Role of Hydrogen Bonding in Ligand Interaction with the *N*-Methyl-D-aspartate Receptor Ion Channel

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Displacement of [^{3}H]MK-801 (dizocilpine, 1) binding to rat brain membranes has been used to evaluate the affinities of novel dibenzocycloalkenimines related to 1 for the ion channel binding site (also known as the phencyclidine or PCP receptor) on the *N*-methyl-D-aspartate (NMDA) subtype of excitory amino acid receptor. In common with many other agents having actions in the central nervous system, these compounds contain a hydrophobic aromatic moiety and a basic nitrogen atom. The conformational rigidity of these ligands provides a unique opportunity to evaluate the importance of specific geometrical properties that influence active-site recognition, in particular the role of the nitrogen atom in hydrogen-bonding interactions. The relative affinities (IC_{50}s) of hydrocarbon-substituted analogues of 1 (Table I) and ring homologated cyclooctenimines (Tables II and III) illustrate the importance of size-limited hydrophobic binding of both aryl rings and of the quaternary C-5 methyl group. Analysis of the binding of a series of the 10 available structurally rigid dibenzoazabicyclo[*x.y.z*]alkanes (Table IV), by using molecular modeling techniques, uncovered a highly significant correlation between affinity and a proposed ligand–active site hydrogen bonding vector ($r = 0.950$, $p < 0.001$). These results are used to generate a pharmacophore of the MK-801 recognition site/PCP receptor, which accounts for the binding of all of the known ligands.

Common structural features have been noted for many classes of molecules having activity in the central nervous system (CNS).[1] Most frequently, the essential pharmacophoric groups are a hydrophobic moiety, usually an aromatic ring, and a basic nitrogen atom which may be charged by protonation or quaternization. On the basis of an analysis of CNS-active substances, Lloyd and Andrews[1] concluded that the optimal distance between the center of the aromatic ring and the nitrogen atom is 5.0 Å. Selectivity for different sites of action in the CNS was proposed to be determined mainly by secondary binding groups. Central to the model was a requirement for a hydrogen-bonding interaction of the amino group with a receptor site situated tetrahedrally 2.8 Å from the nitrogen atom.[1]

A particular problem associated with the generation of aromatic ring-nitrogen based pharmacophores has been the conformational flexibility of many of the ligands used. The general method adopted in most cases[1,2] relies on the generation of the primary pharmacophore by using rigid or semirigid reference molecules, followed by fitting of flexible analogues to the model. While this approach permits molecular matching, the critical spatial relationships between the nitrogens and the aromatic rings of conformationally mobile ligands obviously cannot be uniquely defined. In principle, using a series of structurally rigid congeneric ligands should enable a more precise definition of the relationships between active-site binding and molecular and hydrogen-bonding geometries. In this paper, we have used a series of derivatives of MK-801 (dizocilpine, 1, Table I), a potent and selective uncompetitive antagonist of the *N*-methyl-D-aspartate (NMDA) subtype of excitory amino acid receptor.[3] Analysis of the relative affinities of these conformationally fixed dibenzoazabicyclo[*x.y.z*]alkanes (Tables I–IV) for their active site allows unambiguous evaluation of the specific nitrogen–aryl ring geometry associated with ligand recognition. The results illustrate the importance size-limited hydrophobic binding of both aromatic rings and the quaternary methyl group, coupled with a strictly directional ligand–active site hydrogen bonding interaction.[4] The model is supported by a quantitative correlation between binding affinity and the relative hydrogen bonding vector to a putative active-site group.

Interest in NMDA antagonists has heightened following the discovery that these agents can protect against neuronal degeneration in animal models of cerebral ischemia,[6] and may provide an approach to the treatment of stroke and other neurological disorders.[7] Electrophysiological[5] and ligand binding[5,8,9] studies indicate that 1 acts by binding to a site within the open state of the Ca^{2+} permeable ion channel associated with the NMDA receptor. Inhibition of the accumulation of toxic intracellular concentrations of Ca^{2+} [10] is believed to account for the neuroprotective actions of 1.

In addition to the dibenzocycloalkenimines in Tables I–IV, a wide range of structures are known to act as NMDA receptor ion channel blockers.[3,9,11] These include the psychotomimetic drug phencyclidine (PCP) and related arylcyclohexylamines, benzomorphans and synthetic opiate mimics,[11] dioxalanes including etoxadrol,[12] and tetrahydroisoquinolines.[13] Each of these molecules pos-

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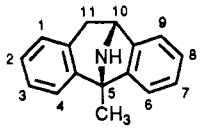
(11) Manallack, D. T.; Wong, M. G.; Costa, M.; Andrews, P. R.; Beart, P. M. *Mol. Pharmacol.* **1988**, *34*, 863.

0022-2623/90/1833-1296$02.50/0

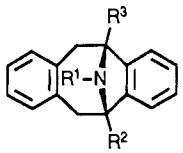
Table I. Substituted MK-801 Analogues



no.[a]	substituent	IC_{50} (μM) vs [^{3}H]MK-801
1		0.056
(+)-1		0.036
(−)-1		0.320
2	1-CH_3	0.80
3	1,2-benzo	5.8
4	2-CH_3	0.64
5	2,3-benzo	89
6	3-CH_3	0.032
7	7-CH_3	0.10
8	7,8-benzo	86
9	8-CH_3	0.44
10	8,9-benzo	2.3
11	9-CH_3	3.0
12	10-CH_3	0.035
13	10-CH_3, 11-*exo*-OH	0.065
14	10-CH_3, 11-*endo*-OH	6.6

[a] All compounds are racemic mixtures except where indicated.

Table II. Bridgehead Substituted Dibenzohomotropanes

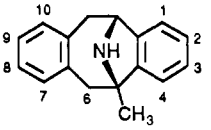


no.[a]	R^1	R^2	R^3	IC_{50} (μM) vs [^{3}H]MK-801
15	H	CH_3	H	0.086
(+)-15	H	CH_3	H	0.104
(−)-15	H	CH_3	H	0.106
16	H	H	H	0.64
17	H	CH_3	CH_3	0.030
18	H	C_2H_5	H	1.36
19	H	*n*-C_4H_9	CH_3	3.6
20	H	OH	CH_3	0.104
21	CH_3	CH_3	H	0.53
22	C_2H_5	CH_3	H	2.51
23	CH_2Ph	CH_3	H	23
24	$(CH_3)_2^+$	CH_3	H	7.1

[a] 16 and 17 are achiral; the remaining compounds are racemic mixtures, except where indicated.

sesses the essential aromatic ring and basic nitrogen atom required of a typical CNS pharmacophore. Modeling studies[11,12] have demonstrated that these compounds can fit to the general pharmacophore proposed by Lloyd and Andrews.[1] In these studies relative activity was determined by ligand binding to a site labeled by [^{3}H]TCP, a PCP analogue.[11] In the present study, we have measured ligand binding by displacement of [^{3}H]MK-801 binding from rat cortical membranes.[5,9] Several lines of evidence, based on in vitro[5,9] and in vivo[14] studies, strongly suggest that the sites of action of PCP and MK-801 are identical. Thus

Table III. Ring-Substituted Dibenzohomotropanes



no.[a]	substituent	IC_{50} (μM) vs [^{3}H]MK-801
15		0.086
25	1-Cl	1.37
26	2-Cl	0.25
27	2-Br	0.27
28	3-Br	0.14
29	6-*endo*-CH_3	0.59
30	8-Br	0.079
31	9-OCH_3	0.22
32	9-OH	0.14
33	9-Cl	0.19
34	9-Br	0.32

[a] All compounds are racemic mixtures.

the classical PCP receptor[15] and the NMDA receptor ion channel are believed to be the same macromolecule, and ligands sharing the biological actions of PCP and MK-801 block the open state of this channel.[3] Use of the term "PCP agonist"[12,13] to describe these molecules is misleading. The mode of action of MK-801 and PCP as open channel blockers means that inhibition of their biological activity is not mechanistically feasible, which accounts for the long-standing failure to identify a "PCP antagonist".

The compounds in Table I are alkyl- and aryl-substituted derivatives of MK-801 which extend the existing series of highly potent halogen-, hydroxy-, and alkoxy-substituted derivatives.[16] These analogues were synthesized specifically to probe active-site bulk tolerance, since the hydrocarbon substituents would be expected to have little or no dipolar properties which could influence ligand recognition. Tables II and III contain a series of structurally novel dibenzo[*a*,*e*]cyclooctenimines (dibenzohomotropanes), which are ring homologated derivatives of MK-801,[17,18] shown here to retain high potency at the MK-801 recognition site. In Table IV, the potencies of the parent compounds[16-26] in the series of dibenzobicyclo[*x*,*y*,*z*]alkenimines are summarized. These conformationally fixed analogues allow the use of molecular

(12) Thurkauf, A.; Zenk, P. C.; Balster, R. L.; May, E. L.; George, C.; Carroll, F. I.; Mascarella, S. W.; Rice, K. C.; Jacobsen, A. E.; Mattson, M. V. *J. Med. Chem.* **1988**, *31*, 2257.

(13) Gray, N. M.; Cheng, B. K.; Mick, S. J.; Lair, C. M.; Conteras, P. C. *J. Med. Chem.* **1989**, *32*, 1242.

(14) (a) Martin, D.; Lodge, D. *Pharmacol. Biochem. Behav.* **1988**, *31*, 279. (b) Price, G. W.; Ahier, R. G.; Middlemiss, D. N.; Singh, L.; Tricklebank, M. D.; Wong, E. H. F. *Eur. J. Pharmacol.* **1988**, *158*, 279.

(15) Zukin, S. R.; Zukin, R. S. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 5372.

(16) Thompson, W. J.; Anderson, P. S.; Britcher, S. F.; Lyle, T. A.; Thies, E. J.; Magill, C. A.; Varga, S. L.; Schwering, J. E.; Lyle, P. A.; Christy, M. E.; Evans, B. E.; Colton, C. D.; Holloway, M. K.; Springer, J. P.; Hirshfield, J. M.; Bell, R. E.; Amato, J. S.; Larsen, R. D.; Wong, E. H. F.; Kemp, J. A.; Tricklebank, M. D.; Singh, L.; Oles, R.; Priestley, T.; Marshall, G. R.; Knight, A. R.; Middlemiss, D. N.; Woodruff, G. N.; Iversen, L. L. *J. Med. Chem.* **1990**, *33*, 789. (b) Woodruff, G. N.; Wong, E. H. F.; Kemp, J. A. Eur. Pat. Appl. 0 230 370 A2, 1987.

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(19) Christy, M. E.; Anderson, P. S.; Britcher, S. F.; Colton, C. D.; Evans, B. E.; Remy, D. E.; Engelhardt, E. L. *J. Org. Chem.* **1979**, *44*, 3117.

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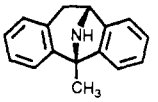
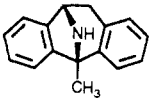
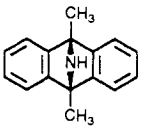
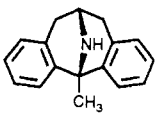
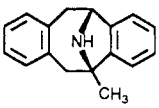
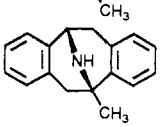
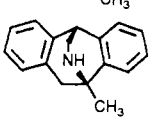
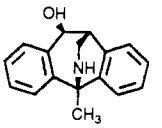
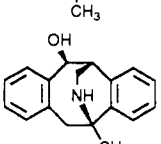
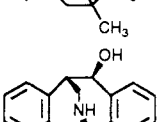
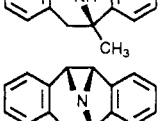
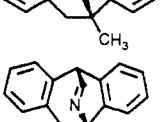
(21) Bender, D. R.; Karady, S.; Rothauser, T. U.S. Patent 4 477 668, 1984.

(22) Baker, R.; Leeson, P. D.; Britcher, S. F. Eur. Pat. Appl. 0 303 512 A1, 1989.

(23) Baker, R.; Carling, W. R.; James, K.; Leeson, P. D. Eur. Pat. Appl. 0 319 073 A2, 1989.

Table IV. Dibenzoazabicyclo[*x.y.z*]alkanes

no.[a]	[*x.y.z*]	structure[b]	IC_{50} (μM) vs [^{3}H]MK-801	*D*[c]	D_1[d]	θ[e]
(+)-1	3.2.1		0.036	3.442	5.890	0.0
(−)-1	3.2.1		0.320	3.687	6.216	13.4
35	2.2.1		0.024	3.335	5.855	3.8
36	3.3.1		0.140	3.673	6.225	14.3
15	4.2.1		0.084	3.391	5.943	4.7
37	3.3.1		0.690	3.755	6.251	15.5
38	3.2.2		0.260	3.756	6.000	9.7
39	3.2.2		0.640	3.774	5.951	16.5
40	4.2.2		0.950	3.632	6.229	16.1
41	3.3.2		8.1	3.824	6.340	28.0
42	3.2.1.0		>100			
43	3.2.2		>100			

[a] All compounds except for (+)- and (−)-1 are racemic mixtures. [b] The absolute stereochemistry of (+)-1 is as shown; both enantiomers of the racemic compounds were used in the modeling studies and the enantiomers used in the preferred fit (Figure 3) are as shown. [c] Distance (in angstroms) between the nitrogen atom and aryl center of the α-methylbenzylamine moiety (**44**) (Figure 2). [d] Distance (in angstroms) between the aryl centroid of the α-methylbenzylamine moiety (**44**) and the common oxygen atom of the hydrogen-bonded water molecule (Figure 3). [e] Hydrogen bond angle relative to MK-801 derived from the active site model (Figures 3 and 4).

modeling techniques to evaluate the importance of molecular geometry and hydrogen bonding in ligand binding to the NMDA receptor ion channel.

Synthesis

The syntheses of many of the compounds in Tables I–IV have been reported previously, including the cycloheptenimines **1**,[16,19–21] **2**, **3**, **6**, **7**, **9**, **10**, and **11**;[22] the dibenzohomotropanes **15**, **16**, **18**, **19**, **21**,[17,18,23] and **25–34**;[23] the anthracenimine **35**;[24] the cyclooctenimine **36**;[19] the pavine analogues **37** and **38**;[17,25] the exo-hydroxylated derivatives **39**, **40**, and **41**;[25,26] and the aziridine **42** and imine **43**.[17,25] Synthesis of the benzo-fused cycloheptenimines **5** and **8** and the 10-methyl derivative **12** utilized the general

(24) Anderson, P. S.; Christy, M. E.; Colton, C. D.; Halczenko, G. S.; Ponticello, G. S.; Shepard, K. L. *J. Org. Chem.* **1979**, *44*, 1519.
(25) Leeson, P. D.; Carling, R. W.; James, K.; Baker, R. *J. Org. Chem.*, in press.
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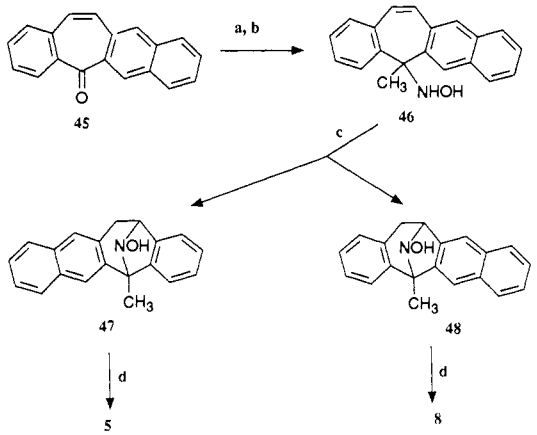
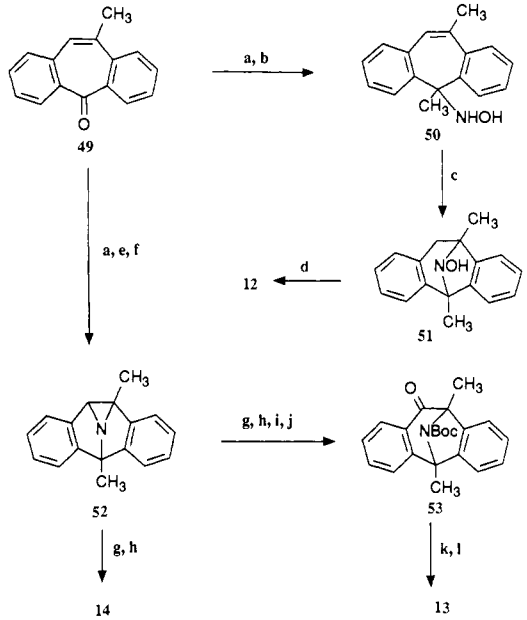
Scheme I[a]



[a] (a) MeLi; (b) NH_2OH·HCl-NaOAc-$CHCl_2CO_2H$; (c) xylene, reflux; (d) Zn-HOAc.

Scheme II[a]

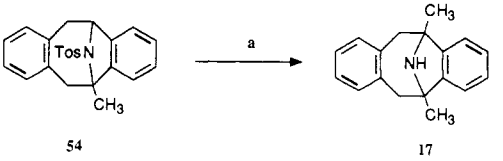


[a] (a) MeLi; (b) NH_2OH·HCl-NaOAc-$CHCl_2CO_2H$; (c) xylene, reflux; (d) Zn-HOAc; (e) NH_2OCH_3·HCl-NaOAc-$CHCl_2CO_2H$; (f) *n*-BuLi; (g) NaOAc-HOAc; (h) NaOH; (i) $(t\text{-BuO})_2CO$-NaOH; (j) pyridinium dichromate; (k) $(i\text{-Bu})_2AlH$; (l) HCl-EtOH.

method[20] of ring closure of C-5 hydroxylamine precursors (**46** and **50**, Schemes I and II). Cyclization of **46** gave mixtures[16] of the regioisomers **47** and **48**, but the 10-methyl derivative **50** formed the bridgehead substituted product **51** exclusively. Extension of the method to provide 4- and 6-alkyl- and aryl-substituted derivatives of **1** was not successful. In these cases, attempts to obtain the required hydroxylamines resulted only in elimination of the precursor tertiary alcohols, forming the exocyclic methylene derivatives. The 11-hydroxylated compounds **13** and **14** were prepared from the aziridine precursor **52** in a manner analogous to the 10-desmethyl derivatives.[27]

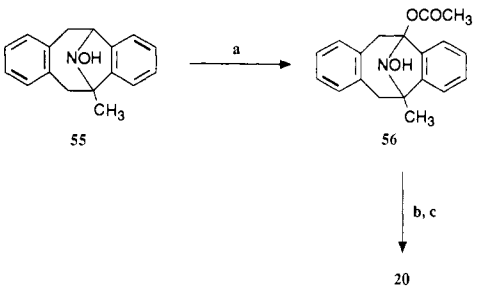
The synthesis of the bridgehead methyl substituted dibenzohomotropane (**17**) was accomplished from the unsubstituted compound (**15**)[17,18] by N-tosylation followed by treatment with methyllithium (Scheme III). The reaction probably proceeds via the anti-Bredt imine[18] derived from elimination of toluenesulfonic acid from **54**. Treatment of the hydroxyimine **55**[17,18] with manganese(III) acetate resulted in selective acetoxylation of the bridgehead methine to give **56** (Scheme IV). Reductive cleavage of the hydroxylamine group followed by basic hydrolysis provided the hydroxy analogue **20**. The N-substituted derivatives **22–24** were readily obtained by alkylation of **15**.

Scheme III[a]



[a] (a) MeLi.

Scheme IV[a]



[a] (a) $Mn(OAc)_3$·$2H_2O$-HOAc-KBr; (b) Zn-HOAc; (c) NaOH.

Structure–Affinity Relationships

The alkyl- and aryl-substituted cycloheptenimines in Table I serve to define the extent of bulk tolerance at the [^{3}H]MK-801 binding site. The results indicate marked positional effects, with methyl substitution at the 1 (**2**), 2 (**4**), 8 (**9**), and 9 (**11**) positions clearly reducing affinity by at least 1 order of magnitude relative to racemic **1**. Benzo substitution also reduces binding, which is especially evident in the 2,3 (**5**) and 7,8 (**8**) analogues. The positions in **1** which tolerate methyl substitution are at 3 (**6**), 7 (**7**), and 10 (**12**). The 3- and 7-positions also tolerate hydroxy, halogen, and methoxy substitution, with larger substituents such as phenyl being allowed at the 3-position.[16] 11-Hydroxylation of the 10-methyl analogue (**12**) retains activity only if the relative stereochemistry is exo (**13**), which is in accord with the results obtained for the 11-hydroxy derivatives of **1**.[16]

The dibenzohomotropanes[17,18] are a new class of cycloalkenimine homologues of **1** which show high affinity for the NMDA receptor ion channel (Tables II and III). The parent racemic analogue **15** is equipotent with racemic **1**, but unlike **1**, the individual enantiomers of **15** have the same binding affinity (Table II). Removal of the quaternary methyl group to give the achiral derivative **16** results in a 6-fold decrease in activity. These results suggested that incorporation of a methyl group at both bridgehead positions should enhance binding. This was shown to be the case by the synthesis of the achiral dimethyl analogue **17**, which proved to be the most potent compound identified in this series (IC_{50} 30 nM). Bridgehead substitution by bulkier groups (**18** and **19**) reduces activity, but hydroxylation (**20**) is tolerated. Substitution on nitrogen reduces affinity in proportion to substituent size (**21** > **22** > **23**).

(27) (a) Britcher, S. F.; Lyle, T. A.; Thompson, W. J.; Varga, S. L. Eur. Pat. Appl. 0 264 183 A1, 1988. (b) Larsen, R. D.; Davis, P.; Corley, E. G.; Reider, P. J.; Lamarec, T. R.; Gragowski, E. J. J. *J. Org. Chem.* **1990**, *55*, 299.

The retention of activity, albeit weak, with the dimethyl quaternary salt **24** suggests that the active site can accept a positively charged group. The loss in potency of **24** can be explained by its inability to donate a hydrogen bond to the active site. In the more active compounds which can readily form protonated quaternary derivatives, the presence of a positive charge could lead to a charge-reinforced increase in energy of the ligand–receptor hydrogen bond.[28] The ionization constants of **15** (pK_a 8.2) and derivatives of **1**[16] suggest that the protonated quaternary derivatives are sufficiently available for active-site binding at physiological pH. Direct evidence for a requirement for binding of the charged species is lacking, but some support is found from the voltage dependence of the binding characteristics of **1**.[29]

A series of aromatic ring substituted derivatives of the parent dibenzohomotropane **15** were prepared for comparison with the corresponding derivatives of **1**. The results (Table III) show that halogen substitution at the 2-, 3-, 8- and 9-positions has little effect on binding affinity, which is in agreement with the relative activities of the **1** analogues.[16] Similarly, the 9-methoxy (**31**) and 9-hydroxy (**32**) analogues retain activity, and the reduction of binding upon 1-substitution (**25**) also parallels the effect of the equivalent 9-substitution in **1** (e.g. **11**, Table I).

The overall structure–affinity relationships for the dibenzohomotropanes in Tables II and III show similar trends to those observed in cycloheptenimines derived from **1**.[16] However, one exception appears to be the effect of ethyl substitution at the quaternary bridgehead position, where **18** is notably less potent than **15**. This contrasts with the equal activities of **1** and its homologated 5-ethyl analogue.[16] Comparisons of the 3-dimensional structures of **1** and **15**, obtained from X-ray crystallographic studies, provide a possible explanation for this difference. These molecular modeling studies are described in the next section.

The results presented in Tables I–III, in combination with the structure–affinity requirements for other NMDA receptor ion channel ligands,[11,12,13,16] suggest an essential requirement for size-limited hydrophobic binding of both aromatic rings, coupled with a hydrogen-bonding interaction requiring donation of a hydrogen from the protonated ligand to an acceptor group in the active site. The molecules in Table IV are alternative ring modified dibenzobicyclo[*x.y.z*]cycloalkenimines related to **1**, which we have used to develop a model for the active site–ligand hydrogen bonding.

Development of the Hydrogen-Bonding Model

The likely importance of a directional hydrogen-bonding interaction of the amino moiety in the binding of these molecules is illustrated by the essential inactivity of the aziridine (**42**) and the imine (**43**) (Table IV). Analogues **42** and **43** have similar overall molecular shapes in comparison with the other compounds, but clearly differ in the directionality of the lone pairs of electrons on their nitrogen atoms relative to the aromatic rings. The loss of activity of **42** and **43** is not due to the presence of tertiary amino groups, since N-alkylation with small groups is tolerated (e.g. **21**), and each of the other structural classes

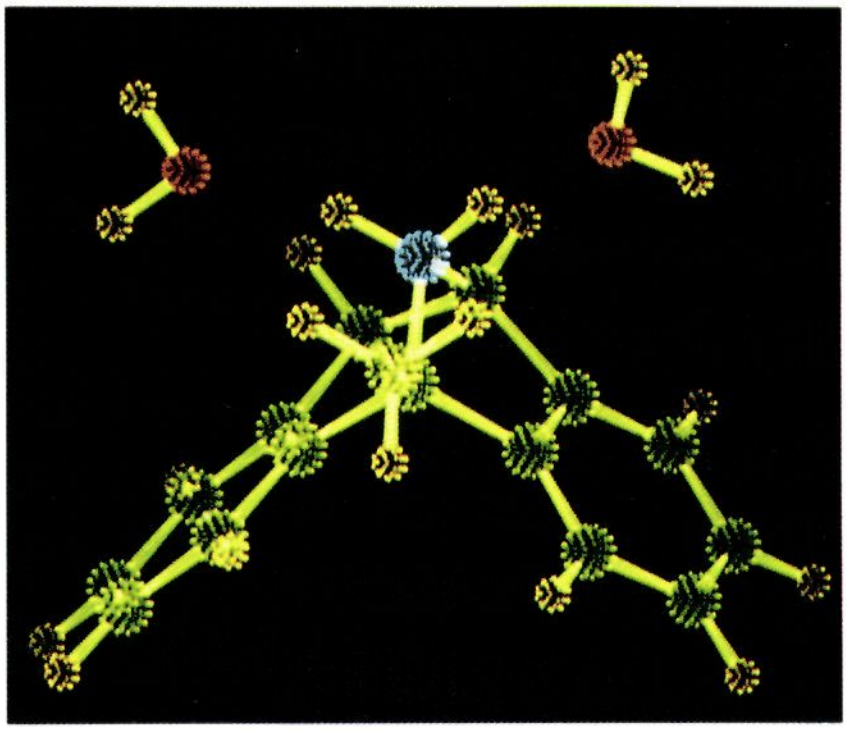
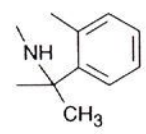


Figure 1. The model of doubly hydrated protonated (+)-**1** used as the basic template for molecular fitting studies. The structure of **1** as determined by X-ray crystallographic studies was modified by addition of water molecules at hydrogen bonding distances, and the trimeric assembly was energy minimized using OPTIMOL.[30]

which bind to the NMDA receptor ion channel are tertiary amines.[11–13]

Conventional molecular modeling techniques were used to generate and compare the 3-dimensional structures of molecules in Table IV. The structures of **1** and its ring homologue **15** were determined by X-ray crystallography.[18] The remaining structures were built and energy minimized with use of the Merck molecular modeling packages MOLEDIT and OPTIMOL.[30] Dummy atoms were added at the center of each aromatic ring using CHEMX[31] and molecules were fitted together in each of the possible orientations using the least squares molecular fitting routine "Compare" within MOLEDIT. The locations of potential hydrogen bonding groups in the active site were modeled by using energy minimized (MM2) doubly hydrated protonated structures, as shown in Figure 1 for (+)-**1**. The absolute stereochemistries of (+)-**1** and its enantiomer are as shown in Table I. Compounds **35** and **36** are not chiral, and both enantiomers of the remaining compounds **15** and **37–41** were individually built and used in the molecular comparison studies. Since the quaternary C-methyl bridgehead group enhances potency, the α-methylbenzylamine moiety (**44**) present in each of the molecules (+)- and (−)-**1**, **15**, and **35–41** (Table IV) was used as a common template in the fitting procedure.



44

The geometrical variables relating to the disposition of aromatic rings and the basic nitrogen in the modeled 3-dimensional structures of the Table IV molecules were explored to seek possible correlations with binding affinity. Among the intramolecular distances and angles which were evaluated, only the distance between the nitrogen and the center of the aryl ring in the common α-methylbenzyl-

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(29) (a) Huettner, J. E.; Bean, B. P. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1307. (b) Javitt, D. C.; Zukin, S. R. *Mol. Pharmacol.* **1989**, *35*, 387.

(30) MSDRL molecular modeling program, written by the Molecular Systems Group, MSDRL Rahway.

(31) Chemical Design Ltd., Oxford, U.K.

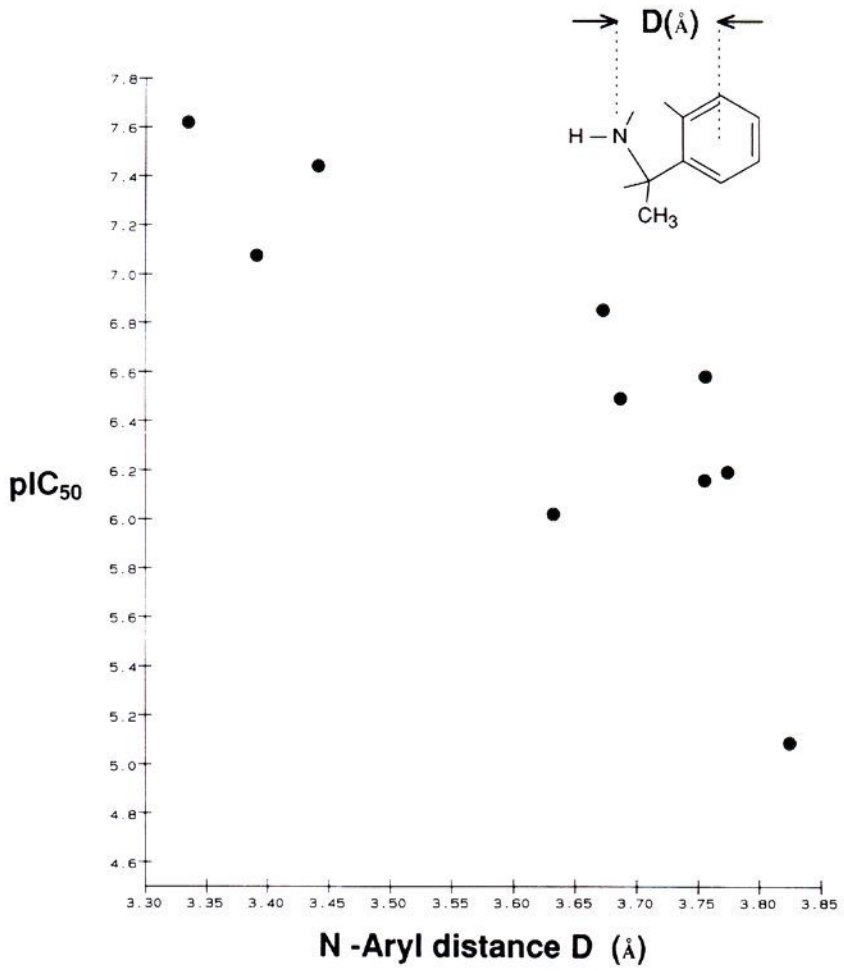


Figure 2. The relationship between binding affinity (pIC_{50}) and N–aryl distance (D) for the compounds in Table IV (see eq 1). D is the distance (in angstroms) between the nitrogen atom and the center of the aryl ring in the common α-methylbenzylamine part structure, determined from the final coordinates of the energy-minimized models of each molecule.

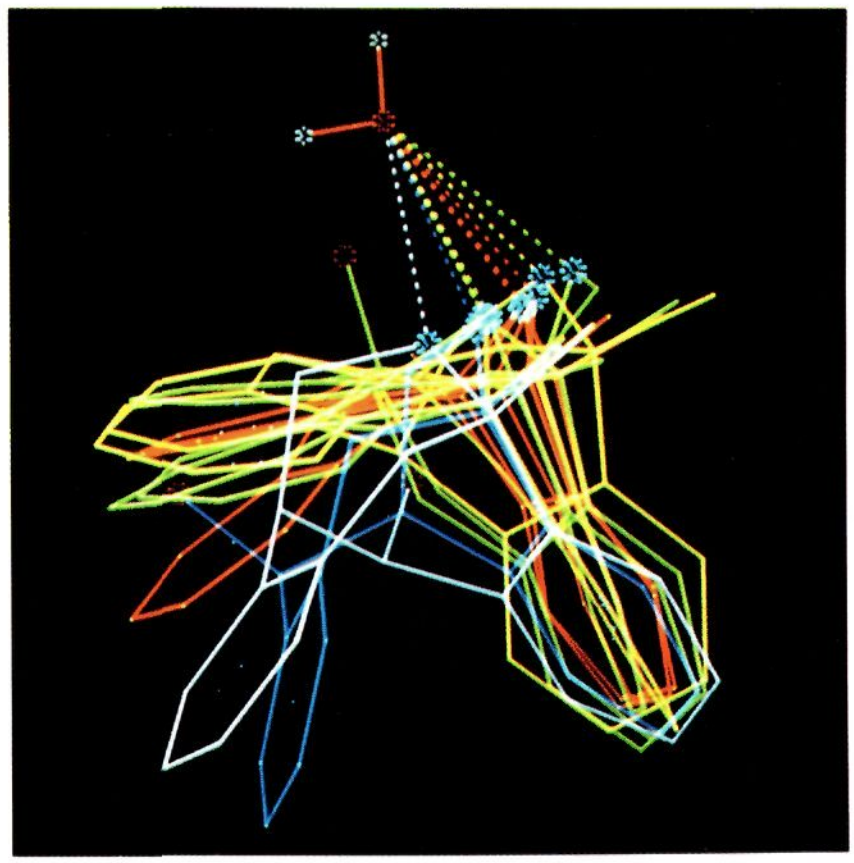


Figure 3. The structures of Table IV compounds fitted to the optimum model obtained by three point fitting of dummy atoms at the centers of both aromatic rings and the water molecule *distal* to the common α-methylbenzylamine moiety. The model was generated by using doubly hydrated protonated structures and for clarity the second water molecule is not shown. Compounds are color coded according to activity: red ((+)-**1**, **15**, **35**) > yellow (**36**, **38**) > green ((−)-**1**, **37**, **39**) > blue (**40**) > white (**41**).

amine moiety (**44**) (D, Table IV) was found to show a statistically significant correlation with binding affinity:

$$pIC_{50} = -3.57D + 19.49 \quad (1)$$

$$n = 10,\ r = 0.836,\ s = 0.434,\ F = 18.51\ (p = 0.00261)$$

In eq 1, n is the number of compounds included, r is the correlation coefficient, s is the standard deviation, F is the variance ratio, and p is the significance level of the correlation. The relationship expressed by eq 1 is shown graphically in Figure 2. The correlation suggests the importance of decreasing the *N*-aryl distance in **44** to enhance potency. In the most potent compounds, namely (+)-**1**, **35**, and **15**, the value of D is ~3.4 Å, which is significantly less than the value of 5.0 Å proposed in the general model[1a] for CNS-active compounds. Neither the alternative *N*-aryl distance nor the possible nitrogen to aromatic ring plane distances showed a significant relationship with binding affinity. The least active of the compounds in Table I (i.e., **41**) has a D value of 3.82 Å and the overall spread in D values of 0.5 Å is linked to a 300-fold change in potency, suggesting there is a stringent geometrical requirement associated with the interaction of this group of ligands at the NMDA receptor ion channel. This result appears surprising in the context of the wide structural tolerance at this binding site.[11,12] However, the compounds in the present study include the most potent of the known ligands, and consequently ligand–active site interactions are likely to be more specific than with the less active and conformationally flexible derivatives used previously.[11,12] Increased specificity coupled with conformational rigidity probably underlies eq 1. In terms of a binding model, eq 1 supports the notion of a strictly directional active site–ligand hydrogen bond being critical for high affinity. Displacement of the nitrogen atom relative to the rigid hydrophobic framework would result in less optimal hydrogen bonding. To test this idea, molecular fitting studies were performed with hydrogen-bonded water molecules (Figure 1) to mimic potential active site groups.

The initial problem in the modeling comparisons was selecting which of the two possible hydrogen-bonded water molecules in the model of (+)-**1** (Figure 1) most reasonably represents the putative active site hydrogen bond acceptor. Models of the doubly hydrated quaternary derivatives of (−)-**1**, **15**, and **35**–**41**, and their enantiomers where appropriate, were matched systematically in each of the four possible ways to the structure of (+)-**1**. The molecular superimposition procedures employed were three-point least-squares fitting of dummy atoms at the two aromatic ring centroids and one water oxygen and four-point fitting by including the quaternary *C*-methyl group. The fitting of the water oxygen atoms in each pair was weighted by 100 relative to the other fitting pairs, to ensure that the putative receptor group represented by the water oxygen would have essentially the same coordinates in each case. Using the enantiomers of **15** and **35**–**41** with the absolute stereochemistries as shown in Table I, fitting to the water oxygen syn to the piperidine ring of (+)-**1** and matching the aryl rings of the key common moiety (**44**) resulted in the best overall fit (Figure 3).

Correlations were sought between binding affinity and geometrical properties relating to the fitted model shown in Figure 3. The distance (D_1, Table IV) between the aryl ring centroid of the common part structure (**44**) and the fitted water oxygen atom (representing the putative receptor hydrogen bond acceptor) provided a correlation with pIC_{50} of comparable significance to eq 1 ($r = 0.776$, $p = 0.0084$). In contrast to the D values (see above), D_1 (5.9–6.3 Å) is similar to the equivalent aryl center to receptor site point distance used in the generation of the common CNS model (6.4 Å, calculated from Figure 1b of ref 1a). How-

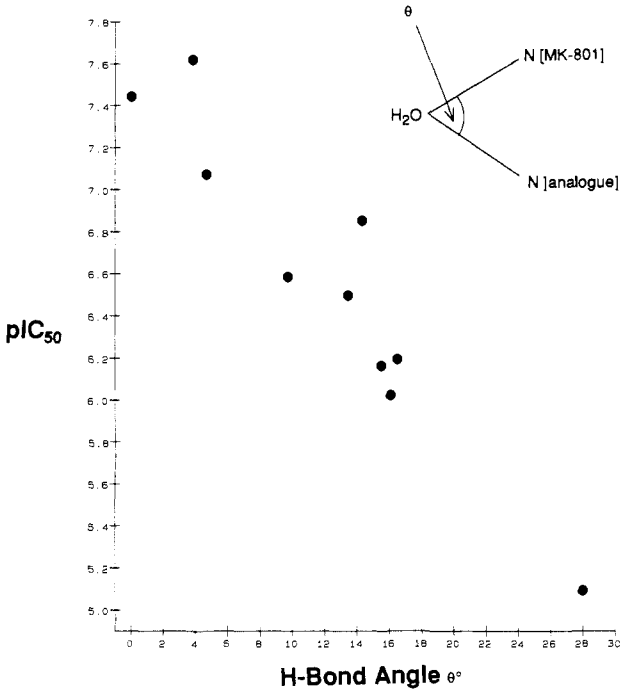


Figure 4. The relationship between binding affinity (pIC_{50}) and the angle of hydrogen bonding relative to (+)-1 (θ, deg) as derived from the model in Figure 3 (see eq 2).

ever, as would be expected from eq 1, higher activity is found with lower D_1 values (Table IV).

The angles of the hydrogen bonds to the water oxygen, relative to (+)-1 (defined by the N–O–N angle θ, Table IV) were determined from the superimposition shown in Figure 3 and were found to provide an improved correlation with binding affinity:

$$pIC_{50} = -0.088\theta + 7.63 \quad (2)$$

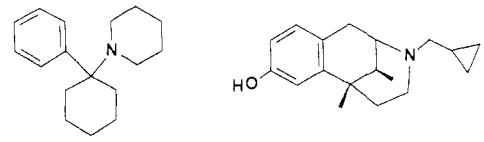
$n = 10$, $r = 0.950$, $s = 0.246$, $F = 74.65$ ($p = 0.000\,025$)

The correlation given by eq 2 is shown graphically in Figure 4, and provides support for a binding model where hydrogen-bond directionality plays a major role in determining the affinity of this class of molecules for the NMDA receptor ion channel. None of the alternative possible superimpositions examined gave correlations of the quality of eq 2. For example, fitting the same molecules to the water oxygen syn to the pyrrolidine ring of (+)-1 gave a statistically insignificant correlation between pIC_{50} and θ ($r = 0.41$, $p = 0.24$). The fit shown in Figure 3 also explains the differences in the effect of homologation of the 5-methyl groups in (+)-1 and **15** (see above), since in this superimposition, the methyl groups occupy distinct regions in space, being separated by 0.84 Å.

Equation 2 implies that in this series binding energy is related exclusively to the geometry of the ligand–receptor hydrogen bond. The analysis assumes that other steric and electronic effects which could influence binding are essentially the same in the Table IV compounds. For example, it is assumed that the *exo*-hydroxyl groups present in **39–41** will have no effect on binding, by analogy with **13** and **20**. Similarly, the double bridgehead methylation in **35** is retained in the active analogues **12** and **17**. The possibility remains that steric interactions of the differently located aromatic rings (Figure 3) at the active site could explain relative activity. However we were unable to uncover correlations of equivalent significance to eq 1, between potency and various geometrical variables describing the relative disposition of the two aromatic rings.

Direct experimental evidence linking the energy of hydrogen bonds to their geometry, which would give credence to eq 2, is lacking.[32] However, some support for the model is found from the extensive studies of the geometrical distributions of hydrogen bonds in the crystalline state.[33] These studies have shown that C=O···HN hydrogen bonds tend to form near the plane of the carbonyl group and in the directions of the oxygen lone pairs.[34] Very few examples were found with C=O—N angles <90°, probably as a consequence of steric as well as electronic effects. Steric inhibition of hydrogen bonding at the active site, together with deviation from the ideal geometry, could therefore explain eq 2. The rigidity of these ligands means that induced fit at the active site will not occur, and a change in the orientation of the active-site acceptor group would be needed to compensate any alteration in directionality of the ligand hydrogen bond donor.

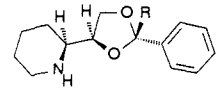
The structures of other compounds having activity at the NMDA receptor ion channel,[9] including phencyclidine (PCP, **57**) and benzomorphan derivatives [e.g. cyclazocine (**58**) and SK&F 10,047], have previously been compared with MK-801 [(+)-1] in a study of PCP receptor ligands.[11]



57 58

The current model can account for the binding of these ligands. Protonated models of **57** and **58** were built from their crystal structures,[35,36] and water molecules were minimized at hydrogen-bonding distances. Fitting of **57** and **58** to (+)-1 was done as described above, using three-point superimposition of (a) the centroids ofthe aryl rings with the part structure (**44**) of **1**, (b) the centroid of the cyclohexyl ring of **57** and a dummy atom centered between the four C–CH_3 carbons of **58** with the other aryl ring of **1**, and (c) the water molecules of each structure (weighted by 100 as before). The values of D, D_1, and θ which resulted were respectively: (**57**) 3.715 Å, 6.243 Å, 5.3°; (**58**) 4.125 Å, 6.246 Å, 18.9°. Relative K_i values (vs [^{3}H]MK-801[9]) and D and θ show the rank order (+)-1 < **57** < **58**, consistent with the trends found in eqs 1 and 2.

This model is also compatible with the binding[9] of more conformationally flexible ligands, such as the dioxolanes etoxadrol (**59**) and dexoxadrol (**60**), where the proposed angle of hydrogen bonding (see Figure 4 of ref 12) appears to show greater divergency from PCP (and **1**) than the compounds of Table IV. The changes in conformation of **59** which have have been suggested to occur on binding[12] imply induced fit to allow optimal hydrogen bonding of the nitrogen to occur in a less sterically demanding receptor environment.



59 R=Et
60 R=Ph

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(34) Taylor, R.; Kennard, O.; Versichel, W. *J. Am. Chem. Soc.* **1983**, *105*, 5761.
(35) Argos, P.; Barr, R. E.; Weber, A. H. *Acta Crystallogr.* **1970**, *B26*, 53.
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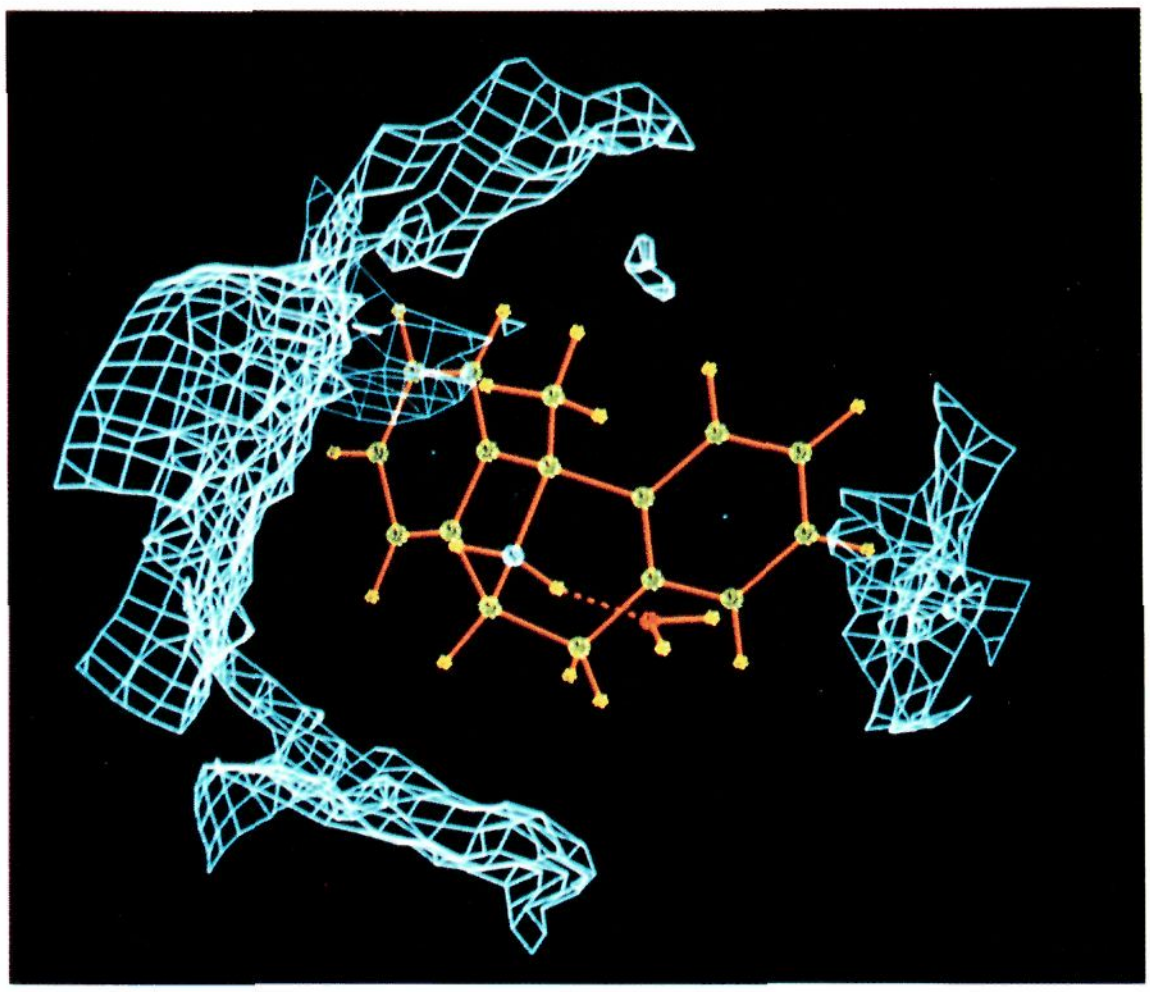

Figure 5. 3-Dimensional pharmacophoric map of the (+)-1 binding site on the ion channel of the NMDA receptor (see text). The structure of (+)-1 is shown, with the hydrogen-bonded water molecule mimicking an essential active site hydrogen bond acceptor group. The map was calculated by using CHEMX[31] and is contoured at 1.0-Å resolution with a 25 × 25 grid.

Active-Site Pharmacophore

The molecules in Tables I–IV were used to generate a 3-dimensional pharmacophore of the active site in the NMDA receptor ion channel. The "active analogue" approach[37] was used, where the combined van der Waals volumes of molecules having high affinity was taken to represent the free or excluded volume of the active site. Hydrocarbon substituted compounds showing reduced affinity were assumed to be competing with the active site for volume. Fitting of the molecules using the procedures outlined earlier and subtraction of the excluded volume from the volume occupied by the less active analogues gave the pharmacophoric map (Figure 5). The map shows that the aromatic rings of **1** are nearly completely engulfed by the active site. Interestingly, aryl ring reduced analogues of **1** retain high potency,[38] and these molecules also fit to the active site depicted in Figure 5, suggesting that the interaction is essentially hydrophobic in origin and is not specific for aromatic compounds. However, the map also shows that there is greater steric freedom in the region of the basic nitrogen atom. The pharmacophore can also accommodate PCP (**57**) and benzomorphan (**58**) analogues, and it explains the differential effect of nitrogen substitution, which in dibenzocycloalkenimines (e.g. **21–23**) reduces binding[16] and in PCP analogues has no effect.[11] The nitrogen substituent in the two series clearly occupy different regions in the active site when their hydrophobic and hydrogen-bonding groups are aligned.

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Conclusions

The binding site for MK-801 [(+)-1] and related molecules[9,11,12] shares the common features of a hydrophobic group and a basic nitrogen which are found for active-site recognition by many different classes of CNS-active substances.[1,2] The optimum nitrogen to aryl distance of 3.4 Å in this series is shorter than the mean distance of 5.0 Å found previously,[1a] but the nitrogen receptor site to aryl distances are more comparable, being 5.9 Å in the present series and 6.4 Å in the common CNS model.[1a] The site of action of **1** is not at a transmitter or hormone binding site, but is in the ion channel of the NMDA subtype of excitatory amino acid receptor. These results imply that ion channel binding sites in the CNS may share some structural resemblance to the binding sites of some transmitter molecules at their physiologically relevant receptors.

The conformational rigidity of the dibenzocycloalkenimines used in this study has provided a unique opportunity to evaluate specific geometrical properties relating to the relative disposition of hydrophobic and hydrogen-bonding groups which is required for active-site binding. The resulting correlation between affinity and a hydrogen-bonding vector (eq 2) suggests the design of alternative ligands could be accomplished by retaining the defined ideal hydrogen bonding angle and optimizing the essential hydrophobic binding interaction.

Experimental Section

Melting points were taken on an Electrothermal apparatus and are uncorrected. Proton NMR were measured on Bruker AM 360 or AC 250 spectrometers and chemical shifts are reported in parts per million (δ) downfield from tetramethylsialne as the internal standard. Mass spectra were recorded on a VG 70/250 spectrometer. Merck Kieselgel (230–400 mesh) was used for column chromatography. Organic solutions were dried with anhydrous sodium or magnesium sulfate. Elemental analyses were done by

the C.H.N. Microanalytical Laboratory, Wigston, Leicester, U.K.

7-Methyl-12,13-dihydro-7*H*-benzo[4,5]cyclohepta[1,2-*b*]naphthalen-7,12-imine (5) and 7-Methyl-12,13-dihydro-7*H*-benzo[4,5]cyclohepta[1,2-*b*]naphthalen-7,13-imine (8). Ketone **45** (2.2 g, 0.0086 mol, prepared from the 12,13-dihydro compound[39] by using established conditions[40]) was dissolved in tetrahydrofuran (THF, 50 mL) and to the cooled (0 °C) stirred solution was added, dropwise, a solution of methyllithium (9.2 mL of a 1.4 M solution in hexane, 0.0129 mol). After 20 min at room temperature the mixture was quenched with water (50 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated to dryness, and the residue was dissolved in dichloromethane (50 mL) and added over 10 min to a stirred suspension which had been preprepared by addition of sodium acetate (7.05 g, 0.086 mol) and hydroxylamine hydrochloride (6.0 g, 0.086 mol) to a cooled (0 °C) solution of dichloroacetic acid (10.64 mL, 0.13 mol) in dichloromethane (10 mL) and stirring of the mixture at room temperature for 1 h. After 2 h sodium hydroxide solution (1 M) was added to give a pH of 8, and the organic layer was removed, dried, and evaporated to give the crude hydroxylamine **46** as a white foam (1.5 g), which was dissolved in dry toluene (200 mL). The solution was heated at reflux for 2 h under a nitrogen atmosphere and then was cooled and evaporated to dryness. The product was purified by chromatography on silica gel, eluting with 10% ethyl acetate in hexane, to give a mixture of the cyclic hydroxylamines **47** and **48** (1.23 g). This mixture (0.62 g) was dissolved in acetic acid (10 mL), zinc powder (1.3 g) was added, and the mixture was stirred and heated at 65 °C under a nitrogen atmosphere. After 14 h the mixture was filtered and evaporated to dryness, and the residue crystallized from ether to give a 9:1 mixture of compounds **5** and **8** (0.063 g). The mixture was treated with a solution of hydrogen chloride in ethyl acetate, the solution concentrated under high vacuum, and the residue crystallized from methanol–acetate to give the pure imine **5** as its hydrochloride salt (0.065 g, 5%): mp >290 °C; ^{1}H NmR (360 MHz, DMSO-d_6) δ 2.28 (3 H, S, CH_3), 3.20 (1 H, d, J = 17.5 Hz, 13-H_{eq}), 3.76 (1 H, dd, J = 17.5 and 5.0 Hz, 13-H_{ax}), 5.40 (1 H, d, J = 5.0 Hz, 12-H), 7.29–7.93 (8 H, m, ArH), 7.69 (1 H, s, 1-H or 6-H), and 8.01 (1 H, s, 6-H or 1-H). Irradiation of 12-H (δ 5.40) gave a nuclear Overhauser enhancement (NOE) to the aromatic multiplet and not to H-1 or H-6 (δ 7.61 or 8.01). MS m/z = 271 (M^+). Anal. ($C_{20}H_{17}N$·1.5HCl) C, H, N.

The ethereal mother liquor from the initial crystallization (containing a 1:2 mixture of **5** and **8**) was evaporated and the residue treated with hydrogen chloride in ethyl acetate. Crystallization from ethyl acetate–methanol gave pure imine **8** as its hydrochloride salt (0.082 g, 6%): mp >290 °C; ^{1}H NMR (360 MHz, DMSO-d_6) δ 2.27 (3 H, s, CH_3), 3.09 (1 H, d, J = 17.7 Hz, 12-H_{eq}), 3.72 (1 H, dd, J = 17.7 and 4.9 Hz, 12-H_{ax}), 5.49 (1 H, d, J = 4.9 Hz, 13-H), 7.14–7.93 (8 H, m, ArH), 7.83 (1 H, s, 6-H), and 8.06 (1 H, s, 1-H). Irradiaiton of 13-H (δ 5.49) gave a NOE to 1-H (δ 8.06). MS m/z = 271 (M^+). Anal. ($C_{20}H_{17}N$·HCl·0.25H_2O) C, H, N.

5,10-Dimethyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene-5,10-imine (12). 10-Methyldibenzosuberenone (**49**)[41] (2.2 g, 0.01 mol) was successively treated with methyllithium and hydroxylamine as described above to give **50**, which was heated in refluxing xylene to give **51** (0.68 g, mp 179–180 °C). Reduction of **51** (0.34 g) with zinc as described above, followed by recrystallization of the hydrochloride from methanol–ethyl acetate–diethyl ether, gave **12** as its hydrochloride salt (0.12 g): mp 280 °C dec; ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.18 (3 H, s, CH_3), 2.38 (3 H, s, CH_3), 2.87 (1 H, d, J = 17.3 Hz, 11-H_{eq}), 3.82 (1 H, d, J = 17.3 Hz, 11-H_{ax}), 6.97–7.30 (8 H, m, ArH), and 10.69 (2 H, br s, NH_2^+); MS m/z = 235 (M^+). Anal. ($C_{17}H_{17}N$·HCl·0.6H_2O) C, H, N.

5,10-Dimethyl-11-*exo*-hydroxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene-5,10-imine (13) was obtained as its hydrochloride salt from the 10-methylsuberenone (**49**) as outlined in Scheme II using the methodology reported for the 10-desmethyl analogue:[27] mp 290–292 °C dec; ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.18 (3 H, s, CH_3), 2.37 (3 H, s, CH_3), 4.44 (1 H, d, J = 10.7 Hz, CH), 4.63 (1 H, d, J = 10.7 Hz, OH), and 7.06–7.46 (8 H, m, ArH); MS m/z = 251 (M^+). Anal. ($C_{17}H_{17}NO$·HCl·0.7H_2O) C, H, N.

5,10-Dimethyl-11-*endo*-hydroxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene-5,10-imine (14) was obtained from the suberenone (**49**)[41] by using reported[27] methods: mp 191–192 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.75 (3 H, s, CH_3), 1.90 (3 H, s, CH_3), 4.79 (1 H, s, CH), and 7.09–7.40 (8 H, m, ArH); MS m/z = 251 (M^+). Anal. ($C_{17}H_{17}NO$) C, H, N.

5,12-Dimethyl-5,6,11,12-tetrahydrodibenzo[*a*,*e*]cyclooctene-5,12-imine (17). To a solution of the imine **15**[17,18] (2.91 g, 0.0124 mol) in dichloromethane (100 mL) were added *p*-toluenesulfonyl chloride (4.72 g, 0.0248 mol) and 4-(dimethylamino)pyridine (3.03 g, 0.0248 mol). The resulting mixture was refluxed under nitrogen for 14 h, cooled, and evaporated to dryness and the residue purified by flash chromatography on silica gel, eluting with 50% dichloromethane in petroleum (bp 60–80 °C). Trituration of the crude product with pentane gave the tosylate **54** (3.85 g), mp 165–167 °C. To a stirred solution of tosylate **54** (0.50 g, 0.001 29 mol) in dry THF (25 mL) maintained under a nitrogen atmosphere was added methyllithium (1.7 mL of a 1.6 M solution in diethyl ether (0.0027 mol)). After 7 min, the reaction was quenched with water, diluted with brine, and extracted with diethyl ether. The procedure was repeated a further four times using a total of 3.2 g of **54**. The combined ether extracts were dried and evaporated, and the residue (2.63 g) was purified by exhaustive chromatography, finally using a Lobar (Merck) silica gel column, eluting with 4% methanol–0.2% ammonia in dichloromethane, to give the title compound (0.28 g). Treatment of the free base with hydrogen chloride in diethyl ether gave the hydrochloride salt of **17**: mp 230–234 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.23 (6 H, s, 2 CH_3), 2.90 (2 H, d, J = 16.4 Hz, 2 CH_{eq}), 4.36 (2 H, d, J = 16.4 Hz, 2 CH_{ax}), and 6.76–7.14 (8 H, m, ArH); MS m/z = 249 (M^+). Anal. ($C_{18}H_{19}N$·HCl·0.15H_2O) C, H, N.

12-Hydroxy-5-methyl-5,6,11,12-tetrahydrodibenzo[*a*,*e*]cycloocten-5,12-imine (20). To a stirred solution of the hydroxylamine **55**[17,18] (0.2 g) in glacial acetic acid (15 mL) at room temperature was added potassium bromide (20 mg) and manganese(III) acetate dihydrate (0.256 g). After 1 h, ethyl acetate (50 mL) was added, and sodium hydroxide solution (2 M) was added until an apparent pH of 12 was obtained. The organic layer was removed, the aqueous was extracted with further ethyl acetate, and the combined organic extracts were washed with water and then with brine, dried, and evaporated. The residue was purified by chromatography on silica gel, eluting with ethyl acetate–hexane, to give the acetate **56**, mp 135–138 °C dec. Compound **56** (0.14 g) was dissolved in acetic acid (5 mL), zinc powder (0.14 g) was added, and the mixture was heated at 60 °C with stirring for 12 h. The cooled solution was filtered, and 2 M sodium hydroxide solution was added to give an apparent pH of 12. The mixture was extracted with ethyl acetate, the combined organic extracts were dried and evaporated, and the residue was purified by chromatography on silica gel, eluting with 0–15% methanol in dichloromethane. Compound **20** was isolated as its hydrochloride salt (0.048 g, 35%): mp >280 °C; ^{1}H NMR (360 MHz, DMSO-d_6) δ 1.88 (3 H, s, CH_3), 3.05 and 3.95 (1 H each, d, J = 16.0 Hz, CH_3CCH_2), 3.27 and 4.18 (1 H each, d, J = 16.4 Hz, $HOCCH_2$), 6.85–6.94 (4 H, m, ArH), 7.19–7.25 (4 H, m, ArH), 8.39 (1 H, br s, OH), and 9.17 and 11.00 (1 H each, br s, NH_2^+); MS m/z = 251 (M^+). Anal. ($C_{17}H_{17}NO$·HCl) C, H, N.

N-Alkylated Derivatives 21–24. To a cooled (0 °C) solution of the imine **15** (0.50 g) in dichloromethane (80 mL) was added methyl iodide (0.132 mL). The solution was stirred at room temperature overnight, and then a further amount (0.66 g) of methyl iodide was added. After 4 h, the mixture was treated with concentrated ammonia (5 mL), and the organic layer was washed with sodium hydroxide solution, dried, and evaporated. Crystallization of the residue gave the quaternary salt **24** (0.090 g), mp 259–261 °C. Anal. ($C_{19}H_{22}IN$) C, H, N. The mother liquors were concentrated, and the residue was chromatographed on silica gel, eluting with 4.5% methanol–0.5% ammonia in dichloromethane to give the *N*-methyl derivative **21** isolated as its hydrochloride salt (0.023 g), mp 272–273 °C. Anal. ($C_{18}H_{19}N$·1.5HCl) C, H, N. Similarly prepared were the *N*-ethyl (**22**) and *N*-benzyl

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(**23**) derivatives. **22**·HCl: mp 164–166 °C. Anal. ($C_{19}H_{21}N$·1.45HCl) C, H, N. **23**: mp 118–122 °C. Anal. ($C_{24}H_{23}N$) C, H, N.

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Supplementary Material Available: Listings of coordinates used in the molecular modeling studies to generate the superimposition shown in Figure 3, i.e. compounds (+)-**1**, (−)-**1**, **15**, and **35–41** (11 pages). Ordering information is given on any current masthead page.