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Syntheses and Evaluation of Anticonvulsant Profile and Teratogenicity of Novel Amide Derivatives of Branched Aliphatic Carboxylic Acids with 4-Aminobenzensulfonamide

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Despite the availability of 14 new antiepileptic drugs (AEDs), about 30% of epileptic patients are not seizure-free. Consequently there is substantial need to develop new effective AEDs. A novel class of aromatic amides composed of phenylacetic acid or branched aliphatic carboxylic acids, with five to nine carbons in their carboxylic moiety, and aminobenzenesulfonamide were synthesized and evaluated in the anticonvulsant rat-maximal electroshock (MES) and subcutaneous metrazol seizure (scMet) tests. Fourteen of the synthesized amides had an anticonvulsant ED_{50} of <50 mg/kg in the rat-MES test. The amides 2-methyl-*N*-(4-sulfamoylphenyl)butyramide (**10**), 2-ethyl-*N*-(4-sulfamoylphenyl)butyramide (**11**), and 3,3-dimethyl-*N*-(4-sulfamoylphenyl)butyramide (**15**) were the most potent compounds possessing MES-ED_{50} values of 7.6, 9.9, and 9.4 mg/kg and remarkable protective index ($PI = TD_{50}/ED_{50}$) values of 65.7, 50.5, and 53.2, respectively. These potent sulfanylamides caused neural tube defects only at doses markedly exceeding their effective dose. The anticonvulsant properties of these compounds make them potential candidates for further development as new, potent, and safe AEDs.

Introduction

Epilepsy is a chronic disorder of the brain characterized by recurrent unprovoked seizures. These seizures are signs or symptoms of abnormal neuronal activity in the brain.[1] About 50 million people worldwide have epilepsy. In spite of the large therapeutic arsenal of old and new antiepileptic drugs (AEDs[a]), approximately 30% of epileptic patients are not seizure-free.[2−4] In many cases the clinical use of AEDs is restricted by their side effect. Therefore, a substantial need remains to discover novel chemical entities for the development of new effective and safer AEDs.[5−7]

In recent years, extensive anticonvulsant structure activity relationship (SAR) studies of numerous branched and nonbranched monocarboxylic fatty acids have been performed.[8−12] The nonbranched small-chain fatty acids were found to be inactive in anticonvulsant tests.[8−11,13−20] Valproic acid (VPA; 2-propylpentanoic acid, **1**, Figure 1) is a branched monocarboxylic acid with eight carbon atoms and an optimal chemical structure with regard to efficacy and safety margin between anticonvulsant activity and sedative/hypnotic adverse effects.[12,18,19] Many analogues and derivatives of **1** have been synthesized over the years in an attempt to find a superior compound that would retain the anticonvulsant activity associated with the basic structure of **1** but would be free of the adverse effects associated with chronic **1** therapy. The design of new derivatives and analogues of **1** is mainly based on previous empirical knowledge coming from SAR studies.[5,6,21] Amidation of **1** leads to the formation of valpromide (VPD, **2**, Figure 1), a compound with improved anticonvulsant potency and lower teratogenicity in animal models.[6,12] Unfortunately, these advantages in animals do not have clinical implications, since in humans **2** serves as a prodrug of **1**.[14] On the other hand amide derivatives of the constitutional isomers of **1** were found to be metabolically stable and significantly more potent as anticonvulsants compared to their corresponding acids.[15,16,22,23]

The urea derivatives of **1** and its constitutional isomers and analogues have been investigated and found to be active as

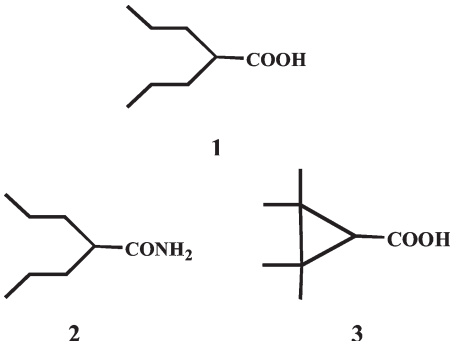

Figure 1. Structures of valproic acid (**1**), its corresponding amide valpromide (**2**), and valproic acid's cyclopropyl analogue 2,2,3,3-tetramethylcyclopropanecarboxylic acid (**3**).

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[a] Abbreviations: CNS, central nervous system; AED, antiepileptic drug; SAR, structure−activity relationship; MES, maximal electroshock seizure; scMet, subcutaneous metrazol; PI, protective index; VPA, valproic acid; VPD, valpromide; TMCA, 2,2,3,3-tetramethylcyclopropanecarboxylic acid; TMCD, 2,2,3,3-tetramethylcyclopropanecarboxamide; LDA, lithium diisopropylamine; BuLi, butyllithium; THF, tetrahydrofuran; NTD, neural tube defects.

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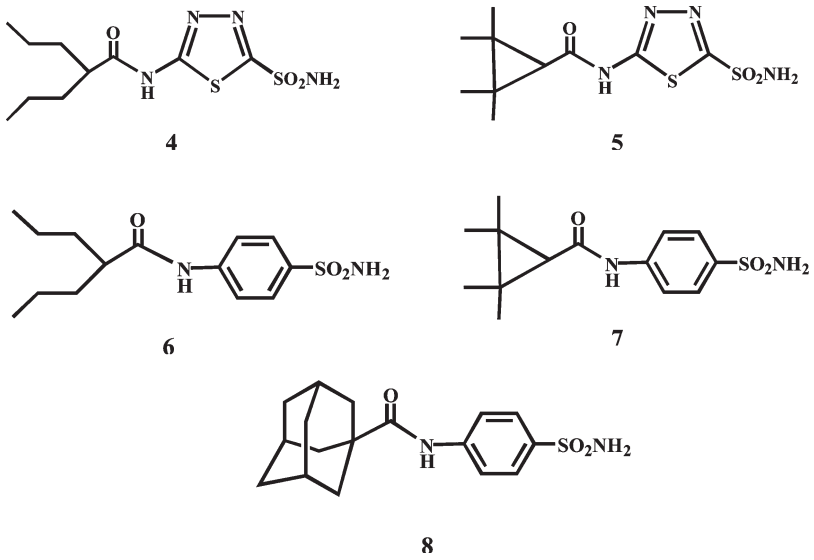


Figure 2. Carboxamide derivatives containing 4-aminobenzenesulfonamide (**6**, **7**, **8**) and thiadiazolesulfonamide (**4**, **5**) in their structures.

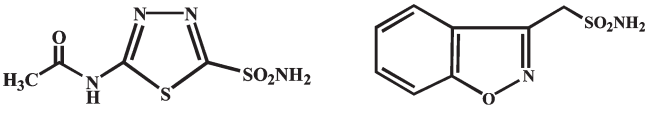


Figure 3. Chemical structures of the AEDs acetazolamide and zonisamide containing sulfonamide in their structures.

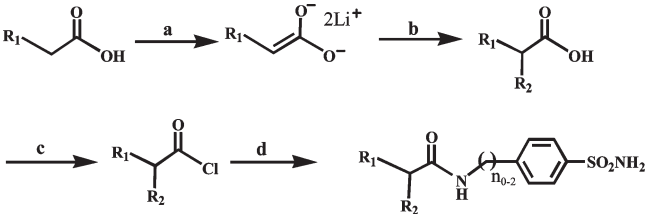
anticonvulsants.[24−27] Derivatives of urea with branched aliphatic carboxylic acids composed of six or seven carbons and possessing a tertiary carbon atom in their structures showed broad-spectrum anticonvulsant activity in maximal electroshock (MES) and subcutaneous metrazol (scMet) seizure tests.[28]

In the late 1970s Ganz and colleagues evaluated the anticonvulsant activity of nine derivatives of 4-aminobenzenesulfonamide (sulfanilamide) in MES and scMet seizure tests and found that they displayed an excellent anticonvulsant activity profile.[29] Acetazolamide (Figure 3) is an old AED, containing a sulfonamide group in its structure.[30] Syntheses of various aromatic sulfonamide derivatives of **1** demonstrated the impact of different sulfonamide moieties on their anticonvulsant activity.[31−34] It was shown that 5-valproylamido-1,3,4-thiamidazole-2-sulfonamide (**4**, Figure 2), a heterocyclic sulfonamide, displayed strong anticonvulsant activity in the MES test in mice.[31] The analogue of **4**, 5-(2,2,3,3-tetramethylcyclopropanecarboxamide)-1,3,4-thiamidazole-2-sulfonamide (**5**, Figure 2), also demonstrated potent anticonvulsant activity and a protective index ($PI = TD_{50}/ED_{50}$) above 50 in the MES seizure test in rats.[32] Tasso et al. have reported that 4-(valproylamido)benzenesulfonamide (**6**, Figure 2) is a potent anticonvulsant in the MES test in mice.[34]

2,2,3,3-Tetramethylcyclopropanecarboxylic acid (TMCA, **3**, Figure 1) is a cyclopropyl analogue of **1** displaying weak anticonvulsant activity and a small safety margin.[35] Recently, we found that 4-(2,2,3,3-tetramethylcyclopropanecarboxamido)-benzenesulfonamide (TMCD-benzenesulfonamide, **7**, Figure 2) was potent in the mice-MES test and exhibited high potency and a wide protective index ($PI = TD_{50}/ED_{50}$) in the rat-MES seizure test.[33]

There is a substantial need to discover novel chemical entities for the development of potent and safe new AEDs.

Scheme 1. Synthesis of Branched Chain Aliphatic Carboxylic Acids and Amide Derivatives[a]



[a] Reagents and conditions: (a) LDA, THF, −15 °C, 20 min; (b) methyl iodide, ethyl iodide, propyl iodide, or isopropyl iodide, THF, 0 °C, 12 h; (c) $SOCl_2$, CH_2Cl_2, 25 °C, 10 h; (d) 4-aminobenzenesulfonamide or 4-aminomethylbenzenesulfonamide or 4-aminoethylbenzenesulfonamide, acetone, pyridine, room temperature, 12 h.

Consequently, in this study we assessed the effect of changing the length and extending the branching of the aliphatic side chains in the carboxylic acids used for the derivatization with aminobenzenesulfonamides. We synthesized and comparatively evaluated the anticonvulsant activity and neurotoxicity of 18 new derivatives of 4-aminobenzenesulfonamide, three derivatives with 4-aminomethylbenezenesulfonamide, and one derivative with 4-aminoethylbenzenesulfonamide with phenylacetic acid or branched-chain aliphatic carboxylic acids possessing five to nine carbons in the structures of the carboxyl moieties. The teratogenic potency of the most potent anticonvulsant (rat-MES) compounds was evaluated. We aimed to determine the SAR of the above-mentioned compounds in an attempt to develop new potent and safe AEDs.

Chemistry

The general syntheses for the 4-aminobenzenesolfonamide derivatives of **1** and its analogues are depicted in Scheme 1. The starting material for compounds **9** and **26** was 2,2-dimethylpropionic acid; for compounds **16**, **17**, **18**, and **22** it was valeric acid; for compound **13**, it was isovaleric acid; for compounds **19**, **20**, **21**, and **24**, it was 3-methylvaleric acid; for compound **23**, it was 4-methylvaleric acid; for compounds **12**, **14**, **15**, and **27**, it was 3,3-dimethylbutyric acid; for compounds **10**, **11**, **28**, and **29**, it was butyric acid; for compound **25**, it was hexanoic acid; and for compound **30**, it was phenylacetic acid.

3-Methylvaleric acid, 2,2-dimethylpropionic acid, 3,3-dimethylbutyric acid, and phenylacetic acid were commercially available (Aldrich, St. Louis, MO). The carboxylic acids with extended branching were prepared by the conversion of the acids mentioned above to the enolates by use of lithium diisopropylamine (LDA), followed by condensation with the appropriate alkyl iodide to yield the corresponding branched carboxylic acids with six to nine carbons in their structures.[36] The carboxylic acids were converted by thionyl chloride to the corresponding acyl chlorides and then coupled with 4-aminobenzenesulfonamide or 4-aminomethylbenzenesulfonamide or 4-aminoethylbenzenesulfonamide in dry acetone and dry pyridine to yield the amides **9**−**30**, as depicted in Scheme 1. Compounds **10**, **12**, **14**, and **16**−**25** are chiral compounds possessing one or two stereogenic carbons in their structures. In this study they were synthesized by nonstereoselective methods and evaluated as racemates. The synthesized products were purified by crystallization. Their chemical structures were identified by ^{1}H NMR spectra measured in dimethyl sulfoxide-d_6 (DMSO), and their purity was established by elemental analyses.

Results and Discussion

A large variety of amide derivatives of the analogues of **1** have been synthesized and assayed as anticonvulsants.[12,14−17,20,37−39] Previous studies have shown that 4-(valproylamido)benzenesulfonamide (**6**, Figure 2) was potent as an anticonvulsant in the mice-MES test (ED_{50} = 21 mg/kg [70.46 μmol/kg]).[34] The 4-(2,2,3,3-tetramethylcyclopropanecarboxamido)benzenesulfonamide (**7**), a cyclic analogue of **6**, showed similar high anticonvulsant potency in the MES test in mice and rats (rat-ED_{50} = 26 mg/kg [87.83 μmol/kg]).[33] This indicates that the 4-aminobenzenesulfonamide derivatives of **1** and **3** had better anticonvulsant potency and higher PI values compared to **1**.[33,34]

In the present study we designed and synthesized a series of aromatic sulfonamides containing aminobenzenesulfonamides coupled with phenylacetic acid or with branched aliphatic carboxylic acids composed of five to nine carbon atoms in their structures and evaluated their anticonvulsant activity and neurotoxicity. Tables 1 and 2 present the results obtained for anticonvulsant potency and the neurotoxicity of compounds **9**−**30** in mice and rats, respectively.

Derivatives of the Analogues of 1 with Five Carbons. Among the synthesized 4-aminobenzenesulfonamide derivatives (**9**, **10**, and **26**, Table 1) containing five carbon atoms in their carboxyl moiety, compounds **9** and **10** were active and had rat-MES-ED_{50} values of 42.2 mg/kg (164.8 μmol/kg) and 7.6 mg/kg ([29.68 μmol/kg), respectively. **10** is the most potent compound discovered in this study, and it is 113 times more potent than **1** (utilizing ED_{50} as μmol/kg). The insertion of a methylene spacer between the carboxamide and the phenylsulfonamide group of **9** yielded **26** that had no anticonvulsant activity (Table 1). Compound **24** is a homologue of **10**. Alkylation at the β carbon adjacent to the carbonyl group (as in **24**) leads to a ∼2-fold decrease in the activity compared to alkylation at the α-carbon with the same substituent (as in **10**) (Table 3).

Derivatives of the Analogues of 1 with Six Carbons. The anticonvulsant potency varied among the compounds **11**, **15**, **16**, **24**, **27**, **28**, and **29**, all containing six carbon atoms in their carboxyl moiety (Table 1). In this group **15** was the most potent anticonvulsant with rat-MES-ED_{50} = 9.4 mg/kg (34.81 μmol/kg) followed by **11**, **16**, and **24** (Tables 1−3). The active anticonvulsant **15** was 97 times more potent than **1**. Insertion of a spacer with one carbon atom between the carboxamide and the phenylsulfonamide groups in **11** yielded **28** which had decreased anticonvulsant activity (9.9 mg/kg [36.66 μmol/kg] for **11** compared to 24.7 mg/kg [86.97 μmol/kg] for **28**), and their respective PI values dropped from 50 (**11**) to 20 (**28**). Insertion of a spacer with two carbon atoms between the same components as above leads to elimination of the anticonvulsant activity (9.9 mg/kg [36.66 μmol/kg] for **11** versus no activity for **29**). A similar phenomenon was noticed in **3** or adamantylcarboxylic acid, coupled with 4-aminobenzenesulfonamide or its analogues with spacers of one or two carbon atoms between the carboxamide and phenylsulfonamide moieties.[33,40]

Derivative of the Analogue of 1 with Seven Carbons. Compound **17**, a homologue of **6**, contains seven carbon atoms in its carboxylic moiety. **17** showed high anticonvulsant potency in the rat-MES test (rat-ED_{50} = 11.6 mg/kg [40.7 μmol/kg]), and it was 83 times more potent than **1**.

Derivatives of the Analogues of 1 with Eight Carbons. The widely used AED, **1**, is a branched (at carbon-2) monocarboxylic acid with eight carbon atoms in its structure (Figure 1). Its coupling product 4-(valproylamido)benzenesulfonamide (**6**, Figure 2) showed remarkable anticonvulsant activity in the mice-MES test.[34] We further explored the anticonvulsant activity of an additional six coupling products of 4-aminobenzenesulfonamide with branched short chain fatty acids, containing eight carbon atoms in their carboxylic moiety (compounds **12**, **13**, **18**, **19**, **23**, and **25**, Tables 1−3). The amide derivative **23** was the most active (rat-MES-ED_{50} = 16.7 mg/kg [56.04 μmol/kg]), being 60 times more potent than **1**. Compounds **18** and **25** were less potent than **23** and its other constitutional isomers; **12**, **13**, and **19** were inactive.

Derivatives of the Analogues of 1 with Nine Carbons. Among the synthesized amide derivatives of the acids containing nine carbon atoms in their carboxyl moiety and 4-aminobenzenesulfonamide (**14**, **20**, **21**, and **22**, Table 1) only **14** and **21** were active with rat-MES-ED_{50} values of 18 mg/kg (57.69 μmol/kg) and 35.8 mg/kg (114.74 μmol/kg), respectively.

In the synthesized series presented in Table 3, 2-methyl-*N*-(4-sulfamoylphenyl)butyramide (**10**) was the most potent compound exhibiting remarkable anticonvulsant properties (rat-MES-ED_{50} = 7.6 mg/kg [29.68 μmol/kg], PI = 66). However, in the scMet test it was inactive at doses up to 250 mg/kg. Zonisamide (Figure 3), a widely used AED containing a sulfonamide group in its structure, is less active than **10** in the rat-MES test (zonisamide-ED_{50} = 21 mg/kg, PI = 9.0).[41] Zonisamide, like all the synthesized compounds presented in Table 1, also did not display anticonvulsant activity at the rat-scMet test.[41]

Phenylacetamide. Phenylacetyl moiety is a lipophilic component in a series of phenylacetylurea derivatives.[42] Phenylacetylurea emerged as the most potent anticonvulsant compound effective in grand mal and petit mal epilepsies as well as in psychomotor seizures.[28,42,43] In the present study we synthesized **30**, a derivative of phenylacetic acid with 4-aminobenzenecarboxamide. **30** structurally differs from all the other amides presented in Table 1, since it contains two aromatic groups in its chemical structure (Table 1). We have found **30** to be more active at the rat-MES test (Table 3) than the previously reported analogous compound *N*-(2-chloro-4-sulfamoylphenyl)-2-phenylacetamide.[29] **30**, like the other

Table 1. Anticonvulsant Activity and Neurotoxicity of Compounds **9**−**30** Administered Intraperitoneally to Mice

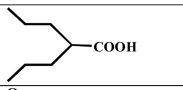
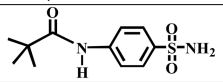
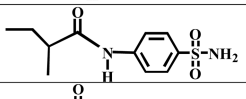
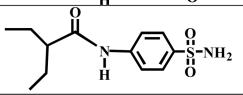
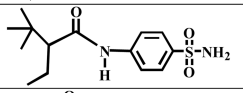
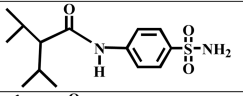
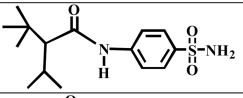
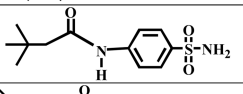
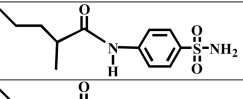
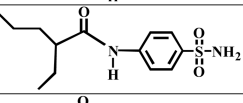
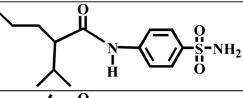
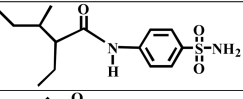
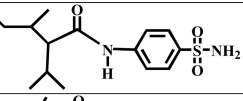
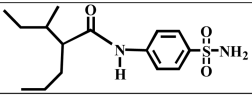
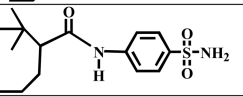
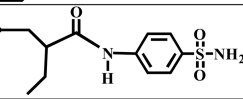
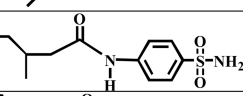
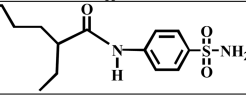
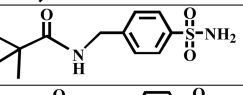
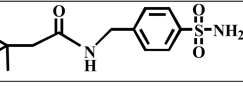
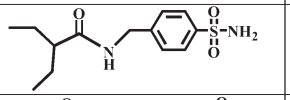
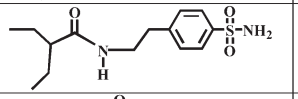
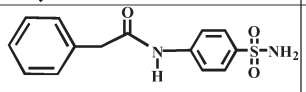
Compd.	Structure	Dose (mg/kg)	MES[a] 0.5h[d]	MES[a] 4h[d]	scMet[b] 0.5h[d]	scMet[b] 4h[d]	TOX[c] 0.5h[d]	TOX[c] 4h[d]
1		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	0/3	0/1	0/1	0/8	0/4
		300	1/1	0/1	1/1	0/1	4/4	0/2
9		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	1/3	2/3	0/1	0/1	2/8	0/4
		300	1/1	1/1	0/1	0/1	2/4	0/2
10		30	1/1	1/1	0/1	0/1	1/4	0/2
		100	3/3	3/3	0/1	0/1	1/8	0/4
		300	1/1	1/1	0/1	0/1	2/4	0/2
11		30	1/1	1/1	0/1	0/1	0/4	0/2
		100	3/3	3/3	0/1	0/1	0/8	0/4
		300	1/1	1/1	0/1	0/1	0/4	0/2
12		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	1/3	0/1	0/1	0/8	0/4
		300	0/1	1/1	0/1	0/1	0/4	0/2
13		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
14		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	1/3	3/3	0/1	0/1	0/8	0/4
		300	0/1	1/1	0/1	0/1	0/4	0/2
15		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	5/7	1/3	0/1	0/1	0/8	0/4
		300	4/5	1/1	0/1	0/1	0/4	0/2
16		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	2/3	0/1	0/1	0/8	0/4
		300	1/1	1/1	0/1	0/1	0/4	0/2
17		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	1/3	0/1	0/1	0/8	0/4
		300	0/1	1/1	0/1	0/1	0/4	0/2
18		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	2/3	0/1	0/1	0/8	0/4
		300	1/1	1/1	0/1	0/1	0/4	0/2
19		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	3/7	3/3	0/1	0/1	0/8	0/4
		300	4/5	1/1	0/1	0/1	0/4	0/2
20		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
21		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	1/3	2/3	0/1	0/1	0/8	0/4
		300	1/1	1/1	0/1	0/1	0/4	0/2
22		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
23		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	1/3	0/1	0/1	0/8	0/4
		300	0/1	1/1	0/1	0/1	0/4	0/2
24		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	0/3	0/1	0/1	0/8	0/4
		300	0/1	0/1	0/1	0/1	0/4	0/2
25		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	3/3	3/3	2/5	0/1	0/8	0/4
		300	1/1	1/1	3/5	0/1	1/4	1/2
26		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
27		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	1/3	0/1	0/1	0/8	0/4
		300	0/1	1/1	0/1	0/1	0/4	0/2

Table 1. Continued

Compd.	Structure	Dose (mg/kg)	MES[a] 0.5h[d]	MES[a] 4h[d]	scMet[b] h[d]4	scMet[b] h[d]0.5	TOX[c] h[d]4	TOX[c] h[d]0.5
28		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	3/3	1/3	0/1	0/1	0/8	0/4
		300	1/1	1/1	0/1	0/1	0/4	0/2
29		30	0/1	1/1	0/1	0/1	0/4	0/2
		100	0/3	1/3	0/1	0/1	0/8	0/4
		300	0/1	1/1	0/1	0/1	0/4	0/2
30		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	3/3	3/3	0/1	0/1	0/8	0/4
		300	1/1	1/1	0/1	0/1	0/4	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 evaluated as motor impairment or sedation (number of animals affected/number of animals tested). [d] Time after drug administration.

Table 2. Anticonvulsant (Anti-MES) Activity and Neurotoxicity of Compounds **9**−**30** Administered Orally to Rats

		number 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] 0.25−4 h
9	30	0/4	0/4	3/4	2/4	4/4	0/4
10	30	2/4	2/4	4/4	4/4	4/4	0/4
11	30	1/4	3/4	4/4	4/4	4/4	0/4
12	30	0/4	0/4	0/4	1/4	2/4	0/4
13	30	0/4	0/4	0/4	0/4	2/4	0/4
14	30	0/4	0/4	0/4	0/4	2/4	0/4
15	30	3/4	1/4	3/4	4/4	4/4	0/4
16	30	1/4	1/4	4/4	4/4	4/4	0/4
17	30	1/4	2/4	2/4	2/4	2/4	0/4
18	30	0/4	0/4	4/4	3/4	2/4	0/4
19	30	0/4	0/4	3/4	4/4	3/4	0/4
20	30	0/4	0/4	1/4	0/4	0/4	0/4
21	30	0/4	1/4	1/4	2/4	1/4	0/4
22	30	1/4	0/4	0/4	0/4	0/4	0/4
23	30	2/4	1/4	3/4	3/4	3/4	0/4
24	30	3/4	4/4	4/4	4/4	4/4	0/4
25	30	0/4	0/4	4/4	3/4	4/4	0/4
27	30	0/4	0/4	1/4	2/4	2/4	0/4
28	30	1/4	1/4	2/4	0/4	0/4	0/4
29	30	1/4	2/4	3/4	1/4	1/4	0/4
30	30	0/4	0/4	4/4	3/4	4/4	0/4

[a] Number of animals protected/number of animals tested. [b] Neurotoxicity evaluated as motor impairment or sedation (number of animals affected/number of animals tested). [c] Time after drug administration.

amide derivatives of branched carboxylic acids presented in Table 1, had no anticonvulsant activity in the rat-scMet test.

The anticonvulsant activity of compounds **9**−**11**, **15**, **16**, **19**, **23**−**25**, and **30** lasted from 1 to 4 h after dosing in all (**10** and **24**) or most of the rats (Table 2). In a survey of 257-marketed CNS drugs a relatively favorable anticonvulsant activity was indicated for compounds displaying optimal ClogP values (between 1 and 2).[44] The compounds with the highest anticonvulsant potencies **10**, **11**, **15**, **16**, **17**, **23**, **24**, and **30** (Table 3) were highly lipophilic (Table 4), which implies that penetration through the blood−brain barrier is an important factor influencing the drugs' efficacy. The correlation between lipophilicity expressed as ClogP (Table 4) and in vivo anticonvulsant activity of the compounds presented in Table 1 is not straightforward. The most active compound in this series was **10**, possessing ClogP of 1.01, whereas **20**, **21**, and **22** with higher ClogP values (Table 4) showed rather limited anticonvulsant activities (**21**) or were inactive (**20**, **22**) (Tables 1 and 2). These compounds possess methyl (**21**) or dimethyl substituents (**20**, **22**) in the β-position to the carbonyl in the carboxamide moieties. The above-mentioned methyl or dimethyl substituents present an additional steric hindrance in comparison to **10** and increase the compounds' bulkiness and thus decrease or prevent optimal receptor (target site) interaction with these compounds. The spatial arrangement of the alkyl groups in the chemical structures of the constitutional isomers **20**, **21**, and **22** may also considerably influence the compounds' anticonvulsant activity *in vivo*. In the homologous series of compounds with branching by an ethyl group at the carbon next to the carbonyl (compounds **10**, **11**, **17**, and **25**), homologues with shorter side chain length were very potent as anticonvulsant, whereas elongation of the side chain led to a decrease in anticonvulsant potency (Table 3).

In recent years the teratogenic properties of several isomers of **1** and their corresponding amides have been extensively studied in NMRI and SWV-mouse models for VPA-induced teratogenicity.[9,17,22,23] **1** has been shown to be highly teratogenic, resulting in a very high prevalence of exencephaly, a severe neural tube defect (NTD) in mice exposed in utero at critical time points of neural tube closure. Branching at the position β to the carbonyl group, as shown in the chemical structures of constitutional isomers of **1** (e.g., valnoctic acid and propylisopropyl acetic acid) as well as amidation of their carboxylic groups, reduces their teratogenic potency.[9,17,22] We have found that amide derivatives of branched aliphatic carboxylic acid with 4-aminobenzenesulfonamide were teratogenic and embryotoxic only at doses 3−73 times higher than their anticonvulsant MES-ED_{50} values (Tables 3 and 5). For example, **11** demonstrated a distinct teratogenicity and embryotoxicity at doses >29 times higher than its anticonvulsant MES-ED_{50} value (Tables 3 and 5).

At the lowest dose (0.55 mmol/kg) used in the teratogenicity study all tested CNS-active compounds presented in Table 5 were found to be nonteratogenic and nonembryotoxic except for **16**, **21**, and **25**. It is emphasized that **1** showed marked teratogenic activity and embryolethality at doses in the same range as its ED_{50} values with no separation between anticonvulsant activity and teratogenicity. In contrast **9**, **10**, **11**, **15**, **17**, **18**, **23**, **24**, **28**, and **30**, the novel CNS-active amide derivatives of aminobenzenesulfonamide with branched aliphatic carboxylic acids analogues to **1**, possessing five, six,

Table 3. Quantitative Anticonvulsant Data (Anti-MES and Anti-scMet) in Rats Dosed Orally

compd	MES[a] ED_{50}[f] (mg/kg) [μmol/kg]	PI[b]	scMet[c] ED_{50}[f] (mg/kg) [μmol/kg]	PI[d]	TD_{50}[e,f] (mg/kg) [μmol/kg]
1[g]	485 (324−677) [3368]	1.6	646 (466−869) [4486]	1.2	784 (503−1176) [5444]
9	42.2 (22.9−86.4) [164.8]	> 11.8			> 500 [1953]
10	7.6 (4.7−10.4) [29.7]	> 65.7	> 250	> 2	> 500 [1953]
11	9.9 (6.5−14.5) [36.7]	> 50.5	> 250	> 2	> 500 [1851]
14	18 (8.2−40) [57.7]	> 27.7	> 250	> 2	> 500 [1602]
15	9.4 (5.3−17.5) [34.8]	> 53.2	> 250	> 2	> 500 [1851]
16	10.8 (7.2−15.5) [40.0]	> 46.3	> 250	> 2	> 500 [1851]
17	11.6 (4.9−18.7) [40.7]	> 43.1	> 250	> 2	> 500 [1754]
18	41.5 (23.9−63.4) [139.3]	> 12.0	> 250	> 2	> 500 [1677]
21	35.8 (25.8−43.9) [114.7]	> 13.9	> 250	> 2	> 500 [1602]
23	16.7 (8.3−25.6) [56.0]	> 30.0			> 500 [1677]
24	16.4 (11.8−21.9) [60.7]	> 30.4	> 250	> 2	> 500 [1851]
25	23.9 (16.3−32.6) [80.2]	> 21.0	> 250	> 2	> 500 [1677]
28	24.7 (16.2−34) [87.0]	> 20.2	> 250	> 2	> 500 [1760]
30	16.1 (8.5−24.8) [55.5]	> 31.0	> 250	> 2	> 500 [1724]

[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] The interval in parentheses stands for the 95% confidence interval. [g] Data taken from ref 41.

Table 4. Lipophilicity Data (ClogP) of the Investigated Compounds

compd	ClogP	compd	ClogP
9	0.883	**20**	2.869
10	1.013	**21**	2.999
11	1.542	**22**	2.869
12	2.34	**23**	2.47
13	2.34	**24**	1.632
14	2.739	**25**	2.6
15	1.502	**26**	0.35
16	1.542	**27**	0.969
17	2.071	**28**	1.009
18	2.47	**29**	1.078
19	2.47	**30**	1.184

seven, and eight carbon atoms or phenylacetic acid in their carboxylic moieties, respectively, showed a marked difference between anticonvulsant activity and teratogenicity (Table 5).

In a recent published article, Enna and Williams discussed the current challenges in the search for new CNS drugs.[45] The authors concluded that because the majority of CNS drugs were discovered empirically, CNS drug discovery is less amenable to target-based approaches than in other therapeutic areas. Improving the success rate in CNS drug discovery requires a renewed emphasis on defining basic CNS functions in intact animals and a more systematic *in vivo* screening of novel structures. This should be in addition to designing second generation drugs of existing drugs with improved efficacy and safety.[45] The second generation approach is currently quite popular in the discovery of new AEDs including CNS-active sulfonamides,[3,6,21,46,47] and consequently, the current research is focused on this approach.

Conclusion

We have reported in this study on a novel class of anticonvulsant aromatic amides obtained by the coupling of 4-aminobenzenesulfonamide or 4-methylaminobenzenesulfonamide or 4-ethylaminobenzenesulfonamide with branched aliphatic carboxylic acids (analogues of **1** with five to nine carbons in their aliphatic carboxylic moiety), or the coupling of phenyl acetic acid with 4-aminobenzenesulfonamide. We have identified 14 compounds possessing anticonvulsant activity with rat (po)-MES-ED_{50} values of less than 50 mg/kg (Table 3). Compound **30** structurally differs from the other 21 compounds presented in Table 1, since it contains an additional aromatic group also in the chemical structure of the carboxamide moiety. It was 61 times more potent than **1** (Table 3). The ED_{50} values of the most active sulfonamides **10**, **11**, and **15** ranged from 7.6 to 10 mg/kg (30.0−35.0 μmol/kg) (Table 3). They are equipotent to the new generation of AEDs[48] and are 92−113 times more potent than **1**. The potent sulfonamides **10**, **11**, and **15** were teratogenic and embryotoxic only at doses >29 times larger than their MES-ED_{50} values, while **1** showed marked teratogenicity and embryo lethality at doses in the same range as its ED_{50} values.[41] **10** was the most potent compound evaluated in this study with anticonvulsant potency 113 times greater than that of **1** (Table 3). It contains only five carbon atoms in its carboxyl moiety, as opposed to eight carbons of **1**. The rat-MES PI value of **10** is 65.7 which is about 41 times higher than that of **1**.[41] **9**, a constitutional isomer of **10**, which contains a quaternary carbon atom α to carbonyl, was 5 times less potent than **10**. **11**, **15**, and **16** are homologues of **10** and possess identical anticonvulsant ED_{50} values (Table 3). Alkylation at the β-carbon adjacent to the carbonyl group versus the alkylation at the α-carbon (**24** versus **10**) by the same substituent leads to a less active homologue **24** (Table 3). We concluded that **10**, **11**, and **15** are the most potent among the compounds investigated in this study (Tables 1−3) and thus are potential candidates for further development to become new, potent, and safe AEDs.

Experimental Section

Chemicals. All common reagents were obtained from Sigma-Aldrich and used without further purification. Acetone, dichloromethane (DCM), tetrahydrofuran (THF), petroleum ether, and ethyl acetate were A.R. grade and purchased from Frutarom Israel. Dry acetone, dichloromethane, and tetrahydrofuran were obtained by their reflux over CaH_2 for 2 h and distillation prior to use. Dry *N*,*N*-dimethylpropyleneurea (DMPU) was obtained by its refluxing over CaH_2 for 2 h and distillation at 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 TLC techniques. TLC analyses were performed on precoated silica gel on aluminum sheets (Kieselgal 60 F_{254}, Merck). ^{1}H NMR spectra were recorded on a Varian Mercury series NMR 300 spectrometer. Chemical shifts (δ scale)

Table 5. Teratogenic Effect in the SWV Mouse Model[a] of Amide Derivatives of Phenylacetic Acid or Branched Aliphatic Carboxylic Acids with 4-Amino- or 4-Methylaminobenzensulfonamide in Comparison to VPA

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 NTDs[b] (%)
control	25% CEL	11	153	8 (5.2)	145 (94.8)	0	0
1[c]	452 (2.7)	13	160	18 (11.3)	141 (88.1)	1 (0.6)	41 (29.1)
1[c]	301 (1.8)	12	154	17 (11.0)	133 (86.4)	4 (2.6)	2 (1.5)
1[c]	181 (1.09)	12	156	12 (7.7)	144 (92.3)	0	0
1[c]	91 (0.55)	12	140	8 (5.7)	131 (93.6)	1 (0.7)	1 (0.8)
9	282 (1.09)	9	123	11 (8.9)	112 (91.1)	0	3 (2.7)
9	141 (0.55)	10	134	10 (7.5)	120 (93.3)	0	1 (3.7)
10	462 (1.8)	9	116	57 (49.1)	59 (50.9)	0	15 (25.4)
10	279 (1.09)	9	125	11 (8.8)	114 (91.2)	0	5 (4.4)
10	141 (0.55)	9	122	10 (8.2)	112 (91.8)	0	0
11	729 (2.7)	10	126	67 (53.2)	59 (46.8)	0	12 (20.3)
11	486 (1.8)	10	121	63 (52.1)	58 (47.9)	0	15 (25.9)
11	294 (1.09)	10	133	21 (15.8)	112 (84.2)	0	5 (4.5)
11	148 (0.55)	10	130	10 (7.7)	120 (93.3)	0	1 (0.8)
15	486 (1.8)	9	124	11 (8.9)	113 (91.1)	0	17 (15.0)
15	294 (1.09)	9	119	10 (8.4)	109 (91.6)	0	4 (3.7)
15	148 (0.55)	9	116	10 (8.6)	106 (91.4)	0	0
16	729 (2.7)	6	67	62 (92.5)	5 (7.5)	0	0
16	486 (1.8)	10	127	74 (58.3)	53 (41.7)	0	9 (17.0)
16	294 (1.09)	10	138	48 (34.8)	90 (65.2)	0	5 (5.6)
16	148 (0.55)	10	140	14 (10.0)	126 (90.0)	0	4 (3.2)
17	511 (1.8)	6	67	66 (98.5)	1 (1.5)	0	0
17	310 (1.09)	9	107	77 (72.0)	30 (28.0)	0	4 (13.3)
17	156 (0.55)	10	115	18 (15.7)	97 (84.3)	0	1 (1.0)
18	325 (1.09)	10	130	46 (35.4)	84 (64.6)	0	2 (2.4)
18	164 (0.55)	9	126	132 (10.3)	113 (100)	0	0
21	562 (1.8)	9	112	56 (50.0)	56 (50.0)	0	4 (7.1)
21	340 (1.09)	9	120	40 (33.3)	80 (66.7)	0	4 (5.0)
21	172 (0.55)	9	117	14 (12.0)	103 (88.0)	0	8 (7.8)
23	805 (2.7)	3	36	36 (100.0)	0	0	0
23	537 (1.8)	9	104	100 (96.2)	4 (3.8)	0	2 (50.0)
23	325 (1.09)	10	131	65 (49.6)	66 (50.4)	0	15 (22.7)
23	164 (0.55)	9	122	8 (6.6)	14 (95.4)	0	1 (0.9)
24	486 (1.8)	8	100	68 (68.0)	32 (32.0)	0	3 (9.4)
24	294 (1.09)	9	120	39 (32.5)	81 (67.5)	0	8 (9.9)
24	148 (0.55)	8	104	11 (10.6)	93 (89.4)	0	1 (1.1)
25	325 (1.09)	10	132	98 (74.2)	34 (25.8)	0	5 (14.7)
25	164 (0.55)	10	120	23 (19.2)	97 (80.8)	0	5 (5.2)
28	511 (1.8)	10	140	9 (6.4)	131 (93.6)	0	4 (3.1)
28	310 (1.09)	9	124	8 (6.5)	116 (93.5)	0	4 (3.4)
28	156 (0.55)	9	126	7 (5.6)	119 (94.4)	0	0
30	783 (2.7)	2	24	20 (83.3)	4 (16.7)	0	0
30	522 (1.8)	9	113	38 (34.5)	72 (63.7)	2 (1.8)	4 (3.0)
30	316 (1.09)	10	138	5 (3.6)	133 (96.4)	0	0
30	159 (0.55)	9	130	8 (6.2)	122 (93.8)	0	0

[a] All dams received the drugs intraperitoneally on the morning of day 8 of gestation. [b] Neural tube defects. [c] **1** was injected as sodium valproate. Data taken from ref 52.

are reported in parts per million (ppm) relative to residual solvent proton ($\delta = 2.50$ ppm). Multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), m (multiplet), dd (doublet of doublet), 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 analysis. Melting point was determined on a 100−230 VAC Mel-Temp capillary melting point apparatus. Elemental analyses were performed on a 2400−2 Perkin-Elmer C, H, N analyzer. C, H, N analyses of all newly synthesized compounds were within ±0.4 of theoretical values and thus were considered satisfactory.

General Procedure for the Syntheses of Compounds 9−30. 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 2,2-dimethylpropionic acid (for the synthesis of compounds **9** and **26**), valeric acid (for the synthesis of compounds **16**, **17**, **18**, and **22**), isovaleric acid (for the synthesis of compound **13**), 3-methylvaleric acid (for the synthesis of compounds **19**, **20**, **21**, and **24**), 4-methylvaleric acid (for the synthesis of compound **23**), 3,3-dimethylbutyric acid (for the synthesis of compounds **12**, **14**, **15**, and **27**), butyric acid (for the synthesis of compounds **10**, **11**, **28**, and **29**), hexanoic acid (for the synthesis of compound **25**), or phenylacetic acid (for the synthesis of compound **30**) were added. The mixture was allowed to stir for an additional 15 min at −5 °C. Then 72 mmol 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 alkyl iodide (methyl iodide, ethyl iodide, propyl iodide, or isopropyl iodide) in 10 mL of anhydrous THF. The reaction mixture was stirred 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 structures of the free carboxylic acids were identified by the ^{1}H NMR and GC/MS. The purified acids were converted with thionyl chloride to acyl chlorides according to a previously published method.[49] For the synthesis of an appropriate amide, the acyl chloride (9 mmol) of an individual acid dissolved in 20 mL of dry acetone was added dropwise to a stirred solution of suitable aromatic aminosulfonamide (9.2 mmol) and pyridine (9.1 mmol) in 50 mL of dry acetone. After addition, the reaction mixture was stirred for 12 h at room temperature and then the solvent was evaporated under reduce pressure. The residue was dissolved in 100 mL ethyl acetate and the organic phase washed three times with 20 mL of distilled water (3 × 20 mL). Then 10% HCl solution was added until pH 1 was reached, and the organic phase was separated from the aqueous phase and washed three times with brine. The aqueous solutions were combined and extracted with ethyl acetate (3 × 50 mL). The ethyl acetate extracts were combined, dried over $MgSO_4$, filtered, and evaporated under reduced pressure.

The obtained products were purified by crystallization using ethanol/petroleum ether mixture (1:3) to give (60−93% yield) white crystals.

2,2-Dimethyl-*N*-(4-sulfamoylphenyl)propionamide (9). White crystals; 88% yield; mp 228−229 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 1.25 (s, 9H), 7.25 (s, 2H, SO_2NH_2), 7.73 (d, J = 9, 2H, H−Ar), 7.85 (d, J = 9, 2H, H−Ar), 9.58 (s, 1H, NH). Anal. ($C_{11}H_{16}N_2O_3S$) C, H, N.

2-Methyl-*N*-(4-sulfamoylphenyl)butyramide (10). White crystals; 82% yield; mp 205−207 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 0.83−0.97 (t, J = 9, 3H), 1.10−1.15 (d, J = 6.3, 3H), 1.31−1.46 (m, 1H), 1.52−1.65 (m, 1H), 2.44 (m, 1H), 7.20 (s, 2H, SO_2NH_2), 7.61−7.83 (m, 4H, H−Ar), 10.21 (s, 1H, NH). Anal. ($C_{11}H_{16}N_2O_3S$) C, H, N.

2-Ethyl-*N*-(4-sulfamoylphenyl)butyramide (11). White crystals; 63% yield; mp 207−209 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 0.88−1.10 (t, J = 6, 6H), 1.43−1.73 (br m, 4H), 2.26 (m, 1H), 7.21 (s, 2H, SO_2NH_2), 7.62−7.80 (m, 4H, H−Ar), 10.22 (s, 1H, NH). Anal. ($C_{12}H_{18}N_2O_3S$) C, H, N.

2-Ethyl-3,3-dimethyl-*N*-(4-sulfamoylphenyl)butyramide (12). White crystals; 81% yield; mp 252−254 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 0.83 (t, J = 7.5, 3H), 0.98 (s, 9H), 1.41−1.71 (br m, 2H), 2.09−2.18 (dd, J = 8.7, 2.7, 1H), 7.22 (s, 2H, SO_2NH_2), 7.61−7.84 (m, 4H, H−Ar), 10.21 (s, 1H, NH). Anal. ($C_{14}H_{22}N_2O_3S$) C, H, N.

2-Isopropyl-3-methyl-*N*-(4-sulfamoylphenyl)butyramide (13). White crystals; 81% yield; mp 244 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 0.85−0.95 (t, J = 6, 12H), 1.81−2.13 (m, 3H), 7.23 (s, 2H, SO_2NH_2), 7.60−7.82 (m, 4H, H−Ar), 10.22 (s, 1H, NH). Anal. ($C_{14}H_{22}N_2O_3S$) C, H, N.

2-Isopropyl-3,3-dimethyl-*N*-(4-sulfamoylphenyl)butyramide (14). White crystals; 76% yield; mp 248−250 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 0.91−1.00 (m, 15H), 1.00−1.14 (s, 1H), 2.05 (m, 1H), 7.25 (s, 2H, SO_2NH_2), 7.62−7.82 (m, 4H, H−Ar), 10.22 (s, 1H, NH). Anal. ($C_{15}H_{24}N_2O_3S$) C, H, N.

3,3-Dimethyl-*N*-(4-sulfamoylphenyl)butyramide (15). White crystals; 82% yield; mp 211−213 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 1.10 (s, 9H), 2.21 (s, 2H), 7.23 (s, 2H, SO_2NH_2), 7.61−7.84 (m, 4H, H−Ar), 10.21 (s, 1H, NH). Anal. ($C_{12}H_{18}N_2O_3S$) C, H, N.

2-Methyl-*N*-(4-sulfamoylphenyl)pentanamide (16). White powder; 93% yield; mp 216−218 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 0.85 (t, J = 6.6, 3H), 1.15 (d, J = 6.9, 3H), 1.18−1.38 (m, 4H), 1.52−1.64 (m, 1H), 7.24 (s, 2H, SO_2NH_2), 7.62−7.82 (m, 4H, H−Ar), 10.22 (s, 1H, NH). Anal. ($C_{12}H_{18}N_2O_3S$) C, H, N.

2-Ethyl-*N*-(4-sulfamoylphenyl)pentanamide (17). White "cotton-like" powder; 83% yield; mp 203−205 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 0.84 (t, J = 6.6, 6H), 1.19−1.62 (br m, 6H), 2.20−2.43 (m, 1H), 7.24 (s, 2H, SO_2NH_2), 7.60−7.83 (m, 4H, H−Ar), 10.20 (s, 1H, NH). Anal. ($C_{13}H_{20}N_2O_3S$) C, H, N.

2-Isopropyl-*N*-(4-sulfamoylphenyl)pentanamide (18). White crystals; 89% yield; mp 217−219 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 0.78−0.90 (m, 9H), 1.12−1.36 (m, 2H), 1.36−1.63 (br m, 2H), 1.65−1.85 (m, 1H), 2.05−2.22 (m, 1H), 7.23 (s, 2H, SO_2NH_2), 7.61−7.84 (m, 4H, H−Ar), 10.20 (s, 1H, NH). Anal. ($C_{14}H_{22}N_2O_3S$) C, H, N.

2-Ethyl-3-methyl-*N*-(4-sulfamoylphenyl)pentanamide (19). White crystals; 71% yield; mp 203−205 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 0.78−0.90 (m, 9H), 1.05−1.22 (m, 1H), 1.40−1.68 (m, 4H), 2.08−2.22 (m, 1H), 7.22 (s, 2H, SO_2NH_2), 7.63−7.82 (m, 4H, H−Ar), 10.21 (s, 1H, NH). Anal. ($C_{14}H_{22}N_2O_3S$) C, H, N.

2-Isopropyl-3-methyl-*N*-(4-sulfamoylphenyl)pentanamide (20). White crystals; 69% yield; mp 230−233 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 0.81−1.00 (m, 12H), 1.03−1.26 (m, 1H), 1.38−1.58 (m, 1H), 1.72−1.80 (m, 1H), 1.90−2.08 (m, 1H), 2.08−2.24 (m, 1H), 7.23 (s, 2H, SO_2NH_2), 7.60−7.83 (m, 4H, H−Ar), 10.20 (s, 1H, NH). Anal. ($C_{15}H_{24}N_2O_3S$) C, H, N.

2-Propyl-3-methyl-*N*-(4-sulfamoylphenyl)pentanamide (21). White crystals; 72% yield; mp 223−226 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 0.81−0.98 (m, 9H), 1.05−1.30 (br m, 2H), 1.30−1.63 (br m, 5H), 2.24−2.30 (m, 1H), 7.23 (s, 2H, SO_2NH_2), 7.60−7.84 (m, 4H, H−Ar), 10.20 (s, 1H, NH). Anal. ($C_{15}H_{24}N_2O_3S$) C, H, N.

2-*tert*-Butyl-*N*-(4-sulfamoylphenyl)pentanamide (22). White crystals; 60% yield; mp 260−264 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 0.82−0.89 (t, J = 6.6, 3H), 0.89−1.08 (s, 9H), 1.08−1.22 (m, 1H), 1.32−1.44 (m, 2H), 1.56−1.78 (m, 1H), 2.26 (dd, J = 9, 3, 1H), 7.22 (s, 2H, SO_2NH_2), 7.63−7.81 (m, 4H, H−Ar), 10.21 (s, 1H, NH). Anal. ($C_{15}H_{24}N_2O_3S$) C, H, N.

2-Ethyl-4-methyl-*N*-(4-sulfamoylphenyl)pentanamide (23). White powder; 87% yield; mp 199−200 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 0.77−0.93 (m, 9H), 1.10−1.38 (m, 1H), 1.38−1.65 (br m, 4H), 2.36−2.45 (m, 1H), 7.21 (s, 2H, SO_2NH_2), 7.60−7.84 (m, 4H, H−Ar), 10.22 (s, 1H, NH). Anal. ($C_{14}H_{22}N_2O_3S$) C, H, N.

3-Methyl-*N*-(4-sulfamoylphenyl)pentanamide (24). White crystals; 76% yield; mp 204−206 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 0.83−0.92 (m, 6H), 1.14−1.23 (m, 1H), 1.23−1.40 (m, 1H), 1.83−1.98 (m, 1H), 2.09−2.23 (dd, J = 15, 7.5, 1H), 2.23−2.36 (dd, J = 15, 6, 1H), 7.21 (s, 2H, SO_2NH_2), 7.61−7.84 (m, 4H, H−Ar), 10.22 (s, 1H, NH). Anal. ($C_{12}H_{18}N_2O_3S$) C, H, N.

2-Ethyl-*N*-(4-sulfamoylphenyl)hexanamide (25). White powder; 90% yield; mp 188−190 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 0.78−0.86 (m, 6H), 1.12−1.37 (m. 4H), 1.37−1.65 (br m, 4H), 2.23−2.46 (m, 1H), 7.20 (s, 2H, SO_2NH_2), 7.64−7.81 (m, 4H, H−Ar), 10.20 (s, 1H, NH). Anal. ($C_{14}H_{22}N_2O_3S$) C, H, N.

2,2-Dimethyl-*N*-(4-sulfamoylbenzyl)propionamide (26). White crystals; 70% yield; mp 157−158 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 1.15 (s, 9H), 4.32 (d, J = 6, 2H), 7.30 (s, 2H, SO_2NH_2), 7.42 (d, J = 7.8, 2H, H−Ar), 7.80 (d, J = 8.1, 2H, H−Ar), 8.17 (t, J = 6.6, 1H, NH). Anal. ($C_{12}H_{18}N_2O_3S$) C, H, N.

3,3-Dimethyl-*N*-(4-sulfamoylbenzyl)butyramide (27). White crystals; 80% yield; mp 205−207 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 0.98 (s, 9H), 2.09 (s, 2H), 4.30 (d, J = 6, 2H), 7.38 (s, 2H, SO_2NH_2), 7.45 (d, J = 9, 2H, H−Ar), 7.80 (d, J = 9, 2H, H−Ar), 8.32 (t, J = 6.7, 1H, NH). Anal. ($C_{13}H_{20}N_2O_3S$) C, H, N.

2-Ethyl-*N*-(4-sulfamoylbenzyl)butyramide (28). White crystals; 62% yield; mp 148−150 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 0.88−1.03 (t, J = 6, 6H), 1.42−1.70 (br m,

4H), 2.11 (m, 1H), 4.38 (d, J = 6, 2H), 7.29 (s, 2H, SO_2NH_2), 7.45 (d, J = 9, 2H, H−Ar), 7.79 (d, J = 9, 2H, H−Ar), 8.30 (t, J = 6.6, 1H, NH). Anal. ($C_{13}H_{20}N_2O_3S$) C, H, N.

2-Ethyl-*N*-[2-(4-sulfamoylphenyl)ethyl]butyramide (29). White crystals; 60% yield; mp 181−183 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 0.76−0.88 (t, J = 6, 6H), 1.20−1.53 (br m, 4H), 1.83−2.00 (m, 1H), 2.85 (t, J = 9, 2H), 3.35 (q, J = 6, 2H), 7.28 (s, 2H, SO_2NH_2), 7.44 (d, J = 9, 2H, H−Ar), 7.80 (d, J = 6, 2H, H−Ar), 7.98 (t, J = 4, 1H, NH). Anal. ($C_{14}H_{22}N_2O_3S$) C, H, N.

2-Phenyl-*N*-(4-sulfamoylphenyl)acetamide (30). White crystals; 85% yield; mp 210−211 °C. ^{1}H NMR (300 MHz, CD_3SOCD_3, δ): 3.63 (s, 2H), 7.25 (s, 2H, SO_2NH_2), 7.38 (m, 4H, H−Ar), 7.78 (s, 4H, H−Ar), 10.50 (s, 1H, NH). Anal. ($C_{14}H_{14}N_2O_3S$) C, H, N.

Biological Testing. The evaluation of anticonvulsant activity in the maximal electroshock seizure test (MES) and subcutaneous metrazol seizure threshold test (scMet) and the determination of neurotoxicity in the rotorod test, positional sense test, and others were performed at the NIH Epilepsy Branch as a part of the Anticonvulsant Drug Development Program according to the protocols described in ref 41.

Preparation of the Compounds for Testing. The tested compounds were suspended in 0.5% methylcellulose and administered (a) intraperitioneally (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 a 0.85% solution for administration to mice and a 2.82% solution for administration to rats.[41]

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 three to four points were established between the dose level of 0% protection and of 100% protection. These data were subjected to the FORTRAN probit analysis program,[41] 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}.[41]

Evaluation of Teratogenicity. The teratogenic properties of the compounds were evaluated in the highly inbred SWV mice strain known from its high susceptibility to VPA-induced neural tube defects (NTDs).[50,51] Two-month-old nulligravid females were mated overnight with males and examined for the presence of vaginal plugs the following morning, and the onset of gestation was considered to be 10 p.m. of the previous night, the midpoint of the dark cycle. At day 8.5 of gestation, each dam received a single ip injection of the tested compounds in a range of 0.55−2.7 mmol/kg or the vehicle (25% water solution of Cremophor EL, Fluka Biochemica Germany). The volume of injection was 10 μL/g of body weight. At gestation day 18.5 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. The teratogenicity data (implantations, resorptions, and NTDs) were evaluated for significant differences between groups by analyzing the contingency table with Fisher's exact test. Statistical analysis was conducted using GraphPad InStat (version 3.06; GraphPad Software, San Diego, CA), and the results of all tests were considered to be statistically significant when p was less than 0.05.

Calculation of ClogP. ClogP was calculated by means of ChemDraw-Ultra Software 8.

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Supporting Information Available: Elemental analysis results for compounds **9**−**30** 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.

References

(1) Fisher, R. S.; van Emde Boas, W.; Blume, W.; Elger, C.; Genton, P.; et al. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia* **2005**, *46*, 470–472.
(2) Holmes, G. L. Animal model studies application to human patients. *Neurology* **2007**, *69*, S28–S32.
(3) Perucca, E.; French, J.; Bialer, M. Development of new antiepileptic drugs: challenges, incentives, and recent advances. *Lancet Neurol.* **2007**, *6*, 793–804.
(4) Smith, M.; Wilcox, K. S.; White, H. S. Discovery of antiepileptic drugs. *Neurotherapeutics* **2007**, *4*, 12–17.
(5) Bialer, M.; Johannessen, S. I.; Kupferberg, H. J.; Levy, R. H.; Perucca, E. Progress report on new antiepileptic drugs: a summary of the eighth Eilat conference (EILAT VIII). *Epilepsy Res.* **2007**, *73*, 1–52.
(6) Bialer, M.; Yagen, B. Valproic acid: second generation. *Neurotherapeutics* **2007**, *4*, 130–137.
(7) Nau, H.; Loscher, W. Pharmacologic evaluation of various metabolites and analogs of valproic acid: teratogenic potencies in mice. *Fundam. Appl. Toxicol.* **1986**, *6*, 669–676.
(8) Abbott, F. S.; Acheampong, A. A. Quantitative structure−anticonvulsant activity relationships of valproic acid, related carboxylic acids and tetrazoles. *Neuropharmacology* **1988**, *27*, 287–294.
(9) Bojic, U.; Elmazar, M. M.; Hauck, R. S.; Nau, H. Further branching of valproate-related carboxylic acids reduces the teratogenic activity, but not the anticonvulsant effect. *Chem. Res. Toxicol.* **1996**, *9*, 866–870.
(10) Isoherranen, N.; Yagen, B.; Bialer, M. New CNS-active drugs which are second-generation valproic acid: can they lead to the development of a magic bullet? *Curr. Opin. Neurol.* **2003**, *16*, 203–211.
(11) Liu, M. J.; Pollack, G. M. Pharmacokinetics and pharmacodynamics of valproate analogues in rats. IV. Anticonvulsant action and neurotoxicity of octanoic acid, cyclohexanecarboxylic acid, and 1-methyl-1-cyclohexanecarboxylic acid. *Epilepsia* **1994**, *35*, 234–243.
(12) Loscher, W.; Nau, H. Pharmacological evaluation of various metabolites and analogues of valproic acid. Anticonvulsant and toxic potencies in mice. *Neuropharmacology* **1985**, *24*, 427–435.
(13) Badir, K.; Haj-Yehia, A.; Vree, T. B.; van der Kleijn, E.; Bialer, M. Pharmacokinetics and anticonvulsant activity of three monoesteric prodrugs of valproic acid. *Pharm. Res.* **1991**, *8*, 750–753.
(14) Bialer, M. Clinical pharmacology of valpromide. *Clin. Pharmacokinet.* **1991**, *20*, 114–122.
(15) Haj-Yehia, A.; Bialer, M. Structure−pharmacokinetic relationships in a series of valpromide derivatives with antiepileptic activity. *Pharm. Res.* **1989**, *6*, 683–689.
(16) Haj-Yehia, A.; Bialer, M. Structure−pharmacokinetic relationships in a series of short fatty acid amides that possess anticonvulsant activity. *J. Pharm. Sci.* **1990**, *79*, 719–724.
(17) Isoherranen, N.; Yagen, B.; Woodhead, J. H.; Spiegelstein, O.; Blotnik, S.; Wilcox, K. S.; Finnell, R. H.; Bennett, G. D.; White, H. S.; Bialer, M. Characterization of the anticonvulsant profile and enantioselective pharmacokinetics of the chiral valproylamide propylisopropyl acetamide in rodents. *Br. J. Pharmacol.* **2003**, *138*, 602–613.
(18) Keane, P. E.; Simiand, J.; Mendes, E.; Santucci, V.; Morre, M. The effects of analogues of valproic acid on seizures induced by pentylenetetrazol and GABA content in brain of mice. *Neuropharmacology* **1983**, *22*, 875–879.

(19) Morre, M.; Keane, P. E.; Vernieres, J. C.; Simiand, J.; Roncucci, R. Valproate: recent findings and perspectives. *Epilepsia* **1984**, *25* (Suppl. 1), S5–S9.
(20) Redecker, C.; Altrup, U.; Hoppe, D.; Dusing, R.; Speckmann, E. J. Effects of valproate derivatives I. Antiepileptic efficacy of amides, structural analogs and esters. *Neuropharmacology* **2000**, *39*, 254–266.
(21) Bialer, M. New antiepileptic drugs that are second generation to existing antiepileptic drugs. *Expert. Opin. Invest. Drugs* **2006**, *15*, 637–647.
(22) Radatz, M.; Ehlers, K.; Yagen, B.; Bialer, M.; Nau, H. Valnoctamide, valpromide and valnoctic acid are much less teratogenic in mice than valproic acid. *Epilepsy Res.* **1998**, *30*, 41–48.
(23) Spiegelstein, O.; Bialer, M.; Radatz, M.; Nau, H.; Yagen, B. Enantioselective synthesis and teratogenicity of propylisopropyl acetamide, a CNS-active chiral amide analogue of valproic acid. *Chirality* **1999**, *11*, 645–650.
(24) Shimshoni, J. A.; Bialer, M.; Wlodarczyk, B.; Finnell, R. H.; Yagen, B. Potent anticonvulsant urea derivatives of constitutional isomers of valproic acid. *J. Med. Chem.* **2007**, *50*, 6419–6427.
(25) Sobol, E.; Bialer, M.; Yagen, B. Tetramethylcyclopropyl analogue of a leading antiepileptic drug, valproic acid. Synthesis and evaluation of anticonvulsant activity of its amide derivatives. *J. Med. Chem.* **2004**, *47*, 4316–4326.
(26) Sobol, E.; Yagen, B.; Steve White, H.; Wilcox, K. S.; Lamb, J. G. Preclinical evaluation of 2,2,3,3-tetramethylcyclopropanecarbonylurea, a novel, second generation to valproic acid, antiepileptic drug. *Neuropharmacology* **2006**, *51*, 933–946.
(27) Tantisira, B.; Tantisira, M. H.; Patarapanich, C.; Sooksawate, T.; Chunngam, T. Preliminary evaluation of the anticonvulsant activity of a valproic acid analog: *N*-(2-propylpentanoyl) urea. *Res. Commun. Mol. Pathol. Pharmacol.* **1997**, *97*, 151–164.
(28) Spielman, M. A.; Geiszler, A. O.; Close, W. J. Anticonvulsant drugs; some acylureas. *J. Am. Chem. Soc.* **1948**, *70*, 4189–4191.
(29) Ganz, A. J.; Waser, P. G.; Pfirrmann, R. W. Development of new antiepileptics. V. Pharmacological activity of some derivatives of sulfanilamide (author's transl). *Arzneimittelforschung* **1978**, *28*, 1331–1334.
(30) McNamara, J. O. *The Pharmacological Basis of Therapeutics*, 11th ed.; McGraw-Hill: New York, 2006; pp 501−525.
(31) Masereel, B.; Rolin, S.; Abbate, F.; Scozzafava, A.; Supuran, C. T. Carbonic anhydrase inhibitors: anticonvulsant sulfonamides incorporating valproyl and other lipophilic moieties. *J. Med. Chem.* **2002**, *45*, 312–320.
(32) Okada, A.; Onishi, Y.; Yagen, B.; Shimshoni, J. A.; Kaufmann, D.; Bialer, M.; Fujiwara, M. Tetramethylcyclopropyl analogue of the leading antiepileptic drug, valproic acid: evaluation of the teratogenic effects of its amide derivatives in NMRI mice. *Birth Defects Res., Part A* **2008**, *82*, 610–621.
(33) Shimshoni, J. A.; Bialer, M.; Yagen, B. Synthesis and anticonvulsant activity of aromatic tetramethylcyclopropanecarboxamide derivatives. *Bioorg. Med. Chem.* **2008**, *16*, 6297–6305.
(34) Tasso, S. M.; Moon, S.; Bruno-Blanch, L. E.; Estiu, G. L. Characterization of the anticonvulsant profile of valpromide derivatives. *Bioorg. Med. Chem.* **2004**, *12*, 3857–3869.
(35) Winkler, I.; Sobol, E.; Yagen, B.; Steinman, A.; Devor, M. Efficacy of antiepileptic tetramethylcyclopropyl analogues of valproic acid amides in a rat model of neuropathic pain. *Neuropharmacology* **2005**, *49*, 1110–1120.
(36) Pfeffer, P. E.; Silbert, L. S.; Chirinko, J. M. Alpha-anions of carboxylic acids. II. The formation and alkylation of alpha-methylated aliphatic acids. *J. Org. Chem.* **1972**, *37*, 451–458.
(37) Bialer, M.; Hadad, S.; Kadry, B.; Abdul-Hai, A.; Haj-Yehia, A. Pharmacokinetic analysis and antiepileptic activity of tetramethylcyclopropane analogues of valpromide. *Pharm. Res.* **1996**, *13*, 284–289.
(38) Isoherranen, N.; White, H. S.; Finnell, R. H.; Yagen, B.; Woodhead, J. H.; Bialer, M. Anticonvulsant profile and teratogenicity of *N*-methyl-tetramethylcyclopropyl carboxamide: a new antiepileptic drug. *Epilepsia* **2002**, *43*, 115–126.
(39) Pisani, F.; Fazio, A.; Oteri, G.; Di Perri, R. Dipropylacetic acid plasma levels; diurnal fluctuations during chronic treatment with dipropylacetamide. *Ther. Drug Monit.* **1981**, *3*, 297–301.
(40) Ilies, M. A.; Masereel, B.; Rolin, S.; Scozzafava, A.; Campeanu, G.; et al. Carbonic anhydrase inhibitors: aromatic and heterocyclic sulfonamides incorporating adamantyl moieties with strong anticonvulsant activity. *Bioorg. Med. Chem.* **2004**, *12*, 2717–2726.
(41) White, H. S.; Woodhead, J. H.; Wilcox, K. S.; Stables, J. P.; Kupferberg, H. J.; Wolf, H. H. *Discovery and Preclinical Development of Antiepileptic Drugs*, 5th ed.; Lippincott Williams & Wilkins: New York, 2002; pp 36−48.
(42) Everett, G. M.; Richards, R. K. Pharmacological studies of phenacetylurea (phenurone) an anticonvulsant drug. *J. Pharmacol. Exp. Ther.* **1952**, *106*, 303–313.
(43) Swinyard, E. A.; Toman, J. E. A comparison of the anticonvulsant actions of some phenylhydantoins and their corresponding phenylacetylureas. *J. Pharmacol. Exp. Ther.* **1950**, *100*, 151–157.
(44) Jezequel, S. G. *General Nervous System Penetration of Drugs: Importance of Phisicochemical Properties*; Taylor & Francis: London, 1992; pp 141−178.
(45) Enna, S. J.; Willaims, M. Challenges in the search for drugs to treat central nervous system disorders. *J. Pharmacol. Exp. Ther.* **2009**, *329*, 404–410.
(46) Bialer, M.; White, H. S. Key factors in the discovery and development of new antiepileptic drugs. *Nat. Rev. Drug Discovery* **2010**, *9*, 68–83.
(47) Supuran, C. T. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. *Nat. Rev. Drug Discovery* **2008**, *7*, 168–181.
(48) Bialer, M.; Johannessen, S. I.; Levy, R. H.; Perucca, E.; Tomson, T. Progress report on new antiepileptic drugs: a summary of the ninth Eilat conference (EILAT IX). *Epilepsy Res.* **2009**, *83*, 1–43.
(49) Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. *Vogel's Textbook of Practical Organic Chemistry*; Prentice Hall: New York, 1989; pp 720−723.
(50) Finnell, R. H.; Bennett, G. D.; Karras, S. B.; Mohl, V. K. Common hierarchies of susceptibility to the induction of neural tube defects by valproic acid and its 4-propyl-4-pentenoic acid metabolite. *Teratology* **1988**, *38*, 313–320.
(51) Wlodarczyk, B. C.; Craig, J. C.; Bennett, G. D.; Calvin, J. A.; Finnell, R. H. Valproic acid-induced changes in gene expression during neurulation in a mouse model. *Teratology* **1996**, *45*, 284–297.
(52) Sobol, E.; Yagen, B.; Lmb, J. G.; White, H. S.; Wlodarczyk, B. J.; Finnell, R. H.; Bialer, M. Anticonvulsant activity, neural tube defect induction, mutagenicity and pharmacokinetics of a new potent antiepileptic drug, *N*-methoxy-2,2,3,3-tetramethylcyclporopance carboxamide. *Epilepsy Res.* **2007**, *73*, 75–84.