Radiosynthesis of 7-chloro-N,N-dimethyl-5-[11C]methyl-4-oxo-3-phenyl-3,5-dihydro-4H-pyridazino[4,5-b]indole-1-acetamide, [11C]SSR180575, a novel radioligand for imaging the TSPO (p...

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Radiosynthesis of 7-chloro-*N*,*N*-dimethyl-5-[^{11}C]methyl-4-oxo-3-phenyl-3,5-dihydro-4*H*-pyridazino[4,5-*b*]indole-1-acetamide, [^{11}C]SSR180575, a novel radioligand for imaging the TSPO (peripheral benzodiazepine receptor) with PET

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SSR180575 (7-chloro-*N*,*N*,5-trimethyl-4-oxo-3-phenyl-3,5-dihydro-4*H*-pyridazino[4,5-*b*]indole-1-acetamide) is the lead compound of an original pyridazinoindole series of potent and highly selective TSPO (peripheral benzodiazepine receptor) ligands. Isotopic labeling of SSR180575 with the short-lived positron-emitter carbon-11 ($T_{1/2}$: 20.38 min) at its 5-methylpyridazino[4,5-*b*]indole moiety as well as at its *N*,*N*-dimethylacetamide function by methylation of the corresponding *nor*-analogues was investigated. Best results in terms of radiochemical yields and purities were obtained for the preparation of [*indole-N-methyl*-^{11}C]SSR180575, where routine production batches of 4.5–5.0 GBq of radiochemically pure (>99%) *i.v.* injectable solutions (specific radioactivities: 50–90 GBq/μmol) could be prepared within a total synthesis time of 25 min (HPLC purification included) starting from a 55 GBq [^{11}C]CO_2 cyclotron production batch (non-decay-corrected overall radiochemical yields: 8–9%). The process comprises (1) trapping at −10°C of [^{11}C]methyl triflate in DMF (300 μl) containing 0.2–0.3 mg of the indole precursor for labeling and 4 mg of K_2CO_3 (excess); (2) heating at 120°C for 3 min; (3) dilution of the residue with 0.5 ml of the HPLC mobile phase and (4) purification using semi-preparative reversed-phase HPLC (Zorbax® SB-C-18). *In vivo* pharmacological properties of [*indole-N-methyl*-^{11}C]SSR180575 as a candidate for imaging neuroinflammation with positron emission tomography are currently evaluated.

Keywords: carbon-11; methylation; SSR180575; PBR; TSPO 18 kDa

Introduction

Microglia activation is a major cellular response during inflammation within the central nervous system,[1,2] a process characterized by a drastic change in the morphology of these cells and by the notable over-expression of the peripheral benzodiazepine receptor (PBR), recently renamed translocator protein (18 kDa) or in short TSPO.[3]

The radiolabeling of the 3-isoquinolinecarboxamide PK11195 with the short-lived positron-emitter carbon-11 ($T_{1/2}$: 20.38 min) in the mid eighties[4] paved the way to the current concept of positron emission tomography (PET) imaging of neuroinflammation using TSPO radioligands.[5] [^{11}C]PK11195 (Figure 1) remains the most cited radiotracer in this field and is still widely used, in spite of its low brain uptake and high level of non-specific

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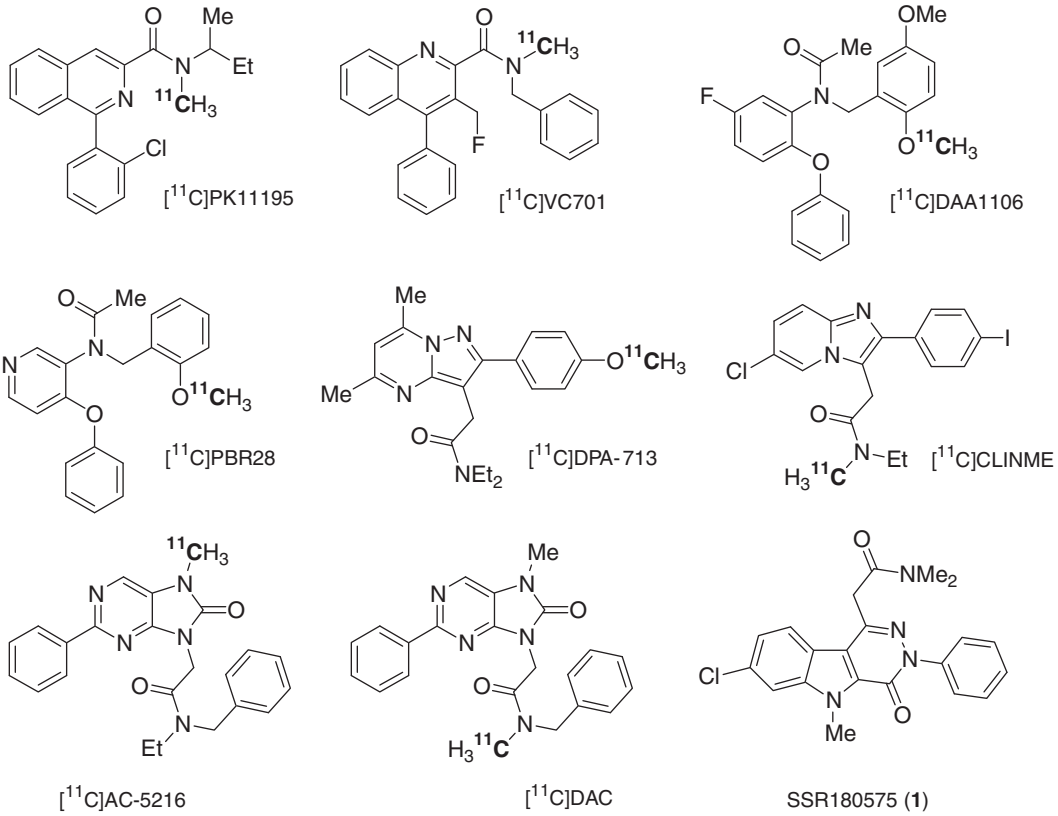


Figure 1. Selected carbon-11-labeled TSPO (PBR) PET-radioligands and SSR180575 (**1**).

binding (both leading to a poor signal-to-noise ratio) and its extensive binding to plasma proteins that complicates quantitative analysis of the receptor density.[6,7]

In the last two decades, extensive efforts have been undertaken to design and synthesize new radioligands that avoid some of the limitations observed with [^{11}C]PK11195.[8–11] Among the compounds labeled with carbon-11 the following are worth mentioning (Figure 1): the quinoline-2-carboxamide [^{11}C]VC701,[12] the *N*-benzyl-*N*-(2-phenoxyaryl)-acetamides [^{11}C]DAA1106[13,14] and [^{11}C]PBR28,[15–18] the 2-phenylpyrazolo [1,5-*a*]pyrimidineacetamide [^{11}C]DPA-713,[19–24] the 2-phenylimidazo[1,2-*a*]pyridineacetamide [^{11}C]CLINME[25–27] and the 2-aryl-8-oxodihydropurines [^{11}C]AC-5216[28–31] and [^{11}C]DAC.[32,33]

Radioligands based on the indoleacetamide moiety have so far remained under-investigated despite reports on several highly potent and specific ligands for the PBR.[34–37] Of particular interest is SSR180575 (7-chloro-*N*,*N*,5-trimethyl-4-oxo-3-phenyl-3,5-dihydro-4*H*-pyridazino[4,5-*b*]indole-1-acetamide, **1**) (Figure 1), the lead compound of a new series of pyridazino[4,5-*b*]indoleacetamides, showing *in vitro* nanomolar affinity for rodent and human PBR,[38] as well as *in vivo* neuroprotective[39] and mitochondria-rescuing[40] properties.

In this work, the isotopic labeling of SSR180575 with the short-lived positron-emitter carbon-11 at two different sites, the 5-methylpyridazino[4,5-*b*]indole moiety and the *N*,*N*-dimethylacetamide function, is reported.

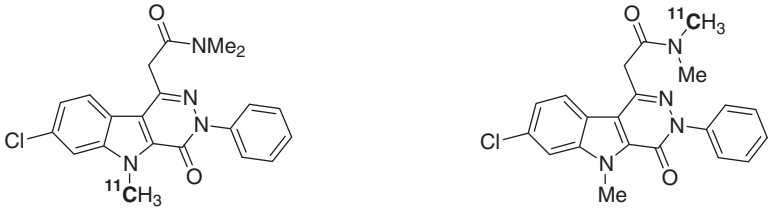
Results and discussion

Carbon-11-labeling of SSR180575 (**1**) was investigated *via* methylation reaction at its 5-methylpyridazino[4,5-*b*]indole moiety as well as at its *N*,*N*-dimethylacetamide function (Figure 2).

The use of the methylation reagent [^{11}C]methyl triflate[41–44] was privileged for the labeling at the 5-methylpyridazino[4,5-*b*]indole moiety (leading to compound **1a**), whereas [^{11}C]methyl iodide[45] was preferred for the labeling at the *N*,*N*-dimethylacetamide function (leading to compound **1b**), a choice based on literature reports as well as on our own experience with similar structures.[4,25,46] The *nor*-derivatives needed as precursors for labeling as well as SSR180575 itself needed as a reference were synthesized at Sanofi-Aventis.[47]

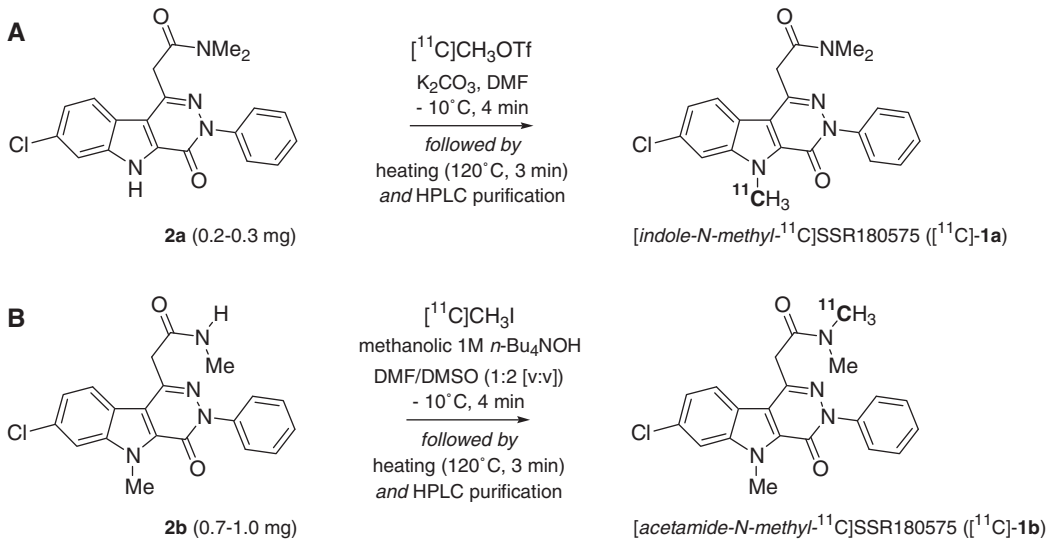
[^{11}C]Methyl iodide was prepared from cyclotron-produced [^{11}C]carbon dioxide using the well-known two step protocol, consisting of the trapping of [^{11}C]carbon dioxide in lithium aluminium hydride forming [^{11}C]methanol, followed by iodination using aqueous hydriodic acid giving [^{11}C]methyl iodide.[45] [^{11}C]Methyl triflate was prepared according to a literature procedure from [^{11}C]methyl iodide using silver triflate.[41] On average, about 24 GBq of [^{11}C]methyl iodide or [^{11}C]methyl triflate were routinely obtained in 6–7 min after end of bombardment (EOB) in 70% decay-corrected yield, based on starting [^{11}C]carbon dioxide.

Carbon-11-labeling of SSR180575 at its 5-methylpyridazino[4,5-*b*]indole moiety was relatively straightforward and based on slightly modified standard conditions routinely used in our laboratory for [^{11}C]methylation reactions.[48–50] Optimized conditions for the preparation of [*indole-N-methyl*-^{11}C]SSR180575 ([^{11}C]-**1a**) (Scheme 1A) include a trapping at −10°C of [^{11}C]methyl triflate in DMF (300 µl) containing 0.2–0.3 mg of the indole precursor **2a** and an excess of powdered K_2CO_3 (4 mg) followed by a 3-min heating period at 120°C. Purification



[*indole-N-methyl*-^{11}C]SSR180575 ([^{11}C]-**1a**) [*acetamide-N-methyl*-^{11}C]SSR180575 ([^{11}C]-**1b**)

Figure 2. [^{11}C]SSR180575: chemical structures resulting from the labeling at the 5-methylpyridazino[4,5-*b*]indole moiety ([^{11}C]-**1a**) and at the *N,N*-dimethylacetamide function ([^{11}C]-**1b**).



Scheme 1. Radiosynthesis of [*indole-N-methyl*-^{11}C]SSR180575 ([^{11}C]-**1a**) and [*acetamide-N-methyl*-^{11}C]SSR180575 ([^{11}C]-**1b**) from [^{11}C]methyl triflate and [^{11}C]methyl iodide, respectively.

of [^{11}C]-**1a** was performed on a semi-preparative Symmetry Prep® C-18 HPLC column, using a 50:50:0.1 [v:v:v] mixture of acetonitrile, water and TFA as the eluent (HPLC A, see Experimental). Using this HPLC system, [^{11}C]-**1a**, which is the only radioactive reaction product present on the radiochromatogram, could be well separated (t_R: 8.0 min) from unlabeled precursor **2a** (t_R: 5.5 min) and residual [^{11}C]methyl triflate (t_R < 3 min), if any. Removal of the HPLC solvents and formulation of [^{11}C]-**1a** as an *i.v.* injectable solution was then performed using a home-made SepPak® Plus C18-based device (see Experimental part). Recovered [^{11}C]-**1a**, as ethanol solution, was diluted with aqueous 0.9% sodium chloride (physiological saline) to an ethanol concentration below 10%. The total procedure, from EOB to final formulation, lasted 33 min.

Purification of [^{11}C]-**1a** could also be performed on a semi-preparative Zorbax® SB-C-18 HPLC column using the eluent described above, without noticeable changes in the retention times ([^{11}C]-**1a:** t_R: 7.5–8.0 min, unlabeled precursor **2a:** t_R: 5.5 min and residual [^{11}C]methyl triflate: t_R < 3 min, HPLC B, see Experimental).

However, finally we chose to purify [^{11}C]-**1a** on a semi-preparative Zorbax® SB-C-18 HPLC column using a 50:50:0.1 [v:v:v] mixture of aqueous 0.9% sodium chloride, ethanol and aqueous 1M phosphate buffer (pH 2.3) as the eluent (HPLC C, see Experimental). The advantage of this system is the compatibility of the mobile phase with an *i.v.* administration. Although the separation is less efficient, it is still good enough to separate the target compound ([^{11}C]-**1a**: t_R: 9.0 min) from unlabeled precursor **2a** (t_R: 7.0–8.0 min) as long as the amount of the latter was kept below 0.3 mg. Formulation of [^{11}C]-**1a** as an *i.v.* injectable solution was simplified and consisted in a further dilution of the HPLC-collected fraction containing [^{11}C]-**1a** with physiological saline to an ethanol concentration below 10%, thus shortening the total radiosynthesis time by 8 min. Using these optimized conditions, routine production batches of 4.5–5.0 GBq of *i.v.* injectable solutions of [*indole-N-methyl*-^{11}C]SSR180575 ([^{11}C]-**1a**) could be prepared within a total synthesis time of 25 min (HPLC purification included) starting from a 55 GBq [^{11}C]CO_2 cyclotron production batch (decay-corrected overall radiochemical yields: 19–21%).

Quality controls were performed on aliquots of the preparations ready for *i.v.* injection. The radiotracer preparations were clear and colorless solutions with a measured pH between 5 and 6. As demonstrated by analytical HPLC analysis (HPLC D, see Experimental), the preparations were >95% chemically and >99% radiochemically pure (**1**, t_R: 2.44 min) with specific radioactivities ranging from 50 to 90 GBq/μmol at the end of synthesis. The preparations were also shown to be free of the non-radioactive precursor (**2a,** t_R: 1.64 min) and were chemically and radiochemically stable for at least 60 min.

No attempts were made to further optimize the preparation of [*indole-N-methyl*-^{11}C]SSR180575 ([^{11}C]-**1a**), as sufficient material was obtained to allow for radiopharmacological characterization. Log *D* (*n*-octanol/buffer pH 7.4 partition

coefficient) of [^{11}C]-**1a** was found to be 1.86 using the shake-flask method.

Carbon-11-labeling of SSR180575 at its *N,N*-dimethylacetamide function surprisingly turned out to be more difficult (Scheme 1B). Initially, we tried the standard conditions we routinely use in our laboratory for the radiosynthesis of [^{11}C]CLINME, a compound similarly labeled with [^{11}C]methyl iodide *via* a methylation reaction at a *N,N*-dimethylacetamide function.[25] These procedures include a trapping at −10°C of [^{11}C]methyl iodide in DMF or a mixture of DMF and DMSO (300 µl) containing up to 2.0 mg of the methylacetamide precursor **2b** and an excess of powdered KOH (3 to 15 mg) followed by a 3-min heating period at 120°C. HPLC purification of the crude reaction mixtures was attempted using the systems described above (HPLC A, B and C, see Experimental).

On the HPLC radiochromatograms recorded using the system initially developed (HPLC A and B) for the purification of [*indole-N-methyl*-^{11}C]SSR180575 ([^{11}C]-**1a**), three minor radioactive peaks (t_R: 4.5, 5.5 and 6.5 min) and one major radioactive peak (t_R: 7.5–8.0 min, corresponding to the expected retention time of [*acetamide-N-methyl*-^{11}C]SSR180575 ([^{11}C]-**1b**)) could be observed beside a peak attributed to residual [^{11}C]methyl iodide (t_R <3 min). Using the third system (HPLC C), only one relatively large radioactive peak (t_R: 7.5–9.0 min, corresponding to the expected retention time of [^{11}C]-**1b**) was observed beside residual [^{11}C]methyl iodide (t_R < 3 min). Regardless the HPLC system used, [^{11}C]-**1b** could well be separated from unlabeled precursor **2b** (t_R: 4.5–5.0 min).

Analytical HPLC analysis using the system dedicated to the quality controls (HPLC D) demonstrated that in all cases [*acetamide-N-methyl*-^{11}C]SSR180575 ([^{11}C]-**1b**) was present within the collected fractions corresponding to the major products (t_R at 7.5–8.0 min when using HPLC A and B and t_R at 7.5–9.0 min when using HPLC C). Identity of [*acetamide-N-methyl*-^{11}C]SSR180575 ([^{11}C]-**1b**) was confirmed by co-chromatography with an authentic standard of **1** (t_R: 2.44 min) but these analyses also showed the presence of an additional radiochemical compound (t_R: 2.78 min) representing 80–85% of the total injected radioactivity.

Further attempts to label SSR180575 at its *N,N*-dimethylacetamide function included the replacement of the base KOH by K_2CO_3 or triethylamine, as well as the use of no base at all, which all gave almost no incorporation of [^{11}C]methyl iodide. Reducing the reaction temperature or the reaction time was not tried in this work, seen the discouraging results obtained with this in the optimization of the labeling of [^{11}C]PK11195 or [^{11}C]CLINME.

The best compromise in terms of final radiochemical yield but also radiochemical purity was found using *n*-Bu_4NOH as the base and the following conditions: (1) Trapping of [^{11}C]methyl iodide at −10°C in a 1/2 (v:v) mixture of DMF/DMSO (300 µl) containing 0.7–1.0 mg of the methylacetamide precursor **2b** and 5 µl of methanolic 1M *n*-Bu_4NOH followed by (2) a 3-minute heating period at 120°C and (3) an HPLC purification performed on a semi-preparative SymmetryPrep® C-18 HPLC column, using a 50:50:0.1 [v:v:v] mixture of acetonitrile, water and TFA as the eluent (HPLC A, see Experimental). Using this HPLC system, [^{11}C]-**1b** could be well separated (t_R: 8.0 min) from precursor **2b** (t_R: 4.5 min) as well as from the three unidentified radiolabeled side-products (4.0 < t_R < 6.5 min) and residual [^{11}C]methyl iodide (t_R<3 min). Although [^{11}C]-**1b** could not be separated from the co-eluting unidentified radiochemical side-product discussed above, the final radiochemical purity could be improved by cutting-off the second half of the peak during collection, but without reproducible results from batch to batch.

Removal of the HPLC solvents and formulation of [^{11}C]-**1b** as an *i.v.* injectable solution was then performed using a home-made SepPak® Plus C18-based device (see Experimental). Production batches of 1.0–1.5 GBq of *i.v.* injectable solutions of [*acetamide-N-methyl*-^{11}C]SSR180575 ([^{11}C]-**1b**) could be prepared within a total synthesis time of 33 min (HPLC purification included) starting from a 55 GBq [^{11}C]CO_2 cyclotron production batch (decay-corrected overall radiochemical yields: 6–8%).

Quality controls were performed on aliquots of the preparations ready for *i.v.* injection, as described above. The radiotracer preparations were clear and colorless solutions with a measured pH between 5 and 7. As demonstrated by analytical HPLC analysis (HPLC D see experimental), radiochemical and chemical purities were in most cases greater than 70% (**1**, t_R: 2.44 min/unidentified side-product, t_R: 2.78 min), however without ever reaching 90%. The preparations were found to be free of the non-radioactive precursor (**2b,** t_R: 1.31 min) with specific radioactivities in the same range as those mentioned above (50–90 GBq/µmol), and were chemically and radiochemically stable for at least 60 min.

Experimental

General

Chemicals

Chemicals were purchased from ABX GmbH (Germany), Aldrich-, Fluka- or Sigma (France) and were used without further purification.

HPLC analysis

[HPLC A]: Equipment: Waters system equipped with a 510 pump, a Shimadzu SPD-10A UV-multiwavelength detector and a miniature ionization chamber probe; column: semi-preparative SymmetryPrep® C-18, Waters (300 × 7.8 mm); porosity: 7 µm; conditions: eluent: CH_3CN/H_2O/TFA: 50/50/0.1 (v:v:v); flow rate: 5 ml/min; temperature: RT; UV detection at λ: 258 nm. [HPLC B]: Equipment: Waters system equipped with a 510 pump, a Shimadzu SPD-10A UV-multiwavelength detector and a miniature ionization chamber probe; column: semi-preparative Zorbax® SB-C-18, Hewlett Packard (250 × 9.4 mm); porosity: 5 µm; conditions: eluent: CH_3CN/H_2O/TFA: 50/50/0.1 (v:v:v); flow rate: 6 ml/min; temperature: RT; UV detection at λ: 258 nm. [HPLC C]: Equipment: Waters system equipped with a 510 pump, a Shimadzu SPD-10A UV-multiwavelength detector and a miniature ionization chamber probe; column: semi-preparative Zorbax® SB-C-18, Hewlett Packard (250 × 9.4 mm); porosity: 5 µm; conditions: eluent: aq 0.9% NaCl/EtOH/aq 1M phosphate buffer (pH 2.3): 50/50/0.5 [v:v:v]; flow rate: 6 ml/min; temperature: RT; UV detection at λ: 258 nm. [HPLC D]: Equipment: Waters Alliance 2690 equipped with a UV spectrophotometer (Photodiode Array Detector; Waters 996) and a Berthold LB509 radioactivity detector; column: analytical Symmetry-M® C-18; Waters (50 × 4.6 mm); porosity: 3.5 µm; conditions: eluent: solvA/solvB: 40/60 (v/v) [solvent A: H_2O containing low-UV PIC® B7 reagent (Waters), 20 ml for 1000 ml; solvent B: H_2O/CH_3CN: 30/70 (v/v) containing low-UV PIC® B7 reagent (Waters), 20 ml for 1000 ml]; flow rate: 2.0 ml/min; temperature: 30°C; UV detection at λ: 258 nm.

Miscellaneous

Radiosyntheses, including the HPLC purifications, were performed remote-controlled in a 5.0-cm-lead shielded cell.

Radiochemistry

Radioisotope production

[^{11}C]CO_2 was produced *via* the ^{14}N[p,α]^{11}C nuclear reaction by irradiation of a N_2/O_2 target mixture (99.5/0.5, ultrapure, Air Liquide) with an 18 MeV proton beam (at 25 μA) on an IBA Cyclone-18/9 cyclotron (8.5 μA.h in about 20 min). At the end of the bombardment, the target contents were transferred to a 5-cm-lead shielded hot cell and passed through a glass P_2O_5-guard (70 mm length, 3 mm internal diameter) in order to remove moisture. [^{11}C]CO_2 was then separated from the target gas by trapping in an empty stainless-steel coil (150 mm length, 0.51 mm internal diameter), cooled at −186°C using liquid argon. On average, at EOB about 45 GBq (1.20 Ci) of [^{11}C]CO_2 is routinely obtained in our laboratory for the irradiation described above.

Preparation of [^{11}C]CH_3I

[^{11}C]CO_2 was released from the trap by raising the stainless-steel coil temperature to ambient, swept away by a flow of nitrogen gas (40 ml/min) and trapped at −10°C (EtOH-ice bath) in 50 μl of THF containing 5 μl of a 1.0 M THF solution of lithium aluminium hydride. Concentration to dryness (evaporation of solvent at 155°C using a stream of nitrogen) followed by hydrolysis (100 μl of deionized water) of the formed lithium aluminium complex afforded [^{11}C]CH_3OH, which was distilled using a flow of nitrogen gas into 0.8 ml of an aqueous 57% HI solution (heating block at 155°C). The [^{11}C]CH_3I thus synthesized was continuously swept away by the same flow of nitrogen gas and passed through a combined 1/1 (v/v) soda lime/P_2O_5-guard (35 mm pathlength each, 3 mm internal diameter).

Preparation of [^{11}C]CH_3OTf

[^{11}C]CH_3I (in a continuous stream of nitrogen gas) was converted into [^{11}C]CH_3OTf by passing it through a glass column (33 cm length, 5 mm internal diameter), heated at 200°C and containing silver triflate-impregnated graphitized carbon (200 mg).

*Preparation of [*indole-N-methyl-^{11}*C]SSR180575 ([^{11}C]-**1a**)*

[^{11}C]CH_3OTf, carried by a flow of nitrogen gas, was trapped (bubbling through) at −10°C (EtOH-ice bath) in a reaction vessel containing 0.2–0.3 mg of 7-chloro-*N*,*N*-dimethyl-4-oxo-3-phenyl-3,5-dihydro-4*H*-pyridazino[4,5-*b*]indole-1-acetamide (**2a**, 0.52 to 0.78 μmol) dissolved in DMF (300 μl) and finely powdered potassium carbonate (4 mg, excess). Trapping of [^{11}C]CH_3OTf was monitored using an ionization-chamber probe. When the reading had reached its maximum (about 3 min), the reaction mixture was heated at 120°C (heating block) for 3 min. The reaction vessel was then cooled (EtOH–ice bath) and the reaction mixture was diluted with 0.5 ml of the HPLC mobile phase and was injected onto the HPLC column (HPLC A, B or C). The product [^{11}C]-**1a** was collected.

*Preparation of [*acetamide-N-methyl-^{11}*C]SSR180575 ([^{11}C]-**1b**)*

[^{11}C]CH_3I, carried by a flow of nitrogen gas, was trapped (bubbling through) at −10°C (EtOH-ice bath) in a reaction vessel containing 0.7–1.0 mg of 7-chloro-*N*,5-dimethyl-4-oxo-3-phenyl-3,5-dihydro-4*H*-pyridazino[4,5-*b*]indole-1-acetamide (**2b**, 1.84 to 2.63 μmol) dissolved in a mixture of DMF/DMSO (100 μl/200 μl) and methanolic 1M *n*-Bu_4NOH (5 μl). Trapping of [^{11}C]CH_3I was monitored using an ionization chamber probe. When the reading had reached its maximum (about 4 min), the reaction mixture was heated at 120°C (heating block) for 3 min. The reaction vessel was then cooled (EtOH-ice bath) and the reaction mixture was diluted with 0.5 ml of the HPLC mobile phase and was injected onto the HPLC column (HPLC A). The product [^{11}C]-**1b** was collected with cutting-off the second half of the peak to enhance final radiochemical purity.

*Formulation of [*indole-N-methyl-^{11}*C]SSR180575 ([^{11}C]-**1a**) and [*acetamide-N-methyl-^{11}*C]SSR180575 ([^{11}C]-**1b**)*

Formulation of the labeled product for *i.v.* injection was effected as follows: the HPLC-collected fraction containing the radiotracer ([^{11}C]-**1a** or [^{11}C]-**1b**) was diluted with water (50 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 helium stream for 10 s. The radiotracer was eluted with 2 ml of EtOH (less than 10% of the total radioactivity was left on the cartridge) followed by 8 ml of physiological saline 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.[51]

*Quality control of [*indole-N-methyl-^{11}*C]SSR180575 ([^{11}C]-**1a**) and [*acetamide-N-methyl-^{11}*C]SSR180575 ([^{11}C]-**1b**)*

The radiotracer preparation was visually inspected for clarity, absence of color 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 D), with a sample of authentic **1** (t_R: 2.44 min). Particular attention was paid to the absence of non-radioactive precursor (**2a**, t_R: 1.64 min or **2b**, t_R: 1.31 min). Chemical and radiochemical stability of the entire preparation were tested by HPLC (D) at regular 10-min intervals during 60 min. Specific radioactivity of the radiotracer was calculated from three consecutive HPLC (D) analyses (average) and determined as follows: the area of the UV absorbance peak corresponding to the radiolabeled product was measured (integrated) on the HPLC chromatogram and compared with a standard curve relating mass to UV absorbance. The HPLC fraction containing the radiolabeled product was collected, its radioactivity was then measured and the corresponding value divided by the found mass to give the specific radioactivity.

Log D *determination*

Log *D* (*n*-octanol/buffer pH 7.4 partition coefficient): [*indole-N-methyl-*^{11}C]SSR180575 ([^{11}C]-**1a**) (1–5 kBq in 50 μl of water) was added to a two-layer system of *n*-octanol (500 μl) and 0.1 M PBS buffer pH 7.4 (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). The partition coefficient (log *D*) was 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.

Conclusions

The novel pyridazinoindole TSPO (PBR) ligand SSR180575 (7-chloro-*N*,*N*,*5*-trimethyl-4-oxo-3-phenyl-3,5-dihydro-4*H*-pyridazino[4,5-*b*]indole-1-acetamide, **1**) has been isotopically labeled with carbon-11 ($T_{1/2}$: 20.38 min) at its *N*,*N*-dimethylacetamide function and at its 5-methylpyridazino[4,5-*b*]indole moiety from the corresponding *nor*-analogues. Best results in terms of radiochemical yields and purities were obtained for the preparation of [*indole-N-methyl*-^{11}C]SSR180575 ([^{11}C]-**1a**). Routine production batches of 4.5–5.0 GBq of radiochemically pure (>99%) *i.v.* injectable solutions (specific radioactivities: 50–90 GBq/µmol) could be prepared within a total synthesis time of 25 min (HPLC purification included) starting from a 55 GBq [^{11}C]CO_2 cyclotron production batch (non-decay-corrected overall radiochemical yields: 8–9%). *In vivo* pharmacological and imaging properties of [*indole-N-methyl*-^{11}C]SSR180575 ([^{11}C]-**1a**) are currently evaluated in a rat-model of neuroinflammation (unilateral intrastriatal injection of AMPA) using a 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.[21,26]

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