Novel Benzo[b]quinolizinium Cations as Uncompetitive N-Methyl-D-aspartic Acid (NMDA) Antagonists: The Relationship between log D and Agonist Independent (Closed) NMDA Channel Block

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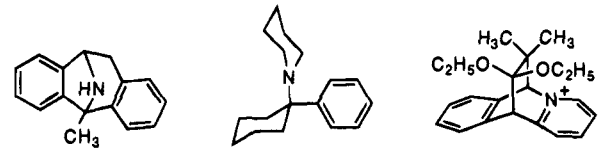
A series of permanently charged benzo[b]quinolizinium cations having lower lipophilicity than MK-801 or phencyclidine (PCP) were synthesized. Data relating agonist independent block of N-methyl-D-aspartic acid (NMDA) ion channels to log D are described. Closed channel access is predicted to result in a more noncompetitive profile of antagonism compared to selective open channel blockers, which are uncompetitive inhibitors. Reduced closed channel block may underlie the absence of PCP or MK-801-like behavioral side effects observed for benzo[b]-quinolizinium cations.

Introduction

Glutamate is the predominant excitatory neurotransmitter in the central nervous system. The associated postsynaptic excitatory amino acid receptors have been differentiated into several classes by pharmacological and molecular biological studies. The ionotropic groups have been named for their selective agonists: N-methyl-D-aspartic acid (NMDA), 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propanoic acid (AMPA), and kainic acid.[1] Ca^{2+} influx into neurons via activation of NMDA receptors has been implicated in acute neurodegeneration, especially ischemic stroke and head trauma, as well as chronic neurodegenerative conditions such as Huntington's disease, Parkinson's disease, AIDS dementia, and Alzheimer's disease.[2]

A site within the NMDA ion channel is specifically identified by [^{3}H]TCP ([1-(2-thienyl)cyclohexyl]piperidine) binding. The flow of Ca^{2+} through the NMDA receptor channel can be inhibited by the binding of TCP-site ligands such as phencyclidine (PCP), ketamine, dextrorphan, MK-801, and CNS 1102.[3] These molecules are neuroprotective in models of focal ischemia,[4] but the clinical advancement of this class of compounds has been hindered by side effects, including cognitive disturbance,[5] neuronal vacuolization,[6] and hemodynamic abnormalities.[7] In a previous paper, we reported on a high-affinity TCP-site ligand (WIN 63480, **1**) (Figure 1) that was shown to be an NMDA antagonist *in vitro* and anti-ischemic *in vivo*.[8] MK-801 and PCP are thought to access the NMDA ion channel by agonist dependent (open channel) and agonist independent (closed channel) routes.[9] In contrast to MK-801 being lipophilic (log D = +1.78), compound **1** has only one polar ionization state and is hydrophilic (log D = −4.08).[10] Herein, we report the relationship between log D and closed NMDA receptor channel access for compounds structurally related to 1, MK-801, and PCP.



MK-801 PCP 1 (WIN 63480)

Figure 1. Structures of NMDA antagonists.

Chemistry

The compounds (12,12-diaryl-6,11-ethanobenzo[b]-quinolizinium cations) chosen for this study were synthesized in a straightforward manner following the procedures described by Bradsher[11] and Fields.[12] The majority of the benzo[b]quinolizinium cations were made following two different methods. The prerequisite starting materials for the preparation of the cycloaddition products, 6,11-ethanobenzo[b]quinolizinium cations **3**,[11b] **4**,[13] **13**, **14**,[14] **21**, **23**,[16b] **24**,[15] **25**,[11d] and **26** (Table 1), were prepared through the acid-catalyzed cyclization of the appropriate pyridinium quaternary compounds. The latter were derived from the reaction of a substituted benzyl bromide and a pyridine-2-carboxaldehyde [2-(1,3)dioxolane] derivative (Scheme 1).[16]

Starting materials for the 6,11-ethanobenzo[b]quinolizinium cations **6**, **8**, **16**, **17**, **27**, and **29** (Table **1**) were made following the method described in a previous article.[17] The appropriate dianion was generated from either 2-bromobenzyl alcohol or 3-methoxybenzyl alcohol and reacted with a substituted pyridine-2-carboxaldehyde, followed by cyclization with $POCl_3$ to the benzo-[b]quinolizinium cation (Scheme 2). One exception to this method was the precursor for the 4-chloro-6,11-benzo[b]quinolizinium cation (**19**). This was prepared following the aforementioned dianion procedure using the appropriate 6-bromopyridine-2-carboxaldehyde derivative as the electrophile. However, the diol intermediate when cyclized with $POCl_3$ gave the halogen exchange product, 4-chloro-6,11-benzo[b]quinolizinium cation. The hydroxyl precursors were made by demethylation of the corresponding methyl ethers with

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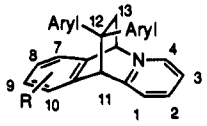
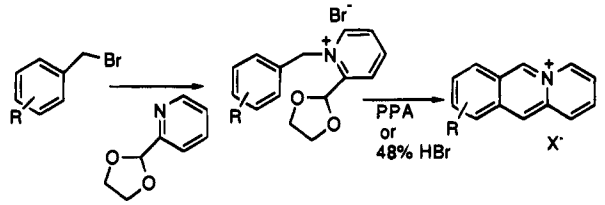
Table 1. [^{3}H]TCP Affinity of Compounds in Rat Brain Membranes and log *D* Values



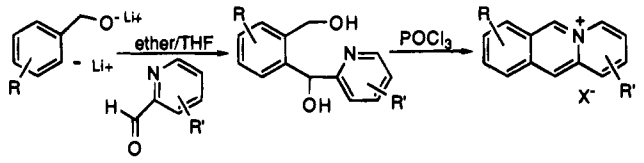
compound	aryl	R	[^{3}H]TCP K_i (nM)	log D[a]
3	3-C_4H_3O	H	2 ± 0.2	−3.41
4	3-C_4H_3O	6-CH_3	1.2 ± 0.2	ND
5	3-C_4H_3O	10,7-Br_2	345 ± 37	ND
6	3-C_4H_3O	10-OCH_3	6.0 ± 0.2	−3.66
7	3-C_4H_3O	10-OH	1.8 ± 0.3	−0.81
8	3-C_4H_3O	1-OCH_3	19 ± 3	ND
9	3-C_4H_3O	1-OH	294 ± 26	1.66
10	3-C_4H_3O	6-CN	10 ± 1	ND
11	3-C_4H_3O	10-OC_8H_{17}	3377 ± 348	ND
12	3-C_4H_3O	10-SO_3^-	>10000	ND
13	3-C_4H_3O	6-$COOCH_3$	39 ± 4	ND
14	3-C_4H_3O	[1,2]-benzo	19 ± 1	ND
15	3-C_4H_3O	10-CH_2OH	159 ± 17	ND
16	3-C_4H_3O	9,10-C_1H_2O	2.7 ± 0.5	−2.66
17	3-C_4H_3O	9-Cl, 10-OCH_3	64 ± 5	ND
18	3-C_4H_3O	9-Cl, 10-OH	24 ± 4	−0.99
19	3-C_4H_3O	4-Cl	633 ± 65	ND
20	3-C_4H_3O	3-OH	1530 ± 47	ND
21	3-C_4H_3O	9-OH	2.1 ± 0.2	0.32
22	3-C_4H_3O	4-OH (4-pyridone)	3785 ± 222	ND
23	3-C_4H_3O	9-F	3.6 ± 0.8	0.75
24	3-C_4H_3O	9-CO_2H	>10,000	1.76
25	3-C_4H_3O	8-OH	29 ± 2	ND
26	3-C_4H_3O	8,10-$(OH)_2$	125 ± 26	ND
27	3-C_4H_3O	8,10-$(OCH_3)_2$	3793 ± 54	ND
28	C_6H_5	H	8.2 ± 1.6	−2.54
29	C_6H_5	10-OH	12 ± 2	1.75

[a] ND = not determined.

Scheme 1



Scheme 2



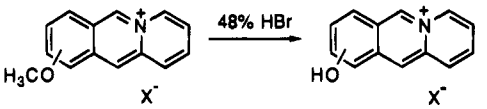
refluxing 48% HBr (Scheme 3). The precursors for **10** and **12** were prepared following the procedures described in the literature.[18,19]

The final cyclic adducts (Scheme 4) were prepared via reaction of an activated olefin with the benzo[*b*]quinolizinium cations in refluxing nitromethane or acetonitrile.[12,16] The diaryl olefins were made following methods previously described.[20]

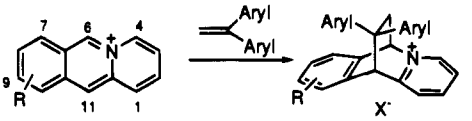
Structure–Activity Relationships

A series of 12,12-difuryl adducts were prepared with hydroxyl substitutions at various positions on the benzo and pyridinium rings (Table 1). These variations yielded a range of potency from K_i = 1.8 nM to K_i = 3785 nM in the binding assay. Hydroxyl substitution

Scheme 3



Scheme 4



2 **4 - 29**

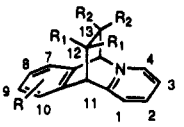
in the pyridinium ring, as in compounds **22** (4-OH, K_i = 3785 nM), **20** (3-OH, K_i = 1503 nM), and **9** (1-OH, K_i = 294 nM), has a detrimental effect on the binding affinity. Compound **22** is a special case since it may exist as a pyridone tautomer instead of a pyridinium cation. Hydroxylation of the benzo ring as in **7** (10-OH, K_i = 1.8 nM), **21** (9-OH, K_i = 2.1 nM), and **25** (8-OH, K_i = 32 nM) maintained the binding affinity. The 10-hydroxymethyl derivative **15** was 80-fold less active than the 10-hydroxy derivative **7**. Dihydroxylation as in **26** led to a loss of activity compared to that of the mono-hydroxyl derivatives. Compound **6**, the 10-methoxy analog of **7**, showed a 3-fold loss of affinity. The 1,3-methylenedioxy derivative **16** maintained affinity for the NMDA receptor channel. Ionized substituents showed the most pronounced effect on binding affinity. Substitution at the 9-position was usually beneficial; however, the 9-CO_2H derivative (**24**) had a 6000-fold lower affinity. A similar effect was observed when position 10 was substituted with SO_3^- (**12**). However, analogs with electron-withdrawing groups at position 6 such as compounds **10** and **13** had only a 5-fold lower affinity than **3** (Table 1). Compound **3** had a log *D* value of −3.41, while the corresponding 10-hydroxy analog, **7**, had a log *D* value of −0.81 yet maintained comparable affinity. Nonionizable structural modifications of the benzo or pyridinium moieties had little or no effect on log *D* values.

Electrophysiology

MK-801 and related TCP-site ligands such as PCP were observed to block NMDA ion channels by agonist dependent (open channel) and agonist independent (closed channel) mechanisms. These effects have previously been observed in both receptor binding and physiological experiments.[9c] Since channel block is due to binding to the TCP site, the affinity of a ligand should be identical for either closed or open channel access. Thus, differences in closed channel block were assessed by the ratio of closed/open channel block. This study indicated a high correlation (r^2 = 0.89) between more positive log *D* and closed channel access (reduced closed/open channel IC_{50} ratio) (Table 2, Figure 2), consistent with partitioning into the lipid membrane being a principal determinant of closed channel inhibition. There was no apparent correlation between compound affinity and closed channel access (r^2 = 0.15).

The ability to block open and closed NMDA channels is predicted to result in a noncompetitive profile of channel block, while a selective open channel inhibitor would be predicted to generate an uncompetitive profile of antagonism. Because of this difference in antago-

Table 2. Comparison of log *D* to Open Channel Selectivity



						NMDA channel block				
entry	R	R_1	R_2	pK_a	log *D*	open channel IC_{50} μM[a]	R^2	closed channel IC_{50} μM[a]	R^2	ratio of closed/open
MK-801				5.81	1.79	0.023	0.976	0.04	0.94	1.6
PCP				9.97	1.76	0.27	0.969	0.45	0.941	1.7
1	H	OC_2H_5	CH_3	12.20	−4.08	0.027	0.994	14.24	0.942	527
28	H	C_6H_5	H	11.7	−2.54	0.032	0.992	18.10	0.952	564
7	10-OH	C_4H_3O	H	8.60	−0.81	0.00913	0.937	3.86	0.981	422
9	1-OH	C_4H_3O	H	4.88/11.99	1.66	0.557	0.970	24.83	0.940	44.6
21	9-OH	C_4H_3O	H	8.80	0.3	0.0073	0.940	1.03	0.947	142

[a] IC_{50} values were calculated using a nonlinear curve fitting program (Graphpad Inc.,[32] see the Experimental Section). Goodness of fit was assessed from the absolute distance of data points from the curve and the coefficient of determination (R^2) computed. A perfect fit results in $R^2 = 1$ and a very poor fit in $R^2 = 0$.

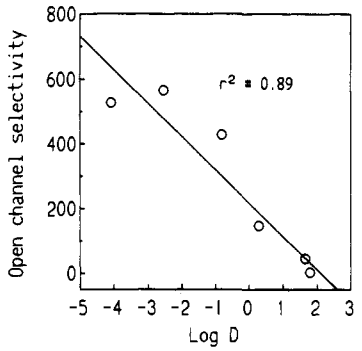


Figure 2. Linear regression correlation of lipophilicity and open channel selectivity (closed NMDA channel IC_{50}/open channel IC_{50}).

nism, especially at low rates of channel stimulation, MK-801 or PCP would be predicted to have greater NMDA antagonist activity (closed and open channel block) than more selective open channel blockers.

An important observation has been that benzo[*b*]-quinolizinium cations, which have closed/open channel inhibition ratios of >40, show no characteristic MK-801- or PCP-like behavioral side effects.[21] This lack of distinctive behavioral side effects for these benzo[*b*]-quinolizinium compounds was observed at anti-ischemic doses in the rat and equivalent drug plasma levels in the dog.[22] Differential antagonism in neural systems, due to varying levels of endogenous NMDA agonists, could explain the lack of MK-801- or PCP-like behavioral side effects observed with **1** and related compounds.

Conclusions

With a limited number of compounds studied, we have shown that there is a good correlation between lipophilicity and closed (agonist independent) NMDA channel block. Benzo[*b*]quinolizinium cations described herein had lower lipophilicity than MK-801 or PCP and reduced closed NMDA channel access. Closed channel access is predicted to produce a more noncompetitive profile of antagonism compared to selective open channel blockers (uncompetitive inhibitors), resulting in greater NMDA antagonism at low levels of agonist stimulation. Reduced closed channel block may underlie the absence of PCP- or MK-801-like behavioral side effects observed for benzo[*b*]quinolizinium cations.

Experimental Section

Infrared spectra were recorded on a Nicolet 20SX FTIR instrument. NMR spectra were acquired in the indicated solvent on a General Electric QE-300 FTNMR instrument. Mass spectra were recorded on a Nermag R10/10 apparatus coupled to a Varian 3400 Gas Chromatograph or on a JEOL JMS-01SC spectrometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Where analyses are indicated only by symbols of the elements, analytical results are within ±0.45% of the theoretical values. Thin layer chromatography (TLC) was performed on E. Merck 5 × 20, Kieselgel 60 F-254 plates. Preparative chromatography was performed using the flash method as described by Still.[23] Columns were packed with Kieselgel 60, 230–400 mesh. High-boiling point solvents were stage-dried over molecular sieves.[24] Anhydrous THF was distilled from sodium benzophenone ketyl. Alkyllithium reagents were titrated with diphenylacetic acid.[25] Other materials and reagents were purified by standard procedures where needed. Known benzo[*b*]-quinolizinium bromides, perchlorates, or hexafluorophosphates were prepared according to the published procedures.[11,16,17] Melting points were determined on a Mel-Temp apparatus and are uncorrected.

General Synthetic Methods. 7,10-Dibromobenzo[*b*]-quinolizinium perchlorate (2a): prepared as in literature;[12] yield 39%; mp >270 °C dec; ^{1}H NMR (DMSO-d_6) δ 10.51 (s, 1H), 9.73 (d, J = 6.9 Hz, 1H), 9.42 (s, 1H), 8.87 (d, J = 6.9 Hz, 1H), 8.33–8.12 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 140.7, 139.1, 137.5, 135.4, 134.9, 134.8, 133.2, 127.2, 125.8, 125.4, 123.6, 120.9, 120.48. Anal. ($C_{13}H_8Br_2ClNO_4$) C, H, Br, N.

General Demethylation Procedure. A solution of methoxybenzo[*b*]quinolizinium perchlorate (4.0 g, 0.013 mol) in 48% HBr (50 mL) was heated at 100 °C for 20 h and cooled to room temperature. The solids that precipitated were collected by filtration, redissolved in hot water, and treated with 20% aqueous $NaClO_4$ (50 mL). The precipitated yellow solid was collected by filtration and crystallized from iPrOH.

9-Chloro-10-hydroxybenzo[*b*]quinolizinium perchlorate (2b): from 9-chloro-10-methoxy derivative[17] following the general demethylation procedure; yield 79%; mp 203–205 °C; ^{1}H NMR (DMSO-d_6) δ 8.00 (m, 3H), 8.10 (t, J = 7.2 Hz, 1H), 8.70 (d, J = 8.5 Hz, 1H), 9.30 (d, J = 7.4 Hz, 1H), 9.30 (s, 1H), 10.28 (s, 1H), 11.60 (bs, 1H). Anal. ($C_{13}H_9Cl_2NO_5$) C, H, Cl, N.

4-Chlorobenzo[*b*]quinolizinium Perchlorate (2c).[17] The diol intermediate was prepared from 6-bromopyridine-2-carboxaldehyde and 2-bromobenzyl alcohol in 26% yield after the purification on a silica gel column, eluting with ethyl acetate: ^{1}H NMR ($CDCl_3$) δ 4.45 (bs, 1H), 4.49 (bs, 1H), 4.67 (bs, 1H), 4.99 (d, J = 15.0 Hz, 1H), 6.09 (s, 1H), 7.2–7.4 (m, 5H), 7.4–7.6 (m, 2H). The diol was then cyclized with $POCl_3$ (15 mL) as described to give **2c** in 15% yield after the purification on a silica gel column from CH_2Cl_2/CH_3OH (9:1):

MS (LISMS) 214 (M^+, 1Cl); ^1H NMR (DMSO-d_6) δ 7.76–7.86 (m, 2H), 7.93 (d, J = 9.0 Hz, 2H), 8.17 (d, J = 9.0 Hz, 1H), 8.38 (d, J = 9.0 Hz, 1H), 8.49 (d, J = 9.0 Hz, 1H), 9.12 (s, 1H), 10.50 (s, 1H). The compound was used directly in the cycloaddition reaction below without any further purification.

3-Hydroxybenzo[b]quinolizinium Hexafluorophosphate (2d). This compound was prepared by modification of the published procedure.[26] The ethylene glycol ketal of isoquinoline-3-carboxaldehyde[27] was quaternized with chloroacetone which was then cyclized with refluxing 48% HBr. The treatment of the resuting 3-hydroxybenzo[b]quinolizinium bromide with KPF_6 gave **2d**: yield 82%; mp 261–263 °C; ^1H NMR (DMSO-d_6) δ 7.73 (m, 1H), 7.97 (m, 2H), 8.26 (dd, J = 3.0, 8.1 Hz, 2H), 8.42 (d, J = 9.7 Hz, 1H), 8.66 (d, J = 1.9 Hz, 1H), 9.09 (s, 1H), 10.20 (s, 1H). Anal. ($C_{13}H_{10}F_6NOP$) C, H, N.

8,10-Dihydroxybenzo[b]quinolizinium bromide (2e): prepared from the corresponding 8,10-dimethoxy derivative[17] following the general demethylation procedure; yield 35%; mp >250 °C dec; ^1H NMR (DMSO-d_6) δ 6.88 (s, 1H), 6.92 (s, 1H), 7.65 (t, J = 9.0 Hz, 1H), 7.70 (t, J = 9.0 Hz, 1H), 8.28 (d, J = 9.0 Hz, 1H), 8.85 (s, 1H), 9.03 (d, J = 9.0 Hz, 1H), 9.85 (s, 1H). Anal. ($C_{13}H_{10}BrNO_2 \cdot 0.9H_2O$) C, H, Br, N.

10-(Chloromethyl)benzo[b]quinolizinium Perchlorate (2f). To a solution of 2,6-bis[(tetrahydro-2*H*-2-pyranyloxy)-methyl]phenyl bromide (prepared from 2,6-bis(hydroxymethyl)phenyl bromide)[28] (15.5 g, 39 mmol) in 200 mL of anhydrous ether at −30 °C was added *n*-BuLi (2.5 M, 16.4 mL, 41 mmol), and the mixture was allowed to warm to room temperature and stirred for 1.5 h. The mixture was cooled to 0 °C, and TMEDA (4.56 g, 37 mmol) was added. The reaction mixture was than cooled to −50 °C, and pyridine-2-carboxaldehyde (6.31 g, 58 mmol) was added. The above mixture was warmed to room temperature over a period of 2 h, the reaction quenched with saturated $NaHCO_3$ solution, and the mixture diluted with ethyl acetate while being stirred. The organic layer was washed with brine and concentrated *in vacuo* to afford 11.7 g (73%) of 1-(2-pyridyl)-1-[2,6-bis[(tetrahydro-2*H*-2-pyranyloxy)methyl]phenyl]methanol. The compound was used directly in the reaction below.

A solution of 1.0 g (2.42 mmol) of the above compound in 14 mL of acetic acid/THF/water (4:2:1) was heated at 100 °C under a nitrogen atmosphere for 6 h. The mixture was concentrated *in vacuo*, and the residue was redissolved in ethyl acetate and concentrated *in vacuo*. The residual solid was triturated with ether to afford 0.26 g (49%) of 1-(2-pyridyl)-1-[2,6-bis(hydroxymethyl)phenyl]methanol: ^1H NMR (DMSO-d_6) δ 4.71 (dd, J = 3.5, 13.7 Hz, 2H), 4.32 (dd, J = 5.3, 13.6 Hz, 2H), 5.19 (t, J = 5.1 Hz, 2H), 6.14–6.21 (m, 2H), 7.17–7.33 (m, 4H), 7.69 (d, J = 7.9 Hz, 1H), 7.81 (dt, J = 1.5, 7.7 Hz, 1H), 8.34 (d, J = 3.4 Hz, 1H).

A mixture of the hydroxymethyl compound (0.6 g, 2.5 mmol) and 10 mL of $POCl_3$ was heated to reflux with stirring for 4 h. The mixture was cooled, poured onto ice, stirred, and treated with 20% aqueous sodium perchlorate solution. The resulting solid was filtered, washed with water, and dried to give **2f**: yield 0.58 g (87%); ^1H NMR (DMSO-d_6) δ 5.42 (s, 2H), 7.97 (m, 2H), 8.11 (m, 1H), 8.24 (d, J = 6.7 Hz, 1H), 8.43 (d, J = 8.6 Hz, 1H), 8.63 (d, J = 8.8 Hz, 1H), 9.27 (d, J = 7.0 Hz, 1H), 9.41 (s, 1H), 10.45 (s, 1H). The compound was used directly in the preparation of **15**.

General Procedure for Cycloaddition Reaction. A mixture of the benzo[b]quinolizinium perchlorate, chloride, or hexafluorophosphate (5 mmol) and 1,1-diaryl ethylene compound (10 mmol) in acetonitrile or nitromethane (40 mL) was heated to reflux in an inert atmosphere (N_2 or Ar) for 8 h or until the reaction was complete as shown by TLC and then stirred at room temperature for 16 h. The volatiles were removed under reduced pressure, and the residue was triturated with ether (75 mL). The resulting solid was collected by filtration, washed successively with water, ether, and then hexanes, and dried under reduced pressure at ambient temperature to afford the crude product. The compounds were purified, and the salts were exchanged by ion exchange chromatography by following methods described previously.[8,20]

(±)-12,12-Di(3-furyl)-6,11-ethano-6-methyl-6,11-dihydrobenzo[b]quinolizinium chloride (4): yield 40%; mp 180–184 °C; ^1H NMR (DMSO-d_6) δ 2.58 (d, J = 14.3 Hz, 1H), 2.82 (d, J = 14.2 Hz, 1H), 3.36 (s, 3H), 5.96 (s, 1H), 6.31 (s, 1H), 6.54 (s, 1H), 7.33 (m, 3H), 7.44 (s, 2H), 7.51 (m, 3H), 7.93 (t, J = 6.7 Hz, 1H), 8.08 (d, J = 7.5 Hz, 1H), 8.41 (t, J = 7.5 Hz, 1H), 9.21 (d, J = 5.9 Hz, 1H). Anal. ($C_{24}H_{20}NO_2Cl \cdot 2.0H_2O$) C, H, N.

(±)-12,12-Di(3-furyl)-7,10-dibromo-6,11-ethano-6,11-dihydrobenzo[b]quinolizinium perchlorate (5): yield 92%; mp 174–179 °C; ^1H NMR (DMSO-d_6) δ 2.85 (dq, J = 2.9, 12.8 Hz, 2H), 5.86 (s, 1H), 6.13 (s, 1H), 6.55 (s, 1H), 6.85 (s, 1H), 7.27 (s, 1H), 7.61–7.47 (m, 4H), 7.74 (s, 1H), 7.98 (t, J = 6.7 Hz, 1H), 8.41 (d, J = 7.7 Hz, 1H), 8.50 (t, J = 7.7 Hz, 1H), 9.42 (d, J = 5.9 Hz, 1H). Anal. ($C_{23}H_{16}Br_2ClNO_6 \cdot 0.5H_2O$) C, H, N.

(±)-12,12-Di(3-furyl)-6,11-ethano-10-methoxy-6,11-dihydrobenzo[b]quinolizinium chloride (6): yield 90%; mp amorphous solid; ^1H NMR (DMSO-d_6) δ 2.52 (d, J = 14.2 Hz, 1H), 3.17 (dd, J = 2.3, 14.2 Hz, 1H), 3.62 (s, 3H), 5.48 (s, 1H), 5.53 (s, 1H), 6.23 (s, 1H), 6.91 (s, 1H), 7.06 (s, 1H), 7.23–7.40 (m, 4H), 7.51 (d, J = 7.9 Hz, 1H), 7.71–7.80 (m, 2H), 8.02 (d, J = 7.7 Hz, 1H), 8.23 (t, J = 7.7 Hz, 1H), 10.30 (d, J = 5.1 Hz, 1H). Anal. ($C_{24}H_{20}ClNO_3 \cdot 1.25H_2O$) C, H, Cl, N.

(±)-12,12-Di(3-furyl)-6,11-ethano-10-hydroxy-6,11-dihydrobenzo[b]quinolizinium chloride (7): yield 71%; mp 264–266 °C; ^1H NMR (DMSO-d_6) δ 2.48 (dd, J = 2.0, 15.0 Hz, 1H), 2.96 (dd, J = 2.0, 15.0 Hz, 1H), 5.93 (s, 1H), 6.14 (s, 1H), 6.47 (s, 1H), 6.64 (brs, 1H), 6.81 (d, J = 9.0 Hz, 1H), 7.02–7.13 (m, 2H), 7.42 (s, 1H), 7.43 (s, 1H), 7.46 (s, 2H), 7.89 (t, J = 9.0 Hz, 1H), 8.10 (t, J = 9 Hz, 1H), 8.27 (t, J = 9.0 Hz, 1H), 9.27 (d, J = 9.0 Hz, 1H). Anal. ($C_{24}H_{18}ClNO_3$) C, H, N.

(±)-12,12-Di(3-furyl)-6,11-ethano-1-methoxy-6,11-dihydrobenzo[b]quinolizinium chloride (8): yield 47%; mp amorphous powder; ^1H NMR (D_2O) δ 2.63 (d, J = 14.2 Hz, 1H), 2.83 (dd, J = 2.9, 14.2 Hz, 1H), 3.92 (s, 1H), 5.75 (s, 1H), 6.02 (s, 1H), 6.31 (s, 1H), 6.42 (brs, 1H), 7.00 (s, 1H), 7.22–7.34 (m, 6H), 7.55 (d, J = 7.0 Hz, 1H), 7.65 (dd, J = 6.0, 8.7 Hz, 1H), 7.84 (d, J = 8.8 Hz, 1H), 8.54 (d, J = 5.9 Hz, 1H). Anal. ($C_{24}H_{20}NO_3Cl \cdot 1.5\ H_2O$), C, H, N.

(±)-12,12-Di(3-furyl)-6,11-ethano-1-hydroxy-6,11-dihydrobenzo[b]quinolizinium chloride (9): yield 60%, mp amorphous powder; ^1H NMR (DMSO-d_6) δ 2.55 (d, J = 14.0 Hz, 1H), 2.82 (d, J = 14.0 Hz, 1H), 5.79 (s, 1H), 6.32 (s, 2H), 6.61 (s, 1H), 7.21–7.70 (m, 10H), 8.63 (d, J = 5.3 Hz, 1H). Anal. ($C_{23}H_{18}NO_3Cl \cdot 1.5H_2O$) C, H, N.

(±)-12,12-Di(3-furyl)-6,11-ethano-6-cyano-6,11-dihydrobenzo[b]quinolizinium chloride (10): yield 25%; mp 168 °C dec; ^1H NMR ($CDCl_3$) δ 2.35 (dd, J = 2.0, 15.0 Hz, 1H), 2.99 (dd, J = 2.0, 15.0 Hz, 1H), 5.55 (s, 1H), 5.63 (s, 1H), 6.43 (s, 1H), 7.46 (s, 1H), 7.50–7.76 (m, 6H), 7.80 (d, J = 9.0 Hz, 1H), 7.94 (t, J = 9.0 Hz, 1H), 8.44 (d, J = 9.0 Hz, 1H), 8.70 (t, J = 9.0 Hz, 1H), 9.19 (d, J = 9.0 Hz, 1H). Anal. ($C_{24}H_{17}ClN_2O_2 \cdot 1.5H_2O$) C, H, N.

(±)-12,12-Di(3-furyl)-6,11-ethano-10-*n*-octyl-6,11-dihydrobenzo[b]quinolizinium chloride (11): yield 63%; mp 143–145 °C; ^1H NMR (DMSO-d_6) δ 0.88 (t, J = 4.0 Hz, 3H), 1.45–1.28 (m, 10H), 1.85–1.80 (m, 2H), 2.50 (dd, J = 1.0, 14.56 Hz, 1H), 3.17 (dd, J = 3.5, 14.2 Hz, 1H), 4.02–3.93 (m, 2H), 5.37 (s, 1H), 5.75 (s, 1H), 6.06 (s, 1H), 6.68 (d, J = 1.1 Hz, 1H), 6.84–6.81 (m, 1H), 6.99 (s, 1H), 7.28–7.156 (m; 5H), 7.62 (d, J = 7.7 Hz, 1H), 7.76 (t, J = 7.0 Hz, 1H), 8.195 (d, J = 7.7 Hz, 1H), 9,40 (d, J = 5.92 Hz, 1H); HMRS for $C_{31}H_{34}NO_3$ calcd 468.254 18, found 468.254 18, dev. −0.67.

(±)-12,12-Di(3-furyl)-6,11-ethano-10-sulfenyl-6,11-dihydrobenzo[b]quinolizinium (12): yield 20%; mp >300 °C dec; ^1H NMR (DMSO-d_6) δ 2.55 (d, J = 14.5 Hz, 1H), 3.09 (dd, J = 1.8, 14.1 Hz, 1H), 6.10 (brs, 2H), 6.38 (s, 1H), 6.60 (s, 1H), 7.25 (m, 2H), 7.30 (s, 1H), 7.45 (s, 1H), 7.48 (d, J = 6.0 Hz, 1H), 7.65 (d, J = 5.7 Hz, 1H), 7.75 (d, J = 5.5 Hz, 1H), 7.85 (t, J = 7.8 Hz, 1H), 8.10 (s, 1H), 8.35 (t, J = 7.8 Hz, 1H), 9.20 (d, J = 5.6 Hz, 1H). Anal. ($C_{23}H_{17}NO_5S \cdot 2.25H_2O$) C, H, N, S.

(±)-12,12-Di(3-furyl)-6-carboxy-6,11-ethano-6,11-dihydrobenzo[b]quinolizinium chloride (13): yield 28%; mp 210–212 °C; ^1H NMR (DMSO-d_6) δ 2.85 (d, J = 14.5 Hz, 1H), 3.39 (d, J = 14.6 Hz, 1H), 4.18 (s, 3H), 6.02 (s, 1H), 6.30 (brs,

1H), 6.46 (s, 1H), 7.29–7.49 (m, 7H), 7.57 (d, J = 7.3 Hz, 1H), 7.95 (t, J = 7.0 Hz, 1H), 8.05 (d, J = 7.4 Hz, 1H), 8.44 (t, J = 7.5 Hz, 1H), 9.43 (d, J = 6.3 Hz, 1H). Anal. ($C_{25}H_{20}ClNO_4 \cdot 0.75H_2O$) C, H, N.

(±)-12,12-Di(3-furyl)[1,2]benzo-6,11-ethano-6,11-dihydrobenzo[*b*]quinolizinium chloride (14): yield 37%; mp 235–237 °C; ^{1}H NMR (DMSO-d_6) δ 2.61 (d, J = 13.8 Hz, 1H), 3.17 (dd, J = 2.0, 14.0 Hz, 1H), 6.55 (s, 1H), 6.73 (s, 1H), 6.96 (s, 2H), 7.17 (s, 1H), 7.21 (s, 1H), 7.30 (m, 2H), 7.47 (s, 1H), 7.67 (m, 3H), 8.00 (t, J = 8.7 Hz, 1H), 8.19 (t, J = 8.5 Hz, 1H), 8.22 (d, J = 7.4 Hz, 1H), 8.41 (d, J = 6.7 Hz, 1H), 9.12 (d, J = 6.8 Hz, 1H), 9.26 (d, J = 8.5 Hz, 1H). Anal. ($C_{27}H_{20}ClNO_2 \cdot 0.75H_2O$) C, H, Cl, N.

(±)-12,12-Di(3-furyl)-6,11-ethano-10-(hydroxymethyl)-6,11-dihydrobenzo[*b*]quinolizinium Perchlorate (15). 10-(Chloromethyl)benzo[*b*]quinolizinium was reacted with difurylethylene to give (±)-12,12-di(3-furyl)-6,11-ethano-10-(chloromethyl)-6,11-dihydrobenzo[*b*]quinolizinium perchlorate in 87% yield: ^{1}H NMR (DMSO-d_6) δ 2.71 (dd, J = 1.13, 14.4 Hz, 1H), 2.88 (dd, J = 2.9, 14.3 Hz, 1H), 4.69 (d, J = 11.5 Hz, 1H), 4.93 (d, J = 11.5 Hz, 1H), 5.90 (s, 1H), 6.70 (s, 1H), 7.07 (s, 1H), 7.40–7.34 (m, 3H), 7.54 (s, 1H), 7.60–7.63 (m, 2H), 7.93 (t, J = 6.4 Hz, 1H), 8.07 (d, J = 7.7 Hz, 1H), 8.42 (t, J = 8.1 Hz, 1H), 9.21 (d, J = 5.9 Hz, 1H). To this intermediate were added acetone (50 mL), acetonitrile (15 mL), and NaI (0.3 g, 1.9 mmol). The solution was heated at 60 °C for 4 h, cooled, and filtered. The filtrate was concentrated to give 0.8 g of a solid (±)-12,12-di(3-furyl)-6,11-ethano-10-(iodomethyl)-6,11-dihydrobenzo[*b*]quinolizinium perchlorate. To this compound were added 150 mL of a mixture of acetone and H_2O (1:1) and Na_2CO_3 (0.8 g, 7.5 mmol). The mixture was heated at 60 °C for 16 h and cooled and the acetone removed *in vacuo*. To the resulting slurry were added CH_3OH (25 mL) and $NaClO_4$ (10.0 g, 81 mmol). The CH_3OH was then removed *in vacuo*, and the mixture was filtered. The solid was dissolved in 2-propanol and CH_3CN, treated with charcoal, and filtered, and the filtrate was concentrated *in vacuo*. The solid was taken up in H_2O, cooled, and filtered to give a white solid **15**: yield 80%; mp 224–226 °C; ^{1}H NMR (DMSO-d_6) δ 2.63 (d, J = 14.1 Hz, 1H), 2.87 (dd, J = 2.9, 14.1 Hz, 1H), 4.22 (dd, J = 5.4, 14.0 Hz, 1H), 4.71 (dd, J = 5.8, 14.0 Hz, 1H), 5.27 (t, J = 5.6 Hz, 1H), 5.79 (s, 1H), 5.95 (s, 1H), 6.62 (s, 1H), 6.65 (s, 1H), 7.02 (s, 1H), 7.29 (s, 1H), 7.31 (s, 1H), 7.41 (s, 1H), 7.51 (m, 2H), 7.63 (s, 1H), 7.90 (t, J = 7.0 Hz, 1H), 8.14 (d, J = 7.7 Hz, 1H), 8.40 (t, J = 7.8 Hz, 1H), 9.19 (d, J = 5.9 Hz, 1H). Anal. ($C_{24}H_{20}ClNO_7$) C, H, N.

(±)-12,12-Di(3-furyl)-6,11-ethano-9,10-methylenedioxy-6,11-dihydrobenzo[*b*]quinolizinium chloride (16): yield 47%; mp 198–200 °C; ^{1}H NMR (DMSO-d_6) δ 2.57 (d, J = 14.0 Hz, 1H), (bd, J = 12.8 Hz, 1H), 5.86 (s, 1H), 5.97 (s, 1H), 6.03 (s, 1H), 6.21 (s, 1H), 6.50 (s, 1H), 6.76 (bs, 1H), 6.84 (d, J = 7.7 Hz, 1H), 7.11 (d, J = 7.8 Hz, 1H), 7.34 (s, 1H), 7.48 (m, 2H), 7.53 (s, 1H), 7.95 (t, J = 6.5 Hz, 1H), 8.16 (d, J = 7.6 Hz, 1H), 8.39 (t, J = 7.7 Hz, 1H), 9.35 (d, J = 4.1 Hz, 1H). Anal. ($C_{24}H_{18}ClNO_4 \cdot 0.75H_2O$) C, H, N.

(±)-12,12-Di(3-furyl)-9-chloro-6,11-ethano-10-methoxy-6,11-dihydrobenzo[*b*]quinolizinium chloride (17): yield 71%; mp foam; ^{1}H NMR ($CDCl_3$) δ 2.55 (d, J = 14.2 Hz, 1H), 3.09 (dd, J = 2.0, 14.4 Hz, 1H), 3.62 (s, 3H), 5.48 (s, 1H), 5.53 (s, 1H), 6.23 (s, 1H), 6.92 (s, 1H), 7.07 (s, 1H), 7.19 (s, 1H), 7.26 (d, J = 8.0 Hz, 1H), 7.31 (s, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.74 (m, 2H), 8.04 (d, J = 7.7 Hz, 1H), 8.25 (t, J = 7.6 Hz, 1H), 10.31 (d, J = 5.0 Hz, 1H). Anal. ($C_{24}H_{19}Cl_2NO_3 \cdot 0.5H_2O$) C, H, Cl, N.

(±)-12,12-Di(3-furyl)-9-chloro-6,11-ethano-10-hydroxy-6,11-dihydrobenzo[*b*]quinolizinium chloride (18): yield 40%; mp 256–258 °C; ^{1}H NMR (DMSO-d_6) δ 2.56 (d, J = 13.4 Hz, 1H), 2.97 (dd, J = 2.6, 14.1 Hz, 1H), 6.30 (s, 1H), 6.51 (s, 1H), 6.65 (s, 1H), 6.67 (s, 1H), 7.08 (d, J = 7.9 Hz, 1H), 7.29 (d, J = 7.8 Hz, 1H), 7.45 (m, 4H), 7.93 (t, J = 7.2 Hz, 1H), 8.06 (d, J = 7.8 Hz, 1H), 8.41 (t, J = 7.2 Hz, 1H), 9.26 (d, J = 5.8 Hz, 1H). Anal. ($C_{23}H_{17}Cl_2NO_3 \cdot 0.5H_2O$) C, H, N.

(±)-12,12-Di(3-furyl)-4-chloro-6,11-ethano-6,11-dihydrobenzo[*b*]quinolizinium chloride (19): yield 15%; mp foam; IR (KBr) 3141, 3096, 1617, 1561, 1466, 1214, 1165 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.77 (dd, J = 3.0, 15.0 Hz, 1H), 2.96 (dd, J = 3.0, 15.0 Hz, 1H), 5.75 (s, 1H), 5.82 (s, 1H), 6.51 (s, 1H), 6.93 (s, 1H), 7.18 (s, 2H), 7.30–7.43 (m, 4H), 7.56 (dd, J = 2.0, 6.0 Hz, 1H), 7.66 (dd, J = 2.0, 6.0 Hz, 1H), 7.93 (dd, J = 2.0, 8.0 Hz, 1H), 8.30 (m, 2H). Anal. ($C_{23}H_{17}Cl_2NO_2$) C, H, N.

(±)-12,12-Di(3-furyl)-6,11-ethano-3-hydroxy-6,11-dihydrobenzo[*b*]quinolizinium chloride (20): yield 20%; mp amorphous powder; ^{1}H NMR (DMSO-d_6) δ 2.51 (d, J = 14.1 Hz, 1H), 2.81 (d, J = 13.8 Hz, 1H), 5.52 (s, 1H), 6.20 (s, 1H), 6.42 (s, 1H), 6.48 (s, 1H), 7.23–7.60 (m, 10H), 8.52 (s, 1H). Anal. ($C_{23}H_{18}NO_3Cl \cdot 0.5H_2O$) C, H, N.

(±)-12,12-Di(3-furyl)-6,11-ethano-9-hydroxy-6,11-dihydrobenzo[*b*]quinolizinium chloride (21): yield 55%; mp 274–276 °C; ^{1}H NMR (DMSO-d_6) δ 2.52 (d, J = 13.5 Hz, 1H), 2.89 (dd, J = 2.5, 13.7 Hz, 1H), 5.77 (s, 1H), 6.22 (s, 1H), 6.51 (s, 1H), 6.65 (d, J = 1.7 Hz, 1H), 6.68 (d, J = 2.0 Hz, 1H), 6.85 (d, J = 1.9 Hz, 1H), 7.27 (s, 1H), 7.37 (d, J = 8.2 Hz, 1H), 7.48 (m, 3H), 7.89 (t, J = 6.7 Hz, 1H), 8.01 (d, J = 7.7 Hz, 1H), 8.36 (t, J = 7.8 Hz, 1H), 9.29 (d, J = 5.8 Hz, 1H), 9.95 (s, 1H). Anal. ($C_{23}H_{18}ClNO_3 \cdot 0.25H_2O$) C, H, Cl, N.

(±)-12,12-Di(3-furyl)-6,11-ethano-4-hydroxy-6,11-dihydrobenzo[*b*]quinolizinium (22). The cycloaddition product **19** (4-chloro) was hydrolyzed from methanolic 2 N sodium hydroxide on a steam bath for 1.5 h to give the crude 4-hydroxy derivative. The product was recrystallized from 2-propanol to give **22** as a yellow solid in 56% yield: mp 185 °C dec; ^{1}H NMR ($CDCl_3$) δ 2.34 (dd, J = 2.0, 15.0 Hz, 1H), 2.72 (dd, J = 3.0, 15.0 Hz, 1H), 4.40 (s, 1H), 5.31 (s, 1H), 5.65 (s, 1H), 6.05 (d, J = 6.0 Hz, 1H), 6.10 (d, J = 2.0 Hz, 1H), 6.38 (dd, J = 1.0, 9.0 Hz, 1H), 6.65 (t, J = 2.0 Hz, 1H), 6.83 (d, J = 1.0 Hz, 1H), 7.10 (s, 1H), 7.12–7.30 (m, 5H), 7.44 (dd, J = 1.0, 6.0 Hz, 1H); HMRS for $C_{23}H_{17}NO_3$ calcd 356.128 67, found 356.128 28, dev. −1.08.

(±)-12,12-Di(3-furyl)-6,11-ethano-9-fluoro-6,11-dihydrobenzo[*b*]quinolizinium chloride (23): yield 45%; mp 163–165 °C; ^{1}H NMR (DMSO-d_6) δ 2.57 (d, J = 13.9 Hz, 1H), 2.95 (dd, J = 2.3, 14.2 Hz, 1H), 5.88 (s, 1H), 6.31 (s, 1H), 6.43 (s, 1H), 6.78 (bs, 1H), 7.18 (m, 1H), 7.26–7.61 (m, 5H), 7.64 (m, 1H), 7.94 (t, J = 6.8 Hz, 1H), 8.03 (d, J = 7.8 Hz, 1H), 8.40 (t, J = 7.9 Hz, 1H), 9.30 (d, J = 5.8 Hz, 1H). Anal. ($C_{23}H_{17}ClFNO_2 \cdot 0.75H_2O$) C, H, Cl, N.

(±)-12,12-Di(3-furyl)-9-carboxy-6,11-ethano-6,11-dihydrobenzo[*b*]quinolizinium hexafluorophosphate (24): yield 5%; mp amorphous powder; ^{1}H NMR (DMSO-d_6) δ 2.67 (d, J = 14.2 Hz, 1H), 2.99 (d, J = 14.0 Hz, 1H), 5.95 (s, 1H), 6.35 (s, 1H), 6.48 (s, 1H), 7.35 (s, 1H), 7.45 (s, 1H), 7.49 (s, 1H), 7.55 (s, 1H), 7.75 (d, J = 7.6 Hz, 1H), 8.04–9.1 (m, 4H), 8.44 (t, J = 7.5 Hz, 1H), 9.26 (d, J = 5.2 Hz, 1H), 13.50 (brs, 1H); HRMS for $C_{24}H_{18}NO_4$ calcd 384.123 58, found 384.122 43, dev. −3.01.

(±)-12,12-Di(3-furyl)-6,11-ethano-8-hydroxy-6,11-dihydrobenzo[*b*]quinolizinium chloride (25): yield 22%; mp amorphous powder; ^{1}H NMR (DMSO-d_6) δ 2.52 (d, J = 13.6 Hz, 1H), 2.88 (dd, J = 3.1, 14.1 Hz, 1H), 5.65 (s, 1H), 6.16 (s, 1H), 6.46 (s, 1H), 6.59 (s, 1H), 6.64 (dd, J = 2.1, 8.9 Hz, 1H), 7.04 (d, J = 2.0 Hz, 1H), 7.15 (d, J = 8.1 Hz, 1H), 7.26 (s, 1H), 7.43 (s, 2H), 7.48 (s, 1H), 7.88 (t, J = 6.5 Hz, 1H), 7.96 (d, J = 7.16 Hz, 1H), 8.36 (t, J = 7.7 Hz, 1H), 9.23 (d, J = 5.9 Hz, 1H), 9.92 (s, 1H). Anal. ($C_{23}H_{18}NO_3Cl \cdot 0.75H_2O$) C, H, Cl, N.

(±)-12,12-Di(3-furyl)-6,11-ethano-8,10-dihydroxy-6,11-dihydrobenzo[*b*]quinolizinium chloride (26): yield 32%; mp amorphous powder; ^{1}H NMR (DMSO-d_6) δ 2.41 (d, J = 15.0 Hz, 1H), 2.91 (dd, J = 15.0, 2.0 Hz, 1H), 5.78 (s, 1H), 6.14 (s, 1H), 6.29 (s, 1H), 6.44 (s, 1H), 6.51 (s, 2H), 7.38–7.48 (m, 4H), 7.84 (t, J = 7.0 Hz, 1H), 8.2 (d, J = 7.0 Hz, 1H), 8.37 (t, J = 7.0 Hz, 1H), 9.25 (d, J = 7.0 Hz, 1H), 9.79 (s, 1H), 10.24 (s, 1H). Anal. ($C_{23}H_{18}ClNO_4 \cdot 1.98H_2O$) C, H, Cl, N.

(±)-12,12-Di(3-furyl)-6,11-ethano-8,10-dimethoxy-6,11-dihydrobenzo[*b*]quinolizinium perchlorate (27): yield 45%; mp amorphous powder; ^{1}H NMR (DMSO-d_6) δ 2.48 (d, J = 15 Hz, 1H), 2.90 (dd, J = 15.0, 2.0 Hz, 1H), 3.76 (s, 3H), 3.77 (s, 3H), 5.73 (s, 1H), 6.10 (s, 1H), 6.45 (s, 1H), 6.52 (d, J = 2.0 Hz, 1H), 6.56 (s, broad, 1H), 6.81 (d, J = 2.0 Hz, 1H), 7.25 (s, 1H), 7.43 (s, 1H), 7.45 (s, 1H), 7.47 (s, 1H), 7.90 (t, J = 7.0 Hz, 1H), 8.17 (d, J = 7.0 Hz, 1H), 8.39 (t, J = 7.0 Hz, 1H), 9.17 (d, J = 7.0 Hz, 1H). Anal. ($C_{25}H_{22}ClNO_8 \cdot 7H_2O$) C, H, Cl, N.

(±)-12,12-Diphenyl-6,11-ethano-6,11-dihydrobenzo[b]quinolizinium chloride (28): yield 40%; mp 169–174 °C; ^{1}H NMR (DMSO-d_6) δ 3.10 (d, J = 15.0 Hz, 1H), 3.45 (dd, J = 3.0, 15.0 Hz, 1H), 6.49 (s, 1H), 6.82 (brs, 1H), 7.04 (t, J = 6.0 Hz, 1H), 7.08 (t, J = 7.0 Hz, 1H), 7.16 (t, J = 7.0 Hz, 2H), 7.20 (t, J = 7.0 Hz, 2H), 7.24–7.30 (m, 4H), 7.36 (d, J = 7.0 Hz, 2H), 7.47 (dd, J = 3.0, 7.0 Hz, 2H), 7.63 (dd, J = 3.0, 7.0 Hz, 1H), 7.93 (t, J = 6.0 Hz, 1H), 8.13 (d, J = 6.0 Hz, 1H), 8.38 (t, J = 6.0 Hz, 1H), 9.36 (d, J = 6.0 Hz, 1H); IR (KBr) 3413, 3053, 1628, 1499, 1446 cm^{-1}. Anal. ($C_{27}H_{22}ClN\cdot1.25H_2O$) C, H, N.

(±)-12,12-Diphenyl-10-hydroxy-6,11-ethano-6,11-dihydrobenzo[b]quinolizinium chloride (29): yield 68%; mp >290 °C; ^{1}H NMR (MeOH-d_4) δ 2.90 (d, J = 14.3 Hz, 1H), 3.66 (dd, J = 4.1, 14.2 Hz, 1H), 6.09 (s, 1H), 6.55 (m, 1H), 6.78 (m, 1H), 7.03–7.20 (m, 10H), 7.36 (d, J = 7.9 Hz, 1H), 7.81 (m, 2H), 8.19 (t, J = 7.2 Hz, 1H), 9.22 (d, J = 5.8 Hz, 1H). Anal. ($C_{27}H_{22}NOCl\cdot0.25H_2O$) C, H, N.

Biological Methods. [^{3}H]TCP Radioreceptor Assay. [^{3}H]TCP binding to PCP recognition sites was performed as described by Vignon et al.[8,29]

Electrophysiology. Receptor Expression in Frog Oocyte. Total RNA was isolated from mouse brain by the use of Trizol reagent (Life Technologies, Gaithersburg, MD), a solution of phenol and guanidinium isothiocyanate. Briefly, mouse brains were removed from females anesthetized with CO_2 and sacrificed by decapitation. Animal procedures were approved by the Sanofi Winthrop Animal Care and Use Committee.[30] Forebrain tissue was homogenized in Trizol (10 mL/g of sample wet weight), followed by addition of chloroform (20% v/v) and centrifugation to separate aqueous and organic phases. RNA was precipitated from the aqueous phase by addition of 2-propanol and subsequent centrifugation, washed with 75% ethanol, and resuspended in water at 1–10 mg/mL.

Female *Xenopus laevis* were anesthetized in 0.17% tricaine (Sigma, St. Louis, MO) and oocytes surgically removed. Oocytes were suspended in OR-2 medium, consisting of the following (in millimolar): NaCl, 82.5; KCl, 2; $MgCl_2$, 1; and HEPES, 5 maintained at pH 7.5. Follicular cells were removed by treatment with 0.2% type A Collagenase (Boehringer). Stage V–VI oocytes[31] were placed in ND96 buffer consisting of the following (in millimolar): NaCl, 96; KCl, 2; $CaCl_2$, 1.8; $MgCl_2$, 2; HEPES, 5; theophylline, 0.5; sodium pyruvate, 2.5; and 50 μg/mL gentamycin, maintained at pH 7.5.

Approximately 50 mL of RNA solution was injected into each oocyte using a microdispenser (Nanoject, Drummond Sci. Co., Broomall, PA) mounted on a micromanipulator. Oocytes were incubated for 48–72 h at 18–22 °C in ND-96 medium, which was changed daily.

Electrophysiological Recording. Oocytes were placed in a small bath and superfused at approximately 4 mL/min with a medium with the following composition (in millimolar): NaCl, 88; KCl, 1; $CaCl_2$, 1.24; Hepes, 10; and $NaHCO_3$, 2.4, at pH 7.4. Two electrode voltage clamp recordings were made using low-resistance microelectrodes (1–2 μΩ) filled with 3 M KCl and either a Dagan 8500 instrument (Dagan Corp., Minneapolis, MN) or Geneclamp 500 instrument (Axon Instruments, Burlingame, CA). Data acquisition was performed using a computer-based program (pCLAMP; Axon Instruments) and an analog chart recorder.

Open channel block was assessed by application of a test compound with NMDA (100 μM) 90 s after the initiation of NMDA superfusion (to establish a relatively stable NMDA response). Single drug applications were made to individual cells due to tachyphylaxis of NMDA responses and slow reversal of channel block by inhibitor. Current at 300 s was normalized to the initial NMDA current, the values were averaged for control cells (NMDA alone), and inhibition produced by a compound (test) was calculated as ((control − test)/control) × 100%. To assess closed channel blockade, control responses were established by applying two pulses of NMDA (100 μM) in 1 mL of medium at 15 min intervals. Cells were then incubated for 30 min with 10 μM 7-chlorokynurenate (KYN) to minimize channel opening and washed for 1–2 min, and the response to 100 μM NMDA was again determined. In preliminary experiments, incubation with 10 μM KYN followed by a wash period did not affect subsequent NMDA responses. Kainate (100 μM) was used as an internal control for changes in response which were independent of NMDA channel block. Inhibitors under study were included with KYN for the 30 min incubation period, and the degree of NMDA inhibition was calculated as above by comparing the NMDA response following the incubation period to the initial control responses. IC_{50} values were calculated using a nonlinear curve fitting program[32] according to the equation % inhibition = 100/(1 + (IC_{50}/DRUG)^n) where IC_{50} is the concentration of inhibitor resulting in 50% inhibition and n is a slope value, which were fit to at least four data points (i.e. % inhibition data for at least four concentrations of inhibitor, which were replicated 2–4 times).

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