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Research paper

# Synthesis, radiolabeling and evaluation of novel amine guanidine derivatives as potential positron emission tomography tracers for the ion channel of the *N*-methyl-D-aspartate receptor

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ABSTRACT

The *N*-Methyl-D-Aspartate receptor (NMDAR) is involved in many neurological and psychiatric disorders including Alzheimer's disease and schizophrenia. The aim of this study was to develop a positron emission tomography (PET) ligand to assess the bio-availability of the NMDAR ion channel *in vivo*. A series of tri-*N*-substituted diarylguanidines was synthesized and their *in vitro* binding affinities for the NMDAR ion channel assessed in rat forebrain membrane fractions. Compounds **21**, **23** and **26** were radiolabeled with either carbon-11 or fluorine-18 and *ex vivo* biodistribution and metabolite studies were performed in Wistar rats. Biodistribution studies showed high uptake especially in prefrontal cortex and lowest uptake in cerebellum. Pre-treatment with MK-801, however, did not decrease uptake of the radiolabeled ligands. In addition, all three ligands showed fast metabolism.



## 1. Introduction

Excitatory neurotransmission in the mammalian central nervous system (CNS) is primarily accountable to the glutamate receptor system. Glutamate receptors are divided in ionotropic and metabotropic receptors. Ionotropic receptors are subdivided into three groups of receptors: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), kainate and *N*-methyl-D-aspartate receptor (NMDAR) [1]. NMDARs are necessary for long term potentiation, and are thought to play an important role in learning and memory [2]. It is hypothesized that hypoactivity of the NMDAR, associated with advancing age, may have a role in developing Alzheimer's disease and other neurodegenerative diseases [3,4]. Imaging of NMDARs using positron emission tomography (PET) could make it possible to assess the NMDAR status *in vivo*. To date, only [$^{18}$F]-GE-179 has been used successfully in human applications [5].

The NMDAR is a heteromultimeric assembly of four subunits. To date, three types of subunits are known: the NR1 subunit with eight splice variants (NR1a–h) [6], the NR2 subunit encoded by four distinct genes (NR2A–D) [7], and the NR3A and NR3B subunits (2 genes) [8]. This assembly together forms the ion channel of the NMDAR.

Recently, several structural classes with NMDAR affinity were reported. Propargylamine and acetylene conjugated polycyclic cage derivatives, β- and γ-carboline derivatives, pentamidine analogs of MK-801 and ifenprodil and (3-hydroxy-pyrazolin-5-yl)glycine based ligands. Unfortunately, all exhibit binding affinities in the micro-molar range [9–12].

Non-competitive ion channel NMDAR antagonists, such as [5*R*,10*S*]-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801), *N*-(1-[thienyl] cyclohexyl)piperidine (TCP) and ketamine are termed “use-dependent” ligands. These non-competitive antagonists gain access to the ion channel site primarily when the NMDAR is in an open state [13,14], i.e. they can bind within the channel when the receptor is activated by the

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agonist glutamate and the co-agonist glycine. Therefore, binding of non-competitive antagonists is proportional to the amount of activated, open receptor channels. Hence, by using non-competitive radiolabeled NMDAR antagonists and PET, the status of regional activation of NMDARs can be assessed *in vivo*.

The class of guanidine compounds with affinity for the NMDAR ion channel is known since 1989 [15–23]. Structure activity relationships studies showed that **1** (GMOM, $K_i$: 5.2–21.7 nM against [$^3$H]MK801) [22,24], **2** (CNS 5161, $K_i$: 1.87 nM against [$^3$H]MK801) [20] and **3** (GE-179, $K_i$: 2.4 nM against [$^3$H]TCP) [5] have superior $K_i$ values towards the ion channel of the NMDAR (Fig. 1). Apart from its $K_i$ value, ligand **1** showed promising characteristics for use as an NMDA PET tracer, although human applications have not been reported yet. [$^{11}$C]**2** has been used in patients with Parkinson's disease, but no significant increase in uptake of [$^{11}$C]**2** was seen in patients as compared with control subjects [25]. Recently, in an initial evaluation of [$^{18}$F]**3** in healthy volunteers high and rapid brain extraction was observed, with a relatively homogeneous distribution in gray matter and rapid peripheral metabolism. Nevertheless, quantification of [$^{18}$F]**3** appeared to be feasible [5].

So far, there have been no reports on *meta* substituted alkylamine analogues of **1**–**3**. Therefore, the aim of this study was to synthesize and evaluate amine and pyridine analogs of **1** and **2** as potential PET ligands for the NMDAR ion channel. Synthesis, binding affinity to the ion channel of the NMDAR, radiolabeling of higher affinity compounds, the Log $D_{7.4}$ value, metabolism and biodistribution in rats were investigated.

## 2. Results and discussion

### 2.1. Chemistry

The synthesis of the pyridine substituted guanidines (**7a**, **c**) is shown in Scheme 1. The *N*-methyl-*N*-(pyridinyl)cyanamides **6a**–**c** were synthesized by a three step procedure adapted from Stanovnik et al. [26] and Huntsman et al. [27]. First, the formamide oximes (**5a**–**c**) were obtained by a two step reaction from the aminopyridines **4a**–**c** with *N*,*N*-dimethylformamide dimethyl acetal (DMF–DMA) in 2-propanol, followed by treatment with hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) in 52–71% yield. Secondly, a reaction with DMF–DMA in toluene yielded the *N*-methylcyanamides **6a**–**c** in 30–61% yield. The pyridine guanidines **7a**, **c** were synthesized by condensation of 2-chloro-5-(methylthio)aniline hydrochloride in chlorobenzene with **6a**–**c** in 34–39% yield, but **7b** could not be obtained using this method. The reason for this is unclear, presumably the *meta* configuration of the pyridine ring deactivates the cyanic moiety too much for condensation. As compound **7a** and **7c** showed affinities less than 10 μM towards the NMDA receptor, no further research was performed to obtain compound **7b**.

Unsubstituted aminophenyl guanidine **14** was synthesized according to Scheme 2 via condensation of **12** with 2-chloro-5-(methylthio)aniline hydrochloride in 46% yield and subsequent quantitative deprotection of the 2,5-dimethylpyrrole group using $NH_2OH{\cdot}HCl$ [28].

Compound **12** was synthesized in 4 steps from 3-nitro-aniline (**8**) in 64% yield. First, **8** was protected with a 2,5-dimethylpyrrole group [29] followed by a reduction of the nitro moiety [30]. Secondly, amine **10** was reacted with cyanogen bromide [31] and then methylated to obtain **12**.

The alkyl substituted amine guanidines **21**, **23**, **26** and **29** were prepared using **17**–**19** as key intermediates. Amine **17** was mono protected to prevent double alkylation in the synthesis of **19** and **27**, and to preclude formation of side products during the guanidine formation of **23** and **29**. Scheme 3 shows the synthesis of mono- and dimethylaminophenyl guanidines **21** and **23**. The *N*-(3-aminophenyl)-*N*-methylcyanamide intermediates **17**–**19** were prepared from 3-nitro-aniline **8**. Cyanamide **15** was obtained in 93% yield [32]. After methylation of the cyanamide moiety and reduction of the nitro moiety, **17** was obtained in 72% overall yield. Subsequent monoprotection of the amine moiety with a trifluoroacetic acid group [33] gave **18** in 86% yield and the subsequent methylation gave **19** in quantitative yield. Compound **17** was dimethylated to obtain **20** in 51% yield. The condensation of **20** with 2-chloro-5-(methylthio)aniline hydrochloride in chlorobenzene gave dimethylaminophenyl guanidine **21** in 59% yield. Synthesis of *N*-trifluoroacetic-*N*-methylaminophenyl guanidine **22** was carried out using the trifluoroacetyl protected cyanamide **19** in 41% yield. Deprotection of **22** under basic aqueous conditions yielded **23** quantitatively.

Intermediate **19** was deprotected and alkylated with fluoroethylbromide to obtain **25** in 30% overall yield (Scheme 4). *N*-methyl-*N*-fluoroethyl guanidine was synthesized in 53% yield. However, by using standard conditions to obtain guanidines **28** and **26**, namely 165 °C in chlorobenzene, an inseparable side product was formed. High resolution mass spectrometry and $^{13}$C NMR measurements revealed an impurity containing a chloroethyl moiety besides the fluoroethyl product. Formation of this chlorinated side product was circumvented by lowering the temperature to 140 °C and using toluene instead of chlorobenzene as solvent.

The fluoroethylamine analogue **29** was synthesized via a similar approach, starting from **18** (Scheme 5). Alkylation with fluoroethylbromide provided **27** in 70% yield. Subsequent condensation with 2-chloro-5-(methylthio)aniline hydrochloride in toluene (**28**, 66%) and quantitative deprotection led to fluoroethylamine guanidine **29**.

### 2.2. Structure activity relationship study

Table 1 summarizes binding affinities of the synthesized guanidines for the ion channel of the NMDAR. The binding affinity was determined by measuring the ability of various concentrations of unlabeled ligand to inhibit specific binding of [$^3$H]MK-801 to rat forebrain plasma membranes. Substitution of a phenyl group (**30**) by a pyridyl group (**7a**, **c**) was not allowed as the affinity of the guanidines **7a** and **c** dropped to values above 10 μM.

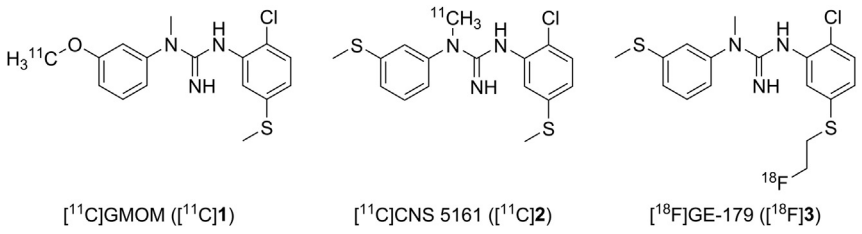


**Fig. 1.** Structures of radiolabeled *N*,*N*′-diaryl-*N*-methylguanidines.