h, 78%; (d) 100 °C, 10 min, 8%; (e) NaH, DMF, 15 °C, 10 min, 86%; (f) pyridine, 25 °C, 4 h, 48%; (g) NaH, DMF, 25 °C, 8 h, 37%; (h) *o*-dichlorobenzene, 130 °C, 5 min, 57%; (i) NaH, DMF, 130 °C, 10 min, 29%.

This success prompted us to design two ^{18}F-labeled analogues of [^{11}C]DAA1106: *N*-(5-fluoro-2-phenoxyphenyl)-*N*-(2-[^{18}F]fluoromethyl-5-methoxybenzyl)acetamide ([^{18}F]-FMDAA1106) and *N*-(5-fluoro-2-phenoxyphenyl)-*N*-(2-[^{18}F]fluoroethyl-5-methoxybenzyl)acetamide ([^{18}F]-FEDAA1106) as putative PET ligands for PBR (Scheme 1). First, because of the molecular similarity and bioisoteric property of *O*–CH_2F and *O*–CH_2CH_2F with *O*–CH_3 group, the two compounds may display similar affinities for PBR with DAA1106. Second, compared with DAA1106, FMDAA1106 and FEDAA1106 were more lipophilic and may readily pass through the blood-brain barrier, which is necessary for a suitable PET tracer over the brain. Third, the [^{18}F]fluoroalkyl substitution may lead to significant improvement in tracer behavior including pharmacokinetics, as can be seen in some examples of PET tracers.[11,12] Finally, since ^{18}F has advantage over ^{11}C, with a longer half-life (110 min vs 20 min) and a lower positron energy (650 KeV vs 960 KeV), ^{18}F is convenient for long-time storage and long-distance transportation and could give a higher quality of images with higher spatial resolution.

In this paper, we report: (1) synthesis, binding affinities of FMDAA1106 and FEDAA1106 to PBR and CBR; (2) radiosynthesis of [^{18}F]FMDAA1106 and [^{18}F]FEDAA1106 and ex vivo autoradiograms of their binding sites in the rat brain.

The non-radioactive FMDAA1106 and FEDAA1106 were prepared according to Scheme 1. The fluoromethylating agent fluoromethyl iodide (FCH_2I) was prepared by reacting diiodomethane (CH_2I_2) with HgF_2 under reduced pressure in a 15% yield.[13] Reaction of DAA1123 with FCH_2I in the presence of NaH at 0 °C was accomplished rapidly to give FMDAA1106[14] in a 78% yield. Reaction of DAA1123 with NaH and 1-fluoro-2-tosyloxyethane (FCH_2CH_2OTs), which was prepared from 2-fluoroethanol and *p*-toluenesulfonyl chloride, gave FEDAA1106[14] in a 75% yield.

[^{18}F]FMDAA1106 and [^{18}F]FEDAA1106 were synthesized as shown in Scheme 1. The labeling intermediate [^{18}F]fluoromethyl iodide ([^{18}F]FCH_2I) or 2-[^{18}F]fluoroethyl bromide ([^{18}F]FCH_2CH_2Br) for the radiosynthesis was prepared by the reaction of [^{18}F]F^- with CH_2I_2 or 2-bromoethyl triflate ($BrCH_2CH_2OTf$) using a newly developed automated system.[15] [^{18}F]FCH_2I or [^{18}F]-FCH_2CH_2Br was purified by distillation and trapped in a solution of DMF containing DAA1123 (1 mg) and NaH (6–8 μL, 0.5 g/20 mL DMF) at −15 °C. After the radioactive reagent trapping ended, the fluoromethylation finished perfectly, whereas the fluoroethylation required a further 10 min at 130 °C. The desired product was purified by HPLC using a reversed phase semi-preparative YMC J'sphere ODS-H80 column (10 mm ID × 250 mm) and a mixture of CH_3CN/H_2O (5.5/4.5 for [^{18}F]FMDAA1106 and 6/4 for [^{18}F]FEDAA1106). Using CAPCELL PAK C_{18} analytic column (4.6 mm ID × 250 mm) and a mixture of CH_3CN/H_2O (7/3), the identity of [^{18}F]FMDAA1106 or [^{18}F]FEDAA1106 was confirmed by co-injecting with the corresponding non-radioactive sample. The retention time was 6.1 min for [^{18}F]FMDAA1106 and 6.3 min for [^{18}F]FEDAA1106 at a flow rate of 2 mL/min, respectively. In the final product solution, no significant DAA1123 peak was determined by HPLC. The radiochemical purity of [^{18}F]FMDAA1106 or [^{18}F]FEDAA1106 was higher

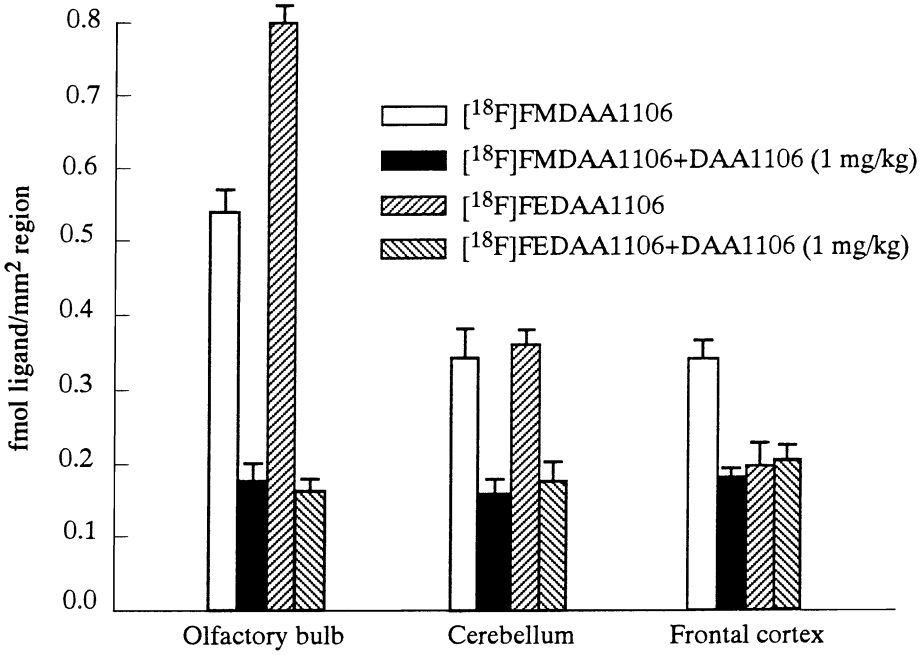

Figure 1. Ex vivo autoradiographic localizations or [^{18}F]FMDAA1106 and [^{18}F]FEDAA1106 in the rat brains at 30 min postinjection (20 MBq, 0.1 nmol). Quantified values (fmol/mm^2) of the ex vivo bindings in the olfactory bulb, cerebellum and frontal cortex ($n = 8$) were calculated by the method.[16,17]

than 98% and the specific activity was >120 GBq/μmol as determined from the mass measured from the HPLC UV analysis. The radiochemical purities of two radioligands remained $>95\%$ after maintenance of the preparations at 25 °C for 4 h, and they were stable for performing evaluation.

The in vitro binding (IC_{50}) studies of FMDAA1106 and FEDAA1106 to PBR were performed using [^{11}C]DAA1106 according to the previous method.[16,17] As shown in Table 1, FEDAA1106 displayed 2-fold higher affinity for PBR than DAA1106, whereas FMDAA1106 displayed similar affinity with DAA1106. This result showed that substituting *O*–CH_3 group with *O*–CH_2CH_2F group augmented the binding affinity, whereas substituting with *O*–CH_2F group did not obviously affect the affinity for PBR. FEDAA1106 was 10-fold more potent than PK11195, the most commonly used ligand for PBR. Competition binding site analyses were also used to evaluate the comparative affinities of FMDAA1106 and FEDAA1106 for CBR labeled by [^{11}C]flumazenil (a selective CBR ligand) (Table 1). The IC_{50} values of FEDAA1106 and FMDAA1106 for CBR in the rat brain were >10 μM, 10^4-fold lower than for PBR. These findings revealed that FEDAA1106 and FMDAA1106 exhibited high potency for PBR and negligible affinities for CBR. The distribution coefficients (log Ps) of [^{18}F]FMDAA1106 and [^{18}F]FEDAA1106 were determined in the water/octanol system at pH 7.4 using the shaking method according to the previous procedure[18] (Table 1).

Table 1. In vitro binding affinity (IC_{50}) for PBR and CBR, and octanol/water distribution coefficient (log *P*)

Ligand	IC_{50} (nM)[a]		Log *P*
	[^{11}C]DAA1106 displacement (PBR)	[^{11}C]Flumazenil displacement (CBR)	
FMDAA1106	1.71	>10,000	3.70
FEDAA1106	0.77	>10,000	3.81
DAA1106	1.62	>10,000	3.65
PK11195	8.26	>10,000	No test

[a]IC_{50} was obtained from 9 concentrations of compound using at least 8 slices of rat brains ($n = 3$).

Figures 1 and 2 show ex vivo autoradiographic localizations of [^{18}F]FMDAA1106 and [^{18}F]FEDAA1106 binding sites in the rat brains at 30 min postinjection. As can be seen in comparison with the sagittal sections of the control rat brain (Figs. 1 and 2a and b), both of these radioligands showed a considerably high brain uptake, indicating that they can pass through the blood-brain barrier, which is a prerequisite for a promising PET tracer. This finding was compatible with their

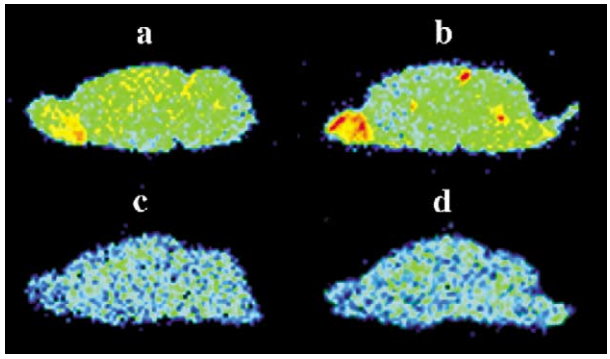

Figure 2. Ex vivo autoradiogram of [^{18}F]FMDAA1106 and [^{18}F]FEDAA1106 in the sagittal sections of rat brains at 30 min postinjection (20 MBq, 0.1 nmol): (a) [^{18}F]FMDAA1106; (b) [^{18}F]FEDAA1106; (c) [^{18}F]FMDAA1106 + DAA1106 (1 mg/kg), (d) [^{18}F]FEDAA1106 + DAA1106 (1 mg/kg).

lipophilicities as shown in Table 1. A significantly-high radioactivity was observed in the olfactory bulb, the high PBR density area in the rat brain.[8,19] Followed by the olfactory bulb, a moderate radioactivity level was observed in the cerebellum, whereas a low uptake was seen in the other brain regions such as frontal cortex. The uptake pattern of radioactivity was not only consistent with [^{3}H]DAA1106 and [^{3}H]PK11195 binding sites in the rat brain,[8,19] but was also in accordance with the regional distribution of PBR in the brain.[1–3] The ratio of radioactivity in the olfactory bulb to that in the frontal cortex was 3.5 for [^{18}F]FEDAA1106 and 1.6 for [^{18}F]-FMDAA1106. The difference between the radioactivity distributions of the two tracers in the olfactory bulb and frontal cortex may be due to their stabilities in the rat brain. Metabolite analysis for the brain homogenate displayed that [^{18}F]FEDAA1106 was not metabolized, whereas [^{18}F]FEDAA1106 was decomposed in the brain at 30 min postinjection. Co-injection with non-radioactive DAA1106 (1 mg/kg) exhibited a significant reduction of [^{18}F]FMDAA1106 or [^{18}F]FEDAA1106 concentration in the brain regions when compared with the control groups (Figs. 1 and 2c and d). As for [^{18}F]FMDAA1106, the radioactivity levels were reduced to 30–50% of control in the brain regions including the olfactory bulb. As for [^{18}F]FEDAA1106, the most significantly reduced uptake (less than 20% of control) was found in the olfactory bulb, whereas a modest decrease (40–60%) was observed in the other regions. These findings revealed that [^{18}F]FMDAA1106 and [^{18}F]FEDAA1106 displayed high specific bindings in the rat brain, especially in the olfactory bulb. Since FMDAA1106 and FEDAA1106 had potent affinities for PBR, these radioligands may have high binding sites to PBR in the rat brain.

In conclusion, [^{18}F]FMDAA1106 and [^{18}F]FEDA-A1106 were designed, synthesized and evaluated as two potent radioligands for PBR. They showed high specific bindings to PBR in the rat brain and may become promising PET tracers for PBR. Further investigation into the binding of [^{18}F]FMDAA1106 and [^{18}F]FEDAA1106 to PBR in the primate brains is currently underway.

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