Synthesis and Pharmacology of the Baclofen Homologues 5-Amino-4-(4-chlorophenyl)pentanoic Acid and the *R*- and *S*-Enantiomers of 5-Amino-3-(4-chlorophenyl)pentanoic Acid

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(*RS*)-5-Amino-4-(4-chlorophenyl)pentanoic acid (**10**) and the *R*-form (**11**) and *S*-form (**12**) of (*RS*)-5-amino-3-(4-chlorophenyl)pentanoic acid, which are homologues of the 4-aminobutanoic acid$_B$ ($GABA_B$) receptor agonist (*RS*)-4-amino-3-(4-chlorophenyl)butanoic acid (baclofen), were synthesized. Compound **10** was synthesized by homologation at the carboxyl end of baclofen using a seven-step reaction sequence. *N*-Boc-protected (4*R*,5*R*)-4-(4-chlorophenyl)-5-hydroxy-2-piperidone (**18**) was deoxygenated via a modified Barton−McCombie reaction to give *N*-Boc-protected (*R*)-4-(4-chlorophenyl)-2-piperidone (**20**), which was ring opened and deprotected to give **11**·HCl. The corresponding *S*-enantiomer, **12**·HCl, was synthesized analogously from the 4*S*,5*S*-enantiomer of **18**, compound **21**. The enantiomeric purities of **11**·HCl (ee = 99.8%) and **12**·HCl (ee = 99.3%) were determined by chiral HPLC. Compound **10** did not show detectable affinity for $GABA_A$ or $GABA_B$ receptor sites and was inactive as an agonist or an antagonist at $GABA_B$ receptors in the guinea pig ileum. Like the enantiomers of baclofen, neither **11** nor **12** showed detectable affinity for $GABA_A$ receptor sites, and in agreement with the findings for (*S*)-baclofen, **12** did not interact significantly with $GABA_B$ receptor sites. Compound **11** (IC_{50} = 7.4 ± 0.6 μM), a homologue of (*R*)-baclofen (**2**), was shown to be some 50 times weaker than **2** (IC_{50} = 0.14 ± 0.01 μM) as an inhibitor of $GABA_B$ binding. Accordingly, **11** (EC_{50} = 150 ± 23 μM) was shown to be weaker than **2** (EC_{50} = 11 ± 1 μM) as an inhibitor of electrically induced contractions of the guinea pig ileum. However, whereas this effect of **2** was sensitive to the $GABA_B$ antagonist, CGP35348 (**4**), the inhibition by **11** was not significantly affected. Furthermore, **12** (EC_{50} = 310 ± 16 μM) was shown to be one-half as potent as **11** in this test system, and this effect of **12** also was insensitive to **4**. The dissimilarities of the pharmacological effects of **2** and compounds **11** and **12** were emphasized by the observation that whereas **2** only inhibits the ileum contraction by 59 ± 5%, **11** as well as **12** were shown to inhibit this response by approximately 94%. Neither **11** nor **12** appeared to affect significantly cholinergic mechanisms in the ileum, and their mechanism(s) of action remain enigmatic.

Introduction

4-Aminobutanoic acid (GABA, **1**) is the major inhibitory neurotransmitter in the central nervous system (CNS), and GABA operates through ionotropic ($GABA_A$ and $GABA_C$) as well as G protein-coupled ($GABA_B$) receptors.[1,2] There is a pharmacological and therapeutic interest in agonists, partial agonists, and antagonists for these receptors and in compounds interacting with modulatory sites associated with GABA receptors.[1−5]

(*R*)-4-Amino-3-(4-chlorophenyl)butanoic acid [(*R*)-baclofen, **2**], which interacts stereospecifically with $GABA_B$ receptors, is the classical $GABA_B$ agonist.[6] Whereas the phosphono amino acid analogue, (*R*)-phaclofen (**3**), is a weak competitive $GABA_B$ antagonist,[7,8] the phosphinic acid analogue of GABA, *P*-(3-aminopropyl)-*P*-(diethoxymethyl)phosphinic acid (CGP35348, **4**) (Figure 1), is a moderately potent $GABA_B$ antagonist capable of penetrating the blood−brain barrier.[2,9,10] The homologous analogue of baclofen, (*RS*)-5-amino-2-(4-chlorophenyl)-pentanoic acid (**5**), does not interact detectably with $GABA_B$ receptors.[11]

Partial GABA receptor agonists of appropriate relative efficacies may have particular therapeutic interest as drugs adapted to regulation of GABA receptor dysfunctions,[5] and we have previously described a number of partial $GABA_A$ receptor agonists, some of which are homologues of GABA.[12,13] Whereas (*R*)-4-amino-3-hydroxybutanoic acid [(*R*)-3-OH-GABA, **6**] is a selective $GABA_B$ agonist,[14] the GABA homologue, 5-aminopentanoic acid (DAVA), is a nonselective $GABA_B$ antagonist.[15] Using these two amino acids as leads, a number of structural hybrids were synthesized and pharmacologically characterized, including (*RS*)-5-amino-3-hydroxypentanoic acid (3-OH-DAVA, **7**), (*S*)-5-amino-2-hydroxypentanoic acid [(*S*)-2-OH-DAVA, **8**], and (*R*)-5-amino-4-hydroxypentanoic acid [(*R*)-4-OH-DAVA, **9**][14,16] (Figure 1). Whereas **7** did not significantly interact with

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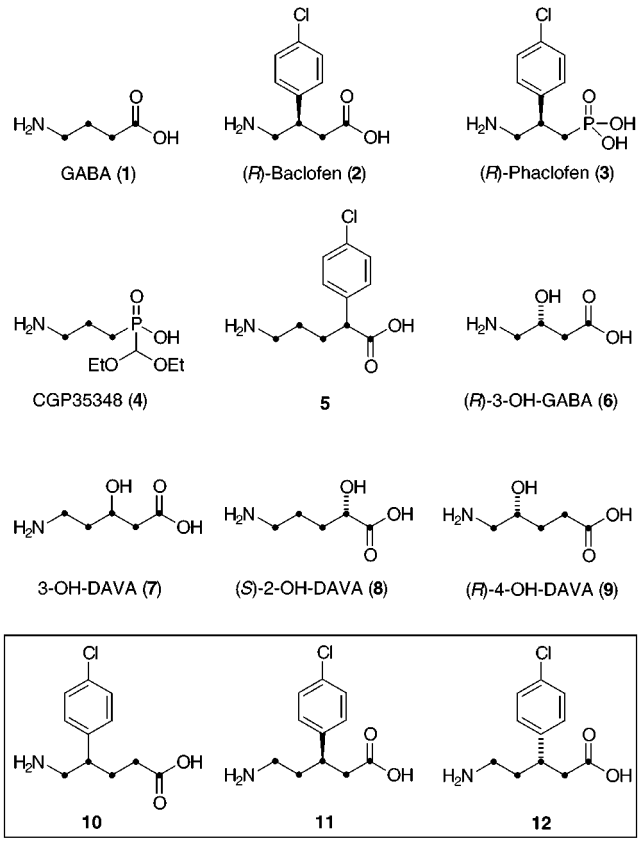


Figure 1. Structures of GABA (**1**), (*R*)-baclofen (**2**), and a number of analogues of GABA, baclofen, and 5-aminopentanoic acid (DAVA).

$GABA_B$ receptors, **8** showed $GABA_B$ antagonism and **9** low-efficacy partial agonism at $GABA_B$ receptors.[14]

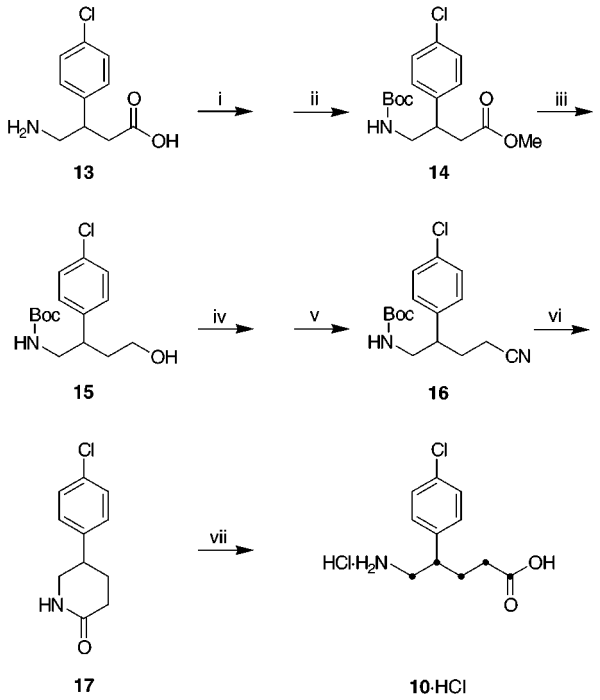
In continuation of this project, the synthesis and pharmacological characterization of (*RS*)-5-amino-4-(4-chlorophenyl)pentanoic acid (**10**) and the *R*-form (**11**) and the *S*-form (**12**) of 5-amino-3-(4-chlorophenyl)-pentanoic acid, which are structural hybrids of baclofen and DAVA, are described (Figure 1).

Results

Chemistry and Stereochemistry. Compound **10**·HCl was synthesized as outlined in Scheme 1. The *N*-Boc-protected methyl ester **14** of (*RS*)-baclofen (**13**) was converted into the primary alcohol **15** using lithium triethylborohydride as the reducing agent, and in two steps, **15** was further transformed into the corresponding nitrile **16**. Methanolysis of the cyano group of **16** and subsequent cyclization of the *N*-deprotected methyl ester formed gave (*RS*)-5-(4-chlorophenyl)-2-piperidone (**17**), which has been synthesized previously using a different procedure.[17] Prolonged treatment of **17** with 6 M hydrochloric acid under reflux gave the target compound **10**·HCl.

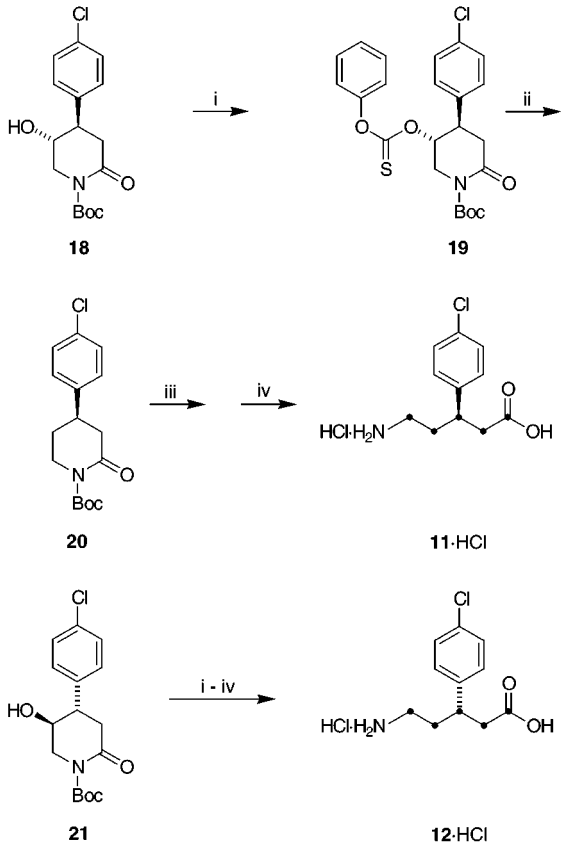
The syntheses of **11** and **12** are outlined in Scheme 2. Compound **11** was synthesized in a four-step reaction sequence, using (4*R*,5*R*)-4-(4-chlorophenyl)-5-hydroxy-2-piperidone (**18**)[18] as the starting material. Removal of the hydroxy group of **18**, which is the key step of the sequence, was unsuccessfully attempted using a variety of reductive substitution reactions, based on zinc as the reducing agent,[19,20] or using platinum-catalyzed hydrogenation conditions.[21] Subsequently, deoxygenation at

Scheme 1[a]



[a] Reagents: (i) MeOH, HCl; (ii) $(Boc)_2O$, Et_3N; (iii) $LiBEt_3H$, THF; (iv) $(C_6H_5)_3P$, CCl_4; (v) NaCN, DMF; (vi) MeOH, HCl, NaOH; (vii) 6 M HCl.

Scheme 2[a]



[a] Reagents: (i) phenyl chlorothionoformate, CH_2Cl_2, DMAP; (ii) $SnBu_3H$, acetone, di-*tert*-butyl peroxyoxalate; (iii) 1 M LiOH, THF, 0.5 M HCl; (iv) 6 M HCl.

C-5 of **18** was attempted using Barton−McCombie reaction conditions. Compound **18** was successfully

Table 1. Receptor Binding and Pharmacological Data (Values ± SEM, $n \geq 5$)

	receptor binding IC_{50} (μM)		guinea pig ileum pharmacology	
compound	$GABA_A$ [^{3}H]muscimol	$GABA_B$ [^{3}H]GABA	EC_{50} (μM)	sensitivity to CGP35348
(*R*)-baclofen (**2**)	>100	0.14 ± 0.1	11 ± 1	yes
(*S*)-baclofen	>100	>100	3300 ± 1300	nd[a]
10	>100	>100	>3000	
11	>100	7.4 ± 0.6	150 ± 23	no
12	>100	>100	310 ± 16	no

[a] nd, not determined.

converted into the thioxocarbonate **19**, but treatment of this intermediate with tributyltin hydride in the presence of AIBN, using previously described conditions,[22] led to extensive decomposition of starting material. Finally, the deoxygenation reaction was completed by treatment of **19** with tributyltin hydride in the presence of small amounts of di-*tert*-butyl peroxyoxalate[23] to give **20** in 94% yield. Ring opening of **20** was accomplished using lithium hydroxide as a base, and treatment of the Boc-protected amino acid formed with hydrochloric acid gave the target compound **11**·HCl. The enantiomer **12**·HCl was synthesized using the 4*S*,5*S*-enantiomer of **18**, namely compound **21**,[18] as the starting material and the reaction conditions described for the synthesis of **11**·HCl (Scheme 2).

The enantiomeric purities of **11** and **12** were determined by chiral HPLC using a Crownpak CR(−) column. The enantiomeric excess (ee) of the more slowly eluting enantiomer, compound **12**, was determined to be 99.8%. Under the analysis conditions used, compound **11** did not contain detectable amounts of **12**, and based on the analysis of a spiked sample of **11**, ee was found to be >99.3%.

Receptor Binding. The affinities of **10**−**12** for $GABA_A$ receptor sites were measured using [^{3}H]muscimol as radioligand,[24] whereas $GABA_B$ receptor affinity was studied as inhibition of baclofen-sensitive and isoguvacine-insensitive [^{3}H]GABA binding.[14] None of the compounds studied showed detectable affinity for $GABA_A$ receptor sites (Table 1). Like (*S*)-baclofen, the *S*-enantiomer, **12**, did not significantly affect the binding of [^{3}H]GABA to $GABA_B$ receptor sites. The *R*-enantiomer, **11**, on the other hand, was shown to be an inhibitor of $GABA_B$ receptor binding, though some 50 times weaker than **2**. Compound **10** was inactive in the $GABA_B$ receptor binding assay.

Guinea Pig Ileum Pharmacology. The isolated longitudinal guinea pig ileum muscle, which previously has been used to characterize $GABA_B$ ligands,[7] was used as a functional test system for comparative studies of (*RS*)-baclofen, baclofen enantiomers, compound **10**, and **11** and **12**. Following published procedures,[7,14] the compounds were characterized as inhibitors of electrically evoked muscle contractions. As shown in Figure 2A,B, **11** and **12** were weak inhibitors of muscle contractions showing EC_{50} values of 150 ± 23 and 310 ± 16 μM, respectively (Table 1). The maximal percentage inhibitions by **11** or **12** were, however, significantly higher (more than 94%) than those produced by (*R*)-baclofen (**2**) or (*RS*)-baclofen (59 ± 5%; *t*-test: $p > 99.5\%$) (Figure 2B). In this functional test system, **2** (EC_{50} = 11 ± 1 μM) was typically some 15 times more potent than **11**, whereas (*S*)-baclofen was essentially inactive (EC_{50} = 3300 ± 1300 μM) (Figure 2A and Table 1). Compound **10** was devoid of affinity for $GABA_B$ receptor sites and was inactive in this functional assay.

The sensitivity of the inhibitory effects of (*RS*)-baclofen, **11**, and **12** to the $GABA_B$ receptor antagonist CGP35348[10] was studied, and the results are illustrated in Figure 2C,D. Whereas the addition of this antagonist caused a parallel shift of the dose−response curve for (*RS*)-baclofen corresponding to a K_i value of approximately 10 μM, the effects of **11** (Figure 2D) or **12** (not illustrated) were not significantly affected by the presence of CGP35348.

Prompted by these latter, quite surprising, observations, the effects of **11** and **12** on acetylcholine-mediated contractions of the guinea pig ileum muscle preparation were studied. At a concentration of 3 μM, **11** was shown to inhibit the acetylcholine-mediated muscle contraction as shown by a shift of the dose−response curve to acetylcholine to the right by a factor of 2 (not illustrated). This inhibitory effect of **11** was, however, concentration-independent, and **12** as well as (*RS*)-baclofen turned out to be inactive at concentrations up to 100 μM.

Discussion

We have previously described the design of partial $GABA_B$ agonists and $GABA_B$ antagonists[14,25,26] as structural hybrids between the $GABA_B$ agonist (*R*)-3-OH-GABA (**6**)[14,27] and the $GABA_B$ antagonist DAVA.[15] Within this series of hydroxylated GABA homologues (Figure 1), 3-OH-DAVA (**7**) turned out to be inactive, whereas (*S*)-2-OH-DAVA (**8**) and (*R*)-4-OH-DAVA (**9**) showed $GABA_B$ antagonist and low-efficacy partial $GABA_B$ agonist effects, respectively.[14] On the basis of computer modeling studies, low-energy conformations of **6**, **8**, and **9** could be superimposed in a flexible four-point fit involving all oxygen and nitrogen atoms.[14]

Although this approach had provided $GABA_B$ receptor ligands showing the desired pharmacological profiles, the key compounds **8** and **9** were not sufficiently potent to be useful as pharmacological tools or potential drugs. This conclusion provoked an analogous approach involving the use of baclofen as a lead. Although both **2** and **6** have the *R*-configuration, the stereochemical orientation of the 4-chlorophenyl group of **2** is "opposite" to that of the hydroxy group of **6** (Figure 1) suggesting that these groups interact with different structural element of the $GABA_B$ receptor recognition site.[25] Furthermore, **2** is somewhat more potent than **6** as a $GABA_B$ agonist, and these aspects prompted us to synthesize structural hybrids between baclofen and DAVA as potential new $GABA_B$ receptor ligands. Soon after the start of this project, the synthesis of (*RS*)-5-amino-2-(4-chlorophenyl)pentanoic acid (**5**) and its inactivity as a $GABA_B$ receptor ligand were reported,[11] and we decided to synthesize the isomeric C-4-substituted DAVA analogue

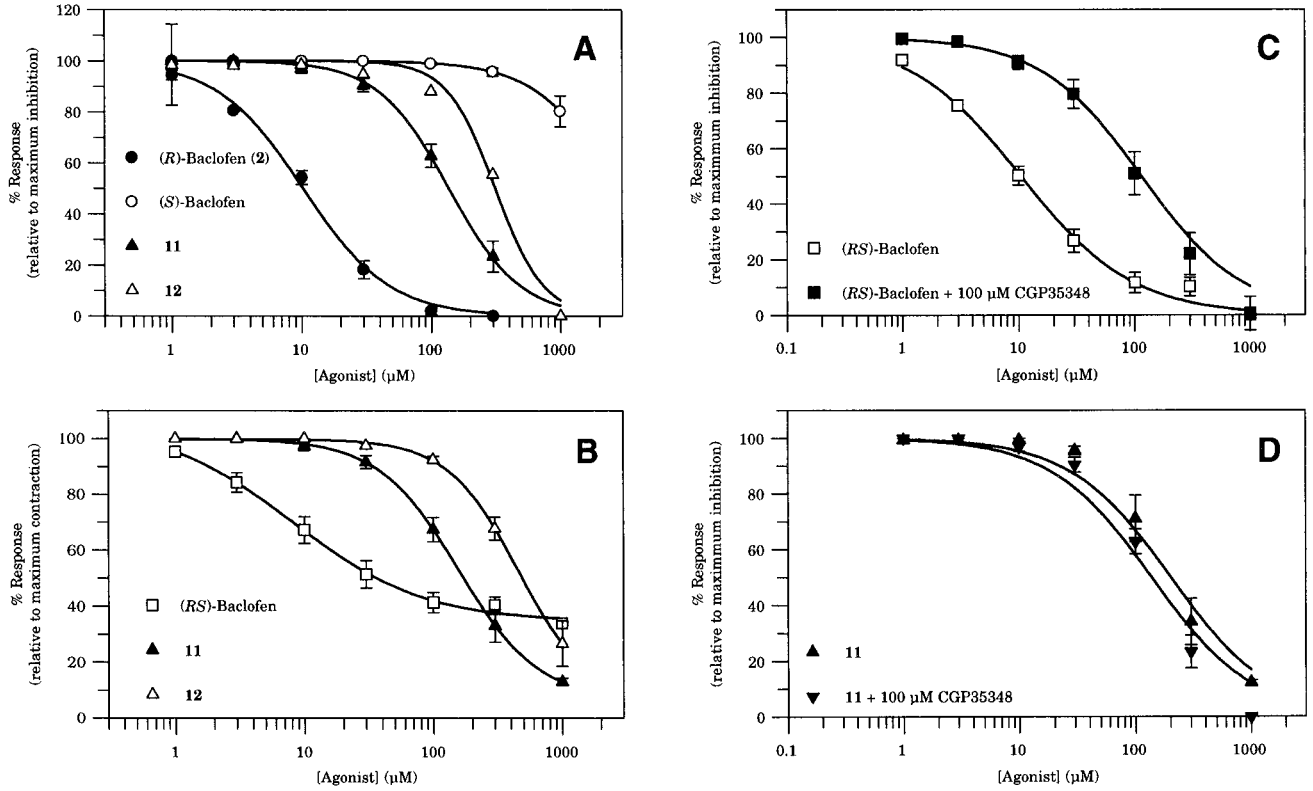


Figure 2. A: Dose-dependent inhibition of contractions of the longitudinal guinea pig ileum. Percent inhibition is expressed relative to maximum inhibition observed for each of the four compounds tested. Data were fitted to the following equation: % inhibition $= 100 \times (1 - [\text{Inh}]^n/([\text{Inh}]^n + EC_{50}{}^n))$, where [Inh] is the concentration of inhibitor and n is the Hill slope of the curve. EC_{50} values are shown in Table 1. B: Inhibition, expressed relative to the maximum muscle contraction obtainable, for each of the three compounds tested. Data were fitted to the following equation: % inhibition $= \max \times (1 - [\text{Inh}]^n/([\text{Inh}]^n + EC_{50}{}^n)) +$ baseline, where max is the maximum muscle contraction, [Inh] is the concentration of inhibitor, n is the Hill slope of the curve, and baseline is the muscle contraction insensitive to the test compound. C: Effects of the competitive $GABA_B$ antagonist CGP35348[9] on the inhibition of muscle contraction by (*RS*)-baclofen, the inhibition being expressed relative to the maximum inhibition obtained. Data were fitted to the following equation: % inhibition $= 100 \times (1 - [\text{Inh}]^n/([\text{Inh}]^n + EC_{50}{}^n))$, where [Inh] is the concentration of inhibitor and n is the Hill slope of the curve. The dose ratio, calculated as the ratio between the concentration of agonist in the presence or absence of 100 μM CGP35348, was transformed into a K_i value by the following equation: $\log(\text{dose ratio} - 1) = -\log K_i - \log [\text{antagonist}]$. D: Effects of the competitive $GABA_B$ antagonist CGP35348 on the inhibition of muscle contraction by **11**, as described above for (*RS*)-baclofen (C). For all of the dose−response data illustrated in panels A−D, values are mean values ± SEM of at least five experiments.

10 and the *R*-form (**11**) and *S*-form (**12**) of the analogue substituted at C-3 of the DAVA backbone (Figure 1).

On the basis of receptor binding studies, the structure−activity relationship (SAR) of **5** and **10**−**12** is complementary to that of the hydroxylated analogues of DAVA, **7**−**9**. Thus, whereas 3-OH-DAVA (**7**) is the only member of the latter series of compounds that is devoid of $GABA_B$ receptor affinity,[14,25] only a C-3-substituted analogue, compound **11**, in the former series of compounds shows significant $GABA_B$ receptor affinity (Table 1). Notably, this receptor affinity, like that of (*R*)-baclofen (**2**), resides in the *R*-enantiomer, **11**, which also shows agonist properties.

Pharmacological data, obtained using the guinea pig ileum $GABA_B$ receptor functional assay, are, however, not fully consistent with the SAR based on receptor binding data (Table 1). Thus, although **12** is devoid of $GABA_B$ receptor affinity, **12** turned out to be approximately one-half as potent as **11** in this ileum assay. Furthermore, both **11** and **12** showed markedly higher efficacy than baclofen in this assay, but in contrast to the effects of baclofen, those of **11** and **12** were not significantly attenuated by the standard $GABA_B$ antagonist, CGP35348 (**4**) (Figure 2).

Thus, compounds **11** and **12** show atypical "$GABA_B$-like" pharmacological effects. These homologues of baclofen appear to interact with $GABA_B$ receptors in a manner different from that of (*R*)-baclofen (**2**), or alternatively, **11** and **12** may show a $GABA_B$ receptor subtype selectivity different from that of **2**. At present, we are not in a position to study the effects of these compounds on cloned subtypes of $GABA_B$ receptors, but such future studies may shed light on the mechanism(s) of action of **11** and **12** and may indicate new directions of $GABA_B$ receptor pharmacology.

Experimental Section

Chemistry. General Procedures. Column chromatography (CC) was performed on Merck silica gel 60. Thin-layer chromatography (TLC) was performed on Merck precoated silica gel 60 F_{254} plates. Compounds were visualized on TLC plates using UV light (254 nm) and/or by iodine vapor. Melting points were determined on a Büchi 510 apparatus and are uncorrected. Evaporations were performed in a vacuum on a rotary evaporator at approximately 15 mmHg. IR spectra were recorded on a Perkin-Elmer spectrophotometer 681. NMR spectra were measured on a Bruker AC 200 instrument (200 MHz for ^{1}H NMR and 50 MHz for ^{13}C NMR). TMS or 1,4-dioxane (δ 3.70) was used as internal standard for spectra

recorded in organic solvents or D_2O, respectively. Electron impact mass spectra (MS) were determined by the Mass Spectrometry Laboratory of the Department of Organic Chemistry at the University of Würzburg on a Finnigan MAT 8200 instrument at 70 eV. Optical rotations were determined with a Perkin-Elmer polarimeter 241 at 589 nm. Elemental analyses were carried out at the Microanalytical Laboratory at the Department of Inorganic Chemistry at the University of Würzburg.

Methyl (*RS*)-4-[*N*-(*tert*-Butyloxycarbonyl)amino]-3-(4-chlorophenyl)butanoate (14). A suspension of (*RS*)-baclofen (**13**) (3.44 g, 16.2 mmol) in MeOH (50 mL) was saturated with HCl_g to give a clear solution. The addition of HCl was continued for a further 10 min, and then the solution was evaporated to give methyl (*RS*)-4-amino-3-(4-chlorophenyl)-butanoate·HCl (3.82 g, 89%) as colorless crystals: mp 182−184 °C; ^{1}H NMR (DMSO-d_6) δ 8.23 (3H, s), 7.50−7.40 (4H, m), 3.56−3.40 (4H, m), 3.07−2.90 (3H, m), 2.90−2.63 (1H, dd, J = 9.5 and 16.4 Hz); ^{13}C NMR ($CDCl_3$) δ 171.4, 139.2, 132.2, 129.9, 128.7, 51.5, 43.2, 39.3, 37.5. Anal. ($C_{11}H_{14}ClNO_2$·HCl) H, N; C: calcd, 50.02; found, 49.43.

Without further purification, this compound (2.19 g, 8.8 mmol) was dissolved in EtOH (100 mL), and to this solution was added at 0 °C a solution of Et_3N (3.61 mL, 26.3 mmol) and di-*tert*-butyl dicarbonate (4.70 g, 21.7 mmol) in C_2H_5OH (20 mL). This solution was left at 0 °C for 1.5 h and then at 25 °C for 30 min, and after evaporation of most of the content of EtOH, the remaining suspension was dissolved in a mixture of H_2O (50 mL) and EtOAc (100 mL). After acidification of the suspension with HOAc, the phases were separated and the aqueous phase was extracted with EtOAc (2 × 50 mL). The combined and dried (Na_2SO_4) organic phases were evaporated, and the residue was subjected to CC (light petroleum−EtOAc) to give **14** (2.40 g, 76%): mp 84.0−85.0 °C; ^{1}H NMR ($CDCl_3$) δ 7.27−7.08 (4H, m), 4.64 (1H, s), 3.54 (3H, s), 3.39−3.17 (3H, m), 2.77−2.55 (1H, dd, J = 5.9 and 15.8 Hz), 2.67−2.42 (1H, dd, J = 7.9 and 15.7 Hz), 1.36 (9H, s); ^{13}C NMR ($CDCl_3$) δ 172.0, 155.6, 139.7, 132.6, 128.8, 128.7, 79.2, 51.5, 45.4, 41.7, 37.8, 28.2. Anal. ($C_{16}H_{22}ClNO_4$) C, H, N.

(*RS*)-4-[*N*-(*tert*-Butyloxycarbonyl)amino]-3-(4-chlorophenyl)butanol (15). To a solution of **14** (1.30 g, 3.9 mmol) in CH_2Cl_2 (100 mL) was added at −30 °C lithium triethylborohydride[28] (11.7 mL, 11.7 mmol, 1 M in THF). The solution was left at −30 °C for 0.5 h and then at 0 °C for 1 h. The reaction was quenched with a saturated aqueous solution of NH_4Cl, and the aqueous phase was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases were dried (Na_2SO_4) and evaporated, and the residue was subjected to CC (light petroleum−EtOAc) to give **15** as a colorless oil (0.92 g, 78%): ^{1}H NMR ($CDCl_3$) δ 7.22−7.01 (4H, m), 4.61 (1H, s), 3.54−3.28 (3H, m), 3.21−2.91 (2H, m), 2.91−2.74 (1H, m), 1.95−1.61 (2H, m), 1.30 (9H, s); ^{13}C NMR ($CDCl_3$) δ 156.0, 140.9, 132.3, 129.1, 128.6, 79.5, 59.9, 45.9, 42.0, 35.9, 28.2. Anal. ($C_{15}H_{22}ClNO_3$) C, H, N.

(*RS*)-4-[*N*-(*tert*-Butyloxycarbonyl)amino]-3-(4-chlorophenyl)butanenitrile (16). A solution of **15** (2.58 g, 8.6 mmol) and triphenylphosphine (2.37 g, 9.0 mmol) in CCl_4[29] (100 mL) was heated to reflux for 5 days. After cooling, triphenylphosphine oxide was filtered. The filtrate was evaporated and the residue subjected to CC (light petroleum−EtOAc) to give (*RS*)-4-[*N*-(*tert*-butyloxycarbonyl)amino]-1-chloro-3-(4-chlorophenyl)butane (3.14 g, 97%): mp 61.0−61.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.24−7.02 (4H, m), 4.58 (1H, t, J = 5.1 Hz), 3.43−3.24 (2H, m), 3.24−3.07 (2H, m), 3.07−2.85 (1H, m), 2.38−1.53 (2H, m), 1.31 (9H, s); ^{13}C NMR ($CDCl_3$) δ 155.6, 139.3, 132.6, 129.1, 128.8, 79.1, 45.5, 42.6, 42.2, 35.8, 28.1. Anal. ($C_{15}H_{21}Cl_2NO_2$) H, N; C: calcd, 56.61; found, 57.27.

Without further purification, a suspension of this compound (1.19 g, 3.6 mmol) and NaCN (0.40 g, 8.2 mmol) in DMF (30 mL) was heated at 90 °C for 4 h. After evaporation of the solvent, the residue was taken up in a mixture of H_2O (50 mL) and EtOAc (100 mL). The organic phase was washed with a saturated aqueous solution of $NaHCO_3$ and then with 1 M HCl and brine, and after drying (Na_2SO_4) this solution was evaporated and the residue subjected to CC (light petroleum−EtOAc) to give **16** (0.91 g, 79%): mp 71.0−72.0 °C; ^{1}H NMR ($CDCl_3$) δ 7.27−7.03 (4H, m), 4.58 (1H, s), 3.49−2.73 (3H, m), 2.31−1.56 (4H, m), 1.33 (9H, s); ^{13}C NMR ($CDCl_3$) δ 155.7, 138.5, 133.0, 129.1, 129.0, 118.9, 79.3, 45.4, 44.6, 28.5, 28.1, 15.0. Anal. ($C_{16}H_{21}ClN_2O_2$) C, H, N.

(*RS*)-5-(4-Chlorophenyl)-2-piperidone (17). A solution of **16** (0.60 g, 1.94 mmol) in MeOH (50 mL) was saturated with HCl_g, and with continuous addition of HCl_g, this solution was refluxed for 2 h. The solvent was evaporated and the crystalline residue taken up in 2 M NaOH (50 mL). This mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were washed with brine, dried (Na_2SO_4), and evaporated. The crystalline residue was subjected to CC (CH_2Cl_2−MeOH) to give **17** (0.35 g, 86%): mp 174 °C (lit. mp 171.5−172.5 °C[17]); ^{1}H NMR ($CDCl_3$) δ 7.34−7.27 (4H, m), 3.59−3.33 (1H, m), 3.34 (1H, dd, J = 10.5 Hz), 3.15−2.92 (1H, ddd, J = 5.0 and 10.3 Hz), 2.63−2.38 (2H, m), 2.16−1.98 (2H, m); ^{13}C NMR ($CDCl_3$) δ 172.1, 140.1, 132.6, 128.7, 128.2, 48.1, 38.7, 30.9, 27.5. Anal. ($C_{11}H_{12}ClNO$) H, N; C: calcd, 63.01; found, 62.42.

(*RS*)-5-Amino-4-(4-chlorophenyl)pentanoic Acid Hydrochloride (10·HCl). A solution of **17** (0.21 g, 1.00 mmol) in 6 M HCl (10 mL) was refluxed for 18 h. After cooling to 25 °C and addition of charcoal (50 mg), the mixture was heated to reflux for 30 min. After cooling to 25 °C, filtration, and evaporation of the solvent, the oily residue was dissolved in EtOH. After addition of acetone and CH_2Cl_2, **10**·HCl (90 mg, 34%) crystallized: mp 163−165 °C; ^{1}H NMR (DMSO-d_6) δ 8.41−7.67 (1H, s), 7.43−7.24 (4H, m), 3.42 (3H, s), 3.35−2.91 (4H, m), 2.21−1.93 (3H, m); ^{13}C NMR (DMSO-d_6) δ 173.9, 139.5, 131.9, 130.1, 128.8, 43.6, 42.2, 31.4, 28.4. Anal. ($C_{11}H_{14}ClNO_2$·HCl·0.5H_2O) C, H, N.

(4*R*,5*R*)-1-(*tert*-Butyloxycarbonyl)-4-(4-chlorophenyl)-5-[*O*-(phenyloxythiocarbonyl)hydroxy]-2-piperidone (19). To a solution of **18**[18] (0.31 g, 0.93 mmol) and phenyl chlorothionoformate (0.20 g, 1.14 mmol) in CH_2Cl_2 (15 mL) was added a solution of 4-(dimethylamino)pyridine (DMAP) (2.17 g, 17.8 mmol) in CH_2Cl_2 (15 mL) under N_2 and within a period of 15 min. After stirring for 18 h at 25 °C, the reaction mixture was quenched with 1 M HCl, and then CH_2Cl_2 (40 mL) was added. The organic phase was washed twice with 1 M HCl and subsequently with a saturated aqueous solution of $NaHCO_3$ and brine. The dried (Na_2SO_4) organic phase was evaporated and the residue subjected to CC (light petroleum−EtOAc) to give **19** (0.37 g, 85%): mp 153−154 °C; $[\alpha]^{20}_D$ = +31° (c = 1.2, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.45−7.02 (9H, m), 5.80−5.58 (1H, m), 4.30−4.07 (1H, dd, J = 4.0 and 14.5 Hz), 3.87−3.65 (1H, dd, J = 3.8 and 14.5 Hz), 3.78−3.53 (1H, m), 3.12−2.90 (1H, dd, J = 6.1 and 17.0 Hz), 2.90−2.68 (1H, dd, J = 7.4 and 17.0 Hz), 1.54 (9H, s); ^{13}C NMR ($CDCl_3$) δ 193.8, 168.9, 153.1, 151.7, 137.4, 133.7, 129.6, 129.3, 128.5, 126.8, 121.6, 83.8, 80.3, 46.2, 41.3, 37.3, 27.9; MS *m/e* (rel intensities) 307 (2), 251 (78), 172 (56), 165 (47), 115 (17), 94 (47), 77 (17), 57 (100), 41 (44). Anal. ($C_{23}H_{24}ClNO_5S$) C, H, N, S.

(*R*)-1-(*tert*-Butyloxycarbonyl)-4-(4-chlorophenyl)-2-piperidone (20). To a solution of **19** (0.35 g, 0.75 mmol) in acetone (50 mL) were added tributyltin hydride (0.30 g, 1.39 mmol) and subsequently di-*tert*-butyl peroxyoxalate (10 mg). The solution was left at 25 °C for 18 h. The opalescent solution was evaporated, and the oily residue was subjected to CC (light petroleum−EtOAc) to give **20** (0.22 g, 94%): mp 95.0−96.0 °C; $[\alpha]^{20}_D$ = +22° (c = 2.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.26−7.06 (4H, m), 3.68−3.44 (1H, dd, J = 4.4 and 10.8 Hz), 3.60−3.36 (1H, dd, J = 4.3 and 10.1 Hz), 3.12−2.93 (1H, m), 2.86−2.63 (1H, ddd, J = 2.0, 5.4, and 17.0 Hz), 2.61−2.38 (1H, dd, J = 11.1 and 17.0 Hz), 2.26−2.03 (1H, ddd, J = 2.0, 4.1, and 13.5 Hz), 1.97−1.80 (1H, m), 1.47 (9H, s); ^{13}C NMR ($CDCl_3$) δ 169.9, 152.6, 141.5, 132.7, 128.9, 127.8, 85.1, 46.7, 41.9, 37.8, 30.2, 28.0; M/S *m/e* (rel intensities) 311 (M^+, 2), 254 (18), 209 (25), 181 (6), 138 (22), 97 (41), 57 (100).

(*R*)-5-Amino-3-(4-chlorophenyl)pentanoic Acid Hydrochloride (11·HCl). To a solution of **20** (0.31 g, 1.0 mmol) in THF (15 mL) was added an aqueous solution of LiOH (1 M,

1.5 mL), and the mixture was stirred for 2 h. After evaporation of the THF, the pH of the aqueous solution was adjusted to 1.5 by addition of 0.5 M HCl. The aqueous layer was extracted with Et_2O (3 × 30 mL), and the combined and dried (Na_2SO_4) organic phases were evaporated. The remaining colorless oil was purified by CC (EtOAc−CH_2Cl_2−AcOH) to give (*R*)-5-[*N*-(*tert*-butyloxycarbonyl)amino]-3-(4-chlorophenyl)pentanoic acid (0.22 g, 64%) as a colorless oil; $[\alpha]^{20}_D = +4°$ ($c = 0.6$, CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 10.88 (1H, s), 7.31−7.14 (4H, m), 4.83 (1H, s), 3.40−3.25 (1H, m), 3.15−3.02 (2H, m), 2.99−2.77 (1H, dd, $J = 6.6$ and 15.5 Hz), 2.87−2.60 (1H, dd, $J = 8.5$ and 15.5 Hz), 2.10−1.86 (2H, m), 1.44 (9H, s); ^{13}C NMR ($CDCl_3$) δ 176.0, 156.0, 141.5, 132.2, 128.7, 128.6, 40.9, 38.7, 38.6, 35.8.

Without further purification, this compound (0.19 g, 0.58 mmol) was mixed with 6 M HCl (10 mL) and the solution heated to reflux for 2 h. After evaporation, the remaining crystalline product was recrystallized (EtOH−acetone−CH_2Cl_2) to give **11**·HCl (0.11 g, 70%): mp 178 °C; $[\alpha]^{20}_D = +12°$ ($c = 0.6$, MeOH); ^{1}H NMR (DMSO-d_6) δ 8.12 (1H, s), 7.38 (4H, m), 3.59 (3H, s), 3.30−3.20 (2H, m), 2.82−2.34 (3H, m), 1.99−1.89 (2H, m); ^{13}C NMR (MeOH-d_4) δ 175.8, 142.2, 133.7, 130.2, 129.8, 41.9, 40.1, 38.9, 24.3. Anal. ($C_{11}H_{14}ClNO_2$·HCl·H_2O) C, N; H: calcd, 6.07; found, 5.65.

(*S*)-5-Amino-3-(4-chlorophenyl)pentanoic Acid Hydrochloride (12·HCl). Compound **12**·HCl was prepared from **21**[18] (0.12 g, 0.36 mmol) following a synthetic sequence and using reaction conditions identical with those described above for the synthesis of **11**·HCl starting with compound **18**. The following intermediates showed IR, ^{1}H NMR, and ^{13}C NMR spectroscopic data essentially identical with those of the corresponding enantiomeric compounds:

(4*S*,5*S*)-1-(*tert*-Butyloxycarbonyl)-4-(4-chlorophenyl)-5-[*O*-(phenyloxythiocarbonyl)hydroxy]-2-piperidone (0.15 g, 89%): mp 154−156 °C; $[\alpha]^{20}_D = -34°$ ($c = 0.9$, CH_2Cl_2). Anal. ($C_{23}H_{24}ClNO_5S$) C, H, N, S.

(*S*)-1-(*tert*-Butyloxycarbonyl)-4-(4-chlorophenyl)-2-piperidone (0.41 g, 90%): mp 95−96 °C; $[\alpha]^{20}_D = -22°$ ($c = 2.0$, CH_2Cl_2); MS *m*/*e* (rel intensities) 311 (M^+, 2), 254 (18), 209 (25), 181 (6), 138 (22), 97 (41), 57 (100).

(*S*)-5-[*N*-(*tert*-Butyloxycarbonyl)amino]-3-(4-chlorophenyl)pentanoic acid (0.15 g, 63%): colorless oil; $[\alpha]^{20}_D = -6°$ ($c = 1.1$, CH_2Cl_2).

12·HCl (0.07 g, 61%): mp 179 °C; $[\alpha]^{20}_D = -12°$ ($c = 0.7$, MeOH). Anal. ($C_{11}H_{14}ClNO_2$·HCl·H_2O) C, H, N.

Determination of the Enantiomeric Purity. A 150- × 4-mm Crownpak CR(−) column (Diacel) was thermostated at 38 °C and eluted at 0.8 mL/min with aqueous perchloric acid, pH 2, using a Waters instrumentation consisting of a M510 pump connected to a UK6 injector and a M991 photodiode array detector with a detection wavelength of 200 nm.

Guinea Pig Ileum Pharmacology. The method used is described in detail by Kristiansen et al.[14] In short, male guinea pigs were stunned and bled; 2−4-cm long segments of the distal ileum, taken 10 to 30 cm from the ileocecal valve, were quickly removed, cleared of intraluminal contents, and placed in a modified Krebs-bicarbonate solution of the composition (mM): NaCl (151.0), KCl (4.7), $MgSO_4$ (0.6), $CaCl_2$ (2.8), $NaHCO_3$ (16.3), NaH_2PO_4 (1.3), and glucose (7.7), which had been equilibrated with a gas mixture of 5% CO_2 and 95% O_2, resulting in a pH of 7.4 at 37 °C. Strips of longitudinal muscle with myenteric plexus attached, obtained by the method of Paton and Zar,[30] were mounted vertically in a 10-mL organ bath containing the modified Krebs-bicarbonate buffer continuously gassed with the mixture of 5% CO_2 and 95% O_2 and maintained at 37 °C. After 60 min of equilibration, the muscle segments were stimulated electrically (0.1 Hz; duration, 5 ms; supramaximal voltage, 100 V) using an HSE stimulator. Isometric muscle contractions were recorded using a force transducer (Grass model FT-03) coupled to a BBC-Goerz Metrawatt recorder. Effects of test compounds were measured as inhibition of the electrically induced contractions and expressed either relative to maximum inhibition induced by the $GABA_B$ agonist (*RS*)-baclofen or relative to maximum inhibition induced by the compound itself. The dose−response curves were obtained by adding cumulative amounts of test compound to the organ bath. When an antagonist was used, it was added to the organ bath 5−7 min prior to the first application of agonist. Between each recorded dose−response curve, the preparations were allowed to recover for 20−30 min until the contractions had returned to baseline level. Each experiment was performed on at least four preparations obtained from two animals. The effect of compounds on contractions elicited by acetylcholine in the absence of electrical stimulation was characterized using the same setup and allowing the compound to equilibrate with the tissue for at least 5 min prior to the first application of agonist.

Receptor Binding. $GABA_A$ and $GABA_B$ receptor binding assays were performed using rat brain synaptic membranes from male Sprague−Dawley rats, tissue preparations being prepared as earlier described.[14] On the day of the assay, the membrane preparation was thawed at room temperature for 45 min, suspended in 75 volumes (w/v) of 5 mM Tris-HCl buffer (pH 7.1) using an Ultra-Turrax homogenizer, and centrifuged at 48.000*g* for 20 min at 4 °C (Sorvall rotor SM34). This step was repeated four times. The final pellet was resuspended in the incubation buffer used in the particular binding assay.

$GABA_A$ binding was studied using [^{3}H]muscimol as radioligand.[24] Membranes were resuspended in 50 mM Tris-HCl buffer (pH 7.4) at 1 mg/mL, and aliquots were incubated with [^{3}H]muscimol (10 nM) for 1 h at 20 °C in a total volume of 1.0 mL in the presence of various $GABA_A$ agonists or antagonists at concentrations ranging from 10^{-10} to 10^{-3} M. Nonspecific binding was defined with 1 mM GABA. Membranes were filtered through Whatman GF/B filters, which were washed three times with ice-cold buffer, and radioactivity was counted by liquid scintillation spectroscopy.

$GABA_B$ receptor binding assays were carried out as previously described.[14] Membranes corresponding to 0.5 mg of protein were incubated in 50 mM Tris-HCl buffer (pH 7.4) containing 2.5 mM $CaCl_2$, in the presence of 20 μM isoguvacine and 50 nM of [^{3}H]GABA. Nonspecific binding was determined using 100 μM (*RS*)-baclofen. Following incubation at 24 °C for 45 min, the membranes were filtered through Whatman GF/B filters, which were washed three times with ice-cold buffer, and radioactivity was counted by liquid scintillation spectroscopy.

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