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DESIGN, SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF α-SUBSTITUTED *N*-BENZYLAMIDES OF γ-HYDROXYBUTYRIC ACID WITH POTENTIAL GABA-ERGIC ACTIVITY. PART 6. SEARCH FOR NEW ANTICONVULSANT COMPOUNDS

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Abstract: In the recent study we have extended our investigations to the new anticonvulsant derivatives of α-substituted *N*-benzylamides of γ-hydroxybutyric acid (GHB). Among the obtained compounds *N*-benzylamide of α-(1,2,3,4-tetrahydroisoquinoline)-GHB (**9**) has demonstrated activity against maximal electroshock (MES) induced seizures in mice (at 100 mg/kg *ip*) and in rats (at 30 mg/kg, *po* dose). Lactone **8** and amide **9** have possessed a weak effect on [^{3}H]-muscimol binding. Molecular modeling studies have revealed that anticonvulsant activity of the α-substituted amides of GHB might partially be explained by the orientation of the terminal benzylamide fragment.

Keywords: α-substituted *N*-benzylamides of γ-hydroxybutyric acid, anticonvulsant activity, GABA-ergic activity, molecular modeling

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter involved in the control of neuronal activity in the mammalian central nervous system (CNS) and in the regulation of many physiological mechanisms. Dysfunctions of the gabaergic system lead to several neurological disorders such as epilepsy, insomnia, anxiety, spasticity, neuropathic pain and others. Therefore, agents acting via GABA-receptor complex might be useful for the treatment of these diseases. GABA interacts with three major classes of receptors, $GABA_A$, $GABA_B$, and $GABA_C$. The $GABA_A$ receptor forms a ligand-gated ion channel controlling neuronal chloride ion flux. In addition to the binding site for GABA itself, and specific agonist such as muscimol, the $GABA_A$ receptor possesses several allosteric modulatory sites. The $GABA_A$ receptor complex is allosterically modulated by benzodiazepines, barbiturates, picrotoxin, neurosteroids, γ-butyrolactones, loreclezole and other compounds (1-5). Many GABA-mimetic compounds such as GABA receptor agonists, GABA reuptake inhibitors and inhibitors of GABA metabolism have been reported to be potent anticonvulsant agents, and some of these have been clinically used for the treatment of epilepsy (tiagabine, gabapentin, topiramate, vigabatrin, valproate). However, the antiepileptic activity of these drugs depends also on other mechanisms, including the influence on voltage-dependent ions channels and/or an excitatory neurotransmission (6-9). On the other hand, some antiepileptic drugs are also clinically used for the treatment of other diseases like: neurogenic pain and anxiety disorders (rufinamide, pregabalin). As a consequence, there is no easy way for searching new antiepileptic drugs, and many targets must be taken into consideration.

In this work we have extended our investigation to the new anticonvulsant derivatives of α-substituted *N*-benzylamides of γ-hydroxybutyric acid (GHB) with potential GABA-ergic activity (10-12). Results of pharmacological, physicochemical, and molecular modeling investigations have enabled us to define a pharmacophore model for anticonvulsant *N*-substituted amides of GHB.

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In this model, the presence of the *N*-benzylamide fragment is essential for their activity, as well as a hydrophobic unit (aryl ring) acting as a distal binding site and H-bond donor. It was shown that *N*-substituted amides of GHB possessed anticonvulsant activity in the maximal electroshock (MES) (*ip*) screens. The most potent anticonvulsant compounds were α-(benzylamino)-γ-hydroxybutyric acid *N*-benzylamide **1** and *N*-(2-chlorobenzylamide) **2** (Figure 1), their median effective doses (ED_{50}) and protective index PI (TD_{50}/ED_{50}) values were 63.0 mg/kg (MES), 2.4 and 54.0 mg/kg (MES), 2.9, respectively. These compounds were less active than commonly used anticonvulsants carbamazepine and phenytoin which exhibited PI values of 4.8 and 6.6, respectively. It is worth noting that amides **1** and **2** were found to be more active than sodium valproate (PI = 1.7). In this group of compounds, amide **1a** also possessed anticonvulsant activity in the MES (*ip*) screens at the dose < 100 mg/kg. The biochemical *in vitro* tests have shown that the active amides act as the allosteric modulators of the $GABA_A$ receptor complex, and have affinity to voltage sensitive calcium channels (VSCC) receptors. The *N*-(4-methylbenzyl)amide of α-(4-phenylpiperazin-1-yl)-γ-hydroxybutyric acid **3** has displaced [^{35}S] TBPS (*tert*-butylbicyclophosphorothionate), a ligand specific for the picrotoxin binding site of the $GABA_A$ receptor complex; its IC_{50} value was 95 mM in comparison with GABA itself for which IC_{50} value was 8 mM. Finally, the results of pharmacological *in vivo* experiments in mice showed, that these amides act weakly via GABA receptors, which is consistent with their protective activity proven in the picrotoxin-induced seizures (12).

In this study, we have designed, prepared and pharmacologically evaluated three series of α-substituted *N*-benzylamides of GHB (**A**, **B**, **C**) based on previous data and on the mentioned above pharmacophore model as follows. First, the α-benzylamine group with an aminomethyl linker of active amides (**1**, **2**) was replaced by a rigid skeleton of tetrahydroisoquinoline (THIQ) (series **A**). This modification allowed us to examine the flexibility of the substituent in the α-position of GHB. Second, a glycine spacer was introduced between the GHB backbone and the α-benzylamine group (series **B**) or *N*-phenylpiperazine fragment (series **C**). The modification was inspired by the discovery

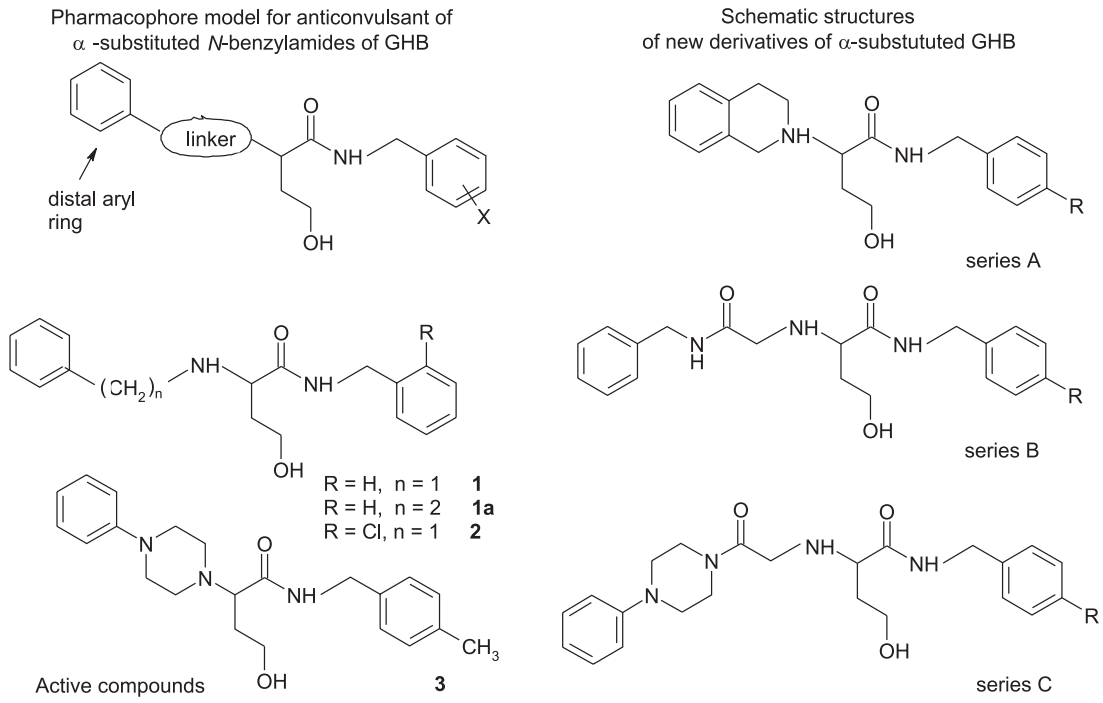


Figure 1. Structures of *N*-benzylamides derivatives of α-substituted GHB.

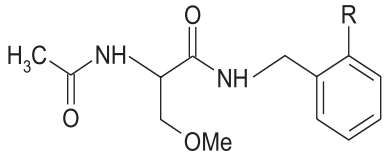


Figure 2. Structure of harkoserid.

of the anticonvulsant activity of the functionalized amino acids (FAA) by H. Kohn and coworkers (13-16). Among these compounds, there is harkoserid [(*R*)-*N*-benzyl-2-acetamido-3-methoxypropionamide] which has entered clinical trials for the treatment of epilepsy and neuropathic pain (Figure 2). Three prepared series **A**-**C** of α-substituted *N*-benzylamides of GHB were evaluated for their anticonvulsant activity at the NIH Epilepsy Branch. Some new compounds have been selected for an *in vitro* evaluation.

EXPERIMENTAL

Chemistry

Melting points were determined with an Electrothermal 9300 apparatus. Reactions were monitored by TLC using silica gel plates (5 × 10 cm, 0.25 mm) Kieselgel 60 F_{254} (Merck) using solvent systems: S_1 chloroform-acetone (1:1, v/v), S_2 chloroform-methanol-acetic acid (60:10:5, v/v/v), S_3 methanol/ammonium hydroxide (25%) (98:2, v/v), S_4 n-hexane-ethanol-triethylamine (7:2:1, v/v/v), with visualization of spots under UV light. 3-Bromobutyrolactone was supplied from Aldrich. ^{1}H-NMR spectra were recorded with a Varian Mercury 300 spectrometer at 300 MHz in $CDCl_3$, with tetramethylsilane as an internal standard. The mass spectra were taken with a Finnigan 95 MAT S apparatus. Elemental analyses (C, H, N) were carried out on a Vario EL III Model Elemental Analyzer and the results are within ± 0.4 % of the theoretical values.

The following abbreviations were used: DMF, dimethylformamide; NMM, *N*-methylmorpholine; TFA, trifluoroacetic acid; TBAB, *tert*-butylammonium bromide;

Glycine *N*-benzylamide (4)

To a cold (-10°C) solution of BOC-glycine (0.05 mol, 8.76 g) in dichloromethane (40 mL) under vigorous stirring NMM (0.05 mol, 5.5. mL) and pivaloyl chloride (0.05 mol, 6.15 mL) were successively added. After 5 min of stirring at -10°C, *N*-benzylamine, 5.25 mL (0.05 mol) in 2 mL of CH_2Cl_2 was added and the mixture was stirred at 0°C for 30 min. After 30 min of standing at room temperature, the solvent was concentrated *in vacuo* and the residue was dissolved in AcOEt (100 mL). The resulting solution was washed with 1 M aqueous $KHSO_4$ (50 mL), saturated sodium bicarbonate (50 mL), water (50 mL), dried over Na_2SO_4, and concentrated *in vacuo* to leave an oily residue which was triturated with n-hexane, collected, and dried *in vacuo*. BOC-glycine benzylamide was obtained in 73 % yield (9.75 g); m.p. 64-68°C; TLC: R_f (S_1) = 0.68, R_f (S_2) = 0.94. Anal. (C H N) calcd. for: $C_{14}H_{20}O_3N_2$; M = 264.325. The resulting BOC-glycine benzylamide 9.25 g (0.035 mol) was deprotected with TFA (20 mL). After standing at room temperature for 1 h, the solvent was concentrated *in vacuo*, and then the residue was diluted with AcOEt (30 mL). The solution was neutralized to pH 8 by addition of 25 % aqueous ammonia, 10 mL of water was added and extracted with AcOEt (3 × 30 mL). The organic phases were combined, dried over sodium sulfate, and concentrated *in vacuo*. The expected compound **4** precipitated upon addition of hexane (10 mL), then it was collected by filtration, and dried *in vacuo*. Yield 4.93 g (86 %), m.p. 125-130°C; TLC: R_f (S_2) = 0.35, R_f (S_3) = 0.49. Anal. (N) calcd. for $C_9H_{12}ON_2$; M = 164.208.

Glycine-4-phenylpiperazin-1-ylamide (5)

Carbonyldiimidazole (0.05 mol, 8.11 g) was added to a solution of BOC-glycine (0.05 mol, 8.76 g) in dry DMF (30 mL). Thirty minutes later 8.60 mL (0.05 mol) of *N*-phenylpiperazine was added and the reaction mixture was stirred at room temperature overnight. The expected compound precipitated upon addition of water. It was collected and dried *in vacuo*. Yield of BOC-glycine-4-phenylpiperazin-1-ylamide 15.5 g (97 %), m.p. 136-139°C; TLC: R_f (S_2) = 0.91, R_f (S_5) = 0.77. Anal. (C H N) calcd. for $C_{17}H_{25}N_3O_3$, M = 319.405. The obtained BOC-glycine-4-phenylpiperazin-1-ylamide 6.5 g (0.02 mol) was treated with TFA (20 mL) as described above. Compound **5** was obtained as TFA salt with yield 3.99 g (60 %); m.p. 170-175° C, TLC: R_f (S_1) = 0.42, R_f (S_2) = 0.22. Anal. (C, H, N) calcd. for $C_{14}H_{18}O_3N_3F$; M = 333.308.

3-(1,2,3,4-Tetrahydroisoquinolin-2-yl)-tetrahydrofuran-2-one (6)

A mixture of 1,2,3,4- tetrahydroisoquinoline (0.10 mol, 13.3 g, 12.5 mL) in acetonitrile (100

mL) and anhydrous K_2CO_3 (0.10 mol, 13.8 g) was stirred at room temperature for 15 min. Then a solution of 3-bromobutyrolactone (0.10 mol, 16.5g, 8.2 mL) in acetonitrile (25 mL) was added dropwise within 15 min, and the stirring was continued for 48 h. The resultant mixture was filtered and the solvent was evaporated. AcOEt (50 mL) and n-hexane (50 mL) were added to the oily residue to obtain crude product which was purified by crystallization from 2-propanol. Yield: 14.5 g (66 %); m.p. 58-63°C; TLC: R_f (S_1) = 0.61, R_f (S_2) = 0.87; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.36-2.45 (m, CH_2), 2.84-2.94 (m, CH_2), 2.91-3.12 (m CH_2), 3.74 (m, CH), 3.95 (s, CH_2), 4.26-4.43 (m, CH_2), 7.00-7.26 (m, 4 PhH); MS (70eV), m/z(%): 217(2) [M^+], 172(7), 158(78), 133(15), 132(100), 117(25), 104(26), 91(5), 56(1). Anal. (C H N) calcd. for $C_{13}H_{15}O_2N$; M = 217.270.

3-(*N*-benzylamidomethylamino)-tetrahydrofuran-2-one (7)

A mixture of glycine *N*-benzylamide (0.05 mol, 8.21 g), anhydrous K_2CO_3 (0.05 mol, 6.91 g) and TBAB (0.005 mol, 1.61 g) in acetonitrile (100 mL) was stirred at room temperature for 30 min. Then a solution of 3-bromobutyrolactone (0.05 mol, 8.25 g, 4.6 mL) in acetonitrile (15 mL) was added dropwise within 15 min and the stirring was continued for 48 h. The resultant mixture was filtered and the solvent was evaporated. The oily residue was dissolved in acetone (50 mL) and acidified with 25% hydrochloric acid. The expected compound precipitated as hydrochloride salt. It was collected by filtration and washed with acetone. Yield: 7.1 g; mp: > 300°C; TLC: R_f (S_2) = 0.68. The obtained *N*-(tetrahydrofuran-2-on-3-yl)-glycine benzylamide hydrochloride was dissolved in small amount of water and the solution was adjusted to pH about 8 with concentrated solution of $NaHCO_3$. Then the product was extracted with AcOEt portions (3 × 50 mL). The combined extracts were dried over anhydrous Na_2SO_4 and the solvent was evaporated *in vacuo* to give a crude oil which crystallized at low temperature. Thus were obtained 5.9 g lactone of **7**; yield (47 %); m.p. 52-56°C (from ethyl acetate); TLC: R_f (S_1) = 0.53, R_f (S_2) = 0.70; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.97-2.10 (m, C*H*$_2$), 2.43-2.60 (m, N*H*), 3.40 (m, C*H*), 3.50-3.60 (m, C*H*$_2$), 4.12-4.20; 4.33-4.39 (m, C*H*$_2$), 4.44-4.50 (m, C*H*$_2$), 7.26-7.45 (m, 5Ph*H* N*H*); MS (70 eV), m/z (%): 248(15) [M^+], 149(96), 147(10), 114(100), 106(13), 91(43), 86(15), 70(10), 56(25). Anal. (C H N) calcd. for $C_{13}H_{16}O_3N_2$; M = 248.28.

3-[(4-Phenylpiperazin-1-yl)-carbonamidomethylamine)]-tetrahydrofuran-2-one (8)

The reaction was carried out as described above for compound **7** using a mixture of glycine-4-phenylpiperazin-1-ylamide (0.025 mol, 5.85 g), anhydrous K_2CO_3 (0.025 mol, 3.45 g) and TBAB (0.0025 mol, 0.81 g) in acetonitrile (100 mL) and then 3-bromobutyrolactone (0.025 mol, 4.1 g, 2.3 mL) in acetonitrile (10 mL). Thus was obtained hydrochloride salt of compound **8** (6.03 g); m.p. 210-215°C; TLC: R_f (S_2) = 0.54. Yield of lactone **8** 5.01 g (62 %); m.p. 110-113°C (from ethyl acetate); TLC: R_f (S_2) = 0.53; R_f (S_4) = 0.14; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.11-2.30, 2.50-2.59 (m, C*H*$_2$ C*H*$_2$), 3.15-3.20 (m, C*H*$_2$NC*H*$_2$), 3.56-3.65 (m, C*H*$_2$NC*H*$_2$), 3.73-3.80 (m, C*H*), 4.18-4.26; 4.39-4.59 (m, C*H*$_2$), 6.90-6.94, 7.26-7.32 (m, 5Ph*H*), 7.44-7.49 (m, N*H*); MS (70 eV), m/z (%): 303(100) [M^+], 304(18), 204(39), 189(14), 162(19), 132(50), 119(17), 114(40), 105(15), 77(13), 56(34). Anal. (C H N) calcd. for $C_{16}H_{21}O_3N_3 + H_2O$; M = 321.377.

***N*-benzylamides of α-substituted γ-hydroxybutyric acid (9-21)**

Lactone **6** (0.02 mol, 4.34 g) was heated at 110°C for 2.5-3 h with appropriate 4-substituted benzylamine (0.04 mol). Crude oily product was purified by crystallization from AcOEt. Thus amides **9** – **14** were obtained. Lactone **7** (0.01 mol) with 4-substituted benzylamine (0.04 mol) was refluxed for 6 h in toluene. The obtained product was crystallized from toluene. Thus amides **15-17** were obtained. Lactone **8** (0.005 mol, 1.52 g) was heated at a temperature of 120°C for 3-5 h with appropriate 4-substituted benzylamine (0.025 mol). The crude product which crystallized at room temperature was purified by crystallization from 2-propanol. Thereby amides **18-21** were obtained.

***N*-benzylamide of α-(1, 2, 3, 4-tetrahydroisoquinoline)-γ-hydroxybutyric amide (9)**

NMR ($CDCl_3$) δ (ppm): 1.95-2.09 (m, C*H*$_2$), 2.70-2.82 (m, C*H*$_2$ C*H*$_2$), 3.39-3.44 (m, C*H*), 3.66-3.94 (m, C*H*$_2$ C*H*$_2$), 4.39-4.55 (m, C*H*$_2$ and O*H*), 6.99-7.35 (m, 5Ph*H* and 4Ph*H*), 7.83 (s, N*H*); MS (70 eV), m/z(%): 191(13), 190(100), 172(10), 158(4), 132(22), 117(4), 105(2), 91(7.19), 77(1), 56(2). Anal. (C H N) calcd. for $C_{20}H_{24}O_2N_2$; M = 324.426.

***N*-(4-chlorobenzylamide) of α-(1,2,3,4-tetrahydroisoquinoline)-γ-hydroxybutyric acid (10)**

Yield: 5.79 g (80%); m.p. 133-136°C (from ethyl acetate); TLC: R_f (S_1) = 0.44, R_f (S_2) = 0.55;

^{1}H-NMR ($CDCl_3$) δ (ppm): 1.94-2.08 (m, CH_2), 2.71-2.83 (m, CH_2 CH_2), 3.38-3.43 (m, C*H*), 3.66-3.93 (m, CH_2 CH_2), 4.34-4.50 (m, CH_2 and O*H*), 6.98-7.29 (m, 4Ph*H* and 4Ph*H*), 7.86 (s, N*H*); MS (70 eV), m/z(%): 191(12), 190(100), 172(12), 158(5), 132(23), 125(8), 117(5), 115(4), 104(4), 56(3). Anal. (C H N) calcd. for $C_{20}H_{23}O_2N_2Cl$; M = 358.872.

***N*-(4-fluorobenzylamide) of α-(1,2,3,4-tetrahydroisoquinoline)-γ-hydroxybutyric acid (11)**

Yield: 4.57 g (67%); m.p. 115-116°C (from ethyl acetate); TLC: R_f (S_1) = 0.46, R_f (S_2) = 0.61; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.94-2.08 (m, CH_2), 2.70-2.85 (m, CH_2 CH_2), 3.38-3.43 (m, C*H*), 3.66-3.93 (m, CH_2 CH_2), 4.35-4.48 (m, CH_2 and O*H*), 6.97-7.26 (m, 4Ph*H* 4Ph*H*), 7.83 (s, N*H*); MS (70 eV), m/z(%): 191(12), 190(100), 172(11), 158(4), 133(3), 132(23), 124(1), 117(4), 109(7), 91(2), 56(3). Anal. (C H N) calcd. for $C_{20}H_{23}O_2N_2F$; M = 342,417.

***N*-(4-methylbenzylamide) of α-(1,2,3,4-tetrahydroisoquinoline)-γ-hydroxybutyric acid (12)**

Yield: 4.90 g (73%); m.p. 111-112°C (from ethyl acetate); TLC: R_f (S_1) = 0.51, R_f (S_2) = 0.69; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.94-2.05(m, CH_2), 2.33 (s, CH_3), 2.69-2.82 (m, CH_2 CH_2), 3.40 (d, *J* = 3.3 Hz C*H*), 3.68-3.80 (m, CH_2), 3.85-3.93 (m, CH_2) 4.39-4.44 (m, CH_2), 6.99-7.26 (m, 4Ph*H* 4Ph*H*), 7.77 (s, N*H*), the signal for the O*H* proton was not detected; MS (70 eV), m/z(%): 338(0.42) [M^+], 191(13), 190(100), 172(9), 158(4), 132(22), 115 (2), 105(8), 91(2), 56(2). Anal. (C H N) calcd. for $C_{21}H_{26}O_2N_2$; M = 338.454.

***N*-(4-methoxybenzylamide) of α-(1,2,3,4-tetrahydroisoquinoline)-γ-hydroxybutyric acid (13)**

Yield: 1.07 g (63%); m.p. 129-130°C (from ethyl acetate); TLC: R_f (S_1) = 0.42, R_f (S_2) = 0.60; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.94-2.04 (m, CH_2), 2.70-2.84 (m, CH_2 CH_2), 3.37-3.41 (m, C*H*), 3.65-3.73 (m, CH_2), 3.79 (s, CH_3), 3.84-3.92 (m, CH_2), 4.32-4.47 (m, CH_2), 6.82-6.86 (m, 2Ph*H*), 6.98-7.25 (m, 6Ph*H*), 7.75 (s, N*H*), the signals for the O*H* proton were not detected; MS (70 eV), m/z(%): 354(5) [M^+], 190(36), 172(35), 158(100), 132(21), 115(19), 104(6), 77(15), 56(7). Anal. (C H N) calcd. for $C_{21}H_{26}O_3N_2$; M = 354.453.

***N*-(2-phenylethylamide of α-(1,2,3,4-tetrahydroisoquinoline)-γ-hydroxybutyric acid (14)**

Yield: 1.73 g (51%); m.p. 92-93°C (from ethyl acetate); TLC: R_f (S_1) = 0.41, R_f (S_2) = 0.55; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.82-2.04 (m, CH_2), 2.55-2.74 m, CH_2 CH_2), 2.79-2.89 (m, CH_2), 3.27-3.49 (m, CH_2), 3.61-3.76 (m, CH_2 CH_2), 3.82-3.89 (m, C*H*), 4.69 (s, O*H*), 6.97-7.27 (m, 5Ph*H* 4Ph*H*), 7.54 (s, N*H*); MS (70eV), *m*/*z* (%): 190(100), 172(9), 158(3), 132(17), 115(2), 104(3), 56(2). Anal. (C H N) calcd. for $C_{21}H_{26}O_2N_2$; M = 338.454.

***N*-benzylamide of α-(*N*-benzylamidomethylamino)-γ-hydroxybutyric acid (15)**

Yield: 6.3 g (84%); m.p: 133-135°C (from toluene); TLC: R_f (S_2) = 0.24; R_f (S_4) = 0.75; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.70-1.94 (m, CH_2), 3.15-3.40 (m, CH_2 C*H*), 3.72-3.80 (m, CH_2), 4.40 (s, CH_2), 4.42 (s, CH_2), 6.80 (t, *J* = 4.9 Hz, N*H*), 7.20-7.35 (m, 5Ph*H* 5Ph*H*), the signal for the O*H* proton was not detected; MS (70 eV), m/z(%): 355(2) [M^+], 221(83), 203(33), 191(54), 149(6), 114(6), 120(6), 106(10), 91(100), 65(4), 56(10). Anal. (C H N) calcd. for $C_{20}H_{25}O_3N_3$; M =355.44.

***N*-(4-chlorobenzylamide) of α-(*N*-benzylamidomethylamino)-γ-hydroxybutyric acid (16)**

Yield: 0.49 g (50%); m.p. 98-101°C (from toluene); TLC: R_f (S_2) = 0.37; R_f (S_4) = 0.75; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.71-1.95 (m, CH_2), 2.3-2.8 (broad O*H*, N*H*), 3.22-3.40 (m, CH_2 C*H*), 3.72-3.84 (m, CH_2), 4.38 (d, *J* = 7.1 Hz, CH_2), 4.42 (dd, *J* = 2.4 Hz, CH_2), 6.66 (s, N*H*), 7.15-7.39 (m, 5Ph*H* 4Ph*H*); MS (70 eV), m/z(%): 289(1) [M^+], 221(96), 203(43), 191(75), 149(26), 140(21), 125(55), 114(30), 106(38), 91(100), 77(12), 56(20). Anal. (C H N) calcd. for $C_{20}H_{24}O_3N_3Cl$; M =389.88.

***N*-(4-methylbenzylamide) of α-(*N*-benzylamidomethylamino)-γ-hydroxybutyric acid (17)**

Yield: 0.69 g (74%); m.p. 129-132°C (from toluene); TLC: R_f (S_2) = 0.47; R_f (S_4) = 0.85; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.69-1.80, 1.82-1.90 (m, CH_2), 2.31 (s, CH_3) 2.5-3.1 (broad O*H*, N*H*), 3.08-3.37 (m, CH_2 C*H*), 3.68-3.78 (m, CH_2), 4.32-4.40 (m, CH_2,CH_2), 6.94 (s, N*H*), 7,10 (s, 4Ph*H*), 7.21-7.35 (m, 5Ph*H*); MS (70 eV), m/z(%): 369(3) [M^+], 221(79), 203(45), 191(82), 189(22), 149(20), 120(22), 114(17), 105(90), 91(100), 77(12), 56(15). Anal. (C H N) calcd. for $C_{21}H_{27}O_3N_3$; M =369.468.

***N*-benzylamide of α-[(4-phenylpiperazin-1-yl)-carbonamidomethylamine]-γ-hydroxybutyric acid (18)**

Yield: 1.52 g (74%); m.p. 79-81°C (from 2-propanol); TLC: R_f (S_2) = 0.78; R_f (S_4) = 0.28; ^{1}H-NMR (CDCl) δ (ppm): 1.74-2.04 (m, C*H*), 3.07-3.21 (m, C*H*NC*H*), 3.25-3.39 (m, C*H*), 3.40-3.52

(m, C*H*), 3.74-3.88 (m, C*H*NC*H* C*H*), 4.40-4.53 (m, CH), 6.90-7.36 (m, 5Ph*H* 5Ph*H*), 7.79(s, N*H*), the signal for the OH proton was not detected; MS (70 eV), m/z(%): 410(19) [M^+], 303(17), 276(100), 258(47), 246(67), 204(21), 163(25), 161(40), 132(47), 120(51), 114(25), 106(43), 91(73), 77(17), 56(38). Anal. (C H N) calcd. for $C_{23}H_{30}O_3N_4$; M = 410.517.

4-Chlorobenzylamide of α-[(4-phenylpiperazin-1-yl)-carbonamidomethylamine]-γ-hydroxybutyric acid (19)

Yield: 1.2 g (54%); m.p. 71-75°C (from 2-propanol); TLC: R_f (S_2) = 0.34, R_f (S_4) = 0.77; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.71-1.83, 1.93-2.04 (m, CH_2), 2.30-3.00 (broad O*H*, N*H*), 3.06-3.22 (m, CH_2NCH_2), 3.24-3.36 (m, C*H*), 3.40-3.55 (m, CH_2), 3.69-3.90 (m, CH_2NCH_2 CH_2), 4.34-4.91 (m, CH_2), 6.89-6.94, 7.18-7.30 (m, 5Ph*H* 4Ph*H*), 7.79 (t, *J* = 5.8 Hz N*H*); MS (70 eV), m/z(%): 444.96(13) [M^+], 303(51), 276(87), 258(43), 246(58), 161(43), 140(43), 132(100), 125(66), 120(78), 114(69), 106(82), 77(35), 56(52). Anal. (C H N) calcd. for $C_{23}H_{29}O_3N_4Cl$; M = 444.962.

4-Fluorobenzylamide of α-[(4-phenylpiperazin-1-yl)-carbonamidomethylamine]-γ-hydroxybutyric acid (20)

Yield: 0.66 g (31%); m.p. 121-123°C (from 2-propanol); TLC: R_f (S_2) = 0.12, R_f (S_4) = 0.53; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.72-1.84, 1.93-2.02 (m, CH_2), 3.07-3.21 (m, CH_2NCH_2), 3.24-3.41 (m, C*H*), 3.44-3.52 (m, CH_2), 3.70-3.82 (m, CH_2NCH_2), 3.83-3.87 (m, CH_2), 4.34-4.51 (m, CH_2), 6.90-6.95, 6.98-7.50, 7.22-7.32 (m, 5Ph*H* 4Ph*H*), 7.76 (t, *J* = 5.9 Hz, N*H*), the signal for the O*H* proton was not detected; MS (70 eV), m/z(%): 428(21) [M^+], 303(35), 258(47), 276(100), 246(67), 204(24), 161(46), 145(23), 132(73), 124(43), 120(62), 109(85), 105(40), 77(19), 56(44). Anal. (C H N) calcd. for $C_{23}H_{29}O_3N_4F$; M = 428.508.

4-Methylbenzylamide of α-[(4-phenylpiperazin-1-yl)-carbonamidomethylamine]-γ-hydroxybutyric acid (21)

Yield: 0.66 g (31%); m p. 98-101°C (from 2-propanol); TLC: R_f (S_2) = 0.34, R_f (S_4) = 0.77; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.73-1.84, 1.94-2.02 (m, CH_2), 2.33 (s, CH_3), 3.08-3.19 (m, CH_2NCH_2), 3.25-3.41 (m, C*H*), 3.44-3.50 (m, CH_2), 3.71-3.80 (m, CH_2NCH_2), 3.83-3.89 (m, CH_2), 4.35-4.71 (m, CH_2), 6.90-6.95, 7.12-7.17, 7.18-7.32 (m, 5Ph*H* 4Ph*H*), 7.68 (t, *J* = 5.7 Hz, N*H*), the signal for the O*H* proton was not detected; MS (70 eV), m/z(%): 424(15) [M^+], 276(77), 258(37), 246(53), 204(14), 189(23), 161(31), 145(15), 132(43), 120(54), 114(24), 105(100), 91(18), 77(15), 56(30.13). Anal. (C H N) calcd. for $C_{24}H_{32}O_3N_4$; M = 424.545.

Molecular modeling study

The compounds were modeled and minimized using PM3 (MOPAC) method of the Alchemy 2000 programme (Alchemy 2000 ver. 2.0, Tripos Inc., St. Luis, USA, 1997). The conformational analyses were carried out by systematic stepwise rotation by 10° four torsion angles marked in Figure 3 as ϕ_1- ϕ_4, common to all molecules. The energy was calculated for all conformations taking into account both the molecular mechanics and Van der Waals interactions. Energy criterion, set to 3 kcal/mol above the lowest energy conformation found, was used to accept new conformation. Distances between atoms, which are not involved in building atomic bonds were above 76% of the sum of their Van der Waal's radii. The RMS routine is a procedure which provided an estimation how closely molecules fit to each other. The quality of the fit is determined via an RMS calculation using the equation:

$$RMS = \sqrt{\frac{\Sigma d^2}{n}}$$

where: *d* is the distance between two paired atoms and *n* is the number of pairs that are fitted.The lower *RMS* fit value is, the better is similarity.

Pharmacology

Preliminary anticonvulsant assays.

Phase I evaluations included three tests: maximal electroshock (MES), subcutaneous pentetrazole (scMet), and rotorod test for neurological toxicity (Tox).

Animals

Male albino, CF No. 1 mice (18-25 g, Charles River, Willimington, MA, USA) and male albino, Sprague–Dawley rats (100-150 g, Charles River, Willimington, MA, USA) were used in the experiment. Compounds were either dissolved in saline or suspended in 0.5% methylcellulose. Mice were administered compound by *ip* injection at three dosage levels (30, 100, and 300 mg/kg) with anticonvulsant activity, and neurotoxicity noted 15, 30, 60 and 240 min after administration.

Maximal electroshock seizure (MES) test (17)

Maximal electroshock seizures were elicited with a 60-cycle alternating current of 50 mA inten-

sity (5-7 times that necessary to elicit minimal electroshock seizures) delivered for 0.2 s via corneal electrodes. A drop of 0.5% tetracaine hydrochloride in 0.9% saline was instilled in the eye prior to application of the electrodes. Protective endpoints were defined as abolition of the hind limb tonic extension component of the seizure. Results are expressed as a ratio of the number of animals protected to number of animals tested.

Pentetrazole inducted seizure (scMet)

A dose of 85 mg/kg of pentetrazole, which produces seizures in more than 95% of mice, was administered as a 0.5% solution subcutaneously in the posterior midline. Animals were observed for 30 min. A failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5 s duration) was defined as protection and the results were expressed as number of animals protected / number of animals tested.

Neurotoxicity (18, 19)

The rotorod test was used to evaluate neurotoxicity in mice. Animals were placed on a 1-inch diameter knurled plastic rod rotating at 6 rpm. Non-toxic (normal animals) mice can remain on a rod rotating at this speed almost indefinitely. Neurological toxicity is defined as the failure of the animal to remain on the rod for 1 min and is expressed as a number of animals exhibiting toxicity per a number of animals tested. Animals are considered toxic if they fail this test on three successive attempts. Rat toxicity was determined using overt evidence of ataxia, abnormal gait or the positional sense test.

Radioligand binding assay

Frozen Wistar rat cortices stored at -80°C were used for radioligand binding assay. Tissues were thawed in 50 volumes of ice-cold 50 mM TRIS-HCl buffer, pH 7.4, homogenized (Ultra-Turrax) and centrifuged at 30,000 × g for 40 min at 4°C. Tissue pellets were resuspended in ice-cold buffer and centrifuged at 30,000 × g for 50 min (4°C). Following three further washes, pellets were stored at -20°C for at least 18 h. On the day of the assay, membrane pellets were thawed at room temperature, resuspended in ice-cold buffer and centrifuged at 30,000 × g for 20 min. Finally, the pellets were resuspended by homogenization in the required volume of assay buffer (~0.5 mg protein/mL). Binding assays were performed in plates (MAFCNOB 10, MultiScreen® – FC, Millipore) and the final incubation mixture (final volume 300 mL) consisted of 240 mL of membrane suspension and 30 mL of buffer containing from seven to eight concentrations (10^{-11} – 10^{-4} M) of tested compounds. Nonspecific binding was determined in the presence of 100 mM GABA. Samples were incubated for 10 min at 0°C. The incubation was terminated by rapid filtration over glass fiber (Whatman GF/C) using a Vacuum manifold (Millipore). The filters were then washed 2 times with 0.1 mL of ice-cold buffer (pH 7.4) and placed in scintillation vials with scintillation counter. All assays were done in duplicates. Radioligand binding data were analyzed using iterative curve fitting routines (GraphPAD/Prism, version 3.0, San Diego, CA, USA).

RESULTS AND DISCUSSION

Chemistry

The route of the synthesis of the new compounds is shown in Scheme 1. The synthesis of the α-substituted *N*-benzylamides of GHB required the preparation of 3-substituted derivatives of tetrahydrofuran-2-ones **6**–**8**, which were prepared from 3-bromobutyrolactone and 1,2,3,4-tetrahydroisoquinoline, glycine *N*-benzylamide (**4**) and glycine-4-phenylpiperazin-1-ylamide (**5**), respectively. The two later compounds were obtained in the reaction of *tert*-butyloxycarbonylglycine with trimethylacetyl chloride in the presence of *N*-methylmorpholine and benzylamine or with carbonyldiimidazole and 4-phenylpiperazine. A deprotection of the obtained BOC-protected glycine amides with TFA, provided the desired glycine amides **4** and **5**. The aminolysis of lactones **6**-**8** with various 4-substituted benzylamines yielded the target compounds **9**-**21**.

Pharmacology

Preliminary assays

Preliminary anticonvulsant evaluation (phase I) of all the synthesized amides **9**-**21** was provided by testing procedures which have been described earlier (17) in protocols designed and sponsored by the National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD, USA, for the Anticonvulsant Screening Program (ASP). Phase I studies of the investigated compounds involved three initial model evaluations: maximal electroshock seizure model (MES), subcutaneous metrazole (scM) model and the rotorod test for assessment of possible neurological toxicities. Phase I is a qualitative assay involving a relatively small number of animals (total 16 mice). All tested

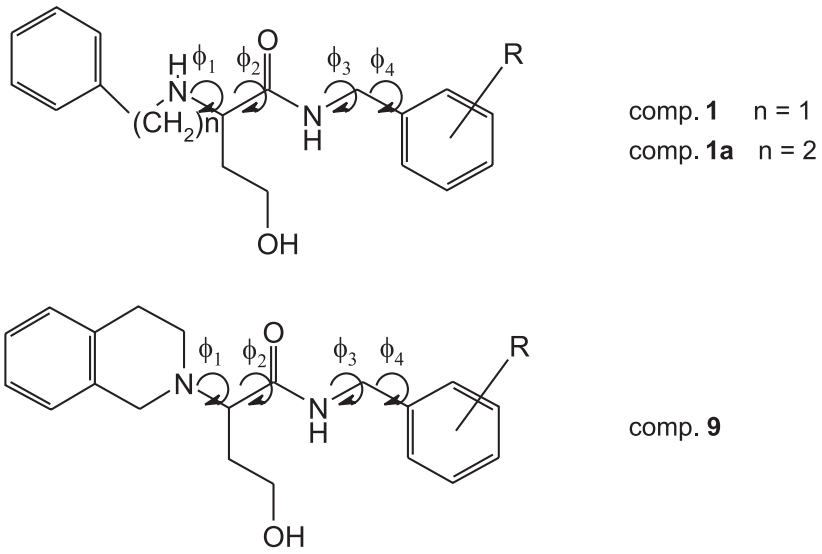
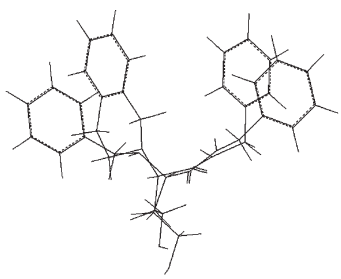
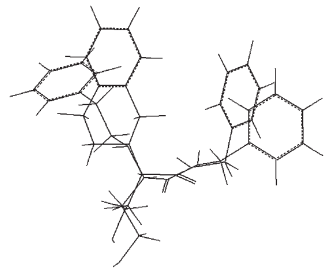


Figure 3. Schematic structure of studied amides.



A

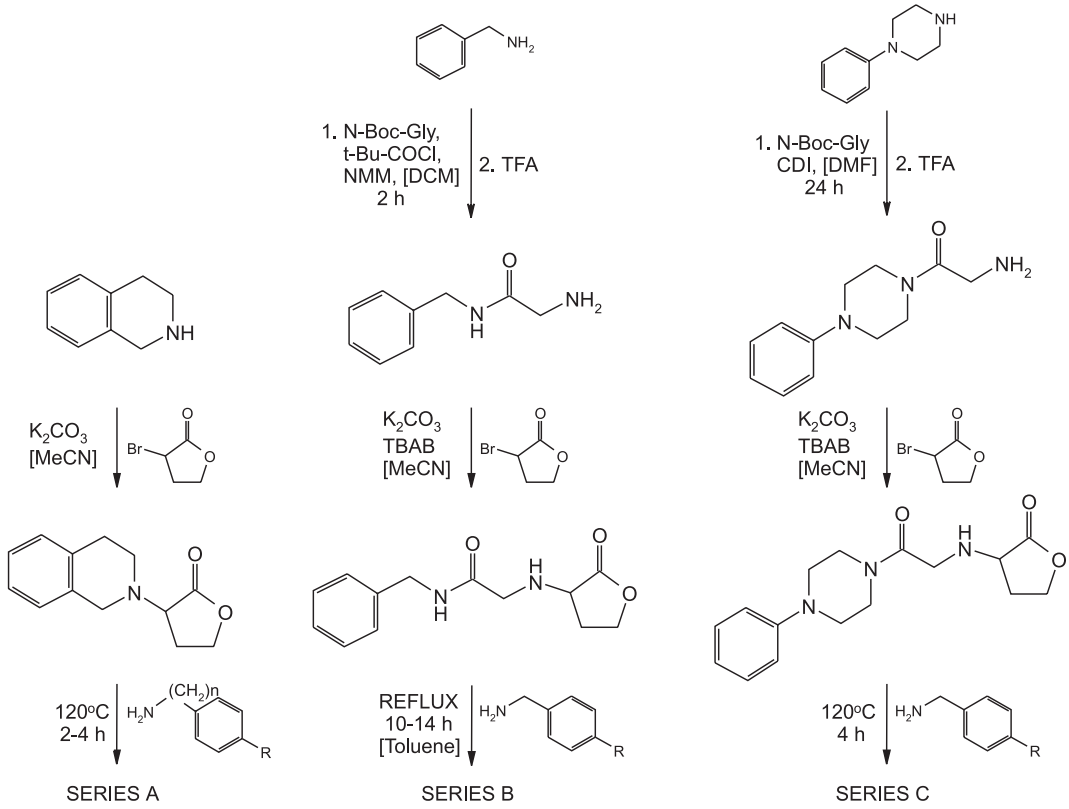
Figure 4a. Superimposition of anticonvulsant active *N*-benzylamide of α-(benzylamino)-γ-hydroxybutyric acid (compound **1**) and new compound **9**.

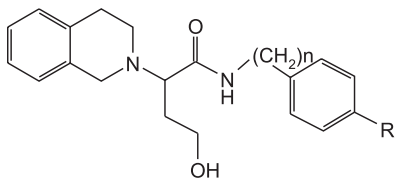


B

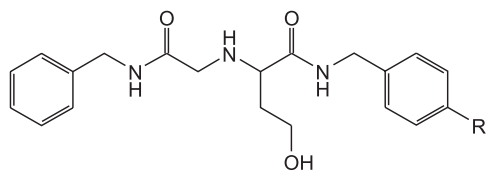
Figure 4b. Superimposition of anticonvulsant active *N*-benzylamide of α-(2-phenyletylamino)-γ-hydroxybutyric acid (compound **1a**) and new compound **9**.

compounds at this stage were administered intraperitoneally. For the MES assay, the investigated compound is administered to one, three and one animals at three respective doses (30, 100 and 300 mg/kg) using 0.25-, 0.5- and 4-h time periods. A similar paradigm for dosing and time period is used for the scMet and toxicity testing. All animals were also examined for potential neurological deficit at each dose and time period utilizing the ASP's standard rotorod model. Three of tested amides (**9**, **11** and **14**) demonstrated protective activity against MES seizures in mice. Compounds **9** and **14** were active at doses of 100 mg/kg (2/3 and 1/3 animals protected) 0.25 h after administration, inactive at later time points. The amides **9**, **11** and **14** were active at 300 mg/kg 0.5 h after administration, however, toxicity was also observed at these doses. Compound **9** displayed a marked MES activity in mice and was subsequently evaluated for oral activity in rats. Amide **9** demonstrated activity in the MES screen at 30 mg/kg (*po* dosing), 1/4 of animals protected at 0.25, 0.5 and 4 h; 1/2 of animals protected at 2 h. Amide **9** was used in rat testing and was not toxic at the doses employed. The results of *in vivo* testing are summarized in Table 1. Amides of series **B** and **C** were not active in these assays nor were they toxic (data not shown). None of the compounds were active in the ScMet mouse screens at the doses and time points evaluated. Based on these preliminary qualitative pharmacological evaluations and for comparison activity of earlier tested derivatives of GHB, the selected compounds were

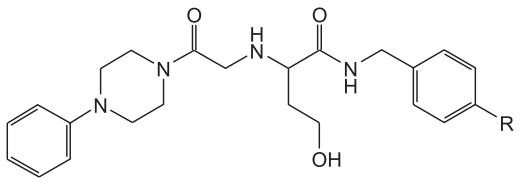




Compd.	n	R
9	1	H
10	1	Cl
11	1	F
12	1	CH_3
13	1	OCH_3
14	2	H



Compd.	R
15	H
16	Cl
17	CH_3



Compd.	R
18	H
19	Cl
20	F
21	CH_3

Scheme1. Synthesis of new compounds.

Table 1. Anticonvulsant screening project (ASP): phase I – test results in mice.

Compound	Dose (mg/kg)	Activity MES[a] time (h)		TOX[b] time (h)	
		0.25	0.5	0.25	0.5
9	100	2/3	0/3	2/3	1/8
	300	-	1/1	-	4/4
11	100	0/3	0/3	0/3	1/8
	300	-	1/1	-	1/4
14	100	1/3	0/3	0/3	0/8
	300	-	1/1	-	3/4

[a] Maximal electroshock test (number of animal protected/number of animals tested).
[b] Rotorod toxicity (number of animals exhibiting toxicity/number of animals tested).

Table 2. Displacement of radioactive ligand of $GABA_A$ receptor from rat brain membranes by selected compounds.

Compound	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**18**	GABA
% Displacement of [^{3}H] -muscimol at 100 μM	0	10	11	7	0	0	6	0	0	0	100

evaluated *in vitro* for their ability to displace radioligand from rat brain membrane preparations.

Radioligand receptor binding assay ($GABA_A$)

Binding to the $GABA_A$ receptor site was determined by displacement of [^{3}H]-muscimol. For this test lactones **7** and **8**, amides of series **A** and one of the representative amides of series **B** (amide **15**) and from series **C** (amide **18**) were selected. The results are presented as a percent of specific displacement of [^{3}H]-muscimol in relation to GABA (100%) at 100 mM concentration (Table 2). Lactone **8** and amides **9**, **10**, **13** weakly displaced (6-11%) [^{3}H]-muscimol from its binding sites, whereas the other tested compounds did not reveal any affinity for $GABA_A$ receptors in this assay.

Molecular modeling study

To study the flexibility of the synthesized amides of series **A**, preliminary molecular modeling investigations along with conformational analysis were performed. Molecular modeling investigations were carried out on two pairs of compounds: **1** – **9** and **1a** – **9**. The conformational analyses were carried out by systematic stepwise rotation of 10° four torsion angles marked in Figure 3 as ϕ_1-ϕ_4, common to all molecules. Energy criterion was set to 3 kcal/mol above the lowest energy conformation. Although this approach explores only a part of all possible conformations, the results give an estimate of the range of energy changes in geometry of isolated molecules. The calculated structures were optimized and each structure for one pair of compounds was constrained to superimpose, taking into account three atoms common to all evaluated molecules. Two nitrogen atoms and the carbon unsubstituted atom from the hydroxyalkyl chain were selected for fitting procedures. Their similarity was calculated as *RMS* fit. The *RMS* routine provided estimates of how closely molecules fit to each other. The lower the *RMS* value, the better the similarity observed. The *RMS* deviations of the two pairs of amides were 0.182 Å (**1**, **9**), and 0.137 Å (**1a**, **9**), respectively. Comparison of molecules from each pair (Figures 4a and 4b) showed similarity in the orientation of the GHB chain, while both the benzylamide moiety and a substituent in the α-position of GHB possessed a different orientation.

Our earlier study indicated similarity in the orientation of both the GHB chain and the *N*-benzylamide moiety for anticonvulsant active amides (**1**, **1a**) (10). Amide **9** with a rigid 1,2,3,4-tetrahydroisoquinoline skeleton was less active than compounds **1** and **1a** (MES screens). The benzylamide moiety appears to be an important pharmacophore group in many anticonvulsant agents such as derivatives of amino acids (14-16, 20-21). These results reveal the importance of the orientation of the terminal benzylamide fragment for anticonvulsant activity of α-substituted amides of GHB.

Conclusion

Considering our previous investigations with analogs of GHB and the pharmacophore model for anticonvulsant *N*-substituted amides of GHB, we analyzed how the modifications influenced the pharmacological action of these compounds. The results of the preliminary anticonvulsant evaluation according to the established procedure by NIH Anticonvulsant Screening Program showed that

among the examined compounds only the series A amides (**9**, **11** and **14**) were active in the MES test. Compound **9** was the most active (but also toxic at the active dose). Amide **9** is a rigid analog of the active amide **1**. The difference observed in the shape of the examined molecules (**1**, **1a** and **9**) appears to predict the loss of anticonvulsant activity for amide **9**. Series **A** possesses a rigid tetrahydoisoquinoline skeleton. We haven't observed an increase in activity after introducing different substituents in the phenyl ring of the benzylamine fragment. The extension of the arylalkylamine fragment in the a position of the GHB by the introduction of the glycine moiety led to a loss of activity in both series **B** and **C**. It is possible that too many acceptor/donor hydrogen bonds might make it difficult for these compounds to act at the target site. A weak displacement of [^{3}H] muscimol by compounds **8 – 10** might indicate their weak influence on $GABA_A$ receptors. Molecular modeling studies revealed that anticonvulsant activity of α-substituted amides of GHB might partially be explained by the orientation of the terminal benzylamide fragment.

In conclusion, we obtained three series of α-substituted *N*-benzylamides of GHB. Three of the tested amides (**9**, **11** and **14**) showed protection against MES seizures in mice. From this data, ideas for future molecular modifications leading to compounds with more favorable pharmacological properties might be obtained.

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