Full Paper

Synthesis and Anticonvulsant Activity of *N*-(2-Hydroxyethyl)amide Derivatives

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A series novel *N*-(2-hydroxyethyl)amide derivatives was synthesized and screened for their anticonvulsant activities by the maximal electroshock (MES) test, and their neurotoxicity was evaluated by the rotarod test (Tox). The maximal electroshock test showed that *N*-(2-hydroxyethyl)decanamide **1g**, *N*-(2-hydroxyethyl)palmitamide **1l**, and *N*-(2-hydroxyeth-yl)stearamide **1n** were found to show a better anticonvulsant activity and also had lower toxicity than the marked anti-epileptic drug valproate. In the anti-MES potency test, these compounds exhibited median effective doses (ED_{50}) of 22.0, 23.3, 20.5 mg/kg, respectively, and median toxicity doses (TD_{50}) of 599.8, >1000, >1000 mg/kg, respectively, resulting in a protective index (PI) of 27.5, >42.9, >48.8, respectively. This is a much better protective index than that of the marked anti-epileptic drug valproate (PI = 1.6). To further investigate the effects of the anticonvulsant activity in several different models, compounds **1g**, **1l**, and **1n** were tested having evoked convulsions with chemical substances, including pentylenetetrazloe, isoniazide, 3-mercaptopropionic acid, bicuculline, thiosemicarbazide, and strychnine.

Keywords: Chemically induced models / *N*-(2-Hydroxyethyl)amide derivatives / Maximal electroshock (MES) / Neurotoxicity

Received: August 27, 2008; accepted: September 22, 2008

DOI 10.1002/ardp.200800153

Introduction

Epilepsy is one of the most common neurological disorders, affecting about 1% of the world population. The currently available anticonvulsants/anti-epileptic drugs (AEDs) are effective in reducing the severity and number of seizures in less than 70% of patients. Moreover, their usage is associated with undesirable side effects ranging from cosmetic (gingival hyperplasia) to life threatening conditions such as megaloblastic anemia [1 – 3]. Therefore, the continued search for safer and more effective AEDs is urgently necessary.

N-Palmitoylethanolamide (PEA) (Fig. 1) or *N*-(2-hydroxyethyl)hexadecanamide belong to a family of endogenous lipid amides, the *N*-acylethanolamines [4, 5], a well-known member of this family is anandamide. Lambert *et al*. [6] reported the effects of PEA on electroshock-induced convulsion in mice and found dose-dependent protection against maximal electroshock (MES) at non-toxic doses.

In light of the description of the anticonvulsant properties of *N*-palmitoylethanolamide (PEA) by Lambert *et al*., and in order to obtain compounds with better anticonvulsant activity, a series of new *N*-(2-hydroxyethyl)amide derivatives was designed and synthesized using PEA as the lead compound. The synthesized compounds were characterized by IR, ^{1}H-NMR, MS, and elemental analysis. The anticonvulsant activity was evaluated by

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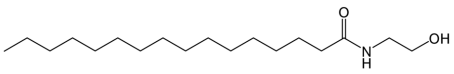
Abbreviations: anti-epileptic drugs (AEDs); gamma-aminobutyric acid (GABA); glutamate decarboxylase (GAD); maximal electroshock (MES); *N*-Palmitoylethanolamide (PEA); pentylenetetrazloe (PTZ)



Palmitoylethanolamide (FEA)

Figure 1. Structure of *N*-palmitoylethanolamide.

using the MES test and several different chemical models test. Neurotoxicity was evaluated by using the rotarod test.

Results and discussion

Synthesis

Target compounds were prepared according to Scheme 1. Compounds **1a–s** were obtained in high yield through a one-step reaction using substituted carboxylic acid, methyl chloroformate, triethylamine, and ethanolamine as the starting material. The reaction mixture was maintained at room temperature for 8–10 h. All compounds were identified by the spectral data. In general, IR spectra showed the C=O peak at 1678–1713 cm^{-1}, the NH stretching vibrations at 3017–3218 cm^{-1}, and the OH stretching vibrations at 3305–3325 cm^{-1}. In the nuclear magnetic resonance spectra (^{1}H-NMR), the signals of the respective protons of the synthesized compounds were verified on the basis of their chemical shifts, mutiplicities, and coupling constants. The spectra showed the amide (NH) proton as a singlet at 5.77–6.23 ppm and the hydroxyethyl proton (OH) at 1.81–2.16 ppm.

Pharmacological evaluations

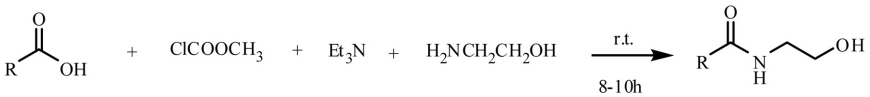
Pharmacological tests of the *N*-(2-hydroxyethyl) amide derivatives **1a–s** were conducted at the Epilepsy Branch of the National Institute of Neurological Disorders and Stroke (NINDS) following the protocol adopted by the Antiepileptic Drug Development (ADD) Program [7, 8].

In the preliminary (phase I) screening, compounds **1a–s** were evaluated in the MES test and the results are summarized in Table 1. All the compounds exhibited anticonvulsant activity, but among those four compounds (**1g**, **1h**, **1l**, and **1n**) possessed anticonvulsant activity against MES-induced seizure at the dose of 30 mg/kg, nine compounds (**1e**, **1f**, **1i–k**, **1m**, and **1q–s**) were active at the dose of 100 mg/kg, and the remaining six compounds (**1a–d**, **1o–p**) were active at the dose of 300 mg/kg. These results indicated that lengthening of the alkyl chain substituent from two to nine carbons led to an increase of anticonvulsant activity, in which the compound with nine carbons in the alkyl chain (**1g**) was found to be the most active (30 mg/kg). However, for a substituted alkyl chain of eleven to twenty-one carbon atoms, the relationship between chain length and activity was zig-zagged, in which compounds **1l** (-n-$C_{15}H_{31}$) and **1n** (-n-$C_{17}H_{35}$) were the most active (30 mg/kg). In addition, compounds with

Table 1. Phase-I evaluation of anticonvulsant activity in mice (i. p.).

Compound	R	Dosage (mg/kg)	MES[a]	
			0.5 h	4 h
1a	-C_2H_5	300	1/5	0/5
1b	-*n*-C_3H_7	300	2/5	0/5
1c	-*n*-C_4H_9	300	2/5	0/5
1d	-*n*-C_5H_{11}	300	1/5	0/5
1e	-*n*-C_7H_{15}	100	2/5	0/5
1f	-*n*-C_8H_{17}	100	2/5	0/5
1g	-*n*-C_9H_{19}	30	4/5	0/5
1h	-*n*-$C_8H_{16}CH{=}CH_2$	30	4/5	0/5
1i	-*n*-$C_{11}H_{23}$	100	2/5	0/5
1j	-*n*-$C_{13}H_{27}$	100	2/5	0/5
1k	-*n*-$C_{14}H_{29}$	100	1/5	0/5
1l	-*n*-$C_{15}H_{31}$	30	3/5	0/5
1m	-*n*-$C_{16}H_{33}$	100	1/5	0/5
1n	-*n*-$C_{17}H_{35}$	30	4/5	0/5
1o	-*n*-$C_{19}H_{39}$	300	1/5	0/5
1p	-*n*-$C_{21}H_{43}$	300	1/5	0/5
1q	-*n*-$CH_3(CH_2)_7CH{=}CH(CH_2)_7$	100	2/5	0/5
1r	-*n*-$CH_3(CH_2)_7CH{=}CH(CH_2)_9$	100	2/5	0/5
1s	-*n*-$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}$	100	1/5	0/5

[a] Maximal electroshock test (number of animals protected / number of animals tested).



1a-s

R:

1a: -C_2H_5 1b: -n-C_3H_7 1c: -n-C_4H_9 1d: -n-C_5H_{11} 1e: -n-C_7H_{15}

1f: -n-C_8H_{17} 1g: -n-C_9H_{19} 1h: -n-$C_8H_{16}CH{=}CH_2$ 1i: -n-$C_{11}H_{23}$ 1j: -n-$C_{13}H_{27}$

1k: -n-$C_{14}H_{29}$ 1l: -n-$C_{15}H_{31}$ 1m: -n-$C_{16}H_{33}$ 1n: -n-$C_{17}H_{35}$ 1o: -n-$C_{19}H_{39}$

1p: -n-$C_{21}H_{43}$ 1q: -n-$CH_3(CH_2)_7CH{=}CH(CH_2)_7$ 1r: -n-$CH_3(CH_2)_7CH{=}CH(CH_2)_9$ 1s:-n-$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}$

Scheme 1. Synthesis of compounds **1a–s**.

Table 2. Phase II quantitative anticonvulsant data in mice (test drug administered i. p.).

Compound	MES, ED_{50}[a)]	TD_{50}[b)]	PI[c)]
1g	22.0 (18.5–26.2)[d)]	599.8	27.5
1h	21.6 (15–31.2)	192.8 (167.1–227.5)	8.9
1l	23.3 (16.3–33)	>1000	>42.9
1n	20.5 (17.1–24.6)	>1000	>48.8
Valproate	272 (247–338)	426 (369–450)	1.6

a) Dose measured in mg/kg.
b) Minimal neurotoxicity was determined by the rotarod test 30 min after the tested compounds were administrated.
c) PI = TD_{50}/ED_{50}.
d) The 95% confidence limits.

a unsaturated alkyl chain of 17 to 21 carbon atoms (**1q–s**) showed similar activity, while a compound with a unsaturated alkyl chain of ten carbon atoms (**1h**) showed stronger activity.

In the phase-II pharmacology test, four compounds were quantitatively evaluated for their anticonvulsant activity (indicated by ED_{50}) and neurotoxicity (indicated by TD_{50}) with the marked anti-epileptic drug valproate as the control (Table 2). The result showed compounds **1g**, **1h**, **1l**, and **1n** possessed similar anti-MES activity with ED_{50} of 22.0, 21.6, 23.3, and 20.5 mg/kg, respectively, and they were all far more potent than valproate (ED_{50} = 272 mg/kg) and also had much higher PI (range from 8.9–48.8) than valproate (PI = 1.6). The compound **1n** (*N*-(2-hydroxyethyl) stearamide), with the lowest ED_{50} and highest PI (>48.8), was found to be the best, and compounds **1h** and **1n** were also considered excellent.

To further investigate the effects of the anticonvulsant activity in several different models, compounds **1g**, **1l**, and **1n** were tested against convulsions induced by chemical substances, including pentylenetetrazloe (PTZ), isoniazid, 3-mercaptopropionic acid, bicuculline, thiosemicarbazide, and strychnine. Compounds **1g**, **1l**, and **1n** were administered into mice i. p. at a dose of 50 mg/kg, which was slightly higher than their $2ED_{50}$ value and far below their TD_{50} value. The reference drug carbamazepine was also administered i. p. at a dose of 50 mg/kg.

In the sc-PTZ model, compounds **1g**, **1l**, and **1n**, and the reference drug carbamazeping did not inhibit the clonic seizures induced by sc-PTZ, but they inhibited the tonic seizures and reduced lethality to a significant degree (Table 3). In the isoniazid model, carbamazeping inhibited the clonic seizures, tonic seizures, and death induced by isoniazid at the rate of 50, 0, and 0%, respectively; and compounds **1g**, **1l**, and **1n** did not affect the clonic seizures and showed partial inhibition of the tonic seizures and death induced by isoniazid (Table 4). Pentylenetetrazole and isoniazid have been reported to produce seizures by inhibiting gamma-aminobutyric acid (GABA) neurotransmission [9, 10]. GABA is the main inhibitory neurotransmitter substance in the brain, and is widely implicated in epilepsy. Inhibition of GABAergic neurotransmission or activity has been shown to promote and facilitate seizures [11], while enhancement of GABAergic neurotransmission is known to inhibit or attenuate seizures. The findings of the present study suggest that the newly synthesized compounds **1g**, **1l**, and **1n** might have not inhibited or attenuated pentylenetetazole-induced and isoniazid-induced seizures in mice by enhancing GABAergic neurotransmission.

In the 3-mercaptopropionic acid-induced seizure model, carbamazepine inhibited the clonic seizures, tonic seizures, and death at the rate of 10, 100, and 90%, respectively. In comparison, compounds **1g**, **1l**, and **1n** showed stronger inhibition of the clonic seizures (40, 50, and 70%, respectively), less inhibition of the tonic seizures (70, 80, and 80%, respectively) and comparable

Table 3. Effect of compounds **1g**, **1l**, and **1n** on PTZ-induced convulsion in mice.

Compound	Doses (mg/kg)	Test time (h)	Clonic seizures (%)	Tonic seizures (%)	Lethality (%)
DMSO :CMC-Na	–	0.5	100	40	40
Carbamazepine	50	0.5	100	0	0
1g	50	0.5	100	20	10
1l	50	0.5	100	40	20
1n	50	0.5	100	60	0

Table 4. Effect of compounds **1g**, **1l**, and **1n** on isoniazid-induced convulsion in mice.

Compound	Doses (mg/kg)	Test time (h)	Clonic seizures (%)	Tonic seizures (%)	Lethality (%)
DMSO : CMC-Na	–	0.5	100	100	75
Carbamazepine	50	0.5	50	0	0
1g	50	0.5	100	100	70
1l	50	0.5	100	80	20
1n	50	0.5	100	60	50

Table 5. Effect of compounds **1g**, **1l,** and **1n** on 3-mercaptopropionic acid-induced convulsion in mice.

Compound	Doses (mg/kg)	Test time (h)	Clonic seizures (%)	Tonic seizures (%)	Lethality (%)
DMSO : CMC-Na	–	0.5	100	100	100
Carbamazepine	50	0.5	90	0	10
1g	50	0.5	60	30	10
1l	50	0.5	50	20	20
1n	50	0.5	30	20	0

Table 6. Effect of compounds **1g**, **1l**, and **1n** on thiosemicarbazide-induced convulsion in mice.

Compound	Doses (mg/kg)	Test time (h)	Clonic seizures (%)	Tonic seizures (%)	Lethality (%)
DMSO: CMC-Na	–	2.5	100	100	100
Carbamazepine	50	2.5	80	0	20
1g	50	2.5	50	30	20
1l	50	2.5	40	30	20
1n	50	2.5	20	20	10

Table 7. Effect of compounds **1g**, **1l**, and **1n** on strychnine-induced convulsion in mice.

Compound	Doses (mg/kg)	Test time (h)	Clonic seizures (%)	Tonic seizures (%)	Lethality (%)
DMSO : CMC-Na	–	0.5	100	100	100
Carbamazepine	50	0.5	100	80	20
1g	50	0.5	100	100	100
1l	50	0.5	100	100	100
1n	50	0.5	100	100	100

inhibition of death (90, 80, 100%, respectively) (Table 5). In the thiosemicarbazide-induced convulsion, the effect pattern is similar to that of the 3-mercaptopropionic acid-induced seizure model where compared to the reference drug, compounds **1g**, **1l**, and **1n** showed stronger inhibition of the clonic seizures, less inhibition of the tonic seizures and comparable inhibition of death (Table 6). 3-Mercaptopropionic acid and thiosemicarbazide are competitive inhibitors of the GABA synthesis enzyme glutamate decarboxylase (GAD); it inhibits the synthesis of GABA resulting in decreased GABA level in the brain [12]. Compounds **1g**, **1l**, and **1n** showed moderate antagonism to 3-mercaptopropionic acid-induced seizures and thiosemicarbazide-induced seizures, suggesting that it might activate GAD or inhibit (GABA)–aoxoglutarate aminotransferase (GABA-T) in the brain.

Next, in the strychnine-induced seizure model, strychnine administration caused 100% lethality in the control mice receiving vesicle only and also all the mice receiving compounds **1g**, **1l**, and **1n**, while mice treated with the reference drug had only 20% lethality (Table 7). It is known that strychnine directly antagonizes the inhibitory spinal reflexes of glycine [13], so the result suggests that compounds **1g**, **1l**, and **1n** could not influence the glycine system.

Finally, in a bicuculline-induced convulsion model, the reference drug carbamazepine, as well as the new compounds **1g**, **1l**, and **1n** sharply inhibited the clonic seizures, tonic seizures, and death induced by bicuculline (between 80 to 100% inhibition for all) (Table 8). Bicuculline (BIC) is a known competitive antagonist of the inhibitory amino acid GABA at the central $GABA_A$ receptors [14]. BIC produces convulsions through its antagonism of $GABA_A$-receptor-mediated inhibition [15]. The anticonvulsant effects of compounds **1g**, **1l**, and **1n** in the scBIC test suggests the presence of compounds capable of interaction with $GABA_A$ receptors or augmenting the functional role of GABA.

Table 8. Effect of compounds **1g**, **1l**, and **1n** on bicuculline-induced convulsion in mice.

Compound	Doses (mg/kg)	Test time (h)	Clonic seizures (%)	Tonic seizures (%)	Lethality (%)
DMSO : CMC-Na	–	0.5	100	100	40
Carbamazepine	50	0.5	20	0	0
1g	50	0.5	10	10	10
1l	50	0.5	20	10	0
1n	50	0.5	10	0	0

Conclusions

In conclusion, the results of this study demonstrat that *N*-(2-hydroxyethyl)amide derivatives have potent anticonvulsant activity. Especially, compounds **1g**, **1l**, and **1n** showed better anticonvulsant activity but also much lower toxicity than a benchmark marketed drug. In addition, compounds **1g**, **1l**, and **1n** demonstrated antagonistic activity against seizures induced by 3-mercaptopropionic acid, bicuculline, thiosemicarbazide, but failed to control those induced by strychnine, pentylenetetrazole, and isoniazid. These experiments suggested that compounds **1g**, **1l**, and **1n** might activate glutamate decarboxylase (GAD) or inhibit (GABA)–aoxoglutarate aminotransferase (GABA-T).

This work was supported by the National Natural Science Foundation of China (No. 30460151 and No. 30760290) and Important Item Foundation of the Ministry of Education P. R. China (No. 20070422029).

The authors have declared no conflict of interest.

Experimental

Chemistry

Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded (in KBr) on a FT-IR1730 (Bruker, Switzerland), ^{1}H-NMR spectra were measured on

a AV-300 (Bruker, Switzerland), and all chemical shifts were given in ppm relative to tetramethysilane. Microanalyses of C, N, and H were performed using a Heraeus CHN Rapid Analyzer (Heraeus, Germany). Mass spectra were measured on an HP1100LC (Agilent Technologies, USA). The major chemicals were purchased from Alderich Chemical Corporation. All other chemicals were the analytical grade.

*General procedure for the preparation of **1a–s***

In a three-necked round-bottomed flask containing substituted carboxylic acid 2 g (0.05 mol), 50 mL dichlormethane and triethylamine (0.1 mol), methyl chloroformate (0.1 mol) was slowly added dropwise with stirring and in an ice bath. The mixture was stirred 2 h at room temperature. Then, ethanolamine (0.1 mol) was dropwise added with stirring (in an ice bath), the mixture was stirred 8–10 h at room temperature. The solvents were removed under reduced pressure. The residue was poured into 100 mL ice water and stirred for 10 min. The solid obtained after filtration was recrystallized in hot water to afford a white solid.

*N-(2-Hydroxyethyl) propionamide **1a***

Yield: 56%; m.p.: 46–48°C; IR (KBr) [cm^{-1}]: 1678 (C=O), 3120 (NH), 3309 (OH); ^{1}H-NMR ($CDCl_3$) [ppm]: 1.15 (t, J = 6.1 Hz, 3H, CH_3), 1.97 (s, 1H, OH), 2.23 (t, J = 7.1 Hz, 2H, CH_2CO), 3.37 (dd, J = 5.1 Hz, 2H, CH_2), 3.79 (t, J = 4.8 Hz, 2H, CH_2), 6.08 (s, 1H, NH); MS m/z: 118 [M + 1]. Anal. Calcd. for $C_5H_{11}NO_2$: C, 51.26; H, 9.46; N, 11.96. Found: C, 51.19; H, 9.34; N, 11.82.

*N-(2-Hydroxyethyl)butyramide **1b***

Yield: 54%; m.p.: 49–51°C; IR (KBr) [cm^{-1}]: 1682 (C=O), 3124 (NH), 3305 (OH); ^{1}H-NMR ($CDCl_3$) [ppm]: 0.95 (t, J = 6.7 Hz, 3H, CH_3), 1.61 (m, 2H, CH_2), 2.03 (s, 1H, OH), 2.19 (t, J = 7.3 Hz, 2H, CH_2CO), 3.41 (dd, J = 4.5 Hz, 2H, CH_2), 3.78 (t, J = 4.9 Hz, 2H, CH_2), 6.09 (s, 1H, NH); MS m/z: 132 [M + 1]. Anal. Calcd. for $C_6H_{13}NO_2$: C, 54.94; H, 9.99; N, 10.68. Found: C, 54.86; H, 9.88; N, 10.54.

*N-(2-Hydroxyethyl)pentanamide **1c***

Yield: 49%; m.p.: 53–55°C; IR (KBr) [cm^{-1}]: 1700 (C=O), 3017 (NH), 3310 (OH); ^{1}H-NMR ($CDCl_3$) [ppm]: 0.92 (t, J = 7.3 Hz, 3H, CH_3), 1.25–1.63 (m, 4H, $(CH_2)_2$), 2.16 (s, 1H, OH), 2.22 (t, J = 7.2 Hz, 2H, CH_2CO), 3.44 (dd, J = 5.1 Hz, 2H, CH_2), 3.74 (t, J = 4.8 Hz, 2H, CH_2), 5.89 (s, 1H, NH); MS m/z: 146 [M + 1]. Anal. Calcd. for $C_7H_{15}NO_2$: C, 57.90; H, 10.41; N, 9.65. Found: C, 57.81; H, 10.28; N, 9.53.

*N-(2-Hydroxyethyl)hexanamide **1d***

Yield: 50%; m.p.: 47–49°C; IR (KBr) [cm^{-1}]: 1702 (C=O), 3204 (NH), 3311(OH); ^{1}H-NMR ($CDCl_3$) [ppm]: 0.89 (t, J = 6.2 Hz, 3H, CH_3), 1.29–1.63 (m, 6H, $(CH_2)_3$), 2.15 (s, 1H, OH), 2.17 (t, J = 7.8 Hz, 2H, CH_2CO), 3.40 (dd, J = 5.2 Hz, 2H, CH_2), 3.73 (t, J = 4.9 Hz, 2H, CH_2), 6.12 (s, 1H, NH); MS m/z: 160 [M + 1]. Anal. Calcd. for $C_8H_{17}NO_2$: C, 60.35; H, 10.76; N, 8.80. Found: C, 60.23; H, 10.61; N, 8.71.

*N-(2-Hydroxyethyl)octanamide **1e***

Yield: 53%; m.p.: 44–46°C; IR (KBr) [cm^{-1}]: 1696 (C=O), 3110 (NH), 3319 (OH); ^{1}H-NMR ($CDCl_3$) [ppm]: 0.89 (t, J = 6.8 Hz, 3H, CH_3), 1.28–1.63 (m, 10H, $(CH_2)_5$), 2.16 (s, 1H, OH), 2.32 (t, J = 7.6 Hz, 2H, CH_2CO), 3.51 (dd, J = 5.4 Hz, 2H, CH_2), 4.13 (t, J = 5.3 Hz, 2H, CH_2), 5.89 (s, 1H, NH); MS m/z: 188 [M + 1]. Anal. Calcd. for $C_{10}H_{21}NO_2$: C, 64.13; H, 11.30; N, 7.48. Found: C, 64.01; H, 11.12; N, 7.29.

*N-(2-Hydroxyethyl)nonanamide **1f***

Yield: 56%; m.p.: 45-47°C; IR (KBr) [cm^{-1}]: 1706 (C=O), 3218 (NH), 3320 (OH); ^{1}H-NMR ($CDCl_3$) [ppm]: 0.87 (t, J = 6.8 Hz, 3H, CH_3), 1.28–1.62 (m, 12H, $(CH_2)_6$), 2.15 (s, 1H, OH), 2.31 (t, J = 7.5 Hz, 2H, CH_2CO), 3.53 (dd, J = 5.3 Hz, 2H, CH_2), 4.16 (t, J = 5.1 Hz, 2H, CH_2), 5.77 (s, 1H, NH); MS m/z: 202 [M + 1]. Anal.Calcd. for $C_{11}H_{23}NO_2$: C, 65.63; H, 11.52; N, 6.96. Found: C, 65.51; H, 11.46; N, 6.79.

*N-(2-Hydroxyethyl)decanamide **1g***

Yield: 81%; m.p.: 78–80°C; IR (KBr) [cm^{-1}]: 1689 (C=O), 3189 (NH), 3321 (OH); ^{1}H-NMR ($CDCl_3$) [ppm]: 0.88 (t, J = 6.7 Hz, 3H,CH_3), 1.28-1.63 (m, 14H, $(CH_2)_7$), 1.81 (s, 1H, OH), 2.20 (t, J = 7.5 Hz, 2H, CH_2CO), 3.41 (dd, J = 5.1 Hz, 2H, CH_2), 3.70 (t, J = 5.2 Hz, 2H, CH_2), 6.07 (s, 1H, NH); MS m/z: 216 [M + 1]. Anal. Calcd. for $C_{12}H_{25}NO_2$: C, 66.93; H, 11.70; N, 6.50. Found: C, 66.84; H, 11.63; N, 6.41.

*N-(2-Hydroxyethyl)undec-10-enamide **1h***

Yield: 79%; m.p.: 56–58°C; IR (KBr) [cm^{-1}]: 1695 (C=O), 3211 (NH), 3319 (OH); ^{1}H-NMR ($CDCl_3$) [ppm]: 1.27–1.60 (m, 14H, $(CH_2)_7$), 1.98 (s, 1H, OH), 2.20 (t, J = 7.4 Hz, 2H, CH_2CO), 3.38 (dd, J = 5.1 Hz, 2H, CH_2), 3.67 (t, J = 5.1 Hz, 2H, CH_2), 4.92 (t, J = 10.6 Hz, 2H, $=CH_2$), 5.77 (m, 1H, =CH), 6.46 (s, 1H, NH); MS m/z: 228 [M + 1]. Anal. Calcd. for $C_{13}H_{25}NO_2$: C, 68.68; H, 11.08; N, 6.16. Found: C, 68.56; H, 10.87; N, 5.91.

*N-(2-Hydroxyethyl)dodecanamide **1i***

Yield: 77%; m.p.: 76–78°C; IR (KBr) [cm^{-1}]: 1692 (C=O), 3123 (NH), 3314 (OH); ^{1}H-NMR ($CDCl_3$) [ppm]: 0.88 (t, J = 6.9 Hz, 3H, CH_3), 1.26-1.64 (m, 18H, $(CH_2)_9$), 1.92 (s, 1H, OH), 2.21 (t, J = 7.5 Hz, 2H, CH_2CO), 3.42 (dd, J = 5.3 Hz, 2H, CH_2), 3.73 (t, J = 4.8 Hz, 2H, CH_2), 6.04 (1H, s, NH); MS m/z: 244 [M + 1]. Anal. Calcd. for $C_{14}H_{29}NO_2$: C, 69.09; H, 12.01; N, 5.75. Found: C, 68.89; H, 11.90; N, 5.63.

*N-(2-Hydroxyethyl)tetradecanamide **1j***

Yield: 75%; m.p.: 88–91°C; IR (KBr) [cm^{-1}]: 1695 (C=O), 3087 (NH), 3321 (OH); ^{1}H-NMR ($CDCl_3$) [ppm]: 0.89 (t, J = 6.9 Hz, 3H, CH_3), 1.26–1.64 (m, 22H, $(CH_2)_{11}$), 1.98 (s, 1H, OH), 2.24 (t, J = 7.6 Hz, 2H, CH_2CO), 3.42 (dd, J = 5.3 Hz, 2H, CH_2), 3.73 (t, J = 4.8 Hz, 2H, CH_2), 6.05 (s, 1H, NH); MS m/z: 272 [M + 1]. Anal. Calcd. for $C_{16}H_{33}NO_2$: C, 70.80; H, 12.25; N, 5.16. Found: C, 70.71; H, 12.10; N, 5.02.

*N-(2-Hydroxyethyl)pentadecanamide **1k***

Yield: 79%; m.p.: 85–88°C; IR (KBr) [cm^{-1}]: 1703 (C=O), 3198 (NH), 3318 (OH); ^{1}H-NMR ($CDCl_3$) [ppm]: 0.89 (t, J = 6.1 Hz, 3H, CH_3), 1.26–1.64 (m, 24H, $(CH_2)_{12}$), 1.97 (s, 1H, OH), 2.22 (t, J = 7.4 Hz, 2H, CH_2CO), 3.43 (dd, J = 5.2 Hz, 2H, CH_2), 3.74 (t, J = 5.0 Hz, 2H, CH_2), 5.97 (s, 1H, NH); MS m/z: 286 [M + 1]. Anal. Calcd. for $C_{17}H_{35}NO_2$: C, 71.53; H, 12.36; N, 4.91. Found: C, 71.41; H, 12.20; N, 4.81.

*N-(2-Hydroxyethyl)palmitamide **1l***

Yield: 82%; m.p.: 95–96°C; IR (KBr) [cm^{-1}]: 1701 (C=O), 3210 (NH), 3320 (OH); ^{1}H-NMR ($CDCl_3$) [ppm]: 0.88 (t, J = 6.1 Hz, 3H, CH_3), 1.25–1.63 (m, 26H, $(CH_2)_{13}$), 1.98 (s, 1H, OH), 2.21 (t, J = 7.5 Hz, 2H, CH_2CO), 3.44 (dd, J = 5.1 Hz, 2H, CH_2), 3.73 (t, J = 4.9 Hz, 2H, CH_2), 5.94 (s, 1H, NH); MS m/z: 300 [M + 1]. Anal. Calcd. for $C_{18}H_{37}NO_2$: C, 72.19; H, 12.45; N, 4.68. Found: C, 72.01; H, 12.34; N, 4.50.

N-(2-Hydroxyethyl)heptadecanamide ***1m***

Yield: 76%; m.p.: 96 – 98°C; IR (KBr) [cm^{-1}]: 1706 (C=O), 3114 (NH), 3316 (OH); ^{1}H-NMR ($CDCl_3$) [ppm]: 0.88 (t, *J* = 5.9 Hz, 3H, CH_3), 1.25-1.63 (m, 28H, $(CH_2)_{14}$), 1.97 (s, 1H, OH), 2.20 (t, *J* = 7.5 Hz, 2H, CH_2CO), 3.41 (dd, *J* = 5.2 Hz, 2H, CH_2), 3.72 (t, *J* = 5.0 Hz, 2H, CH_2), 6.04 (s, 1H, NH); MS m/z: 314 [M + 1]. Anal. Calcd. for $C_{19}H_{39}NO_2$: C, 72.79; H, 12.54; N, 4.47. Found: C, 72.65; H, 12.44; N, 4.35.

N-(2-Hydroxyethyl)stearamide ***1n***

Yield: 81%; m.p.: 90 – 92°C; IR (KBr) [cm^{-1}]: 1706 (C=O), 3124 (NH), 3321 (OH); ^{1}H-NMR ($CDCl_3$) [ppm]: 0.88 (t, *J* = 5.8 Hz, 3H, CH_3), 1.25 – 1.63 (m, 30H, $(CH_2)_{15}$), 2.10 (s, 1H, OH), 2.24 (t, *J* = 7.5 Hz, 2H, CH_2CO), 3.43 (dd, *J* = 5.1 Hz, 2H, CH_2), 3.72 (t, *J* = 5.1 Hz, 2H, CH_2), 6.23 (s, 1H, NH); MS m/z: 328 [M + 1]. Anal. Calcd. for $C_{20}H_{41}NO_2$: C, 73.34; H, 12.62; N, 4.28. Found: C, 73.27; H, 12.58; N, 4.09.

N-(2-Hydroxyethyl)icosanamide ***1o***

Yield: 78%; m.p.: 96 – 99°C; IR (KBr) [cm^{-1}]: 1678 (C=O), 3121 (NH), 3311 (OH); ^{1}H-NMR ($CDCl_3$) [ppm]: 0.88 (t, *J* = 6.0 Hz, 3H, CH_3), 1.24 – 1.62 (m, 34H, $(CH_2)_{17}$), 2.03 (s, 1H, OH), 2.23 (t, *J* = 7.6 Hz, 2H, CH_2CO), 3.43 (dd, *J* = 5.3 Hz, 2H, CH_2), 3.74 (t, *J* = 4.9 Hz, 2H, CH_2), 6.06 (s, 1H, NH); MS m/z: 356 [M + 1]. Anal. Calcd. for $C_{22}H_{45}NO_2$: C, 74.31; H, 12.76; N, 3.94. Found: C, 74.24; H, 12.65; N, 3.81.

N-(2-Hydroxyethyl)docosanamide ***1p***

Yield: 70%; m.p.: 102 – 104°C; IR (KBr) [cm^{-1}]: 1712 (C=O), 3127 (NH), 3319 (OH); ^{1}H-NMR ($CDCl_3$) [ppm]: 0.87 (t, *J* = 6.9 Hz, 3H, CH_3), 1.25-1.63 (m, 38H, $(CH_2)_{19}$), 1.98 (s, 1H, OH), 2.21 (t, *J* = 7.4 Hz, 2H, CH_2CO), 3.46 (dd, *J* = 5.3 Hz, 2H, CH_2), 3.72 (t, *J* = 5.0 Hz, 2H, CH_2), 6.05 (s, 1H, NH); MS m/z: 384 [M + 1]. Anal. Calcd. for $C_{24}H_{49}NO_2$: C, 75.14; H, 12.87; N, 3.65. Found: C, 74.03; H, 12.61; N, 3.49.

(E)-N-(2-Hydroxyethyl)octadec-9-enamide ***1q***

Yield: 67%; oil; IR (KBr) [cm^{-1}]: 1713 (C=O), 3022 (NH), 3325 (OH); ^{1}H-NMR ($CDCl_3$) [ppm]: 0.87 (t, *J* = 6.5 Hz, 3H, CH_3), 1.26 – 1.63 (m, 26H, $(CH_2)_{13}$), 2.00 (s, 1H, OH), 2.22 (t, *J* = 7.4 Hz, 2H, CH_2CO), 3.40 (dd, *J* = 5.2 Hz, 2H, CH_2), 3.72 (t, *J* = 5.0 Hz, 2H, CH_2), 5.33 (m, 1H, =CH), 5.35 (m, 1H, =CH), 6.23 (s, 1H, NH); MS m/z: 326 [M + 1]. Anal. Calcd. for $C_{20}H_{39}NO_2$: C, 73.79; H, 12.08; N, 4.30. Found: C, 73.67; H, 11.83; N, 4.16.

(E)-N-(2-Hydroxyethyl)icos-11-enamide ***1r***

Yield: 62%; oil; IR (KBr) [cm^{-1}]: 1711 (C=O), 3123 (NH), 3315 (OH); ^{1}H-NMR ($CDCl_3$) [ppm]: 0.88 (t, *J* = 6.7 Hz, 3H, CH_3), 1.25 – 1.65 (m, 30H, $(CH_2)_{15}$), 2.01 (s, 1H, OH), 2.23 (t, *J* = 7.5 Hz, 2H, CH_2CO), 3.41 (dd, *J* = 5.2 Hz, 2H, CH_2), 3.74 (t, *J* = 5.1 Hz, 2H, CH_2), 5.35 (m, 1H, =CH), 5.37 (m, 1H, =CH), 6.21 (s, 1H, NH); MS m/z: 354 [M + 1]. Anal. Calcd. for $C_{22}H_{43}NO_2$: C, 74.73; H, 12.26; N, 3.96. Found: C, 74.65; H, 12.12; N, 3.86.

(E)-N-(2-Hydroxyethyl)docos-13-enamide ***1s***

Yield: 59%; oil; IR (KBr) [cm^{-1}]: 1701 (C=O), 3115 (NH), 3317 (OH); ^{1}H-NMR ($CDCl_3$) [ppm]: 0.86 (t, *J* = 6.5 Hz, 3H, CH_3), 1.25 – 1.64 (m, 34H, $(CH_2)_{17}$), 2.02 (s, 1H, OH), 2.21 (t, *J* = 7.4 Hz, 2H, CH_2CO), 3.43 (dd, *J* = 5.3 Hz, 2H, CH_2), 3.72 (t, *J* = 5.2 Hz, 2H, CH_2), 5.32 (m, 1H, =CH), 5.34 (m, 1H, =CH), 6.18 (s, 1H, NH); MS m/z: 382 [M + 1]. Anal. Calcd. for $C_{24}H_{47}NO_2$: C, 75.53; H, 12.41; N, 3.67. Found: C, 75.45; H, 12.32; N, 3.54.

Pharmacology

The MES test and the rotarod test were carried out by the standard described in the Antiepileptic Drug Development Program (ADD) of the National Institutes of Health (USA) [7, 8]. All compounds were tested for anticonvulsant activities with C57B/6 mice in the 18 to 25 g weight range purchased from the Laboratory of Animal Research, College of Pharmacy, Yanbian University. The tested compounds were prepared as a suspension in a mixture of 1 : 19 (vol / vol) dimethylsulfoxide / saline (0.9% NaCl) containing 0.5% methylcellulose.

In phase-I screening (Table 1), each compound was administered at the dose levels of 30, 100, and 300 mg/kg for evaluating the anticonvulsant activity, and its neurotoxicity was measured at 30 min and 4 h intervals after administration. Anticonvulsant efficacy was measured in the MES test. In the MES test, seizures were elicited with a 60 Hz alternating current of 50 mA intensity in mice. The current was applied via corneal electrodes for 0.2 s. The protection against the spread of MES-induced seizures was defined as the abolition of the hind leg and tonic maximal extension component of the seizure. Anticonvulsant drug-induced neurologic deficit was detected in mice by using the rotorod ataxia test.

The pharmacologic parameters estimated in phase-I screening were quantified for compounds **1a – s** in phase-II screening (Table 2). Anticonvulsant activity was expressed in terms of the median effective dose (ED_{50}), and neurotoxicity was expressed as the median toxic dose (TD_{50}). For determination of the ED_{50} and TD_{50} values, groups of ten mice were given a range of intraperitoneal doses of the tested compound until at least three points were established in the range of 10 – 90% seizure protection or minimal observed neurotoxicity. From the plot of this data, the respective ED_{50} and TD_{50} values, 95% confidence intervals, slope of the regression line, and the standard error of the slope were calculated by means of a computer program written at National Institute of Neurological Disorders and Stroke.

In chemically induced seizures (see Tables 3 to 8), mice were given doses of convulsant drugs that could induce seizures at least 97% of animals. The doses used were: PTZ, 85 mg/kg; isoniazid, 250 mg/kg; 3-mercaptopropionic acid, 40 mg/kg; strychnine, 1.2 mg/kg; thiosemicarbazide, 50 mg/kg; and bicuculline, 30 mg/kg. The test compounds and standard AEDs were administered i. p. in a volume of 50 mg/kg to groups of ten mice 30 min before either i. p. administration of isoniazid and bicuculline or s. c. injection of PTZ, 3-mercaptopropionic acid, and strychnine. The mice were placed in individual cages and observed for 30 min, the number of clonic seizures, tonic seizures and the lethality were recorded [16 – 19]. Thiosemicarbazide also was administered i. p., and the test compounds were administered i. p. to mice 30 min before thiosemicarbazide. The mice were placed in individual cages and observed for 2 h 30 min, the number of clonic seizures, tonic seizures, and the lethality were noted [20].

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