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Synthesis and Evaluation of Antiallodynic and Anticonvulsant Activity of Novel Amide and Urea Derivatives of Valproic Acid Analogues

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Valproic acid (VPA, **1**) is a major broad spectrum antiepileptic and central nervous system drug widely used to treat epilepsy, bipolar disorder, and migraine. VPA's clinical use is limited by two severe and life-threatening side effects, teratogenicity and hepatotoxicity. A number of VPA analogues and their amide, *N*-methylamide and urea derivatives, were synthesized and evaluated in animal models of neuropathic pain and epilepsy. Among these, two amide and two urea derivatives of **1** showed the highest potency as antineuropathic pain compounds, with ED_{50} values of 49 and 51 mg/kg for the amides (**19** and **20**) and 49 and 74 mg/kg for the urea derivatives (**29** and **33**), respectively. **19**, **20**, and **29** were equipotent to gabapentin, a leading drug for the treatment of neuropathic pain. These data indicate strong potential for the above-mentioned novel compounds as candidates for future drug development for the treatment of neuropathic pain.

Introduction

Neuropathic pain is a neurological disorder affecting between 3% and 8% of adults. It often has devastating effects on patients' quality of life, general mood, and occupational functioning and is associated with a heavy financial burden to family and to society.[1,2] Many patients are resistant to current therapy, and thus, there is a substantial need for further development of novel medications for the treatment of neuropathic pain.[1,3,4] Among the pharmacotherapy currently used to treat neuropathic pain conditions are antiepileptic drugs (AEDs[a]), e.g., carbamazepine, gabapentin, and pregabalin. Indeed, these are among the first line treatment options for many neuropathic pain conditions.[2,5−7] It is believed that epilepsy and neuropathic pain share aspects of their underlying pathophysiology[8] and that this is the reason that some AEDs are useful for the treatment of neuropathic pain.[5]

Valproic acid (VPA, **1**, Figure 1) is a broad spectrum AED widely used today for treating various types of epileptic seizures, bipolar disorder, and migraine.[9−11] It was also shown to be effective for treating some forms of neuropathic pain. However, its efficacy as a pain reliever, including as a reliever of neuropathic allodynia (antiallodynic drug), is controversial.[12−15] VPA's clinical use is limited by two severe and life threatening side effects, teratogenicity and hepatotoxicity.[16−19] Hepatotoxicity induced by **1** is caused by the formation of metabolite(s) possessing a terminal double bond (e.g., 4-ene-VPA). Teratogenicity induced by **1** is caused by the parent compound.[20−24] As **1** is a small branched fatty acid having eight carbons in its structure and a wide range of CNS activity, it is a good target for structure modification and structure−activity relationship (SAR) studies.

Many studies have been conducted in an attempt to find derivatives superior to **1**, with a similarly broad spectrum of activity and an improved side effects profile, by synthesizing analogues and derivatives of **1** and evaluating their anticonvulsant activity.[25−40] Studies conducted by our group also evaluated the antiallodynic activity of such derivatives in animal models for neuropathic pain. Among the compounds tested for this activity are the corresponding amide of **1** and its constitutional isomers and cyclopropyl analogue.[41,42,49] Valpromide (VPD, **2**), the corresponding amide of **1**, and the constitutional isomers of **2** valnoctamide (VCD, **3**), diisopropylacetamide (DID, **4**), and propylisopropylacetamide (PID, **5**) (Figure 1), were 10 times more potent than **1** as anticonvulsants, and 4 times more potent as antineuropathic pain compounds than **1**. In addition, they were also more potent than their corresponding acids in animal models of both anticonvulsant and antiallodynic activity.[41,42]

In the present study, 17 new amide derivatives of analogues of **1**, including amide and uride derivatives, were synthesized

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[a]Abbreviations: VPA, valproic acid; AEDs, antiepileptic drugs; CNS, central nervous system; VPD, valpromide; VCD, valnoctamide; DID, diisopropylacetamide; PID, propylisopropylacetamide; SNL, spinal nerve ligation; MES, maximal electroshock seizure; scMet, subcutaneous pentylentetrazole; LDA, lithium diisopropylamine; NMR, nuclear magnetic resonance; GC−MS, gas chromatography−mass spectrometry; VFF, von Frey filaments; TLC, thin layer chromatograpy; DMPU, 1,3-dimethyl-3,4,5,6-tetrahydro-2-pyrimidinone; TMCA, 2,2,3,3-tetramethylcyclopropylcarboxylic acid; TMCD, 2,2,3,3-tetramethylcyclopropylcarboxamide; MTMCD, *N*-methyl-2,2,3,3-tetramethylcyclopropyl carboxamide.

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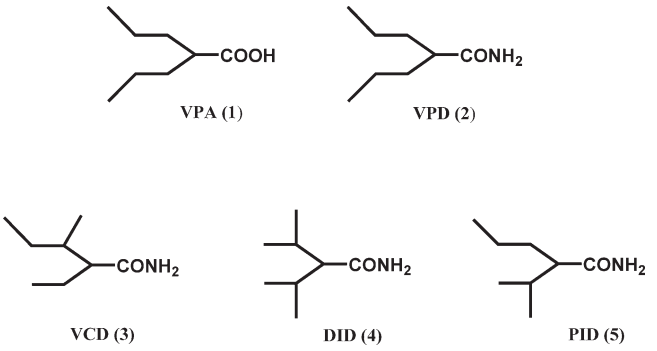


Figure 1. Chemical structure of valproic acid (VPA, **1**), its amide derivative (VPD, **2**), and the amide derivatives of its constitutional isomers.

Table 1. Antiallodynic Activity of Compounds **6**−**34** 30−240 min after Intraperitoneal Administration to Rats at 80 mg/kg

	SNL[a]				
compd	30 min[b]	60 min[b]	120 min[b]	180 min[b]	240 min[b]
6	1/9	3/9	4/9	4/9	2/9
7	0/9	0/9	1/9	1/9	1/9
8	1/9	1/9	2/9	2/9	2/9
9	1/8	1/8	3/8	3/8	1/8
10	0/9	0/9	2/9	2/9	1/9
11	0/8	0/8	0/8	2/8	1/8
12	0/9	0/9	0/9	0/9	0/9
13	1/9	1/9	1/9	1/9	0/9
14	1/9	3/9	3/9	4/9	2/9
15	0/8	1/8	1/8	3/8	2/8
16	2/10	1/10	2/10	2/10	0/10
17	1/8	1/8	1/8	1/8	0/8
18	1/10	2/10	2/10	2/10	0/10
19	6/9	7/9	8/9	2/9	2/9
20	3/9	7/9	4/9	4/9	1/9
21	4/9	6/9	4/9	3/9	1/9
22	5/9	6/9	7/9	6/9	5/9
23	0/10	2/10	2/10	2/10	2/10
24	0/10	2/10	6/10	5/10	5/10
25	0/10	1/10	1/10	1/10	1/10
26	0/8	2/8	1/8	2/8	2/8
27	0/9	0/9	0/9	0/9	0/9
28	2/7	2/7	2/7	1/7	1/7
29	5/8	6/8	6/8	4/8	3/8
30	2/9	2/9	3/9	3/9	3/9
31	0/9	0/9	0/9	0/9	2/9
32	0/9	0/9	0/9	0/9	0/9
33	1/9	3/9	2/9	4/9	4/9
34	0/9	1/9	1/9	1/9	0/9

[a] Spinal nerve ligation model of neuropathic pain (number of animals protected/total number of animals tested). [b] Time after drug administration.

by modifying the alkyl side chain(s) of the carboxyl moiety of the appropriate acids. We aimed to determine the SAR of these derivatives in an attempt to develop additional novel and more potent compounds for the treatment of neuropathic pain. We further synthesized 12 *N*-methyl and urea derivatives by replacing the amino group in those amides that yielded the strongest antiallodynic effect among the ones evaluated in this study. The aim was to determine the effect on the antiallodynic and anticonvulsant potencies of substitution of the NH_2 (in the amide moiety) with *N*-methyl and urea substituents.

The antiallodynic activity for all 29 compounds mentioned above was evaluated utilizing the spinal nerve ligation (SNL) model of neuropathic pain (Table 1). Antiallodynic activity of these compounds was compared to their antiepileptic activity in the maximal electroshock (MES) and subcutaneous pentylenetetrazol (scMet) seizure tests (Tables 2−4).

Table 2. Anticonvulsant Activity and Toxicity (Tox) of Compounds **6**−**13** Administered Intraperitoneally to Mice

		MES[a]		scMet[b]		Tox[c]	
compd	dose (mg/kg)	0.5 h[d]	4 h[d]	0.5 h[d]	4 h[d]	0.5 h[d]	4 h[d]
6	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	1/3	0/3	0/1	0/1	0/8	0/4
	300	1/1	1/1	1/1	0/1	3/4	0/2
7	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	0/1	0/1	0/1	0/1	0/4	0/2
8	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	1/1	0/1	0/1	0/1	1/4	0/2
9	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	0/1	0/1	0/1	0/1	0/4	0/2
10	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	1/3	0/3	0/1	0/1	1/8	0/4
	300	1/1	0/1	0/1	0/1	0/4	0/2
11	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	0/1	0/1	0/1	0/1	0/4	0/2
12	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	1/5	0/8	0/4
	300	0/1	0/1	0/1	0/1	3/4	1/2
13	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	1/5	0/1	0/8	0/4
	300	0/1	0/1	0/1	0/1	0/4	0/2

[a] Maximal electroshock test (number of animals protected/number of animals tested). [b] Subcutanous metrazol test (number of animals protected/number of animals tested). [c] Neurotoxicity evaluated as motor impariment or sedation (number of animals protected/number of animals tested). [d] Time after drug administration.

Chemistry

The general synthesis for the analogues of **1** and their corresponding amides, *N*-methylamides and urea derivatives, is depicted in Figure 3. Stated briefly, the starting material for compounds **6**, **7**, **25**, **28**, **31** was valeric acid; for compounds **8**, **9**, **24**, **30** isovaleric acid; for compounds **19**, **20**, **23**, **29** 3-methylvaleric acid; for compounds **15**, **17**, **18** 4-methylvaleric acid; for compounds **12**, **16**, **21**, **22**, **27**, **33** dimethylbutyric acid; for compounds **13**, **34** pivaloylacetic acid. In order to produce the corresponding branched acids, the above-mentioned acids were converted to the corresponding enolates by the use of lithium diisopropylamine (LDA), followed by substitution of a hydrogen atom on the carbon α to the carboxyl with the appropriate alkyl by using specific alkyl iodides.[43] The carboxylic acids were treated with thionyl chloride in order to produce the corresponding acyl chloride followed by treatment with 28−30% ammonium hydroxide or 41% methylamine solution in water at 0 °C for 2 h to yield the corresponding amide or *N*-methylamide derivative, respectively. The urea derivatives of the corresponding acids were synthesized by coupling the acyl chloride moiety with urea according to a previously published procedure.[34,44] The chemical structures of the synthesized amides, *N*-methylamides, and urea derivatives were identified by ^{1}H NMR and GC−MS, while purity was established using elemental analysis.

Table 3. Anticonvulsant Activity and Toxicity of Compounds **14**−**22** Administered Intraperitoneally to Mice

		MES[a]		scMet[b]		Tox[c]	
compd	dose (mg/kg)	0.5 h[d]	4 h[d]	0.5 h[d]	4 h[d]	0.5 h[d]	4 h[d]
14[e]	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	3/3	0/3	0/1	0/1	0/8	0/4
	300	NT[f]	0/1	NT[f]	NT[f]	4/4	1/2
15[g]	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/3	0/1	0/8	0/4
	300	1/1	0/1	0/0	1/1	4/4	0/2
16	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	0/1	1/1	1/1	0/1	2/4	0/2
17	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	0/1	0/1	1/1	0/1	2/4	0/2
18	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	1/1	0/1	1/1	0/1	4/4	0/2
19	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	3/3	0/3	1/1	0/1	8/8	0/4
	300	1/1	0/1	1/1	0/1	4/4	1/2
20	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	2/3	0/3	0/1	0/1	0/8	0/4
	300	1/1	0/1	1/1	0/1	3/4	0/2
21	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	2/3	0/3	1/1	0/1	2/8	0/4
	300	1/1	1/1	1/1	1/1	4/4	2/2
22	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	2/3	1/3	0/1	0/1	6/8	0/4
	300	1/1	1/1	1/1	0/1	3/4	1/2

[a] Maximal electroshock test (number of animals protected/number of animals tested). [b] Subcutanous metrazol test (number of animals protected/number of animals tested). [c] Neurotoxicity evaluated as motor impariment or sedation (number of animals protected/number of animals tested). [d] Time after drug administration. [e] Data taken from ref 27. [f] NT: not tested. [g] Data taken from ref 28.

Table 4. Anticonvulsant Activity and Toxicity of Compounds **23**−**34** Administered Intraperitoneally to Mice

		MES[a]		scMet[b]		Tox[c]	
compd	dose (mg/kg)	0.5 h[d]	4 h[d]	0.5 h[d]	4 h[d]	0.5 h[d]	4 h[d]
23	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	1/8	0/4
	300	0/1	0/1	1/1	0/1	3/4	0/2
24	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	1/8	0/4
	300	1/1	0/1	4/5	0/1	2/4	0/2
25	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	0/1	0/1	1/1	0/1	4/4	0/2
26	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	2/3	1/3	0/1	0/1	0/8	0/4
	300	1/1	1/1	1/1	0/1	4/4	0/2
27	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	0/1	0/1	0/1	0/1	0/4	0/2
28[e]	30	0/1	0/1	0/1	0/1	1/4	1/2
	100	3/3	0/3	1/1	0/1	6/8	0/4
	300	1/1	1/1	1/1	0/1	3/4	1/2
29[e]	30	0/1	0/1	0/1	0/1	1/4	0/2
	100	3/3	0/3	1/1	0/1	8/8	0/4
	300	1/1	1/1	1/1	1/1	4/4	2/2
30[e]	30	0/1	0/1	0/1	0/1	1/4	0/2
	100	2/3	0/3	1/1	0/1	6/8	0/4
	300	1/1	1/1	1/1	0/1	4/4	2/2
31[e]	30	0/1	0/1	0/1	0/1	1/4	1/2
	100	0/3	0/3	1/1	0/1	6/8	1/4
	300	0/1	1/1	1/1	0/1	3/4	0/2
32	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	1/3	0/3	0/1	0/1	0/8	0/4
	300	1/1	0/1	1/1	0/1	4/4	0/2
33[g]	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	4/5	0/1	3/8	0/4
	300	1/1	0/1	1/1	0/1	4/4	0/2
34[g]	30	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]
	100	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]
	300	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]

[a] Maximal electroshock test (number of animals protected/number of animals tested). [b] Subcutanous metrazol test (number of animals protected/number of animals tested). [c] Neurotoxicity (number of animals protected/number of animals tested). [d] Time after drug administration. [e] Data taken from ref 34. [f] NA: not available. [g] Data taken from ref 58.

Biology

In Vivo. The surgical procedure used to produce allodynia was previously described by Kim and Chung.[45] Briefly, rats were anesthetized using intraperitoneal (ip) administration of 85 mg/kg ketamine and 15 mg/kg xylazine. With the rats in prone position the paraspinal muscles on the left were carefully separated from the L4 to the S2 transverse processes followed by removal of the L6 transverse process in order to visualize the L5−L6 spinal nerves. These were tightly ligated with a 5−0 silk thread and cut just distal to the ligature. The paraspinal muscles were closed with sutures, and then the skin was closed with Michel clips. A bacteriostatic powder was applied topically followed by intramuscular administration of ampicillin. We allowed at least 5 days for recovery after surgery before commencement of the behavioral tests.

The rat's foot withdrawal in response to a tactile stimulus was used to detect tactile allodynia using a set of nine nylon von Frey filaments (VFF). The VFF used produced an initial bending force of 5.8, 13.7, 19.6, 39.2, 58.7, 78.3, 97.9, 146.9, and 254.5 mN, equivalent to a mass of 0.6, 1.4, 2, 4, 6, 8, 10, 15, and 26 g. The same set was calibrated and used in all experiments. All compounds were administered ip at 7, 14, and 21 days after surgery using a Latin square design protocol where the experimenter who performed the behavioral tests was not aware of the dose or substance given to the animals tested. Compounds containing one or two chiral centers were evaluated as their racemates or diastereomeric mixtures. The VFF were applied briefly just before and 30, 60, 120, 180, 240 min after injection, at 1−2 s intervals, to the mid-plantar skin of the hind paw. Stimulation began with the 0.6 g VFF, using a perpendicular force to the skin that was just sufficient to bend the monofilament. If the animal failed to respond with a brief paw withdrawal to at least three out of five stimuli, the next monofilament was tested using an ascending staircase protocol. The response threshold was set as the average of the minimal force required to obtain a criterion response on the two repeats. Rats were considered "protected" from allodynia if they failed to respond to the 15 g VFF. All compounds were administered at 80 mg/kg ip, a dose previously shown to produce full protection from allodynia for compounds **2**−**5**. Compounds displaying significant antiallodynic activity at 80 mg/kg ip had their ED_{50} value and 95% confidence interval (CI) calculated utilizing two additional doses with a minimum of 8 rats per dose.

The antiepileptic potential of the tested compounds was established using the MES, scMet seizure, and pilocarpine tests. In the MES test, 60 Hz 50 mA of alternating current

was delivered through corneal electrodes for 0.2 s. During the time of administration of the test substance a drop of 0.5% tetracaine in saline is applied to the eyes of the animals. Animals were restrained by hand during administration of the electrical stimulus and then released for observation of the seizure throughout its entire course. A test substance/dose that is able to abolish the hind limb tonic extensor component of the seizure indicates prevention of the MES induced seizure spread and thus is considered "active". Tonic extension was considered abolished if the hind limbs were not fully extended at 180° to the plane of the body.

In the scMet seizure test, a convulsive dose of pentylenetetrazole was injected subcutaneously (85 mg/kg in mice) at the time of peak effect of the test substance, followed by observation of seizure occurrence. Absence of seizures indicates that the tested compound/dose can elevate the pentylenetetrazole seizure induction threshold.

Systemic administration of pilocarpine, a cholinergic agonist, has been used to induce status epilepticus. This seizure state is clinically defined as continuous seizure activity or multiple seizures without regaining consciousness, lasting more than 30 min. To determine if a test substance can prevent acute pilocarpine induced status, candidate drugs were administered to male Sprague−Dawley rats via the ip route, followed by administration of a challenge dose of pilocarpine immediately (0 min) and 30 min after treatment with a candidate drug. The outcome measures were "protection" or "no protection" from epileptic seizures. In addition, morbidity was also determined 24 h after each test was completed. Quantitative determination of the protective effect was undertaken for compounds found to possess significant protection. This included calculations of the peak time response as well as determination of ED_{50} and 95% confidence limits.

Results and Discussion

Neuropathic pain and epilepsy share similar underlying pathophysiology. This is presumed to be the reason that AEDs have become a mainstay of treatment for various neuropathic pain syndromes.[2,3,7,8,46] Both epilepsy and neuropathic pain have a debilitating effect on patient's daily performance, mood, and quality of life. Current therapy options are often insufficient and limited by severe side effects.[1,4,47,48] Relatively few SAR studies have been performed in order to develop new potent compounds for neuropathic pain.[41,49]

In this study we designed and synthesized a new series of amide, *N*-methylamide and urea derivatives of closely related analogues of **1**, and examined their antiallodynic activity in the SNL (Chung) model of neuropathic pain. We also evaluated their anticonvulsant activity in the MES, scMet, and pilocarpine seizure models. Previously we evaluated the efficacy of compounds **2**−**5** (Figure 1) and their corresponding acids in the SNL model of neuropathic pain.[41,49] Compounds **2**−**5** (Figure 1) were 4−6 times more potent in the neuropathic pain assay and 10−20 times more potent in the anticonvulsant assay than **1**. On the basis of their ED_{50} values (Table 5), they are equipotent to gabapentin (ED_{50} = 32 mg/kg, $p > 0.05$), one the most widely used drugs today in the treatment of neuropathic pain.[5,50−52] The corresponding acids of compounds **2**−**5** were synthesized and evaluated as well.[41] These were inactive in antiallodynic and anticonvulsant animal models.

Table 5. Comparison of ED_{50} Values in Models of Epilepsy (MES) and Neuropahtic Pain (SNL)[a]

	ED_{50}, mg/kg (95 % confidence interval)		
compd	SNL[b]	MES[c]	MES/SNL
1	269 (227−310)[d]	485 (324−677)[d]	1.8
2	61 (44−77)[d]	32 (22−42)[d]	0.52
3	52 (9−78)[d]	29 (19−38)[d]	0.56
4	58 (18−97)[d]	51 (34−66)[d]	0.88
5	42 (14−52)[e]	22 (15−28)[e]	0.52
19	50 (35−61)	NA	
20	49 (9−61)	NA	
29	49 (16−59)	24 (16−35)[f]	0.49
33	74 (31−84)	64 (55−74)[f]	0.86

[a] ED_{50} values and 95% confidence intervals of the log transforms were calculated by the probit analysis. [b] Spinal nerve ligation test. [c] Maximal electroshock test. [d] Data taken from ref 42. [e] Data taken from ref 49. [f] Data taken from ref 34.

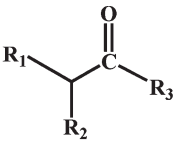
In the present study we evaluated the antiallodynic activity of amide derivatives of **1** (**6**−**22**) and several of their *N*-methyl (**23**−**27**) and urea derivatives (**28**−**34**) (Figure 2) in the SNL model for neuropathic pain. All tested compounds were given at a dose of 80 mg/kg (ip). The dose was chosen as the highest tolerated dose that induced complete reversal of tactile allodynia in all rats for compounds **2**−**5**.[41,49] We also evaluated the correlation between the antiallodynic and anticonvulsant activities of the synthesized compounds in the MES, scMet, and pilocarpine models for epilepsy.

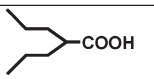
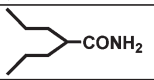
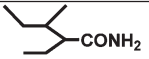
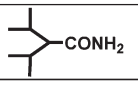
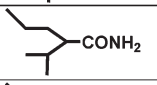
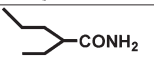
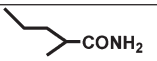
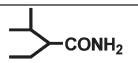
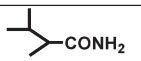
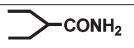
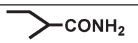
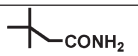
Figure 2 lists the synthetic amide derivatives of the analogues of **1** that were tested: (1) Compounds **6**−**13** possessed five to seven carbons in the carboxylic moiety. (2) Constitutional isomers of VPD had eight carbons in their carboxylic moiety (compounds **14**−**16**), and (3) compounds **17**−**22** had nine carbons in their carboxylic moiety (Figure 2).

We also synthesized several *N*-methylamide derivatives (compounds **23**−**27**) and urea derivatives (compounds **28**−**34**) of those acids that yielded the strongest antiallodynic effect as amide derivatives.

The results obtained for antiallodynic effect evaluated in the rat SNL model of neuropathic pain are presented in Table 1. The results for anticonvulsant activity in the MES and scMet seizure tests in mice are presented in Tables 2−4, and the results obtained in the rat pilocarpine model of status epilepticus are presented in Tables 6 and 7.

Amide Derivatives of the Analogues of 1 with Five to Seven Carbons. Amides **6**−**13**, having five to seven carbons in their carboxylic moiety, showed a decrease in antiallodynic activity compared to the amides **2**−**5** which had eight carbons. Compound **6** had the highest antiallodynic activity in this group of compounds. By use of this compound, four out of nine rats tested were considered protected after 120 and 180 min (Table 1). Compounds **6**−**13** evaluated in the anticonvulsant tests on mice were either inactive or showed no therapeutic window between activity and toxicity (Table 2). ClogP values are an important indicator of the balance between the lipophilic and hydrophilic moieties in a given molecule. It was previously shown that there is a correlation between anticonvulsant activity and ClogP values, where an increase in ClogP is correlated with increased anticon vulsant properties.[40,53,54] Relatively favorable anticonvulsant activity was indicated in one study for compounds displaying ClogP values between 1.84 and 2.64.[40] Since the ClogP values of **6**−**13** (0.123−1.311) were the lowest among the compounds presented in Figure 2 this might explain the weaker anticonvulsant effect of this group



		Substituents		
Compound	Structure	R_1	R_2	R_3
(1)		n-propyl	n-propyl	OH
(2)		n-propyl	n-propyl	NH_2
(3)		secbutyl	ethyl	NH_2
(4)		isopropyl	isopropyl	NH_2
(5)		propyl	isopropyl	NH_2
(6)		n-propyl	ethyl	NH_2
(7)		n-propyl	methyl	NH_2
(8)		isopropyl	ethyl	NH_2
(9)		isopropyl	methyl	NH_2
(10)		ethyl	ethyl	NH_2
(11)		ethyl	methyl	NH_2
(12)		tertbutyl	H	NH_2
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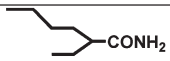
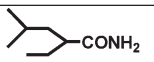
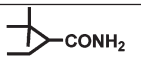
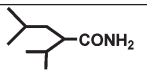
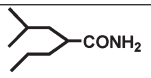
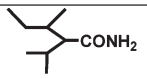
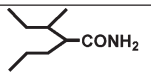
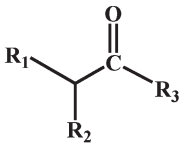
(14)		n-butyl	ethyl	NH_2
(15)		isobutyl	ethyl	NH_2
(16)		tertbutyl	ethyl	NH_2
(17)		isobutyl	isopropyl	NH_2
(18)		isobutyl	propyl	NH_2
(19)		secbutyl	isopropyl	NH_2
(20)		secbutyl	propyl	NH_2

Figure 2 (continued on the next page)



		Substituents		
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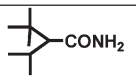
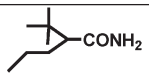
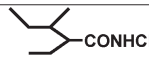
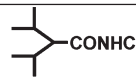
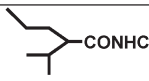
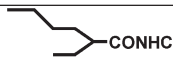
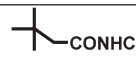
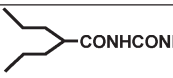
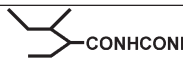
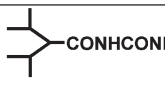
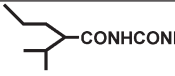
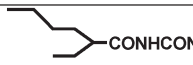
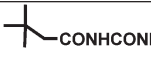
(21)		tertbutyl	isopropyl	NH_2
(22)		tertbutyl	propyl	NH_2
(23)		secbutyl	ethyl	$NHCH_3$
(24)		isopropyl	isopropyl	$NHCH_3$
(25)		propyl	isopropyl	$NHCH_3$
(26)		n-butyl	ethyl	$NHCH_3$
(27)		tertbutyl	H	$NHCH_3$
(28)		n-propyl	n-propyl	$NHCONH_2$
(29)		secbutyl	ethyl	$NHCONH_2$
(30)		isopropyl	isopropyl	$NHCONH_2$
(31)		propyl	isopropyl	$NHCONH_2$
(32)		n-butyl	ethyl	$NHCONH_2$
(33)		tertbutyl	H	$NHCONH_2$
(34)		dimethyl	methyl	$NHCONH_2$

Figure 2. Structures of valproic acid (VPA) and the amide, *N*-methylamide, and urea derivatives of VPA analogues.

of compounds and their correspondingly weaker antiallodynic activity.[40,53]

Constitutional Isomers of 2. Consistent with previously synthesized constitutional isomers of **2**,[35,37,42,55] three amide derivatives **14**−**16** (Figure 2), containing eight carbons in their carboxylic moiety, were synthesized and evaluated for antiallodynic and anticonvulsant activities. They showed lower activity as antiallodynic compounds at 80 mg/kg ip than their constitutional isomers **2**−**5**. Compound **14** was moderately active at all time points compared to **15**−**16**, compounds that were almost inactive. When administered at a higher dose, 100 mg/kg ip, **14** remained partially active as an antiallodynic, but only half of the animals reached the criterion for protection (data not shown). An even higher dose, 120 mg/kg, caused sedation in the rats, and thus, **14** was excluded from further testing. **14** was also previously evaluated in the anticonvulsant test in mice and was found to have ED_{50} values of 78 and 103 mg/kg in the MES and scMet

Table 6. Anticonvulsant Activity of Compounds in the Pilocarpine Test of Status Epilepticus after po Administration to Rats[a]

compd	dose (mg/kg)	time (h)	pilocarpine[b]	deaths
9	450	0.0	8/8	0
	600	0.5	0/7	0
14	100	0.5	1/8	1
16	200	0.0	8/8	0
	400	0.5	7/7	1
20	65	0.0	8/8	0
	130	0.5	8/8	0
22	65	0.0	7/7	0
	130	0.5	0/8	0
24	450	0.0	0/8	8
29	37.5	0.0	6/8	0
	75	0.0	8/8	0
31	200	0.5	0/8	0
32	300	0.5	10/13	3
33	100	0.0	8/8	0
	200	0.5	2/6	1

[a] A challenge dose of pilocarpine is given 0 and 30 min following ip administration of a candidate drug to male Spragu−Dawley rats. [b] Pilocarpine test (number of animal protected/total number of rats tested).

Table 7. ED_{50} in the Pilocarpine Test of Status Epilepticus[a]

compd	time (h)	ED_{50}, mg/kg (95% CI)	ED_{97}, mg/kg (95% CI)
16	0.5	257 (192−317)	451 (345−1269)
20	0.5	84 (62−103)	149 (114−393)
29	0.0	23 (4−33)	NT
32	0.5	247 (207−295)	439

[a] Analysis of statistical significance was done by means of probit analysis. ED_{50} and ED_{97} values and 95% confidence intervals of the log of the transforms were calculated by the probit analysis.

tests, respectively, 2−3 times more potent than **1**.[27] This compound was not evaluated in rats as an anticonvulsant. **15** and **16** were considered inactive at 80 mg/kg ip, as only a small fraction of animals were protected at this dose compared to full protection provided by **2**−**5** in this model (Table 1). **15** was previously reported to have only partial activity in the anticonvulsant tests in mice,[28] and thus, it was not evaluated further. **16**, however, was active as an anticonvulsant at the highest dose tested (300 mg/kg), although a mild sedative effect was observed after 0.5 h (Table 3). It is worth mentioning that at these doses compound **16**, unlike other compounds in this group, was active in the pilocarpine test for status epilepticus having an ED_{50} value of 256 mg/kg. Overall, compounds **14**−**16** were less potent as antiallodynic and anticonvulsant compounds than their structural isomers, compounds **2**−**5**, despite the fact that they had similar ClogP values. The exception was activity in the pilocarpine model for status epilepticus which was an attribute of compound **16** alone (Tables 6 and 7).

Amide Derivatives of the Analogues of 1 with Nine Carbons. In order to increase the lipophilicity of the analogues of **1** and thus increase their cell membrane penetration and brain-to-plasma ratio, amide derivatives having nine carbons in their carboxylic moieties were synthesized (compounds **17**−**22**) (Figure 2). Four of them (**19**−**22**) proved to be the most active of the compounds tested in the SNL model (Table 1). However, compounds **19**−**22** were found to be mildly sedative at the highest dose tested in the SNL model, 80 mg/kg ip (at 0.5 h), more so than compounds with five to eight carbons in their carboxylic moiety (data not shown). The sedative effect started to dissipate 1 h after dosing, and no sedation

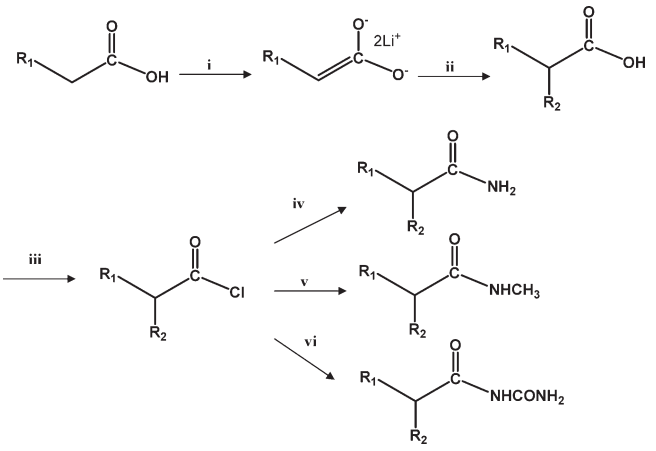


Figure 3. Synthesis of VPA analogues, and their amide (**2**−**22**), *N*-methylamide (**23**−**27**), and urea (**28**−**34**) derivatives. Reagents and conditions are as follows: (i) LDA, THF, −15 °C, 20 min; (ii) propyl iodide, isopropyl iodide, ethyl iodide, or methyl iodide, THF, 0 °C, 30 min; (iii) $SOCl_2$, CH_2Cl_2, 25 °C,12 h; (iv) 28−30% NH_4OH, acetonitrile, 0 °C, 2 h; (v) 41% methylamine, acetonitrile, 0 °C, 2 h; (vi) urea, acetonitrile, 80 °C, 2 h.

was observed at 2 h, the time of the peak antiallodynic effect of **19**, **20**, and **22**. Compounds **19**, **20**, and **22** were further tested at two additional doses (60 and 40 mg/kg ip) in order to determine their ED_{50} values in the SNL model. Compound **21**, the least potent among the four active compounds **19**−**22**, was not tested further. The ED_{50} values of compounds **19** and **20** were 50 and 49 mg/kg, respectively (Table 5). **22**, although active at 80, 60, and 40 mg/kg (data not shown), showed a nonlinear dose−response curve, and thus, its ED_{50} value could not be calculated. On the basis of ED_{50} values, **19** and **20** are equipotent to gabapentin ($p > 0.05$),[50−52] indicating the potential for these compounds to become candidates for further investigation as analgesic drugs. Although **19**−**22** were active in the mouse MES and scMet seizure tests (Table 3), they did not show separation between activity and toxicity, and thus, these compounds were not evaluated further. **20** was shown to be active in the pilocarpine status model, having an ED_{50} value of 84 mg/kg. It was thus the most potent compound in both epilepsy and neuropathic pain models among the group with nine carbon atoms in the carboxylic moiety. Interestingly **19**−**22** showed a correlation between antiallodynic and anticonvulsant activity, but neurotoxicity was evident starting at 100 mg/kg (Table 3). Neurotoxicity was not seen in compounds **6**−**13** having five to seven carbons in their carboxylic moiety. The decreased safety margin between activity and toxicity for the acids containing more than eight carbons in their structures was already noted in the literature for analogues of **1** (2-propylhexanoic acid, 2-pentylheptanoic acid, and 2-hexyloctanoic acid) having 9, 12, and 14 carbons in their carboxylic moiety.[56] For this reason amide derivatives of analogues of **1** having more than 9 carbons in their carboxylic moiety were not synthesized and evaluated.

2,2,3,3-Tetramethylcyclopropanecarboxylic acid (TMCA) is a cyclic analogue of **1**, and its amide (TMCD) and *N*-methylamide derivatives (MTMCD) (Figure 4) were evaluated previously in our laboratory as antiallodynic and anticonvulsant compounds.[49] TMCD was much more potent than its corresponding acid. However, its *N*-methylamide derivative, MTMCD, was even more potent than TMCD in

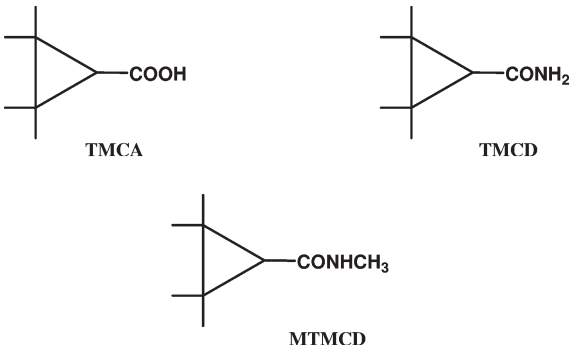


Figure 4. Cyclic analogue of **1** (TMCA), its amide derivative (TMCD), and its *N*-methyl derivative (MTMCD).

both epilepsy and neuropathic pain models.[41] Therefore, in order to evaluate the effect of substitution of NH_2 in the amide with the *N*-methyl group, we synthesized five *N*-methylamides corresponding to compounds **23**−**27** (Figure 1) and evaluated their antiallodynic and anticonvulsant activities (Tables 1 and 4).

***N*-Methyl Derivatives of the Analogues of 1. 23**−**25** are the *N*-methyl derivatives of the acids, synthesized by replacing the NH_2 group in the amides **3**−**5**, respectively. The antiallodynic data indicated that the *N*-methyl derivatives were either less active or inactive as analgesic compounds compared to their amide counterparts. Compound **25** was active in the rat MES model; its ED_{50} value was 63 mg/kg. Thus, **25** is less active as an anticonvulsant than **5**, the amide derivative of the same acid (ED_{50} = 22 mg/kg), but it is 8 times more potent than **1** in the rat MES test. **25** was not active at all in the SNL model of neuropathic pain, compared to its amide analogue **5** which was previously found to be highly active and equipotent to gabapentin.[42] **24**, was the most active as an antiallodynic among the *N*-methyl derivatives **23**−**27** (Table 1). However, at 80 mg/kg ip it produced unfavorable side effects such as sedation and severe wet dog shake reminiscent of the "serotonin syndrome",[57] unlike its amide counterpart **4**. For this reason it was not evaluated further. **26** and **27** were inactive in the SNL model (Table 1), but **26** was active in both the MES and scMet models in mice with ED_{50} values of 134 and 155 mg/kg for the MES and scMet tests, respectively. Compound **27**, having only six carbons in its carboxylic moiety, was not active in either the anticonvulsant or the antiallodynic animal models, and thus no other *N*-methylamide derivative of acids with side chains shorter than seven carbons were synthesized or evaluated. **23**−**25** had ClogP values similar to those of their amide counterparts **3**−**5**. However, they were less active in both antiallodynic and anticonvulsant tests, indicating that other requirements, such as spatial arrangement of the compounds, dictate the antiallodynic and anticonvulsant activities in addition to ClogP values. These results indicate that the *N*-methylamide moiety in **23**−**27** decreased or abolished potency in both antiallodynic and anticonvulsant animal models compared to the amide derivatives **3**−**5**, **12**, and **14** of their corresponding acids (Tables 1 and 4, respectively). The results obtained with the derivatives of the branched aliphatic carboxylic acids contrasted with those seen with TMCD and MTMCD, the amide and *N*-methylamide derivatives of TMCA, the cyclopropyl analogue of **1**.[49]

Urea Derivatives of the Analogues of 1. Urea derivatives of **1** and its constitutional isomers and analogues have previously been synthesized in our lab and were found to be very potent as anticonvulsant compounds in the MES and scMet tests, as well as in other models of epilepsy.[34−36,58] It is worth mentioning that the urea moiety is found as a fused ring in three leading antiepileptic drugs: carbamazepine, phenytoin, and phenobarbital. We decided to synthesize seven urea derivatives of acids with five, six, and mainly eight carbons in their structures (**28**−**34**) and to evaluate their antiallodynic activity (Figure 2). **29**, a urea derivative of 2-ethyl-3-methylpentanoic acid, was the most active compound with an ED_{50} value of 49 mg/kg in the SNL model of neuropathic pain. Although mild sedation was witnessed at 80 mg/kg, the highest dose tested, the two lower doses, 60 and 40 mg/kg, did not produce sedation or motor impairment. Compared to **29**, 1-(3,3-dimethylbutanoyl) urea (**33**), a urea derivative of butyric acid containing two methyl groups at the β-position to the carbonyl, was found to be the second most active compound in the urea group. **33** was evaluated at two high doses, 100 and 110 mg/kg, and its ED_{50} value was found to be 74 mg/kg. This indicates significantly less potency than gabapentin ($p < 0.05$). At a higher dose, 120 mg/kg, **33** was sedative, and thus, it was not evaluated further. These results indicate that substitution of NH_2 by urea in compounds **2**−**5** and **12**−**14** (Figure 2) decreases antiallodynic activity. Only **29**, the urea derivative of 2-ethyl-3-methylpentanoic acid, retained the antiallodynic activity seen in compound **3**, the corresponding amide of this acid (Table 5). The ClogP values of these compounds were 1.71 and 1.79 for **3** and **29**, respectively (Table 8). Although **33** was found to have moderate antiallodynic activity (Table 1 and 5), it was highly active as an anticonvulsant compound (Table 5). The amide (**12**) and *N*-methylamide (**27**) derivatives of 3,3-dimethylbutanoic acid, having similar ClogP values (around 0.8, Table 8), were not active as antiallodynic or anticonvulsant compounds (Tables 1 and 4, respectively). One of the most remarkable observations stemming from this research was the marked effect of the location of a single methyl group in the acyl moiety of the constitutional isomers of amides **17**−**22**, and the constitutional isomers of the urea derivatives **28**−**34**, on the antiallodynic activity of the tested compounds (Figure 2, Table 1).

Table 8. ClogP Values of Valproic Acid and the Amide and Urea Derivatives of Its Analogues[a]

compd	ClogP	compd	ClogP
1	2.58	**18**	2.239
2	1.4	**19**	2.109
3	1.71	**20**	2.239
4	1.58	**21**	1.979
5	1.71	**22**	2.109
6	1.311	**23**	1.746
7	0.782	**24**	1.616
8	1.181	**25**	1.746
9	0.652	**26**	1.876
10	0.782	**27**	0.778
11	0.253	**28**	1.92
12	0.742	**29**	1.79
13	0.123	**30**	1.66
14	1.84	**31**	1.79
15	1.71	**32**	1.92
16	1.58	**33**	0.822
17	2.109	**34**	0.203

[a] ClogP was calculated by utilizing the ChemDraw Ultra software, version 8.

Conclusion

We have reported in this study a novel class of amide, *N*-methylamide and urea derivatives of several new analogues of **1**. It appears that amide derivatives of the analogues that have eight carbons in their carboxylic moiety are both active as antiallodynic and anticonvulsant agents and have a relatively wide safety margin between activity and toxicity. Amide derivatives of analogues of **1** with fewer than eight carbons in their carboxylic moiety were the least potent of the compounds evaluated, in both antiallodynic and anticonvulsant animal models. The amide derivatives from the analogues of **1** that contained nine carbons in their carboxylic moiety were more potent than those containing eight carbons, but they had a narrower safety margin between antiallodynic activity and undesirable motor impairment or sedation. Compounds **19** (ED_{50} = 50 mg/kg), **20** (ED_{50} = 49 mg/kg), and **29** (ED_{50} = 49 mg/kg) were the most promising compounds for neuropathic pain evaluated in this study, with potencies equal to that of gabapentin. 3-Methyl-2-propylpentanamide (**20**) was also active in the pilocarpine model of epilepsy, thus making it a superior candidate for future drug development as both an antiallodynic and an anticonvulsant drug. Interestingly **20** is a close analogue of **3**, previously evaluated and found to be active in both models for epilepsy and neuropathic pain. **20** differs from **3** by having a propyl rather than an ethyl side chain (Figure 2). Substitution of hydrogen by a methyl group on the amide nitrogen (**23**−**27**) reduced the antiallodynic and the anticonvulsant activity in all compounds tested in this study. 1-(2-Ethyl-3-methylpentanoyl)urea (**29**) was the most active compound among the group **28**−**34** (the urea derivatives of carboxylic acids with five, six, and mainly eight carbons in their structures) in both the antiallodynic and the anticonvulsant tests. However, all of these induced mild sedation at the highest dose tested. We conclude that **20** and **29** offer the best combination of safety and efficacy among the active compounds evaluated in this study. Thus, they are potential candidates for further development as both anticonvulsant and analgesic compounds.

Experimental Section

Chemicals. All chemicals were purchased from Sigma-Aldrich. Tetrahydrofuran (THF), acetonitrile (ACN), thionyl chloride, dichloromethane (DCM), petroleum ether, and ethyl acetate were purchased from Frutarom Israel. Ammonium hydroxide, 28−30% in water, methylamine, 41% in water, and urea were purchased from Acros Organics Company Inc. Dry dichloromethane, tetrahydrofuran, acetonitrile, and DMPU were obtained by refluxing over CaH_2 for 2 h and distillation, fresh prior to use. DMPU was refluxed over CaH_2 for 2 h, distilled under reduced pressure, and stored over 4 Å molecular sieves (8−12 mesh) under nitrogen atmosphere. Compounds **6**−**34** were prepared according to a method described further in this section.

Materials and Methods. Product formation follow-up was performed by means of ^{1}H NMR and TLC. TLC analyses were performed on precoated silica gel on aluminun sheets (Kieselgel 60 F_{254}, Merck). ^{1}H NMR spectra were recorded on a Varian Mercury series NMR 300 spectrometer. Chemical shifts (δ scale) are reported in parts per million (ppm) relative to the indicated reference. Coupling constants are given in Hz.

Chemical structure and purity of the compounds newly synthesized were assessed by TLC, ^{1}H NMR, GC/MS, and elemental analysis. Melting point was determined on a 1002−230 VAC Mel-Temp capillary melting point apparatus. A gas chromatography−mass spectrometry assay was preformed on a HP5890 series II GC equipped with a Hewlett-Packard MS engine (HP5989A) single quadrupole mass spectrometer, HP7673 autosampler, HP MS-DOS Chemstation, and HP-5MS capillary column (0.25 μm × 15 m × 0.25 mm). The temperature program was as follows: injector temperature, 180 °C; initial temperature, 40 °C for 3 min; gradient of 3 °C/min until 140 °C; gradient of 20 °C/min until 190 °C; hold time of 3 min. The MS parameters were set as follows: source temperature 180 °C; transfer line temperature 280 °C; positive ion monitoring; EI-MS (70 eV). The molecular ion and the five most pronounced ions are provided. Elemental analyses were preformed on a 2400-2 Perkin-Elmer C, H, N analyzer. C, H, N analyses of all newly synthesized compounds were within ±0.4 of theoretical values and thus were considered satisfactory.

General Procedure for the Synthesis of Compounds 6−9 and 15−22. Anhydrous THF (70 mL) and 160 mmol of diisopropylamine were added to a round-bottomed flask cooled to −15 °C under nitrogen (N_2) atmosphere, followed by dropwise addition of 160 mmol *n*-butyllithium in order to prepare 160 mmol of lithium diisopropylamine (LDA). The reaction mixture was stirred for 30 min, and a 1:1 mixture of 10 mL of dry THF and 72 mmol of valeric acid (for the synthesis of compounds **6** and **7**), isovaleric acid (for the synthesis of compounds **8** and **9**), 3-methylvaleric acid (for the synthesis of compounds **19** and **20**), 4-methylvaleric acid (for the synthesis of compounds **15**, **17**, and **18**), or tertbutylacetic acid (for the synthesis of compounds **16**, **21**, and **22**) was added and allowed to stir for an additional 15 min below 0 °C. Then 72 mmol of DMPU was added dropwise after maintaining a temperature of 5 °C and allowed to stir for additional 30 min followed by a slow dropwise addition of a 1:1 solution of 160 mmol of the corresponding alkyl iodide (either methyl iodide, ethyl iodide, propyl iodide, or isopropyl iodide) in 10 mL of anhydrous THF. The reaction mixture was allowed to stir at room temperature for 2 h. THF was distilled from the reaction mixture at 60−80 °C at normal pressure, and the oily product was dispersed in petroleum ether. A 10% HCl solution was added until pH 1 was reached, and the organic phase was separated from the aqueous phase and washed three times with brine. The aqueous phase was combined and extracted with petroleum ether (3 × 50 mL). The petroleum ether extracts were combined, dried over $MgSO_4$, filtered, and evaporated to yield 97% oily product. The oily product was further distilled under reduced pressure to yield the pure corresponding acid. The free carboxylic acids produced were chlorinated with thionyl chloride according to a previously published method.[59] The acyl chloride (44 mmol) obtained was dissolved in 20 mL of dry ACN and was added dropwise to a 50 mL ammonium hydroxide solution (28−30% in water) at 0 °C and was left to stir for 2 h. Reaction mixture was extracted with ethyl acetate (3 × 30 mL), the organic phase was washed with 2 N NaOH, dried over $MgSO_4$, and evaporated, and the oily product was recrystallized with ethyl acetate petroleum ether (3:1) to obtain the desired product.

2-Ethylpentanamide (6). White powder. Mp 104−106 °C. MS-EI, *m*/*z* (%): 128 ($M^+ - 1$, 0.17), 100 (19), 87 (47), 72 (100), 55 (17). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.91 (t, J = 7, 6H), 1.26−1.45 (m, 3H) 1.46−1.66 (m, 3H), 2.0−2.11 (m, 1H), 5.5−5.8 (br d, J = 60, 2H). Anal. ($C_7H_{15}NO$) C, H, N. Yield 40%.

2-Methylpentanamide (7). Silver powder. Mp 82−84 °C. MS-EI, *m*/*z* (%): 100 ($M^+ - 15$, 4.5), 86 (18), 73 (100), 72 (21), 55 (13). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.89 (t, J = 7.2, 3H), 1.14 (d, J = 6.9 3H) 1.28−1.42 (m, 3H), 1.56−1.68 (m, 1H), 2.2−2.34 (m, 1H), 5.5−5.7 (br s, 1H), 5.8−6.0 (br s, 1H). Anal. ($C_6H_{13}NO$) C, H, N. Yield 55%.

2-Ethyl-3-methylbutanamide (8). White "cotton-like" powder. Mp 138−140 °C. MS-EI, *m*/*z* (%): 114 ($M^+ - 15$), 1.7), 100 (18), 87 (68), 72 (100), 55 (16). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.88−1.0 (m, 9H), 1.52−1.64 (m, 2H), 1.5−1.86 (m,

2H), 5.38−5.5 (br s, 1H), 5.6−5.8 (br s, 1H). Anal. ($C_7H_{15}NO$) C, H, N. Yield 65%.

2,3-Dimethylbutanamide (9). Silver powder. Mp 131−133 °C. MS-EI, *m*/*z* (%): 115 (M^+, 0.35), 100 (15), 73 (100), 72 (24), 55 (14). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.95 (dt, J = 6.6, 8.1 6H), 1.12 (d, J = 6.9 3H), 1.76−1.9 (m, 1H), 1.94−2.04 (m, 1H), 5.4−5.7 (br s, 1H), 5.7−6.0 (br s, 1H). Anal. ($C_6H_{13}NO$) C, H, N. Yield 62%.

2-Ethyl-4-methylpentanamide (15). White needles. Mp 86−88 °C. MS-EI, *m*/*z* (%): 128 ($M^+ - 15$, 2.5), 100 (19), 87 (51), 72 (100), 57 (27). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.86−1.0 (m, 9H), 1.18−1.3 (m, 1H), 1.4−1.66 (br m, 4H), 2.1−2.2 (m, 1H), 5.38−5.62 (br d, J = 36, 2H). Anal. ($C_8H_{17}NO$) C, H, N. Yield 65%.

2-Ethyl-3,3,-dimethylbutanamide (16). White powder. Mp 136−138 °C. MS-EI, *m*/*z* (%): 128 ($M^+ - 15$, 1), 87 (71), 72 (100), 57 (23), 55 (11). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.91 (t, J = 7.2, 3H), 0.98 (s, 9H), 1.46−1.7 (br m, 2H), 1.72−1.79 (dd, J = 97.5, 3, 1H), 5.3−5.6 (br d, J = 36, 1H). Anal. ($C_8H_{17}NO$) C, H, N. Yield 56%.

2-Isopropyl-4-methylpentanamide (17). White powder. Mp 108−110 °C. MS-EI, *m*/*z* (%): 142 ($M^+ - 15$, 2), 115 (21), 101 (28), 86 (67), 72 (100). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.89 (t, J = 6.6, 6H), 0.94−0.98 (dd, J = 6.6, 1.2 6H), 1.18−1.3 (m, 1H), 1.5−1.64 (br m, 2H), 1.7−1.82 (m, 1H), 1.88−1.96 (m, 1H), 5.38−5.45 (br s, 1H), 5.6−5.7 (br s, 1H). Anal. ($C_9H_{19}NO$) C, H, N. Yield 50%.

4-Methyl-2-propylpentanamide (18). White "needle-like" powder. Mp 120−121 °C. MS-EI, *m*/*z* (%): 142 ($M^+ - 15$, 1), 114 (12), 101 (17), 72 (100), 55 (13). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.8−0.96 (m, 9H), 1.16−1.42 (br m, 4H), 1.5−1.68 (br m, 3H), 2.18−2.28 (m, 1H), 5.4−5.6 (br s, 1H), 5.7−5.9 (br s, 1H). Anal. ($C_9H_{19}NO$) C, H, N. Yield 67%.

2-Isopropyl-3-methylpentanamide (19). White "cotton-like" crystals. Mp 105−107 °C. MS-EI, *m*/*z* (%): 128 ($M^+ - 29$, 0.4), 115 (8), 101 (24), 86 (100), 69 (11). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.84−1.0 (m, 12H), 1.00−1.3 (br m, 1H), 1.36−1.62 (br dm, 1H), 1.66−1.82 (m, 2H), 1.94−2.1 (br m, 1H), 5.4−5.6 (br s, 1H), 5.7−5.9 (br s, 1H). Anal. ($C_9H_{19}NO$) C, H, N. Yield 35%.

3-Methyl-2-propylpentanamide (20). White "needle-like" powder. Mp 120−121 °C. MS-EI, *m*/*z* (%): 142 ($M^+ - 15$, 0.35), 101 (35), 86 (32), 72 (100), 55 (16). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.8−0.96 (m, 9H), 1.06−1.3 (br m, 2H), 1.3−1.64 (br m, 5H), 0.9−2.0 (m, 1H), 5.4−5.54 (br s, 1H), 5.7−5.9 (br s, 1H). Anal. ($C_9H_{19}NO$) C, H, N. Yield 45%.

2-Isopropyl-3,3-dimethylbutanamide (21). White powder. Mp 128−130 °C. MS-EI, *m*/*z* (%): 142 ($M^+ - 15$, 0.68), 101 (36), 100 (30), 86 (100), 57 (22). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.9−1.1 (m, 15H), 1.7 (d, J = 6, 1H) 2.0−2.1 (m, 1H), 5.3−5.5 (br s, 1H). Anal. ($C_9H_{19}NO$) C, H, N. Yield 45%.

2-*tert*-Butylpentanamide (22). White powder. Mp 118−119 °C. MS-EI, *m*/*z* (%): 142 ($M^+ - 15$, 0.88), 101 (35), 100 (9), 72 (100), 57 (21). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.86−0.94 (t, 3H), 0.94−1.0 (s, 9H), 1.1−1.22 (m, 1H), 1.3−1.44 (m, 2H), 1.56−1.78 (m, 1H), 1.8−1.86 (dd, J = 12, 1H), 5.4−5.6 (br s, 1H), 5.8−6.0 (br s, 1H). Anal. ($C_9H_{19}NO$) C, H, N. Yield 40%.

General Procedure for the Syntheis of Compounds 10−14. The corresponding acid (ethylbutyric acid, methylbutyic acid, *tert*-butylacetic acid, and pivalic acid corresponding to compounds **6**−**9**, respectively) was chlorinated with thionyl chloride according to a previously published method.[59] The acyl chloride (44 mmol) obtained was dissolved in 20 mL of dry ACN and was added dropwise to a 50 mL ammonium hydroxide solution (28−30% in water) at 0 °C and then left to stir for 2 h. Reaction mixture was extracted with ethyl acetate (3 × 30 mL), the organic phase was washed with 2 N NaOH, dried over $MgSO_4$, and evaporated, and the oily product was recrystallized with ethyl acetate petroleum ether (3:1) to obtain the desired product.

2-Ethylbutanamide (10). White crystals. Mp 113−115 °C. MS-EI, *m*/*z* (%): 100 ($M^+ - 15$, 7), 87 (85), 86 (44), 72 (100), 55 (25). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.88−1.0 (t, J = 9, 6H), 1.4−1.68 (br m, 4H), 1.9−2.4 (br m, 1H), 5.4−5.6 (br s, 1H), 5.8−6.0 (br s, 1H). Anal. ($C_6H_{13}NO$) C, H, N. Yield 60%.

2-Methylbutanamide (11). Silver crystals. Mp 112−114 °C. MS-EI, *m*/*z* (%): 86 ($M^+ - 15$, 26), 73 (100), 72 (17), 57 (30), 55 (12). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.9−1.0 (t, J = 9, 3H), 1.14−1.22 (d, J = 6.3, 3H), 1.38−1.54 (m, 1H), 1.6−1.78 (m, 1H), 2.12−2.28 (m, 1H), 5.4−5.8 (br s, 2H). Anal. ($C_5H_{11}NO$) C, H, N. Yield 65%.

3,3-Dimethylbutanamide (12). Silver crystals. Mp 135−137 °C. MS-EI, *m*/*z* (%): 115 (M^+, 0.43), 59 (100), 58 (8), 57 (33), 55 (4). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 1.0 (s, 9H), 2.04 (s, 2H), 1.9−2.4 (br m, 1H), 5.4−5.6 (br s, 1H), 5.8−6.0 (br s, 1H). Anal. ($C_6H_{13}NO$) C, H, N. Yield 60%.

Pivalamide (13). Silver crystals. Mp 156−158 °C. MS-EI, *m*/*z* (%): 101 (M^+, 65), 100 (84), 86 (52), 85 (60), 68 (100). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 1.0 (s, 9H), 5.4−5.8 (br s, 2H). Anal. ($C_5H_{11}NO$) C, H, N. Yield 65%.

2-Ethylhexanamide (14). White crystals. Mp 79−80 °C. MS-EI, *m*/*z* (%): 143 (M^+, 0.2), 115 (11), 87 (58), 72 (100), 57 (25). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.8−0.98 (m, 5H), 1.2−1.38 (m, 4H), 1.4−1.68 (m, 4H), 1.96−2.8 (m, 1H), 5.36−5.7 (ds, 2H). Anal. ($C_8H_{17}NO$) C, H, N. Yield 95%.

General Procedure for the Synthesis of Compounds 23−27. The corresponding acids were prepared according to the above-mentioned procedure for compounds **2**−**5** and **15**−**22**. The corresponding acids (propyl isopropylacetic acid, diisopropylacetic acid, valnoctic acid, ethyl butylacetic acid, and *tert*-butylacetic acid, corresponding to compounds **23**−**27**, respectively) were chlorinated with thionyl chloride according to a previously published method.[59] The obtained acyl chloride (33 mmol) was dissolved in 10 mL of dry DCM and 5 mL of a 41% methylamine solution in water, and was left to stir for 2 h. The reaction mixture was extracted with ethyl acetate (3 × 30 mL), the organic phase was washed with 2 N HCl, dried over $MgSO_4$, and evaporated, and the product was recrystallized with ethyl acetate/petroleum ether (3:1) to obtain the desired product.

2-Ethyl-*N*,3-dimethylpentanamide (23). White powder. Mp 69−72 °C. MS-EI, *m*/*z* (%): 157 (M^+, 0.33), 101 (72), 86 (100), 58 (42), 57 (36). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.76−0.94 (m, 9H), 1.0−1.2 (m, 2H), 1.4−1.62 (br m, 3H), 1.7−1.8 (m, 1H), 2.79 (d, J = 4.8, 3H), 5.6−5.8 (br s, 1H). Anal. ($C_9H_{19}NO$) C, H, N. Yield 75%.

2-Isopropyl-*N*,3-dimethylbutanamide (24). White needles. Mp 102−104 °C. MS-EI, *m*/*z* (%): 157 (M^+, 0.14), 115 (39), 100 (100), 58 (24), 57 (25). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.86−0.96 (dd, J = 6.75, 12H), 1.549 (t, J = 15, 1H), 1.9−2.6 (m, 2H), 2.77 (d, J = 4.8, 3H), 5.6 (br s, 1H). Anal. ($C_9H_{19}NO$) C, H, N. Yield 73%.

2-Isopropyl-*N*-methylpentanamide (25). White powder. Mp 97−99 °C. MS-EI, *m*/*z* (%): 157 (M^+, 0.40), 115 (56), 100 (53), 86 (100), 58 (45). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.8−0.96 (m, 9H), 1.06−1.26 (br m, 2H), 1.4−1.6 (br m, 2H), 1.6−1.82 (br m, 2H), 2.8 (d, J = 4.5, 3H), 5.44−5.6 (br s, 1H). Anal. ($C_9H_{19}NO$) C, H, N. Yield 70%.

2-Ethyl-*N*-methylhexanamide (26). White powder. Mp 73−76 °C. MS-EI, *m*/*z* (%): 157 (M^+, 0.74), 101 (59), 86 (100), 58 (53), 57 (37). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.86−0.96 (dd, J = 6.75, 12H), 1.549 (t, J = 15, 1H), 1.9−2.6 (m, 2H), 2.77 (d, J = 4.8, 3H), 5.6 (br s, 1H). Anal. ($C_9H_{19}NO$) C, H, N. Yield 70%.

***N*,3,3-Trimethylbutanamide (27).** White needles. Mp 73−75 °C. MS-EI, *m*/*z* (%): 129 (M^+, 0.33), 114 (2.2), 73 (100), 58 (42), 57 (42). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 1.0 (s, 9H), 2.04 (s, 2H), 2.8 (d, J = 4.5, 3H), 5.4 (br s, 1H). Anal. ($C_7H_{16}NO$) C, H, N. Yield 70%.

General Procedure for the Synthesis of Compounds 28−34. The chemical synthesis for compounds **28**−**34** was identical to

that previously published by Shimshoni et al. in 2008. Briefly, the corresponding acyl chlorides were prepared as previously described for compounds **6**−**22**. They were dissolved in dry acetonitrile (50 mL) and slowly added to a boiling acetonitrile solution (100 mL) of 0.14 mol of urea and refluxed for 2 h. The organic solvent was evaporated under reduced pressure, followed by dissolving the products in 100 mL of ethyl acetate. The products were washed three times with 20 mL of distilled water. The organic fraction was dried over magnesium sulfate and evaporated, and products were purified by crystallization from ethyl acetate.

1-(2-Propylpentanoyl)urea (28). White crystals. Mp 217−220 °C. MS-EI, *m*/*z* (%): 157 (2), 144 (36), 115 (100), 72 (30), 61 (21). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.86−0.74 (t, J = 15, 6H), 1.2−1.74 (m, 8H), 2.24−2.4 (m, 1H), 5.62 (s, 1H), 8.4 (s, 1H), 9.42 (s, 1H). Anal. ($C_9H_{18}N_2O_2$) C, H, N. Yield 83%.

1-(2-Ethyl-3-methylpentanoyl)urea (29). White crystals. Mp 147−148 °C. MS-EI, *m*/*z* (%): 157 (6), 130 (100), 115 (69), 72 (32), 61 (36). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.86−0.96 (m, 9H), 1.08−1.24 (m, 1H), 1.38−1.74 (m, 4H), 1.96−2.06 (m, 1H), 5.35 (s, 1H), 8.37 (s, 1H), 8.7 (s, 1H). Anal. ($C_9H_{18}N_2O_2$) C, H, N. Yield 61%.

1-(2-Isopropyl-3-methylbutanoyl)urea (30). White crystals. Mp 199−200 °C. MS-EI, *m*/*z* (%): 144 (59), 129 (100), 86 (27), 69 (34), 61 (25). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.91−0.98 (t, J = 6, 12H), 1.78−1.84 (t, J = 6, 1H), 1.98−2.1 (m, 2H), 5.49 (s, 1H), 8.41 (s, 1H), 8.9 (s, 1H). Anal. ($C_9H_{18}N_2O_2$) C, H, N. Yield 51%.

1-(2-Isopropylpentanoyl)urea (31). White crystals. Mp 213 °C. MS-EI, *m*/*z* (%): 144 (81), 129 (65), 115 (100), 72 (35), 61 (33). ^{1}H NMR (300 MHz, DMSO-d_6): 0.78−0.88 (m, 9), 1.06−1.22 (m, 2H), 1.28−1.54 (m, 2H), 1.62−1.74 (m, 1H), 2.1−2.2 (m, 1H), 7.21 (s, 1H), 7.86 (s, 1H), 10.14 (s, 1H). Anal. ($C_9H_{18}N_2O_2$) C, H, N. Yield 71%.

1-(2-Ethylhexanoyl)urea (32). White powder. Mp 164−166 °C. MS-EI, *m*/*z* (%): 158 ($M^+ - 28$, 7.7), 130 (67), 115 (70), 72 (100), 57 (63). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 0.8−0.93 (m, 6H), 1.22−1.3 (m, 4H), 1.3−1.7 (br m, 4H), 2.17 (m, 1H), 5.5 (br s, 1H), 8.39 (br s, 1H), 9.15 (br s, 1H). Anal. ($C_9H_{18}N_2O_2$) C, H, N. Yield 73%.

1-(3,3-Dimethylbutanoyl)urea (33). White crystals. Mp 175 °C. MS-EI, *m*/*z* (%): 143 ($M^+ - 15$, 0.9), 102 (100), 83 (18), 59 (79), 57 (45). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 1.0 (s, 9H), 2.18 (s, 2H), 5.62 (br s, 1H), 8.4 (br s, 1H), 9.55 (br s, 1H). Anal. ($C_7H_{14}N_2O_2$) C, H, N. Yield 70%.

1-(Pivaloyl)urea (34). White crystals. Mp 151−153 °C. MS-EI, *m*/*z* (%): 144 (3), 129 (14), 89 (22), 88(16), 57 (100). ^{1}H NMR (300 MHz, $CDCl_3$, δ TMS): 1.25 (s, 9H), 5.6 (br s, 1H), 8.3 (br s, 2H). Anal. ($C_6H_{12}N_2O_2$) C, H, N. Yield 70%.

Biological Testing. The surgical procedure intended to induce allodynia was done utilizing the spinal nerve ligation model as described previously.[45] Spinal nerve ligation was performed 7 days prior to the behavioral test. Mechanical allodynia was evaluated using the von Frey monofilaments according to a protocol previously described.[42] Briefly, the tested compounds were suspended in 0.5% solution of methylcellulose (MC) in doubly distilled water (DDW) and injected intraperitoneally (ip) into male albino rats (Sprague−Dawley, 200−250 g) utilizing a volume of 4 mL/kg of body weight.

The evaluation of the anticonvulsant activity in the maximal electroshock seizure test (MES) and subcutaneous pentylenetetrazole seizure threshold test (scMet) as well as determination of neurotoxicity using the rotorod test was performed in mice at the NIH Epilepsy Branch as a part of the Anticonvulsant Drug Development Program according to the protocols previously described.[60] Generally, tested compounds were suspended in a 0.5% solution of MC in DDW and were injected ip into adult male albino mice (CF-1, 18−25 g) in a volume of 10 mL/kg of body weight. The pentylenetetrazole solution at a convulsant dose was prepared according to a previously described standard procedure.

Determination of the Median Effective Dose (ED_{50}). In order to determine the ED_{50} for the respective antiallodynic and anticonvulsant protocols, doses were varied until three to four points were established between the dose levels producing 0% protection and 100% protection in both protocols. The data were subjected to FORTRAN probit analysis,[60] resulting in a calculated ED_{50} and 95% confidence interval.

Evaluation of Rat Motor Dysfunction or Sedation. Compounds **19**−**22**, **29**, and **33**, which were found active in the antiallodynic protocol, were evaluated in the accelerating rotorod apparatus using the highest dose administered in the antiallodynic sensory test (80 mg/kg). Naive male albino rats (n = 5) (Sprague−Dawley, 200−250 g) were injected with **19**−**22**, **29**, or **33** and were placed on the rotorod after 60 and 120 min in accordance with the time to peak antiallodynic effect previously measured. The time before falling off the apparatus was measured, with a maximum cutoff time spent of 120 s.

Calculation of ClogP. ClogP was calculated by means of ChemDraw Ultra software, version 8.

Statistical Analysis. The results are presented as either the ED_{50} or 95% confidence intervals. A Student's t test was used for calculating significant difference between ED_{50} values. A p value of < 0.05 was considered significant.

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Supporting Information Available: Purity determination of the synthesized compounds by combustion analysis; description of the protocols of the animal models used for the screening of the investigational anticonvulsant and antiallodynic compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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