Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 2773–2785

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis of Novel GABA Uptake Inhibitors. Part 6†: Preparation and Evaluation of *N*-Ω Asymmetrically Substituted Nipecotic Acid Derivatives

Knud Erik Andersen,* Jesper Lau, Behrend F. Lundt, Hans Petersen, Per O. Huusfeldt, Peter D. Suzdak and Michael D.B. Swedberg

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

Received 26 March 2001; accepted 4 April 2001

Abstract—In a previous series of potent GABA uptake inhibitors published from this laboratory, we noticed that asymmetry in the substitution pattern of the bis-aromatic moiety in known GABA uptake inhibitors such as **4** [1-(4,4-diphenyl-3-butenyl)-3-piperidinecarboxylic acid] and **5** [(*R*)-1-(4,4-bis(3-methyl-2-thienyl)-3-butenyl)-3-piperidinecarboxylic acid] was beneficial for high affinity. This led us to investigate asymmetric analogues of known symmetric GABA uptake inhibitors in which one of the aryl groups has been exchanged with an alkyl, alkylene or cycloalkylene moiety as well as other modifications in the lipophilic part. The in vitro values for inhibition of [^{3}H]-GABA uptake in rat synaptosomes was determined for each compound, and it was found that several of the novel compounds inhibit GABA uptake as potently as their known symmetrical reference analogues. Several of the novel compounds were also evaluated for their ability to inhibit clonic seizures induced by a 15 mg/kg (ip) dose of methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) in vivo. Some of the compounds, for example **18** [(*R*)-1-(2-(((1,2-bis(2-fluorophenyl)ethylidene)amino)oxy)ethyl)-3-piperidinecarboxylic acid], show a high in vivo potency and protective index comparable with that of our recently launched anticonvulsant product, **5** [(*R*)-1-(4,4-bis(3-methyl-2-thienyl)-3-butenyl)-3-piperidinecarboxylic acid], and may therefore serve as second-generation drug candidates.

Introduction

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the mammalian central nervous system (CNS).[2–6] GABA has been estimated to be present in 60–70% of all synapses in the CNS.[7] A decrease in GABA'ergic neurotransmission appears to be involved in the etiology of several neurological disorders, including anxiety, pain and epilepsy.[4,5,8–10] Thus, numerous investigations have focused on finding novel approaches to modulate GABA'ergic function in man. These approaches include direct agonism of the GABA receptors,[11,12] the inhibition of enzymatic breakdown of GABA,[13,14] or by the inhibition of the uptake of GABA into neuronal and glial cell bodies.[5,15] It is well documented that GABA agonists are responsible for a number of unacceptable side effects in man.[16] However, in principle, GABA uptake inhibitors should exert a more therapeutically useful influence than GABA agonists. This is because a major enhancement of GABA'ergic neurotransmission would only take place under conditions where GABA is already being released physiologically. GABA can be removed from the synapse by either a high-affinity sodium-dependent GABA uptake carrier into neuronal or glial cells or by diffusion from the synapse. The GABA uptake system has traditionally been classified as either neuronal or glial on the basis of pharmacological selectivity of cyclic amino acid GABA uptake inhibitors.[5] However, several investigators have recently cloned and sequenced subtypes of the GABA uptake carrier and the selectivity of novel and hitherto known GABA uptake inhibitors on these subtypes has been investigated.[17,18] It was found that the type of GABA uptake inhibitors described in this paper is binding to the GAT-1 subtype.

Nipecotic acid **1**, guvacine **2** and homo-β-proline **3**[19,20] (Fig. 1) which can be considered as conformationally-restricted GABA analogues[21] display in vitro activity as inhibitors of [^{3}H]-GABA uptake. However, compounds **1**–**3** do not readily cross the blood–brain barrier.[19,22,23] Recently, novel series of lipophilic GABA uptake inhibitors that possess potent activity in vitro and in vivo were described.[24–27] These compounds differ from

*Corresponding author. Tel.: +45-4443-4898; fax: +45-4466-3450; e-mail: kea@novonordisk.com

†See ref 1.

0968-0896/01/$ - see front matter
PII: S0968-0896(01)00148-1

compounds **1**–**3** in that they readily cross the blood–brain barrier due to the attachment of a lipophilic anchor to the nitrogen in compounds **1**.

In the early 1980s, such lipophilic derivatives of amino acids **1**–**3** were described for the first time. These compounds, an example of which is **4** (SKF 89976A, $IC_{50} = 328$ nM,[48] Fig. 2), exhibited promising seizure protection[24,28,29] in some animal models predictive of anticonvulsant activity.[30] The compounds also displayed reduced CNS depressant effects compared with some commonly used anticonvulsant drugs, such as diazepam.[31] These observations have also stimulated others to investigate this field,[32–40] leading to the discovery of a highly lipophilic GABA uptake inhibitor with CNS activity, **6** (CI-966, $IC_{50} = 440$ nM,[48] Fig. 2). This compound was discovered at Parke–Davis/Warner–Lambert, and has been investigated in a phase I clinical trial.[41] From this laboratory, we have previously reported on the structure–activity studies leading to the choice of **5** (tiagabine, NO-328, NNC 05-0328, $IC_{50} = 67$ nM,[48] Fig. 2) as an anticonvulsant drug candidate.[25–27,42,43] Extensive pharmacological[44,45] and clinical investigations have been completed on **5** with proven anticonvulsant efficacy in man.[46,47] This novel anti-epileptic agent has now been launched for add-on therapy in the treatment of epilepsy.

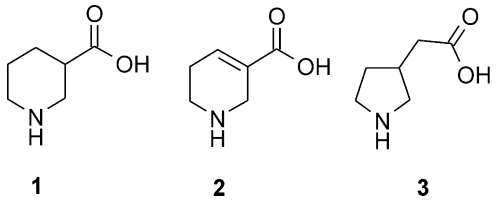


Figure 1. Three cyclic amino acids which act as GABA uptake inhibitors, nipecotic acid **1**, guvacine **2** and homo-β-proline **3**.

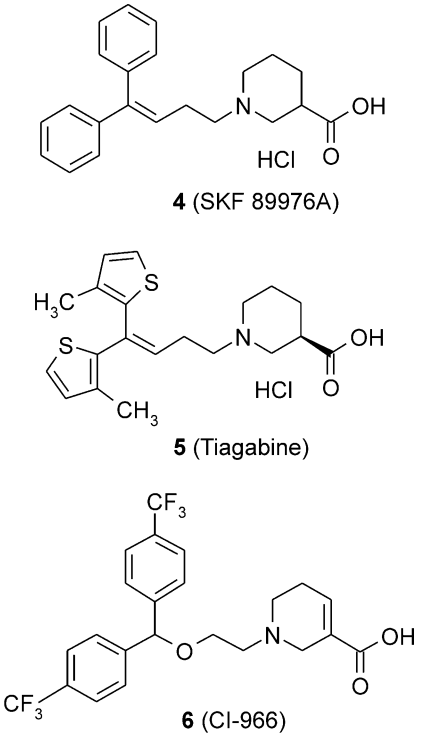


Figure 2. Lipophilic reference GABA uptake inhibitors.

With this new drug as a benchmark we have continuously searched for novel GABA uptake inhibitors with improved properties as second-generation compounds. This has now resulted in several series of highly potent GABA uptake inhibitors of which **7** ($IC_{50} = 137$ nM[48]), **8** ($IC_{50} = 55$ nM[49]), and **9** ($IC_{50} = 184$ nM[1]) are examples (Fig. 3). In this type of compound, the electronegative principle was applied in the chain by introduction of heteroatoms bearing lone pairs and thereby considerably improved GABA uptake inhibition compared to compounds such as **4**, **5** and **6**. Among these new series of compounds, we have previously reported on the selection of compound **9** as a potential second-generation compound to **5**.[1] In order to identify even better compounds, the search for novel potent GABA uptake inhibitors with improved biological properties was continued. In the series of potent GABA uptake inhibitors published earlier from this laboratory,[1,42,48,49] we have noticed that asymmetry in the substitution pattern of the bis-aromatic systems represented in the reference compounds **4**–**9** was beneficial for high affinity for the GABA uptake site. This allowed asymmetry in the bis-aromatic part of the molecule has led us to investigate asymmetric analogues of the reference compounds **4**. Such analogues include (a) monoaryl compounds in which one of the aryl groups has been exchanged with an alkyl, alkylene or cycloalkylene moiety, (b) biphenyl or aralkylaryl analogues or (c) the two aryl groups have been placed in an asymmetric pattern to each other either directly on the linker or in the more constrained 2-phenyl-1,2,3,4-tetrahydronaphalene or 3-phenyl-1,2-dihydroindene ring systems. The synthesis[50–53] and biological activity of these series of novel and selective GABA uptake inhibitors will now be reported (Tables 1 and 2).

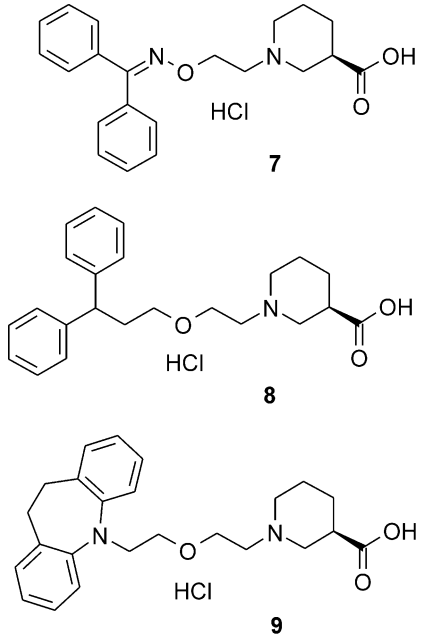
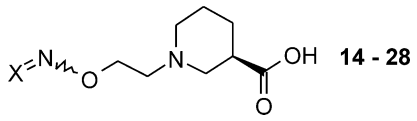
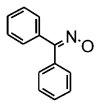
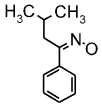
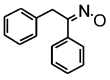
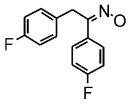
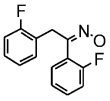
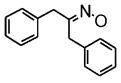
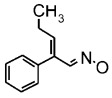
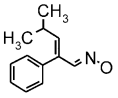
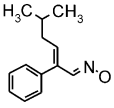
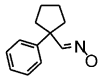
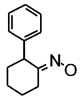
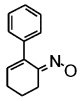
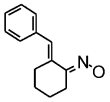


Figure 3. Potent GABA uptake inhibitors with an electronegative moiety introduced in the chain.

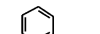
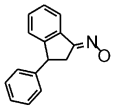
Table 1. Chemical and biological data of oxime derivatives **14–28**



No.	$X=N_{\sim}O$	Method	Mp. (°C)	Formula	Microanalyses[a]	GABA uptake IC_{50} (nM)[b]
7		[c]				137
14		A^1C	130[d]	$C_{18}H_{24}N_2O_3$,HCl,0.25H_2O	C,H,N	1241
15		A^2B^1C	142–145[d]	$C_{19}H_{28}N_2O_3$,HCl	C,H,N	284
16		A^2B^1C	Amorph.	$C_{22}H_{26}N_2O_3$,HCl	C,H,N	135
17		A^2B^1C	Amorph.	$C_{22}H_{24}F_2N_2O_3$,HCl,0.5H_2O	C,H,N	923
18		A^2B^1C	125–130[d]	$C_{22}H_{24}F_2N_2O_3$,HCl	C,H,N	80
19		A^1C	Amorph.	$C_{23}H_{28}N_2O_3$,HCl,0.5H_2O	C,H,N	1027
20		A^1C	Amorph.	$C_{19}H_{26}N_2O_3$,HCl,1.25H_2O	C,H,N	751
21		A^1C	Amorph.	$C_{20}H_{28}N_2O_3$,HCl,1.25H_2O	C,H,N	268
22		A^1C	Amorph.	$C_{21}H_{30}N_2O_3$,HCl,1.25H_2O	C,H,N	290
23		A^1C	150–151[e]	$C_{20}H_{28}N_2O_3$,1.5HCl,0.5H_2O	C,H,N	462
24		A^1C	43–45[d]	$C_{20}H_{28}N_2O_3$,HCl,0.25H_2O	C,H,N	324
25		A^1C	171–172[e]	$C_{20}H_{26}N_2O_3$,HCl,0.25H_2O	C,H,N	349
26		A^1C	116 dec[e]	$C_{21}H_{30}N_2O_3$,HCl	C,H,N	9700

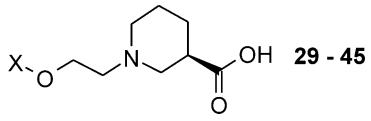
(continued)

Table 1 (*continued*)

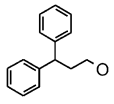
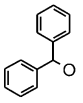
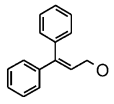
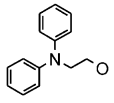
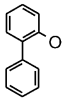
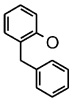
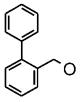
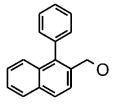
No.		Method	Mp. (°C)	Formula	Microanalyses[a]	GABA uptake IC_{50} (nM)[b]
27		A^1C	160–162[e]	$C_{24}H_{28}N_2O_3$,HCl,0.25H_2O	C,H,N	874
28		A^1C	204–205[e]	$C_{23}H_{26}N_2O_3$,HCl	C,H,N	1405

[a]All new compounds were analysed for C, H, and N and are within ± 0.4% of the theoretical values. Microanalysis on the following compounds showed deviations of N larger than ±0.4% calcd (found): **19**: 6.58 (6.03); **22**: 6.71 (7.18).
[b]Inhibition of GABA uptake in synaptosomes: IC_{50} (nM). Mean of two determinations.
[c]Ref 48.
[d]Compounds were crystallised from CH_2Cl_2.
[e]Compounds were crystallised from acetone.

Table 2. Chemical and biological data of ether derivatives **29–45**

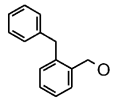
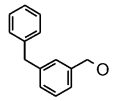
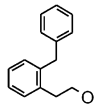
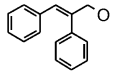
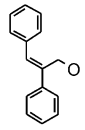
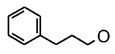
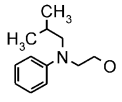
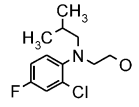


29 - 45

No.		Method	Mp.°C	Formula	Microanalyses[a]	GABA uptake IC_{50} (nM)[b]
8		e				55
29		c,d				1370
30		e				258
31		e				90
32		B^2C	169–170[f]	$C_{14}H_{19}NO_3$,HCl	C,H,N	>9000
33		A^3C	171–172[f]	$C_{20}H_{23}NO_3$,HCl	C,H,N	5823
34		A^3C	165–167[f]	$C_{21}H_{25}NO_3$,HCl	C,H,N	6946
35		A^4B^3C	148–149[f]	$C_{21}H_{25}NO_3$,HCl	C,H,N	334
36		A^4B^3C	153–155[f]	$C_{25}H_{27}NO_3$,HCl	C,H,N	7977

(continued)

Table 2 (*continued*)

No.	X—O	Method	Mp.°C	Formula	Microanalyses[a]	GABA uptake IC_{50} (nM)[b]
37		A^3C	Amorph.	$C_{22}H_{27}NO_3$,HCl	C,H,N	322
38		A^4B^3C	135–137[f]	$C_{22}H_{27}NO_3$,HCl	C,H,N	>3000
39		A^5B^3C	122–124[f]	$C_{22}H_{27}NO_3$,HCl	C,H,N	111
40		A^5B^3C	144–145[f]	$C_{23}H_{29}NO_3$,HCl	C,H,N	101
41		A^4B^3C	214–215[f]	$C_{23}H_{27}NO_3$,HCl	C,H,N	505
42		A^4B^3C	Amorph.	$C_{23}H_{27}NO_3$,HCl,0.25H_2O	C,H,N	>3000
43		A^3C	115–117[g]	$C_{17}H_{25}NO_3$,HCl,0.25H_2O	C,H,N	4500
44		DB^3C	Amorph.	$C_{20}H_{32}N_2O_3$,2HCl,3H_2O	C,H,N	282
45		DB^3C	112–114[f]	$C_{20}H_{30}ClFN_2O_3$,HCl	C,H,N	149

[a]All new compounds were analysed for C, H, and N and are within ±0.4% of the theoretical values. Microanalysis on the following compound showed deviations of C larger than ±0.4% calcd (found): **44:** 50.52 (51.15).
[b]Inhibition of GABA uptake in synaptosomes: IC_{50} (nM) Mean of two determinations.
[c]Ref 42.
[d]Ref 37.
[e]Ref 49.
[f]Compounds were crystallised from acetone.
[g]Compounds were crystallised from EtOAc/acetone.

Only a few examples of this type of *N*-Ω asymmetrically substituted or *N*-Ω monosubstituted derivatives of nipecotic acid or guvacine are known from literature, and these examples (**10**,[54] **11**,[34] **12**,[32] and **13**[55]) are listed in Figure 4. Except for compound **12** (IC_{50} = 1.1 μM[32]) these compounds show no significant activity (IC_{50} > 10 μM) for inhibiting synaptosomal GABA uptake.

Results

Two general strategies were used for preparation of the new compounds presented in Tables 1 and 2. One strategy was via *N*-alkylation of the parent cyclic amino acid **1**, with the appropriate halogenide (Schemes 1 and 2; method B^1 or B^2) or mesylate (Schemes 2 and 3; method B^3). The second strategy was via *O*-alkylation of an oxime (Scheme 1; method A^1) or an alcohol (Scheme 2; method A^3) with (*R*)-1-(2-bromoethyl)-3-piperidinecarboxylic acid ethyl ester.[56]

The parent cyclic amino acids were protected as their ester derivatives for this reaction. The separate enantiomers of **1** could be prepared by the published procedure involving resolution with either L-(+)- or with D-(−)-tartaric acid giving (*R*)- or (*S*)-ethyl nipecotate, respectively.[57,58]

The *N*-alkylated amino acid ester derivatives were saponified under basic conditions (Schemes 1–3; method C), to provide the free *N*-alkylated amino acids featured in Tables 1 and 2, isolated generally as their crystalline hydrochloride salts.

Starting from an oxime two methods were employed to produce the *O*-alkylated oxime derivatives **14**–**28** (Scheme 1). When asymmetric ketones were used the final oxime derivatives were generally obtained as a mixture of geometric isomers that were difficult to separate. *O*-Alkylation of the appropriate oximes with (*R*)-1-(2-bromoethyl)-3-piperidinecarboxylic acid ethyl ester[56] in acetone/K_2CO_3 at rt furnished the corresponding amino acid ester derivatives directly (method A^1). In this alkylation reaction it is crucial to avoid heating in order to reduce the quaternisation of (*R*)-1-(2-bromoethyl)-3-piperidinecarboxylic acid ethyl ester or the products by this very reactive bromide. Quaternised by-products could easily be removed by column chromatography and the purified ethyl esters were then converted into the acids **14**, **19**–**28** (Table 1) by the general method C described above.

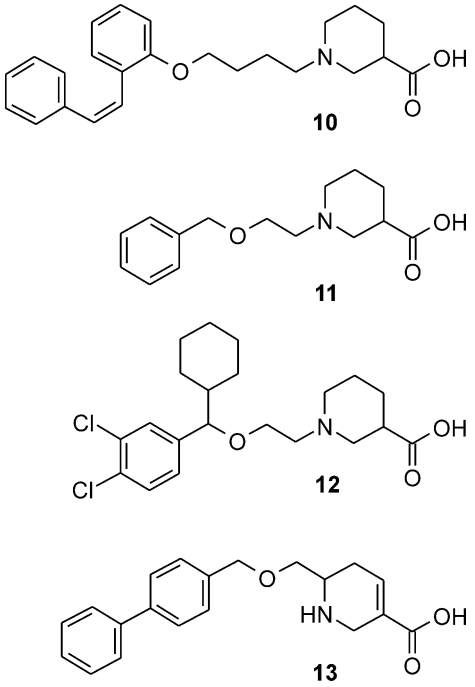


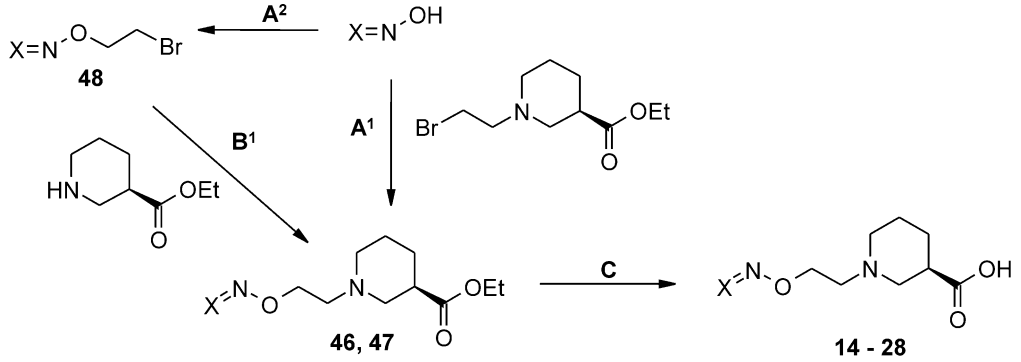
Figure 4. Various known derivatives of nipecotic acid (**1**) and guvacine (**2**) which does not possess any significant affinity for the GABA uptake site.

Alternatively, *O*-alkylation of the appropriate oximes with 1,2-dibromoethane in acetone/K_2CO_3 gave the corresponding 2-bromoethoxyimino derivatives (method A^2). These bromo derivatives were converted via *N*-alkylation of (*R*)-3-piperidinecarboxylic acid ethyl ester into the acids **15**–**18** (Table 1) by the general methods B^1 and C described above.

The ether derivatives **32**–**43** (Table 2) were prepared by several different routes as outlined in Scheme 2. Direct *N*-alkylation of (*R*)-3-piperidinecarboxylic acid ethyl ester with 2-phenoxyethylbromide in MIBK/K_2CO_3 (method B^2) furnished the ester derivative **54** which was then converted into the acid **32** (Table 2) by the general method C described above. Another direct method is the *O*-alkylation of the appropriate phenol or alcohol with (*R*)-1-(2-bromoethyl)-3-piperidinecarboxylic acid ethyl ester in the presence of a strong base like NaH which produced the corresponding amino acid ester derivatives, that is **49** (method A^3). As described above for the oxime derivatives heating must be avoided in order to reduce quaternisation by-products. The amino acid ester derivatives were then converted into the acids **33**, **34**, **37** and **43** (Table 2) by the general method C.

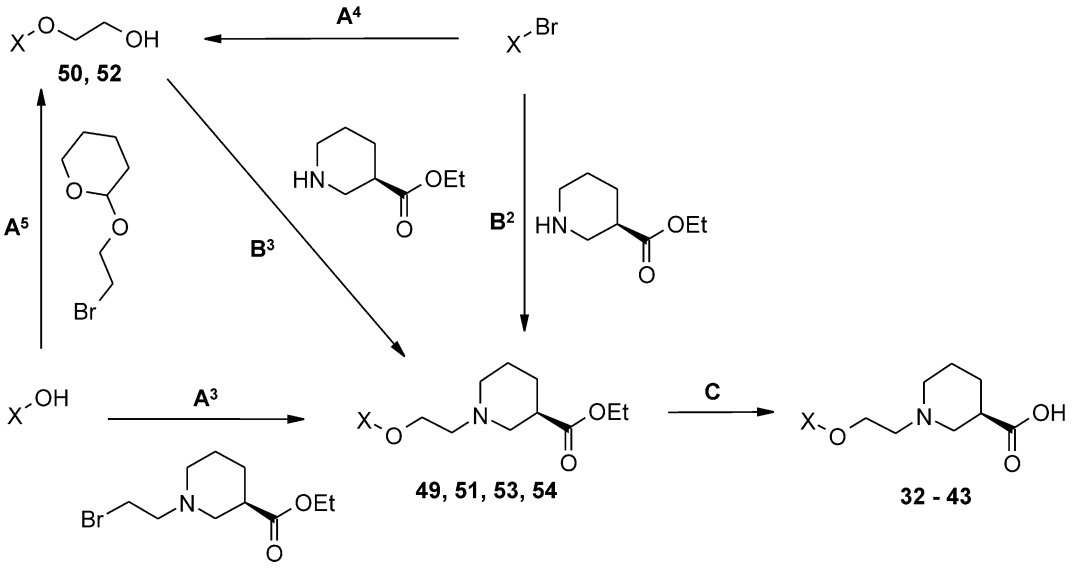
Alternative routes to the ether derivatives were via elongation of the chain (method A^4 or A^5). In method A^4, the appropriate benzyl- or allylbromide derivative was elongated by the Lindy method. Addition of *n*-Buli at 10 °C to ethylene glycol afforded the ethylene glycolate which was *O*-alkylated with the appropriate benzylbromide. This afforded the corresponding 2-hydroxyethylether derivatives, that is **50**. In method A^5, the appropriate alcohol was *O*-alkylated with 2-bromoethyltetrahydro-2-pyranyl ether in KOH/DMSO followed by hydrolysis in dilute acid of the THP protection group to give the corresponding 2-hydroxyethylether derivatives, that is **52**. The 2-hydroxyethylether intermediates, prepared by methods A^4 and A^5, were then converted via their mesylates by *N*-alkylation of (*R*)-3-piperidinecarboxylic acid ethyl ester into the acids **35**, **36, 38**–**42** (Table 2) by the general methods B^3 and C described above.

The general synthesis of the aniline derivatives **44** and **45** is outlined in Scheme 3. Starting from the appropriate substituted *N*-(2-methyl-1-propyl)aniline[59] the

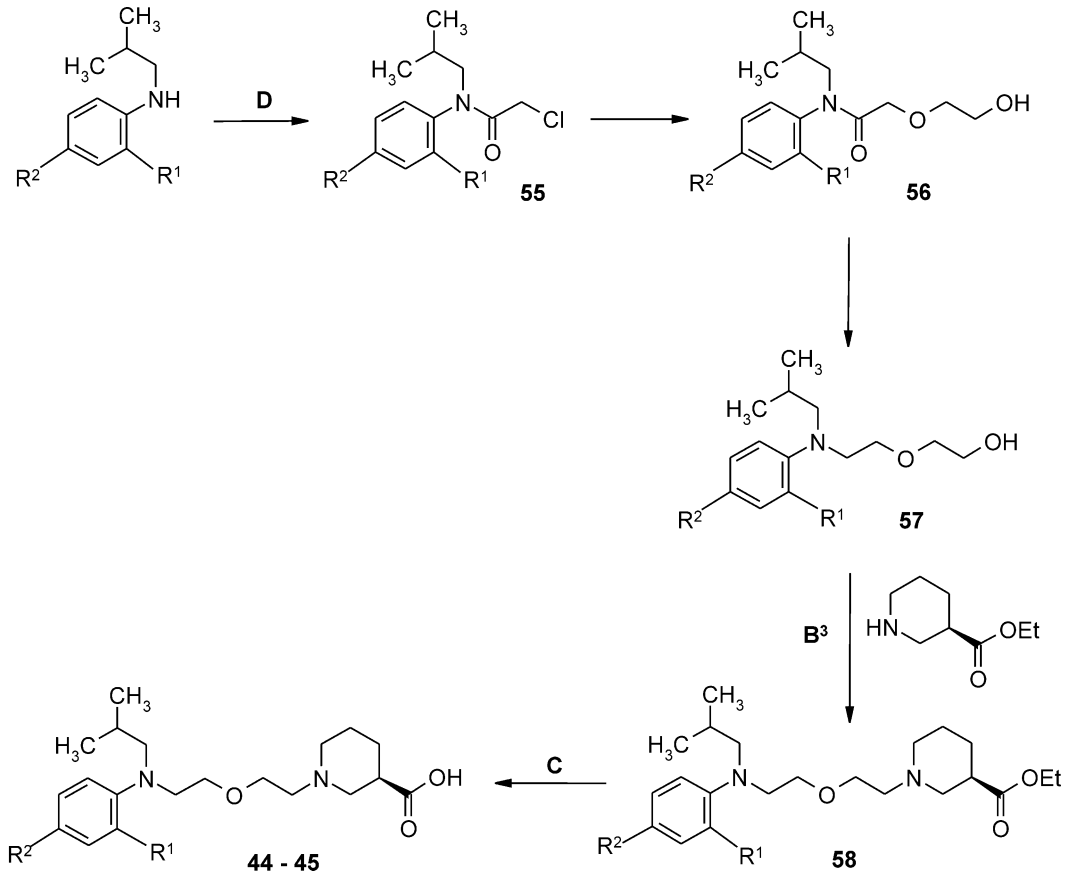


Scheme 1. Synthesis of oxime-ether derivatives **14**–**28.** For definition of X, see Table 1. Method A^1: (1) K_2CO_3, acetone, rt; method A^2: K_2CO_3, acetone, $BrCH_2CH_2Br$, reflux; method B^1: K_2CO_3, acetone, reflux; method C: (1) NaOH, EtOH, rt; (2) HCl.

chain was built in a three step reaction (method D). Acylation of the aniline with chloroacetic anhydride produced the 2-chloroacetamide derivatives. Chain elongation of these chlorides were then accomplished by *O*-alkylation of ethylene glycol/DMF in the presence of K*t*OBu to give the corresponding 2-(2-hydroxyethoxy)acetamide derivatives. Reduction of these amides with $LiAlH_4$ furnished the corresponding 2-(2-hydroxyethoxy)ethylamino derivatives which were then converted via their mesylates by *N*-alkylation of (*R*)-3-



Scheme 2. Synthesis of ether derivatives **32**–**43**. For definition of X, see Table 1. Method A^3: NaH, toluene or THF, rt; method A^4: *n*-BuLi, ethyleneglycol, rt. Method A^5: DMSO, KOH, 100 °C; method B^2: K_2CO_3, MIBK, reflux; method B^3: (1) TEA, Et_2O, MsCl, reflux or *n*-BuLi, THF, MsCl, reflux; method C: (1) NaOH, EtOH, rt; (2) HCl.



Scheme 3. Synthesis of anilinoalkylethers **44**–**45**. R^1 and R^2, see Table 1. Method D: (1) $(ClCH_2CO)_2O$, H_2O, 70 °C; (2) ethyleneglycol, DMF, KO*t*Bu, rt; (3) $LiAlH_4$, toluene, THF, rt; method B^3: (1) TEA, CH_2Cl_2, MsCl, −10 to 0 °C; (2) Li_2CO_3, isopropyl acetate, reflux, method C: (1) NaOH, EtOH, rt; (2) HCl.

piperidinecarboxylic acid ethyl ester into the acids **44** and **45** (Table 2) by the general methods B[3] and C described above.

Discussion

In our continued effort to identify novel GABA uptake inhibitors with improved potency and selectivity, a range of such new inhibitors has been prepared.[50–53] Some representative examples of these structures are included in Tables 1 and 2. Their IC_{50} values for in vitro inhibition of [^{3}H]-GABA uptake determined essentially by Fjalland's method[60] are included (mean of two determinations is given).

In these examples, mainly steric properties of the lipophilic moiety as well as the chain linking the lipophilic moiety and the amino acid part of the molecules have been varied in order to probe the requirements for inhibiting GABA transport at the site[61–64] involved in the uptake of GABA from the synaptic cleft into neuronal and glial cell bodies.

In general, inspection of the data presented in Tables 1 and 2 show that two aryl groups, as in the hitherto known GABA uptake inhibitors (see Figs 2 and 3), are not crucial in order to obtain high potency as exemplified by compounds **15**, **21**–**25** and **44**. However, modifying one of the two aryl groups in the known GABA uptake inhibitors is very delicate. Complete removal of one aryl group seems not to be allowed as shown by example **43** compared to example **8**. Therefore, some residue must be left when one of the two aryl groups is modified. Comparison of example **14** with example **15** and example **20** with examples **21**–**24** further indicate that the non-aromatic residue should represent some bulkiness.

Distortion of the attachment point of the two aryl groups in the known and symmetrical GABA uptake inhibitors is also allowed to some extent. Comparison of example **7** with example **16** and example **30** with example **41** show that affinity for the GABA uptake site is not reduced significantly by such a distortion. However, introduction of this asymmetry results in geometric isomers. Generally, in the oxime series (Table 1) such geometric isomers could not readily be separated, but in the allyl ether derivatives **41** and **42** this was accomplished through column chromatography. In these two examples the most conformationally extended *E*-isomer **42** showed the lowest potency. This tendency of low or weak potency of compounds with two possible extended aryl groups are further illustrated by the examples **19** and **38**. As we have reported previously,[42] *ortho* substitution in one or both aryl groups increase GABA uptake inhibition. This is further illustrated by the examples **18** and **45** when compared to the examples **16** and **44**, respectively.

From the data in Table 2, it can be seen that the phenolether examples **32**–**34** are very poor GABA uptake inhibitors. This may be due to the short length of the chain, and even the more flexible example **34** in which the benzyl group can adapt several more extended orientations is a poor GABA uptake inhibitor. However, extending the chain length with one carbon atom as in the examples **35** and **37** improves potency by a factor of 20 compared to examples **33** and **34**, respectively. The potency of examples **35** and **37** has even been significantly improved compared to their closest symmetrical reference compound **29**. Additional extension of the chain length with one carbon atom as in the examples **39** and **40** gives a further improvement in potency, although only 3-fold. This very significant improvement in potency when the chain is extended can also be observed from example **29** to example **8**.

In Table 3, the in vivo anticonvulsant effect in mice of representative compounds is illustrated, expressed as ED_{50} values in milligram per kilogram. The convulsion model used is based on observing the inhibition of clonic seizures induced by a 15 mg/kg intraperitoneal (ip) dose of the chemoconvulsant methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM), an inverse benzodiazepine agonist. The experimental procedure has been described previously.[27] In Table 3, we have also listed the ED_{50} values found in the rotarod performance test and calculated the protective index as the ratio between ED_{50} values for inhibition of rotarod and inhibition of DMCM induced convulsions in mice. As can be seen from Table 3 there is no correlation between GABA uptake inhibition and in vivo potency which is probably due to the diversity and difference in lipophilicity of the compounds in this investigation. As the in

Table 3. Anticonvulsant properties of selected compounds

No.	DMCM ED_{50} (mg/kg)[a]	Rotarod ED_{50} (mg/kg)[b]	Protective index[c]
4	3.1	—	—
5	1.2	5.5	4.6
7	3.2	—	—
8	1.7	9.0	5.3
31	10	16	1.6
14	15.7	80	5.1
15	1.8	—	—
16	4.1	21	5.1
18	1.3	6.8	5.2
20	5.5	24	4.4
22	7.4	>30	>4.1
23	1.4	5.4	3.9
24	7.4	15	2.0
25	3.0	17.5	5.8
26	35	—	—
27	23	60	2.6
28	>60	>60	—
35	60	>150	>2.5
36	47	>60	>1.3
37	4.9	10	2.0
40	7.9	7.9	1.0
41	5.4	24	4.4
42	38	85	2.2
44	6.2	22	3.5
45	1.7	3.0	1.8

[a]Inhibition of DMCM-induced seizures in mice: ED_{50} (mg/kg) after ip administration.
[b]Rotarod in mice: ED_{50} (mg/kg) after ip administration.
[c]Ratio for ED_{50} values for inhibition of rotarod and inhibition of DMCM induced convulsions in mice.

vivo potency of these novel compounds in general is high these results are encouraging for the selection of an efficacious compound with good kinetic properties. Further, several examples show an in vivo potency and protective index which are comparable with that of potent reference compounds such as **5** and **8**. Even more examples show a comparable protective index with these reference compounds, although they are less potent in vivo.

Conclusion

Previously,[42] we have focussed on the selection of compound **5** as an anticonvulsant drug candidate. Following this selection we continued the search for a second-generation compound with a longer duration of action and broader therapeutic window. In this publication, we have described a new series of GABA uptake inhibitors in which the presence of two aryl groups, as in the hitherto known GABA uptake inhibitors, are not crucial in order to obtain high potency. However, modifying one of the two aryl groups in the known GABA uptake inhibitors is very delicate. Complete removal of one of the aryl groups seems not to be allowed, and some bulky residue must be left when one of the two aryl groups is modified. Distortion of the attachment point of the two aryl groups in the known and symmetrical GABA uptake inhibitors is also allowed to some extent. *Ortho* substitution in one or both aryl groups increase GABA uptake inhibition as reported previously[42] for other series of GABA uptake inhibitors.

In this investigation, no correlation between GABA uptake inhibition and in vivo potency could be found, which is probably due to the diversity and difference in lipophilicity of the compounds. However, several examples, that is **18**, **23** and **25**, show an in vivo potency and protective index that are comparable with that of potent reference compounds such as **5** and **8**.

Experimental

General

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 analyser) were obtained within $\pm 0.4\%$ of theoretical values where given. ^{1}H NMR spectra were recorded on a Bruker WM400 spectrometer with TMS as internal standard, with illustrative 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.[59,65–73] Column chromatography was carried out using the technique described by W.C. Still et al.,[74] on Merck silica gel 60 (Art 9385) using thick-walled glass columns columns and TLCs on Merck silica gel 60, 5×20 cm plates (Art 5714).

Synaptosomal [^{3}H]-GABA uptake

Uptake of [^{3}H]-GABA into synaptosomal preparations was assayed by a filtration assay.[60] Rat forebrain was rapidly excised and homogenised in 20 mL of ice cold 0.32 M sucrose with a hand-driven Teflon/glass Potter-Elvehjem homogeniser. The homogenate was centrifuged for 10 min at 600*g* at 4 °C. The pellet was resuspended in 50 volumes 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 microlitres of this synaptosomal suspension (0.1 mg protein), diluted into 300 μL of phosphate buffer and 100 μL of test substance solutions in water, were pre-incubated for 8 min at 30 °C. Then 50 μL of [^{3}H]-GABA (final concentration 0.9 nM) and unlabelled GABA (final concentration 0.9 nM) were added before continuing incubation for another 8 min. Synaptosomes were then recovered by rapid filtration through Whatman GF/F glass fibre 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 Filter-Count (Packard). Non-carrier-mediated uptake was determined in the presence of nipecotic acid (500 μM) and was subtracted from total binding to give carrier-mediated [^{3}H]-GABA uptake. The IC_{50} value obtained for each example is shown in Table 1.

Antagonism of seizures induced by methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) in mice.[27] Female NMRI mice were used. The test drug was injected ip 30 min prior to the seizure test. In this test the animals were injected ip with 15 mg/kg of DMCM and were observed for the next 30 min for the presence of clonic seizures and death ($N = 5$–10/dose). The ED_{50} value obtained for each example is shown in Table 3.

Rotarod test in mice.[27] The animals were pretrained in the rotarod apparatus (Ugo-Basile, Italy) for 2 min before to testing (speed: 6 rpm). The rod diameter was 3 cm. In the test procedure, the animals were placed on the rotating rod. If the animal fell from the rod, the animal was immediately picked up by the tail and again placed on the rod. Testing was stopped when a total of 10 failures were obtained or 2 min had elapsed.

Chemistry. Each of the methods A–D are illustrated by the preparation of the following derivatives. Although the methods are illustrated for specific compounds, the methods have been found to be general for the examples in Tables 1 and 2.

(Method A[1]): (*R*)-1-(2-(((2-(Benzylidene)cyclohexylidene)amino)oxy)ethyl)-3-piperidinecarboxylic acid ethyl ester (46). A mixture of 2-benzylidenecyclohexanone oxime[69] (3.7 g, 18.4 mmol), (*R*)-1-(2-bromoethyl)-3-piperidinecarboxylic acid ethyl ester hydrobromide[56] (7.0 g, 20.2 mmol), K_2CO_3 (10.2 g, 73.5 mmol) and acetone (150 mL) was stirred at rt for 11 days. The mixture was filtered and the solvent evaporated. The residue was purified by column chromatography on

silica gel (200 g, heptane/EtOAc = 3:2) to give 2.5 g (35%) of **46** as an oil. TLC: R_f : 0.27 (SiO_2; heptane/EtOAc = 2:3). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.20–7.35 (m, 5H, Ar*H*); 6.91 (s, 1H, C=C*H*); 4.28 (t, 2H, J = 5 Hz, OCH_2CH_2N); 4.12 (q, 2H, J = 7 Hz, CH_2CH$_3$); 3.06–3.12 (m, 1H, NCH_2CH); 2.80–2.87 (m, 1H, NCH_2CH); 2.75 (t, 2H, J = 5 Hz, OCH$_2$CH_2N); 2.55–2.70 (m, 5H, C*H*CO$_2$Et and =CCH_2CH$_2$CH$_2$CH_2C=); 2.25–2.33 (m, 1H, NCH_2CH_2CH_2CH); 2.10–2.17 (m, 1H, NCH_2CH_2CH_2CH); 1.90–2.00 (m, 1H, NCH_2CH_2CH_2CH); 1.45–1.75 (m, 7H, NCH_2CH_2-CH_2CH and =CCH$_2$CH_2CH_2CH$_2$C=); 1.25 (t, 3H, J = 7 Hz, CH$_2$CH_3).

(Method A^{2}B^1): (*R*)-1-(2-(((1,2-Diphenylethylidene)amino)oxy)ethyl)-3-piperidine-carboxylic acid ethyl ester (47). A suspension of 1,2-diphenylethanone oxime[66,67] (5.0 g, 24 mmol), 1,2-dibromoethane (25 mL) and K_2CO_3 (10.0 g, 72 mmol) in acetone (25 mL) was heated at reflux for 1 h, then stirred at rt for 72 h and finally heated at reflux for 4.5 h. KI (0.5 g) was added and refluxing was continued for an additional 16 h. H_2O (100 mL) was added and the resulting mixture was extracted with CH_2Cl_2 (2×100 mL). The combined organic extracts were washed with H_2O (100 mL), brine (10 mL) and dried ($MgSO_4$). The solvent was evaporated and the residue was purified by column chromatography on silica gel (60 g, *n*-heptane/EtOAc = 10:1) to give 3.6 g of 1,2-diphenylethanone *O*-(2-bromoethyl)oxime (**48**) as an oil. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.20–7.70 (m, 10H, Ar*H*); 4.45 (t, 2H, J = 5 Hz, OCH_2CH$_2$Br); 4.21 (s, 2H, CH_2Ar); 3.60 (t, 2H, J = 5 Hz, OCH$_2$CH_2Br).

A mixture of **48** (3.5 g, 11 mmol), (*R*)-3-piperidinecarboxylic acid ethyl ester (3.1 g, 20 mmol), K_2CO_3 (4.1 g, 30 mmol), NaI (0.15 g, 1 mmol) and acetone (100 mL) was heated at reflux for 16 h. H_2O (100 mL) was added and the resulting mixture was extracted with CH_2Cl_2 (2×100 mL). The combined organic extracts were washed with H_2O (100 mL), brine (10 mL) and dried ($MgSO_4$). The solvent was evaporated to give 5.0 g of **47** as an oil. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.15.65 (m, 10H, Ar*H*); 4.37 (t, 2H, J = 5 Hz, OCH_2CH$_2$N); 4.13 (s, 2H, CH_2Ar); 4.08 (q, 2H, J = 7 Hz, CH_2CH$_3$); 3.00–3.09 (m, 1H, NCH_2CH); 2.82.–9090 (m, 1H, NCH_2CH); 2.75 (t, 2H, J = 5 Hz, OCH$_2$CH_2N); 2.55–2.60 (m, 1H, C*H*CO$_2$Et); 2.23–2.33 (m, 1H, NCH_2CH_2CH_2CH); 1.85.–215 (m, 2H, NCH_2CH_2-CH_2CH); 1.35.–175 (m, 3H, NCH_2CH_2CH_2CH); 1.22 (t, 3H, J = 7 Hz, CH$_2$CH_3).

(Method A^3): (*R*)-1-(2-(2-Benzylphenoxy)ethyl)-3-piperidinecarboxylic acid ethyl ester (49). NaH (0.8 g, 20 mmol, 60% oil dispersion) was added portionwise to a stirred solution of 2-benzylphenol (1.8 g, 10 mmol) in toluene (30 mL) placed under N_2. The mixture was stirred for 0.5 h and (*R*)-1-(2-bromoethyl)-3-piperidinecarboxylic acid ethyl ester hydrobromide (3.5 g, 10 mmol) was added portionwise. The reaction mixture was stirred for 2 h at rt and H_2O (50 mL) was added. The phases were separated and the organic phase was extracted with a 10% citric acid solution (200 mL). This acidic extract was washed with a small portion of toluene and the organic phases were discarded. The acidic aqueous solution was neutralised with 4 N NaOH and the mixture was extracted with EtOAc (150 mL). The organic extract was dried (Na_2SO_4) and the solvent was evaporated to give 1.7 g (46%) of **49** as an oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.80–7.25 (m, 9H, Ar*H*); 4.10 (q, 2H, J = 7 Hz, CH_2CH$_3$); 4.06 (t, 2H, J = 5 Hz, OCH_2CH$_2$N); 3.96 (s, 2H, ArCH_2Ar); 3.03–3.08 (m, 1H, NCH_2CH); 2.75–2.85 (m, 3H, OCH$_2$CH_2N and NCH_2CH); 2.50.60 (m, 1H, C*H*CO$_2$Et); 2.30–2.40 (m, 1H, NCH_2CH_2CH_2CH); 2.10–2.18 (m, 1H, NCH_2CH_2-CH_2CH); 1.88.95 (m, 1H, NCH_2CH_2CH_2CH); 1.42–1.68 (m, 3H, NCH_2CH_2CH_2CH); 1.25 (t, 3H, J = 7 Hz, CH$_2$CH_3).

(Method A^{4}B^3): 2-((3-Benzylbenzyloxy)ethanol (50). A 2.5 M solution of *n*-BuLi in hexanes (36 mL) was added drop wise to dry ethylene glycol (120 mL) placed on an ice-bath under N_2. When addition was complete the mixture was stirred for 0.5 h and 3-benzylbenzylbromide[73] was added in one portion and the reaction mixture was stirred at rt for 72 h. H_2O (200 mL) was added and the resulting mixture was extracted with Et_2O (2×150 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent was evaporated to give a residue which was purified by column chromatography on silica gel (600 mL, heptane/EtOAc = 3:2). This afforded 8.0 g (55%) of **50**. TLC: R_f 0.30 (SiO_2; heptane/EtOAc = 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.15–7.30 (m, 9H, Ar*H*); 4.62 (t, 1H, J = 5 Hz, *H*OCH$_2$CH$_2$O); 4.45 (s, 2H, OCH_2Ar); 3.94 (s, 2H, ArCH_2Ar); 3.50 (q, 2H, J = 5 Hz, HOCH_2CH$_2$O); 3.44 (t, 2H, J = 5 Hz, HOCH$_2$CH_2O).

(*R*)-1-(2-(3-Benzylbenzyloxy)ethyl)-3-piperidinecarboxylic acid ethyl ester (51). To a solution of **50** (5.0 g, 21 mmol) in dry Et_2O (100 mL), TEA (7.3 mL) was added. A solution of MsCl (3.5 g, 31 mmol) in dry Et_2O (20 mL) was added dropwise at rt. When addition was complete, the reaction mixture was left overnight. H_2O (50 mL) was added and the mixture was stirred for 10 min. The phases were separated, the organic phase was dried ($MgSO_4$) and the solvent was evaporated to give a residue which was dissolved in acetone (150 mL). To this solution (*R*)-3-piperidinecarboxylic acid ethyl ester tartrate (9.5 g, 31 mmol) and K_2CO_3 (7.3 g, 53 mmol) were added and the mixture was heated at reflux for 96 h. The mixture was allowed to cool, diluted with acetone (150 mL) and filtered. The solvent was evaporated to give a residue which was purified by column chromatography on silica gel (500 mL, heptane/EtOAc = 1:1). This afforded 4.3 g (54%) of **51** as an oil. TLC: R_f 0.19 (SiO_2; heptane/EtOAc = 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.10–7.30 (m, 9H, Ar*H*); 4.42 (s, 2H, OCH_2Ar); 4.03 (q, 2H, J = 7 Hz, CH_2CH$_3$); 3.94 (s, 2H, ArCH_2Ar); 3.48 (t, 2H, J = 5 Hz, OCH_2CH$_2$N); 2.82–2.90 (m, 1H, NCH_2CH); 2.60–2.68 (m, 1H, NCH_2CH); 2.45–2.55 (m, 3H, C*H*CO$_2$Et and OCH$_2$CH_2N); 2.15–2.20 (m, 1H, NCH_2CH_2CH_2CH); 1.98.–207 (m, 1H, NCH_2CH_2CH_2CH); 1.72–1.80 (m, 1H, NCH_2CH_2CH_2CH); 1.30–1.60 (m, 3H, NCH_2CH_2-CH_2CH); 1.15 (t, 3H, J = 7 Hz, CH$_2$CH_3).

(Method A[5]B[3]): 2-(2-(2-Benzylphenyl)ethoxy)ethanol (52). To a solution of 2-benzylphenylacetic acid[72] (5.5 g, 24 mmol) in THF (100 mL) a solution of $LiAlH_4$ in THF (24 mL, 24 mmol, 1 M) was added dropwise on an ice-bath. When addition was complete the mixture was stirred for 1 h at rt. H_2O (1 mL), 4 N NaOH (2 mL) and H_2O (4 mL) were added successively. The mixture was filtered and the filtrate diluted with Et_2O (250 mL). The phases were separated and the organic phase was washed with saturated NH_4Cl (3×100 mL) and dried ($MgSO_4$). The solvent was evaporated to give 4.8 g of crude 2-(2-benzylphenyl)ethanol as an oil. To a mixture of this alcohol (4.8 g, 23 mmol), 2-bromoethyltetrahydro-2-pyranyl ether (9.6 g, 46 mmol) and DMSO (100 mL), KOH (2.6 g, 46 mmol) was added. The reaction mixture was stirred at rt for 20 h and then heated at 100 °C for 3 h. The reaction mixture was allowed to cool, poured into ice-water (200 mL) and extracted with Et_2O (2×150 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent was evaporated to give 4.6 g of an oily residue. This oil was purified by column chromatography on silica gel (3×40 cm, heptane/EtOAc = 1:4). This afforded 1.3 g of **52** as an oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.10–7.30 (m, 9H, Ar*H*); 4.05 (s, 2H, ArCH_2Ar); 3.66 (q, 2H, J = 5 Hz, HOCH_2-CH_2O); 3.53 (t, 2H, J = 5 Hz, OCH_2CH_2Ar); 3.45 (t, 2H, J = 5 Hz, HOCH_2CH_2O); 2.90 (t, 2H, J = 5 Hz, OCH_2CH_2Ar); 1.92 (t, 1H, J = 5 Hz, *H*OCH_2CH_2O).

(*R*)-1-(2-(2-(2-Benzylphenyl)ethoxy)ethyl)-3-piperidinecarboxylic acid ethyl ester (53). TEA (10 mL) was added to a solution of **52** (1.3 g, 6.1 mmol) in CH_2Cl_2 (50 mL) and then MsCl (0.77 g, 6.7 mmol) was added dropwise at rt. When addition was complete the reaction mixture was diluted with CH_2Cl_2 (100 mL), washed with H_2O (3×50 mL) and dried ($MgSO_4$). The solvent was evaporated to give a residue which was dissolved in acetone (100 mL). To this solution (*R*)-3-piperidinecarboxylic acid ethyl ester tartrate (2.8 g, 9.5 mmol) and K_2CO_3 (2.1 g, 15.3 mmol) were added and the mixture was heated at reflux for 72 h. An additional portion of (*R*)-3-piperidinecarboxylic acid ethyl ester tartrate (1.4 g, 4.8 mmol) was added and heating was continued for another 24 h. The mixture was allowed to cool and then filtered. The solvent was evaporated to give a residue which was purified by column chromatography on silica gel (3×30 cm, heptane/EtOAc = 1:1→3:7). This afforded 0.9 g of **53** as an oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.10–7.30 (m, 9H, Ar*H*); 4.12 (q, 2H, J = 7 Hz, CH_2CH$_3$); 4.05 (s, 2H, ArCH_2Ar); 3.50 (2×t, 4H, J = 5 Hz, CH_2OCH_2); 2.98–3.05 (m, 1H, NCH_2CH); 2.88 (t, 2H, J = 5 Hz, OCH$_2$CH_2Ar); 2.74–2.82 (m, 1H, NCH_2CH); 2.50–2.60 (m, 3H, C*H*CO$_2$Et and OCH$_2$CH_2N); 2.12–2.20 (m, 1H, NCH_2CH_2-CH_2CH); 1.96–2.04 (m, 1H, NCH_2CH_2CH_2CH); 1.92 (m, 1H, NCH_2CH_2CH_2CH); 1.35–1.70 (m, 3H, NCH_2CH_2CH_2CH); 1.25 (t, 3H, J = 7 Hz, CH$_2$CH_3).

(Method B[2]): (*R*)-1-(2-Phenoxyethyl)-3-piperidinecarboxylic acid ethyl ester (54). A mixture of 2-phenoxyethylbromide (20 g, 100 mmol), (*R*)-3-piperidinecarboxylic acid ethyl ester (34 g, 112 mmol), K_2CO_3 (41 g, 300 mmol) and MIBK (200 mL) was heated at reflux overnight. The reaction mixture was allowed to cool and then filtered. The solvent was evaporated and the residue was purified by column chromatography on silica gel (800 g, heptane/EtOAc = 4:1) to give 17.1 g (62%) of **54** as an oil. TLC: R_f 0.20 (SiO_2; heptane/EtOAc = 3:2). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.27 (t, 2H, J = 8 Hz, m-Ar*H*); 6.95 (t, 1H, J = 8 Hz, p-Ar*H*); 6.90 (d, 2H, J = 8 Hz, o-Ar*H*); 4.14 (q, 2H, J = 7 Hz, CH_2CH$_3$); 4.08 (t, 2H, J = 7 Hz, OCH_2CH$_2$N); 3.05–3.12 (m, 1H, NCH_2CH); 2.80–2.90 (m, 3H, OCH$_2$CH_2N and NCH_2CH); 2.55–2.63 (m, 1H, C*H*CO$_2$Et); 2.28–2.37 (m, 1H, NCH_2CH_2CH_2CH); 2.11–2.20 (m, 1H, NCH_2CH_2CH_2CH); 1.90–2.00 (m, 1H, NCH_2CH_2CH_2CH); 1.45–1.75 (m, 3H, NCH_2CH_2-CH_2CH); 1.25 (t, 3H, J = 7 Hz, CH$_2$CH_3).

(Method C): Hydrolysis of 3-piperidinecarboxylic acid ester derivatives. General method. The ester under consideration (1.0 mmol) was dissolved in ethanol (3 mL) and 3 mmol of 4 or 12 N NaOH was added. The reaction mixture was stirred at rt until TLC indicated complete reaction (3–6 h). A concentrated aqueous HCl solution was added with cooling on an ice-bath until pH 1. Then CH_2Cl_2 (100 mL) was added and the resulting emulsion was dried (Na_2SO_4). The solvent was evaporated to give a residue which was crystallized. Recrystallization afforded the pure acid hydrochlorides for which the data are shown in Tables 1 and 2.

(Method DB[3]): 2-Chloro-*N*-(2-methyl-1-propyl)-*N*-phenylacetamide (55). A mixture of *N*-(2-methyl-1-propyl)aniline[59] (14.9 g, 100 mmol), chloroacetic anhydride (19.0 g, 111 mmol) and H_2O (100 mL) was heated at 70 °C for 1 h. The reaction mixture was poured into ice-water (300 mL) and concentrated HCl was added until pH 2. The precipitate was collected and dried to give 11.3 g (50%) of **55**. TLC: R_f 0.37 (SiO_2; heptane/EtOAc = 4:1). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.20–7.50 (m, 5H, Ar*H*); 3.80 (s, 2H, CH_2Cl); 3.60 (d, 2H, NCH_2); 1.78 (hept., 1H, C*H*Me$_2$); 0.93 (d, 6H, 2×CH_3).

2-(2-Hydroxyethoxy)-*N*-(2-methyl-1-propyl)-*N*-phenylacetamide (56). To a solution of dry ethylene glycol (12.4 g, 200 mmol) in DMF (50 mL) was carefully added KO*t*Bu (16.8 g, 150 mmol) while the temperature was kept below 70 °C. The resulting mixture was stirred at rt for 0.5 h, then a solution of **55** (11.3 g, 50 mmol) in DMF (5 mL) was added in one portion and the mixture was stirred at rt for 1.5 h. The reaction mixture was poured into ice-water (400 mL) and extracted with CH_2Cl_2 (3×50 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent was evaporated to give 12.5 g (99%) of **56** as an oil. TLC: R_f 0.47 (SiO_2; EtOAc). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.15–7.45 (m, 5H, Ar*H*); 3.85 (s, 2H, OCH_2C=O); 3.50–3.70 (m, 6H, OCH_2CH_2OH and NCH_2); 1.75 (hept., 1H, C*H*Me$_2$); 0.91 (d, 6H, 2×CH_3).

2-(2-(*N*-(2-Methyl-1-propyl)-*N*-phenylamino)ethoxy)ethanol (57). To a suspension of $LiAlH_4$ (2.8 g, 75 mmol) in dry toluene (16 mL), dry THF (20 mL) was added dropwise. A solution of **56** (12.5 g, 49 mmol) in dry toluene (5 mL) was added dropwise while the temperature was kept

below 75 °C. When addition was complete, the mixture was stirred at rt for 1 h. H_2O (10 mL) was carefully added and when the initial exotherm had subsided K_2CO_3 (10 g) was added in one portion. The mixture was stirred for 15 min and then filtered. The filterpad was washed with CH_2Cl_2 (2×100 mL) and the phases were separated. The organic phase was dried ($MgSO_4$) and the solvent evaporated to give 10.6 g (91%) of **57** as an oil. TLC: R_f 0.75 (SiO_2; EtOAc).

(*R*)-1-(2-(2-(*N*-(2-Methyl-1-propyl)-*N*-phenylamino)ethoxy)-ethyl)-3-piperidinecarboxylic acid ethyl ester (58). A mixture of **57** (10.6 g, 45 mmol), TEA (7.3 mL, 53 mmol) and CH_2Cl_2 (100 mL) was cooled to −10 °C and MsCl (3.3 mL, 42 mmol) was added dropwise while the temperature was kept below −5 °C. When addition was complete the mixture was stirred at 0 °C for 1 h and then at rt for 0.5 h. The reaction mixture was washed with 0.1 N HCl (2×50 mL), H_2O (50 mL) and brine (10 mL). The organic phase was dried ($MgSO_4$) and the solvent was evaporated. A mixture of the residue, (*R*)-3-piperidinecarboxylic acid ethyl ester (10.0 g, 64 mmol), Li_2CO_3 (3.1 g, 43 mmol) and isopropyl acetate (100 mL) was stirred at rt for 16 h and then heated at reflux for 24 h. The reaction mixture was filtered and the filtrate was concentrated. The residue was dissolved in CH_2Cl_2 (100 mL), dried ($MgSO_4$) and the solvent evaporated. The residue was purified by column chromatography on silica gel (100 g, *n*-heptane/EtOAc = 4:1) to give 8.0 g (47%) of **58** as an oil. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.15–7.25 (dd, 2H, m-Ar*H*); 6.60–6.70 (m, 3H, o,p-Ar*H*); 4.12 (q, 2H, $CO_2CH_2CH_3$); 4.40 (m, 6H, CH_2-$CH_2OCH_2CH_2$NAr); 3.12 (d, 2H, $CHCH_2$NAr); 3.02–3.09 (m, 1H, NCH_2CHCO_2Et); 2.77–2.84 (m, 1H, NCH_2CHCO_2Et); 2.50–2.65 (m, 3H, $CHCO_2Et$ and $OCH_2CH_2N(CH_2)_2$); 1.90–2.25 (m, 4H, NCH_2CH_2-CH_2CH and $CHMe_2$); 1.35–1.75 (m, 4H, NCH_2-CH_2CH_2CH); 1.25 (t, 3H, CH_2CH_3); 0.90 (d, 6H, 2× CH_3).

Acknowledgements

The authors wish to acknowledge the contribution of Karina Madsen, Freddy Petersen and Paw Bloch for skilled synthesis of target compounds and thank Dorthe Andersen for determining IC_{50} values.

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