Structure-Activity Studies on Benzhydrol-Containing Nipecotic Acid and Guvacine Derivatives as Potent, Orally-Active Inhibitors of GABA Uptake

Michael R. Pavia,*,† Sandra J. Lobbestael,† David Nugiel,† Daniel R. Mayhugh,† Vlad E. Gregor,† Charles P. Taylor,‡ Roy D. Schwarz,‡ Laura Brahce,‡ and Mark G. Vartanian‡

Departments of Chemistry and Pharmacology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, Michigan 48105-2430

Received February 19, 1992

The introduction of lipophilic groups onto the ring nitrogen of nipecotic acid and guvacine, two known GABA uptake inhibitors, afforded potent, orally-active anticonvulsant drugs. A series of compounds is reported which explores the structure-activity relationships (SAR) in this series. Among the areas explored: side-chain SAR (aromatic-, heterocyclic-, and tricyclic-containing side chains) and modifications to the tetrahydropyridine ring. The benzhydrol ether-containing side chains afforded the most potent compounds with several exhibiting in vitro IC_{50} values for GABA uptake of <1 μM (including **5**, Table I; **37**, **43**, Table IV; and **44**, Table V). Compound **44** was selected for extensive evaluation and subsequently progressed to Phase 1 clinical trials with severe adverse effects seen after single dose administration to humans.

Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system.[1-3] Malfunctions of the central GABA system have been suggested to contribute to the development of certain psychiatric and neurological diseases,[4,5] and there is considerable evidence that impaired operation of GABAergic synapses may be an important causative factor in seizure disorders. Consequently, compounds which enhance GABAergic inhibition in the brain are of interest as possible therapeutic agents.[6-9] There are several methods by which GABAergic transmission could be enhanced, including GABA receptor agonists and inhibitors of GABA metabolism. In addition, since the synaptic actions of GABA are primarily terminated by reuptake into nerve terminals and glia cells,[10,11] an additional strategy to enhance the synaptic actions of GABA is to block its reuptake.[12-14] The relevance of GABA uptake inhibition to antiepileptic drug research has recently been reviewed.[15]

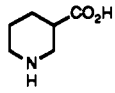
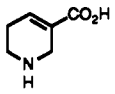
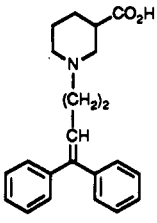
Small amino acids such as nipecotic acid and guvacine (Table I) are potent inhibitors of GABA uptake with no affinity for GABA receptors.[11,16] There are also a number of additional amino acid derivatives which have been reported to be GABA uptake inhibitors such as guanidinoethanesulfonic acid[17] and 3-pyrrolidineacetic and carboxylic acids.[14] These compounds do not readily enter the CNS following peripheral administration, presumably due to their hydrophilic nature.[18-20] A number of more lipophilic analogs of these GABA uptake inhibitors have been reported in an attempt to increase their ability to cross the blood-brain barrier, although their limited

* Present address: Genesis Pharmaceuticals, Inc., 840 Memorial Drive, Cambridge, MA 02139.
† Department of Chemistry.
‡ Department of Pharmacology.

(1) Curtis, D. R. Gabergic Transmission in the Mammalian Central Nervous System. In *GABA Neurotransmiters. Pharmacochemical, Biochemical and Pharmacological Aspects*; Krogsgaard-Larsen, P., Scheel-Kruger, J., Kofod, H., Eds.; Munksgaard: Copenhagen, 1979; pp 17-27.
(2) Roberts, E.; Chase, T. N.; Tower, D. B., Eds. *GABA in Nervous System Function*; Raven Press: New York, 1976.
(3) Young, A. B.; Chu, D. Distribution of $GABA_A$ and $GABA_B$ Receptors in Mammalian Brain: Potential Targets for Drug Development. *Drug Dev. Res.* 1990, *21*, 161-8.
(4) Krnjevic, K. GABA Mediated Inhibitory Mechanisms in Relation to Epileptic Discharges. In *Basic Mechanisms of Neuronal Hyperexcitability*; Jasper, H. H., Van Gelder, N. M., Eds.; Alan R. Liss: New York, 1983, pp 249-80.
(5) Taylor, C. P. GABA Receptors and GABAergic Synapses as Targets for Drug Development. *Drug Dev. Res.* 1990, *21*, 151-60.
(6) Krogsgaard-Larsen, P. γ-Aminobutyric Acid Agonist, Antagonist, and Uptake Inhibitors. Design and Therapeutic Aspects. *J. Med. Chem.* 1981, *24*, 1377-83.
(7) Allan, R. D.; Johnston, G. A., Synthetic Analogs for the Study of GABA as a Neurotransmitter. *Med. Res. Rev.* 1983, *3*, 91-118.
(8) Kaplan, J.-P.; Raizon, B. M.; Desarmenien, M.; Feltz, P.; Headley, P. M.; Worms, P. M.; Lloyd, K. E.; Bartholini, G. New Anticonvulsant Schiff Bases of γ-Aminobutyric acid and γ-Aminobutyramide. *J. Med. Chem.* 1980, *23*, 702-704.
(9) Meldrum, B. M. GABAergic Mechanisms in the Pathogenesis and Treatment of Epilepsy. *Br. J. Clin. Pharmacol.* 1989, *27* (Suppl. 1), 3S-11S.
(10) Iversen, L. L.; Kelly, J. S. Uptake and Metabolism of Gamma-aminobutyric acid by Neurons and Glial Cells. *Biochem. Pharmacol.* 1975, *24*, 933-8.
(11) Krogsgaard-Larsen, P. In *Specific GABA Receptor Agonist and Uptake Inhibitors: Design, Development, and Structure-Activity Studies*; adl's Forlag: Copenhagen, 1980.
(12) Krogsgaard-Larsen, P.; Falch, E.; Larsson, O. M.; Schousboe, A. GABA Uptake Inhibitors: Relevance to Antiepileptic Drug Research. *Epilepsy Res.* 1987, *1*, 77-93.
(13) Wood, J. D. GABA-Uptake Inhibitors as Anticonvulsant agents. In *Problems in GABA Research from Brain to Bacteria*; Okada, Y.; Roberts, E.; Excerpta Medica: Amsterdam, 1982; pp 173-194.
(14) Krogsgaard-Larsen, P.; Labouta, I.; Meldrum, B. S.; Croucher, M.; Schousboe, A. GABA Uptake Inhibitors as Experimental Tools and Potential Drugs in Epilepsy. In *Neurotransmitters, Seizures and Epilepsy*; Morselli, P. L., Lloyd, K. G., Loscher, W., Meldrum, B. S., Reynolds, E. H., Eds.; Raven Press: New York, 1981; pp 23-36.
(15) Larsson, O. M.; Falch, E.; Krogsgaard-Larsen, P.; Schousboe, A. GABA Uptake Inhibitors: Relevance to Antiepileptic Drug Research. *Epilepsy Res.* 1987, *1*, 77-93.
(16) Schousboe, A.; Thorbek, P.; Hertz, L.; Krogsgaard-Larsen, P., Effects of GABA Analogues of Restricted Conformation on GABA Transport in Astrocytes and Brain Cortex Slices and on GABA Receptor Binding. *J. Neurochem.* 1979, *33*, 181-9.
(17) Li, Y. P.; Lombardini, J. B., Guanidinoethanesulfonic acid—Inhibitor of GABA uptake in Rat Cortical Synaptosomes. *Brain Res.* 1990, *510*, 147-9.
(18) Lodge, D.; Johnston, G. A. R.; Curtis, D. R.; Brand, S. J. Effects of the Areca Nut Constituents Acrecaidine and Guvacine on the Action of GABA in the Cat Central Nervous System. *Brain Res.* 1977, *136*, 513-22.
(19) Frey, H. H.; Popp, C.; Loscher, W., Influence of Inhibitors of High Affinity GABA Uptake on Seizure Threshold in Mice. *Neuropharmacology* 1979, *18*, 581-90.
(20) Croucher, M. J.; Meldrum, B. S.; Krogsgaard-Larsen, P. Anticonvulsant Activity of GABA Uptake Inhibitors and Their Prodrugs Following Central or Systemic Administration. *Eur. J. Pharmacol.* 1983, *89*, 217-28.

0022-2623/92/1835-4238$03.00/0

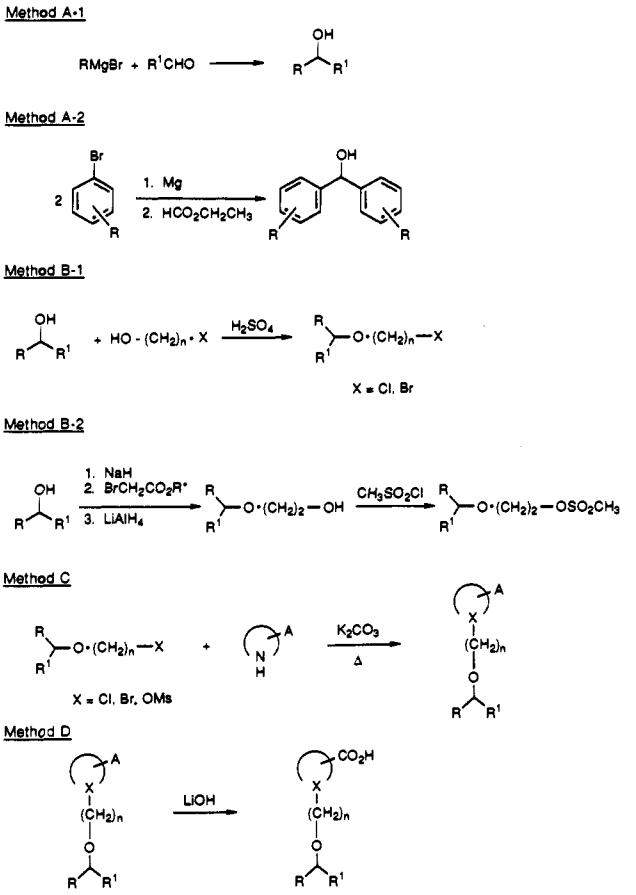
Table I. Inhibition of [^{3}H]GABA Uptake into Rat Hippocampal Slices

compound	IC_{50} ± SEM, μM
 GABA	8.6 ± 0.94
 nipecotic acid	*R*(−) 5.2 ± 0.29 *S*(+) 55 ± 4.7
 guvacine	7.3 ± 0.69
 SKF 89976A	10 ± 0.083
compound 44	0.34 ± 0.064
phenytoin	>50
carbamazepine	>50
valproic acid	>50

success as orally-active anticonvulsant agents suggested that improved derivatives are needed.[21-23]

We hoped to increase the entry of the known GABA uptake inhibitors guvacine and nipecotic acid into the CNS by introducing lipophilic groups onto the nitrogen atom of the six-membered ring. A similar strategy was reported while this work was in progress by a group at SmithKline which resulted in the identification of SKF 89976A (Table I),[24-30] a compound shown to be a potent, nonsedative anticonvulsant drug in an animal model of complex-partial seizures in rats. More recently, Novo[31,32] has disclosed a related analog, NO-328, which is a potent anticonvulsant

Scheme I



in rats and mice,[33] and Krogsgaard-Larsen reported a limited series of GABA, β-alanine, nipecotic acid, and guvacine analogs containing the benzhydryl ethyl ether side chain.[34]

In this paper we report extensive structure–activity studies of a series of *N*-(benzhydryl ethyl ether) nipecotic acid or guvacine derivatives which afforded GABA uptake inhibitors that are more potent and lipophilic than the parent amino acids and possess potent anticonvulsant activity after oral administration.[35,36]

Chemistry

In most cases the target compounds were prepared from commercially available starting materials as described in methods A–D (Scheme I). In the first step, the benzhydrols

(21) Gonsalves, S. F.; Twitchell, B.; Harbaugh, R. E.; Krogsgaard-Larsen, P.; Schousboe, A. Anticonvulsant Activity of the Glial GABA Uptake Inhibitor, THAO, in Chemical Seizures. *Eur. J. Pharm.* **1989**, *168*, 265–8.

(22) Gonsalves, S. F.; Twitchell, B.; Harbaugh, R. E.; Krogsgaard-Larsen, P.; Schousboe, A. Anticonvulsant Activity of Intracerebroventricularly Administered Glial GABA Uptake Inhibitors and Other GABA Mimetics in Chemical Seizure Models. *Epilepsy Res.* **1989**, *4*, 34–41.

(23) Schousboe, A.; Larsson, O. M.; Hertz, L.; Krogsgaard-Larsen, P. Heterocyclic GABA Analogues as New Selective Inhibitors of Astroglial GABA Transport. *Drug Dev. Res.* **1981**, *1*, 115–128.

(24) Ali, F. E.; Bondinell, W. E.; Dandridge, P. A.; Frazee, J. S.; Garvey, E.; Girard, G. R.; Kaiser, C.; Ku, T. W.; Lafferty, J. J.; Moonsammy, G. I.; Oh, H.-J.; Rush, J. A.; Setler, P. E.; Stringer, O. D.; Venslavsky, J. W.; Volpe, B. W.; Yunger, L. M.; Zirkle, C. L. Orally Active and Potent Inhibitors of γ-Aminobutyric Acid Uptake. *J. Med. Chem.* **1985**, *28*, 653–60.

(25) Loscher, W. SK&F-89976-A. *Drugs Future*, **1986**, *11*, 36–43.

(26) Yunger, L. M.; Fowler, P. J.; Zarevics, P.; Setler, P. E. Novel Inhibitors of GABA Uptake: Anticonvulsant Action in Rats and Mice. *J. Pharmacol. Exp. Ther.* **1984**, *228*, 109–15.

(27) Bondinell, W.; Lafferty, J. J.; Firkle, C. L. Inhibition of GABA Uptake by N-Substituted Azaheterocyclic Carboxylic Acids and their Esters. U.S. Patent 4,383,999.

(28) Schwark, W. S.; Loscher, W. Comparison of the Anticonvulsant Effects of Two Novel GABA Uptake Inhibitors and Diazepam in Amygdaloid Kindled Rats. *Naunyn-Schmiedebergs Arch. Pharmacol.* **1985**, *329*, 367–71.

(29) Loscher, W. Anticonvulsant Action in the Epileptic Gerbil of Novel Inhibitors of GABA Uptake. *Eur. J. Pharmacol.* **1985**, *110*, 103–8.

(30) Heit, M. C.; Schwark, W. S. Pharmacological Studies with a GABA Uptake Inhibitor in Rats with Kindled Seizures in the Amygdala. *Neuropharmacology*, **1988**, *27*, 367–74.

(31) Braestrup, C.; Nielsen, E. B.; Wolffbrandt, K. H.; Andersen, K. C.; Knutsen, L. J. S.; Sonnewald, U. Modulation of GABA Receptor Interactions with GABA Uptake Inhibitors. *Int. Congr. Ser. Excerpta Med.* **1987**, *750*, 125–128.

(32) Sonnewald, U., Preparation of Heterocyclylbutenylpyridycarboxylates as Gamma-Aminobutyric acid Uptake Inhibitors, 1987, EP 231996.

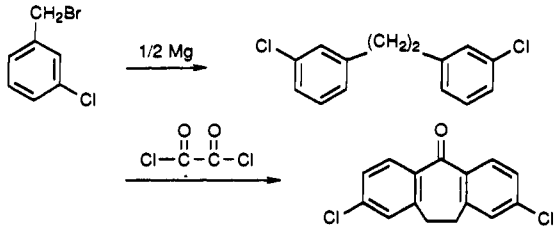
(33) Braestrup, C.; Nielsen, E. B.; Sonnewald, U.; Knutsen, L. J.; Andersen, K. E.; Jansen, J. A.; Frederiksen, K.; Andersen, P. H.; Mortensen, A.; Suzdak, P. D. (R)-N-[4,4bis(3-methyl-2-thienyl)but-3-en-1-yl]nipecotic Acid Binds with High Affinity to the Brain Gamma-Aminobutyric Acid Uptake Carrier. *J. Neurochem.* **1990**, *54*, 639–47.

(34) Falch, E.; Krogsgaard-Larsen, P. GABA Uptake Inhibitors. Syntheses and Structure–Activity Studies on GABA Analogues Containing Diarylbutenyl and Diarylmethoxyalkyl N-Substituents. *Eur. J. Med. Chem.* **1991**, *26*, 69–78.

(35) Pavia, M. R. Various N-Substituted 3-Piperidinecarboxylic Acids or N-Substituted 3-Pyridinecarboxylic Acids and Derivatives Thereof, 1988, U.S. Patent 4,772,615.

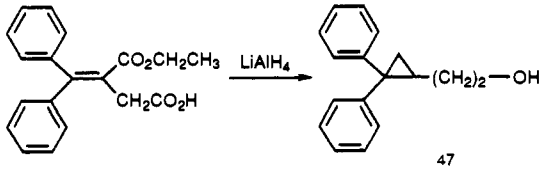
(36) Bjorge, S.; Black, A.; Bockbrader, H.; Chang, T.; Gregor, V. E.; Lobbestael, S. J.; Nugiel, D.; Pavia, M. R.; Radulovic, L.; Woolf, T. Synthesis and Metabolic Profile of CI-966: A Potent, Orally-Active Inhibitor of GABA Uptake. *Drug Dev. Res.* **1990**, *21*, 189–94.

Scheme II



46

Scheme III

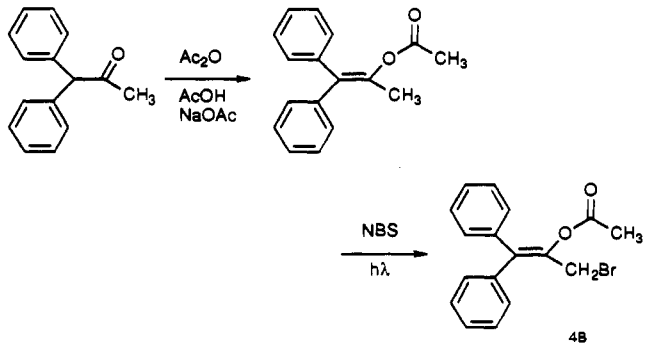


47

were prepared by reaction of a Grignard reagent with an aldehyde (method A-1) or in the case of several symmetrical benzhydrols, by reaction of 2 equiv of Grignard reagent with 1 equiv of ethyl or methyl formate (method A-2). In a few cases the benzhydrols were commercially available or the commercially available benzophenones were reduced with sodium borohydride (method A-3). The side chain was added to the benzhydrols by either reaction with chloro- or bromoethanol in the presence of sulfuric acid (method B-1, Scheme I) or for acid-sensitive benzhydrols, reaction with sodium hydride and ethyl or *tert*-butyl bromoacetate, reduction to the alcohol with lithium aluminum hydride, and formation of the mesylate (method B-2). The intermediates prepared by method B were reacted with secondary amines in the presence of K_2CO_3 (method C), and the final products were generated by lithium hydroxide hydrolysis (method D).

Several of the final products required unique synthetic routes. For the preparation of **3**, the side chain was prepared from benzhydrol chloride and thiourea followed by alkaline hydrolysis to afford the thiol derivative[37] which was further elaborated by reaction with chloroethanol and potassium *tert*-butoxide. 4,4-Dichlorodibenzosuberone (Scheme II, **46**) was prepared by Wurtz-like[38] coupling of 3-chlorobenzyl bromide followed by reaction with oxalyl chloride. The side chain for **21** was prepared from the anion of 3-phenylbenzofuran[39] followed by quenching with ethylene oxide. The epoxy alcohol **47** (Scheme III) was prepared by a novel reaction.[40] The Stobbe condensation product of benzophenone and the half ester of succinic acid[41] was reacted with lithium aluminum hydride to afford **47** as the sole product in 77% yield. For the preparation **48** (Scheme IV) 1,1-diphenylacetone was converted to the enol acetate followed by selective bromination with bromine and light. This material, **48**, was further reacted with ethyl nipecotate followed by hydrolysis resulting in

Scheme IV



48

ester and enol acetate cleavage to afford **23**. For the preparation of compounds **27** and **28**, methyl 4-oxo-3-piperidinecarboxylate was reduced to a mixture of 4-cis and 4-trans alcohols with sodium borohydride.[42] After introduction of the side chain, the cis and trans compounds were separated by chromatography and carried on separately. Compound **29** was prepared from **38** by deprotonation with LDA and reaction with acetyl chloride. Alkaline hydrolysis and decarboxylation afforded **29**. The primary amide **40** was reacted with phosphorus oxychloride to afford **30**. DIBAL reduction of **39** gave **31**. The five-membered ring analogs **33** and **34** were prepared via side-chain alkylation of the required pyrrolidinecarboxylic acid ester.[43-47] For the preparation of **36**, the racemic acid **5** was resolved with (*S*)-(−)-α-methylbenzylamine. The mother liquor which was enriched in **37** was further enriched by regenerating the free base and resolution with (*R*)-(+)-α-methylbenzylamine. Compound **40** was prepared from the acid **5** by reaction with carbonyldiimidazole and ammonia. The secondary, **41**, and tertiary, **42**, amides were prepared from **39**, using methylamine and Weinreb's chemistry,[48] respectively. The syntheses of **43–45** required guvacine methyl ester which was prepared from arecoline by demethylation.[49]

Results and Discussion

To prepare potent, orally-active GABA uptake inhibitors we began with (±)-nipecotic acid, a known inhibitor (IC_{50} = 8 μM for GABA uptake inhibition), and introduced lipophilic side chains onto the nitrogen atom to increase lipophilicity and oral activity of this polar amino acid. A large series of compounds were tested for their ability to inhibit GABA uptake in vitro, and the results with forty-five of these compounds are presented herein to illustrate

(37) Urquhart, G. G.; Gates, J. W., Jr.; Connor, R. n-Docecyl Mercaptan. *Organic Syntheses*; John Wiley & Sons, Inc.: New York, 1955; Collect. Vol. 3, pp 363–5.

(38) Kharasch, H. J.; Reinmuth, A. C. *Grignard Reactions of Nonmetallic Substances*; Prentice-Hall: Englewood Cliffs, New Jersey, 1954; pp 1046–1165.

(39) Chatterjea, J. N.; Srivastava, S.; Bhakta, C.; Lal, S. Synthesis of Furano Compounds. Part XL. Cyclization of 3-Phenylbenzo(b)furan-2-acetic acid. *J. Ind. Chem. Soc.* 1976, *53*, 295–9.

(40) Manuscript in preparation.

(41) Johnson, W. S.; Daub, G. H. The Stobbe Condensation. *Organic Reactions*; John Wiley & Son Inc.: New York, 1951; Vol. 6, pp 1–73.

(42) Jacobsen, P.; Labouta, I. M.; Schaumburg, K.; Falch, E.; Krogsgaard-Larsen, P. Hydroxy- and Amino-Substituted Piperidinecarboxylic Acids as γ-Aminobutyric Acid Agonists and Uptake Inhibitors. *J. Med. Chem.* 1982, *25*, 1157–62.

(43) Morgans, D. J.; Stork, G. An Intramolecular Diels-Alder Approach to the Galanthin Ring System. *Tetrahedron Lett.* 1979, 1959–62.

(44) Kornet, M. J.; Thio, P. A.; Tan, S. I. Borane Reduction of Amido Esters. *J. Org. Chem.* 1968, *33*, 3637–9.

(45) Bondinell, W. C.; Lafferty, J. J.; Zirkle, C. L. N-Substituted Pyrrolidineacetic Acids and Their Esters, 1985, U.S. Patent 4,514,414.

(46) Lindborg, B.; Cron, K.; Dahlbom, R.; Ross, S. B. Troxonium-Like Inhibitors of the High Affinity Uptake of Choline in Mouse Brain Synaptosomes in Vitro. *Acta. Pharm. Sci.* 1984, *21*, 271–94.

(47) Wu, Y-H.; Feldkamp, R. F. 1-Substituted-3-Pyrrolidinylmethyl Alcohols and Chlorides. *J. Org. Chem.* 1961, *26*, 1519–24.

(48) Weinreb, S. M.; Staib, R. R. Synthetic Aspects of Diels-Alder Cycloadditions with Heterodienophiles. *Synth. Commun.* 1982, *38*, 3087–128.

(49) Wolf-Pflugmann, M.; Lambrecht, G.; Wess, J.; Mutschler, E. Synthesis and Muscarinic Activity of a Series of Tertiary and Quaternary N-Substituted Guvacine Esters Structurally Related to Arecoline and Arecaidine Propargyl Ester. *Arzneim. Forsch.* 1989, *39*, 539–44.

the structure–activity relationships observed. Among the areas explored: side-chain SAR (aromatic, heterocyclic, tricyclic) and modifications to the tetrahydropyridine ring.

The (diphenylmethoxy)ethyl-containing analog 1 (Table II; IC_{50} = 3.4 μM) demonstrated that large groups could be introduced onto the nipecotate nucleus with no loss of activity, and 1 showed anticonvulsant activity after systemic administration in the kindled rat model of partial-complex seizures.[50] Lengthening the side chain to afford 2 or replacing the oxygen with a sulfur, 3 caused a loss of GABA uptake inhibition. Aromatic substitution resulted in a wide range of activities (Table II; 4–13). Substitution on only one ring introduces a chiral center at the diphenylmethane carbon which with the chiral center at C-3 of the tetrahydropyridine ring results in four possible diastereomers. This complication was avoided when the symmetrically substituted derivative 5 was found to be greater than 10-fold more active than 4. Replacement of one phenyl ring with a cycloalkyl group, compound 14, or heterocycles, compounds 15 and 16, gave a moderate loss of activity. Compounds 17 and 18, where the two phenyl rings of 1 are in a seven-membered ring resembling the anticonvulsant carbamazepine, resulted in a slight loss of activity while introduction of a third phenyl ring with either an ethoxy or propoxy spacer (compounds 19 and 20) afforded less active derivatives.

The benzofuran derivative 21, a hybrid of the SKF-type compound and our benzhydrol derivatives, was nearly inactive as was replacement of the oxygen linkage with a cyclopropyl ring, 22, or a carbonyl group, 23. In summary, the results in Table II illustrate that two phenyl rings both substituted in the 4-position results in the best activity profile.

Modifications to the nipecotate nucleus were made with the side chain found in 5 placed on each compound to allow direct comparison to 5 (Table III). Changing the position of the carboxylic acid (compound 26) gave an inactive analog in agreement with previous studies on isonipecotic acid GABA uptake inhibitors.[11] *cis*- and *trans*-4-hydroxynipecotic acids have been reported to be excellent GABA uptake inhibitors, and while the cis compound 27 was nearly 7 times more potent as a GABA uptake inhibitor than the corresponding trans compound 28, both were considerably less potent than 5. Conversion of the carboxylic acid to the methyl ketone 29, nitrile 30, alcohol 31, or its complete removal, 32, resulted in greatly reduced activity. It is interesting to note that the piperidine derivative 32 (or the 2-pyrrolidinone analog 35) retained GABA uptake inhibitory activity, suggesting that the side chain may have some activity on its own. Ring contraction to the five-membered ring without (compound 33) or with a one-carbon spacer (compound 34) was carried out. In the former compound almost all activity was abolished suggesting that the spatial relationship between the nitrogen and carboxylic acid groups was changed beyond allowed limits but was restored to some degree when the carbon spacer was introduced. Compound 34 was tested in vivo and had significant anticonvulsant activity at doses of 30 and 100 mg/kg iP in mice, but because of its relatively low potency was not studied further.

Separation of the C-3 enantiomers of compound 5 afforded the *S*-(+) derivative 36 (Table IV), which was approximately 50 times less potent than the *R*-(−) enantiomer 37. Preparation of the esters (38 and 39) and amides (40–42) afforded less potent derivatives which had no apparent in vivo benefits. The one standout, 38, was approximately 5-fold less potent than 5 as an anticonvulsant in kindled rats[50] (ED_{50} = 25 mg/kg ip) and had a very slow onset of action after ip administration consistent with a slow conversion of the ester to a free acid in vivo. The unsaturated analog 43 afforded a potent inhibitor which contains no chiral center, removing the need for resolution of enantiomers and affording a much simpler chemical synthesis. In vivo evaluation of 43 revealed anticonvulsant activity, with an ED_{50} value of 14 mg/kg ip for elevation of afterdischarge threshold in kindled rats,[50] but ataxia at only slightly higher doses (ED_{50} = 17 mg/kg) precluding further development. This undesirable effect was not observed with 44 (Table V), which exhibited anticonvulsant effects in mice and kindled rats at doses that did not cause ataxia. Compound 44 is a potent GABA uptake inhibitor producing a concentration-related blockade of sodium-dependent, high-affinity [^{3}H]GABA uptake into rat hippocampal slices. The concentration which inhibits uptake by 50% (IC_{50} value) was 340 ± 64 nM for 44 as compared to 5200 ± 290 nM for (−)-nipecotic acid and 10 000 ± 83 nM for SKF 89976A in our hands (Table I). The value obtained for SKF 89976A is approximately 40-fold higher than that previously reported in the literature.[25] The reason for this discrepancy is unclear but may relate to the different in vitro conditions and tissue preparations used and the relative proportion of neuronal vs glial GABA uptake inhibition sites present. The 10–20-fold increase in in vitro potency of 44 compared to *R*-(−)-nipecotic acid or SKF 89976A might result from several factors including the hydrogen bonding ability of the side-chain oxygen atom, a loss of electron delocalization upon olefin removal, or change in conformational constraints upon removal of the olefin which affords a diphenyl conformation that better matches the three-dimensional topography of the GABA uptake site. The results of our structure–activity studies on the heterocyclic ring analogs are consistant with conclusions previously reported that the (*R*)-(−)-nipecotic acid and guvacine nuclei place the nitrogen and carboxylic acid in the correct relationship for optimal GABA uptake inhibition.[51] Furthermore, a recent paper[52] in which the authors used published SAR data[24] to establish a pharmacaphore model is also in full accord with the compounds described in this paper.

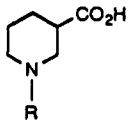
Compound 44, which was selected for further evaluation, specifically blocked GABA uptake since no significant inhibition of D-aspartate, dopamine, norepinephrine, or serotonin uptake was observed at concentrations producing maximal effects on GABA. Using receptor binding techniques, 44 showed no affinity to a variety of neurotransmitter receptors including adenosine-1 and -2, α1 and -2, β-adrenergic, benzodiazepine, bradykinin, dihydropyridine-sensitive calcium channels, dopamine-1, ex-

(50) Schwark, W. S.; Loscher, W. Comparison of the Anticonvulsant Effects of Two Novel GABA Uptake Inhibitors and Diazepam in Amygdaloid Kindled Rats. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1985, *329*, 367–71.

(51) Lapuyade, G.; Schlewer, G.; N'goka, V.; Vernieres, J.-C.; Chambon, J.-P.; Lagrange, J.; Lagrange, P.; Wermuth, C. G. Alkyl and Aryl Derivatives of Nipecotic Acid: Synthesis and Evaluation of Inhibition of GABA Uptake as a Function of Conformational Parameters and Bioavailability. *Eur. J. Med. Chem.* 22, 1987, 383–91.

(52) N'Goka, V.; Schlewer, G.; Linget, J. M.; Chambon, J. P.; Wermuth, C. G. GABA Uptake Inhibitors: Construction of a General Pharmacaphore Model and Successful Prediction of a New Representative. *J. Med. Chem.* 1991, *34*, 2547–57.

Table II



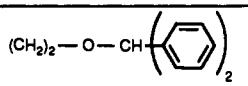
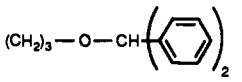
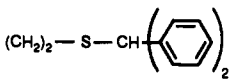
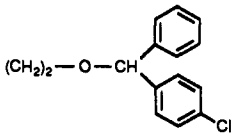
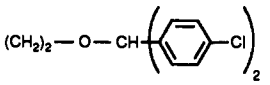
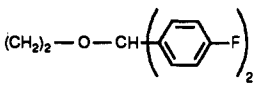
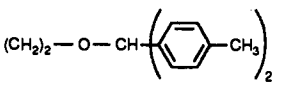
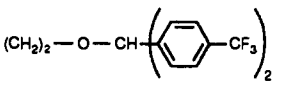
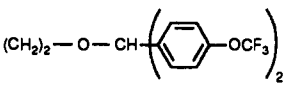
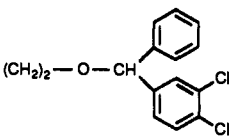
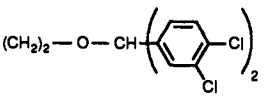
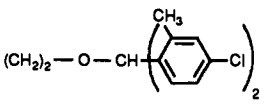
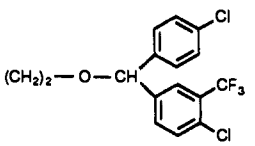
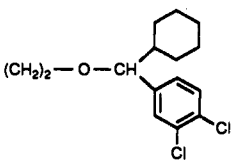
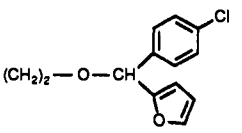
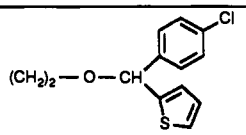
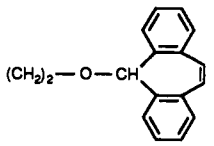
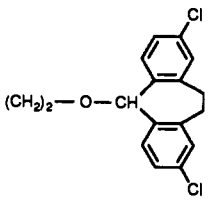
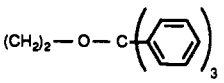
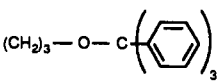
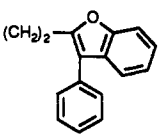
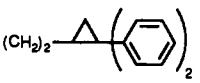
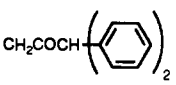
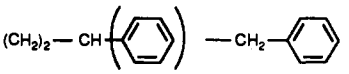
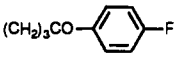
entry	R	mp (°C)	molecular formula	general synthetic methods	GABA uptake inhibition: $IC_{50} \pm SEM$, μM
1		180–182	$C_{21}H_{25}NO_3 \cdot HCl$	B-1, C, D	3.4 ± 0.26
2		–	$C_{22}H_{27}NO_3$	B-1, C, D	12 ± 1.0
3		58–65	$C_{21}H_{25}NO_2S \cdot HCl$	B-2, C, D	60 (est)
4		–	$C_{21}H_{24}ClNO_3 \cdot 1.2HCl$	A-3, B-1, C, D	20 (est)
5		148–152	$C_{21}H_{23}Cl_2NO_3 \cdot HCl$	B-1, C, D	0.41 ± 0.076
6		–	$C_{21}H_{23}F_2NO_3 \cdot HCl$	B-1, C, D	7.8 ± 1.0
7		125–140	$C_{23}H_{29}NO_3 \cdot HCl$	A-3, B-1, C, D	5.7 ± 0.26
8		211–215	$C_{23}H_{23}F_6NO_3 \cdot HCl$	A-1, B-1, C, D	0.71 ± 0.34
9		151–152	$C_{23}H_{23}F_6NO_5 \cdot HCl$	A-2, B-1, C, D	0.92 ± 0.069
10		131–138	$C_{21}H_{23}Cl_2NO_3 \cdot HCl$	A-3, B-1, C, D	1.2 ± 0.73
11		166–167	$C_{21}H_{21}Cl_4NO_3 \cdot 0.8HCl$	A-2, B-1, C, D	2.5 ± 0.43
12		224–225	$C_{23}H_{27}Cl_2NO_3 \cdot HCl$	A-2, B-1, C, D	3.2 ± 0.77
13		157–160	$C_{22}H_{22}Cl_2F_3NO_3 \cdot 0.9HCl$	A-1, B-1, C, D	1.4 ± 0.34
14		162–164	$C_{21}H_{29}Cl_2NO_3 \cdot HCl$	A-1, B-2, C, D	1.1 ± 0.14
15		48–65	$C_{19}H_{22}ClNO_4$	A-1, B-2, C, D	8.7 ± 0.54

Table II (Continued)

entry	R	mp (°C)	molecular formula	general synthetic methods	GABA uptake inhibition: $IC_{50} \pm SEM$, μM
16		97–105	$C_{19}H_{22}ClNO_3S$	A-1, B-2, C, D	3.6 ± 0.36
17		152–153	$C_{23}H_{25}NO_3 \cdot 0.6H_2O$	A-3, B-2, C, D	2.5 ± 0.29
18		182–183	$C_{23}H_{25}Cl_2NO_3 \cdot HCl$	A-3, B-2, C, D	1.6 ± 0.29
19		-	$C_{27}H_{29}NO_3$	B-1, C, D	3.1 ± 0.49
20		-	$C_{28}H_{31}NO_3$	B-1, C, D	7.9 ± 2.0
21		251–256	$C_{22}H_{23}NO_3 \cdot HCl$	B-2, C, D	22 (est)
22		-	$C_{23}H_{27}NO_2 \cdot HCl$	B-2, C, D	>50
23		-	$C_{21}H_{23}NO_3 \cdot HCl$	C, D	>50
24		85–97	$C_{22}H_{27}NO_2 \cdot HCl$	B-2, C, D	50 (est)
25		189–194	$C_{16}H_{20}FNO_3 \cdot HCl$	C, D	>50

citatory amino acid (glutamate, quisqualate, kainate, NMDA, and strychnine-insensitive glycine), $GABA_A$ and $GABA_B$, muscarinic, nicotinic, μ, δ, and κ opiate and phencyclidine binding sites. Weak binding was observed at dopamine-2 receptors, histamine-1 receptors, σ sites, and site 2 of the voltage-sensitive sodium channel; however, these effects occurred at μM concentrations compared to the nM potency observed for GABA uptake inhibition.

The spontaneous release of preloaded [^{3}H]GABA from neuronal tissue is increased by nipecotic acid[53] but not by **5** or **44** (unpublished data). The increased GABA release caused by application of nipecotic acid has been explained on the basis of heteroexchange of nipecotic acid for cystolic GABA via the GABA transporter. However, this process is only possible because nipecotic acid is a substrate for the GABA carrier. The related bulky GABA uptake inhibitor NO-326 has been reported to lack substrate activity for the GABA carrier.[31] On the basis of this information, most of the bulky compounds described in this report are unlikely to be substrates for the GABA carrier, although all of them are apparently competitive inhibitors of GABA uptake. Experiments with radiolabeled compounds are necessary to rigorously explore this idea.

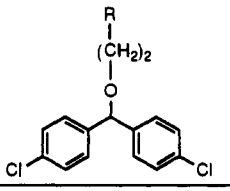
Compound **44** inhibited veratridine-stimulated [^{14}C]-guanidine influx into rat cortical slices with an IC_{50} value of 10 μM. This action is shared by several other anticonvulsants (e.g. phenytoin) which are thought to act on voltage-sensitive sodium channels. This appears to be a minor mode of action for **44** since 10 μM is approximately 35 times the IC_{50} for GABA uptake blockade.

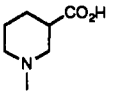
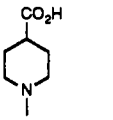
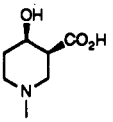
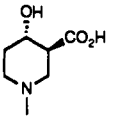
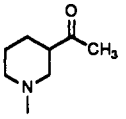
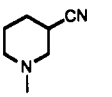
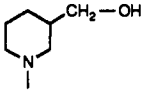
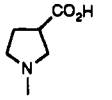
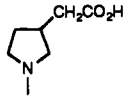
In vivo studies with **44** have previously been described in detail.[54,55] In summary, **44** was effective in rodents following oral administration in several seizure models at doses that did not cause ataxia. Clonic seizures induced

(53) Szerb, J. C. Effect of Nipecotic Acid, a Gamma-Aminobutyric Acid Transport Inhibitor, on the Turnover and Release of Gamma Aminobutyric Acid in Rat Cortical Slices. *J. Neurochem.* 1982, *39*, 850–8.

(54) Taylor, C. P.; Vartanian, M. G.; Schwarz, R. D.; Rock, D. M.; Callahan, M. J.; Davis, M. D. Pharmacology of CI-966: A Potent GABA Uptake Inhibitor, In Vitro and in Experimental Animals. *Drug Dev. Res.* 1990, *21*, 195–216.

Table III



entry	R	mp (°C)	molecular formula	general[a] synthetic methods	GABA uptake inhibition: $IC_{50} \pm SEM$, μM
5		148–152	$C_{21}H_{23}Cl_2NO_3 \cdot HCl$	–	0.41 ± 0.076
26		109–110	$C_{21}H_{23}Cl_2NO_3$	C, D	>50
27		120–122	$C_{21}H_{23}Cl_2NO_4 \cdot HCl$	C, D	1.5 ± 0.15
28		92–100	$C_{21}H_{23}Cl_2NO_4 \cdot HCl$	C, D	1.2 ± 0.91
29		91–94	$C_{22}H_{25}Cl_2NO_2 \cdot HCl$	E	22 (est)
30		123–126	$C_{21}H_{22}Cl_2N_2O \cdot HCl$	E	>50
31		160–162	$C_{21}H_{25}Cl_2NO_2 \cdot HCl$	E	47 (est)
32		171–173	$C_{20}H_{23}Cl_2NO \cdot HCl$	C	21 (est)
33		51–63	$C_{20}H_{21}Cl_2NO_3 \cdot HCl$	C, D	>50
34		–	$C_{21}H_{23}Cl_2NO_3$	C, D	2.7 ± 1.7
35		–	$C_{19}H_{19}Cl_2NO_2$	E	26 ± 2.9

[a] E refers to individual description in Experimental Section.

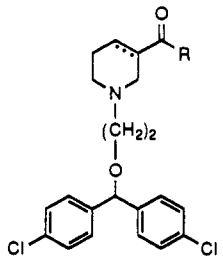
by the GABA antagonist pentylenetetrazole (PTZ)[56] in mice were prevented by 44 with an ED_{50} of 0.4 mg/kg po (0.2–0.6 mg/kg po, 95% confidence interval, Figure 1) as were threshold clonic seizures from picrotoxin (ED_{50} = 1.7 mg/kg ip, 1.9–2.6 mg/kg 95% confidence interval). Tonic extensor seizures from low-intensity corneal electroshock in mice[57] were effectively blocked with 44 (Figure 1; ED_{50} = 1.0 mg/kg po, 0.6–1.6 mg/kg 95% confidence limits) whereas conventional maximal electroshock seizures[56] were prevented only by high doses of 44 in mice or rats, with approximately 50% of the mice protected after doses of 30–250 mg/kg po. Finally, 44 potently inhibited focal seizures in the rat hippocampus preventing hippocampal afterdischarges in a dose-related manner (ED_{50} = 2.6 mg/kg po, 1.4–4.8 mg/kg, 95% confidence

(55) Taylor, C. P.; Sedman, A. J. Pharmacology of the Gamma-Aminobutyric Acid-Uptake Inhibitor CI-966 and Its Metabolites: Preclinical and Clinical Studies. In *Transmitter Amino Acid Receptors: Structures, Transduction and Models for Drug Development*; Barnard, E. A., Costa, E., Eds.; Thieme Medical Publ., Inc., New York, 1991.

(56) Krall, R. L.; Penry, J. K.; White, B. G.; Kupferberg, H. J.; Swinyard, E. A. Antiepileptic Drug Development: II. Anticonvulsant Drug Screening. *Epilepsia* 1978, *19*, 409–28.

(57) Piredda, S. G.; Woodhead, J. H.; Swinyard, E. A. Effects of Stimulus Intensity on the Profile of Anticonvulsant Activity of Phenytoin, Ethosuximide, and Valproate. *J. Pharmacol. Exp. Ther.* 1985, *232*, 741–5.

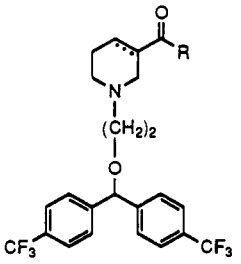
Table IV



entry	R	mp (°C)	molecular formula	general[a] synthetic methods	GABA uptake inhibition: IC_{50} ± SEM, μM
5	OH	148–152	$C_{21}H_{23}Cl_2NO_3$·HCl	–	0.41 ± 0.076
36	OH; *S*-(+)	182–183	$C_{21}H_{23}Cl_2NO_3$·HCl	E	14 ± 0.83
37	OH; *R*-(−)	181–184	$C_{21}H_{23}Cl_2NO_3$·HCl	E	0.18 ± 0.0062
38	OCH_3	87–89	$C_{22}H_{25}Cl_2NO_3$·HCl	E	0.70 ± 0.050
39	OCH_2CH_3	–	$C_{23}H_{27}Cl_2NO_3$·HCl	B-1, C	11 ± 1.9
40	NH_2	211–213	$C_{21}H_{24}Cl_2N_2O_2$·HCl	E	35 (est)
41	$NHCH_3$	100–103	$C_{22}H_{26}Cl_2N_2O_2$·0.9HCl	E	40 (est)
42	$N(CH_3)_2$	178–179	$C_{23}H_{28}Cl_2N_2O_2$·HCl	E	25 ± 7.1
43	OH; Δ-3,4	183–185	$C_{21}H_{21}Cl_2NO_3$·HCl	C, D	0.41 ± 0.046

[a] E refers to individual description in Experimental Section.

Table V



entry	R	mp (°C)	molecular formula	general[a] synthetic methods	GABA uptake inhibition: IC_{50} ± SEM, μM
44	OH; Δ-3,4	234–237	$C_{23}H_{21}F_6NO_3$·HCl	C, D	0.34 ± 0.064
45	OCH_3; Δ-3,4	–	$C_{24}H_{23}F_6NO_3$	C	40 ± 11

[a] E refers to individual description in Experimental Section.

interval, Figure 2). Ataxia (ED_{50} = 64 mg/kg po, 29–155 mg/kg 95% confidence interval, mice) and other behavioral side effects of 44 were observed at doses greater than those producing anticonvulsant effects (against PTZ or after discharges).

We can speculate that the oral effectiveness of 44 results from its ability to efficiently cross the blood–brain barrier (BBB), however its mechanism of transport is uncertain. Unrestricted diffusion through the BBB is possible for substances that can cross biological membranes by virtue of their lipophilic character, and to a large extent this can be predicted from an in vitro lipid–water partition coefficient. The partition coefficient (octanol–water) of 44 was determined by the shake-flash method. At pH 7.4, 4, and 1 the partition coefficient was >3 indicating sufficient lipophilicity to anticipate BBB transport. Alternatively, there are at least seven specific carier systems which transport compounds into the brain.[56] For example, acidic drugs such as salicylic, benzoic, nicotinic, and

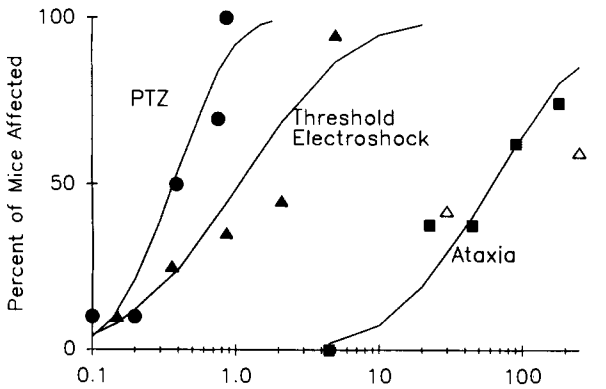


Figure 1. Anticonvulsant and ataxia-producing effects of 44 in mice after various oral doses. Data are the percent of eight to ten mice per dose that exhibited each effect. PTZ (filled circles) denotes protection from clonic pentylenetetrazole (85 mg/kg sc) seizures. Threshold electroshock (filled triangles) denotes mice protected from tonic extensor seizures from 14-mA corneal electroshock. Ataxia (filled squares) denotes mice that fell from a rotating rod.[56] Mice protected from tonic extensor seizures from maximal electroshock are shown as open triangles. All data were obtained at the approximate time of peak effect after oral dosing (PTZ = 2.0 h, threshold electroshock = 2.0 h, maximal electroshock = 1.5–2.0 h, ataxia = 1.0 h). The solid lines are the best-fit curves from probit analyses.[61]

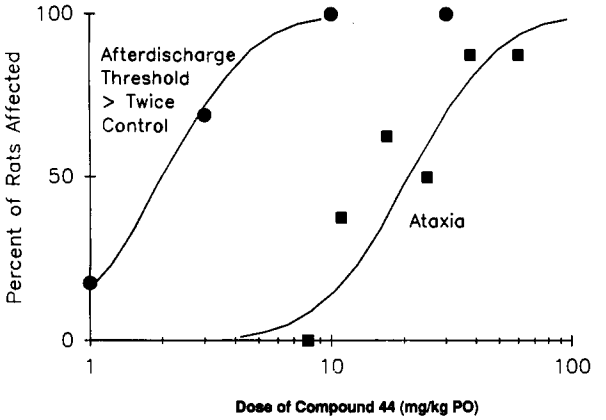


Figure 2. Anticonvulsant and ataxia-producing effects of oral 44 in rats. Anticonvulsant action (filled circles) is the percent of eight rats that failed to exhibit hippocampal afterdischarge. Ataxia (filled squares) is the percent of rats ataxic when observed on a level surface. Time of testing after dose was 2.5 h (anticonvulsant action) or 2.0 h (ataxia). Solid lines are from probit analyses.[61]

valproic acids are transported across the BBB in a carrier-mediated manner via a monocarboxylic acid transport system.[59] While the lipophilic nature of 44 is likely to explain the oral activity of this compound alternate mechanisms can not be ruled out.

On the basis of our results, single doses of 44 were administered to healthy human volunteers in a preliminary tolerance and safety study. Unfortunately, the highest dose administered (50 mg; n = 2) was associated with unanticipated severe neurological and psychological side effects. These clinical findings have been reported in detail elsewhere.[60] Further clinical evaluation of 44 was terminated because of these adverse effects.

(58) Pardridge, W. M.; Olendorf, W. H. Transport of Metabolic Substances Through the Blood-Brain Barrier. *J. Neurochem.* **1977**, *28*, 5–12.

(59) Kang, Y. S.; Terasaki, T.; Tsuji, A. Acidic Drug Transport in vivo Through the Blood-Brain Barrier. A Role of the Transport Carrier for Monocarboxylic Acids. *J. Pharmacobio-Dyn.* **1990**, *13*, 158–63.

(60) Sedman, A. J.; Gilmet, G. P.; Sayed, A. J.; Posvar, E. L. Initial Human Safety and Tolerance Study of a GABA Uptake Inhibitor, CI-966: Potential Role of GABA as a Mediator in the Pathogenesis of Schizophrenia and Mania. *Drug. Dev. Res.* **1990**, *21*, 235–42.

Experimental Section

All melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded with a Varian EM-390 spectrometer using Me_4Si as the internal standard and deuteriochloroform or Me_2SO-d_6 as solvent. NMR values are reported in δ values. Purity was determined by microanalysis and by TLC with 0.25-mm thick plates coated with silica gel G as the stationary phase. IR spectra were recorded with a Nicolet XS-20 FT-IR spectrometer using KBr pellets. All compounds possessed microanalytical and spectral data consistent with the proposed structures.

Method A-1. 4-(Trifluoromethyl)-α-[4-(trifluoromethyl)phenyl]benzenemethanol. The Grignard reagent derived from 4-bromobenzotrifluoride (Aldrich, 40 g, 0.18 mol) was prepared in ether and cooled to 5 °C, and α,α,α-trifluoro-*p*-tolualdehyde (Aldrich, 29.6 g, 0.17 mol) was added dropwise in ether. The reaction mixture was stirred for 1.5 h at reflux and 16 h at room temperature and the solid alkoxide precipitate isolated by filtration, washed with 1:1 ether–pentane, dried, and slurried with 150 mL of $CHCl_3$ and 120 mL of saturated ammonium chloride solution. The $CHCl_3$ layer was separated and the aqueous layer extracted with $CHCl_3$ (2 × 50 mL) to afford 34.25 g of product. The mother liquors from the filtration were evaporated and reextracted to afford an additional 8.2 g of product giving a total of 42.3 g (77.7% yield): TLC (4:1 pentane–ether), R_f = 0.3; ^{1}H-NMR ($CDCl_3$) 7.61 (d, 4 H, aromatic), 7.52 (d, 4 H, aromatic), 5.95 (d, 1 H, $CHPh_2$), 2.38 (d, 1 H, OH). Anal. ($C_{15}H_{10}F_6O$)C, H.

Method A-2. 3,4-Dichloro-α-(3,4-dichlorophenyl)benzenemethanol. The Grignard of 3,4-dichlorobromobenzene (21 mmol) was cooled to 5 °C, ethyl formate (0.78 g, 10.5 mmol) in 5 mL of THF added over 5 min, and the reaction refluxed for 4 h and poured onto 40 mL of cold 1 N HCl. The organic layer was separated, the aqueous layer extracted with ether (2 × 30 mL), and the organic-soluble material purified by chromatography (silica, hexane–ethyl acetate–methanol; 80:20:1) to afford 2.75 g of the desired carbinol (74% yield).

Method A-3. 4-Methyl-α-(4-methylphenyl)benzenemethanol. To 4,4′-dimethylbenzophenone (12.0 g, 57 mmol) in 2-propanol (130 mL) at room temperature was added sodium borohydride (0.57 g, 15 mmol). After 16 h an additional 0.57 g of sodium borohydride was added and the reaction stirred for 3 h at room temperature. Five milliliters of water was added, and evaporation left a residue which was taken up in ether, washed with water and saturated NaCl, dried, and filtered to afford 11.70 g of the desired product as a white solid (97% yield), mp 68–70 °C.

Method B-1. 1-[[(2-Bromoethoxy)-4-(trifluoromethyl)phenyl]methyl]-4-(trifluoromethyl)benzene (49). To 2-bromoethanol (46.3 g, 0.37 mol) in 300 mL toluene was added 7 mL of concentrated sulfuric acid and the mixture warmed to 60 °C. 4-(Trifluoromethyl)-α-[4-(trifluoromethyl)phenyl]benzenemeth anol (79.32 g, 0.25 mol) in warm toluene (285 mL) was added dropwise. The reaction temperature was maintained at 85–90 °C for 6 h, cooled, and diluted with 75 mL of toluene, and the layers were separated. The toluene layer was washed with H_2O (2 × 75 mL) and saturated NaCl and dried to afford an oil which was dissolved in 20 mL of hexane. Cooling to −70 °C gave the desired product (76.6 g, 72.6% yield): mp 35–38 °C; R_f = 0.2 (40:1 pentane–ether); ^{1}H-NMR ($CDCl_3$) 7.60 (d, 4 H, aromatic), 7.52 (d, 4 H, aromatic), 5.53 (s, 1 H, $CHPh_2$), 3.81 (t, 2 H, CH_2), 3.55 (t, 2 H, CH_2). Anal. ($C_{17}H_{13}BrF_6O$) C, H, F, Br.

Method B-2. 2-Furfuryl-(4-chlorophenyl)carbinol (2.0 g, 9.58 mmol) was dissolved in dry THF (50 mL) at 0 °C. Sodium hydride (0.38 g of 60% mineral oil dispersion, 9.58 mmol) was added portionwise at 0 °C and then refluxed for 30 min. *tert*-Butyl bromoacetate (1.8 g, 9.23 mmol) in THF (20 mL) was added over 20 min followed by a 16-h reflux. The product was added dropwise to an ether (25 mL) suspension of lithium aluminum hydride (0.18 g, 4.6 mmol) and the reaction stirred 16 h at room temperature, quenched with water, cooled, and filtered through Celite. The ether solution was washed with saturated NaCl, dried, and purified by chromatography (silica, 1:3; ethyl acetate–hexane) to afford a light brown oil (1.8 g, 6.7 mmol) which was dissolved in methylene chloride (15 mL) and cooled in an ice bath. Addition of diisopropylethylamine (1.2 g, 9.4 mmol) followed by dropwise addition of methanesulfonyl chloride (0.92 g, 8.0 mmol) in 5 mL of methylene chloride followed. The reaction was stirred for 30 min, water (0.5 mL) added, the mixture evaporated, and the oil dissolved in methylene chloride (30 mL), washed with water, dried, and used in the next reaction.

Method C. 1,2,5,6-Tetrahydro-1-[2-[bis[4-(trifluoromethyl)phenyl]methoxy]ethyl]-3-pyridinecarboxylic Acid, Methyl Ester (45). Guvacine methyl ester (30.1 g, 0.21 mol), compound **49** (76.0 g, 0.18 mol; see method B-1), acetonitrile (150 mL), and anhydrous K_2CO_3 (29.0 g, 0.21 mol) were combined and stirred 10 min at room temperature, refluxed for 15 h under nitrogen, cooled, and filtered through powdered K_2CO_3, and the solids were washed with acetonitrile (2 × 60 mL). Evaporation gave 97.2 g of an oil which was purified by chromatography (silica, 3:7; ethyl acetate–hexane, R_f = 0.18): ^{1}H-NMR ($CDCl_3$) 7.33–7.19 (m, 8 H, aromatic), 7.00 (s, 1 H, CH═C), 5.33 (s, 1 H, $CHPh_2$), 3.73 (s, 3 H, CO_2CH_3), 3.62 (t, 2 H, J = 5.85 Hz, OCH_2CH_2N), 3.25 (s, 2 H, piperidine C-2), 2.79 (t, 2 H, J = 5.84 Hz, OCH_2CH_2N), 2.61 (t, 2 H, J = 5.65 Hz, piperidine C-6), 2.34 (bs, 2 H, piperidine C-5); IR (liquid film) 2949, 1715, 1659, 1615, 1491 cm^{-1}. Anal. ($C_{24}H_{23}F_6NO_3$ C, H, N.

Method D. 1,2,5,6-Tetrahydro-1-[2-[bis[4-(trifluoromethyl)phenyl]methoxy]ethyl]-3-pyridinecarboxylic Acid, Monohydrochloride (44). The ester from method C was dissolved in methanol (150 mL) and aqueous 2 N LiOH solution (150 mL, 0.3 mol) added. The reaction was stirred for 64 h at room temperature under nitrogen. Evaporation afforded a semisolid which was dissolved in 1200 mL of water and cooled, and 100 mL cold concentrated HCl was added. The solid was filtered, washed with 500 mL of cold 3 N HCl and 500 mL of ether and dried under N_2. Crystallization from 2:1 acetonitrile–water gave 82.3 g of the hydrochloride salt: R_f = 0.15, 7:2.5:0.5 toluene–2-propanol–acetic acid; ^{1}H-NMR (DMSO-d_6) 7.55–7.81 (2 doublets, 8 H, aromatic), 7.01 (s, 1 H, CH═C), 5.91 (s, 1 H, $CHPh_2$), 4.10–2.60 (4 multiplets, 11 H, piperidine ring CH_2). Anal. ($C_{23}H_{21}F_6NO_3HCl$). C, H, N, Cl.

1-[3-(Diphenylmethoxy)propyl]-3-piperdinecarboxylic acid (2): ^{1}H-NMR ($CDCl_3$) 7.24 (s, 10 H, aromatic), 5.31 (s, 1 H, $CHPh_2$), 3.48 (t, 2 H, J = 5.7 Hz, CH_2O), 3.31–2.35 (m, 7 H, NCH_3, $CHCO_2H$), 2.14–1.60 (m, 6 H, piperidine C-4,5, OCH_2CH_2); IR (KBr) 3430, 3030, 2930, 2870, 1712, 1589, 1495, 1455, 1392, 1307 cm^{-1}.

1-[2-[(Diphenylmethyl)thio]ethyl]-3-piperidinecarboxylic Acid (3). Diphenylmethyl mercaptan (4.0 g, 20 mmol)[37] was dissolved in ethanol (40 mL), and potassium *tert*-butoxide (2.24 g, 20 mmol) was added. The reaction was heated to reflux and chloroethanol (1.61 g, 20 mmol) added over 50 min. After 30 additional min, the mixture was cooled and evaporated and the oil purified by chromatography (ethyl acetate–hexane; 3:7) to afford product, a precursor of **3** (3.6 g, 75% yield, mp = 35 °C).

1-[2-[(4-Chlorophenyl)phenylmethoxy]ethyl]-3-piperidinecarboxylic acid (4): ^{1}H-NMR ($CDCl_3$) 12.7 (bs, 1 H, acid), 7.28 (m, 9 H, aromatic), 5.45 (s, 1 H, $CHPh_2$), 4.15–3.20 (m, 6 H, OCH_2CH_2N, piperidine C-2), 2.95–1.10 (m, 7 H, piperidine C-3,4,5,6); IR ($CHCl_3$) 2875, 1733, 1604 cm^{-1}.

1-[2-[Bis(4-fluorophenyl)methoxy]ethyl]-3-piperidinecarboxylic acid (6): ^{1}H-NMR ($CDCl_3$) 7.55–7.45 (m, 4 H, aromatic), 7.14–7.04 (m, 4 H, aromatic), 5.66 (s, 1 H, $CHPh_2$), 4.38–2.94 (m, 9 H, OCH_2CH_2N, piperidine C-2,3,6), 2.35–1.35 (m, 4 H, piperidine C-4,5); IR (KBr) 2874, 1673, 1601 cm^{-1}.

1-[2-[Bis[4-(trifluoromethoxy)phenyl]methoxy]ethyl]-3-piperidinecarboxylic acid, monohydrochloride (9) was prepared from 4-(trifluoromethoxy)bromobenzene obtained by cooling 4-bromophenol (Kodak, 34.6 g, 0.2 mol) to −70 °C and adding COF_2 (20 g, 0.3 mol). The reaction was sealed and heated to 100 °C for 1 h and then 140 °C for 2 h. The reaction was cooled to 0 °C, excess COF_2 vented, and the reaction cooled to −78 °C. SF_4 (24 g, 0.22 mol) was added, and the reactor was sealed and heated to 140 °C for 4 h and then cooled. The vessel was vented and the residue dissolved in CH_2Cl_2 (100 mL) and poured into a saturated $NaHCO_3$ solution. The organic layer was evaporated and the residue distilled at 58–62 °C, 20 mmHg to afford 25.0 g of product (52% yield).

1-[2-[(2,8-Dichloro-10,11-dihydro-5*H*-dibenzo[*ad*]cyclohepten-5-yl)oxy]ethyl]-3-piperidinecarboxylic acid, monohydrochloride (18) was prepared from 4,4'-dichlorodibenzosuberone (**46**, Scheme II). The Grignard of 3-chlorobenzyl bromide (10.0 g, 48 mmol, Aldrich) was prepared, Li_2CuCl_4 (Alfa, 0.1 M in THF, 0.2 mL) added, and the mixture refluxed for 15 min. The reaction was poured into dilute aqueous HCl–ice and extracted with ether (100 mL), and the product was crystallized from hexane to afford 5.2 g of the biphenyl product (86% yield, mp = 48–9 °C). To CS_2 (50 mL) was added $AlCl_3$ (17.4 g, 130.0 mmol) and the mixture cooled to −78 °C. Oxalyl chloride (6.5 g, 50 mmol) was added dropwise and the reaction warmed to room temperature. The biphenyl product (3.2 g, 130 mmol) was added in CS_2 (10 mL) and stirred 16 h at 35 °C. Water was added, CS_2 evaporated, the mixture extracted with $CHCl_3$, and the product purified by chromatography (silica, 9:1; hexane–ethyl acetate) to afford 0.8 g of product (22% yield).

1-[2-(Triphenylmethoxy)ethyl]-3-piperidinecarboxylic acid (19): ^{1}H-NMR ($CDCl_3$) 7.40–7.20 (m, 15 H, aromatic), 3.01 (t, 2 H, J = 5.6 Hz, CH_2O), 2.81 (m, 1 H, $CHCO_2H$), 2.60–1.95 (m, 6 H, CH_2N), 1.77–1.30 (m, 4 H, piperidine C-4,5); IR (KBr) 3420, 3057, 2960, 1716, 1597, 1491, 1450 cm^{-1}.

1-[3-(Triphenylmethoxy)propyl]-3-piperidinecarboxylic acid (20): ^{1}H-NMR ($CDCl_3$) 7.42–7.22 (m, 15 H, aromatic), 3.02 (t, 2 H, J = 3.7 Hz, CH_2O), 2.94–2.00 (m, 7 H, CH_2N, $CHCO_2H$), 2.00–1.29 (m, 6 H, piperidine C-4,5, OCH_2CH_2); IR (KBr) 3445, 3057, 2972, 1716, 1597, 1491, 1450, 1389 cm^{-1}.

1-[2-(3-Phenyl-2-benzofuranyl)ethyl]-3-piperidinecarboxylic acid, monohydrochloride (21) was prepared from 3-phenylbenzo[2,3]furan[39] (8.96 g, 46.2 mmol). The anion was formed with *n*-BuLi (20.6 mL, 48.4 mmol) in THF (120 mL). After 7 h the reaction was cooled to −78 °C, oxirane (6 mL, 8.53 M in THF, 51.3 mmol) added, and after 10 min CuBr·Me_2S (0.47 g in 2 mL of Me_2S) added, stirred 2 h at −78 °C, and warmed to room temperature over 24 h. The reaction was quenched by addition of water and saturated $Na_2S_2O_3$. The organic layer was extracted with ether (3 × 30 mL) and the resulting oil purified by chromatography (silica, 25:2.5:1; CH_2Cl_2–hexane–2-propanol) to afford 3-phenyl-2-benzo[2,3]furanyl-2-ethanol as light yellow crystals (6.7 g, 61% yield, mp = 81–2 °C).

1-[2-(2,2-Diphenylcyclopropyl)ethyl]-3-piperidinecarboxylic Acid, Hydrochloride (22) (Scheme III). To lithium aluminum hydride (4.25 g, 112 mmol in 100 mL of THF) was added the Stobbe condensation[41] product (17.35 g, 55.9 mmol) of benzophenone and the half-ester of succinic acid in 100 mL of THF over 30 min. The reaction was refluxed for 19 h and cooled and ether (100 mL), hexane (100 mL), and 10 mL of 20% aqueous NaOH were added. The ether layer was dried (K_2CO_3) and the product purified by chromatography (silica, 1:1; pentane–ether) to afford 10.2 g (77% yield) of **47**: ^{1}H-NMR (d_6-DMSO) 12.8 (bs, 1 H, HCl), 10.6 (bs, 1 H, COOH), 7.30–7.08 (m, 10 H, aromatic), 3.80–3.00 (m, 7 H, CH_2N, piperidine C-3), 2.05–1.00 (m, 9 H, piperidine C-4,5, cyclopropyl, CH_2O); IR (KBr) 1725, 1600, 1496, 1447, 1401, 1276 cm^{-1}.

1-(2-Oxo-3,3-diphenylpropyl)-3-piperidinecarboxylic acid (23) was prepared from **48** (Scheme IV). 1,1-Diphenyl-2-propanone (Aldrich, 7.73 g, 36.8 mmol in 25 mL of acetic acid) and 7 g of sodium acetate were refluxed for 4 days, the mixture was cooled to 80 °C, and excess acetic anhydride was decomposed with 15 mL of water. The mixture was poured onto 100 g of ice, stirred 16 h at room temperature, neutralized with solid $NaHCO_3$, extracted (3 × 100 mL ethyl acetate), and distilled (<0.1 mmHg) to afford the desired enol ether (3.28 g, 13.0 mmol) which was dissolved in CCl_4 (50 mL) and to which *N*-bromosuccinimide (2.5 g, 14 mmol) was added. The mixture was heated at reflux and 50 mg of AIBN added, and the mixture was irradiated with a 275-W sunlamp with reflux continuing for 15 min to afford **48** as an oil which was used directly in methods C and D: ^{1}H-NMR (d_6-DMSO) 8.20–7.80 (bs, 1 H, COOH), 7.32 (s, 10 H, aromatic), 5.55 (s, 1 H, $CHPh_2$), 4.56 (s, 2 H, $COCH_2N$), 4.00–1.20 (m, 9 H, piperidine); IR (KBr) 1730, 1599, 1497, 1454, 1400 cm^{-1}.

1-(3,4-Diphenylbutyl)-3-piperidinecarboxylic acid, monohydrochloride (24) was prepared from 3,4-diphenylbutan-1-ol, in turn prepared by placing sodium hydride (1.24 g, 50% suspension in mineral oil, 26 mmol) in THF (50 mL), and adding triethyl phosphonoacetate (Aldrich, 5.82 g, 26 mmol) dropwise with stirring. 2-Phenylacetophenone (Aldrich, 5.0 g, 26 mmol) was added in 10 mL of THF and the reaction stirred at reflux for 16 h, cooled, quenched with water (5 mL), and extracted with ether. The product (a mixture of 2,3 and 3,4 olefins) was purified by chromatography (silica, 1:9; ethyl acetate–hexane) to afford 5.0 g of product (72% yield) which was dissolved in ethyl acetate (100 mL) and 1 g of 20% Pd on carbon added. The reaction was stirred 16 h under a hydrogen atmosphere and the product purified by chromatography (silica, 1:4; ethyl acetate–hexane) to afford 3.54 g (70% yield) of the desired saturated product which was dissolved in 100 mL of CH_2Cl_2 and cooled to −78 °C. Diisobutylaluminum hydride (84 mL of a 1 M solution in hexane) was added dropwise and the reaction stirred for 3 h at −78 °C. Methanol (1 mL) was added and the mixture poured into ether, washed with saturated sodium potassium tartrate solution, dried, and purified by chromatography (silica, 1:1, ethyl acetate–hexane) to afford 2.56 g (85% yield) of product.

1-[1-[2-[Bis(4-chlorophenyl)methoxy]ethyl]-3-piperidinyl]-ethanone (29) was prepared from **38** (5.56 g, 13.2 mmol) which was added to a THF solution of lithium diisopropylamine (14.5 mmol) over 15 min at −78 °C. After 30 min, acetyl chloride (1.03 g, 13.2 mmol) was added in 5 mL of THF. The reaction was slowly warmed to room temperature over 16 h. Evaporation afforded an oil which was dissolved in $CHCl_3$ (50 mL), washed with water and saturated NaCl, and evaporated. Purification by chromatography (silica, 1:4; ethyl acetate–hexane) furnished 3.4 g of desired keto ester (56% yield) which was dissolved in methanol (60 mL) and 1 N LiOH (12.5 mL) and stirred at room temperature for 72 h. The methanol was evaporated and 35 mL of water added followed by 30 mL of 5% NaH_2PO_4 and 25 mL of ethanol. The reaction was heated at reflux for 2 h and cooled, solid K_2CO_3 added to pH 8, the solution extracted with $CHCl_3$ (3 × 50 mL), and the resulting oil purified by chromatography (silica, gradient of 10–20% acetone in $CHCl_3$ with 1% triethylamine added) to afford 1.6 g of **29**.

1-[2-[Bis(4-chlorophenyl)methoxy]ethyl]-3-piperidinecarbonitrile, monohydrochloride (30) was prepared from **40** (2.6 g, 6.4 mmol) which was dissolved in pyridine (10 mL). $POCl_3$ (2.0 g, 12.8 mmol) was added dropwise, the reaction stirred at 90 °C for 90 min and then at 45 °C for 40 h and cooled, and ether (100 mL) added followed by solid $NaHCO_3$. The ether layer was separated, washed with water and saturated NaCl, and evaporated to afford 1.5 g of **30**.

1-[2-[Bis(4-chlorophenyl)methoxy]ethyl]-3-piperidinemethanol, monohydrochloride (31) was prepared from **39** (3.0 g, 6.87 mmol) dissolved in CH_2Cl_2 (20 mL) and cooled to −70 °C. To this was added diisobutylaluminum hydride (20.62 mL of a 1 M solution in hexane, 20.62 mmol) over 20 min. The reaction was allowed to warm to room temperature over 16 h, then diluted with CH_2Cl_2 (100 mL) and 20 mL of 1.2 N NaOH, and shaken vigorously. The CH_2Cl_2 layer was separated, the aqueous layer extracted with CH_2Cl_2 (2x), and the organic layers evaporated to afford an oil which was purified by chromatography (silica, 98:2; $CHCl_3$–methanol) to afford 2.18 g of **31**.

1-[2-[Bis(4-chlorophenyl)methoxy]ethyl]-2-pyrrolidinone (35). To sodium hydride (0.55 g, 60% in oil, 13.75 mmol) in DMF (35 mL) was added 2-pyrrolidinone (0.85 g, 10 mmol) and the reaction stirred for 2 h followed by addition of bromo side chain (3.15 g, 10 mmol). After 18 h at 40 °C, standard workup gave 3.3 g of solid which was purified by chromatography (silica, 1:18; ether–hexane) to afford 0.9 g of **35** (25% yield): ^{1}H-NMR ($CDCl_3$) 7.32–7.20 (m, 8 H, aromatic), 5.30 (s, 1 H, $CHPh_2$), 3.68–3.45 (m, 6 H, NCH_2CH_2O, $NCOCH_2$), 2.36 (t, 2 H, J = 7.90 Hz, pyrrolidinone C-4), 2.00 (m, 2 H, pyrrolidinone C-3).

(*S*)-(+)-1-[2-[Bis(4-chlorophenyl)methoxy]ethyl]-3-piperidinecarboxylic acid, hydrochloride (36) was prepared from **5** by resolution with (*R*)-(+)-α-methylbenzylamine. The salt was formed in ethyl acetate followed by three recrystallizations from hexane–ethyl acetate and further treated with 25% aqueous HCl, and the HCl salt was recrystallized from ethyl acetate–acetone to afford **36**: $[\alpha]_D$ = +8.50 (c = 2.04, DMF); HPLC, >98% optically pure (glycoprotein column, 3% ethanol in 0.05 M phosphate buffer, 0.002 M DMOA, pH 7.0, 0.4 mL/min).

(*R*)-(−)-1-[2-[Bis(4-chlorophenyl)methoxy]ethyl]-3-piperidinecarboxylic acid, hydrochloride (37) was obtained from the filtrate from the first crystallization in the preparation of **36**

which was evaporated to a solid enriched in the (*R*)-(−) enantiomer. The residue was taken up in acetone and treated with ice-cold 25% aqueous HCl. The salt was removed by filtration and dissolved in 1 N NaOH and $CHCl_3$, and the pH was adjusted to 6.5 with saturated NaH_2PO_4. The organic layer was separated, evaporated, and redissolved in ethyl acetate, and 1 equiv of (*S*)-(−)-α-methylbenzylamine was added. The mixture was evaporated to dryness and the resulting salt recrystallized three times and converted to the HCl salt as described for **36**. The HCL salt was crystallized from ethyl acetate–acetone to obtain **37**: HPLC (as for **36**) showed 100% optical purity); $[\alpha]_D = -8.71$ ($c = 2.06$, DMF).

1-[2-[Bis(4-chlorophenyl)methoxy]ethyl]-3-piperidinecarboxylic acid, methyl ester, hydrochloride (38) was prepared from the free base of **5** using excess diazomethane in ether to afford 0.72 g (86% yield) of **38**.

1-[2-[Bis(4-chlorophenyl)methoxy]ethyl]-3-piperidinecarboxylic acid, ethyl ester (39): ^{1}H-NMR ($CDCl_3$) 7.33–7.22 (m, 8 H, aromatic), 5.32 (s, 1 H, CH_2Ph_2), 4.12 (q, 2 H, $J = 7.17$ Hz, CO_2CH_2Me), 3.57 (t, 2 H, $J = 5.98$ Hz, CH_2O), 3.07–2.74 (m, 2 H, pip C-2), 2.67 (t, 2 H, $J = 5.98$ Hz, CH_2N), 2.62–1.38 (m, 7 H, pip C-3,4,5,6), 1.23 (t, 3 H, $J = 7.17$ Hz, Me); IR (liquid film) 2941, 1730, 1491, 1091, 1016 cm^{-1}.

1-[2-[Bis(4-chlorophenyl)methoxy]ethyl]-3-piperidinecarboxamide, monohydrochloride (40) was prepared by dissolving **5** (21.56 g, 52.8 mmol) in THF and adding carbonyldiimidazole (9.42 g, 58.1 mmol). The reaction was heated at reflux for 24 h and cooled slightly, and a stream of anhydrous ammonia was bubbled through the reaction for 5 min. The mixture was then heated at reflux for 16 h, ammonia again bubbled for 10 min, and heating continued for an additional 4 h. The solvent was evaporated and the oil dissolved in 250 mL of ether, washed with saturated NaHCO3 and saturated sodium chloride, and purified by chromatography (silica, 5.5:0.5:0.1; ether–methanol–triethylamine) to yield **40**.

1-[2-[Bis(4-chlorophenyl)methoxy]ethyl]-*N*-methyl-3-piperidinecarboxamide, hydrochloride (41) was prepared from **39** (1.86 g, 4.27 mmol) dissolved in methanol (100 mL) and methylamine gas (10 g). The reaction vessel was sealed and heated at 60 °C for 18 h. Evaporation afforded an oil which was dissolved in ether, washed with water, evaporated, and purified by chromatography (silica, 98:2; ether–methanol) to give **41**.

1-[2-[Bis(4-chlorophenyl)methoxy]ethyl]-*N,N*-dimethyl-3-piperidinecarboxylic acid, monohydrochloride (42) was prepared from **39** (2.18 g, 5 mmol) in 10 mL of toluene to which methylchloroaluminum *N,N*-dimethylamide[48] was added. The reaction was refluxed for 5 h, cooled, and quenched with 5% aqueous HCl. The organic layer was washed with water and saturated $NaHCO_3$, concentrated, and purified by chromatography (silica, acetone) to obtain 0.70 g of **42** (32% yield).

1-[2-[Bis(4-chlorophenyl)methoxy]ethyl]-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid, methyl ester (45): ^{1}H-NMR ($CDCl_3$) 7.33–7.19 (m, 8 H, aromatic), 7.00 (s, 1 H, CH=C), 5.33 (s, 1 H, $CHPh_2$), 3.73 (s, 3 H, CO_2CH_3), 3.62 (t, 2 H, $J = 5.85$ Hz, OCH_2CH_2N), 3.25 (s, 2 H, piperidine C-2), 2.79 (t, 2 H, $J = 5.84$ Hz, OCH_2CH_2N), 2.61 (t, 2 H, $J = 5.65$ Hz, piperidine C-6), 2.34 (bs, 2 H, piperidine C-5); IR (liquid film) 2949, 1715, 1659, 1615, 1491, 1437, 1267, 1091 cm^{-1}.

Pharmacological Methods. Pharmacological methods have been previously described in detail.[54]

In Vitro GABA Uptake Inhibition. Rat hippocampal prisms (0.1 mm cuboidal) were incubated for 15 min in the presence or absence of test compound. [^{3}H]GABA was added and samples incubated for an additional 2 min before rapid filtration through Whatman GF/F filters. Samples were then washed with 5 mL of ice-chilled 0.9% saline. Distilled water was added and samples were allowed to sit at least 60 min before being measured for radioactivity by scintillation counting. Blanks were treated in an identical manner but were left on ice throughout the incubation. IC_{50} values were determined by an iterative nonlinear curve-fitting routine to the logistic equation.

Threshold Pentylenetetrazole Seizures in Mice. Pentylenetetrazole (85 mg/kg) was given subcutaneously and animals were observed for 30 min afterwards for the appearance of clonic convulsive seizures of the forelimbs.[56] The appearance of more than 3 sec of clonus was scored as a seizure. The number of mice with seizures was scored at a number of different doses of **44**, and ED_{50} was determined by probit analysis.[61]

Electroshock Seizures in Mice. Low-intensity electroshock (14 mA) was delivered via corneal electrodes, and the number of mice with tonic extensor seizures was scored.[57] Standard maximal electroshock seizures were produced in the same manner, but with a 50-mA stimulus.[56]

Focal Seizures in Rats. Wire electrodes were implanted in the dorsal hippocampus of rats and stimulated (1-ms biphasic pulses, 500 μA, 10 Hz for 10 s) repeatedly in four separate day-long sessions. At least 1 week later, threshold electrical current for a focal afterdischarge was determined by incrementing stimulus current by 20%, starting at 40 μA. The test stimuli were 1-ms biphasic pulses, 60 Hz for 1 sec. Threshold was determined in three sequential trials before drug treatment. Following drug treatment, stimulation was given at twice the mean predrug threshold. If no afterdischarge developed, an anticonvulsant effect was scored.

Ataxia. Ataxia in mice was determined by the rotorod procedure.[56] Mice were placed on a 1-in. rod rotating at 6 revolutions per minute. Failure to maintain balance in each of three 30-s trials was scored as ataxia. Ataxia in rats was scored by observation of markedly abnormal posture or gait in three consecutive trials on a flat table top.

Acknowledgment. We gratefully acknowledge the help of the NINCDS Antiepileptic Drug Discovery Program (H. Kupferberg, J. Stables) with some of the pharmacological data.

(61) Litchfield, J. T.; Wilcoxin, F. A. Simplified Method of Evaluating Dose-Effect Experiments. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99–113.