Synthesis of Novel GABA Uptake Inhibitors. 3. Diaryloxime and Diarylvinyl Ether Derivatives of Nipecotic Acid and Guvacine as Anticonvulsant Agents1

ARTICLE *in* JOURNAL OF MEDICINAL CHEMISTRY · AUGUST 1999

Impact Factor: 5.45 · DOI: 10.1021/jm981027k

Articles

Synthesis of Novel GABA Uptake Inhibitors. 3. Diaryloxime and Diarylvinyl Ether Derivatives of Nipecotic Acid and Guvacine as Anticonvulsant Agents[1]

Lars J. S. Knutsen,* Knud Erik Andersen, Jesper Lau, Behrend F. Lundt, Rodger F. Henry,[‡] Howard E. Morton,[‡] Lars Nærum, Hans Petersen,[§] Henrik Stephensen, Peter D. Suzdak,[∥] Michael D. B. Swedberg,[⊥] Christian Thomsen, and Per O. Sørensen[▽]

Health Care Discovery and Development, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Måløv, Denmark

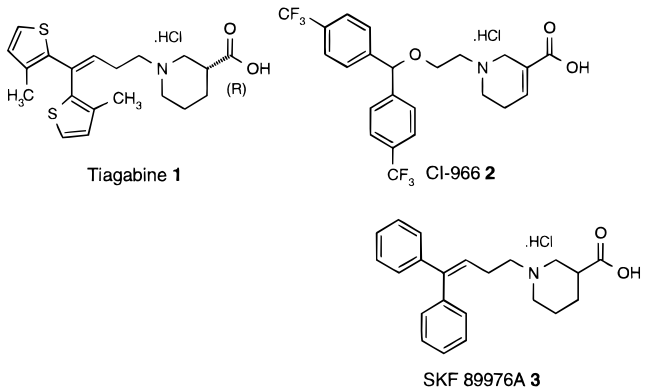
Received April 21, 1998

(3*R*)-1-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-3-piperidinecarboxylic acid **1** (tiagabine, Gabitril) is a potent and selective γ-aminobutyric acid (GABA) uptake inhibitor with proven anticonvulsant efficacy in humans. This drug, which has a unique mechanism of action among marketed anticonvulsant agents, has been launched for add-on treatment of partial seizures with or without secondary generalization in patients >12 years of age. Using this new agent as a benchmark, we have designed two series of novel GABA uptake inhibitors of remarkable potency, using a putative new model of ligand interaction at the GABA transporter type 1 (GAT-1) uptake site. This model involves the postulated interaction of an electronegative region in the GABA uptake inhibitor with a positively charged domain in the protein structure of the GAT-1 site. These two novel series of anticonvulsant agents contain diaryloxime or diarylvinyl ether functionalities linked to cyclic amino acid moieties and were derived utilizing the new model, via a series of design steps from the known 4,4-diarylbutenyl GABA uptake inhibitors. The new compounds are potent inhibitors of [^{3}H]-GABA uptake in rat brain synaptosomes in vitro, and their antiepileptic potential was demonstrated in vivo by their ability to protect against seizures induced by the benzodiazepine receptor inverse agonist methyl 4-ethyl-6,7-dimethoxy-β-carboline-3-carboxylate (DMCM) in mice. From structure−activity studies of these new GABA uptake inhibitors, we have shown that insertion of an ether oxygen in conjugation with the double bond in tiagabine (K_i = 67 nM) improves in vitro potency by 5-fold to 14 nM.

Introduction

γ-Aminobutyric acid (GABA) is recognized as the principal brain inhibitory neurotransmitter,[2] and this amino acid is intrinsically involved in the mechanism of action of a number of marketed and development stage anticonvulsant drugs.[3−9] Two cyclic amino acid derivatives which act via inhibition of the uptake of GABA in the central nervous system (CNS) have been investigated in human clinical trials. These are (3*R*)-1-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-3-piperidinecarboxylic acid[10,11] **1** (tiagabine, NNC 05-0328) and 1-[2-[bis[4-(trifluoromethyl)phenyl]methoxy]ethyl]-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid (CI-966)[12−16] **2** (Chart 1). In preclinical rodent models of epilepsy, both of these relatively lipophilic amino acid derivatives exhibit a promising seizure protection profile, with ED_{50} values of 2.6 and 3.0 mg/kg, respectively, following intraperitoneal (i.p.) dosing in amygdala kindled rats.[17] Tiagabine has now been more extensively investigated.[18−24]

Chart 1. Structures of the Main Reference GABA Uptake Inhibitors



Evidence is mounting that tiagabine, now a marketed drug, is acting centrally as an inhibitor of GABA uptake modulating the removal of GABA from the synaptic cleft,[24,25] and clinical findings indicating effectiveness as an add-on treatment of partial seizures with or without secondary generalization[26−28] have recently been summarized.[29] Tiagabine appears to show some

* Current address for correspondence: Lars J. S. Knutsen, Ph.D., Cerebrus Limited, Oakdene Court, Winnersh, WOKINGHAM, Berkshire, RG41 5UA, U.K. Tel: +44-(0)-118-989-9303. Fax: +44-(0)-118-989-9300. E-mail: L.Knutsen@Cerebrus.ltd.uk.

‡ Abbott Laboratories, 1401 Sheridan Road, North Chicago, IL 60064-4000.

§ Current address: H. Lundbeck A/S, Ottiliavej 9, DK-2500 Copenhagen - Valby, Denmark.

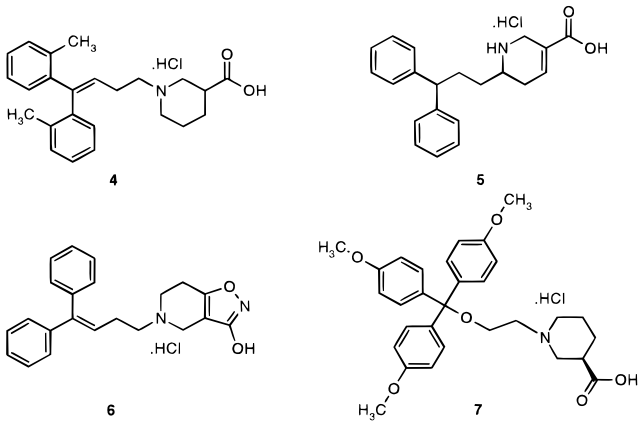
∥ Current address: Guilford Pharmaceuticals, 6611 Tributary Street, Baltimore, MD 21224.

⊥ Current address: Astra Pain Control, Department of Pharmacology, S-15185 Sodertalje, Sweden.

▽ P.O.S. has now changed name to Per O. Huusfeldt.

10.1021/jm981027k CCC: $18.00
Published on Web 08/05/1999

Chart 2. Structures of Other GABA Uptake Inhibitors



clinical advantages over current epilepsy therapy, particularly in terms of safety and side-effect profile.[30−34] The observations[24] from the preclinical animal models have therefore been borne out.

At the outset of our investigations in the GABA uptake and/or re-uptake field only one structural type of CNS-active GABA uptake inhibitor was known, illustrated by 1-(4,4-diphenyl-3-butenyl)-3-piperidinecarboxylic acid[35−38] **3** (SKF 89976A, Chart 1). This diphenylbutenyl derivative formed the starting point for the design of **1**, but simple isosteric replacement of phenyl with thiophene is not the whole explanation behind the improved activity of **1** when compared to **3**. We found subsequently that the presence of the two "ortho" methyl groups in **1** is very significant in progressing to drugs of higher potency than **3**. Correspondingly, the introduction of "ortho" methyl groups in the diaryl moiety of **3**, giving the novel diarylbutene **4** (Chart 2), increases in vitro potency 5-fold compared to **3**, when compared in Fjalland's assay[39] measuring inhibition of [^{3}H]-GABA uptake in vitro.

The 4,4-diarylbutenyl structural class was followed chronologically by diarylmethoxyethyl derivatives[10,13−15,40] of which **2** is a representative. More recently, N'Goka et al.[41] have prepared some structural analogues of **3**. The most potent of these, **5** (Chart 2), has a diarylpropyl system bonded directly to a carbon of the cyclic amino acid and exhibits in vitro potency similar to that of **3**. Compound **6**[42] (Chart 2), an analogue of **3** containing a hydroxyisoxazole as an acid isostere, exhibited a certain degree of seizure suppression in vivo. The triphenylmethyl derivative **7** (Chart 2), with some structural similarity to **2**, has been shown to have micromolar affinity for the GABA transporter type 4 (GAT-4).[43]

We report here the synthesis and pharmacological profiles of two new series of GABA uptake inhibitors, conceptually related to each other and which incorporate novel structural elements. These structural features have resulted in a leap in potency in these new agents when compared to currently available compounds. These new anticonvulsants are comprised of diaryl/heteroaryl-oxime[44] and -vinyl ether[45] moieties joined by an aliphatic "linker" to a cyclic amino acid (Figure 1).

New Proposal of GAT-1 Binding Mode

The objectives of this study were to provide structurally novel GABA uptake inhibitors with increased in vitro potency, and correspondingly improved anticonvulsant efficacy, compared to **1**−**3**. Two series of novel GABA uptake inhibitors with general structures **8**, **9**, and **10** have been derived using a new proposed model of the GABA uptake inhibitor binding mode. This new model postulates the presence of a positively charged domain in the protein structure of the site controlling the GABA transporter type 1 (GAT-1).[48−51] We suggest that this domain interacts with an electronegative moiety which is part of the linker (Figure 1) in the potent CNS-active compounds comprising cyclic amino acids *N*-substituted via this linker to two aromatic functions. The lipophilic *N*-substituents have previously typically contained 1,1-diarylethylene and diarylmethyl ether functions as the electronegative moieties (Chart 1).

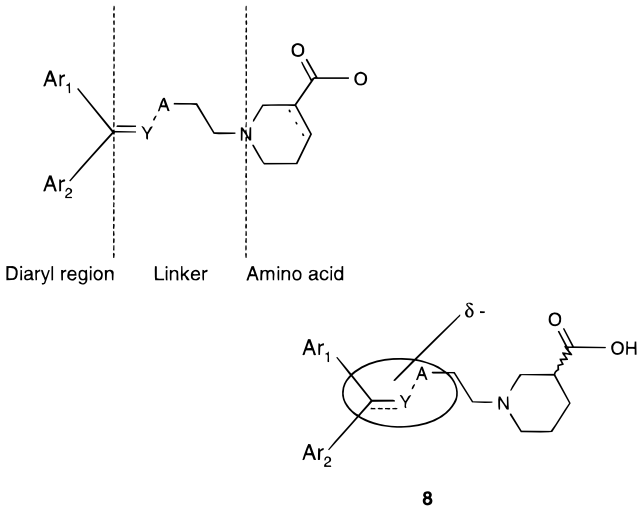


Figure 1. Features of diaryloxime and diarylvinyl ether GABA uptake inhibitors.

The increasing electronegative character of the linker (Figure 1) is coupled to the increase in potency of the compounds as inhibitors of [^{3}H]-GABA uptake in vitro.[39] Five-atom linker molecules **11**, **12**, and **13** show a corresponding increase in linker electronegativity, illustrated by the red surface shading which is apparent in the electrostatic potential diagrams for **12** and **13** in Figure 2.[52]

Another conclusion from the above structural analysis is that extension of the electronegative region further into the middle of the linker leads to greater in vitro potency when compared to butenyl and diarylmethyl ether derivatives. These new insights into the understanding of the structure of GABA uptake inhibitors have led to the logical design of the new agents represented by the general structures **9** and **10** (Chart 3). These diaryloximes and diarylvinyl ethers with improved potency show promise as a potential treatment for partial clonic/tonic seizures in humans.

Synthetic Chemistry

Two main synthetic routes were used for the preparation of diaryl/heteroaryl oximes[44] of general structure **9** (Chart 3, Schemes 1 and 2). The same overall logic, with detail changes, was followed for preparation of the diaryl/heteroaryl vinyl ether derivatives[45] of general structure **10** (Chart 3, Schemes 3 and 4), and one new synthesis unique for vinyl ether GABA uptake inhibitors is illustrated (Scheme 5). We refer to one of the methods

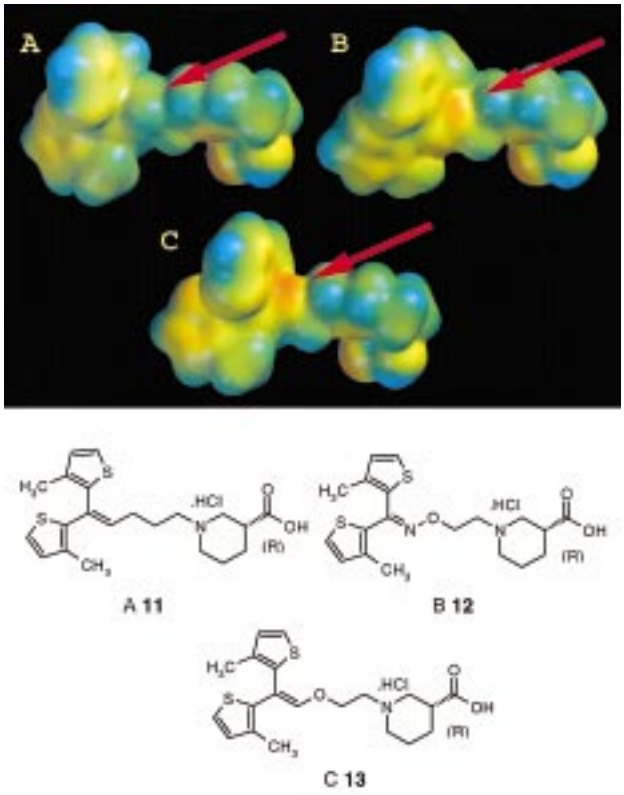
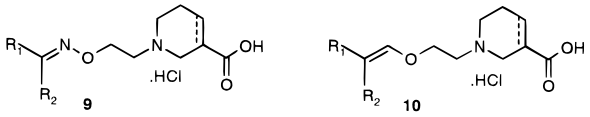
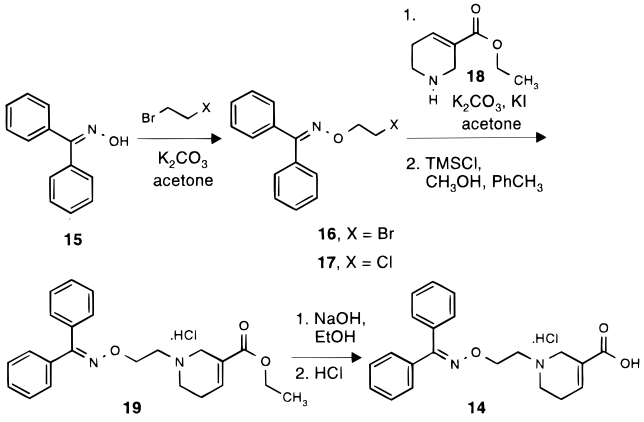


Figure 2. Electrostatic potential calculations[52] for molecules **11**, **12**, and **13**. The most electronegative surface is represented by the red shading (the linker is indicated by the red arrows), graduating toward the electropositive via yellow and green to blue as the most electropositive. As proposed, the oxime **12** has a less electronegative region in the linker than the vinyl ether **13**; both are significantly different from the pentenyl analogue **11** of tiagabine. This is reflected in their activities as inhibitors of [^{3}H]-GABA uptake in vitro, which are 335, 41, and 14 nM, respectively.

Chart 3. General Structures of Diaryloxime and Diarylvinyl Ether GABA Uptake Inhibitors



Scheme 1



A−E, used to prepare each of the target compounds, in the structure−activity relationship (SAR) analysis (Tables 2−4). The underlying principle behind each of the first four syntheses (methods A−D) is the base-catalyzed *O*-alkylation of a diaryl oxime or a diarylacetaldehyde (in its enolic anion form) with either a 1,2-dihaloethane or a *N*-2-(haloethyl)nipecotic acid ester.

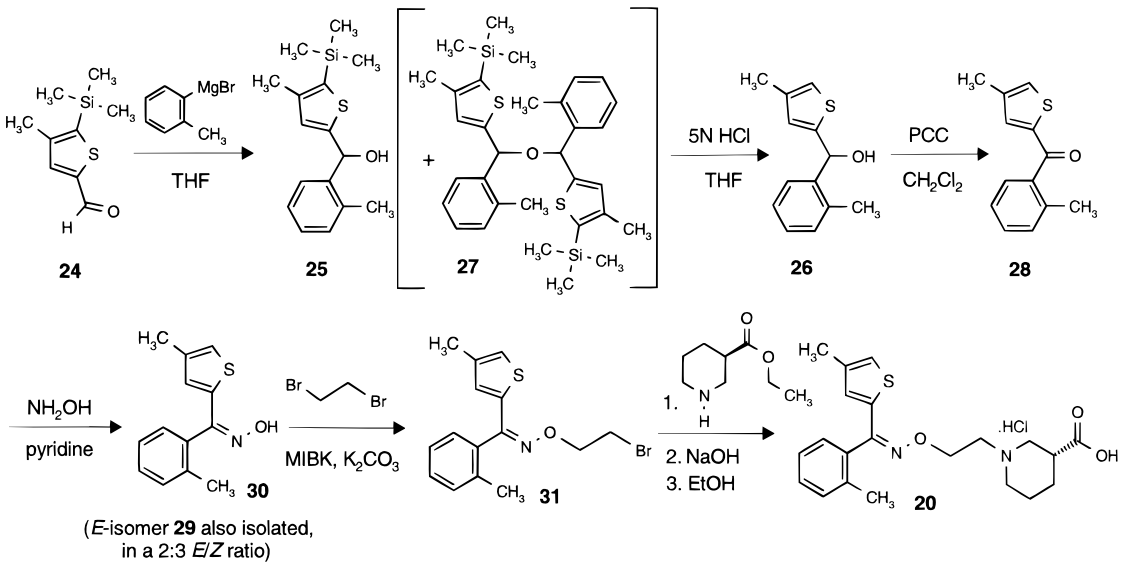
Method A (Oximes). This method is illustrated (Scheme 1) by the synthesis of 1,2,5,6-tetrahydro-1-[2-[[(diphenylmethylene)aminooxy]ethyl]-3-pyridinecarboxylic acid **14**[44,47,50,53,54] (NNC 05-0711; also referred to as NNC-711 or NO-711). Benzophenone oxime **15** could be alkylated under mildly basic conditions using either 1,2-dibromoethane or 1-bromo-2-chloroethane to provide either **16** or **17** as oils. These halides could both be used to alkylate the nitrogen of guvacine ethyl ester **18** to give **19**, with the bromide **16** providing a higher yield as would be predicted. The ester **19** was characterized as its crystalline hydrochloride salt. NNC 05-0711 hydrochloride **14**[47] was obtained following saponification and hydrochloride salt formation.

The synthesis of compounds **20**−**23** (Table 3) was carried out using a modification (Scheme 2) of method A. To prepare 4-methyl-2-thienyl derivatives it was found necessary to block the 5-position with a suitable group, and we opted for a trimethylsilyl group. The aldehyde **24** was prepared from the Grignard reagent **36** derived from 2-bromo-3-methylthiophene followed by formylation. Carbinol **25** was obtained by reaction with 2-methylphenylmagnesium bromide, but this reaction was plagued by the formation of an unwanted ether byproduct, presumably owing to the stability of the carbonium ion derived from dehydration of protonated **25**. Some recovery of carbinol was obtained during acid hydrolysis of **25** and **27**, the main function of which was to effect desilylation prior to pyridinium chlorochromate oxidation of **26** to the ketone **28**. Oxime formation provided the *E*- and *Z*-geometric isomers **29** and **30** in a ratio of 3:2, and these were separated by column chromatography and converted via a bromide such as **31** into the target oximes **20**−**23**, using the same procedure as described in method A.

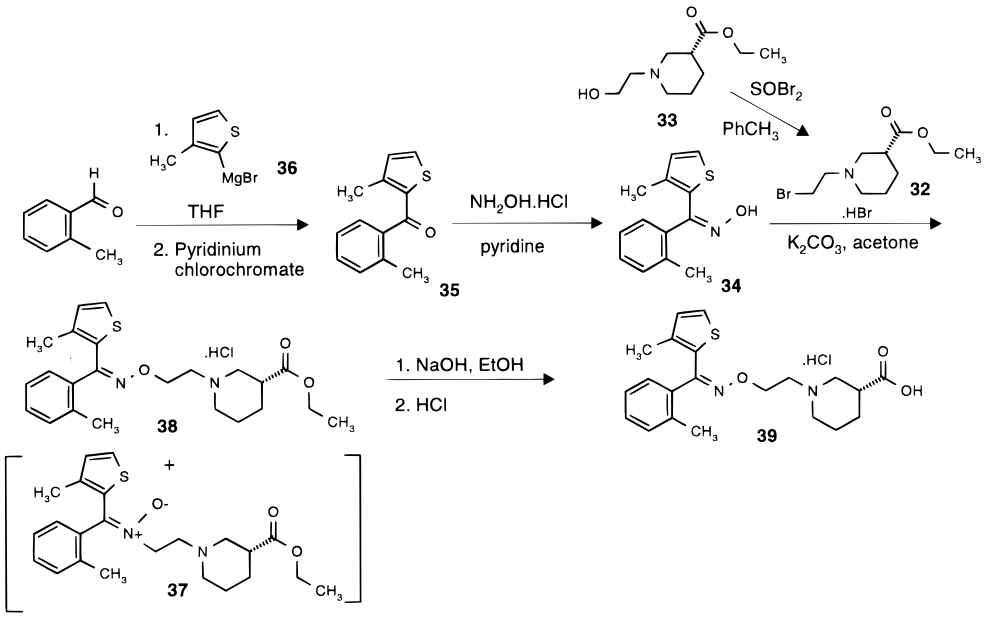
All of the single enantiomer *R*- and *S*-nipecotic acid derivatives described herein were derived by *N*-alkylation of the corresponding *R*- and *S*-nipecotic acid ethyl esters. These esters were prepared in high enantiomeric purity by resolution of commercially available ethyl nipecotate by procedures involving recrystallization of the respective diastereomeric dibenzoyl tartaric acid salts, as utilized in the synthesis of tiagabine **1**.[10,55]

Method B (Oximes). This method (Scheme 3) involves the pivotal *R*-*N*-(2-bromoethyl)nipecotic acid ethyl ester **32**, derived from the corresponding alcohol **33** which has been described previously.[10] Preparation of alcohol **33** relies on the availability of resolved nipecotic acid, ethyl ester.[55] The bromide **32** could conveniently be utilized in the *O*-alkylation of the oxime **34**; the oxime precursor, ketone **35**, was formed in two steps by reaction of *o*-tolualdehyde and the Grignard reagent **36** derived from 2-bromo-3-methylthiophene,[10,56] followed by oxidation of the resultant alcohol. In some cases we observed an *N*-alkylation which competed with the desired *O*-alkylation, providing what was apparently the *N*-oxide or nitrone **37**.[57] However, this relatively polar byproduct was readily removed during column chromatography. The desired ester **38** was characterized as a crystalline hydrochloride salt, and saponification afforded **39**. This preparation illustrated the possibilities

Scheme 2



Scheme 3



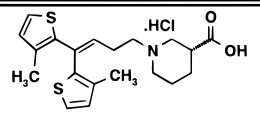
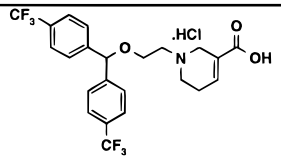
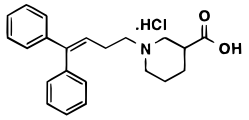
for *E*- and *Z*-geometric isomers in the final product **39** or its opposite geometric isomer **40** (Table 2). Fractional crystallization of the oxime **34** from cyclohexane provided a single *E*- or *Z*-isomer, and either of these could be carried through to the appropriate isomer of final product, **39** or **40**. Unambiguous spectroscopic geometric isomer identification was not straightforward despite previous investigations in this field[58] (see Results and Discussion section). Observations recorded in Table 2 indicate that structures **39** and **40**, related as geometric isomers, possess very similar biological activities.

Method C (Vinyl Ethers). A number of different methods exist for the preparation of diarylacetaldehydes.[59] However, we opted for the classical Darzen's glycidic ester condensation[60] (Scheme 4) for a number of reasons. First, the synthesis utilized ubiquitous diaryl ketones as starting points, as there are a range of convenient methods available for diaryl ketone preparation (for example, Scheme 3) and the diaryl ketones could also be used as starting points for diaryl oximes. Second, the intermediate glycidic acid, e.g., **41**, obtained from saponification of **42**, was convenient for practical reasons because a base extraction prior to the decarboxylation step helped to improve the purity of the diarylacetaldehyde **43**. Under basic conditions, in this case using phase transfer catalysis, the stabilized enol form of acetaldehyde **43** could be *O*-alkylated to give the bromoethylvinyl ether **44** (Scheme 4). This ether was reacted further to afford an intermediate ester and subsequently the amino acid **45** by the method described for compound **14** (Scheme 1).

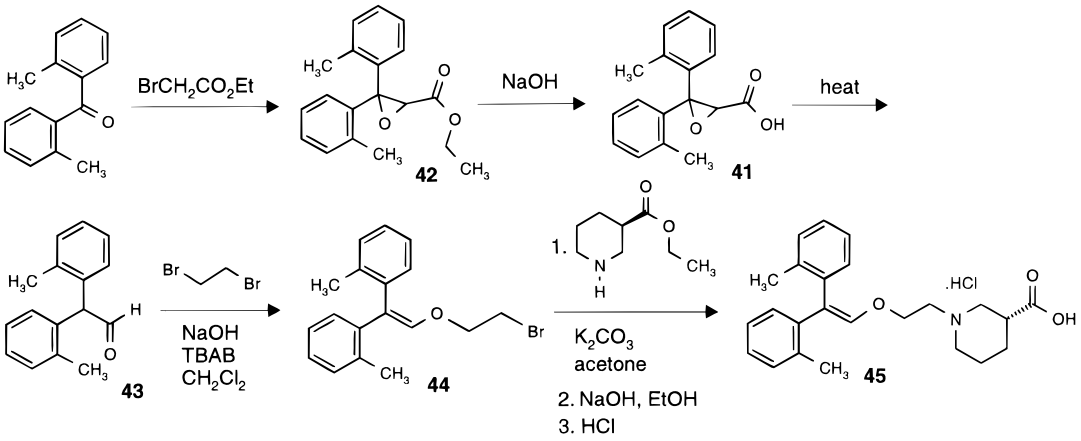
Method D (Vinyl Ethers). In this very direct synthetic approach, the *R*-*N*-(2-bromoethyl)nipecotic acid ester hydrobromide **32** was used in alkylation of the enol anion of diphenylacetaldehyde to provide the ester **46** and subsequently the acid **47** (Scheme 5).

Method E (Vinyl Ethers). This method (Scheme 6)

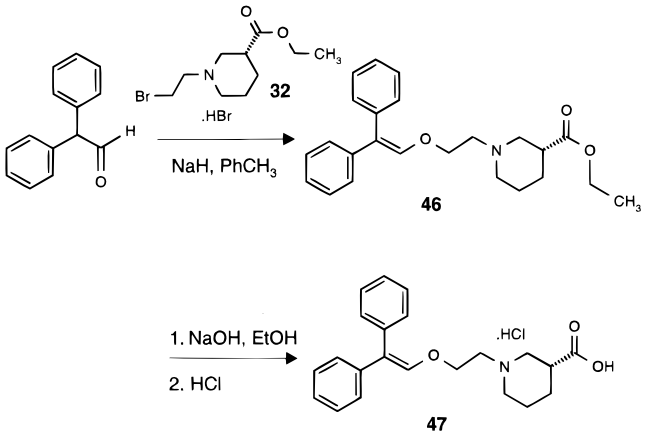
Table 1. Pharmacological Data for Reference GABA Uptake Inhibitors

Cpd #	Structure	K_i (nM) (Mean ± SE)	ED_{50} (mg/kg) DMCM-induced Seizures in mice	ED_{50} (mg/kg) rotarod (mice)
1		67 ± 5	1.2	4.5
2		440 ± 15	2.1	10.0
3		328 ± 36	3.1	-

Scheme 4



Scheme 5



involves as its crucial step the reaction of a Grignard reagent **36** with the ethyl ester **48** of the trityloxyethoxyacetic acid **49**. The resultant alcohol **50** was converted into the tosylate ester **51** and was reacted as described previously for **44** and **16** to provide the ester **52**. The synthesis illustrated is that of **13** (Figure 2 and Chart 4), one of the most potent GABA uptake inhibitors in this vinyl ether series, which in turn comprises the most potent published examples of such agents.

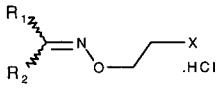
Results and Discussion

As inhibitors of rat synaptosome [^{3}H]-GABA uptake in vitro, the new compounds described herein exhibit remarkable potency, with K_i values as low as 12 nM in some cases (see Tables 2−4), in the assay run essentially as described by Fjalland.[39] This represents a 5-fold potency improvement over tiagabine **1**, the most potent of the currently available reference compounds (Table 1). The compounds, besides being investigated for their in vitro profiles, have had their in vivo effects evaluated in a mouse convulsion model, in which seizures were induced by the inverse benzodiazepine receptor agonist methyl 4-ethyl-6,7-dimethoxy-β-carboline-3-carboxylate (DMCM).[46,47] Ataxia (or neurological toxicity) in rodents was assessed for selected compounds using a mouse rotarod apparatus.[47]

The two new series of related, structurally novel inhibitors of GABA uptake are illustrated by the representative structures **13** (Figure 2, Table 4) and **14** (Scheme 1, Table 2), which have K_i values[39] of 14 ± 1 and 47 ± 3 nM, respectively (Chart 4). The latter compound is commercially available,[53] and its pharmacological profile has been described separately.[47,54]

Before the discovery of these oxime and vinyl ether

Table 2. Pharmacological and Physical Data for Oxime GABA Uptake Inhibitors



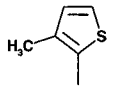
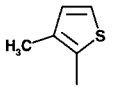
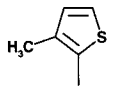
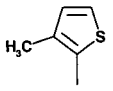
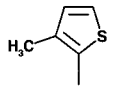
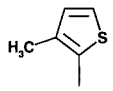
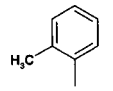
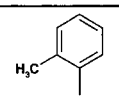
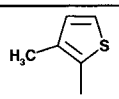
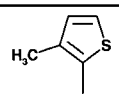
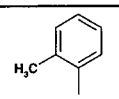
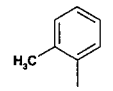
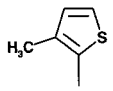
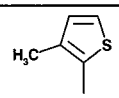
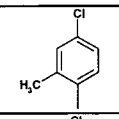
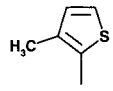
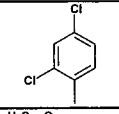
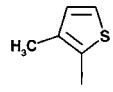
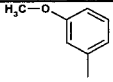
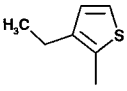
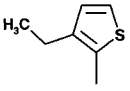
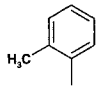
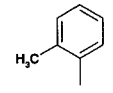
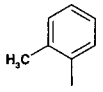
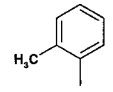
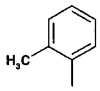
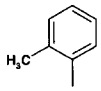
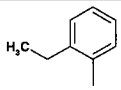
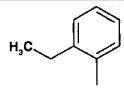
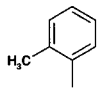
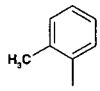
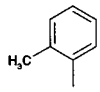
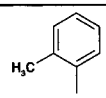
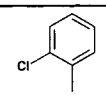
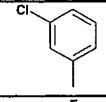
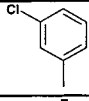
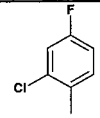
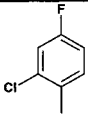
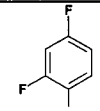
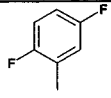
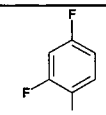
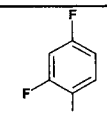
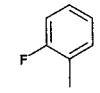
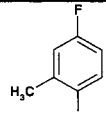
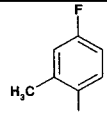
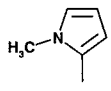
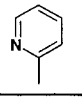
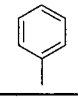
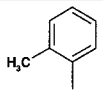
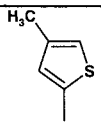
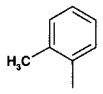
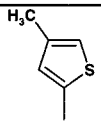
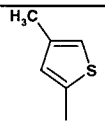
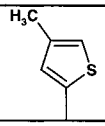
Cpd #	R_1	R_2	X	Method	mp (°C)	Empirical Fomula	Analysis	K_i (nM) (Mean± SE)	ED_{50} (mg/kg) DMCM-induced seizures in mice
12			(R)-Nip	B	179-181 f/h	$C_{19}H_{24}NO_3S_2$.HCl	C,H,N	41 ± 7	1.7
80			(S)-Nip	A	176-178 f/h	$C_{19}H_{24}NO_3S_2$.HCl 0.75 H_2O	C,H,N	82 ± 17	3.6
81			(R)-Nip	B	210-216 d	$C_{18}H_{22}N_2O_3S_2$.0.8HCl.H_2O	C,H,N	74 ± 11	6.1
39			(R)-Nip	B	204-205 e/h	$C_{21}H_{26}N_2O_3S$.0.6 HCl	C,H,N	70 ± 16	1.7
40			(R)-Nip	B	199-201 f/h	$C_{21}H_{26}N_2O_3S$.HCl	C,H,N	88 ± 14	2.5 (6.2)
82			(S)-Nip	A	198-200 e	$C_{21}H_{26}N_2O_3S$.HCl.0.25 H_2O	C,H,N,Cl	83 ± 12	1.5
83			(S)-Nip	A	198-200 e	$C_{21}H_{26}N_2O_3S$.HCl	C,H,N,Cl,S	129 ± 39	5.5 (11.0)
84			(R)-Nip	B	170-175 i	$C_{21}H_{25}N_2ClO_3$.HCl	C,H,N,Cl	90 ± 9	1.7
85			(R)-Nip	B	amorph.	$C_{20}H_{22}N_2Cl_2O_3S$.HCl	C,H,N	32 ± 14	1.7
86			(R)-Nip	B	183-185 f/h	$C_{21}H_{26}N_2O_4S$.HCl	C,H,N	87 ± 21	4.0 (11.0)
87			(R)-Nip	B	164-166 f/h	$C_{21}H_{28}N_3O_5S_2$.0.7 HCl. 0.75H_2O	C,H,N	87 ± 20	4.5
60			(R)-Nip	B	251-253 f/h	$C_{21}H_{24}N_2O_3$.HCl	C,H,N	81 ± 1	3.2
61			(S)-Nip	B	amorph.	$C_{21}H_{24}N_2O_3$.0.75 HCl .0.5H_2O	C,H,N	422 ± 27	4.8
14			Guv	A	214-216 b	$C_{21}H_{22}N_2O_3$.HCl	C,H,N,Cl	47 ± 3	1.6 (6.8)
62			(R)-Nip	A	182-183 f/h	$C_{23}H_{28}N_2O_3$.HCl	C,H,N	71 ± 15	1.2 (4.4)
63			(S)-Nip	A	181-182 f/h	$C_{23}H_{28}N_2O_3$.HCl	C,H,N	126 ± 17	3.6

Table 2 (Continued)

Cpd #	R_1	R_2	X	Method	mp (°C)	Empirical Formula	Analysis	K_i (nM) (Mean± SE)	ED_{50} (mg/kg) DMCM-induced seizures in mice
64			Guv	A	214-218 e	$C_{23}H_{26}N_2O_3$.HCl	C,H,N,Cl	55 ± 4	0.8
88			Guv	A	130-135 e	$C_{23}H_{30}N_2O_3$.0.4 HCl	C,H,N,Cl	183 ± 16	1.2
89			(R)-Nip	B	164-166 a	$C_{22}H_{25}N_2FO_3$.HCl. 0.25 H_2O	C,H,N,Cl	41 ± 1	2.1 (5.0)
90			(R)-Nip	B	Amorph.	$C_{22}H_{25}N_2ClO_3$.0.8HCl. 0.6 H_2O	C,H,N,Cl	202 ± 62	2.5
91			(S)-Nip	A	195 f	$C_{22}H_{25}N_2ClO_3$.HCl	C,H,N,Cl	93 ± 2	4.8
92			Guv	A	223-225 b	$C_{22}H_{23}N_2ClO_3$.HCl	C,H,N,Cl	147 ± 15	1.8 (5.6)
74			(R)-Nip	B	175-185 e	$C_{21}H_{22}N_2Cl_2O_3$.HCl. . 2 H_2O	C,H,N	39 ± 4	3.9 (17.0)
93			(R)-Nip	A	165-166.5 b/j	$C_{21}H_{22}N_2Cl_2O_3$.HCl. 0.5 H_2O	C,H,N,Cl	305 ± 116	6.7 (5.5)
94			(R)-Nip	B	186-187 a	$C_{21}H_{20}N_2Cl_2$ F_2O_3.HCl	C,H,N	100 ± 16	1.3 (4.5)
67			(R)-Nip	B	149-151 a	$C_{21}H_{20}N_2$ F_4O_3.HCl. 0.5 H_2O	C,H,N	52 ± 18	0.6 (1.9)
68			(R)-Nip	B	amorph.	$C_{21}H_{20}N_2O_3$.HCl	C,H,N	103 ± 11	0.7 (2.4)
75			(R)-Nip	A	184-185 b/j	$C_{21}H_{22}N_2$ F_2O_3.HCl	C,H,N	215 ± 16	3.0 (14.5)
66			Hom	A	131-133 f	$C_{23}H_{26}N_2Cl_2$ F_2O_3.HCl	C,H,N	408 ± 17	5.1 (19.5)
95			(R)-Nip	B	224-226 f	$C_{23}H_{25}N_2O_3$.HCl. 0.5 H_2O	C,H,N	59 ± 3	5.3 (6.4)
65			Nip	B	61-63 d	$C_{20}H_{23}N_3O_3$.0.75HCl.H_2O	C,H,N	2069 ± 579	>100 (>100)

structures, it was assumed that the optimal length of the linker (Figure 1) joining the diaryl moiety and the cyclic amino acid in lipophilic GABA uptake inhibitors was four atoms. This was certainly the case for the three most prominent agents **1**, **2**, and **3** (Chart 1). Indeed, early preparation of the 5,5-diphenylpentenyl derivative **53**[31] (Chart 5) provided only a relatively weak (K_i > 1 μM) in vitro inhibitor of [^{3}H]-GABA uptake. The same trend was observed when **1** was extended from a 4,4-dithienylbutenyl linker to the 5,5-dithienylpentenyl derivative **11** (Figure 2 and Chart 5). The in vitro value for inhibition of [^{3}H]-GABA uptake was likewise shown

Table 3. Data for 4-Methyl-2-thienyl/2-Methylphenyl Oxime GABA Uptake Inhibitors

Cpd #	R_1	R2	X	Method	mp (°C)	Empirical Formula	Analysis	K_i (nM) (Mean± SE)	ED_{50} (mg/kg) DMCM-induced seizures in mice
20			(R)-Nip	A	239	$C_{21}H_{26}N_2O_3S.HCl$	C,H,N,Cl,S	783 ± 63	4.5 (50)
21			(S)-Nip	A	239	$C_{21}H_{26}N_2O_3S.HCl$	C,H,N,Cl,S	8674 ± 870	38 (>60)
22			(R)-Nip	A	175	$C_{21}H_{26}N_5O_3S.HCl$	C,H,N,Cl	261 ± 29	5.5 (60)
23			(S)-Nip	A	165	$C_{21}H_{26}N_5O_3S.1.3\ HCl.\ 0.2\ H_2O$	C,H,N,Cl,S	1209 ± 165	18

to be considerably higher (K_i = 335 nM) for the homologue **11**; the corresponding K_i value for **1** is 67 nM.

It was therefore surprising to us when five-atom linker compounds such as **13** and **14** (Chart 4) were found to possess greater in vitro potency than the known butenyl derivatives **1**, **2**, and **3**. The step to a five-atom linker was initially taken in the above oximes and vinyl ethers because we found that synthesis of the shorter (i.e. four-atom linker) oxime analogues was not achievable. We further demonstrated that five was the optimum linker length in the oxime and vinyl ether series by preparing six-atom linker compounds **54** and **55**. These novel structures were found to be only very weak inhibitors of [^{3}H]-GABA uptake in vitro, with K_i values in the micromolar range (Chart 5).

The oxime ether functionality has been used previously in drug design to provide novel biologically active molecules. Some selected examples include Ridogrel **56**,[61] a thromboxane synthetase inhibitor, the antineoplastic agent Amidox **57**,[62] the phosphodiesterase inhibitor **58**,[63] muscarinic agonists such as **59**[64] (Chart 6), as well as a range of oxime-based cephalosporin antibiotics.

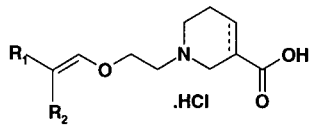
The novel anticonvulsants described herein[44,45] have been examined for their in vitro effects as inhibitors of [^{3}H]-GABA uptake, expressed as K_i values in nM.[39] Representative compounds from each series, ordered by their substitution pattern, are shown in Tables 2−4, with selected reference GABA uptake inhibitors featured in Table 1. The tables also contain ED_{50} values for protection against DMCM-induced seizures,[46,47] utilizing a 30 min pretreatment time in mice. Since new anticonvulsant agents to be considered as back-ups to **1** should ideally have minimized sedative effects, neurological toxicity in mice assessed in a standard rotarod test[47] has also been included for selected compounds in Tables 2−4. The ED_{50} values in this latter paradigm are included in parentheses below the ED_{50} values for DMCM-induced seizures.

The relatively facile synthesis of diaryloxime ether-based GABA uptake inhibitors by *O*-alkylation of an oxime derived from the corresponding ketone, allowed the synthesis of a relatively large range of compounds for the examination of SAR in this series[44] (Tables 2 and 3). The conclusions of this study are that in terms of in vitro potency the oximes are generally more potent than diarylbutenes with a corresponding diaryl/heteroaryl substitution. Although the linker now comprises five atoms, the bis(3-methyl-2-thienyl) oxime derivative **12** with *R*-nipecotic acid as amino acid, corresponding to **1**, is among the most potent in vitro from this oxime series, with a K_i value of 41 nM for inhibition of [^{3}H]-GABA uptake. The rank order of potency among the different cyclic amino acid derivatives (for example, in Table 2, **60**, **61**, **14**, and **62**, **63**, **64**[47]) is that the *R*-nipecotic acid derivatives are substantially more potent than the corresponding *S*-nipecotic acid derivatives,[55] and that the guvacines[10,69] have generally similar in vitro potency to the *R*-nipecotic acid derivatives. The "ortho" effect of aryl/heteroaryl methyl groups, which is apparent in **1**,[10] is not as prominent in this oxime series compared to the butenyl series, illustrated by the observation that the ditoluyl derivative **64**[47] (K_i = 55 ± 4 nM) is not more potent than **14**[47] (K_i = 47 ± 3 nM). Although exceptions do exist, this may reflect the longer linker, which presumably affects the binding of the diaryl moiety at the uptake site. Furthermore, the increased electronegativity of the oxime linker compared to a butenyl linker may also have an effect on binding conformation.

Significantly, the *R*-nipecotic acid derivatives **39** and **40**, which are geometric isomers, have very similar biological activity, which implies that thiophenes and phenyl rings are isosterically interchangeable in this oxime series. Pyridine/phenyl isosterism is however not permitted, illustrated by the poor in vitro actvity of **65** (K_i of >2 μM) compared to **60** (K_i of 81 nM). One β-homoproline derivative,[65] **66**, is featured in Table 2; this compound has an unremarkable in vitro effect, but performs comparatively well against the seizure in vivo measure.

The vinyl ether series (Table 4) has provided the most

Table 4. Pharmacological and Physical Data for Vinyl Ether GABA Uptake Inhibitors[a]



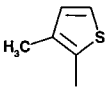
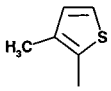
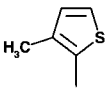
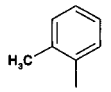
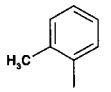
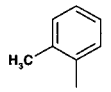
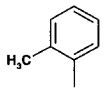
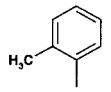
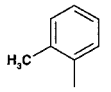
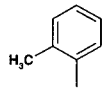
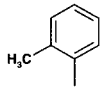
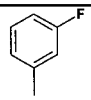
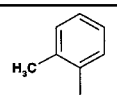
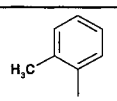
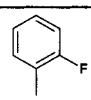
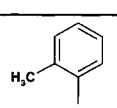
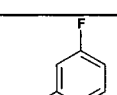
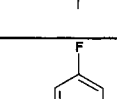
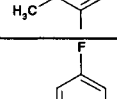
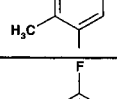
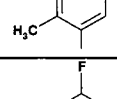
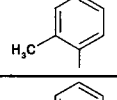
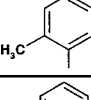
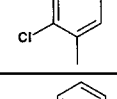
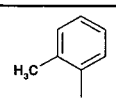
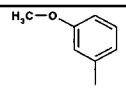
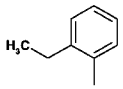
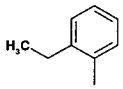
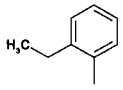
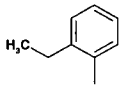
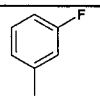
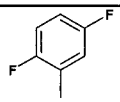
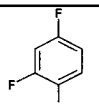
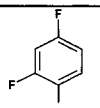
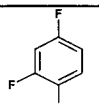
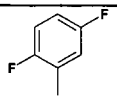
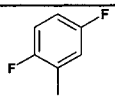
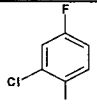
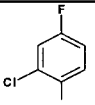
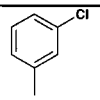
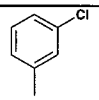
Cpd #	R_1	R2	X	Method	mp (°C)	Empirical Formula	Analysis	K_i (nM) (Mean± SE)	ED_{50} (mg/kg) DMCM-induced seizures in mice
13			(R)-Nip	E	55-70 (dec.) e	$C_{20}H_{25}NO_3S_2.0.3$ HCl. 0.75 H_2O	C,H,N,Cl,S	14 ± 1	0.6
69			(R)-Nip	D	207-212 f/h	$C_{22}H_{28}NO_3S$.HCl. 0.33 $PhCH_3$	C,H,N	17 ± 2	0.6
47			(R)-Nip	D	217-218 e	$C_{22}H_{25}NO_3$.HCl.H_2O	C,H,N	86 ± 19	1.5
96			(R)-Nip	D	195-196 a	$C_{23}H_{27}NO_3$.HCl .0.25 H_2O	C,H,N	21 ± 1	0.5 (1.5)
97			(R)-Nip	D	206-211 a	$C_{23}H_{27}NO_3$.HCl .0.25 H_2O	C,H,N	49 ± 2	1.5 (6.7)
45			(R)-Nip	C	217-222 e	$C_{24}H_{29}NO_3$.HCl .0.75 H_2O	C,H,N,Cl	19 ± 0.4	0.8
70			Guv	C	195-198 b/f	$C_{22}H_{27}NO_3$.HCl 0.33 $PhCH_3$	C,H,N	26 ± 0.1	0.6 (1.8)
71			(R)-Nip	D	185-187 a	$C_{23}H_{26}$ FNO_3.HCl	C,H,N,Cl	28 ± 0.1	0.5 (1.5)
76			(R)-Nip	D	193-195 a	$C_{23}H_{26}FNO_3$.HCl	C,H,N,Cl	48 ± 1.1	1.6 (10.0)
72			Guv	C	183-185 f	$C_{23}H_{24}FNO_3.1.25$ HCl	C,H,N,Cl	19 ± 1.3	0.6
99			(R)-Nip	C	195-210 f	$C_{24}H_{28}FNO_3$.HCl	C,H,N,Cl	30 ± 3	1.8
77			(R)-Nip	D	187-190 a	$C_{24}H_{27}F_2NO_3$.HCl	C,H,N	61 ± 2	3.0 (14.2)
100			Guv	C	softens 195 melts 209 f/h	$C_{24}H_{25}F_2NO_3$.HCl	C,H,N,Cl	79 ± 4	2.5
101			(R)-Nip	C	231-234 f/h	$C_{23}H_{27}ClNO_3$.HCl	C,H,N,Cl	12 ± 0.1	1.7
78			Guv	C	214-215 a/i	$C_{23}H_{25}ClNO_3$.HCl	C,H,N,Cl	64 ± 18	1.6 (16.7)

Table 4 (Continued)

Cpd #	R_1	R2	X	Method	mp (°C)	Empirical Formula	Analysis	K_i (nM) (Mean± SE)	ED_{50} (mg/kg) DMCM-induced seizures in mice
102			(R)-Nip	C	188-192 a	$C_{24}H_{29}NO_4.HCl$	C,H,N,Cl	83 ± 1	1.6 (3.8)
103			(R)-Nip	C	205-206 a	$C_{26}H_{33}NO_3.HCl$	C,H,N,Cl	32 ± 0.9	1.9
104			Guv	C	162-165 a	$C_{26}H_{31}NO_3.HCl$	C,H,N,Cl	47 ± 4	1.7
105			Guv	C	158-159 e	$C_{22}H_{21}F_2NO_3.HCl.0.33\ H_2O$	C,H,N,Cl	346 ± 17	>30 (>30)
106			(R)-Nip	D	162-164 a/c	$C_{22}H_{21}F_4NO_3.HCl.0.5\ H_2O$	C,H,N,Cl	51 ± 5	1.9 (5.0)
79			(R)-Nip	D	148-150 a	$C_{22}H_{21}F_4NO_3.HCl.0.75\ H_2O$	C,H,N	132 ± 37	1.3 (5.5)
107			(R)-Nip	D	215-217 e	$C_{22}H_{21}F_4NO_3.HCl$	C,H,N	179 ± 14	2.3 (8.0)
73			(R)-Nip	D	softens 170 melts 198 f	$C_{22}H_{24}ClNO_3.HCl$	C,H,N,Cl	12 ± 0.3	0.7 (1.8)
108			(R)-Nip	D	227-228 f/h	$C_{22}H_{24}ClNO_3.HCl$	C,H,N,Cl	52 ± 3	2.3
109			(R)-Nip	C	243-245 a	$C_{22}H_{23}Cl_2NO_3.HCl$	C,H,N,Cl	52 ± 5	-
110			Guv	C	200-203 f	$C_{22}H_{21}Cl_2NO_3.HCl$	C,H,N,Cl	12 ± 1.3	1.7 (6.6)
111			(R)-Nip	D	180-182	$C_{22}H_{21}Cl_2F_2NO_3.HCl$	C,H,N	66 ± 8	2.4 (6.0)
112			(R)-Nip	C	232-233 d	$C_{22}H_{23}Cl_2NO_3.HCl$	C,H,N	201 ± 17	-

[a] Crystallization solvents: a, acetone; b, 2-propanol; c, Et_2O; d, CH_2Cl_2; e, H_2O; f, $PhCH_3$; g, EtOH; h, CH_3OH; i, EtOAC; j, *n*-heptane. Amino acids (X): NIP represents nipecotic acid (see **1**); Guv, guvacine (see **2**); Hom, *β*-homoproline; all are alkylated on nitrogen. Figures in parentheses below ED_{50} values for DMCM-induced seizures relate to mouse rotarod ED_{50} values in mg/kg.[47]

potent in vitro inhibitors of GAT-1 so far discovered. Again, the special activity of the vinyl ether containing the bis(3-methyl-2-thienyl) heteroaromatic moieties is borne out, with compound **13** exhibiting an K_i value of 14 nM for inhibition of [^{3}H]-GABA uptake. Several other structures in Table 4, particularly those containing 2-chlorophenyl derivatives also exhibit high in vitro potency. Generally, we conclude that the vinyl ether derivatives, of a particular aromatic/heteroaromatic substitution have a greater in vitro potency than the corresponding oxime structures. The rank order of potency among the different amino acid derivatives is as for the oxime series, the *R*-nipecotic acid derivatives being generally more potent in vitro than the corresponding guvacines, although exceptions exist.

Clearly, when one is moving from an in vitro assay for inhibition of [^{3}H]-GABA uptake to an in vivo paradigm where compounds are investigated for seizure control, many more variables come into play. Issues such as penetration across the blood−brain barrier, plasma half-life, distribution, and bioavailability become important. Despite these potential variables, the com-

Scheme 6

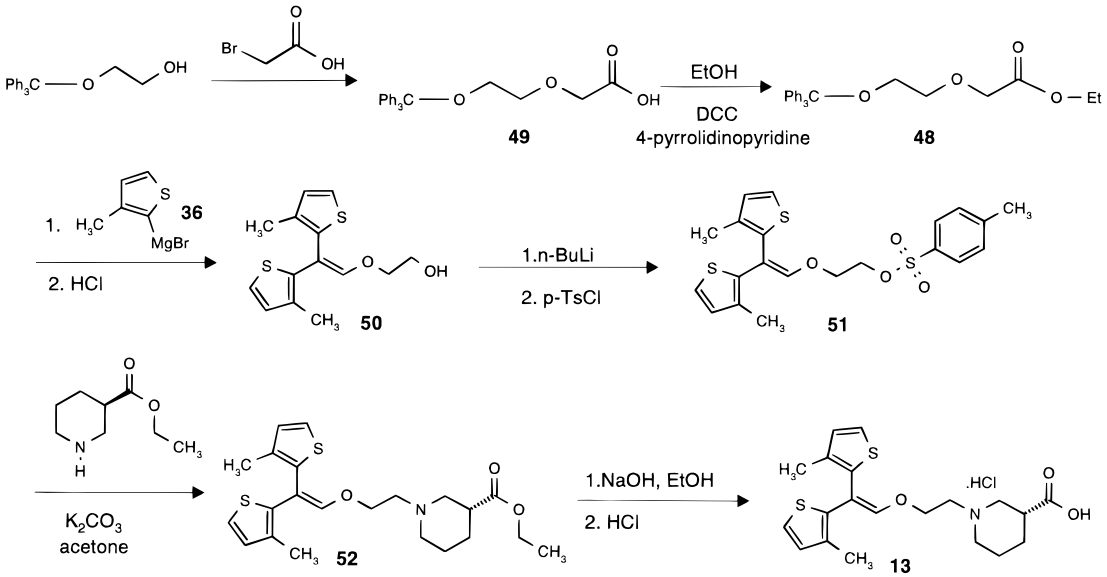


Chart 4. Structures of NNC 05-1010 **13** and NNC 05-0711 **14**

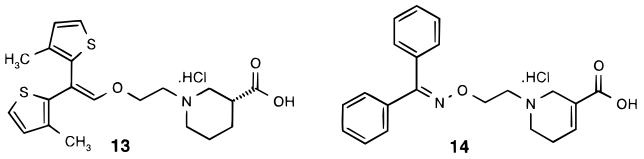
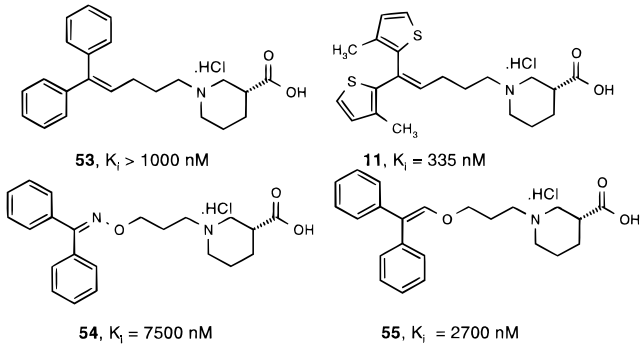


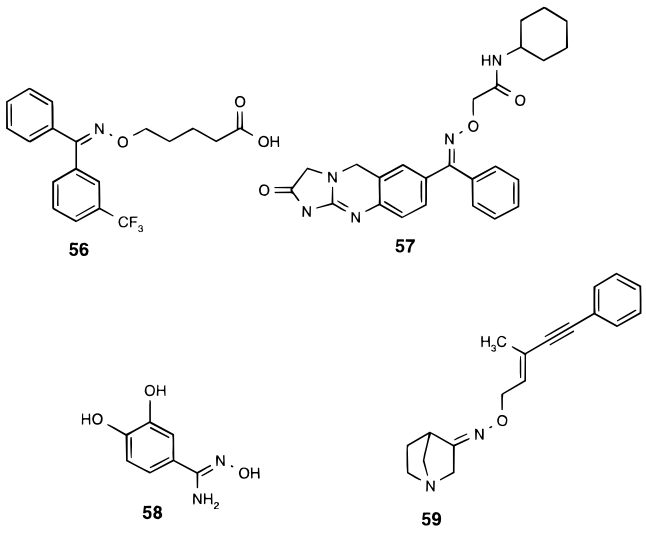
Chart 5. GABA Uptake Inhibitors with 5- and 6-Atom Linkers



pounds featured in Tables 2−4 have a remarkably consistent effect in protection against seizures. A range of these novel anticonvulsants have ED_{50} values of <2 mg/kg in this paradigm. Three oxime derivatives, **64**, **67,** and **68** (Table 2) have ED_{50} values below 1 mg/kg, as do six compounds, **13**, **45**, and **69**−**73**, in the vinyl ether series (Table 4).

Tiagabine has been extensively investigated in human subjects after oral dosing as add-on therapy for the control of partial seizures. The usual maintenance dose in human is 30−50 mg/day, with doses of up to 70 mg/day being well tolerated. The ED_{50} value for tiagabine against DMCM-induced seizures in mice is 1.2 mg/kg intraperitoneally (i.p.), so the total daily dose in humans is of a similar order to the rodent anticonvulsant ED_{50} in this paradigm, which gives some prediction of potential human doses. To provide a superior drug candidate to **1**, it is important to improve upon the therapeutic ratio of ED_{50}(rotarod)/ED_{50}(DMCM seizures), which is 3.75 for **1** (4.5/1.2) (Table 1). Of the oxime derivatives

Chart 6. Examples of Biologically Active Molecules Containing the Oxime Functionality



in Table 2 which have been examined in mouse rotarod, those with improved ratios compared to the marketed drug are **14**, **74**, and **75**, with ratios of between 4 and 5. In Table 4, vinyl ethers such as **76**−**79** also exhibited improved therapeutic ratios compared to **1**. However, during our investigations, we identified a series of 4-methyl-2-thienyl/2-methylphenyloxime derivatives, **20**−**23** (Table 3, Scheme 2), which became the subject of a special study. We wished to evaluate these ligands in detail in order to compare the pharmacological activity of the *E*- and *Z*-isomers and both the *R*- and *S*-enantiomers.

The oximes **20**−**23** are not outstandingly potent as inhibitors of [^{3}H]-GABA uptake in vitro, as the *R*-nipecotic acid derivatives **20** (*Z*) and **22** (*E*) had K_i values of 783 and 261 nM. However, in view of this in vitro profile, **20** and **23** are remarkably potent in terms of their in vivo anticonvulsant activity, with ED_{50} values of 4.5 and 5.5 mg/kg, respectively. Rather like the other *E*- and *Z*-isomers such as **39** and **40**, there was little observed difference in anticonvulsant potency between **20** and **22**, but these two examples exhibited an im-

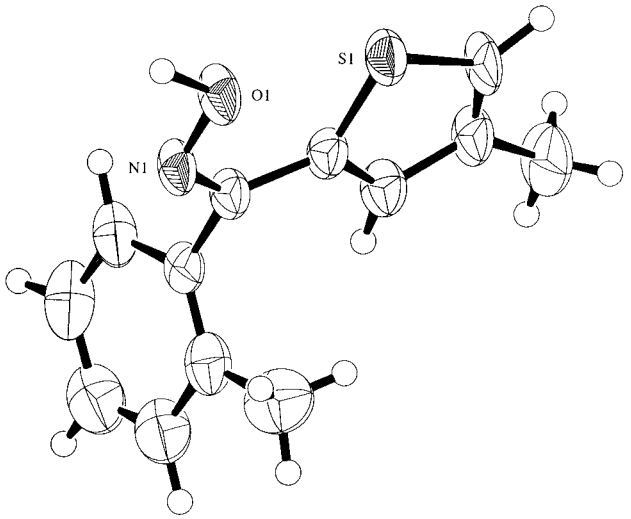


Figure 3. X-ray structure of 2-methylphenyl, 4-methyl-2-thienylmethanone oxime, **30**.

proved therapeutic ratio of 11 when compared to other GABA uptake inhibitors. The compounds featured in Table 3 are all oximes derived from 2-methylphenyl(4-methyl-2-thienyl)-methanone, and because of this promising therapeutic ratio, all of the four combinations of isomers, i.e., the *E*- and *Z*-oxime isomers as well as the *R*- and *S*-nipecotic acid derivatives, were prepared and tested. The precursor to compounds **20** and **21**, 2-methylphenyl, 4-methyl-2-thienylmethanone oxime, **30**, was subjected to X-ray crystallographic analysis, confirming that it was a *Z*-isomer (Figure 3).

It is difficult to assign the cis or trans geometry across the oxime double bond in these four *O*-alkylated oxime GABA uptake inhibitors by NMR even though all four *E*/*Z* and *R*/*S* combinations **20**−**23** were isolated; this is a simpler matter when monoaryl oxime ethers are involved since the vicinal proton NMR coupling can be readily analyzed.[58] Hence we opted for an X-ray analysis of **30**, allowing assignment of *E*- and *Z*-isomer identity to the pharmacologically promising series, **20**−**23**.

Conclusion

We have demonstrated that improved anticonvulsant efficacy in mice can be achieved by rational design of novel uptake inhibitors at the GABA transporter type 1 (GAT-1) site with the goal of providing new clinically effective drugs for treatment of partial seizures.

Given the background of **1** as a benchmark GABA uptake inhibitor and a clinically effective anticonvulsant drug, we have prepared novel compounds of increased potency, using a new putative model of ligand interaction at the GABA uptake site GAT-1 (Figure 1). This model involves the postulated interaction of an electronegative region in the small molecule GABA uptake inhibitor with a positive domain in the protein structure of the site controlling GABA uptake. The new agents synthesized on the basis of this new model have potent anticonvulsant properties in rodent seizure models, and some have an improved therapeutic ratio.

By a series of design steps, **12** (Table 2) was derived from **1**, and in turn led to the discovery of **13** (Table 2), which is one of the most potent known inhibitors of [^{3}H]-GABA uptake in vitro. An insertion of an ether oxygen (Chart 3) in **1** thereby improves in vitro potency by 5-fold. These highly potent GABA uptake inhibitors may have potential in the treatment of epilepsy in humans, and further structural modifications in this series of anticonvulsant agents are planned.

Future challenges in this field include the design of new ligands which are specific for other subtypes of GABA transporters.[48−51] These transporters have a different brain distribution to GAT-1, and inhibitors with subtype selectivity, which are now emerging,[66,67] may show a different therapeutic and side-effect profile.

Experimental Section

Chemistry. Melting points were determined in open capillary tubes on a Büchi 535 melting point apparatus and are uncorrected. The structures of all compounds are consistent with spectroscopic data, and satisfactory elemental analyses (for C, H, N with a Perkin-Elmer model 240 elemental analyzer; S and Cl were determined by the Schöniger combustion method) were obtained within ±0.4% of theoretical values where given. ^{1}H NMR spectra were recorded on a Bruker WM400 spectrometer with TMS as standard, with selected representative chemical shifts quoted in ppm (δ) in the solvents indicated. Compounds used as starting materials are either known compounds or compounds which can be prepared by methods known per se. Column chromatography was carried out using the technique described by W. C. Still et al.,[68] on Merck silica gel 60 (Art 9385) using thick-walled glass columns. HPLC was carried out on a Waters model 510 chromatograph interfaced via a system module to a Waters 490 multiwavelength detector to a reversed phase C18 column (250 × 4 mm, 5 mm, 100 Å; eluent flow rate 1 mL/min at 35 °C). Retention times are given in minutes.

1-[2-[[(Diphenyl)imino]oxy]ethyl]-3-(1,2,5,6-tetrahydropyridin-1-yl)carboxylic acid (14) (Method A, Scheme 1). Diphenylmethanone, *O*-(2-chloroethyl)oxime (17). Benzophenone oxime **15** (3.94 g, 20 mmol), 1-bromo-2-chloroethane (28.7 g, 200 mmol), and dried, powdered K_2CO_3 (5.53 g, 80 mmol) were heated at reflux in acetone (60 mL) for 72 h. The reaction mixture was cooled and filtered and the filtrate was evaporated to an oily residue. Flash chromatography (95:5 *n*-heptane/EtOAc) provided diphenylmethanone, *O*-(2-chloroethyl)oxime **17** (3.82 g, 73%) as an oil: R_f = 0.36 (SiO_2, 90:10 *n*-heptane/EtOAc); ^{1}H NMR ($CDCl_3$) 3.79 (t, 2H), 4.38 (t, 2H), 7.29−7.50 (m, 10H).

Ethyl 1-[2-[[(Diphenyl)imino]oxy]ethyl]-3-(1,2,5,6-tetrahydropyridin-1-yl)carboxylate (19). To a solution of **17** (13.09 g, 50 mmol) in $PhCH_3$ (500 mL) were introduced guvacine ethyl ester[69] **18** (7.76 g, 50 mmol) and powdered, dried K_2CO_3 (13.82 g, 100 mmol). The reaction mixture was heated at reflux for 90 h and cooled. Filtration and evaporation provided an oil which was purified by flash chromatography (9:1 cyclohexane/EtOAc initially, later increasing polarity to 1:1) to provide ethyl 1-[2-[[(diphenyl)imino]oxy]ethyl]-3-(1,2,5,6-tetrahydropyridin-1-yl) carboxylate **19** (5.63 g, 30%) as a gum: R_f = 0.26 (SiO_2, 1:1 cyclohexane/EtOAc); ^{1}H NMR ($CDCl_3$) 2.26−3.34 (m, 2H), 2.57 (t, 2H), 2.87 (t, 2H), 3.26 (q, 2H), 3.72 (s, 3H), 4.38 (t, 2H), 6.99 (m, 1H), 7.29−7.52 (m, 10H). Starting material **17** (8.87 g, 67%) was also isolated. The ethyl ester **19** was converted into a hydrochloride salt by dissolving the gum in $PhCH_3$ at 75 °C and precpitating by treatment with chlorotrimethylsilane and CH_3OH (1 equiv), a method we have described previously.[10] The white solid hydrochloride had a melting point of 110−116 °C. Anal. ($C_{23}H_{26}N_2O_3$·HCl) C, H, N, Cl.

1-[2-[[(Diphenyl)imino]oxy]ethyl]-3-(1,2,5,6-tetrahydropyridin-1-yl)carboxylic Acid (14). To a solution of **19** hydrochloride salt (16.3 g, 43 mmol) in EtOH (100 mL) was added 10 N aqueous NaOH solution (43 mL). The mixture was stirred for 2.5 h at room temperature and evaporated in vacuo. The creamy residue was dissolved in water (250 mL) and cooled to ca. 5 °C. The pH was adjusted to ca. 2 with 2 N aqueous HCl, and the mixture was extracted with CH_2Cl_2 (4

× 200 mL). The combined organic extracts were dried ($MgSO_4$). The residue after evaporation was crystallized first from CH_2Cl_2/cyclohexane, followed by recrystallization from 2-propanol to provide **14** as a white solid (9.4 g, 56%): mp 214.5−216.5 °C; ^{1}H NMR ($CDCl_3$) 2.46−2.56 (m, 2H), 2.57 (t, 2H), 2.87 (t, 2H), 3.26 (q, 2H), 3.72 (s, 3H), 4.38 (t, 2H), 6.99 (m, 1H), 7.29−7.52 (m, 10H). Anal. ($C_{23}H_{26}N_2O_3$·HCl) C, H, N, Cl.

(3*R*)1-[2-[[2-(2-Methylphenyl)-2-(3-methyl-2-thienyl)-imino]oxy]ethyl]-3-piperidinecarboxylic acid (39) (Method B, Scheme 3). (3*R*)-*N*-(2-Bromoethyl)nipecotic Acid Ethyl Ester (32). To ethyl *R*-3-piperidine carboxylate (ethyl nipecotate)[55] (100 g, 640 mmol) in anhydrous acetone (400 mL) were added 2-bromoethanol (95.4 g, 760 mmol), dried, powdered K_2CO_3 (175.8 g, 1270 mmol), and KI (21 g, 127 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was filtered, and the filtrate was concentrated in vacuo to a residue which was fractionated to provide *R*-*N*-(2-hydroxyethyl)nipecotic acid ethyl ester **33** (95 g, 74%): bp 110−115 °C/0.1 mmHg; ^{1}H NMR ($CDCl_3$) 1.28 (t, 3H); 1.6 (m, 2H); 1.75 (m, 1H); 1.9 (m, 1H); 2.2 (m, 1H); 2.4 (m, 1H); 2.55 (m, 3H); 2.75 (m, 1H); 2.9 (m, 1H); 3.10 (br s, 1H); 3.63 (m, 2H); 4.16 (q, 2H). This alcohol **33** (40.3 g, 200 mmol) was dissolved in $PhCH_3$ (300 mL). A solution of $SOBr_2$ (41.6 g, 200 mol) in $PhCH_3$ (50 mL) was added dropwise, and the reaction mixture was stirred at room temperature for 2 h. Et_2O (300 mL) was added, and the solid product (65 g) was collected by filtration. The bromide **32** (46.6 g, 68%) was thereby obtained as a white solid hydrobromide salt, mp 187.5−194.5 °C, by recrystallization from 2-propanol: ^{1}H NMR ($CDCl_3$) 1.27 (t, 3H); 1.58 (q, 1H); 2.02 (d, 1H); 2.30−2.50 (m, 2H); 2.84−3.04 (m, 2H); 3.42−3.58 (m, 3H); 3.70 (d, 1H); 3.80 (d, 1H); 3.95 (m, 2H); 4.17 (q, 2H); 11.6 (br s, 1H).

2-Methylphenyl(3-methyl-2-thienyl)methanone Oxime (34). 2-Methylphenyl(3-methyl-2-thienyl)methanone **35** was prepared from 2-bromo-3-methylthiophene[56] and 2-methylbenzaldehyde by the standard method described previously for bis(3-methyl-2-thienyl)methanone.[10] Compound **35** (2.28 g, 10.5 mmol) was dissolved in pyridine (70 mL), NH_2OH·HCl (1.50 g, 21.5 mmol) was introduced, and the reaction mixture was heated at reflux for 66 h before being concentrated in vacuo to a residue. EtOAc (100 mL) and water (100 mL) were introduced, and the EtOAc phase was separated before being extracted with water (100 mL). The organic phase was dried ($MgSO_4$) and evaporated to an oil which was purified by flash chromatography (19:1 *n*-heptane/EtOAc) to provide a mixture of geometric isomers which was recrystallized from cyclohexane to provide **34** (0.7 g, 29%) as a single geometric isomer: mp 99−100 °C; ^{1}H NMR [$(CD_3)_2SO$] 1.73 (s, 3H), 2.07 (s, 3H), 6.88 (d, 1H), 7.17−7.33 (m, 4H), 7.61 (d, 1H), 11.81 (s, 1H). By crystallization of the mother liquors, the other geometric isomer of **34** was obtained (0.5 g, 21%): mp 136−138 °C; ^{1}H NMR [$(CD_3)_2SO$] 1.87 (s, 3H), 2.15 (s, 3H), 6.87 (d, 1H), 7.17−7.32 (m, 4H), 7.39 (d, 1H), 11.19 (s, 1H).

Ethyl (3*R*)-1-[2-[[2-(2-Methylphenyl)-2-(3-methyl-2-thienyl)imino]oxy]ethyl]-3-piperidinecarboxylate (38). To a solution of the lower-melting oxime **34** (9.75 g, 42 mmol) in acetone (100 mL) was added **32** (22.5 g, 65.2 mmol), $PhCH_3$ (200 mL), and dried, powdered K_2CO_3 (23.22 g, 168 mmol). The reaction mixture was heated at reflux for 2 h, cooled, and filtered. The filtrate was evaporated to an oil, which was purified by flash chromatography (9:1 *n*-heptane/EtOAc). The ester **38** (12.1 g, 69%) was converted into the corresponding hydrochloride salt, using the method ($(CH_3)_3SiCl/CH_3OH$) described for the ester **19**. The resultant solid was recrystallized from $PhCH_3$/cyclohexane to afford white crystals: mp 124−125.5 °C; ^{1}H NMR ($CDCl_3$) 1.22 (t, 3H), 1.42 (q, 1H), 1.72 (s, 3H), 2.16 (s, 3H), 3.46 (t, 2H), 4.12 (q, 2H), 4.79 (t, 2H), 6.81 (d, 1H), 7.20−7.38 (m, 4H), 7.41 (d, 1H). Anal. ($C_{23}H_{30}N_2O_3S$·HCl) C, H, N, Cl, S.

(3*R*)-1-[2-[[2-(2-Methylphenyl)-2-(3-methyl-2-thienyl)-imino]oxy]ethyl]-3-piperidinecarboxylic Acid (39). The hydrochloride salt of ester **38** (4.87 g, 10.8 mmol) was hydrolyzed as described for compound **19**. The residue after evaporation was recrystallized from H_2O/CH_3OH to provide **39** as a white solid hydrochloride salt (3.25 g, 71%): mp 204−205 °C; $[\alpha]^{20}_D$ −6.8° (*c* = 1.0, CH_3OH); ^{1}H NMR [$(CD_3)_2SO$] 1.76 (s, 3H), 2.09 (s, 3H), 2.96 (t, 2H), 4.39 (t, 2H), 6.92 (d, 1H), 7.20−7.38 (m, 4H), 7.68 (d, 1H). Anal. ($C_{23}H_{26}N_2O_3$·HCl) C, H, N.

(3*R*)-1-[2-[[2,2-Bis(2-methylphenyl)ethenyl]oxy]ethyl]-3-piperidinecarboxylic Acid (45) (Method C, Scheme 4). Bis(2-methylphenyl)acetaldehyde (43). Hexamethyldisilazane (1.55 g, 96 mmol, 1.99 mL) in dry THF (150 mL) was cooled to −70 °C. *n*-BuLi (2.5 M in hexanes) (40 mL, 100 mmol) was added dropwise while keeping the temperature below −65 °C, and the solution was stirred for 1 h at −70 °C. Ethyl bromoacetate (16.0 g, 96 mmol, 10.65 mL) was introduced dropwise over a 10 min period, followed by bis(2-methylphenyl)methanone[10] (16.8 g, 80 mmol) in dry THF (200 mL), again keeping the temperature below −65 °C. After being stirred for 20 min at this temperature, the reaction mixture was allowed to reach 0 °C, and it was poured into a vigorously stirred mixture of H_2O (500 mL) and Et_2O (200 mL). The phases were separated, the aqueous phase was extracted with Et_2O (200 mL), and the combined ether extracts were dried ($MgSO_4$). The residue on evaporation, which contained ethyl 3,3-bis(2-methylphenyl)-2-oxiranecarboxylate **42**, was dissolved in EtOH (100 mL) and saponified by stirring with 10 N aqueous NaOH (40 mL) at room temperature for 0.5 h. The reaction mixture was acidified to pH 2 with aqueous HCl and was extracted with Et_2O (400 mL). The Et_2O phase was extracted twice with a mixture of 10 N aqueous NaOH (8 mL) and H_2O (300 mL). The combined aqueous extracts were acidified with 0.5 M aqueous citric acid, and the mixture was extracted with Et_2O (2 × 200 mL). The combined Et_2O extracts were dried ($MgSO_4$) and evaporated to afford the residual solid 3,3-bis(2-methylphenyl)-2-oxiranecarboxylic acid **41** which was dissolved in 1,4-dioxan (50 mL). Decarboxylation was performed by heating this solution at 130 °C for 2 h, and **43** was obtained as an oil (10.1 g, 56%) following evaporation: ^{1}H NMR ($CDCl_3$) 2.25 (s, 6H), 5.19 (s, 1H), 6.95 (d, 2H), 7.15−7.28 (m, 6H), 10.0 (1H, s).

2,2-Bis(2-methylphenyl)ethenyl 2-Bromoethyl Ether (44). 1,2-Dibromoethane (16.9 g, 90 mmol, 7.75 mL), tetrabutylammonium bromide (1.45 g, 4.5 mmol) and 12 N aqueous NaOH solution (25 mL) were added to a solution of **43** (10.0 g, 45 mmol) in CH_2Cl_2 (50 mL). The two phases were stirred vigorously for 20 h, and the mixture was acidified with 4 N aqueous HCl (75 mL). H_2O (200 mL) was added, and the organic phase was separated before being washed with H_2O (200 mL) and dried ($MgSO_4$). Evaporation (coevaporation with $PhCH_3$ and $PhCH_2CH_3$) provided **44** as an oil (12.8 g, 86%): ^{1}H NMR ($CDCl_3$) 2.20 (s, 6H), 3.37 (2H, t), 4.08 (2H, t) 6.28 (s, 1H), 6.90−7.28 (m, 8H).

(3*R*)-1-[2-[[2,2-Bis(2-methylphenyl)ethenyl]oxy]ethyl]-3-piperidinecarboxylic Acid (45). Ethyl *R*-3-piperidine carboxylate hydrochloride[55] (2.25 g, 11.6 mmol) and powdered, dried K_2CO_3 (6.4 g, 46.3 mmol) were added to a solution of **44** (12.8 g, 38.6 mmol) in acetone (100 mL). The reaction mixture was heated at reflux for 18 h and cooled. Filtration and evaporation provided an oil which was purified by flash chromatography on silica gel (9:1 cyclohexane/EtOAc initially, increasing polarity to 1:1) to provide ethyl (3*R*)-1-[2-[[2,2-bis-(2-methylphenyl)ethenyl]oxy]ethyl]-3-piperidine carboxylate (2.23 g, 47%) as a gum. This ethyl ester was hydrolyzed by dissolution in EtOH (20 mL) and treatment with 10 N aqueous NaOH solution (7 mL) at ambient temperature for 3 h. Water (300 mL) was added, and the mixture was washed with Et_2O (3 × 50 mL) to remove unsaponified material. The aqueous solution was adjusted to pH 5 with 2 N aqueous HCl and then extracted with CH_2Cl_2 (5 × 50 mL), dried ($MgSO_4$), and evaporated to a gum. The residual **45** (1.9 g) was converted into a crystalline hydrochloride salt by the method described for compound **19**. The salt was collected by filtration and recrystallized from H_2O to provide analytically pure **45** as the hydrochloride salt (1.4 g, 62%): mp 217−222 °C; $[\alpha]^{20}_D$ −23.5° (*c* = 0.02, CH_3OH); ^{1}H NMR [$(CD_3)_2SO$] 2.06 (s, 3H), 2.12 (s,

3H), 4.34 (br t, 2H), 6.54 (s, 1H), 7.02−7.20 (m, 8H). Anal. ($C_{22}H_{29}NO_3$·HCl) C, H, N, Cl.

(3*R*)-1-[2-[(2,2-Diphenylethenyl)oxy]ethyl]-3-piperidinecarboxylic Acid (47) (Method D, Scheme 5). Ethyl (3*R*)-1-[2-[(2,2-Diphenylethenyl)oxy]ethyl]-3-piperidinecarboxylate (46). Diphenylacetaldehyde (4.9 g, 25 mmol) was added dropwise to a mixture of NaH (1.5 g, 5 mmol, 80% oil dispersion) and dry $PhCH_3$ (25 mL) at 0 °C. This mixture was stirred at room temperature for 0.5 h, heated to 50 °C, and allowed to cool again to room temperature. *R*-1-(2-Bromoethyl)-3-ethoxycarbonylpiperidine hydrobromide **32** (8.6 g, 25 mmol) was introduced portionwise while the temperature was kept below 30 °C with an ice−water bath. After being stirred for 1 h, the reaction mixture was filtered, and the filtrate was evaporated to a residue. Flash chromatography (4:1 heptane/THF) provided **46** (6.6 g, 69%) as a gum: ^{1}H NMR ($CDCl_3$) 1.22 (t, 3H), 1.43 (q, 1H), 1.55 (dq, 1H), 2.11 (dt, 1H), 2.30 (t, 3H), 2.69 (t, 2H), 2.99 (br d, 1H), 4.03 (t, 2H), 4.10 (q, 2H), 6.51 (s, 1H), 7.18−7.32 (m, 8H), 7.41 (d, 2H).

(3*R*)-1-[2-[[2,2-Diphenylethenyl)oxy]ethyl]-3-piperidinecarboxylic Acid (47). Ethyl (3*R*)-1-[2-[(2,2-Diphenylethenyl)-oxy]ethyl]-3-piperidine carboxylate (3.0 g, 79 mmol) was hydrolyzed as described for compound **14**. The crystalline residue after evaporation was triturated with Et_2O to provide **47** as a white solid (2.65 g, 86%): mp 217−218 °C; $[\alpha]^{20}_D$ −7.6° (c = 0.02, CH_3OH); 1H NMR [$(CD_3)_2SO$] 2.82−3.05 (br m, 2H), 4.42 (dt, 2H), 6.86 (s, 1H), 7.18−7.38 (m, 10H). Anal. ($C_{22}H_{25}ClNO_3$·HCl) C, H, N, Cl.

(3*R*)-1-[2-[[2,2-Bis(3-methyl-2-thienyl)ethenyl]oxy]ethyl]-3-piperidinecarboxylic Acid (13) (Method E, Scheme 6). Ethyl 2-(Trityloxy)ethoxyacetate (48). 2-(Trityloxy)ethanol (3.98 g, 13 mmol) was dissolved in dry THF (50 mL), and a 2.5 M solution of *n*-BuLi in hexanes (5.5 mL, 13.7 mmol) was added at 5 °C. In a separate vessel, a solution of $BrCH_2CO_2H$ (1.81 g, 13 mmol) was treated with a 2.5 M solution of *n*-BuLi in hexanes (5.5 mL, 13.7 mmol) at 5 °C before the two solutions were mixed. This reaction mixture was heated at reflux for 68 h, cooled, and water (200 mL) was added. The reaction mixture was washed with EtOAc (100 mL), and the aqueous phase was acidified with 0.5 M citric acid solution (50 mL). Extraction with EtOAc (2 × 100 mL) and drying of the combined extracts ($MgSO_4$) provided crude 2-(trityloxy)ethoxyacetic acid **49** (2.78 g, 58%). This acid was dissolved in CH_2Cl_2 (30 mL), and dicyclohexyl carbodiimide (1.72 g, 8.3 mmol) was introduced, followed by 4-pyrrolidinopyridine[70] (0.11 g, 0.74 mmol) and ethanol (0.89 mL, 2 equiv). The reaction mixture was stirred for 16 h at room temperature and filtered to remove dicyclohexyl urea. The filtrate was evaporated, and the residue was purified by flash chromatography (100:1 cyclohexane/EtOAc initially, then 30:1) to provide **48** (1.5 g, 50%) as an oil: ^{1}H NMR ($CDCl_3$) 1.24 (t, 3H), 3.24 (t, 2H), 3.66 (t, 2H), 4.06 (s, 2H), 4.08 (q, 2H), 7.12−7.58 (m, 15H).

Ethyl (3*R*)-1-[2-[[2,2-Bis(3-methyl-2-thienyl)ethenyl]-oxy]ethyl]-3-piperidinecarboxylate (52). 2-Bromo-3-methylthiophene[56] (1.5 g, 8.5 mmol) and Mg turnings (0.22 g) were heated gently in anhydrous THF (30 mL), and the reaction rapidly became exothermic. After the resultant Grignard reagent **36** was stirred for 0.2 h under a nitrogen atmosphere, the reaction mixture was heated at reflux for 0.5 h, and ester **48** (1.5 g, 3.8 mmol) was introduced as a solution in anhydrous THF (20 mL). The mixture was again heated at reflux for 0.5 h and then cooled, and saturated aqueous NH_4Cl (100 mL) was carefully added. Stirring for 0.5 h at room temperature was followed by extraction with EtOAc (3 × 70 mL). The combined extracts were dried ($MgSO_4$) and evaporated. The resultant residue was dissolved in a mixture of 2 N aqueous HCl (50 mL), THF (50 mL), and EtOH (50 mL), and the solution was heated at 50 °C for 1 h (to ensure deprotection and alkene formation), before basification to pH 9.5 with aqueous NaOH. The organic solvents were evaporated, and the aqueous residue was extracted with EtOAc (3 × 75 mL). Drying of the combined organic extracts ($MgSO_4$) and evaporation provided an oil, which was purified by flash chromatography (9:1 cyclohexane/EtOAc) to afford 2-[2-(2-hydroxyethoxy)-1-(3-methyl-2-thienyl)ethenyl]-3-methylthiophene **50** (0.54 g, 50%) as a gum. Compound **50** (0.53 g, 19 mmol) was dissolved in dry toluene (20 mL), and the solution was cooled to 0 °C. A solution of *n*-BuLi (2.5 M in hexanes) (0.9 mL, 23 mmol) was introduced, the reaction mixture was allowed to stand at 0 °C for 1 h, and a solution of *p*-toluenesulfonyl chloride (0.47 g, 25 mmol) in $PhCH_3$ (10 mL) was added. The mixture was stirred at room temperature for 20 h, and to the resultant solution of tosylate **51** were added ethyl *R*-3-piperidine carboxylate hydrochloride (0.59 g, 3.8 mmol) and powdered, dried K_2CO_3 (1.04 g, 7.5 mmol). The temperature was increased to 80 °C, and stirring was maintained for 50 h. The reaction mixture was cooled, and water (50 mL) was added. The $PhCH_3$ phase was separated, and the water phase was extracted with EtOAc (50 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated to give an oil, which was purified by flash chromatography (19:1 cyclohexane/EtOAc initially, later increasing polarity to 5:1) to provide **52** (0.25 g, 31%) as a gum: ^{1}H NMR ($CDCl_3$) 1.20 (t, 3H), 1.93 (s, 3H), 1.96 (s, 3H), 2.06 (t, 1H), 2.24 (t, 1H), 2.66 (t, 2H), 4.01 (t, 2H), 4.09 (q, 2H), 6.50 (s, 1H), 6.75 (d, 1H), 6.77 (d, 1H), 7.02 (d, 1H), 7.14 (d, 1H).

(3*R*)-1-[2-[[2,2-Bis(3-methyl-2-thienyl)ethenyl]oxy]ethyl]-3-piperidinecarboxylic Acid (13). Compound **52** (420 mg, 1 mmol) was dissolved in ethanol (20 mL) and hydrolyzed as described for compound **19**. The residue after evaporation was recrystallized from H_2O to provide **13** as a white solid hydrochloride salt: mp 55−70 °C (dec); ^{1}H NMR ($CDCl_3$) 1.26 (q, 1H), 1.40 (q, 1H), 1.91 (s, 3H), 1.93 (s, 3H), 1.97 (t, 1H), 2.09 (t, 1H), 2.33 (br t, 1H), 2.24 (t, 1H), 2.54 (t, 2H), 2.70 (br d, 1H), 2.90 (br d, 1H), 3.41 (t, 1H), 4.02 (t, 2H), 4.09 (q, 2H), 6.67 (s, 1H), 6.79 (d, 1H), 6.81 (d, 1H), 7.23 (d, 1H), 7.32 (d, 1H). Anal. ($C_{20}H_{25}NO_3S_2$·0.75H_2O) C, H, N, S, Cl.

Electrostatic Potential Calculations. Geometry optimization was performed by using the semiempirical method AM1 (Figure 2). Electrostatic potentials were based upon the Hartree−Fock 6-31G** ab initio procedure[52] implemented in the program Spartan.

Pharmacological Methods. Synaptosomal [^{3}H]-GABA Uptake. Uptake of [^{3}H]-GABA into synaptosomal preparations was assayed by a filtration assay.[39] Rat forebrain was rapidly excised and homogenized in 20 mL of ice-cold 0.32 M sucrose with a hand-driven Teflon/glass Potter-Elvehjem homogenizer. The homogenate was centrifuged for 10 min at 600*g* at 4 °C. The pellet was resuspended in 50 volumes of ice-cold buffer (120 mM NaCl, 0.18 mM KCl, 2.30 mM $CaCl_2$, 4.0 mM $MgSO_4$, 12.66 mM Na_2HPO_4, 2.97 mM NaH_2PO_4, and 10.0 mM glucose, pH 7.4) at 4 °C. Fifty microliters of this synaptosomal suspension (0.1 mg protein), diluted into 300 mL of phosphate buffer and 100 mL of test substance solutions in water, was preincubated for 8 min at 30 °C. Then 50 mL of [^{3}H]-GABA (final concentration 0.9 nM) and unlabeled GABA (final concentration 0.9 nM) was added before continuing incubation for another 8 min. Synaptosomes were then recovered by rapid filtration through Whatman GF/F glass fiber filters under vacuum. Filters were washed twice, each time with 10 mL of ice-cold isotonic saline, and the tritium trapped on the filters was assessed by conventional scintillation counting in 4 mL of FilterCount (Packard). Noncarrier-mediated uptake was determined in the presence of nipecotic acid (500 mM) and was subtracted from total binding to give carrier-mediated [^{3}H]-GABA uptake. IC_{50} values were calculated from dose−response curves (3 points minimum), and inhibitory constants (K_i) were calculated using the equation: $K_i = IC_{50}/(1 + [L]/K_m)$, where [L] is the concentration of [^{3}H]-GABA (25 nM) and K_m the affinity constant for GABA (3700 nM).[71] The K_i value (with standard error) obtained for each compound is shown in Tables 1−4.

DMCM-Induced Seizures. These tests were conducted as described previously.[46,47] Briefly, male or female NMRI mice (Bomholdtgaard, Ry, Denmark) weighing 20−25 g were dosed with 15 mg/kg DMCM i.p. 30 min after i.p. administration of test compound. Eight mice were tested per dose. The number

of mice failing to exhibit clonic seizures within 15 min were considered to be protected.

Motor Impairment. These tests were conducted as described previously.[47] Briefly, NMRI mice were trained for 2 min to stay on top of a rotating rod (6 rev/min) as the rod rotated toward the animal. After a 2 min training period mice (n = 8/dose) were administered the test compound, as described in the DMCM seizure tests above, and were then placed on the rotating rod. Each mouse was observed for 2 min, and a mouse that fell off the rod twice or more was considered ataxic.

Drugs. Compounds were suspended in duphasol-x or dissolved in distilled water and were administered in a volume of 10 mL/kg. DMCM (a generous gift from Schering AG, Berlin, Germany, courtesy of Dr. Sprzagala) was dissolved in 0.02 N saline and was delivered in a volume of 15 mL/kg.

Data Analysis. ED_{50} values were the doses (mg/kg) that protected 50% of the mice against seizures or the dose producing falls from the rotating rod in 50% of the mice, respectively.

Acknowledgment. We acknowledge the contribution of Claus Bruun Jensen, Jan Sørensen, Freddy Pedersen, Paw Bloch, Charlotte Gade, and Karina Madsen for synthesis of novel compounds, Dorthe Andersen for determination of K_i values, and Hanne Nielsen and Wei Liu for in vivo pharmacology assistance. The encouragement and helpful comments of Prof. Claus Braestrup are acknowledged.

Supporting Information Available: X-ray structural data for 2-methylphenyl, 4-methyl-2-thienylmethanone oxime, **30**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) This work was initially presented in part as posters at the 33rd. National Organic Chemistry Symposium in Bozeman, Montana, June 1993, and at the Gordon Conference, Medicinal Chemistry, New London, New Hampshire, August 1997. For part 2, see: Andersen, K. E.; Begtrup, M.; Chorgade, M. S.; Lee, E. C.; Lau, J.; Lundt, B. F.; Petersen, H.; Sørensen, P. O.; Thøgersen, H. The Synthesis of Novel GABA uptake inhibitors. Part 2. Synthesis of 5-hydroxytiagabine, a Human Metabolite of the GABA Reuptake Inhibitor Tiagabine. *Tetrahedron* **1994**, *50*, 8699−8710.

(2) Costa, E. Building a Bridge Between Neurobiology and Mental Illness. *J. Psychiat. Res.* **1992**, *26*, 449−460.

(3) DeLorenzo, R. Mechanisms of Action of Anticonvulsant Drugs. *Epilepsia* **1988**, *29*, S35−S47.

(4) Natsch, S.; Hekster, Y. A.; Keyser, A.; Deckers, C. L. P.; Meinardi, H.; Renier, W. O. Newer anticonvulsant drugs: role of pharmacology, drug interactions and adverse reactions in drug choice. *Drug Saf.* **1997**, *17*, 228−240.

(5) Walker, M. C.; Patsalos, P. N. Update on novel antiepileptic drugs. *Emerging Drugs* **1997**, *2*, 381−393.

(6) Gram, L. Tiagabine: A Novel Drug with a GABAergic Mechanism of Action. *Epilepsia* **1994**, *35*, S85−S87.

(7) Rogawski, M. A.; Porter, R. J. Antiepileptic Drugs: Pharmacological Mechanisms and Clinical Efficacy with Consideration of Promising Development Stage Compounds. *Pharmacol. Rev.* **1990**, *42*, 223−286.

(8) Upton, N. Mechanisms of Action of New Antiepileptic Drugs: Rational Design and Serendipitous Findings. *TIPS* **1994**, *15*, 456−462

(9) Chadwick, D. W. An overview of the efficacy and tolerability of new antiepileptic drugs. *Epilepsia* **1997**, *38*, S59−S62.

(10) Andersen, K. E.; Braestrup, C.; Grønwald, F. C.; Jørgensen, A. S.; Nielsen, E. B.; Sonnewald, U.; Sørensen, P. O.; Suzdak, P. D.; Knutsen, L. J. S. The Synthesis of Novel GABA uptake inhibitors, 1. Elucidation of the Structure−Activity Studies leading to the choice of *R*1[4,4-bis(3-Methyl-2-thienyl)-3-piperidine carboxylic acid (Tiagabine) as an Anticonvulsant Drug Candidate. *J. Med. Chem.* **1993**, *36*, 1716 1725.

(11) Mengel, H. Tiagabine. *Epilepsia* **1994**, *29*, S81−S84.

(12) Taylor, C. P. GABA Receptors and GABAergic Synapses as Targets for Drug Development. *Drug Dev. Res.* **1990**, *21*, 151−160.

(13) 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 CI966: A Potent, Orally-Active Inhibitor of GABA Uptake. *Drug Dev. Res.* **1990**, *21*, 189193.

(14) Pavia, M. R.; Lobbestael, S. J.; Nugiel, D.; Mayhugh, D. R.; Gregor, V. E.; Taylor, C. P.; Schwarz, R. D.; Brahce, L.; Vartanian, M. G. Structure−activity studies on benzhydrol-containing nipecotic acid and guvacine derivatives as potent, orally active inhibitors of GABA uptake. *J. Med. Chem.* **1992**, 35, 4238−4248.

(15) Taylor, C. P.; Vartanian, M. G.; Schwarz, R. D.; Rock, D. M.; Callahan, M. J.; Davis, M. D. Pharmacology of CI966: A Potent GABA Uptake Inhibitor, In Vitro and in Experimental Animals. *Drug Dev. Res.* **1990**, *21*, 195−215.

(16) Sedman, A. J.; Gilmet, G. P.; Sayed, A. J.; Posvar, E. L. Initial Human Safety and Tolerance Study of a GABA Uptake Inhibitor, CI966: Potential Role of GABA as a Mediator in the Pathogenesis of Schizophrenia and Mania. *Drug Dev. Res.* **1990**, *21*, 235−242.

(17) Suzdak, P. Lipophilic GABA Uptake Inhibitors: Biochemistry, Pharmacology and Therapeutic Potential. *Drugs Fut.* **1993**, *18*, 1129−1136.

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

(19) Nielsen, E. B.; Suzdak, P. D.; Andersen, K. E.; Knutsen, L. J. S.; Sonnewald, U.; Braestrup, C. Characterization of Tiagabine (NO-328), a New Potent and Selective GABA Uptake Inhibitor. *Eur. J. Pharmacol.* **1991**, *196*, 257266.

(20) Suzdak, P. D.; Swedberg, M. D. B.; Andersen, K. E.; Knutsen, L. J. S.; Braestrup, C. In vivo Labeling of the Central GABA Uptake Carrier with ^{3}H-Tiagabine. *Life Sci.* **1992**, *51*, 1857−1868.

(21) Smith, S. E.; Parvez, N. S.; Chapman, A. G.; Meldrum, B. S. The γ-Aminobutyric Acid Uptake Inhibitor, Tiagabine, is Anticonvulsant in Two Animal Models of Reflex Epilepsy. *Eur. J. Pharmacol.* **1995**, *273*, 259−265.

(22) Halonen, T.; Nissinen, J.; Jansen, J. A.; Pitkanen, A. Tiagabine prevents seizures, neuronal damage and memory impairment in experimental status epilepticus. *Eur. J. Pharmacol.* **1996**, *299*, 69−81.

(23) Dalby, N. O.; Nielsen, E. B. Tiagabine exerts an epileptogenic effect in amygdala kindling epileptogenesis in the rat. *Neurosci. Lett.* **1997**, *229*, 135−137. Morimoto, K.; Sato, H.; Yamamoto, Y.; Watanabe, T.; Suwaki, H. Antiepileptic effects of tiagabine, a selective GABA uptake inhibitor, in the rat kindling model of temporal lobe epilepsy. *Epilepsia* **1997**, *38*, 966−974.

(24) Suzdak, P. D.; Jansen, J. A. A review of the preclinical pharmacology of tiagabine − a potent and selective anticonvulsant GABA uptake inhibitor. *Epilepsia* **1995**, *36*, 612−626.

(25) Schousboe, A.; Larsson, O. M.; Krogsgaard-Larsen, P. GABA Uptake Inhibitors as Anticonvulsants. In *GABA Mechanisms in Epilepsy*; Tunnicliff, G., Raess, B. U., Eds.; Wiley-Liss: New York, 1991; p 165−187. Update on the mechanism of action of antiepileptic drugs.

(26) Bauer, J.; Stawowy, B.; Lenders, T.; Bettig, U.; Elger, C. E. Efficacy and tolerability of tiagabine − results of an add-on study in patients with refractory partial seizures. *J. Epilepsy* **1995**, *8*, 83−86.

(27) Meldrum, B. S. Update on the mechanism of action of antiepileptic drugs. *Epilepsia* **1996**, *37*, S4−S11.

(28) Benmenachem, E. International experience with tiagabine add-on therapy. *Epilepsia* **1995**, *36*, S14−S21.

(29) Tiagabine, Gabitril, Tiabex. *Drugs Fut.* **1997**, *22*, 1396−1397.

(30) Sachdeo, R. C.; Biton, V.; Boellner, S. W.; Schachter, S. C.; Alto, G. H.; Kaply, C. W.; Phillips, H. B.; Kardatzke, D.; Pixton, G.; Sommerville, K. W. Long-term safety of tiagabine·HCl. *Epilepsia* **1995**, *36*, 283−283.

(31) Uthman, B.; Rowan, A. J.; Ahmann, P.; Wannamaker, B.; Schachter, S.; Rask, C. Safety and efficacy of 3 dose levels of tiagabine·HCl versus placebo as adjunctive treatment for complex partial seizures. *Ann. Neurol.* **1993**, *34*, 272.

(32) Dodrill, C. B.; Arnett, J. L.; Sommerville, K. W.; Shu, V. Cognitive and quality-of-life effects of differing dosages of tiagabine in epilepsy. *Neurology* **1997**, *48*, 1025−1031.

(33) Mengel, H. B.; Pierce, M. W.; Mant, T.; Christensen, M. S.; Gustafson, L. Tiagabine Safety and Tolerance During 2-weeks Multiple Dosing to Healthy Volunteers. *Epilepsia* **1991**, *32* (Suppl. 1).

(34) Chadwick, D.; Richens, A.; Duncan, A.; Dam, M.; Gram, L.; Morrow, J.; Mengel, H.; Shu, V.; Mc. Kelvy, J. F.; Pierce, M. W. Tiagabine Hydrochloride. Safety and Efficacy as Adjunctive Treatment for Complex Partial Seizures. *Epilepsia* **1991**, *32* (Suppl. 3).

(35) Ali, F. E.; Bondinell, W. E.; Danbridge, 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 Gamma Aminobutyric Acid uptake. *J. Med. Chem.* **1985**, *28*, 653−660.

(36) Yunger, 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. Pharm. Exp. Ther.* **1984**, *228*, 109−115.

(37) Krogsgaard-Larsen, P.; Hjeds, H.; Falch, E.; Joergensen, F. S.; Nielsen, L. Recent Advances in GABA Agonists, Antagonists and Uptake Inhibitors: Structure−Activity Relationships and Therapeutic Potential. *Adv. Drug. Res.* **1988**, *17*, 381−456.

(38) Krogsgaard-Larsen, P.; Falch, E.; Larsson, O. M.; Schousboe, A. GABA Uptake Inhibitors; Relevance to Antiepileptic Drug Research. *Epilepsy Res.* **1987**, *1*, 7793.

(39) Fjalland, B. Inhibition by Neuroleptics of Uptake of [^{3}H]-GABA into Rat Brain Synaptosomes. *Acta Pharmacol. Toxicol.* **1978**, *42*, 7376.

(40) Falch, E.; Krogsgaard-Larsen, P. GABA Uptake Inhibitors. Syntheses and Structureactivity Studies on GABA Analogues Containing Diarybutenyl and Diarylmethoxyalkyl N-substituents. *Eur. J. Med. Chem.* **1991**, *26*, 6978.

(41) N'Goka, V.; Schlewer, G.; Linget, J.-M.; Chambon, J.-P.; Wermuth, C.-G. GABA-Uptake Inhibitors: Construction of a General Pharmacophore Model and Successful Prediction of a New Representative. *J. Med. Chem.* **1991**, *34*, 2547−2557.

(42) Falch, E.; Larsson, O. M.; Schousboe, A.; Krogsgaard-Larsen, P. GABA-A agonists and GABA Uptake Inhibitors: Structure−Activity Relationships. *Drug Dev. Res.* **1990**, *21*, 169−188.

(43) Dhar, T. G. M.; Borden, L. A.; Tyagarajan, S.; Smith, K. E.; Branchek, T. A.; Weinshank, R. L.; Gluchowski, C. Design, Synthesis and Evaluation of Substituted Triarylnipecotic Acid Derivatives as GABA Uptake Inhibitors: Identification of a Ligand with Moderate Affinity and Selectivity for the Cloned Human GABA Transporter GAT-3. *J. Med. Chem.* **1994**, *37*, 2334. See also refs 48−51 and Dhar, T. G. M.; Nakanishi, H.; Borden, L. A.; Gluchowski, C. On the bioactive conformation of the GABA uptake inhibitor SK&F 89976A. *BioMed. Chem. Lett.* **1996**, 1535−1540.

(44) Knutsen, L. J. S.; Andersen, K. E.; Jørgensen, A. S.; Sonnewald, U. Azacyclic Carboxylic Acid Derivatives, Their Preparation and Use. Novo Nordisk A/S, U.S. Patent 5,039,685, August 13, 1991.

(45) Knutsen, L. J. S.; Jørgensen, A. S.; Andersen, K. E.; Sonnewald, U. N-Substituted Azaheterocyclic Carboxylic Acids and Pharmaceutical Uses. Novo Nordisk A/S, U.S. Patent 5,071,859, December 10, 1991.

(46) Klitgaard, H.; Knutsen, L. J. S.; Thomsen, C. Contrasting effects of adenosine A_1 and A_2 receptor ligands in different chemoconvulsive models. *Eur. J. Pharmacol.* **1993**, *224*, 221−228.

(47) Suzdak, P. D.; Frederiksen, K.; Andersen, K. E.; Sørensen, P. O.; Knutsen, L. J. S.; Nielsen, E. B. NNC-711, a Novel Potent and Selective GABA Uptake Inhibitor: Pharmacological Characterization. *Eur. J. Pharmacol.* **1992**, *223*, 189−198. For DMCM method descriptions, see also ref 19 and Swedberg, M. D. B.; Jacobsen, P.; Honoré, T. Anticonvulsant, anxiolytic and discriminative effects of the AMPA antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[*f*]quinoxaline (NBQX). *J. Pharmacol. Exp. Ther.* **1995**, *274*, 1113−1121.

(48) Borden, L. A. GABA transporter heterogeneity: pharmacology and cellular localization. *Neurochem. Int.* **1996**, *29*, 335−356. Note that this article and ref 43 utilize different terminology for the GAT transporters from that in ref 49.

(49) Liu, Q. R.; López-Corcurera, B.; Mandiyan, S.; Nelson, H.; Nelson, N. Molecular Characterization of Four Pharmacologically Distinct γ-Aminobutyric Acid Transporters in Mouse Brain. *J. Biol. Chem.* **1993**, *268*, 2106-2112.

(50) Borden, L. A.; Dhar, T. G. M.; Smith, K. E.; Weinshank, R. L.; Branchek, T. A.; Gluchowski, C. Tiagabine, SK&F 89976A, CI-966, and NNC-711 are selective for the cloned GABA transporter GAT-1. *Eur. J. Pharmacol.* **1994**, *269*, 219−224.

(51) Bennett, E. R.; Kanner, B. I. The membrane topology of GAT-1, a (Na^+Cl^-) -coupled gamma-aminobutyric acid transporter from rat brain. *J. Biol Chem.* **1997**, *272*, 1203−1210.

(52) Program Spartan Version 4.0, available from Wave Function, Inc., 18401 Von Karman, Suite 370, Irvine, CA 92715. Ab initio level used for the calculations was HF 6-31G**.

(53) NNC 05-0711[47] is marketed as a pharmacological tool by Research Biochemicals, Inc., One Strathmore Road, Natick, MA 017602418.

(54) Richards, D. A.; Bowery, N. G. Comparative effects of the GABA uptake inhibitors, tiagabine and NNC-711, on extracellular GABA levels in the rat ventrolateral thalamus. *Neurochem. Res.* **1996**, *21*, 135−140.

(55) Akkerman, A. M.; De Jongh, D. K.; Veldstra, H. Synthetic Oxytocics. I. 3-(Piperidyl-(N)-Methyl)indoles and Related Compounds. *Rec. Trav. Chim.* **1951**, *70*, 899−916. Bettoni, G.; Duranti, E.; Tortorella, V. Absolute Configuration and Optical Purity of 3-Substituted Piperidines. *Gazz. Chim. Ital.* **1972**, *102*, 189195.

(56) Kellogg, R. M.; Schaap, A. P.; Harper, E. T.; Wynberg, H. Acid-Catalysed Brominations, Deuterations, Rearrangements of Thiophenes under Mild Conditions. *J. Org. Chem.* **1968**, *33*, 2902−2909.

(57) Buehler, E. Alkylation of *syn*- and *anti*-Benzaldoximes. *J. Org. Chem.* **1967**, *32*, 261−264.

(58) See, for example: Laforest, J.; Thuiller, G. Etude de la configuration d'oximes a activite cardiotrope. *J. Heterocycl. Chem.* **1977**, *14*, 793−796; Haney, W. G.; Brown, R. G.; Isaacson, E. I.; Delgado, J. N. Synthesis and Structure Activity Relationships of Selected Isomeric Oxime *O*-Ethers as anticholinergic agents. *J. Pharm. Sci.* **1977**, *66*, 1602−1606. Conde, S.; Corral, C.; Lissavetzky, J. *E*- and *Z*-isomerism of 2-acetylthiophene oximes. *J. Heterocycl. Chem.* **1985**, *22*, 301−304.

(59) (a) Ashwood, M. S.; Bell, L. A.; Houghton, P. G.; Wright, S. H. B. Synthesis of 1,1-Diaryl-2,-2-dimethoxyethanes. *Synthesis* **1988**, 379−381. (b) Borch, R. F. A New Procedure for the Darzen's Synthesis of Glycidic Esters. *Tetrahedron Lett.* **1972**, *36*, 3761−3763. (c) Martin, S. F. Synthesis of Aldehydes, Ketones and Carboxylic Acids from Lower Carbonyl Compounds by C−C Coupling Reactions. *Synthesis* **1979**, 633−665. (d) Maruoka, K.; Nagahara S.; Ooi, T.; Yamamoto, H. An Efficient, Catalytic Procedure for Epoxide Rearrangement. *Tetrahedron Lett.* **1989**, *30*, 5607−5610. (e) Meyers, A. I.; Jagdmann, G. E. Enamidines. Versatile Vehicles for Homologation of Carbonyl Compounds. *J. Am. Chem. Soc.* **1982**, *104*, 877−879. (f) Mateson, D. S.; Moody, R. J. Homologation of Carbonyl Compounds to Aldehydes with Lithium Bis(ethylenedioxyboryl)methide. *J. Org. Chem.* **1980**, *45*, 1091−1095.

(60) Blicke, F. F.; Faust, J. A. Antispasmodics. XV. β-Diethylaminoethyl Esters of β,β-Diphenylglycidic, β,β-Diphenyllactic and β,β-Diphenylglyceric Acids. *J. Am. Chem. Soc.* **1954**, *76*, 3156−3159.

(61) Ridogrel (R-68070). *Drugs Fut.* **1991**, *16*, 488−489.

(62) vant Reit, B.; Elford, H. L. Amidox. *Drugs Fut.* **1991**, *16*, 990−991.

(63) De Chaffoy de Courcelles, D.; De Loore, K.; Freyne, E.; Janssen, P. A. J. Inhibition of human cardiac cyclic AMP-phosphodiesterases by R 80122, a new selective cyclic AMP-phosphodiesterase III inhibitor: a comparison with other cardiotonic compounds**.** *J. Pharmacol. Exp. Ther.* **1992**, *263*, 6−14.

(64) Schwarz, R. D.; Davis, R. E.; Jaen, J. C.; Spencer, C. J.; Tecle, H.; Thomas, A. J. Characterization of muscarinic agonists in recombinant cell lines. *Life Sci.* **1993**, *52*, 465−72.

(65) Nielsen, L.; Brehm, L.; Krogsgaard-Larsen, P. GABA agonists and uptake inhibitors. Synthesis, absolute stereochemistry, and enantioselectivity of (*R*)-(−)- and (*S*)-(+)-homo-β-proline *J. Med. Chem.* **1990**, *33*, 71−7.

(66) Dalby, N. O.; Thomsen, C.; Fink Jensen, A.; Lundbeck, J.; Sokilde, B.; Man, C. M.; Sorensen, P. O.; Meldrum, B. Anticonvulsant properties of two GABA uptake inhibitors NNC 05-2045 and NNC 05-2090, not acting preferentially on GAT-1. *Epilepsy Res.* **1997**, *28*, 51−61.

(67) Thomsen, C.; Sorensen, P. O.; Egebjerg, J. 1-(3-(9H-Carbazol-9-yl)-1-propyl)-4-(2-methoxyphenyl)-4-piperidinol, a novel subtype-selective inhibitor of the mouse type-II GABA transporter. *Br. J. Pharmacol.* **1997**, *120*, 983−985.

(68) Still, W. C.; Kahn, M.; Mitra, A. Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. *J. Org. Chem.* **1978**, *43*, 29235.

(69) Morlacchi, F.; Cardellini, M.; Liberatore, F. Preparation of unsaturated heterocyclic compounds, II. Synthesis of guvacine. *Ann. Chim.* **1967**, *57*, 1456−1461.

(70) Hassner, A.; Alexanian, V. Direct room temperature esterification of carboxylic acids. *Tetrahedron Lett.* **1978**, *46*, 4475−4478.

(71) Braestrup, C.; Nielsen, E. B.; Sonnewald, U.; Knutsen, L. J. S.; Andersen, K. E.; Jansen, J. A.; Frederiksen, K.; Andersen, P. H.; Mortensen, A.; Suzdak, P. D. (R)-*N*-(4,4-Bis-(3-Methyl-2-Thienyl)-but-3-en-1-yl)nipecotic acid binds with high affinity to the brain γ-aminobutyric acid uptake carrier. *J. Neurochem.* **1990**, *54*, 639−647.

JM981027K