

114772-45-1; 103, 114772-60-0; 104, 124750-36-3; 105, 37455-55-3; 106, 68282-41-7; 107, 68282-59-7; 108, 124750-54-5; 109, 124750-55-6; 110, 133379-10-9; 111, 114772-46-2; 112, 114772-62-2; 113, 114772-63-3; 114, 114772-83-7; 115, 114772-84-8; 116, 114772-85-9; 117, 124779-26-6; 118, 114772-77-9; 119, 124750-43-2; 120, 114772-51-9; 121, 114772-70-2; 122, 114772-75-7; 123, 114772-76-8; 124, 120568-15-2; 125, 120568-16-3; 126, 124750-65-8; 127, 133910-04-0; 128, 16191-28-9; 129, 124750-63-6; 130, 124750-64-7; 131, 133910-05-1; 132, 133910-06-2; 133, 133910-07-3; 134, 124750-42-1; 135, 114772-81-5; 136, 114772-82-6; methyl 2-iodo-

benzoate, 610-97-9; 4-iodotoluene, 624-31-7; 1-bromo-2-nitrobenzene, 577-19-5; 2-methoxybenzoic acid, 579-75-9; 2-amino-2-methyl-1-propanol, 124-68-5; 4-bromotoluene, 106-38-7; 3-bromobenzonitrile, 6952-59-6; 2-butyl-4(5)-chloro-5(4)-(hydroxymethyl)imidazole, 79047-41-9; 4-[[3-[*N*-(*tert*-butoxycarbonyl)-*N*-isopropylamino]-2-hydroxypropyl]oxy]indole-2-carboxylic acid, 103221-84-7; 2-butyl-4(5)-(hydroxymethyl)imidazole, 68283-19-2; butyraldehyde, 123-72-8; acetol, 116-09-6; (phenylsulfonyl)acetonitrile, 7605-28-9; ethyl 3-(4-methylphenyl)-3-oxopropanoate, 7116-41-8; allyl bromide, 106-95-6.

GABA-Uptake Inhibitors: Construction of a General Pharmacophore Model and Successful Prediction of a New Representative

Victor N'Goka,[†] Gilbert Schlexer,^{*†} Jean-Michel Linget,[†] Jean-Pierre Chambon,[‡] and Camille-Georges Wermuth[†]

Laboratoire de Pharmacochimie Moléculaire, Centre de Neurochimie du CNRS, 5, rue Blaise Pascal, 67084 Strasbourg Cedex, France, and Sanofi Recherche, Rue du Professeur J. Blayac, 34082 Montpellier Cedex, France. Received March 18, 1991

A model for the pharmacophore of GABA-uptake inhibitors was established using published structure-activity data and molecular modeling. The model accounted for the activities of different classes of GABA-uptake inhibitors. Analogues of guvacine substituted at position 6 were synthesized in order to confirm the model. 6-(3,3-Diphenylpropyl)guvacine (30f), which fit well with the pharmacophore, had an in vitro IC₅₀ of 0.1 μM. This value is as good as those of the best GABA-uptake inhibitors known today.

γ-Aminobutyric acid (GABA, 1) is a major neurotransmitter in mammals.¹⁻⁹ Dysfunctioning of GABA-ergic synapses has been invoked for diseases such as Parkinson's disease,¹⁰⁻¹³ Huntington's chorea,¹⁴ epilepsy,^{15,16} and some forms of schizophrenia.¹⁷⁻¹⁹ One of the possible ways to palliate GABA deficiency lies in the inhibition of uptake mechanisms of this neurotransmitter.²⁰⁻²⁴ Taking in account the results of various studies devoted to this GABA-ergic regulation approach²⁵⁻²⁷ allowed us to establish a model pharmacophore by means of computer modeling. The validity of the model pharmacophore was confirmed through its ability to rationalize the activity of some newly described uptake inhibitors and to guide the design of a novel, potent GABA uptake inhibitor.

Identification and Construction of the Pharmacophore

Studies on GABA-uptake inhibitors have been developed in several directions. Analogues of GABA itself have been prepared in order to modulate the chain length or to introduce substituents or unsaturation. Although these modifications yielded active compounds, only 2-fluoro-GABA (2)²⁸ (Figure 1) showed uptake-inhibitory potency comparable to that of GABA itself. Most of these acyclic GABA analogues are not selective for GABA-uptake inhibition but also possess agonist properties.

Conformationally restricted cyclic GABA analogues have also been studied, e.g., where the methylenes have been incorporated into homocyclic systems such as cyclopropyl,²⁹ cyclobutyl,²⁹ cyclopentyl,³⁰ cyclopentenyl,³⁰ cyclohexyl,^{29,31} cyclohexenyl,²⁹ and cyclohexadienyl²⁹ rings. The best activities were found for cyclopentyl and cyclopentenyl rings, and high stereoselectivity was observed for the (+)-4(*S*)- versus the (-)-4(*R*)-aminocyclopentene-1-carboxylic acids³⁰ 3 and 4, respectively (Figure 1). Some

heterocyclic amino acids were also described as GABA-uptake inhibitors, especially in the pyrrolidine²⁹ and pi-

- (1) Roberts, E.; Chase, T. R.; Tower, D. B., Eds. *GABA in Nervous System Function*; Raven Press: New York, 1976, 554 pp.
- (2) Awapara, J.; Landau, A. UJ.; Fuerst, R.; Seale, B. Free γ-aminobutyric acid in brain. *J. Biol. Chem.* 1950, 187, 35-39.
- (3) Roberts, E.; Frankel, S. γ-Aminobutyric acid in brain: Its formation from glutamic acid. *J. Biol. Chem.* 1950, 187, 55-64.
- (4) Udenfriend, S. Identification of γ-aminobutyric acid in brain by the isotope derivative method.
- (5) Oja, S. S.; Kontro, P.; Lähdesmäki, P. Amino Acids as Inhibitory Neurotransmitters. *Progress in Pharmacology*; G. Fischer Verlag: Stuttgart, 1977; Vol. 1, No. 3, 120 pp.
- (6) Curtis, D. R.; Johnston, G. A. R. Amino acid transmitters in the mammalian nervous system. *Ergeb. Physiol. Biol. Chem. Exp. Pharmacol.* 1974, 69, 97-188.
- (7) Enna, S. J.; Gallaghe, J. P. Biochemical and electrophysiological characteristics of mammalian GABA receptors.
- (8) Okada, Y.; Ozawa, S. The concentration of GABA required for its inhibitory action in the hippocampal pyramidal cell in vitro. *Excerpta Med., Sect. 1* 1982, 565, 87-95.
- (9) Olsen, R. W.; Yang, J.; King, R. G.; Dilber, A.; Stauber, G. B.; Ransom, R. W. Barbiturate and benzodiazepine modulation of GABA receptor-binding and function. *Life Sci.* 1986, 39, 1969-1976.
- (10) McGeer, P. L.; McGeer, E. G. Enzymes associated with penetration of catecholamines, acetylcholine and GABA in human controls and patients with Parkinson's disease and Huntington's chorea. *J. Neurochem.* 1976, 261, 65-76.
- (11) Lloyd, K. G.; Möhler, H.; Heitz, P.; Bartholini, G. Distribution of choline acetyltransferase and glutamate decarboxylase within substantia nigra and in other brain-regions from control and Parkinson's patients. *J. Neurochem.* 1975, 25, 789-795.
- (12) Lloyd, K. G.; Shemen, L.; Hornykiewicz, O. Distribution of high affinity sodium-independent [³H]γ-aminobutyric acid [³H]GABA binding in human brain. Alteration in Parkinson's disease. *Brain Res.* 1977, 127, 269-278.
- (13) Tower, D. B. GABA and seizures. Clinical correlates in man. In *GABA in Nervous System Function*; Roberts, E., Chase, T. R., Tower, D. B., Eds.; Raven: New York, 1976; pp 461-478.
- (14) Lloyd, K. G.; Dreksler, S.; Bird, E. D. Alteration in [³H]GABA binding in Huntington's chorea. *Life Sci.* 1977, 21, 747-754.

[†]Laboratoire de Pharmacochimie Moléculaire, Centre de Neurochimie du CNRS.

[‡]Sanofi Recherche.

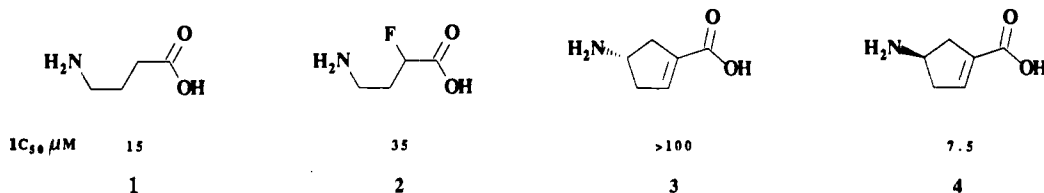


Figure 1. GABA uptake inhibitory potency of GABA (1), 2-fluoro-GABA (2), and (S)- and (R)-4-aminocyclopentene-1-carboxylic acids (3 and 4).^{28,30}

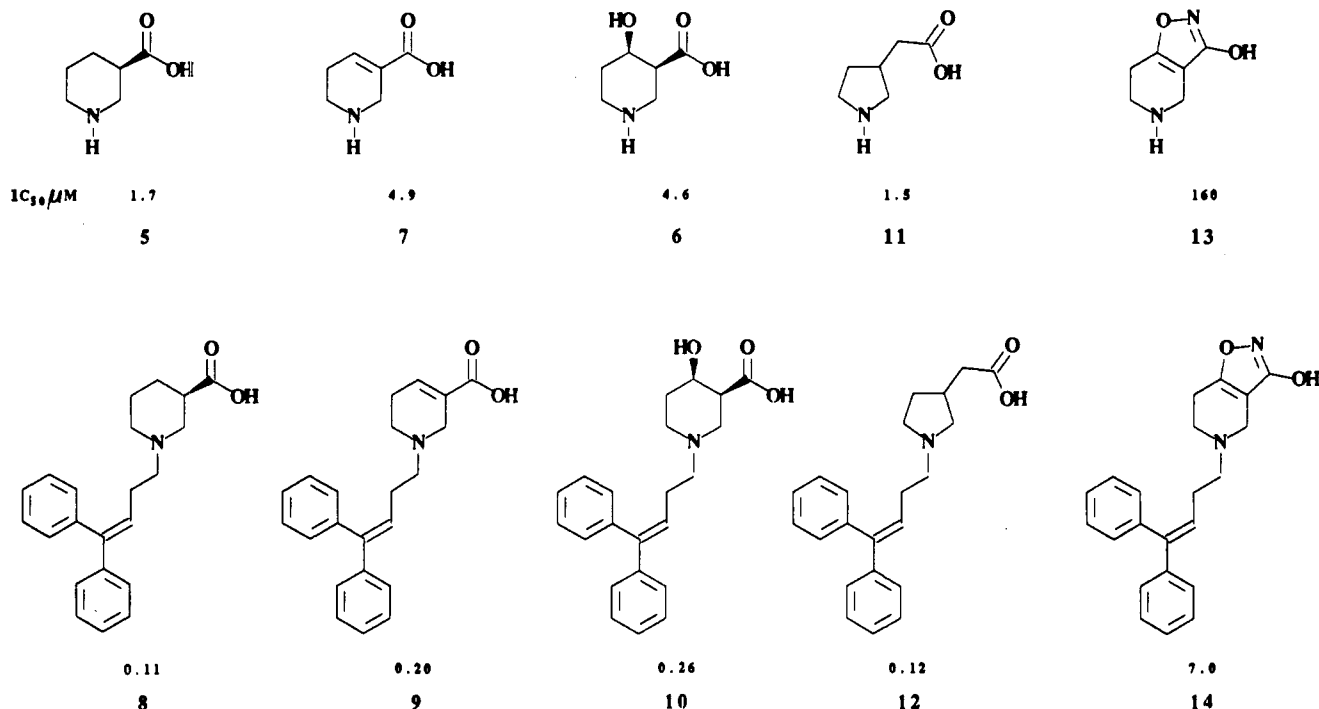


Figure 2. GABA uptake inhibitory potency of (R)-nipecotic acid (5), guvacine (7), cis-4-hydroxynipecotic acid (6), 3-pyrrolidineacetic acid (11), THPO (13), and their N-4,4-diphenyl-3-butenyl derivatives.³³

peridine.^{27,29,32-34} series. The most active compound in the piperidine series, (-)-(*R*)-nipecotic acid (5) (Figure 2), has

- (15) Nitsch, C.; Okada, Y. Differential decrease of GABA in substantia nigra and other discrete regions of rabbit brain during preictal period of methoxypyridoxine-induced seizures. *Brain Res.* 1976, 105, 173-178.
- (16) Meldrum, B. S. Convulsant drugs, anticonvulsants and GABA-mediated neuronal inhibition. In *GABA-Neurotransmitters: Pharmacological, Biochemical and Pharmacological Aspects*; Krogsgaard-Larsen, P., Scheel-Kruger, J., Kofod, H., Eds.; Munksgaard: Copenhagen, 1978; pp 390-405.
- (17) Roberts, E. GABA in nervous system function. An overview. In *Nervous System*; Tower, D. B., Ed.; Raven: New York, 1975; Vol. 1, pp 541-542.
- (18) Vankammen, D. P. Gamma-aminobutyric acid (GABA) and dopamine hypothesis of schizophrenia. *Am. J. Psychiatry* 1977, 134, 138-143.
- (19) Fuxe, K.; Andersson, K.; Ogren, S. O.; Perez de la Mora, M.; Schwarcz, R.; Hkpelt, T.; Eneroth, P.; Gustafsson, J. A.; Skett, P. GABA neurons and their interaction with monoamine neurons. An anatomical, pharmacological and functional analysis. In *GABA-Neurotransmitters: Pharmacological, Biochemical and Pharmacological Aspects*; Krogsgaard-Larsen, P., Scheel-Kruger, J., Kofod, H., Eds.; Munksgaard: Copenhagen, 1978; pp 74-94.
- (20) Shousboe, A. Significance of the glial transport system for the interaction of GABA and effects of structural analogues on the uptake. In *GABA-Neurotransmitters: Pharmacological, Biochemical and Pharmacological Aspects*; Krogsgaard-Larsen, P., Scheel-Kruger, J., Kofod, H., Eds.; Munksgaard: Copenhagen, 1978; pp 263-280.
- (21) Cohen, S. R.; Lajtha, A. Amino acid transport. *Handbook of Neurochemistry*; Lajtha, A., Ed.; Plenum: New York, 1972; Vol. 7, pp 543-572.

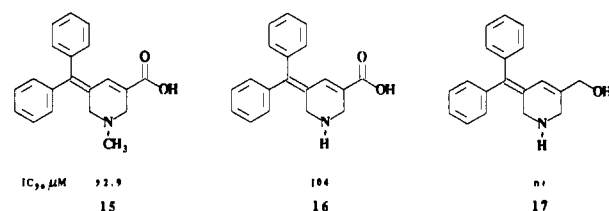


Figure 3. GABA uptake inhibitory potency of 5-benzhydrylidene-guvacine derivatives.⁴⁴

an IC_{50} value of 1.7 μM on GABA-uptake inhibition.^{33,34} For this substance again some enantiomeric selectivity was

- (22) Iversen, L. L.; Kelly, J. S. Uptake and metabolism of γ -aminobutyric acid by neurones and glial cells. *Biochem. Pharmacol.* 1975, 24, 933-938.
- (23) Martin, D. L. Carrier-mediated transport and removal of GABA from synaptic regions. In *GABA in Nervous System Function*; Roberts, E., Chase, T. R., Tower, D. B., Eds.; Raven Press: New York, 1976; pp 347-386.
- (24) Storm-Mathisen, J.; Fonnum, F.; Malthe-Sorensen, D. Gaba uptake in nerve terminals. In *GABA in Nervous System Function*; Roberts, E., Chase, T. R., Tower, D. B., Eds.; Raven Press: New York, 1976; pp 387-394.
- (25) Krogsgaard-Larsen, P., Ed. *Specific GABA receptors agonists and uptake inhibitors: Design, development and structure activity studies*; Fald's Forlag: Copenhagen, 1980; 198 pp.
- (26) Shousboe, A.; Thorbek, P.; Hertz, L.; Krogsgaard-Larsen, P. Effects of GABA analogues of restricted conformations on GABA transport in astrocytes and brain cortex slices and on GABA receptor binding. *J. Neurochem.* 1979, 33, 181-189.
- (27) Krogsgaard-Larsen, P.; Johnston, G. A. R. Inhibition of GABA uptake in rat brain slices by nipecotic acid, various isoxazoles and related compounds. *J. Neurochem.* 1975, 25, 797-802.

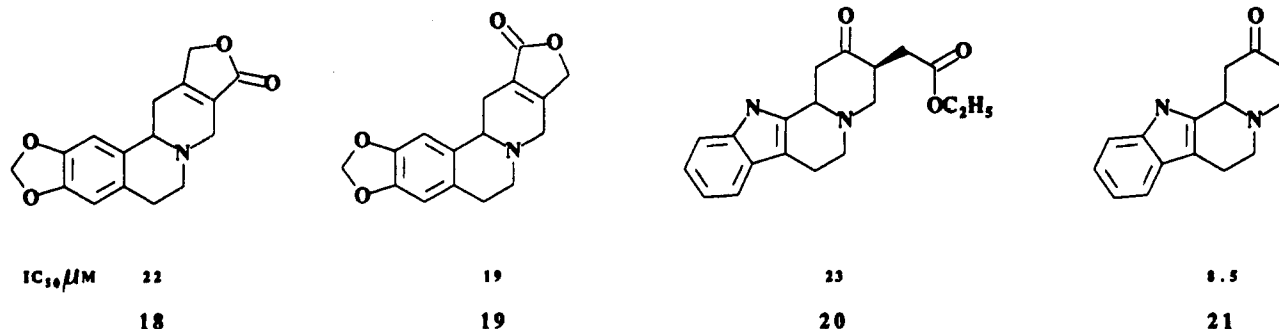


Figure 4. GABA uptake inhibitory potency of polycyclic compounds.⁴⁵

observed insofar that (+)-(*S*)-nipecotic acid is approximately 6 times less potent than the (–)-(*R*) enantiomer.³⁴ X-ray analyses, as well as NMR studies, demonstrated that the carboxylic function of (–)-(*R*)-nipecotic acid stayed in an equatorial position.³⁵ In such an orientation, the acidic group is coplanar with the mean plane of the molecule.

On the basis of its potency and selectivity as a GABA-uptake inhibitor, nipecotic acid became the focus of additional research. Thus, substitution^{29,36} or unsaturation³¹ of nipecotic acid resulted in (±)-*cis*-4-hydroxynipecotic acid²⁹ (6) (Figure 2) and the 3,4-unsaturated analogue, guvacine²⁹ (7) (Figure 2), which showed potencies comparable to that of nipecotic acid.

The carboxylic acid function of GABA was also replaced by bioisosteric functions such as isoxazoles³⁷ or tetrazoles,³⁸

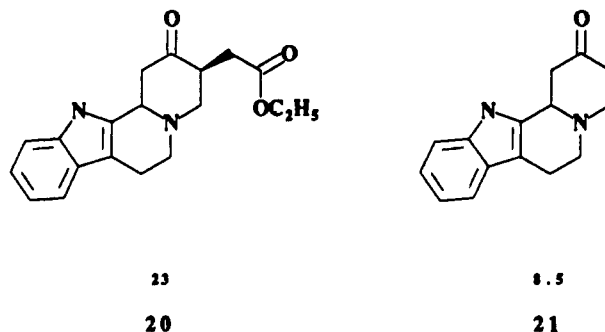


Figure 5. Superimposition of SKF 89976-A (8) and *N*-methyl-5-benzhydrylidene-guvacine (15).

leading to less active compounds. In 1984, a group at Smith Kline and French Laboratories reported the potent GABA uptake inhibitory properties of nipecotic acid, guvacine, *cis*-4-hydroxynipecotic acid, and homo-β-proline analogues, *N*-substituted with a diphenylbutenyl group.^{34,39} These compounds, 8 (SKF 89976-A), 9 (SKF 100330-A), 10 (SKF 100591), and 12 (SKF 100561) (Figure 2), are approximately 20 times more potent than the unsubstituted amino acids. The authors explained the observed activity increase by the existence of additional binding sites to which the aromatic rings could fit. Lengthening or shortening the alkenyl chain, which links the piperidine ring to the aromatic groups, as well as saturation of the double bond in the side chain of 8 leads to an important loss of activity.³³ Further studies demonstrated that the replacement of one or both of the benzene rings by furan, pyrrole, or thiophene rings leads to slightly more active molecules.^{40,41} Also, the replacement of one of the sp² carbon atoms of the side chain by an oxygen atom, bearing two p doublets, led to an equipotent product.⁴²

Krogsgaard-Larsen et al. grafted the diphenylbutenyl chain to other GABA-uptake inhibitors such as tetra-

- (28) Iversen, L. L.; Johnston, G. A. R. GABA uptake in rat central nervous system: comparison of uptake in slices and homogenates and effects of some inhibitors. *J. Neurochem.* 1971, 18, 1939–1950.
- (29) Johnston, G. A. R.; Allen, R. D.; Kennedy, S. M. G.; Twitchin, B. Systematic study of GABA analogues of restricted conformation. In *GABA-Neurotransmitters: Pharmacological, Biochemical and Pharmacological Aspects*; Krogsgaard-Larsen, P., Scheel-Krüger, J., Kofod, H., Eds.; Munksgaard: Copenhagen, 1978; pp 147–164.
- (30) Allan, R. D.; Dickenson, H. W.; Fong, J. Structure-activity studies on the activity of cyclopentane GABA analogs on GABA receptors and GABA uptake. *Eur. J. Pharmacol.* 1986, 122, 339–348.
- (31) Beart, P. M.; Johnston, G. A. R.; Uhr, M. L. Competitive inhibition of GABA uptake in rat brain slices by some GABA analogues of restricted conformation. *J. Neurochem.* 1972, 19, 1855–1861.
- (32) Johnston, G. A. R.; Stephanson, A. L.; Twitchin, B. Uptake and release of nipecotic acid by rat brain slices. *J. Neurochem.* 1976, 26, 83–87.
- (33) 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.; Moosammy, G. I.; Oh, H. J.; Rush, J. A.; Setler, P. E.; Stringer, O. D.; Venslavsky, J. W.; Volpe, B. W.; Yungler, L. M.; Zirkle, C. L. Orally active and potent inhibitors of γ-aminobutyric acid uptake. *J. Med. Chem.* 1985, 28, 653–660.
- (34) Johnston, G. A. R.; Krogsgaard-Larsen, P.; Stephanson, A. L.; Twitchin, B. Inhibition of the uptake of GABA and related amino acids in rat brain slices by the optical isomers of nipecotic acid. *J. Neurochem.* 1976, 26, 1029–1032.
- (35) Brehm, L.; Krogsgaard-Larsen, P.; Johnston, G. A. R.; Schaumburg, K. X-Ray crystallographic and proton magnetic resonance spectroscopic investigations of nipecotic acid, a potent inhibitor of γ-aminobutyric acid (GABA) uptake. *Acta Chem. Scand.* 1976, B30, 542–548.
- (36) Lapuyade, G.; Schlewer, G.; N'Goka, V.; Vernières, J. C.; Chambon, J. P.; Lagrange, J.; Lagrange, P.; Wermuth, C. G. Dérivés alkylés et arylés de l'acide nipécotique: synthèse et appréciation de l'activité inhibitrice de la capture du GABA en fonction de paramètres conformationnels et de lipophilie. *Eur. J. Med. Chem.* 1987, 22, 383–391.
- (37) Krogsgaard-Larsen, P.; Fälch, E.; Larsson, O.; Schousboe, A. GABA Uptake Inhibitors: Relevance to Antiepileptic Drug Research. *Epilepsy Res.* 1987, 1, 77–93.

- (38) Schlewer, G.; Wermuth, C. G.; Chambon, J. P. Analogues tétrazoliques d'agents GABA mimétiques. *Eur. J. Med. Chem.* 1984, 19, 181–186.
- (39) Yungler, L. M.; Fowler, P. J.; Zarevics, P.; Setler, P. E. Novel inhibitors of γ-aminobutyric acid (GABA) uptake: Anticonvulsant actions in rats and mice. *J. Pharmacol. Exp. Ther.* 1984, 228, 109–115.
- (40) Groenvald, F. C.; Braestrup, C. Diheterocyclbutenylamino acid as GABA uptake inhibitors. Patent (Novo Industri A/S), PCT int. Appl. W.O. 8700,171 (Cl.C07D401/14), 15 Jan 1987.
- (41) Sonnewald, U. Preparation of heterocyclbutenyl pyridyl-carboxylates as GABA uptake inhibitors. Patent (Novo Industri A/S) Eur. Pat. 0231996/A3 (Cl 07D401/06), 12 Aug 1987.
- (42) Pavia, M. R. Preparation and formulation of *N*-substituted 3-piperidine- or 3-pyridine-carboxylic acid and their derivatives for the treatment of epilepsy. Warner-Lambert Company, Eur. Pat. E.P. 221,572 (Cl. C07 D211/60), 13 May 1987.

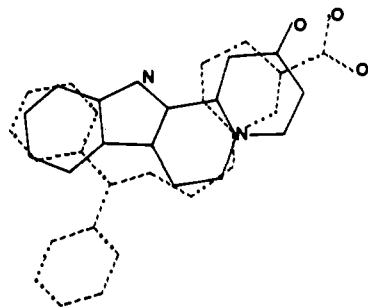


Figure 6. Superimposition of SKF 89976-A (8) in the same conformation as in Figure 5 with piperidone 21.

hydroisoxazopyridinol (THPO) 13 to give 14. These authors again noted an activity increase comparable to that observed for 8–10 and 12³⁷ (Figure 2). All the above results support the hypothesis of the existence of additional binding sites for the aromatic rings. However, the side-chain flexibility of these molecules allows a large number of energetically equivalent conformations. With the help of the SYBYL program⁴³ we searched the optimal spatial localization for the additional binding sites.

Two papers devoted to rigid GABA-uptake inhibitors were helpful for this study. The paper by Müller-Uri et al.⁴⁴ describes guvacine derivatives substituted by a benzhydrylidene group in the 5-position, 15–17 (compound 15 is also N-methylated) (Figure 3). Compound 15 shows activities similar to those of the unsubstituted guvacine.

The second paper, by Kardos et al.,⁴⁵ observed that the benzo[*a*]perhydroquinolizines 18 and 19 and the indolo-[3,2-*d*]perhydroquinolizines 20 and 21 have inhibitory activities similar to that of nipecotic acid (Figure 4). If we consider these published results as signifying GABA-uptake activity, they suggest that the presence of aromatic rings in the vicinity of the 6-position of the piperidine ring of nipecotic acid analogues is well tolerated by the GABA-uptake system.

On the contrary, substitution at the 2-position of the nipecotic acid skeleton seems to be detrimental. Thus, during earlier studies on GABA-uptake inhibitors, we observed that substituting nipecotic acid with a methyl or a phenyl group at the 2-position yielded practically inactive compounds.³⁸

Applied to 8 and 9 these observations suggest placement of the aromatic rings of the diphenylbutenyl side chain in the vicinity of position 6 rather than position 2 of the piperidinic ring.

Molecular modeling shows that there are unstrained conformations of 8 and 9 in which the phenyl rings have a partial overlap with the benzhydrylidene group of the guvacine derivative 15 when the amine functions and the carboxylic acid groups are overlaid (Figure 5). The lower activities observed for the benzhydrylidene guvacine derivative 15 in comparison with derivatives 8 and 9 can be attributed at least to two factors: (i) Compound 15 could only partially occupy the binding sites reached by the side chain of 8–10 and 12. (ii) The larger volume at the top of position 5 of compound 15 could be inappropriate with regard to the additional binding site prerequisites. The same unstrained conformation of the SKF compounds

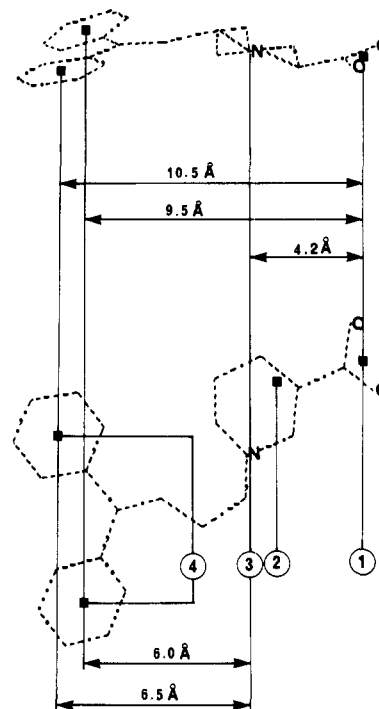


Figure 7. Parameters of the proposed pharmacophore for GABA-uptake inhibitors (---) SKF 89976-A (8) in its active conformation (see text for explanations).

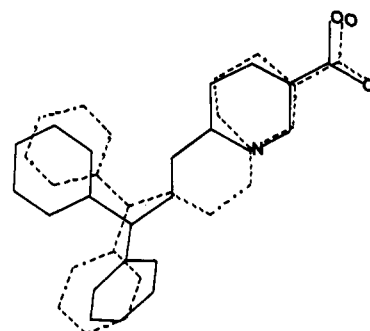


Figure 8. Fitting of 6-(3,3-diphenylpropyl)guvacine with the proposed pharmacophore.

8–10 and 12 also allowed a good overlap of one of their aromatic groups with the end of the aromatic region of the perhydroquinolizines 18–21 (Figure 6).

These observations, integrated with the recent findings of Wise⁴⁶ and Krogsgaard-Larsen,³⁷ allowed us to define important parameters associated with a possible GABA uptake inhibition pharmacophore (Figure 7):

1. The acidic group in position 3 of the piperidino ring should be situated in the pharmacophore mean plane.

2. A double bond in position 3,4 is often beneficial for the activity. Such an unsaturation also forces the carboxylic function into an orientation toward the general pharmacophore plane.

3. The amine function should be located at a distance of 4.2 Å from the center of the acidic group.

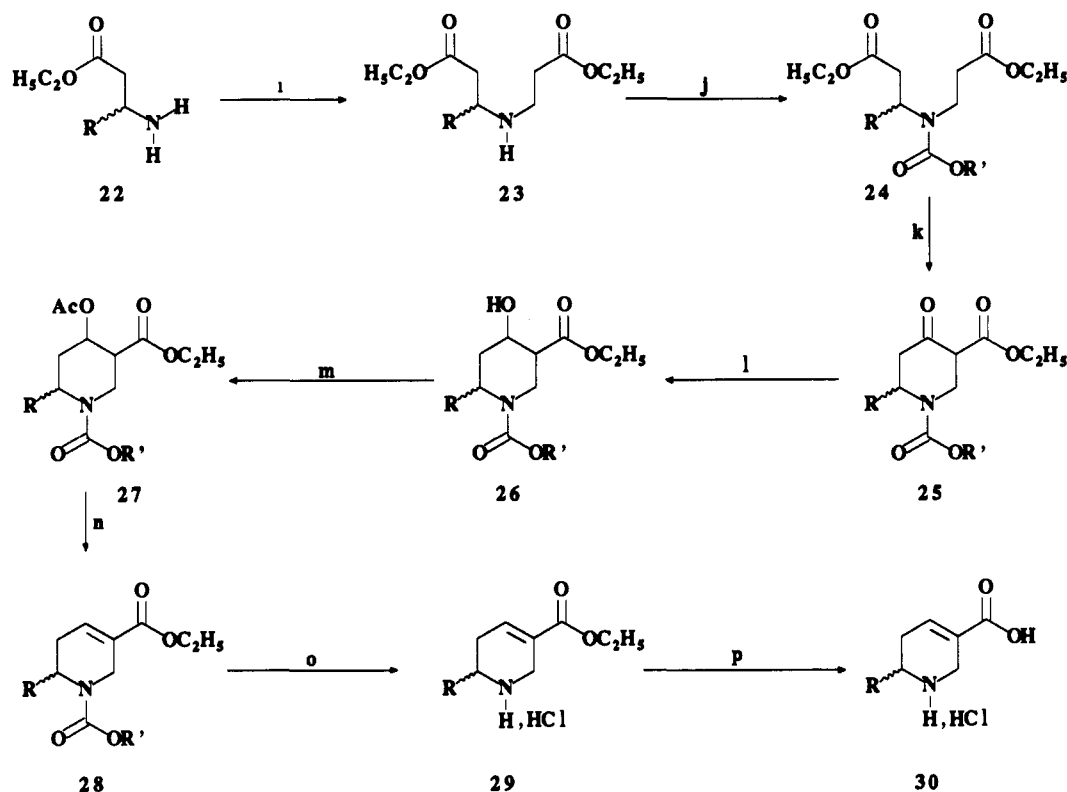
4. The two aromatic rings should occupy a region of space essentially coplanar with the piperidine average plane. The centers of these aromatic rings are then located 9.5 Å from the acidic center and 6.0 Å from the amino group for the first ring, and 10.5 Å from the acidic center and 6.5 Å from the amine for the second ring.

(43) SYBYL Program, Tripos Associates, St. Louis, MO 63117.

(44) Müller-Uri, C.; Singer, E. A.; Fleischhacker, W. Synthesis of Alkyl-Substituted Arecoline Derivatives as γ -Aminobutyric Acid Uptake Inhibitors. *J. Med. Chem.* 1986, 29, 125–132.

(45) Kardos, J.; Blaskó, G.; Simony, M.; Szántay, C. Octahydro-[2,3-*a*]quinolizin-2-one, a novel structure of γ -aminobutyric acid (GABA) uptake inhibitor. *Eur. J. Med. Chem.* 1986, 21, 151–154.

(46) Wise, M. Application of molecular graphics to the design of γ -aminobutyric acid uptake inhibitors. *Top. Mol. Pharmacol.* 1986, 3, 183–194.

Scheme I^{a,b}

^a (i) $\text{CH}_2=\text{CHCO}_2\text{Et}$, EtOH; (j) ClCO_2Et , K_2CO_3 , H_2O or $(\text{BOC})_2\text{O}$, K_2CO_3 , dioxane- H_2O ; (k) Na, EtOH, C_6H_6 ; (l) H_2 -Raney Ni EtOH; (m) $(\text{CH}_3\text{CO})_2\text{O}$; (n) DBN, THF; (o) HCl, EtOH; (p) HCl, H_2O . ^b R = (a) methyl, (b) ethyl, (c) propyl, (d) phenyl, (e) phenethyl, (f) 3,3-diphenylpropyl; R' = Et or tBu.

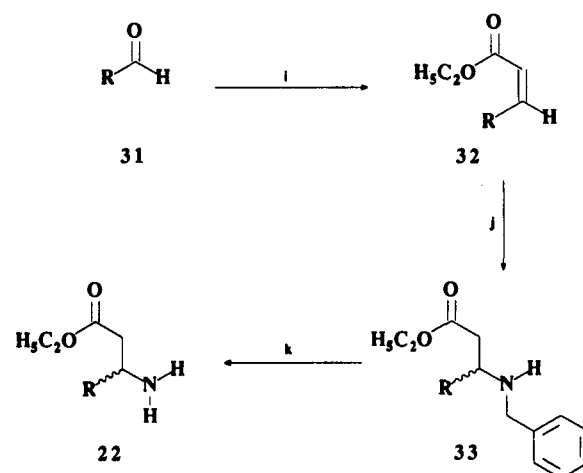
Predictive Value of the Pharmacophore Model

To confirm the real predictive value of our model, we prepared a series of guvacine derivatives in which the π binding sites of the pharmacophore were filled by substituting on position 6 of the guvacine ring (Table I). By adjustment of the parameters of our model pharmacophore and for synthetic reasons, simple hydrocarbon chains with different lengths and chains substituted with one or two phenyl groups were chosen. Molecular modeling suggested that a 3,3-diphenylpropyl side chain would represent a good fit with the pharmacophore (Figure 8), whereas a monophenylethyl side chain should give less potent derivatives comparable to the benzhydrylidene-guvacine 15 or the quinolizine derivatives 18–21.

Synthesis

The syntheses of the 6-substituted guvacine derivatives can be divided into two main parts: (1) the construction of the piperidino ring using appropriate β -substituted β -amino esters and (2) the preparation of the β -substituted β -amino esters. These latter compounds were prepared by a simple and general synthetic method starting from aldehydes. The starting aldehydes are generally commercially available except for β,β -diphenylbutanal, whose preparation will also be described here.

The piperidino ring construction starting from β -substituted β -amino esters was done by analogy with the work of Bishop⁴⁷ and Krogsgaard-Larsen.⁴⁸ The addition of

Scheme II^{a,b}

^a (i) $(\text{C}_6\text{H}_5)_3\text{P}^+\text{CH}_2\text{CO}_2\text{EtBr}^-$, C_6H_6 ; (j) $\text{C}_6\text{H}_5\text{CH}_2\text{NH}_2$, EtOH; (k) H_2 -Pd/C, EtOH. ^b R = (a) methyl, (b) ethyl, (c) propyl, (d) phenyl, (e) phenethyl, (f) 3,3-diphenylpropyl.

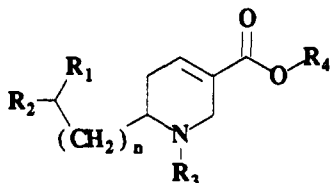
amino ester 22 to ethyl acrylate yielded diester 23. The amine function was then protected as a carbamate 24 by the action of ethyl chloroformate under the conditions reported by Krogsgaard-Larsen⁴⁸ or as a BOC derivative by reaction with di-*tert*-butyl pyrocarbonate, as described by Grzonka.⁴⁹ N-Protected diester 24 was cyclized to 3-carbethoxy-4-piperidone 25 by a selective Dieckmann reaction. The catalytic hydrogenation of piperidone 25 gave hydroxypiperidone 26. After acetylation of the hy-

(47) Bishop, M. J. Compounds related to arecoline. I Synthesis of 5-ethoxycarbonyl-1,2-dimethyl-1,2,3,6-tetrahydropyridine. *Z. Naturforsch.* 1970, 25B, 1248–1250.

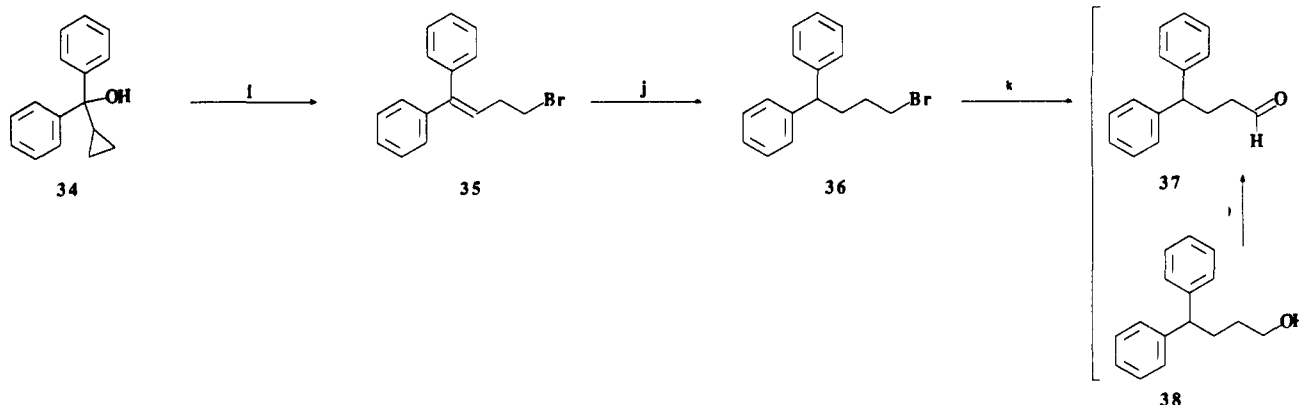
(48) Krogsgaard-Larsen, P.; Thyssen, K.; Schaumburg, K. Inhibitors of GABA uptake. Synthesis and ¹H NMR spectroscopic investigations of guvacine, (3RS, 4SR)-4-hydroxypiperidine-3-carboxylic acid and related compounds. *Acta Chem. Scand.* 1978, B32, 327–334.

(49) Grzonka, Z.; Liberek, B. Tetrazole analogs of amino-acids and peptides. I Preparation of tetrazole analogs of amino-acids from amino-acids. *Rocz. Chem.* 1971, 45, 967–980.

Table I. 6-Substituted Guvacine: Biochemical Data



R ₁	R ₂	R ₃	R ₄	n	no.	anal.	IC ₅₀ , μM
H	H	H	H	0	(37)		40
H	H	H	H	1	30b	C ₈ H ₁₃ NO ₂ ·HCl	>100
H	H	H	C ₂ H ₅	1	29b	C ₁₀ H ₁₇ NO ₂ ·HCl	>100
C ₆ H ₅	H	H	H	1	30e	C ₁₄ H ₁₇ NO ₂ ·HCl	100
H	H	H	H	2	30c	C ₉ H ₁₅ NO ₂ ·HCl	>100
H	H	H	C ₂ H ₅	2	29c	C ₁₁ H ₁₉ NO ₂ ·HCl	>100
C ₆ H ₅	C ₆ H ₅	H	H	2	30f	C ₂₁ H ₂₃ NO ₂ ·HCl	0.1
C ₆ H ₅	C ₆ H ₅	H	C ₂ H ₅	2	29f	C ₂₃ H ₂₇ NO ₂ ·HCl· ¹ / ₂ H ₂ O	37
C ₆ H ₅	H	H	H	3	30g	C ₁₆ H ₂₁ NO ₂ ·HCl	>100
C ₆ H ₅	H	CH ₃	H	3	39	C ₁₇ H ₂₃ NO ₂ ·HCl	>100

Scheme III^a

^a (i) HBr, H₂O; (j) H₂, 5% Pd/charcoal, EtOH; (k) DMSO, NaI, NaHCO₃; (l) DMSO, (CO)₂Cl₂, CH₂Cl₂.

droxy group, treatment of acetate 27 with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) created the conjugated double bond. Total deprotection using hydrochloric acid yielded the expected 6-substituted guvacine 30 (Scheme I). The synthesis described above (Scheme I) uses β-substituted β-amino esters as starting material. The published methods for the preparation of these amino esters are often very long and/or tedious as well as suffering from a lack of generality.⁵⁰⁻⁵⁵ We have found a simple and general method for the preparation of β-substituted β-amino esters in three steps starting from aldehydes^{48,56} (Scheme II).

The reaction of aldehydes 31 with (carbethoxymethyl)-triphenylphosphonium bromide according to Delmas and Lebigot⁵⁷ gave β-substituted unsaturated esters 32. The addition of benzylamine to conjugated ester 32 according to Morsch^{57,58} yielded N-benzylamino esters 33, which were submitted to hydrogenolysis to produce the expected β-amino esters 22.

4,4-Diphenylbutanal was prepared by starting from cyclopropyldiphenylmethanol (34). The action of hydrobromic acid upon this alcohol gave unsaturated bromide 35. Catalytic hydrogenation in the presence of palladium on charcoal gave 4,4-diphenyl-1-bromobutane (36), which was oxidized to aldehyde 37 via a Kornblum modified reaction.⁵⁹ The expected aldehyde was accompanied by 22% of the corresponding alcohol 38. This side product could also be oxidized into the expected aldehyde via a Swern reaction⁶⁰ (Scheme III).

- (50) Slopianka, M.; Gossauer, A. Thiocarbonyl olefination 4. Preparation of beta-amino-acids from N-acetyl thioamides. Total synthesis of iturinic acid. *Liebigs Ann. Chem.* 1981, 2258-2265.
- (51) Slopianka, M.; Gossauer, A. Thiocarbonyl olefination 3. A new synthesis of beta-amino-acid from thioacylurethanes. *Synth. Commun.* 1981, 11, 95-99.
- (52) Lamon, R. W. Synthesis of 4-thiouracils. *J. Heterocycl. Chem.* 1968, 5, 837-844.
- (53) Decombe, J. Acylacetic esters. *Ann. Chim. (Paris)* 1932, 18, 81-187.
- (54) Rodionov, V. M.; Postovskii, E. A. Mechanism of formation of β-aryl-β-amino fatty acids by the condensation of aromatic aldehydes with malonique acid and its derivatives. *J. Am. Chem. Soc.* 1929, 51, 841-847.
- (55) Johnson, T. B.; Livak, J. E. Pyrimidines. CXLIX. The synthesis of aryl-substituted dihydrouracils and their conversion into uracil derivatives. *J. Am. Chem. Soc.* 1936, 58, 299-303.
- (56) Delmas, M.; Le Bigot, Y. A simplified Wittig synthesis using solid/liquid transfer processes. *Synth. Commun.* 1981, 11, 125-132.

- (57) Morsch, K. Action of ammonia and amines on esters of unsaturated acids. I. Action of ammonia, methyl amine and diethylamine on ethyl crotonate. *Monatsh. Chem.* 1932, 60, 50-68.
- (58) McEvain, S. M.; Stork, G. Piperidine derivatives. XV. The preparation of 1-benzoyl-3-carbethoxy-4-piperidone. A synthesis of guvacine. *J. Am. Chem. Soc.* 1946, 68, 1049-1053.
- (59) Dave, P.; Byun, H. S.; Engel, R. An improved direct oxidation of alkylhalides to aldehydes. *Synth. Commun.* 1986, 16, 1343-1346.
- (60) Mancuso, A. J.; Huang, S. L.; Swern, D. Oxidation of Long-Chain and Related Alcohols to Carbonyls by Dimethyl Sulfoxide "Activated" by Oxalyl Chloride. *J. Org. Chem.* 1978, 43, 2480-2482.

Table II

compd	pK _{a1} ^a	pK _{a2} ^a	pK _a ^{a,b} ester	I/U ratio	log P _{oct} ^c	log P _{cyc} ^c	ΔP
30f	3.79	9.25	7.64	7000	0.71	-2.00	2.71
SKF 89976-A	3.57 ^d	9.23 ^d	7.36	6000	0.99 ^e	-0.43	1.43

^a Determined by potentiometric titration. ^b pK_a of the corresponding ester. ^c Measured by a conventional shake flask technique at room temperature. The concentrations of the compound in the aqueous phase (0.15 M phosphate buffer, pH 7.4) were determined spectrophotometrically. ^d pK_{a1} = 3.32 and pK_{a2} = 9.36.³⁶ ^e Log P_{oct} = 1.14.³⁶ All the values are the means of at least two determinations.

Biochemistry

The in vitro studies on GABA uptake were conducted by using rat brain synaptosome preparations.⁶¹ The IC₅₀ values were established by using concentration ranges up to 10⁻⁴ M in tested compound. The results of the in vitro tests are given in Table I.

Discussion

The results expressed in Table I demonstrate the predictive value of the proposed pharmacophore. In the guvacine series we had previously observed that position 6 tolerates a methyl substituent. However, replacing the methyl group with an ethyl or a propyl chain (30b and 30c) led to a loss of activity. Presumably the ethyl or propyl groups cannot occupy the π binding area to provide additional binding, and the net result is a loss of potency. However, the phenethyl derivative 30e showed some activity, presumably reflecting the ability of the phenyl to partially fill the π area. A further, important increase in potency was seen with the diphenylpropyl derivative 30f wherein the phenyl rings are quite ideally positioned to fill the π site. A further lengthening of the alkyl chain to give the phenylbutyl derivative 30g led to a loss of activity; this can be interpreted by assuming that the longer side chain interacts negatively with an area of bulk intolerance on the GABA-uptake carrier. Yunger et al. observed the same variations of activity related to alkyl chain length in their series of N-substituted nipecotic acids.³⁹

Compound 30f was inactive in classical in vivo screening models for anticonvulsants, particularly in antagonizing bicuculline-induced convulsions. It is generally accepted that, in the absence of a specific carrier, the rate and extent of entry of a compound into the brain are primarily related to its proton dissociation constant (K_a),⁶² partition coefficient P,^{63,64} and molecular size.⁶⁵ The inactivity in vivo of compound 30f in comparison to its N-substituted analogue SKF 89976-A cannot be attributed to a difference in molecular size; therefore, we measured the dissociation constants and the partition coefficients of both derivatives. As seen in Table II, very similar pK_a values are found for 30f and SKF 89976-A (8). The I/U ratios between the

zwitterionic (I) and the unionized (U) species, calculated according to Wegscheider,⁶⁶ do not furnish a satisfactory explanation for the in vivo inactivity of 30f, even if they are slightly in favor of compound 8.

The partition coefficients for 30f and 8 were then measured in two solvent systems: 1-octanol/phosphate buffer pH 7.4 (P_{oct}) and cyclohexane/phosphate buffer pH 7.4 (P_{cyc}). The P_{oct} coefficient accounts for protein binding in the bloodstream⁶⁷ whereas P_{cyc} accounts for the permeability through lipid membranes or for the partitioning process into nonpolar brain areas.⁶⁸ Thus, the difference between log P_{oct} and log P_{cyc}, defined as ΔP,⁶⁹ can be correlated with the logarithm of the brain/blood concentration ratio⁶⁹ and accounts for the overall hydrogen-binding ability of the drug.⁶⁹ High ΔP values limit permeability across the blood-brain barrier. As shown in Table II, the P_{oct} value of 30f is slightly lower than that of 8. The P_{cyc} of 30f shows, however, a value 40 times lower than that of 8, which gives a greater ΔP for 30f than for 8. This ratio corresponds to an insufficient brain/blood concentration ratio, thus accounting for the lack of in vivo activity.

Besides these physicochemical considerations, one cannot exclude the possibility of metabolic inactivation as another contribution to in vivo inactivity.

Experimental Section

Melting points were measured on a calibrated Kofler hot-stage apparatus and are uncorrected. IR spectra were measured with a Beckman Acculab-4 spectrophotometer, and ¹H NMR spectra were recorded on Bruker WP-60 or AC 200 spectrometers using the δ scale with reference to Me₄Si. All the new compounds gave satisfactory C,H,N analyses.

Ethyl N-(2-Carboxyethyl)-3-aminoalkanoates or -aralkanoates 23. General Procedure. A solution of 0.12 mol of the appropriate β-substituted β-amino ester 22 and 0.13 mol of ethyl acrylate in 200 mL of absolute ethanol was stirred overnight at room temperature. The solvent was evaporated under reduced pressure. Water (100 mL) was added to the residue and the solution acidified (pH 4) by addition of diluted hydrochloric acid. The aqueous phase was washed with ethyl acetate (2 × 200 mL), neutralized with a saturated solution of sodium hydrogen carbonate, and extracted with ethyl acetate (2 × 200 mL). The organic layer was washed with water, dried over magnesium sulfate, and evaporated to dryness. The product was pure enough to be used for the next step.

Ethyl N-(2-carboxyethyl)-3-aminohydrocinnamate (23d): starting from ethyl 3-aminohydrocinnamate (22d); yield, 90%; IR (CHCl₃) 1720 (C=O); ¹H NMR (60 MHz, CDCl₃) 1.22

- (61) Ramsay, P. B.; Krigman, M. E.; Morel, P. Developmental studies of the uptake of choline GABA and dopamine by crude synaptosomal preparations after in vivo or in vitro lead treatment. *Brain Res.* 1980, 187, 383-402.
- (62) Krosgaard-Larsen, P.; Falch, E.; Mikkelsen, H.; Jacobsen, P. Development of structural analogs and pro-drugs of GABA agonists with desirable pharmacokinetic properties. In *Optimization of drug delivery*; Alfred Benzon Symposium 17; Bundgaard, H., Hansen, A. B., Kofod, H., Eds.; Munksgaard: Copenhagen, 1982; pp 225-234.
- (63) Cornford, E. M.; Braun, L. D.; Oldendorf, W. H.; Hill, M. A. Comparison of lipid-mediated blood-brain-barrier penetrability in neonates and adults. *Am. J. Physiol.* 1982, 243, C161-C168.
- (64) Hansch, C.; Björkroth, J. P.; Leo, A. Hydrophobicity and central nervous system agents: On the principle of minimal hydrophobicity in drug design. *J. Pharm. Sci.* 1987, 76, 663-687.
- (65) Levin, V. A. Relationship of octanol/water partition and molecular weight to rat brain capillary permeability. *J. Med. Chem.* 1980, 23, 682-684.

- (66) Edsall, J. T.; Wyman, J. *Biophysical Chemistry*; Academic Press: New York, 1958; Vol. 1, pp 485-486.
- (67) Vandebelt, J. M.; Hantsch, C.; Church, C. Binding of Apolar Molecules by Serum Albumin. *J. Med. Chem.* 1972, 15, 787-789.
- (68) Young, R. C.; Mitchell, R. C.; Brown, T. C.; Ganellin, R. C.; Griffiths, R.; Jones, M.; Rana, K. K.; Saunders, D.; Smith, I. R.; Sore, E. S.; Wilks, T. J. Development of a new physicochemical model for brain penetration and its application to the design of centrally acting H₂ receptor histamine antagonists. *J. Med. Chem.* 1988, 31, 656-671.
- (69) Seiler, P. Interconversion of lipophilicities from hydrocarbon/water systems into the octanol/water system. *Eur. J. Med. Chem. (Chim. Ther.)* 1974, 9, 473-479.

(t, $J = 6.8$, 3 H, OCH_2CH_3), 1.24 (t, $J = 6.8$, 3 H, OCH_2CH_3), 2.0–3.0 [m, 7 H, containing at 2.14 (br s, exchangeable with D_2O , 1 H, NH), 6 H, $\text{O}=\text{CCH}_2\text{CH}_2$, $\text{O}=\text{CCH}_2$], 3.9–4.5 [m, 5 H, containing at 4.12 (g, $J = 6.8$, 2 H, OCH_2CH_3), at 4.14 (q, $J = 6.8$, 2 H, OCH_2CH_3), 1 H, $\text{O}=\text{CCH}_2\text{CH}$], 7.1–7.6 (m, 5 H, C_6H_5).

Ethyl *N*-(2-carbethoxyethyl)-3-amino-5-phenylvalerate (23e): starting from ethyl 3-amino-5-phenylvalerate (22e); yield, 88%; IR (CHCl_3) 1720 (C=O); $^1\text{H NMR}$ (60 MHz, CDCl_3) 1.24 (t, $J = 6.8$, 6 H, 2 OCH_2CH_3), 1.57 (br s, exchangeable with D_2O , 1 H, NH), 1.6–2.0 (m, 2 H, $\text{C}_6\text{H}_5\text{CH}_2\text{CH}_2$), 2.3–3.1 (m, 9 H, $\text{O}=\text{CCH}_2\text{CHNHCH}_2\text{CH}_2\text{C}=\text{O}$, $\text{CH}_2\text{C}_6\text{H}_5$), 4.12 (q, $J = 6.8$, 4 H, 2 OCH_2CH_3), 7.21 (s, 5 H, C_6H_5).

Ethyl *N*-(2-carbethoxyethyl)-3-amino-6,6-diphenylcaproate (23f): starting from ethyl 3-amino-6,6-phenylcaproate (22f); yield, 95%; IR (CHCl_3) 1725 (C=O); $^1\text{H NMR}$ (200 MHz, CDCl_3) 1.21 (t, $J = 6.8$, 3 H, OCH_2CH_3), 1.23 (t, $J = 6.8$, 3 H, OCH_2CH_3), 1.3–1.5 (m, 2 H, $\text{CH}_2\text{CH}_2\text{CHNH}$), 1.59 (br s, exchangeable with D_2O , 1 H, NH), 2.0–2.2 (m, 2 H, $\text{CHCH}_2\text{CH}_2\text{CHNH}$), 2.37 (d, $J = 6.8$, 2 H, $\text{O}=\text{CCH}_2\text{CH}$), 2.43 (t, $J = 6.8$, 2 H, $\text{O}=\text{CCH}_2\text{CH}_2$), 2.78 (t, $J = 6.8$, 2 H, $\text{O}=\text{CCH}_2\text{CH}_2\text{NH}$), 3.00 (qu, $J = 6.8$, 1 H, CH_2CHCH_2), 3.85 (t, $J = 6.8$, 1 H, $\text{C}_6\text{H}_5\text{CH}(\text{C}_6\text{H}_5)\text{CH}_2$), 4.08 (q, $J = 6.8$, 2 H, OCH_2CH_3), 4.10 (q, $J = 6.8$, 2 H, OCH_2CH_3), 7.0–7.4 (m, 10 H, 2 C_6H_5).

Ethyl *N*-(*tert*-Butyloxycarbonyl)-*N*-(2-carbethoxyethyl)-3-aminoalkanoates or -aralkanoates 24. General Procedure. A mixture containing 0.1 mol of a secondary amine 23, 54 mL of dioxane, 108 mL of water, and 54 mL of 5% potassium carbonate solution was cooled in an ice bath. Di-*tert*-butyl pyrocarbonate (0.13 mL) was added slowly with stirring. The stirring was maintained for 15 min at 0 °C and then continued at room temperature for 3 h more. After concentration under reduced pressure, the residue was extracted with ether (2 × 200 mL). The ethereal phase was washed with 1 N hydrochloric acid (50 mL) and water and dried over magnesium sulfate. After evaporation of the solvent, the crude product was pure enough to be used in the next step. The product was crystallized in a mixture of hexane–ethyl acetate. The yield was >95%.

Ethyl *N*-(*tert*-butyloxycarbonyl)-*N*-(2-carbethoxyethyl)-3-aminovalerate (24b): IR (CHCl_3) 1725 (C=O ester), 1680 (C=O carbamate); $^1\text{H NMR}$ (200 MHz, CDCl_3) 0.87 (t, $J = 6.8$, 3 H, CHCH_2CH_3), 1.24 (t, $J = 6.8$, 3 H, OCH_2CH_3), 1.25 (t, $J = 6.8$, 3 H, OCH_2CH_3), 1.4–1.8 [m, 11 H, containing at 1.45 and 1.51 (2 s, 9 H, $(\text{CH}_3)_3\text{C}$), 2 H, CHCH_2CH_3], 2.3–2.7 (m, 4 H, $\text{O}=\text{CCH}_2$, $\text{O}=\text{CCH}_2$), 3.3–3.7 (m, 3 H, $\text{CH}_2\text{CH}_2\text{N}$, CH_2CHCH_2), 4.12 (q, $J = 6.8$, 2 H, OCH_2CH_3), 4.13 (q, $J = 6.8$, 2 H, OCH_2CH_3).

Ethyl *N*-(*tert*-butyloxycarbonyl)-*N*-(2-carbethoxyethyl)-3-aminocaproate (24c): IR (CHCl_3) 1725 (C=O ester), 1680 (C=O carbamate); $^1\text{H NMR}$ (60 MHz, CDCl_3) 0.7–1.7 [m, 22 H, containing at 1.26 (t, $J = 6.8$, 6 H, 2 OCH_2CH_3), at 1.46 (s, 9 H, $(\text{CH}_3)_3\text{C}$), 7 H, $\text{CHCH}_2\text{CH}_2\text{CH}_3$], 2.3–2.8 (m, 4 H, $\text{O}=\text{CCH}_2$, $\text{O}=\text{CCH}_2\text{CH}$), 3.2–3.7 (m, 3 H, $\text{O}=\text{CCH}_2\text{CH}_2\text{N}$, CH_2CHCH_2), 4.13 (q, $J = 6.8$, 4 H, 2 OCH_2CH_3).

Ethyl *N*-(*tert*-butyloxycarbonyl)-*N*-(2-carbethoxyethyl)-3-amino-5-phenylvalerate (24e): IR (CHCl_3) 1730 (C=O ester), 1685 (C=O carbamate); $^1\text{H NMR}$ (60 MHz, CDCl_3) 1.24 (t, $J = 6.8$, 6 H, 2 OCH_2CH_3), 1.46 (s, 9 H, $(\text{CH}_3)_3\text{C}$), 1.6–2.2 (m, 2 H, $\text{CHCH}_2\text{CH}_2\text{C}_6\text{H}_5$), 2.2–2.7 (m, 6 H, $\text{CH}_2\text{C}_6\text{H}_5$, $\text{O}=\text{CCH}_2\text{CH}_2$, $\text{O}=\text{CCH}_2\text{CH}$), 3.2–3.7 (m, 2 H, NCH_2CH_2), 3.7–4.4 [m, 5 H, containing at 4.13 (q, $J = 6.8$, 4 H, 2 OCH_2CH_3), 1 H, CH_2CHCH_2], 7.22 (s, 5 H, C_6H_5).

Ethyl *N*-(*tert*-butyloxycarbonyl)-*N*-(2-carbethoxyethyl)-3-amino-6,6-diphenylcaproate (24f): IR (CHCl_3) 1725 (C=O ester), 1680 (C=O carbamate); $^1\text{H NMR}$ (200 MHz, CDCl_3) 1.20 (t, $J = 6.8$, 3 H, OCH_2CH_3), 1.23 (t, $J = 6.8$, 3 H, OCH_2CH_3), 1.42 and 1.52 (2 s, 9 H, $(\text{CH}_3)_3\text{C}$), 2.00 (q, $J = 6.8$, 2 H, $\text{CHCH}_2\text{CH}_2\text{CHN}$), 2.3–2.6 (m, 4 H, $\text{O}=\text{CCH}_2\text{CH}_2$, $\text{O}=\text{CCH}_2\text{CH}$), 3.2–3.6 [m, 4 H, containing at 3.47 (q, $J = 6.8$, 2 H, $\text{CHCH}_2\text{CH}_2\text{CHN}$) 2 H, $\text{O}=\text{CCH}_2\text{CH}_2\text{N}$], 3.8–4.5 [m, 6 H, containing at 4.09 (q, $J = 6.8$, 4 H, 2 OCH_2CH_3), 1 H, $\text{C}_6\text{H}_5\text{CHCH}_2$, and 1 H, CH_2CHCH_2], 7.0–7.4 (m, 10 H, 2 C_6H_5).

Ethyl *N*-Carbethoxy-*N*-(2-carbethoxyethyl)-3-aminoalkanoates 24. General Procedure. A 50% solution of secondary amine 23 in water was cooled at 0 °C. A 5% potassium carbonate solution (1.1 equiv) was cooled and added with vigorous stirring. Ethyl chloroformate (1.1 equiv) was added, and stirring was maintained for 30 min at 0 °C and 1 h at room temperature.

After the same workup as for ethyl *N*-(*tert*-butyloxycarbonyl)-*N*-(2-carbethoxyethyl)-3-aminoalkanoate, the ethyl carbamate was obtained with a yield greater than 90%.

Ethyl *N*-carbethoxy-*N*-(2-carbethoxyethyl)-3-amino-butyrates (24a): IR (CHCl_3) 1725 (C=O ester), 1680 (C=O carbamate); $^1\text{H NMR}$ (60 MHz, CDCl_3) 0.8–1.4 [m, 12 H, containing at 1.24 (t, $J = 6.8$, 3 H, OCH_2CH_3), at 1.26 (t, $J = 6.8$, 6 H, 2 OCH_2CH_3), 1.26 (d, $J = 6.8$, 3 H, CHCH_3), 2.5–2.8 (m, 4 H, $\text{O}=\text{CCH}_2\text{CH}$, $\text{O}=\text{CCH}_2\text{CH}_2$), 3.4–3.9 (m, 2 H, $\text{O}=\text{CCH}_2\text{CH}_2\text{N}$), 4.0–4.3 [m, 7 H, containing at 4.14 (q, $J = 6.8$, 2 H, OCH_2CH_3), at 4.16 (q, $J = 6.8$, 4 H, 2 OCH_2CH_3), 1 H, CH_3CHCH_2].

Ethyl *N*-carbethoxy-*N*-(2-carbethoxyethyl)-3-amino-hydrocinnamate (24d): IR (CHCl_3) 1725 (C=O ester), 1680 (C=O carbamate); $^1\text{H NMR}$ (200 MHz, CDCl_3) 1.1–1.4 [m, 9 H, containing at 1.21 and 1.22 (2 t, $J = 7.5$, 6 H, 2 OCH_2CH_3), at 1.27 (t, $J = 6.8$, 3 H, OCH_2CH_3), 2.0–2.6 (m, 2 H, $\text{O}=\text{CCH}_2\text{CH}_2$), 2.8–3.1 (d, $J = 6.8$, 2 H, $\text{O}=\text{CCH}_2\text{CH}$), 3.2–3.5 (m, 2 H, CH_2N), 3.9–4.3 [m, 7 H, containing at 4.06 (q, $J = 7.5$, 4 H, 2 OCH_2CH_3), 4.18 (q, $J = 6.8$, 2 H, OCH_2CH_3), 1 H, CH_2CHCH_2], 7.32 (br s, 5 H, C_6H_5).

***N*-Carbalkoxy-2-alkyl- or -2-aralkyl-5-carbethoxy-4-piperidones 25. General Procedure.** A mixture of 0.095 mol of diester 24, 0.096 g-atom of sodium, and 0.2 mL of absolute ethanol in 200 mL of dry benzene was stirred at room temperature for 24–48 h. The benzene phase was washed with 100 mL of 1 N hydrochloric acid and dried over potassium carbonate. The solvents were evaporated under reduced pressure, and the crude product was purified by silica gel column chromatography with a mixture of hexane–ethyl acetate, 9/1, as eluent.

***N*-Carbethoxy-2-methyl-5-carbethoxy-4-piperidone (25a):** yield, 90%; IR (CHCl_3) 1720 (C=O ester), 1690 (C=O carbamate), 1620 (C=O); $^1\text{H NMR}$ (60 MHz, CDCl_3) 1.14 (d, $J = 6.8$, 3 H, CHCH_3), 1.25 (t, $J = 6.8$, 3 H, OCH_2CH_3), 1.26 (t, $J = 6.8$, 3 H, OCH_2CH_3), 1.5–3.8 (m, 4 H, $\text{O}=\text{CCH}_2\text{CH}$, CHCH_2N), 3.9–5.0 [m, 5 H, containing at 4.12 (q, $J = 6.8$, 2 H, OCH_2CH_3), at 4.20 (q, $J = 6.8$, 2 H, OCH_2CH_3), 1 H, CH_2CHCH_3], 12.00 and 12.27 (2 s, exchangeable with D_2O , 1 H, OH).

***N*-(*tert*-Butyloxycarbonyl)-2-ethyl-5-carbethoxy-4-piperidone (25b):** yield, 91%; IR (CHCl_3) 1725 (C=O ester), 1700 (C=O ketone), 1680 (C=O carbamate), 1650 (C=C); $^1\text{H NMR}$ (200 MHz, CDCl_3) 0.8–1.0 (m, 3 H, CHCH_2CH_3), 1.2–1.9 [m, 14 H, containing at 1.27 (t, $J = 6.8$, 3 H, OCH_2CH_3), at 1.45 (s, 9 H, $(\text{CH}_3)_3\text{C}$), 2 H, CHCH_2CH_3], 2.3–2.8 (m, 2 H, $\text{O}=\text{CCH}_2$), 3.2–3.8 (m, 2 H, CH_2N), 4.0–5.2 [m, 3 H, containing at 4.20 (q, $J = 6.8$, 2 H, OCH_2CH_3), 1 H, CH_2CHCH_2], 12.04 and 12.23 (2 s, exchangeable with D_2O , 1 H, OH).

***N*-(*tert*-Butyloxycarbonyl)-2-propyl-5-carbethoxy-4-piperidone (25c):** yield, 85%; IR (CHCl_3) 1620–1700 (C=O ester, ketone, carbamate; C=C enol); $^1\text{H NMR}$ (60 MHz, CDCl_3) 0.8–1.8 [m, 19 H, containing at 1.30 (t, 6.8, 3 H, OCH_2CH_3), at 1.47 (s, 9 H, $(\text{CH}_3)_3\text{C}$), 7 H, $\text{CHCH}_2\text{CH}_2\text{CH}_3$], 2.0–3.8 (m, 4 H, CH_2N , $\text{O}=\text{CCH}_2$), 4.0–5.0 [m, 3 H, containing at 4.25 (q, $J = 6.8$, 2 H, OCH_2CH_3), 1 H, CH_2CHCH_2], 11.90 and 12.10 (2 s, exchangeable with D_2O , 1 H, OH).

***N*-(*tert*-Butyloxycarbonyl)-2-phenethyl-5-carbethoxy-4-piperidone (25e):** yield, 80%; IR (CHCl_3) 1620–1730 (C=O, ester, ketone, carbamate; C=C, enol); $^1\text{H NMR}$ (200 MHz, CDCl_3) 1.1–1.6 [m, 12 H, containing at 1.30 (t, $J = 6.8$, 3 H, OCH_2CH_3), at 1.44 (s, 9 H, $(\text{CH}_3)_3\text{C}$), 1.6–1.8 (m, 2 H, CHCH_2CH_2), 2.2–2.8 (m, 4 H, CHCH_2CH_2 , $\text{O}=\text{CCH}_2\text{CH}$), 3.3–3.8 (m, 2 H, CH_2N), 4.0–5.2 [m, 3 H, containing at 4.28 (q, $J = 6.8$, 2 H, OCH_2CH_3), 1 H, CH_2CHCH_2], 7.0–7.3 (m, 5 H, C_6H_5), 12.06 and 12.36 (2 s, exchangeable with D_2O , 1 H, OH).

***N*-(*tert*-Butyloxycarbonyl)-2-(3,3-diphenylpropyl)-5-carbethoxy-4-piperidone (25f):** yield, 87%; IR (CHCl_3) 1620–1730 (C=O, ester, ketone, carbamate; C=C, enol); $^1\text{H NMR}$ (200 MHz, CDCl_3) 1.0–1.6 [m, 14 H, containing at 1.25 and 1.26 (2 t, $J = 6.8$, 3 H, OCH_2CH_3), at 1.42 and 1.47 (2 s, 9 H, $(\text{CH}_3)_3\text{C}$), 2 H, $\text{CHCH}_2\text{CH}_2\text{CHN}$], 1.9–3.1 (m, 4 H, $\text{O}=\text{CCH}_2\text{CHCH}_2\text{CH}_2\text{CH}$), 3.3–5.0 [m, 5 H, containing at 4.15 and 4.16 (2 q, $J = 6.8$, 2 H, OCH_2CH_3), 3 H, CH_2N , $\text{C}_6\text{H}_5\text{CHCH}_2$], 7.1–7.4 (m, 10 H, 2 C_6H_5), 11.98 and 12.26 (2 s, exchangeable with D_2O , 1 H, OH).

***N*-Carbalkoxy-2-alkyl- or -2-aralkyl-5-carbethoxy-4-piperidinols 26. General Procedure.** A 20% ethanolic solution

of a piperidone 25 was hydrogenated at 80 °C under 60 atm in the presence of 20% Raney nickel. The cooled mixture was filtered through a Celite pad. Complete reaction was indicated by a negative enol test using ferric chloride. The solvent was evaporated under reduced pressure. The infrared spectrum did not show ketone or enol absorption but only the ester carbonyl band at 1720 cm^{-1} and the carbamate carbonyl band at 1685 cm^{-1} . The ^1H NMR spectra, measured at 200 MHz, were very complex due to the presence of diastereoisomers and could not be interpreted.

4-Acetoxy-N-carbalkoxy-2-alkyl- or -2-aralkyl-5-carb-ethoxy-5-piperidines 27. General Procedure. A 10% solution of the appropriate piperidinol 25 in acetic anhydride was refluxed overnight. After evaporation under reduced pressure, water was added to the residue. The aqueous solution was alkalized by addition of saturated sodium hydrogen carbonate and extracted with ethyl acetate. The organic layer was washed with water and dried over magnesium sulfate. After evaporation of the solvents under reduced pressure, the crude product was purified by silica gel column chromatography with a mixture of hexane-ethyl acetate, 2/1, as eluent. The IR spectra showed the esters and carbamate bonds at 1735 and 1685 cm^{-1} . The ^1H NMR spectra, measured at 200 MHz, were too complex to be interpreted.

N-Carbalkoxy-2-alkyl- or -2-aralkyl-5-carb-ethoxy-1,2,3,6-tetrahydropyridines 28. General Procedure. A 10% solution of an acetate 27 in freshly distilled THF was stirred at room temperature for 2-3 h in the presence of 1 equiv of 1,5-diazabicyclo[4.3.0]non-5-ene (DBN). The solvent was evaporated and water added to the residue. The aqueous solution was acidified with 1 N hydrochloric acid and extracted with ethyl ether. The organic layer was washed with water and dried over magnesium sulfate. The crude product obtained after evaporation of the solvent was purified by silica gel column chromatography with a mixture of hexane-ethyl acetate, 1/1, as eluent.

N-Carb-ethoxy-2-methyl-5-carb-ethoxy-1,2,3,6-tetrahydropyridine (28a): yield, 94%; IR (CHCl_3) 1700 ($\text{C}=\text{O}$), 1690 ($\text{C}=\text{C}$); ^1H NMR (60 MHz, CDCl_3) 0.9-1.5 (m, 9 H, CHCH_3 , 2 OCH_2CH_3), 2.0-3.0 (m, 2 H, $\text{C}=\text{CHCH}_2$), 3.4-5.0 [m, 7 H, containing at 4.03 (q, $J = 6.8$, 2 H, OCH_2CH_3), at 4.07 (q, $J = 6.8$, 2 H, OCH_2CH_3), 6.8-7.2 (m, 1 H, $\text{CH}_2\text{CH}=\text{C}$).

N-(tert-Butyloxycarbonyl)-2-ethyl-5-carb-ethoxy-1,2,3,6-tetrahydropyridine (28b): yield, 80%; IR (CHCl_3) 1710 ($\text{C}=\text{O}$ ester), 1680 ($\text{C}=\text{O}$ carbamate), 1650 ($\text{C}=\text{C}$); ^1H NMR (60 MHz, CDCl_3) 0.6-1.6 [m, 17 H, containing at 0.90 (t, $J = 6.8$, 3 H, CHCH_2CH_3), at 1.30 (t, $J = 6.8$, 3 H, OCH_2CH_3), at 1.45 (s, 9 H, $(\text{CH}_3)_3\text{C}$), 2 H, CHCH_2CH_3], 2.0-2.6 (m, 2 H, $\text{C}=\text{CHCH}_2$), 3.2-3.8 (m, 2 H, CH_2N), 4.0-4.8 [m, 3 H, containing at 4.20 (q, $J = 6.8$, 2 H, OCH_2CH_3), 1 H, CH_2CHCH_2], 6.8-7.2 (m, 1 H, $\text{CH}_2\text{CH}=\text{C}$).

N-(tert-Butyloxycarbonyl)-2-propyl-5-carb-ethoxy-1,2,3,6-tetrahydropyridine (28c): yield, 80%; IR (CHCl_3) 1705 ($\text{C}=\text{O}$ ester), 1675 ($\text{C}=\text{O}$ carbamate), 1640 ($\text{C}=\text{C}$); ^1H NMR (60 MHz, CDCl_3) 0.7-1.7 [m, 19 H, containing at 1.29 (t, $J = 6.8$, 3 H, OCH_2CH_3), at 1.46 (s, 9 H, $(\text{CH}_3)_3\text{C}$), 7 H, $\text{CHCH}_2\text{CH}_2\text{CH}_3$], 2.0-2.6 (m, 2 H, $\text{C}=\text{CHCH}_2$), 3.2-3.8 (m, 2 H, CH_2N), 4.0-4.8 [m, 3 H, containing at 4.21 (q, $J = 6.8$, 2 H, OCH_2CH_3), 1 H, CH_2CHCH_2], 6.8-7.2 (m, 1 H, $\text{CH}_2\text{CH}=\text{C}$).

N-(tert-Butyloxycarbonyl)-2-phenethyl-5-carb-ethoxy-1,2,3,6-tetrahydropyridine (28e): yield, 75%; IR (CHCl_3) 1705 ($\text{C}=\text{O}$ ester), 1680 ($\text{C}=\text{O}$ carbamate), 1650 ($\text{C}=\text{C}$); ^1H NMR (60 MHz, CDCl_3) 0.7-3.0 [m, 18 H, containing at 1.29 (t, $J = 6.8$, 3 H, OCH_2CH_3), at 1.47 (s, 9 H, $(\text{CH}_3)_3\text{C}$), at 1.59 (t, $J = 6.8$, 2 H, $\text{CH}_2\text{CH}_2\text{C}_6\text{H}_5$), 4 H, $-\text{CHCH}_2\text{CHCH}_2$], 3.2-3.8 (m, 2 H, CH_2N), 4.0-4.8 [m, 3 H, containing at 4.20 (q, $J = 6.8$, 2 H, OCH_2CH_3), 1 H, CH_2CCH_2], 7.0-7.3 (m, 1 H, $\text{C}=\text{CHCH}_2$).

N-(tert-Butyloxycarbonyl)-2-(3,3-diphenylpropyl)-5-carb-ethoxy-1,2,3,6-tetrahydropyridine (28f): yield, 90%; IR (CHCl_3) 1705-1680 ($\text{C}=\text{O}$, $\text{C}=\text{C}$); ^1H NMR (200 MHz, CDCl_3) 0.7-1.8 [m, 16 H, containing at 1.26 (t, $J = 6.8$, 3 H, OCH_2CH_3), at 1.46 (s, 9 H, $(\text{CH}_3)_3\text{C}$), 4 H, $\text{CHCH}_2\text{CH}_2\text{CHN}$] 1.8-2.6 (m, 2 H, $\text{C}=\text{CHCH}_2$), 3.3-4.5 (m, 2 H, CH_2N), 3.87 (t, $J = 6.8$, 1 H, $(\text{C}_6\text{H}_5)_2\text{CHCH}_2$), 4.2-4.7 [m, 3 H, containing at 4.20 (q, $J = 6.8$, 2 H, OCH_2CH_3), 1 H, CH_2CHCH_2], 6.8-7.0 (m, 1 H, $\text{C}=\text{CHCH}_2$), 7.1-7.4 (m, 10 H, 2 C_6H_5).

2-Alkyl- or -2-aralkyl-5-carb-ethoxy-1,2,3,6-tetrahydropyridine Hydrochlorides 29. General Procedure. A protected amine 28 as a 20% solution in absolute ethanol was treated with

an equal volume of a saturated solution of hydrogen chloride in ethanol. The solution was heated under reflux for 2 h. Evaporation of the solvent under reduced pressure and crystallization with a mixture of 2-propanol and ethyl ether gave the expected hydrochlorides.

2-Ethyl-5-carb-ethoxy-1,2,3,6-tetrahydropyridine hydrochloride (29b): yield, 60%; mp 189 °C; IR (KBr) 1725 ($\text{C}=\text{O}$), 1660 ($\text{C}=\text{C}$); ^1H NMR (200 MHz, D_2O) 1.03 (t, $J = 6.8$, 3 H, CHCH_2CH_3), 1.30 and 1.31 (2 t, $J = 6.8$, 3 H, OCH_2CH_3), 1.6-1.9 (m, 2 H, CHCH_2CH_3), 2.40 (ddq, $J = 20.0$, $J = 9.5$, $J = 3.0$, 1 H, H_{3a}), 2.75 (dt, $J = 20.0$, $J = 3.0$, 1 H, H_{3a}), 3.3-3.5 (m, 1 H, H_2), 3.9-4.0 (m, 2 H, containing the AB corresponding to H_{6a} and H_{6b}), 4.27 and 4.28 (2 q, $J = 6.8$, 2 H, OCH_2CH_3), 7.0-7.3 (m, 1 H, H_4).

2-Propyl-5-carb-ethoxy-1,2,3,6-tetrahydropyridine hydrochloride (29c): yield, 64%; mp 132.8 °C; IR (KBr) 1725 ($\text{C}=\text{O}$), 1660 ($\text{C}=\text{C}$); ^1H NMR (200 MHz, D_2O) 0.95 (t, $J = 6.8$, 3 H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 1.30 (t, $J = 6.8$, 3 H, OCH_2CH_3), 1.45 (q, $J = 6.8$, 2 H, $\text{CHCH}_2\text{CH}_2\text{CH}_3$), 1.70 (sext, $J = 6.8$, 2 H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 2.40 (ddq, $J = 20.0$, $J = 9.5$, $J = 3.0$, 1 H, H_{3a}), 2.75 (dt, $J = 20.0$, $J = 3.0$, 1 H, H_{3a}), 3.3-3.5 (m, 1 H, H_2), 3.8-4.1 (m, 2 H, H_{6a} , H_{6b}), 4.27 (q, $J = 6.8$, OCH_2CH_3), 7.0-7.3 (m, 1 H, H_4).

2-(3,3-Diphenylpropyl)-5-carb-ethoxy-1,2,3,6-tetrahydropyridine hydrochloride (29f): yield, 58%; mp 114 °C; IR (KBr) 1725 ($\text{C}=\text{O}$), 1660 ($\text{C}=\text{C}$); ^1H NMR (200 MHz, D_2O) 1.29 (t, $J = 6.8$, 3 H, OCH_2CH_3), 1.5-1.9 (m, 2 H, $\text{CHCH}_2\text{CH}_2\text{CHN}$), 2.0-2.6 (m, 3 H, H_{3a} , $\text{CHCH}_2\text{CH}_2\text{CHN}$), 2.75 (dt, $J = 20.0$, $J = 3.0$, H_{3a}), 3.3-3.6 (m, 1 H, H_2), 3.6-4.2 [m, 3 H, containing at 4.07 (t, $J = 6.8$, 1 H, $\text{C}_6\text{H}_5\text{CHCH}_2$), 2 H, H_{6a} , H_{6b}], 4.24 (q, $J = 6.8$, 2 H, OCH_2CH_3), 7.0-7.3 (m, 1 H, H_4), 7.3-7.5 (m, 10 H, 2 C_6H_5).

6-Alkyl- or -6-aralkyl-1,2,5,6-tetrahydropyridine-3-carboxylic Acid Hydrochlorides 30. General Procedure. A 3 N hydrochloric acid solution containing ester 28 or 29 was refluxed for 3 h. After evaporation of the solvent under reduced pressure, the crude product was recrystallized in a mixture of 2-propanol and ethyl ether.

6-Ethyl-1,2,5,6-tetrahydropyridine-3-carboxylic acid hydrochloride (6-ethylguvacine hydrochloride) (30b): yield, 46%; mp 218 °C dec; IR (KBr) 1725 ($\text{C}=\text{O}$), 1660 ($\text{C}=\text{C}$); ^1H NMR (200 MHz, D_2O) 1.01 (t, $J = 6.8$, 3 H, CHCH_2CH_3), 1.6-1.9 (m, 2 H, CHCH_2CH_3), 2.40 (ddq, $J = 20.0$, $J = 9.5$, $J = 3.0$, 1 H, H_{5a}), 2.72 (dt, $J = 20$, $J = 3.0$, 1 H, H_{5a}), 3.2-3.4 (m, 1 H, H_6), 3.7-4.1 (m, 2 H, H_{2a} and H_{2b}), 7.0-7.3 (m, 1 H, H_4).

6-Propyl-1,2,5,6-tetrahydropyridine-3-carboxylic acid hydrochloride (6-propylguvacine hydrochloride) (30c): yield, 50%; mp 220-222 °C dec; IR (KBr) 1720 ($\text{C}=\text{O}$), 1665 ($\text{C}=\text{C}$); ^1H NMR (200 MHz, D_2O) 0.93 (t, $J = 6.8$, 3 H, CH_2CH_3), 1.45 (q, $J = 6.8$, 2 H, $\text{CHCH}_2\text{CH}_2\text{CH}_3$), 1.70 (sext, $J = 6.8$, 2 H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 2.36 (ddq, $J = 20.0$, $J = 9.5$, $J = 3.0$, 1 H, H_{5a}), 2.70 (dt, $J = 20.0$, $J = 3.0$, 1 H, H_{5a}), 3.3-3.6 (m, 1 H, H_6), 3.6-4.2 (m, 2 H, H_{2a} and H_{2b}), 7.0-7.4 (m, 1 H, H_4).

6-Phenethyl-1,2,5,6-tetrahydropyridine-3-carboxylic acid hydrochloride (6-phenethylguvacine hydrochloride) (30e): yield, 63%; mp 220 °C; IR (KBr) 1700 ($\text{C}=\text{O}$), 1650 ($\text{C}=\text{C}$); ^1H NMR (200 MHz, D_2O) 1.9-2.3 (m, 2 H, $\text{CH}_2\text{CH}_2\text{CH}$), 2.3-3.0 [m, 4 H, containing at 2.45 (ddq, $J = 20.0$, $J = 9.5$, $J = 3.0$, 1 H, H_{5a}), $\text{CH}_2\text{CH}_2\text{CH}$], 3.2-3.5 (m, 1.4 H, 0.8 H_2 and 0.6 H_{6a}), 3.6-4.1 (m, 1.2 H, H_{2a} and H_{2b}), 4.30 (dd, $J = 7.5$, $J = 1.5$, 0.4 H_{6a}), 7.0-7.2 (m, 1 H, H_4), 7.2-7.5 (m, 5 H, C_6H_5).

6-(3,3-Diphenylpropyl)-1,2,5,6-tetrahydropyridine-3-carboxylic acid hydrochloride [6-(3,3-diphenylpropyl)guvacine hydrochloride] (30f): yield, 70%; mp 263 °C; IR (KBr) 1710 ($\text{C}=\text{O}$), 1655 ($\text{C}=\text{C}$); ^1H NMR (200 MHz, D_2O) 1.5-2.0 (m, 2 H, $\text{CHCH}_2\text{CH}_2\text{CHN}$), 2.1-2.7 (m, 4 H, $\text{CHCH}_2\text{CH}_2\text{CHN}$, H_{5a} , H_{5b}), 3.2-3.5 (m, 1.4 H, 0.8 H_2 and 0.6 H_{6a}), 3.6-4.2 [m, 2.2 H, containing at 4.00 and 4.01 (2 t, $J = 6.8$, 1 H, $\text{C}_6\text{H}_5\text{CHCH}_2$), 1.2 H, H_{2a} and H_{2b}], 4.3-4.4 (dd, $J = 7.5$, $J = 1.5$, 0.4 H_{6a}), 6.8-7.1 (m, 1 H, H_4), 7.2-7.4 (m, 10 H, 2 C_6H_5).

α,β -Unsaturated Esters 32. General Procedure. A flask containing 1 L of benzene, 0.9 mol of aldehyde 31, 0.9 mol of triphenyl(carb-ethoxymethyl)phosphonium bromide, and 0.92 mol of crushed potassium hydroxide was heated at 40 °C for 2 h with stirring. The mixture was filtered, and the filtrate was evaporated under reduced pressure. The residue was taken up in 500 mL of hexane, slightly heated with stirring, and filtered again. The filtrate was cleaned by filtration through silica gel rinsed with a mixture of hexane-ethyl acetate, 1/3. The evaporation of the

solvent yielded the expected compounds.

Ethyl 2-pentenoate (32b):⁷⁰ yield, 60%; IR (CHCl₃) 1705 (C=O), 1655 (C=C); ¹H NMR (60 MHz, CDCl₃) 1.05 (t, *J* = 6.8, 3 H, CCH₂CH₃), 1.25 (t, *J* = 6.8, 3 H, OCH₂CH₃), 2.15 (q, *J* = 6.8, 2 H, CCH₂CH₃), 4.18 (q, *J* = 6.8, 2 H, OCH₂CH₃), 5.80 (dt, *J* = 15.0, *J* = 1.5, 1 H, O=CCH=CH trans), 7.02 (dt, *J* = 15.0, *J* = 6.8, 1 H, O=CCH=CHCH₂ trans).

Ethyl 2-hexenoate (32c):⁷¹ yield, 65%; IR (CHCl₃) 1705 (C=O), 1655 (C=C); ¹H NMR (60 MHz, CDCl₃) 0.7–2.5 [m, 7 H, containing at 1.28 (t, *J* = 6.8, 3 H, OCH₂CH₃), 4 H, CH₃CH₂CH₂CH], 4.24 (q, *J* = 6.8, 2 H, OCH₂CH₃), 5.75 (dt, *J* = 15.0, *J* = 1.5, 1 H, O=CCH=CH), 7.00 (dt, *J* = 15.0, *J* = 6.8, 1 H, O=CCH=CHCH₂).

Ethyl 5-phenyl-2-pentenoate (32e): yield, 80%; IR (CHCl₃) 1715 (C=O), 1660 (C=C); ¹H NMR (60 MHz, CDCl₃) 1.28 (t, *J* = 6.8, 3 H, OCH₂CH₃), 2.3–3.0 (m, 4 H, CH₂CH₂), 4.18 (q, *J* = 6.8, 2 H, OCH₂CH₃), 5.83 (dt, *J* = 15.0, *J* = 1.5, 1 H, O=CCH=CH), 7.07 (dt, *J* = 15.0, *J* = 1.5, 1 H, O=CCH=CHCH₂), 7.24 (s, 5 H, C₆H₅).

Ethyl 6,6-diphenyl-2-hexenoate (32f): yield, 85%; IR (CHCl₃) 1710 (C=O), 1660 (C=C); ¹H NMR (200 MHz, CDCl₃) 1.26 (t, *J* = 6.8, 3 H, OCH₂CH₃), 2.0–2.2 (m, 4 H, CHCH₂CH₂CH=), 3.92 (t, *J* = 6.8, 1 H, CHCH₂), 4.15 (q, *J* = 6.8, 2 H, OCH₂CH₃), 5.80 (dt, *J* = 15.0, *J* = 1.5, 1 H, O=CCH=CH), 7.03 (dt, *J* = 15.0, *J* = 6.8, 1 H, O=CCH=CHCH₂), 7.0–7.4 (m, 10 H, 2 C₆H₅).

Ethyl 3-(Benzylamino)alkanoates or -aralkanoates 33. General Procedure. A solution of 0.4 mol of the α,β -unsaturated ester 32 and 0.5 mol of freshly distilled benzylamine in 150 mL of absolute ethanol was heated at reflux for 48 h. After evaporation of the solvent under reduced pressure, the product was purified by silica gel column chromatography with a mixture of hexane-ethyl acetate, 1/1, as eluent.

Ethyl 3-(benzylamino)butyrate (33a): yield, 80%; IR (CHCl₃) 1720 (C=O); ¹H NMR (60 MHz, CDCl₃) 1.15 (d, *J* = 6.8, 3 H, CHCH₃), 1.24 (t, *J* = 6.8, 3 H, OCH₂CH₃), 1.60 (br s, exchangeable with D₂O, 1 H, NH), 2.40 (dd, *J* = 6.8, *J* = 1.5, 2 H, O=CCH₂CH), 2.8–3.4 (m, 1 H, CH₂CHCH₃), 3.80 (s, 2 H, NHCH₂C₆H₅), 4.12 (q, *J* = 6.8, 2 H, OCH₂CH₃), 7.30 (s, 5 H, C₆H₅).

Ethyl 3-(benzylamino)valerate (33b): yield, 40%; IR (CHCl₃) 1725 (C=O); ¹H NMR (60 MHz, CDCl₃) 0.6–1.8 [m, 9 H, containing at 0.91 (t, *J* = 6.8, 3 H, CHCH₂CH₃), at 1.24 (t, *J* = 6.8, 3 H, OCH₂CH₃), at 1.49 (q, *J* = 6.8, 2 H, CHCH₂CH₃), at 1.57 (br s, exchangeable with D₂O, 1 H, NH)], 2.43 (d, *J* = 6.0, 2 H, O=CCH₂C₆H₅), 2.97 (qu, *J* = 6.0, 1 H, CH₂CHCH₃), 3.77 (s, 2 H, CH₂C₆H₅), 4.18 (q, *J* = 6.8, 2 H, OCH₂CH₃), 7.29 (s, 5 H, C₆H₅).

Ethyl 3-(benzylamino)caproate (33c): yield, 55%; IR (CHCl₃) 1725 (C=O); ¹H NMR (60 MHz, CDCl₃) 0.6–1.6 [m, 11 H, containing at 1.24 (t, *J* = 6.8, 3 H, OCH₂CH₃), at 1.51 (br s, exchangeable with D₂O, 1 H, NH) 7 H, CHCH₂CH₂CH₃], 2.43 (d, *J* = 6.0, 2 H, CHCH₂C=O), 2.8–3.2 (m, 1 H, CH₂CHCH₃), 3.77 (s, 2 H, CH₂C₆H₅), 4.13 (q, *J* = 6.8, 2 H, OCH₂CH₃), 7.29 (s, 5 H, C₆H₅).

Ethyl 3-(benzylamino)-5-phenylvalerate (33e): yield, 50%; IR (CHCl₃) 1720 (C=O); ¹H NMR (60 MHz, CDCl₃) 1.24 (t, *J* = 6.8, 3 H, OCH₂CH₃), 1.5–2.0 (m, 3 H, CH₂CH₂CHNH), 2.3–3.3 (m, 5 H, CH₂CH₂CHCH₂C=O), 3.77 (s, 2 H, NCH₂C₆H₅), 4.12 (q, *J* = 6.8, 2 H, OCH₂CH₃), 7.21 (s, 5 H, C₆H₅), 7.30 (s, 5 H, C₆H₅).

Ethyl 3-(benzylamino)-6,6-diphenylcaproate (33f): yield, 62%; IR (CHCl₃) 1725 (C=O); ¹H NMR (200 MHz, CDCl₃) 1.20 (t, *J* = 6.8, 3 H, OCH₂CH₃), 1.45 (q, *J* = 6.8, 2 H, CHCH₂CH₂CHNH), 1.58 (br s, exchangeable with D₂O, 1 H, NH), 2.10 (q, *J* = 6.8, 2 H, CHCH₂CH₂CHNH), 2.42 (d, *J* = 6.8, 2 H, O=CCH₂CH), 3.04 (qu, *J* = 6.8, 1 H, CH₂CHCH₃), 3.68 (s, 2 H, CH₂C₆H₅), 3.83 (t, *J* = 6.8, 1 H, C₆H₅CHCH₂), 4.08 (q, *J* = 6.8, 2 H, OCH₂CH₃), 7.0–7.4 (m, 10 H, 2 C₆H₅).

Ethyl 3-Aminoalkanoates or -aralkanoates 22. General Procedure. An ethanolic solution of the benzylamino derivative 33 was hydrogenated under a pressure of 4 atm for 3 h in the presence of 5% Pd/C. After filtration of the catalyst through a Celite pad, the solvents were evaporated under reduced pressure.

The resulting residue was chromatographed through a silica gel column with ethyl acetate as eluent. The yield of the free secondary amine was generally around 95%.

Ethyl 3-aminobutyrate (22a):⁷² IR (CHCl₃) 1725 (C=O); ¹H NMR (60 MHz, CDCl₃) 1.12 (d, *J* = 6.8, 3 H, CHCH₃), 1.27 (t, *J* = 6.8, 3 H, OCH₂CH₃), 1.73 (br s, exchangeable with D₂O, 2 H, NH₂), 2.2–2.5 (m, 2 H, O=CCH₂CH), 3.1–3.8 (m, 1 H, CH₂CHCH₂), 4.16 (q, *J* = 6.8, 2 H, OCH₂CH₃).

Ethyl 3-aminocaproate (22c):⁷³ IR (CHCl₃) 1725 (C=O); ¹H NMR (60 MHz, CDCl₃) 0.7–1.7 [m, 12 H, containing at 1.26 (t, *J* = 6.8, 3 H, OCH₂CH₃), at 1.47 (br s, exchangeable with D₂O, 2 H, NH₂), 7 H, CHCH₂CH₂CH₃], 2.2–2.5 (m, 2 H, O=CCH₂CH), 3.0–3.8 (m, 1 H, CHCH₂CH₂), 4.14 (q, *J* = 6.8, 2 H, OCH₂CH₃).

Ethyl 3-amino-6,6-diphenylcaproate (22f): IR (CHCl₃) 1725 (C=O); ¹H NMR (200 MHz, CDCl₃) 1.22 (t, *J* = 6.8, 3 H, OCH₂CH₃), 1.3–1.5 (m, 2 H, CHCH₂CH₂CHNH₂), 1.65 (br s, exchangeable with D₂O, 2 H, NH₂), 1.8–2.8 (m, 4 H, O=CCH₂CHCH₂CH₂CH), 3.0–3.4 (m, 1 H, CH₂CHCH₃), 3.87 (t, *J* = 6.8, 1 H, C₆H₅CH₂CH₂), 4.12 (q, *J* = 6.8, 2 H, OCH₂CH₃), 7.0–7.4 (m, 10 H, 2 C₆H₅).

4,4-Diphenylbutanal (37). Method A. A mixture containing 90 g (0.31 mol) of bromide 36, 600 mL of DMSO, 67.5 g (0.46 mol) of sodium iodide, and 77.8 g (0.93 mol) of sodium hydrogen carbonate was heated at 110 °C with stirring for 2 h. After cooling, water was added (200 mL) and the product was extracted with ethyl ether. The ethereal phase was dried over magnesium sulfate, and the solvents were vaporated under reduced pressure. Aldehyde 37 was purified by silica gel column chromatography with a mixture of hexane-ethyl acetate, 3/1, as eluent. The yield of aldehyde was 60%: IR (CHCl₃) 1720 (C=O); ¹H NMR (200 MHz, CDCl₃) 2.3–2.5 (m, 4 H, CH₂CH₂), 3.92 (t, *J* = 6.8, 1 H, C₆H₅CHCH₂), 7.0–7.4 (m, 10 H, C₆H₅), 9.73 (s, 1 H, O=CH).

Method B. A flask equipped with a mechanical stirrer and an argon intake, containing 325 mL of dichloromethane and 13 mL (0.15 mol) of oxalyl chloride, was cooled at –60 °C. DMSO (23 mL, 0.30 mol) in 68 mL of dichloromethane was slowly added. After 3 min, a solution of 30 g (0.14 mol) of alcohol 38 in 150 mL of dichloromethane was added during 5 min. After 15 min of stirring, triethylamine (95.5 mL, 0.68 mol) was added. The stirring was maintained for 5 min at –60 °C, and then the reaction mixture was allowed to warm to room temperature. Water was added (750 mL), and the organic layer was separated. The aqueous phase was extracted again with dichloromethane. The organic phases were combined, washed with water and brine, and dried over magnesium sulfate. The solvents were evaporated under reduced pressure, and the crude aldehyde was purified by silica gel column chromatography with ethyl acetate as eluent. The yield of pure aldehyde was 95%.

4,4-Diphenylbutanol (38). This compound was obtained as a side product (22%) in the preparation of 4,4-diphenylbutanal (37) using method A described above: ¹H NMR (200 MHz, CDCl₃) 1.35 (br s, exchangeable with D₂O, 1 H, OH), 1.53 (qu, *J* = 6.8, 2 H, CH₂CH₂OH), 2.14 (q, *J* = 6.8, 2 H, CHCH₂CH₂), 3.65 (t, *J* = 6.8, 2 H, CH₂OH), 3.93 (t, *J* = 6.8, 2 H, C₆H₅CHCH₂), 7.0–7.4 (m, 10 H, 2 C₆H₅).

[³H]GABA Synaptosomal Uptake. Experiments were slightly modified from Ramsey et al.⁶¹ Rats were killed by decapitation, and corpora striata were rapidly dissected. Tissues were pooled and homogenized in 20 volumes of 0.32 M sucrose on a Potter Elvehjem tissue grinder. Homogenates were centrifuged at 1000g at 4 °C for 15 min. The supernatant was centrifuged at 20000g at 4 °C, and the resulting pellet was resuspended in cold 0.32 M sucrose.

Incubation was carried out at 37 °C for 2 min in glass tubes containing 50 μ L of synaptosomes (1 mg of protein), 750 μ L of pH 7.4 Krebs-Ringer phosphate buffer supplemented with NaCl (0.15 M), and 100 μ L of [³H]GABA (25–40 Ci/mmol, New England Nuclear, Boston, MA), in a final concentration of 1.1 μ mol, and 100 μ L of compound to be tested. Blanks were treated identically

(70) La Combe, E. M.; Stewart, B. A new reaction of allylic sulfones. *J. Am. Chem. Soc.* 1961, 83, 3457–3461.

(71) Arens, J. F.; Tjakob, H. J.; Viergege, H. α,β -Unsaturated carbonyl compounds. Organon Inc., U.S. Pat. 3,030,359, 17 April 1962.

(72) Biemann, K.; Seibl, J.; Gapp, F. Mass spectra of organic molecules. I. Ethyl esters of amino acids. *J. Am. Chem. Soc.* 1961, 83, 3795–3801.

(73) Testa, E.; Fontanella, L.; Aresi, V. Weitere Untersuchungen über die 2-substituierten Azetidine. *Justus Liebigs Ann. Chem.* 1964, 673, 60.

except that NaCl was not added in the incubation medium. Uptake was determined by dilution with 5 mL of incubation medium without NaCl. Samples were centrifuged at 20000g at 4 °C for 15 min, and radioactivity was evaluated in pellets after dilution in 1 mL of Proposol (New England Nuclear).

Acknowledgment. We thank Marlyse Wernert for typing this manuscript and Alain Jacob for his help during his stay in the laboratory. Also Professor B. Spiess for measuring the pK_a 's and Dr. Robert Kerr for manuscript corrections.

Registry No. 1, 56-12-2; 2, 5130-17-6; 3, 102629-73-2; 4, 102629-74-3; 5, 498-95-3; 6, 68464-00-6; 7, 498-96-4; 8, 85375-85-5; 9, 85375-88-8; 10, 134525-28-3; 11, 89203-64-5; 12, 89203-55-4; 13, 53602-00-9; 14, 132033-96-6; 15, 98943-12-5; 16, 98976-55-7; 17,

134420-65-8; 18, 71622-29-2; 19, 71622-34-9; 20, 134455-45-1; 21, 1217-82-9; 22a, 5303-65-1; 22c, 59663-70-6; 22d, 10039-64-2; 22e, 134420-70-5; 22f, 134420-71-6; 23b, 134420-67-0; 23e, 134420-72-7; 23f, 134420-73-8; 24a, 95629-08-6; 24b, 134420-74-9; 24c, 134420-75-0; 24d, 134420-76-1; 24e, 134420-77-2; 24f, 134420-78-3; 25a, 116140-33-1; 25b, 134420-79-4; 25c, 134420-80-7; 25e, 134420-81-8; 25f, 134420-82-9; 28a, 116140-36-4; 28b, 134420-83-0; 28c, 134420-84-1; 28e, 134420-85-2; 28f, 134420-86-3; 29b, 134420-68-1; 29c, 134420-87-4; 29f, 134420-88-5; 30b, 134420-69-2; 30c, 134420-89-6; 30e, 134420-90-9; 30f, 134420-91-0; 32b, 24410-84-2; 32c, 1552-67-6; 32e, 6048-08-4; 32f, 134420-92-1; 33a, 6335-80-4; 33b, 134420-93-2; 33c, 134455-46-2; 33e, 134420-94-3; 33f, 134420-95-4; cyclopropyldiphenylmethanol, 5785-66-0; 4,4-diphenylbutanol, 56740-71-7; ethyl acrylate, 140-88-5; triphenyl(carbethoxymethyl)phosphonium bromide, 1530-45-6; benzylamine, 100-46-9.

3-Thienyl- and 3-Furylaminobutyric Acids. Synthesis and Binding GABA_B Receptor Studies¹

Pascal Berthelot,[†] Claude Vaccher,[†] Nathalie Flouquet,[†] Michel Debaert,^{*†} Michel Luyckx,[†] and Claude Brunet[†]

Laboratoires de Pharmacie Chimique et de Pharmacodynamie, Faculté de Pharmacie, 3, rue du Professeur Laguesse, 59045 Lille Cedex, France. Received December 27, 1990

Baclofen (β -(*p*-chlorophenyl)-GABA) is a selective agonist for the bicuculline-insensitive GABA_B receptor. The search for new compounds that bind to the GABA_B receptor is very important to clarify structural requirements. We report herein the synthesis and the binding studies of variously substituted 3-thienyl- and 3-furylaminobutyric acids. 4-Amino-3-(5-methyl-2-thienyl)butyric acid (**5d**) and 4-amino-3-(5-chloro-2-thienyl)butyric acid (**5h**) are potent and specific ligands for GABA_B receptor. The IC₅₀ values for the displacement of (*R*)-(-)-[³H]baclofen are 1.34 and 0.61 μ M for **5d** and **5h**, respectively, as compared to 0.33 μ M for baclofen.

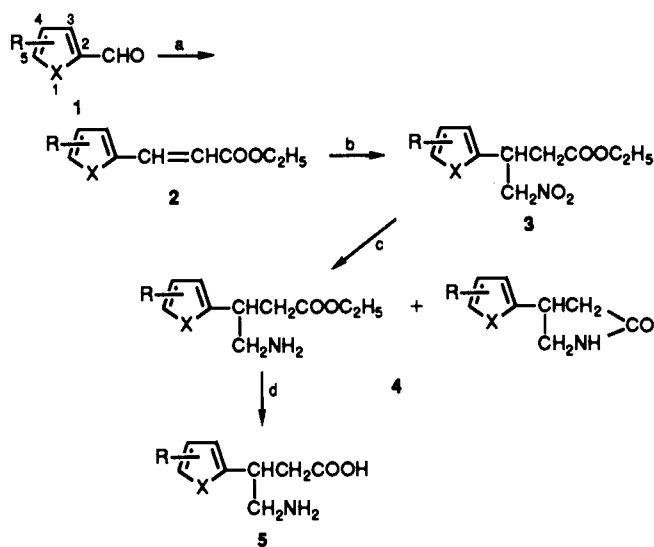
The neutral amino acid γ -aminobutyric acid (GABA) is an inhibitory neurotransmitter concerned with the control of neuronal activity in the mammalian central nervous system and with the regulation of many physiological mechanisms.² Within the central and peripheral nervous system, GABA has been shown to act through at least two distinctly different receptor sites,³ termed GABA_A and GABA_B receptors, with different binding properties.^{4,5} Accumulating evidence suggests that GABA_B receptors are predominantly located presynaptically.⁶ However, in a recent report, postsynaptically located GABA_B receptor have been described.⁷ GABA_B receptors have also been detected and characterized in a variety of tissue preparations of peripheral origin.⁸

Until now, β -(*p*-chlorophenyl)-GABA (baclofen) was the selective agonist for the GABA_B receptor. Analogues of baclofen, saturated or unsaturated, have been synthesized and tested for GABA_B receptor affinity. These compounds showed no selective action at GABA_B receptor sites in vitro.⁹ The only new agonist available is (3-amino-propyl)phosphonic acid, which is a potent displacer of baclofen in binding studies.¹⁰ However, this compound can act as a partial agonist under certain conditions.¹¹

In recent papers, the phosphonic analogue of baclofen (phaclofen) and two sulfonic analogues (saclofen and 2-hydroxysaclofen) have been shown to be antagonists at GABA_B receptors.^{12,13} We recently described the synthesis of 3-(benzo[*b*]furan-2-yl)-GABA, new selective ligands of GABA_B sites,¹⁴ which are specific GABA_B receptor antagonists.^{15,16}

In the course of our work and in attempts to elucidate the structural requirements for access to the GABA_B receptor,¹⁴ we report the synthesis and the binding studies

Scheme I^a



	R	X
a	H	O
b	(CH ₃) ₅	O
c	H	S
d	(CH ₃) ₅	S
e	(CH ₃) ₃	S
f	(Br) ₅	S
g	(Br) ₄	S
h	(Cl) ₅	S
i	(Cl) ₄	S
j	H	S
	and (GABA chain) ₃	

^a Reagents: (a) (C₆H₅)₂PCHCOOC₂H₅/C₆H₆/reflux; (b) CH₃NO₂/Triton B/85 °C; (c) Raney Ni/H₂; (d) NaOH/EtOH/reflux.

of new 3-heteroaryl-GABA analogues. These racemic compounds, especially **5d** and **5h**, are potent and specific

[†]Laboratoire de Pharmacie Chimique.

[†]Laboratoire de Pharmacodynamie.