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Syntheses and Evaluation of Anticonvulsant Activity of Novel Branched Alkyl Carbamates

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Supporting Information

ABSTRACT: A novel class of 19 carbamates was synthesized, and their anticonvulsant activity was comparatively evaluated in the rat maximal electroshock (MES) and subcutaneous metrazol (scMet) seizure tests and pilocarpine-induced status epilepticus (SE) model. In spite of the alkyl-carbamates' close structural features, only compounds **34**, **38**, and **40** were active at the MES test. The analogues 2-ethyl-3-methyl-butyl-carbamate (**34**) and 2-ethyl-3-methyl-pentyl-carbamate (**38**) also exhibited potent activity in the pilocarpine-SE model 30 min postseizure onset. Extending the aliphatic side chains of homologous carbamates from 7 to 8 (**34** to **35**) and from 8 to 9 carbons in the homologues **38** and **43** decreased the activity in the pilocarpine-SE model from ED_{50} = 81 mg/kg (**34**) to 94 mg/kg (**35**) and from 96 mg/kg (**38**) to 114 mg/kg (**43**), respectively. The most potent carbamate, phenyl-ethyl-carbamate (**47**) (MES ED_{50} = 16 mg/kg) contains an aromatic moiety in its structure. Compounds **34**, **38**, **40**, and **47** offer the optimal efficacy−safety profile and, consequently, are promising candidates for development as new antiepileptics.

INTRODUCTION

Epilepsy is a common neurological disorder characterized by the onset of spontaneous convulsant and nonconvulsant seizures that result from neuronal hyperexcitability and hypersynchronous neuronal firing. Over 50 million people worldwide are affected by epilepsy, and it is estimated that about 30% of the patients suffer from therapy-resistant epilepsy. Resistance to antiepileptic drugs (AEDs) and the side effects associated with the current AEDs are the most serious problems in the treatment of epilepsy.[1−6] Therefore, there is a substantial need to design anticonvulsants for the development of more effective and safer AEDs.[7−9]

The discovery in 1951 of the dicarbamate, meprobamate, as a new anxiolytic that also possesses anticonvulsant activity triggered the search for new central nervous system (CNS)-active carbamates.[10,11] In 1961, Close and Spielman reviewed a series of 96 carbamates that were tested in anticonvulsant animal models.[12] Two aliphatic (2-ethyl-butyl carbamate and 2,2-dimethyl-propyl carbamate) and one aromatic (phenyl-ethyl carbamate) carbamates exhibited anticonvulsant activity in the mice maximal electroshock (MES) and subcutaneous metrazol (scMet) seizure tests.[12] A structure−activity relationship (SAR) study of carbamic acid esters with the general formula of R−O−$CONH_2$ showed that the anticonvulsant activity in the mice MES test increased as the alkyl substituent (R) in the ester moiety extended from methyl to pentyl but decreased with the higher analogues.[13,14] It was concluded that the reported esters of the carbamic acid exhibit anticonvulsant activity coupled with low neural and general toxicity.[14]

Taillandier et al. evaluated the anticonvulsant activity of six branched alkyl carbamate derivatives of carbamic acid and concluded that carbamic esters possess tranquilizing activity but at a level lower than that of meprobamate [mice MES dose effective in 50% of test subjects (ED_{50}) = 127 mg/kg].[15] Yamagami et al. studied the quantitative structure−activity relationship (QSAR) of benzyl *N*,*N*-dimethyl carbamates,[16] phenylacetanilides,[17] and arylalkyl and alkylcarbamtes[18] and reported that their anticonvulsant activity depended parabolically on log *P*.[17,18]

Felbamate (**2**, Figure 1) is an AED containing dicarbamate moiety in its chemical structure. In spite of its broad spectrum of antiepileptic activity,[19] **2** is currently seldom used clinically due to the fatal aplastic anemia and hepatotoxicity associated with its therapy.[20,21] Carisbamate (**3**, Figure 1), a new AED possessing a carbamate moiety in its chemical structure, was submitted in 2009 for regulatory approval to the Food and Drug Administration (FDA) and the European Medicines Agency (EMA).[22] Although **3** is generally well tolerated, it

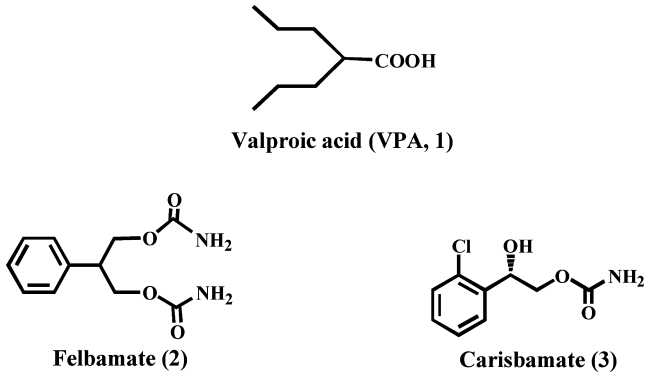

Figure 1. Chemical structures of the AEDs: valproic acid (**1**), felbamate (**2**), and carisbamate (**3**).

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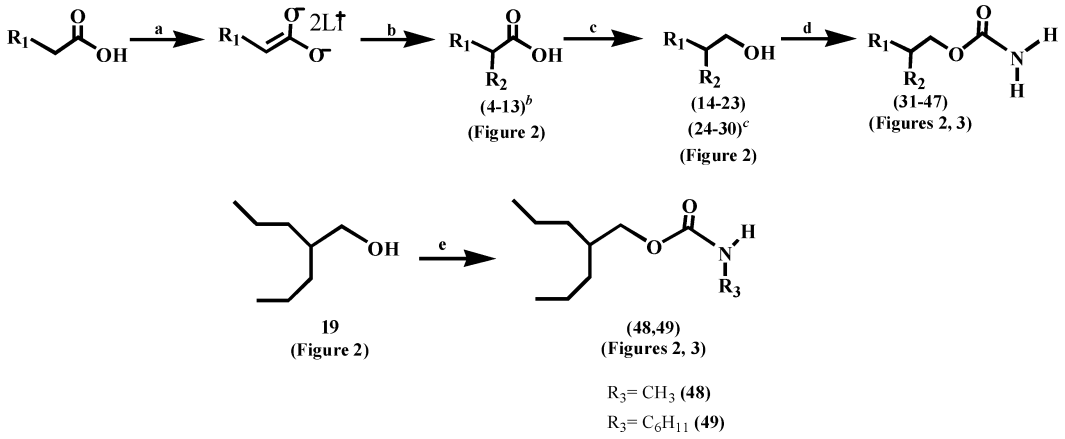
Scheme 1. A. Synthesis of alkyl carbamates[a]



[a]Reagents and conditions: (a) LDA, THF, −15 °C, 30 min. (b) Methyliodide or ethyliodide or propyliodide or isopropyliodide, THF, rt, 2 h. (c) $LiAlH_4$, DCM, reflux, 24 h. (d) Sodium isocyanate, TFA, DCM, 5 h. (e) Methyl isocyante or cyclohexyl isocyanate, TFA, DCM, 5 h. [b]2,2,3,3-Tetramethylcyclopropanecarboxylic acid (**13**) is commercially available. [c]Seven commercially available alcohols (**24**−**30**) (see the Materials and Methods) were utilized for the syntheses of carbamates **31**, **33**, **36**, **37**, **44**, **45**, and **47**, respectively (Figures 2 and 3).

failed to demonstrate consistent efficacy across the dose range. Consequently, its applications to the FDA and EMA were withdrawn in January, 2010, and its clinical program in epilepsy was discontinued.[7,22] Recently, Kung and Kwon reported on a series of carbamate derivatives of **2** that exhibited anticonvulsant activity with a wide range of ED_{50} values (15−300 mg/kg) in the mice MES test.[23]

There is a substantial need to discover novel chemical entities for the development of potent and safe new AEDs. In light of the extensive involvement of a carbamate moiety in the structures of antiepileptics and CNS drugs, the rationale of the current study was to synthesize carbamates containing various alkyl substituents analogous to the alkyl backbone of valproic acid (VPA) (**1**, Figure 1) and its analogues in the ester moiety of the carbamates. In 2007, we showed that very minor structural changes in amide and urea derivatives of **1** constitutional isomers (e.g., location of a single methyl group in the acyl moiety) had a significant influence on the pharmacological profile of the tested compounds.[24] Consequently, in this study, we assessed the effect of modification of the length and branching in the ester moieties of the investigated carbamates. In our current study, 16 new branched alkyl esters of carbamic acid, a phenyl alkyl ester of a carbamic acid, and *N*-methyl and *N*-cyclohexyl of 2-propyl-pentyl carbamate were synthesized, and their anticonvulsant activity and neurotoxicity were comparatively evaluated to establish if some of these carbamates might become new potential candidates for further development as new AEDs.

CHEMISTRY

The general synthesis of the alkyl carbamate is depicted in Scheme 1. Besides the seven commercially available alcohols (**24**−**30**), the remaining alcohols for the syntheses of carbamates were obtained by reduction of appropriate acids as depicted in Figure 2. The following acids served as a starting material for synthesis of the branched acids (**4**−**12**): isovaleric acid for the syntheses of acids **4**−**6**; valeric acid for the syntheses of acids **9** and **11**; 3-methyl-valeric acid for the syntheses of acids **7**, **10**, and **12**; and 4-methyl-valeric acid for the syntheses of acid **8**. The above acids were converted to the corresponding enolates by lithium diisopropylamine (LDA), followed by an alkyl substitution of the hydrogen on the carbon in α-position to the carboxyl by using alkyl iodides[25] (Scheme 1). The carboxylic acids (**4**−**12**) and 2,2,3,3-tetramethylcyclopropanecarboxylic acid (TMCA, **13**) were treated with lithium aluminum hydride (LAH), to produce the corresponding alcohols (**14**−**23**).[26] These alcohols were subsequently coupled with sodium isocyanate in dry dichloromethane (DCM) with trifluoroacetic acid (TFA) for 5 h to yield the carbamates **31**−**47** (Scheme 1). The following alcohols were utilized for the syntheses of appropriate carbamates: **14** → **32**; **15** → **34**; **16** → **35**; **17** → **38**; **18** → **39**; **19** → **40**; **20** → **41**; **21** → **42**; **22** → **43**; and **23** → **46** (Figures 2 and 3). The carbamates **48** and **49** were prepared by coupling of 2-propyl-pentanol with methyl isocyanate or cyclohexyl isocyanate, respectively, at the same reaction conditions. Compounds **32**, **34**, **37**−**39**, and **41**−**45** are chiral molecules possessing one or two stereogenic carbons in their chemical structure (Figure 2). These chiral carbamates were synthesized by nonstereoselective methods and evaluated pharmacologically as racemates. The synthesized liquid products were purified by distillation and the solid products by crystallization. ^1H nuclear magnetic resonance (NMR) spectra of the synthesized compounds were measured in $CDCl_3$ using TMS as an internal standard. Elemental analyses were performed for all of the synthesized compounds.

RESULTS AND DISCUSSION

Currently, about 30% of patients with epilepsy are not seizure-free with the existing medications. In addition, AED therapy particularly with **1** is associated with severe side effects including teratogenicity that restrict the clinical use of major AEDs in women of child-bearing age or in children due to heaptotoxicity.[27−32] There are three major approaches for designing and developing new AEDs. The first (mechanism-based) is associated with the pathophysiological processes underlying the generation of seizures through a better understanding of the molecular mechanism responsible for the various seizure types.[28,33,34] The second approach is the design of follow-up or second generation compounds to existing AEDs, which will circumvent problems associated with the first generation AEDs.[8,28,35] The third (empirical or mechanism-unbiased) approach is based on the screening of

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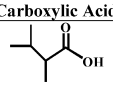
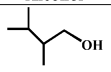
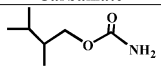
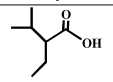
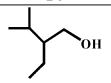
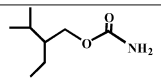
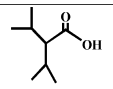
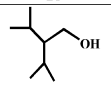
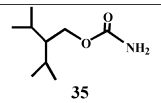
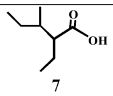
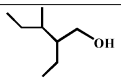
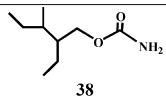
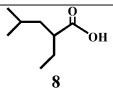
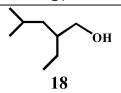
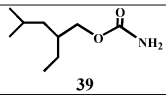
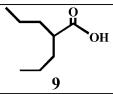
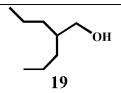
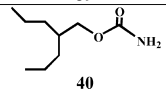
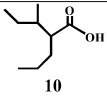
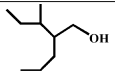
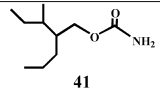
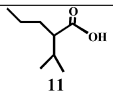
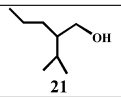
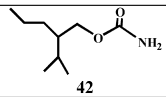
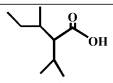
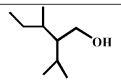
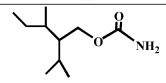
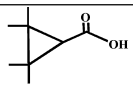
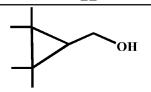
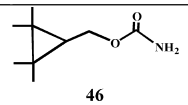
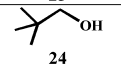
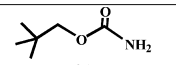
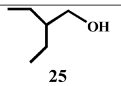
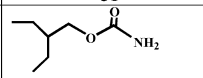
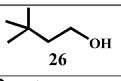
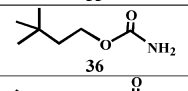
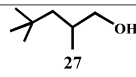
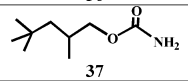
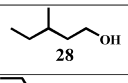
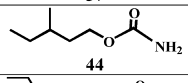
Carboxylic Acid	Alcohol	Carbamate
 4	 14	 32
 5	 15	 34
 6	 16	 35
 7	 17	 38
 8	 18	 39
 9	 19	 40
 10	 20	 41
 11	 21	 42
 12	 22	 43
 13	 23	 46
	 24	 31
	 25	 33
	 26	 36
	 27	 37
	 28	 44
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	19	48
	19	49

Figure 2. Structures of carboxylic acids and alcohols that served as the starting materials for the syntheses of various carbamates.

		Substituents		
Compound	Structure	R_1	R_2	R_3
31		methyl	dimethyl	H
32		isopropyl	methyl	H
33		ethyl	ethyl	H
34		isopropyl	ethyl	H
35		isopropyl	isopropyl	H
36		isobutyl	H	H
37		isobutyl	methyl	H
38		secbutyl	ethyl	H
39		isobutyl	ethyl	H
40		n-propyl	n-propyl	H
41		secbutyl	n-propyl	H
42		n-propyl	isopropyl	H
43		secbutyl	isopropyl	H
44		secbutyl	H	H
45		n-butyl	ethyl	H
46		2,2,3,3,-tetramethyl cyclopropyl	H	H
47		phenyl	H	H
48		n-propyl	n-propyl	methyl
49		n-propyl	n-propyl	cyclohexyl

Figure 3. Structures of synthesized and tested carbamates.

drug candidates in anticonvulsant animal (rodent) models of epilepsy that have established their predictability since the discovery of phenytoin in 1938.[3,4,28]

In this study, we combined the last two approaches by designing, synthesizing, and evaluating various novel branched alkyl carbamates and exploring the effect of modification of the

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Table 1. Anticonvulsant Activity and Neurotoxicity of Compounds 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]
31	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
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	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	4/4	0/2
34	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
35	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	0/0	0/1	0/0	0/1	3/4	0/2
36	30	1/1	0/0			4/4	0/0
	100	1/1	0/0			8/8	0/0
	300					4/4	0/0
37	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	1/1	1/1	0/1	4/4	0/2
38	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	3/3	0/3	1/1	0/1	5/8	0/4
	300	1/1	0/1	1/1	0/1	3/4	0/2
39	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
40	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	3/3	0/3	0/1	0/1	4/8	0/4
	300	1/1	1/1	1/1	1/1	4/4	2/2
41	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	2/3	0/3	0/1	0/1	3/8	0/4
	300	1/1	1/1	1/1	0/1	3/4	0/2
42	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	1/1	0/1	1/1	0/1	4/4	0/2
43	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	3/3	0/3	1/1	0/1	7/8	0/4
	300	1/1	0/1	1/1	0/1	4/4	0/2
44	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	2/3	0/3	0/1	0/1	1/8	0/4
	300	1/1	0/1	0/1	0/1	4/4	0/2
45	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	1/1	1/1	1/1	0/1	4/4	0/2
46	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	4/4	0/2
47	3	0/4	0/0			0/4	0/0
	10	0/4	0/0			0/4	0/0
	30	1/1	0/1	0/1	0/1	0/4	0/2
	100	3/3	0/3	3/5	0/1	2/8	0/4
	300	1/1	1/1	1/1	0/1	4/4	0/2
48	30	0/1	0/1	0/1	0/1	0/4	1/2
	100	2/3	0/3	0/1	0/1	3/8	1/4
	300	1/1	1/1	1/1	0/1	3/4	0/2
49	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	2/8	0/4
	300	0/1	0/1	0/1	0/1	2/4	0/2

[a]Maximal. [b]scMet test (no. of animals protected/no. of animals tested). [c]Neurotoxicity evaluated as motor impairment or sedation (no. of animals affected/no. of animals tested). [d]Time after drug administration.

length and branching of the aliphatic side chains in the carbamates ester moiety on the anticonvulsant profile. Some of the analyzed carbamates (Figure 3) are carbamic acid esters formed by alcohols with the alkyl side chain similar to the alkyl backbone of **1** or its analogues. This combination between an alkyl side chain of 2-propyl-pentanol or its analogues with the carbamate pharmacophore possessed by **2** and **3** (Figure 1) may lead to a novel molecule with improved anticonvulsant potency.

A reasonable prediction of a compound's potential as a new AED candidate is based on the characterization of the compound's anticonvulsant profile in a variety of anticonvulsant animal models.[27,28,36,37] Although there is a variety of animal models for epilepsy, the MES and scMet seizure models remain the "gold standards" in early stages of discovery of new AEDs and have gained an appreciable degree of predictability since the MES was first utilized in the discovery of the anticonvulsant activity of phenytoin (1938).[27,28,35−37]

Although AEDs are routinely categorized according to a single mechanism of action (e.g., sodium channel blockade etc.) to which their antiepileptic activity is attributed, all major AEDs have multiple mechanisms of action (MOA).[38] Compound **1** is an example of a major AED with multiple MOA (including hitsone deacetylase inhibition) that is highly effective in both partial and generalized seizures and is the first drug of choice in generalized epilepsy.[38,39] Because of these multiple MOA, **1** is also effective and FDA/EMA-approved for the treatment of bipolar disorder and migraine prophylaxis and might have a potential in Alzheimer's disease and cancer therapy.[40] Similarly, the active branched alkyl (derivatives of **1**) and aryl carbamates emerged from this study are likely to have multiple MOA.

Table 1 presents the anticonvulsant activity and neurotoxicity of compounds **31**−**49** at the mouse MES and scMet tests (ip). The results showed no significant difference between efficacy and neurotoxicity at 300 mg/kg (highest tested dose) for most of the compounds. With the exception of **36** and **47**, none of the tested compounds showed anticonvulsant (MES) activity at 30 mg/kg. Although **36** was active at 30 mg/kg, there was no difference between its anticonvulsant activity and neurotoxicity, while **47** showed a separation or safety margin at 30 and 100 mg/kg (Table 1). At the scMet test, all compounds were inactive at 30 mg/kg; however, most of them demonstrated activity at 300 mg/kg.

Table 2 summarizes the qualitative anticonvulsant activity and neurotoxicity of compounds **31**−**49** at the rat-MES test (po). All tested compounds showed no neurotoxicity at 30 mg/kg, and the most potent compounds were **47**, which was also active at the scMet test, followed by **38** and **45**. Comparative quantitative evaluation at the rat-MES (po) test showed that compounds **34**, **38**, **40**, and **47** had ED_{50} values of ≤64 mg/kg and high protective index (PI = TD_{50}/ED_{50}) values and thus were more potent and had a wider safety margin than **1** (Table 3).

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Table 2. Anticonvulsant (Anti-MES) Activity and Neurotoxicity of Compounds Administered Orally to Rats

		no. of tested rats per time after drug administered[a]					
compd	dose (mg/kg)	15 min[c]	30 min[c]	1 h[c]	2 h[c]	4 h[c]	TOX[b]
31	30	1/4	0/4	0/4	0/4	1/4	0/4
33	30	0/4	0/4	0/4	0/4	0/4	0/4
34	30	2/4	0/4	0/4	0/4	0/4	0/4
35	30	0/4	0/4	0/4	0/4	0/4	0/4
37	30	1/4	1/4	0/4	0/4	0/4	0/4
38	30	2/4	1/4	0/4	0/4	0/4	0/4
39	30	0/4	0/4	1/4	1/4	0/4	0/4
40	30	0/4	0/4	0/4	0/4	0/4	0/4
	100	1/4	4/4	1/4	2/4	0/4	0/8
41	30	0/4	0/4	0/4	0/4	0/4	0/4
42	30	0/4	1/4	1/4	0/4	0/4	0/4
44	30	0/4	0/4	0/4	0/4	0/4	0/4
45	30	2/4	0/4	0/4	0/4	0/4	0/4
46	30	0/4	0/4	1/4	0/4	0/4	0/4
47[d]	30	4/4	4/4	4/4	3/4	0/4	0/4
48	30	0/4	0/4	0/4	0/4	0/4	0/4

[a]No. of animals protected/no. of animals tested. [b]Neurotoxicity evaluated as motor impairment or sedation (no. of animals affected/no. of animals tested). [c]Time after drug administration. [d]Compound **47** was also active at the scMet test with 2/4 rats protected at 15 min at 50 mg/kg.

Compound **34** also demonstrated anticonvulsant activity following ip administration to mice and rats with a rat ED_{50} (ip) value four times more potent than its rat ED_{50} (po) (Table 3).

A carbamate with a phenyl-ethyl group (**47**) in the ester moiety showed increased MES potency by 2−4-fold as compared to **34**, **38**, and **40**, thus making **47** as the most potent compound (ED_{50} = 16 mg/kg). Compounds **40** (valproyl carbamate) and **47** were previously synthesized, but only **47** showed activity at the mice MES test.[12−14,18] Close and Spielman reported that **47** was active at the mice MES with no separation between anticonvulsant activity and side effects.[12] Subsequently, Tanaka et al. reported that **47** exhibited in mice MES-ED_{50} (ip) and TD_{50} values of 18 and 114 mg/kg, respectively, with no activity at the scMet test.[18] Because anticonvulsant tests are notoriously sensitive to experimental conditions, consequently, it is difficult to compare results from different laboratories. Therefore, we synthesized compounds **40** and **47** and evaluated their anticonvulsant activity under the same conditions used for the rest of the carbamates investigated in this study.

Substitution of a hydrogen atom of the amine (NH_2) in the amide moiety with a methyl (**48**) or cyclohexyl (**49**) resulted in decreased anticonvulsant potency as compared to the underivatized carbamate **40** (Tables 1 and 2). Compounds **34** and **35** were active at the 6 Hz (32 mA) psychometric test in mice with ED_{50} values of 33 and 41 mg/kg and PI values of 4.4 and 3.4, respectively (Table 3).

Administration of lithium-pilocarpine induces status epilepticus (SE) characterized by convulsive and nonconvulsive seizures that can last for several hours.[41] SE is then followed by a latent phase, characterized by synaptic remodeling and neuronal plasticity, extensive neuronal loss, and subsequent cognitive deficits and the precipitation of spontaneous recurrent seizures, the hall mark of epilepsy. From a behavioral perspective, the number and severity of the observed convulsive seizures following pilocarpine administration were similar in the two treatment groups (pilocarpine alone and pilocarpine + carbamate). Each of the tested carbamates (Table 4) were administered at 0 and 30 min after the first observed Racine stage 3 motor seizure (which marked the onset of SE).[42]

Table 4 shows the anticonvulsant activity and neurotoxicity of compounds **31**−**48** at the rat-pilocarpine SE model. Significant anticonvulsant protection at the pilocarpine-induced SE model was observed for most of the carbamates. Comparative analysis shows that out of the carbamate tested, compounds **34**, **35**, and **38** exhibited the highest level of seizure protection in the pilocarpine-SE model (Table 5). SAR studies showed that extending the branched aliphatic side chains moiety from seven (**34**) to nine carbons (**43**) decreased the anti-SE activity from ED_{50} = 81 to 114 mg/kg (Table 5). The only other AED found to exert an effect similar to **34** when administered under the same experimental conditions was carbamazepine (ED_{50} = 50 mg/kg) and the AED candidate *sec*-butyl-propylacetamide (ED_{50} = 84 mg/kg).[41] The other comparative prototypical AEDs tested in this model, clonazepam, diazepam, **1**, and phenobarbital, were all ineffective at the highest dose tested, that is, 40, 100, 300, and 40 mg/kg, respectively.[41]

SE is not a disease but is a manifestation of an underlying CNS insult or systemic pathology that affects CNS function. SE results when there is a failure of those inherent factors that would normally function to stop seizures. SE can result when

Table 3. Quantitative Anticonvulsant Data (Anti-MES and Anti-scMet) in Rats Dosed Orally and in Mice (ip) at the 6 Hz−32 mA Psychomotor Seizure Test

compd	MES[a] ED_{50}^{f} (mg/kg)	PI[b]	scMet[c] ED_{50}^{f} (mg/kg)	PI[d]	$TD_{50}^{e,f}$ (mg/kg)	6 Hz ED_{50}^{j} (mg/kg)
1[g]	485 (324−677)	1.6	646 (466−869)	1.2	784 (503−1176)	
2[h]	25 (19−30)	20	>250	>2	>500	
3[i]	8	18	58	2.4	137	
34	64 (39−92)	7.8	35 (20−73)	14	>500	33 (23−47)
	rats ip, 16 (11−21)	3.6	rats ip, 19 (13−28)	1.3	rats ip, 58 (45−70)	
	mice ip, 103 (90−124)[j]	1.5	mice ip, 120(108−131)	2.9	mice ip, 157 (136−185)	
					144 (130−168)	
35					139 (125−150)	41 (22−75)
38	52 (37−70)	5.5	68 (46−97)	4.3	291 (218−342)	
40	35 (25−44)	>14	>125	>4	>500	
47	16 (11−21)	<25			<400	

[a]Maximal electroshock test. [b]PI (PI = TD_{50}/ED_{50}) in the MES test. [c]scMet test. [d]PI (PI = TD_{50}/ED_{50}) in the scMet test. [e]Neurotoxicity. [f]The interval in parentheses stands for the 95% confidence interval. [g]Data taken from ref 8. [h]Data taken from ref 19. [i]Data taken from ref 22. [j]6 Hz (32 mA) psychomotor seizure test.

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Table 4. Anticonvulsant Activity in the Pilocarpine-Induced SE Model after ip Administration to Rats[a]

	response data			toxicity test					
compd	dose (mg/kg)	time (min)	N/F[b]	dose (mg/kg)	0.25 (h)	0.5 (h)	1.0 (h)	2.0 (h)	4.0 (h)
31	200	0	8/8	100	0/2	0/2	0/2	0/2	0/2
				300	2/2	2/2	2/2	2/2	1/2
32	200	0	8/8	100	0/2	0/2	0/2	0/2	0/2
				300	2/2	2/2	1/2	0/2	0/2
	400	0.5	4/7						
33	200	0	2/8	100	1/2	1/2	0/2	0/2	0/2
				300	2/2	2/2	0/2	0/2	0/2
34	65	0	8/8	30	0/2	0/2	0/2	0/2	0/2
				100	2/2	2/2	1/2	0/2	0/2
				300	2/2	1/2			
	130	0.5	7/7						
35	65	0	7/7	30	0/2	0/2	0/2	0/2	0/2
				100	2/2	2/2	0/2	0/2	0/2
				300	2/2	2/2			
36	65	0	1/8	100	0/2	0/2	0/2	0/2	0/2
				300	2/2	2/2	0/2	0/2	0/2
37	65	0	6/8	30	0/2	0/2	0/2	0/2	0/2
				100	2/2	2/2	1/2	0/2	0/2
				300	2/2	2/2	1/1	1/1	0/1
	130	0.5	3/8						
38	65	0	8/8	30	0/2	0/2	0/2	0/2	0/2
				100	2/2	2/2	1/2	0/2	0/2
				300	2/2	2/2	2/2	2/2	2/2
39	65	0	6/8	30	0/2	0/2	0/2	0/2	0/2
				100	2/2	2/2	1/2	0/2	0/2
				300	2/2	2/2	2/2	1/2	0/2
	130	0.5	2/8						
40	200	0	2/8	100	1/2	0/2	0/2	0/2	0/2
				300	2/2	2/2	2/2	2/2	2/2
41	65	0	6/7	30	2/2	2/2	2/2	2/2	2/2
				100	2/2	2/2	1/2	0/2	0/2
				300	0/2	0/2	0/2	0/2	0/2
	130	0.5	3/8						
42	65	0	8/8	30	0/2	0/2	0/2	0/2	0/2
				100	2/2	2/2	2/2	0/2	0/2
				300	2/2	2/2	2/2	2/2	2/2
	130	0.5	6/7						
43	65	0	8/8	30	0/2	0/2	0/2	0/2	0/2
				100	2/2	2/2	1/2	0/2	0/2
44	65	0	7/7	30	0/2	0/2	0/2	0/2	0/2
				100	2/2	2/2	0/2	0/2	0/2
				300	2/2	2/2	1/2	0/2	0/2
45	200	0	4/7	100	0/2	0/2	0/2	0/2	0/2
				300	2/2	2/2	2/2	2/2	2/2
46	65	0	6/8	30	0/2	0/2	0/2	0/2	0/2
				100	2/2	2/2	2/2	0/2	0/2
				300	2/2	2/2	2/2	2/2	0/2
	130	0.5	0/8						
48	65	0	4/8	30	0/2	0/2	0/2	0/2	0/2
				100	2/2	2/2	2/2	0/2	0/2
				300	2/2	2/2	2/2	2/2	2/2

[a]A challenge dose of pilocarpine is given 0 and 30 min following ip administration of a candidate drug to male Sprague–Dawley rats. [b]Pilocarpine test (no. of animal protected/total no. of rats tested).

there is a decrease in inhibition or an increase in excitation or a combination of both.[41] Treatment of SE is aimed at controlling convulsive seizures as quickly as possible before compensatory mechanisms fail and the patient enters into a "refractory" state. The benzodiazepines (lorazepam and diazepam), phenytoin or its parenteral prodrug phosphenytoin, and phenobarbital are generally considered the first line AEDs for the early treatment of SE. Second line AEDs to treat SE include **1**, levetiracetam, and lacosamide. SE can quickly become pharmacologically refractory when initial attempts to control the seizures fail despite adequate

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Table 5. Quantitative Anticonvulsant Data (in the Pilocarpine-Induced SE Model) in Rats Dosed ip (Time, 30 min)

compd	time (h)	PILO ED_{50} (mg/kg)	PILO ED_{97} (mg/kg)	dose (mg/kg)	time (h)	N/F[a]
34	0.5	81 (66−95)	114 (95−200)	65	0.5	1/8
				100	0.5	7/8
35	0.5	94 (76−113)	184 (141−339)	50	0.5	0/8
				65	0.5	2/8
				100	0.5	4/8
				130	0.5	6/8
				160	0.5	8/8
38	0.5	96 (73−120)	174 (130−628)	65	0.5	1/8
				100	0.5	4/8
42	0.5	107 (82−129)	151 (126−692)	65	0.5	0/8
				100	0.5	3/8
43	0.5	114 (92−136)	199 (155−500)	65	0.5	0/8
				100	0.5	3/8
				150	0.5	6/8
47	0.5	105 (80−133)	221.3 (158.0−655.2)	65	0.5	1/8
				100	0.5	4/8
				200	0.5	8/8

[a]No. of rats affected/no. of rats tested.

treatment. There is a clear need for more effective treatments for refractory SE that display rapid onset and effective seizure control without producing dose-limiting sedation and respiratory depression. Furthermore, the development of an effective therapy that attenuates refractory SE offers some neuroprotective potential and prevents the cognitive decline associated with SE would represent an important advance in the treatment of SE.

The optimal balance between lipophilic and hydrophilic moieties (log *P*) is a very important consideration in designing AEDs.[43] Previous studies showed that a direct correlation between anticonvulsant activity and Clog *P* values exists, mainly as a result of an increased blood−brain barrier (BBB) penetration.[44,45]

The branched aliphatic carbamates with the highest anticonvulsant potencies **34**, **35**, **38**, and **40**−**43** (Tables 3 and 5) were highly lipophilic (Table 6), which implies that penetration through the BBB is an important factor influencing the drugs' efficacy.[46] Compounds **40** and **38** were the most active alkyl-carbamates in the rat MES model with an ED_{50} values of 35 and 53 mg/kg, respectively, whereas **34** and **35** were the most potent compounds in the pilocarpine-SE test (ED_{50} = 81 and 94 mg/kg, respectively).

Table 6. Lipophilicity Data (Clog *P*) of the Investigated Compounds[a]

compd	Clog *P*	compd	Clog *P*
31	1.152	**41**	3.268
32	1.681	**42**	2.739
33	1.811	**43**	3.138
34	2.21	**44**	1.282
35	2.609	**45**	2.869
36	1.681	**46**	2.865
37	2.609	**47**	1.393
38	2.739	**48**	3.415
39	2.739	**49**	5.446
40	2.869		

[a]Clog *P* was calculated by utilizing the ChemDraw Ultra software, version 8.

Screening animal models is essential in AED discovery particularly if, like most current AEDs, the drug candidate has multiple mechanisms of action.[47] In addition, animal models provide an insight on pharmacokinetic−pharmacodynamic correlation of the investigational drug. Given the highly heterogeneous nature of seizure disorders in humans, the complexity of the seizure, and the syndrome involved, it is unlikely that a single anticonvulsant animal model will predict the full therapeutic potential of a drug candidate.[28,47]

CONCLUSION

In this study, we report the synthesis and comparative evaluation of the anticonvulsant activity and neurotoxicity of a novel series of aromatic-carbamate (**47**) and 18 alkyl-carbamates with ester backbone containing the aliphatic side chain of 2-propyl pentanol (valproyl alcohol) and its analogues.

In spite of the similarity of the alkyl group in the carbamates chemical structure presented in this study (Figure 3), only a few synthesized carbamates showed potent ED_{50} values in both the rat MES and the pilocarpine SE tests. Compound **35** was active at the 6 Hz and SE models, while **40** was active only at the MES model. Compounds **42** and **43** were only active at the SE models. The carbamates with the widest anticonvulsant spectrum of activity were **34** and **38** showing activity at the rat MES, scMet, and pilocarpine SE models with ED_{50} values ranging between 35 and 96 mg/kg. Carbamate **47** differs structurally from the other 18 compounds presented in Figure 3, since it contains an aromatic group in the ester moiety instead of the branched aliphatic groups. On the basis of rat MES ED_{50} values, **47** was the most potent compound with an anticonvulsant potency 30 times greater than **1** (Table 3) and with a rat MES PI value ∼15 times higher than **1**. Compound **47** was previously tested only qualitatively in mice,[12−14,18] while the current quantitative evaluation demonstrated its potent activity in both the rat MES and the pilocarpine-SE models. In conclusion, the carbamates **34**, **38**, and **47** offer an optimal anticonvulsant efficacy and safety profile and consequently are potential candidates for further development as new antiepileptics and CNS drugs.

EXPERIMENTAL SECTION

Chemicals. Valeric acid, isovaleric acid, 3-methylvaleric acid, 4-methylvaleric acid, 2,2,3,3-tetramethylcyclopropanecarboxylic acid (TMCA) (**13**), 2,2-dimethyl-1-propanol (**24**), 2-ethyl-butanol (**25**),

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3,3-dimethyl-1-butanol (**26**), 2,4,4-trimethyl-1-pentanol (**27**), 3-methyl-1-pentanol (**28**), 2-ethyl-1-hexanol (**29**), and 2-phenyl-ethanol (**30**) and all common reagents were obtained from Sigma-Aldrich (United States) and used without further purification. DCM, tetrahydrofuran (THF), petroleum ether, and ethyl acetate were A.R. grade and obtained from Frutarom Israel. DCM was dried by refluxing over CaH_2 for 2 h and distillation prior to use. THF was dried by refluxing over CaH_2 for 2 h, distillation, and refluxing over LAH for 2 h and distillation prior to use. *N*,*N*-Dimethylpropyleneurea (DMPU) was dried by its refluxing over CaH_2 for 2 h and distillation under reduced pressure. It was stored over 4 Å molecular sieves under a nitrogen atmosphere.

Materials and Methods. Product formation follow-up was performed by means of gas chromatography−mass spectroscopy (GC-MS) and thin-layer chromatography (TLC). TLC analyses were performed on precoated silica gel on aluminum sheets (Kieselgal 60 F_{254}, Merck). A GC-MS assay was performed on a HP5890 Series II GC equipped with a Hewlett-Packard MS Engine (HP5989A) single quadrupole MS, 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 6 min; gradient of 20 °C/min until 140 °C; gradient of 10 °C/min until 200 °C; and hold time, 3 min. The MS parameters were set as follows: source temperature, 180 °C; transfer line, 280 °C; positive ion monitoring; and EI-MS (70 eV). The molecular ion and the five most-pronounced ions are provided.

^{1}H NMR spectra, in $CDCl_3$ using TMS as the internal standard, were recorded on a Varian Mercury series NMR 300 spectrometer. Chemical shifts (δ scale) are reported in parts per million (ppm) relative to residual TMS. Multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), m (multiplet), dd (doublet of doublet), and br m (broad multiplet). Coupling constants (J) are given in (Hz).

Chemical structures of the newly synthesized compounds were assessed by ^{1}H NMR and elemental analyses. The melting point was determined on a Buchi 530 capillary melting point apparatus. Elemental analyses were performed on a 2400-2 Perkin-Elmer C, H, N analyzer. Elemental analysis (C, H, N) were used to confirm the purity of all newly synthesized compounds (>95%), results within ±0.4 of theoretical values (see the Supporting Information).

General Procedure for the Synthesis of Compounds 32, 34, 35, 38−43, and 46. To a solution of 160 mmol of diisopropylamine in 70 mL of anhydrous THF kept at −15 °C under nitrogen (N_2) atmosphere was added dropwise 160 mmol of *n*-butyllithium. The reaction mixture was stirred for 30 min, and then, 10 mL of dry THF and 72 mmol of valeric acid (for the synthesis of compounds **9** and **11**), isovaleric acid (for the synthesis of compound **4**−**6**), 3-methylvaleric acid (for the synthesis of compounds **7**, **10**, and **12**), or 4-methylvaleric acid (for the synthesis of compound **8**), were added and allowed to stir for an additional 15 min at −5 °C. A 72 mmol amount of DMPU was added dropwise, and the reaction mixture was stirred for 30 min at 5 °C followed by the dropwise addition of 160 mmol of the corresponding alkyliodide (either methyliodide, ethyliodide, propyliodide, or isopropyliodide) in 10 mL of anhydrous THF. The reaction mixture was allowed to stir at room temperature for 2 h, and THF was evaporated. The oily product was dispersed in petroleum ether (50 mL), and 10% HCl solution was added until pH = 1 was reached. The organic phase was separated from the aqueous phase and washed three times with brine. The aqueous solutions were combined and extracted with petroleum ether (3 × 50 mL). The petroleum ether extracts were combined, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The oily products were further distilled under reduced pressure to yield the pure corresponding acids. The synthesized carboxylic acids (**4**−**13**) were converted by LAH ($LiAlH_4$) in DCM to primary alcohols by using 2 equiv of $LiAlH_4$ for each equiv of an acid according to a previously published procedure.[26] The oily crude product was distilled under reduced pressure to afford the desire primary alcohols. TFA (105 mmol) dissolved in 50 mL of dry DCM was added dropwise to a stirred mixture of primary alcohol (50 mmol) and sodium isocyanate (100 mmol) in 100 mL of dry DCM. The reaction mixture was stirred for 5 h at room temperature. Water (15 mL) was added, and the organic layer was separated. The aqueous phase washed three times with 20 mL of DCM. The organic extracts were combined and washed three times with brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The obtained products were purified by crystallization using ethyl acetate/hexane mixture (1:3) to give (42−98% yield) of white crystals.

2,3-Dimethyl-butyl Carbamate (***32***). Silver plates; 60% yield, mp 55−57 °C. MS-EI, m/z (%): 146 (M^+ + 1, 0.12), 102 (36), 84 (73), 69 (100), 55 (20). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.80−0.98 (m, 9H), 1.61−1.79 (m, 2H), 3.88 (dd, J = 6, 11.4, 1H), 4.02 (dd, J = 6, 11.4, 1H), 4.50−4.80 (br s, 2H: NH). Anal. ($C_7H_{15}NO_2$) C, H, N.

2-Ethyl-3-methyl-butyl Carbamate (***34***). White crystals; 54% yield, mp 61−63 °C. MS-EI, m/z (%): 158 (M^+ − 1, 0.14), 116 (22), 98 (34), 83 (54), 62 (100). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.87−0.95 (m, 9H), 1.22−1.47 (m, 3H), 1.77 (m, 1H), 4.33 (m, 2H), 4.42−4.68 (br s, 2H: NH). Anal. ($C_8H_{17}NO_2$) C, H, N.

2-Isopropyl-3-methyl-butyl Carbamate (***35***). White crystals; 77% yield, mp 49−52 °C. MS-EI, m/z (%): 130 (M^+ − 43, 7), 112 (12), 97 (30), 69 (100), 57 (86). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.86−0.98 (dd, J = 9, 18 12H), 1.22 (m, 1H), 1.93 (m, 2H), 4.15 (d, J = 5.1, 2H), 4.42−4.68 (br s, 2H: NH). Anal. ($C_9H_{19}NO_2$) C, H, N.

2-Ethyl-3-methyl-pentyl Carbamate (***38***). Oil; 67% yield. MS-EI, m/z (%): 144 (M^+ − 29, 0.4), 112 (14), 83 (100), 62 (96), 55 (96). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.81−0.94 (br m, 9H), 1.18 (m, 1H), 1.36 (m, 4H), 1.51 (m, 1H), 3.92−4.12 (br m, 2H), 4.46−4.72 (br s, 2H: NH). Anal. ($C_9H_{19}NO_2$) C, H, N.

2-Ethyl-4-methyl-pentyl Carbamate (***39***). White crystals; 68% yield, mp 39−42 °C. MS-EI, m/z (%): 144 (M^+ − 29, 0.27), 130 (6), 83 (39), 75 (33), 57 (100). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.89 (m, 9H), 1.14 (m, 1H), 1.34 (m, 4H), 1.63 (m, 1H), 3.90−4.09 (br m, 2H), 4.42−4.72 (br s, 2H: NH). Anal. ($C_9H_{19}NO_2$) C, H, N.

2-Propyl-pentyl Carbamate (***40***). White crystals; 98% yield, mp 68−70 °C. MS-EI, m/z (%): 130 (M^+ − 43, 0.68), 112 (18), 84 (47), 70 (100), 57 (96). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.88 (m, 6H), 1.24−1.36 (br m, 8H), 1.63 (m, 1H), 3.96 (d, J = 5.7, 2H), 4.48−4.70 (br s, 2H: NH). Anal. ($C_9H_{19}NO_2$) C, H, N.

2-Propyl-3-methyl-pentyl Carbamate (***41***). Oil; 89% yield. MS-EI, m/z (%): 158 (M^+ − 29, 0.36), 130 (9), 97 (74), 70 (77), 55 (100). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.80−0.94 (br m, 9H), 1.08−1.56 (br m, 7H), 1.61 (m, 1H), 3.82−4.12 (br m, 2H), 4.44−4.70 (br s, 2H: NH). Anal. ($C_{10}H_{21}NO_2$) C, H, N.

2-Isopropyl-pentyl Carbamate (***42***). White crystals; 42% yield, mp 66−68 °C. MS-EI, m/z (%): 130 (M^+ − 43, 9), 112 (14), 83 (31), 69 (95), 57 (100). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.89 (t, J = 8.7, 9H), 1.16−1.42 (br m, 7H), 1.48 (m, 1H), 1.76 (m, 1H), 4.02 (dd, J = 1.5, 5.7, 2H), 4.44−4.68 (br s, 2H: NH). Anal. ($C_9H_{19}NO_2$) C, H, N.

2-Isopropyl-3-methyl-pentyl Carbamate (***43***). Oil; 76% yield. MS-EI, m/z (%): 158 (M^+ − 29, 0.13), 126 (4), 83 (43), 70 (100), 55 (84). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.82−0.98 (br m, 12H), 1.20−1.52 (br m, 4H), 1.81 (m, 1H), 4.09 (m, 2H), 4.46−4.68 (br s, 2H: NH). Anal. ($C_{10}H_{21}NO_2$) C, H, N.

2,2,3,3-Tetramethylcyclopropylmethylen Carbamate (***46***). White crystals; 68% yield, mp 77−80 °C. MS-EI, m/z (%): 171 (M^+, 0.2), 110 (29), 95 (100), 67 (38), 55 (62). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.58 (t, J = 9, 1H), 1.05 (S, 6H), 1.18 (S, 6H), 4.10 (d, J = 9, 2H), 4.46−4.68 (br s, 2H: NH). Anal. ($C_9H_{17}NO_2$) C, H, N.

General Procedure for the Synthesis of Compounds 31, 33, 36, 37, 44, 45, and 47. TFA (105 mmol) dissolved in 50 mL of dry DCM was added dropwise to a stirred mixture of the commercially available primary alcohol (**24**−**30**) (50 mmol) and sodium isocyanate (100 mmol) in 100 mL of dry DCM. The reaction mixture was stirred for 5 h at room temperature. Water (15 mL) was added, and the organic layer was separated. The aqueous phase washed three times with 20 mL of DCM. The organic extracts were combined and washed three times with brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The obtained products were purified by crystallization using ethyl acetate/hexane mixture (1:3) to give (79−98% yield) of white crystals.

*2,2-Dimethyl-propyl Carbamate (**31**).* White crystals; 92% yield, mp 75−78 °C. MS-EI, *m*/*z* (%): 116 (M^+ − 15, 2), 75 (100), 87 (14), 70 (16), 57 (81). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.82−0.93 (br s, 9H), 3.78 (s, 2H), 4.50−4.80 (br s, 2H: NH). Anal. ($C_6H_{13}NO_2$) C, H, N.

*2-Ethyl-butyl Carbamate (**33**).* White crystals; 98% yield, mp 83 °C. MS-EI, *m*/*z* (%): 116 (M^+ − 29, 10), 101 (7), 84 (89), 75 (92), 55 (100). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.85−0.94 (t, *J* = 6.9, 6H), 1.38 (m, 4H) 1.50 (m, 1H), 3.98 (d, *J* = 5.7, 2H), 4.52−4.65 (br s, 2H: NH). Anal. ($C_7H_{15}NO_2$) C, H, N.

*3,3-Dimethyl-butyl Carbamate (**36**).* White crystals; 88% yield, mp 57−60 °C. MS-EI, *m*/*z* (%); 145 (M^+, 0.58), 88 (50), 69 (100), 62 (60), 57 (34). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.82−0.93 (s, 9H), 1.58 (t, *J* = 6.6, 2H), 4.12 (t, *J* = 8.4, 2H), 4.50−4.80 (br s, 2H: NH). Anal. ($C_7H_{15}NO_2$) C, H, N.

*2-Methyl-4,4-dimethyl-pentyl Carbamate (**37**).* White crystals; 79% yield, mp 46 °C. MS-EI, *m*/*z* (%); 117 (M^+ − 56, 10), 97 (45), 70 (27), 62 (100), 57 (79). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.88−1.09 (br m, 12H), 1.22−1.30 (dd, *J* = 3, 15, 2H), 1.83 (m, 1H), 3.76 (dd, *J* = 6, 11.4, 1H), 3.90 (dd, *J* = 6, 11.4, 1H), 4.48−4.78 (br s, 2H: NH). Anal. ($C_9H_{19}NO_2$) C, H, N.

*3-Methyl-pentyl Carbamate (**44**).* White crystals; 89% yield, mp 38 °C. MS-EI, *m*/*z* (%); 116 (M^+ − 39, 5), 84 (55), 69 (76), 62 (100), 55 (67). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.86 (m, 6H), 1.18 (m, 1H), 1.41 (br m, 3H), 1.64 (m, 1H), 4.08 (m, 2H), 4.51−4.78 (br s, 2H: NH). Anal. ($C_7H_{15}NO_2$) C, H, N.

*2-Ethyl-hexyl Carbamate (**45**).* White crystals; 86% yield, mp 39−42 °C. MS-EI, *m*/*z* (%): 144 (M^+ − 29, 0.4), 112 (12), 83 (38), 70 (100), 55 (72). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.89 (t, *J* = 8.4, 6H), 1.24−1.44 (br m, 8H), 1.56 (m, 1H), 3.9−4.02 (br m, 2H), 4.51−4.78 (br s, 2H: NH). Anal. ($C_9H_{19}NO_2$) C, H, N.

*Phenylethyl Carbamate (**47**).* White crystals; 80% yield, mp 93−95 °C. MS-EI, *m*/*z* (%): 123 (M^+ − 42, 0.16), 122 (2), 91 (100), 65 (33), 51 (20). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 2.91 (t, *J* = 6, 2H), 4.30 (t, *J* = 6, 2H), 4.46−4.68 (br s, 2H: NH), 7.26 (m, 5H: PH). Anal. ($C_9H_{11}NO_2$) C, H, N.

General Procedure for the Synthesis of Compounds 48 and 49. TFA (105 mmol) dissolved in 50 mL of dry DCM was added dropwise to a stirred mixture of the 2- propyl-1-pentanol (**19**) (50 mmol) and methyl isocyanate (for the synthesis of compound **48**) or cyclohexyl isocyanate (for the synthesis of compound **49**) (100 mmol) in 100 mL of dry DCM. The reaction mixture was stirred for 5 h at room temperature. Water (15 mL) was added, and the organic layer was separated. The aqueous phase washed three times with 20 mL of DCM. The organic extracts were combined and washed three times with brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The obtained products were purified by crystallization using ethyl acetate/hexane mixture (1:3) to give (87−98% yield) of white crystals.

*N-Methyl-2-propyl-pentyl Carbamate (**48**).* White crystals; 87% yield, mp 68−70 °C. MS-EI, *m*/*z* (%): 157 (M^+ − 30, 0.46), 112 (42), 76 (100), 70 (54), 58 (81). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.86 (m, 6H), 1.22−1.35 (br m, 8H), 1.60 (m, 1H), 2.79 (d, J = 5.1, 3H), 3.95 (d, *J* = 5.7, 2H), 4.48−4.70 (br s, 2H: NH). Anal. ($C_{10}H_{21}NO_2$) C, H, N.

*N-Cyclohexyl-2-propyl-pentyl Carbamate (**49**).* White crystals; 98% yield, mp 60−61 °C. MS-EI, *m*/*z* (%): 255 (M^+, 0.67), 212 (7), 144 (100), 71 (64), 57 (95). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.88 (t, *J* = 7.2, 6H), 1.09−1.20 (br m, 4H), 1.31 (m, 10H), 1.57−1.67 (br m, 4H), 1.94 (m, 1H), 3.44 (s, 1H), 3.93 (d, *J* = 5.4, 2H), 4.48−4.70 (br s, 1H: NH). Anal. ($C_{15}H_{29}NO_2$) C, H, N.

Biological Testing/Anticonvulsant Activity. The evaluation of anticonvulsant activity in the MES, scMet, pilocarpine-induced status test and 6 Hz test, and the determination of neurotoxicity in the rotorod test were performed at the National Institute of Health (NIH) Epilepsy Branch as a part of the Anticonvulsant Drug Development Program according to the protocols described in ref 36.

Preparation of the Compounds for Testing. The tested compounds were suspended in 0.5% methylcellulose and administered (a) intraperitoneally (ip) to adult male CF no. 1 albino mice (18−25 g) in a volume of 0.01 mL/g body weight and (b) orally to adult male Sprague−Dawley albino rats (100−150 g) in a volume of 0.04 mL per 10 g of body weight. The pentylenetetrazol solution at convulsing dose was prepared by sufficient dissolution of pentylenetetrazol in 0.9% saline to make 0.85% solution for administration to mice and 2.82% solution for administration to rats.[36]

Determination of the Median Effective Dose (ED_{50}) and the Median Neurotoxic Dose (TD_{50}). For the determination of the ED_{50} by the respective anticonvulsant procedure, doses of the tested compounds were varied until a minimum of 3−4 points was established between the dose level of 0% protection and 100% protection. These data were subjected to the FORTRAN probit analysis program,[36] and the ED_{50} and 95% confidence intervals were calculated. The TD_{50} was determined by varying the dose of the tested compounds until four points were established between the dose level that induced no signs of minimal motor impairment in any of the animals and the dose at which all of the animals were considered impaired. The TD_{50} and the 95% confidence intervals were calculated by FORTRAN probit analysis. The PIs were calculated by dividing the TD_{50} by the ED_{50}.[36]

To determine if the test substance could prevent acute pilocarpine-induced status, the compound was given ip to male albino Sprague−Dawley rats (150−180 g). A challenge dose of pilocarpine was then administered, and the treatment effects of the candidate drug were observed. The outcome measures were "protection" or "no protection". The seizure severity was determined by using the established Racine scale. Compounds found to possess significant protection at time zero (time from the first stage III seizure) were further evaluated in a sustained seizure model where the drug candidate was given 30 min after pilocarpine status induction (or after first stage III seizure).[41]

Calculation of Clog *P*. Clog *P* was calculated by means of ChemDraw-Ultra Software 8.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Purity determination of the carbamates of the constitutional isomers and analogue of **1** by combustion analysis and the material and description of the protocols of the animal models used for the screening of investigational AED. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare the following competing financial interest(s): Dr. Meir Bialer has received in the last three years speakers or consultancy fees from Bial, CTS Chemicals, Desitin, Janssen-Cilag, Johnson & Johnson, Medgenics, Rekah, Sepracor, Teva, UCB Pharma and Upsher-Smith. In the last five years, the author received research grants from Jazz Pharmaceuticals, Johnson & Johnson and The Epilepsy Therapy Development Project and has been involved in the design and development of new antiepileptics and CNS drugs as well as new formulations of existing drugs. None of the other authors has any conflict of interest to disclose. We, the authors, confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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■ ABBREVIATIONS USED

CNS, central nervous system; AED, antiepileptic drug; EMA, European Medicines Agency; FDA, Food and Drug Administration; NIH, National Institute of Health; SAR, structure−activity relationship; QSAR, quantitative structure−activity relationship; MES, maximal electroshock seizure; scMet, subcutaneous metrazol; SE, status epilepticus; ED_{50}, dose effective in 50% of test subjects; TD_{50}, dose neurotoxic in 50% of test subjects; PI, protective index; BBB, blood−brain barrier; ip, intraperitoneally; VPA, valproic acid; LDA, lithium diisopropylamide; LAH, lithium aluminum hydride; TFA, trifluoroacetic acid; DCM, dichloromethane; THF, tetrahydrofuran; DMPU, 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone; NMR, nuclear magnetic resonance; GC-MS, gas chromatography−mass spectrometry; TLC, thin-layer chromatography; mp, melting point

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