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Synthesis, radiolabeling and preclinical evaluation of a [^{11}C]GMOM derivative as PET radiotracer for the ion channel of the N-methyl-D-aspartate receptor

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ABSTRACT

Introduction: Presently available PET ligands for the NMDAr ion channel generally suffer from fast metabolism. The purpose of this study was to develop a metabolically more stable ligand for the NMDAr ion channel, taking [^{11}C]GMOM ([^{11}C]**1**) as the lead compound.
Methods: [^{11}C]**1**, its fluoralkyl analogue [^{18}F]PK209 ([^{18}F]**2**) and the newly synthesized fluorovinyloxy analogue [^{11}C]**7b** were evaluated ex vivo in male Wistar rats for metabolic stability. In addition, [^{11}C]**7b** was subjected to a biodistribution study and its affinity (K_i) and lipophilicity ($logD_{7.4}$) values were determined.
Results: The addition of a vinyl chain in the fluoromethoxy moiety did not negatively alter the affinity of [^{11}C]**7b** for the NMDAr, while lipophilicity was increased. Biodistribution studies showed higher uptake of [^{11}C]**7b** in forebrain regions compared with cerebellum. Pre-treatment with MK-801 decreased the overall brain uptake significantly, but not in a region-specific manner. 45 min after injection 78, 90 and 87% of activity in the brain was due to parent compound for [^{11}C]**1**, [^{18}F]**2** and [^{11}C]**7b**, respectively. In plasma, 26–31% of activity was due to parent compound.
Conclusion: Complete substitution of the alpha-carbon increased lipophilicity to more favorable values. Substitution of one or more hydrogens of the alpha-carbon atom in the methoxy moiety improved metabolic stability. In plasma, more parent compound was found for [^{18}F]**2** and [^{11}C]**7b** then for [^{11}C]**1**, although differences were not significant. At 45 min, significantly more parent [^{18}F]**2** and [^{11}C]**7b** was measured in the brain compared with [^{11}C]**1**.

1. Introduction

The N-methyl-D-aspartate receptor (NMDAr) is beside the AMPA and kainate receptors one of the three members of the ionotropic glutamate receptor family, named after their corresponding agonists. The NMDArs are formed as tetraheteromers, and contain an obligatory glycine-binding NR1 subunit, in complex with glutamate-binding NR2A-D, and occasionally NR3A-B subunits. These subunits together form a ligand-gated ion channel, within which a magnesium binding site exists. At resting state, the ion channel is blocked by Mg^{2+}, which inhibits ion flow (Na^+, K^+ and Ca^{2+}) through the channel. This Mg^{2+} block can be removed by depolarization of the plasma membrane, a physiological process underlying the important role of NMDArs in synaptic plasticity, learning and memory. Deregulation of NMDArs is involved in several neurological and neuropsychiatric disorders [1–3].

Phenylcyclidine (PCP), thienylcyclohexyl piperidine (TCP), ketamine, memantine and MK-801 are compounds known to bind within the ion-channel site of NMDArs and block the channel pore. These compounds were initially synthesized for therapeutic purposes, and later radiolabeled to develop PET or SPECT radiotracers for the NMDArs [4–7].

Another class of interesting compounds that interacts at the ion channel binding site are the *N*,*N*′-diarylguanidines. To date, several *N*-(2,5-disubstited phenyl)-*N*′-(3-substituted phenyl)-*N*′-methylguanidines, such as [^{11}C]GMOM, [^{11}C]CNS-5161, [^{18}F]GE-179 and [^{18}F]PK209 (See Fig. 1.), have been reported, all with promising characteristics, such as high affinity, moderate lipophilicity, and selectivity for the NMDA ion channel over the sigma receptor.

[^{11}C]GMOM, ([^{11}C]**1**, K_i: 5.2–21.7 nM vs. [^{3}H]MK-801; log P: 2.34; $logD_{7.4}$: 1.72) [8,18] demonstrated regional brain uptake in awake rats ranging from 0.75 ± 0.13% injected dose per gram (ID/g) in the medulla and pons to 1.15 ± 0.17% ID/g in the occipital cortex. Pre-treatment with MK-801 (1 mg·kg^{-1}) significantly reduced [^{11}C]**1** uptake in all regions (24–28%), while D-serine, a glycine site co-agonist, increased uptake in

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all regions (10–24%). Administering the NR2B subunit antagonist RO 25–6981 reduced uptake of [^{11}C]**1** by 24–38% over control. In isoflurane anesthetized baboons a fairly uniform distribution volume across the brain was observed. Pre-treatment with MK-801 (0.5 and 1.0 mg·kg^{-1}) did not alter regional distribution volumes, indicating a lack of saturable binding. During scans the amount of [^{11}C]**1** in plasma was, on average, 18.6 ± 5.9%, and only polar metabolites were observed [8,9].

In human, highest uptake of [^{11}C]GMOM was observed in NMDAr rich regions, such as hippocampus and thalamus. Lowest values were found in temporal cortex and cerebellum. [^{11}C]GMOM showed rapid metabolism, after 20 min. 50% of the radioactivity in the plasma was due to parent compound. After administrating S-ketamine, a significant reduction in uptake was observed for the entire brain suggesting specific binding of [^{11}C]GMOM to NMDAr. [^{11}C]GMOM could be used for imaging and quantifying the NMDAr [10].

[^{11}C]CNS-5161, (K_i: 1.87 nM vs. [^{3}H]MK-801) a thiomethyl analogue of GMOM, with a log$D_{7.4}$ of 2.68, demonstrated uptake in human brain tissues with the lowest uptake in cerebellum and the highest in putamen and thalamus. This compound, however, suffered from fast metabolism and specific uptake could not be demonstrated [11–15].

In humans, [^{18}F]GE-179 (K_i: 2.35 nM vs. [^{3}H]TCP), a fluoroethyl analogue of CNS-5161 with a log$D_{7.4}$ of 2.49, showed rapid plasma metabolism. After 16 min 50% of the radioactivity in the plasma could be attributed to radiolabeled metabolites. Nevertheless, the rate of metabolism was slower than for [^{11}C]CNS-5161. Uptake in gray matter was relatively homogeneous [16,17].

Recently, [^{18}F]PK209 ([^{18}F]**2**, K_i: 18 nM vs., [^{3}H]MK-801), a fluoromethoxy analogue of GMOM with a log$D_{7.4}$ of 1.45 was reported. Preclinical evaluation in rhesus monkeys showed retention in NMDAr rich cortical regions relative to cerebellum. In two out of three subjects, a reduction in signal relative to baseline was seen after MK-801 pretreatment. However, [^{18}F]**2** suffered from fast metabolism, as 10 min post injection only 30 ± 6% of the radioactivity in the plasma was due to parent compound [18,19].

All compounds described above consist of the same guanidine core structure, and show fast peripheral metabolism. Structural alterations exist in one or more short alkyl chains. It is known that the P450 cytochrome system is responsible for *O*-, *N*- and *S*-demethylation and/or oxidation [20]. A common procedure to prevent metabolism of short alkyl chains is the replacement of hydrogen by deuterium or fluoride [21]. The CH_2F group can be used as a bio-isostere of a methyl group, preventing metabolic oxidation by its electron withdrawing effect [22].

Since [^{18}F]**2** next to [^{11}C]**1** still showed relatively fast metabolism in rhesus monkeys, an obvious choice was to introduce deuterium or more fluorine atoms in the methoxy moiety. From previous SAR studies it was known that there is some spatial freedom for alterating the methoxy moiety without losing affinity toward the NMDAr. The methoxy moiety can be altered with two or three fluorine atoms, but the resulting molecules showed less promising characteristics for use as a PET tracer [18]. A vinylic moiety could be an alternative way to prevent metabolic oxidation of the methoxy moiety of [^{18}F]**2**.

The aim of the present study was to develop an analogue of [^{11}C]GMOM with improved metabolic stability and to evaluate its potential as an NMDAr ion channel ligand.

2. Materials and methods

2.1. General

Nuclear magnetic resonance (NMR) spectra (^{1}H, ^{13}C, ^{19}F NMR) were recorded on a Bruker Avance 250 (250.13, 62.90, 235.36 MHz respectively) (Billerica, USA). Chemical shifts of the NMR spectra are reported in parts per million (ppm) relative to the solvent residual peak. Description of signals: s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, ddd = doublet of dd, dt = doublet of triplets, tt = triplet of triplets, dq = double quartet. Thin-layer chromatography (TLC) was performed on Merck DC-alufolien, silica gel 60, F254. Flash column chromatography was performed on silica gel 60 Å, 230–400 Mesh. All chemicals were used without further purification, unless stated otherwise. The high-performance liquid chromatography (HPLC) analysis 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 and a Raytest Na(I) radioactivity detector (Raytest, Straubenhardt, Germany). HPLC data were collected and integrated with the software package GINA 5.01. For high-resolution mass spectrometry (HRMS), a Bruker MicroTOFQ with ESI (electrospray ionization) in a positive mode (Billerica, USA) was used. Samples were injected (10 μL) in a liquid flow of methanol/water (1/1) at a rate of 100 μL·min^{-1}. [^{11}C]CO_2 was produced by the ^{14}N(p,α)^{11}C nuclear reaction with an IBA Cyclone 18/9 cyclotron (Louvain-La-Neuve, Belgium). Radioactivity was measured with a Veenstra VDC-405 dose calibrator (Comecer, Joure, the Netherlands). Radiochemistry was carried out in homemade, remotely controlled devices [23].

2.2. Chemistry

2.2.1. *N-(3-hydroxyphenyl)cyanamide (**5a**) and N-(3-hydroxyphenyl)-N-methylcyanamide (**5b**)*

N-(3-hydroxyphenyl)cyanamide (**5a**) and *N*-(3-hydroxyphenyl)-*N*-methylcyanamide (**5b**) were prepared according to published procedures [18].

2.2.2. *1-(2-chloro-5-(methylthio)phenyl)-3-(3-hydroxyphenyl)guanidine (**6a**)*

A screw cap reaction vessel was charged with *N*-(3-hydroxyphenyl)cyanamide (**5a**) (0.68 g, 5.08 mmol), 2-chloro-5-(methylthio)aniline hydrochloride (1.16 g, 5.51 mmol) and 200 μL chlorobenzene. Next, this vessel was flushed with nitrogen, closed and stirred at 165 °C for 12 h. The reaction mixture was cooled down to 20 °C and dissolved in ethyl acetate (25 mL) and washed with 0.1 M HCl (2 × 25 mL) followed by water (25 mL). The pH of the combined aqueous layers was adjusted with potassium carbonate to pH ≥ 10 and extracted with ethyl acetate (2 × 25 mL). The organic layers were collected and dried over anhydrous $MgSO_4$, filtrated and evaporated to dryness under reduced pressure. The residue was purified by column chromatography using ethyl acetate/triethylamine (99:1, *v*/v) to obtain the title compound as a white solid (1.16 g, 3.77 mmol, 74%). R_f 0.13 (ethyl acetate/triethylamine 99:1 *v*/v). ^{1}H NMR ($CDCl_3$) δ 7.23 (d, J = 8.40 Hz, 1H, H_{Aryl}), 7.05 (t, J = 8.03 Hz, 1H, H_{Aryl}), 6.96 (d, J = 2.21 Hz, 1H, H_{Aryl}), 6.84 (dd, J = 8.43 Hz, J = 2.25 Hz, 1H, H_{Aryl}), 6.74–6.72 (m, 1H, H_{Aryl}), 6.61–6.57 (m, 2H, H_{Aryl}), 5.96 (bs, 4H, 3 x NH, OH), 2.38 (s, 3H, SCH_3); ^{13}C NMR ($CDCl_3$) δ 157.76 (Ar-NH), 150.74 (NCN), 143.98 (Ar-O), 140.83 (Ar-NH), 137.93 (Ar-S), 130.02 (Ar), 129.99 (Ar), 124.60 (Ar-Cl), 122.55 (Ar), 121.87 (Ar), 113.67 (Ar), 111.43 (Ar), 109.69 (Ar), 15.55 (CH_3).

2.2.3. *3-(2-chloro-5-(methylthio)phenyl)-1-(3-hydroxyphenyl)-1-methylguanidine (**6b**)*

3-(2-chloro-5-(methylthio)phenyl)-1-(3-hydroxyphenyl)-1-methylguanidine (**6b**) was prepared according to a published procedure [18].

2.2.4. *3-(2-chloro-5-(methylthio)phenyl)-1-(3-(1-fluorovinyloxy)phenyl)-guanidine (**7a**)*

A screw cap reaction vessel containing a solution of sodium hydride (60%) (53 mg, 1.33 mmol) in 1,2-dimethoxyethane (2 mL) and *N*,*N*-dimethylformamide (0.15 mL) was stirred at 0 °C. 1-(2-chloro-5-(methylthio)phenyl)-3-(3-hydroxyphenyl)guanidine (**6a**) (194 mg, 0.63 mmol) and sodium iodide (19 mg, 0.13 mmol) was added. After 30 min 1-bromo-1-fluoroethene (150 μL, 2.00 mmol) dissolved in 1,2-