Notes

Novel NMDA Antagonists: Replacement of the Pyridinium Ring of 6,11-Ethanobenzo[*b*]quinolizinium Cations with Heteroisoquinolinium Cations

Virendra Kumar,*,† Phil M. Carabateas,† John A. Dority, Jr.,† William G. Earley,† John P. Mallamo,†,⊥ Chakrapani Subramanyam,†,⊥ Lisa D. Aimone,§,⊥ Brian Ault,§ Diane L. DeHaven Hudkins,‡ and Matthew S. Miller§,⊥

Departments of Medicinal Chemistry, Biochemistry, and Vascular and Neurodegenerative Pharmacology, Sterling Winthrop Pharmaceuticals Research Division, Collegeville, Pennsylvania 19426-0900

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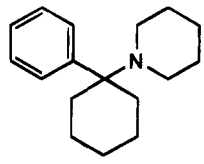
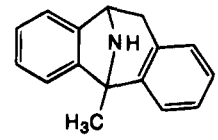
Replacement of the pyridinium ring of 6,11-ethanobenzo[*b*]quinolizinium cations with thiazolium (**4a** and **4b**)and *N*-methylimidazolium (**4c** and **4d**) resulted in equipotent compounds in the [^{3}H]TCP binding assay. The corresponding *N*-methyl-1,2,4-triazolium analogs were less potent in this assay. The thiazolium derivative **4b,** with a K_i = 2.9 nM, is being evaluated as a possible neuroprotective *N*-methyl-D-aspartic acid (NMDA) antagonist.

Introduction

Agents which prevent Ca^{2+} flux through *N*-methyl-D-aspartate (NMDA) regulated ion channels, such as phencyclidne (PCP, **1**) and the noncompetitive antagonist dizocilpine (MK-801, **2**), are effective antiischemic agents in several animal models.[1] The antiischemic efficacy of these prototypical compounds has been associated with long-lasting behavioral effects and hemodynamic effects which halted the therapeutic development of these compounds for the treatment of neurodegenerative diseases.[2,3] However, the effectiveness of NMDA ion channel blockers in neuroprotection experiments supports the hypothesis that NMDA receptors play a significant role in plasticity processes, pathophysiology, and excitotoxicity of neurons. These agents have been shown to work at a specific site within the ion channel labeled with [^{3}H]TCP. Thus the separation of the psychotomimetic and neuroprotective effects of new NMDA antagonists acting at the [^{3}H]TCP site could result in clinically useful neuroprotective agents.

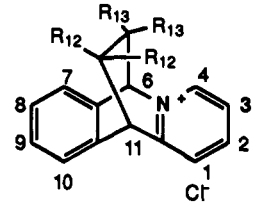


1, Phencyclidine (PCP)



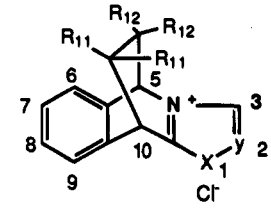
2, Dizocipline (MK-801)

We have recently published[4,5] data on two closely related unique series (**3a** and **3b**) of compounds which are highly selective uncompetitive NMDA antagonists acting at the [^{3}H]TCP site of the ion channel. Both series of compounds inhibit NMDA-induced current and NMDA-induced cell death in cultured cortical neuronal cells but are devoid of the psychotomimetic effects demonstrated by previously reported PCP ligands. Compounds **3a** and **3b** were potent and selective inhibitors with K_is of 4.0 and 2.0 nM (vs [^{3}H]TCP), respectively. These compounds had an uncompetitive profile of inhibiton, consistent with their selectivity for the open NMDA ion channels, which may be one of the reasons for the lack of PCP like side effects. In addition, both of these compounds were active in the middle cerebral artery occlusion (MCAO) focal ischemia model in rats.[4,5] In order to expand the strcture–activity relationship (SAR) of these novel NMDA antagonists, we were interested in preparing the quaternary compounds in which the pyridinium ring is replaced with other heterocycles without compromising the pharmacological pfofile shown by compounds **3a** and **3b**. Herein, we describe the replacement of the pyridinium ring with the five-membered heterocycles which will also change the electronic character of this part of the molecule as well. The resulting 6,11-ethanoheteroquinolinium cation derivatives (**4**) are potent and selective antagonists at the PCP site of NMDA receptor complex.



3a, R_{12} = OC_2H_5; R_{13} = CH_3

3b, R_{12} =  ; R_{13} = H



4, R_{11} = OC_2H_5 , 

R_{12} = H, CH_3

X = S, N-R; Y = CH, N

R = alkyl, aryl

Chemistry

The requisite heteroisoquinolinium cations (**9a–j**) were prepared from the corresponding aldehydes (**6**) following either Fields or Bradsher methods[6–8] (Scheme 1). The synthesis of imidazo[1,2-*b*]isoquinolinium cations **9h** [X = NCH_2(1-naphthyl), Y = CH] and **9i** [X = NCH_2(2-naphthyl), Y = CH] was reported by us in a previous paper.[9] The dianion, prepared from 2-bro-

† Department of Medicinal Chemistry.
‡ Department of Biochemistry.
§ Department of Vascular and Neurodegeneration Pharmacology.
⊥ Present address: Cephalon Inc., West Chester, PA 19380-4245.
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0022-2623/95/1838-1826$09.00/0

Scheme 1

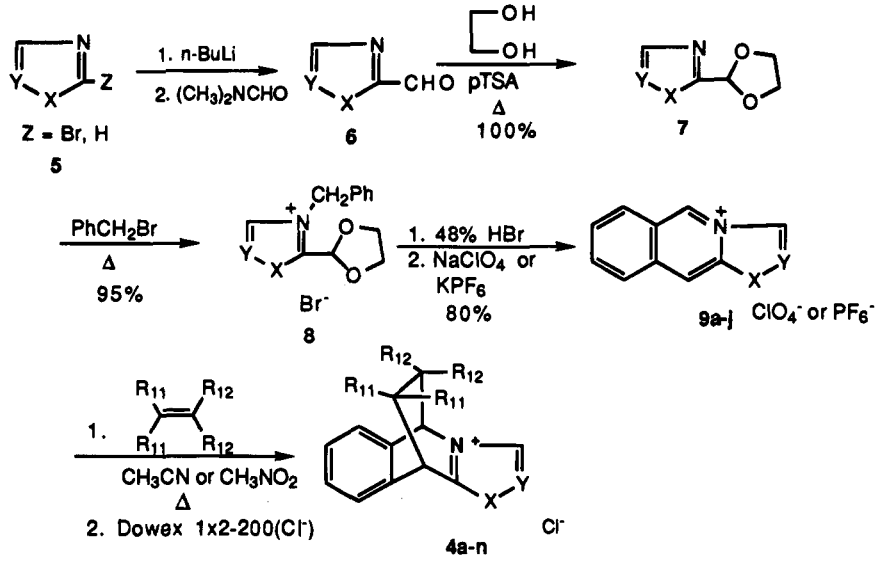
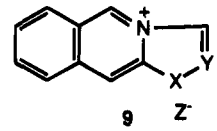


Table 1. Physical Properties of New Heteroisoquinolinium Cations



compd	X	Y	molecular formula/Z^-	anal.	mp (°C)	yield (%)
9a	S	CH	$C_{11}H_8NO_4SClO_4^-$	C, H, N, S	220–222 dec	90
9b	NCH_3	CH	$C_{12}H_{11}N_2PF_6^-$	C, H, F, N	198–200	60
9c	N-*n*-C_4H_9	CH	$C_{15}H_{17}N_2PF_6^-$	C, H, F, N	142–144	62
9d	NPh	CH	$C_{17}H_{13}N_2ClO_4^-$	C, H, Cl, N	208–210 dec	67
9e	NCH_2Ph	CH	$C_{11}H_8N_2 \cdot 0.25H_2O/Br^-$	C, H, N	175–177	90
9f[a]	$NCH(Ph)_2$	CH	$C_{24}H_{19}N_2/PF_6^-$			10
9g	$N(CH_2)_3Ph$	CH	$C_{20}H_{19}N_2PF_6^-$	C, H, F, N	160–162 dec	36
9j	NCH_3	N	$C_{11}H_{10}N_3/ClO_4^-$	HRMS 184.08723 (M − ClO_4^-)	148–150 dec	45

[a] Satisfactory elemental analysis was not obtained.

mobenzyl alcohol/*n*-BuLi, was reacted with the appropriate 2-imidazolecarboxaldehyde followed by dehydration of the diol with $POCl_3$ resulting in the imidazo[1,2-*b*]isoquinolinium cations. Thiazole aldehyde **6** (X = S, Y = CH) was prepared from 2-bromothiazole (**5**, Z = Br) as described in the literature.[10] The 2-imidazolyl (**6**, X = NCH_3 and others, Y = CH) and 2-triazolyl (**6**, X = NCH_3, Y = N)[11] aldehydes were prepared from the corresponding 2-unsubstituted compounds following the literature methods.[12] The physical properties of the new heteroisoquinolinium cations are described in Table 1.

The inverse electron demand Diels–Alder reactions of **9** were performed in either CH_3CN or CH_3NO_2 as solvents with dienophiles such as ketene acetal[4] and 1,1-di-3-furylethylene as reported before.[5] The initial adduct was then converted to the chloride counterion using the ion-exchange method, and the list of compounds (**4a–n**) prepared along with the biological results are shown in Table 2.

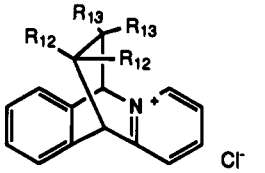
Biological Results and Discussions

As depicted in Table 2, the binding affinity for the [^{3}H]TCP site of the NMDA ion channel for the pyridinium replacement compounds **4a–n** was dependent upon both the heteroisoquinolinium portion of the molecule and the substituents at the R_{11} and R_{12} positions. The diethoxy ketal derivatives **4a** (thiazole) and **4c** (*N*-methylimidazole) have similar binding affinity at the PCP site of the channel with K_is of 6.1 and 8.2 nM, respectively, comparable to **3a** (K_i = 5.3 nM). Similarly, the 3-furyl analogs of the thiazolium (**4b**, K_i = 2.9 nM) and *N*-methylimidazolium (**4d**, K_i = 2.9 nM) derivatives were equipotent in the binding assay compared to the pyridinium derivative **3b** (K_i = 1.8 nM). However the *N*-methyltriazolium analog **4m** was nearly 6-fold less active (K_i = 30.0 nM) than **3a** (K_i = 5.3 nM). One may speculate that the NMDA channel complex is sensitive to the presence of a heteroatom at this part of the molecule which could participate in a hydrogen binding in a nondesirable fashion. A similar loss in potency was also observed for the 3-furyl compound (**4n**) when compared to **3b**, **4b**, and **4d**, which were single digit nanomolar potent compounds at TCP site.

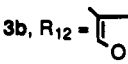
The imidazolium replacement of pyridinium ring presented opportunity to vary the substituents at the N-1 position. As shown in Table 2, steric bulk (**4i**, **4k**, and **4l**) and increasing length of the alkyl chain (**4j**) were detrimental to the binding affinity. The *N*-benzylimidazole derivative (**4h**), with a K_i of 7.7 nM, was the only other potent analog.

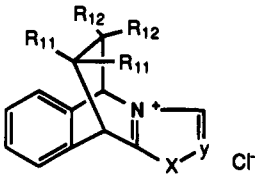
From these data it became apparent that for compounds **4**, the best substitution at R_{11} was 3-furyl and at R_{12} was the hydrogen. Also, the thiazolium and imidazolium were comparable replacements of the pyridinium ring of **3a** and **3b**. Analogs from each series

Table 2. [^{3}H]TCP Binding Affinity of Compounds in Rat Brain Homogenate



3a, R_{12} = OC_2H_5; R_{13} = CH_3

3b, R_{12} =  ; R_{13} = H



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compd	X	Y	R_{11}	R_{12}	mp (°C)	yield (%)	K_i (nM)[b]	NMDA induced cell death: IC_{50} (nM)[c]
2 (MK-801)							2.2 ± 0.2	67.0
3a							5.3 ± 0.9	60.0
3b							1.8 ± 0.2	45.0
4a	S	CH	OC_2H_5	CH_3	122–124 dec	54	6.1 ± 0.8	173.0
4b	S	CH	3-furyl	H	amorphous	82	2.9 ± 0.4	50.0
4c	NCH_3	CH	OC_2H_5	CH_3	amorphous	69	8.2 ± 1.2	53.0
4d	NCH_3	CH	3-furyl	H	amorphous	81	2.9 ± 0.1	257.0
4e	N-*n*-C_4H_9	CH	3-furyl	H	amorphous	52	36 ± 1.4	ND
4f	NPh	CH	3-furyl	H	amorphous	64	52 ± 6	ND
4g	NCH_2Ph	CH	OC_2H_5	CH_3	215–217	70	10 ± 1	ND
4h	NCH_2Ph	CH	3-furyl	H	240–242	65	7.7 ± 0.4	42.0
4i	$NCH(Ph)_2$	CH	3-furyl	H	amorphous	30	306 ± 17	ND
4j	$N(CH_2)_3Ph$	CH	3-furyl	H	amorphous	57	68 ± 11	ND
4k	CH_2-(1-naphthyl)	CH	3-furyl	H	165–167	40	329 ± 21	ND
4l	NCH_2- (2-naphthyl)	CH	3-furyl	H	220–222	45	330 ± 38	ND
4m	NCH_3	N	OC_2H_5	CH_3	188–190	88	30 ± 2	ND
4n	NCH_3	N	3-furyl	H	222–224	37	14 ± 1	ND

[a] The [^{3}H]TCP binding to the PCP site was performed as described by Vignon et. al.[13] [b] Mean of at least of three separate determinations in triplicate. [c] Neuroprotection in cultured mouse cortical neurons (see the Experimental Section). ND = not determined.

of compounds were also neuroprotective in primary cultures of cortical neurons in the presence of excitotoxic concentrations of NMDA (Table 2). As previously demonstrated for these cationic compounds,[4,5] the potency in the cell death assay correlate with the binding affinity at the PCP site of the NMDA ion channel complex.

Thus, the obejctive of the replacement of pyridinium ring of 6,11-ethanobenzo[*b*]quinolizinium cations with other heterocyclics, such as thiazolium and *N*-methylimidazolium, was ascertained without any deleterious effects on the binding affinity. Among these pyridinium replacements, the thiazolium analog **4b** (K_i = 2.9 nM) was found to be the best compound of this series. It displayed selectivity for the open state of the NMDA channel complex, consistent with a log D = −3.08[13] which is similar to compound **3b** (log D = −3.41) of the pyridinium series.[5] Both of these compound are undergoing further evauation as NMDA antagonist.

Conclusions

In summary, we have shown that the replacement of the pyridinium ring of 6,11-ethanobenzoquinolinium cations with thiazolium and imidazolium moieties resulted in compounds with nanomolar affinity potency at the PCP site of the NMDA channel. The thiazolium compound **4b** is undergoing further evaluation as a possible neuroprotective agent and will be the subject of future publication.

Experimental Section

Melting points are uncorrected. ^{1}H-NMR were recorded on a JEOL-FX270 or General Electric QE-300 spectrometer with tetramethylsilane as an internal standard. Infrared spectra were measured on a Perkin-Elmer model 467 or Nicolet 20 SX FT IR instrument. Mass spectra were determined using a JOEL JMS-O1SC model instrument. Elemental analyses were performed by Galbraith Laboratories of Knoxville, TN, Instranal Laboratories of Rensselaer, NY, or QTI of Whitehouse, NJ. Where analyses are indicated only by symbols of the elements, analytical results are within ±0.4% of the theoretical values. Thin-layer chromatography (TLC) was performed on E. Merck 5 × 20, Kieselgel 60 F-254 plates. Column chromatography was performed with Whatman LP52 (37–53 μm) SiO_2 or Kieselgel 60 (230–400 mesh). Preparative HPLC was performed on a Waters Prep 500 instrument using two standard silica Prep-pak cartridges. Most of the yields reported here are from single experiments and are unoptimized.

General Method. A mixture of the heteroisoquinolinium perchlorate or hexafluorophosphate (5 mmol), dimethylketene diethyl acetal,[4] or 3-furylethylene[5] (10 mmol) in acetonitrile or nitromethane (40 mL) was heated to reflux in an inert atmosphere (N_2 or Ar) for 8–48 h or until the reaction is complete by TLC (CH_2Cl_2:CH_3OH, 9:1). The solvent was removed under reduced pressure, and the residue was treated with diethyl ether (75 mL) for trituration in a sonication bath. 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. In most instances, the TLC of the crude showed a single product. Minor impurities were removed on a silica gel column, eluting with CH_2Cl_2:CH_3OH (4:1).

Ion-Exchange Procedure. A column of Dowex 1×2-200 ion-exchange resin (300 g) was eluted with 0.5 N HCl until the eluant was clear and was then washed with distilled water until a pH of about 6.5–7.5 was obtained. A solution of the appropriate crude 5,10-ethano-5,10-dihydroheteroisoquinoquinolinium perchlorate, hexafluorophosphate, in a minimun amount of acetonitrile or methanol was loaded and the column rinsed with distilled water until the eluant no longer contains the product as detected by TLC. The water was removed under reduced pressure (lyophilization or rotary evaporation) to provide the pure chloride salt in near quantitative yield.

11,11-Diethoxy-12,12-dimethyl-5,10-ethano-5,10-dihydrothiazole[3,2-*b*]isoquinolin-4-ium chloride (4a): Beige-colored solid (CH_2Cl_2:Et_2O, 1:1); MS (LSIMS, *m/z*) 230 (M^+ −

Cl); IR (KBr) 3408, 3088, 3048, 2976, 2900, 1639, 1542, 1219 cm^{-1}; ^1H-NMR (300 MHz, $CDCl_3$) δ 0.92 (s, 3H), 1.05 (m, 9H), 3.48 (q, J = 3.8 Hz, 2H), 3.60 (q, J = 4.0 Hz, 2H), 5.58 (s, 1H), 6.75 (s, 1H), 7.30 (m, 2H), 7.50 (m, 1H), 7.68 (m, 1H), 7.94 (d, J = 3.5 Hz, 1H), 9.46 (d, J = 3.3 Hz, 1H). Anal. ($C_{19}H_{24}ClNO_2S \cdot 0.75H_2O$) C, H, Cl, N.

11,11-Di-3-furyl-5,10-ethano-5,10-dihydrothiazol[3,2-*b*]isoquinolin-4-ium chloride (4b): white powder (H_2O); MS (LSIMS, m/z) 348 (M-Cl); ^1H-NMR (300 MHz, DMSO-d_6) δ 2.53 (d, J = 13.5 Hz, 1H), 2.92 (d, J = 12.8 Hz, 1H), 6.11 (s, 1H), 6.35 (s, 1H), 6.55 (s, 1H), 6.85 (s, 1H), 7.25–7.61 (m, 8H), 8.02 (d, J = 2.7 Hz, 1H), 8.67 (d, J = 2.6 Hz, 1H). Anal. ($C_{21}H_{16}ClNO_2S \cdot 0.75H_2O$) C, H, N, S.

11,11-Diethoxy-12,12-dimethyl-5,10-ethano-5,10-dihydro-1-methylimidazo[1,2-*b*]isoquinolin-4-ium chloride (4c): white powder (CH_2Cl_2:Et_2O, 1:1); MS (LSIMS, m/z) 327 (M^+ – Cl); ^1H-NMR (300 MHz, $CDCl_3$) δ 0.85 (s, 3H), 0.93 (s, 3H), 1.05 (m, 6H), 3.45 (m, 2H), 3.65 (m, 2H), 4.10 (s, 3H), 5.40 (s, 1H), 5.72 (s, 1H), 7.30 (m, 2H), 7.48 (d, J = 2.3 Hz, 1H), 7.55 (d, J = 6.5 Hz, 1H), 7.78 (d, J = 6.2 Hz, 1H), 8.10 (d, J = 2.2 Hz, 1H). Anal. ($C_{20}H_{27}ClN_2O_2 \cdot 0.75H_2O$) C, H, Cl, N.

11,11-Di-3-furyl-5,10-ethano-5,10-dihydro-1-methylimidazo[1,2-*b*]isoquinolin-4-ium chloride (4d): white powder (H_2O); MS (LSIMS, m/z) 343 (M^+ – Cl); ^1H-NMR (300 MHz, DMSO-d_6) δ 2.38 (d, J = 13.0 Hz, 1H), 2.84 (dd, J = 3.5 Hz, J = 12.9 Hz, 1H), 3.63 (s, 3H), 6.11 (s, 1H), 6.32 (s, 1H), 6.42 (bs, 1H), 7.26 (m, 2H), 7.38–7.53 (m, 7H), 7.60 (s, 1H), 7.84 (d, J = 1.7 Hz, 1H). Anal. ($C_{22}H_{19}ClN_2O_2 \cdot 1.5H_2O$) C, H, Cl, N.

11,11-Di-3-furyl-5,10-ethano-5,10-dihydro-1-butylimidazo[1,2-*b*]isoquinolin-4-ium chloride (4e): white powder (H_2O); MS (LSIMS, m/z) 385 (M^+ – Cl); ^1H-NMR (300 MHz, DMSO-d_6) δ 0.82 (m, 3H), 1.25 (m, 4H), 2.48 (d, J = 12.0 Hz, 1H), 3.00 (d, J = 12.5 Hz, 1H), 4.18 (m, 2H), 6.35 (s, 1H), 6.40 (bs, 1H), 6.60 (bs, 2H), 7.25 (s, 2H), 7.50 (m, 9H), 7.95 (s, 1H). Anal. ($C_{25}H_{25}ClN_2O_2 \cdot 2H_2O$) C, H, Cl, N.

11,11-Di-3-furyl-5,10-ethano-5,10-dihydro-1-phenylimidazo[1,2-*b*]isoquinolin-4-ium chloride (4f): cream-colored powder (H_2O); MS (LSIMS, m/z) 405 (M – Cl); ^1H-NMR (300 MHz, DMSO-d_6) δ 2.44 (d, J = 13.0 Hz, 1H), 3.05 (dd, J = 3.0 Hz, J = 12.6 Hz, 1H), 5.60 (s, 1H), 5.85 (s, 1H), 6.38 (s, 1H), 6.55 (s, 1H), 7.15 (m, 3H), 7.30 (m, 2H), 7.42 (m, 2H), 7.60 (m, 5H), 7.68 (m, 1H), 7.90 (d, J = 2.0 Hz, 1H), 8.20 (d, J = 2.3 Hz, 1H). Anal. ($C_{27}H_{21}ClN_2O_2 \cdot H_2O$) C, H, Cl, N.

11,11-Diethoxy-12,12-dimethyl-5,10-ethano-5,10-dihydro-1-(phenylmethyl)imidazo[1,2-*b*]isoquinolin-4-ium chloride (4g): white solid (CH_3CN:EtOAc, 1:1); MS (LSIMS, m/z) 403 (M^+ – Cl); IR (KBr) 3394, 3090, 3030, 2975, 2938, 1577, 1542, 1475, 1455, 1059 cm^{-1}; ^1H-NMR (300 MHz, DMSO-d_6) δ 0.79 (s, 3H), 0.85 (s, 3H), 0.911 (t, J = 12.2 Hz, 6H), 3.37 (q, J = 12.0 Hz, 2H), 3.61 (q, J = 12.5 Hz, 2H), 5.40 (d, J = 14.0 Hz, 1H), 5.68 (s, 1H), 5.77 (d, J = 14.5 Hz, 1H), 5.92 (s, 1H), 7.25–7.70 (m, 10H), 7.94 (d, J = 1.3 Hz, 1H). Anal. ($C_{26}H_{31}ClN_2O_2 \cdot 0.5H_2O$) C, H, Cl, N.

11,11-Di-3-furyl-5,10-ethano-5,10-dihydro-1-(phenylmethyl)imidazo[1,2-*b*]isoquinolin-4-ium chloride (4h): cream-colored solid (CH_3CN); MS (LSIMS, m/z) 419 (M^+ – Cl); IR (KBr) 3423, 3362, 3087, 2913, 1580, 1454, 1162, 1028 cm^{-1}; ^1H-NMR (300 MHz, DMSO-d_6) δ 2.45 (d, J = 12.0 Hz, 1H), 2.98 (dd, J = 2.0 Hz, J = 12.4 Hz, 1H), 5.25 (d, J = 12.5 Hz, 1H), 5.48 (d, J = 13.0 Hz, 1H), 6.30 (s, 1H), 6.35 (s, 1H), 6.50 (s, 1H), 6.62 (s, 1H), 7.10–7.60 (m, 14H), 7.90 (d, J = 2.0 Hz, 1H). Anal. ($C_{28}H_{23}ClN_2O_2 \cdot 0.5H_2O$)C, H, N.

11,11-Di-3-furyl-5,10-ethano-5,10-dihydro-1-(diphenylmethyl)imidazo[1,2-*b*]isoquinolin-4-ium hexafluorophosphate (4i): beige-colored powder (CH_2Cl_2:Et_2O, 1:1); MS (LSIMS, m/z) 495 ($M^+ - PF_6$); ^1H-NMR (300 MHz, $CDCl_3$) δ 2.40 (d, J = 12.4 Hz, 1H), 3.25 (dd, J = 2.0 Hz, J = 12.0 Hz, 1H), 4.70 (s, 1H), 5.65 (s, 1H), 6.30 (s, 1H), 6.35 (s, 1H), 6.38 (d, J = 2.4 Hz, 1H), 6.63 (d, J = 2.2 Hz, 1H), 6.80–7.50 (m, 18H), 8.20 (d, J = 2.5 Hz, 1H); HRMS $C_{34}H_{27}F_6N_2O_2P$ ($M - PF_6^-$) calcd 495.202 75, found 495.207 04.

11,11-Di-3-furyl-5,10-ethano-5,10-dihydro-1-(phenylpropyl)imidazo[1,2-*b*]isoquinolin-4-ium chloride (4j): cream-colored powder (CH_2Cl_2:Et_2O, 1:1); MS (LSIMS, m/z) 447 (M^+ – Cl); ^1H-NMR (300 MHz, $CDCl_3$) δ 1.95 (m, 2H), 2.40 (d, J = 12.0 Hz, 1H), 2.38 (m, 1H), 2.50 (m, 1H), 3.10 (d, J = 12.4 Hz, 1H), 4.25 (m, 2H), 6.10 (s, 1H), 6.19 (s, 1H), 6.38 (s, 1H), 6.85 (bs, 1H), 7.00–7.40 (m, 12H), 7.55 (bd, J = 3.5 Hz, 1H), 7.70 (m, 1H), 8.40 (bs, 1H). Anal. ($C_{30}H_{27}ClN_2O_2 \cdot 0.25H_2O$) C, H, Cl, N.

11,11-Di-3-furyl-5,10-ethano-5,10-dihydro-1-(1-naphthylmethyl)imidazo[1,2-*b*]isoquinolin-4-ium chloride (4k): beige solid (EtOAc); MS (LSIMS, m/z) 469 (M^+ – Cl); ^1H-NMR (300 MHz, $CDCl_3$) δ 2.28 (d, J = 12.4 Hz, 1H), 2.98 (d, J = 12.2 Hz, 1H), 5.18 (s, 1H), 5.30 (d, J = 13.5 Hz, 1H), 5.45 (s, 1H), 5.62 (d, J = 12.8 Hz, 1H), 6.20 (bs, 2H), 6.28 (d, J = 2.5 Hz, 1H), 7.00–7.58 (m, 14H), 7.88 (bs, 1H). Anal. ($C_{32}H_{25}ClN_2O_2 \cdot 1.5H_2O$) C, H, Cl, N.

11,11-Di-3-furyl-5,10-ethano-5,10-dihydro-1-(2-naphthylmethyl)imidazo[1,2-*b*]isoquinolin-4-ium chloride (4l): white powder (H_2O); Ms (LSIMS, m/z) 469 (M^+ – Cl); ^1H-NMR (300 MHz, DMSO-d_6) δ 2.35 (d, J = 12.3 Hz, 1H), 2.90 (d, J = 12.0 Hz, 1H), 5.30 (m, 2H), 5.78 (s, 1H), 5.82 (s, 1H), 6.15 (s, 1H), 6.50 (bs, 1H), 6.90–7.70 (m, 16H), 8.00 (bs, 1H). Anal. ($C_{32}H_{25}ClN_2O_2 \cdot 1.25H_2O$) C, H, Cl, N.

11,11-Diethoxy-12,12-dimethyl-5,10-ethano-5,10-dihydro-1-methyl-1,2,4-triazolo[1,2-*b*]isoquinolin-4-ium chloride (4m): white powder (CH_2Cl_2:Et_2O, 1:1); MS (LSIMS, m/z) 328 (M^+ – Cl); IR (KBr) 3420, 2976, 1610, 1604, 1539, 1362, 1181, 1084, 1060 cm^{-1}; ^1H-NMR (300 MHz, DMSO-d_6) δ 1.25 (s, 3H), 1.36 (s, 3H), 1.45 (m, 6H), 3.94 (q, J = 7.0 Hz, 2H), 4.15 (q, J = 7.4 Hz, 2H), 4.63 (s, 3H), 6.39 (s, 1H), 6.47 (s, 1H), 7.90 (m, 2H), 8.06 (d, J = 5.8 Hz, 1H), 9.87 (s, 1H). Anal. ($C_{19}H_{26}ClN_3O_2 \cdot H_2O$) C, H, Cl, N.

11,11-Di-3-furyl-5,10-ethano-5,10-dihydro-1-methyl-1,2,4-triazolo[1,2-*b*]isoquinolin-4-ium chloride (4n): white solid (CH_3CN); MS (LSIMS, m/z) 344 (M^+ – Cl); ^1H-NMR (300 MHz, DMSO-d_6) δ 2.41 (d, J = 13.0 Hz, 1H), 3.00 (dd, J = 3.4 Hz, J = 12.5 Hz, 1H), 3.84 (s, 3H), 6.40 (bs, 2H), 6.50 (s, 1H), 6.64 (s, 1H), 7.30 (m, 2H), 7.40–7.61 (m, 6H), 9.15 (s, 1H). Anal. ($C_{21}H_{18}ClN_3O_2 \cdot 0.25H_2O$) C, H, N.

[^{3}H]TCP Receptor Binding Assay. [^{3}H]TCP binding to PCP recognition sites was performed as described by Vignon.[14] Male Sprague–Dawley rats were sacrificed by decapitation, and whole brains were homogenized in 10 volumes (wt/vol) of cold Tris-HCl buffer (50 mM, pH 7.7) using a Brinkmann Polytron (setting 6, 30 s). The homogenate was centrifuged at 40000g for 10 min at 4 °C. The supernatant was decanted, and the homogenization and centrifugation steps were repeated twice as described above. Following this, the pellet was resuspended in Tris-HCl (5 mM, pH 7.7) at a tissue concentration of 0.5–0.75 g/mL, and 1 mL aliquots were frozen at −70 °C until use. The binding characteristics for PCP recognition sites were not altered by the freezing of membrane suspensions.

On the day of the assay, membrane aliquots were thawed, resuspended in fresh 5 mM Tris-HCl buffer at a tissue concentration of 1 mg/mL, and stored on ice until use. Each assay tube contained 100 μL of [^{3}H]TCP at a final concentration of approximately 1 nM, 100 μL of various concentrations of the compounds of interest, 500 μL of the tissue suspension, and 300 μL of buffer to a final assay volume of 1 mL and a final protein concentration of 0.5 mg/tube. Nonspecific binding was defined by addition of a final concentration of 100 μM PCP to blank tubes. All tubes were incubated at room temperature for 25 min before termination of the reaction by rapid filtration over Whatman GF/B glass fiber filters that had been presoaked in a solution of 0.5% polyethylenimine for at least 1 h prior to use. Filters were washed with three 4 mL volumes of cold Tris buffer. Following addition of scintillation cocktail, the amount of bound radioactivity was determined by liquid scintillation spectrometry using a Beckman LS 5000TA liquid scintillation counter with an efficiency for tritium of approximately 55%. Inhibition constants (K_i values) were calculated using the EBDA/LIGAND program,[15] purchased from Elsevier/Biosoft, Inc.

Neuroprotection in Cultured Mouse Cortical Neurons. Pregnant, Swiss-Webster mice were obtained from Taconic Farms and killed 16 days post conception. Fetuses were removed and placed in a sterile dish containing Hank's balanced salt solution (HBSS), pH 7.4. Brain cortices were

dissected, meninges removed, and then the tissue was minced and placed into a solution of HBSS containing 0.25% (w/v) trypsin at 37 °C for 15 min. Tissue was then triturated with a sterile pasteur pipet, diluted with minimal essential media (Gibco 330-1430), pH 7.4, supplemented with 10% horse serum, 10% fetal calf serum, 2 mM L-glutamine, 21 mM d-glucose, 2.2 g/L sodium bicarbonate, 1000 units/mL penicillin, and 1000 μg/mL streptomycin. Cells were plated onto Falcon Primaria 96-well plates at a final density of 50 000 cells/well and incubated at 37 °C in the presence of 5% (v/v) carbon dioxide. After 5 days, plating media was replaced with maintenance media containing minimal essential media (Gibco 330-1430), pH 7.4, supplemented with 10% horse serum, 10% L-glutamine, 21 mM D-glucose, 2.2 g/L sodium bicarbonate, 1000 units/mL penicillin, 1000 μg/mL streptomycin, and 10 μM cytosine arabinoside. On days 7 and 10, media was replaced with maintenance media as above lacking the cytosine arabinoside. Experiments were conducted on day 13.

Day 13 cultured cortical neurons were washed twice with minimal essential media, pH 7.4, and then exposed for 30 min to 500 μM *N*-methyl-D-aspartic acid (NMDA) with or without varying concentrations of test agents. MK-801 at a final concentration of 10 μM was routinely included as a positive control. MK-801 and test agents were prepared in minimal essential media supplemented with 21 mM D-glucose and 2.2 g/L sodium bicarbonate (MEM). After 30 min, media was replaced with MEM alone. Exposure of neurons to test agents was limited to the NMDA treatment period. Twenty-four hours after removal of NMDA, an aliquot of media from each well was removed for assessment of cell injury by determining lactate dehydrogenase (LDH) activity by the method of Wroblewski and LaDue.[16,17]

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