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Radiosynthesis of [^{18}F]DPA-714, a selective radioligand for imaging the translocator protein (18 kDa) with PET

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Recently, a novel series of 2-phenylpyrazolo[1,5-*a*]pyrimidineacetamides has been reported as selective ligands of the translocator protein (18 kDa). Within this series, DPA-714 (*N,N*-diethyl-2-(2-(4-(2-fluoroethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl)acetamide, $K_i = 7.0$ nM) is a compound, which had been designed with a fluorine atom in its structure, allowing labelling with fluorine-18 (half-life: 109.8 min) and *in vivo* imaging using positron emission tomography. DPA-714 and its tosyloxy derivative (*N,N*-diethyl-2-(2-(4-(2-toluenesulfonyloxyethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl)acetamide) as precursor for the labelling with fluorine-18 were synthesized in two steps from DPA-713 (*N,N*-diethyl-2-(2-(4-methoxyphenyl)-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl)acetamide) and obtained in 32 and 42% yields, respectively. [^{18}F]DPA-714 was synthesized using a simple one-step process (a tosyloxy-for-fluorine nucleophilic aliphatic substitution), which has been fully automated on our Zymate-XP robotic system. It involves: (A) reaction of K[^{18}F]F-Kryptofix® 222 with the tosyloxy precursor (4.5–5.0 mg, 8.2–9.1 μmol) at 165°C for 5 min in dimethyl sufloxide (0.6 mL) followed by (B) C18 PrepSep cartridge pre-purification and finally (C) semi-preparative high-performance liquid chromatography (HPLC) purification on a Waters X-Terra™ RP18. Typically, 5.6–7.4 GBq of [^{18}F]DPA-714 (> 95% chemically and radiochemically pure) could be obtained with specific radioactivities ranging from 37 to 111 GBq/μmol within 85–90 min (HPLC purification and SepPak®-based formulation included), starting from a 37 GBq [^{18}F]fluoride batch (overall non-decay-corrected and isolated radiochemical yield: 15–20%).

Keywords: fluorine-18; DPA-714; TSPO; PBR

Introduction

The translocator protein (18 kDa) (TSPO,[1,2] formerly known as the peripheral benzodiazepine receptor[3] (PBR)) is associated with the outer mitochondrial membrane. The best characterized functional role of the TSPO is its ability to facilitate transport of cholesterol from the outer to the inner mitochondrial membrane, where it is metabolized to pregnenolone, a key derivative for the production of various steroids in mammals.[4,5] Consistent with this involvement in steroidogenesis, the TSPO is mostly present in steroid-producing endocrine organs, such as kidney, heart, adrenal cortex, testis or ovary. In the brain, TSPO expression is very low and restricted to the olfactory bulbs and the glial cells.[6,7]

In the central nervous system (CNS), glial cells (commonly called neuroglia) are non-neuronal cells that provide support and nutrition, maintain homeostasis, form myelin and participate in signal transmission. They comprise macroglia (astrocytes, oligodendrocytes and ependymal cells) and microglia (the intrinsic macrophage population of the CNS). Microglia respond quickly with activation to virtually any kind of CNS injury and the term 'sensors of pathology' has been often proposed for their pivotal role in neuroinflammatory processes.[8]

Positron emission tomography (PET) is a radiotracer-based non-invasive medical imaging technique and currently the most advanced technology available for studying molecular interactions *in vivo*. Since TSPO expression reflects the extent of microglial activation, PET could play a key role in elucidating the involvement of microglia in various neurodegenerative diseases such as Alzheimer's disease, stroke and multiple sclerosis[3,8,9] provided that a TSPO-radioligand is available.

The first PET-radioligand synthesized for imaging the TSPO was [^{11}C]PK11195 (Figure 1, compound **A**), in the early 1980s.[10] It is still today the most widely used TSPO-ligand in spite of its

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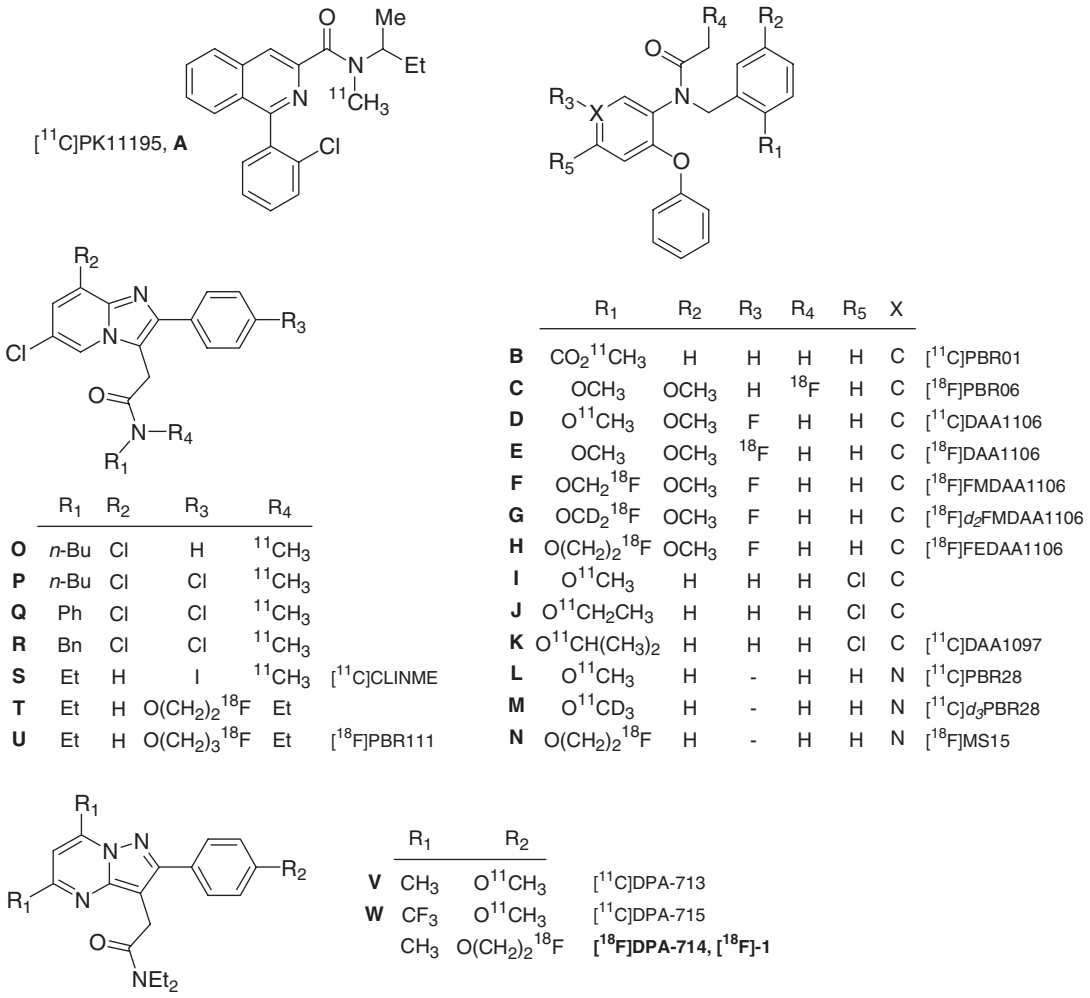


	R_1	R_2	R_3	R_4	R_5	X	
B	$CO_2{}^{11}CH_3$	H	H	H	H	C	[^{11}C]PBR01
C	OCH_3	OCH_3	H	^{18}F	H	C	[^{18}F]PBR06
D	$O^{11}CH_3$	OCH_3	F	H	H	C	[^{11}C]DAA1106
E	OCH_3	OCH_3	^{18}F	H	H	C	[^{18}F]DAA1106
F	$OCH_2{}^{18}F$	OCH_3	F	H	H	C	[^{18}F]FMDAA1106
G	$OCD_2{}^{18}F$	OCH_3	F	H	H	C	[^{18}F]d_2FMDAA1106
H	$O(CH_2)_2{}^{18}F$	OCH_3	F	H	H	C	[^{18}F]FEDAA1106
I	$O^{11}CH_3$	H	H	H	Cl	C	
J	$O^{11}CH_2CH_3$	H	H	H	Cl	C	
K	$O^{11}CH(CH_3)_2$	H	H	H	Cl	C	[^{11}C]DAA1097
L	$O^{11}CH_3$	H	-	H	H	N	[^{11}C]PBR28
M	$O^{11}CD_3$	H	-	H	H	N	[^{11}C]d_3PBR28
N	$O(CH_2)_2{}^{18}F$	H	-	H	H	N	[^{18}F]MS15

	R_1	R_2	
O	*n*-Bu	Cl	H	$^{11}CH_3$	
P	*n*-Bu	Cl	Cl	$^{11}CH_3$	
Q	Ph	Cl	Cl	$^{11}CH_3$	
R	Bn	Cl	Cl	$^{11}CH_3$	
S	Et	H	I	$^{11}CH_3$	[^{11}C]CLINME
T	Et	H	$O(CH_2)_2{}^{18}F$	Et	
U	Et	H	$O(CH_2)_3{}^{18}F$	Et	[^{18}F]PBR111

	R_1	R_2	
V	CH_3	$O^{11}CH_3$	[^{11}C]DPA-713
W	CF_3	$O^{11}CH_3$	[^{11}C]DPA-715
	CH_3	$O(CH_2)_2{}^{18}F$	**[^{18}F]DPA-714, [^{18}F]-1**

Figure 1. Selected TSPO PET-radioligands including DPA-714 (**1**).

low brain uptake and extensive binding to plasma proteins, which complicate a quantitative analysis of the receptor density.[11–14] Meanwhile, more than 30 other TSPO-ligands have been radiolabelled with the positron emitters carbon-11 (half-life: 20.38 min) or fluorine-18 (half-life: 109.8 min).[15,16] They belong to different chemical classes among which there are two dominant families. The first one is that of the *N*-benzyl-*N*-(2-phenoxyaryl)acetamides (Figure 1, compounds **B**–**N**), a series resulting from the ring-opening of the atypical benzodiazepine 4′-chlorodiazepam (Ro5-4864), which includes [^{18}F]FEDAA1106[17–25] (compound **H**) as well as the recently reported pyridinylacetamide [^{11}C]PBR28[26–30] (compound **L**). Both derivatives seem to be a promising alternative to [^{11}C]PK11195, based on preliminary data obtained in humans.[23,25,28,30] The second family comprises the 2-phenylimidazo[1,2-*a*]pyridineacetamides (Figure 1, compounds **O**–**U**), a series derived from alpidem (a compound known to bind both to the peripheral and central sites), with [^{11}C]CLINME[31–34] (compound **S**) and [^{18}F]PBR-111[35] (compound **U**) being the most recently reported compounds. Another promising class consists of a series of 2-phenylpyrazolo[1,5-*a*]pyrimidineacetamides[36,37] and includes [^{11}C]DPA-713[31,38–42] (compound **V**) for which exceptional *in vivo* binding properties have been reported, the latter being currently further evaluated in non-human primates.[39] DPA-714 (**1**, *N,N*-diethyl-2-(2-(4-(2-fluoroethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-a]pyrimidin-3-yl)acetamide, $K_i = 7.0$ nM[43–45]) is structurally closely related to DPA-713 and has been designed with a fluorine atom in its structure, allowing labelling with fluorine-18, today one of the most attractive PET-isotopes for radiopharmaceutical chemistry.[46–49]

In the present study, the synthesis of DPA-714 (**1**) and its tosyloxy derivative (*N,N*-diethyl-2-(2-(4-(2-toluenesulfonyloxyethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-a]pyrimidin-3-yl)acetamide) as precursors for the labelling with fluorine-18 is reported as well as the radiosynthesis of [^{18}F]DPA-714 ([^{18}F]-**1**) using a simple, one-step, robot-assisted process.

Results and discussion

Chemistry

The preparation of DPA-714 (**1**, *N,N*-diethyl-2-(2-(4-(2-fluoroethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl)acetamide) as a reference compound and the tosylate **2** (*N,N*-diethyl-2-(2-(4-(2-toluenesulfonyloxyethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-a]pyrimidin-3-yl)acetamide) as a labelling precursor is illustrated in Scheme 1. Both compounds were synthesized in

two steps from DPA-713 (**3**)[39] and obtained in 32 and 42% yield, respectively.

Briefly, DPA-713 (**3**) was *O*-demethylated in a 1 M boron tribromide solution in dichloromethane at a low temperature (−60 to −40°C) for 2 h, giving the expected phenol **4** in moderate yield (55%) as light-yellow crystals. Subsequent reaction with 1,2-di(tosyloxy)ethane (**5**, commercially available) or 1-fluoro-2-tosyloxyethane (**6**, synthesized as described below) in tetrahydrofuran (THF) containing 1.1–1.8 eq of powdered NaH gave the target DPA-714 (**1**) and its tosyloxy derivative (**2**) in 58 and 77%, respectively. 1-Fluoro-2-tosyloxyethane (**6**) was quantitatively synthesized from commercially available 2-fluoroethanol and *p*-toluenesulfonyl chloride (1.4 eq) in dichloromethane containing triethylamine (1.4 eq) and a catalytic amount of 4-(*N*,*N*-dimethylamino)pyridine (DMAP) at room temperature (RT) for 2 h.

Radiochemistry

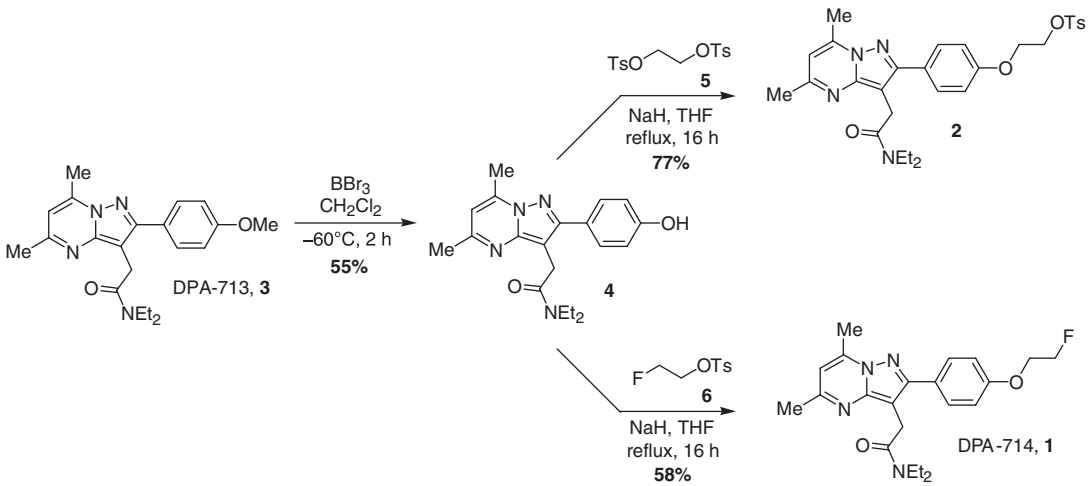
DPA-714 (**1**) was labelled with fluorine-18 at its 2-fluoroethyl moiety from the corresponding tosyloxy analog **2** using a one-step optimized radiochemical process outlined in Scheme 2.

Optimized fluorination with the cyclotron-produced [^{18}F]fluoride as the, no-carrier-added, activated K[^{18}F]F-Kryptofix®222 complex[50,51] was performed in dimethyl sulfoxide (DMSO) using 8.2–9.1 μmol (4.5–5.0 mg) of the tosylate **2** at 165°C for 5 min without stirring the contents in an unsealed tube. After cooling, 97% to over 99% of the initial radioactivity was still present. Thin layer chromatography (TLC) analyses showed only one radioactive peak (R_f: 0.6, see Experimental), co-migrating with DPA-714 (**1**), beside unreacted [^{18}F]fluoride (R_f: 0). The radiochemical yields (RCY) of fluorine-18 incorporation, calculated from the TLC-radiochromatogram and defined as the ratio of radioactivity area of [^{18}F]-**1** over total radioactivity area, were about 50–70%.

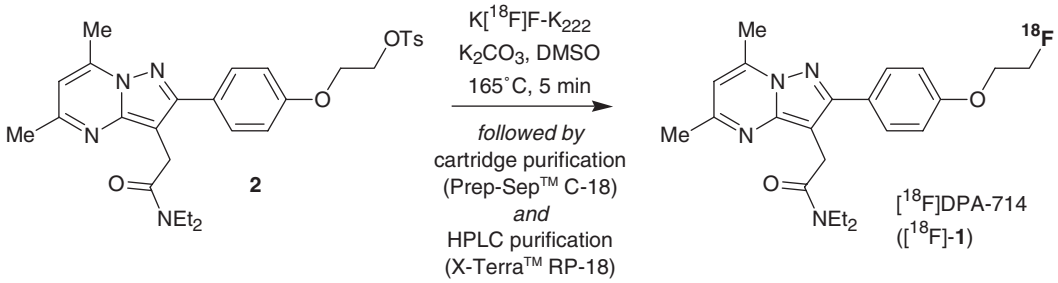
At this stage, a C18 PrepSep™ cartridge was used to rapidly trap and isolate [^{18}F]-**1** (with a radiochemical purity >95%, according to radio-TLC) from the reaction mixture. It represented 45–65% of the total radioactivity amount engaged in the fluorination process, whereas most likely unreacted [^{18}F]fluoride was eluted off the cartridge as waste. These values clearly confirmed the RCY measured by radio-TLC. Noteworthy, less than 2% of the total radioactivity amount engaged in the fluorination process was left behind in the initial fluorination reactor tube.

[^{18}F]-**1** was then simply eluted from the cartridge with CH_2Cl_2 (about 4–7% remaining trapped and being lost on the cartridge) and purified by high-performance liquid chromatography (HPLC) on a semi-preparative X-Terra™ RP18 column (HPLC A, see Experimental), using a mixture of 1 mM aqueous NH_4OAc (pH 10) and CH_3CN as the eluent. Using these conditions, [^{18}F]-**1** (t_R: 10–11 min) could be obtained with a >95% chemical and radiochemical purity and was completely separated from the remaining tosylate **2** (t_R: >30 min).

Decreasing the amount of starting labelling precursor **2** (7.3 μmol and down to 2.7 μmol) gave lower incorporation yields (calculated from the TLC-radiochromatogram or after the C18 PrepSep™ cartridge purification). Introduction of fluorine-18



Scheme 1.



Scheme 2.

was also attempted in CH_3CN (with 2.7–9.1 μmol of **2**) at 120°C for 5–15 min also resulting in lower incorporation yields (RCY < 10%). The use of dimethylformamide (DMF) as solvent was also disappointing (RCY < 10%). Final HPLC purification of the crude reaction mixture was also tried with a classical mixture of H_2O, CH_3CN and trifluoroacetic acid as the eluent as well as with a semi-preparative Symmetry® C18 column with, however, poor results in terms of final chemical purity (< 75%).

Formulation of [^{18}F]DPA-714 ([^{18}F]-**1**) as an i.v. injectable solution was performed using a home-made SepPak® Plus C18 device. The HPLC-collected fraction containing the radiotracer was diluted with water and the resulting solution was passed through a C18 SepPak® cartridge. The cartridge was then washed with water, partially dried with nitrogen and finally eluted with ethanol followed by physiological saline. The solution was then sterile-filtered and diluted with physiological saline to an ethanol concentration below 10%.

Typically, using the one-step synthesis described herein, 5.6–7.4 GBq (150–200 mCi) of [^{18}F]DPA-714 ([^{18}F]-**1**) ready to use could be obtained with a specific radioactivity of 37–111 GBq/μmol (1–3 Ci/μmol) within 85–90 min (HPLC purification and SepPak®-based formulation included), starting from a 37.0 GBq (1.0 Ci) [^{18}F]fluoride batch. Calculated overall non-decay-corrected isolated yields were 15–20% (26–35% decay-corrected).

Quality controls of [^{18}F]DPA-714 ([^{18}F]-**1**) were performed on an aliquot of the preparation ready for i.v. injection. The radiotracer preparation was a clear and colourless solution with a measured pH between 5 and 7. As demonstrated by analytical HPLC analysis (HPLC B, see Experimental), the radiotracer preparation was found to be > 95% chemically and radiochemically pure (**1**, t_R: 1.98 min). The preparation was also shown to be free of the non-radioactive precursor, the tosylate **2** (t_R: 9.60 min), and was chemically and radiochemically stable for at least 120 min. Log *P* (*n*-octanol/water partition coefficient) and log *D* (*n*-octanol/buffer pH 7.4 partition coefficient) of [^{18}F]DPA-714 ([^{18}F]-**1**) were measured using the shake-flask method and values of 1.66 and 1.74 were found, respectively.

Experimental

General

Chemicals, flash chromatography and TLC analysis

Chemicals were purchased from Aldrich, Fluka or Sigma, France, and were used without further purification, unless otherwise stated. Flash chromatographies were conducted on silica gel or alumina gel (0.63–0.200 mm, VWR) columns. TLCs were run on pre-coated plates of silica gel $60F_{254}$ (VWR). The compounds were localized when possible at 254 nm using a UV-lamp and/or by dipping the TLC-plates in a 1% ethanolic ninhydrin solution or a 1% MeOH/H_2O (1/1, v:v) $FeCl_3$ solution and heating on a hot plate. Radioactive spots were detected using a Berthold TraceMaster 20 automatic TLC linear analyser.

HPLC analysis

[HPLC A]: Equipment: System equipped with a Waters 600 pump and a Waters 600 controller, a Shimadzu SPD10-AVP UV-multiwavelength detector and a miniature ionization chamber probe; column: semi-preparative X-Terra™ RP18, Waters (300 × 7.8 mm); porosity: 7 μm; eluent 1 mM aqueous NH_4OAc (pH 10)/CH_3CN: 60/40 (v:v); flow rate: 5 mL/min; temperature: RT; absorbance detection at $\lambda = 263$ nm. [HPLC B]: Equipment: Waters Alliance 2690 (or a Waters binary HPLC pump 1525) equipped with a UV spectrophotometer (Photodiode Array Detector, Waters 996) and a Berthold LB509 radioactivity detector; column: analytical Symmetry-M® C18, Waters (50 × 4.6 mm); porosity: 5.0 μm; conditions: isocratic elution with solvA/solvB: 40/60 (v:v) (solvent A: H_2O containing Low-UV PIC® B7 reagent (20 mL for 1000 mL); solvent B: H_2O/CH_3CN: 30:70 (v:v) containing Low-UV PIC® B7 reagent (20 mL for 1000 mL)); flow rate: 2.0 mL/min; temperature: RT; absorbance detection at $\lambda = 263$ nm.

Spectroscopies

NMR spectra were recorded on a Bruker (Wissembourg, France) Avance (400 MHz) apparatus using the hydrogenated residue of the deuterated solvent CD_2Cl_2 ($\delta = 5.32$ ppm) or trimethylsilyl (TMS) ($\delta = 0.0$ ppm) as internal standards for ^{1}H-NMR as well as the deuterated solvent CD_2Cl_2 ($\delta = 54.0$ ppm) as an internal standard for ^{13}C-NMR. The chemical shifts are reported in ppm, downfield from TMS (s, d, t, q, m, b for singlet, doublet, triplet, quadruplet, multiplet and broad, respectively). The mass spectra (MS) were measured on a Thermo Electron (Les Ulis, France) Ion Trap LCQ Deca XP+ spectrometer (positive electrospray ionization (ESI+)).

Radioisotope production

No-carrier-added fluorine-18 (half-life: 109.8 min) was produced via the [^{18}O(p,n)^{18}F] nuclear reaction by irradiation of a 2-mL [^{18}O]water (> 97% enriched, Rotem (CortecNet, Paris, France)) target on an IBA Cyclone-18/9 (IBA, Louvain-la-Neuve, Belgium) cyclotron (18 MeV proton beam) and the aqueous radioactive solution was then transferred to the appropriate hot cell. *Target hardware*: Commercial, 2-mL, two-port, stainless steel target holder equipped with a domed-end niobium cylinder insert. *Target to hot cell liquid-transfer system*: 60 m polytetrafluoroethylene line (0.8 mm internal diameter; $\frac{1}{16}$-in external diameter), 2.0 bar helium drive pressure, transfer time 3–6 min. Typical production of [^{18}F]fluoride at the end of bombardment for a 20 μA, 30 min (10 μA. h) irradiation: 27.7–29.6 GBq (750–800 mCi).

Miscellaneous

Radiosyntheses using fluorine-18, including the HPLC purifications, were performed in a shielded cell (7.5 cm lead) using a computer-assisted Zymate-XP robot system (Zymark corporation, USA).

Chemistry

N,N-*diethyl-2-(2-(4-methoxyphenyl)-5,7-dimethylpyrazolo[1,5-a]pyrimidin-3-yl)acetamide (DPA-713,* ***3****)*

Synthesized according to Reference 39. R_f (CH_2Cl_2/MeOH: 95/5 v:v): 0.4. ^{1}H-NMR (CD_2Cl_2, 400 MHz) δ 7.75 (d, 2H, $J = 8.8$ Hz, Ph), 6.99 (d, 2H, $J = 8.8$ Hz, Ph), 6.54 (s, 1H, Ph), 3.88 (s, 2H, $C\underline{H}_2$(C=O)), 3.85 (s, 3H, OCH_3), 3.51 (q, 2H, $J = 7.2$ Hz, N($C\underline{H}_2CH_3$)), 3.39 (q, 2H, $J = 7.2$ Hz, N($C\underline{H}_2CH_3$)), 2.72 (s, 3H, 7-CH_3), 2.53 (s, 3H, 5-CH_3), 1.23 (t, 3H, $J = 7.2$ Hz, N($CH_2C\underline{H}_3$)), 1.12 (t, 3H, $J = 7.2$ Hz, N($CH_2C\underline{H}_3$)). ^{13}C-NMR (CH_2Cl_2, 400 MHz) δ 170.3 [C], 160.4 [C], 158.1 [C], 155.0 [C], 148.3 [C], 145.4 [C], 130.2 [2 × CH], 127.0 [C], 114.4 [2 × CH],

108.7 [CH], 101.3 [C], 55.8 [CH_3], 42.8 [CH_2], 41.0 [CH_2], 28.6 [CH_2], 24.9 [CH_3], 17.2 [CH_3], 14.7 [CH_3], 13.4 [CH_3]. MS (ESI+): *m/z*: 367 $(M+H)^+$.

*1-Fluoro-2-tosyloxyethane (**6**)*

To a solution of 2-fluoroethanol (60 mg, 0.94 mmol) in dry dichloromethane (2 mL) were successively added *p*-toluenesulfonyl chloride (270 mg, 1.41 mmol), triethylamine (200 µL, 1.41 mmol) and a catalytic amount of DMAP (13 mg, 0.11 mmol). The reaction mixture was stirred for 2 h at RT. The mixture was then partitioned between 1 M aqueous HCl (10 mL) and CH_2Cl_2 (20 mL). The organic layer was then separated, washed with a sat. aqueous K_2CO_3 solution, dried over $MgSO_4$, filtered and evaporated to dryness. The residue was finally purified by flash chromatography on silica gel (heptane/EtOAc: 9/1 (v:v)) to afford pure 1-fluoro-2-tosyloxyethane (**6**) (200 mg, 98%) as a colourless oil. R_f (heptane/EtOAc: 80/20 v:v): 0.3. ^{1}H-NMR (CD_2Cl_2, 400 MHz) δ 7.79 (d, 2H, J = 8.0 Hz, Ph), 7.39 (d, 2H, J = 8.0 Hz, Ph), 4.56 (dt, 2H, J^2_{H-F} = 47.2 Hz and J^3_{H-H} = 4.0 Hz, CH_2F), 4.23 (dt, 2H, J^3_{H-F} = 28.0 Hz and J^3_{H-H} = 4.0 Hz, OCH_2CH_2F), 2.45 (s, 3H, CH_3). ^{13}C-NMR (CD_2Cl_2, 400 MHz) δ 146.0 [C], 133.1 [C], 130.5 [2 × CH], 128.4 [2 × CH], 81.4 [d, J^1_{F-C} = 172 Hz, CH_2], 69.3 [d, J^2_{F-C} = 20 Hz, CH_2], 21.9 [CH_3].

N,N-*Diethyl-2-(2-(4-hydroxyphenyl)-5,7-dimethylpyrazolo[1,5-a]pyrimidin-3-yl)acetamide (**4**)*

To a solution of compound **3** (1 g, 2.73 mmol) in CH_2Cl_2 (12 mL) was added dropwise at −60°C a 1 M solution of boron tribromide in THF (13.7 mL, 13.7 mmol). The reaction mixture was stirred for 2 h at −60°C, then poured over ice and extracted twice with CH_2Cl_2 (100 mL). The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and evaporated to dryness. The residue was crystallized from Et_2O/EtOH to afford pure **4** (640 mg, 55%) as light-yellow crystals. R_f (CH_2Cl_2/MeOH: 95/5 v:v): 0.35. ^{1}H-NMR (CD_2Cl_2, 400 MHz) δ 8.62 (bs, 1H, OH), 7.54 (d, 2H, J = 8.4 Hz, Ph), 6.81 (d, 2H, J = 8.4 Hz, Ph), 6.54 (s, 1H, H-6), 3.90 (s, 2H, CH_2), 3.47 (q, 2H, J = 6.8 Hz, $N(CH_2CH_3)$), 3.37 (q, 2H, J = 6.8 Hz, $N(CH_2CH_3)$), 2.71 (s, 3H, 7-CH_3), 2.52 (s, 3H, 5-CH_3), 1.18 (t, 3H, J = 6.8 Hz, $N(CH_2CH_3)$), 1.09 (t, 3H, J = 6.8 Hz, $N(CH_2CH_3)$). ^{13}C-NMR (CD_2Cl_2, 400 MHz) δ 171.2 [C], 158.7 [C], 158.2 [C], 155.8 [C], 148.2 [C], 145.8 [C], 130.4 [2 × CH], 125.3 [C], 115.9 [2 × CH], 108.9 [CH], 100.8 [C], 43.0 [CH_2], 41.4 [CH_2], 28.7 [CH_2], 24.7 [CH_3], 17.2 [CH_3], 14.4 [CH_3], 13.3 [CH_3]. MS (ESI+): *m/z*: 353 $(M+H)^+$.

N,N-*Diethyl-2-(2-(4-(2-toluenesulfonyloxyethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-a]pyrimidin-3-yl)acetamide (**2**)*

To a suspension of NaH (38 mg, 1.55 mmol) in dry THF (8 mL) was added a dry solution of THF (1 mL) containing compound **3** (500 mg, 1.41 mmol). The reaction mixture was stirred for 10 min at 5°C and then allowed to warm up to RT before addition of a solution of 1,2-di(tosyloxy)ethane (**5**, 1.04 g, 2.82 mmol) in CH_2Cl_2 (2 mL). The reaction mixture was refluxed overnight. The mixture was then partitioned between 1 M aqueous HCl (100 mL) and CH_2Cl_2 (100 mL). The organic layer was then separated, washed with brine, dried over $MgSO_4$, filtered and evaporated to dryness. The residue was finally purified by flash chromatography on silica gel (CH_2Cl_2/MeOH: 98/2–95/5 (v:v)) to afford **2** (600 mg, 77%) as a white powder. R_f (CH_2Cl_2/MeOH: 98/2 v:v): 0.38. ^{1}H-NMR (CD_2Cl_2, 400 MHz) δ 7.82 (d, 2H, J = 8.3 Hz, Ph), 7.72 (d, 2H, J = 8.8 Hz, Ph), 7.39 (d, 2H, J = 8.3 Hz, Ph), 6.88 (d, 2H, J = 8.8 Hz, Ph), 6.55 (s, 1H, Ph), 4.37 (dd, 2H, J = 6.2 and 4.4 Hz, OCH_2), 4.19 (dd, 2H, J = 6.2 and 4.4 Hz, CH_2OTs), 3.87 (s, 2H, $CH_2(C{=}O)$), 3.50 (q, 2H, J = 7.1 Hz, $N(CH_2CH_3)$), 3.38 (q, 2H, J = 7.1 Hz, $N(CH_2CH_3)$), 2.72 (s, 3H, 7-CH_3), 2.53 (s, 3H, 5-CH_3), 2.45 (s, 3H, $PhCH_3$), 1.22 (t, 3H, J = 7.1 Hz, $N(CH_2CH_3)$), 1.11 (t, 3H, J = 7.1 Hz, $N(CH_2CH_3)$). ^{13}C-NMR (CD_2Cl_2, 400 MHz) δ 170.3 [C], 158.7 [C], 158.2 [C], 154.7 [C], 148.2 [C], 145.8 [C], 145.4 [C], 133.3 [C], 130.5 [2 × CH], 130.2 [2 × CH], 128.4 [2 × CH], 127.7 [C], 115.0 [2 × CH], 108.8 [CH], 101.3 [C], 68.9 [CH_2], 66.1 [CH_2], 42.8 [CH_2], 41.0 [CH_2], 28.5 [CH_2], 24.9 [CH_3], 21.9 [CH_3], 17.1 [CH_3], 14.7 [CH_3], 13.4 [CH_3]. MS (ESI+): *m/z*: 573 $(M+Na)^+$, 551 $(M+H)^+$.

N,N-*diethyl-2-(2-(4-(2-fluoroethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-a]pyrimidin-3-yl)acetamide (DPA-714, **1**)*

To a suspension of NaH (12 mg, 0.50 mmol) in dry THF (8 mL) was added a dry solution of THF (1 mL) containing compound **3** (100 mg, 0.28 mmol). The reaction mixture was stirred for 10 min at 5°C and then allowed to warm up to RT before addition of a solution of 1-fluoro-2-tosyloxyethane (**6**, 200 mg, 0.92 mmol) in CH_2Cl_2 (1 mL). The reaction mixture was heated overnight at 60°C with stirring. The mixture was then partitioned between 1 M aqueous HCl (100 mL) and CH_2Cl_2 (100 mL). The organic layer was then separated, washed with brine, dried over $MgSO_4$, filtered and evaporated to dryness. The residue was finally purified by flash chromatography on silica gel (CH_2Cl_2/MeOH: 98/2–95/5 (v:v)) to afford **1** (65 mg, 58%) as white crystals after crystallization from Et_2O. R_f (CH_2Cl_2/MeOH: 98/2 v:v): 0.33. ^{1}H-NMR (CD_2Cl_2, 400 MHz) δ 7.77 (d, 2H, J = 8.8 Hz, Ph), 7.01 (d, 2H, J = 8.8 Hz, Ph), 6.55 (s, 1H, Ph), 4.77 (dt, 2H, J^2_{H-F} = 47.6 Hz and J^3_{H-H} = 4.0 Hz, CH_2F), 4.26 (dt, 2H, J^3_{H-F} = 28.8 Hz and J^3_{H-H} = 4.0 Hz, OCH_2CH_2F), 3.88 (s, 2H, $CH_2(C{=}O)$), 3.50 (q, 2H, J = 7.1 Hz, NCH_2CH_3), 3.38 (q, 2H, J = 7.1 Hz, NCH_2CH_3), 2.72 (s, 3H, 7-CH_3), 2.53 (s, 3H, 5-CH_3), 1.22 (t, 3H, J = 7.1 Hz, NCH_2CH_3), 1.11 (t, 3H, J = 7.1 Hz, NCH_2CH_3). ^{13}C-NMR (CD_2Cl_2, 400 MHz) δ 170.3 [C], 159.2 [C], 158.2 [C], 155.0 [C], 148.3 [C], 145.3 [C], 130.3 [2 × CH], 127.6 [C], 115.0 [2 × CH], 108.7 [CH], 101.3 [C], 82.7 [d, J^1_{F-C} = 170 Hz, CH_2], 67.8 [d, J^2_{F-C} = 20 Hz, CH_2], 42.8 [CH_2], 41.0 [CH_2], 28.5 [CH_2], 24.9 [CH_3], 17.1 [CH_3], 14.7 [CH_3], 13.4 [CH_3]. MS (ESI+): *m/z*: 399 $(M+H)^+$.

Radiochemistry

Preparation of the K[^{18}F]F-K_{222} complex

No-carrier-added cyclotron-produced fluorine-18 was isolated as [^{18}F]fluoride ion by passing the irradiated [^{18}O]water target, using helium pressure (1.5–2.0 bar), through an anion exchange resin (SepPak® Light Waters Accell™ Plus QMA cartridge (chloride form, beforehand washed with 1 M aqueous $NaHCO_3$ (2 mL) and rinsed with water (20 mL) and CH_3CN (10 mL))). Helium was blown through the column to maximally extract [^{18}O]water. The [^{18}F]fluoride ion was then eluted from the resin, using an aqueous K_2CO_3 solution (1.0 mL of a 4.5 mg/mL solution), into a Vacutainer® tube containing Kryptofix®222 (K_{222}: 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane, 12.0–15.0 mg). The resulting solution was then gently concentrated to dryness at 145–150°C under a nitrogen stream for 10 min to give no-carrier-added K[^{18}F]F-K_{222} complex as a white semi-solid residue.

Preparation of *N,N*-diethyl-2-(2-[4-(2-[^{18}F]fluoroethoxy)-phenyl]-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl)acetamide ([^{18}F]DPA-714, [^{18}F]-1)

Fluorine-18 incorporation studies

Solvent (DMSO, acetonitrile or DMF, 600 μL) containing **2** (1.5–5.0 mg, 2.7-9.1 μmol) was added into the Vacutainer® tube containing the dried K[^{18}F]F-K_{222} complex. The tube (not sealed) was thoroughly vortexed (30 s) and then placed in a heating block (at 120 or 165°C, for 5–15 min) without stirring the contents. The reaction vessel was then cooled using an ice–water bath; the remaining radioactivity was measured and the reaction mixture was analysed by radio-TLC. The reaction yield was calculated from the TLC-radiochromatogram and is defined as the ratio of radioactivity area of [^{18}F]DPA-714 ([^{18}F]-**1**) over total fluorine-18 radioactivity area (SiO_2-TLC: R_f: [^{18}F]-**1**: 0.2 (EtOAc) and 0.6 (CH_2Cl_2/MeOH: 90/10 (v:v)) – R_f: [^{18}F]fluoride ion : 0.0 (both eluents)). The reaction mixture was then diluted with water (1 mL) and transferred onto a C18 cartridge (PrepSep™ R-C18 Extraction Column, Fisher Scientific, activated beforehand with EtOH (2 mL) and then rinsed with water (10 mL)), pre-filled with water (2 mL). The tube was rinsed twice with water (1 mL), which was also transferred and added to the diluted reaction mixture on top of the cartridge. An additional portion of water (2 mL) was further added to the diluted reaction mixture on top of the cartridge. The whole was then passed through the cartridge, which was then washed with water (3 mL) and partially dried for 0.5 min by applying a nitrogen stream. [^{18}F]-**1** was eluted from the cartridge with CH_2Cl_2 (3 mL) into an empty 5-mL reaction vial. Elution was repeated twice with 1 mL of CH_2Cl_2 for maximal transfer of [^{18}F]-**1**. The incorporation yield was estimated after the C18 cartridge elution by the CH_2Cl_2- over total eluted radioactivity (DMSO/H_2O+CH_2Cl_2) ratio.

Optimized conditions

DMSO (600 μL) containing **2** (4.5–5.0 mg, 8.2–9.1 μmol) was added into the Vacutainer® tube containing the dried K[^{18}F]F-K_{222} complex. The tube (not sealed) was thoroughly vortexed (30 s) and then placed in a heating block (at 165°C, for 5 min) without stirring the contents. The reaction vessel was then cooled using an ice–water bath; the reaction mixture was diluted with water (1 mL) and transferred onto a C18 cartridge (PrepSep™ R-C18 Extraction Column, activated beforehand as described above). The cartridge pre-purification procedure was performed as mentioned above. The CH_2Cl_2 solution containing [^{18}F]-**1** was concentrated to dryness at 65–75°c under a gentle nitrogen stream for 3–5min. Finally, the residue was redissolved in the HPLC solvent used for purification (1.0 mL) and the crude was injected onto HPLC (HPLC A).

Formulation

Formulation of the labelled product for i.v. injection was effected as follows: The HPLC-collected fraction containing the radiotracer was diluted with water (30 mL). The resulting solution was passed through a SepPak® Plus C18 cartridge (Waters, washed with 2 mL of EtOH and then rinsed with 10 mL of water prior to use). The cartridge was washed with water (10 mL) and partially dried by applying a nitrogen stream for 10 s. The radiotracer was eluted with 2 mL of EtOH followed by 8 mL of physiological saline (less than 10% of the total radioactivity was left on the cartridge) and filtered on a 0.22 μm GS-Millipore filter (vented). Finally, physiological saline was added to take the EtOH concentration below 10%. This whole process was performed using a remote-controlled dedicated home-made device based on a literature procedure.[52]

Quality control

The radiotracer preparation was visually inspected for clarity, absence of colour and particulates. An aliquot of the preparation was removed for determination of pH using standard pH paper. Chemical and radiochemical purities were also assessed on this aliquot by HPLC (HPLC B), with a sample of authentic **1**. Particular attention was paid to the absence of non-radioactive precursor **2**. Chemical and radiochemical stabilities of the entire preparation were tested by HPLC (HPLC B) at regular 15-min intervals during 150 min. Specific radioactivity of the radiotracer was calculated from three consecutive HPLC (HPLC B) analyses (average) and determined as follows: the area of the UV absorbance peak corresponding to the radiolabelled product was measured (integrated) on the HPLC-chromatogram and compared with a standard curve relating mass to UV absorbance.

Log P*/log* D *determination*

Log *P* (*n*-octanol/water partition coefficient): [^{18}F]-**1** (1–5 kBq in 50 μL of water) was added to a two-layer system of *n*-octanol (500 μL) and water (450 μL) in an Eppendorf cap. The vessel was strongly vortexed for 3 min and then quickly centrifuged at 3000 rpm for 2 min. An aliquot of each layer (100 μL) was assessed for radioactivity in a cross-calibrated Perkin-Elmer Cobra Quantum γ-counter (Les Ulis, France). Log *D* (*n*-octanol/buffer pH 7.4 partition coefficient): The procedure described above was repeated by replacing water (450 μL) by 0.1 M phosphate-buffered saline pH 7.4 (450 μL). The partition coefficients (log *P* and log *D*) were calculated as the decimal logarithm of the ratio between the counted radioactivity in the *n*-octanol phase and the counted radioactivity in the aqueous phase.

Conclusion

DPA-714 (**1**), a fluorine-containing 2-phenylpyrazolo[1,5-*a*]pyrimidineacetamide and selective ligand of the TSPO (K_i = 7.0 nM), has been labelled with fluorine-18 using a simple one-step process (a tosyloxy-for-fluorine nucleophilic aliphatic substitution), the latter being fully automatized on our Zymate-XP robotic system. *In vivo* pharmacological and imaging properties of [^{18}F]DPA-714 are currently evaluated in a rat model of neuroinflammation (unilateral intrastriatal injection of AMPA) using our small-animal dedicated PET tomograph (Focus Concorde 220) and data will be compared with that already reported for [^{11}C]PK11195, [^{11}C]DPA-713 and [^{11}C]CLINME.[34,41]

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