Potent Anticonvulsant Urea Derivatives of Constitutional Isomers of Valproic Acid

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Valproic acid (VPA) is a major antiepileptic drug (AED); however, its use is limited by two life-threatening side effects: teratogenicity and hepatotoxicity. Several constitutional isomers of VPA and their amide and urea derivatives were synthesized and evaluated in three different anticonvulsant animal models and a mouse model for AED-induced teratogenicity. The urea derivatives of three VPA constitutional isomers propylisopropylacetylurea, diisopropylacetylurea, and 2-ethyl-3-methyl-pentanoylurea displayed a broad spectrum of anticonvulsant activity in rats with a clear superiority over their corresponding amides and acids. Enanatiomers of propylisopropylacetylurea and propylisopropylacetamide revealed enantioselective anticonvulsant activity, whereas only enantiomers of propylisopropylacetylurea displayed enantioselective teratogenicity. These potent urea derivatives caused neural tube defects, but only at doses markedly exceeding their effective dose, whereas VPA showed no separation between its anticonvulsant activity and teratogenicity. The broad spectrum of anticonvulsant activity of the urea derivatives coupled with their wide safety margin make them potential candidates to become new, potent AEDs.

Introduction

Epilepsy, characterized by recurrent seizure attacks, is one of the most common neurological conditions, occurring in about 1% of the global population.[1] Valproic acid (VPA[a], **1**, Figure 1), one of the leading antiepileptic drugs (AEDs), is utilized to treat various types of epileptic seizures.[2] In spite of the broad spectrum of antiepileptic activity, the clinical use of VPA is restricted by its two rare but life-threatening side effects, i.e., hepatotoxicity and teratogenicity.[3–5] Although VPA-induced hepatotoxicity is associated with the formation of metabolite(s) with a terminal double bond,[6,7] VPA-induced teratogenicity is caused by the parent compound.[8] It was shown with various VPA derivatives and analogues that the teratogenicity caused by these compounds is structure-dependent and stereoselective.[8–10] A clear stereoselective teratogenicity was demonstrated in a series of VPA analogues with a terminal double (4-ene) or triple (4-yne) bond.[10]

Numerous analogues of VPA with nonbranched, branched, and unsaturated alkyl moieties were prepared and evaluated for their anticonvulsant activity.[11–23] The nonbranched fatty acids (butyric, pentanoic, hexanoic, and octanoic acids) were found to be inactive in anticonvulsant animal models.[13–25] In a series of branched monocarboxylic acids, VPA was found to have the optimal chemical structure with regard to its efficacy and the margin between anticonvulsant potency and sedative/hypnotic side effects.[14,15] Some VPA analogues containing additional methyl in their structures maintained their anticonvulsant activity but exhibited lower teratogenicity.[16] Numerous cyclic analogues of VPA were evaluated as potential anticonvulsants; some of them, such as 1-methylcyclohexanecarboxylic acid and cyclooctylidenacetic acid, were more potent than VPA.[13] Esters of VPA have been found to be prodrugs of VPA in dogs.[18,21] The amide derivative of **1**, valpromide (VPD, **8**), and the corresponding amides of VPA's constitutional isomers, propylisopropylacetamide (PID, **11**), diisopropylacetamide (DID, **10**), and 2-ethyl-3-methyl-valeramide or valnoctamide (VCD, **9**) (Figure 2), have been synthesized and their anticonvulsant potential was

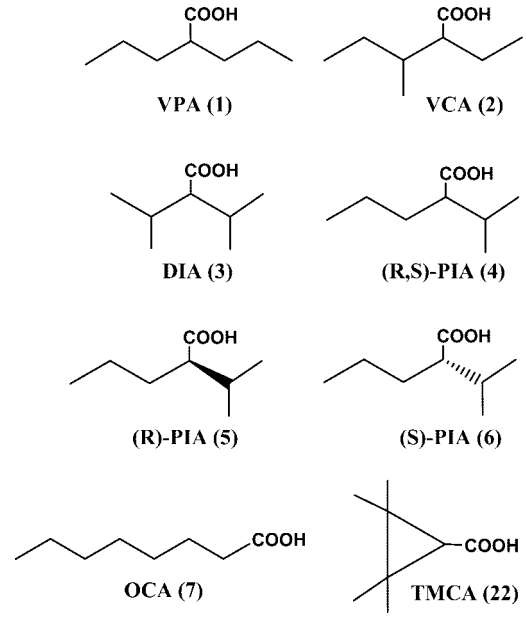

Figure 1. Structures of valproic acid (VPA), its constitutional isomers, and VPA's analogue 2,2,3,3-tetramethylcyclpropane carboxylic acid (TMCA).

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[a] Abbreviations: AED, antiepileptic drug; NTD, neural tube defects; MES, maximal electroshock seizure; PI, protective index; SAR, structure-activity relationship; scMet, subcutaneous pentylenetetrazole; SI, stereoselective index; VPA, valproic acid.

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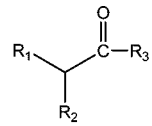


Compd	Substituent R_1	R_2	R_3
VPA (1)	n-propyl	n-propyl	OH
VCA (2)	sec-butyl	ethyl	OH
DIA (3)	isopropyl	isopropyl	OH
(R,S)-PIA (4)	isopropyl	n-propyl	OH
(R)-PIA (5)	isopropyl	n-propyl	OH
(S)-PIA (6)	isopropyl	n-propyl	OH
OCA (7)	n-hexyl	H	OH
VPD (8)	n-propyl	n-propyl	NH_2
VCD (9)	sec-butyl	ethyl	NH_2
DID (10)	isopropyl	isopropyl	NH_2
(R,S)-PID (11)	isopropyl	n-propyl	NH_2
(R)-PID (12)	isopropyl	n-propyl	NH_2
(S)-PID (13)	isopropyl	n-propyl	NH_2
OCD (14)	n-hexyl	H	NH_2
VPU (15)	n-propyl	n-propyl	$NHCONH_2$
VCU (16)	sec-butyl	ethyl	$NHCONH_2$
DIU (17)	isopropyl	isopropyl	$NHCONH_2$
(R,S)-PIU (18)	isopropyl	n-propyl	$NHCONH_2$
(R)-PIU (19)	isopropyl	n-propyl	$NHCONH_2$
(S)-PIU (20)	isopropyl	n-propyl	$NHCONH_2$
OCU (21)	n-hexyl	H	$NHCONH_2$

Figure 2. Structures of valproic acid (VPA), constitutional isomers of VPA, and their corresponding amide and urea derivatives.

evaluated.[12,19–22] In various anticonvulsant models in rats, VPD (**8**) was 10–15 times more potent than **1** and was nonteratogenic in a mouse model for VPA-induced teratogenicity.[22] However, these advantages have no clinical value, because in humans, unlike rodents and dogs, VPD (**8**) serves as a prodrug to VPA.[22] VPD's constitutional isomers VCD (**9**) and PID (**11**) demonstrated in rats a anticonvulsant profile similar to that of VPD and all three were nonteratogenic in a mouse model.[24–26] In Europe, VCD (**9**) has been approved as a tranquilizer and is currently undergoing phase-II clinical trials as a potential antimania agent.[27,28] Unlike VPD (**8**), VCD (**9**) acts in humans as a drug on its own and not as a prodrug for valnoctic acid (VCA, **2**).[29] Analogously, PID (**11**) did not undergo in dogs and rats metabolic biotransformation to propylisopropylacetic acid (PIA, **4**).[24,26]

The synthesis of (*R*)- and (*S*)-enantiomers of PIA and their amides (Figures 1 and 2) has been reported, and it was found that (*R*)-PID (**12**) was more potent than (*S*)-PID (**13**) in numerous anticonvulsant tests in mice, including the 6 Hz psychomotor seizure model.[24] In the rat, the MES- and scMet-ED_{50} and the TD_{50} of the racemic (*R*,*S*)-PID (**11**) were not significantly different from the ED_{50} and TD_{50} of the individual PID enantiomers (**12** and **13**).[24,26]

A series of VPA conjugates with N-alkylated amides, amino acids, and glycinamide has been extensively explored.[23,30–32] Valproylglycinamide (valrocemide) was found to be the most active compound and is currently undergoing phase II clinical trials.[32,33]

Urea is an integral part of the heterocyclic chemical structure of the major AEDs phenobarbital, phenytoin, and carbamazepine.[1] Six decades ago, Spielman and co-workers evaluated the anticonvulsant activity of urea derivatives of numerous branched aliphatic monocarboxylic acids, including VPA, and found valproylurea (VPU, **15,** Figure 2) to be inactive in mouse MES and scMet anticonvulsant models.[34] In contrast, a decade ago, Tantisira et al. showed VPU (**15**) to possess anticonvulsant activity with a favorable PI value in these models.[35]

2,2,3,3-Tetramethylcyclopropane carboxylic acid (TMCA, **22**, Figure 1), a cyclopropyl analogue of VPA, was found to be inactive in the rat MES and scMet models.[36] As part of our ongoing research program, we synthesized the urea derivative of TMCA, 2,2,3,3-tetramethylcyclopropanecarbonylurea (TMCU). It was shown to possess a highly effective anticonvulsant potency with a wide safety margin in the rat-MES, scMet, and hippocampal kindling models.[37,38] With regard to teratogenicity testing, TMCU displayed no significant increase in the incidence of neural tube defects (NTD) in an AED-induced mouse model for teratogenicity.[38] The amide derivative of TMCA, 2,2,3,3-tetramethylcyclopropanecarboxamide (TMCD) was active only in the scMet test and showed a better anticonvulsant potency and protective index (PI) than VPA.[39]

In the current work, we aimed to assess the urea derivatives of VPA and the constitutional isomers of VPA: octanoylurea (OCU, **21**), DIU (**17**), PIU (**18**) and its two enantiomers (**19** and **20**) and VCU (**16**) in three animal models for anticonvulsant activity and in a mouse model for AED-induced teratogenicity (Figure 2, Tables 1–3). We also aimed to determine whether the two individual enantiomers (R)-PIU (**19**) and (S)-PIU (**20**) exhibit significantly different anticonvulsant activity in the MES, scMet and 6 Hz models and/or teratogenicity. The results obtained in this study were compared to the anticonvulsant activity of the respective acids of the corresponding constitutional isomers of VPA and to their amides.

Chemistry

The synthesis of the racemic valproic acid isomers, their corresponding amides, and urea derivatives is outlined in Scheme 1. The starting material for the synthesis of (*R*,*S*)-PIA or DIA was isovaleric acid and for VCA, 3-methylvaleric acid. These acids were converted to the enolates by use of lithium diisopropylamine (LDA), followed by condensation with the appropriate alkyliodide to yield the corresponding branched carboxylic acids.[40] The carboxylic acids were converted by thionyl chloride to the corresponding acyl chlorides[41] and treated with 25% ammonium hydroxide in aqueous solution at 0 °C to yield VCD, PID, and DID. The urea derivatives of PIA, DIA, and VCA were synthesized by coupling the appropriate acylchlorides with urea according to the procedure reported in the literature.[37] The structures of the carboxylic acids, their amide, and urea derivatives were identified by ^{1}H NMR and GC-MS, and their purity was established by elemental analyses.

The enantiomerically pure (*R*)-PIA (**5**) and (*S*)-PIA (**6**) were synthesized by a procedure previously published by our group.[42] In brief, the enantioselective synthesis of PIA enantiomers was achieved by conversion of valeric acid to acylchloride followed by coupling of valeroylchloride with optically pure chiral auxiliaries (4*S*)- or (4*R*)-benzyl-2-oxazolidinones in order to prepare (4*S*)- or (4*R*)-3-(1′-oxopentyl)-4-benzyl-2-oxazolidinones respectively. The two oxazolidinone enolates were alkylated with isopropyltriflate to obtain (4*S*, 2′*R*)- or (4*R*, 2′*S*)-3-(2′-isopropyl-1′-oxopentyl)-4-benzyl-2-oxazolidinones, which further hydrolyzed with lithium hydroperoxide to yield (*R*)- and (*S*)-PIA enantiomers respectively with optical purity above 99.4%. The syntheses of amide and urea derivatives of (*R*)- and (*S*)-PIA were accomplished as described above for the syntheses of the racemic PID and PIU.

Results and Discussion

In spite of the large therapeutic arsenal including both old and new AEDs, about 30% of epileptic patients are still not seizure-free.[1] Consequently there is a substantial need to develop

Table 1. Quantitative Anticonvulsant Data in Rats Dosed Orally with Compounds 1 and 8–21[a]

compound	MES[b] (ED_{50} mg/kg)	scMet[c] (ED_{50} mg/kg)	neurotoxicity (TD_{50} mg/kg)	PI[d] (MES)	PI (scMet)
VPA[e] (**1**)	485 (324–677)[f]	646 (466–869)	784 (503–1176)	1.6	1.2
VPD[g] (**8**)	32 (22–42)	59 (44–77)	87 (68–107)	2.7	1.5
VCD[h] (**9**)	29 (19–38)	54 (46–63)	58 (47–66)	2.0	1.1
DID (**10**)	51 (34–66)	60 (54–65)	72 (62–85)	1.4	1.2
(*R*,*S*)-PID (**11**)	50 (33–73)	27 (20–40)	87 (69–108)	1.7	3.2
(*R*)-PID (**12**)	47 (39–62)	22[k] (15–33)	82 (67–92)	1.7	3.7
(*S*)-PID (**13**)	63 (39–91)	38 (29–46)	83 (75–94)	1.3	2.2
OCD[I] (**14**)					
VPU (**15**)	54 (38–66)	77 (55–107)	232 (193–365)	4.3	3.0
VCU (**16**)	24 (16–35)	14[j] (11–18)	97 (75–122)	4	6.9
DIU (**17**)	33[j] (18–51)	16[j] (10–24)	56 (45–66)	1.7	3.5
(*R*,*S*)-PIU (**18**)	16[j] (11–23)	45 (35–61)	95 (71–124)	5.9	2.1
(*R*)-PIU (**19**)	36[k] (25–52)	22[k] (14–32)	124 (93–182)	3.4	5.6
(*S*)-PIU (**20**)	18[j] (10–29)	37 (32–45)	118 (82–154)	6.5	3.2
OCU (**21**)	>300	>300	>300		

[a] Analysis for statistical significance was done by means of probits. ED_{50} values were compared using 95% confidence intervals of the log transforms provided by the probit analysis. [b] Maximal electroshock test. [c] Subcutaneous pentylentetrazole test. [d] Protective index (TD_{50}/ED_{50} ratio). [e] Data taken from ref 49. [f] Data in parentheses stand for 95% confidence intervals. [g] Data taken from ref 23. [h] Data taken from ref 59. [I] Not active because of extensive metabolic clearance.[25] [j] Significantly different than the corresponding amide ($P < 0.05$). [k] Significantly different than the (S)-enantiomer ($P < 0.05$).

Table 2. Anticonvulsant Activity in the 6 Hz Psychomotor Seizure Test of Compounds **1** and **8–20** Administered Intraperitoneally to Mice[a]

compound	6 Hz test at 32 mA (ED_{50} mg/kg)	6 Hz test at 44 mA (ED_{50} mg/kg)	neurotoxicity (TD_{50} mg/kg)
VPA[b] (**1**)	126 (95–152)[c] (PI[d] = 3.2)	310 (258–335) (PI = 1.3)	398 (356–445)
(*R*,*S*)-PIA (**4**)	149 (87–239) (PI > 2)	NT[e]	>300
VPD[b] (**8**)	57 (47–65) (PI = 1.4)	66 (29–87) (PI = 1.2)	81 (74–91)
VCD[b] (**9**)	37 (26–50) (PI = 2.1)	67 (61–72) (PI = 1.1)	77 (69–88)
DID (**10**)	>100	>100	57[f]
(*R*,*S*)-PID[g] (**11**)	44 (35–57) (PI = 2.5)	73 (60–100) (PI = 1.5)	112 (106–118)
(*R*)-PID[g] (**12**)	46[I] (31–65) (PI = 2.4)	57[I] (41–69) (PI = 1.9)	111 (92–122)
(*S*)-PID[g] (**13**)	73 (56–92) (PI = 1.3)	81 (70–95) (PI = 1.2)	97 (89–137)
VPU (**15**)	58 (49–71) (PI < 1.7)	105[h] (83–139) (PI < 0)	100 > TD_{50} > 50
VCU (**16**)	21[j] (17–25) (PI < 4.7)	48[j] (43–51) (PI < 2.1)	100 > TD_{50} > 50
DIU (**17**)	43[j](31–53) (PI < 2.3)	49[j](45–59) (PI < 2)	100 > TD_{50} > 50
(*R*,*S*)-PIU (**18**)	42 (39–45) (PI < 2.4)	71 (58–79) (PI < 1.4)	100 > TD_{50} > 50
(*R*)-PIU (**19**)	43 (30–60) (PI = 2.7)	56[I] (28–75) (PI = 2.1)	118 (94–147)
(*S*)-PIU (**20**)	46[j](36–59) (PI < 6.5)	75 (54–93) (PI < 4)	300 > TD_{50} > 100

[a] Analysis for statistical significance was done by means of probits. ED_{50} values were compared using 95% confidence intervals of the log transforms provided by the probit analysis. [b] Data taken from ref 25. [c] Data in parentheses stand for 95% confidence intervals. [d] Protective index. [e] Not tested. [f] Data taken from ref 20. [g] Data taken from ref 24. [h] VPU-ED_{50} approached the TD_{50} and therefore was obtained at the expense of some rotorod impairment. [I] Significantly different than the (*S*)-enantiomer ($P < 0.05$). [j] Significantly different than the corresponding amide ($P < 0.05$).

new AEDs that might provide better seizure control. Extensive structure–activity relationship (SAR) studies have been performed on the VPA molecule with a strong incentive to develop new VPA analogoues and derivatives with better potency that are free of any teratogenicity and hepatotoxicity.[11–26] We have synthesized the following VPA constitutional isomers, VCA (**2**), DIA (**3**), and (R,S)-PIA (**4**), as well as its two individual enantiomers (*R*)- and (*S*)-PIA (**5** and **6**), and evaluated their pharmacological potency for anticonvulsant activity. In this series of acids, there were no differences (in mice) between the anticonvulsant ED_{50} and minimal neurotoxicity (TD_{50}) (with the exception of DIA in the scMet model). Subsequently, the anticonvulsant activity of the amides (**9**–**13**) of the VPA's constitutional isomers was assessed in electrically (MES) and chemically (scMet) induced seizures in rats with the results presented in Table 1. In the MES and scMet models for generalized seizures, the amides were more potent as anticonvulsants than their corresponding acids. In the case of DID, the increase in the anticonvulsant potencies at the MES and scMet models was parallel with a decrease in their TD_{50}, resulting in no significant change in DID's protective indexes (PI = TD_{50}/ED_{50}) (Table 1). However, the MES-PI value of VPD and the scMet-PI values of PID (racemate or individual enantiomers) were larger than those of VPA, DID, and VCD (Table 1).

Numerous acylureas have been evaluated as potential anticonvulsants. The urea derivative of VPA, 2-*n*-propyl-pentanoylurea (valproylurea, VPU, **15**) was reported to be inactive.[34] In contrast, the urea derivatives of the VPA analogues, 2-ethyl-3-methyl-valeroylurea (VCU, **16**), 2-ethyl-caproylurea, and 2-isopropyl-Δ^4-pentenoylurea demonstrated potent MES anticonvulsant activity.[43–45] VCU (**16**) underwent clinical trials more than 30 years ago as a potential anxiolytic and a short-acting hypnotic drug with a rapid onset of pharmacologic activity but is not currently commercially available.[43–45] However, its unsaturated analogue, 2-isopropyl-Δ^4-pentenoylurea, is available in Japan as an over-the-counter (OTC) hypnotic that is also used as an analgesic and antipyretic agent.[46]

We have shown (Table 1) that VPU is less potent in the MES model than VPD, but it possesses a greater PI value (Table 1) and thus our results are in agreement with those of Tantisira et al.[38]

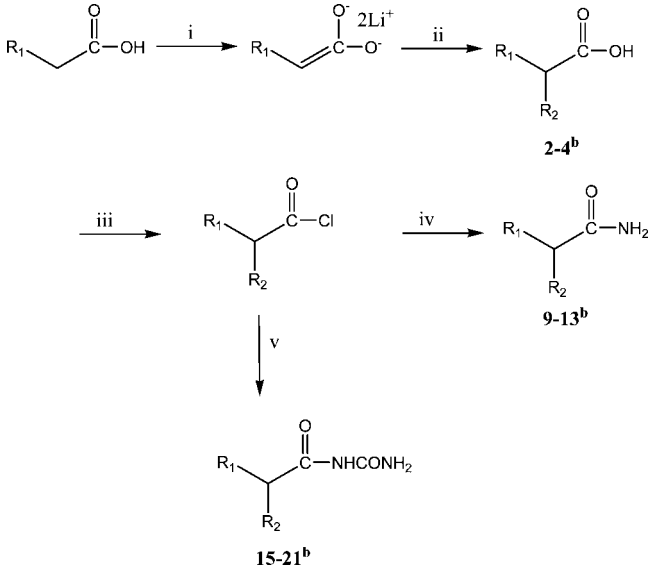
From the anticonvulsant profile of the urea derivatives of VPA's constitutional isomers analyzed in this study (Table 1), we can conclude that VCU (**16**) and DIU (**17**) are the most active compounds in the scMet model (ED_{50} = 14 and 16 mg/kg, respectively), whereas (*R*,*S*)-PIU (**18**) and (*S*)-PIU (**20**) are the most potent compounds in the MES test (ED_{50} = 16 and 18 mg/kg, respectively).

Table 3. Teratogenic Effect in a Mouse Model of VPA and the Amide (**8**–**13**) and the Urea Derivatives (**15**–**20**) of VPA's constitutional Isomers (**1**, **2**, **4**–**6**)

treatment group	route[a]	dose mg/kg (mmol/kg)	mouse strain	no. of litters	no. of live fetuses	embryo/ feto-lethality (%)	exencephaly (%)
control	i.p.	25% CEL[c]	SWV	15	188	6	0
VPA (**1**)	i.p.	600 (3.6)	SWV	13	107	18.3	53.3[g]
VPA	i.p.	452 (2.7)	SWV	13	141	11.9	29.1[g]
VCA (**2**)[b]	s.c.	500 (3.5)	NMRI	10	124	3	1
(*R*,*S*)-PIA (**4**)	i.p.	520 (3.6)	SWV	11	120	5.5	0
(*R*)-PIA (**5**)	i.p.	520 (3.6)	SWV	12	157	4.3	0
(*S*)-PIA (**6**)	i.p.	520 (3.6)	SWV	12	145	5.8	0
VPD (**8**)[b]	s.c	430 (3.0)	NMRI	9	115	7	6[g]
VCD (**9**)[b]	s.c.	430 (3.0)	NMRI	10	129	2	1
(*R*,*S*)-PID (**11**)[d]	i.p.	600 (4.2)	SWV	10	118	6.3	0
(*R*)-PID (**12**)[d]	i.p	600 (4.2)	SWV	10	120	10.4	0
(*S*)-PID (**13**)[d]	i.p.	500 (3.5)	SWV	10	123	6.1	0.8
VPU[e] (**15**)	i.p.	261 (1.4)	SWV	10	80	41.2[g]	13.7[g]
VPU	i.p.	167 (0.9)	SWV	10	115	12.9	0.9
VCU[f] (**16**)	i.p.	205 (1.1)	SWV	10	75	39[g]	2.7
(*R*)-PIU (**19**)	i.p.	503 (2.7)	SWV	13	57	65.7[g,h]	17.5[g,h]
(*R*)-PIU	i.p.	336 (1.8)	SWV	9	52	54.4[g h]	15.4[g,h]
(*R*)-PIU	i.p.	205 (1.1)	SWV	9	104	7.1	0
(*S*)-PIU (**20**)	i.p.	503 (2.7)	SWV	9	70	40.7[g]	4.3[g]
(*S*)-PIU	i.p.	336 (1.8)	SWV	10	123	10.2	5.7[g]
(*S*)-PIU	i.p.	205 (1.1)	SWV	10	124	6.8	0.8

[a] All dams received the drugs intraperitoneally or subcutaneously on the morning of day 8 of gestation, as indicated for each treatment. [b] Data taken from ref 53. [c] 25% water solution of Cremophor EL. [d] Data taken from ref 26. [e] VPU in the highest tested dose was 3.6 mmol/kg was lethal to the dams for 24 h postinjection. [f] VCU at the highest tested doses, 2.7 mmol/kg and 1.8 mmol/kg, was lethal to the dams for 24 h postinjection. [g] Significantly different from control, $p < 0.05$, Fisher exact test. [h] Significantly different from the *S*-enantiomer, $p < 0.05$, Fisher exact test.

Scheme 1. Synthesis of Constitutional Isomers of VPA (2–4), Their Amides (9−13) and Urea Derivatives (15–21)[a]



[a] Reagents: (i) LDA, THF, −15°C, 20 min; (ii) propyliodide or isopropyliodide or ethyliodide, THF, 0°C, 30 min; (iii) $SOCl_2$, CH_2Cl_2, 25°C, 10 h; (iv) 25% NH_4OH, 0°C, 10 min; (v) urea, acetonitrile, 80°C, 2 h.

The anticonvulsant activity of PIU (**18**) was enantioselective in the MES model with (S)-PIU (**20**) exhibiting a higher potency and a wider PI value than (*R*)-PIU (**19**). (*R*)-PIU (**19**) was significantly more potent than (*S*)-PIU (**20**) in the scMet model and more potent in the scMet model than in the MES model (Table 1).

Although Spielman et al. described the ineffectiveness of 2-ethyl-3-methyl-valeroylurea (VCU, **16**) in the MES test,[34] our quantitative evaluation of VCU anticonvulsant activity showed that it is a very potent compound in the MES and scMet models. In both tests, VCU (**16**) had a wide safety margin (PI) (Table 1).

PID (**11**) in its racemic form as well as its individual enantiomers demonstrated anticonvulsant activity in the MES and scMet models. Following oral administration to rats, (*R*)-PID was found to be more potent than (*S*)-PID in the scMet model (Table 1). Similarly, (*R*)-PID was more active at the 6 Hz model at 44 mA current, thus exhibiting enantioselective anticonvulsant activity (Table 2).

The success and wide acceptance of levetiracetam (LEV) as a new AED for therapy-resistant patients with epilepsy generated much interest in the 6 Hz model, because LEV was active in this model despite its lack of anticonvulsant activity in the classical MES and scMet models.[47] The 6 Hz model undoubtedly displays a different pattern of seizure-induced neuronal activation from that observed in the MES and scMet seizure models and therefore may have a potential as an AED for therapy-resistant patients.[48–50]

In the current study, to our surprise, all of the urea derivatives tested demonstrated remarkable anticonvulsant activity at both 32 and 44 mA currents (Table 2). In general, as the stimulus intensity in the 6 Hz model is increased from 22 to 44 mA, some AEDs lose their activity (e.g., phenytoin, ethosuximide, and lamotrigine), even LEV's ED_{50} increases from 19 mg/kg (at 32 mA) to 1089 mg/kg (at 44 mA).[48–50] In the present study, enantioselective anticonvulsant activity was observed in the 6 Hz model for the individual enantiomers of both PID and PIU (Table 2). (*R*)-PIU (**19**) showed better potency than (*S*)-PIU (**20**) in the 6 Hz (44mA) model and had a PI value of 2.1. On the other hand, (*R*)-PID (**12**) emerged as the more potent enantiomer than (*S*)-PID (**13**) with an ED_{50} of 46 mg/kg (32 mA) and 57 mg/kg (44 mA), respectively.

Unlike VPU, VCU and DIU showed separation between their 6 Hz ED_{50} (32mA) and TD_{50} values in mice (Table 2). It is important to emphasize that VCU (**16**) and DIU (**17**) possessed the highest anticonvulsant potency at 44 mA (ED_{50} = 48 and 49 mg/kg, respectively), a value significantly exceeding the ED_{50} of the two marketed AEDs, LEV (ED_{50} = 1089 mg/kg) and VPA (ED_{50} = 310 mg/kg) (Table 2). Therefore, both compounds (**16** and **17**) are promising candidates for development as novel AEDs that would provide more potent and better seizure

suppression than LEV in patients with therapy-resistant epilepsy.[49,50]

We have thus far found several structure–activity requirements concerning VPA's constitutional isomers and their amide and urea derivatives (Table 1). Octanoic acid (OCA, **7**), a straight chain constitutional isomer of VPA, octanoylamide (OCD, **14**), and octanoylurea (OCU, **21**) (Figure 1) did not possess anticonvulsant activity at doses up to 300 mg/kg (Table 1). The lack of anticonvulsant activity of **7**, its amide **14**, and its urea derivatives **21** may be due to their high (metabolic) clearance.[20] Introducing a methyl group at the β-position to the carbonyl, as reflected in the structures of VCA, DIA, and PIA (Figure 1), significantly increased the anticonvulsant activity of their urea and amide derivatives and improved their safety margin. In all three anticonvulsant animal models utilized in this study, the urea derivatives VCU, DIU, racemic PIU, and (*R*)- and (*S*)-PIU displayed an improved anticonvulsant profile with a broad spectrum of activity compared to their amide derivatives (Tables 1 and 2).

From the enantioselective anticonvulsant activity of (*R*)- and (*S*)-PID and (*R*)- and (*S*)-PIU (Tables 1 and 2), it can be claimed that enantioselectivity is test specific. The degree of stereoselectivity or stereoselective index (SI) (SI = less potent ED_{50}/more potent ED_{50}) for a pair of enantiomers depends on the anticonvulsant model or test.[51] For example, (*S*)-PIU (**20**) was more potent than its enantiomer in the MES test, whereas (*R*)-PIU (**19**) was more potent in the scMet and 6 Hz (44 mA) tests (Tables 1 and 2). It seems that the enantioselective potency depends on the kind of derivative, amide or urea, of the corresponding acids of the VPA's constitutional isomers. The reason for the enantioselective activity of the individual PIA's amide and urea derivatives is unknown, although it may be due to stereospecific binding of the derivatives of the individual enantiomers to specific binding sites present on synaptic vesicles and neuroendocrine cells in the brain.[52] We presume that these tested compounds have more than one target and mechanism of action with a higher affinity than VPA. Multiple mechanisms of action may explain why (*S*)-PIU (**20**) is more active in the MES test, whereas (*R*)-PIU (**19**) is more active in the scMet test (Table 1).

In recent years, the teratogenic properties of several VPA isomers and their corresponding amides have been extensively studied in NMRI and SWV mouse models for VPA-induced teratogenicity.[16,24,26,53,54] VPA has been shown to be highly teratogenic, resulting in a very high percentage of exencephaly, a severe neural tube defect (NTD) in mice exposed in utero at critical time points of neural tube closure. Branching at the β-position to the carbonyl group, as shown in the chemical structures of VCA, DIA, and PIA (Figure 1), as well as amidation of their carboxylic groups (compounds **9**–**13**), reduces their teratogenic potency.[16,24,29,53,54] We have found that the urea derivatives of the VPA's isomers (**16**–**20**) were teratogenic and embryotoxic in mice at doses 6 and 3 times more than the lowest anticonvulsant ED_{50} value found in the mice 6 Hz test at 32 and 44 mA, respectively. Consequently, the urea derivatives (**16**–**20**) possess a broader safety margin than VPA (Tables 2 and 3). VCU (**16**) revealed the largest safety margin, possessing a greater PI value than that of VPA in both currents used (PI = 16 and 7 at 32 and 44 mA, respectively) (Table 2). PIU enantiomers (**19**, **20**) demonstrated a distinct enantioselective teratogenicity and embryotoxicity at doses 5−8 times higher than their anticonvulsant ED_{50} values in the 6 Hz model at 32 and 44 mA. At a dose of 336 mg/kg, which is 8 and 6 times higher than its ED_{50} at 32 and 44 mA respectively, (*R*)-PIU (**19**) was significantly more embryotoxic than (*S*)-PIU (**20**), causing 54% of embryotoxicity vs 10% as established for **20**. The rate of NTD was also higher in fetuses treated with (*R*)-PIU (15%) than with (*S*)-PIU (6%) (Table 3). The lowest dose (205 mg/kg) of the urea derivatives (**19**, **20**) used for the teratogenic evaluation was 2.5 and 4.5 times larger than their 6 Hz ED_{50} and did not cause any teratogenic or embryotoxic effects in mice, as compared to control (Table 3).

Table 4. Physical and Chemical Properties of VPA and the Amide and Urea Derivatives of Its Constitutional Isomers

compound	water solubility (mg/mL)	*C* log *P*[a]	MW	mp (°C)
VPA (**1**)	1.3	2.76	144	liquid
VPD (**8**)	3.7	1.84	143	125–126
VCD (**9**)	8.7[b]	1.71	143	113–114
DID (**10**)	4.3[b]	1.58	143	142–144
(R,S)-PID (**11**)	3.5[c]	1.71	143	130–131
OCD (**14**)	1.2[b]	2.06	143	105–106
VPU (**15**)	0.12	1.92	186	218–220
VCU (**16**)	0.28	1.79	186	147–148
DIU (**17**)	0.27	1.66	186	199–200
(*R*,*S*)-PIU (**18**)	0.13	1.79	186	213–214
OCU (**21**)	<0.05	2.14	186	184–185

[a] Octanol/water partition coefficient. *C* log *P* was calculated by utilizing the ChemDraw Ultra-Software 8. [b] Data taken from ref 20. [c] Data taken from ref 19.

In contrast to PIU, of the five dams treated with 336 mg/kg VPU (**15**), three died within 24 h of treatment and of the two dams that survived, their entire litters were resorbed. Lowering VPU dose to 251 mg/kg still caused embryotoxicity and teratogenicity (41% embryolethality and 13% NTDs). VCU (**16**) at a dose of 205 mg/kg produced resorption in 39% of the conceived embryos and 3% were affected with NTDs (two fetuses with exencephaly in two litters). Although there was no significant statistical difference between the VCU and the control group with respect to the rate of NTDs, these two fetuses with exencephaly possibly represent the very low teratogenic potential of VCU. In the group treated with 167 mg/kg VPU, the resorption rate was twice as high as in the control group, although not statistically significant, and the rate of exencephaly did not differ from control (1% NTDs). VPU (**15**) was significantly more embryotoxic and teratogenic at the lower equimolar doses than VPA (1.35 vs 1.8 mmol/kg, Table 3). On the other hand, VPU displayed a pronounced separation between its anticonvulsant activity (MES-ED_{50} = 54 mg/kg and scMet-ED_{50} = 77 mg/kg) and its teratogenic and embryotoxic activity in contrast to VPA, whereas both PIU enantiomers (**19**, **20**) displayed more favorable separation between anticonvulsant activity and embryotoxic/teratogenic activity (Tables 2 and 3). It is worth emphasizing that VPA showed marked teratogenic activity and embryolethality at doses in the same range as its ED_{50} values, thereby revealing no separation between anticonvulsant activity and teratogenicity in contrast to the amide and urea derivatives of VPA's constitutional isomers (Table 3).

PID (**11**) and VCD (**9**) were also nonteratogenic and did not biotransform in dogs to their corresponding acids.[19] In humans, VCD underwent only minimal metabolism to the nonteratogenic VCA (**2**).[29,53] Even in humans, if PID enantiomers were partially metabolized to their corresponding acids, it would not lead to any teratogenic effect, because PIA (**4**–**6**; racemate or individual enantiomers) is nonteratogenic (Table 3). VPD (**8**) is the only amide in the above-mentioned series, demonstrating a low teratogenic potential with 6% exencephaly, which might result from partial biotransformation of VPD to VPA.[53]

Comparative analysis of the physical and chemical properties of the CNS-active urea and amide derivatives of VPA consti-

tutional isomers evaluated in this study (Table 4) shows that these amides and the urea derivatives have similar ClogP in the range of 1.6–2.1. However, the urea derivatives (**15**–**21**) are less water-soluble than the corresponding amide derivatives (**8**–**14**) and are still highly potent following oral and i.p. administration to rats and mice. Thus, both groups of compounds belong to class 2 of the biopharmaceutics classification system (BCS), namely, compounds with high permeability and low solubility.[55]

Conclusions

The aims of this study were to evaluate the anticonvulsant profile of urea derivatives of VPA's constitutional isomers and to explore if the presence of a chiral center at the C-2 position, like in PID (**11**) and PIU (**18**), may lead to enantioselectivity in their anticonvulsant activities and teratogenicity. VCU and DIU are the most potent anticonvulsant compounds to emerge from this study. (*R*)- and (*S*)-PIU enantiomers displayed stereoselective anticonvulsant activity in the MES, scMet, and 6 Hz (32 and 44 mA) models, whereas (*R*)- and (*S*)-PID showed stereoselectivity in the 6 Hz and scMet models. The teratogenicity study revealed that at a dose (335 mg/kg) 3−16 times larger than their ED_{50} values in the 6 Hz model, all of the urea derivatives were highly teratogenic and embryotoxic. Still, at both currents used in the 6 Hz model (32 and 44 mA), all the urea derivatives were more potent and displayed a broader safety margin than VPA.

One of the most striking observations that stemmed from this study is the effect of very minor structural changes such as the location of a single methyl group in the acyl moiety in the derivatives of VPA's constitutional isomers, on the pharmacologic profile of the resulting compounds. The broad spectrum of anticonvulsant activity of the urea derivatives VCU (**16**), DIU (**17**), racemic-PIU (**18**), and its individual enantiomers (**19**, **20**), coupled with their wide safety margin, make them potential candidates to become new, potent AEDs.

Experimental Section

Materials and Methods. All reagents were purchased from Sigma-Aldrich. 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 $F_{254,}$ Merck). A gas chromatography–mass spectroscopy assay was performed on a HP5890 Series II GC equipped with a Hewlett-Packard MS Engine (HP5989A) single quadrupole MS, HP7673 autosampler, HP MS-DOS Chemstation, and HP-5MS capillary column (0.25 μm × 15 m × 0.25 mm). The temperature program was as follows: injector temperature, 180 °C; initial temperature, 60 °C for 3 min; gradient of 20 °C/min until 140 °C; gradient of 10 °C until 190 °C; hold time, 3 min. The MS parameters were set as follows: source temperature, 180 °C; transfer line, 280 °C; positive ion monitoring; EI-MS (70 eV). The molecular ion and the five most-pronounced ions are provided.

The chemical structure and purity of the newly synthesized compounds were assessed by TLC, GC/MS, NMR, and elemental analyses. Melting points were determined on a Buchi 530 capillary melting point apparatus. ^{1}H NMR spectra were recorded on a Varian Mercury series NMR 300 spectrometer. Chemical shifts (δ scale) are reported in parts per million (ppm) relative to the indicated reference. Coupling constants (J values) are given in hertz (Hz). Elemental analyses were performed on a 2400–2 Perkin-Elmer C, H, N analyzer. C, H, N analyses of all newly synthesized compounds had satisfactory results (within ±0.4 of theoretical values).

Solvents. Tetrahydrofurane and acetonitrile were dried by refluxing for 2 h over CaH_2 and distilling freshly prior to use. HMPA was refluxed over CaH_2 for 5 h and distilled at reduced pressure and stored over 4A molecular sieves under a nitrogen atmosphere. Diisopropylamine was distilled from NaH and stored over 4A molecular sieves prior to use.

General Procedure for the Synthesis of Compounds 2–4. A solution of 0.126 mol of lithiumdiisopropylamine (LDA) was prepared by mixing 17.8 mL of diisopropylamine in 90 mL of dry tertahydrofuran under nitrogen. The mixture was cooled to −20 °C and 0.126 mol of butyl lithium (BuLi) was added slowly. After the addition of BuLi was completed, the reaction mixture was stirred at 0 °C for 30 min. The LDA solution was cooled again to −20 °C followed by the addition of 0.06 mol of isovaleric acid (for the synthesis of compounds **3** and **4**) or 0.06 mol of methylpentanoic acid (for the synthesis of compound **2**). After the addition of the carboxylic acid was completed, the mixture was stirred for 15 min and a solution of 0.06 mol of HMPA was added rapidly to the reaction mixture and stirred for 5 min at 4 °C. Following the enolate formation, 0.12 mol of an appropriate alkyliodide was added dropwise to the reaction mixture at 0 °C and stirred for 1 h at room temperature. After the reaction was completed, the reaction mixture was acidified with 10% HCl to pH 1–2 and the product was extracted three times with 300 mL of petroleum ether. The combined petroleum ether fractions were washed with HCl (1N), water, and brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to yield pure branched carboxylic acids.

2-Ethyl-3-methylpentanoic acid (Valnoctic Acid, 2). Colorless oil. MS-EI, *m*/*z* (%): 145 (M^+, 0.1), 115 (7), 88 (100), 73 (97), 57 (17). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.85–0.96 (m, 9H), 1.1–1.3 (m, 1H), 1.38–1.76 (m, 4H), 2.1–2.24 (m, 1H) 11.2 (br s, 1H). Anal. ($C_8H_{16}O_2$) C, H. Yield 75%.

2-Isopropyl-3-methylbutanoic acid (Diisopropylacetic Acid, 3). Bright yellow oil. MS-EI, *m*/*z* (%): 145 (M^+, 0.06), 102 (35), 87 (100), 69 (11), 57 (4). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.9–1 (dd, J =0.01, 12H), 1.92–2.06 (m, 3H), 11.8 (br s, 1H). Anal. ($C_8H_{16}O_2$) C, H. Yield 72%.

2-Isopropylpentanoic acid (Propylisopropylacetic Acid, 4). Bright yellow oil. MS-EI, *m*/*z* (%): 145 (M^+, 0.08), 102 (53), 87 (23), 73 (100), 55 (16). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.84–1.06 (m, 9H), 1.2–1.66 (m, 4H), 1.8–1.95 (m, 1H), 2.08–2,18 (m, 1H), 11.6 (br s, 1H). Anal. ($C_8H_{16}O_2$) C, H. Yield 88%.

General Procedure for the Synthesis of Compounds 5–6. Enantiomerically pure (*R*)-PIA (**5**) and (*S*)-PIA (**6**) were synthesized by previously described procedures.[45]

(*R*)-2-Isopropylpentanoic Acid or (*R*)-Propylisopropylacetic Acid (5). Bright yellow oil. MS-EI, *m*/*z* (%): 145 (M^+, 0.06), 102 (50), 87 (22), 73 (100), 55 (15). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.84–1.06 (m, 9H), 1.2–1.66 (m, 4H), 1.8–1.95 (m, 1H), 2.08–2,18 (m, 1H), 11.6 (br s, 1H). Anal. ($C_8H_{16}O_2$) C, H. Yield 83%.

(*S*)-2-Isopropylpentanoic Acid or (*S*)-Propylisopropylacetic Acid (6). Bright yellow oil. MS-EI, *m*/*z* (%): 145 (M^+, 0.03), 102 (49), 87 (22), 73 (100), 55 (15). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.84–1.06 (m, 9H), 1.2–1.66 (m, 4H), 1.8–1.95 (m, 1H), 2.08–2,18 (m, 1H), 11.6 (br s, 1H). Anal. ($C_8H_{16}O_2$) C, H. Yield 89%.

General Procedure for the Synthesis of Compounds 9–13. The free carboxylic acids (**2–6**) were chlorinated with thionylchloride according to the published method.[44] Obtained acylchloride (0.03 mol) was dissolved in 50 mL of dry acetonitrile and slowly added to 100 mL of 25% NH_4OH solution cooled to 0 °C and stirred for 10 min. The product was extracted three times with 100 mL of ethylacetate, and the combined organic phases were washed three times with 30 mL of distilled water, 30 mL of 0.1 M HCl, and brine. The organic fraction was dried over $MgSO_4$, filtered, and evaporated. The obtained products were purified by crystallization from ethylacetate.

2-Ethyl-3-methylpentanamide (Valnoctamide, 9). White crystals. Mp 113–114 °C. MS-EI, *m*/*z* (%): 143 (M^+, 0.1), 114 (13), 87 (76), 72 (100), 57 (22). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.83–0.77 (m, 9H), 1.13–0.95 (m, 1H), 1.54–1.29 (m, 4H), 1.93–1.82 (m, 1H), 5.18–5.55 (br d, J = 0.39, 2H). Anal. ($C_8H_{17}NO$) C, H, N. Yield 71%.

2-Isopropyl-3-methylbutanamide (Diisopropylacetamide,10). White crystals. Mp 141.5–143.5 °C. MS-EI, *m/z* (%): 143 (M^+, 0.05), 101 (40), 86 (100), 69 (13), 57 (17). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.94–0.97 (d, $J = 0.025$, 12H), 1.65–1.74 (m, 1H), 1.92–2.04 (m, 2H), 5.38–5.76 (br d, $J = 0.38$, 2H). Anal. ($C_8H_{17}NO$) C, H, N. Yield 83%.

2-Isopropylpentanamide (Propylisopropylacetamide, 11). White crystals. Mp 130 °C. MS-EI, *m/z* (%): 143 (M^+, 0.2), 101 (57), 86 (46), 72 (100), 69 (7), 57 (30). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.88–0.98 (m, 9H), 1.14–1.62 (m, 4H), 1.72–1.86 (m, 2H), 5.3–5.58 (br d, $J = 0.1$, 2H). Anal. ($C_8H_{17}NO$) C, H, N. Yield 84%.

(*R*)-2-Isopropylpentanamide or (*R*)-Propylisopropylacetamide (12). White crystals. Mp 151–153 °C. MS-EI, *m/z* (%): 143 (M^+, 0.2), 101 (56), 86 (46), 72 (100), 69 (7), 57 (26). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.88–0.98 (m, 9H), 1.14–1.62 (m, 4H), 1.72–1.86 (m, 2H), 5.3–5.58 (br d, $J = 0.1$, 2H). Anal. ($C_8H_{17}NO$) C, H, N, O. Yield 79%.

(*S*)-2-Isopropylpentantamide or (*S*)-Propylisopropylacetamide (13). White crystals. Mp 151–153 °C. MS-EI, *m/z* (%): 143 (M^+, 0.2), 101 (59), 86 (47), 72 (100), 69 (6), 57 (28). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.88–0.98 (m, 9H), 1.14–1.62 (m, 4H), 1.72–1.86 (m, 2H), 5.3–5.58 (br d, $J = 0.1$, 2H). Anal. ($C_8H_{17}NO$) C, H, N. Yield 84%.

General Procedure for the Synthesis of Compounds 15–21. The acylchlorides (0.057 mol), prepared from the appropriate carboxylic acids with thionylchloride according to a published method,[41] were dissolved in dry acetonitrile (50 mL), and slowly added to boiling acetonitrile solution (100 mL) of 0.14 mol urea, and refluxed for 2 h. The organic solvent was then evaporated under a vacuum; the products were dissolved in 100 mL of ethylacetate and washed three times with 20 mL of distilled water. The organic fraction was dried over $MgSO_4$, filtered, and evaporated. The products were purified by crystallization from ethylacetate.

2-Propylpentanoyl Urea (15). White crystals. Mp 217–220 °C. MS-EI, *m/z* (%): 157 (2), 144 (36), 115 (100), 72 (30), 61 (21). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.86–0.94 (t, $J = 0.05$, 6H), 1.2–1.74 (m, 8H), 2.24–2.4 (m, 1H), 5.62 (s, 1H), 8.4 (s, 1H), 9.42 (s, 1H). Anal. ($C_9H_{18}N_2O_2$) C, H, N. Yield 83%.

2-Ethyl-3-methyl-pentanoyl Urea (16). White crystals. Mp 147–148 °C. MS-EI, *m/z* (%): 157 (6),130 (100), 115 (69), 72 (32), 61 (36). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.86–0.96 (m, 9H), 1.08–1.24 (m, 1H), 1.38–1.74 (m, 4H), 1.96–2.06 (m, 1H), 5.35 (s, 1H), 8.37 (s, 1H).. 8.7 (s, 1H) Anal. ($C_9H_{18}N_2O_2$) C, H, N. Yield 61%.

2-Isopropyl-3-methylbutanoyl Urea (17). White crystals. Mp 199–200 °C. MS-EI, *m/z* (%): 144 (59), 129 (100), 86 (27), 69 (34), 61 (25). ^{1}H NMR (300 MHz, $CDCl_3$ δ TMS): 0.91–0.98 (t, $J = 0.02$, 12H), 1.78–1.84 (t, $J = 0.02$, 1H), 1.98–2.1 (m, 2H), 5.49 (s, 1H), 8.41 (s, 1H). 8.9 (s, 1H). Anal. ($C_9H_{18}N_2O_2$) C, H, N. Yield 51%.

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

(*R*)-2-Isopropylpentanoyl Urea (19). White crystals. Mp 203–204 °C. MS-EI, *m/z* (%): 144 (78), 129 (63), 115 (100), 72 (37), 61 (33). ^{1}H NMR (300 MHz, DMSO-d_6): 0.76–0.88 (m, 9H), 1.06–1.22 (m, 2H), 1.26–1.52 (m, 2H), 1.6–1.74 (m, 1H), 2.08–2.2 (m, 1H), 7.21 (s, 1H), 7.86 (s, 1H), 10.14 (s, 1H). Anal. ($C_9H_{18}N_2O_2$) C, H, N. Yield 66%.

(*S*)-2-Isopropylpentanoyl Urea (20). White crystals. Mp 203–204 °C. MS-EI, *m/z* (%): 144 (73), 129 (62), 115 (100), 72 (35), 61 (34). ^{1}H NMR (300 MHz, DMSO-d_6): 0.74–0.88 (m, 9H), 1.04–1.22 (m, 2H), 1.26–1.52 (m, 2H), 1.6–1.74 (m, 1H), 2.08–2.2 (m, 1H), 7.19 (s, 1H), 7.85 (s, 1H), 10.13 (s, 1H). Anal. ($C_9H_{18}N_2O_2$) C, H, N. Yield 71%.

Octanoylurea (21). White crystals. Mp 184–185 °C. MS-EI, *m/z* (%): 157 (1), 115 (15), 102 (100), 72 (8), 59 (41). ^{1}H NMR (300 MHz, DMSO-d_6): 0.78–0.9 (t, $J = 0.02$, 3H), 1.21 (s, 8H), 1.42–1.54 (m, 2H), 2.18–2.28 (t, $J = 0.025$, 2H), 2.08–2.2 (m, 1H), 7.17 (s, 1H), 7.74 (s, 1H), 10.09 (s, 1H). Anal. ($C_9H_{18}N_2O_2$) C: calcd, 58.03; found, 57.25. H, N: calcd, 15.04; found, 14.94. Yield 85%.

Biological Testing. The evaluation of anticonvulsant activity in the maximal electroshock seizure test (MES), subcutaneous pentylenetetrazole seizure threshold test (scMet), and the 6-Hz psychomotor seizure test, as well as the determination of toxicity in the rotorod test, was performed at the NIH Epilepsy Branch as a part of the Anticonvulsant Drug Development Program according to the protocols described in ref 52.

In general, the tested compounds were suspended in 0.5% methylcellulose and administered intraperitoneally (i.p.) to male albino mice (CF-1 strain, 18–25 g) in a volume of 0.01 mL/g body weight and in a volume of 0.04 mL per 10 g of body weight orally to male albino rats (Sprague–Dawley, 100–150 g). The pentylenetetrazole solution at convulsant dose was prepared according to a previously described standard procedure.[49]

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 at least four points were established between the dose level of no protection and 100% protection. These data were subjected to probit analysis[59] 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 probit analysis.[56] The protective index was calculated by dividing the TD_{50} by the ED_{50}.

Evaluation of Teratogenicity. The teratogenicity of the compounds was evaluated in the highly inbred SWV mice strain on the basis of its known susceptibility to VPA-induced neural tube defects (NTDs) according to a published procedure.[57,58] At day 8.5 of gestation, each dam was exposed to a single i.p. injection of the tested compounds in a range of 1.1−3.6 mmol/kg or the vehicle (25% water solution of Cremophor EL, Fluka Biochemica, Germany). At 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.

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

Water Solubility of the Tested Compunds. Water solubility of a compound was assessed by stirring 10 mg of the tested compound for 2 h in 5 mL of water at room temperature and centrifuging it at 3000 g for 10 min. A 0.25 mL aliquot was taken from the supernatant for evaluation of the concentration of the dissolved compound in water by GC/MS.

Statistical Analysis. Results are presented as either the ED_{50} or TD_{50} and 95% confidence intervals. The teratogenicity data were evaluated for significance by analyzing the contingency table with Fisher's exact test. A p value <0.05 was considered significant.

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Supporting Information Available: Purity determination of urea derivatives of the constitutional isomer of VPA by combustion

analysis (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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