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Research Article

Synthesis of [^{11}C] *N*-(2-chloro-5-thiomethylphenyl)-*N*′-(3-methoxyphenyl)-*N*′-methylguanidine ([^{11}C]GMOM): a candidate PET tracer for imaging the PCP site of the NMDA ion channel

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Summary

The *N*-methyl-D-aspartate (NMDA) ion channel plays an important role in a number of neurodegenerative disorders including stroke, Parkinson's disease, Huntington's Chorea, Alzheimer's disease, schizophrenia and epilepsy. To provide effective radioligands for imaging the PCP binding site of the NMDA ion channel, we synthesized and characterized *in vitro* the candidate PCP site ligand *N*-(2-chloro-5-thiomethylphenyl)-*N*′-(3-methoxyphenyl)-*N*′-methylguanidine (GMOM: $K_i = 5.2 \pm 0.3$ nM, $\log P = 2.34$). The corresponding PET radiotracer [^{11}C]GMOM was synthesized with a radiochemical yield of $8.4 \pm 3.2\%$ EOS and with a specific activity of 1.23 ± 0.25 Ci/μmol EOS ($n = 5$). The average time required for synthesis, purification and formulation was 52 ± 5 min. The final product was prepared in a sterile saline solution suitable for *in vivo* use. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: NMDA; glutamate; phencyclidine; PET; radiotracer

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Introduction

The *N*-methyl-D-aspartate (NMDA) ion channel, a major site of action for glutamate, is known to play a central role in many biological functions including neuroprotection, neurodegeneration, long-term potentiation, memory, and cognition.[1–5] Alterations in normal NMDA channel composition, densities and function have been implicated in the pathophysiology of certain neurological and neuropsychiatric disorders such as Parkinson's disease, Huntington's Chorea, schizophrenia, alcoholism and stroke.[6–12] This ion channel is complex in respect to both its structure and function and is comprised of at least one NR-1 subunit along with one or more NR-2 subunits (NR-2A, -2B, -2C, or -2D).[13–15] In addition to the site of binding for the agonist L-glutamate, receptors for the known co-agonist glycine also exist. Several NMDA receptor modulatory sites have also been well characterized, including those for polyamines, Zn^{2-}, Mg^{2+} and phencyclidine (PCP). It has been established that the specific subunit composition of a particular NMDA ion channel, and the nature of modulation by NMDA receptor ligands, dictate subsequent biological responses.

Non-invasive methods for imaging the PCP site *in vivo* would allow for quantification of these sites in living subjects and, in addition, would make possible drug occupancy studies to evaluate new NMDA receptor targeted drugs that might be tested clinically as neuroprotective or anti-stroke agents. In addition, it is known that the *in vitro* and *in vivo* binding of [^{3}H]MK801 is affected by the state of activation of NMDA receptors.[16–18] Agonists and antagonists at the glutamate or glycine sites increase and decrease, respectively, [^{3}H]MK801 binding to the PCP site which is located inside the ion channel. Therefore, *in vivo* binding of a PET PCP site ligand might report on the state of activation of NMDA receptors *in vivo*.

To date, several candidate PCP site radioligands have been reported. Examples include [^{11}C]ketamine,[19,20] ^{123}I, and ^{125}I-labeled dizocilpine (MK 801),[21–23] ^{123}I, ^{18}F and ^{11}C-labeled derivatives of phencyclidine (PCP), such as [^{18}F]fluorothienylcyclohexylpiperidine ([^{18}F]FETCP),[24] and adamantane derivatives including [^{18}F]1-amino-3-fluoromethyl-5-methyl-adamantane ([^{18}F]AFA).[25] Although many of these compounds show promise *in vitro* for labeling the PCP site, all suffer *in vivo* from either fast metabolism and subsequent fast brain clearance, as is the case for [^{11}C]ketamine, or a lack of demonstrable specific binding. A low target-to-background ratio is possibly a result of non-selectivity, as with

[^{18}F]AFA and other ligands that also binds sigma receptors, or high lipophilicity as exemplified by [^{123}I]MK801. We have chosen as a starting point a group of selective non-competitive antagonists consisting of *N*-(2,5-disubstitutedphenyl)-*N′*-(3-substitutedphenyl)-*N′*-methylguanidines.[26,27] Compounds of this series comprise some of the highest affinity PCP site ligands reported to date, and these also exhibit specificity for the PCP site vs sigma receptors. Herein, we report the synthesis, *in vitro* characterization, lipophilicity estimation and radiolabeling of the first novel PET tracer of this series, [^{11}C]*N*-(2-chloro-5-thiomethylphenyl)-*N′*-(3-methoxyphenyl)-*N′*-methylguanidine ([^{11}C]GMOM).

Materials and methods

General

Proton NMR spectra were recorded on a Brucker 400 MHz FT-NMR spectrometer (Department of Chemistry, Columbia University). Chemical shifts were recorded in ppm (δ) from an internal tetramethylsilane standard in either chloroform-d_3 or methylsulfoxide-d_6 and coupling constants (*J*) are reported in Hz. Chromatographic purification of unlabeled compounds was performed using silica gel (Aldrich, 70–230 mesh, ASTM) using the solvent systems indicated in the text. For mixed solvent systems, the ratios are given with respect to volumes. All reagents and solvents were purchased from commercial sources and were used without further purification. The 3-substituted anilines purchased from Aldrich Chemical Company (Milwaukee Wisconsin). [^{11}C]Methyl iodide is synthesized routinely at Columbia University Radioligand Laboratory. This radioactive synthon is prepared by the lithium aluminum hydride reduction of [^{11}C]carbon dioxide to form [^{11}C]methanol, followed by treatment of the [^{11}C]methanol with hydrogen iodide and distillation of newly formed [^{11}C]methyl iodide. HPLC analysis of the target radioligand [^{11}C]GMOM was performed using a Waters 515 HPLC pump, a Waters PDA UV detector, and a Bicron Flow-Scint radiation detector. The column used was a reverse-phase base-deactivated column (Phenomenex, ODS, analytical: 4×250 mm, 5 μm particle size; semi-preparative: 10×250 mm, 10 μm particle size) and the mobile phases and flow rates used are indicated in the text below.

Synthesis

The synthesis of *N*-(2-chloro-5-thiomethylphenyl)-*N′*-(3-ethylphenyl)-*N′*-methylguanidine (**1**), *N*-(2-chloro-5-thiomethylphenyl)-*N′*-(3-thiomethylphenyl)-*N′*-methylguanidine were synthesized according to established methods (**2**). The novel compounds **7** and **8** were prepared using the general method of Hu et al. with modifications as required.[26]

The *N*-methylated 3-substituted cyanamides were prepared using the general methods described in Hu *et al*. and the crude products obtained were subjected to column chromatography (hexanes/ethyl acetate 2:1 v/v) to provide the desired purified compounds.

N-methyl-3-hydroxyphenylcyanamide: mp = 92–94°C; ^{1}H-NMR: 7.22 (t, 1H, Ar), 6.66 (t, 1H, Ar), 6.59 (m, 2H, Ar), 3.32 (s, 3H, *N*-C**H**$_3$).

N-methyl-3-methoxyphenylcyanamide: oil; ^{1}H-NMR: 7.28 (t, 1H, Ar), 6.62–6.71 (t, 3H, Ar), 3.83 (s, 3H, *O*-CH_3), 3.33 (s, 3H, *N*-CH_3).

Synthesis of **7** *and* **8** *(GMOM)*

A mixture of the appropriate *N*-methyl-3-substituted phenylcyanamide and 2-chloro-5-thiomethylaniline hydrochloride (1.1 equivalents) in chlorobenzene was heated at 140°C under a nitrogen atmosphere for 4 h. The mixture was diluted with aqueous HCl solution (0.1 M) and the product was extracted into dichloromethane. The organic layer was separated, dried over sodium sulfate, and the solvent was evaporated under reduced pressure. Column chromatography (silica; dichloromethane/methanol 95:5 v/v) provided the desired substituted guanidines in good yield (40–68%).

N-(2-chloro-5-thiomethylphenyl)-*N′*-(3-hydroxyphenyl)-*N′*-methylguanidine, **7**: mp = 160–164°C; ^{1}H–NMR: 9.72 (br s, 1H) 8.33 (br s, 2H), 6.65–7.20 (m, 7H, Ar), 3.65 (s, 3H, *N*-CH_3), 2.42 (s, 3H, *S*-CH_3); MS *m/z*: 322.

N-(2-chloro-5-thiomethylphenyl)-*N′*-(3-methoxyphenyl)-*N′*-methylguanidine (GMOM) **8**: mp = 149–151°C; ^{1}H-NMR: 9.80 (br s, 1H) 8.4 (br s, 2H), 6.85–7.30 (7H), 3.82 (s, 3H, *O*-CH_3), 3.65 (s, 3H, *N*-CH_3), 2.45 (s, 3H, *S*-CH_3); MS *m/z* found (and theory), 336.0953 (336.0937).

Ligand binding assay

Compounds **1**, **2** and **8** were evaluated for binding to the PCP site through receptor binding assays using established methods.[16,8] Male

Sprague–Dawley rats were decapitated under CO_2 anesthesia, and the frontal cortex was removed and homogenized with a PowerGen 125 tissue homogenizer (Fisher Scientific; 15 s, half-maximum speed) in a 30-fold (w/v) excess of ice-cold Tris–HCl buffer (50 nM, pH = 7.4). The resulting homogenate was centrifuged at 10000*g* for 10 min at 4°C using an Allegra 64R ultracentrifuge (Beckman Coulter). The pellet was collected and resuspended in 30 volumes (w/v) of the same buffer, and was centrifuged a second time. The process was repeated once more. The final pellet was resuspended in ice-cold Tris–HCl buffer (50 mm, pH = 7.4) containing glutamate (10 mM) and glycine (10 mM) to provide a final concentration of 30 mg membrane per 1 ml solution.

[^{3}H]MK801 (10 mM) was incubated for 1.5 h in a total volume of 1.0 ml Tris acetate buffer (pH = 7.4, 22°C) including glutamate (10 mM) and glycine (10 mM) in the presence of rat cortical membranes (3 mg/tube). The effect of different concentrations of test ligand (10^{-12}–10^{-4} M) GMOM was examined, and all assays were performed in triplicate. Experiments were terminated by the addition of 3 ml ice-cold buffer, and the unbound tracer was removed by washing twice more (approx. 4 ml/wash) cold buffer using a 48 well cell harvester (Brandel). The membranes were incubated in 4 ml scintillation fluid (Packard Ultrascint) and the radioactivity measured with a Tricarb 1500 scintillation counter (Packard).

Lipophilicity estimations

The lipophilicity of **1**, **2** and **8** was examined by determination of the log *P* value using a HPLC method previously described.[28] Briefly, samples were analyzed using an analytical C18 column (Phenominex Protegy 10 μm, 4.6 × 250 mm) and a mobile phase of methanol and phosphate buffer (85:15 v/v, pH = 7.5) at 1.0 ml/min. Lipophilicity was determined by comparison of the retention time of the compound to that of standards having known log *P* values. Relative retention times (RRT) were calculated, and a calibration curve of log *P* vs log RRT was generated. The calibration equations were polynomial with r^2 of 0.986. All standard and sample injections were done in triplicate and the results averaged to provide the final values.

Radiolabeling

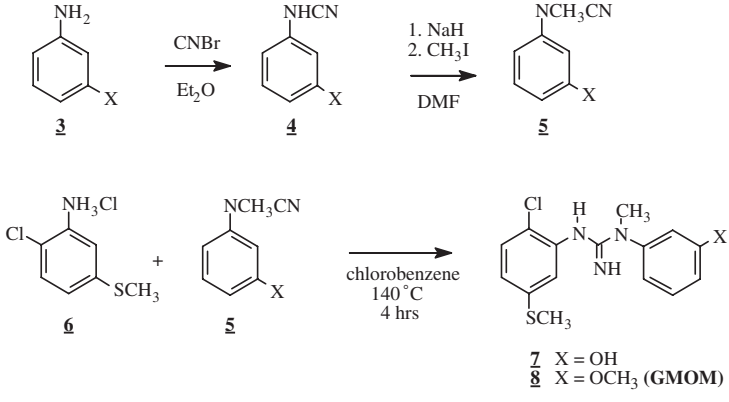
[^{11}C]*N*-(2-chloro-5-thiomethylphenyl)-*N*′-(3-methoxyphenyl)-*N*′-methyl-guanidine, [^{11}C]GMOM. Into a 1 ml reaction vial was added 1 mg **7**,

5 μL aqueous sodium hydroxide (5 N) and dimethylformamide (400 μL). The vessel was sealed and [^{11}C]methyl iodide was transferred into the solution via a stream of argon. The vessel radioactivity was monitored, and once maximized, the mixture was heated at 70°C for 5 min. The crude product was diluted with 1 ml mobile phase, and then injected onto a C-18 HPLC column (mobile phase: ammonium acetate (0.1 M, pH = 5)/acetonitrile 30:70 (v/v); flow rate = 12 ml/min). The product eluted with a retention time between 12 and 14 min.

To obtain preparations of the radiotracer suitable for use *in vivo*, the eluted radioactive peak corresponding to [^{11}C]GMOM was collected and diluted with 100 ml de-ionized water, passed through a solid phase extraction cartridges (Water C-18 Sep-Pak) and the product was eluted with 1 ml ethanol. A 10–50 μl sample of the ethanol solution was analyzed to determine radiotracer purity and specific activity.

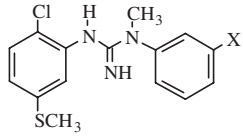
Results and discussion

The synthesis of the 3-methoxy derivative **8** (GMOM) and the precursor **7** was carried out using reported methods with modifications as required,[26] and the general method is outlined in Scheme 1. For our initial studies, we chose to prepare and characterize the 3-methoxy analog GMOM as a PET radiotracer instead of the 3–thiomethyl derivative **2** due to the ease of synthesis and expected stability of the precursor **7** required to prepare [^{11}C]GMOM. *In vitro* characterization was carried out in rat forebrain membranes using a standard PCP site



Scheme 1. Synthesis of the target compounds 7 and 8 (GMOM)

Table 1. *In vitro* inhibition constants and experimental log *P* values for selected substituted *N*, *N*′-diphenyl-*N*′-methylguanidines

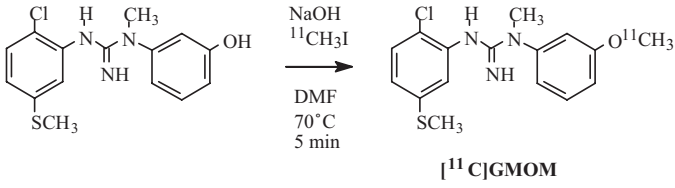


	x	K_i (PCP site)[a]	K_i (PCP site)[b]	log *P*
1	CH_2CH_3	5.1 ± 0.3	6.28 ± 0.28	3.00
2	SCH_3	1.87 ± 0.61	1.87 ± 0.25	2.68
8	OCH_3		5.2 ± 0.3	2.34

[a] See References 26.
[b] Performed in house by methods outlined in Reference 16.

assay with [^{3}H]MK801 as the radioligand.[16,18] Analysis of GMOM provided a K_i value of 5.2 ± 0.3 nM ($n = 3$), indicating that the 3-methoxy group provided an affinity similar to the 3-ethyl derivative **1** but somewhat lower than the 3-thiomethyl compound **2** (Table 1). Compounds **1** and **2** were also analyzed to ensure our assay provided K_i values close to those already reported in the literature. The K_i values of 6.28 ± 0.28 and 1.87 ± 0.25 nM for **1** and **2**, respectively, compared very well with those reported by Hu *et al.*[26] In addition, an HPLC method was used to determine the log *P* value of these ligands at physiological pH (pH = 7.5). GMOM was found to have the lowest lipophilicity compared to the 3-ethyl and 3-thiomethyl derivatives (Table 1). Its log *P* value of 2.34 is sufficient to allow passage across the blood/brain barrier, but not so high as to suggest that a large degree of non-specific binding would occur, as is often associated with radioligands such as 3-Iodo-MK801 and phencyclidine-based tracers with log *P* values above 3.0.

The corresponding PET tracer [^{11}C]GMOM was synthesized by methylation of the 3-hydroxyphenyl derivative **7** with [^{11}C]methyl iodide in dimethylformamide in the presence of aqueous sodium hydroxide (Scheme 2). This mixture was heated briefly at 70°C, and [^{11}C]GMOM was purified by reversed phase semi-preparative HPLC. After complete work-up as described in detail in the Experimental section, [^{11}C]GMOM was provided in radiochemical yields of 5.8–13.0% (8.4 ± 3.2%, $n = 5$, EOS) and high specific activity (1.25 ± 0.25 Ci/μmol, $n = 5$, EOS). The radiochemical purity was 96.7 ± 1.5% and up to 39 mCi of product was



[11C]GMOM

Scheme 2. Radiosynthesis of [^{11}C]GMOM

obtained. The chemical purity was demonstrated by HPLC analysis to be >95%, as no UV associated peaks other than those attributed to GMOM and ethanol were noted. Diluted solution of the tracer was made with sterile saline to provide approximately 200 μCi/100 μl solution, and the final preparation was passed through a sterile 0.22 μM filter into a sterile vial. The radiochemical and chemical purity of the final formulation was >95% as determined by the analytical HPLC method described in the experimental section. The average time required for synthesis, purification and formulation was 52 ± 5 min. The final formulation was deemed suitable for *in vivo* use.

Conclusions

The novel high-affinity guanidine-based NMDA/PCP site radioligand [^{11}C]GMOM has been synthesized. This compound exhibits high affinity ($K_i = 5.2 \pm 0.3$ nM) for the PCP site as well as a log *P* value (2.34) suitable for good brain penetration and low non-specific binding. The corresponding desmethyl precursor was labeled with the positron emitting isotope carbon-11 in good yield and high specific activity, and [^{11}C]GMOM was subsequently prepared as a formulation suitable for use in *in vivo* studies.

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