α-Fluoro-2,2,3,3-Tetramethylcyclopropanecarboxamide, a Novel Potent Anticonvulsant Derivative of a Cyclic Analogue of Valproic Acid

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2,2,3,3-Tetramethylcyclopropanecarboxylic acid (TMCA, **4**) is a cyclic analogue of the antiepileptic drug (AED) valproic acid (VPA) (**1**). α-F, α-Cl, α-Br, and α-methyl derivatives of **4** and their amides were synthesized and tested in rodent models for anticonvulsant potency and AED-induced teratogenicity. In the anticonvulsant rat-maximal electroshock (MES) and subcutaneous metrazol (scMet) tests, α-Cl-TMCD (**17**) had ED_{50} values of 97 and 27 mg/kg, respectively. α-F-TMCD (**11**) was 120 times more potent than VPA in the rat-scMet test (ED_{50} = 6 mg/kg) and had a protective index (PI = TD_{50}/ED_{50}) of 20. In the 6 Hz psychomotor mouse model **11** had ED_{50} values of 57 mg/kg (32 mA) and 59 mg/kg (44 mA). The ED_{50} values of **11** in the hippocampal-kindled rat model and in the pilocarpine-induced-status rat model were 30 and 23 mg/kg, respectively. Unlike **1**, **11** was nonteratogenic in mice. This novel compound has the potential to become a candidate for development as a new potent and safe antiepileptic and CNS drug.

Introduction

Epilepsy is a common neurological disorder characterized by recurrent seizures that affects about 1% of the world's population.[1] Despite the availability of >20 antiepileptic drugs (AEDs[a]), there is still a substantial need for the development of more effective and safer AEDs, since about 30% of epileptic patients are not seizure-free with the existing AEDs.[2] Furthermore, most of the currently utilized AEDs have side effects, which are sometimes severe and in some cases even fatal.[3]

Valproic acid (VPA, **1**, Figure 1), an eight-carbon branched short chain fatty acid, has a broad spectrum of anticonvulsant activities and is highly efficient in the treatment of various types of epilepsies. **1**, one of the four most prescribed AEDs, is also used for the treatment of bipolar disorders and migraine prophylaxis and is being currently tested as a possible anticancer drug.[4−7] The clinical utilization of **1** is limited by different side effects with the most serious of those being teratogenicity[8−10] and life-threatening hepatotoxicity.[11,12]

The induced teratogenicity of **1** is caused primarily by the parent compound,[13,14] but its induced hepatotoxicity is caused primarily by **1**'s metabolites 4-ene-VPA (**2**) and 2,4-diene-VPA

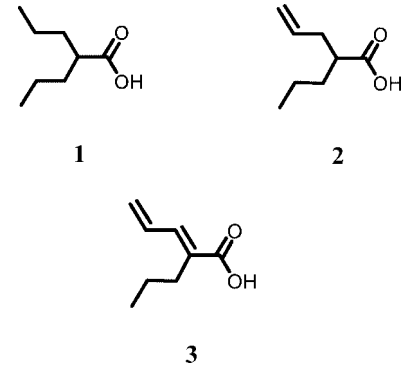

Figure 1. Structures of compounds **1**−**3**.

(**3**) (Figure 1), possessing a terminal double bond in their structure.[15,16] These metabolites are further biotransformed in vivo to chemically reactive intermediates that bind to cellular macromolecules and enzymes involved in fatty acids metabolism.[16−18]

Since the teratogenicity of these compounds is strictly structure dependent, numerous analogues and derivatives of **1** were investigated in an attempt to find new nonteratogenic and nonhepatotoxic CNS-active follow-up compounds that can become second generation to VPA.[13,14,19−28] Recent studies have revealed that further branching of **1** by the addition of a methyl group in the α position to the carboxyl group or to one of its side chains reduced the teratogenic and embryotoxic potency of these branched analogues but did not decrease their anticonvulsant activity.[14] The α-methylation of **1** led to a compound with improved anticonvulsant activity in the subcutaneous metrazol seizure threshold test (scMet).[14] The α-fluorination of **1** resulted in a reduced anticonvulsant activity profile and loss of hepatotoxicity.[15,17] α-Fluoro-VPA hydroxamic acid was found to be a nonteratogenic derivative of **1** with improved anticonvulsant activity compared to α-fluoro-VPA (**20**, Figure 4).[29]

2,2,3,3-Tetramethylcyclopropanecarboxylic acid (TMCA, **4**, Figure 2) is a cyclic analogue of **1**. The two quaternary carbons in the *β*-position to the carboxyl group prevent its biotransfor-

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[a] Abbreviations: AED, antiepileptic drug; VPA, valproic acid; CNS, central nervous system; SAR, structure−activity relationship; scMet, subcutaneous metrazol; MES, maximal electroshock seizure; SE, status epilepticus; PI, protective index; LEV, levetiracetam; TMCA, 2,2,3,3-tetramethylcyclopropanecarboxylic acid; TMCD, 2,2,3,3-tetramethylcyclopropanecarboxamide; TMCU, 2,2,3,3-tetramethylcyclopropanecarbonyl urea; VPD, valpromide; THF, tetrahydrofuran; LDA, lithiumdiisopropylamine; NFSI, *n*-fluorobenzenesulfonimide; BuLi, butyllithium; NTD, neural tube defects.

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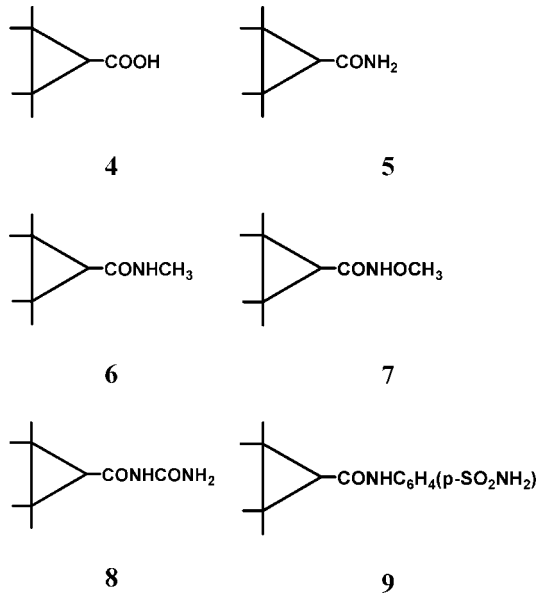


Figure 2. Structures of compound **4** and its amide derivatives **5**−**9**.

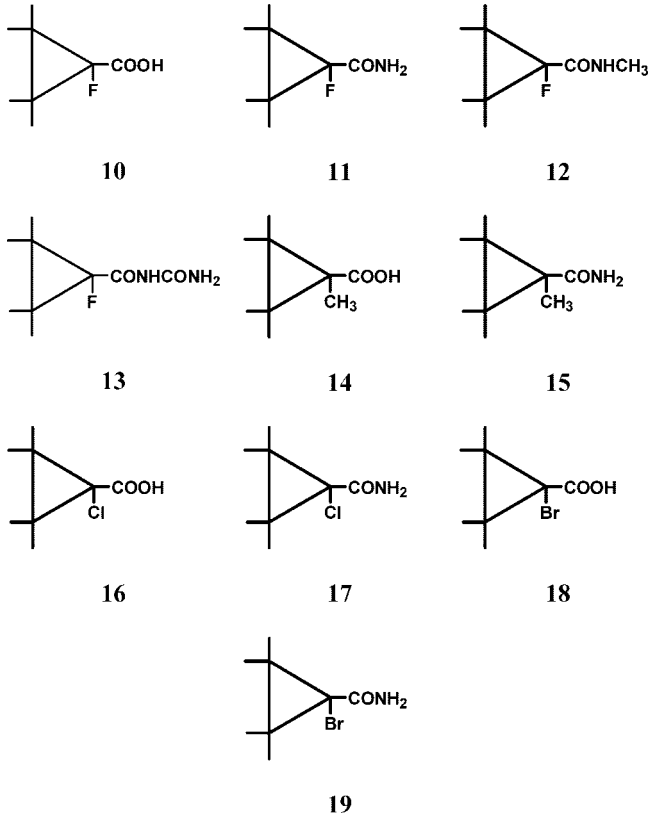


Figure 3. Structures of α substituted (by F, methyl group, Cl, or Br) derivatives of **4** (**10**, **14**, **16**, **18**) and their amides (**11**−**13**, **15**, **17**, and **19**).

mation to hepatotoxic metabolite(s) with a terminal double bond. **4** is inactive at the rat anticonvulsant maximal electroshock MES (ED_{50} > 150 mg/kg) model.[30] In contrast the following amide derivatives of **4**, 2,2,3,3-tetramethylcyclopropanecarboxamide (TMCD, **5**),[24,31] *N*-methyl-TMCD (MTMCD, **6**),[23,24,31] *N*-methoxy-TMCD (*N*-OCH_3-TMCD, **7**),[28] TMC urea[28] (TMCU, **8**), and *N*-(2,2,3,3,-tetramethylcyclopropanecarboxamide)-*p*-phenylsulfonamide (**9**)[32] (Figure 2), had potent anticonvulsant activity (ED_{50} values ranging between 10 and 90 mg/kg) in the rat-MES or scMet tests models. Even when administered to a

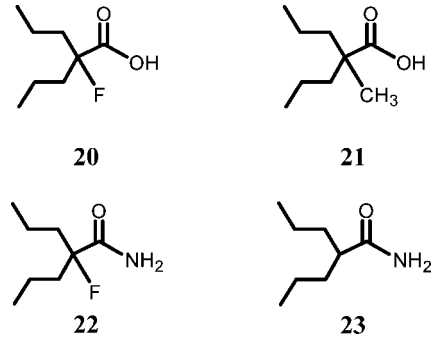


Figure 4. Structures of compounds **20**, **21**, **22**, and **23**.

mice strain sensitive to VPA-associated teratogenicity at doses several times higher than their anticonvulsant effective dose (ED_{50}) values, none of the above-mentioned derivatives of **4** (Figure 2) appeared to share the degree of teratogenicity caused by **1**.[23,33]

The amides of **20** and of its analogues have improved the anticonvulsant profile compared to the corresponding nonfluorinated amides[29,34] The present study dealt with the syntheses of the α-F, α-Cl, α-Br, and α-methyl derivatives of **4** and **5** and with the evaluation of their potency in two anticonvulsant model tests.

α-F-TMCD (**11**), the most potent of the synthesized novel compounds emerging from this study, was further investigated for quantification of its anticonvulsant activity values in the scMet test, in the 6 Hz psychomotor seizure model, in the hippocampal kindled rat model, and in the pilocarpine induced status model in rats. Its teratogenic and embryotoxic risks were determined using NMRI mice. The results are reported here.

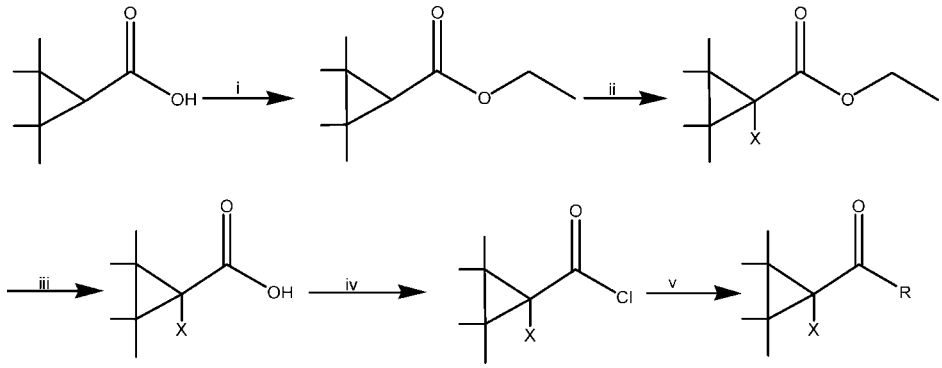
Chemistry

Compound **4** was the starting material for the syntheses of compounds **10**−**15** (Figure 3). **4** was converted to its corresponding ethyl 2,2,3,3-tetramethylcyclopropanecarboxylate (TMCE) with ethyl alcohol and sulfuric acid by a standard esterification method.[35] The hydrogen atom at the α-position to the carbonyl in TMCE was substituted by a fluorine atom using lithiumdiisopropylamine (LDA) as a base and *N*-fluorobenzensulfonimide (NFSI) as the substituting reagent. α-Methyl-TMCE was synthesized using LDA and methyl iodide. Both reactions were performed in dry tetrahydrofuran (THF) under nitrogen at −8 °C (Scheme 1).

The fluorinated or methylated esters were hydrolyzed by potassium hydroxide (0.045 mol in water/ethanol (1:1)). The ethanol was evaporated, the aqueous mixture was acidified to pH 1 using 1 N HCl, and the corresponding acids were extracted with ethyl acetate. Purification of the acids was performed by extraction of the ethyl acetate solution three times with a saturated solution of sodium bicarbonate, combining the extracts and acidification of the basic aqueous solution to pH 1 using 1 N HCl, followed by the extraction of the acidic solution with dichloromethane, yielding the corresponding acids **10** or **14**.

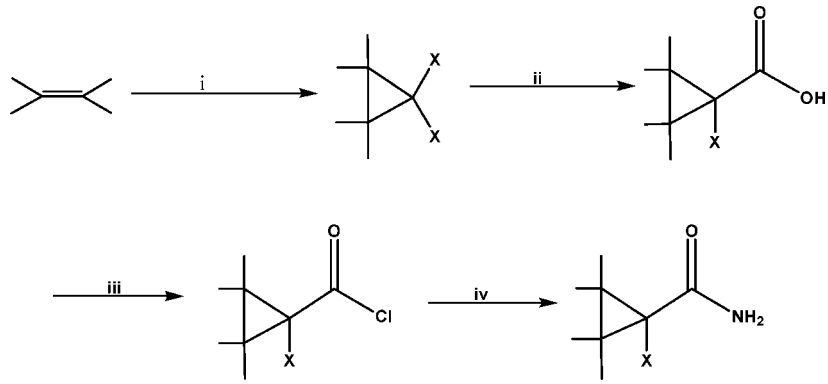
2,3-Dimethyl-2-butene was the starting material for compounds **16**−**19** (Figure 3). This olefin was reacted at 4 °C with chloroform or bromoform in the presence of potassium *tert*-butoxide in *tert*-butanol (via a dihalo carbene), yielding 1,1-dichloro or 1,1-dibromo-2,2,3,3-tetramethylcyclopropane correspondingly. The dihalo cyclopropanes were then reacted with butyllithium (BuLi) and carbon dioxide at −78 °C, yielding the desired α-chloro or α-bromo-2,2,3,3-tertramethylcyclopropane carboxylic acid **16** or **18** (Scheme 2).

Scheme 1. Synthesis of Compounds **10** and **14** and Their Amide Derivatives **11**−**13** and **15**[a]



[a] Reagents and conditions: (i) EtOH, H_2SO_4, reflux, 48 h. (ii) In the preparation of α-F-TMC ethyl ester (X = F): LDA, −8 °C, 40 min, NFSI, −8°C, 30 min. In the preparation of α-methyl-TMC ethyl ester (X = CH_3): LDA, −8°C, 40 min, CH_3I, −8°C, 30 min. (iii) 0.5 M KOH in H_2O/EtOH. (iv) $SOCl_2$, CH_2Cl_2, 0°C, 10 h. (v) In preparation of amides **11** and **15** (R = NH_2): 25% NH_4OH, 0 °C. In preparation of amide **12** (R = $NHCH_3$): methylamine, 0 °C. In preparation of amide **13** (R = $NHCONH_2$): NH_2CONH_2 in acetonitrile, 80 °C.

Scheme 2. Synthesis of Compounds **16** and **18** and Their Amide Derivatives **17** and **19**[a]



[a] Reagents and conditions. In the preparation of **16** (X = Cl): (i) $CHCl_3$, $^tBuO^-K^+$, ^tBuOH, 0 °C, 10 h, room temp; (ii) BuLi, −78 °C, 40 min, CO_2, 2 h, −78°C. In the preparation of **18** (X = Br): (i) $CHBr_3$, $^tBuO^-K^+$, ^tBuOH, 0 °C, 10 h, room temp; (ii) BuLi, −78 °C, 40 min, CO_2, 2 h, −78°C. In the preparation of **17** (X = Cl): (iii) $SOCl_2$, CH_2Cl_2, 0°C, 10 h; (iv) 25% NH_4OH, 0 °C. In the preparation of **19** (X = Br): (iii) $SOCl_2$, CH_2Cl_2, 0°C, 10 h; (iv) 25% NH_4OH, 0 °C.

Compounds **10**, **14**, **16**, and **18** were converted by $SOCl_2$ to the corresponding acyl chlorides and then coupled with the suitable amine in dry acetonitrile or dry dichloromethane to yield compounds **11**, **12**, **13**, **15**, **17**, and **19**. The synthesized products were purified by crystallization. ^1H NMR spectra of the synthesized compounds were measured in $CDCl_3$ using TMS as the internal standard. Elemental analyses were performed for all the synthesized compounds.

Results and Discussion

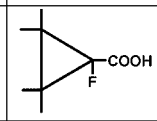
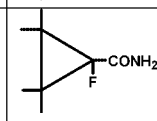
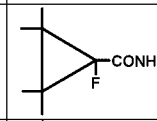
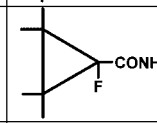
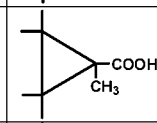
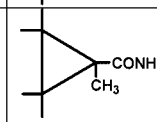
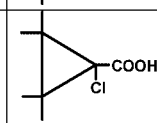
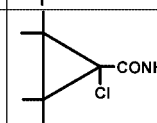
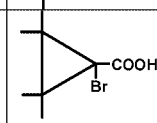
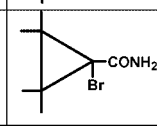
In spite of a large number of existing AEDs, about 30% of epileptic patients are still not seizure-free.[1−3] Because of multiple mechanisms of action, discovery of new potent analogues and derivatives of **1** is based on a structure−activity relationship (SAR) approach utilizing structural modification of the molecule (**1**) and comparative evaluation of the potency and safety margin of the new synthesized compunds in established anticonvulsant animal models. Compound **4**, a cyclic analogue of **1**, does not possess anticonvulsant activity in the MES test model.[30] Recently a number of amide derivatives of **4** were synthesized by our research group[23,24,28,31−33] and their anticonvulsant potency was evaluated in the MES and in the scMet tests in mice and in rats. These models predict the efficacy of a drug to treat human generalized tonic-clonic seizures (MES) and myoclonic seizures (scMet).[36] Compounds **5**−**9** (Figure 2) showed improved anticonvulsant activities relative to **1** in the MES and scMet tests, both in mice and in rats.[23,24,28,31−33] In mice, the protective index (PI) values of these compounds were of the same magnitude as that of **1**. However, in the rat, the PI values improved significantly and were much wider than those of **1**, especially for compound **8**.[28] All the CNS-active amide derivatives of **4** reported in the literature to date represented substitution of the hydroxyl group in the carboxyl moiety of **4** by different amine or amide groups, without modification of the tetramethylcyclopropane moiety.[23,24,28,31−33,37]

The present study explored the anticonvulsant activity of TMCA derivatives that are halogenated or methylated at the α-position to the carboxyl or carboxamide moiety (compounds **10**−**19**, Figure 3).

The positive preliminary results obtained for **11** in the rat scMet anticonvulsant model led us to synthesize additional TMCA derivatives (**12**−**19**, Figure 3) and explore SAR of these compounds.

By utilizing the modified procedure for the synthesis of α-fluoro-VPA (**20**, Figure 4),[17] we were able to obtain α-fluorotetramethylcyclopropanecarboxylic acid (α-F-TMCA, **10**) in high yields. Chlorination and bromination of **4** resulted in very low yields of the halogenated products α-Cl-TMCA (**16**) and α-Br-TMCA (**18**) using common literature procedures, such as the Hell−Volhard−Zelinski reaction frequently used for α-halogenations of carboxylic acids.[38,39] Therefore, syntheses of **18** was performed by a two-step reaction as described in Scheme

Table 1. Anticonvulsant Activity and Toxicity of Compounds **10**−**19** Administered Intraperitoneally to Mice

Compd	Structure	Dose (mg/kg)	MES[a] 0.5h[d]	MES[a] 4h[d]	scMet[b] 0.5h[d]	scMet[b] 4h[d]	Tox[c] 0.5h[d]	Tox[c] 4h[d]
10		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	0/3	0/1	0/1	3/8	0/4
		300	0/1	0/1	1/1	0/1	4/4	0/2
11		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	0/3	1/1	0/1	5/8	1/4
		300	1/1	0/1	1/1	0/1	4/4	0/2
12		30	0/1	0/1	0/1	0/1	1/4	1/2
		100	0/3	0/3	5/5	0/1	3/8	0/4
		300	1/1	0/1	0/1	0/1	4/4	0/2
13		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	0/3	0/1	2/5	0/8	0/4
		300	0/1	0/1	0/1	0/1	0/4	0/2
14		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	0/3	0/8	0/4	0/8	0/4
		300	1/1	0/1	0/8	0/4	1/4	0/2
15		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
16		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	0/3	0/1	0/1	2/8	1/4
		300	0/1	0/1	0/1	0/1	1/4	0/2
17		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	0/3	3/5	0/1	1/8	0/4
		300	1/1	0/1	1/1	0/1	4/4	0/2
18		50	0/1	0/1	0/1	0/1	0/4	0/2
		150	0/3	0/3	0/1	0/1	0/8	0/4
		450	0/1	0/1	0/1	0/1	0/4	0/2
19		50	0/1	0/1	0/1	0/1	1/4	0/2
		150	0/3	0/3	0/1	0/1	2/8	1/4
		450	0/1	0/1	0/1	0/1	4/4	0/2

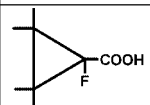
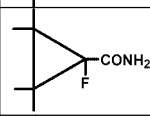
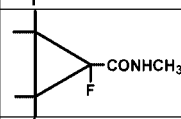
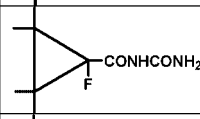
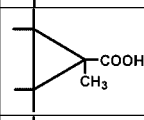
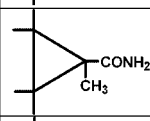
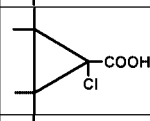
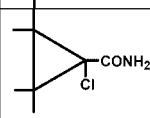
[a] Maximal electroshock test (number of animals protected/number of animals tested). [b] Subcutaneous metrazol test (number of animals protected/number of animals tested). [c] Neurotoxicity (number of animals exhibiting neurotoxicity/number of animals tested). [d] Time after drug administration.

2.[40,41] In the first step a 1,1-dibromo-2,2,3,3-tetramethylcyclopropane was obtained by reaction of 2,3-dimethyl-2-butene with a dibromocarbene, produced by the reaction of bromoform with potassium *tert*-butoxide.[40] In the second step, a metallodehalogenation reaction was carried out using butyllithium (BuLi) followed by passing carbon dioxide gas through the reaction mixture to yield the desired compound **18**. We utilized the above-described procedure for the synthesis of **16** by the reaction of dichloro carbene with 2,3-dimethyl-2-butene, followed by metallodehalogenation and carboxylation reaction as described above to yield compound **16**.

The anticonvulsant activity and toxicity profiles of the synthesized α-halogenated TMCA and their amide derivatives (compounds **10**−**19**, Figure 3) are presented in Tables 1−6. Marginal activity for compounds **11**, **12**, and **17** was obtained in the scMet test in mice using a 100 mg/kg dose (Table 1). The remaining compounds presented in Table 1 were inactive in the MES and scMet models tests.

With the exception of **17**, none of the tested compounds presented in Table 2 showed anticonvulsant activity in the rat MES test. Compounds **18** and **19** were not tested in this model because of a very low activity obtained by their screening in mice (Table 1). In the scMet model test in rats (Table 3) compound **11** exhibited excellent activity with no toxicity at 50 mg/kg. Compounds **10** and **15** showed marginal anticonvulsant activity, and **17** showed partial anticonvulsant activity at this dose. Compound **13** showed partial anticonvulsant activity at 30 mg/kg but was toxic and caused myoclonic jerks in some of the tested animals and therefore was not further investigated. In general, the α-halogenated and α-methyl free acids **10**, **14**, **16**, and **18** were inactive in the anticonvulsant scMet and MES tests (Tables 1−3). α-F-MTMCD (**12**) and α-F-TMCU (**13**)

Table 2. Anticonvulsant [Anti-MES] Activity and Toxicity of Compounds **10**−**17** Administered Orally to Rats[a]

Compd	Structure	Dose (mg/kg)	Activity & no of tested rats per time after drug administration					Tox[b]
			15min	30min	1h	2h	4h	
10		50	- 0/4	- 0/4	- 0/4	- 0/4	- 0/4	- 0/8
11		50	- 0/4	- 0/4	- 0/4	- 0/4	- 1/4	- 0/8
12		50	NT[c]	NT	NT	NT	NT	++++ 4/4
13		30	- 0/4	- 0/4	- 0/4	- 0/4	- 0/4	- 0/4
14		50	- 0/4	- 0/4	- 0/4	- 0/4	- 0/4	- 0/8
15		50	- 0/4	- 0/4	- 0/4	- 0/4	- 0/4	- 0/4
16		75	+ 1/4	+ 1/4	- 0/4	- 0/4	+ 1/4	- 0/8
17		50	+ 1/4	+ 1/4	+++ 3/4	+++ 3/4	- 0/4	- 0/8

[a] Symbols are as follows: ++++, 100% of the animals were protected; +++, 75% of the animals were protected; ++, 50% of the animals were protected; +, 25% of the animals were protected; −, no protection. In the case of toxicity: ++++, 100% of the animals exhibited neurotoxicity; +++, 75% of the animals exhibited neurotoxicity; ++, 50% of the animals exhibited neurotoxicity; +, 25% of the animals exhibited neurotoxicity; −, no neurotoxicity. [b] Neurotoxicity. [c] Not tested.

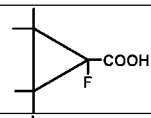
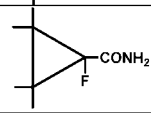
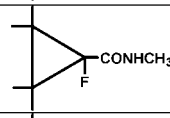
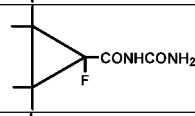
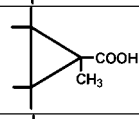
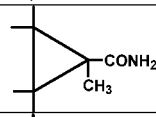
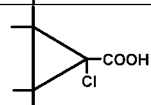
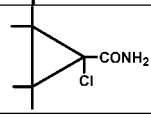
possess significantly reduced anticonvulsant activity in the MES and scMet models in mice (Table 1), compared to their parent compounds **6** and **8**.[23,24,28,31] Compound **12** was inactive at 50 mg/kg in the scMet model test in rats (Table 3). Compound **13** was slightly active in scMet (Table 3) and inactive in the MES test (Table 2). We assume that **12** and **13**, the fluorinated derivatives of **6** and **8**, activate one or more of their presumed targets with lower affinity than their nonfluorinated counterpartners.

α-Cl-TMCD (**17**) was active in the MES and scMet tests in rats at 50 mg/kg and showed no toxicity at this dose (Tables 2 and 3). The insertion of a chlorine atom (compound **17**) instead of the fluorine atom (compound **11**) at the α-position to the carboxamide **5** has a critical influence on the anticonvulsant activity in the rat MES test. Compound **19** was inactive in the MES and scMet tests in mice and was toxic at 450 mg/kg (Table 1). Furthermore, compound **15** showed reduced anticonvulsant activities compared to **5** (Tables 1 and 2), although α-methyl-VPA (**21**, Figure 4) was found to be more potent relative to **1**.[14] Compound **11** was more potent than **5** in the scMet model test both in mice and in rats (Tables 4 and 6). In a similar manner, α-fluoro-VPD (**22**, Figure 4) was more potent in the scMet test in mice than VPD (**23**),[29,34] exhibiting the same positive effect on anticonvulsant potency of an α-fluoro substituent in this compound. However, compound **20** (Figure 4) was less potent than **1** in the scMet anticonvulsant model test in mice.[17] At a dose of 50 mg/kg, **14** was inactive in the MES and in the scMet model tests in rats (Tables 2 and 3), and compound **15** was also inactive at the same dose (Tables 2 and 3) compared to **5** (Table 6).

The excellent potency of **11** in the rat scMet test (Table 2) led us to further investigation and quantification of its pharmacological properties in the following additional animal models for anticonvulsant activity: the 6 Hz psychomotor seizure model (Table 5), the rat-kindling model, and the pilocarpine-induced status model which is used to discover active compounds for the treatment of status epilepticus (SE).[42] SE is one of the most severe epileptic seizure conditions in which seizure duration lasts for 30 min or more and consciousness is not always regained.[43,44]

In the mice scMet model **11** was ∼7 times more potent than **1** and it was also more potent than compounds **23** and **5** (Table 4). In the 6 Hz model tests in mice at the currents of 32 and 44 mA, **11** was also more potent than **1**, **23**, and **5** and had relatively high PI (PI = TD_{50}/ED_{50}) values (Table 5). The high potency of the new AED levetiracetam (LEV) in the 6 Hz psychomotor

Table 3. Anticonvulsant [Anti-scMet] Activity and Toxicity of Compounds **10**−**17** Administered Orally to Rats[a]

Compd	Structure	Dose (mg/kg)	Times after drug administration 15min	30min	1h	2h	4h	Tox[b]
10		50	++ 2/4	- 0/4	- 0/4	- 0/4	- 0/4	- 0/8
11		50	++++ 4/4	+++ 3/4	+++ 3/4	+++ 3/4	++ 2/4	- 0/8
12		50	- 0/4	- 0/4	- 0/4	- 0/4	- 0/4	++++ 4/4
13		30	+ 1/4	+ 1/4	+ 1/4	++ 2/4	- 0/4	- 0/4
14		50	- 0/4	- 0/4	- 0/4	- 0/4	+ 1/4	- 0/8
15		50	- 0/4	- 0/4	+ 1/4	+ 1/4	- 0/4	- 0/4
16		75	- 0/4	- 0/4	- 0/3	+ 1/4	- 0/4	- 0/8
17		50	- 0/4	++ 2/4	++ 2/4	+ 1/4	- 0/4	- 0/8

[a] Symbols are as follows: ++++, 100% of the animals were protected; +++, 75% of the animals were protected; ++, 50% of the animals were protected; +, 25% of the animals were protected; −, no protection. In the case of toxicity: ++++, 100% of the animals exhibited neurotoxicity; +++, 75% of the animals exhibited neurotoxicity; ++, 50% of the animals exhibited neurotoxicity; +, 25% of the animals exhibited neurotoxicity; −, no neurotoxicity. [b] Neurotoxicity.

Table 4. Quantitative Anticonvulsant Data (Anti-MES and Anti-scMet) in Mice Dosed Intraperitoneally with Compound **11** Compared to **1**, **5**, **22**, and **23**

compd	MES[a] ED_{50}[g] (mg/kg)	PI[b]	scMet[c] ED_{50}[g] (mg/kg)	PI[d]	TD_{50}[e,g] (mg/kg)
1[f]	263 (237−282)	1.5	220 (177−268)	1.8	398 (356−445)
23[h]	56 (51−64)	1.4	55 (45−63)	1.5	81 (74−91)
22[i]	NT	NT	23	3.8	0.46
11	>200[j]		38[k] (26−54)	3.2	120[l] (108−135)
5[m]	>120		57 (39−76)	1.7	99 (85−109)

[a] Maximal electroshock test. [b] Protective index (PI = TD_{50}/ED_{50}) in the MES test. [c] Subcutaneous metrazol test. [d] Protective index (PI = TD_{50}/ED_{50}) in the scMet test. [e] Neurotoxicity. [f] Data taken from ref 36. [g] The interval in parentheses stands for the 95% confidence interval. [h] Data taken from ref 53. [i] Data taken from refs 29 and 34. [j] The ratio of protected-to-tested mice was 0/8 (60 and 150 mg/kg), 1/4 (200 mg/kg), 4/4 (400 mg/kg). [k] The ratio of protected-to-tested mice was 1/8 (25 mg/kg) and 8/8 (50 and 100 mg/kg). [l] The ratio of neurotoxic-to-tested mice was 0/8 (60 mg/kg), 1/8 (100 mg/kg), 3/8 (120 mg/kg), and 8/8 (150 mg/kg). [m] Data taken from ref 23.

Table 5. Quantitative Anticonvulsant Data in the 6 Hz Psychomotor Test in Mice Dosed Intraperitoneally with Compound **11** Compared to **1**, **5**, and **23**

compd	6 Hz test at 32 mA, ED_{50}[d] (mg/kg)	PI[a]	6 Hz test at 44 mA, ED_{50}[d] (mg/kg)	PI[b]
1[c]	126 (95−152)	3.2	310 (258−335)	1.3
23[e]	57 (47−65)	1.4	66 (29−87)	1.2
11	57[f] (32−85)	2.1	59[g] (42−75)	2
5[h]	72 (46−97)	1.4	>200	

[a] Protective index (TD_{50}/ED_{50} ratio) in the 32 mA current 6 Hz test. [b] Protective index (TD_{50}/ED_{50} ratio) in the 44 mA current 6 Hz test. [c] Data taken from ref 36. [d] The interval in parentheses stands for 95% confidence interval. [e] Data taken from ref 53. [f] The ratio of protected-to-tested rats was 1/8 (25 mg/kg), 4/8 (70 mg/kg), and 8/8 (130 mg/kg). [g] The ratio of protected-to-tested rats was 0/8 (25 mg/kg), 3/8 (50 mg/kg), 6/8 (75 mg/kg), and 7/8 (100 mg/kg). [h] Data taken from ref 23.

seizure test and its lack of anticonvulsant activity in the MES and scMet tests certify that LEV has anticonvulsant activity in seizures with different neuronal mechanisms than those induced by the MES and scMet models.[36] In the 6 Hz seizure test at 32 mA, LEV ED_{50} = 19 mg/kg, and at 44 mA its potency decreases significantly (ED_{50} = 1089 mg/kg).[36,45] We have shown that **11** is efficacious in the 6 Hz psychomotor model both at the 32mA current (ED_{50} = 57 mg/kg) and at the 44 mA current (ED_{50} = 59 mg/kg) with similar potency (ED_{50}) (Table 5). Compound **11**'s potency at 44 mA is by far better than that of LEV.[36] The unique pharmacological profile of this compound in the 6 Hz seizure test suggests it as a potential candidate for treatment of partial seizures and secondary generalized seizures in refractory epileptic patients, in analogy to LEV.[36,46]

The ED_{50} of **11** in the rat scMet test was 6 mg/kg (Table 6), being 120 and 10 times more potent than **1** and **23**, respectively. Compound **11** possesses an excellent safety margin with a PI

Table 6. Quantitative Anticonvulsant Data in Rats Dosed Orally with Compounds **11** and **17** Compared to Compounds **1**, **5**, **6**, **8**, and **23**

compd	MES[a] ED_{50}[g] (mg/kg)	PI[b]	scMet[c] ED_{50}[g] (mg/kg)	PI[d]	TD_{50c}[e,g] (mg/kg)
1[f]	485 (324−677)	1.6	646 (466−869)	1.2	784 (503−1176)
23[h]	32 (22−42)	2.7	59 (44−47)	1.5	87 (68−107)
5[i]	>250		52 (42−163)	7.3	381 (355−418)
6[i]	82 (64−102)	2.0	45 (31−55)	3.6	163 (138−179)
8[j]	29 (18−47)	18.5	92 (50−151)	5.9	538 (437−664)
11	>100[k]		6.4[l] (3.5−9.5)	20	117[m] (71−170)
17	97[n] (43−244)	>2.1	27[o] (16−39)	>7.8	>200[p]

[a] Maximal electroshock test. [b] Protective index (TD_{50}/ED_{50} ratio) in the MES test. [c] Subcutaneous metrazol test. [d] Protective index (TD_{50}/ED_{50} ratio) in the scMet test. [e] Neurotoxicity. [f] Data from ref 36. [g] The interval in parentheses stands for 95% confidence interval. [h] Data from ref 25. [i] Data from ref 23. [j] Data from ref 28. [k] The ratio of protected-to-tested rats was 0/8 (100 mg/kg). [l] The ratio of protected-to-tested rats was 0/8 (2 mg/kg), 5/8 (6 mg/kg), 6/8 (12.5 mg/kg), 8/8 (25 mg/kg). [m] The ratio of neurotoxic-to-tested rats was 1/8 (50 mg/kg), 3/8 (100 mg/kg), 6/8 (175 mg/kg), and 7/8 (250 mg/kg). [n] The ratio of protected-to-tested rats was 1/8 (25 mg/kg), 2/8 (50 mg/kg), 6/8 (100 mg/kg), 6/8 (150 mg/kg), and 4/8 (200 mg/kg). [o] The ratio of protected-to-tested rats was 1/8 (10 mg/kg), 3/8 (25 mg/kg), 4/7 (40 mg/kg), 8/8 (50 mg/kg). [p] The ratio of neurotoxic-to-tested rats was 0/8 (200 mg/kg).

value of 20 that is much wider than those of **1** (PI = 1.2) and **23** (PI = 1.5). Compound **11** was further tested in the hippocampal kindled rat model and was active with an ED_{50} of 30 mg/kg. It was also active in the rat pilocarpine-induced status model.[47] The main feature of the model consists of a large number of spontaneous recurrent seizures of both acute induced SE and chronic spontaneous seizures. At the time of SE induction by pilocarpine injection, **11** significantly reduced the development of seizures in the tested rats (status at time 0 test) and exhibited an ED_{50} of 23 mg/kg. It also showed protection against pilocarpine-induced SE 30 min after status induction (after first stage III seizure[54]) and exhibited an ED_{50} of 80 mg/kg.

The ability of **11** to prevent and to reduce seizure threshold in several types of seizures in a broad range of animal models of epilepsy suggests that this compound may have a multiple mechanism of action and, like **1**, it may exhibit a broad spectrum of action for epilepsy treatment.[48] The relatively good potency of this compound in the 6 Hz model at both 32 and 44 mA currents (Table 5) and in the rat kindling model may also imply that in analogy to LEV it can be useful for the treatment of therapy-resistant epileptic patients.

The teratogenicity and embryotoxicity of **11** was tested in the SWV mouse model at three different doses, and the results were compared to those obtained for **1** at similar doses (Table 7). The teratogenicity of **1** is one of its major side effects limiting its use in women of childbearing age.[10,49] Compound **1** induces high percentages of severe malformations in animal models[8,10,13,49] and in humans.[8,10,49] Evaluation of the teratogenic potential of derivatives and analogues of compound **1** is highly important for their development as new improved AEDs. Unlike **1**, compound **11** caused no malformations and was found to be nonteratogenic in the three doses administered: 671, 502, and 336 mg/kg. This compound, however, was toxic at 671 and 502 mg/kg both to the pregnant dams and to the embryos (Table 7). At the lower dose, 336 mg/kg, however, no toxic effect was observed. The doses of **11** presented in Table 7 are similar to the doses used for establishing the teratogenic effect of **1**.[23] It is important to emphasize that in the case of compound **11** the three tested doses are 18, 14, and 9 times higher than its ED_{50} values in the mice scMet test (Table 4) and 12, 9, and 6 times higher than its ED_{50} values in the 6 Hz model (Table 5), respectively. Therefore, a safety margin of at least 6 times is still maintained after administration of **11** at the 336 mg/kg dose.

Conclusions

The engine that has driven AED discovery is screening in animal models. Although there is a large number of models, the MES and scMet remain the "gold standard" in early stages of testing, since compounds active in the MES and scMet tests have generally been efficacious in clinical trials.[50,51] Among the corresponding α-halo-TMCD derivatives emerging from this study compound **11** was the most active compound. Its anticonvulsant potency, 120 times greater in the rat scMet test (Table 6) than that of the most widely used AED **1**, and its high potency in the 6 Hz psychomotor seizure test in mice, in the hippocampal-kindled rat model and in the pilocarpine induced status model in rats make it a good candidate for development as a new CNS drug. Compound **17** was found to be potent in the rat scMet test (ED_{50} = 27 mg/kg) and, unlike **11**, showed anticonvulsant activity also in the rat MES test (ED_{50} = 97 mg/kg, Table 6) and is also 4 times more potent than **1** in the rat MES test.[36] None of the α-halo- and α-methyltetramethylcyclopropanecarboxylic acids were found to be active in the MES and scMet tests both in mice and in rats (Tables 1−3). In addition, compounds **12**, **13**, **15**, and **19** exhibited no anticonvulsant activity in the mice MES and scMet tests (Table 1).

Compound **11**, a potent CNS-active derivative of **4**, possesses two quaternary carbons in the β-position to the carboxamide group and therefore cannot be converted to metabolites with a terminal double bond, analogues to hepatotoxic compound **2**.[12,15] It is also not teratogenic and is a much more potent compound possessing a much wider safety margin than **1**.[52] Since existing AEDs fail to control seizures in about 30% of epileptic patients and since all frontline AEDs exhibit teratogenic effects in humans, **11** could be a promising candidate for further development as a new, potent, and safe antiepileptic and CNS drug, taking into account its high potency also in the 6 Hz psychomotor seizure test, the hippocampal-kindled rat model, and the pilocarpine-induced status model in rats.

Experimental Section

Chemicals. All reagents were purchased from Sigma-Aldrich. The NFSI was purchased from Fluorochem U.K. Compounds **10**−**19** were prepared according to the methods described further in this section.

Materials and Methods. Product formation follow-up was performed by means of GC/MS and TLC techniques. TLC analyses were performed on precoated silica gel on aluminum sheets (Kieselgel 60 F254, Merck). Gas chromatography−mass spectroscopy assays were performed on an HP5890 series II GC instrument equipped with a Hewlett-Packard MS engine (HP5989A) single quadrupole MS spectrometer, HP7673 autosampler, HP MS-DOS Chemstation, and HP-5MS capillary column (0.25 μm × 15m × 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 until 200 °C; hold time, 3 min. The MS parameters were set as follows: source temperature, 180 °C (140 °C for compounds **18** and **19**); transfer line, 280 °C; positive ion monitoring, EI-MS (70 eV).

The chemical structure and purity of the synthesized compounds were assessed by GC/MS, NMR, and combustion elemental analysis. Melting points were determined on a Buchi 530 capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra, in $CDCl_3$ using TMS as 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 TMS. Coupling

Table 7. Teratogenic Effect of **11** Compared to **1** in the SWV Mouse Model[a]

compd	dose, mg/kg (mmol/kg)	no. of litters	no. of implants	no. of resorptions (%)	no. of live fetuses (%)	no. of dead fetuses (%)	no. of fetuses with NTD[b] (%)
control	0	11	128	0	128	0	0
1[c]	600 (3.6)	13	131	10 (7.6)	107 (81.7)	14 (10.7)[d]	57 (53.3)
1	452 (2.7)	13	160	18 (11.3)	141 (88.1)	1 (0.6)	41 (29.1)
1	301 (1.8)	12	154	17 (11.0)	133 (86.4)	4 (2.6)[d]	2 (1.5)
11	671 (4.2)	4	55	43 (78.2)[d,e]	12 (21.8)	0[d]	0[d]
11	502 (3.2)	10	127	34 (26.8)[d,e]	93 (73.2)	0	0[d]
11	336 (2.1)	8	112	11 (9.8)	101 (90.2)	0	0

[a] All dams received the drugs intraperitoneally on the morning of day 8 of gestation. [b] Neural tube defects. [c] Compound **1** was administered in the form of its sodium salt. [d] Significantly different when compared to the control group: $p < 0.05$, Fishers exact test. [e] Significantly different when compared to group treated with similar dose of **1**: $p < 0.05$, Fishers exact test.

constants (*J*) are given in hertz (Hz). Elemental analyses were performed on a 2400-2 Perkin-Elmer C, H, N, F, Br, Cl analyzer. Analyses of all newly synthesized compounds had satisfactory results indicating ≥98% purity.

General Procedure for the Synthesis of Compounds 10 and 14. Compound **4** was refluxed for 48 h with ethyl alcohol in a ratio of 1:15 in the presence of a catalytic amount of sulfuric acid. The excess of alcohol was removed under reduced pressure and the residue dissolved in 100 mL of hexane, washed with saturated sodium bicarbonate solution following by brine, dried over magnesium sulfate ($MgSO_4$), and filtered, and the hexane was evaporated. The ester, a colorless liquid, was further purified by vacuum distillation.

A solution of lithium diisopropylamine (LDA, 0.032 mol) was prepared by dissolving diisopropylamine in THF distilled over calcium hydride. The reaction mixture kept under nitrogen was cooled to −15 °C, and BuLi (1.6 M in hexanes, 0.032 mol) was added slowly while stirring and maintaining the temperature at −15 °C. The resulting mixture was stirred for additional 10 min, allowed to warm up to 0 °C, following by stirring for an additional 10 min. The reaction mixture cooled to −15 °C, TMCA ethyl ester (0.03 mol) dissolved in 5 mL of dry THF was added dropwise, and the mixture was stirred for 40 min at −15 °C.

The temperature was elevated to −8 °C, and an amount of 1.5 equiv of the α-substituiting reagent (NFSI for the fluorination and methyl iodide for the methylation) dissolved in dry THF was added to the reaction mixture. The reaction mixture was stirred for an additional 30 min while the formation of α-fluoro or α-methyl TMCA ethyl esters was monitored by GC−MS. After the reaction was completed the organic solvent was removed under reduced pressure and the oily residue dispersed in ethyl acetate and filtered by using a Buchner funnel and a vacuum pump. The ethyl acetate solution was washed with water, 1 N HCl, and brine, dried over $MgSO_4$, and filtered. The solvent was evaporated to yield α-fluoro or α-methyl TMCA ethyl ester as yellow oils.

The obtained ethyl esters were hydrolyzed to the corresponding acids using potassium hydroxide (0.045 mol) in a water/ethanol mixture (1:1, v/v), while monitoring the hydrolysis progress by GC−MS. Once the reaction was completed, the ethanol was evaporated and the remaining aqueous solution washed with hexane. The aqueous solution was cooled and slowly acidified to pH 1 using 1 N HCl and thereafter extracted three times with ethyl acetate. The organic fractions were combined and extracted three times with 5% sodium bicarbonate solution. The combined sodium bicarbonate extracts were acidified to pH 1 and extracted three times with ethyl acetate. The organic fraction was dried over $MgSO_4$ and filtered and the solvent was evaporated under vacuum to yield α-substituted TMCA typically as a white powder. Compounds **10** and **14** were purified by crystallization from ethyl acetate.

General Procedure for the Synthesis of Compounds 16 and 18. Chloroform or bromoform (0.053 mol) in the presence of potassium *tert*-butoxide solution in *tert*-butanol (0.064 mol) was reacted with 2,3-dimethyl-2-butene (0.053 mol) at 0 °C. The reaction mixture was allowed to warm up and stirred overnight at room temperature. The solvent was evaporated under reduced pressure and the residue dissolved in petroleum ether, washed three times with water and brine, dried over $MgSO_4$, and filtered. The solvent was evaporated under reduced pressure to yield the 1,1-dihalo-2,2,3,3-tetramethylcyclopropane as a white solid.

Under nitrogen atmosphere, 1,1-dihalo-2,2,3,3-tetramethylcyclopropane (0.02 mol) was dissolved in 30 mL of THF (freshly distilled over lithium aluminum hydride) and cooled to −78 °C. BuLi (1.6 M in hexane, 0.025 mol) diluted by dry THF (1:1, v:v) was slowly added dropwise and the reaction mixture stirred for 30 min at −78 °C following by passing carbon dioxide gas for 2 h through the reaction mixture. The reaction mixture was allowed to warm up to room temperature. The organic solvent was evaporated under reduced pressure and the residue dissolved in petroleum ether and extracted three times with 5% sodium bicarbonate in water. The water extracts were combined, cooled in an ice bath, acidified by 1 N HCl to pH 1, and extracted three times with dichloromethane. The organic extracts were combined, dried over $MgSO_4$, and filtered and the solvent was evaporated to yield **16** or **18** as white solids. They were further purified by crystallization from ethyl acetate.

General Procedure for the Synthesis of Compounds 11, 12, 15, 17, and 19. Thionyl chloride was added dropwise to the α-substituted TMC acids (**10**, **14**, **16**, **18**) dissolved in dry dichloromethane (DCM) and kept at 0 °C. The reaction mixture was stirred for 10 h, and the solvent and excess of thionyl chloride were removed by distillation. The appropriate acyl chlorides were used without further purification. The corresponding acyl chlorides (0.01 mol), dissolved in dry dichloromehane (4 mL), were slowly added at 0 °C to a stirred solution of 25% NH_4OH in water (10−20 mL) and dichloromethane (8 mL) or to a solution of methylamine (10 mL, 2 M in THF). The mixtures were kept at 0 °C for 60 min and then extracted three times with 25 mL of ethyl acetate. The extracts were combined and washed with 25 mL of water, 25 mL of HCl (1 N), and 25 mL of brine. The organic layer was dried over $MgSO_4$, filtered, and evaporated. The obtained amides were purified by crystallization from ethyl acetate.

1-Fluoro-2,2,3,3-tetramethylcyclopropanelcarboxylic Acid (10). Yield 54%; white powder; mp 121−123 °C; MS-EI, *m*/*z* (%) 160 (M^+, 0.46), 145 (69), 115 (100), 73 (38), 61 (70); R_f = 0.24 (DCM/MeOH, 97:3); ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS) 1.20−1.26 (dd, J = 2.1, 15.5 Hz, 18H); Anal. ($C_8H_{13}FO_2$) C, H, F.

1-Fluoro-2,2,3,3-tetramethylcyclopropanecarboxamide (11). Yield 76%; white needles; mp 99 °C; MS-EI, *m*/*z* (%) 159 (M^+, 0.5), 144 (100), 124 (20), 81 (29), 61 (26); R_f= 0.3 (DCM/MeOH, 97:3); ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS) 1.27−1.16 (bd, J = 33 Hz, 18H), 5.43 (1H) 6.3 (1H). Anal. ($C_8H_{14}FNO$) C, H, N, F.

***N*-Methyl-1-fluoro-2,2,3,3-tetramethylcyclopropanecarboxamide (12).** Yield 94%; yellow oil; MS-EI, *m*/*z* (%) 173 (M^+, 0.01) 158 (100), 138 (24), 81 (40), 58 (69); R_f = 0.15 (DCM/MeOH, 98:2); ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS) 1.13−1.32 (ddd, J = 42 Hz, 11, 6, 18H), 2.8 (d, J = 9 Hz, 3H) 6.43 (s, 1H). Anal. ($C_9H_{16}FNO$) C, H, N, F.

Synthesis of 1-Fluoro-2,2,3,3-tetramethylcyclopropanecarbonyl Urea (13). Urea (2.7 g, 0.045 mol), in dry acetonitrile (40 mL), was refluxed for 1 h, followed by dropwise addition of α-fluoro-TMC-acyl chloride (3.2 g, 0.018 mol). The reaction mixture was stirred under reflux for additional 2 h and cooled to room temperature, and the solvent was evaporated under reduced pressure. Chloroform (**40** mL) was added to the remaining oily residue, and

the solids were filtered by vacuum, dissolved in ethyl acetate, and washed with water. The organic extracts were dried over magnesium sulfate, filtered, and evaporated to yield the pure α-F-TMCU. Yield 62%; white crystals; mp 234 °C; MS-EI, *m/z* (%) 202 (M^+, 4), 187 (46), 144 (100), 81 (51), 61 (56); $R_f = 0.5$ (DCM/MeOH, 96:4); ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS) 1.206−1.271 (d, $J = 19.5$ Hz, 18H), 5.179 (1H), 8.068−8.293 (bd, $J = 67.5$ Hz, 2H). Anal. ($C_9H_{15}FN_2O_2$) C, H, N, F.

1-Methyl-2,2,3,3-tetramethylcyclopropanelcarboxylic Acid (α-Methyl-TMCA, 14). Yield 57%; white crystals; mp 86 °C; MS-EI, *m/z* (%) 156 (M^+, 0.3), 141 (100), 123 (30), 95 (55), 69 (23); $R_f = 0.33$ (DCM/MeOH, 98:2); ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS) 1.05 (S, 6H), 1.2 (S, 6H), 1.27 (3H). Anal. ($C_9H_{16}O_2$) C, H.

1-Methyl-2,2,3,3 tetramethylcyclopropanecarboxamide (α-Methyl-TMCD, 15). Yield 30%; white crystals; mp 111−112 °C; MS-EI, *m/z* (%) 156 (M^+, 0.4), 141 (100), 123 (28), 95 (53). 69 (26); $R_f = 0.29$ (DCM/MeOH, 98:2); ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS) 1.02 (6H), 1.15 (6H) 1.3 (3H) 5.28 (bd, $J = 30$ Hz, 2H). Anal. ($C_9H_{17}NO$) C, H, N.

1-Chloro-2,2,3,3-tetramethylcyclopropanelcarboxylic Acid (16). Yield 28%; white crystals; mp 128 °C; MS-EI, *m/z* (%) 176 (M^+, 1.1), 161 (100), 83 (85), 67 (33), 59 (70); $R_f = 0.27$ (DCM/MeOH, 97:3); ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS) 1.24−1.28 (d, $J = 11$ Hz, 12H). Anal. ($C_8H_{13}ClO_2$) C, H, Cl.

1-Chloro-2,2,3,3-tetramethylcyclopropanecarboxamide (17). Yield 86%; white crystals; mp 128 °C; MS-EI, *m/z* (%) 159 (M − 15, 100), 143 (28), 124 (23), 81 (24), 58 (54); $R_f = 0.67$ (DCM/MeOH, 97:3); ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS) 1.22−1.3 (d, $J = 25$ Hz, 12H), 5.43 (1H) 6.23 (1H). Anal. ($C_8H_{14}ClNO$) C, H, N, Cl.

1-Bromo-2,2,3,3-tetramethylcyclopropanecarboxylic Acid (18). Yield 33%; white crystals; mp 161−162 °C; MS-EI, *m/z* (%) 205, 207 (M − 15, 19,18), 123, 125 (30,34), 83 (100), 67 (40), 59 (59); $R_f = 0.19$ (PE/ether, 85:15); ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS) 1.24−1.26 (d, $J = 4$ Hz, 12H). Anal. ($C_8H_{13}BrO_2$) C, H, Br.

1-Bromo-2,2,3,3-tetramethylcyclopropanecarboxamide (α-Br-TMCD, 19). Yield 87%; white crystals; mp 157 °C; MS-EI, *m/z* (%) 204, 206 (M − 15, 53, 51), 124 (21), 81 (28), 67 (20), 58 (100); $R_f = 0.75$ (DCM/MeOH, 96:4); ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS) 1.25−1.26 (d, $J = 2.7$, 12H), 5.47−5.7 (bd, $J = 69$, 2H). Anal. ($C_8H_{14}BrNO$) C, H, N, Br.

Biological Testing. The evaluation of anticonvulsant activities in the MES, scMet, the 6 Hz psychomotor seizure test, the kindled rat seizure model, and the pilocarpine-induced status model, as well as the determination of toxicity in the rotorod test, positional sense test, and others, was performed at the NIH Epilepsy Branch (ADDP) as a part of the Anticonvulsant Drug Development Program according to the protocols described in ref 36. In general, 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 a 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 procedures, doses of the tested compounds were varied until a minimum of three to four points are established between the dose level of 0% protection and of 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 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 can prevent acute pilocarpine-induced status, the compound was given ip to male albino Sprague−Dawley rats (150−180 g). Then a challenge dose of pilocarpine was 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.[54] Compounds found to possess significant protection at time zero (time from the first stage III seizure) were proceeded to further evaluation in a sustained seizure model where the drug candidate was given 30 min after pilocarpine status induction (or after first stage III seizure). To calculate **11**'s ED_{50} at time 0, eight rats per dose were utilized at the following doses: 12, 25, 50, and 100 mg/kg. To calculate **11**'s ED_{50} at 30 min, 50, 75, and 100 mg/kg doses were utilized and the number of rats per dose was 7, 6, and 7, respectively.

Evaluation of Teratogenicity. The teratogenicity of the compounds was evaluated in the highly inbred SWV mice strain highly susceptible to VPA-induced neural tube defects (NTDs) according to a published procedure.[23] On day 8.5 of gestation, each dam received a single ip injection of the tested compounds in a range of 1.8−4.2 mmol/kg or the control (25% water solution of Cremophor EL, Fluka Biochemica, Germany). On day 18.5 of gestation, the dams were sacrificed by carbon dioxide asphyxiation, the location of all viable fetuses and resorption sites were recorded, and the fetuses were examined for the presence of exencephaly or other gross congenital abnormalities.

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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 investigational AED are available free of charge via the Internet at http://pubs.acs.org.

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