Synthesis of GABA-Valproic Acid Derivatives and Evaluation of Their Anticonvulsant and Antioxidant Activity

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Summary

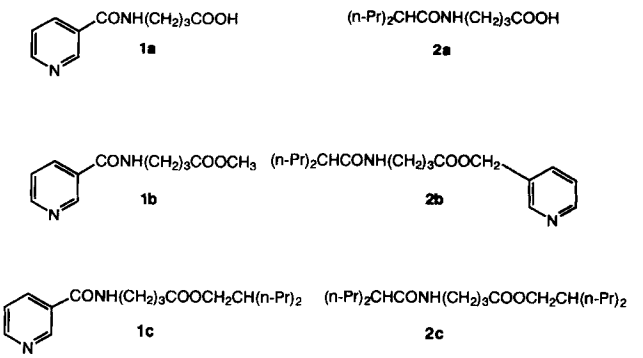
The synthesis and the anticonvulsant activity of a number of GABA and valproic acid derivatives are reported. The lipophilicity of these compounds and their inhibitory effect on lipid peroxidation were also investigated, in an effort to correlate the anticonvulsant activity with lipophilicity and inhibitory effect on lipid peroxidation. The synthesized compounds exhibited anticonvulsant effects which were stronger for the more lipophilic derivatives. One of the active anticonvulsants showed appreciable antioxidant properties. Finally, a good correlation was found between the experimentally derived (R_M) and calculated (Σf and log P_{SK}) lipophilicity for this series of compounds.

Introduction

The neurotransmitter GABA (γ-aminobutyric acid) has been implicated, both directly and indirectly, with several neurologic and psychiatric disorders such as epilepsy, Huntington's chorea, and parkinsonism[1]. Various antiepileptics may develop their action either by interacting directly with certain binding sites of the GABA receptors (e.g. barbiturates and benzodiazepines)[2] or by acting as prodrugs of GABA (e.g. progabide)[3]. Another antiepileptic, valproic acid, acts possibly by enhancing GABA accumulation mainly by stimulating the activity of glutamic acid decarboxylase and inhibiting GABA transaminase. These actions are mediated either by valproic acid itself or by several active metabolites of the former[2].

GABA passes the blood-brain barrier (BBB) to a lesser extent than any other putative neurotransmitter[4]; thus, extremely high doses are required to force it across the BBB. GABA derivatives, more lipophilic than the parent molecule, have been developed in attempts to facilitate penetration through the BBB. Examples of such active derivatives are the amides of GABA with several fatty acids (e.g. dodecanoic[5], linoleic, steatic and palmitic[6]), Schiff bases of GABA (e.g. progabide[3]) and the ester of GABA with cholesterol[7]. *N*-nicotinoyl-GABA (**1a**, **Scheme 1**) has also been synthesized but was found inactive as an anticonvulsant[8]. This inactivity could be attributed to its overall low lipophilicity ($\Sigma f = -0.413$, see **Table I**) and increased polarity.

Considering the above, we designed and synthesized the compounds **1b–c** and **2a–c** (**Scheme 1**) containing the moieties of GABA, valproic acid and 2-propyl-1-pentanol (which could be metabolized to valproic acid) as mutual prodrugs of these bioactive compounds and possible anticonvulsants.



Scheme 1: Structures of the synthesized GABA derivatives.

Table I: R_M, Σf, logP_{SK} values and % inhibition of lipid peroxidation of the synthesized compounds.

Compound	R_M	Σf (Rekker)	logP_{SK}	% inhibition of lipid peroxidation[a]
1a	–0.53±0.032	–0.413	–0.617	21.7
1b	–0.39±0.043	0.051	–0.334	1.4
1c	0.29±0.023	3.677	3.292	81.2
2a	–0.51±0.036	2.007	1.899	3.0
2b	–0.24±0.027	2.801	2.243	2.8
2c	0.66±0.075	6.207	5.808	0.4

[a]Concentration: 1mM; incubation time: 45 min.

Compounds **1b–1c** and **2b** contain also the moieties of nicotinic acid or the respective nicotinyl alcohol (3-pyridylmethanol) which *in vivo* could be reoxidized to the nicotinic acid. We chose the nicotinic acid functionality because it is present in a number of biomolecules. Both the acid and the alcohol moieties will have also a favorable effect on lipophilicity (estimated Σf values: amine group –1.340, nicotinamide group –1.025, carboxylic group –0.942, nicotinyl ester group –0.147).

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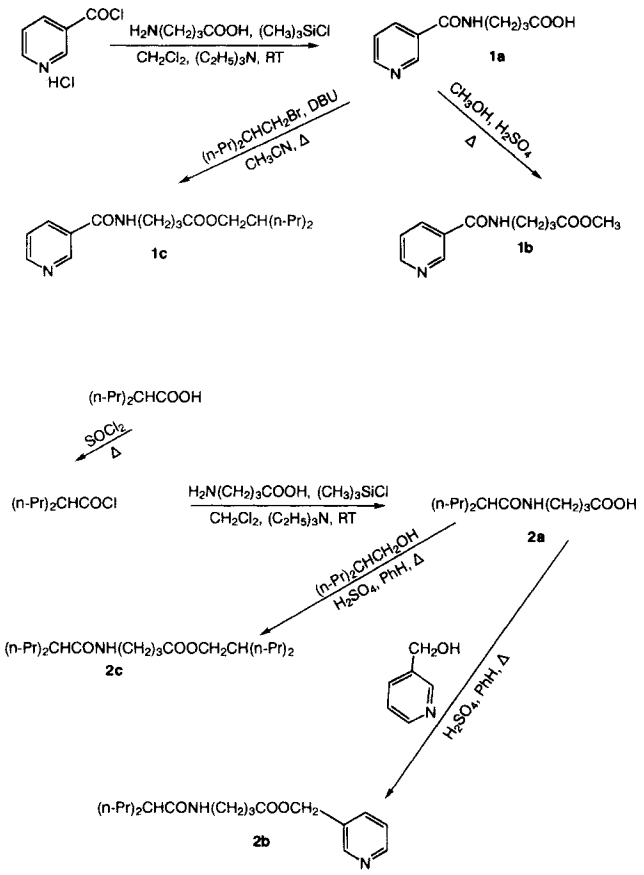
All the synthesized target compounds were tested *in vivo* against picrotoxin induced seizures in rats and the most active (**1c**) was also evaluated in the pentylenetetrazole model. Furthermore, since a connection of epileptic convulsions with the development of oxidative stress and lipid peroxidation in the brain tissues has been indicated[9,10], we tested the synthesized compounds for their ability to inhibit lipid peroxidation *in vitro*.

It is well established that lipophilicity is a physicochemical property that influences both the blood-brain permeability[11] and the antioxidant potential[12] of a compound. Thus, the lipophilicity of the synthesized compounds was determined and related to the observed biological activities.

Synthesis of Compounds

The synthetic routes for the preparation of the intermediate and target compounds are outlined in **Scheme 2**.

The literature procedure for the preparation of **1a** involves condensation of GABA with the intermediate formed by the oxidation of nicotinic acid hydrazide with sodium nitrite and is low yielded (33%)[8]. In this work, compound **1a** was synthesized, in excellent yield, by the amidation of GABA with nicotinoyl chloride in the presence of triethylamine and chlorotrimethylsilane in dichloromethane. The mechanism probably involves solubilization of GABA in the form of its trimethylsilyl ester followed by the amide forming reaction.



Scheme 2: Synthetic route of the synthesized GABA derivatives.

This method is a modification of a patent in which the amidation of GABA with 4-chlorobutyryl chloride is reported[13].

Compound **1b** was synthesized by the esterification of **1a** in methanol under acidic conditions. Sulfuric acid was used in small excess forming the salt with **1a**, which is more soluble in methanol than **1a** itself and also acting as the catalyst of the esterification. Initially, compound **1b** was synthesized by converting GABA to its methyl ester[14] followed by amidation with nicotinic acid in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC) in pyridine, as described for analogous compounds[15]. This technique, however, proved to be tedious, having also the disadvantage of giving low yield.

Compound **1c** was synthesized by the reaction of **1a** with 2-propyl-1-bromopentane in acetonitrile in the presence of DBU according to a general procedure[16]. The following routes were also investigated for the synthesis of **1c**: **i)** Transesterification of **1b** with 2-propyl-1-pentanol in toluene under basic conditions in a Soxhlet apparatus[17]. A significant disadvantage of this procedure was the fact that the yield was not constant with the formation, in various amounts, of at least two byproducts. **ii)** Direct esterification of **1a** with 2-propyl-1-pentanol in a Dean-Stark apparatus using benzene as the solvent and concd. sulfuric acid or 4-toluenesulfonic acid as the acid catalyst. The yield was low and this could be attributed to the low solubility in benzene of the formed salts of **1a** with the acid catalysts. The same reaction was also carried out in toluene using 4-toluenesulfonic acid as the catalyst. In this case a byproduct was isolated, in considerable yield, and identified as the 1-nicotinoyl-2-pyrrolidinone which could be formed by an intramolecular cyclization of **1a** facilitated by the relatively higher boiling point of toluene, compared to that of benzene. **iii)** Esterification of **1a** with 2-propyl-1-pentanol at room temperature, in the presence of DCC using dichloromethane as solvent[7]. The yield was low and purification of the product proved to be tedious.

Compound **2a** was synthesized by the amidation of GABA with 2-propyl-1-pentanoyl chloride in the presence of triethylamine and chlorotrimethylsilane in dichloromethane. An attempt to synthesize **2a** by the amidation of GABA with 2-propyl-1-pentanoyl chloride in aqueous alkaline solution as described for analogous compounds[5], led to a mixture which made the isolation of the product very difficult.

Compound **2b** was synthesized by the esterification of **2a** with 3-pyridylcarbinol in the presence of a small excess of concd. sulfuric acid in refluxing benzene. In a similar manner, **2c** was synthesized by esterifying **2a** with 2-propyl-1-pentanol in the presence of a catalytic amount of concd. sulfuric acid. We observed that by replacing benzene with toluene and sulfuric acid with 4-toluenesulfonic acid in the synthesis of **2b** or **2c**, a byproduct (1-valproyl-2-pyrrolidinone) was formed in considerable yield, by an intramolecular cyclization of **2a**. Finally, attempts to synthesize **2b** or **2c** by esterifying **2a** in the presence of DCC in dichloromethane were not promising.

Results and Discussion

The lipophilicity of the synthesized compounds was both determined experimentally by the reversed phase thin layer chromatographic technique[18] and calculated according to Rekker's fragmental constant (Σf) method[19] and Suzuki/Kudo ($\log P_{SK}$) atom-based procedure[20,21]. Results are shown in **Table I**. It has been reported that a linear correlation could exist between the R_M and the $\log P$[18] or Σf[22] values in a congeneric series of compounds. In the present study, a good correlation was derived between the R_M values and the two expressions of calculated lipophilicity for the synthesized compounds:

i) $\Sigma f = 4.55(\pm 1.06)R_M + 2.93(\pm 0.49)$
$n = 6$, $r = 0.906$, $s = 1.159$, $F = 18.246$

ii) $\log P_{SK} = 4.40(\pm 1.08)R_M + 2.58(\pm 0.50)$
$n = 6$, $r = 0.897$, $s = 1.180$, $F = 16.496$

The anticonvulsant activity of compounds **1b–c** and **2a–c** was evaluated *in vivo* (rats) against picrotoxin-induced seizures. Picrotoxin selectively antagonizes the effects of GABA[23]. The measured parameters were three (onset of seizures, survival time and survival ratio)[5] and results are shown in **Table II**. Compound **1c** exhibited a significant and dose-related anticonvulsant effect. The less lipophilic methyl ester **1b** exhibited some anticonvulsant activity, only at the highest tested dose (1.6 mmol/kg). The hydrophilic parent carboxylic acid **1a** was not tested because it has been reported as inactive, up to a dose of 1000 mg/kg, in the picrotoxin model in mice[8]. Lipophilicity seems to play also a role in the expression of activity in the derivatives of the series **2**. The highly lipophilic ester **2c** showed anticonvulsant properties expressed in all of the three measured parameters, even at the lower tested dose (0.4 mmol/kg). However, compounds **2a** and **2b**, although more lipophilic than **1b**, did not exhibit significantly better anticonvulsant effect. Thus, concerning the structural characteristics of the active anticonvulsants, we could conclude that the nicotinoyl moiety is a suitable amide group to confer activity, in conjugation with lipophilicity, in this type of GABA derivatives.

The most active compound **1c** was also tested (1.6 mmol/kg, i.p.) against pentylenetetrazole-induced seizures in rats and exhibited complete protection against pentylenetetrazole under our experimental conditions i.e. no convulsions were observed in the group of rats ($n = 7$) that received **1c** within the 2 h of the observation period while convulsions in the control animals, which received only pentylenetetrazole, appeared at 9.5±3.15 min ($n = 6$). Pentylenetetrazole is a non-specific CNS stimulant that may act either by blockade of the inhibitory pathways or by direct neuronal excitation[23].

The antioxidant potential of compounds **1a–c** and **2a–c** was studied in the non-enzymatic lipid peroxidation assay. Rat hepatic microsomal preparation, which is a known good peroxidizable substrate[24], was used for the assays. **Table I** shows the percent inhibition of lipid peroxidation of these compounds at a concentration of 1 mM after 45 min incubation. **1c** was the only compound which exhibited significant antioxidant activity; the time course of lipid peroxidation, as affected by various concentrations of **1c**, is shown in **Fig. 1**.

Table II: Anticonvulsant effect of the synthesized compounds.

Compound	Onset of ^ seizure (min)	% of control	Survival time (min)	% of control	Survival ratio (%)
Control	26.2±6.7	100	40.0±8.1	100	0
1b (0.8mmol/kg)	23.7±7.1^{NS}	90	41.0±8.2^{NS}	102	0
(1.6mmol/kg)	51.4±9.1***	196	69.6±12.3***	174	25
1c (0.4mmol/kg)	38.5±11.3***	147	57.8±16.7***	144	0
(0.8mmol/kg)	51.3±9.6***	196	64.9±7.8***	162	8
(1.6mmol/kg)	62.4±18.4***	238	87.5±20.9***	219	65
2a (0.4mmol/kg)	20.1±9.0*	77	40.0±6.1^{NS}	100	0
(0.8mmol/kg)	31.9±5.6*	122	47.6±9.3*	119	0
(1.6mmol/kg)	41.6±16.4**	159	61.2±17.3***	153	17
2b (0.8mmol/kg)	28.0±4.5^{NS}	107	41.5±9.2^{NS}	104	0
(1.6mmol/kg)	36.0±7.0*	137	52.2±6.4*	130	0
2c (0.4mmol/kg)	52.9±10.4***	202	71.7±16.5***	179	12
(0.8mmol/kg)	41.0±18.9**	156	54.2±7.7**	135	65
Valproic acid (0.8mmol/kg)	33.0±11.9*	126	57.1±16.5***	143	0

$n = 6$, NS: non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

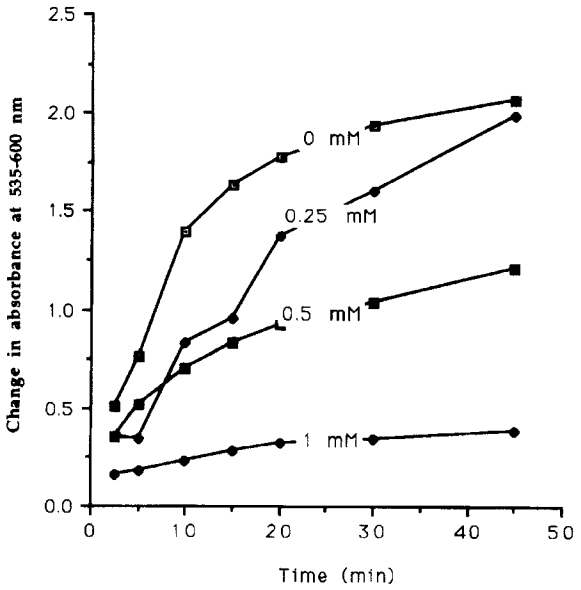


Figure 1: Time course of lipid peroxidation as affected by **1c**.

In order to ascertain the contribution of the structural moieties to the activity in this series of GABA derivatives, we also tested GABA, valproic acid, 2-propyl-1-pentanol, nicotinic acid and 3-pyridylcarbinol. These compounds showed practically no inhibition of lipid peroxidation at concentrations of 1 mM (their inhibitory effect ranged from 0.1 to 1.9%).

A number of experimental results indicate that a linear correlation could exist between lipophilicity and antioxidant activity[12]. Although the active antioxidant **1c** is relatively lipophilic, compound **2c** which has double the lipophilicity of the former is virtually inactive. Therefore, in addition to lipophilicity, other properties, such as favorable electronic distribution for reacting rapidly with radicals or the ability to affect membrane fluidity[25], may contribute to the observed variations in the antioxidant activity in this series of compounds.

Superoxide production has been observed in cerebral vessels of experimental animals subjected to prolonged seizures[26]. Also, hyperoxia was shown to lead to peroxidation of rat brain membrane lipids which coincided with an increase of epileptiform seizures[27]; similar results have been demonstrated in the intracerebral ferrous chloride model of epilepsy[28]. Thus, it is probable that oxygen radicals may be involved in the causation of prolonged seizures in man (status epilepticus)[9,10]. On the other hand, a significant reduction of seizures in 10 of 12 patients was observed in a clinical trial of add-on with the antioxidant vitamin E therapy[29]. Therefore, the ability of an anticonvulsant agent to act as a free radical scavenger and to inhibit lipid peroxidation is a desirable property, since it could prevent further tissue damage during the seizures. Compound **1c** exerts considerable anticonvulsant as well as antioxidant activity, therefore, it may serve as a useful lead structure.

Aknowledgement

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Experimental

Melting points are uncorrected and were determined in open glass capillaries using a Mel-Temp II apparatus. UV measurements were recorded with a Perkin-Elmer 554 spectrophotometer, IR spectra were recorded with a Shimadzu FTIR-8101 M spectrophotometer, ^{1}H-NMR spectra with a Bruker AW-80 spectrometer with internal Me_4Si reference. Elemental analyses were performed on a Perkin-Elmer 2400 CHN analyzer. Flash chromatography was carried out using Merck 9385 silica gel. Thin layer chromatography plates were visualized under UV light or by dipping into a solution of ammonium molybdate (25 g) and ceric sulfate (1 g) in concd. sulfuric acid/water (10/90 ml) and heating on a hot plate[30]. Petroleum ether refers to the fraction of bp 40–60 °C. 2-propyl-1-pentanoyl chloride was obtained by reaction of valproic acid with thionyl chloride and 2-propyl-1-bromopentane by reacting the corresponding alcohol with phosphorus tribromide. They were both purified by distillation under reduced pressure.

4-[(Pyridine-3-carbonyl)amino]butanoic acid (**1a**)

To a stirred suspension of 4-aminobutanoic acid (6.19 g, 60 mmol) in methylene chloride (600 ml), triethylamine (30.0 ml, 215 mmol) and chlorotrimethylsilane (8.2 ml, 65 mmol) were added. The reaction mixture was stirred at room temperature for 2 h, then it was cooled (ice bath) and nicotinoyl chloride hydrochloride (7.12 g, 40 mmol) was added. After stirring for 24 h at room temperature, the volatile material was evaporated under reduced pressure. To the residue, water (400 ml) was added and stirred for 30 min, filtered and the residue washed with 3 × 30 ml of water and 30 ml of ether. After drying, it was recrystallized from methanol (yield 6.94 g, 83%). mp 210–211 °C (ref.[8] 210–212 °C). IR (nujol): ν = 3325 cm^{-1} (NH), 1709 cm^{-1} (O=COH), 1628 cm^{-1} (O=CN). ^{1}H-NMR ([D$_6$]DMSO): δ 8.8-9.1 (m, 1H, pyridine 2-H), 8.4–8.8 (m, 2H, pyridine 4-H, CONH), 8.1–8.3 (m, 1H, pyridine 6-H), 7.3–7.6 (m, 1H, pyridine 5-H), 3.3 (m, 2H, CONHC*H*$_2$CH$_2$), 2.3 (t, 2H, CH$_2$C*H*$_2$COO), 1.8 (m, 2H, CH$_2$C*H*$_2$CH$_2$). Anal. ($C_{10}H_{12}N_2O_3$).

4-[(Pyridine-3-carbonyl)amino]butanoic acid methyl ester (**1b**)

To a stirred solution of concd. sulfuric acid (2.3 ml, 41.4 mmol) in methanol (50 ml, 1.23 mol), **1a** (3.12 g, 15 mmol) was added. After refluxing for 6 h it was cooled in an ice bath, triethylamine (15 ml, 108 mmol) was added and the volatile material was evaporated under reduced pressure. The residue was flash chromatographed using ethyl acetate-petroleum ether (1:1) as the eluent (yield 3.19 g, 96%). M.p. 64–65 °C (dichloromethane/petroleum ether). IR (nujol): ν = 3325 cm^{-1} (NH), 1726 cm^{-1} (O=COR), 1628 cm^{-1} (O=CN). ^{1}H-NMR ($CDCl_3$): δ 9.0 (m, 1H, pyridine 2-H), 8.5–8.8 (m, 1H, pyridine 4-H), 8.0–8.3 (m, 1H, pyridine 6-H), 6.8–7.5 (m, 2H, CONH, pyridine 5-H), 3.3–3.8 (m, 5H, CONHC*H*$_2$CH$_2$, COOCH$_3$), 2.3–2.6 (m, 2H, CH$_2$C*H*$_2$COO), 1.7–2.2 (m, 2H, CH$_2$C*H*$_2$CH$_2$). Anal. ($C_{11}H_{14}N_2O_3$).

4-[(Pyridine-3-carbonyl)amino]butanoic acid 2-propyl-1-pentyl ester (**1c**)

1a (2.08 g, 10 mmol) was added to a stirred solution of 2-propyl-1-bromopentane (1.54 g, 8 mmol) in acetonitrile (40 ml) and a suspension was formed. To this, DBU (1.50 ml, 10 mmol) was added and a solution was formed, which was refluxed for 24 h. Then, the solvent was evaporated under reduced pressure, the residue was taken up in 100 ml of chloroform and washed successively with water (2 × 50 ml), 10% sodium bicarbonate solution (50 ml) and satd. aqueous sodium chloride solution. After drying ($CaCl_2$), the chloroform was evaporated under reduced pressure and the residue was flash chromatographed, using ethyl acetate-petroleum ether (1.5:1) as the eluent, to yield 2.30 g (90%) of a viscous oil. IR (neat): ν = 3325 cm^{-1} (NH), 1731 cm^{-1} (O=COR), 1632 cm^{-1} (O=CN). ^{1}H-NMR ($CDCl_3$): δ 9.0 (m, 1H, pyridine 2-H), 8.5–8.8 (m, 1H, pyridine 4-H), 8.0–8.3 (m, 1H, pyridine 6-H), 6.9–7.5 (m, 2H, pyridine 5-H, CONH), 3.9 (d, J = 6 Hz, 2H, COOC*H*$_2$CH), 3.3–3.7 (m, 2H, CONHC*H*$_2$CH$_2$), 2.3–2.6 (m, 2H, CH$_2$C*H*$_2$COO), 1.7–2.2 (m, 3H, CH$_2$C*H*$_2$CH$_2$, (CH$_3$CH$_2$CH$_2$)$_2$C*H*CH$_2$) 0.7–1.7 (m, 14H, 2CH$_2$CH$_2$CH$_3$). Anal. ($C_{18}H_{28}N_2O_3$).

*4-(2-Propylpentanoylamino)butanoic acid (**2a**)*

To a stirred suspension of 4-aminobutanoic acid (8.25 g, 80 mmol) in dichloromethane (700 ml) triethylamine (30.0 ml, 215 mmol) and trimethylchlorosilane (12.0 ml, 95 mmol) were added. After stirring for 2 h, 2-propyl-1-pentanoyl chloride (6.51 g, 40 mmol) was added and the stirring was continued for 46 h at room temperature. The volatile material was evaporated under reduced pressure and the residue was dissolved in 500 ml of an aqueous 3% potassium carbonate solution. After extraction with 3 × 100 ml of ether, the aqueous solution was carefully acidified with 6 M HCl solution and a white, bulky precipitate was formed. The latter was dissolved with the addition of 100 ml of ethyl acetate and the aqueous phase was extracted with 2 × 100 ml of ethyl acetate. The organic extracts were combined and washed with 50 ml of satd. aqueous sodium chloride solution and dried (Na_2SO_4). After evaporation of the solvent, the residue was recrystallized from ether (yield 6.90 g, 75%). mp 84–86 °C (ether). IR (nujol): ν = 3303 cm^{-1} (NH), 1696 cm^{-1} (O=COH), 1636 cm^{-1} (O=CN). ^{1}H-NMR ($CDCl_3$): δ 9.9 (bs, 1H, COOH), 5.8–6.2 (m, 1H, CONH), 3.1–3.5 (m, 2H, CONHCH_2CH$_2$), 2.1–2.5 (m, 2H, CH$_2$CH_2COO), 0.6–2.1 (m, 17H, CH$_2$CH_2CH$_2$, (CH$_3$CH$_2$CH$_2$)$_2$CHCO, 2CH$_2$CH$_2$CH$_3$). Anal. ($C_{12}H_{23}NO_3$). A material of mp 155–156 °C has been previously claimed as being the 4-(2-propylpentanoylamino)butanoic acid[31]. However, insufficient spectroscopic and analytical data were reported.

*4-(2-Propylpentanoylamino)butanoic acid pyridin-3-yl methyl ester (**2b**)*

2a (2.75g, 12 mmol) was added to a stirred solution of 3-pyridylcarbinol (1.73 ml, 18 mmol) in 180 ml of benzene. Concd. sulphuric acid (2.7 ml, 49 mmol) was then added and the mixture refluxed, connected to a Dean-Stark apparatus, for 15 h. The benzene was evaporated at room temperature under reduced pressure and 100 ml of chloroform were added to the residue. To this, triethylamine (17.0 ml, 122 mmol) was added under stirring and cooling and a solution was formed. The latter was washed successively with 2 × 50 ml of water and 30 ml of satd. aqueous sodium chloride solution and dried (Na_2SO_4). The product was isolated by flash chromatography using ethyl acetate as the eluent (yield 2.80 g, 73%). mp 72–73 °C (acetone/petroleum ether). IR (nujol): ν = 3282 cm^{-1} (NH), 1722 cm^{-1} (O=COR), 1637 cm^{-1} (O=CN). ^{1}H-NMR ($CDCl_3$): δ 8.3–8.7 (m, 2H, pyridine 6-H, 2-H), 7.4–7.8 (m, 1H, pyridine 4-H), 7.0–7.4 (m, 1H, pyridine 5-H), 5.6 (bs, 1H, CONH), 5.1 (s, 2H, COOCH$_2$), 3.0–3.5 (m, 2H, CONHCH_2CH$_2$), 2.2–2.6 (m, 2H, CH$_2$CH_2CH$_2$), 0.6–2.2 (m, 17H, CH$_2$CH_2COO, (CH$_3$CH$_2$CH$_2$)$_2$CHCO, 2CH$_2$CH$_2$CH$_3$). Anal. calcd. for $C_{18}H_{28}N_2O_3$: C, 67.47; H, 8.81; N, 8.74. Found: C, 67.72; H, 8.83; N, 8.33.

*4-(2-Propylpentanoylamino)butanoic acid 2-propylpentyl ester (**2c**)*

2a (2.06g, 9 mmol) was added to a solution of 2-propyl-1-pentanol (2.1 ml, 13.4 mmol) in 180 ml of benzene. After the addition of 3 drops of concd. sulfuric acid, the mixture was refluxed, connected to a Dean-Stark apparatus, for 20 h. Then the mixture was basified with triethylamine and concentrated under reduced pressure. The product was purified by flash chromatography using ethyl acetate/petroleum ether (1:10 → 1:5) as eluent (yield 1.88 g, 61%). mp 44–45 °C (petroleum ether). IR (nujol): ν = 3282 cm^{-1} (NH), 1735 cm^{-1} (O=COR), 1641 cm^{-1} (O=CN). ^{1}H-NMR ($CDCl_3$): δ 5.5–5.9 (m, 1H, CONH), 4.0 (d, J = 6 Hz, 2H, COOCH_2CH), 3.1–3.4 (m, 2H, CONHCH_2CH$_2$), 2.2–2.5 (m, 2H, CH$_2$CH_2CH$_2$), 0.7–2.1 (32H, CH$_2$CH_2COO, (CH$_3$CH$_2$CH$_2$)$_2$CHCO, (CH$_3$CH$_2$CH$_2$)$_2$CHCH$_2$, 4CH$_2$CH$_2$CH$_3$). Anal. ($C_{20}H_{39}NO_3$).

Anticonvulsant Activity

a. The Picrotoxin Model

A method previously reported[5] was adapted with the following modifications: Picrotoxin (9 mg/kg) was administered s.c. to male Fischer-344 rats (220–250 g) and after 30 s the tested compound was administered i.p. (0.4, 0.8 or 1.6 mmol/kg) in the form of an aqueous solution of the hydrochloride (**1b**, **1c**, **2b**), or sodium salt (**2a**) or a suspension in water (**2c**) with the addition of few drops of Tween–80. The behavioral changes of the rats were observed for 2 h. The latency to the onset of generalized clonic seizures, the survival time and the survival ratio were measured and evaluated as a percent of the control. The control rats received only picrotoxin. Valproic acid (0.8 mmol/kg) was used as the positive control.

b. The pentylenetetrazole Model

A method previously reported[32] was adapted with the following modifications: Pentylenetetrazole (110 mg/kg) was administered s.c. to male Fischer-344 rats (180–220 g) and after 30 s the tested compound **1c** was administered i.p. (1.6 mmol/kg) in the form of an aqueous solution of the hydrochloride salt. The behavioral changes of the rats were observed for 2 h. The control rats received only pentylenetetrazole.

Antioxidant Activity

Heat-inactivated (90 °C for 90 s) hepatic microsomes corresponding to 0.125 g liver/ml from Fischer-344 rats were used as the peroxidizable material. The incubation mixture also contained ascorbic acid (0.2 mM) in Tris-HCl/KCl buffer (50 mM/150 mM, pH 7.4). The tested compounds were added to the incubation mixture as DMSO solutions at various concentrations. The reaction was started by the addition of a freshly prepared $FeSO_4$ solution (10 μM) and the mixture was incubated at 37 °C. Aliquots (0.3 ml) were taken at various time intervals for 45 min. Lipid peroxidation, induced by the ascorbic acid (0.2 mM)/Fe^{2+} (10 μM) system, was assayed spectrophotometrically as the 2-thiobarbituric acid reactive material at 535 nm against 600 nm[24]. DL-α-Tocopherol acetate was used as a positive control and at a concentration of 0.5 mM was found to inhibit lipid peroxidation by 100, 75, 19, and 9% after 5, 15, 30 and 45 min of incubation respectively.

Determination of R_M Values from Reversed Phase Thin Layer Chromatography

Silica gel normal phase plates (Merck 5715), impregnated with 5% (*v/v*) liquid paraffin in light petroleum ether, were used as the stationary phase. As a mobile phase, a methanol/water mixture (67/33 *v/v*, pH = 6.5) was used. The plates were developed in closed chromatography tanks, saturated with the polar phase. Spots were detected under UV light or by oxidation with a ceric sulphate/ammonium molybdate solution. R_f values were determined from at least eight individual measurements. R_M values were calculated from the corresponding R_f values[18], applying the equation: $R_M = \log[(1/R_f) - 1]$.

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