Epilepsy & Behavior 22 (2011) 461–468

Contents lists available at SciVerse ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Design and pharmacological activity of glycinamide and *N*-methoxy amide derivatives of analogs and constitutional isomers of valproic acid

Neta Pessah [a], Boris Yagen [a,b], Naama Hen [a], Jakob A. Shimshoni [a], Bogdan Wlodarczyk [c], Richard H. Finnell [c], Meir Bialer [a,b,*]

[a] Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel
[b] The David R. Bloom Centre for Pharmacy, The Hebrew University of Jerusalem, Jerusalem, Israel
[c] Center for Environmental and Genetic Medicine, Institute of Biosciences and Technology, Texas A&M Health Science Center, Texas A&M University, Houston, TX, USA

ARTICLE INFO

Article history:
Received 21 July 2011
Revised 22 August 2011
Accepted 23 August 2011
Available online 29 September 2011

Keywords:
Valproic acid isomers and analogues
Maximal electroshock seizure test
Subcutaneous metrazol seizure test
6-Hz psychomotor seizure test
Glycinamide conjugates
N-methoxy derivatives

ABSTRACT

A series of glycinamide conjugates and *N*-methoxy amide derivatives of valproic acid (VPA) analogs and constitutional isomers were synthesized and evaluated for anticonvulsant activity. Of all compounds synthesized and tested, only *N*-methoxy-valnoctamide (*N*-methoxy-VCD) possessed better activity than VPA in the following anticonvulsant tests: maximal electroshock, subcutaneous metrazol, and 6-Hz (32-mA) seizure tests. In mice, the ED_{50} values of *N*-methoxy-VCD were 142 mg/kg (maximal electroshock test), 70 mg/kg (subcutaneous metrazol test), and 35 mg/kg (6-Hz test), and its neurotoxicity TD_{50} was 118 mg/kg. In rats, the ED_{50} of *N*-methoxy-VCD in the subcutaneous metrazol test was 36 mg/kg and its protective index ($PI = TD_{50}/ED_{50}$) was >5.5. In the rat pilocarpine-induced status epilepticus model, *N*-methoxy-VCD demonstrated full protection at 200 mg/kg, without any neurotoxicity. *N*-Methoxy-VCD was tested for its ability to induce teratogenicity in a mouse strain susceptible to VPA-induced teratogenicity and was found to be non-teratogenic, although it caused some resorptions. Nevertheless, a safety margin was still maintained between the ED_{50} values of *N*-methoxy-VCD in the mouse subcutaneous metrazol test and the doses that caused the resorptions. On the basis of these results, *N*-methoxy-VCD is a good candidate for further evaluation as a new anticonvulsant and central nervous system drug.

1. Introduction

Valproic acid (VPA) (**1**, Fig. 1), a major antiepileptic drug (AED), is used for the treatment of various types of epileptic seizures [1–3]. Yet VPA is the least potent AED as reflected by its higher ED_{50} and higher doses compared with other AEDs [4]. In addition, its clinical use is limited because of two severe side effects: teratogenicity and hepatotoxicity [5, 6]. Consequently, there is a substantial need for the development of new second-generation drugs to VPA that preserve its broad-spectrum efficacy at lower doses and lack hepatotoxicity and teratogenicity [5–12]. Both teratogenicity and hepatotoxicity are related to structure, although, unlike teratogenicity, hepatotoxicity results from a minor metabolite(s) of VPA with a terminal double bond (e.g., 4-ene-VPA) [13]. Following extensive structure–activity relationship (SAR) studies in mouse strains prone to VPA-associated teratogenicity, it has been found that an analog of VPA is likely to be teratogenic if it contains tertiary carbon bound to a carboxylic group, a hydrogen atom, and two alky chains [8, 14, 15]. Forming a VPA CoA ester is the first step in the formation of a VPA hepatotoxic metabolite(s). Therefore, a free carboxylic group is mandatory for VPA hepatotoxicity [16].

Valpromide (VPD) (**2**, Fig. 1), the corresponding amide of VPA, is 4–10 times more potent than VPA in anticonvulsant animal (rodent) models and is nonteratogenic (mouse). VPD has been clinically used in Europe since the 1970s as an antiepileptic and antipsychotic agent [17]. Nevertheless, VPD's lack of teratogenicity and its better potency (compared with VPA) in animal models has no clinical implications, because in humans, VPD is rapidly and presystemically metabolized to VPA and thus acts as a VPA prodrug [17].

Various amide derivatives of VPA, other than VPD, have been synthesized and evaluated in our laboratory and found to possess potent anticonvulsant activity in animal models with minimal metabolism to their corresponding acids [18–20].

Glycine is a neuroinhibitory amino acid and, when co-administered with other AEDs, demonstrated improved anticonvulsant potency in rats as a result of the synergism between the AED and glycine [21–23]. Therefore, it was hypothesized that conjugation of VPA to inhibitory neurotransmitters such as glycine, taurine, and GABA would produce potent anticonvulsants. Instead, it resulted in nonactive compounds [20]. However, conjugation of VPA to the corresponding amides of glycine and taurine led to valproylglycinamide (VGD) (**3**,

* Corresponding author at: Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, POB 12065 Ein Karem, Jerusalem 91120, Israel. Fax: +972 2 6757246.
E-mail address: bialer@md.huji.ac.il (M. Bialer).

1525-5050/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.yebeh.2011.08.026

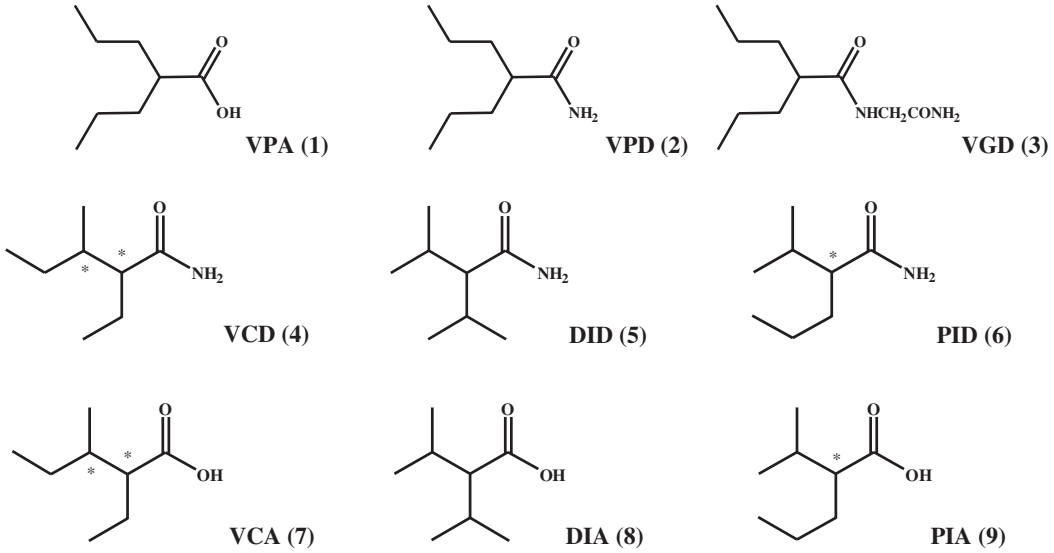


Fig. 1. Chemical structures of VPA (**1**), VPD (**2**), VGD (**3**), VCD (**4**), DID (**5**), and PID (**6**) and their corresponding acids VCA (7), DIA (**8**), and PIA (**9**).

Fig. 1) and valproyltaurinamide, respectively, which possessed potent anticonvulsant activity [24]. In mice, VGD was slightly more potent than VPA and had better protective index (PI) values in the maximal electroshock (MES) and subcutaneous metrazol (scMet) seizure tests [25]. In the rat, VGD was 14 times more potent than VPA in the MES test, but was inactive in the scMet test [18]. VGD was also more potent than VPA in the bicuculline, picrotoxin, and 6-Hz psychomotor seizure tests and in audiogenic seizure-susceptible mice [24]. VGD is currently in phase IIa clinical trials [26, 27]. Three constitutional isomers of VPD—valnoctamide (VCD) (**4**, Fig. 1), diisopropylacetamide (DID) (**5**, Fig. 1), and propylisopropylacetamide (PID) (**6**, Fig. 1)—have been synthesized and found to possess potent activity in various anticonvulsant animal models [12, 28]. VCD has been used in Europe as an anxiolytic drug. Unlike VPD, VCD underwent minimal biotransformation to its corresponding acid valnoctic acid (VCA) [29, 30]. Recently, VCD completed successful phase IIa clinical trials as an add-on therapy for risperidone in the treatment of patients with bipolar disorder [31]. VCD, DID, and PID are not teratogenic (mice) and do not metabolize in dogs and rats to their respective corresponding acids: valnoctic acid (VCA, **7**), diisopropyl acetic acid (DIA, **8**), and propylisopropylacetic acid (PIA, **9**) (Fig. 1) [20, 32–35].

Conjugates of VCA and DIA with glycinamide were synthesized and evaluated for their anticonvulsant activity in mice [25]. On a molar basis, valnoctylglycinamide (VCGD) (**10**, Fig. 2) was slightly more potent than VPA (1 mmol/kg vs 1.4 mmol/kg, respectively) in the MES test and had a PI similar to that of VPA. In the scMet test, VCGD was more potent than VPA (0.54 mmol/kg vs 1 mmol/kg) and had a better PI. Diisopropylglycinamide (DIGD) (**11**, Fig. 2) was less potent than VCGD in the MES test, but still slightly more potent than VPA (1.15 mmol/kg vs 1.4 mmol/kg). Like VCGD, DIGD was more potent and had a better PI in the scMet test (ED_{50} = 0.46 mmol/kg, PI = 3.6)

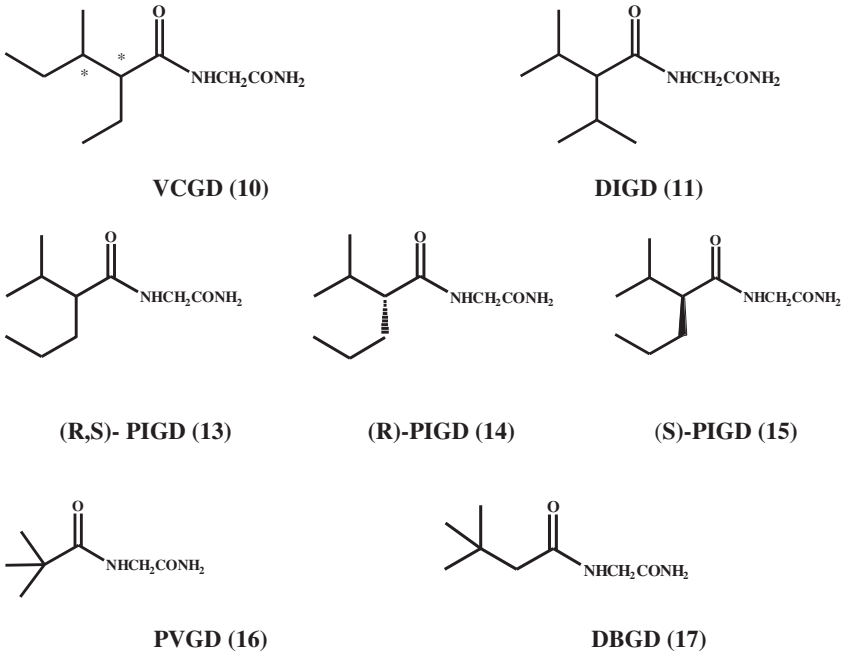


Fig. 2. Chemical structures of VGD's constitutional isomers VCGD (**10**), DIGD (**11**), (R,S)-PIGD (**13**), (R)-PIGD (**14**), and (S)-PIGD (**15**), and VGD's analogs PVGD (**16**), and DBUGD (**17**).

[25]. VCGD and DIGD were tested only in the mouse MES and scMet models.

Conjugation of VPA to hydroxamic acid and its *N*-methoxylamine derivative to yield *N*-methoxy-VPD (**12**, Fig. 3) led to two new compounds that possess better anticonvulsant activity than VPA in the MES test in mice. Valproyl hydroxamic acid was metabolically stable and was not hydrolyzed to VPA in dogs [19]. The *N*-methoxy amide derivative of 2,2,3,3-tetramethylcyclopropanecarboxylic acid (TMCA), *N*-methoxy-TMCD, was found to be highly potent in several animal models of epilepsy [36–38].

In a study conducted previously in our laboratory, urea derivatives of VPA analogs with short alkyl chain moieties were synthesized and tested as anticonvulsants [39]. The urea derivatives of pivalic acid (2,2-dimethylpropionic acid, PVU) and 3,3-dimethylbutyric acid (DBU) were found to possess anticonvulsant activity in mice [39].

In this study we synthesized VCGD and DIGD to evaluate their anticonvulsant activity in the rat following oral dosing, the likely mode of administration of new AEDs. Furthermore, as a continuation of our previous studies on developing new central nervous system-active VPA derivatives, we aimed to explore a series of glycinamides and *N*-methoxy amide derivatives of VPA constitutional isomers (including PIA) and short-chain analogs. PIA is a chiral constitutional isomer of VPA with one asymmetric carbon in its structure; it is thus composed of two enantiomers: (*R*)-PIA and (*S*)-PIA. In this study we synthesized racemic PIA-glycinamide ((*R,S*)-PIGD, **13**), and its two individual enantiomers (*R*)-PIGD (**14**) and (*S*)-PIGD (**15**) (Fig. 2). In addition, we also synthesized and evaluated pivaloylglycinamide (PVGD, **16**) and 3,3-dimethylbutyrylglycinamide (DBUGD, **17**), two glycinamide conjugates of VPA analogs with short side chains and the N-methoxy derivatives of PID (**18**), DID (**19**), VCD (**20**), PVD (**21**), and DBD (**22**).

2. Materials and Methods

2.1. Chemicals

Chemicals were purchased from Sigma–Aldrich. The pure enantiomers of propylisopropylacetic acid ((*R*)-PIA and (*S*)-PIA) were purchased from Zyfine-Cadilla Healthcare (Ahmedaba, India). Tetrahydrofuran (THF), acetonitrile (ACN), dichloromethane (DCM), petroleum ether, ammonium hydroxide, 25% NH_3 in water, and ethyl acetate were purchased from Frutarom Israel. Dry dichloromethane, tetrahydrofuran, acetonitrile, and DMPU were obtained by reflux over CaH_2 for 2 hours and fresh distillation prior to use. DMPU was refluxed over CaH_2 for 2 hours, distilled under reduced pressure, and stored over 4-Å molecular sieves (8–12 mesh) under a nitrogen atmosphere.

2.2. Animals

Adult male CF No.1 albino mice (18–25 g) and adult male Sprague–Dawley albino rats (100–150 g) were used for evaluation of anticonvulsant activity and neurotoxicity. Animals were maintained in the animal facilities of the University of Utah. Animals were allowed to acclimate for 24 to 48 hours before testing. A 12-hour light, 12-hour dark cycle was maintained, and the animals were allowed free access to food and water, with the exception of the testing times. The animals were maintained and handled according to the recommendations of the U.S. Department of Health, Education and Welfare publication (NIH) No. 8623, *Guide for the Care and Use of Laboratory Animals*. All animal experiments were approved by the Institutional Animal Care and Use Committee of the University of Utah.

Virgin SWV/Fnn female mice (2–3 months of age, 20–25 g) and SWV/Fnn male mice (3–4 months of age, 25–35 g) raised from a breeding colony at the Institute of Biosciences and Technology, Houston, TX, USA, were used in the teratogenicity studies. The mice were housed in clear polycarbonate cages, and were allowed free access to food and water (Harlan Tekad, Madison, WI, USA; Rodent Diet 8604), and maintained on a 12-hour light, 12-hour dark cycle in the vivarium at the Institute of Biosciences and Technology, Houston. The teratogenicity studies were approved by the Institutional Animal Care and Use Committee of Texas A&M University.

2.3. Anticonvulsant activity

The protocols used by the NIH Anticonvulsant Drug Development Program were followed [40]. Anticonvulsant activity was identified first in mice (intraperitoneally) using the MES and scMet seizure tests and, in some cases, also the 6-Hz psychomotor seizure test. The active compounds were subsequently evaluated in rats (orally).

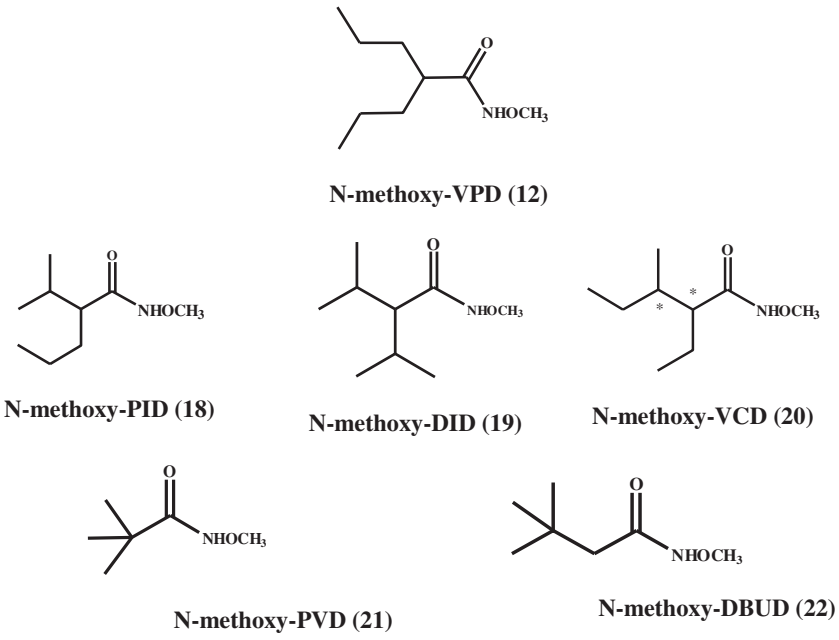


Fig. 3. Chemical structures of the *N*-methoxy derivatives of VPA's constitutional isomers and analogs: *N*-methoxy-VPD (**12**), *N*-methoxy-PID (**18**), *N*-methoxy-DID (**19**), *N*-methoxy-VCD (**20**), *N*-methoxy-PVD (**21**), and *N*-methoxy-DBUD (**22**).

Table 1
Anticonvulsant activity and toxicity of compounds **13–22** after intraperitoneal administration in mice.

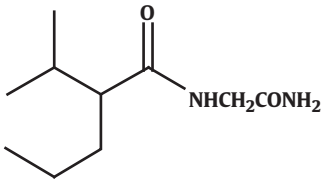
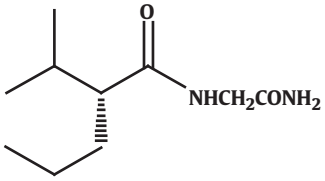
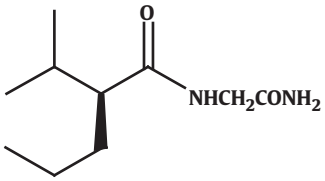
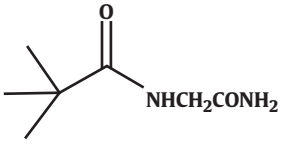
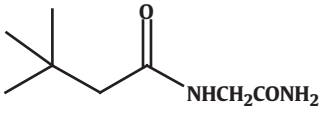
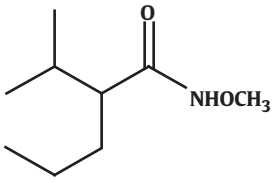
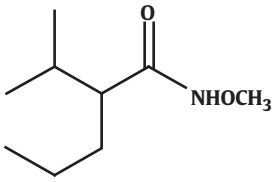
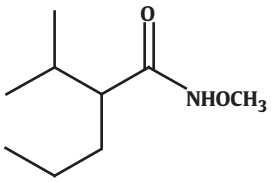
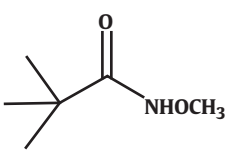
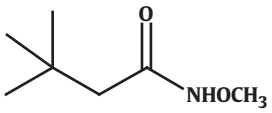
Compound	Structure	Dose (mg/kg)	MES test		scMet test		Neurotoxicity	
			0.5 h	4h	0.5 h	4h	0.5 h	4h
(*R,S*)-PIGD (**13**)		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	1/3	0/3	0/1	0/1	0/8	0/4
		300	1/1	0/1	0/1	0/1	0/4	0/2
(*R*)-PIGD (**14**)		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	0/3	0/1	0/1	0/8	0/4
		300	1/1	0/1	0/1	0/1	0/4	0/2
(*S*)-PIGD (**15**)		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	0/3	0/1	0/1	0/8	0/4
		300	1/1	0/1	0/1	0/1	1/4	0/2
PVGD (**16**)		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	0/3	0/1	0/1	0/8	0/4
		300	0/1	0/1	0/1	0/1	0/4	0/2
TBUGD (**17**)		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	0/3	0/1	0/1	0/8	0/4
		300	0/1	0/1	0/1	0/1	0/4	0/2
N-Methoxy-PID (**18**)		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	1/3	1/3	1/5	0/1	2/8	0/4
		300	1/1	1/1	1/1	0/1	3/4	0/2
N-Methoxy-DID (**19**)		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	3/3	0/3	0/1	0/1	1/8	0/4
		300	1/1	0/1	1/1	0/1	4/4	0/2
N-Methoxy-VCD (**20**)		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	3/3	0/3	0/1	0/1	0/8	0/4
		300	1/1	1/1	1/1	0/1	4/4	0/2
N-Methoxy-PVD (**21**)		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

Table 1 (*continued*)

Compound	Structure	Dose (mg/kg)	MES test		scMet test		Neurotoxicity	
			0.5 h	4h	0.5 h	4h	0.5 h	4h
N-Methoxy-DBUD (**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	5/5	1/1	0/4	0/2

Neurotoxicity was assessed in mice with the rotorod ataxia test, and in rats, with positional sense, gait, and stance tests. After the initial qualitative analysis, the ED_{50} and 95% confidence interval (95% CI) of the active compounds were determined using probit analysis [40].

2.3.1. Maximal electroshock seizure test

In this test an alternating current of 50 mA for adult male CF No. 1 albino mice and 150 mA for adult male Sprague–Dawley albino rats is delivered for a period of 0.2 second through a corneal electrode: A drop of 0.5% tetracaine solution in saline is applied to the subject animal's eyes immediately after the test substance is administered. Saline is placed in each eye and immediately the corneal electrodes are placed. After stimulation, the animals are released and observed throughout the seizure. The endpoint of the MES test is the abolition of the hindlimb tonic extensor component. When the hindlimbs are not fully extended at 180° with the body plane, tonic extension is considered abolished. If such extension does not occur, then the tested substance is able to prevent the spread of seizure discharge through neural tissues.

2.3.2. Subcutaneous metrazol seizure threshold test

In this test a convulsive dose of metrazol is injected subcutaneously into CF No. 1 albino mice (85 mg/kg) and adult male Sprague–Dawley albino rats (56.4 mg/kg). The animals are isolated and observed for the presence or absence of clonic spasms that persist at least 5 seconds. When clonic seizures are not observed, the test compound is considered effective in raising the seizure threshold.

2.3.3. 6-Hz psychomotor seizure test

This test is performed in CF No. 1 albino mice only. The mice are first injected intraperitoneally with the test substance. Then, at varying times (0.25, 0.5, 1, 2, and 4 hours), a sufficient current of 32 mA is delivered at 6 Hz for 3 seconds through corneal electrodes to induce a psychomotor seizure, characterized by a minimal clonic phase followed by stereotyped, automatistic behavior. The test compound is considered effective when the tested animals do not display such behavior.

2.3.4. Pilocarpine-induced status epilepticus test

To determine if *N*-methoxy-VCD can prevent acute pilocarpine-induced status, a challenge dose of pilocarpine was administered to male albino Sprague–Dawley rats (150–180 g), and *N*-methoxy–VCD was administered immediately after the first stage 3 convulsive seizure was observed. Seizure severity was determined by using the established Racine scale [41]. Rats were considered protected if no additional stage 3 (or greater) seizures were observed over the remaining observation period. *N*-Methoxy-VCD was tested only at doses of 200 mg/kg at seizure onset and 400 mg/kg 30 minutes after seizure onset.

Table 2
Comparative ED_{50} values of VPA (**1**), *N*-methoxy-VPD (**12**), and *N*-methoxy-VCD determined after intraperitoneal administration in mice.

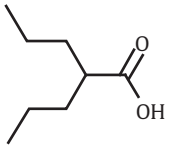
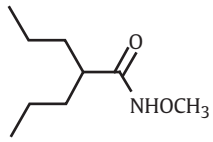
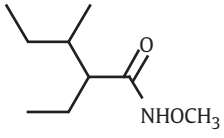
Compound	MES test		scMET test		6-Hz test		Neurotoxicity
	ED_{50}	PI	ED_{50}	PI	ED_{50}	PI	
 VPA (**1**)	263	1.5	220	1.8	126	3.2	398
 N-methoxy-VPD (**12**)	80	2	180	0.87	NT	—	157
 *N*-Methoxy-VCD (**20**)	143	0.8	70	1.7	35	3.4	118

Table 3
Anticonvulsant activity and neurotoxicity of compounds **10**, **11**, 1**3**, **20**, and **22** administered orally to rats.

Compound	Test	Dose (mg/kg)	0.25 h	0.5 h	1.0 h	2.0 h	4.0 h
VCGD (**10**)	MES test	50	0/4	0/4	0/4	1/4	0/4
	scMet test	50	—	0/3	0/4	0/4	—
	Neurotoxicity	50	0/4	0/4	0/4	0/4	0/4
DIGD (**11**)	MES test	50	0/4	2/4	0/4	0/4	0/4
	scMet test	50	0/4	0/4	0/4	—	—
	Neurotoxicity	50	0/4	0/4	0/4	0/4	0/4
PIGD (**13**)	MES	50	1/4	1/4	0/4	0/4	1/4
	scMet	100	1/4	2/4	1/4	0/4	0/4
	Neurotoxicity	100	0/4	0/4	0/4	0/4	0/4
N-Methoxy-VCD (**20**)	scMet test	75	2/4	4/4	3/4	—	—
	scMet test	100	3/4	2/4	3/4	0/4	0/4
N-Methoxy-DBUD (**22**)	scMet test	50	0/4	1/4	0/4	1/4	1/4
	Neurotoxicity	50	0/4	0/4	0/4	0/4	0/4

2.4. Teratogenicity analysis: Induction of neural tube defect

For this study, the highly inbred SWV/Fnn mouse strain with a known susceptibility to AED-induced neural tube defects (NTDs) [42] was used, according to the previously published procedure [43]. Dams were allowed to mate overnight with male mice and were examined the following morning for the presence of vaginal plugs. The onset of gestation was set at 10 PM of the previous night. On gestational day 8.5, pregnant females received a single intraperitoneal injection (10 μL/g body wt) of sodium valproate 3.6, 2.7, or 1.8 mmol/kg or *N*-methoxy-VCD 1.8 or 1.1 mmol/kg. Dams injected intraperitoneally with 25% Cremophor EL water solution constituted the control group. On gestation day 8.5, dams were sacrificed by CO_2 asphyxiation, the abdomens opened, and the gravid uteri removed. Locations of all viable, dead, and resorbed fetuses were recorded, and the fetuses were grossly examined for the presence of exencephaly.

3. Results

3.1. Anticonvulsant activity

Comparison in mice of the qualitative anticonvulsant activity of PIGD (racemate or individual enantiomer) with that of its constitutional isomers VCGD and DIGD is outlined in Table 1, along with the results for compounds **13**–**22**. Despite the activity of VCGD (**10**) and DIGD (**11**) in the mouse MES and scMet tests, PIGD (racemate and individual enantiomers) showed minimal anticonvulsant activity at doses up to 300 mg/kg.

N-Methoxy-PVD (**21**) and *N*-methoxy-DBUD (**22**) were inactive in the MES and scMet tests and exhibited no neurotoxicity in mice. *N*-Methoxy-VCD was active at 100 and at 300 mg/kg and its mouse ED_{50} values (Table 2) were (MES test) 142 mg/kg (95% CI = 116–175 mg/kg), (scMet test) 70 mg/kg (95% CI = 61–95 mg/kg), and (6-Hz test) 35 mg/kg (95% CI = 22–50 mg/kg). Its neurotoxicity TD_{50} was 118 mg/kg (95% CI = 94–136 mg/kg). Consequently, its PI values were 0.8 (MES), 1.7 (scMet), and 3.4 (6 Hz). In the MES test no separation was observed between activity and neurotoxicity. *N*-Methoxy-VCD was more potent than VPA in the scMet and 6-Hz tests and had PI values similar to those of VPA. In contrast to its constitutional isomer, *N*-methoxy-VPD, which was relatively potent in the MES test (80 mg/kg), *N*-methoxy-VCD was more potent in the scMet test and had a better PI (Table 2).

PIGD (racemate, **13**), *N*-methoxy-VCD (**20**), and *N*-methoxy-DBUD (**22**) were tested in rats, and their anticonvulsant activity in comparison to that of VCGD (**10**) and DIGD (**11**) is summarized in Table 3. Despite relatively potent activity (compared with VPA) in mice, the constitutional isomers of VGD exhibited minimal anticonvulsant activity in the rat MES and scMet tests. VCGD and DIGD were inactive in both tests at 50 mg/kg with the exception of one or two of four rats in the MES test. In contrast to its two isomers, PIGD exhibited partial anticonvulsant activity in both the MES (50 mg/kg) and scMet (100 mg/kg) tests. All three constitutional isomers did not demonstrate any neurotoxicity at the tested doses, but the ED_{50} value was >200 mg/kg.

In contrast to the glycinamide derivatives of VPA constitutional isomers, *N*-methoxy-VCD was active in the rat scMet test and, at a dose of 75 mg/kg, afforded full protection 30 minutes after oral dosing in rats. Consequently, its ED_{50} was assessed at 30 minutes, and the ratios of protected to treated rats were 1/8 (12.5 mg/kg), 2/8 (25 mg/kg), 6/8 (50 mg/kg), and 7/8 (100 mg/kg). The slope of the dose–response curve was 2.8, and the ED_{50} of *N*-methoxy-VCD was 36 mg/kg (95% CI = 21–59 mg/kg). *N*-Methoxy-VCD exhibited no neurotoxicity in the rat at doses >200 mg/kg; therefore, its scMet PI is at least 5.5. The ability of *N*-methoxy-VCD to protect rats against chemically induced status epilepticus was evaluated in the pilocarpine-induced status model. *N*-Methoxy-VCD (200 mg/kg) prevented status epilepticus in all eight rats tested at the indicating ED_{50} values <100mg/kg, with only one rat observed to be sedated.

The teratogenic potential of *N*-methoxy-VCD was tested in a mouse model highly susceptible to VPA-induced NTDs. The results of the teratogenicity analysis compared with VPA are summarized in Table 4. At doses of 312 and 189 mg/kg, *N*-methoxy-VCD did not cause NTDs, but did result in 7–9% resorption.

Table 4
Teratogenic effect of *N*-methoxy-VCD (**20**) compared with VPA (**1**) in the SWV mouse model.

Compound	Dose mg/kg (mmol/kg)	Number of litters	Number of implants	Number of resorptions (%)	Number of live fetuses (%)	Number of fetuses with NTD[a] (%)
Control	0	11	128	0	128	0
VPA (**1**)	600 (3.6)	13	131	10 (7.6)	107 (81.7)	57 (53.3)
VPA (**1**)	452 (2.7)	13	160	18 (11.3)	141 (88.1)	41 (29.1)
VPA (**1**)	301 (1.8)	12	154	17 (11.0)	133 (86.4)	2 (1.5)
N-Methoxy-VCD (**20**)	312 (1.8)	10	145	13 (9.0)	132 (91)	1 (0.8)
N-Methoxy-VCD (**20**)	189 (1.1)	11	141	10 (7.1)	131 (92.9)	0

[a] Neural tube defect.

4. Discussion

The aim of this study was to find potent anticonvulsants that might be follow-up compounds to VPA.

Responses to VCGD and DIGD, previously synthesized and tested in mice, were evaluated in rats following oral administration. Testing in rat models of epilepsy following oral administration is important as it may better predict potential antiepileptic activity in humans. A better correlation was demonstrated between rat MES ED_{50} values (compared with those of mice) and AED therapeutic plasma levels in patients [4]. Despite their activity in the mouse MES and scMet models and in contrast to the better activity in rats demonstrated by other VPA amide derivatives [28], VCGD and DIGD exhibited no consistent anticonvulsant activity when tested in the rat. Similarly PIGD, the constitutional isomer of VCGD and DIGD, also exhibited minor activity in the rat MES and scMet models. The reason could be the relatively low solubility of the compounds or erratic absorption of the compounds following oral dosing in rats.

Despite the high potency of the urea derivatives of PVA and dimethylbutyrylacetic acid [39], the glycinamide conjugates and *N*-methoxy derivatives PVGD (**16**), DBUGD (**17**), *N*-methoxy-PVD (**21**), and *N*-methoxy-DBUGD (**22**) were inactive in both MES and scMet tests. It is impossible to predict the anticonvulsant potential of a new compound even when its structure is based on well-established pharmacophores, namely, glycinamide and *N*-methoxy moieties linked to an aliphatic side chain or an "alkyl carrier" as in the case of PVA and DBUA. What is important is the combination of the alkyl moiety along with the different amide moiety to create a new chemical entity with optimal pharmacological qualities. Therefore, screening in animal models is inevitable and essential in the search for new anticonvulsants, particularly if these anticonvulsants have multiple mechanisms of action [12, 44]. Of all the compounds tested, only *N*-methoxy-VCD demonstrated quantitative anticonvulsant activity in both mice and rats, and was found to be more potent than VPA (Table 2). *N*-Methoxy-VCD was 4 times more potent than VPA in the mouse scMet and 6-Hz models and 20 times more potent in the rat scMet test.

Status epilepticus is one of the most severe pathological conditions. It is usually defined as continuous seizure activity lasting at least 30 minutes or intermittent seizure activity lasting 30 minutes or longer during which consciousness is not regained. Overall mortality from status epilepticus in adults is about 25% [45] and it is very important to find new compounds that prevent its occurrence or stop it if already started. *N*-Methoxy-VCD (200 mg/kg) prevented status epilepticus in all eight rats tested at 200 mg/kg, and thus, its ED_{50} is <200 mg/kg. In a subsequent study, *N*-methoxy-VCD (400 mg/kg) administered 30 minutes after seizure onset was tested in the pilocarpine SE model. Unfortunately, no protection was observed and all eight animals died during testing.

The major AEDs—VPA, carbamazepine, phenytoin, phenobarbital, and lamotrigine—are associated with teratogenicity [11, 46]. It is essential to develop new AEDs that are not teratogenic. Despite the fact that the degree of teratogenicity in humans cannot be precisely predicted from animal models, it is very probable that drugs that cause malformations in animals will also be harmful in humans.

N-Methoxy-VCD was found to be nonteratogenic in a mouse model highly susceptible to VPA-induced teratogenicity (Table 4). Despite the 7–9% rate of resorptions, the lower dose tested for teratogenicity (189 mg/kg) is 2.7–5.4 times higher than the ED_{50} of *N*-methoxy-VCD in the mouse scMet and 6-Hz tests.

5. Conclusions

The main pathway in the discovery of new chemical entities for treatment of epilepsy is the use of structure–activity relationship studies and comparative analysis in different anticonvulsant animal models [12, 44, 47]. The aim of this study was to design and synthesize new nonteratogenic derivatives of the constitutional isomers and analogs of VPA that possess better anticonvulsant activity and safety margins (PI value) than VPA. On the basis of previous structure–activity relationship studies we synthesized and evaluated glycinamides and *N*-methoxy amides of the constitutional isomers and analogs of VPA. The most active compound that emerged from these groups of compounds was *N*-methoxy-VCD, which was not teratogenic in a mouse model. These results, along with the ability of *N*-methoxy-VCD to block the emergence of status epilepticus in the pilocarpine model in the rat, make this compound a good candidate for further investigation as a new anticonvulsant and a central nervous system-active compound.

References

[1] Bourgeois FDB. Valproic acid: clinical efficacy and use in epilepsy. In: Levy RH, Mattson RH, Meldrum BS, Perucca E, editors. Antiepileptic drugs. 5th ed. New York: Lippincott Williams & Wilkins; 2002. p. 808–17.
[2] Brodie MJ. Do we need any more new antiepileptic drugs? Epilepsy Res 2001;45:3–6.
[3] Loscher W. Basic pharmacology of valproate. CNS Drugs 2002;16:669–94.
[4] Bialer M, Twyman RE, White HS. Correlation analysis between anticonvulsant-ED50 values of antiepileptic drugs in mice and rats and their therapeutic doses and plasma levels. Epilepsy Behav 2004;5:866–72.
[5] Kaneko S, Battino D, Andermann E, et al. Congenital malformations due to antiepileptic drugs. Epilepsy Res 1999;33:145–58.
[6] Zimmerman HJ, Ishak KG. Valproate-Induced hepatic injury: analyses of 23 fatal cases. Hepatology 1982;2:591–7.
[7] Sussman NM, Mclain Jr LW. A direct hepatotoxic effect of valproic acid. JAMA 1979;242:1173–4.
[8] Nau H, Hauck RS, Ehlers K. Valproic acid-induced neural tube defects in mouse and human: aspects of chirality, alternative drug development, pharmacokinetics and possible mechanisms. Pharmacol Toxicol 1991;69:310–21.
[9] Perucca E. Birth defects after prenatal exposure to antiepileptic drugs. Lancet Neurol 2005;4:781–6.
[10] Koenig SA, Buesing D, Longin E, et al. Valproic acid-induced hepatopathy: nine new fatalities in Germany from 1994 to 2003. Epilepsia 2006;47:2027–31.
[11] Battino D, Tomson T. Management of epilepsy during pregnancy. Drugs 2007;67:2727–46.
[12] Bialer M, White HS. Key factors in the discovery and development of new antiepileptic drugs (AEDs). Nat Rev Drug Discov 2010;9:68–83.
[13] Granneman GR, Wang S, Kesterson JW, McHinist JM. The hepatotoxicity of valproic acid and its metabolism in rats: II. Intermediary and valproic acid metabolism. Hepatology 1984;4:1153–8.
[14] Bojic U, Elmazar MMA, 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;9:866–70.
[15] Bojic U, Ehlers K, Ellerbeck U, et al. Studies on the teratogen pharmacophore of valproic acid analogues: evidence of interactions at a hydrophobic centre. Eur J Pharmacol 1998;354:289–99.
[16] Tang W, Abbott FS. Characterization of thiol-conjugated metabolites of 2-propyl-4-pentenoic acid (4-ene-VPA), a toxic metabolite of valproic acid, by electrospray tandem mass spectrometry. J Mass Spectrom 1996;13:926–36.
[17] Bialer M. Clinical pharmacology of valpromide. Clin Pharmacokinet 1991;20:114–22.
[18] Hadad S, Bialer M. Pharmacokinetic analysis and antiepileptic activity of *N*-valproyl derivatives of GABA and glycine. Pharm Res 1995;12:905–10.
[19] Levi M, Yagen B, Bialer M. Pharmacokinetics and antiepileptic activity of valproyl hydroxamic acid derivatives. Pharm Res 1997;14:213–7.
[20] 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;16:203–11.
[21] Seiler N, Sarhan S. Synergistic anticonvulsant effects of a gaba agonist and glycine. Gen Pharmacol 1984;15:367–9.
[22] Toth E, Lajtha A. Glycine potentiates the action of some anticonvulsant drugs in some seizure models. Neurochem Res 1984;9:1711–8.
[23] Liu Z, Seiler N, Marescaux C, Depaulis A, Vergnes M. Potentiation of gamma-vinyl GABA (vigabatrin) effects by glycine. Eur J Pharmacol 1990;182:109–15.
[24] Isoherranen N, Woodhead JH, White HS, Bialer M. Anticonvulsant profile of valrocemide (TV1901): a new antiepileptic drug. Epilepsia 2001;42:831–6.
[25] Hadad S, Bialer M. Pharmacokinetic analysis and antiepileptic activity of two new isomers of *N*-valproyl glycinamide. Biopharm Drug Dispos 1997;18:557–66.
[26] Hovinga CA. Novel anticonvulsant medications in development. Expert Opin Investig Drugs 2002;11:1387–406.
[27] Bialer M, Johannessen SI, Kupferberg HJ, Levy RH, Perucca E, Tomson T. Progress report on new antiepileptic drugs: a summary of the eighth Eilat conference (Eilat VIII). Epilepsy Res 2007;73:1–52.
[28] Bialer M, Yagen B. Valproic acid: second generation. Neurotherapeutics 2007;4:130–7.

[29] Bialer M, Haj-Yehia, Barzaghi N, Pisani F, Perucca E. Pharmacokinetics of a valpromide isomer, valnoctamide, in healthy subjects. Eur J Clin Pharmacol 1990;38:289–91.
[30] Barel S, Yagen B, Schurig V, et al. Stereoselective pharmacokinetic analysis of valnoctamide in healthy subjects and in patients with epilepsy. Clin Pharmacol Ther 1997;61:442–9.
[31] Bersudsky Y, Applebaum J, Gaiduk Y, et al. Valnoctamide as a valproate substitute with low teratogenic potential in mania: a double-blind, controlled, add-on clinical trial. Bipolar Disord 2010;12:376–82.
[32] Haj-Yehia A, Bialer M. Pharmacokinetics of a valpromide isomer, valnoctamide, in dogs. J Pharm Sci 1988;77:831–4.
[33] Haj-Yehia A, Hadad S, Bialer M. Pharmacokinetic analysis of the structural requirements for forming "stable" analogues of valpromide. Pharm Res 1992;9:1058–63.
[34] Bialer M, Haj-Yehia A, Badir K, Hadad S. Can we develop improved derivatives of valproic acid? Pharm World Sci 1994;16:2–6.
[35] Kaufmann D, Yagen B, Minert A, et al. Evaluation of the antiallodynic, teratogenic and pharmacokinetic profile of stereoisomers of valnoctamide, an amide derivative of a chiral isomer of valproic acid. Neuropharmacology 2010;52:1228–36.
[36] Pessah N, Bialer M, Wlodarczyk B, Finnell RH, Yagen B. Alpha-fluoro-2,2,3,3-tetramethylcyclopropanecarboxamide, a novel potent anticonvulsant derivative of a cyclic analogue of valproic acid. J Med Chem 2009;52:2233–42.
[37] 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;47:4316–26.
[38] Sobol E, Yagen B, Lamb JG, et al. Anticonvulsant activity, neural tube defect induction, mutagenicity and pharmacokinetics of a new potent antiepileptic drug, *N*-methoxy-2,2,3,3-tetramethylcyclopropane carboxamide. Epilepsy Res 2007;73:75–84.
[39] Shimshoni JA, Yagen B, Pessah N, Wlodarczyk B, Finnell RH, Bialer M. Anticonvulsant profile and teratogenicity of 3,3-dimethylbutanoylurea: a potential for a second generation drug to valproic acid. Epilepsia 2008;49:1202–12.
[40] White HS, Woodhead JH, Wilcox KS, Stables JP, Kupferberg HJ, Wolf HN. Discovery and preclinical development of antiepileptic drugs. In: Levy RH, Mattson RH, Meldrum BS, Perucca E, editors. Antiepileptic drugs. 5th ed. New York: Lippincott Williams & Wilkins; 2002. p. 36–48.
[41] Racine RJ. Modification of seizure activity by electrical stimulation: II. Motor seizure. Electroencephalogr Clin Neurophysiol 1972;32:281–94.
[42] Finnell RH, Bennett GD, Karras SB, Mohl VK. Common hierarchies of susceptibility to the induction of neural tube defects in mouse embryos by valproic acid and its 4-propyl-4-pentenoic acid metabolite. Teratology 1988;38:313–20.
[43] Finnell RH, Wlodarczyk B, Craig JC, Piedrahita H, Bennett GD. Strain dependent alteration in the expression of folate pathway genes following teratogenic exposure to valproic acid in mouse model. Am J Med Genet 1997;70:303–11.
[44] Emma SJ, Williams M. Challenges in the search for drugs to treat central nerve systems disorders. J Pharmacol Exp Ther 2009;329:401–11.
[45] Chapman MG, Smith M, Hirsch NP. Status epilepticus. Anaesthesia 2001;56:648–59.
[46] Morrell MJ. The new antiepileptic drugs and women: efficacy, reproductive health, pregnancy, and fetal outcome. Epilepsia 1996;37(Suppl. 6):S34–44.
[47] Rogawski MA. Diverse mechanisms of antiepileptic drugs in the development pipeline. Epilepsy Res 2006;69:273–94.