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Investigation into new anticonvulsant derivatives of α-substituted *N*-benzylamides of γ-hydroxy- and γ-acetoxybutyric acid. Part 5: Search for new anticonvulsant compounds

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Abstract—A series of four *N*-benzylamides of γ-hydroxybutyric acid (GHB), that contain *N*-(4-phenylpiperazine)-, *N*-(4-benzylpiperazine)rings, *N*-benzylamino-, or *N*-(2-phenylethylamine)-groups in the α-position of GHB were selected as model compounds, for determining the structural elements responsible for their potential anticonvulsant action. Based on the results of pharmacological, physicochemical, and molecular modelling investigations, the pharmacophore model for anticonvulsant *N*-substituted amides of GHB was defined. In this model, the presence of the *N*-benzylamide fragment is essential for activity. In addition, all of the amides contained another hydrophobic unit (aryl ring) as a distal binding site and H-bond donor. In consideration of these model parameters, a number of *N*-substituted amides of GHB, containing a hydrophobic moiety such as: *N*-benzylamino or *N*-(4-chlorobenzylamino) group in the α-position of GHB, and a lipophilic substituent in the amide portion, were prepared. It has been shown that the anticonvulsant activities of the newly synthesized compounds might partially be explained on the basis of their lipophilicity (calculated log P values) and the presence of a hydroxyl group in the molecule.

1. Introduction

In recent years, several new antiepileptic drugs (AEDs) such as lamotrigine, oxcarbazepine, felbamate, gabapentin, topiramate, fosphenytoin sodium, tiagabine, zonisamide and levetiracetam have been approved. Others like losigamone, pregabaline, remacemide, rufinamide, retigabine, ganaxolone, soretolide, stiripentol, milacemide, valrocemide, carabersat, harkoseride and talampanel have been or are currently in human studies.[1–4] Some of the newer AEDs represent structural modifications of pre-existing compounds, others were developed with the specific objective of modifying targets such as neurotransmitter function. Some of the mechanisms of AED action include: potentiation of GABA-ergic transmission, blockade of voltage-dependent sodium channels, attenuation of excitatory neurotransmission, and/or modulation of voltage-sensitive calcium channels. Some of the clinically used drugs have not been directly linked with any specific binding site within the brain; therefore, drug identification is partially conducted via in vivo screening tests using whole animal systems. The chemical diversity of compounds, along with multiple mechanism of action of effective medications, makes it difficult to identify any one common pharmacophore responsible for prevention or arrest of seizure activity in all patients.[5–8] This infers that control of unstable electrical environments in the brain resulting in seizure activity involves a host of physiological processes and may require multiple mechanisms to achieve cessation.

In our search for new anticonvulsants, a study of GABA and γ-hydroxybutyric acid (GHB) analogues was initiated. Derivatives of α-substituted γ-amino-, γ-phthalimido-, and γ-hydroxy butyric acid such as: acids, esters and amides were investigated.[9–13] It was shown that α-substituted *N*-benzylamides of γ-hydroxybutyric acid were the most potent compounds and possessed anticonvulsant activities in the maximal electroshock (MES) (ip) screens. The most potent anticonvulsant compounds were α-(benzylamino) - γ - hydroxybutyric

Keywords: Anticonvulsant activity; *N*-Benzylamides of γ-hydroxy- or γ-acetoxybutyric acid; Molecular modelling; SAR.
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acid *N*-benzylamide **1a** and *N*-(2-chlorobenzylamide) **1b** (Fig. 1), their median effective dose (ED_{50}) were respectively 63.0 and 54.0 mg/kg (MES).

In the MES Screen, these compounds were less active than the commonly used anticonvulsants: carbamazepine and phenytoin, but possess higher activity than sodium valproate. The biochemical tests indicate that the active amides act as allosteric modulators of the γ-aminobutyric acid, $GABA_A$ complex, and have an affinity to voltage sensitive calcium channels (VSCC) receptors. The *N*-(4-methylbenzyl)amide of α-(4-phenylpiperazin-1-yl)-γ-hydroxybutyric acid **12** displaced the binding of [^{35}S] TBPS ([^{35}S] *tert*-buthylbicyclophosphorothionate) to the chloride channel of the $GABA_A$ receptor complex ($IC_{50} = 95\ \mu M$).[14,15] This may be the possible mechanism mediating the anticonvulsant effect of these compounds.

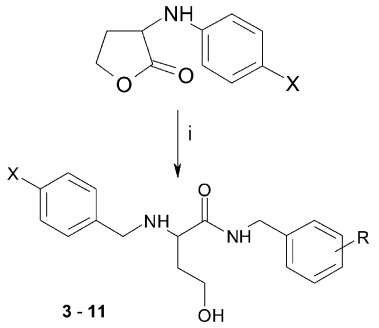
The aim of our study was determination of the structural elements and/or physicochemical parameters necessary for anticonvulsant activity. GHB derivatives were investigated, and based on these findings a series of new compounds were synthesized.

2. Investigations

2.1. Results and discussion

As a part of our search for a potential anticonvulsant agent, we have explored compounds structurally related to GHB. A series of four *N*-benzylamides of GHB (Fig. 2) that contain the *N*-(4-phenylpiperazine)- (series **A**), *N*-(4-benzylpiperazine)- (series **B**), *N*-benzylamino-(series **C**), and *N*-(2-phenylethylamine)- (series **D**), all grouped in the α-position of GHB were selected as models to determine the structural elements and/or physicochemical properties responsible for their anticonvulsant action.

To study the flexibility of synthesized compounds, molecular modelling investigations and their conformational analysis were performed. The conformational analyses were carried out by systematic stepwise rotation of 10° four torsion angles marked in Figure 2 as ϕ_1–ϕ_4, common to all molecules. Energy criterion, set to 3 kcal/mol above the lowest energy conformation found, was used to accept new 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 compared within a particular series, taking into account five atoms



Compd.	X	R	ASP class.*	clog *P*
3	Cl	H	4	2.32
4	Cl	4-CH_3	3	2.75
5	Cl	2-Cl	1	3.03
6	Cl	2-CH_3	4	2.76
7	Cl	2-CF_3	1	3.47
8	Cl	2-OCH_3	2	2.28
9	H	2-CH_3	2	2.04
10	H	2-CF_3	2	2.74
11	H	2-OCH_3	4	1.56

Scheme 1. Reagents and conditions: (i) relevant substituted benzylamine, toluene, reflux, 12 h. *The classifications are as follows; **1**: anticonvulsant activity at 100 mg/kg or less; **2**: anticonvulsant activity at doses greater than 100 mg/kg; **3**: compound inactive at 300 mg/kg; **4**: compound inactive at 300 mg/kg and toxic at 30 mg/kg or less.

common to all evaluated molecules. 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 similarity observed. The *RMS* deviations for each series are as follows: 0.330 Å (series **A**), 0.328 Å (series **B**), 0.197 Å (series **C**) (Fig. 3), and 0.599 Å (series **D**).

In order to obtain more general information concerning the shape of investigated molecules, a representative compound from each series (R = H) was selected to superimpose. Two nitrogen atoms and the first carbon atom from the hydroxyalkyl chain were selected for the fitting procedures. The *RMS* deviations for those four compounds were 0.087 Å (Fig. 4). The molecular

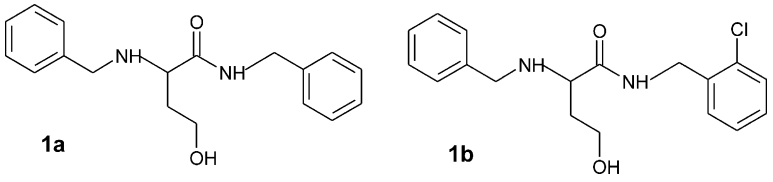


Figure 1. Anticonvulsant active α-(benzylamino)-γ-hydroxybutyric acid *N*-benzylamides.

modelling investigations indicated that features of molecules from each series were similar. Comparison of the representative compounds (Fig. 4) showed similarity in the orientation of both the GHB chain and the benzylamide moiety, while a substituent in the α-position of GHB possessed a different orientation. These results indicate that the *N*-benzylamide moiety may be an important structural unit for their activity.

Based on the results of pharmacological, physicochemical, roentgenostructural and molecular modelling investigations a pharmacophore-model for anticonvulsant activity of *N*-substituted amides of GHB was defined. In this model, the presence of the *N*-benzylamide fragment is essential for that activity. All active amides should contain a hydrophobic unit (aryl ring) as a distal binding site and a group which could act as a H-bond donor (Fig. 5).

For verification of this model, a number of *N*-substituted amides of GHB, containing a hydrophobic moiety such as *N*-benzylamino or *N*-(4-chlorobenzylamino) group in the α-position of GHB, and lipophylic substituent in the amide group, were synthesized. In our research we were particularly interested in the influence of arylalkylamino substituent in the α position and lipophylic substituent such as methyl-, trifluoromethyl-, or chloro- in the *ortho*-position in the phenyl ring in benzylamide fragment on anticonvulsant activity. Introduction of the acetyl substituent in the hydroxyl group allowed us to examine the role of a hydroxyl group in the proposed model.

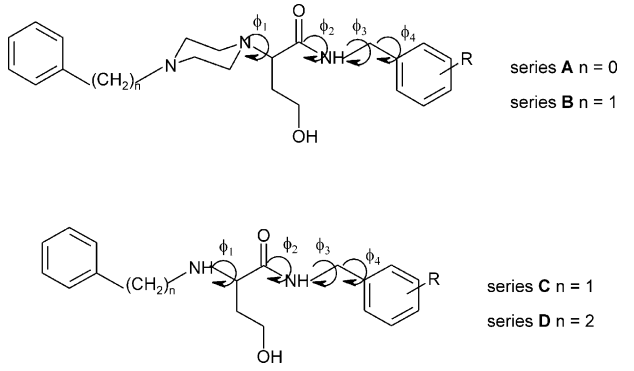


Figure 2. Schematic structure of *N*-benzylamides derivatives of α-substituted GHB.

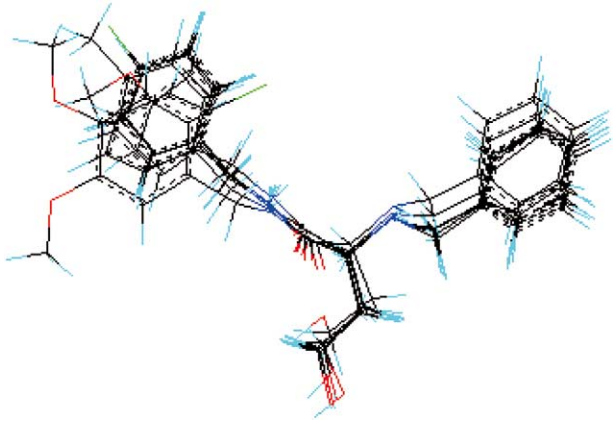


Figure 3. Superimposition of the seven anticonvulsant active compounds of series **C**.

The *N*-substituted amides of α-(benzylamino)- or α-(4-chlorobenzylamino)-GHB (**3–11**) were synthesized according to the procedures shown in Scheme 1. 3-Substituted derivatives of tetrahydrofuran-2-one were synthesized from α-bromo-γ-butyrolactone and benzyl- or 4-chlorobenzylamine by method which was previously described.[9] The aminolysis of lactones with various 2- or 4-substituted benzylamine yielded target compounds (**3–11**).

Acylation of the active anticonvulsants, derivatives of α-(4-phenylpiperazine)-GHB with acetic anhydride led to γ-acetoxy-GHB derivatives (**13–16**) (Scheme 2).

Preliminary anticonvulsant evaluation (phase I) of all the synthesized compounds **3–11**, **13–16** was provided by testing procedures which have been described earlier.[16] Phase I studies of the investigated compounds involved three tests: maximal electroshock seizure (MES), subcutaneous metrazole (scM), and rotorod test for neurological toxicity. Phase I is a qualitative assay involving a small number of mice (total 16). For the MES assay, experimental compound is administered to one, three and one animals at three respective doses (30, 100, and 300 mg/kg) at both 0.5- and 4-h time periods. The same similar paradigm for dosing and time is used

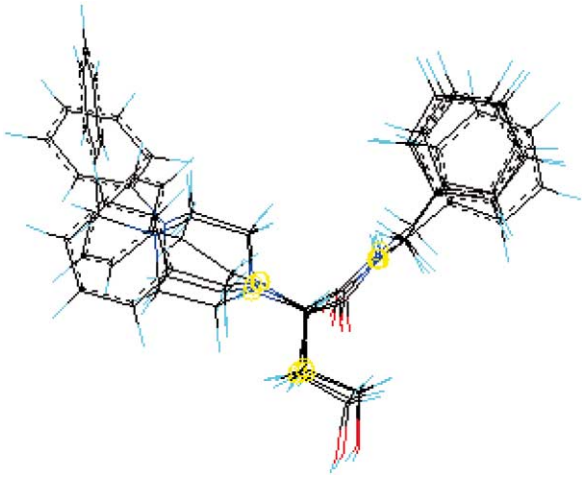


Figure 4. Superimposition of the four anticonvulsant active representative compounds of each series.

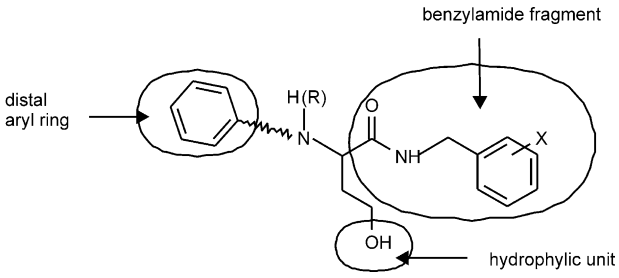


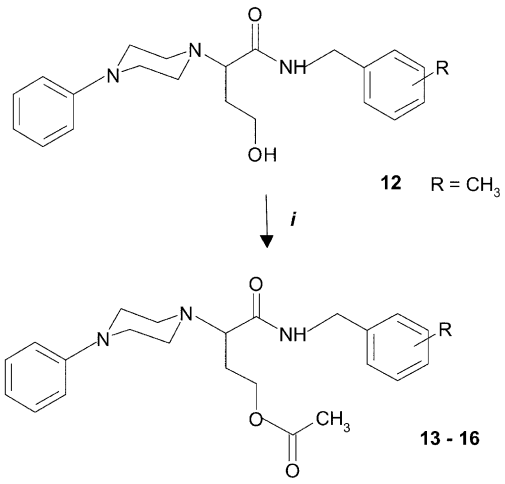
Figure 5. Pharmacophore model for anticonvulsant of α-substituted *N*-benzylamides of γ-hydroxybutyric acid.

for the scM testing. All animals were observed for potential neurological deficit at each dose and time period.

According to the results compounds were classified into four classes. The classification is as follows; (1) anticonvulsant activity at 100 mg/kg or less; (2) anticonvulsant activity at doses higher than 100 mg/kg; (3) compound inactive at any dose up to 300 mg/kg; (4) compound inactive at 300 mg/kg and toxic at 30 mg/kg or less. For the identification of anticonvulsant activity in mice, test compounds were administered intraperitoneally and challenged by maximal electroshock and subcutaneous metrazol. Compounds found to be effective in these seizure challenges are generally regarded to be potentially useful candidates in treatment of partial, generalized and even absence seizures. Neurotoxicity of the test compounds was determined using the rotorod toxicity test (TOX). The results of in the *vivo* tests are summarized in Table 1.

N-Substituted amides of α-(benzylamino)- and α-(4-chlorobenzylamino)-GHB showed some protection against MES seizures in mice. The most potent anticonvulsant compounds were **5**, **7**, **9** and **10**. Compounds **3**, **9**, **10** and **13** were active at doses of 100 mg//kg (1/3, 2/3, 3/3 and 1/3 animals protected) 0.25 h after administration, inactive at later time points. The amides **3**, **5**, **7–10** and **13** were active at 300 mg/kg 0.5 h after administering drug, however, toxicity was also observed at these doses. The compounds **9** and **10** that displayed a marked MES activity in mice, were evaluated for oral activity in rats (Table 2). Amides **9** and **10** demonstrated only weak activity in the MES screen at 30 mg/kg (po dose) (amide **9**: 1/4 animals protected at 0.5 and 2 h; amide **10**: 1/4 animals protected both at 0.25 and 4 h). Amides **9** and **10** used in the tests in rats were not toxic at the doses used. Compounds **4**, **11**, **14–16** were inactive in the MES and scMet mouse screens at these doses and time points. The investigated *N*-benzylamides of 2-(4-phenylpiperazine) - γ - acetoxy-GHB (**14–16**) were inactive and amide **17** was marginally active in the MES screen in mice (not shown). Compounds in which the hydroxyl group was replaced by acetoxy substituent were not active **13–16** (not shown). The results obtained showed that the presence of a hydroxy group in the molecule was necessary for MES activity.

Taking into account that physicochemical properties of chemical compounds have influence on their pharmacological properties, the partition coefficients (clog P) of the amides **3–16** were calculated[17] (Schemes 1 and 2). The predicted clog P values were in the range 1.58–3.47 for amides **3–16**, and for most compounds this value was near 2. Among the investigated compounds, amides **5** and **7** displayed the highest lipophilicity.



Compd.	R	ASP class.*	c log *P*
13	H	**2**	1.96
14	Cl	**3**	2.94
15	F	**3**	2.11
16	CH_3	**3**	2.4

Scheme 2. Reagents and conditions: (i) $(CH_3CO)_2O$, pyridine, 90 °C, 4 h. *Classification as for Scheme 1.

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

Compd	Dose (mg/kg)	Activity MES[a] time (h)				TOX[b] time (h)			
		0.25	0.5	1	4	0.25	0.5	1	4
3	30	—	0/1	—	0/1	—	1/4	—	0/2
	100	1/3	0/3	0/3	0/3	0/3	5/8	0/3	1/4
	300	—	1/1	—	0/1	—	4/4*	—	1/2
5	30	—	0/1	—	0/1	—	0/4	—	0/2
	100	—	1/3	—	0/3	—	2/8	—	0/4
	300	—	1/1	—	0/1	—	2/4	—	0/2
6	30	—	0/1	—	0/1	—	1/4	—	0/2
	100	—	1/6	—	0/3	—	3/8	—	0/4
	300	—	0/4	—	0/1	—	3/4	—	1/2
7	30	—	0/1	—	0/1	—	0/4	—	0/2
	100	—	1/3	—	0/3	—	3/8	—	0/4
	300	—	1/1	—	1/1	—	4/4*	—	0/2
8	30	—	0/1	—	0/1	—	0/4	—	0/2
	100	0/3	0/3	0/3	0/3	0/3	1/8	0/3	0/4
	300	—	1/1	—	0/1	—	2/4*	—	0/2
9	30	—	0/1	—	0/1	—	0/4	—	0/2
	100	2/3	0/3	0/3	0/3	0/3	0/8	0/3	0/4
	300	—	1/1	—	0/1	—	2/4	—	0/2
10	30	—	0/1	—	0/1	—	0/4	—	0/2
	100	3/3	0/3	0/3	0/3	0/3	2/8	0/3	0/4
	300	—	1/1	—	0/1	—	4/4*	—	1/2
13	30	—	0/1	—	0/1	—	0/4	—	0/2
	100	1/3	0/3	0/3	0/3	0/3	0/8	0/3	0/4
	300	—	1/1	—	0/1	—	4/4*	—	0/2

[a] Maximal electroshock test (number of animal protected/number of animals tested).
[b] Rotorod toxicity (number of animals exhibiting toxicity/number of animals tested) endpoint: unable to maintain balance on rotorod.

Table 2. Anticonvulsant screening project; test results in rats; po identification (dose 30 mg/kg)

Compd.	Test	Time (h)				
		0.25	0.5	1.00	2.00	4.00
9	MES[a]	0/4	1/4	0/4	1/4	0/4
9	TOX[b]	0/4	0/4	0/4	0/4	0/4
10	MES[a]	1/4	0/4	0/4	0/4	1/4
10	TOX[b]	0/4	0/4	0/4	0/4	0/4

[a] Maximal electroshock test (number of animal protected/number of animals tested).
[b] Rotorod toxicity (number of animals exhibiting toxicity/number of animals tested) endpoint: unable to maintain balance on rotorod.

The most active compounds **5** and **7** were derivatives with 4-chlorobenzyl substituent in the α position of GHB and the 2-chloro- or 2-trifluoromethyl- substituent in the benzylamide fragment. These amides had the highest lipophilicity, clog $P > 3$. This indicated that the difference in their efficacy might be partially explained on the basis of lipophilicity.

3. Conclusion

Based on the results of pharmacological, physicochemical, and molecular modelling investigations the pharmacophore model for anticonvulsant *N*-substituted amides of GHB was defined. In this model, the presence of the *N*-benzylamide fragment is essential for activity. Beside this, all of the amides contained another hydrophobic unit (aryl ring) as a distal binding site and H-bond donor. To support the suggested pharmacophore model we have synthesized a series of *N*-benzylamides of GHB containing a hydrophobic moiety such as *N*-benzylamino or a *N*-(4-chlorobenzylamino)- group in the α-position of GHB and a lipophylic substituent in the *ortho*-position in the phenyl ring in benzylamide fragment. The 2-chloro- and 2-trifluoromethylbenzylamides of α-(4-chlorobenzylamine)-GHB (**5**, **7**) were the most potent in the MES test. Among derivatives, the introduction of the lipophilic substituent at the *ortho*-position of phenyl ring was sufficient for their anticonvulsant activity. We have also synthesized a series of (**13**–**16**) γ-acetoxy analogues of corresponding anticonvulsant active γ-hydroxy derivatives. In comparison with hydroxy derivatives, γ-acetoxy derivatives of α-substitutes butyric acid did not exhibit anticonvulsant activity. These findings led us to conclude that hydroxy group was necessary for MES activity, and more lipophylic compounds showed better anticonvulsant properties.

From these data, ideas for future molecular modifications leading to compounds with greater favorable pharmacological properties may be derived.

4. Experimental

4.1. Chemistry

Melting points were determinated with a Büchi 535 and were uncorrected. Thin layer-chromatography (TLC) was performed on silica gel plates (5×10 cm, 0.25 mm) Kiselgel 60 F_{254} (Merck) using solvent systems: S_1, chloroform/acetone (1:1); S_2, chloroform/methanol/acetic acid (60:10:5); S_3, methanol/ammonium (25%) (98:2) with visualisation by UV light. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Gemini spectrometer at 200 MHz in $CDCl_3$ with tetramethylsilane as an internal standard. The mass spectra at 70 eV were taken with an LKB 2091 GCMS spectrometer.

4.1.1. 3-(4 - Chlorobenzylamino) - tetrahydrofuran-2-one hydrochloride (2). To a solution of 4-chlorobenzylamine (0.10 mol, 14.1 g, 12.2 mL) in acetonitrile (170 mL) anhydrous K_2CO_3 (0.2 mol, 27.6 g) and tetrabutylammonium bromide (0.01 mol, 3.2 g) were added. The mixture was stirred at room temperature for 0.5 h. Then a solution of 3-bromobutyrolactone (0.1 mol, 16.5 g, 8.2 mL) in acetonitrile (30 mL), the stirring was continued for 48 h. The resultant mixture was filtered and the solvent evaporated. The oily residue was dissolved in anhydrous methanol and acidified with 25% hydrochloric acid. The solvent was evaporated, the product was washed with anhydrous methanol and acetone. The obtained salt was dried in vacuo.

Yield 14.38 g (53.5%); mp 246–249 °C; TLC; R_f (S_1) = 0.47, R_f (S_2) = 0.28. Anal. calcd for: $C_{11}H_{12}O_2NCl$ HCl; M_r = 262.1, C: 50.40; H: 5.00; N: 5.34 found: C: 50.54, H: 4.99, N: 5.43.

4.1.2. Synthesis of *N*-substituted amides of γ-hydroxybutyric acid (3–11). 3-(Substituted benzylamin)-tetrahydrofuran-2-one hydrochloride (0.005 mol) was dissolved in water (10 mL) and a saturated solution of $NaHCO_3$ was added till the mixture become basic. The product was extracted in AcOEt (3 × 25 mL). The organic layers were combined, dried over Na_2SO_4 and evaporated. The obtained oil was dissolved in toluene (30 mL) and an appropriate amine (0.007 mol) was added. The mixture was refluxed for 12 h. After evaporation of solvent, the crude oil which crystallized at room temperature was purified by crystallization.

4.1.3. *N*-Benzylamide of α-(4-chlorobenzylamine)-γ-hydroxybutyric acid (3). Yield: 1.50 g (92.8%); mp 89–91 °C (from toluene); TLC: R_f (S_1) = 0.42, R_f (S_3) = 0.89; ^{1}H NMR ($CDCl_3$): 1.80–1.86 (2H, m, CH_2), 3.14 (2H, s *wide*, OH, NH), 3.27 (1H, t, CH), 3.60–3.75 (4H, m, $2CH_2$), 4.36–4.39 (2H, m, CH_2), 7.10–7.31 (9H, m, arom.), 7.61 (1H, t, NH amid); ^{13}C NMR ($CDCl_3$): 174.14 (C=O), 127.38–137.99 (arom), 61.36 (C-2), 60.66 (C-4), 51.67 (C-12), 43.02 (C-5), 35.36 (C-3). Anal. calcd for: $C_{18}H_{21}O_2N_2Cl$, M_r = 332.8, C: 64.96; H: 6.36; N: 8.42 found: C: 64.43, H: 6.18, N: 8.22.

4.1.4. *N*-4 - Methylbenzylamide of α - (4 - chlorobenzylamine)-γ-hydroxybutyric acid (4). Yield: 0.99 g (57.22%); mp 104–106 °C (from toluene); TLC: R_f (S_2) = 0.39, R_f (S_3) = 0.83; ^{1}H NMR ($CDCl_3$): 1.79–1.88 (2H, m, CH_2), 2.35 (3H, s, CH_3), 3.26 (2H, s *wide*, OH, NH), 3.32 (1H, t, CH), 3.58–3.66 (2H, m, CH_2), 3.69–3.75 (2H, m, CH_2), 4.36–4.39 (2H, m, CH_2), 7.13–7.28 (8H, m, arom), 7.53 (1H, t, NH amid); ^{13}C NMR

($CDCl_3$): 174.04 (C=O), 127.64–137.70 (arom), 61.43 (C-2), 60.76 (C-4), 51.71 (C-12), 42.83 (C-5), 35.62 (C-3), 20.95 (C-19). Anal. calcd for $C_{19}H_{23}O_2N_2Cl$; $M_r = 346.9$, C: 65.79, H: 6.68, N: 8.07 found: C: 66.66, H: 6.51, N: 7.88.

4.1.5. *N*-2-Chlorobenzylamide of α-(4-chlorobenzylamine)-γ-hydroxybutyric acid (5). Yield: 0.75 g (40.98%); mp 76–78 °C (from *n*-hexane and ethyl acetate); TLC: R_f (S_1) = 0.97, R_f (S_3) = 0.85; ^{1}H NMR ($CDCl_3$): 1.80–1.89 (2H, m, CH_2), 2.84 (2H, s *wide*, OH, NH), 3.27 (1H, t, CH), 3.65–3.96 (4H, m, $2CH_2$), 4.51–4.54- (2H, m, CH_2), 7.13–7.39 (8H, m, arom), 7.55 (1H, t, NH amid); ^{13}C NMR ($CDCl_3$): 174.06 (C=O), 127.11–137.66 (arom), 61.52 (C-2), 60.96 (C-4), 51.86 (C-12), 41.29 (C-5), 35.67 (C-3). Anal. calcd for $C_{18}H_{20}O_2N_2Cl$; $M_r = 367.3$, C: 58.86, H: 5.48, N: 7.63, found: C: 58.35, H: 5.38, N: 7.28.

4.1.6. *N*-2-Methylbenzylamide of α-(4-chlorobenzylamine)-γ-hydroxybutyric acid (6). Yield: 1.21 g (69.94%); mp 100–104 °C (from toluene); TLC: R_f (S_2) = 0.26, R_f (S_3) = 0.90; ^{1}H NMR ($CDCl_3$): 1.81–1.90 (2H, m, CH_2), 2.31 (3H, s, CH_3), 2.90 (2H, s *wide*, OH, NH), 3.28 (1H, t, CH), 3.67–3.77 (4H, m, $2CH_2$), 4.41–4.44 (2H, m CH_2), 7.11–7.30 (9H, m, arom, NH); ^{13}C NMR ($CDCl_3$): 173.86 (C=O), 126.20–137.67 (arom), 61.55 (C-2), 60.94 (C-4), 51.86 (C-12), 41.29 (C-5), 35.72 (C-3), 18.89 (C-19). Anal. calcd for $C_{19}H_{23}O_2N_2Cl$; $M_r = 346.9$, C: 65.79, H: 6.68, N: 8.07 found: C: 65.09, H: 6.41, N: 7.71.

4.1.7. *N*-2-Trifluoromethylbenzylamide of α-(4-chlorobenzylamine)-γ-hydroxybutyric acid (7). Yield: 0.70 g (34.90%); mp 72–76 °C (from *n*-hexane and ethyl acetate); TLC: R_f (S_2) = 0.63, R_f (S_3) = 0.86; ^{1}H NMR ($CDCl_3$): 1.79–1.88 (2H, m, CH_2), 2.90 (2H, s *wide*, OH, NH), 3.28 (1H, t, CH), 3.64–3.75 (4H, m, $2CH_2$), 4.59–4.62 (2H, m, CH_2), 7.11–7.51 (8H, m, arom), 7.63 (1H, t, NH amid); ^{13}C NMR ($CDCl_3$): 174.20 (C=O), 121.63–137.63 (arom), 61.41 (C-2), 60.80 (C-4), 51.86 (C-12), 39.83 (C-5), 39.79 (C-12), 35.57 (C-3). Anal. calcd for: $C_{19}H_{20}O_2N_2ClF_3$; $M_r = 400.826$, C: 56.93, H: 5.03, N: 6.98, found: C: 56.13, H: 5.05, N: 7.38.

4.1.8. *N*-2-Methoxybenzylamide of α-(4-chlorobenzylamine)-γ-hydroxybutyric acid (8). Yield: 0.73 g (40.33%); mp 63–68 °C (from cyclohexane and ethyl acetate); TLC: R_f (S_2) = 0.46, R_f (S_3) = 0.87; ^{1}H NMR ($CDCl_3$): 1.78–1.87 (2H, m, CH_2), 3.02 (2H, s *wide*, OH, NH), 3.25 (1H, t, CH), 3.62–3.72 (4H, m, $2CH_2$), 3.79 (3H, s, OCH_3), 4.42–4.46 (2H, m, CH_2), 6.84–6.94 (2H, m, arom), 7.12–7.31 (6H, m, arom), 7.51 (1H, s, NH amid); ^{13}C NMR ($CDCl_3$): 173.70 (C=O), 110.25–157.43 (arom), 61.60 (C-2), 60.89 (C-4), 55.20 (C-19), 51.62 (C-12), 39.11 (C-5), 35.68 (C-3). Anal. calcd for: $C_{19}H_{23}O_3N_2Cl$; $M_r = 362.9$, C: 62.89, H: 6.38, N: 7.72, found: C: 63.03, H: 6.51, N: 7.81.

4.1.9. *N*-2-Methylbenzylamide of α-benzylamine-γ-hydroxybutyric acid (9). Yield: 0.77 g (49.35%); mp 70–75 °C (from ethyl acetate); TLC: R_f (S_2) = 0.44, R_f (S_3) = 0.88; ^{1}H NMR ($CDCl_3$): 1.81–1.90 (2H, m, CH_2), 2.32 (3H, s, CH_3), 3.10 (2H, s *wide*, OH, NH), 3.33 (1H, t, CH), 3.70–3.76 (4H, m, $2CH_2$), 4.41–4.44 (2H, CH_2), 7.19–7.30 (9H, m, arom), 7.45 (1H, t, NH amid); ^{13}C NMR ($CDCl_3$): 174.04 (C=O), 126.10–139.16 (arom), 61.52 (C-2), 60.86 (C-4), 52.56 (C-12), 41.19 (C-5), 35.73 (C-3), 18.86 (C-19). Anal. calcd for $C_{19}H_{24}O_2N_2$; $M_r = 312.4$, C: 73.04, H: 7.74, N: 8.96, found: C: 70.95, H: 6.08, N: 8.80.

4.1.10. *N*-2-Trifluoromethylbenzylamide of α-benzylamine-γ-hydroxybutyric acid (10). Yield: 1.14 g (39.89%); mp 83–85 °C (from cyclohexane and ethyl acetate); TLC: R_f (S_2) = 0.31, R_f (S_3) = 0.83; ^{1}H NMR ($CDCl_3$): 1.79–1.88 (2H, m, CH_2), 2.90 (2H, s *wide*, OH, NH), 3.32 (1H, t, CH), 3.67–3.73 (4H, m, $2CH_2$), 4.59–4.62 (2H, m, CH_2), 7.23–7.48 (9H, m, arom), 7.67 (1H, t, NH amid); ^{13}C NMR ($CDCl_3$): 174.43 (C=O), 121.62–139.12 (arom), 61.37 (C-2), 60.72 (C-4), 52.61 (C-12), 39.73 (C-5), 39.68 (C-19), 35.62 (C-3). Anal. calcd for $C_{19}H_{21}O_2N_2F_3$; $M_r = 366.4$, C: 62.28, H: 5.77, N: 7.64, found: C: 62.99, H: 5.55, N: 7.39.

4.1.11. *N*-2-Methoxybenzylamide of α-benzylamine-γ-hydroxybutyric acid (11). Yield: 1.14 g (64.02%); mp 61–64 °C (from cyclohexane and ethyl acetate); TLC: R_f (S_2) = 0.30, R_f (S_3) = 0.80; ^{1}H NMR ($CDCl_3$): 1.78–1.86 (2H, m, CH_2), 3.07 (2H, s *wide*, OH, NH), 3.28 (1H, t, CH), 3.65–3.73 (4H, m, $2CH_2$), 3.79 (3H, s, OCH_3), 4.43–4.46 (2H, m, CH_2), 6.84–6.94 (3H, m, arom), 7.22–7.25 (6H, m, arom), 7.65 (1H, t, NH amid); ^{13}C NMR ($CDCl_3$): 173.88 (C=O), 110.15–157.37 (arom), 61.55 (C-2), 60.77 (C-4), 55.09 (C-19), 52.31 (C-12), 38.97 (C-5), 35.67 (C-3). Anal. calcd for: $C_{19}H_{24}O_2N_2$; $M_r = 328.4$, C: 69.48, H: 7.36, N: 8.53, found: C: 69.13, H: 7.30, N: 8.66.

4.1.12. Synthesis of *N*-substituted amides of γ-acetoxybutyric acid (13–16). *N*-Substituted benzylamide of α-phenylpiperazin-1-yl-γ-hydroxybutyric acid (0.002 mol), acetic anhydride (2 mL) and piridine (2 mL) was stirred in 100 °C for 15 min. The obtained mixture was cooled down, dissolved in water (30 mL) and a 10% solution of HCl was added in drops till the mixture become acidic. The product was extracted in methylene chloride (3×25 mL). The organic layers were combined, dried over Na_2SO_4 and evaporated. After evaporation of solvent, the crude oil which crystallized at room temperature was purified by crystallization or by column chromatography.

4.1.13. *N*-Benzylamide of α-phenyl-piperazin-1-yl-γ-acetoxybutyric acid (13). Yield: 0.28 g (25.04%); mp 82–83 °C (from *n*-hexane and ethyl acetate); TLC: R_f (S_1) = 0.87, R_f (S_2) = 0.95; ^{1}H NMR ($CDCl_3$): 2.05–2.15 (2H, m, CH_2), 2.06 (3H, s, CH_3), 2.68–2.76 (4H, m, piper.), 3.13–3.24 (5H, m, piper., CH), 4.23–4.31 (2H, m, CH_2), 4.46–4.50 (2H, d-d, CH_2), 6.88–6.93 (3H, m, arom.), 7.24–7.42 (8H, m, arom., NH); MS (70 eV), *m*/*z* (%): 395 (2.47) [M^+], 261(25.47), 202(15.16), 201(100), 132(13.68), 105(8.28), 104(11.53), 96(5.03), 91(13.30), 77(6.84), 43(5.42). Anal. calcd for: $C_{23}H_{29}O_3N_3$; $M_r = 395.5$, C: 69.85, H: 7.39, N: 10.62, found: C: 70.64, H: 7.26, N: 10.82.

4.1.14. *N*-4-Chlorobenzylamide of α-phenyl-piperazin-1-yl-γ-acetoxybutyric acid (14). Yield: 0.28 g (25.05%); mp 101–103 °C (from *n*-hexane and ethyl acetate); TLC: R_f (S_1) = 0.80, R_f (S_2) = 0.92; ^{1}H NMR ($CDCl_3$): 2.04 (2H, m, CH_2), 2.06 (3H, s, CH_3), 2.67–2.75 (4H, m, piper.), 3.13–3.19 (4H, m, piper.), 3.21–3.24 (1H, m, CH), 4.23–4.31 (2H, m, CH_2), 4.41–4.45 (2H, d-d, CH_2), 6.89–6.93 (3H, m, arom.), 7.20–7.34 (6H, m, arom.), 7.43 (1H, t, NH amid); MS (70 eV), *m*/*z* (%): 429 (2.61) [M^+], 261(37.76), 201(100), 159(5.03), 132(10.28), 104(6.83), 77(4.29). Anal. calcd for: $C_{23}H_{28}O_3N_3$; M_r = 429.9, C: 64.25, H: 6.56, N: 9.77, found: C: 64.31, H: 6.11, N: 10.10.

4.1.15. *N*-4-Fluorobenzylamide of α-phenyl-piperazin-1-yl-γ-acetoxybutyric acid (15). Yield: 0.64 g (57.48%); mp 90–92 °C (from cyclohexane and ethyl acetate); TLC: R_f (S_1) = 0.88, R_f (S_2) = 0.82; ^{1}H NMR ($CDCl_3$): 1.98–2.14 (2H, m, CH_2), 2.05 (3H, s, CH_3), 2.62–2.77 (4H, m, piper.), 3.08–3.22 (5H, m, piper. CH), 4.16–4.24 (2H, m, CH_2), 4.29–4.46 (2H, m, CH_2), 6.85–7.05 (4H, m, arom.), 7.34–7.27 (5H, m, arom.), 7.39 (1H, t, NH amid.); MS (70 eV), *m*/*z* (%): 413 (2.84) [M^+], 261(28.79), 202(15.01), 201(100), 159(6.93), 132(12.65), 109(9.24), 104(10.21), 77(6.30). Anal. calcd for: $C_{23}H_{28}O_3N_3F$; M_r = 413.5, C: 66.81, H: 6.82, N: 10.16, found: 66.51, H: 6.71, N: 10.05.

4.1.16. *N*-4-Methylbenzylamide of α-phenyl-piperazin-1-yl-γ-acetoxybutyric acid (16). Yield: 0.52 g (41.90%); mp 113–114 °C (from cyclohexane and ethyl acetate); TLC: R_f (S_1) = 0.80, R_f (S_2) = 0.89; ^{1}H NMR ($CDCl_3$): 1.96–2.16 (2H, m, CH_2), 2.05 (3H, s, CH_3), 2.33 (3H, s, CH_3), 2.63–2.78 (4H, m, piper.), 3.09–3.21 (5H, m, piper., CH), 4.11–4.25 (2H, m, CH_2), 4.30–4.43 (2H, m, CH_2), 6.84–6.91 (3H, m, arom), 7.12–7.26 (6H, m, arom), 7.33 (1H, t, NH amid); MS (70 eV), *m*/*z* (%): 409 (2.20) [M^+], 261(28.13), 201(100), 160(4.14), 132(11.83), 105(15.6), 96(4.29), 91(3.93) 77(6.91). Anal. calcd for: $C_{24}H_{31}O_3N_3$; M_r = 409.5, C: 70.39, H: 7.63, N: 10.26, found: C: 70.03, H:7.80, N: 10.09.

4.2. Molecular modelling study

The compounds were modelled and minimized using PM3 (MOPAC) method of the Alchemy 2000 programme (Alchemy 2000 ver. 2.0, Tripos Inc., St. Louis, USA, 1997). The conformational analyses were carried out by systematic stepwise rotation of 10^0 four torsion angles marked in Figure 2 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 sum their Van der Waal's radius. 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:

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

where *d* is the distance between two paired atoms, *n* is the number of pairs that are fitted.

The lower *RMS* fit value is, the better similarity is.

4.3. Pharmacology

4.3.1. Anticonvulsant assays. Phase I of the evaluation included three tests: maximal electroshock (MES), subcutaneus pentylenetrazole (scMet), and rotorod test for neurological toxicity (Tox). 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 saline or suspended in 0.5% methylcellulose, and were administered by ip injection at three dosage levels (30, 100, and 300 mg/kg) with anticonvulsant activity, and neurotoxicity noted 0.5 h and 4 h after administration.

4.4. Maximal electroshock seizure (MES) test[18]

Maximal electroshock seizures were elicited with a 60-cycle alternating current of 50 mA intensity (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/number of animals tested.

4.5. Neurotoxicity[19,20]

The rotorod test was used to evaluate neurotoxicity in mice. The animal was 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 indefinitely. Neurologic toxicity is defined as the failure of the animal to remain on the rod for 1 min and is expressed as number of animals exhibiting toxicity/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.

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