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Synthesis and preclinical evaluation of carbon-11 labelled N-((5-(4-fluoro-2-[^{11}C]methoxyphenyl)pyridin-3-yl)methyl) cyclopentanamine as a PET tracer for NR2B subunit-containing NMDA receptors

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ABSTRACT

Introduction: The N-methyl-D-Aspartate (NMDA) receptor plays an important role in learning and memory. Overactivation is thought to play an important role in neurodegenerative disorders such as Alzheimer's disease. Currently, it is not possible to assess N-methyl-D-aspartate receptor (NMDAr) bio-availability in vivo. The purpose of this study was to develop a positron emission tomography (PET) ligand for the NR2B binding site of the NMDA receptor.

Methods: N-((5-(4-fluoro-2-methoxyphenyl)pyridin-3-yl)methyl)cyclopentanamine was radiolabelled with carbon-11 in the phenyl moiety. Biodistribution and blocking studies were carried out in anaesthetized mice and in non-anaesthetized rats.

Results: N-((5-(4-fluoro-2-[^{11}C]methoxyphenyl)pyridin-3-yl)methyl)cyclopentanamine was prepared in 49 ± 3% (decay-corrected) yield, affording 4.1 ± 0.3 GBq of formulated product at the end of synthesis with a radiochemical purity of >99% and with a specific activity of 78 ± 10 GBq/μmol.

Conclusion: A new NR2B PET ligand was developed in high yield. [^{11}C]4 readily enters the brain and binds to the NR2B subunit-containing NMDAr in the rodent brain. High sigma-1 receptor binding may, however, limit its future application as a PET probe for imaging the NR2B subunit-containing NMDAr. Anaesthesia has an effect on NMDAr function and therefore can complicate interpretation of preclinical in vivo results. In addition, effects of endogenous compounds cannot be excluded. Despite these potential limitations, further studies are warranted to investigate the values of [^{11}C]4 as an NR2B PET ligand.

1. Introduction

The *N*-methyl-D-aspartate receptor (NMDAr) belongs to the class of ionotropic glutamate receptors (iGluRs). These iGluRs are named after their selective agonists, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainic acid and *N*-methyl-D-aspartate (NMDA). The structure and function of the NMDAr have been reviewed extensively over the years [1–3]. NMDAr dysfunction is present in a wide range of neurological and psychiatric diseases [4–7]. In neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and schizophrenia, NMDAr overexpression may be present, which in turn could lead to excitotoxicity [8,9]. In Alzheimer's disease, NMDAr dysfunction can also occur due to amyloid-β peptide (Aβ) oligomers [10]. (See Schemes 1 and 2.)

In the last decade, a major effort in NMDAr drug development has focussed on antagonists for the *N*-methyl-D-aspartate receptor subtype 2B (NR2B) binding site as possible therapeutics in a wide range of CNS pathologies, including acute and chronic pain, stroke, head trauma, drug induced dyskinesias, and dementias [11]. It has been speculated that selective antagonists of the NR2B subtype might provide a cleaner side effect profile compared with antagonists of the glycine binding site or blockers of the ion channel [12].

In vivo quantification of the NMDAr using positron emission tomography (PET) would be a useful tool for assessing both NMDAr availability and its aetiology in these neurodegenerative diseases. Furthermore, *in vivo* imaging of the NMDAr could be an instrument for image guided pharmacotherapy. Recently, the development of PET and SPECT tracers for imaging the NMDAr system has been reviewed [13]. Unfortunately, the majority of radiotracers listed exhibited poor *in vivo* applicability. Common problems were poor brain penetration, non-specific uptake in the brain and extensive metabolism [14–16].

To develop a radiotracer for the NMDAr, that can successfully be applied *in vivo* in man, several factors come into play. First, there are

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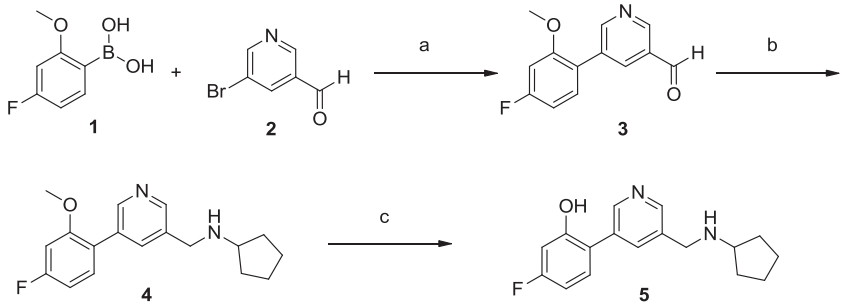


Scheme 1. Synthesis of precursor **5**. a: Cs_2CO_3, $PdCl_2$(dppf)-CH_2Cl_2, 1,2-dimethoxyethane/ethanol/H_2O (7/2/1/v/v/v), 12 h, 80 °C; b: cyclopentanamine, sodium triacetoxyborohydride, dichloromethane (DCM), 16 h, 0 °C to room temperature; c: boron tribromide, DCM, 16 h, −78 °C to room temperature (RT).

the usual requirements for radiosynthesis and the radioligand itself, which needs to have suitable properties for uptake in the brain, receptor binding kinetics and metabolic stability. Second, there is the complex system of NMDAr activation, for which several endogenous ligands (e.g. glutamate, glycine, serine, agents acting at the spermidine site) play a role in regulating channel opening. Exogenous ligands can bind to several sites at the NMDAr and their binding may be dependent on the activation of the receptor by endogenous ligands. Finally, *in vivo* testing of radiotracers in laboratory animals requires anaesthesia, which might interfere with NMDAr functioning.

At present, there is no well validated NMDAr NR2B PET radioligand available. Recently, a new class of NR2B antagonists with a wide range of potential 2,6-disubstituted aromatic and heteroaromatic compounds was described, which may be useful for treating depression [17]. This class of NR2B antagonists offers a good opportunity to develop a radiotracer for investigating the NMDAr complex.

The aim of the present study was to synthesise N-((5-(4-fluoro-2-[^{11}C]methoxyphenyl)pyridin-3-yl)methyl)cyclopentanamine and evaluate its potential as an NMDAr-NR2B selective PET ligand.

2. Materials and methods

2.1. Materials

Chemicals were obtained from Sigma Aldrich (Zwijndrecht, the Netherlands) or Acros Organics (Geel, Belgium) and were used without further purification. Ro25-6981 maleate was purchased from TEBU-Bio (Heerhuogwaard, the Netherlands). Ifenprodil tartrate and GBR12909 dihydrochloride were purchased from Sigma Aldrich (Zwijndrecht, the Netherlands). [^{3}H]Ifenprodil was purchased from Perkin Elmer (Groningen, the Netherlands). High-performance liquid chromatography (HPLC) solvents (HPLC grade) were purchased from J.T. Baker (Valkenswaard, the Netherlands).

All reactions were carried out under argon atmosphere, unless stated otherwise. [^{11}C]CO_2 was produced using an IBA Cyclone 18/9 cyclotron (Louvain-La-Neuve, Belgium) and [^{11}C]methyl iodide was produced as described previously [18]. Radiosyntheses were performed using home-made synthesis modules [19].

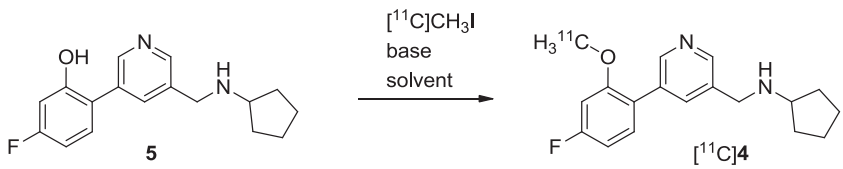
Nuclear magnetic resonance (NMR) spectra were obtained using a Bruker AC 250 or 500 (Billerica, USA). Chemical shifts (δ) were defined relative to the signal of the solvent (7.27 for CDCl3, 2.50 for DMSO-d6). High resolution mass spectrometry (HRMS) was performed using a Bruker MicroTOFQ with ESI (electrospray ionisation) in a positive mode (Billerica, USA). Samples were injected (10 μL) in a liquid flow of methanol/water (1/1) at a flow of 100 μL · min^{-1}.

The preparative high-performance liquid chromatography (HPLC) system consisted of a Jasco PU-2089 HPLC pump (Jasco Benelux, de Meern, the Netherlands), a Rheodyne injector with a 20 μl loop (Thermo Fischer Scientific, Breda, the Netherlands), a Jasco UV-2075 Plus UV detector set at a wavelength of 254 nm (Jasco Benelux, de Meern, the Netherlands) and a Raytest Na(I) radioactivity detector (Raytest, Straubenhardt, Germany). The HPLC data were acquired and integrated using the software package ChromNAV 1.14 (Jasco Benelux, de Meern, the Netherlands). A Reprosphere 100 C18-DE 5 μm, 50 × 8 mm column (Dr Maisch GmbH, Ammerbuch-Entringen, Germany) at a wavelength of 254 nm and an eluent of acetonitrile/water/N,N-diisopropylethylamine 40/60/0.05 (v/v/v) was used with a flow of 3 mL · min^{-1} (method A).

The analytical HPLC system was the same as the preparative HPLC system. The HPLC data were acquired and integrated using the software package GINA 5.01 (Raytest, Straubenhardt, Germany). A GraceSmart RP18 5u, 250 × 4.6 mm (Grace Alltech, Breda, the Netherlands) column at a wavelength of 254 nm with an eluent containing acetonitrile/water/trifluoroacetic acid 15/85/0.15 (v/v/v) and a flow of 1 mL · min^{-1} was used (method B).

For metabolite analysis, a Phenomenex Gemini C18, 10 * 250 mm, 5 μm (Phenomenex, Utrecht, the Netherlands) column was used. The mobile phase was A = acetonitrile B = H_2O/0.1% ammonium acetate in a gradient system from 70% B to 10% B in 9 min at a flow of 3 mL · min^{-1} (method C). The HPLC system consisted of Dionex Ulitmate 3000 HPLC system (Dionex, Breda, the Netherlands), equipped with a Rheodyne injector with a 1 mL loop (Thermo Fischer Scientific, Breda, the Netherlands) and a Raytest Na(I) radioactivity detector (Raytest, Straubenhardt, Germany). The HPLC system was controlled using the software package Chromeleon 6.80 (Dionex, Breda, the Netherlands). Fractions of 30 s were collected and counted using a Perking Elmer 2480 Singlewell gammacounter (Groningen, the Netherlands). Chromatograms were generated using Microsoft Excel version 2007.

For *in vivo* studies, Wistar rats were obtained from Harlan (the Netherlands) and housed in groups of four to six per cage until treatment. B6C3F1/J mice were obtained from the Jackson Laboratory (USA) and were housed in groups of four to six per cage until treatment. All animals were kept at a constant temperature of 21 °C



Scheme 2. Synthesis of [^{11}C]**4**. Conditions listed in Table 2.