Design and Synthesis of Phenoxypyridyl Acetamide or Aryl-oxodihydropurine Derivatives for the Development of Novel PET Ligands Targeting the Translocator Protein 18 kDa (TSPO)

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Since last decades, the neuroinflammation has been elucidated to develop the methods of therapy or diagnosis for neurodegenerative disease including Alzheimer's disease, Parkinson's disease, multiple sclerosis and so on.[1] The translocator protein 18 kDa (TSPO) is mainly found on the outer mitochondrial membrane as intramitochondrial cholesterol transporter. In central nervous system, the overexpression of TSPO represents the activation of microglia, which involves initial stage of the neuroinflammation.[2] Development of TSPO ligands with high-binding affinity and specificity emerged in the mid-1980s, and the first generation TSPO ligands, Ro5-4864[3] and PK11195[4] were investigated as a positron emission tomography (PET) ligand (Figure 1(a)).

Because PET ligands are very useful for sensitive, non-invasive, and pathological diagnosis, many researchers have developed the various structures of TSPO ligands as PET tracers. [^{11}C]PK11195 is the first generation of TSPO PET ligand showing high-binding affinity (K_i = 3–5 nM) and widely used in clinical study, but non-specific binding and slow pharmacokinetic properties are the limitation for *in vivo* quantification of inflammatory process in brain.[2b] Later, C-11 labeled phenoxyphenyl acetamide [^{11}C]PBR28[5] was introduced in 2007 and showed higher contrast between inflammatory region and normal region, which is derived from its relatively lower lipophilicity (*c* log *P* = 2.98).[5a] In the same year, another C-11 labeled TSPO PET ligand [^{11}C]AC-5216 was reported, which has aryl-oxodihydropurine as a core structure.[6]

But still these PET ligands have limitations for clinical use, because they have short-lived radioisotope C-11 (half-life, 20 min), which is not suitable for multi-dose production or remote supply to PET center. In addition, C-11 has higher positron energy, which might lower the PET images resolution compared to F-18. Consequently, several groups have put their efforts on the development of F-18 labeled TSPO PET ligands (Figure 1(b) and (c)).[7–10] Among them, 6-[^{18}F]fluoro-PBR28 showed the enhanced binding affinity compared to PK11195 (2.3–5.9 times in rat and human) and PBR28 (1.6 times in rat).[11] The introduction of fluorine into the 6 position on pyridine ring of PBR28 is much beneficial to obtain higher binding affinity against TSPO.

In this paper, as a continuing effort to search novel TSPO PET ligands, we designed and prepared phenoxypyridyl acetamide and aryl-oxodihydropurine derivatives, and also evaluated their binding affinity. To examine the chemical and biological properties, we prepared non-radioactive compounds (Figure 1(b) and (c)).

As shown in Figure 1(b), the synthesis and evaluation of *O*-[^{18}F]fluoroethyl derivatives of [^{11}C]methyl compounds have been continuously studied and reported. And in several reports, *O*-[^{18}F]fluoroethyl derivatives showed improved properties than [^{11}C]methyl compounds, especially in binding affinity to TSPO.[7,8,10] These results led us to design **6b**, which is the direct derivative of 6-F-PBR28 having F-18 on *O*-[^{18}F]fluoroethyl moiety instead of 6-[^{18}F]fluoropyridyl. And also we designed **6a** having phenolic OH without any alkyl substituent, as there is no precedent report on this specific compound. The synthetic methods for **6a** and **6b** are described in Scheme 1.[11a] Through the linear six-step reaction, **6a** and **6b** were prepared in 2.8 and 4.7% overall yield from the commercially available 4-chloro-3-nitropyridine. Compound **6a** will be able to be used as a precursor for the synthesis of [^{18}F]**6b** in further study.

Next, we designed fluorophenyl derivatives of AC-5216 (**14a–c**), as aromatic [^{18}F]fluorination is one of the well-

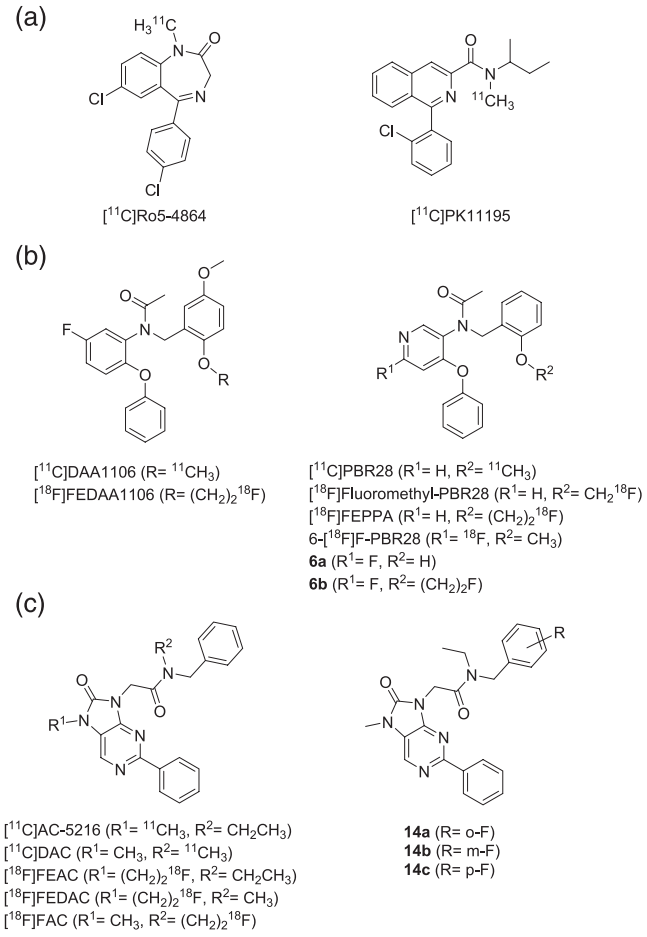
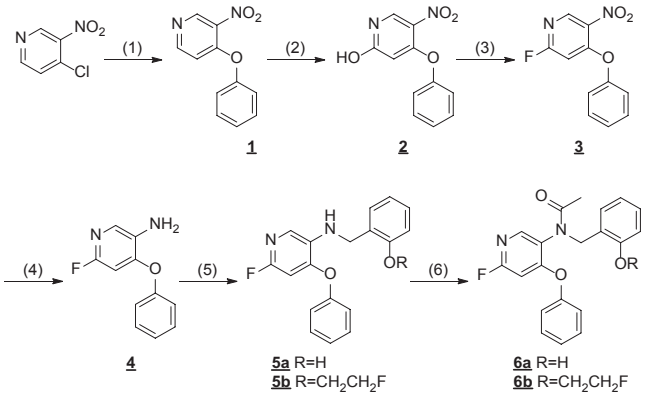


Figure 1. Selected TSPO radioligands and the design of new derivatives: (a) pioneer TSPO PET ligands, (b) phenoxyaryl acetamide (DAA1106 and PBR28) derivatives, (c) aryl-oxodihydropurine (AC-5216) derivatives.

known approach for F-18 radioligands. As shown in Figure 1(c),[6,12] several [^{18}F]fluoroethyl derivatives of AC-5216 ([^{18}F]FEAC, [^{18}F]FEDAC, and [^{18}F]FAC) were reported, but there is no approach to prepare any [^{18}F]fluorophenyl derivatives up to date. The AC-5216 derivatives, **14a–c**, were prepared from benzamidine hydrochloride in 8.8, 17.1, and 16.8% overall yield for linear eight steps, respectively (Scheme 2).[12b] And also we obtained the enough amount of compound **13** as a precursor for several F-18-labeled compounds for further study.

Last, we prepared the known compound **19** (FAC) as an internal standard for binding assay (Scheme 3).[12b] We used the common intermediate **9** and followed same synthetic strategy for **14a–c** to give the compound **19** in 22.4% overall yield. We also obtained the enough amount of compound **18** as a precursor for further study.

The binding affinity (IC_{50}) was measured by competition with [^{3}H]PK11195 in a membrane of human leukocytes,[9,13] and *c* log *P* of each compound was calculated using ACD/ChemSketch software (ACD, Inc., Toronto, Canada). As mentioned above, we compared the newly prepared derivatives



Scheme 1. Synthesis of **6a** and **6b**. Reagents and conditions: (1) PhOH, K_2CO_3, DMF, 70°C, 5 h, 78%; (2) *t*-BuOOH, *t*-BuOK, DMF, −40°C, 1.5 h, 19%; (3) DAST, MeCN, 90°C, 6 h, 46%; (4) Fe powder, AcOH, 90°C, 40 min, 86%; (5) (a) salicylaldehyde , CH_2Cl_2, reflux, 1 h; (b) $NaBH_4$, MeOH, rt, 2 h, 68% (**5a**), 86% (**5b**); (6) Ac_2O, AcOH, 75°C, 1 h, 71% (**6a**), 93% (**6b**). DMF, dimethylformamide; DAST, diethylaminosulfur trifluoride.

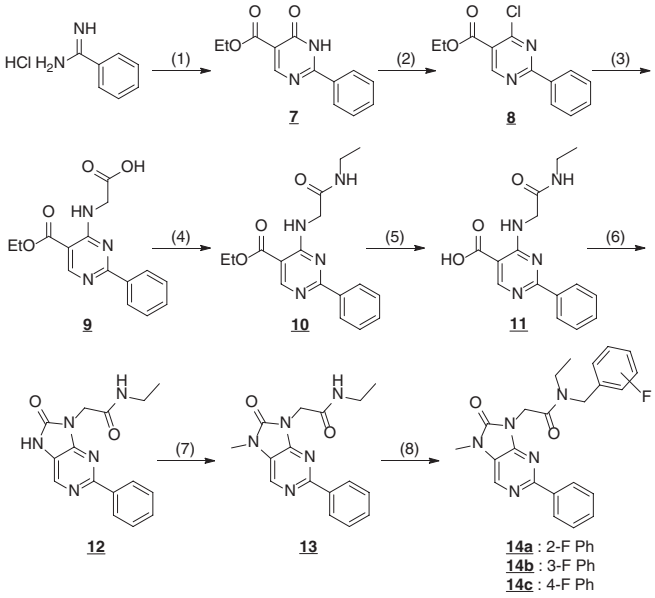
(**6a–b** and **14a–c**) with compound **19**, which has a reported value for K_i and log *D*.[12b]

As shown in Table 1, **6a** (24.85 ± 6.55 nM) and **6b** (29.14 ± 7.12 nM) showed the better affinity than **19** (47.72 ± 4.60 nM). The obtained IC_{50} value itself was not very impressive, but when we compare our experimental data with the reported K_i for **19** (0.51 ± 0.06 nM),[12b] we postulated both **6a** and **6b** might be the quite promising candidate for TSPO imaging. More interestingly, less lipophilic **6a** (*c* log *P* = 3.13 ± 0.58) showed better affinity than **6b**, which has somewhat higher *c* log *P* (4.01 ± 0.60) considered as exceeding lipophilicity for brain imaging.[14] AC-5216 derivatives **14a–c**, which have the fluoro-aryl moiety, did not show any good binding affinity. *meta*-Fluoro derivative **14b** showed moderate affinity (289 ± 35.55 nM), but not sufficient to pursue a following study.

In summary, we designed and successfully prepared novel phenoxypyridyl acetamide and aryl-oxodihydropurine derivatives, which are the current lead compounds for TSPO PET imaging. The 6-F-PBR28 derivatives, **6a** and **6b**, showed promising binding affinity compared with the AC-5216 derivatives, **14a–c**. Especially, the less lipophilic compound **6a** showed the best binding affinity in our new series compound. Based on these results, further investigation such as F-18 labeling on **6a** and **6b** is currently underway.

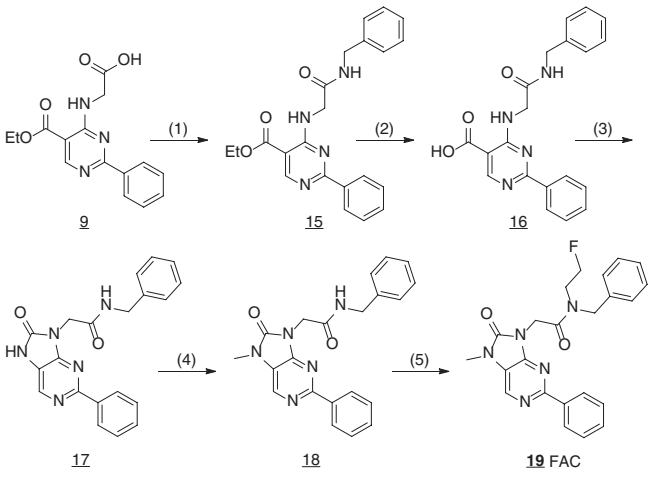
Experimental

***N*-(6-Fluoro-4-phenoxypyridin-3-yl)-*N*-(2-hydroxybenzyl)acetamide** (6a)**.** ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 9.12 (s, 1H), 7.38–7.44 (m, 2H), 7.23–7.32 (m, 2H), 6.92–6.96 (m, 1H), 6.67–6.79 (m, 4H), 6.17 (d, *J* = 1.2 Hz, 1H), 4.79 (dd, *J* = 16.3 Hz, 4.7 Hz, 2H), 2.03 (s, 3H); ^{13}C



Scheme 2. Synthesis of 14a-c. Reagents and conditions: (1) -2-ethoxy-methylene malonate, Na, abs EtOH, 80°C, 6 h, 75%; (2) $POCl_3$, 90°C, 4 h, 93%; (3) glycine, TEA, abs EtOH, 80°C, 4 h, 96%; (4) $EtNH_2$ HCl, BOP, TEA, DMF, rt, 1 h, 93%; (5) 1 M NaOH, EtOH, 80°C, 2 h, 93%; (6) DPPA, TEA, DMF, 100°C, 2 h, 66%; (7) MeI, K_2CO_3, DMF, rt, 1 h, 72%; (8) *o*,*m*,*p*-F-C_4H_4Br, NaH, DMF, rt, 1 h, 32% (**14a**), 62% (**14b**), 61% (**14c**). BOP, (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; TEA, triethylamine; DPPA, diphenylphosphoryl azide.

NMR (100 MHz, DMSO-d_6) δ (ppm): 170.6, 164.3, 163.2 (d, J = 249 Hz, 1C), 155.9, 153.0, 149.2, 149.0, 131.2, 131.1, 129.2, 126.9, 123.0, 121.1, 119.3, 115.5, 96.1 (d, J = 44 Hz, 1C), 46.1, 22.4; ^{19}F NMR (282 MHz, $CDCl_3$) δ (ppm): −62.11; ESI-MS *m*/*z*: calculated for $C_{20}H_{18}FN_2O_3$ ([M+H]): 353.1. Found: 353.1.



Scheme 3. Synthesis of 19 (FAC). Reagents and conditions: (1) $BnNH_2$, BOP, TEA, DMF, rt, 1 h, 91%; (2) 1 M NaOH, EtOH, 80°C, 2 h, 72%; (3) DPPA, TEA, DMF, 100°C, 2 h, 72%; (4) MeI, K_2CO_3, DMF, rt, 1 h, 71%; (5) 1-bromo-2-fluoro-ethane, NaH, DMF, 1 h, 67% (**19**).

***N*-(6-Fluoro-4-phenoxypyridin-3-yl)-*N*-(2-(2-fluoroethoxy)benzyl)acetamide** (6b)**.** ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.78 (s, 1H), 7.19–7.46 (m, 5H), 6.89–6.94 (m, 3H), 6.73–6.76 (m, 1H), 6.10 (d, J = 1.2 Hz, 1H), 5.23 (d, J = 14.1 Hz), 1H), 4.82 (d, J = 14.1, 1H), 4.43–4.73 (m, 1H), 3.90–4.16 (m, 2H), 2.44 (s, minor, 3H), 1.98 (s, major, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 170.1, 164.1, 163.1 (d, J = 235 Hz, 1C), 156.6, 153.0, 149.1, 148.9, 131.1, 130.9, 129.4, 126.9, 125.2, 121.2, 120.9, 112.3, 96.3 (d, J = 45 Hz, 1C), 82.5 (d, J = 166 Hz, 1C), 67.7 (d, J = 19 Hz, 1C), 45.8, 22.4; ^{19}F NMR (282 MHz, $CDCl_3$) δ (ppm): −62.11; ESI-MS *m*/*z*: calculated for $C_{22}H_{21}F_2N_2O_3$ ([M+H]): 399.2. Found: 399.1.

***N*-Ethyl-*N*-(2-fluorobenzyl)-2-(7-methyl-8-oxo-2-phenyl-7,8-dihydro-9*H*-purin-9-yl)acetamide** (14a)**.** ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.45 (s, 1H), 8.23–8.28 (m, 2H), 7.40–7.49 (m, 4H), 6.93–7.30 (m, 3H), 4.86–4.91 (m, 2H), 4.54–4.75 (m, 2H), 3.23–3.55 (m, 5H), 0.93–1.27 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 198.7, 166.1, 165.8, 153.4, 150.0, 133.2, 133.1, 130.3, 129.7, 129.5, 129.0, 127.6, 124.7, 122.6, 116.2, 115.7, 41.4, 41.2, 27.8, 14.3, 12.9; ESI-MS *m*/*z*: calculated for $C_{23}H_{23}FN_5O_2$ ([M+H]): 420.2. Found: 420.1.

***N*-Ethyl-*N*-(3-fluorobenzyl)-2-(7-methyl-8-oxo-2-phenyl-7,8-dihydro-9*H*-purin-9-yl)acetamide** (14b)**.** ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.33–8.37 (m, 2H), 8.23–8.27 (m, 1H), 7.41–7.47 (m, 3H), 6.91–7.24 (m, 4H), 4.92–4.82 (m, 2H), 4.61–4.66 (m, 2H), 3.44–3.52 (m, 5H), 1.12–1.38 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.2, 165.8, 156.6, 153.4, 150.0, 137.9, 137.8, 131.2, 130.7, 130.3, 128.9, 127.5, 123.7, 122.5, 114.5, 113.8, 41.9, 41.1, 27.8, 14.3, 13.0; ESI-MS *m*/*z*: calculated for $C_{23}H_{23}FN_5O_2$ ([M+H]): 420.2. Found: 420.1.

***N*-Ethyl-*N*-(4-fluorobenzyl)-2-(7-methyl-8-oxo-2-phenyl-7,8-dihydro-9*H*-purin-9-yl)acetamide** (14c)**.** ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.33–8.35 (m, 2H), 8.24–8.27 (m, 1H), 7.44–7.50 (m, 3H), 7.34–7.38 (m, 1H), 7.11–7.24 (m, 2H), 6.91–6.97 (m, 1H), 4.83–4.90 (m, 2H), 4.58–4.64

Table 1. *In vitro* binding affinity and lipophilicity of new derivatives.

Ligand	IC_{50} (nM)[a]	*c* log *P*[b]
6a	24.85 ± 6.55	3.13 ± 0.58
6b	29.14 ± 7.12	4.01 ± 0.60
14a	>10 000	3.19 ± 0.95
14b	289 ± 35.55	3.19 ± 0.95
14c	>10 000	3.19 ± 0.95
19	47.72 ± 4.60[c]	3.01 ± 0.95[d]

[a] Affinity of compounds determined by displacement of [^{3}H]PK11195 from isolated human leukocytes. The data are expressed as the mean values and standard deviations in duplicate.
[b] The *c* log *P* was calculated with ACD/ChemSketch software.
[c] The reported K_i value of FAC for PBR was 0.51 ± 0.06 nM.[12b]
[d] The reported log *D* value was 3.[12b]

(m, 2H), 3.41–3.52 (m, 5H), 1.11–1.37 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.0, 165.7, 156.6, 153.5, 150.0, 127.9, 133.2, 129.8, 129.0, 127.5, 122.6, 116.1, 115.6, 110.0, 41.7, 41.2, 27.8, 14.3, 13.0; ESI-MS *m/z*: calculated for $C_{23}H_{23}FN_5O_2$ ([M+H]): 420.2. Found: 420.1.

***N*-Benzyl-*N*-(2-fluoroethyl)-2-(7-methyl-8-oxo-2-phenyl-7,8-dihydro-9*H*-purin-9-yl)acetamide (19).** ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.34–8.38 (m, 2H), 8.23–8.26 (m, 1H), 7.45–7.50 (m, 5H), 7.36–7.41 (m, 2H), 7.26–7.27 (m, 1H), 4.91–4.97 (m, 2H), 4.76–4.82 (m, 2H), 4.62 (dt, *J* = 47.5 Hz, 4.9 Hz, 2H), 3.63–3.79 (m, 2H), 3.49–3.52 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.8, 156.6, 153.4, 150.0, 137.7, 137.3, 133.1, 130.3, 129.3, 128.9, 128.0, 127.5, 126.8, 122.6, 82.7, 50.8, 48.7, 41.7, 27.8; ESI-MS *m/z*: calculated for $C_{23}H_{23}FN_5O_2$ ([M+H]): 420.2. Found: 420.2.

***In Vitro* TSPO Binding Assay.** The prepared TSPO-targeted compounds, **6a** and **b** and **14a–c**, were assayed for their binding affinity to TSPO using isolated human leukocytes and the standard [^{3}H]PK11195. These experiments were carried out by the previously reported method.[9,15]

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Supporting Information. Additional supporting information (experimental procedure and spectra for all compounds) is available in the online version of this article.

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