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Synthesis and In Vitro Evaluation of *N,N′*-Diphenyl and *N*-Naphthyl-*N′*-phenylguanidines as *N*-Methyl-D-aspartate Receptor Ion-Channel Ligands

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Abstract—A series of *N,N′*-diphenyl and *N*-naphthyl-*N′*-phenyl guanidine derivatives was synthesized as potential *N*-methyl-D-aspartate (NMDA) receptor positron emission tomography (PET) ligands. The affinity of the different compounds was determined using in vitro receptor binding assays, and their log P values were estimated using HPLC analysis. The effect of N′-3 and N′-3,5 substitution on affinity and lipophilicity was examined. The K_i values ranged from 1.87 to 839 nM, while log P values between 1.22 and 2.88 were observed.

The ionotropic *N*-methyl-D-aspartate (NMDA) receptor is a major site of action for glutamate, the most prevalent neurotransmitter in the mammalian brain. The receptor plays a role in many biological functions, such as long-term potentiation (LTP), memory, and cognition. In addition, evidence exists for a role in glutamate toxicity, which is thought to be involved in a variety of neurodegenerative processes, including stroke, Parkinson's disease, Alzheimer's disease and epilepsy, and in schizophrenia.[1–9] The NMDA receptor is a receptor complex, and in addition to binding sites for glutamate and glycine (co-agonist), there are also several modulatory binding sites, including the PCP site that lies within the ion channel.[10,11] Since the PCP site is located within the NMDA ion channel, a PET PCP site radioligand could prove useful for reporting on the activation state of the channel in vivo.

To date, several radioligands for this site have been synthesized and labeled with C-11, F-18, or I-123. Although these compounds showed promising results in vitro, their in vivo use was restricted due to fast metabolism or a high lipophilicity (and thus, a high degree of nonspecific binding).[12–22] Structure–activity relationship (SAR) studies of symmetrical (*N,N′*-diphenyl) and unsymmetrical (*N*-phenyl-*N′*-naphthyl) *N,N′*-diarylguanidine derivatives have shown that these compounds act at the NMDA receptor ion channel.[23–25]

An iodinated derivative, *N*-(1-naphthyl)-*N′*-(3-iodophenyl)guanidine (CNS 1261), was synthesized as a potential tracer for single photon emission computerized tomography (SPECT) studies of the NMDA receptor.[26] Our interest in using a high affinity ligand for in vivo positron emission tomography (PET) studies prompted us to synthesize a series of *N,N′*-diphenyl and *N*-naphthyl-*N′*-phenyl guanidines containing functional groups amenable to labeling PET isotopes (*O*- and *S*-methylation reactions with C-11 methyl iodide are commonly used in radiochemistry to form labeled methoxy or thiomethyl groups). The affinity (K_i) of the new compounds was determined using in vitro receptor binding assays, and their lipophilicity (log P value) was estimated using HPLC analysis. The effect of *N′*-3 and *N′*-3,5 substitution on the affinity and lipophilicity was examined.

N,N′-Diphenyl and *N*-naphthyl-*N′*-phenyl guanidines were synthesized by reacting *N*-methyl-*N*-arylcyanamides with the requisite amine hydrochloride salts in refluxing chlorobenzene (*N,N′*-diphenyl guanidines) or neat (*N*-naphthyl-*N′*-phenyl guanidines) (Scheme 1).[24,25] The cyanamides were prepared from a reaction of cyanogen bromide (CAUTION: highly toxic) with the primary amine in diethyl ether, followed by alkylation of the arylcyanamide with sodium hydride/methyl iodide

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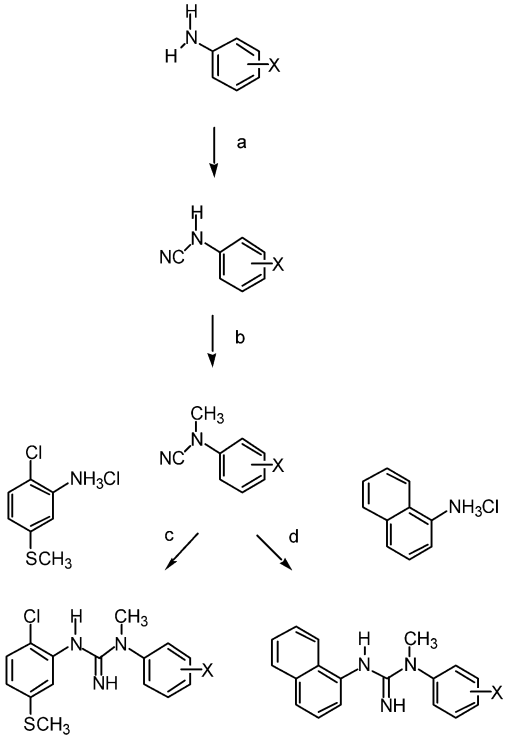


Scheme 1. Synthesis of *N,N′*-diphenyl and *N*-naphthyl-*N′*-phenyl guanidines. Reagents and conditions: (a) BrCN (CAUTION—highly toxic), Et_2O, ambient temperature, overnight; (b) NaH, THF, 65 °C, 30 min; CH_3I, ambient temperature, overnight; (c) C_6H_5Cl, 140–150 °C, 4–12 h; (d) neat, 140–150 °C, 4–12 h.

in tetrahydrofuran. The primary amines were commercially available. All of the compounds were purified by column chromatography.

The structures were determined by ^1H NMR and mass spectrometry. Melting point analysis was performed for each solid. The purity of the compounds was checked by ^1H NMR and by a HPLC system, used for the estimation of the lipophilicity.[27–30]

In vitro radioligand binding assays for the NMDA receptor ion-channel sites were performed according to reported methods using [^3H] MK-801 (1 nM) and rat brain membrane suspensions.[31] Relative affinities of compounds were determined as IC_{50} values from displacement curves. K_i values were calculated from the IC_{50} values using the method of Cheng and Prusoff.[32]

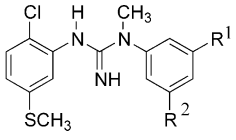
The lipophilicity of the compounds was examined by determination of the log P value using a HPLC method previously described.[27–30] Briefly, the lipophilicity of each compound was estimated by a comparison of its retention time to that of standards having known log P values. Relative retention times, RRT (to catechol), were calculated, and a calibration curve of log P versus log RRT was generated.

Since it was observed that, for the *N,N′*-diphenyl derivatives, the *N*-(2-chloro-5-thiomethylphenyl) combination is one of the most favorable for PCP site binding,[25] we only focused on the effect of the substitution on the *N′*-phenyl ring, particularly on *N′*-3 and *N′*-3,5 substitution. Seven novel and three known *N,N′*-diarylguanidine derivatives were synthesized. The calculated and found exact masses of the various derivatives are listed in Table 1.

Table 1. Calculated and found exact masses (FAB+) of guanidine derivatives

Compd	Molecular formula	Found (and calculated) exact masses
1	$C_{16}H_{18}N_3S_2Cl$	352.0728 (352.0709)
2	$C_{17}H_{20}N_3SCl$	334.1143 (334.1145)
3	$C_{16}H_{18}N_3OSCl$	336.0953 (336.0937)
4	$C_{15}H_{15}N_3FSCl$	324.0747 (324.0737)
5	$C_{15}H_{15}N_3ISCl$	431.9776 (431.9800)
6	$C_{17}H_{20}N_3SCl$	334.1143 (334.1145)
7	$C_{19}H_{19}N_3O$	306.1583 (306.1606)
8	$C_{18}H_{16}N_3F$	294.1385 (294.1406)
10	$C_{20}H_{21}N_3$	304.1812 (304.1814)

Table 2. Inhibition constant (K_i) and log P values of *N,N′*-diphenyl guanidine derivatives



Compd	R^1	R^2	K_i (nM)[a,b]	Log P[c]
MK-801	—	—	1.3	
1	SCH_3	H	1.87 (±0.25)	2.68
2	C_2H_5	H	6.28 (±0.28)	3.00
3	OCH_3	H	5.20 (±0.30)	2.34
4	F	H	19.0 (±3.2)	2.34
5	I	H	3.48 (±0.60)	2.91
6	CH_3	CH_3	14.0 (±3.9)	3.12

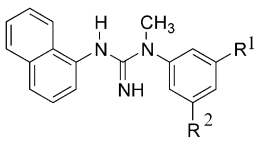
[a]Values are means of at least three experiments, standard deviation is given in parentheses.
[b]Values obtained by method of Cheng and Prusoff.[32]
[c]Values obtained by method of Waterhouse et al.[27–30]

In the *N,N′*-diphenylguanidine series, the inhibition constants (K_i vs [^3H]MK-801—listed in Table 2) of the 3-iodo, 3-fluoro, 3-methoxy, and 3,5-dimethyl derivatives were 3.48, 19, 5.2, and 14 nM, respectively. The K_i values for the 3-thiomethyl and the 3-ethyl compounds were similar to these given in literature: 1.87 versus 1.87 and 6.28 versus 5.10, respectively.[25]

In the *N*-naphthyl-*N′*-phenyl guanidine series, the inhibition constants (K_i vs [^3H]MK-801—listed in Table 3) of the 3-fluoro, 3-methoxy and 3,5-dimethyl derivatives were 839, 43.5, and 21.9 nM, respectively. The K_i value we obtained for the 3-iodo compound (4.65 nM) was similar to the one given in literature (4.21 nM).[26]

Comparison of the affinities of the two groups reveals that the compounds of the *N,N′*-diphenylguanidine series are more potent PCP/NMDA antagonists than the *N*-naphthyl-*N′*-phenyl guanidine derivatives. Within both groups it is observed that for halogen substitution a larger, less electronegative halogen is preferable. This is in accordance with previous results where the K_i value

Table 3. Inhibition constant (K_i) and log P values of *N*-naphthyl-*N'*-phenyl guanidine derivatives



Compd	R^1	R^2	K_i (nM)[a,b]	Log P[c]
MK-801	—	—	1.3	
7	OCH_3	H	43.5 (±5.6)	2.26
8	F	H	839 (±423)	2.31
9[d]	I	H	4.65 (±0.38)	2.94
10	CH_3	CH_3	21.9 (±10.1)	2.94

[a]Values are means of at least three experiments, standard deviation is given in parentheses.
[b]Values obtained by method of Cheng and Prusoff.[32]
[c]Values obtained by method of Waterhouse et al.[27–30]
[d]Owens et al.[26]

of the 3-bromo *N,N'*-diphenyl derivative was measured to be 3.85 nM.[25] A 3-methoxy group in the *N,N'*-diphenylguanidine series provided an affinity similar to the 3-ethyl derivative but somewhat lower than the 3-thiomethyl compound. 3,5-Dimethyl substitution in both series on the other hand afforded compounds with lower affinity.

The lipophilicity (log P value) of the different *N,N'*-diphenyl and *N*-naphthyl-*N'*-phenyl is also listed in Tables 2 and 3. All compounds have a suitable log P value for in vivo use.[33] The values for the methoxy (2.26 and 2.34 for *N*-naphthyl-*N'*-phenyl and *N,N'*-diphenyl derivatives, respectively) and the fluoro compounds (2.31 and 2.34) are nearly equal. They are the most hydrophilic compounds of both series. The 3-methoxy derivative was found to have the lowest lipophilicity compared to the 3-ethyl (3.00) and 3-thiomethyl (2.68) derivatives. This lower log P value could be an advantage for the derivative over many other reported compounds having log P values of 3 or more. The iodinated (2.94 and 2.91) and dimethyl (2.94 and 3.12) derivatives have the highest lipophilicity values. The log P value we observed for the CNS-1261 (compound **9**, 2.94) was somewhat higher than the log D value reported in the literature (2.19).[26]

In an effort to develop a high affinity PET ligand for the PCP site of the NMDA receptor, seven novel *N,N'*-diarylguanidine derivatives, with groups amenable to labeling PET isotopes, were synthesized using reported methods. All derivatives have lipophilicity values that enable their use in in vivo studies, with log P values ranging from 1.94 to 2.88. With the exception of *N*-(1-naphthyl)-*N'*-(3-fluorophenyl)guanidine (K_i value: 839 nM), all derivatives exhibited also high affinity for the ion channel PCP site of the receptor: the K_i values for the various derivatives ranged from 1.87–43.5 nM. The compounds with the best pharmacological profile (**1**, **3**, and **5**) will be selected for radiolabeling and in vivo studies.

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