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[^{18}F]FEAC and [^{18}F]FEDAC: Two novel positron emission tomography ligands for peripheral-type benzodiazepine receptor in the brain

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ABSTRACT

[^{18}F]FEAC ([^{18}F]**4a**) and [^{18}F]FEDAC ([^{18}F]**4b**) were developed as two novel positron emission tomography (PET) ligands for peripheral-type benzodiazepine receptor (PBR). [^{18}F]**4a** and [^{18}F]**4b** were synthesized by fluoroethylation of precursors **8a** and **8b** with [^{18}F]FCH_2CH_2Br ([^{18}F]**9**), respectively. Small-animal PET scan for a neuroinflammatory rat model showed that the two radioligands had high uptakes of radioactivity in the kainic acid-infused striatum, a brain region where PBR density was increased.

The peripheral-type benzodiazepine receptor (PBR), which was initially identified in the peripheral organs, including the kidney, nasal epithelium, lung, heart, and endocrine organs, such as the adrenal, testis, and pituitary gland,[1–3] was subsequently found in the central nervous system (CNS). In the CNS, PBR is mainly located in glial cells, and PBR density was increased in glial cells activated by brain injury and neuroinflammation.[4] Many studies have documented the relationship between PBR and brain injury or neuroinflammation in experimental animals and in human neurodegenerative diseases, such as Alzheimer's disease,[5] Huntington's disease,[6] multiple sclerosis,[7] and stroke-induced brain injury.[8] These results have prompted the development of positron emission tomography (PET) ligands labeled by positron-emitting radioisotopes, which has made it possible to visualize the distribution of PBR in the animal and human brain.

[^{11}C]PK11195 ([^{11}C]**1**, Scheme 1) was the first ligand used for clinical PET imaging of PBR. However, [^{11}C]**1** has several limitations, such as relatively low brain uptake,[9] high nonspecific binding,[10] high plasma protein binding,[11] and too high lipophilicity. To characterize PBR precisely using a PET ligand with advantages over [^{11}C]**1**, nearly 20 new ligands labeled with ^{11}C or ^{18}F for the PBR study have been reported.[12] Recently, we have successfully developed [^{11}C]DAA1106 ([^{11}C]**2a**),[9,13] [^{18}F]FEDAA1106 ([^{18}F]**2b**),[14] and [^{11}C]AC-5216 ([^{11}C]**3**)[15] as useful PET ligands for PBR imaging (Scheme 1). As [^{11}C]**2a**, [^{18}F]**2b**, and [^{11}C]**3** have higher uptake and higher in vivo specific binding to PBR in rodent and primate brains than [^{11}C]**1**, these ligands are being used for the clinical imaging of PBR in the human brain. However, these PET ligands display slow clearance from the brain, which requires time to reach equilibrium and to gain maximum specific binding. Thus, PET studies with these ligands are not simple to perform the quantitative analysis.[16,17]

Here, using **3** as a lead compound, we designed three novel fluorinated ligands: FEAC (**4a**, Scheme 1), FEDAC (**4b**), and FAC (**4c**). These compounds can be labeled with ^{18}F as putative PET ligands for PBR imaging. Since ^{18}F has an advantage over ^{11}C, with a longer half-life (110 min vs 20 min) and lower positron energy (633 keV vs 960 keV), ^{18}F is convenient for long-time storage and long-distance transportation and could give higher quality images with higher spatial resolution. More importantly, because the kinetics and metabolism of some [^{18}F]fluoroalkylated ligands were improved over the corresponding [^{11}C]methylated version,[18] [^{18}F]**4a–c** could be expected to display more rapid clearance of radioactivity from the brain than [^{11}C]**3** as well as [^{11}C]**2a** and [^{18}F]**2b**.

In this paper, we report: (1) chemical synthesis and in vitro binding affinity of **4a–c** to PBR and central benzodiazepine receptor (CBR); (2) radiosynthesis of [^{18}F]**4a–c**; (3) uptake and kinetics of [^{18}F]**4a** and [^{18}F]**4b** in the brain of a neuroinflammatory rat model using small-animal PET. For comparison, PET scan using [^{18}F]**2b** was performed for the same rat model. The animal experimental procedures were approved by the Animal Ethics Committee of the National Institute of Radiological Sciences (NIRS).

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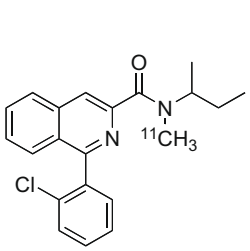
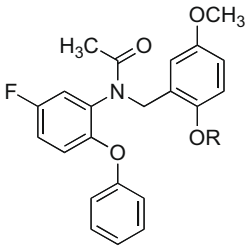
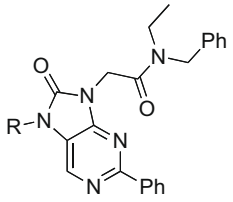


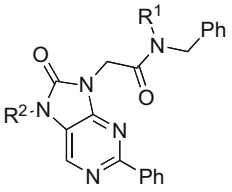
[^{11}C]PK11195 ([^{11}C]**1**)



[^{11}C]DAA1106 ([^{11}C]**2a**): R=^{11}CH$_3$
[^{18}F]FEDAA1106 ([^{18}F]**2b**): R= CH$_2$CH$_2$^{18}F

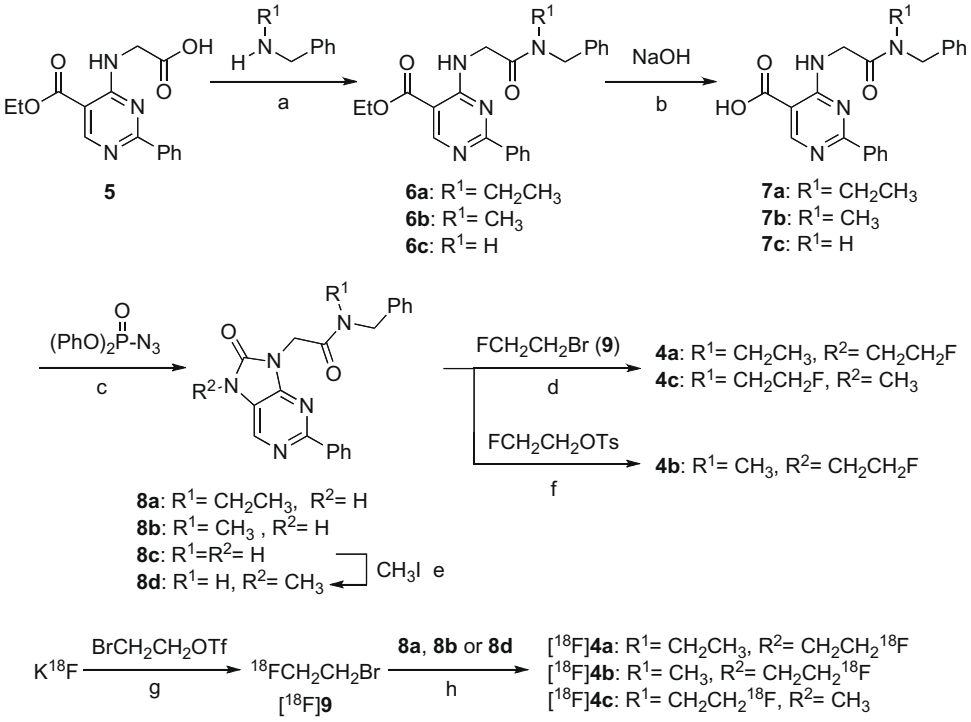


AC-5216 (**3**): R= CH$_3$
[^{11}C]AC-5216 ([^{11}C]**3**): R= ^{11}CH$_3$



FEAC (**4a**): R^1= CH$_2$CH$_3$, R^2= CH$_2$CH$_2$F
[^{18}F]FEAC ([^{18}F]**4a**): R^1= CH$_2$CH$_3$, R^2= CH$_2$CH$_2$^{18}F
FEDAC (**4b**): R^1= CH$_3$, R^2= CH$_2$CH$_2$F
[^{18}F]FEDAC ([^{18}F]**4b**): R^1= CH$_3$, R^2= CH$_2$CH$_2$^{18}F
FAC (**4c**): R^1= CH$_2$CH$_2$F, R^2= CH$_3$
[^{18}F]FAC ([^{18}F]**4c**): R^1= CH$_2$CH$_2$^{18}F, R^2= CH$_3$

Scheme 1. Chemical structures of PET ligands for PBR.



Scheme 2. Chemical synthesis and radiosynthesis. Reagents and conditions: (a) BOP reagent, Et$_3$N, DMF, 30 °C, 3 h, 53% (**6a**), 73% (**6b**), 65% (**6c**); (b) 5 N NaOH, EtOH, 80 °C, 30 min, quant. (**7a**), 83% (**7b**), 92% (**7c**); (c) Et$_3$ N, DMF, 100 °C, 6 h, 33% (**8a**), 42% (**8b**), 83% (**8c**); (d) NaH, DMF, 50 °C, 5 h, 57% (**4a**), 43% (**4c**); (e) K$_2$CO$_3$, DMF, 25 °C, 1 h, 97% (**8d**); (f) K$_2$CO$_3$, DMF, 80 °C, 3 h, 53% (**4b**); (g) *o*-dichlorobenzene, 130 °C, 2 min, 57%; (h) 0.5 N NaOH, DMF, 90 °C, 15 min, 64% ([^{18}F]**4a**), 69% ([^{18}F]**4b**), 3.2% ([^{18}F]**4c**).

The fluoroalkyl ligands **4a–c** and precursors (**8a**, **8b**, and **8d**) for radiosynthesis were synthesized according to the reaction sequences delineated in Scheme 2. The glycine derivative (**5**)[19] was coupled with benzylamines in the presence of BOP reagent to give amides **6a–c**, which were then hydrolyzed with NaOH to afford carboloxyic acids **7a–c**. Curtius rearrangement of **7a–c** using diphenylphosphinic azide produced **8a–c** in high chemical yield. The final desired products **4a** and **4b** were prepared by the reaction

of **8a** and **8b** with FCH_2CH_2Br (**9**) and FCH_2CH_2OTs in the presence of K_2CO_3, respectively. Methylation of **8c** with CH_3I gave **8d**, followed by reaction with **9** to give **4c**.

Radioligands [^{18}F]**4a–c** were synthesized as shown in Scheme 2. The labeling method was a two-step reaction sequence which involved the preparation of the radioactive intermediate [^{18}F]FCH_2CH_2Br ([^{18}F]**9**, bp: 71.5 °C), followed by the alkylation of **8a**, **8b**, and **8d** with [^{18}F]**9**. Reagent [^{18}F]**9** was prepared by the reaction of [^{18}F]F^- with $TfOCH_2CH_2Br$ using an automated system developed in our institute.[20] After the fluorinating reaction, [^{18}F]**9** was distilled from the reaction mixture and trapped in a mixture of precursor and base to accomplish [^{18}F]fluoroethylation. This purification of [^{18}F]**9** by distillation was effective for radiosynthesis, because this treatment left behind all non-volatile impurities such as metal ions from the cyclotron target, unreacted [^{18}F]F^- and the phase transfer reagent Kryptofix 222/K_2CO_3.

[^{18}F]fluroroethylation was performed by reacting **8a**, **b**, and **d** (0.8–1.2 mg, about 2.5 μmol) with [^{18}F]**9** in DMF (300 μL) using NaOH (0.5 N, 6 μL) as a base at 90 °C for 15 min, respectively. The radiochemical yield of [^{18}F]**4a–c** was measured with analytic HPLC (CAPCELL PAK C_{18} column: 4.6 mm ID × 150 mm, CH_3CN/H_2O: 60/40) based on the HPLC-injected radioactivity of each reaction mixture. The yields of [^{18}F]**4a** and [^{18}F]**4b** were 64% and 69%, whereas that of [^{18}F]**4c** was less than 10%. To increase the [^{18}F]fluoroethylating efficiency of **8d**, in place of [^{18}F]**9**, [^{18}F]FCH_2CH_2I, [^{18}F]FCH_2CH_2OTs or [^{18}F]FCH_2CH_2OTf[21] was reacted with **8d** and NaH was used instead of NaOH; however, these treatments could not augment the radiochemical yield of [^{18}F]**4c**. The result suggested that attachment of the fluoroethyl group to benzylamide in **8d** was difficult. The putatively rigid conformation of this amide, which was assumed from the NMR data (4.87 ppm, d, J = 19.1 Hz, 2H) corresponding to methylene of benzyl in **8d**, could not provide enough space for the approach of [^{18}F]**9**.

Purification of the reaction mixtures using reversed phase semi-preparative HPLC (CAPCELL PAK C_{18} column: 4.6 mm ID × 250 mm, CH_3CN/H_2O: 60/40) gave the desired radioactive products [^{18}F]**4a–c** in 35 ± 10% (n = 4), 46 ± 9% (n = 4) and <3% radiochemical yield based on the total [^{18}F]F^-, corrected for physical decay in a synthesis time of 45 ± 2 min from the end of bombardment, respectively. The identity of these radioactive products was confirmed by co-injection with the corresponding non-radioactive **4a–c** on analytic HPLC. In the final product solutions, the radiochemical purity of [^{18}F]**4a–c** was higher than 98% and the specific activity was 30–95 GBq/μmol. No significant UV peak corresponding to **8a**, **8b**, and **8d** and other chemical impurities was observed on HPLC charts of the final products. Moreover, the radiochemical purity of [^{18}F]**4a–c** remained >95% after 180 min at 25 °C, and these ligands were stable for the time of a PET scan.

The in vitro binding affinities (K_i) of **4a–c** for PBR were determined from competition for the [^{11}C]**1** binding to PBR using rat brain homogenate. As shown in Table 1, **4a** showed high affinity for PBR, although its potency was slightly weaker than that of **1** and **3**. This result suggested that substituting N-CH_3 with the N-CH_2CH_2F group in the purine moiety did not have a significant effect on the affinity for PBR. Substitution of N-CH_2CH_3 of the amide moiety (**4a**) by the N-CH_3 group (**4b**) weakened the affinity for PBR, indicating that this replacement might have a negative influence on the binding affinity. Introduction of a fluorine atom (**4c**) to the CH_2CH_3 group (**3**) induced a slight decrease in the affinity, which might be slightly due to the mimic effect of fluorine on the hydrogen atom. The affinity of **4a–c** for CBR was measured using [^{11}C]flumazenil, as shown in Table 1. These ligands did not show significant inhibitory effects (K_i > 1 μM) on [^{11}C]flumazenil binding in the rat brain homogenate. As shown in Table 1, the lipophilicity of these ligands was decreased in the following order: **1**, **2b** > **4a** > **3**, **4b**, and **4c**. These results revealed that **4a–c** were potent and selective ligands for PBR, and had moderate lipophilicity.

Table 1
In vitro binding affinity and lipophilicity of PBR ligands

	Ligand	K_i (nM)[a]		Lipophilicity	
		PBR[b]	CBR[c]	Log D[d]	c Log D[e]
4a	FEAC	0.49 ± 0.05	>8400	3.6	3.6
4b	FEDAC	1.34 ± 0.15	8400	3.2	3.3
4c	FAC	0.51 ± 0.06	>8400	3.1	3.3
3	AC-5216	0.20 ± 0.02	>8400	3.3	3.5
1	PK11195	0.31 ± 0.03	>8400	3.7	5.1
2b	FEDAA1106	0.08 ± 0.01[f]	>1000[f]	3.8[f]	4.3

[a] Values are means ± SD determined in duplicate rat brain homogenates.
[b] [^{11}C]**1** was incubated in the presence of the ligands examined except for **2b**. [^{11}C]**2a** was used to measure the affinity of **2b**.
[c] [^{11}C]Flumazenil was incubated in the presence of the ligands examined.
[d] Log D were determined in the octanol/phosphate buffer (pH 7.4) system by shaking flash method.
[e] The c Log D was calculated with Pallas 3.4 software (CompuDrug).
[f] Data from Ref. 14.

We determined the uptake (expressed as standardized uptake value: SUV) and kinetics of [^{18}F]**4a** and [^{18}F]**4b** in the neuroinflammatory rat brain using a small-animal PET scanner, Inveon™ (Siemens Medical Solutions USA). As the normal rat brain has lower PBR density than peripheral tissues, we used a kainicacid (KA)-lesioned rat with excess density of PBR in the brain for evaluation.[4] To induce neuroinflammation, KA (5 nmol in PBS of 2 μL) was infused into the right striatum of male SD rats (9 weeks old). PET scans were performed at 8–10 days after KA infusion. To examine whether the kinetics of these ligands were improved, we also performed the PET scan using [^{18}F]**2b**.[14] Because the radiochemical yield of [^{18}F]**4c** was too low, the PET scan using [^{18}F]**4c** was not performed this time.

The brain kinetics of [^{18}F]**4a**, [^{18}F]**4b**, and [^{18}F]**2b** are shown in Figure 1. These ligands entered the brain rapidly and the radioactivity level peaked within 1–3 min after injection. Higher uptake of these ligands was observed in the lesioned striatum than in the non-lesioned striatum. This result suggested that the increased binding of these ligands was caused by overexpression of PBR in the rat brain with neuroinflammation. The kinetics of [^{18}F]**4a** and [^{18}F]**4b** differed from that of [^{18}F]**2b**. [^{18}F]**4a** and [^{18}F]**4b** exhibited relatively rapid clearance in the lesioned and non-lesioned striatum. In contrast, [^{18}F]**2b** showed little clearance in the lesioned striatum. The maximum SUV in the lesioned and non-lesioned striatum was about 1.2 and 0.8 ([^{18}F]**4a**), 1.6 and 0.9 ([^{18}F]**4b**), and 1.5 and 1.3 ([^{18}F]**2b**), respectively. The radioactivity level of these ligands was similar to or higher than those of two PBR-selective ligands ([^{11}C]DPA-713, [^{11}C]CLINME) in the neuroinflammatory rat brain.[22,23]

[^{18}F]**4a** and [^{18}F]**4b** displayed the maximum ratio (2.5–3.0) of radioactivity between lesioned and non-lesioned striatum within 10–20 min, whereas [^{18}F]**2b** displayed the maximum (1.5) at 60 min until the end of the PET scan. The ratios of [^{18}F]**4a** and [^{18}F]**4b** were about 2-fold higher than that of [^{18}F]**2b**. Moreover, the time required to achieve the maximum ratio of [^{18}F]**4a** and [^{18}F]**4b** was earlier than that of [^{18}F]**2b**. From these results, the kinetics of [^{18}F]**4a** and [^{18}F]**4b** were more favorable for quantitative analysis to elucidate receptor density and occupancy, and to rapidly gain a high ratio of radioactivity between lesioned and non-lesioned sides. The improved kinetics of [^{18}F]**4a** and [^{18}F]**4b** may be mainly attributed to the suitable binding affinity for PBR. The affinity of [^{18}F]**2b** may be too high, so it is difficult to dissociate from PBR in the brain. The higher lipophilicity of [^{18}F]**2b** may also be related to its slow kinetics.

In conclusion, we synthesized and evaluated [^{18}F]**4a** and [^{18}F]**4b** as potent ligands for PBR using small-animal PET and neuroinflam-

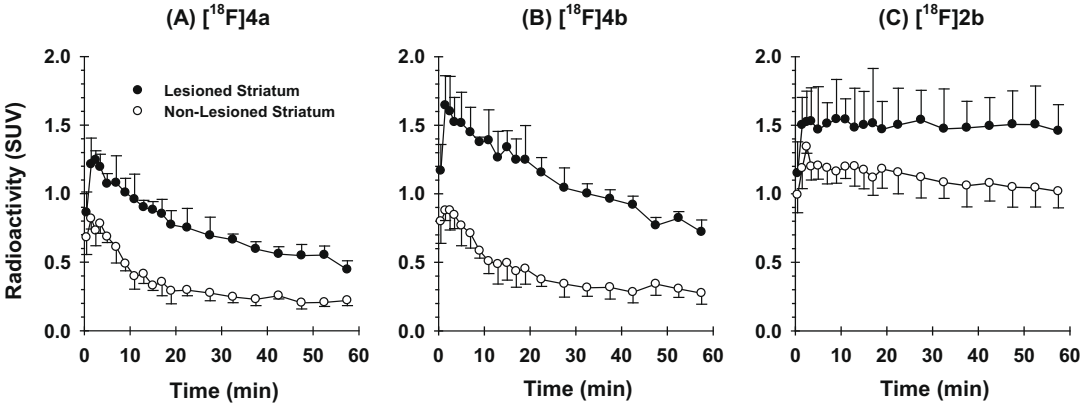


Figure 1. Time-activity curves for [^{18}F]**4a** (A, $n = 4$), [^{18}F]**4b** (B, $n = 4$), and [^{18}F]**2b** (C, $n = 2$) in lesioned (filled circles) and non-lesioned (open circles) striatum of neuroinflammatory rats. Brain uptake of radioactivity was expressed as the standardized uptake value (SUV), normalized for injected radioactivity and body weight. SUV = (radioactivity per cubic centimeter tissue/injected radioactivity) × gram body weight. The results are means ± SD.

matory rats. [^{18}F]**4a** and [^{18}F]**4b** have potent binding affinity and selectivity for PBR, high signal to neuroinflammation, and rapid kinetics in the brain. Therefore, they are promising PET ligands for PBR imaging and could be better in detecting smaller changes in PBR expression than [^{18}F]**2b**. Further investigation into the binding of [^{18}F]**4a** and [^{18}F]**4b** to PBR in the primate brain is currently underway.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.01.093.

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