Articles

N-(Benzyloxycarbonyl)glycine Esters and Amides as New Anticonvulsants

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Glycine is a small neutral amino acid exhibiting weak anticonvulsant activities in vivo. Recently, studies have demonstrated that *N*-(benzyloxycarbonyl)glycine (**1**) antagonized seizures superior to glycine in addition to activity in the maximal electroshock (MES) test, a convulsive model where glycine is inactive. In the present study a series of ester and amide derivatives of **1** as well as esters of *N*-(3-phenylpropanoyl)glycine (**5**) have been prepared. The compounds were evaluated in the MES test as well as in several chemically induced seizure models. Among the derivatives investigated, *N*-(benzyloxycarbonyl)glycine benzylamide (**16**) was the most potent compound exhibiting an anticonvulsant activity in the MES test comparable to the drug phenytoin. Median effective doses (ED_{50}) of 4.8 and 11.6 mg/kg were determined at 30 min and 3 h after ip administration, respectively. Compound **16** also effectively suppressed tonic seizures in different chemically induced models such as the strychnine, 3-mercaptopropionic acid, and pentylenetetrazole tests. Moreover, the compound studied here did not show acute neurotoxicity in the rotorod test up to a dose of 150 mg/kg. It is concluded that *N*-(benzyloxycarbonyl)glycine amides, especially **16**, are potent anticonvulsant agents.

Introduction

In the central nervous system (CNS) glycine exhibits unique properties as one of the major inhibitory neurotransmitters[1] and simultaneously as a coagonist of the excitatory neurotransmitter glutamate.[2] The inhibitory activity is mediated via the strychnine-sensitive glycine receptor, a ligand-gated ion channel, localized mainly in the brain stem and the spinal cord.[3] Interaction with the strychnine-insensitive glycine binding site of the *N*-methyl-D-aspartate (NMDA) receptor complex potentiates the excitatory action of the neurotransmitter glutamate.[4]

In vivo, glycine displays weak anticonvulsant activity in several seizure models including the 3-mercaptopropionic acid test and the strychnine test, while it is inactive in the maximal electroshock (MES) test and the pentylenetetrazole (ScMet) test.[5−11] Moreover, glycine exhibits analgesic[12] and neuroprotective[13] effects. As other agonists of the two glycine receptors, such as L-alanine, L-serine, β-alanine, and taurine for the strychnine-sensitive receptor and D-serine and D-alanine for the strychnine-insensitive glycine receptor, glycine does not easily cross the blood−brain barrier (BBB)[14−16] due to the zwitterionic character and the absence of active transport mechanisms at the luminal side of the endothelial cells. Thus, intracerebroventricular administration or systemic administration of large doses of glycine (10−40 mmol/kg) are necessary for a significant increase in CNS glycine levels.[5]

Recently, we have shown that *N*-(benzyloxycarbonyl)-glycine[17] (**1**) exhibits a higher anticonvulsant activity than glycine after ip administration especially in the MES test.[18] Carbamate derivatives such as *N*-(benzyloxycarbonyl)valine, *N*-(benzyloxycarbonyl)phenylalanine, and *N*-(benzyloxycarbonyl)ethanolamine were devoid of anticonvulsant activity indicating that the *N*-benzyloxycarbonyl group is not responsible for the activity.[19] A log $P = 1.47$ of **1**[19] is in agreement with values known to allow sufficient penetration of the BBB. Besides parameters such as the molecular size, hydrogen bonding, and protein binding, an optimal lipophilicity for brain penetration appears to exist at about log $P = 2$.[20] Intravenous administration of *N*-(benzyloxycarbonyl)[^{14}C]glycine displayed a 13-fold higher brain uptake index compared to glycine.[21] *N*-(Benzyloxycarbonyl)glycine is hydrolyzed in vitro by brain homogenate suggesting a prodrug mechanism.[21,22] Moreover, **1** did not bind to either of the two glycine receptors.[19]

To increase the brain uptake ester- and amide-type lipid conjugates of glycine and *N*-(benzyloxycarbonyl)-glycine (**1**) have been developed.[23] Some of these lipids antagonized MES-induced seizures as well as 3-mercaptopropionic acid- and strychnine-induced seizures. Amide lipids were generally more active than the corresponding ester lipids.

The present study was conducted in order to further evaluate the potential of *N*-acylglycine derivatives as anticonvulsant agents. A series of ester and amide

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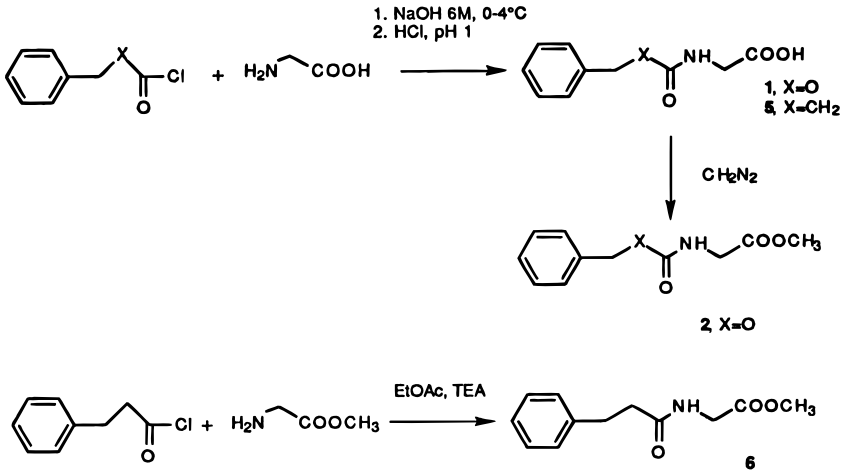
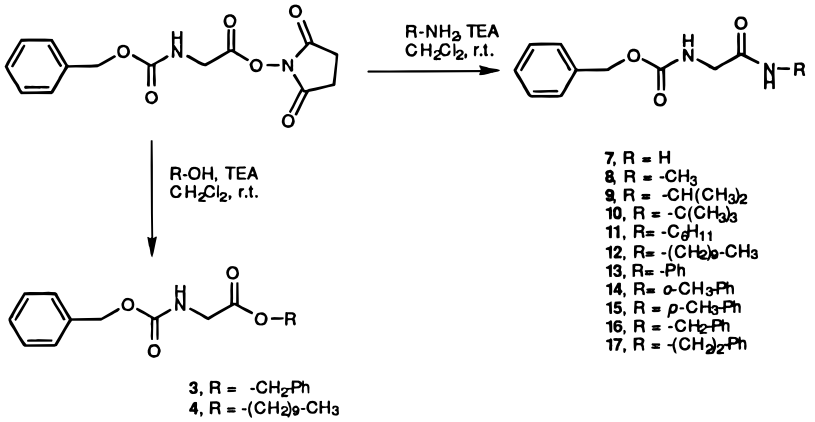
Scheme 1



Scheme 2



derivatives of *N*-(benzyloxycarbonyl)glycine and *N*-(3-phenylpropanoyl)glycine[24] were prepared. The anticonvulsant activity of the compounds was tested in several seizure models.

Results and Discussion

Chemistry. *N*-(Benzyloxycarbonyl)glycine[17] (**1**), *N*-(benzyloxycarbonyl)glycine methyl ester (**2**), *N*-(3-phenylpropanoyl)glycine[24] (**5**), and *N*-(3-phenylpropanoyl)-glycine methyl ester (**6**) were prepared as outlined in Scheme 1. The *N*-(benzyloxycarbonyl)glycine esters **3** and **4** as well as amides **8**−**17** were readily obtained by treatment of *N*-(benzyloxycarbonyl)glycine *N*′-hydroxy-succinimidyl ester with the respective alcohols or amines (Scheme 2). The lipophilicity of the compounds (Table 1) was estimated by calculation using the ClogP program[25] and measured by the determination of isocratic capacity factors, log k_o, using a RP-HPLC assay slightly modified from Bechalany et al.[26] The HPLC method generally gave higher values than the computational method. Between the calculated values and the log k_o values, a good correlation ($r^2 = 0.98$) was obtained (ClogP $= 1.08 \times \log k_o - 1.37$).

Pharmacology. Preliminary evaluation of *N*-(benzyloxycarbonyl)glycine (**1**), *N*-(3-phenylpropanoyl)glycine (**5**), and their respective methyl esters **2** and **6** in comparison to glycine was performed in four different seizure models. The strychnine and the 3-mercaptopropionic acid tests were selected, because glycine showed anticonvulsant effects in these tests though only at very high doses.[5] In addition, the MES test and the ScMet test were selected in which glycine itself is inactive,[18,22] while the acute neurotoxicity was assayed by the rotorod test. None of the compounds exhibited any acute neurotoxicity up to doses of 150 mg/kg. The results of the anticonvulsant testing are summarized in Tables 1 and 2. The *N*-benzyloxycarbonyl derivatives **1** and **2** displayed anticonvulsant activity in the MES test as well as in the strychnine- and 3-mercaptopropionic acid-induced seizures models. The *N*-3-phenylpropanoyl derivatives **5** and **6** were essentially inactive in the chemically induced seizure models while displaying moderate activity in the MES test. None of the compounds showed activity in the ScMet test. The higher anticonvulsant activity of **2** compared to **1** can be explained by an increased BBB penetration of **2** due to its higher lipophilicity (log $P = 1.90$[19]) compared to the parent compound **1**.

Due to the superiority of the carbamates **1** and **2** compared to the amides **5** and **6**, additional benzyl and *n*-decyl esters of **1** were prepared. The benzyl ester **3**[27] was chosen because this derivatization greatly enhanced the CNS activity of the peptide-derived drug *S*-acetylthiorphan,[28−30] while the *n*-decyl ester **4** was designed to further facilitate membrane penetration due to a resemblance to fatty acid ester. However, both esters **3** and **4** displayed only poor activity in the MES test (Table 1).

To increase the metabolic stability of the derivatives, several aliphatic and aromatic amides of **1** were pre-

Table 1. Median Effective Dose (ED_{50}) and Lipophilicity Values (log k_o and ClogP) of Compounds **1**−**17**, Glycine, and Standard Anticonvulsant Drugs in the MES Test after ip Administration to Mice

			ED_{50} (mg/kg)[a] or animals protected/animals tested	
compd	log k_o	ClogP	30 min	3 h
glycine			0/8 at 750 mg/kg[b]	0/8 at 750 mg/kg[b]
1			2/8 at 84 mg/kg[c]	56.5 (40.8−78.1)
2	2.64	1.54	2/8 at 89 mg/kg[c]	16.5 (8.3−32.6)
3	4.35	3.25	4/10 at 120 mg/kg[d]	6/9 at 120 mg/kg[d]
4	>5	6.30	1/8 at 150 mg/kg[c]	5/10 at 150 mg/kg[c]
5			3/9 at 166 mg/kg[c]	6/9 at 166 mg/kg[c]
6	2.37	1.16	3/8 at 89 mg/kg[c]	4/10 at 89 mg/kg[c]
7	1.52	0.34	15.2 (8.5−27.7)	8.3 (4.0−17.7)
8	2.03	0.68	13.6 (6.9−26.4)	7.8 (2.7−21.8)
9	2.65	1.52	14.0 (6.8−28.5)	9.5 (3.5−25.0)
10	3.25	1.92	39.4 (20.9−74.0)	4/8 at 106 mg/kg[c]
11	3.70	2.72	33.4 (13.9−80.4)	3/8 at 116 mg/kg[c]
12	>5	5.45	2/10 at 150 mg/kg[c]	3/8 at 150 mg/kg[c]
13	3.49	2.54	3/10 at 114 mg/kg[c]	4/8 at 114 mg/kg[c]
14	3.49	2.54	1/8 at 120 mg/kgc	74.3 (10.4−130)
15	3.99	3.04	0/8 at 120 mg/kg[c]	27.4 (6.3−121)
16	3.5	2.40	4.8 (2.7−8.6)	11.6 (3.0−33.4)
17	3.88	2.63	8.1 (4.1−16.6)	16.6 (5.3−50.6)
milacemide			2/10 at 58 mg/kg[c]	30.2 (10.1−41.8)
valpromide			14.9 (11.7−17.2)	11.4 (5.7−21.5)
sodium valproate			44.5 (22.1−86.4)	80.6 (50.0−128)
phenytoin			3.5 (2.5−4.9)	3.0 (1.8−5.3)

[a] The data were calculated from 6−7 doses (n = 8−10 animals/dose). The values are expressed in mg/kg; 95% confidence intervals are given in parentheses. [b] Corresponds to 10 mmol/kg. [c] Corresponds to 0.4 mmol/kg. [d] Corresponds to 0.8 mmol/kg.

Table 2. Evaluation of Glycine and Compounds **1**, **2**, **5**, and **6** in the Strychnine and 3-Mercaptopropionic Acid Tests after ip Administration to Mice

		strychnine test[a]		3-mercaptopropionic acid test[b]	
compd	dose (mg/kg)	30 min	3 h	30 min	3 h
vehicle		100 ± 10	100 ± 7	95 ± 7	90 ± 8
1	209	150 ± 27**	142 ± 12***	55 ± 6*	45 ±7*
	418	nd	nd	33 ± 4**	27 ± 4**
2	220	154 ± 10**	139 ± 15**	nd	nd
5	207	99 ± 8	96 ± 14	80 ± 9	100 ± 8
6	220	107 ± 7	92 ± 12	100 ± 5	100 ±7
glycine	150	101 ± 7	131 ± 10 *	60 ±15	53 ± 7*
	300	121 ± 7**	136 ± 10**	nd	nd

[a] The data are expressed as percent of the average latency of the onset of the seizures compared to controls (mean ± SD, n = 8−10 animals). [b] The data are expressed as percent of the tonic seizures of the controls. Statistical evaluation was performed using the t-test for unpaired observations (strychnine test) or Pearson chi square test (3-mercaptopropionic test): *p < 0.1,** p < 0.05,*** p < 0.01.

pared. The results of the MES test are summarized in Table 1. Compared to the respective esters, the amides exhibited a generally higher anticonvulsant activity except for the *n*-decylamide **12**. This may be explained by the poor solubility of the compound even in the vehicle dimethyl sulfoxide which might also result in insufficient absorption from the injection site.

N-(Benzyloxycarbonyl)glycine amide (**7**) and the short-chain alkylamides **8**[31] and **9** displayed high anticonvulsant activity in the MES test (Table 1). All three compounds are highly active 30 min and 3 h after ip administration. Based on a molar ratio the median effective doses (ED_{50}) are superior to those of the anticonvulsant drugs sodium valproate, milacemide, and valpromide (Table 1). Increase of the steric hindrance by introduction of a bulky aliphatic substituent

Table 3. Anticonvulsant Activity of **16** in the Strychnine, 3-Mercaptopropionic Acid, and Pentylenetetrazole Tests after ip Administration of 119 mg/kg to Mice[a]

compd	test time (h)	clonic seizures (%)	tonic seizures (%)	lethality (%)
pentylenetetrazole test (85 mg/kg)				
controls		100 ± 8	67 ± 7	67 ± 9
16	0.5	67 ± 7	0 ± 2***	22 ± 4**
	3	80 ± 11	70 ± 15	60 ± 16
pentylenetetrazole test (120 mg/kg)				
controls		100 ± 7	100 ± 11	100 ± 8
16	0.5	83 ± 9	0 ± 1***	50 ± 6*
3-mercaptopropionic acid test				
controls		100 ± 9	67 ± 8	67 ± 14
16	0.5	83 ± 10	17 ± 5*	0 ± 2**
	3	100 ± 10	0 ± 2**	30 ± 12
strychnine test				
controls			100 ± 7	100 ± 6
16	0.5		33 ± 8**	17 ± 4**
	3		80 ± 8	80 ± 7

[a] Pearson chi square test: *p < 0.1, **p < 0.05, ***p < 0.01.

(compounds **10**[32] and **11**[33]) led to a decreased activity as did a phenyl ring or substituted phenyl groups (compounds **13**,[34] **14**,[35] and **15**[31]). However, separation of the amide bond and the aromatic ring by one or two methylene groups, compounds **16**[31] and **17**,[36] respectively, resulted in highly MES active derivatives. *N*-(Benzyloxycarbonyl)glycine benzylamide (**16**) was the most active derivative of the present series. On a molar basis, the MES activity of **16** can be compared to the MES activity of the drug phenytoin (Table 1).

Because of the high MES activity, compound **16** was selected for further pharmacological evaluations in chemically induced seizure models. The benzylamide **16** antagonized tonic seizures in the strychnine, 3-mercaptopropionic acid, and pentylenetetrazole tests (Table 3). Compared to the parent compound *N*-(benzyloxycarbonyl)glycine (**1**), **16** was by far superior even at lower doses. Moreover, **16** also reduced the lethality consecutive to these tests.

The time curves of anticonvulsant activities in the MES test of *N*-(benzyloxycarbonyl)glycine (**1**), *N*-(benzyloxycarbonyl)glycine methyl ester (**2**), and the benzylamide **16** are summarized in Figure 1. While **1** and **2** exhibit essentially the same behavior with a peak of the activity at 3 h, **16** is highly active 15 min to 1 h after the application with a gradual decrease thereafter. Besides a pharmacokinetic difference, this might be explained by a different mechanism. Neither the amides nor the esters studied displayed significant binding to the strychnine-sensitive glycine receptor or to the glycine binding site of the NMDA receptor. Compounds **1**−**16** failed to displace either [^{3}H]strychnine or [^{3}H]-5,7-dichlorokynurenic acid at concentrations of up to 100 mM. However, while a prodrug mechanism can be assumed for **1**,[21,22] inhibition of esterases by treatment of the animals with bis(*p*-nitrophenyl phosphate), an acylamidase and carboxylesterase inhibitor, did not affect the anticonvulsant potency of compound **16** in the MES test (data not shown). The MES activity of the amides **7**−**17** did not correlate with their lipophilicity as estimated by ClogP or log k_o. Comparable lipophilicities were calculated for **13**, **14**, and **16**, but only **16** displayed high pharmacological activity. The less lipo-

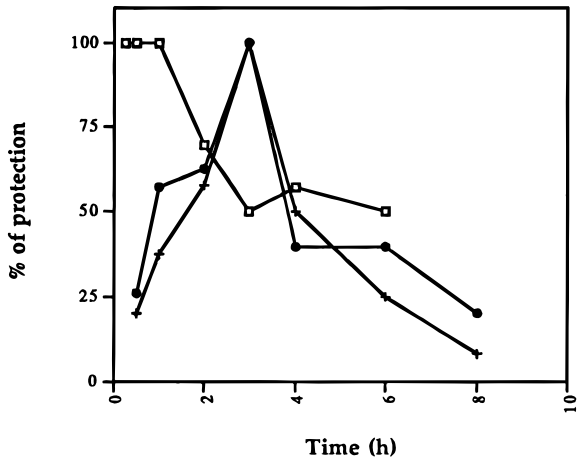


Figure 1. Time curves of anticonvulsant activity in the MES test of compounds (□) **16** (24 mg/kg, 0.08 mmol/kg), (×) **1** (84 mg/kg, 0.4 mmol/kg), and (●) **2** (89 mg/kg, 0.4 mmol/kg) after ip administration to mice (n = 6−8 aminals per time point and compound).

philic derivatives **7**−**9** were also active in the test. Thus, the exact mechanism of action of the *N*-(benzyloxycarbonyl)glycine amides remains to be elucidated. However, the structural similarity to the 2-substituted *N*-acyl amino acid benzylamides developed by Kohn and co-workers[37] suggests that especially **16** might be an effective anticonvulsant agent due to its broad activity in various seizure models.

In conclusion, this paper reports the anticonvulsant activity of esters and amides of *N*-(benzyloxycarbonyl)-glycine and *N*-(3-phenylpropanoyl)glycine. From this series, *N*′-benzyl-*N*-(benzyloxycarbonyl)glycine amide (**16**) displayed high anticonvulsant activity in the MES test comparable to the drug phenytoin. Moreover, **16** also effectively antagonized seizures in several chemically induced models.

Experimental Section

General Methods. Melting points were determined in open capillary tubes with an electrothermal melting point apparatus and are uncorrected. Elemental analyses were performed on a Carlo Erba EA 1108 Analyzer (Carlo Erba, Milano, Italy) and are within ±0.4% of the theoretical values. ^{13}C NMR spectra were recorded at ambient temperature on a AC-300 Bruker spectrometer. The chemical shifts are reported as δ (ppm) values relative to tetramethylsilane. Mass spectra were obtained on a Kratos MS-80 (FAB mode) or LKB GC−MS 9000S instrument. IR spectra were run on a Perkin-Elmer 457 spectrophotometer. Absorption values are expressed as wavenumbers (cm^{-1}). Column chromatography was carried out on silica gel 60 (230−400 mesh; Merck-Belgolabo, Overijse, Belgium). All TLC data were determined using aluminum-backed sheets with silica gel 60 F_{254} (Merck-Belgolabo). Phenytoin was obtained from Sigma (Boornem, Belgium), bis-(*p*-nitrophenyl phosphate) from Aldrich (Boornem, Belgium), and milacemide from Continental Pharma (Mont-Saint Guibert, Belgium). Sodium valproate and valpromide were purchased from Sanofi (Brussels, Belgium); *N*-(benzyloxycarbonyl)-glycine *N*′-hydroxysuccinimidyl ester and *N*-(benzyloxycarbonyl)glycine amide (**7**) were from Fluka (Boornem, Belgium). *N*-(Benzyloxycarbonyl)glycine (**1**) was synthesized as described.[17] Ethyl acetate and methylene chloride were distilled over P_2O_5; triethylamine was distilled over KOH.

***N*-(Benzyloxycarbonyl)glycine Methyl Ester (2).** Diazomethane in diethyl ether was prepared by reaction of KOH and [(*p*-tolylsulfonyl)methyl]nitrosamide (Diazald Kit, Aldrich). Diazomethane was slowly added at 0−4 °C to an ethanolic solution of **1** (6 g, 28.7 mmol) until the mixture became slightly yellowish, and it was stirred at room temperature for 4 h. After removal of the solvents under reduced pressure, the residue was dissolved in 75 mL of ethyl acetate and washed three times with saturated $NaHCO_3$. The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure to give a white solid: yield 5.1 g, 80%; mp 22−25 °C; TLC R_f 0.72 (methylene chloride/acetone, 1:1); ^{13}C NMR ($CDCl_3$) 42.7 (CH_2), 52.3 ($COOCH_3$), 67.1 (C_6H_5-CH_2), 128.1, 128.2, 128.5 (CH arom), 136.3 (C arom), 156.4 (O-CO-NH), 171.5 ($COOCH_3$); MS *m*/*z* 224 (MH^+); IR 1740−1735 ($COOCH_3$). Anal. ($C_{11}H_{13}NO_4$) C,H,N.

***N*-(3-Phenylpropanoyl)glycine (5).** Glycine (3.7 g 50 mmol) was dissolved in 15 mL of 1 M NaOH; 16.8 g of 3-phenylpropanoyl chloride (100 mmol) dissolved in 25 mL of ethyl acetate was added under vigorous stirring and ice cooling. The mixture was stirred at 0−4 °C for 1 h and allowed to warm to room temperature. Excess 3-phenylpropanoyl chloride was removed by extraction with diethyl ether. The aqueous layer was acidified to pH 1 by addition of 6 M HCl and extracted with $CHCl_3$. The organic layer was dried (Na_2SO_4) and evaporated to yield a yellowish solid. Washing with diisopropyl ether gave analytically pure **5**: yield 5.2 g, 51%; mp 113−114 °C (lit.[24] mp 110−111 °C); TLC R_f 0.1 (methylene chloride/acetone, 1:1); ^{13}C NMR ($CDCl_3$) 30.9 ($C_6H_5CH_2$), 36.8 (CH_2-CH_2-CO), 40.9 (NH-CH_2-CO), 126.3, 128.2, 128.5 (CH arom), 140.4 (C arom), 173.3 (CONH), 176.1 (COOH); MS *m*/*z* 207 (M^+); IR 1640 (CO−NH). Anal. ($C_{11}H_{13}NO_3$) C,H,N.

***N*-(3-Phenylpropanoyl)glycine Methyl Ester (6).** Glycine methyl ester (3.3 g, 26 mmol) and 6.6 g of triethylamine (65.7 mmol) were dissolved in 350 mL of ethyl acetate; 6.7 g of phenylpropanoyl chloride (40 mmol) dissolved in 25 mL of ethyl acetate was added dropwise to the mixture at room temperature, and it stirred for 20 h. Following removal of triethylamine hydrochloride by filtration, the ethyl acetate solution was washed successively with 10% citric acid, water, and brine. The organic layer was dried and removed under reduced pressure. Crude **6** was purified by column chromatography using acetone/methylene chloride (1:1) and recrystallized from diethyl ether: yield 2.6 g, 44%; mp 71−72 °C; TLC R_f 0.4 (hexane/propan-2-ol, 4:1); ^{13}C NMR ($CDCl_3$) 31.4 ($C_6H_5CH_2$), 38.0 (CH_2-CH_2-CO), 41.2 (NH-CH_2-CO), 52.4 ($COOCH_3$), 126.3, 128.3, 128.6 (CH arom), 140.3 (C arom), 170.4 ($COOCH_3$), 173.9 (CO-NH); MS *m*/*z* 222 (MH^+); IR 1740−1735 ($COOCH_3$), 1635 (CO−NH). Anal. ($C_{12}H_{15}NO_3$) C,H,N.

General Procedure for the Preparation of Esters 3′ and 4 and Amides 8−17. To a solution of *N*-(benzyloxycarbonyl)glycine *N*′-hydroxysuccinimidyl ester (3 g, 9.8 mmol) and freshly distilled triethylamine (1 g, 10 mmol) in methylene chloride (200 mL) was added 10 mmol of the amine or the alcohol, and the mixture was stirred overnight at room temperature. The mixture was successively washed with water and 10% aqueous citric acid. The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure. The compounds were recrystallized from water/ethanol.

***N*-(Benzyloxycarbonyl)glycine Benzyl Ester (3).** This compound was synthesized according to the procedure described above. After evaporation of the organic layer, a yellowish oil was obtained and purified by chromatography (silica gel, 70−230 mesh; eluent methylene chloride/acetone, 9:1): yield 2.1 g, 70%; mp 71−73 °C (lit.[27] mp 70.5−72 °C); TLC R_f 0.76 (methylene chloride/acetone, 1:1); ^{13}C NMR ($CDCl_3$) 42.8 (CH_2), 67.1 (C_6H_5-CH_2-O), 67.2 (C_6H_5-CH_2-O), 127.8, 128.1, 128.2, 128.4, 128.5, 128.6 (CH arom), 135.2, 136.2 (C arom), 156.3 (O-CO-NH), 169.9 (CH_2-CO-NH); MS *m*/*z* 300 (MH^+); IR 3340 (CH arom), 1720 (COO), 1700 (OCONH). Anal. ($C_{17}H_{17}NO_4$) C,H,N.

***N*-(Benzyloxycarbonyl)glycine decyl ester (4):** yield 2.7 g, 73%; mp 55−57 °C; TLC R_f 0.73 (hexane/2-propanol, 8:2); ^{13}C NMR ($CDCl_3$) 13.8 (CH_3), 22.4, 25.5, 28.2, 28.9, 29.0, 29.2, 29.2, 29.3, 31.6 (CH_2), 42.5 (NH-CH_2-CO), 65.4 ((CH_2)$_{10}$-CH_2-O), 66. 8 (C_6H_5-CH_2-O), 127.8, 127.9, 128.2 (CH arom), 135.9 (C arom), 156.0 (O-CO-NH), 169.7 (CH_2-CO-NH); MS *m*/*z* 377

(MH^+); IR 3350 (CH arom), 2920−2850 (CH alkyl), 1735 (COO), 1690 (OCONH). Anal. ($C_{22}H_{35}NO_4$) C,H,N.

***N*-(Benzyloxycarbonyl)glycine methylamide (8):** yield 0.6 g, 16%; mp 105−107 °C (lit.[31] mp 106−107 °C); TLC R_f 0.46 (methylene chloride/acetone, 1:1); ^{13}C NMR ($CDCl_3$) 26.2 (CH_3), 44.6 (NH-CH_2), 67.2 (C_6H_5-CH_2), 128.1, 128.3, 128.6 (CH arom), 136.1 (C arom), 156.8 (O-CO-NH), 169.8 (CH_2-*C*O-NH); MS *m*/*z* 223 (MH^+); IR 3320−3040 (CH arom), 1710 (OCO-NH), 1660 (CO-NH). Anal. ($C_{11}H_{14}N_2O_3$·0.33H_2O) C,H,N.

***N*-(Benzyloxycarbonyl)glycine isopropylamide (9):** yield 3 g, 74%; mp 96−97 °C; TLC R_f 0.76 (methylene chloride/acetone, 1:1); ^{13}C NMR ($CDCl_3$) 22.2 (CH_3), 41.3 (NH-CH), 44.4 (NH-CH_2), 66.6 (C_6H_5-CH_2), 128.0, 128.1, 128.2 (CH arom), 135.9 (C arom), 156.4 (O-CO-NH), 167.7 (CH_2-*C*O-NH); MS *m*/*z* 251 (MH^+); IR 3280 (CH arom), 1690 (OCONH), 1540 (NHCO). Anal. ($C_{13}H_{18}N_2O_3$) C,H,N.

***N*-(Benzyloxycarbonyl)glycine *tert*-butylamide (10):** yield 1.3 g, 48%; mp 68−70 °C (lit.[32] mp 68.5−71 °C); TLC R_f 0.87 (methylene chloride/acetone, 1:1); ^{13}C NMR ($CDCl_3$) 28.7 (CH_3), 45.1 (NH-CH_2), 51.5 (NH-C), 67.1 (C_6H_5-CH_2), 128.2, 128.3, 128.5 (CH arom), 136.3 (C arom), 156.7 (O-CO-NH), 168.1 (CH_2-*C*O-NH); MS *m*/*z* 265 (MH^+); IR 3310−3250 (CH arom), 1715 (OCONH), 1650 (NHCO). Anal. ($C_{14}H_{20}N_2O_3$·0.5H_2O) C,H,N.

***N*-(Benzyloxycarbonyl)glycine cyclohexylamide (11):** yield 1 g, 41%; mp 108−110 °C (lit.[33] mp 108−110 °C); TLC R_f 0.28 (methylene chloride/acetone, 9:1); ^{13}C NMR ($CDCl_3$) 24.8, 25.5, 33.0 (NH-C_6H_{11}), 44.8 (NH-CH_2), 48.4 (NH-C_6H_{11}), 67.2 (C_6H_5-CH_2), 128.1, 128.3, 128.6 (CH arom), 136.2 (C arom), 156.7 (O-CO-NH), 168.0 (CH_2-*C*O-NH); MS *m*/*z* 291 (MH^+); IR 3320−3260 (CH arom), 2900 (CH alkyl), 1710 (OCONH), 1650−1640 (NHCO). Anal. ($C_{16}H_{22}N_2O_3$) C,H,N.

***N*-(Benzyloxycarbonyl)glycine decylamide (12):** yield 1.3 g, 64%; mp 115−116 °C; TLC R_f 0.85 (methylene chloride/acetone, 1:1); ^{13}C NMR ($CDCl_3$) 14.1 (CH_3), 22.7, 26.9, 29.3, 29.4, 29.5, 29.6, 29.6, 29.67, 31.9, 39.7 (CH_2), 44.8 (NH-CH_2), 67.3 (C_6H_5-CH_2), 128.1, 128.3, 128.7 (CH arom), 136.2 (C arom), 156.6 (O-CO-NH), 168.7 (CH_2-*C*O-NH); MS *m*/*z* 377 (MH^+); IR 3340 (CH arom), 2920−2860 (CH alkyl), 1690 (OCONH), 1640 (NHCO). Anal. ($C_{22}H_{36}N_2O_3$) C,H,N.

***N*-(Benzyloxycarbonyl)glycine anilide (13):** yield 2 g, 44%; mp 148−149 °C (lit.[34] mp 145−149 °C); TLC R_f 0.90 (methylene chloride/acetone, 1:1); ^{13}C NMR ($CDCl_3$) 45.7 (NH-CH_2), 67.5 (C_6H_5-CH_2), 120.0, 124.7, 128.1, 128.4, 128.6, 129.0 (CH arom), 135.9, 137.3 (C arom), 157.0 (O-CO-NH), 167.1 (CH_2-*C*O-NH); MS *m*/*z* 285 (MH^+); IR 3340−3280 (CH arom), 1700 (OCONH), 1610 (NHCO). Anal. ($C_{16}H_{16}N_2O_3$) C,H,N.

***N*-(Benzyloxycarbonyl)glycine *o*-toluidide (14):** yield 2.4 g, 64%; mp 122−124 °C (lit.[35] mp 114−116°C); TLC R_f 0.82 (hexane/2-propanol, 1:1); ^{13}C NMR ($CDCl_3$) 17.6 (CH_3), 45.8 (NH-CH_2), 67.5 (C_6H_5-CH_2), 123.0, 125.4, 126.8, 128.2, 128.4, 128.6, 129.2, 130.5 (CH arom), 135.2, 136.0 (C arom), 157.0 (O-CO-NH), 167.4 (CH_2-*C*O-NH); MS *m*/*z* 299 (MH^+); IR 3340−3270 (CH arom), 1690 (OCONH), 1660 (NHCO). Anal. ($C_{17}H_{18}N_2O_3$) C,H,N.

***N*-(Benzyloxycarbonyl)glycine *p*-toluidide (15):** yield 2 g, 72%; mp 160−162 °C (lit.[31] mp 158−160 °C); TLC R_f 0.9 (methylene chloride/acetone, 1:1); ^{13}C NMR ($CDCl_3$) 20.9 (CH_3), 45.6 (NH-CH_2), 67.5 (C_6H_5-CH_2), 120.1, 128.2, 128.4, 128.7, 129.6, 134.4 (CH arom), 134.8, 136.0 (C arom), 156.9 (O-CO-NH), 167.0 (CH_2-*C*O-NH); MS *m*/*z* 299 (MH^+); IR 3320 (CH arom), 1690 (OCONH), 1610 (NHCO). Anal. ($C_{17}H_{18}N_2O_3$) C,H,N.

***N*-(Benzyloxycarbonyl)glycine benzylamide (16):** yield 5 g, 92%; mp 119−120 °C (lit.[31] mp 116−118 °C); TLC R_f 0.76 (methylene chloride/acetone, 1:1); ^{13}C NMR ($CDCl_3$) 43.6 (CH_2-C_6H_5), 44.8 (NH-CH_2-CO), 67.3 (C_6H_5-CH_2-O), 127.7, 127.8, 128.1, 128.3, 128.6, 128.8 (CH arom), 136.0, 137.7 (C arom), 156.6 (O-CO-NH), 166.7 (CH_2-*C*O-NH); MS *m*/*z* 299 (MH^+); IR 3320−3300 (CH arom), 1690 (OCONH), 1640 (NHCO). Anal. ($C_{17}H_{18}N_2O_3$) C,H,N.

***N*-(Benzyloxycarbonyl)glycine phenethylamide (17):** yield 2.3 g, 46%; mp 109−110 °C (lit.[31] mp 104−107 °C); TLC R_f 0.82 (methylene chloride/acetone, 1:1); ^{13}C NMR ($CDCl_3$) 35.6 (CH_2), 40.6 (CH_2), 44.7 (NH-CH_2-CO), 67.3 (C_6H_5-CH_2-O), 126.6, 128.1, 128.3, 128.6, 128.7, 128.7 (CH arom), 136.1, 138.6 (C arom), 156.5 (O-CO-NH), 168.8 (CH_2-*C*O-NH); MS *m*/*z* 313 (MH^+); IR 3320−3300 (CH arom), 1700 (OCONH), 1650 (NHCO). Anal. ($C_{18}H_{20}N_2O_3$) C,H,N.

Lipophilicity. 1. log k_o Determinations: The log k_o values were measured by RP-HPLC according to the method of Bechalany[26] with slight modifications. The HPLC apparatus consisted of a Perkin-Elmer series 10 liquid chromatograph injector, a UV detector Perkin-Elmer LC-85B variable wavelength spectrophotometer (set at 254 nm), and a Perkin-Elmer LCI-100 laboratory computing integrator. The lipophilic stationary phase was a Bio-Sil C18 HL 90-5 S column, 250 × 4.6 mm, particle size 5 μm (Bio-Rad, Nazareth, Belgium), and the flow rate was set to 1.0 mL/min. The mobile phases were prepared volumetrically from a combination of 10−50% water and 50−90% aqueous phosphate buffer (0.01 M, pH 7.4). All solutions were purified by filtration using a Whatman filtration unit. The logarithms of isocratic capacity factors, log k_o, were defined as $\log k_o = \log(t_r - t_o)/t_o$, where t_r is the retention time of the solute and t_o is the column dead time (methanol was used as the nonretained compound). Assays were made in triplicates with each eluent. log k_o values were extrapolated from at least four determinations using different eluents.

2. log *P* Calculations: The log *P* calculated values were calculated on a Macintosh Power PC using the ClogP 2.0 program[25] (Biobyte Corp., Claremont, CA).

Pharmacology. 1. Electrically Induced Seizures (MES) Test: Male OF1 mice, 15−25 g, were obtained from Iffa-Credo (Les Oncins, France) and housed in colony cages in a 12-h light−dark cycle with free access to commercial rodent chow and water. During the experiments the animals were only allowed free access to water. Maximal electroshock seizures were induced by delivering an electrical stimulus of 50 mA for 0.2 s via corneal electrodes. Blockade of the tonic extension of the hind limbs was considered as protection against seizures.[38] The compounds were dissolved in dimethyl sulfoxide. A volume of 2 mL/kg of the freshly made solutions was injected intraperitoneally. Typically, 8−10 animals were used per compound, dose, and time point. All compounds were screened at an initial dose corresponding to 0.4 mmol/kg. ED_{50} values were determined when the compounds exhibited more than 50% protection at the initial dose of 0.4 mmol/kg and were calculated according to Lietchfield and Wilcoxon.[39] For esterase inhibition experiments, 200 mg/kg bis(*p*-nitrophenyl phosphate) dissolved in 10% Tween 80 saline solution was injected subcutaneously 30 min prior to the MES test or simultaneously with compound **16**.

2. Chemically Induced Seizures: Male NMRI mice (bred at the animal facilities at UCL), weighing 18−25 g, were housed as described above. The compounds were dissolved in dimethyl sulfoxide and injected ip in a constant volume of 2 mL/kg.

3. Strychnine-Induced Seizures: At 30 min or 3 h after the administration of the compounds, the animals received a sc injection of a solution of strychnine chlorhydrate in saline (1.2 mg/mL, 1 mL/kg). The mice were placed in individual cages and observed for 30 min. The time of onset of the seizure,[6] the number of tonic seizures,[38] and the lethality were recorded.

4. Pentylenetetrazole-Induced Seizures: At 30 min and 3 h after the administration of the compounds, 85 or 120 mg/kg pentylenetetrazole dissolved in saline was administered sc. The animals were placed in individual cages and observed for 30 min. The number of clonic and tonic seizures as well as the number of deaths was noted.

5. 3-Mercaptopropionic Acid-Induced Seizures: At 30 min and 3 h after the administration of the compounds, 40 mg/kg 3-mercaptopropionic acid in saline solution were injected ip. The animals were placed in individual cages and observed for 30 min. The number of clonic and tonic seizures as well as the number of deaths was recorded.

6. Acute Neurotoxicity: At the time of the peak effect of the MES test, the animal was subjected to the rotorod test.[38]

Briefly, neurotoxicity is defined as the failure of the animals to maintain equilibrium on a rod rotating at 6 rpm for 1 min in each of three trials.

7. Radioligand Binding Assays: Male Wistar rats (bred at UCL) were sacrificed by decapitation. The cerebral cortex and spinal cord were carefully removed and stored at −80 °C for no more than 24 h. Protein concentration was determined using the Coomassie blue method[40] (Bio-Rad). Stock solutions of the compounds were prepared in absolute ethanol. After filtration, filters were placed in plastic scintillation vials with 7 mL of Aqualuma (Lumac, Schaesberg, The Netherlands), shaken vigorously, and counted in a Pharmacia Wallac 1410 scintillation counter. All assays were performed in triplicate.

8. Strychnine-Insensitive Glycine Binding Site of the NMDA Receptor: Buffy-coat membranes from rat cerebral cortex were prepared according to Canton[41] with minor modifications. Membranes (0.4 mg of protein/mL, final volume 1 mL) were incubated for 30 min at 4 °C with [^{3}H]-5,7-dichlorokynurenic and (New England Nuclear; specific activity 614.2 GBq/mmol, 16.6 Ci/mmol, final concentration 20 nM) in HEPES−KOH buffer (50 mM, pH 7.5). Nonspecific binding was determined with 1 mM glycine. Following the incubation, the homogenate was filtered through Whatman GF/C filters treated with 0.5% poly(ethylenimine) and rinsed three times with 4 mL of ice-cold HEPES−KOH buffer (50 mM) containing 10 mM $MgSO_4$.

9. Strychnine-Sensitive Glycine Receptor: Binding experiments using membranes from rat spinal cord were performed according to Marvizon.[42] The membrane preparations (0.3 mg of protein/mL, final volume 1 mL) were incubated for 15 min at 4 °C in sodium phosphate buffer (50 mM, pH 7.4) containing [^{3}H]strychnine (New England Nuclear; specific activity 906.5 GBq/mmol, 24.5 Ci/mmol, final concentration 2 nM). Nonspecific binding was determined with 10 mM unlabeled strychnine. Following the incubation, the homogenate was filtered through Whatman GF/B glass filters and rinsed three times with 4 mL of ice-cold 0.15 M NaCl.

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