

Synthesis and Structure-Activity Relationships of *N,N'*-Di-*o*-tolylguanidine Analogues, High-Affinity Ligands for the Haloperidol-Sensitive σ Receptor

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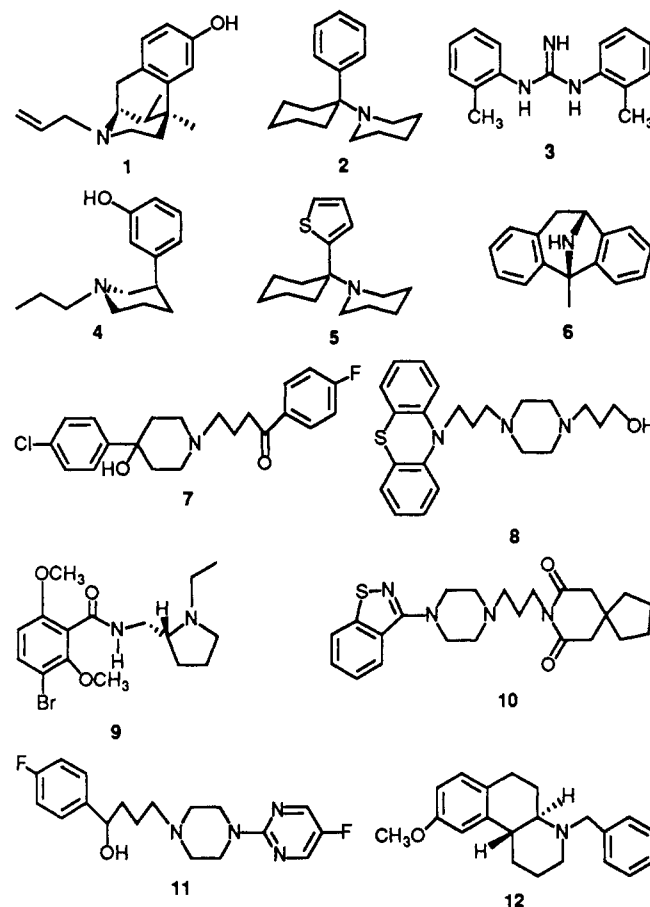
With an eye toward the development of novel atypical antipsychotic agents, we have studied the structure-affinity relationships of *N,N'*-di-*o*-tolylguanidine (DTG, **3**) and its congeners at the haloperidol-sensitive σ receptor. A number of DTG analogues were synthesized and evaluated in *in vitro* radioligand displacement experiments with guinea pig brain membrane homogenates, using the highly σ -specific radioligands [³H]-**3** and [³H]-(+)-**3**-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine and the phencyclidine (PCP) receptor specific compounds [³H]-*N*-[1-(2-thienyl)cyclohexyl]piperidine and [³H]-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine. The affinity of *N,N'*-diarylguanidines for the σ receptor decreases with increasing steric bulk of ortho substituents larger than C₂H₅. Hydrophobic substituents are generally preferred over similarly positioned hydrophilic ones. Furthermore, electroneutral substituents are preferred over strongly electron donating or withdrawing groups. Significant binding to the σ receptor is usually retained as long as at least one side of the guanidine bears a preferred group (e.g. 2-CH₃C₆H₅). Replacement of one or both aryl rings with certain saturated carbocycles (e.g. cyclohexyl, norbornyl, or adamantyl) leads to a significant increase in affinity. By combining the best aromatic and best saturated carbocyclic substituents in the same molecule, we arrived at some of the most potent σ ligands described to date (e.g. *N*-*exo*-2-norbornyl-*N'*-(2-iodophenyl)guanidine, IC₅₀ = 3 nM vs [³H]-**3**). All of the compounds tested were several orders of magnitude more potent at the σ receptor than at the PCP receptor, with a few notable exceptions. This series of disubstituted guanidines may be of value in the development of potential antipsychotics and in the further pharmacological and biochemical characterization of the σ receptor.

Introduction

Certain benzomorphan opioids, represented by (+)-*N*-allylnormetazocine ((+) SKF-10,047, **1**) (Chart I), cause hallucinations and other bizarre behavioral effects in mammals.¹ A similar syndrome is elicited by phencyclidine (PCP, **2**),²⁻⁴ which has been described as the best available drug model for schizophrenia.⁵ *In vitro* radioligand binding and brain distribution experiments have distinguished two receptors which may mediate the psychotomimetic syndrome.⁶⁻¹⁹ They have been termed the haloperidol-sensitive σ receptor,²⁰ characterized by the selective ligands [³H]-*N,N'*-di-*o*-tolylguanidine ([³H]DTG, [³H]-**3**)²¹ and [³H]-(+)-**3**-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine ([³H]-(+)-**3**-PPP, [³H]-**4**),^{15,22} and the PCP receptor, characterized by its selective ligands [³H]-*N*-[1-(2-thienyl)cyclohexyl]piperidine ([³H]TCP, [³H]-**5**)²³ and [³H]-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine ([³H]-(+)-MK-801, [³H]-**6**).²⁴⁻²⁶ Benzomorphan and PCP (**2**) can bind to both σ and PCP receptors. Although drug-discrimination studies in animals indicate that a significant part of the behavioral effects of PCP (**2**) and benzomorphan are mediated by PCP receptors, it remains to be established which of the two receptors mediates the psychotomimetic syndrome caused by these drugs in humans.^{27,28}

Antipsychotic neuroleptic drugs, widely used in the treatment of schizophrenia, act as antagonists of the dopamine D₂ receptor. Antagonist actions at this site are thought to mediate the therapeutic effects as well as the serious extrapyramidal side effects of these drugs. Interestingly, some of the most potent and clinically useful neuroleptics, such as haloperidol (**7**) and perphenazine (**8**), have high affinity for the σ receptors.⁶⁻⁹ Furthermore, several atypical antipsychotic drugs, including remoxipride (**9**) and tiospirone (**10**), have recently been shown to bind

Chart I



tightly to the σ receptor, and may exert their beneficial effects through this site.²⁹⁻³⁵ In clinical trials and in animal

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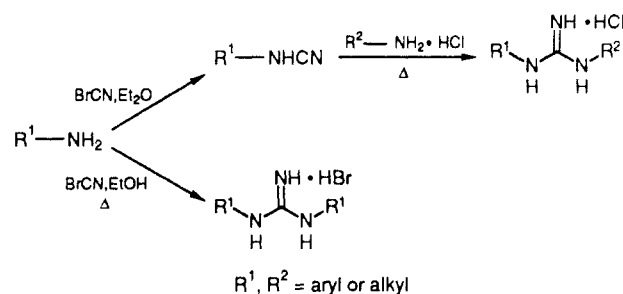
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models predictive of antipsychotic efficacy, these and certain other non-dopaminergic antipsychotics (e.g. [α -(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazinebutanol, BMY 14802, 11] are devoid of the severe extrapyramidal side effects typically associated with D_2 receptor antagonism.^{30,31} These findings suggest that the σ receptor may provide a novel therapeutic target in the treatment of schizophrenia. The biochemical function of the σ receptor is, however, still unclear.

The σ receptor is evidently not a dopamine receptor,^{6,8,9} but it does appear to be involved in catecholamine release. Su et al.^{36,37} and Campbell et al.³⁸ have described bio-

Scheme I. Synthetic Routes to Unsymmetrical (Top) and Symmetrical (Bottom) N,N' -Disubstituted Guanidines



chemically functional σ receptors in rodent vasa deferentia, in which (+)-3-PPP (4) enhances the electrically stimulated release of norepinephrine. Steinfelds and Tam have reported that microinjected (+)-3-PPP (4) dose dependently inhibits the firing of dopaminergic neurons in anesthetized rats, and that 11 dose dependently antagonizes this response.³⁹ In experiments in a guinea pig ileum longitudinal muscle/myenteric plexus (LMMP) preparation, Campbell et al. have shown that certain σ receptor ligands dose dependently inhibit electrochemically or serotonin induced contractions of the LMMP via an opioid receptor independent mechanism.⁴⁰ These results suggest that the σ receptor may mediate its antipsychotic effects by inhibiting neurotransmitter release.

The biological function of the PCP receptor is now well characterized. In *in vitro* electrophysiological experiments, PCP receptor ligands such as 6,⁴¹⁻⁴³ TCP (5),^{44,45} PCP (2),⁴⁶ and certain N,N' -disubstituted guanidines⁴⁷⁻⁴⁹ (vide infra) potentially obstruct the *N*-methyl-D-aspartate (NMDA) class of glutamate-gated, nonselective cation channels.⁵⁰⁻⁵⁴ As a result, PCP receptor ligands are powerful neuroprotective agents against glutamate-induced neuronal cell death.⁵⁵⁻⁵⁸

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Such compounds have considerable therapeutic potential in the treatment of stroke, heart attack, brain trauma, or any other acute disorder involving ischemia.^{51,59,60} NMDA receptor antagonism may also be responsible for the psychotomimetic effects of PCP receptor ligands.^{14,27,61-64} These unwanted side effects may limit the usefulness of NMDA receptor antagonists as neuroprotective agents.

Our eventual goal is the complete biochemical characterization of the σ receptor. The combination of both the physiological and biochemical characterization of the σ receptor may yield new medicinal strategies for the development of novel antipsychotic agents. The design and synthesis of potent, highly specific probes for the σ receptor plays a central role in our efforts. Structurally simple derivatives of DTG (3) have already provided powerful tools for the characterization and isolation of the σ receptor.^{19,21,65,66}

Herein, we report the preparation of more than 70 DTG congeners (Tables I-V) and their affinities for the σ receptor, as measured by their ability to displace [³H]-3 and/or [³H]-4 from guinea pig brain membrane suspensions. Given the tendency of other σ receptor ligands to cross-react with the PCP receptor, and the therapeutic potential of PCP receptor ligands, we also compiled displacement data for all compounds against [³H]-5 and/or [³H]-6.⁶⁷ We discuss structure-activity relationships in this series of guanidines and compare our results with recent efforts by Largent et al.⁶⁸⁻⁷⁰ and Manallack et al.^{28,71,72} to define the topographical requirements for high affinity binding to the σ receptor.

Chemistry

Unsymmetrical N,N'-disubstituted guanidines were prepared by the following known methods (Scheme I): (a) coupling an aryl or alkyl cyanamide with the appropriate amine hydrohalide salt either in refluxing chlorobenzene,⁷³ or (b) directly in a 1:1 melt without solvent.⁷⁴ The re-

quisite cyanamides were synthesized from the corresponding amines by treatment with cyanogen bromide (BrCN) in dry ethereal solution,^{73,74} or in the case of deactivated aromatic amines, in aqueous solution.⁷³ Symmetrical N,N'-disubstituted guanidines were readily obtained by directly reacting 2 equiv of the amine with 1 equiv of BrCN in ethanol (EtOH), without isolating the intermediate cyanamide. The N,N',N''-trisubstituted guanidines were prepared by reacting dicyclohexylcarbodiimide with an amine in tetrahydrofuran (THF).⁷⁵ Cyclization of the appropriate diamine with BrCN in EtOH gave rise to the rigid guanidines 82,⁷⁶ 84,⁷⁷ 85,⁷⁸ 86,⁷⁹ and 87.⁸⁰ Catalytic hydrogenation¹⁹ of nitrophenylguanidines 19, 26, 27, and 41 gave the corresponding aminophenylguanidines smoothly. The recent report of steroid binding at the σ receptor⁸¹ prompted us to prepare several steroidal guanidines (Table V). The preparation of the requisite aminosteroids was achieved by published procedures.⁸²

Binding Studies

In vitro radioligand binding assays using guinea pig brain membrane suspensions provided a rank order of potency of all compounds at the σ receptor and the PCP receptor, as determined by their IC₅₀ vs [³H]-3 and [³H]-4, or [³H]-5, and [³H]-6, respectively. The data are compiled in Tables I-V in order of decreasing potency at the σ receptor (i.e. increasing IC₅₀ versus [³H]-3). In general, all compounds are orders of magnitude more potent at the σ receptor than at the PCP receptor, with several notable exceptions (vide infra).

Results and Discussion

σ Receptor Affinity. The affinity of N,N'-diarylguanidines for the σ receptor is a sensitive function of their substitution pattern. Of the metasubstituted guanidines, N,N'-bis(3-ethylphenyl)guanidine (13) is the most potent (IC₅₀ of 8.3 nM vs [³H]-3). Modification of the meta substituent, either by decreasing (21) or increasing (24) steric bulk or by introducing iodine (29) or an hydroxyl group (30), results in reduced affinity.

The ortho position appears to be more tolerant to structural modifications. Small alkyl substituents such as CH₃ (DTG, 3) or C₂H₅ (14), or especially iodine (cf. 15 and 16), bestow high σ affinity. Thus, N,N'-bis(2-iodophenyl)guanidine (15, IC₅₀ 14 nM vs [³H]-3) proved to be among the most potent σ ligands of the N,N'-diarylguanidines (Table I) series. σ affinity decreases with increasing steric bulk in the ortho position: cf. ethyl (14) vs isopropyl (22) vs *tert*-butyl (35). Larger unsaturated substituents, such as phenyl (47) or styryl (41 and 44) result in a sharp drop in potency. Strongly electron withdrawing or donating ortho substituents such as nitro (26), trifluoromethyl (31), amino (37), or methoxy (45) decrease binding significantly.

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Table I. *N,N'*-Diarylguanidines and Their IC₅₀s against [³H]-3, [³H]-4, [³H]-5, and/or [³H]-6

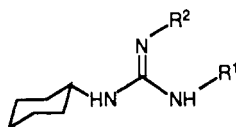
compd	X ^b	Y	mp, °C	proced (yield, %) ^c	formula ^d	IC ₅₀ (nM) against ^e			
						[³ H]-3	[³ H]-4	[³ H]-5	[³ H]-6
13	3-C ₂ H ₅	3-C ₂ H ₅	96-98	B (20)	C ₁₇ H ₂₁ N ₃	8.3 ± 0.2	30	82 ± 10	168 ± 38
14	2-C ₂ H ₅	2-C ₂ H ₅	158-161 ^e	B (59)	C ₁₇ H ₂₁ N ₃	13.8 ± 0.9	44	358 ± 53	820 ± 66
15	2-I	2-I	161-162	B (39)	C ₁₃ H ₁₁ I ₂ N ₃	14 ± 1	16 ± 1	210 ± 60	240 ± 60
16	2-CH ₃	2-I	163-165	A (57)	C ₁₄ H ₁₄ IN ₃	21 ± 1	23 ± 2	2050 ± 50	nd
3	2-CH ₃	2-CH ₃	148-150	f	C ₁₃ H ₁₇ N ₃	32 ± 1	38 ± 6	7800 ± 400	10 700 ± 2100
17	3-CH ₃ (CH ₂) ₂	3-CH ₃ (CH ₂) ₂	69-70	B (20)	C ₁₉ H ₂₅ N ₃	36 ± 2	nd	nd	2110 ± 10
18	4-Br-2-CH ₃	4-Br-2-CH ₃	209-210	A (8) ^g	C ₁₅ H ₁₅ Br ₂ N ₃	37 ± 3	32 ± 1	>10 000	nd
19	2-CH ₃	2-CH ₃ -4-NO ₂	177-179	A (91) ^h	C ₁₅ H ₁₆ N ₄ O ₂	37 ± 5	39 ± 2	5700 ± 495	nd
20			217-218	B (48)	C ₂₁ H ₂₆ N ₃ ⁱ	58.8 ± 3.2	nd	nd	1570 ± 210
21	3-CH ₃	3-CH ₃	105-107 ^j	B (38)	C ₁₅ H ₁₇ N ₃	59 ± 4	84 ± 3	370 ± 30	330 ± 30
22	2-(CH ₃) ₂ CH	2-(CH ₃) ₂ CH	175-177 ^k	B (53)	C ₁₉ H ₂₅ N ₃	65 ± 7	nd	nd	275 ± 69
23	2-CH ₃	2,6-CH ₃	216-218	A (47)	C ₁₆ H ₁₉ N ₃	70 ± 5	42 ± 7	1900	nd
24	3-(CH ₃) ₂ CH	3-(CH ₃) ₂ CH	118-119	B (32) ^l	C ₁₉ H ₂₅ N ₃	77 ± 23	nd	nd	498
25	2,6-CH ₃	2,6-CH ₃	245-246 ^m	B (25)	C ₁₇ H ₂₁ N ₃	90 ± 18	66 ± 8	32 000	nd
26	2-CH ₃	2-NO ₂	124-125	A (67)	C ₁₄ H ₁₄ N ₄ O ₂	113 ± 15	nd	2100 ± 71	nd
27	2-CH ₃ -4-NO ₂	3-OH	222-223	A (58)	C ₁₄ H ₁₄ N ₄ O ₃	118 ± 5	94 ± 4	37 000	nd
28			210-211 ⁿ	B (30)	C ₂₁ H ₁₇ N ₃	133 ± 39	nd	nd	134 ± 39
29	3-I	3-I	172-173	B (15)	C ₁₃ H ₁₁ I ₂ N ₃ ^o	173 ± 42	nd	1100 ± 88	nd
30	2-CH ₃	3-OH	157-159	A (85)	C ₁₄ H ₁₅ N ₃ O·HCl· 0.25H ₂ O	207 ± 19	155 ± 25	2500 ± 212	nd
31	2-CF ₃	2-CF ₃	149-150	B (3)	C ₁₅ H ₁₁ F ₆ N ₃	215 ± 7	766 ± 119	4150 ± 350	nd
32	4-C ₂ H ₅	4-C ₂ H ₅	136-138 ^p	B (38)	C ₁₇ H ₂₁ N ₃	245 ± 38	nd	nd	10 300 ± 600
33	4-(CH ₃) ₂ CH	4-(CH ₃) ₂ CH	137-139	B (27)	C ₁₉ H ₂₅ N ₃	270 ± 26	nd	nd	26 800 ± 15 100
34	2-CH ₃	2-CH ₃ -4-NH ₂	232.5-234.5	(78) ^q	C ₁₅ H ₁₈ N ₄ ·HCl	280 ± 14	220 ± 14	4350 ± 389	nd
35	2-(CH ₃) ₃ C	2-(CH ₃) ₃ C	204-205	B (33)	C ₂₁ H ₂₉ N ₃	356 ± 63	nd	nd	38 300 ± 14 900
36	H	H	176-178	f	C ₁₅ H ₁₇ N ₃	397 ± 21	400	3450 ± 318	nd
37	2-CH ₃	2-NH ₂	161-162	(91) ^r	C ₁₄ H ₁₆ N ₄	463 ± 15	440 ± 35	12 000	nd
38	4-CH ₃	4-CH ₃	168-170 ^s	B (20)	C ₁₅ H ₁₇ N ₃ ^t	535 ± 62	nd	31 000	13 300 ± 3300
39	4-Br	4-Br	166-168	B (27)	C ₁₅ H ₁₁ Br ₂ N ₃	540 ± 25	350 ± 35	34 000	nd
40	2-CH ₃ -4-NO ₂	2-CH ₃ -4-NO ₂	248-249	A (63)	C ₁₅ H ₁₅ N ₅ O ₄ ·HCl	760 ± 169	850	4300	nd
41	2-CH ₃	2-(E-CHCHC ₆ H ₅)	168-169	A (41) ^u	C ₂₂ H ₂₁ N ₃ ^v 0.25H ₂ O	920 ± 201	nd	nd	>10 000
42			190-191	A (36)	C ₂₃ H ₁₉ N ₃	935 ± 44	nd	nd	>10 000
43	2-CH ₃ -4-NH ₂	3-OH	242-248	(100) ^w	C ₁₄ H ₁₆ N ₄ O· 2HCl	962 ± 89	nd	nd	>10 000
44	2-I	2-(E-CHCHC ₆ H ₅)	159-161	A (45)	C ₂₁ H ₁₈ IN ₃	1200 ± 200	nd	nd	>10 000
45	2-CH ₃ O	2-CH ₃ O	117-119 ^x	B (35)	C ₁₅ H ₁₇ N ₃ O ₂	2200	3800	nd	1600
46	2-CH ₃ -4-NH ₂	2-CH ₃ -4-NH ₂	>350, dec	(100) ^y	C ₁₅ H ₁₉ N ₃ ·3HCl	7150 ± 106	5100 ± 353	>100 000	nd
47	C ₆ H ₅	C ₆ H ₅	181-182	B (62)	C ₂₅ H ₂₁ N ₃	8113 ± 43	nd	nd	>10 000

^a [³H]-3, [³H]*N,N'*-Di-*o*-tolylguanidine; [³H]-4, [³H]-(+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine; [³H]-5, [³H]-*N*-[1-(2-thienyl)cyclohexyl]piperidine; [³H]-6, [³H]-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohept-5,10-imine; nd, not determined. IC₅₀ values are mean ± SEM and, with a few exceptions, are the result of three to seven determinations. Values without error limits were obtained from single determinations. ^b For those compounds, in which X ≠ Y, the guanidine was prepared by coupling the cyanamid derived from the X entry with the amine hydrohalide salt of the Y entry. For compounds 20, 28, and 42 the entire structure is shown. See the Experimental Section for details. ^c Yields refer to analytically pure products. ^d Elemental analyses for all new compounds were obtained for C, H, and N, and were within 0.4% of the theoretical values for the indicated molecular formula. ^e Lit. mp 161-162 °C. Beaver, D. J. U.S. Patent 2 633 474, 1953; *Chem. Abstr.* 1953, 47, 6171f. ^f Purchased from Aldrich Chemical Co. ^g Reference 21. ^h Reference 19. ⁱ C: calcd, 78.96; found, 78.40. ^j Lit. mp 110 °C. Ali, M. U.; Paranjpe, M. G. *J. Indian Chem. Soc.* 1986, 63, 253. ^k Ikeda, T.; Imai, E.; Fukumoto, H.; Tanaka, K.; Suematsu, K.; Urawa, M.; Takenouchi, M. Eur. Patent 179 642, 1986; *Chem. Abstr.* 1986, 105, 181470h. ^l 3-Isopropylaniline was prepared according to the literature procedure: Saxena, A. K.; Arunamurthy, V.; Patnaik, G. K.; Jain, P. C.; Anand, N. *Indian J. Chem.* 1980, 19B, 873. ^m Lit. mp 252-253 °C. Lempert, K.; Puskas, J.; Bekassy, S. *Period. Polytech., Chem. Eng. (Budapest)* 1968, 12, 123; *Chem. Abstr.* 1969, 70, 11239f. ⁿ Lit. mp 197.5-198 °C. Naunton, W. J. S. *J. Soc. Chem. Ind.* 1926, 45, 376; *Chem. Abstr.* 1927, 21, 672. ^o N: calcd, 9.07; found, 8.54. ^p Lit. mp 137-138 °C. Pauksch, H. *Chem. Ber.* 1884, 17, 2804; *Beilstein* 12, 1091c. ^q Prepared by catalytic hydrogenation of 26, as described in ref 19. ^r Lit. mp 166.5-167.5 °C. Nauton, W. J. S. *J. Soc. Chem. Ind.* 1926, 45, 376; *Chem. Abstr.* 1927, 21, 672. ^s C: calcd, 75.28; found, 74.79. ^t 2-Aminostilbene was prepared according to the literature procedure: Ziegler, C. B.; Heck, R. F. *J. Org. Chem.* 1978, 43, 2941. ^u Prepared by catalytic hydrogenation of 27, as described in ref 19. ^v Lit. mp 116-117 °C. Schotte, G. Ger. Patent 509 264, 1927; *Chem. Abstr.* 1931, 25, 712.⁵ ^w Prepared by catalytic hydrogenation of 40, as described in ref 19.

Para substituents of increasing steric bulk lead to a rise in binding affinity: cf. methyl (38) vs ethyl (32) and isopropyl (33). High affinity for the σ receptor is usually but not always (e.g. 37, 41, and 44) retained as long as one of the two aryl rings bears a preferred group such as 2-CH₃ or 2-I. Thus, a para-situated electron withdrawing group is well tolerated in *N*-(2-methylphenyl)-*N'*-(2-methyl-4-

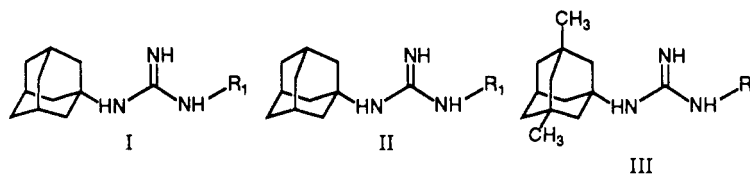
nitrophenyl)guanidine (19), but not in the symmetrical *N,N'*-bis(2-methyl-4-nitrophenyl)guanidine (40). A similar trend is illustrated by the decreasing potency of 3 vs 34 vs 46.

Replacement of one or both of the aryl rings with certain saturated carbocycles such as cyclohexyl (Table II), norbornyl (Table III), or adamantyl (Table IV) leads to a

Table II. Cyclohexyl-Substituted Guanidines and Their IC₅₀s against [³H]-3, [³H]-4, [³H]-5, and/or [³H]-6

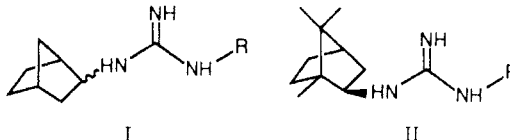
compd	R ₁	R ₂	mp °C	proced (yield, %) ^b	formula ^c	IC ₅₀ (nM) against ^a			
						[³ H]-3	[³ H]-4	[³ H]-5	[³ H]-6
48	2-CH ₃ -C ₆ H ₄	H	145-146	A (22)	C ₁₄ H ₂₁ N ₃	12 ± 3	15 ± 1	>10000	10000
49	adamant-1-yl	H	269-271 ^d	(44) ^e	C ₁₇ H ₂₉ N ₃ ·HCl	13 ± 2	8	>10000	nd
50	C ₆ H ₁₁	H	181-182 ^f	B (30)	C ₁₃ H ₂₅ N ₃	71 ± 7	48 ± 5	>10000	nd
51	C ₆ H ₁₁	OH	123-124 ^g	C	C ₁₃ H ₂₅ N ₃ O	217 ± 36	140 ± 17	>10000	nd
52	C ₆ H ₁₁	CH ₃	278-279 ^h	C (18)	C ₁₄ H ₂₇ N ₃ ·HCl ⁱ	237 ± 56	145 ± 24	>10000	>10000
53	C ₆ H ₁₁	n-C ₈ H ₁₇	174-178	C (37)	C ₂₁ H ₄₁ N ₃ ·HCl	238 ± 76	237 ± 16	nd	>10000
54	C ₆ H ₁₁	CH ₂ CHCH ₂	238-241	C (15)	C ₁₆ H ₂₉ N ₃	513 ± 57	163 ± 15	>10000	>10000

^a See footnote a in Table I. ^b See footnote c in Table I. ^c See footnote d in Table I. ^d Lit.⁷⁴ mp 267-268 °C. ^e Prepared according to the literature procedure (ref 74). ^f Lit. mp 181-182 °C. Chambers, R. W.; Moffatt, J. G.; Khorana, H. G. *J. Am. Chem. Soc.* 1957, 79, 4240. ^g Lit.⁷⁵ mp 123-124 °C. ^h Ouchi, S.; Hayashi, E. *Jap. Patent* 46/27781, 1971; *Chem. Abstr.* 1971, 77, 36249s. ⁱ N: calcd, 15.34; found, 14.81.

Table III. Various Substituted Adamantylguanidines and Their IC₅₀s against [³H]-3, [³H]-4, [³H]-5, and/or [³H]-6

compd	structure	R ₁	mp, °C	proced (yield, %) ^b	formula ^c	IC ₅₀ (nM) against ^a			
						[³ H]-3	[³ H]-4	[³ H]-5	[³ H]-6
55	II	2-I-C ₆ H ₄	248-250	A (43)	C ₁₇ H ₂₂ IN ₃ ·HCl	5.2 ± 0.4	nd	nd	>10000
56	II	2-CH ₃ -C ₆ H ₄	166-167	A (64)	C ₁₈ H ₂₅ N ₃ ^d	6 ± 2	nd	nd	>10000
57	I	2-I-C ₆ H ₄	264-265	A (66)	C ₁₇ H ₂₂ IN ₃ ·HCl	6 ± 5	5	nd	>10000
58	I	2-CH ₃ -C ₆ H ₄	160-161	A (71)	C ₁₈ H ₂₅ N ₃	8 ± 0.3	8 ± 0.3	32000	nd
49	I	C ₆ H ₁₁	269-271	(44) ^e	C ₁₇ H ₂₉ N ₃ ·HCl	13 ± 2	8	>10000	nd
59	III	2-CH ₃ -C ₆ H ₄	248-250	A (59) ^f	C ₂₀ H ₂₉ N ₃ ·HCl	15 ± 7	nd	nd	2000
60	I	adamant-1-yl	289-291 ^g	(15) ^e	C ₂₁ H ₃₃ N ₃ ·HCl	16 ± 1	11	>10000	nd
61	III	2-I-C ₆ H ₄	264-266	A (46)	C ₁₉ H ₂₆ IN ₃ ·HCl	16 ± 6	nd	nd	4000
62	I	2-NO ₂ -C ₆ H ₄	135-136	A (48)	C ₁₇ H ₂₂ N ₄ O ₂	30	20	nd	>10000
63	I		235-236	(80) ^h	C ₂₅ H ₃₁ N ₃ ·HCl ⁱ	112 ± 40	nd	nd	>10000
64	I		254-255	A (49)	C ₂₀ H ₂₇ N ₃ ·HCl	120 ± 8	nd	nd	>10000
65	II	adamant-2-yl	336-338	(48) ^j	C ₂₁ H ₃₃ N ₃ ·HCl	203 ± 80	nd	>10000	nd
66	I		257-258	A (45)	C ₂₅ H ₂₄ F ₅ N ₃ ·HCl·0.8H ₂ O	245 ± 35	nd	nd	>10000
67	I		173-174	A (76)	C ₂₃ H ₂₇ N ₃	300 ± 101	nd	nd	>10000
68	I		234-235	A (60)	C ₂₅ H ₂₉ N ₃ ·HCl	345 ± 136	nd	nd	3500 ± 1314
69	II		205-207	A (58)	C ₂₅ H ₂₉ N ₃ ·HCl·0.5H ₂ O	370 ± 2	nd	nd	>10000
70	III		240-241	A (47)	C ₂₇ H ₃₃ N ₃ ·HCl ^k	970 ± 47	nd	nd	>10000

^a See footnote a in Table I. ^b See footnote c in Table I. The guanidines were prepared from the corresponding adamantyl cyanamides. ^c See footnote d in Table I. ^d C: calcd, 76.32; found, 75.85. ^e See footnote e in Table II. ^f 1-Amino-3,5-dimethyladamantane was prepared according to the literature procedure: Stetter, H.; Mayer, J.; Schwarz, M.; Wulff, K. *Chem. Ber.* 1960, 93, 226. ^g Lit.⁷⁴ mp 288-290 °C. ^h Prepared by catalytic hydrogenation of 68, following the procedure in ref 19. ⁱ C: calcd, 73.28; found, 72.82. ^j Prepared following the literature⁷⁴ procedure for the preparation of 60. ^k C: calcd, 74.39; found, 73.92.

Table IV. Norbornyl-Substituted (I) and Isobornyl-Substituted (II) Guanidines and Their IC₅₀s against [³H]-3, [³H]-4, [³H]-5, and/or [³H]-6


compd	structure (endo/exo)	R	mp, °C	proced (yield, %) ^b	formula ^c	IC ₅₀ (nM) against ^a			
						[³ H]-3	[³ H]-4	[³ H]-5	[³ H]-6
71	I (exo)	2-I-C ₆ H ₄	170-171	A (24)	C ₁₄ H ₁₈ IN ₃	4 ± 1	nd	nd	>10 000
72	I (endo)	2-I-C ₆ H ₄	158-159	A (77)	C ₁₄ H ₁₈ IN ₃	5 ± 2	nd	nd	>10 000
73	I (endo)	2-CH ₃ -C ₆ H ₄	156-158	A (68)	C ₁₅ H ₂₁ N ₃	6 ± 1	nd	nd	>10 000
74	I (exo)	2-CH ₃ -C ₆ H ₄	169-170	A (86)	C ₁₅ H ₂₁ N ₃ ·0.15H ₂ O	7.7 ± 0.2	nd	nd	>10 000
75	I (endo)	endo-norborn-2-yl	248-250	A (68)	C ₁₅ H ₂₅ N ₃ ·HCl	16 ± 1	nd	nd	>10 000
76	II	2-I-C ₆ H ₄	248-250	A (25)	C ₁₈ H ₂₇ N ₃ ·HCl	18 ± 6	nd	nd	>10 000
77	I (exo)	exo-norborn-2-yl	293-295	A (80)	C ₁₅ H ₂₁ N ₃ ·HBr	22	nd	nd	5 000
78	II	2-CH ₃ -C ₆ H ₄	156-157	A (68)	C ₁₅ H ₂₅ N ₃	25 ± 4	nd	nd	5 000

^a See footnote a in Table I. ^b See footnote c in Table I. The guanidines were prepared from the corresponding (±)-norbornyl or (R)-(-)-isobornyl amines. ^c See footnote d in Table I.

significant increase in affinity for the σ receptor. For example *N*-cyclohexyl-*N'*-(2-methylphenyl)guanidine (48) exhibits an IC₅₀ of 12 nM vs [³H]-3, compared to 32 nM for DTG (3). Symmetrical *N,N'*-diadamantyl-(60) and *N,N'*-dinorbornylguanidines (75 and 77) are potent σ receptor ligands, with affinities in the low nanomolar range. The structure-activity relationships described above in the *N,N'*-diarylsubstituted guanidines series are also born out in the *N*-aryl-*N'*-norbornyl (Table IV), and *N*-aryl-*N'*-adamantyl (Table III) guanidines. Thus, large aromatic ortho substituents greatly reduce affinity (63, 64, 66-70). We were gratified to find that ortho substitution with preferred groups (55-58 and 71-74) provides some of the most potent σ receptor ligands reported to date (e.g. *N*-(*exo*-norborn-2-yl)-*N'*-(2-iodophenyl)guanidine (71), IC₅₀ 4 nM vs [³H]-3). The sheer steric bulk of the saturated carbocycles does not appear to be solely responsible for the high affinity of these ligands, since increasing the steric bulk further does not result in increased σ receptor affinity. Thus, the 1- or 2-adamantylguanidines (57 and 58, 55 and 56, respectively) are by a factor of approximately 3 more potent than their 3,5-dimethyladamant-1-yl analogues (59 and 61). Similarly, the *exo*- or *endo*-2-norbornylguanidines (71 and 74, 72 and 73, respectively) are approximately 4 times more potent than their *exo*-2-isobornyl-substituted analogues (76 and 78). In summary, this series of cycloalkyl-substituted guanidines includes excellent σ receptor ligands, with several compounds approaching subnanomolar IC₅₀s against [³H]-3.

Several *N,N'*-dicyclohexyl-*N''*-substituted-guanidines (51-54) were prepared (Table II). Although they are all less potent than the parent *N,N'*-dicyclohexylguanidine (50), they retain significant binding to the σ receptor.

Recently, Su et al. have reported the binding of several steroids, notably progesterone, to the σ receptor.⁸¹ This prompted us to prepare the steroidal guanidines 81 and 88 (Table V). We chose these particular steroids for their structural similarity to progesterone and their availability. The pregnen-20-one derivative 81 proved to have moderate affinity for the σ receptor. The structural requirements for steroid binding at the σ receptor are not known. It is interesting to note that the octahydrobenzo[*f*]quinoline (OHBQ) series of σ receptor ligands⁶⁸⁻⁷⁰ (e.g. (±)-*trans*-9-methoxy-*N*-benzyl-OHBQ, 12) share the same ring system with the A, B, and C rings of the steroid nucleus. Unlike the OHBQ series however, the steroids reported to bind at the σ receptor lack of nitrogen atom.

Rigid Analogues. We have prepared (Table V) several rigid guanidines as an approach toward defining the con-

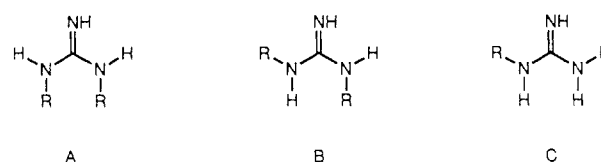


Figure 1. The three possible conformations of symmetrical *N,N'*-disubstituted guanidines. Conformation A is designated as anti,anti; B as syn,anti; C as syn,syn.

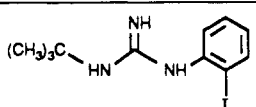
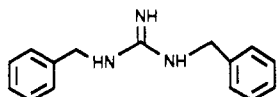
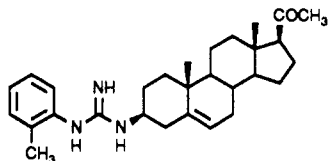
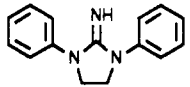
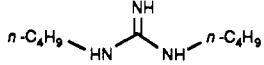
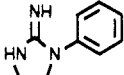
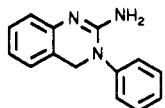
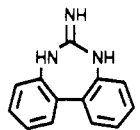
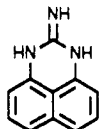
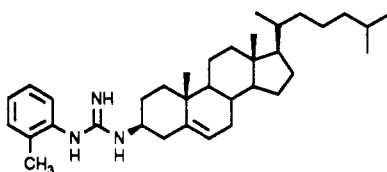
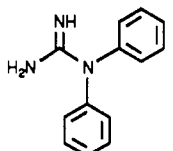
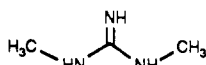
formation in which *N,N'*-disubstituted guanidines bind at the σ receptor. Space-filling molecular models clearly indicate that steric crowding precludes those rotational isomers around the guanidine carbon-nitrogen bond in which both guanidine substituents simultaneously occupy the positions anti to the unsubstituted nitrogen (i.e. conformation A in Figure 1). Therefore, two possible rotomers around a symmetrically *N,N'*-disubstituted guanidine function remain (Figure 1): either a syn,anti conformation (B), or a syn,syn conformation (C). In the single-crystal X-ray crystallographic analysis of both the free base and the hydrochloride salt of *N*-adamant-1-yl-*N'*-(2-iodophenyl)guanidine (57), we found that the former crystallized in the syn,anti, and the latter in the syn,syn conformation.⁸³ In both cases the phenyl ring was essentially perpendicular to the plane of the guanidine function. This solid state analysis suggests that both syn,syn and syn,anti conformations are energetically accessible to the *N,N'*-disubstituted guanidines.

We prepared rigid guanidines which were designed to test all three of these conformations. Amino diazepine 86 and amino perimidine 87 are analogues of the anti,anti conformation, and proved to be, as expected, completely devoid of affinity for the σ receptor. The amino quinazoline 85 simulates the syn,anti conformation, and shows poor σ binding (IC₅₀ 4100 nM vs [³H]-3); imino imidazolidine 82 mimics the syn,syn conformation and has an IC₅₀ of 340 nM vs [³H]-3, slightly better than its nonrigid analogue, *N,N'*-diphenylguanidine (36). Removal of one of the phenyl rings (84) results in a large drop of binding affinity. These results suggest that the *N,N'*-diarylguanidines may prefer to bind in a syn,syn conformation at the σ receptor, and that the ethylene bridge in 82 does not interfere with high-affinity binding.

σ Receptor Site Model. The structural requirements for high affinity binding to the σ receptor have been dis-

(83) Weakley, T. J. R.; Scherz, M. W.; Keana, J. F. W. *Acta Crystallogr.* 1990, in press.

Table V. Miscellaneous and Rigid Guanidines and Their IC₅₀s against [³H]-3, [³H]-4, [³H]-5, and/or [³H]-6

compd	structure	mp, °C	proced (yield, %) ^b	formula ^c	IC ₅₀ (nM) against ^a			
					[³ H]-3	[³ H]-4	[³ H]-5	[³ H]-6
79		184-186	A (49) ^d	C ₁₁ H ₁₆ IN ₃ ·HCl	21.1 ± 1.5	nd	nd	>9600
80		143-145 ^e	B (20)	C ₁₅ H ₁₇ N ₃ ·HBr	90	33 ± 1	6800 ± 300	nd
81		274-276	A (10) ^f	C ₂₉ H ₄₁ N ₃ O·HCl	250 ± 100	nd	nd	>10000
82		159-161 ^g	B (43)	C ₁₅ H ₁₅ N ₃	340 ± 43	nd	nd	>10000
83		121-122	(37) ^h	C ₉ H ₂₁ N ₃ ·C ₆ H ₃ N ₃ ·O ₇	750 ± 33	800	49000 ± 700	nd
84		172-174	B (25)	C ₉ H ₁₁ N ₃ ·HBr	3,500	nd	>10000	nd
85		174-180	<i>i</i>	C ₁₄ H ₁₃ N ₃	4100	nd	>10000	nd
86		238-240 ^j	B (20)	C ₁₃ H ₁₁ N ₃	>10000	nd	nd	>10000
87		297-299	<i>k</i>	C ₁₁ H ₉ N ₃	>10000	nd	nd	>10000
88		228-230	A (41) ^f	C ₃₆ H ₅₅ N ₃ ·HCl	>10000	nd	nd	>10000
89		140-141	(21) ^l	C ₁₃ H ₁₃ N ₃	>100000	>100000	nd	nd
90		142-144 ^m	B (20)	C ₃ H ₉ N ₃ ·HBr	>100000	>10000	>10000	nd

^a See footnote a in Table I. ^b See footnote c in Table I. ^c See footnote d in Table I. ^d Prepared by reaction of 2-iodophenylcyanamide and *tert*-butylamine. ^e Lit. mp 186 °C (HCl salt). Braun, C. E.; Randall, W. M. *J. Am. Chem. Soc.* 1934, 56, 2134. ^f Purified by preparative TLC (CHCl₃/EtOH, 95:5). 3 α -Amino-5-cholestene and 3 α -amino-5-pregnen-20-one were prepared according to the literature⁷⁹ procedure. ^g Lit.⁷⁶ mp 162 °C. ^h Prepared and characterized as the picrate salt (lit. mp 122 °C) according to the literature procedure: Mold, J. D.; Ladino, J. M.; Scantz, E. J. *J. Am. Chem. Soc.* 1953, 75, 6321. ⁱ Prepared according to the literature⁷⁸ procedure (lit. mp 175-178 °C). ^j Lit.⁷⁹ mp 208-209 °C. ^k Purchased from Aldrich Chemical Co. ^l Prepared according to the literature procedure: Arndt, F. *Chem. Ber.* 1917, 50, 1261. ^m Lit. mp 144 °C. Mold, J.; Ladino, J.; Schantz, E. *J. Am. Chem. Soc.* 1953, 75, 6321.

cussed by Wikström and Largent et al.⁶⁸⁻⁷⁰ They find that a 3- or 4-phenylpiperidine ring system and a lipophilic N-substituent are key features in most (but not all) classes of potent σ receptor ligands. We suggest that the diarylguanidines mimic this feature (Figure 2) when binding to the σ receptor. Our data are also in line with the finding

of Largent et al. that σ affinity increases with increasing lipophilicity of the N-substituent.⁶⁸⁻⁷⁰ The σ receptor is not very structure sensitive, since aryl-, cyclohexyl-, norbornyl-, and adamantyl-substituted guanidines all can exhibit high affinity. The σ receptor site model proposed by Manallack et al.^{28,71,72} is consistent with our finding that

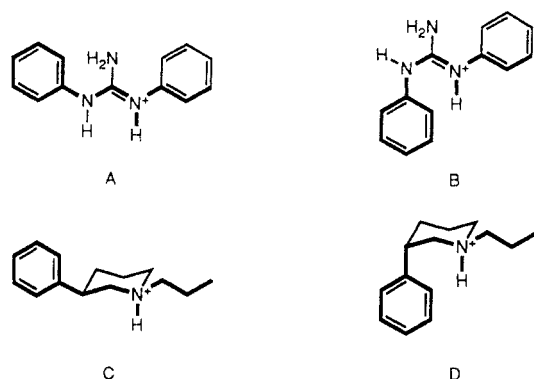


Figure 2. Structural similarities between different conformations of the ring systems of the protonated forms of the σ receptor ligands DTG (A and B) and 3-PPP (C and D). The heavy bonds indicate shared connectivities between A and C, and between B and D. They are not meant to imply that the phenyl rings lie in the plane of the guanidine.

good σ affinity is usually retained as long as one side of the guanidine bears a preferred group. Thus, the 2-methylphenylguanidine substituent presents to the receptor the "primary pharmacophor", and the proposed "lipophilic cleft" accepts the variously modified guanidine substituents, e.g. cyclohexyl, norbornyl, or adamantyl. However, the model of Manallack et al. must be revised to accommodate the N,N' -dinorbornyl- and N,N' -diadamantylguanidines (75 and 77 and 60, respectively), which bind tightly to the σ receptor but are too bulky to be accommodated within the hypothesized narrow hydrophobic pocket of the primary pharmacophor. Furthermore, within our series of compounds, we find no evidence for the proposed secondary binding site for phenolic substituents (cf. 30 vs 3) within the primary pharmacophor.⁷²

PCP Receptor Affinity. In general, all of the guanidines we report here are poor ligands for the PCP receptor (IC_{50} vs [3H]-5 or [3H]-6 > 5000 nM), with several notable exceptions. The ortho- or meta-substituted N,N' -diarylguanidines 13–15, 21, 22, 24, and 28 have submicromolar IC_{50} s at the PCP receptor, as well as at the σ receptor. The structural requirements for high-affinity binding of N,N' -disubstituted guanidines to the PCP receptor are more restrictive than for the σ receptor. Only small, nonpolar ortho- and meta-substituents are well tolerated. The most potent PCP receptor ligand in this series is N,N' -bis(1-naphthyl)guanidine (28) (IC_{50} 134 nM vs [3H]-6), which also binds with equal affinity at the σ receptor (IC_{50} vs [3H]-3 133 nM). Cycloalkyl-substituted guanidines (Tables II–IV) are devoid of significant affinity for the PCP receptor.

In separate studies, we have found that N,N' -diarylguanidines which bind to the PCP receptor have powerful neuroprotective properties against glutamate-induced neuronal cell death.^{48,49} Whether or not they possess psychotomimetic properties similar to other PCP receptor ligands is currently under active investigation. These compounds constitute a novel structural class of noncompetitive N -methyl-D-aspartate antagonists, which are of considerable interest as potential therapeutic neuroprotective agents.^{55–58}

Conclusions

We have described a new series of substituted guanidines, which includes some of the most potent ligands for the haloperidol-sensitive σ receptor described to date. Their structure and synthesis are uncomplicated, and a

wide array of structural variations result in high σ receptor affinity. These compounds are candidates for the development of novel antipsychotics and for the further characterization of the σ receptor through affinity labeling approaches. We have also shown that certain N,N' -diarylguanidines cross-react with the PCP receptor. Separate studies have demonstrated that these compounds make up a new class of noncompetitive NMDA antagonists, which are of interest as potential therapeutic agents against glutamate-induced neuronal cell death.

Experimental Section

Melting points were determined in open capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Thin-layer chromatography was performed on Merck silica gel 60 F₂₅₄ (0.2 mm) plastic-coated sheets. Guanidines were visualized on TLC sheets with 254-nM UV light or as a blue spot with bromocresol spray reagent (Aldrich Chemical Co.) Preparative TLC was performed on Analtech GF precoated silica gel (1000 μ m) glass-backed plates (20 \times 20 cm). The IR and 1H and ^{13}C NMR spectra of all compounds were consistent with their assigned structure. NMR spectra were recorded on a General Electric QE-300, and chemical shifts are reported in ppm (δ) relative to the residual signal of the deuterated solvent ($CHCl_3$, δ 7.26; CHD_2OD , δ 3.30). Infrared spectra were recorded in KBr (unless otherwise noted) on a Nicolet 5DXB FT-IR. C, H, and N elemental analyses for all new compounds were performed by Desert Analytics (Tucson, AZ) or Galbraith Laboratories (Knoxville, TN). Dicyclohexylcarbodiimide, BrCN, N,N' -di-*o*-tolylguanidine (3), N,N' -diphenylguanidine (36), and perimidine (87) were obtained from Aldrich Chemical Co. and were recrystallized from aqueous EtOH before use, except dicyclohexylcarbodiimide and BrCN, which were used as received. All starting amines were obtained from commercial sources and were purified by standard procedures before use or, where noted, were prepared by published procedures. (\pm)-*Endo*-2- and (\pm)-*exo*-2-aminonorbornane and *R*-(-)-isobornylamine hydrochloride served as precursors for the respective norbornyl- and isobornyl-substituted guanidines. Chlorobenzene was freshly distilled from CaH_2 . Ether (Et_2O) and THF were refluxed over sodium/benzophenone ketyl radical and freshly distilled under N_2 . All other solvents were reagent grade. Alkyl- and arylcyanamides were prepared according to published procedures by reaction of the amines with BrCN in Et_2O ,^{73,74} or, in the case of (2-methyl-4-nitrophenyl)cyanamide, in H_2O ,⁷³ and were used without further purification.

General Procedure for the Synthesis of Unsymmetrical N,N' -Disubstituted Guanidines. Method A. A stirred mixture of the appropriate cyanamide⁸⁴ (10 mmol) and amine hydrohalide salt (10 mmol) in chlorobenzene (30 mL) was heated at 90–130 $^{\circ}C$ under N_2 for 2–10 h. The reaction was monitored by TLC ($CHCl_3/EtOH/Et_3N$, 75:20:5). On cooling to 25 $^{\circ}C$, the title compounds precipitated from solution as their hydrohalide salts, were filtered off, and washed with dichloromethane (CH_2Cl_2) (3 \times 5 mL) to remove residual chlorobenzene. When the guanidine hydrohalide did not precipitate from the cooled reaction mixture, the solvent was evaporated, and the residue was taken up in aqueous 1 N HCl (15 mL). The solution was basified with 1 N NaOH, and the precipitated guanidine free base was filtered off. The guanidine free base was crystallized by dissolution in EtOH (20–30 mg/mL), followed by slow addition of H_2O (30–50% volume). The analytical sample was obtained after two further recrystallizations. Typically, the guanidine hydrohalide salts were crystallized inside a closed Et_2O -containing chamber, by the slow diffusion of Et_2O into a loosely covered flask containing a solution of the guanidine salt in absolute EtOH (20–40 mg/mL). Two such recrystallizations provided the analytical material.

N -(Adamant-1-yl)- N' -(2-iodophenyl)guanidine Hydrochloride (57). Method A. A suspension of adamant-1-ylcyanamide (4.09 g, 16.0 mmol), 2-iodoaniline hydrochloride (2.82 g, 16.0 mmol), and 2-iodoaniline (50 mg, 0.288 mmol) in chlorobenzene (50 mL) was heated at reflux for 2 days. The resulting white precipitate was filtered off, washed with CH_2Cl_2 (3 \times 30 mL), and dried to give 57 (6.45 g, 93%) as a white powder, mp

(84) See footnote b in Table I.

255–257 °C. After crystallization from EtOH/Et₂O, the analytical sample was obtained as white needles (3.67 g, 66%): 264–265 °C dec. ¹H NMR (CD₃OD): δ 1.779 (s, 6 H), 2.090 (s, 6 H), 2.165 (s, 3 H) 7.168 (t, 1 H, *J* = 8.1 Hz), 7.388 (d, 1 H, *J* = 8.1 Hz), 7.503 (t, 1 H, *J* = 7.8), 8.004 (d, 1 H, *J* = 7.8 Hz). IR: 3442, 3160, 2909, 1653, 1634 cm⁻¹. Anal. (C₁₇H₂₃ClIN₃) C, H, N.

General Procedure for the Synthesis of Symmetrical N,N'-Disubstituted Guanidines. Method B. To a stirred solution of the appropriate amine (10 mmol) in EtOH (3–5 mL) at 0 °C was carefully added a solution of BrCN (11 mmol, 1.1 eq) in EtOH (1–2 mL). After the exotherm subsided, the reaction mixture was allowed to warm to 25 °C and was then heated at 150 °C for 15–30 min, while N₂ was swept through the flask to completely remove the boiling solvent. The fused reaction mixture was allowed to cool to 25 °C, and the resulting glassy solid was taken up in hot EtOH (10–15 mL), treated with decolorizing charcoal (50–60 mg), and filtered through Celite. The filtrate was diluted with aqueous 1 N NaOH (10–20 mL), and the precipitated guanidine free base was filtered off. The analytical sample was obtained by repeated crystallizations from aqueous EtOH, as described in Method A.

N,N'-Bis(3-ethylphenyl)guanidine (13). Method B. A solution of BrCN (650 mg, 6.14 mmol) in Et₂O (1 mL) was added to neat 3-ethylaniline (1.42 g, 11.7 mmol). After the exothermic reaction subsided, the resulting viscous oil was heated under a stream of N₂ at 150 °C for 15 min and then was allowed to cool to 25 °C. The resulting solid was dissolved in EtOH (20 mL), and 10% NaOH (20 mL) was added. A white precipitate was filtered off and recrystallized twice from aqueous 50% EtOH, to give 13 (620 mg, 20%) as white needles, mp 96–98 °C. ¹H NMR (CDCl₃): δ 1.216 (t, 6 H, *J* = 7.5), 2.608 (q, 4 H, *J* = 7.5), 6.937 (m, 6 H), 7.222 (t, 2 H, *J* = 7.8 Hz). IR (CDCl₃): 2971, 1629, 1589, 1490, 1417, 1217 cm⁻¹. Anal. (C₁₇H₂₁N₃) C, H.

Preparation of N,N'-Dicyclohexyl-N''-substituted-guanidines. Method C. To a stirred solution of dicyclohexylcarbodiimide (10 mmol) in THF (10 mL) under N₂ was added the appropriate amine (9.8 mmol). The resulting solution was stirred at 25 °C for several days and then evaporated. The residue was taken up in absolute EtOH (15 mL). Excess ethanolic HCl was added, and any precipitated dicyclohexylurea was filtered off. The filtrate was evaporated, and the residue was crystallized from EtOH/Et₂O as described in Method A.

Radioligand Binding Assays. Frozen whole guinea pig brains (Pel-Freez, Rodgers, AR, and Biotrol, Indianapolis, IN) were homogenized in 10 volumes (w/v) of ice-cold 0.32 M sucrose with use of a Polytron (Brinkman). The homogenate was centrifuged at 1,000g for 20 min at 4 °C. The homogenate was then centrifuged again at 20,000g for 20 min at 4 °C. The resulting pellet was resuspended in 10 volumes of 50 mM Tris-HCl buffer (pH 7.4) and centrifuged at 20,000g for 20 min at 4 °C. The resulting pellet was then resuspended in 5 volumes of 50 mM Tris-HCl (pH 7.4), and the final volume was adjusted to yield a protein concentration of 3 mg/mL, as determined by dye-binding protein assay (Biorad); 20-mL aliquots were stored at -70 °C until used.

For [³H]-3, [³H]-4, and [³H]-5 radioligand binding assays, 20-mL aliquots of the frozen membrane suspension were thawed and diluted 1:3 in 50 mM Tris-HCl (pH 7.4). To 12 × 75-mm polystyrene test tubes were added 0.8 mL of diluted membrane suspension, 0.1 mL of [³H]-3 (52 Ci/mmol)²¹ or [³H]-4 (104 Ci/mmol, New England Nuclear) or [³H]-5 (100 Ci/mmol, New England Nuclear) to yield a final concentration of 1.4, 0.96, or 1.8 nM, respectively, and 0.1 mL of unlabeled compound or buffer. The protein content in the 1-mL final incubation volume was 800 μg, corresponding to 32 mg of brain tissue (original wet weight) and to a tissue concentration within the linear range for specific binding. Nonspecific binding for both [³H]-3 and [³H]-4 assays was defined as that remaining in the presence of 10 μM haloperidol, and for [³H]-5 in the presence of 10 μM PCP. Specific binding constituted 92.1 ± 0.4% SEM (*n* = 7) of total [³H]-3

binding, 91.5 ± 0.4% SEM (*n* = 4) of total [³H]-4 binding and 94.6 ± 0.8% (*n* = 6) of total [³H]-5 binding. Incubations were terminated after 90 min (45 min for [³H]-5) at room temperature by addition of 4 mL of 50 mM Tris-HCl (pH 7.4) and rapid filtration of the membrane suspension through Whatman GF/B glass-fiber filters (or Schleicher & Schueller No. 32 filters) under vacuum, using a 48-well cell harvester (Brandel, Gaithersburg, MD). The filters were washed two times with 4 mL of 50 mM Tris-HCl (pH 7.4). Total filtration and washing time was less than 10 s. Each filter was suspended in 10 mL of Cytosint (Westchem, Sand Diego, CA), and radioactivity was measured by liquid scintillation spectrometry at a counting efficiency of approximately 50%. Saturation data were evaluated by Scatchard analysis using the EBDA (MacPherson, 1983) data analysis program on an IBM PC-AT. IC₅₀ values were determined by interpolation from displacement-curve plots on semilogarithmic graph paper.

[³H]-6 (97 Ci/mmol)⁶⁷ radioligand assays were performed in a fashion similar to [³H]-3, [³H]-4, and [³H]-5 radioligand assays but with the following modifications. Final concentration of [³H]-6 used was 1 nM and protein concentration was 150 μg/mL. Tris-HCl (5 mM; pH 7.4) was used as assay buffer and for filtration. Incubation time was 4 h at 25 °C. Nonspecific binding was defined as that remaining in the presence of 10 μM 5 or 6 and was ≤10% of total binding.

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Registry No. 3, 106916-81-8; 4, 85976-54-1; 5, 21500-98-1; 6, 77086-21-6; 13, 123403-56-5; 14, 101577-96-2; 15, 123403-55-4; 16, 128413-36-5; 17, 128413-37-6; 18, 106916-80-7; 19, 114828-36-3; 20, 128413-38-7; 21, 51131-78-3; 22, 104919-97-3; 23, 128413-39-8; 24, 128413-40-1; 25, 87-34-3; 26, 128413-41-2; 27, 128413-42-3; 28, 7469-00-3; 29, 128413-43-4; 30, 128413-44-5; 30-HCl, 128413-45-6; 31, 128413-46-7; 32, 128413-47-8; 33, 128413-48-9; 34, 124190-35-8; 34-HCl, 111858-08-3; 35, 128413-49-0; 36, 102-06-7; 37, 128413-50-3; 38, 54116-98-2; 39, 54434-03-6; 40, 128413-51-4; 40-HCl, 128413-52-5; 41, 128413-53-6; 42, 128413-54-7; 43, 128413-55-8; 43-2HCl, 128413-56-9; 44, 128413-57-0; 45, 6268-03-7; 46, 128413-58-1; 46-3HCl, 128413-59-2; 47, 104919-90-6; 48, 124190-34-7; 49, 124190-33-6; 49-HCl, 23166-33-8; 50, 35168-15-1; 51, 34147-57-4; 52, 35168-16-2; 52-HCl, 128413-60-5; 53, 128413-61-6; 53-HCl, 128413-62-7; 54, 128413-63-8; 55, 128413-65-0; 55-HCl, 128413-64-9; 56, 128413-66-1; 57, 124190-30-3; 57-HCl, 128413-67-2; 58, 124190-31-4; 59, 128413-68-3; 59-HCl, 128413-69-4; 60, 124190-32-5; 60-HCl, 23265-92-1; 61, 128413-70-7; 61-HCl, 128413-71-8; 62, 128413-72-9; 63, 128413-73-0; 63-HCl, 128413-74-1; 64, 128413-75-2; 64-HCl, 128413-76-3; 65, 128413-77-4; 65-HCl, 128413-78-5; 66, 128413-79-6; 66-HCl, 128413-80-9; 67, 128413-81-0; 68, 128413-82-1; 68-HCl, 128413-83-2; 69, 128413-84-3; 69-HCl, 128413-85-4; 70, 128413-86-5; 70-HCl, 128413-87-6; 71, 128413-88-7; 72, 128413-89-8; 73, 128413-90-1; 74, 128413-91-2; 75, 128413-92-3; 75-HCl, 128413-93-4; 76, 128413-94-5; 76-HCl, 128524-21-0; 77-HBr, 128413-95-6; 78, 128413-96-7; 79, 128413-97-8; 79-HCl, 128413-98-9; 80, 25709-42-6; 80-HBr, 73571-55-8; 81, 128413-99-0; 81-HCl, 128414-00-6; 82, 4044-91-1; 83, 34331-58-3; 83-C₆H₅N₃O₇, 69724-23-8; 84, 41213-54-1; 84-HBr, 128414-01-7; 85, 75063-89-7; 86, 2849-03-8; 87, 28832-64-6; 88, 128414-02-8; 88-HCl, 128414-03-9; 89, 20277-92-3; 90, 3324-71-8; 90-HBr, 13314-44-8; 2-IC₆H₄NH₂HI, 128414-04-0; BrCN, 506-68-3; 3-C₂H₅C₆H₄NH₂, 587-02-0; *n*-C₈H₁₇NH₂, 111-86-4; 2-IC₆H₄NHCN, 128414-05-1; (CH₃)₃CNH₂, 75-64-9; 1-adamantylcyanamide, 15784-82-4; dicyclohexylcarbodiimide, 538-75-0.