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GABA uptake inhibitors. Syntheses and structure–activity studies on GABA analogues containing diarylbutenyl and diarylmethoxyalkyl *N*-substituents

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Summary — A number of analogues of GABA or ß-alanine containing 4,4-diphenyl-3-butenyl (DPB), benzhydryl ethyl ether (BEE), or benzhydryl propyl ether *N*-substituents have been synthesized and tested as inhibitors of synaptosomal GABA uptake. Whereas the *N*-DPB and *N*-BEE analogues of GABA are markedly less potent than GABA itself as inhibitors of GABA uptake, *N*-methylation of these analogues resulted in increased potency and reduced p*K*a II values of the reaction products **4a** and **8a**, respectively. Incorporation of the alkyl groups of the benzhydryl alkyl ether moieties of *N*-methyl-*N*-BEE-ß-alanine (**12**), *N*-methyl-*N*-BEE-GABA (**8a**), or the benzhydryl propyl ether analogue of *N*-methyl-GABA (**10**) into the cyclized piperidine analogues gave the less active compounds **17**, **18**, and **19**, respectively. This loss of *in vitro* activity was most pronounced for the GABA analogues **18** and **19**. These results suggest that the basic character of the amino groups as well as the conformational flexibility of the spacer-arm connecting the amino acid 'heads' and the aromatic moieties of this class of GABA uptake inhibitors are factors of importance for GABA uptake affinity.

Résumé — Inhibiteurs de capture du GABA. Synthèse et étude de structure–activité d'analogues du GABA renfermant des substituants diarylbutényle et diarylméthoxyalkyle à l'azote. *Un certain nombre d'analogues du GABA ou de la ß-alanine présentant des substituants à l'azote tels que 4,4-diphényl-3-butényle (DPB), benzhydryl éthyl éther (BEE) ou benzhydryl propyl éther ont été synthétisés et essayés comme inhibiteurs de la capture du GABA synaptosomial. Tandis que les analogues N-DPB et BEE sont nettement moins puissants que le GABA lui-même comme inhibiteurs de la capture du GABA, la* N-*méthylation de ces analogues augmente la puissance et réduit les valeurs de* p*K*a *II des produits* ***4a*** *et* ***8a****. L'incorporation des groupes alkyles des parties benzhydryl alkyl éther de la* N-*méthyl-*N-*BEE-ß-alanine* (***12***), N-*méthyl-*N'-*BEE-GABA* (***8a***) *ou l'analogue benzhydryl propyl éther du* N-*méthylGABA* (***10***) *dans les analogues cyclisés pipéridiniques donnait respectivement les composés moins actifs* ***17***, ***18*** *et* ***19****. Cette perte d'activité* in vitro *est plus prononcée pour les analogues du GABA* ***18*** *et* ***19****. Ces résultats suggèrent que le caractère basique des groupes aminés aussi bien que la flexibilité conformationnelle du bras reliant les têtes aminoacides et les parties aromatiques de cette classe d'inhibiteurs de capture du GABA sont des facteurs importants.*

GABA uptake / GABA uptake inhibitors / nipecotic acid / *N*-4,4-diphenyl-3-butenyl analogues / *N*-diarylmethoxyalkyl analogues / *N*-benzhydryl ether analogues / ether-alkene bioisosterism / structure–activity studies / active conformations

Introduction

Several lines of direct and indirect evidence support the view that dysfunction(s) of central inhibitory 4-aminobutyric acid (GABA) synapses are major causative factors in epilepsy[1–6]. Although the nature of the apparent abnormalities in GABAergic mechanisms in epileptic human tissues are not fully understood [7], these aspects have focused much interest on GABA synaptic mechanisms as potential targets for clinically useful anticonvulsants [8, 9].

Direct stimulation of the postsynaptic $GABA_A$ receptors by agonists may not represent the most suitable therapeutic approach to epileptic diseases [8–13]. Central $GABA_A$ receptors are of the fast type (class I receptors) [14], and it is apparently difficult to maintain relatively normal $GABA_A$ synaptic function and to avoid rapid detector desensitization by administration of $GABA_A$ receptor agonists. Partial $GABA_A$ agonists may have better prospects as future antiepileptic drugs than full agonists [9].

Traditionally used antiepileptic drugs such as benzodiazepines, barbiturates, and phenytoin have been shown to interact with and facilitate the function of the postsynaptic $GABA_A$ receptor complex, and

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these compounds have proved to be useful, though not ideal, anti-epileptic drugs [1–5, 7, 15]. In principle, the most flexible attempt to re-establish normal function of hypoactive GABA synapses may be inhibition of the GABA uptake mechanisms [9, 16], which are responsible for the termination of GABA neurotransmission processes [16, 17].

The potent GABA uptake inhibitors nipecotic acid (fig 1) and related cyclic amino acids [9, 16] do not easily penetrate the blood-brain barrier (BBB) [9, 16, 18, 19]. We have shown earlier that certain types of ester pro-drugs of nipecotic acid are systemically active in different experimental models of epilepsy [16, 20, 21]. In an alternative approach to convert amino acid GABA uptake inhibitors into compounds capable of penetrating the BBB, incorporation of the bulky and lipophilic 4,4-diphenyl-3-butenyl (DPB) substituent on the amino groups of nipecotic acid and related cyclic GABA uptake inhibitors [22] (fig 1) provided GABA uptake inhibitors such as SKF-89976-A, which were not only systemically active but also 10-20 times more potent *in vitro* than the parent amino acids [23]. The mechanism(s) underlying this latter effect are enigmatic. Structural modifications on the DPB substituent was shown to result in substantial loss of activity [22, 23].

Whereas reduction of the double bond in the side chain of SKF-89976-A results in an almost complete loss of effect on GABA uptake [22], replacement of the double bond of this uptake inhibitor by an ether

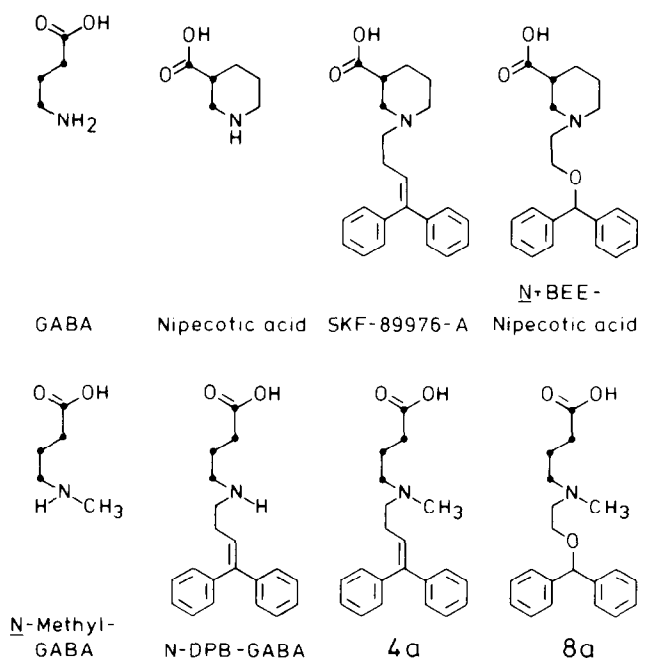


Fig 1. The structures of GABA and some GABA uptake inhibitors.

group to give the benzhydryl ethyl ether (BEE) analogue of nipecotic acid had relatively little effect on biological activity [24]. Thus, *N*-BEE-nipecotic acid (fig 1) was only 7 times weaker than SKF-89976-A as an inhibitor of GABA uptake [24] (table III).

Quite surprisingly, the *N*-DPB analogue of GABA [22] (fig 1) as well as the *N*-BEE analogue of GABA [24] proved to be less active than GABA itself as an inhibitor of GABA uptake (table III). These observations suggest that the presence of a tertiary amino group in the *N*-DPB and *N*-BEE analogues of GABA uptake inhibitors is a pre-requisite for effective interaction with the GABA uptake systems. Other structural parameters may, however, also be of importance in this regard. We have synthesized and tested *in vitro* a number of analogues of *N*-DPB-GABA and, in particular, of *N*-BEE-GABA in an attempt to shed some light on the relationship between the affinities for the GABA uptake systems of these compounds and their protolytic properties, structures, and conformational mobilities.

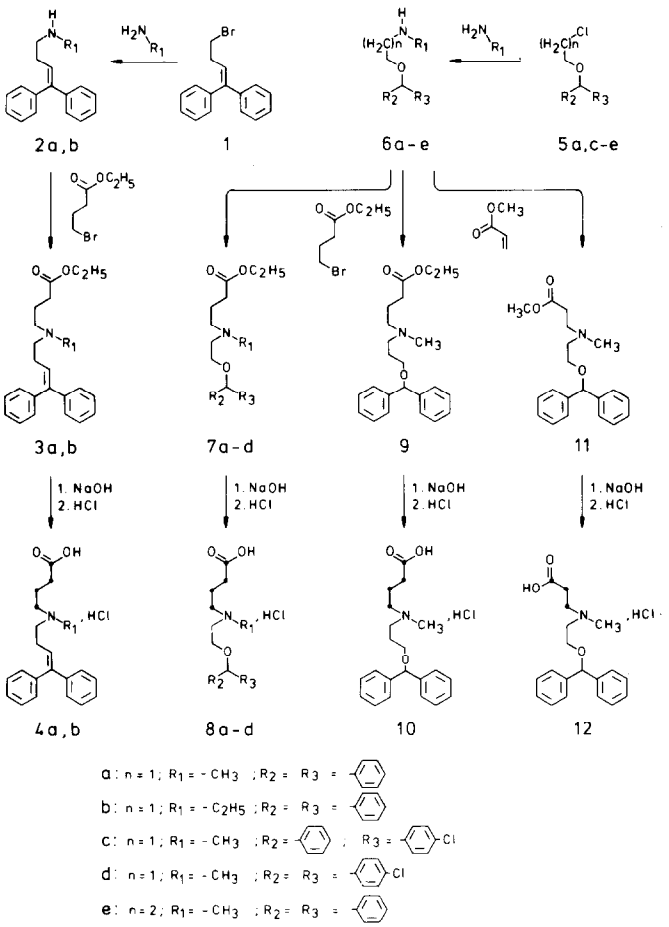
Chemistry

N-Methyl-*N*-(4,4-diphenyl-3-butenyl)-4-aminobutyric acid hydrochloride (**4a**) and the corresponding *N*-ethyl analogue (**4b**) were synthesized *via* alkylation of the secondary amines **2a**, **b** with ethyl 4-bromobutyrate (scheme 1). Whereas attempts to hydrolyze **3a**, **b** by aqueous hydrochloric or hydrobromic acid caused pronounced decomposition, basic hydrolysis led to the final products, the hydrochlorides of which were extractable from aqueous solutions using methylene chloride.

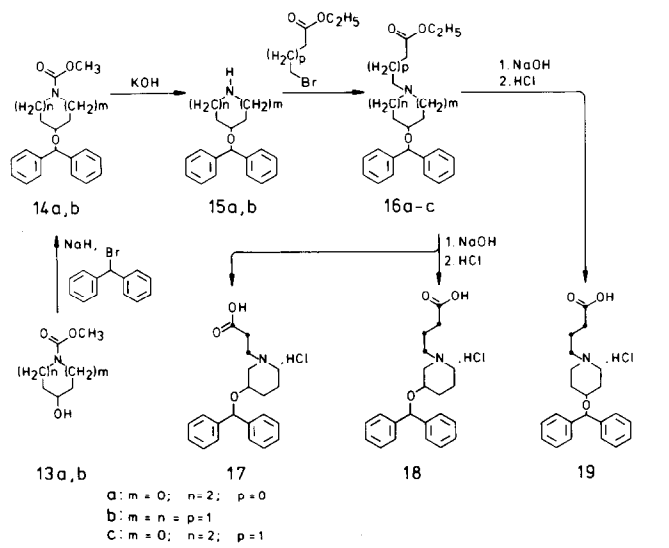
Using the *N*-alkyl-diarylmethoxyalkylamines **6a–e** (table I) as intermediates, analogous alkylation reactions and subsequent hydrolyses of the intermediates **7a–d** and **9** under basic conditions afforded the *N*-alkyl-*N*-BEE-GABA analogues **8a–d** and **10**, respectively, which were also isolated as hydrochlorides (table II). Treatment of the amino acid esters **7a–d** and **9** with mineral acids (scheme 1) led to even more extensive decomposition than in the case of **3a**, **b**. Conjugate addition of methyl acrylate to **6a** and subsequent base-catalyzed hydrolysis of the product **11** gave *N*-methyl-*N*-BEE-ß-alanine (**12**).

The *N*-protected hydroxypiperidines **13a**, **b** were converted into the benzhydryl ethers **14a**, **b**, which upon treatment with potassium hydroxide gave the secondary amines **15a**, **b** (scheme 2). The final products **17–19** (table II) were synthesized following reaction sequences analogous to those outlined in scheme 1.

The structures of all new compounds were confirmed by IR and ^{1}H NMR spectroscopic data supported by elemental analyses (tables I and II).



Scheme 1.



Scheme 2.

In vitro pharmacology

The affinity of the compounds synthesized and the reference compounds GABA, *N*-methyl-GABA [25], *N*-DPB-GABA [22], *N*-BEE-GABA [24], nipecotic acid [16, 26], *N*-DPB-nipecotic acid (SKF-89976-A) [22, 23], and *N*-BEE-nipecotic acid [24] were tested as inhibitors of GABA uptake *in vitro* (table III). A crude synaptosomal preparation isolated from rat brains was used as the test system [27, 28].

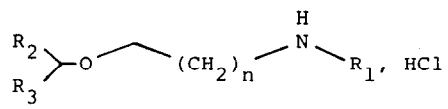
Whereas SKF-89976-A (*N*-DPB-nipecotic acid) is markedly and *N*-BEE-nipecotic acid significantly more potent than nipecotic acid itself, *N*-DPB- as well as *N*-BEE-GABA are weaker than GABA as inhibitors of synaptosomal GABA uptake (table III). *N*-Methyl-GABA, on the other hand, is much weaker than GABA in this test system, but introduction of the DPB or the BEE substituent onto the amino group of this GABA analogue gave compounds **4a** and **8a**, respectively, with substantially increased GABA uptake affinity. Compounds **4b** and **8b**, which contain ethyl substituents on the amino groups, are weaker than the corresponding *N*-methylated analogues **4a** and **8a**.

Introduction of an additional methylene group into the spacer-arm between the amino acid 'head' and the aromatic moiety of SKF-89976-A results in a marked loss of GABA uptake affinity [22]. An analogous homologation of **8a** has less dramatic consequences, compound **10** being about 3-fold weaker than **8a** (table III). Replacement of 1 of the phenyl groups of **4a** by a 4-chlorophenyl group also reduces activity, **8c** being 5 times weaker than **8a**, but, quite surprisingly, repetition of this substitution to give **8d** does not reduce activity further. In the series of *N*-BEE-nipecotic acid analogues, replacement of the phenyl groups by 4-chlorophenyl groups increased potency 5 times [24].

Compound **12**, in which the GABA structure element has been replaced by ß-alanine, is almost an order of magnitude weaker than **8a** as an inhibitor of GABA uptake. However, ring closure of **12** to the piperidine analogue **17** is only accompanied by a modest loss of effect on GABA uptake (table III). These structure-activity relationships for **12** and **17** sharply contrast those observed for **8a** and **10** and their respective cyclized analogues **18** and **19**, **18** being some 30 times less potent than **8a**, and **19** at least 13 times weaker than **10**.

All of the new compounds were tested as inhibitors of the binding of ^{3}H-GABA to $GABA_A$ receptor sites [29]. None of the compounds showed detectable affinity for these receptor sites (table III). Furthermore, none of the compounds under study showed any effect on the binding of ^{3}H-quinuclidinyl benzilate (^{3}H-QNB) to muscarinic acetylcholine receptors in rat cortical membranes.

Table I. Chemical characteristics of some *N*-alkyl-diarylmethoxyalkylamine hydrochlorides.



Compound	R_1	R_2	R_3	n	Crystallization solvent	Mp (°C)	Yield (%)	Analyses[a]
6a	CH_3	C_6H_5	C_6H_5	1	EtOH/EtOAc/ether	155-156	25	C,H,N,Cl
6b	C_2H_5	C_6H_5	C_6H_5	1	EtOH/EtOAc	167-169	51	C,H,N,Cl
6c	CH_3	C_6H_5	4-Cl-C_6H_4	1	EtOH/EtOAc/ether	154-156	28	C,H,N,Cl
6d	CH_3	4-Cl-C_6H_4	4-Cl-C_6H_4	1	EtOH/EtOAc/ether	179-180	39	C,H,N,Cl
6e	CH_3	C_6H_5	C_6H_5	2	EtOH/EtOAc/ether	116-117	40	C,H,N,Cl

[a]Microanalytical data for C, H, N, Cl were within ± 0.4% of theory

Table II. Chemical characteristics of the hydrochlorides of some *N*, *N*-disubstituted GABA analogues.

Compound	Molecular formula[a]	Crystallization solvent	Mp (°C)	Yield[b]	Yield of ester[c]	Scheme No.
4a	$C_{21}H_{25}NO_2 \cdot HCl$	CH_3CN/Me_2CO/ether	140-141	40	89	1
4b	$C_{22}H_{27}NO_2 \cdot HCl$	CH_3CN/Me_2CO/ether	96-98	56	95	1
8a	$C_{20}H_{25}NO_3 \cdot HCl$	i-PrOH/Me_2CO/ether	137-139	45	74	1
8b	$C_{21}H_{27}NO_3 \cdot HCl$	i-PrOH/Me_2CO/ether	101-103	66	86	1
8c	$C_{20}H_{24}ClNO_3 \cdot HCl$	CH_3CN/ether	130-132	37	53	1
8d	$C_{20}H_{23}Cl_2NO_3 \cdot HCl$	CH_3CN/ether	139-141	72	42	1
10	$C_{21}H_{27}NO_3 \cdot HCl$	EtOH/Me_2CO/ether	141-144	74	56	1
12	$C_{19}H_{23}NO_3 \cdot HCl$	Me_2CO/ether	103-106	71	75	1
17	$C_{21}H_{25}NO_3 \cdot HCl \cdot 0.25\ H_2O$	CH_3CN/ether	185-187	44	49	2
18	$C_{22}H_{27}NO_3 \cdot HCl \cdot 0.5\ H_2O$	CH_3CN/ether	114-116	51	71	2
19	$C_{22}H_{27}NO_3 \cdot HCl$	CH_3CN/ether	192-194	47	66	2

[a]Microanalytical data for C, H, N, Cl were within ± 0.4% of theory; [b]Yields of amino acid hydrochlorides from corresponding esters; [c]Yields of the respective esters **3a, b, 7a–d, 9, 11** and **16a–c**.

Table III. Effects *in vitro* on GABA uptake.

Compound	Structure: Parent amino acid	Structure: N-substituents	GABA uptake into synaptosomes (IC_{50}, μM)	$GABA_A$ receptor binding
Nipecotic acid	Nipecotic acid	H	3.6	>100
SKF-89976-A	-	$(C_6H_5)_2$-C=CH-$(CH_2)_2$	0.23	>100
N-BEE-Nipecotic acid	-	$(C_6H_5)_2$-CH-O-$(CH_2)_2$	1.6	>100
GABA	GABA	H; H	3.0	0.03
N-Methyl-GABA	-	H; CH_3	65	67
N-DPB-GABA	-	H; $(C_6H_5)_2$-C=CH-$(CH_2)_2$	18	>100
4a	-	CH_3; $(C_6H_5)_2$-C=CH-$(CH_2)_2$	4.0	>100
4b	-	C_2H_5; $(C_6H_5)_2$-C=CH-$(CH_2)_2$	28	>100
N-BEE-GABA	-	H; $(C_6H_5)_2$-CH-O-$(CH_2)_2$	22	>100
8a	-	CH_3; $(C_6H_5)_2$-CH-O-$(CH_2)_2$	8.5	>100
8b	-	C_2H_5; $(C_6H_5)_2$-CH-O-$(CH_2)_2$	21	>100
8c	-	CH_3; $(C_6H_5)(4\text{-}Cl\text{-}C_6H_4)$-CH-O-$(CH_2)_2$	43	>100
8d	-	CH_3; $(4\text{-}Cl\text{-}C_6H_4)_2$-CH-O-$(CH_2)_2$	45	>100
10	-	CH_3; $(C_6H_5)_2$-CH-O-$(CH_2)_3$	23	>100
12	β-Alanine	CH_3; $(C_6H_5)_2$-CH-O-$(CH_2)_2$	60	>100
17	-	$(C_6H_5)_2$-CH-O-CH<$^{CH_2}_{(CH_2)_3}$	90	>100
18	GABA	$(C_6H_5)_2$-CH-O-CH<$^{CH_2}_{(CH)_3}$	240	>100
19	-	$(C_6H_5)_2$-CH-O-CH<$^{(CH_2)_2}_{(CH_2)_2}$	>300	>100

Discussion

Introduction of the bulky DPB substituent onto the amino group of the classical GABA uptake inhibitors/substrates nipecotic acid and related cyclic amino acids [16, 18, 26] provided a series of potent and lipophilic GABA uptake inhibitors [22, 23], notably SKF-89976-A (fig 1). Whereas saturation of the spacer-arm between the amino acid 'head' and the aromatic moiety of SKF-89976-A is accompanied by a dramatic loss of GABA uptake affinity [22], recovery of activity can be largely achieved by replacement of the C(3) in the spacer-arm of dihydro-SKF-89976-A by oxygen, *N*-BEE-nipecotic acid (fig 1) being only 7 times weaker than SKF-89976-A [24] (table III). Thus, the C(4)-O bond of *N*-BEE-nipecotic acid is a rather effective bioisostere of the alkene group of SKF-89976-A.

Neither nipecotic acid, SKF-89976-A, nor *N*-BEE-nipecotic acid are structural analogues of GABA, but these compounds contain a ß-alanine structure element (fig 1). Since the GABA analogues **4a** and **8a** are considerably more potent than the corresponding ß-alanine analogue **12** (fig 2, table III), the ß-alanine part of nipecotic acid and its analogues does not seem to be of decisive importance *per se* for potent GABA uptake inhibition. It is the structure of the entire molecule of nipecotic acid [26] and of the amino acid 'head' of its analogues, which is a pre-requisite for effective binding to the GABA uptake mechanisms.

Like *N*-DPB-GABA [22], *N*-BEE-GABA [24] is weaker than GABA as an inhibitor of synaptosomal GABA uptake (table III). We have now shown that conversion of the secondary amino group of both of these compounds into tertiary amino groups by *N*-methylation results in increased potency, the respect-

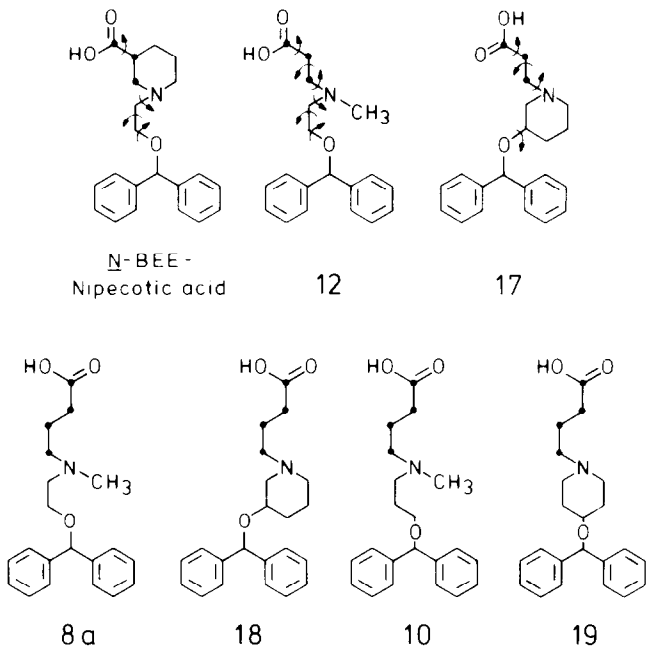


Fig 2. The structures of some GABA uptake inhibitors. The approximate conformational flexibility of *N*-BEE-nipecotic acid and compounds **12** and **17** is indicated.

ive products **4a** and **8a** showing GABA uptake affinities comparable with that of GABA. This increase in activity may be explained by a stabilizing effect of the methyl groups on the active conformation of the inhibitors. However, the observation that substitutions of ethyl groups for the methyl groups of **4a** and **8a** give less active inhibitors, **4b** and **8b** respectively, does not seem to be consistent with such a positive effect, but rather with a negative steric effect.

The increased potency of **4a** and **8a** as compared with those of the respective non-methylated compounds, *N*-DPB-GABA and *N*-BEE-GABA may, at least to some extent, be explained on the basis of the protolytic properties of the amino groups of the compounds under study. The *N*-methylated analogues of **4a** and **8a** of *N*-DPB-GABA and *N*-BEE-GABA, respectively, have less basic amino groups than the parent compounds (table IV). Although the amino groups of the compounds tested are largely protonated at physiological pH (7.4), calculations based on the equation

% protonated = 100/[1 + antilog (pH — p*K*a II)]

indicate that the fractions of molecules containing free amino groups of **4a** and **8a** are approximately 4 and 2 times larger than the corresponding fractions of the parent compounds *N*-DPB-GABA and *N*-BEE-GABA, respectively. These factors are very similar to

Table IV. Relationship between GABA uptake affinity and protolytic properties of some inhibitors.

Compound	*Effect on GABA uptake (IC_{50}, μM)*	*p*K*a I*	*p*K*a II*
N-DPB-GABA	18	3.72	10.05
4a	4.0	3.85	9.48
N-BEE-GABA	22	3.37	9.41
8a	8.5	3.91	9.05
SKF-89976-A	0.23	—	9.36[a]
N-BEE-Nipecotic acid	1.6	2.90	8.81

[a]From [22]

the potency ratios between **4a** and *N*-DPB-GABA and between **8a** and *N*-BEE-GABA. Thus, although the compounds under study are undoubtedly bound to the GABA uptake sites/carriers in the ionized forms, the fraction of molecules containing free amino groups may play an important role in activity, perhaps for the access of the compounds to the active sites in the lipophilic membrane. The carboxylate groups of all of the compounds studied are virtually fully ionized at pH 7.4. More extensive studies of these aspects on series of structurally very similar compounds may shed some light on the molecular mechanisms underlying the effects of this class of inhibitors on the GABA uptake systems. Studies along these lines using molecular modeling and NMR spectroscopic techniques are in progress.

Other structural parameters, such as conformational flexibility, obviously play important roles in the structure-activity relationships of *N*-DPB- or *N*-BEE-substituted GABA uptake inhibitors. In general, inhibitors of the former type are more sensitive to structural variations of the *N*-substituents. Thus, homologation of the DPB substituent of SKF-89976-A results in a pronounced loss of activity [22], whereas the decrease in activity following homologation of the more flexible BEE substituent of *N*-BEE-nipecotic acid is only approximately 50% [24]. Similarly, the potency ratio between **4a** and *N*-DPB-GABA is lower than that between **8a** and *N*-BEE-GABA as mentioned above.

Incorporation of part of the spacer-arm of **8a** or **10** into piperidine rings to give **18** and **19**, respectively (fig 2), is accompanied by a marked loss of activities (table III). These observations suggest that the preferred conformations of **18** or **19** only represent to a limited extent the active conformations of the respective non-cyclized analogues, or, alternatively, that a high degree of conformational mobility of the spacer-arms of **8a** or **10** is essential for activity. Compound **12** and

the corresponding ring-closed analogue, **17** (fig 2), do, however, show comparable activity (table III). Provided that the molecular mechanism for inhibition of GABA uptake of the ß-alanine analogue **12** and those of the GABA analogues **8a** and **10** are similar, these findings for **12** and **17** may indicate that the reduced conformational flexibility of **18** and **19** is not of primary importance *per se* for their virtual lack of activity. A more likely explanation appears to be that the active conformations of **8a** and **10** are not easily accessible to their cyclized analogues **18** and **19**.

The structure-activity studies described here and further studies in progress may contribute to an understanding of the molecular mechanisms underlying the biological activity of these classes of pharmacologically and, perhaps, therapeutically important GABA uptake inhibitors [9, 16, 30].

Experimental protocols

Chemistry

Melting points, determined in capillary tubes, are uncorrected. Analyses, indicated by elemental symbols, were within ± 0.4% of the theoretical values and were performed by G Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, Denmark. Column chromatography (CC) was performed on silica gel 60 (70-230 mesh, ASTM, Merck). ^{1}H NMR spectra were recorded on a Varian 360L instrument, and chemical shift data are given as δ (ppm) values. IR spectra were recorded on a Perkin-Elmer grating infrared spectrophotometer, model 781. p*K*a values were determined as described in [31].

*Halides **1**, **5a**, **5c–5e***
Compound **1** and compounds **5a**, **5d–e** [24, 32] were prepared by published procedures. Compound **5c**, bp (0.5 mmHg) = 154–158°C, was prepared by a method analogous to that described for **5a** [32].

N-*Alkyl-4,4-diphenyl-3-butenylamines **2a**, **2b***
A mixture of **1** (3.0 g, 10.4 mmol) and a 33% solution of methylamine in ethanol (10 ml) or a 70% solution of ethylamine in water (10 ml) diluted with ethanol (10 ml) was stirred at room temperature for 3 d. The mixture was evaporated and water (25 ml) was added to the residue. After acidification with 4 N HBr the mixture was extracted with 3 50 ml portions of CH_2Cl_2. The combined extracts were dried and evaporated. The residue was recrystallized from ethanol-ethyl acetate-ether to give **2a**, **HBr** (1.37 g, 48%), mp = 150–152°C, anal ($C_{17}H_{19}N$·HBr) C, H, N, Br or **2b**, **HBr** (1.99 g, 57%) mp = 163–164°C, anal ($C_{18}H_{21}N$·HBr) C, H, N, Br.

N-*Alkyl-diarylmethoxyalkylamine hydrochlorides **6a–6e***
A mixture of the chloride (**5a** or **5c–5e**) and the alkylamine in water or ethanol was stirred at room temperature for 3–5 d. The mixture was evaporated and water added to the residue. After acidification with 4 N HCl the mixture was extracted 3 times with CH_2Cl_2. The combined extracts were washed with water, dried and evaporated. The residue was recrystallized (table I).

N-*Substituted-4-alkylaminobutyric acid hydrochlorides **4a**, **4b**, **8a–d**, **10***
A mixture of the amine salt **2a**, **2b** or **6a–e** (1 eq), ethyl 4-bromobutyrate (1.1 eq) and K_2CO_3 (3 eq) in DMF (3 ml/mmol) was stirred at 100°C for 20 h. The mixture was evaporated at 80°C and 1 mmHg. Water was added to the residue and the ester was extracted with ethyl acetate. The extracts were dried and evaporated. After CC of the residue (eluent: ethyl acetate) the ester was isolated as an oil. The yields of the esters (**3a**, **3b**, **7a–d**, **9**) are listed in table II. The ^{1}N NMR spectra of the esters were consistent with their structures.

A mixture of the ester, 20% NaOH (8 ml/mmol) and methanol (4 ml/mmol) was refluxed for 2 h. The reaction mixture was acidified with 4 N HCl and evaporated to half volumes. After extractions with CH_2Cl_2, the extracts were dried and evaporated. The residue was recrystallized (table II).

Methyl N-*[2-(diphenylmethoxy)ethyl]*-N-*methyl-3-aminopropionate hydrochloride **11***
The hydrochloride of **6a** (385 mg, 1.38 mmol) was dissolved in water (5 ml) and 2 N NaOH (3 ml) was added. The mixture was extracted with 3 10 ml-portions of ether. The extracts were dried and evaporated. The residue was dissolved in methanol (5 ml) and methyl acrylate (0.13 ml, 1.44 mmol) was added. The mixture was stirred at room temperature for 20 h and evaporated. The residue was dissolved in ether and excess of a 10% solution of HCl in methanol was added. The salt was collected and recrystallized from acetonitrile-ether to give 377 mg of **11**, mp = 126–127°C. Anal ($C_{20}H_{25}NO_3$·HCl) C, H, N, Cl.

N-*[2-(Diphenylmethoxy)ethyl]*-N-*methyl-3-aminopropionic acid hydrochloride **12***
Hydrolysis of **11** with NaOH in aqueous methanol was carried out as described above for the preparations of compounds **4** and **8**.

*(R,S)-Methyl 3-hydroxypiperidine-1-carboxylate **13a** and methyl 4-hydroxypiperidine-1-carboxylate **13b***
To an ice-cold solution of 3- or 4-hydroxypiperidine (1 eq, Fluka) in water, an aqueous solution of K_2CO_3 (4 eq) and methyl chloroformate (4 eq) was added dropwise. The mixture was stirred vigorously at room temperature for 20 h. The reaction mixture was acidified with 4 N HCl and extracted 3 times with CH_2Cl_2. The extracts were dried and evaporated. The compounds were isolated in 90–95% yields as oils. The compounds were previously prepared by other routes [33, 34].

*(R,S)-Methyl 3-diphenylmethoxypiperidine-1-carboxylate **14a***
A mixture of **13a** (636 mg, 4 mmol), diphenylmethyl bromide (1.48 g, 6 mmol) and K_2CO_3 (690 mg, 5 mmol) in xylene (10 ml) was refluxed for 20 h. The mixture was washed twice with water, dried and evaporated. After cc of the residue (eluent: toluene) **14a** (910 mg, 70%) was isolated as an oil. ^{1}H NMR ($CDCl_3$): 7.30 (s, 10 H); 5.55 (s, 1 H); 3.55 (s, 3 H); 2.8–3.7 (m, 5 H); 1.3–1.9 (m, 4 H).

*Methyl 4-diphenylmethoxypiperidine-1-carboxylate **14b***
Sodium hydride (312 mg, 80% in oil, 13 mmol) was added to a solution of **13b** (1.75 g, 11 mmol) in toluene (30 ml). The mixture was refluxed for 30 min and a solution of diphenylmethyl bromide (4.1 g, 16 mmol) in toluene (40 ml) was added. The mixture was refluxed for 20 h, washed twice with water, dried and evaporated. After cc of the residue (eluent: toluene)

14b (780 mg, 22%) was isolated as an oil. ^{1}H NMR ($CDCl_3$): 7.30 (s, 10 H); 5.50 (s, 1 H); 3.65 (s, 3 H); 3.0–3.9 (m, 5 H); 1.2–1.9 (m, 4 H).

*3- and 4-Diphenylmethoxypiperidine **15a** and **15b***

To a solution of **14a** or **14b** (1 eq) in 80% aqueous methanol (7.5 ml/mmol) was added KOH (10 eq) and the mixture was refluxed for 3 d. After evaporation, water was added and the mixture was extracted with ether. The extracts were dried and evaporated to give **15a** or **15b** as oils in yields of 90 and 92%, respectively.

The hydrochlorides of **15a** and **15b** were prepared by adding excess of a solution of HCl in ethyl acetate. The salts were recrystallized from acetonitrile-ether.

15a, HCl: mp = 171–173°C. Anal ($C_{18}H_{21}NO$•HCl) C, H, N, Cl.
15b, HCl: mp = 202–204°C. Anal ($C_{18}H_{21}NO$•HCl) C, H, N, Cl.

*3- or 4-Diphenylmethoxypiperidin-1-yl alkanoic acid hydrochlorides **17–19***

A mixture of **15a** or **15b** (1 eq), K_2CO_3 (3 eq), ethyl 3-bromopropionate or ethyl 4-bromobutyrate (1.1 eq) in DMF (10 ml/mmol) was stirred at 100°C for 24 h. The ethyl esters **16a–c** were isolated as described above for the compounds **3**, **7** and **9**. The yield of the esters are listed in table II. The ^{1}H NMR spectra of **16a–c** were consistent with their structures.

Hydrolysis of **16a–c** with NaOH in aqueous methanol was carried out as described above for the preparation of the compounds **4**, **8** and **10**. The hydrochlorides were crystallized from acetonitrile-ether (table II).

In vitro *receptor binding*

Inhibition of synaptosomal GABA uptake

Inhibition of ^{3}H-GABA uptake was performed by a previously described method [28]. A crude synaptosomal suspension was prepared from rat brains [27] and incubated at 25°C in phosphate medium containing the test substance. Then ^{3}H-GABA was added (final concentration 50 nM) and after incubation the synaptosomes were isolated by rapid filtration (Whatman GF/C). The filters were washed with ice-cold phosphate medium, transferred to scintillation vials, and the radioactivity was measured by conventional scintillation counting methods.

Inhibition of $GABA_A$ receptor binding

The ^{3}H-GABA binding assay was performed with rat brain synaptic membranes as previously described in detail [29]. Aliquots of synaptic membranes (0.8–1.2 mg of protein) were incubated in triplicate at 2°C in 2 ml of Tris-citrate buffer (pH 7.1) containing 5 nM ^{3}H-GABA. Test substances were added in various concentrations. The samples were incubated for 15 min at 2°C, followed by centrifugation. The pellets were rinsed twice with 5 ml portions of cold water and suspended in water (0.4 ml). The IC_{50} values were estimated by measuring the inhibition of at least 4 different concentrations. Nonspecific binding in the presence of 1 mM GABA was subtracted.

Inhibition of muscarinic acetylcholine receptor binding

^{3}H-Quinuclidinyl benzilate (^{3}H-QNB) binding to muscarinic receptor sites on membrane fractions prepared from rat brains was performed essentially as described by Watson *et al* [35]. Briefly, rat brains were homogenized in 100 vol (w/v) 10 mM sodium potassium phosphate buffer (pH 7.4) and diluted 1/10 with the same buffer. Aliquots (0.5 mg of tissue) were incubated with 0.12 nM ^{3}H-QNB (46 Ci/mmol, Amersham) alone or in the presence of test compound in a total volume of 5 ml for 30 min at 37°C. The reaction was stopped by adding 5 ml of ice-cold buffer and rapid filtration through Whatman GF/B filters soaked previously in 0.1% polyethylenimine (Sigma) for a minimum of 30 min. The filters were washed twice with the same volume of buffer and bound radioactivity estimated by liquid scintillation counting methods. Each compound was tested in 5 different concentrations, and nonspecific binding estimated at 20 μM atropine. All estimations were made in triplicate, and each displacement experiment was repeated at least twice. The dissociation constant (K_d) for the binding of ^{3}H-QNB to rat brain membranes was determined to 13.7 ± 0.9 pM based on Scatchard analysis following a previously described procedure [35].

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