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Synthesis, Radiosynthesis and In Vivo Evaluation of 5-[3-(4-Benzylpiperidin-1-yl)prop-1-ynyl]-1,3-dihydrobenzoimidazol-2-[^{11}C]one, as a Potent NR_{1A}/2B Subtype Selective NMDA PET Radiotracer

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Abstract—Recently, a new series of potent and highly subtype-selective 1-(heteroarylalkynyl)-4-benzylpiperidine antagonists of the NMDA receptors has been described by Pfizer Laboratories. In this series, 5-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]-1,3-dihydrobenzoimidazol-2-one (**1**) was identified as a selective antagonist for the $NR1_A$/2B subtype, displaying IC_{50} values for inhibition of the NMDA responses of 5.3 nM for this subtype (compared to $NR1_A$/2A: 35 μM and $NR1_A$/2C > 100 μM) and was active in rat at a relatively low dosage (10 mg/kg po). Derivative **1** has been synthesized in four chemical steps in good overall yield and labelled with carbon-11 ($T_{1/2}$: 20.4 min) at its benzoimidazolone ring using [^{11}C]phosgene. The pharmacological profile of [^{11}C]-**1** was evaluated in vivo in rats with biodistribution studies and brain radioactivity monitored with intracerebral radiosensitive β-microprobes. The brain uptake of [^{11}C]-**1** was extremely low (0.07% I.D./mL on average at 30 min) and rather uniform across the different brain structures. This in vivo brain regional distribution of [^{11}C]-**1** did not match with autoradiographic or binding data obtained with other NR2B subtype-selective NMDA ligands. Competition studies with ifenprodil (20 mg/kg, ip, 30 min before the radiotracer injection) failed to demonstrate specific binding of the radiotracer in the brain. In view of these results, and especially considering the low brain penetration of the radiotracer, [^{11}C]-**1** does not have the required properties for imaging NMDA receptors using positron emission tomography.

Introduction

The *N*-methyl-D-aspartate (NMDA) receptor is a glutamate-gated ion channel, playing a fundamental and complex role in an array of physiological processes.[1] It is one of the three families of ionotropic receptors (besides AMPA and Kainate) that is activated by glutamate,[2] the principal excitatory neurotransmitter in the CNS. It is strongly implicated in neuroprotection, neurodegeneration, long-term potentiation, memory and cognition.[3–7] The hypothesis that aberrant activation of NMDA receptors underlines a number of neurological and neuropsychiatric disorders, such as Parkinson's disease, Huntington's chorea, schizophrenia, alcoholism and stroke,[1] has generated considerable interest in the NMDA receptor as a target for new pharmacotherapies. First generation NMDA receptor antagonists fall into three main classes: (A) competitive antagonist at the glutamate binding site such as selfotel (CGS 19755);[8,9] (B) non-competitive antagonists at a channel site, such as dizocilpine (MK 801)[10] or phencyclidine (PCP),[11] and (C) glycine site antagonists, such as licostinel (ACEA 1021).[12,13] Antagonists acting at these sites were shown to be neuroprotective, but suffer from a too high level of associated side effects for the treatment of neurodegenerative diseases where chronic drug administration is required.[1]

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From a structural point of view, the NMDA receptor is a hetero-oligomeric cation channel complex, comprising of one or more NR1 subunits, of which there are eight isoforms (NR1a–h) and at least one NR2 subunit (called NR2: NR2A–2D), with different subunit combinations conferring diverse functional properties.[1,2,14] The discovery of the existence of distinct NMDA receptor subtypes has led to the development of new classes of NMDA receptor antagonists, retaining a therapeutic efficacy but exhibiting a reduced side-effect profile. For example, NR2B-selective NMDA antagonists have been developed, such as the prototypic compounds ifenprodil, Ro25-6981, CP-101,606 and CI-1041 and have shown efficacy in neuroprotection, anti-hyperalgesic and Parkinson animal models.[15,16]

Imaging the NMDA receptors using positron emission tomography (PET) would provide useful information on the integrity of the NMDA system in vivo and could play a key role both in elucidating the involvement of these receptors in neurological diseases and in the follow-up of therapies with already established pharmaceuticals or with drugs under development. Recently, several carbon-11-labelled radioligands ($T_{1/2}$: 20.4 min) have been developed for imaging NMDA receptors at the glycine site, including the high affinity quinolinones [^{11}C]L701,324, [^{11}C]L703,717[17,18] and the methoxy derivative of gavestinel (GV150526A), [^{11}C]3MPICA.[19] [^{11}C]L701,324 and [^{11}C]L703,717 have not been proven, until now, to be clinically useful in vivo. The low brain penetration of these radioligands is a common feature, believed to be due to their excessive acidic character, high lipophilicity and binding to the warfarin binding site of serum albumin (for review, see ref 20). Also, [^{11}C]3MPICA does not appear to be a promising PET radiotracer.[19]

Starting from the prototypic selective NR2B subtype antagonist ifenprodil, Pfizer Laboratories recently described a new series of potent and highly subtype-selective 1 - (heteroarylalkynyl) - 4 - benzylpiperidine antagonists for NMDA receptors.[21,22] In this series, derivative **1**, namely 5-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]-1,3-dihydrobenzoimidazol-2-one, was identified as a selective antagonist for the NR1$_A$/2B subtype, displaying IC_{50} values for inhibition of the NMDA responses of 5.3 nM for this subtype (compared to NR1$_A$/2A: 35 μM and NR1$_A$/2C > 100 μM). This compound is also devoid of acidic function and was active in 6-OHDA lesioned rat[22] at a relatively low dosage (10 mg/kg po). Moreover, due to its chemical structure, labelling with carbon-11 at the benzoimidazolone ring using [^{11}C]phosgene was attractive and based on its preliminary reported pharmacological data, this compound was therefore considered a good candidate for imaging the NMDA receptors (Fig. 1).

The present work represents (a) a concise synthesis of derivative **1**, as the non-radioactive reference and its precursor **4** for radiolabelling; (b) the labelling of **1** with carbon-11 at the benzoimidazolone ring using [^{11}C]phosgene; (c) the first in vivo evaluation of this radioligand in rodents with biodistribution studies and brain radioactivity monitoring with intracerebral radiosensitive β-microprobes.

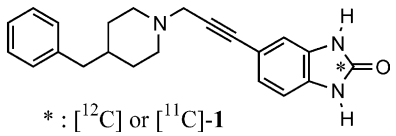


Figure 1. Chemical structure of 5-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]-1,3-dihydrobenzoimidazol-2-one (**1**) and its carbon-11-labelled analogue [^{11}*C*]-**1**.

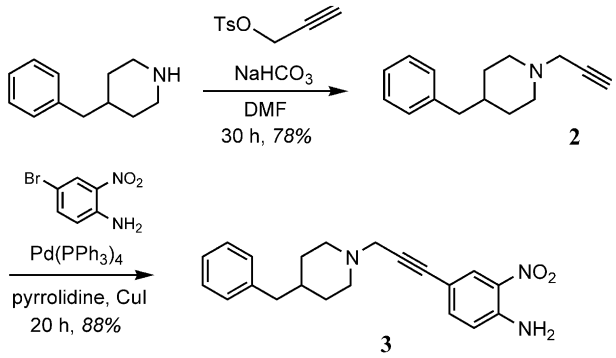
Results and Discussion

Chemistry

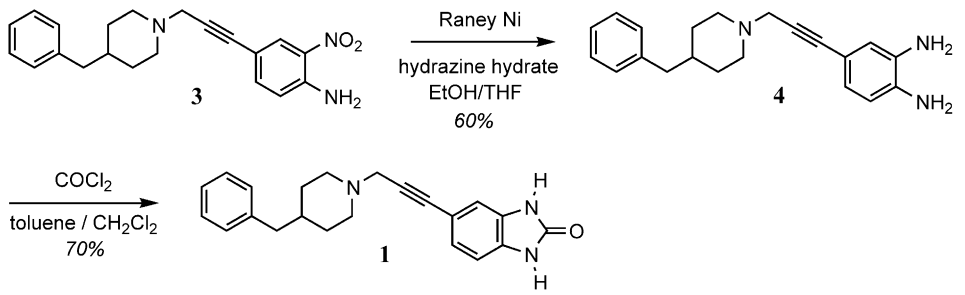
5 - [3 - (4 - Benzylpiperidin - 1 - yl)prop - 1 - ynyl] - 1,3 - dihydrobenzoimidazol-2-one (**1**, as a standard) and 4-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]benzene-1,2-diamine (**4**, as the precursor for labelling with carbon-11) were synthesized in four and three chemical steps in 41 and 29% overall yield, respectively (Schemes 1 and 2). Both compounds were synthesized according to literature procedures.[21,22]

Commercially available 4-benzylpiperidine and propargyl tosylate were first reacted at 80 °C in DMF containing $NaHCO_3$ (argon atmosphere, 30 h) to give the acetylene **2** in 78% yield (Scheme 1).[22] The key step of the preparation of both **1** and **4** is the Sonogashira coupling[23] between commercially available 4-bromo-2-nitroaniline and the acetylene **2**, using $Pd(PPh_3)_4$ as catalyst.[21] Pyrrolidine as base and solvent was more effective than triethylamine in various solvents such as THF and CH_3CN. The use of CuI (10% mol) improved the yield of the reaction. Using our optimized conditions [$Pd(PPh_3)_4$ and CuI in pyrrolidine], the aminonitrophenyl **2** could be obtained in up to 88% yield. Yields were however variable and often several purifications steps were necessary to obtain pure **3**.

Reduction of the nitro function at room temperature (< 25 °C) with Raney–Ni and hydrazine hydrate gave the diaminophenyl derivative **4** in 60% yield (Scheme 2).[24] Noteworthy, if the reaction mixture was slightly heated (40 °C), the acetylene function of **4** was reduced



Scheme 1. Synthesis of 4-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]-2-nitrobenzeneamine (**3**).



Scheme 2. Synthesis of 5-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]-1,3-dihydrobenzoimidazol-2-one (**1**).

at the same time and the corresponding reduced vinyl (*Z*)-enantiomer was obtained as the predominant product. Treatment of **3** with iron powder and HCl in ethanol was not successful[21] and **4** could not be isolated. Cyclisation of the diaminophenyl derivative **4** with phosgene in a mixture of toluene and dichloromethane at room temperature gave rapidly (<15 min) **1** in 70% isolated yield.[25–27] The use of CDI also yielded the expected benzoimidazolone **1** in only 50% when reacted for 3 days.[21]

Radiochemistry

The benzoimidazolone **1** was labelled with carbon-11 ($T_{1/2}$: 20.4 min) using no-carrier-added [^{11}C]phosgene and the corresponding ring-opened diaminophenyl precursor **4** (4-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]benzene-1,2-diamine) (Schemes 3 and 4).

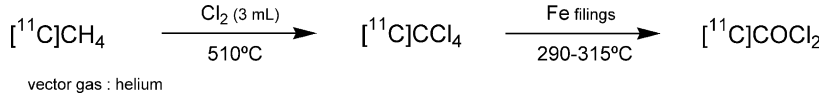
[^{11}C]Phosgene ([^{11}C]$COCl_2$) was synthesized from cyclotron-produced [^{11}C]methane ([^{11}C]CH_4) via [^{11}C]carbon tetrachloride ([^{11}C]CCl_4) using minor modifications of published processes (Scheme 3).[28–31] Briefly, [^{11}C]CH_4 was separated from the target contents, trapped and concentrated on Porapak-Q. [^{11}C]CH_4 was then carried off by a flow of helium gas, mixed with 3 mL of chlorine and the mixture passed through an empty linear horizontal glass tube at a temperature of 510 °C.[30] The on-line synthesized [^{11}C]CCl_4 was continuously swept away by the same helium vector gas and finally passed through a glass U-tube containing iron filings at a temperature of 290–310 °C, without intentional addition of oxygen to the system, giving [^{11}C]$COCl_2$ in 12–13 min radiosynthesis time and an average of 30–50% decay-corrected radiochemical yield, based on starting [^{11}C]CH_4.

[^{11}C]$COCl_2$ was trapped (bubbling through) at room temperature in CH_2Cl_2 (500 μL) containing the labelling precursor **4** (1–2 mg) and 2 μL of triethylamine (Scheme 4). The cyclization reaction of [^{11}C]phosgene was fast (almost instantaneously), but gave [^{11}C]-**1** in low radiochemical yields. Typically, starting from a 1.2 Ci (44.4 GBq) [^{11}C]CH_4 production batch, 15–30 mCi (0.55–1.10 GBq) of [^{11}C]-**1** with a radiochemical and chemical purity of more than 98% were routinely obtained within 30 min of radiosynthesis, including HPLC purification. The total decay-corrected radiochemical yield of [^{11}C]-**1**, based on starting [^{11}C]CH_4, was only 3.5–7.0% ($n=5$). No further efforts were engaged in order to increase the observed low yields. The specific radioactivity measured at the end of the radiosynthesis was 1–2 Ci/μmol (37–74 GBq/μmol).

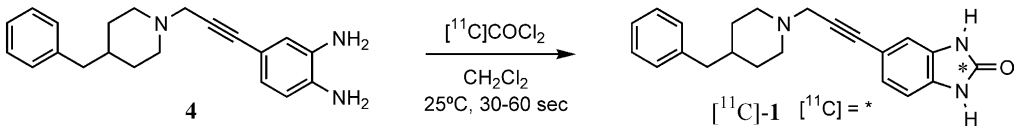
Formulation of labelled product for iv injection was effected as follows: (1) HPLC solvent removal by evaporation; (2) Taking up the residue in 5 mL of physiological saline containing 10% of ethanol; (3) Sterile filtration through a 0.22-μm filter. The solution for injection was a clear and colourless solution and its pH was between 5 and 7. The preparation was found to be >98% chemically and radiochemically pure, as demonstrated by HPLC analysis. The preparation was free from starting labelling precursor and was shown to be chemically and radiochemically stable for at least 120 min. Administration to animals were done within 15 min after end of synthesis.

Pharmacology

Radiosensitive β-microprobe acquisition. Kinetics of the brain uptake of [^{11}C]-**1** was investigated in rats using an intracerebral radiosensitive-β-microprobe device[32,33]



Scheme 3. Preparation of [^{11}C]phosgen from [^{11}C]methane.



Scheme 4. Radiosynthesis of 5-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]-1,3-dihydrobenzoimidazol-2-[^{11}C]one ([^{11}C]-**1**).

(Fig. 2). Following iv injection of [^{11}C]-**1** (0.5–0.7 mCi with specific radioactivity greater than 1 Ci/μmol), the uptake in the cerebellum and in the striata were low: 0.1% I.D./mL in the cerebellum and 0.04% I.D./mL in the striata (Fig. 2). In both structures, the radioactive concentration was maximal 2 min post-injection. The washout phase was very slow and similar in both structures.

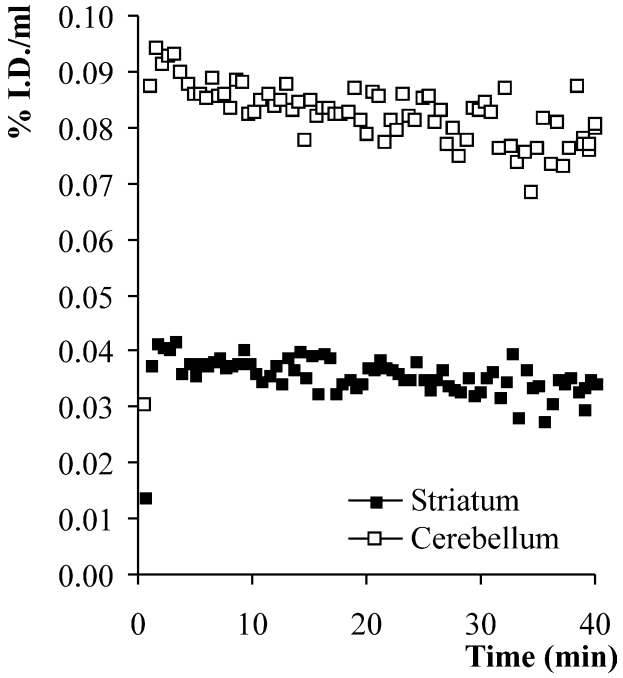


Figure 2. Radiosensitive β-microprobe acquisition. Kinetics of 5-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]-1,3-dihydrobenzoimidazol-2-[^{11}C]one ([^{11}C]-**1**) uptake into striatum and cerebellum (rat).

Biodistribution studies. Kinetics of the rat brain uptake of [^{11}C]-**1** was also determined in biodistribution experiments (Fig. 3A and B). Following the iv injection of [^{11}C]-**1** (22–26 μCi with specific radioactivity greater than 1 Ci/μmol), the brain uptake was extremely low (0.07% I.D./mL on average at 30 min) and rather uniform across the different structures, the cerebellum having the highest uptake and the striata the lowest (Fig. 3A). This was consistent with that observed using the radiosensitive-β-microprobes. The clearance from the blood was also slow, the blood radioactivity being higher than the brain radioactivity until 15 min post-injection. The in vivo brain regional distribution of [^{11}C]-**1** did not match with the autoradiographic or in vitro binding data obtained with other NR2B subtype selective ligands. Using the NR2B selective compound [^{3}H]Ro 25-6981 and autoradiography[34] or using an antibody that recognizes a portion of the C-terminal of the NR2B subunit,[35] a high density of binding sites was detected in the forebrain structures (cortex, hippocampus, striata), moderate levels in the midbrain (thalamus), whereas the cerebellum had low expression level. Furthermore, striata contain NR2B subunits while cerebellum contains NR2A and NR2C.[36] In the peripheral organs, the liver, the lung and the kidneys showed the highest radioactivity (Fig. 3B).

In vivo receptor blocking studies were also performed on three rats by administration of ifenprodil (20 mg/kg, ip) followed 30 min later by iv injection of the radiotracer ([^{11}C]-**1**, 20 μCi in 0.1 mL saline) (Fig. 4). In all brain tissues examined, the uptake of the radiotracer increased compared to control animals ($n=3$). This observation suggests that the binding of [^{11}C]-**1** in peripheral binding sites or to plasma proteins is prevented, resulting in an increased availability for uptake in the brain.

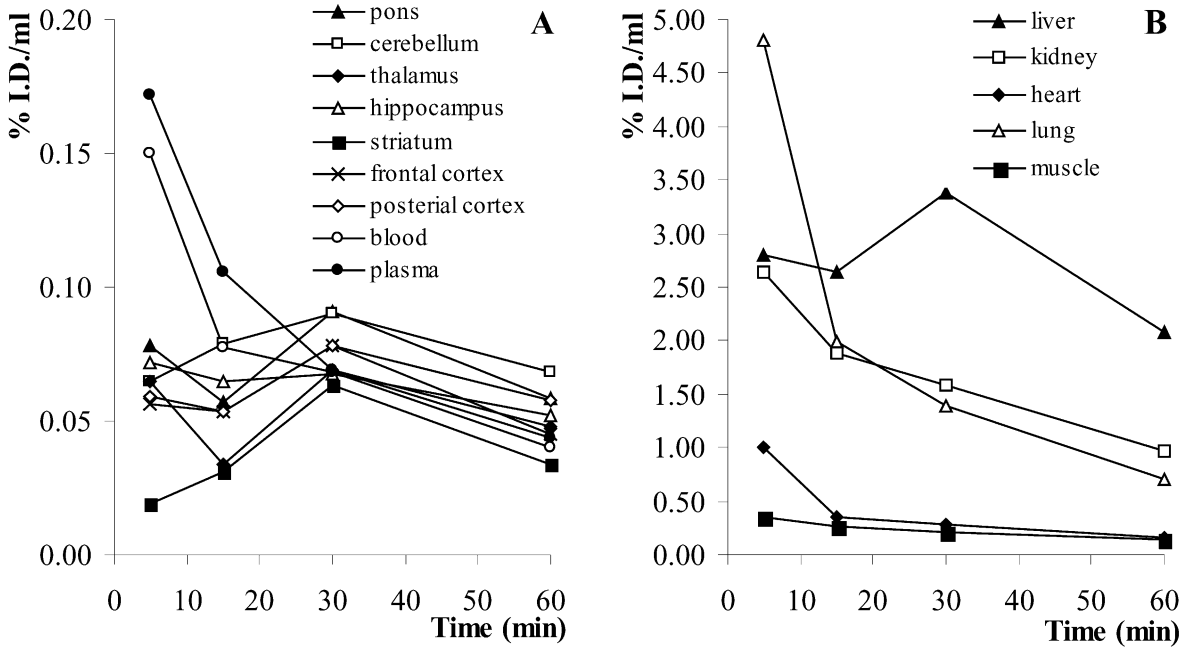


Figure 3. Biodistribution studies in rats: (A) kinetics of 5-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]-1,3-dihydrobenzoimidazol-2-[^{11}C]one ([^{11}C]-**1**) uptake into various brain regions; (B) kinetics of [^{11}C]-**1** uptake in peripheral organs.

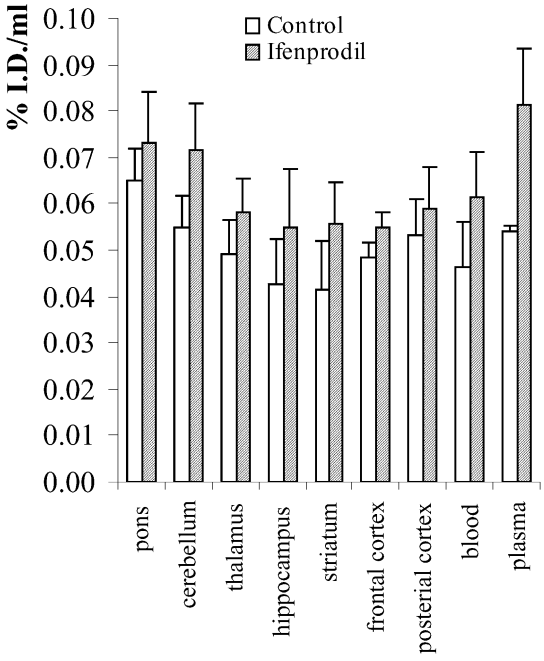


Figure 4. Effect of ifenprodil on 5-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]-1,3-dihydrobenzoimidazol-2-[^{11}C]one ([^{11}C]-**1**) binding in the rat brain. Data are means ± SD ($n = 3$).

Conclusion

The lead compound of a new series of potent and highly $NR1_A/2B$ subtype-selective antagonists of the NMDA receptors, namely 5-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]-1,3-dihydrobenzoimidazol-2-one (**1**), has been synthesized in four chemical steps in good overall yield and labelled with carbon-11 ($T_{1/2}$: 20.4 min) at its benzoimidazolone ring using [^{11}C]phosgene. The evaluation of its in vivo pharmacological profile (biodistribution studies and brain radioactivity monitoring using intracerebral radiosensitive β-microprobes), clearly indicate that the radiotracer does not have the required properties for imaging NMDA receptors using positron emission tomography.

Experimental

General

Chemicals, TLCs and HPLCs. Chemicals were purchased from Aldrich, Fluka or Sigma France and were used without further purification. TLCs were run on pre-coated plates of silica gel $60F_{254}$ (Merck). The compounds were localized (1) when possible at 254 nm using a UV-lamp and/or (2) by dipping the TLC plates in a 1% ethanolic ninhydrin solution and heating on a hot plate. HPLCs (Equipment: Waters and/or Shimadzu systems): (HPLC A): Equipment: system equipped with a Waters 600 Pump and Waters 600 Controller, a Shimadzu SPD10-AVP UV-multi-wavelength detector ; column: semipreparative SiO_2, Merck Lichrosorb® (250 × 10 mm); porosity: 7 μm; eluent CH_2Cl_2/MeOH/NH_3: 95:5:0.5 (v/v/v) ; flow rate: 8 mL/min; temperature: rt; absorbance detection at λ = 254 nm. (HPLC B): 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 (4.6 × 50 mm); porosity: 3.5 μm; conditions: isocratic elution with solvent A/solvent B: 45:55 (v/v) [solvent A: H_2O containing Low-UV PIC® B7 reagent (Waters), 20 mL for 1000 mL; solvent B: H_2O/CH_3CN: 50:50 (v:v) containing Low-UV PIC® B7 reagent (% by weight: methanol (18–22%), heptane sulfonic acid–sodium salts (4–6%), phosphate buffer solution (3–7%), water (65–75%), pH 3, Waters), 20 mL for 1000 mL]; flow rate: 2.0 mL/min; temperature: 30 °C; absorbance detection at λ = 254 nm.

Spectroscopies. NMR spectra were recorded on a Bruker AMX (300 MHz) apparatus using the hydrogenated residue of the deuteriated solvents (CD_2Cl_2, δ = 5.32 ppm; DMSO-d_6, δ = 2.50 ppm) and/or TMS as internal standards for ^{1}H NMR as well as the deuteriated solvents (CD_2Cl_2, δ = 53.8 ppm; DMSO-d_6, δ = 39.5 ppm) and/or TMS as internal standards for ^{13}C NMR. The chemical shifts are reported in ppm, downfield from TMS (s, d, t, dd, b for singlet, doublet, triplet, doublet of doublet and broad, respectively). The mass spectra (MS), DCI/NH_4^+, were measured on a Nermag R10-10 apparatus.

Radioisotope production. No-carrier-added [^{11}C]CH_4 was produced on a CGR-MeV 520 cyclotron by irradiation of a target consisting of an ultrapure Air Liquide 95/5 mixture of N_2/H_2 using a 20-MeV proton beam by the ^{14}N[p,α]^{11}C nuclear reaction. Typical production: 1.20 Ci (44.40 GBq) of [^{11}C]CH_4 at the end of bombardment (EOB) for a 30 μA, 30 min (54,000 μC) irradiation.

Miscellaneous. Radiosyntheses using carbon-11, including the HPLC purifications, were performed in a 5-cm lead shielded cell. Specific radioactivity was determined as follows: the area of the absorbance peak corresponding to the radiolabelled product was measured on the HPLC chromatogram and compared to a standard curve relating mass to absorbance.

Chemistry

4-Benzyl-1-prop-2-ynylpiperidine (2). A mixture of 7.8 mL of 4-benzylpiperidine (44.36 mmol, MW: 175.28, d: 0.997), 10.0 g of propargyl *p*-toluene-sulfonate (47.56 mmol, 1.07 equiv, Mw: 210.25) and 4.7 g of $NaHCO_3$ (55.95 mmol, 1.26 equiv, Mw: 84.01) in 115 mL of DMF was stirred at 80 °C under argon atmosphere for 30 h. The reaction mixture was then concentrated to dryness, dissolved in water (250 mL), extracted with ether (2 × 250 mL), and washed with saturated brine. The solution was dried over $MgSO_4$, filtered, and concentrated to dryness to give a brown oil. This oil was taken up in EtOAc and extracted several times with aq 1 N HCl. The combined acidic layers were then basified by addition of an 1 M aq NaOH solution until pH = 8, and extracted several times with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and evaporated to give 7.44 g of **2** as a pale-brown oil (79%), which was used without further purification.

^{1}H NMR (CD_2Cl_2, 298 K): δ: 7.27 (t, J = 7.5 Hz, 2H); 7.16 (t, J = 7.5 Hz, 3H); 3.22 (s, 2H); 2.85 (dt, J = 11.4 Hz and < 2.0 Hz, 2H); 2.54 (d, J = 6.9 Hz, 2H); 2.27 (t, J = 2.4 Hz, 1H); 2.15 (td, J = 11.4 Hz and < 2 Hz, 2H); 1.64 (d, J = 12.6 Hz, 2H); 1.52 (m, 1H); 1.33 (qd, J = 12.0 Hz and 3.3 Hz, 2H). ^{13}C NMR (CD_2Cl_2, 298 K): δ: 141.2 [C]; 129.4 [2 CH]; 128.4 [2 CH]; 126.0 [CH]; 79.4 [CH]; 73.0 [CH_2]; 52.7 [2 CH_2]; 47.2 [CH_2]; 43.3 [CH_2]; 37.7 [CH]; 32.5 [2 CH_2]. MS (DCI/NH_4^+): $C_{15}H_{19}N$: 214 [M + H$^+$].

4-[3-(4-Benzylpiperidin-1-yl)prop-1-ynyl]-2-nitrobenzeneamine (3). A mixture of 1.8 g of 4-bromo-2-nitroaniline (**7**, 8.29 mmol, Mw: 217.02), 2.0 g of 4-benzyl-1-prop-2-ynylpiperidine (**2**, 9.38 mmol, 1.13 equiv, Mw: 213.32), 500 mg of $Pd(PPh_3)_4$ (0.42 mmol, 5% mol, Mw: 1200) and 160 mg of CuI (0.84 mmol, 10% mol, Mw: 190.45) was stirred in 300 mL of pyrrolidine and deoxygenated by bubbling argon through the solution for 10 min. The mixture was stirred at 50 °C under argon atmosphere for a further 20 h. Then the pyrrolidine was evaporated and the residue purified by chromatography [eluant: CH_2Cl_2/EtOAc: 70:30 (v/v)] to give 1.54 g of **3** as a brown solid (88%, corrected from recovered starting material, about 40%).

^{1}H NMR (CD_2Cl_2, 298 K): δ: 8.16 (s, 1H); 7.36 (dd, J = 8.7 Hz and 2.1 Hz, 1H); 7.26 (t, J = 7.5 Hz, 2H); 7.16 (t, J = 7.5 Hz, 3H); 6.76 (d, J = 8.7 Hz, 1H); 6.23 (b, 2H); 3.42 (s, 2H); 2.89 (d, J = 11.4 Hz, 2H); 2.53 (d, J = 7.0 Hz, 2H); 2.15 (dt, J = 11.0 Hz and < 2.0 Hz, 2H); 1.64 (d, J = 12.0 Hz, 2H); 1.52 (m, 1H); 1.30 (dq, J = 12.0 Hz and 3.0 Hz, 2H). ^{13}C NMR (CD_2Cl_2, 298 K): δ: 144.8 [C]; 141.1 [C]; 138.6 [CH]; 131.6 [C]; 129.4 [3 CH]; 128.4 [2 CH]; 126.0 [CH]; 119.2 [CH]; 111.8 [C]; 84.7 [C]; 83.2 [C]; 53.1 [2 CH_2]; 48.1 [CH_2]; 43.3 [CH_2]; 37.8 [CH]; 32.4 [2 CH_2]. MS (DCI/NH_4^+): $C_{21}H_{23}N_3O_2$: 350 [M + H$^+$].

4-[3-(4-Benzylpiperidin-1-yl)prop-1-ynyl]benzene-1,2-diamine (4). 220 mg of Ni–Raney was filtered, washed with EtOH (not dried) and added to 2 mL of EtOH. This solution was stirred at 65 °C for 15 min. A solution of 220 mg of 4-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]-2-nitrobenzeneamine (**3**, 0.63 mmol, Mw: 349.43) in 3 mL of THF and a solution of 306 μL of hydrazine hydrate (9.82 μmol, 0.016 equiv, Mw: 32.05, d: 1.029) in 3 mL of EtOH were added to the mixture at room temperature. The mixture was stirred until decolourisation of the solution. It was filtered through Celite, washed with EtOH and concentrated to dryness. The residue was taken up in diethylether and activated carbon was added to the solution. The mixture was stirred at room temperature for 15 min, then filtered through Celite and concentrated to dryness to give **4** as a yellow oil. Purification by chromatography [eluant: pure CH_2Cl_2 to CH_2Cl_2/MeOH 50:50 containing 0.5% of aq 28% NH_3 (v/v/v)] gave 107 mg of **2** as a pale yellow solid (60%).

^{1}H NMR (DMSO, 298 K): δ: 7.26 (t, J = 7.5 Hz, 2H); 7.16 (t, J = 7.5 Hz, 3H); 6.55 (s, 1H); 6.43 (q, J = 7.8 Hz, 2H); 4.75 (b, 2H); 4.52 (b, 2H); 3.35 (s, 2H); 2.79 (d, J = 10.8 Hz, 2H); 2.50 (d, J = 6.9 Hz, 2H); 2.06 (t, J = 10.8 Hz, 2H); 1.55 (d, J = 12.0 Hz, 2H); 1.45 (m, 1H); 1.22 (q, J = 11.7 Hz, 2H). ^{13}C NMR (DMSO, 298 K): δ: 140.4 [C]; 135.9 [C]; 134.6 [C]; 129.0 [2 CH]; 128.1 [2 CH]; 125.7 [CH]; 121.2 [CH]; 117.1 [CH]; 117.1 [CH]; 113.9 [CH]; 110.2 [C]; 86.6 [C]; 81.4 [C]; 52.0 [2CH_2]; 47.5 [CH_2]; 42.4 [CH_2]; 38.7 [CH_2]; 31.8 [2CH_2]. MS (DCI/NH_4^+): $C_{21}H_{25}N_3$: 320 [M + H$^+$].

5-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]-1.3-dihydrobenzoimidazol-2-one (1). 67.6 mg of 4-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]benzene-1,2-diamine (**2**, 0.21 mmol, Mw: 319.45) in 2 mL of CH_2Cl_2 and 7 mL of toluene was stirred at room temperature under argon atmosphere. 258 μL of $COCl_2$ (20% in toluene, 0.49 mmol, 2.3 equiv, Mw: 98.92, d: 0.935) was added slowly to the mixture. A pale pink precipitate appeared immediately, which was filtered off. The precipitate was re-dissolved in EtOH and the solution was concentrated to dryness. Purification by chromatography (eluant: CH_2Cl_2/MeOH 98:2 to 50:50 (v:v) gave 44 mg of **1** as a white solid (64%).

^{1}H NMR (DMSO, 298 K): δ: 10.78 (b, 1H); 10.68 (b, 1H); 7.26 (t, J = 7.2 Hz, 2H); 7.16 (t, J = 7.5 Hz, 3H); 7.0 (dd, J = 7.8 Hz and < 2.0 Hz, 1H); 6.89 (t, J = 8.1 Hz, 2H); 3.42 (s, 2H); 2.82 (d, J = 10.8 Hz, 2H); 2.50 (d, J = 6.9 Hz, 2H); 2.10 (t, J = 10.8 Hz, 2H); 1.56 (d, J = 12.3 Hz, 2H); 1.45 (m, 1H); 1.20 (q, J = 11.7 Hz, 2H). ^{13}C NMR (DMSO, 298 K): δ: 155.3 [C]; 140.4 [C]; 129.9 [C]; 129.7 [C]; 129.0 [2 CH]; 128.1 [2 CH]; 125.8 [CH]; 124.5 [CH]; 114.4 [C]; 111.1 [CH]; 108.6 [CH]; 85.4 [C]; 83.5 [C]; 52.0 [2 CH_2]; 47.3 [CH_2]; 42.4 [CH_2]; 37.1 [CH_2]; 31.7 [2 CH_2]. MS (DCI/NH_4^+): $C_{22}H_{23}N_3O$: 346 [M + H$^+$].

Radiochemistry

Preparation of [^{11}C]CCl_4. At the end of the bombardment, the target contents were transferred to the 5-cm lead shielded hot cell dedicated to the radiosynthesis of the tracer and passed firstly through an empty tube (stainless steel coil, 500 mm length, 4 mm internal diameter, cooled at −186 °C using liquid argon) in order to remove traces of ammonia (produced during the irradiation) and secondly through a guard of P_2O_5 (glass tube, 70 mm length, 3 mm internal diameter) in order to remove residual moisture. [^{11}C]CH_4 was then separated from the target gas by trapping in a copper-U-tube (150 mm length, 4 mm internal diameter) filled with Porapak-Q (80–100 mesh, Waters) and cooled at −186 °C (liquid argon). [^{11}C]CH_4 was released from the trap by warming the copper-U-tube to room temperature (hot air) and swept away by a flow of helium gas (40 mL/min). [^{11}C]CH_4 was then passed through a guard of P_2O_5 (glass tube, 70 mm length, 10 mm internal diameter) and concentrated in a second copper U-tube (150 mm length, 2 mm internal diameter) filled with Porapak-Q (80–100 mesh, Waters) and cooled at −186 °C (liquid argon). On average, about 1.20 Ci or 44.40 GBq (at EOB) of [^{11}C]CH_4 is routinely produced for a 30 μA, 30 min (54,000 μC) irradiation and then transferred and concentrated in 4–5 min using the process described above. [^{11}C]CH_4 was released from the

trap by warming the latter to room temperature and swept (15 mL/min) in a volume of 1–2 mL of helium into a gas mixing chamber containing 3 mL of chlorine (99.99%, Air Liquide). Using the same helium as vector gas (15 mL/min), the [^{11}C]CH_4–chlorine mixture was passed through an empty horizontal glass tube (215 mm length, 7 mm internal diameter) at a temperature of 510 °C converting it into [^{11}C]CCl_4.

Preparation of [^{11}C]$COCl_2$. The on-line synthesized [^{11}C]CCl_4 was then passed through a glass U-tube (200 mm length, 4 mm internal diameter) containing 1.5 g of iron filings (Telar 57, Weber) at a temperature of 290–310 °C (using the [^{11}C]CCl_4 vector gas) converting it into [^{11}C]$COCl_2$ which was then passed through an antimony-trap (glass tube, 70 mm length, 3 mm internal diameter) containing a 2/1 ratio (v/v) of antimony (400 mg) and glass beads (1 mm diameter) in order to remove the excess chlorine.

Preparation of 5-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]-1.3-dihydrobenzoimidazol-2-[^{11}C]one ([^{11}C]-1). The on-line synthesized [^{11}C]$COCl_2$ was trapped (bubbling through) at room temperature in a reaction vessel containing 1 to 2 mg of the labelling precursor 4-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]benzene-1,2-diamine (**4**, 3.13–6.26 μmol) dissolved in 500 μL of CH_2Cl_2 containing 2–4 μL NEt_3. Trapping of [^{11}C]$COCl_2$ was monitored using an ionisation-chamber probe. When the reading had reached its maximum (2–3 min usually), the reaction mixture was diluted with 1.0 mL of HPLC solvent and injected onto the column. (HPLC A; Rt: [^{11}C]-**1**: 7.0–7.5 min).

Formulation of 5-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]-1.3-dihydrobenzoimidazol-2-[^{11}C]one ([^{11}C]-1). Formulation of labelled product for iv injection was effected as follows: The HPLC-collected fraction containing [^{11}C]-**1** was concentrated to dryness (using a rotavapor, water bath temperature: 40–60 °C or using a helium gas stream, oil bath temperature: 70–80 °C). The residue was taken up in 2–5 mL of physiological saline containing 10% of ethanol and filtered through a sterile 0.22 μm filter into a sterile, pyrogen free evacuated vial.

Quality control of 5-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]-1.3-dihydrobenzoimidazol-2-[^{11}C]one ([^{11}C]-1). The radiopharmaceutical preparation is a clear and colourless solution and its pH is between 5 and 7. As demonstrated by HPLC analysis (HPLC B), the radiolabelled product was found to be >98% radiochemically pure (HPLC B; retention time: 2.68 min). The preparation was shown to be free of non-radioactive precursor and radiochemically stable for at least 100 min. Specific radioactivity was calculated from three consecutive HPLC analyses 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 to a standard curve relating mass to UV absorbance. The first injection in animal experiments was done within 15 min after the end of synthesis.

Kinetics, regional distribution and radiopharmacological characterization in rodents

Animals. Animals use procedures were in accordance with the recommendations of the EEC (86/609/CEE) and the French National Committee (decret 87/848) for the care and use of laboratory animals. Sprague–Dawley male rats weighing 250–350 g were used in all experiments

Intracerebral radiosensitive β-microprobes acquisition. Anesthesia of animals was induced with 5% isoflurane in a gas mixture of O_2/N_2O (30%/70%) and maintained with 1.5–2.5% isoflurane during the entire surgical procedure. Body temperature was monitored rectally and maintained by means of a thermoregulated blanket. Catheters were placed in both femoral vein and artery. Animals were then mounted in a stereotaxic frame. Craniotomies was performed in order to implant the radiosensitive β-microprobes (Biospace Mesures, Paris, France). Two probes were implanted, the first one in the left striatum and the second one in the cerebellum using the coordinates of implantation according to the atlas of Paxinos and Watson.[37] Data were acquired 30 min after probes implantation. Local radioactivity count rates were acquired for 30 min prior to the radiotracer injection to evaluate environmental background (approximately 5 cps) with a temporal resolution of 1 s. The radiotracer was injected in the femoral vein ([^{11}C]-**1**, 0.8–1.0 mCi) in a volume of 1 mL over 1 min. Data were acquired for another 40–80 min with a temporal resolution of 1 s. Finally, mean background noise was subtracted from the raw data and radioactive decay correction for carbon-11 was applied to obtain quantitative time–activity curves.

Biodistribution studies. Each animal received 22–26 μCi of the radiotracer ([^{11}C]-**1**), dissolved in 0.1 mL saline, by injection in the tail vein. At designated times (15, 30, 60, 120 min) after injection of the radiotracer, animals ($n=3$ per time point) were killed by decapitation, the brains as well as peripheral organs were quickly removed, dissected, weighed and assayed for regional radioactivity in a γ-counter (Cobra Quantum, Packard). Samples of liver, kidney, heart, lung, muscle, blood, plasma and for the brain, pons, cerebellum, diencephalon, hippocampus, striata, frontal cortex, posterior cortex were obtained for each animal. In vivo receptor blocking studies were also performed on three rats by administration of ifenprodil (20 mg/kg, ip), followed 30 min later by iv injection of the radiotracer ([^{11}C]-**1**, 20 μCi in 0.1 mL saline). Animals were sacrified 15 min after injection of the radiotracer and the brain dissected as mentioned above. Results were expressed as% injected dose per mL (% I.D./mL) after correction for the physical decay of the radioisotope.

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