Synthesis and Pharmacological Evaluation of *N*-(2,5-Disubstituted phenyl)-*N*′-(3-substituted phenyl)-*N*′-methylguanidines As *N*-Methyl-D-aspartate Receptor Ion-Channel Blockers

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Received July 15, 1997[⊗]

In the mammalian central nervous system, the *N*-methyl-D-aspartate (NMDA) subclass of glutamate receptors may play an important role in brain diseases such as stroke, brain or spinal cord trauma, epilepsy, and certain neurodegenerative diseases. Compounds which specifically antagonize the actions of the neurotransmitter glutamate at the NMDA receptor ion-channel site offer a novel approach to treating these disorders. CERESTAT (**4**, aptiganel CNS 1102) is currently undergoing clinical trial for the treatment of traumatic brain injury and stroke. Previously, we reported that analogues of *N*-1-naphthyl-*N*′-(3-ethylphenyl)-*N*′-methylguanidine (**4**) bound to the NMDA receptor ion-channel site with high potency and selectivity. Recently, molecules active at both σ receptors and NMDA receptor sites were investigated. A series of substituted diphenylguanidines **6** which are structurally related to *N*-1-naphthyl-*N*′-(3-ethylphenyl)-*N*′-methylguanidine was prepared. Compounds containing appropriate substitution pattern in one of the phenyl rings of diphenylguanidines displayed high affinity. For example, *N*-(2,5-dibromophenyl)-*N*′-(3-ethylphenyl)-*N*′-methylguanidine (**27b**, $R_2 = R_5 = Br$, $R_3 = C_2H_5$) exhibited potency at both σ receptors and NMDA receptor sites; **27b** also showed high efficacy in vivo in a neonatal rat excitotoxicity model. Further studies indicated that substituent effects were important in this compound series, and 2,5-disubstituted phenyl was the preferred substitution pattern for high-affinity binding at NMDA receptor sites. Bromo and methylthio were the optimal substituents for the R_2 and R_5 positions of the 2,5-disubstituted phenyl group, respectively. *N*-(2-Bromo-5-(methylthio)phenyl)-*N*′-(3-ethylphenyl)-*N*′-methylguanidine (**34b**, $R_2 = Br$, $R_5 = SMe$, $R_3 = C_2H_5$) was highly active at NMDA receptor sites. We found that the binding affinity of guanidines of type **6** could be further enhanced with the appropriate substitution at R_3. Optimal activity in this series are afforded by **43b** and **44b** ($R_2 =$ Cl or Br, $R_5 = R_3 = SCH_3$). Both **43b** and **44b** bound to NMDA receptor sites with high potency and selectivity (K_i vs [^{3}H]MK-801: 1.87 and 1.65 nM, respectively); these compounds are active in vivo in various animal models of neuroprotection. The structure−activity relationships for these compounds at the NMDA receptor ion-channel site are discussed.

Introduction

NMDA receptors are widely distributed in mammalian brain and spinal cord; the receptor is a ligand-gated ion channel composed of at least two different protein subunits: NR1 and NR2. The NMDA receptor complex has been suggested to play an important role in normal brain functions such as learning and memory. Prolonged stimulation of the receptor complex can cause excitotoxicity or cell death by mechanisms that may include irreversible overload of intracellular free Ca^{2+}; activation of calcium-sensitive proteases (e.g. calpain and lipases); and stimulation of nitric oxide synthase and the subsequent generation of toxic free radicals. Such excitotoxic mechanisms may contribute to brain disorders and neurodegenerative diseases in the mammalian central nervous system.[1] Ligands which specifically antagonize the actions of the neurotransmitter glutamate at the NMDA receptor−channel complex offer a novel approach to treating disorders such as stroke, brain or spinal cord trauma, epilepsy, Alzheimer's disease, and Huntington's disease.[1]

In the past decade, efforts have been made to develop potent ligands for a binding site within the NMDA receptor associated ion channel. This site has also been termed the PCP binding site. Compounds such as PCP (phencyclidine, **1a**; Chart 1)[2] as well as TCP (*N*-[1-(2-thienyl)cyclohexyl]piperidine, **1b**),[3] (+)-SKF 10047 (**2a**),[4a] hexahydro-9,4-(iminomethano)-1*H*-benz[*f*]indenes (**2b**), WIN 67870-2 (**2c**, 6,11-ethanobenzo[*b*]quinolizium cation)[4b,] PD 134365 (**3**),[5] *N*-(1-naphthyl)-*N*′-(3-ethylphenyl)-*N*′-methylguanidine (**4**),[6] dizocilpine (MK-801, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine, **5a**),[7] and (+)-IDDC ((+)-10,5-(iminomethano)-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene, **5b**)[8] are known noncompetitive antagonists of NMDA receptors. *N*-(1-Naphthyl)-*N*′-(3-ethylphenyl)-*N*′-methylguanidine (**4**, CNS 1102, CERESTAT) is a potential therapeutic agent for the treatment of stroke and traumatic brain injury and is currently undergoing clinical trials.[6d]

Many NMDA receptor ion-channel blockers also bind to σ receptors in mammalian brain membranes.[9] Although the function of the σ receptor is poorly understood, σ antagonists have been shown to be neuroprotective. It has been suggested that σ activity might attenuate the neurological and behavioral toxicity associated with high doses of NMDA antagonists.[10] In

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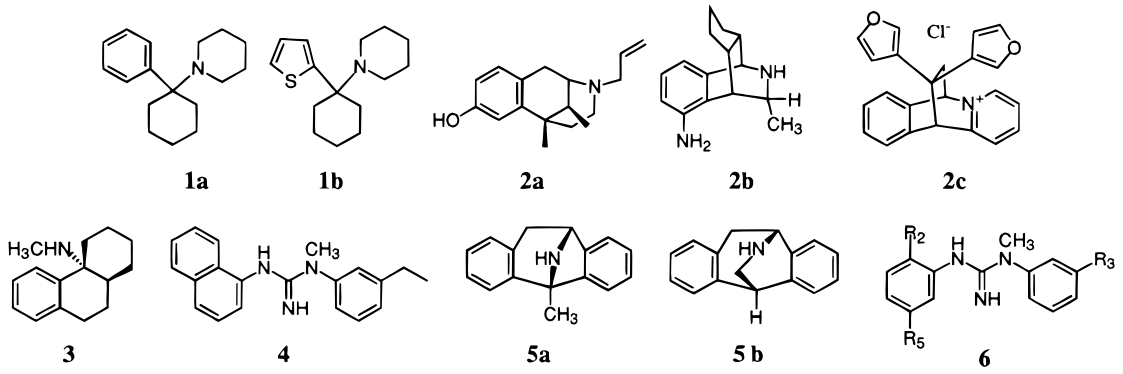
⊗ Abstract published in *Advance ACS Abstracts,* December 1, 1997.

S0022-2623(97)00459-7 CCC: $14.00

Chart 1



previous studies, we identified *N*-1-naphthyl-*N*′-(3-ethylphenyl)-*N*′-methylguanidine analogues with high affinity and selectivity for the NMDA receptor ion-channel site. We have now synthesized either molecules with high selectivity at NMDA receptor or molecules active for both the NMDA receptor ion-channel site and the σ receptor.

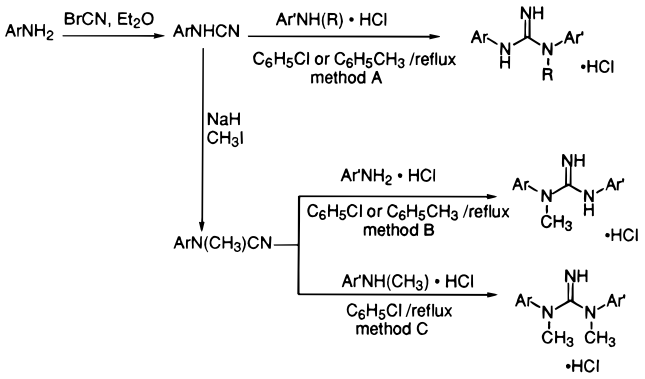
Previous structure−activity relationship (SAR) studies of symmetrical bis(substituted phenyl)guanidines suggested that substitution was preferred in the ortho or meta position on the phenyl ring and that small alkyl groups are especially advantageous.[6a] In unsymmetrical *N*-1-naphthyl-*N*′-phenylguanidine analogues, a lower alkyl group was preferred as a 3-substituent on this phenyl ring.[6a] Further investigation of substituents in this ring indicated that activity was correlated with substituent size and hydrophobicity: ethyl was found to be optimal, while halogen (iodo or bromo), trifluoromethyl, or methylthio had comparable activity. Examination of substituent effects using Cl atoms as probes indicated that a further enhancement in potency could be achieved with appropriate 2,5-disubstituents (e.g. 2-bromo-5-ethyl) in *N*-1-naphthyl-*N*′-phenylguanidine analogues.

We hypothesized that the 1-naphthyl substituent of **4** could be replaced by a substituted phenyl group to give an *N*,*N*′-diphenylguanidine that retains high affinity at NMDA receptor ion-channel sites. This, and our interest in comparing molecules active at both NMDA receptor ion-channel and σ receptor sites, prompted us to make a detailed investigation of a series of substituted diphenylguanidines. In this paper, we describe a series of novel, high-affinity NMDA receptor ion-channel blockers with varying potencies and selectivities (Tables 2−5). The SAR was systematically examined in the following five groups: *N*-(2- or 3-substituted phenyl)-*N*′-(3-ethylphenyl)guanidines, *N*-(monochloro or dichlorophenyl)-*N*′-(3-ethylphenyl)-guanidines, *N*-(2,5-disubstituted phenyl)-*N*′-(3-ethylphenyl)guanidines, *N*-(2,5-disubstituted phenyl)-*N*′-(3-substituted phenyl)guanidines, and di-, tri-, and tetrasubstituted guanidines. Herein, the synthesis and SAR of this series of NMDA ion-channel blocking agents is discussed.

Chemistry

N,N′-Disubstituted or N,N′,N′-trisubstituted guanidines were synthesized (Scheme 1, method A or B) by reacting *N*-arylcyanamides (or *N*-methyl-*N*-arylcyanamides) with the requisite amine hydrochloride salts in refluxing chlorobenzene (or toluene). The starting cyanamides

Scheme 1



were prepared either from a reaction of cyanogen bromide with the primary amine in diethyl ether or by the alkylation of an arylcyanamide with sodium hydride/alkyl halide in tetrahydrofuran. The requisite amines were either commercially available or prepared according to a literature procedure. The *N*-methyl-*N*-arylamine hydrochloride salts were prepared by a sequence of steps involving formation of the formamide of the substituted aniline, reduction of the formamide into the substituted *N*-methylanilines, and conversion of the amines into the hydrochloride salts. The syntheses of N,N,N′,N′-tetrasubstituted guanidines is outlined in Scheme 1 (method C) by reacting *N*-methyl-*N*-arylcyanamides with the *N*-methyl-*N*-arylamine hydrochloride salts in refluxing chlorobenzene (or toluene) in the presence of aluminum chloride. The compound characterizations and reaction yields are summarized in Table 1.

Binding Studies

In vitro radioligand binding assays for the NMDA receptor ion-channel sites were performed by using [^{3}H]-MK-801 and rat brain membrane suspensions.[11] Binding assays for the σ receptor sites were performed by utilizing [^{3}H]-*N*,*N*′-di-*o*-tolylguanidine ([^{3}H]DTG) and guinea pig brain membrane suspensions.[11] Relative affinities of compounds were determined as IC_{50} values from displacement curves. K_i values were derived from the IC_{50} values as described by Cheng and Prusoff.[12] The results are presented in Tables 2−5.

Results and Discussion

Previous SAR studies of analogues related to **4** indicated that *N*,*N*′-diaryl substitution on the guanidinium group was important for achieving high affinity and selectivity at NMDA receptor ion-channel binding

Table 1. Characterization of Guanidine Derivatives

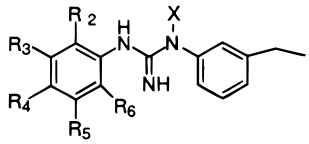
no.	method	yield, %	mp, °C	molecular formula	MS (M^+)	anal.
7a	A	53	89−90	$C_{15}H_{17}N_3$·HCl	239	C,H,N
7b	B	78	83−84	$C_{16}H_{19}N_3$·HCl	253	C,H,N
8a	A	65	87−88	$C_{15}H_{16}N_3Cl$·HCl·0.25H_2O	273	C,H,N
8b	B	63	154−155	$C_{16}H_{18}N_3Cl$·HCl·0.25H_2O	287	C,H,N
9b	B	88	164−165	$C_{16}H_{18}N_3Br$·HCl·0.5H_2O	331	C,H,N
10a	A	87	167−168	$C_{16}H_{18}N_3I$·HCl	379	C,H,N
11a	A	70	76−77	$C_{16}H_{19}N_3$·HCl·0.5H_2O	253	C,H,N
12a	A	58	78−79	$C_{17}H_{21}N_3$·HCl·0.25H_2O	267	C,H,N
13a	A	90	62−63	$C_{18}H_{23}N_3$·HCl·H_2O	281	C,H,N
13b	B	74	78−79	$C_{19}H_{25}N_3$·HCl·0.25H_2O	295	C,H,N
14a	A	91	155−156	$C_{15}H_{16}N_3Br$·HCl	317	C,H,N
14b	B	55	146−147	$C_{16}H_{18}N_3Br$·HCl	331	C,H,N
16a	A	82	154−155	$C_{15}H_{16}N_3Cl$·HCl	274	C,H,N
16b	B	66	78−80	$C_{16}H_{18}N_3Cl$·HCl·H_2O	288	C,H,N
17a	A	45	117−119	$C_{15}H_{16}N_3Cl$·HCl·0.25H_2O	273	C,H,N
17b	B	46	167−168	$C_{16}H_{18}N_3Cl$·HCl·H_2O	288	C,H,N
18a	A	69	104−105	$C_{15}H_{15}N_3Cl_2$·HCl	307	C,H,N
18b	B	64	184−185	$C_{16}H_{17}N_3Cl_2$·HCl·0.25H_2O	321	C,H,N
19a	A	52	133−134	$C_{15}H_{15}N_3Cl_2$·HCl	307	C,H,N
20a	A	58	111−112	$C_{15}H_{15}N_3Cl_2$·HCl	307	C,H,N
20b	B	72	162−163	$C_{16}H_{17}N_3Cl_2$·HCl	321	C,H,N
20c	B	74	92−93	$C_{16}H_{17}N_3Cl_2$·HCl	321	C,H,N
20d	C	63	161−162	$C_{17}H_{19}N_3Cl_2$·HCl·H_2O	334	C,H,N
21a	A	52	112−113	$C_{15}H_{15}N_3Cl_2$·HCl	307	C,H,N
22a	A	64	173−174	$C_{15}H_{15}N_3Cl_2$·HCl	307	C,H,N
22b	B	64	190−191	$C_{16}H_{17}N_3Cl_2$·HCl	321	C,H,N
23a	A	69	185−186	$C_{15}H_{15}N_3Cl_2$·HCl	307	C,H,N
24a	A	88	52−53	$C_{17}H_{20}N_3F$·HCl	285	C,H,N
24b	B	90	171−172	$C_{18}H_{22}N_3F$·HCl	299	C,H,N
25a	A	83	77−78	$C_{17}H_{20}N_3Cl$·HCl	301	C,H,N
25b	B	76	190−191	$C_{18}H_{22}N_3Cl$·HCl	315	C,H,N
25c	B	78	131−132	$C_{18}H_{22}N_3Cl$·HCl	315	C,H,N
25d	C	72	129−130	$C_{19}H_{24}N_3Cl$·HCl·2H_2O	330	C,H,N
26a	A	52	70−71	$C_{17}H_{20}N_3Br$·HCl	345	C,H,N
26b	B	76	189−190	$C_{18}H_{22}N_3Br$·HCl	360	C,H,N
27a	A	75	76−77	$C_{15}H_{15}N_3Br_2$·HCl	397	*a*
27b	B	60	217−218	$C_{16}H_{17}N_3Br_2$·HCl	411	C,H,N
27d	C	60	179−180	$C_{17}H_{19}N_3Br_2$·HCl	425	C,H,N
28a	A	60	105−106	$C_{16}H_{15}N_3F_4$·HCl	325	*a*
28b	B	62	158−159	$C_{17}H_{17}N_3F_4$·HCl	339	C,H,N
29a	A	53	73−74	$C_{16}H_{15}N_3ClF_3$·HCl	341	*a*
29b	B	64	180−181	$C_{17}H_{17}N_3ClF_3$·HCl	355	C,H,N
30a	A	30	109−110	$C_{16}H_{15}N_3BrF_3$·HCl	386	*a*
30b	B	80	184−185	$C_{17}H_{17}N_3BrF_3$·HCl	400	C,H,N
31a	A	70	88−89	$C_{16}H_{18}N_3Cl$·HCl·0.5H_2O	288	C,H,N
31b	B	61	204−205	$C_{17}H_{20}N_3Cl$·HCl	302	C,H,N
32a	A	73	132−133	$C_{16}H_{18}N_3ClS$·HCl	319	C,H,N
32b	B	81	198−199	$C_{17}H_{20}N_3ClS$·HCl	333	C,H,N
33b	B	61	64−65	$C_{17}H_{21}N_3S_1$·HCl	300	C,H,N
34b	B	40	90−92	$C_{17}H_{20}N_3Br_1S_1$·HCl	377	C,H,N
35b	B	53	164−165	$C_{17}H_{17}N_3ClF_3$·HCl	355	C,H,N
36b	B	20	95−96	$C_{17}H_{17}N_3BrF_3$·HCl	399	C,H,N
37b	B	31	156−157	$C_{16}H_{15}N_3ClF_3S$·HCl	373	C,H,N
38b	B	50	121−122	$C_{16}H_{15}N_3BrF_3S$·HCl	417	C,H,N
39b	B	88	233−234	$C_{15}H_{12}N_3Br_2F_3$·HCl	449	C,H,N
40b	B	81	209−210	$C_{15}H_{12}N_3Cl_2F_3$·HCl	361	C,H,N
41b	B	95	212−213	$C_{17}H_{20}N_3ClS$·HCl	333	C,H,N
42b	B	75	199−200	$C_{17}H_{20}N_3BrS$·HCl	378	C,H,N
43b	B	70	203−204	$C_{16}H_{18}N_3ClS_2$·HCl	351	C,H,N
44b	B	46	204−205	$C_{16}H_{18}N_3BrS_2$·HCl·0.25H_2O	395	C,H,N
45b	B	27	239−240	$C_{15}H_{15}N_3Br_2S$·HCl	429	C,H,N
46b	B	63	200−201	$C_{15}H_{15}N_3Cl_2S$·HCl	339	C,H,N
47b	B	64	245−246	$C_{15}H_{15}N_3Br_2SO_2$·HCl	461	C,H,N
48b	B	10	169−170	$C_{15}H_{15}N_3BrSO$·HCl	499	C,H,N
49b	B	27	188−189	$C_{15}H_{12}N_3Br_2F_3O$·HCl	467	C,H,N
50b	B	15	74−75	$C_{17}H_{17}N_3BrF_3O$·HCl	416	C,H,N
51b	B	43	244−245	$C_{15}H_{15}N_3BrClS$·HCl	384	C,H,N

[a] Accurate mass measured in EI mode for the guanidine free base and HPLC purity: **27a** (394.9618, 97%), **28a** (325.1212, 98%), **29a** (341.0907, 95%), **30a** (385.0391, 96%).

sites. The 1-naphthyl and 3-ethylphenyl groups were preferred substituents for high potency.[6] In this report, we first focused on the effect of replacing the 1-naphthyl group of **4** with various substituted phenyl groups while retaining the *N*′-(3-ethylphenyl) group. Both disubstituted (*N*-phenyl-*N*′-(3-ethylphenyl)guanidine) and trisubstituted guanidines (*N*-phenyl-*N*′-(3-ethylphenyl)-*N*′-methylguanidine) were explored.

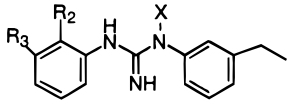
Initially, the 1-naphthyl group of **4** was replaced by a monosubstituted benzene. Chlorine atoms were used as probes to systematically investigate the preferred substitution pattern among *N*-(monochlorophenyl)-*N*′-

Table 2. *N*-(Mono- or Dichlorophenyl)-*N*′-(3-ethylphenyl)guanidines



					X = H			X = CH_3		
R_2	R_3	R_4	R_5	R_6	no.	[^{3}H]MK-801 K_i (nM)	[^{3}H]DTG IC_{50} (nM)	no.	[^{3}H]MK-801 K_i (nM)	[^{3}H]DTG IC_{50} (nM)
H	H	H	H	H	**7a**	732 ± 54	30.1 ± 15.1	**7b**	4470 ± 260	42.8 ± 0.4
Cl					**8a**	249 ± 42	15.9 ± 2.1	**8b**	386 ± 20	71.8 ± 10.8
	Cl				**16a**	112 ± 27	27 ± 0	**16b**	497 ± 23	43.5 ± 2.6
		Cl			**17a**	738 ± 188	20 ± 1.1	**17b**	3338 ± 563	45.7 ± 3.3
Cl	Cl				**18a**	156 ± 16	13.3 ± 1	**18b**	116 ± 4	97 ± 18.8
Cl		Cl			**19a**	140 ± 41	36.5 ± 19			
Cl			Cl		**20a**	68 ± 9	11.8 ± 1.9	**20b**	65 ± 9	28.5 ± 6.7
Cl				Cl	**21a**	133 ± 2	14.4 ± 0.3			
	Cl	Cl			**22a**	204 ± 37	31.8 ± 0.5	**22b**	1280 ± 16	39 ± 7.2
	Cl		Cl		**23a**	115 ± 3	69.9 ± 28.4			

Table 3. *N*-(Substituted phenyl)-*N*′-3-(ethylphenyl)guanidines



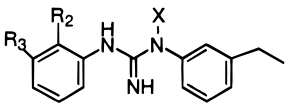
		X = H			X = CH_3		
R_2	R_3	no.	[^{3}H]MK-801 K_i (nM)	[^{3}H]DTG IC_{50} (nM)	no.	[^{3}H]MK-801 K_i (nM)	[^{3}H]DTG IC_{50} (nM)
H	H	**7a**	722 ± 54	30.1 ± 15.1	**7b**	4466 ± 355	42.8 ± 0.4
Cl	H	**8a**	249 ± 42	15.9 ± 2.1	**8b**	386 ± 20	71.8 ± 10.8
Br	H				**9b**	265 ± 45	125 ± 64
I	H				**10b**	101 ± 8	234 ± 96
CH_3	H	**11a**	768 ± 193	10.8 ± 3			
C_2H_5	H	**12a**	350 ± 107	18.5 ± 8			
i-Pr	H	**13a**	230 ± 28	20.6 ± 2	**13b**	564 ± 62	362 ± 131
H	Br	**14a**	51 ± 18	10.3 ± 3.3	**14b**	81 ± 36	40 ± 10.4
H	Et	**15a**[17]	129 ± 29	8 ± 2	**15b**[17]	164 ± 28	82 ± 10
H	Cl	**16a**	112 ± 27	27 ± 0	**16b**	497 ± 23	43.5 ± 2.6
H	SMe				**33b**	6.4 ± 0.6	164 ± 27

(3-ethylphenyl)guanidines (Table 2). The 2- or 3-substituted analogues (**8b** or **16b**) displayed 6−8-fold higher activity than the 4-substituted or unsubstituted derivatives (**17b** or **7b**) in vitro. The 2- or 3-substituent was preferred for the diphenylguanidines and was consistent with previous SAR results.[6] Subsequently, we investigated a series of 2-substituents: 2-hydrogen, 2-chloro, 2-bromo, 2-iodo, 2-methyl, 2-ethyl, and 2-isopropyl (Table 3). A beneficial steric effect of the 2-substituent was observed; large substituents were preferred for high NMDA receptor affinity: 2-iodo (**10b**) > 2-bromo (**9b**) > 2-chloro (**8b**) > 2-hydrogen (**7b**). Consistent with this for 2-alkyl substitution, the affinity declined as the size of the substituent on 2-position decreased: 2-isopropyl (**13a**) > 2-ethyl (**12a**) > 2-methyl (**11a**) = 2-hydrogen (**7a**). The effect of additional substitution on the benzene ring was examined by comparing the 3-chloro, 3-bromo, 3-hydrogen, 3-ethyl, and 3-methylthio analogues. Surprisingly, replacement of the 3-hydrogen (**7b**) with a bromo atom (**14b**) or a 3-methylthio group (**33b**) enhanced the binding affinity 55- and 220-fold, respectively. It is likely that the size, lipophilicity, and inductive effects of the substituents are associated to NMDA receptor affinity. The 3-methylthio group (**33b**, K_i vs [^{3}H]MK-801: 6.4 nM) was found to be the optimal substituent among the meta-substituted phenyl derivatives studied.

We then investigated whether the multichlorophenylguanidines might function as high-affinity NMDA receptor ligands. In part, this was prompted by the possibility that 2,3-dichlorophenyl substitution could mimick the 1-naphthyl group present in **4** and therefore be beneficial for binding affinity. *N*-(Dichlorophenyl)-*N*′-(3-ethylphenyl)guanidines incorporating two chloro atoms with various substitution patterns on the phenyl ring were investigated (Table 2). Optimal NMDA affinity was obtained with 2,5-dichloro-substituted analogue **20a**. Compound **20a** showed higher activity than all other monochloro or dichloro analogues (**8a**, **16a**, **17a**, **18a**, **19a**, **21a**, **22a**, **23a**). The 2,5-dichloro analogue (**20a**) enhanced potency at least 2-fold relative to the 3-chloro analogue (**16a**), whereas 3,5- and 3,4-dichloro analogues (**23a**, **22a**) showed no enhancement. These results indicated that the 2,5-disubstituted combination is the most favorable for NMDA-receptor binding.

Subsequent syntheses of various *N*-(2,5-disubstituted phenyl)-*N*′-(3-ethylphenyl)guanidines (**24**−**34**) was directed toward optimizing NMDA receptor site activity (Table 4). Binding affinities in this series ranged from 2 to 200 nM for NMDA receptor site and suggested that ligand binding affinity is strongly influenced by substituent effects. For *N*-(2-halo-5-ethylphenyl)guanidines (**15**, **24**, **25**, **26**), the 2-bromo-substituted compounds (**26**) are consistently more potent than 2-chloro (**25**), 2-fluoro (**24**), and 2-hydrogen (**15**) analogues; this observation was also made in 2-halo-5-(trifluoromethyl)phenyl (**28**−**30**) and 2-halo-5-(methylthio)phenyl series (**32b**, **33b**,

Table 4. *N*-(2,5-Disubstituted phenyl)-*N*′-(3-substituted phenyl)guanidines



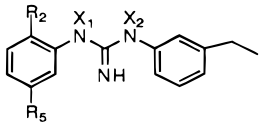
			X= H			X = CH_3		
R_2	R_5	R_3	no.	[^{3}H]MK-801 K_i (nM)	[^{3}H]DTG IC_{50} (nM)	no.	[^{3}H]MK-801 K_i (nM)	[^{3}H]DTG IC_{50} (nM)
H	H	Et	**7a**	722 ± 54	30.1 ± 15.1	**7b**	4466 ± 355	42.8 ± 0.4
H	Et	Et	**15a**	129 ± 29	8 ± 2	**15b**	164 ± 28	82 ± 10
F	Et	Et	**24a**	50 ± 7	7.4 ± 2.1	**24b**	97 ± 16	113 ± 4.5
Cl	Et	Et	**25a**	35 ± 7	4.5 ± 1	**25b**	41 ± 9	112 ± 21
Br	Et	Et	**26a**	29 ± 5	5.7 ± 2.4	**26b**	13 ± 1	203 ± 28.5
F	CF_3	Et	**28a**	76 ± 5	23.9 ± 4.5	**28b**	194 ± 39	79.0 ± 19.1
Cl	CF_3	Et	**29a**	44 ± 10	17.3 ± 2.9	**29b**	101 ± 7	115 ± 29.6
Br	CF_3	Et	**30a**	67 ± 8	19.6 ± 3.6	**30b**	78 ± 1	205 ± 36.2
Cl	CH_3	Et	**31a**	77 ± 18	30.7 ± 10.3	**31b**	78 ± 5	45.3 ± 4.3
H	SMe	Et				**33b**	6.4 ± 1.2	164 ± 27
Cl	SMe	Et	**32a**	11.8 ± 3	15.8 ± 4	**32b**	5.1 ± 0.3	309 ± 125
Br	SMe	Et				**34b**	2.45 ± 0.4	299 ± 26
H	Br	Et	**14a**	51 ± 18	10.3 ± 3.3	**14b**	81 ± 36	40 ± 10.4
Br	Br	Et	**27a**	67 ± 14	18.2 ± 7.5	**27b**	37 ± 8	40.2 ± 4.1
Cl	Cl	Et	**20a**	68 ± 9	11.8 ± 1.9	**20b**	65 ± 9	28.5 ± 6.7
Cl	Et	CF_3				**35b**	59.2 ± 10.2	200 ± 64
Br	Et	CF_3				**36b**	15.7 ± 1.8	221 ± 55
Cl	SMe	CF_3				**37b**	6.9 ± 1.6	421 ± 117
Br	SMe	CF_3				**38b**	4.27 ± 0.6	755 ± 69
Br	Br	CF_3				**39b**	26.1 ± 2.7	224 ± 96
Cl	Cl	CF_3				**40b**	155 ± 27	110 ± 36
Cl	Et	SMe				**41b**	9.69 ± 1.15	178 ± 48
Br	Et	SMe				**42b**	3.92 ± 0.63	180 ± 20
Cl	SMe	SMe				**43b**	1.87 ± 0.61	480 ± 54
Br	SMe	SMe				**44b**	1.65 ± 0.19	1034 ± 96
Br	Br	SMe				**45b**	4.13 ± 1.93	113 ± 22
Cl	Cl	SMe				**46b**	45.6 ± 1.6	130 ± 43
Br	Br	SO_2Me				**47b**	408 ± 98	3378 ± 675
Br	Br	SOMe				**48b**	248 ± 68	1947 ± 259
Br	Br	OCF_3				**49b**	19.7 ± 3.3	113 ± 9
Br	Et	OCF_3				**50b**	12.3 ± 1.2	261 ± 4
Cl	SMe	Br				**51b**	3.85 ± 0.56	494 ± 10
			5a	1.7 ± 0.1				
			4	29 ± 5	2535 ± 670			

34b) (Table 4). Comparison revealed a graded preference for 2-bromo over 2-chloro, 2-fluoro, or 2-hydrogen among the *N*-(2-halo-5-substituted phenyl)-*N*′-(3-ethylphenyl)-guanidines that were studied. This preference for a bulky substituent in the 2-position of the phenyl ring is consistent with our previous described observations for compounds reported in Table 3.

Binding affinities for *N*-(2-bromo or 2-chloro, 5-substituted phenyl)-*N*′-(3-ethylphenyl)-*N*′-methylguanidines (**9b**, **26b**, **27b**, **30b**, **34b**) (**8b**, **20b**, **25b**, **29b**, **32b**) varied widely (K_i values ranged from 2.5 to 386 nM). Analysis of these ligands suggested that the R_5-substituents are important for activity and that the size of R_5 correlates with ligand potency at NMDA receptor sites. In *N*-(2-bromo-5-substituted phenyl) analogues, binding affinities for 5-(methylthio) (**34b**), 5-ethyl (**26b**), 5-bromo (**27b**), 5-(trifluoromethyl) (**30b**), and 5-hydrogen (**9b**) were 2.45, 13, 37, 78, and 265 nM, respectively, and correlated to the sizes of R_5 as indicated by MR values (13.8, 10.3, 8.9, 5.0, and 1.0, respectively).[13] In the *N*-2-chloro-5-substituted phenyl series, a similar pattern was observed; binding affinities of 5-(methylthio) (**32b**), 5-ethyl (**25b**), 5-chloro (**20b**), 5-(trifluoromethyl) (**29b**), and 5-hydrogen (**8b**) declined (K_i: 5.1, 41, 65, 101, and 386 nM, respectively) as the sizes of R_5 (MR values) decreased. A larger substituent at R_5, such as methylthio, is preferred for the enhanced binding affinity. The other four groups: bromo, chloro, trifluoromethyl, and ethyl were effective but less active. The influence of lipophilicity and inductive effects of the R_5-substituent on ligand potency is unclear but is likely to contribute to affinity. Combining optimal substituents (2-bromo and 5-methylthio) afforded analogue **34b** which is a highly active NMDA receptor ion-channel blocker (K_i vs [^{3}H]MK-801: 2.4 nM) and displays a 12-fold greater affinity than **4**. We conclude that 2-bromo-5-(methylthio)phenyl is the optimal substituent combination for binding affinity among all the *N*-(2,5-disubstituted phenyl)-*N*′-(3-ethylphenyl)guanidines investigated.

N-(1-Naphthyl)-*N*′-(3-ethylphenyl)-*N*′-methylguanidine analogues bind to NMDA receptor ion-channel sites with high potency and selectivity; previous SAR indicated that lower alkyl group was preferred as a 3-substituent on the phenyl ring among analogues of **4**.[6a] Further SAR of substituents in this ring indicated that activity was correlated with substituent size and hydrophobicity: ethyl was found to be optimal, with halogen (iodo or bromo), trifluoromethyl, or methylthio also being comparably active. Herein, we report, that in the *N*-(2,5-disubstituted phenyl)guanidine series, the 3-substituent (R_3) on the *N*′-phenyl ring of ligand **6** has a significant effect on binding affinity, and this effect is different from that of the above 1-naphthyl series. Four sets of active NMDA receptor ligands, containing *N*-(2,5-disubstituted phenyl)-*N*′-(3-substituted phenyl)-guanidine structures, were investigated (Table 4). A consistent trend was observed in this SAR: methylthio is the optimal R_3 substituent for high-affinity binding,

Table 5. The Comparison of Di-, Tri-, and Tetrasubstituted Guanidines



		$X_1 = X_2 = H$			$X_1 = H, X_2 = CH_3$			$X_1 = CH_3, X_2 = H$			$X_1 = X_2 = CH_3$		
R_2	R_5	no.	[^{3}H]-**5** K_i (nM)	[^{3}H]DTG IC_{50} (nM)	no.	[^{3}H]-**5** K_i (nM)	[^{3}H]DTG IC_{50} (nM)	no.	[^{3}H]-**5** K_i (nM)	[^{3}H]DTG IC_{50} (nM)	no.	[^{3}H]-**5** K_i (nM)	[^{3}H]DTG IC_{50} (nM)
Cl	Cl	**20a**	68 ± 9	11.8 ± 1.9	**20b**	65 ± 9	28.5 ± 6.7	**20c**	118 ± 20	64 ± 38	**20d**	72 ± 9	39.3 ± 17.5
Br	Br	**27a**	67 ± 14	18.2 ± 7.5	**27b**	37 ± 8	40.4 ± 4.1				**27d**	27 ± 4	381 ±149
Cl	Et	**25a**	35 ± 7	4.5 ± 1	**25b**	41 ± 9	112 ± 21	**25c**	65 ± 10	156 ± 4.7	**25d**	111 ± 16	241 ± 49.5

with trifluoromethyl, bromo, ethyl, or trifluoromethoxy being up to 6-fold less active; both methylsulfonyl and methylsulfoxy derivatives are less potent. In the *N*-(2,5-dibromophenyl)-*N*′-(3-substituted phenyl)-*N*′-methylguanidine series, the K_i values (vs [^{3}H]MK-801) of methylthio (**45b**), ethyl (**27b**), trifluoromethyl (**39b**), trifluoromethoxy (**49b**), methylsulfonyl (**48b**), and methylsulfoxy (**47b**) are 4.13, 37, 26.1, 19.7, 248, and 408 nM, respectively. In the *N*-(2-chloro-5-methylthiophenyl)-*N*′-(3-substituted phenyl)guanidines series, the K_i values of methylthio (**43b**), bromo (**51b**), ethyl (**32b**), and trifluoromethyl (**37b**) derivatives are 1.87, 3.85, 5.1, and 6.9 nM, respectively. Thus, methylthio has been found to be the optimal substituent for R_3. Two compounds that displayed high selectivity and affinity for NMDA receptor ion-channel sites are *N*-(2-chloro-5-(methylthio)phenyl)-*N*′-(3-(methylthio)phenyl)-*N*′-methylguanidine (**43b**) and *N*-(2-bromo-5-(methylthio)phenyl)-*N*′-(3-(methylthio)-phenyl)-*N*′-methylguanidine (**44b**) (K_i: 1.87 and 1.65 nM vs [^{3}H]MK-801; 480 and 1034 nM vs [^{3}H]DTG) (Table 4). Compounds **43b** and **44b** are the leading compounds of the *N*,*N*′-diaryl-*N*′-methylguanidines prepared to date and were selected for further in vivo studies.

Finally, exploration of the effect of substitution on the N or N′ positions of the *N*,*N*′-diphenylguanidine derivatives was pursued by synthesizing a series of di-, tri-, and tetrasubstituted analogues. In the *N*-(2,5-disubstituted phenyl)-*N*′-(3-ethylphenyl)guanidines series, no large differences were found in pharmacological activities at the NMDA receptor site for di-, tri-, or tetrasubstitution guanidines (Table 5). Analogues of *N*-(2-chloro-5-ethylphenyl)guanidines, the *N*,*N*′-desmethyl (**25a**), *N*′-methyl (**25b**), *N*-methyl (**25c**), and *N*,*N*′-dimethyl (**25d**), showed similar binding affinities. Within the other two series, **20** and **27** were also not influenced to any large extent by these substitution changes onguanidine nitrogen. The relatively high level of potency retained by the *N*-methyl derivatives is of particular interest, and the result is in contrast with the SAR exhibited by the analogues of **4**, where a simple methyl substituent appeared to be better tolerated by at least 1 order of magnitude on the nitrogen atom bearing 3-ethylphenyl moiety (N′ nitrogen atom) than on the nitrogen atom bearing the naphthyl substituent (N nitrogen atom).[6a] Diarylguanidines are flexible molecules; they could exist in various conformations with different relative energies. Possibly, *N*-(2,5-disubstituted phenyl)-*N*′-(3-ethylphenyl)guanidines (**6**) are less rigid than *N*-(1-naphthyl)-*N*′-(3-ethylphenyl)-*N*′-methylguanidine (**4**), and they might provide the preferred ligand conformations which could interact with the binding sites maximally. This would provide a rationale for why very slight differences in potency at the NMDA receptor site among di-, tri-, or tetrasubstituted derivatives were observed in this series.

All of the compounds presented here showed moderate to high affinities for the σ binding site. We were able to obtain compounds with high selectivity at NMDA receptors or alternately molecules active at both the NMDA receptor ion-channel site and σ site for in vivo comparison. These results will be reported elsewhere. Several of the compounds included in Table 4 have been determined to be efficacious in a range of anticonvulsant and excitotoxicity models. These include protection against audiogenic seizures in DBA/2 mice, protection against necrotic effects of exogenous NMDA and hypoxia-ischemia in neonatal rats, and neuroprotection in middle cerebral artery occlusion (MCAO) in adult rats.

Conclusion

Sixty-eight novel *N*,*N*′-diphenylguanidines with activity and selectivity at NMDA receptor ion-channel sites were explored. Notably, *N*-(2,5-dibromophenyl)-*N*′-(3-ethylphenyl)-*N*′-methylguanidine (**27b**) showed in vitro activity at both NMDA receptor and σ receptor binding sites and was an efficacious neuroprotective agent in a neonatal rat excitotoxiciy model.[16] *N*-(2-Chloro-5-(methylthio)phenyl)-*N*′-(3-(methylthio)phenyl)-*N*′-methylguanidine (**43b**) and *N*-(2-bromo-5-(methylthio)phenyl)-*N*′-(3-(methylthio)phenyl)-*N*′-methylguanidine (**44b**) were highly active and selective NMDA receptor blockers.

Consistent with literature reports that described a tight SAR for other classes of NMDA receptor ion-channel blockers, our series of compounds demonstrated a wide range of affinities with fairly subtle changes in the substitution pattern. We discovered that 2-bromo-5-(methylthio) was the optimal substituent for R_2 and R_5 of ligand **6** and that the methylthio group was the preferred substituent at the R_3-position of ligand **6**. In addition, the structure−activity relationships revealed that the 2,5-disubstituted phenyl derivatives might interact with the NMDA receptor site differently from the 1-naphthyl analogues. Certain of the trisubstituted guanidines may be useful for potential neuroprotective drug development as well as further pharmacological and biochemical characterization of the NMDA receptor ion channel. On the basis of its favorable pharmacological profile and safety, **43b** (CNS 5161) was selected as a clinical candidate for the prevention of neuropathic pain and neuropsychological deficits resulting from cardiac surgery (CABG).

Experimental Section

Binding Studies. In vitro radioligand binding assays for the NMDA receptor ion-channel sites were performed by using

[^{3}H]MK-801 and rat brain membrane suspensions.[13] In this assay, thrawed crude synaptic membranes, 1 mg of protein/mL with 0.01% Triton X-100 were incubated at 37 °C for 15 min and then washed by centrifugation three times. Glycine (1 μM) and L-glutamate were added to the binding assays to promote the opening of the ion channel and enhance binding. [^{3}H]MK-801 (1 nM, 97 Ci/mmol) was incubated with about 100 mg of membrane protein for 4 h at room temperature. The in vitro radioligand binding assays for the σ receptor sites were performed by utilizing [^{3}H]DTG and guinea pig brain membrane suspensions.[11] Relative affinities of compounds were determined as IC_{50} values from displacement curves, and K_i values were derived from the IC_{50} values as described by Cheng and Prusoff.[12] The results are presented in Tables 2−5.

Chemistry. Melting points were determined in open capillary tubes on a Thomas-Hoover apparatus. Thin-layer chromatography was performed on Merck silica gel 60 F_{254} (0.2 mm) or baker-flex 1B2-F silica gel plates. Guanidines were visualized on TLC with 254-nM UV light or as a blue spot with bromocresol spray reagent (Sigma Chemical Co.). The ^{1}H NMR spectra of all compounds were consistent with their assigned structure. NMR spectra were recorded on a General Electric QE-300, and the chemical shifts were reported in ppm (δ) relative to trimethylsilane (0 ppm). All new compounds were analyzed either for C, H, and N elemental analyses or for exact mass. Compounds analyzed for exact mass were further checked by HPLC. Elemental analyses were performed by M-H-W Laboratories (Phoenix, AZ) or Galbraith Laboratories (Knoxville, TN). High-resolution mass spectra (HRMS) were recorded on a Finnegan MAT 90. 2-Fluoro-5-(trifluoromethyl)aniline was purchased from Maybridge. 2-Chloro-5-(trifluoromethyl)aniline, 2-bromo-5-(trifluoromethyl)-aniline, various dichloroanilines, and monochloroanilines were purchased from Aldrich. The preparation of 2-fluoro-5-ethylaniline, 2-chloro-5-ethylaniline, 2-bromo-5-ethylaniline, 2-chloro-5-(methylthio)aniline, 2-bromo-5-(methylthio)aniline, *N*-methyl-3-methylthio)aniline hydrochloride, *N*-methyl-3-(methylsulfoxy)aniline hydrochloride, and *N*-methyl-3-(methylsulfonyl)-aniline hydrochloride are described below.

Preparation of the Requisite Amines. 2-Fluoro-5-ethylaniline: Step 1:3′-Nitro-4′-fluoroacetophenone. To stirred, precooled fuming nitric acid (40 mL) at −10 °C was added dropwise 4′-fluoroacetophenone (Aldrich; 75 g, 54.3 mmol) over a period of 10 min. The temperature was strictly maintained at −9 to −10 °C for a total of 8 h. The reaction mixture flask was then transferred to the freezer (−10 °C) for storage overnight. Then the reaction mixture was poured onto ice (1.5 kg). The resultant mixture was extracted three times with ether (400 mL). The organic layer was washed four times with NaOH (1 N, 300 mL) as well as brine and concentrated. The crude product was purified by column chromatography (silica gel, eluting with a gradient of hexanes−ethyl acetate, 10:1 to 3:1) to afford 3′-nitro-4′-fluoroacetophenone (27.6 g) as a light yellow liquid: ^{1}H NMR ($CDCl_3$) δ (ppm) 8.2−7.0 (m, 3H, Ar-H), 2.1 (s, 3H, CH_3); MS (EI) *m/e* 183 (M^+ for $C_8H_6NO_3F$).

Step 2. 3′-Amino-4′-fluoroacetophenone. To a stirred mixture of 3′-nitro-4′-fluoroacetophenone (10.04 g, 55 mmol) in 72 mL of concentrated hydrochloric acid was added tin(II) chloride dihydrate (37 g), in portions. After approximately one-third of the material had been added, a rapid rise in the internal reaction temperature (to 95 °C) was noted. The mixture was then heated to reflux for 10 min, resulting in the dissolution of all solids to give a solution. The mixture was then cooled to room temperature and poured onto an ice/water mixture (150 g). The mixture was then further cooled in an ice bath while 50% sodium hydroxide was added until pH 12 was reached. The aqueous layer was extracted twice with ether (50 mL). The combined organic extracts were washed with brine and then dried over sodium sulfate. Removal of the drying agent and in vacuo concentration of the filtrate afforded a yellow-orange oil (8.73 g) which recrystallized on standing. This material was of sufficient purity to be used directly in the next step. ^{1}H NMR ($CDCl_3$) δ (ppm) 8.0−7.0 (m, 3H, Ar-H), 2.1 (s, 3H, CH_3); MS (EI) *m/e* 153 (M^+ for C_8H_8NOF).

Step 3. 2-Fluoro-5-ethylaniline: To a stirred mixture of 3′-amino-4′-fluoroacetophenone (7.56 g, 49.4 mmol) in triethylene glycol (60 mL) was added 4.94 g of sodium hydroxide. Neat hydrazine hydrate (7.2 mL) was added to the mixture in one portion via a syringe. This addition resulted in a slight exotherm (temperature around 50 °C). The reaction flask (three neck, equipped with claisen adapter and receiving flask) was then equipped with a heating mantle and the reaction heated to 100 °C for 1 h. The starting materials were removed by distillation at 150−180 °C, and a single major product remained in the reaction mixture. The reaction mixture was cooled to room temperature with an ice bath and poured into 100 mL of water. The aqueous mixture was extracted three times with ether (125 mL). The combined organic extracts were washed once with water, and once with brine and then dried over potassium carbonate. Concentration of the organic extracts in vacuo afforded the crude product as an amber liquid. This material was further purified by column chromatogtaphy (silica gel, hexanes/ethyl acetate, 2/1) to give 7.11 g of product as a viscous liquid: ^{1}H NMR ($CDCl_3$) δ (ppm) 8.0−7.0 (m, 3H, Ar-H), 2.5 (q, J = 7.6 Hz, 2H, CH_2), 1.2 (t, J = 7.6 Hz, 2H, CH_3); MS (EI) *m/e* 139 (M^+ for $C_8H_{10}NF$). Anal. ($C_8H_{10}NF$) C, H, N.

2-Chloro-5-ethylaniline[14] was prepared in accordance with the process of 2-fluoro-5-ethylaniline, except 3′-nitro-4′-chloroacetophenone was used instead of 3′-nitro-4′-fluoroacetophenone (yield 40% for three steps): viscous liquid; ^{1}H NMR ($CDCl_3$) δ (ppm) 8.1−7.0 (m, 3H, Ar−H), 2.6 (q, J = 7.6 Hz, 2H, CH_2), 1.2 (t, J = 7.6 Hz, CH_3); MS (EI) *m/e* 155 (M^+ for $C_8H_{10}NCl$).

2-Bromo-5-ethylaniline[15] was prepared in accordance with the process of 2-fluoro-5-ethylaniline, except 3′-nitro-4′-bromoacetophenone was used instead of 3′-nitro-4′-fluoroacetophenone (yield 64% for three steps): viscous liquid; ^{1}H NMR ($CDCl_3$) δ (ppm) 8.1−7.1 (m, 3H, Ar-H), 2.5 (q, J = 7.5 Hz, 2H, CH_2), 1.2 (t, J = 7.5 Hz, 3H, CH_3); MS (EI) *m/e* 200 (M^+ for $C_8H_{10}NBr$).

2-Bromo-5-methylmercaptoaniline Hydrochloride. To a stirred solution (cooled to 16−19 °C) of 2-bromo-5-(methylthio)benzoic acid (1.5 g, 6.07 mmol)[16] in DMF (17 mL) was added triethylamine (1.05 mL, 7.28 mmol). After stirring briefly, diphenyl phosphor azidate (1.7 mL, 7.59 mmol) was added by an addition funnel over a 15 min period. After 2 h of stirring at ambient temperature, TLC (SiO_2, cyclohexane/ethyl acetate, 8:1) showed the reaction was completed. To this solution was added distilled water (7 mL), and the mixture was then heated to 65 °C for 2 h. The reaction mixture was concentrated in vacuo at 45 °C to afford a light yellow, syrupy residue. After water (50 mL) was added to this residue, saturated potassium carbonate was added until pH 9. The mixture was then extracted with 40 mL of methylene chloride two times. The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo to yield a yellow oil. The yellow oil was dissolved in 10 mL of ether, and HCl/ether (10 mL, 1 N) was added to provide a white precipitate. The solid was collected by filtration and further purified by column chromatography (SiO_2, hexanes/EtOAc, 100% to 80%). The final product was a white solid (0.6 g, 39% yield): mp: 181−182 °C; ^{1}H NMR (CD_3OD) δ (ppm) 7.8−7.2 (m, 3H, Ar-H), 2.5 (s, 3H, CH_3); MS (EI) *m/e* 218 (M^+ for C_7H_8BrNS). Anal. ($C_7H_8BrNS\cdot HCl$) C, H, N.

2-Chloro-5-(methylthio)aniline Hydrochloride was prepared in accordance with the process of 2-chloro-5-(methylthio)aniline, except 2-chloro-5-(methylthio)benzoic acid (Aldrich) was used instead 2-bromo-5-(methylthio)benzoic acid (yield 56%): mp 180−181 °C; ^{1}H (CD_3OD) δ (ppm) 7.7−7.2 (m, 3H, Ar-H), 2.5 (s, 3H, CH_3); MS (EI) *m/e* 174 (M^+ for C_7H_8ClNS). Anal. ($C_7H_8ClNS\cdot HCl\cdot 0.25H_2O$) C, H, N.

***N*-Methyl-(3-(methylthio)phenyl)amine Hydrochloride:** 3-(Methylthio)aniline (Aldrich; 5 g, 34.8 mmol) was dissolved in formic acid (1.92 mL, 49 mmol) and heated to 100−105 °C under argon overnight. The reaction mixture was cooled to room temperature and extracted with CH_2Cl_2 (75 mL). The organic layer was washed with saturated Na_2CO_3 (30 mL) three times, dried over $MgSO_4$, filtered to remove $MgSO_4$, and concentrated to afford the formamide. The

formamide was dissolved in anhydrous THF (30 mL) under argon. To this solution was added slowly $LiAlH_4$ in THF (50 mL, 1 M) at 0−5 °C. The reaction was warmed to room temperature and stirred for 20 h. To this reaction mixture was added 50 mL of saturated aqueous $MgSO_4$. The organic layer was saved. The water layer was further extracted with EtOAc (50 mL) three times, and the combined organic solution was washed with H_2O (50 mL) and brine (50 mL) and dried over $MgSO_4$. Filtration to remove $MgSO_4$ and concentration to yield the crude product which was purified by column chromatography (SiO_2, hexanes/EtOAc, 8/1). The fractions which contained the product were collected, concentrated, and dried over vacuum to yield *N*-methyl-(3-(methylthio)phenyl)-amine (5.25 g, 98% in yield). It was further converted to its hydrochloride salt (5.5 g, 97% in yield): mp 123−124 °C; ^{1}H NMR (CD_3OD) δ (ppm) 7.9−7.5 (m, 4H, ArH), 3.1 (s, 3H, CH_3), 2.9 (s, 3H, $SOCH_3$); MS (EI) *m/e* 153 (M^+ for $C_8H_{11}NS$).

***N*-Methyl-3-(methylsulfoxy)aniline Hydrochloride.** Hydrogen peroxide (30% in water, 10.22 mL, 1 mol; Aldrich) was added to a solution of *N*-methyl-3-(methylthio)aniline (3.0 g, 19.6 mmol) in acetone (17 mL) at 0−5 °C. The reaction was stirred overnight. Then acetone was removed. To the water layer was added 1 N NaOH until pH 12, and the mixture was further extracted with ether (30 mL) three times. The combined organic layers were dried over $MgSO_4$. Filtration to remove $MgSO_4$ and concentration yielded the crude product which was purified by column chromatography (silica gel, eluted by EtOAc/MeOH,100% to 90%). *N*-Methyl-3-(methylsulfoxy)aniline was obtained and was further converted to its hydrochloride salt (1.82 g, 45% in yield): mp 137−138 °C; ^{1}H NMR (CD_3OD) δ (ppm) 7.9−7.6 (m, 4H, ArH), 3.1 (s, 3H, NCH_3), 2.9 (s, 3H, $SOCH_3$); MS (EI) *m/e* 169 (M^+ for $C_8H_{11}NSO$).

***N*-Methyl-3-(methylsulfonyl)aniline hydrochloride** was a side product obtained from the prepartion of *N*-methyl-3-(methylsulfoxy)aniline; *N*-methyl-3-(methylsulfonyl)aniline was further converted to its hydrochloride salt (1.8 g, 42% in yield): mp 169−170 °C; ^{1}H NMR (CD_3OD) δ (ppm) 7.9−7.5 (m, 4H, ArH), 3.2 (s, 3H, SO_2CH_3), 3.1 (s, 3H, NCH_3); MS (EI) *m/e* 185 (M^+ for $C_8H_{11}NSO_2$).

General Method for the Preparation of Di-, Tri-, or Tetrasubstituted Guanidines. Method A: Illustrated by the Synthesis of *N*-(2,5-Disubstituted phenyl)-*N*′-(3-ethylphenyl)guanidines. Step 1. Synthesis of (3-ethylphenyl)cyanamide. A solution of cyanogen bromide (11.36 g, 107 mmol) in anhydrous diethyl ether (50 mL) was added slowly to a stirred solution of 3-ethylaniline (20.8 g, 171 mmol) in diethyl ether at 4 °C. After the addition, the reaction mixture was stirred at 24 °C for 12 h and became a brown solution with a white precipitate. The precipitate was filtered off; the filtrate was washed with aqueous HCl (1 N, 3 × 150 mL) and brine (60 mL). Then the ether solution was dried over $MgSO_4$, filtered, and concentrated to yield a thick liquid. The crude product was further purified by chromatography (SiO_2, a gradiant of hexanes/CH_2Cl_2) to afford (3-ethylphenyl)cyanamide (11.6 g, 76% in yield) as a liquid: ^{1}H NMR ($CDCl_3$) δ (ppm) 8.1−7.1 (m, 4H, Ar-H), 2.5 (q, J = 7.5 Hz, 2H, CH_2), 1.2 (t, J = 7.5 Hz, 3H, CH_3); MS (EI) *m/e* 146 (M^+ for $C_9H_{10}N_2$).

Step 2. Preparation of the Requisite Substituted Aniline Hydrochloride. To a solution of substituted aniline (9 mmol) in methanol (10 mL) was added methanolic HCl (1 M, 30 mL) at 4 °C, and then the reaction mixture was stirred at 25 °C for 30 min. The resulting solution was then evaporated and dried under vacuum to afford the substituted aniline hydrochloride (85−98% yield).

Step 3. Synthesis of *N*-(2,5-Disubstituted phenyl)-*N*′-(3-ethylphenyl)guanidine. A solution of the appropriate 2,5-disubstituted aniline hydrochloride (3 mmol) and (3-substituted)phenylcyanamide (3.3 mmol) in 3 mL of chlorobenzene under nitrogen was heated to 140−150 °C with stirring for 2−15 h with occasional TLC monitoring. The reaction mixture was cooled to 23 °C. The pure product was obtained either by crystallization from chlorobenzene/diethyl ether or column chromatography (SiO_2, gradient elution by 0−10% MeOH in CH_2Cl_2).

Method B: Illustrated by the Synthesis of *N*-(2,5-Disubstituted phenyl)-*N*′-(3-ethylphenyl)-*N*′-methylguanidines. Step 1. Synthesis of *N*-(3-Ethylphenyl)-*N*-methylcyanamide. A suspension of 3-ethylphenyl cyanamide (4.65 g, 31.8 mmol) and sodium hydride (2.55 g of 80% NaH in mineral oil suspension, 63.6 mmol of NaH) in dried THF was heated at reflux for 3 h. The reaction mixture was cooled in an ice bath, and methyl iodide (11.28 g, 79.5 mmol) was added dropwise with stirring to the mixture. The reaction mixture was then allowed to stir for 15 h, followed by the addition of MeOH (10 mL). The reaction mixture was then concentrated to dryness to give the crude product. To this crude product was added distilled water (40 mL), and the mixture was extracted with CH_2Cl_2 (4 × 40 mL). The combined organic extracts were washed with water (3 × 30 mL) and then dried over $MgSO_4$. The solvent was removed to afford the crude product as an amber syrup. Column chromatography (SiO_2, CH_2Cl_2) of the crude product afforded 4.2 g (75% yield) of the desired product.

Step 2. Synthesis of *N*-(2,5-Disubstituted phenyl)-*N*′-(3-ethylphenyl)-*N*′-Methylguanidine. A solution of the appropriate 2,5-disubstituted aniline hydrochloride (3 mmol) and *N*-(3-ethylphenyl)-*N*-methylcyanamide (3.3 mmol) in 2−3 mL of chlorobenzene under nitrogen was heated to 140−150 °C with stirring for 2−15 h with occasional TLC monitoring. The reaction mixture was cooled to 23 °C. The pure product was obtained either by crystallization from chlorobenzene/diethyl ether or column chromatography (SiO_2, gradient elution by 0−10% MeOH in CH_2Cl_2).

Method C: Illustrated by the Synthesis of *N*-(2,5-Disubstituted phenyl)-*N*′-(3-ethylphenyl)-*N*,*N*′-dimethylguanidine. Step 1. Preparation of *N*-Methyl-2,5-disubstituted Aniline Hydrochloride. 2,5-Disubstituted aniline (28.7 mmol) was dissolved in formic acid (97%, 1.85 g, 40.2 mmol) and magnetically stirred at 100−105 °C under argon. After 6 h, the reaction mixture was cooled to 25 °C and diluted with dichloromethane (40 mL). Then the mixture was washed with saturated sodium bicarbonate (3 × 30 mL) as well as brine; the organic extracts were further dried over $MgSO_4$ and evaporated to yield the formamide (22 mmol, 76%) as an amber syrup, which was used in the next step without further purification. $LiAlH_4$−THF solution (1.0 M, 26 mmol) was added slowly into the solution of formamide (22 mmol) in THF (23 mL) at 0−4 °C, and then the mixture was brought to 25 °C and stirred for 20 h. Then a saturated sodium sulfate solution was added and the THF was evaporated. Subsequently, the reaction mixture was extracted by EtOAc, and the organic layers were dried over $MgSO_4$ and evaporated. The crude product was purified by column chromatography to yield the pure *N*-methyl-2,5-disubstituted aniline (50%). The *N*-methyl-2,5-disubstituted aniline was further converted into its hydrochloiride described in method A.

Step 2. Synthesis of *N*-(2,5-Disubstituted phenyl)-*N*-(3-ethylphenyl)-*N*,*N*′-dimethylguanidine. Aluminum chloride (3.5 mmol) was added to a stirred solution of *N*-(3-ethylphenyl)-*N*-methylcyanamide (3.3 mmol) in chlorobenzene at 25 °C and stirred for 5 min. Then the appropriate *N*-methyl-2,5-disubtituted aniline hydrochloride (3 mmol) was added, and the mixture was heated at 100−120 °C for 5−10 h to yield the crude product. The product was further purified by column chromatography (SiO_2, gradient elution by 0−10% MeOH in CH_2Cl_2) to afford the desired guanidine hydrochloride.

Acknowledgment. We thank Dr. Michael Perlman and Mrs. Lu Zhang for providing analytical support and physical chemical data to assist our studies. We acknowledge Dr. William Holt for identifying CNS 5161 as a clinical candidate. We also thank Dr. Robert McBurney and William Holt for their helpful discussion in this research.

Supporting Information Available: Experimental data for compounds 7−51 (12 pages). Ordering information is given on any current masthead page.

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JM970459C