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Pharmacodynamic and pharmacokinetic analysis of CNS-active constitutional isomers of valnoctamide and *sec*-butylpropylacetamide — Amide derivatives of valproic acid

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ABSTRACT

Valnoctamide (VCD) and *sec*-butylpropylacetamide (SPD) are CNS-active closely related amide derivatives of valproic acid with unique anticonvulsant activity. This study evaluated how small chemical changes affect the pharmacodynamics (PD; anticonvulsant activity and teratogenicity) and pharmacokinetics (PK) of three constitutional isomers of SPD [*sec*-butylisopropylacetamide (SID) and *tert*-butylisopropylacetamide (TID)] and of VCD [*tert*-butylethylacetamide (TED)]. The anticonvulsant activity of SID, TID, and TED was comparatively evaluated in several rodent anticonvulsant models. The PK–PD relationship of SID, TID, and TED was evaluated in rats, and their teratogenicity was evaluated in a mouse strain highly susceptible to teratogen-induced neural tube defects (NTDs). *sec*-Butylisopropylacetamide and TID have a similar PK profile to SPD which may contribute to their similar anticonvulsant activity. *tert*-Butylethylacetamide had a better PK profile than VCD (and SPD); however, this did not lead to a superior anticonvulsant activity. *sec*-Butylisopropylacetamide and TED did not cause NTDs at doses 4–7 times higher than their anticonvulsant ED_{50} values. In rats, SID, TID (ip), and TED exhibited a broad spectrum of anticonvulsant activity. However, combined anticonvulsant analysis in mice and rats shows SID as the most potent compound with similar activity to that of SPD, demonstrating that substitution of the isobutyl moiety in the SPD or VCD molecule by *tert*-butyl as well as a propyl-to-isopropyl replacement in the SPD molecule did not majorly affect the anticonvulsant activity.

1. Introduction

Valnoctamide (VCD, Fig. 1) is a CNS-active chiral constitutional isomer of valpromide (VPD, Fig. 1), the corresponding amide of valproic acid (VPA) [1–3]. Unlike VPD that in humans acts as a prodrug to VPA, VCD acts as a drug on its own with minimal biotransformation to its corresponding acid, valnoctic acid (VCA) [1,4–6]. *sec*-Butylpropylacetamide (SPD, Fig. 1) is a one-carbon homologue of VCD currently in the preclinical stage that has unique activity against status epilepticus and organophosphate neuronal damage [7–9]. Both VCD and SPD (Fig. 1) possess two stereogenic carbons in their structure and exist as racemic mixture of 4 stereoisomers.

Valnoctamide (racemate) was commercially available as an anxiolytic drug (Nirvanil®) in several European countries from 1964 until as recently as 2005 [1–3]. Following a successful phase IIa study in patients with mania funded by the Stanley Medical Research Institute (SMRI) [10], teratogenicity studies (by the SMRI) comparing racemic to VPA (head-to-head) in mice, rats, and rabbits at Covance Laboratories were conducted. In these additional studies, VCD, in contrast to VPA, failed to demonstrate teratogenic potential in mice and rabbits. In rats, a modest teratogenic signal was observed at plasma concentrations 15 times higher than VCD therapeutic plasma levels [11]. Consequently, VCD recently underwent a 3-week SMRI-funded phase IIb, randomized, double-blind, multicenter, monotherapy study in patients with bipolar manic episodes [11]. Interim analysis of the primary study endpoint for the modified intent-to-treat (mITT) cohort demonstrated the superiority of the risperidone treatment compared to placebo ($p = 0.0446$) while VCD did not. In contrast, the Completers Cohort analysis demonstrated the superiority of both VCD ($p = 0.0159$) and risperidone ($p = 0.0188$) over the placebo treatment. The difference in the VCD effect between the mITT and Completers Cohorts is unclear and is in contrast to the phase IIa results, but it shows that patients with bipolar manic episodes who stayed on VCD benefitted from it [12]. Because of the mITT interim analysis results, the SMRI decided to early terminate the phase IIb study.

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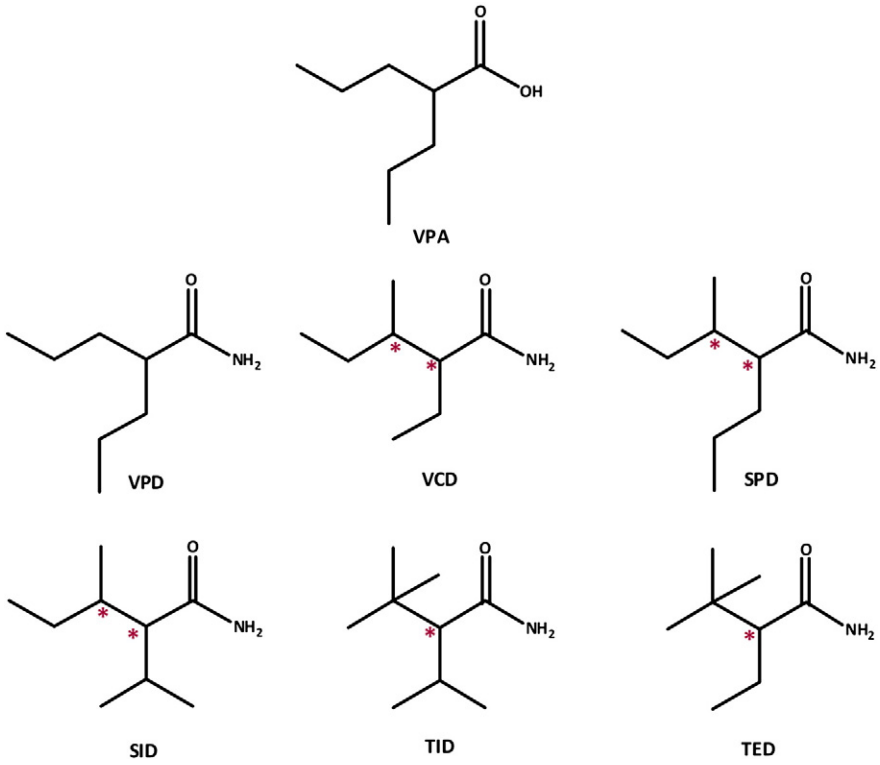

Fig. 1. Chemical structure of valproic acid (VPA), valpromide (VPD), valnoctamide (VCD), *sec*-butylpropylacetamide (SPD), *sec*-butylisopropylacetamide (SID), *tert*-butylisopropylacetamide (TID), and *tert*-butylethylacetamide (TED).

Racemic VCD and racemic SPD have a wide spectrum of anticonvulsant activity, and their ED_{50} values are 2–16 times (depending on the model) more potent than those of VPA [7,9,13–15]. Valnoctamide (65 mg/kg, ip) also provided a full protection in the pilocarpine-induced status epilepticus (SE) rat model when administered at seizure onset. However, in contrast to its one-carbon homologue, SPD, VCD lost its behavioral SE protection when administered (80 mg/kg) 30 min after seizure onset [14], but it did block pilocarpine-induced electrographic SE at a higher dose (180 mg/kg) [8]. Following a single dose (600 mg/kg, ip) at day 8.5 of gestation, VCD and SPD and their individual stereoisomers failed to exert any significant teratogenic effect in Swiss Vancouver (SWV)/Fnn mice, an inbred mouse strain that is highly susceptible to VPA-induced teratogenicity [9,14].

All studies so far have focused on VCD and SPD and their individual stereoisomers [7,9,13,14]. The current study comparatively evaluated the pharmacodynamics (PD; anticonvulsant activity and teratogenicity) and pharmacokinetics (PK) of three constitutional isomers of SPD [*sec*-butylisopropylacetamide (SID) and *tert*-butylisopropylacetamide (TID)] and of VCD [*tert*-butylethylacetamide (TED)]. *tert*-Butylethylacetamide and TID are the two new constitutional isomers of VCD and SPD, respectively, where the *sec*-butyl moiety was substituted with *tert*-butyl. *sec*-Butylisopropylacetamide is an SPD constitutional isomer where SPD's n-prorpyl moiety was replaced by isopropyl. The study also aimed to explore the impact of small chemical changes on the unique anticonvulsant activity of VCD and SPD.

2. Materials and methods

2.1. Animals and test substances used for seizure testing

Male and female albino CF1 mice (18–25 g, Charles River, Portage, MI) and male albino Sprague–Dawley rats (100–150 g, Charles River, Wilmington, MA) were used as experimental animals. Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-accredited temperature- and humidity-controlled facility and maintained on a standard 12 h/12 h light–dark (lights on at 06:00) cycle with free access to standard laboratory chow (Prolab RMH 3000, Charles River, Wilmington, MA, USA) and water ad libitum. All animal experiments were performed in accordance with the guidelines set by the National Institutes of Health and the University of Utah Institutional Animal Care and Use Committee (IACUC). All animals were allowed free access to both food and water except when they were removed from their cages for the experimental procedure. Except for the kindling studies, animals were used once. All animals were euthanized in accordance with the Institute of Laboratory Resources policies on the humane care of laboratory animals. In 0.5% methylcellulose, TID, SID, or TED was administered (ip or po) in a volume of 0.04 mL/10 g body weight to rats.

2.2. Biological testing/anticonvulsant activity

2.2.1. Pharmacokinetic studies: analysis of TID, SID, and TED in plasma

The plasma concentrations of each compound were quantified by a GC/MS assay. Plasma (200 μL) was added to the test tubes, followed by 25 μL of methanol and 25 μL of internal standard solution [α-fluoro-2,2,3,3-tetramethylcyclopropanecarboxamide (α-F-TMCD) 250 μg/mL in methanol] [14], and the tubes were thoroughly vortexed. Chloroform (2 mL) was used for the extraction of the compounds. The dry residues obtained after evaporation of 1.8 mL chloroform were reconstituted with 50 μL methanol, of which 1 μL was injected into the GC/MS apparatus. The temperature program was as follows: injector temperature of 200 °C, initial temperature of 60 °C for 5 min, gradient of 15 °C/min until 180 °C, gradient of 20 °C until 310 °C, and hold time of 1 min. The MS parameters were set as follows: source temperature of 200 °C, transfer line of 280 °C, and positive ion monitoring using EI-MS at 70 eV. The pressure of the carrier gas, helium, was set

Table 1
Anticonvulsant activity (ED_{50}) and neurotoxicity (TD_{50}) of TED, TID, and SID in comparison to their respective constitutional isomers (VCD and SPD) following ip and oral administration to rats.[a]

Anticonvulsant test		ED_{50} (95% confidence interval) (mg/kg)				
		SPD	TID	SID	VCD	TED
po administration	Maximal electroshock seizure (MES)	29 (18–53)	>60	25 (20–30)	29 (19–38)	68 (45–104)
	Metrazol-induced seizure (scMet)	18 (13–25)	–[b]	38 (27–46)	54 (46–63)	29 (22–38)
	Neurotoxicity (TD_{50})	154 (124–192)	>60	57 (45–66)	58 (47–66)	178 (162–192)
ip administration	Maximal electroshock seizure (MES)	20 (15–27)	30 (14–48)	32 (24–36)	66 (50–74)	59 (41–77)
	Metrazol-induced seizure (scMet)	21 (15–27)	15 (7–25)	23 (19–28)	17 (10–24)	15 (6–27)
	Pilocarpine-induced status epilepticus (0 min)	8/8 protected at 65 mg/kg[c]	4/8 protected at 65 mg/kg	8/8 protected at 65 mg/kg	8/8 protected at 65 mg/kg[d]	8/8 protected at 200 mg/kg[c]
	Pilocarpine-induced status epilepticus (30 min)	84 (62–103)[c]	–[b]	73 (50–94)	0/8 at 80 mg/kg[d]	257 (192–317)[c]
	Neurotoxicity (TD_{50})	49 (43–55)	48 (32–36)	35 (30–39)	72 (63–80)	124 (115–134)

[a] Four to 8 rats were used to determine the ED_{50} and TD_{50} values.
[b] Not determined.
[c] Data are taken from ref. [7].
[d] Data are taken from ref. [18].

at 5 psi. For EI analysis, the ionization energy was 70 eV with a source pressure of 10^{-6} Torr. Retention times of SID, TED, TID, and the internal standard were 12.5, 12.7, 11.2, and 10.1 min, respectively. Calibration curves were constructed for each analytical run and were linear on the concentration range between 1 and 100 μg/mL.

2.2.2. Calculation of pharmacokinetic (PK) parameters

The PK parameters of SID, TED, and TID were calculated by noncompartmental analysis based on statistical moment theory using PK software Phoenix Winnonlin Tripos L.P. version 6.3 (Pharsight Co., Mountain View, CA) as previously described [7,9,14].

2.2.3. Anticonvulsant tests in animals

The antiepileptic potential of the tested compounds was established in mice and/or rats using the following seizure models: maximal electroshock (MES), chemically induced shock with subcutaneous pentylenetetrazole (metrazol) (scMet), bicuculline (scBIC), picrotoxin (scPIC), the 6-Hz psychomotor test, the corneally kindled mouse, Frings audiogenic seizure mouse, and rat pilocarpine tests. In the MES test, 60 Hz 50 mA of alternating current was delivered through corneal electrodes for 0.2 s. Four to 8 mice or rats were used to determine the ED_{50} and TD_{50} values. During the time of administration of the test substance, a drop of 0.5% tetracaine in saline is applied to the eyes of the animals. Animals were restrained by hand during administration of the electrical stimulus and then released for observation of the seizure throughout its entire course. A test substance/dose that is able to abolish the hind limb tonic extensor component of the seizure, indicating prevention of the MES-induced seizure spread, is considered "active". Tonic extension was considered abolished if the hind limbs were not fully extended at 180° to the plane of the body. In the scMet seizure test, a convulsant dose of pentylenetetrazole was injected subcutaneously (85 mg/kg in mice) at the time to peak effect of the test substance, followed by observation of seizure occurrence. Absence of seizures indicates that the tested compound/dose can elevate the pentylenetetrazole seizure induction threshold. Systemic administration of pilocarpine, a cholinergic agonist, has been used to induce status epilepticus. This seizure state is clinically defined as continuous seizure activity or multiple seizures without regaining consciousness lasting more than 30 min. To determine if a test substance can prevent acute pilocarpine-induced status epilepticus, candidate drugs were administered to male Sprague–Dawley rats via the ip route, followed by administration of a challenge dose of pilocarpine immediately (0 min) and 30 min after treatment with a candidate drug. The outcome measures were "protection" or "no protection" from epileptic seizures. In addition, morbidity was also determined 24 h after each test was completed. Quantitative determination of the protective effect was undertaken for compounds found to possess significant protection. This included calculations of the peak time response as well as determination of ED_{50} and 95% confidence limits.

2.2.4. Minimal behavioral toxicity tests

Minimal toxicity was identified in rats and mice as minimal motor impairment (MMI) as determined by overt evidence of ataxia, abnormal gait, and stance.

2.2.5. Teratogenicity

For this study, the highly inbred SWV/Fnn mouse strain with a known susceptibility to AED-induced neural tube defects (NTDs) was used according to the previously published procedure [16,17]. Dams were allowed to mate overnight with male mice and were examined

Table 2
Anticonvulsant activity and neurotoxicity of TED, TID, and SID in comparison to their respective constitutional isomers (VCD and SPD) following ip administration to mice.[a]

Anticonvulsant test	ED_{50} (95% confidence interval) (mg/kg)				
	SPD	TID	SID	VCD	TED
6 Hz 32 mA	27 (24–30)	118 (81–162)	40 (31–52)	37 (26–50)	153 (125–174)
6 Hz 44 mA	45 (40–49)	Negative	53 (34–81)	67 (61–72)	~150
Maximal electroshock seizure (MES)	71 (55–90)	100 (91–109)	35 (23–50)	58 (41–71)	163 (155–173)
Metrazol-induced seizure (scMet)	62 (47–71)	70 (60–78)	39 (32–47)	32 (22–45)	58 (50–76)
Bicuculline-induced seizure (scBIC)	94 (87–103)	>140	60 (46–76)	162 (130–211)	>210
Picrotoxin-induced seizure (scPIC)	17 (9–28)	>210	39 (24–56)	>200	>210
Corneally kindled mouse	39 (31–45)	–[b]	15 (8–25)	35 (29–48)	–
Frings audiogenic seizures	20 (18–22)	44 (38–56)	10 (9–12)	10 (7–12)	30 (24–35)
Neurotoxicity (TD_{50})	114 (100–134)	127 (113–143)	49 (46–54)	81 (72–87)	202 (183–230)

[a] Four to 8 rats were used to determine the ED_{50} and TD_{50} values.
[b] Not determined.

Table 3
Solubility (mg/mL) and lipophilicity data (ClogP) of VCD, TED, SPD, SID, and TID.

Solvent	SPD	TID	SID	VCD	TED
Water	1.5	3	6	7	9
Saline:PG:EtOH (5:4:1)	5	16	39	27	41
PG:water:EtOH (4:5:1)	27	17	33	50	31
PG:water:EtOH (5:4:1)	50	22	66	80	63
ClogP	2.2	2.0	2.1	1.7	1.6

PG—propylene glycol and EtOH—ethanol.
ClogP was calculated by utilizing the ChemDraw Ultra version 8 software.

on the following morning for the presence of vaginal plugs. The onset of gestation was set at 1 a.m. of the previous night. On gestational day 8.5, pregnant females received a single ip injection (10 μL per gram of body weight) of sodium valproate at a dose of 1.1, 1.8, or 2.7 mmol/kg or TID, SID, and TED at a dose of 1.8 or 2.7 mmol/kg. A 25% Cremophor EL water solution was injected ip to dams constituting the control group. On gestation day 18.5, the dams were euthanized by CO_2 asphyxiation, the abdomen opened, and the gravid uteri removed. The locations of all viable, dead, and resorbed fetuses were recorded, and the fetuses were grossly examined for the presence of exencephaly. For statistical purposes, either ANOVA with Tukey's posttest multiple comparison (fetus weight) or contingency table analysis with Fisher's exact test (number of resorptions and NTDs) was performed. The p-value was set at 0.05.

2.3. Methods

A gas chromatography–mass spectrometry (GC/MS) assay was performed on a HP5890 series II GC equipped with a Hewlett-Packard MS engine (HP5989A) single quadrupole mass spectrometer, 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 of 180 °C, initial temperature of 60 °C for 5 min, gradient of 20 °C/min until 180 °C, gradient of 10 °C/min until 300 °C, and hold time of 3 min. The MS parameters were set as follows: source temperature of 180 °C, transfer line temperature of 280 °C, and positive ion monitoring using electron ionization-mass spectrometry (EI-MS) at 70 eV. The molecular ion and the five most pronounced ions are provided. Elemental analyses were preformed on a 2400-2 Perkin-Elmer CHN analyzer. The C, H, and N analyses of all newly synthesized compounds were within ±0.4 of theoretical values and, thus, were considered satisfactory.

2.4. Water solubility and ClogP calculations

The solubility of SID, SPD, TED, TID, and VCD in water and various other solvents was determined by adding gradually 10–100 mg of each compound to 1 mL of double-distilled water or any other solvent until the compound precipitated. The solution was stirred for 2 h. At the end of the 2-h period, the sample was centrifuged and 1-μL aliquots were taken for the GC analysis.

ClogP was calculated by utilizing the ChemDraw Ultra version 12 software.

3. Results

3.1. Chemistry

The syntheses of SID, TID, and TED were previously described by Kaufmann et al. [18]. Briefly, the starting material for SID was 3-methylvaleric acid and for TED and TID, dimethylbutyric acid. The acids were converted to their corresponding enolates by LDA, followed by substitution of a hydrogen atom on the α-carbon by the appropriate alkyl using specific alkyl iodide. The carboxylic acids were treated with thionyl chloride in order to produce the corresponding acyl chloride followed by treatment with NH_4OH. The chemical structures of the synthesized amides were identified by ^1H NMR GC–MS, while purity was established using elemental analysis.

3.2. Time to peak effect (TPE) of SID, TID, and TED and determination of their median effective (ED_{50}) or behavioral toxic (TD_{50}) dose

All quantitative in vivo anticonvulsant/behavioral neurotoxicity studies were conducted at time to peak effect (TPE) values previously determined in a qualitative analysis. The TPE of SID, TED, and TID was 0.25 h following ip and oral administration. Groups of 4–8 mice or rats were tested with various doses until at least two points were established between the limits of 100% protection or minimal toxicity and 0% protection or minimal toxicity. The dose of drug required to produce the desired endpoint in 50% of animals (ED_{50} or TD_{50}) in each test, the 95% confidence interval, the slope of the regression line, and the SEM of the slope were then calculated by a computer program based on the method described by Finney [19].

3.3. Anticonvulsant efficacy of SID, TED, and TID in seizure and epilepsy rodent models

The anticonvulsant activity of SID, TID, and TED in comparison to their respective isomers (SPD and VCD) is depicted in Tables 1 (rats) and 2

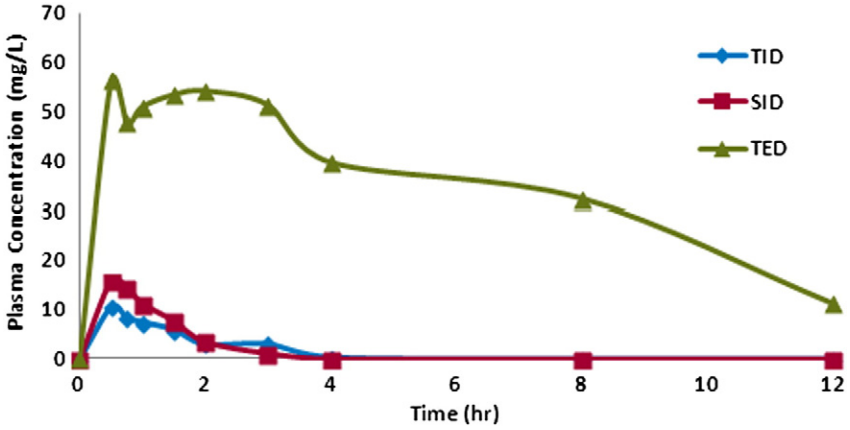

Fig. 2. Plasma concentration–time plots of SID (50 mg/kg), TID (50 mg/kg), and TED (70 mg/kg) following ip administration to rats. Each time point represents a mean from three rats with % coefficient of variation (%CV) of 20–30%.

(mice). In rats, SID and TED had a wide spectrum of anticonvulsant activity and were equipotent to their respective isomers (SPD and VCD) following ip administration to rats. In mice, SID was more potent than TED and TID and exhibited a similar wide spectrum of anticonvulsant activity as SPD. *tert*-Butylisopropylacetamide was the least active among the tested compounds.

3.4. Solubility, lipophilicity, and pharmacokinetics of SID, TED, and TID in rats

The solubility of SID, TED, and TID (in comparison to SPD and VCD) in water and other solvents is depicted in Table 3 which also includes their lipophilicity expressed as ClogP.

The PK of SID, TED, and TID was studied following ip administration (50, 70, and 50 mg/kg, respectively) of each amide to rats. The doses chosen for the PK study were the intermediate doses among the various ED_{50} values. The plasma concentration–time plots of SID, TED, and TID are presented in Fig. 2. Each time point in Fig. 2 represents a mean of three rats with % coefficient of variation (%CV) of 20–30%.

The PK parameters, calculated by noncompartmental analysis, are summarized in Table 4. *tert*-Butylisopropylacetamide and SID had similar clearance and volume of distribution values: 2.7 L/h/kg and 2–3.3 L/kg, respectively (Table 4). Consequently, the half-life of TID and SID was rather short and ranged between 0.6 and 0.8 h. *tert*-Butylethylacetamide had a different PK profile compared to TID and SID, its clearance (0.13 L/h/kg) was the smallest, and consequently, its half-life (4.9 h) was the longest. The t_{max} value of SID, TID, and TED was 0.5 h, a similar value to that obtained previously with SPD and VCD and their individual stereoisomers [7,9,14].

3.5. Teratogenicity

The teratogenic potential of SID, TED, and TID was assessed for their ability to induce gross morphological defects in the SWV/Fnn mice that are highly susceptible to VPA-induced exencephaly. Valproic acid, at a dose of 2.7 mmol/kg, was embryotoxic and teratogenic, causing almost a twofold increase in the resorption rate compared to the control group (11.9% vs 6.3%, respectively) and NTDs in 29.1% of live fetuses. At a lower dose of 1.8 mmol/kg, VPA was still embryotoxic (13.6% of resorptions), but the number of fetuses with exencephaly (2) was not statistically significant. In contrast to VPA, SID and TED did not cause a statistically significant increase of NTDs at doses of 141 and 283 mg/kg (Table 4). These doses are 4–7 times higher than their anticonvulsant ED_{50} values. *tert*-Butylisopropylacetamide (283 mg/kg) was embryotoxic and induced resorptions in 14.8% of conceptions (Table 5).

4. Discussion

Antiepileptic drug therapy, particularly with VPA, is associated with severe side effects (e.g., teratogenicity). In addition, currently, approximately 30% of patients with epilepsy are not seizure-free despite therapy with the existing medications [6].

Table 4
PK parameters of SPD and VCD and their constitutional isomers TID, SID, and TED as obtained after ip administration to rats.

PK parameter	SPD[a]	TID	SID	VCD[b]	TED
$t_{1/2}$ (h)	1.1	0.8	0.6	1.6	4.9
CL/F (L/h/kg)	1.8	2.7	2.7	0.18	0.13
V/F (L/kg)	3.0	3.3	2.0	0.41	0.96
AUC (mg/L/h^{-1})	31	18	22	413	515
C_{max} (mg/L)	14	11	16	94	71
t_{max} (h)	0.75	0.5	0.5	–	0.5
MRT (h)	1.8	1.5	1.2	3.6	6.8

[a] Data taken from ref. [7].
[b] Data following iv administration (74 mg/kg) taken from ref. [20].

Table 5
Teratogenic effect in the SWV mouse model of the constitutional isomers of VCD and SPD.

Compound	Dose, mg/kg (mmol/kg)	No. of litters	No. of implants	No. of resorptions (%)	No of live fetuses (%)	No. of fetuses with NTDs (%)
Control 7	25% CEL	10	140	9 (6.4)	131 (93.6)	0
NaVPA [a]	452 (2.7)	13	160	19 (11.9)	141 (88.1)	41 (29.1)[b]
NaVPA [a]	301 (1.8)	12	154	21 (13.6)[b]	133 (86.4)	2 (1.5)
NaVPA [c]	181 (1.1)	12	156	12 (7.7)	144 (92.3)	0
SPD	283 (1.8)	12	179	24 (13.4)[b]	155 (86.6)	0
SPD	141 (0.9)	11	160	12 (7.5)	148 (92.5)	0
VCD	389 (2.7)	9	119	25 (21.0)[b]	94 (79.0)	0[d]
VCD	257 (1.8)	10	132	5 (3.8)[d]	127 (96.2)	1 (0.8)
TID	424 (2.7)	10	113	94 (83.2)[b,d]	19 (16.8)	6 (31.6)[b]
TID	283 (1.8)	10	142	21 (14.8)[b]	121 (85.2)	5 (4.1)[b]
TED	386 (2.7)	10	127	10 (7.9)	117 (92.1)	2 (1.7)[d]
TED	257 (1.8)	10	142	13 (9.1)	129 (90.9)	1 (0.8)
SID	283 (1.8)	10	142	13 (9.1)	129 (90.9)	0
SID	141 (0.9)	10	141	10 (7.1)	131 (92.9)	0

For statistical purposes, either ANOVA with Tukey's posttest multiple comparison (fetus weight) or contingency table analysis with Fisher's exact test (number of resorptions and NTDs) was performed. p-Value was set at 0.05.
SID (2.7 mmol/kg) was a lethal dose (2 pregnant mice died in 2 h after injection).
[a] Results from ref. [21].
[b] Significantly different when compared to the control group.
[c] Results from ref. [22].
[d] Significantly different when compared to the group treated with an equimolar dose of VPA.

Recently, Spampanato and Dudek showed that VCD induces a specific, rapid, dose-dependent, and reversible slowing of the decay of miniature inhibitory postsynaptic current in CA1 pyramidal cells [23]. This effect was similar to that of benzodiazepines (BZDs), but the effect of VCD persisted in the presence of flumazenil (BZD-binding site antagonist) and was additive to the effect of diazepam. These results indicate that VCD acts through a different binding site than BZDs, a fact which may contribute the effect of VCD in BZD-refractory SE [23].

tert-Butylethylacetamide (a VCD isomer) and its one-carbon homologue, SID, are active in the BZD-resistant pilocarpine-induced SE model when given 30 min after seizure onset and may thus share a similar mechanism of action as VCD or SPD. In the rat (ip) MES and scMet models, TED was found to be equipotent to VCD and less neurotoxic. However, following oral administration, TED was found to be less potent than VCD in the MES test but was more potent at the scMet model as well as less neurotoxic. TED's best PK profile compared to all other closely related investigated compounds did not translate into a better anticonvulsant activity. TED's constitutional isomer in which the *tert*-butyl moiety was substituted by n-butyl as well as its nonbranched isomer, octanamide, were previously found to be less potent than TED [18,24,25]. In contrast, another constitutional isomer of TED and VCD, propylispopropyl acetamide, in which the isobutyl (VCD) or *tert*-butyl (TED) moiety was replaced by isopropyl and one additional methyl was added to the ethyl side chain (shared by TED and VCD), was previously found to be equipotent to VCD [26].

In mice and rats (ip), SID exhibited a similar broad spectrum of anticonvulsant activity as SPD but was less potent in the rat (po) scMet and mouse 6-Hz (32 mA) tests and was more neurotoxic. In contrast, SID was two times more potent than SPD in the corneal kindled and Frings audiogenic seizure mouse models. *tert*-Butylisopropylacetamide was less active than SPD in the mouse MES and 6-Hz tests but was equipotent to SPD in the rat MES and mouse and rat scMet tests. *tert*-Butylisopropylacetamide was less potent than SPD in the pilocarpine-induced SE model when given at seizure onset (0 time) but was not tested at 30 min. *tert*-Butylisopropylacetamide was less potent in the rat MES model following oral dosing than after ip administration. This might be caused by low water solubility (3 mg/mL) coupled with a first-pass effect. As the rat liver blood flow (Q) is 4 L/h/kg and assuming a blood-to-plasma ratio of about 1, TID's liver extraction ratio (E) is 68%.

This high E value makes TID susceptible to hepatic first-pass effect after oral administration. Although SID had a similar clearance value as TID (Table 4), its solubility was 2–3 times higher than that of TID. The better solubility of SID may contribute to its similar rat MES and scMet ED_{50} values following oral and ip administration (Table 1). In the pilocarpine-induced SE model, SID was equipotent to SPD at 0 and 30 min after seizure onset but was more potent than TID, TED, and VCD. Although SID, SPD, TED, TID, and VCD have similar lipophilicity, SPD had the highest ClogP value and the lowest water solubility (Table 3), a fact that may contribute to its wide and potent spectrum of anticonvulsant activity.

Since numerous commercially available AEDs are teratogenic, it is essential to develop new AEDs that are nonteratogenic [27,28]. This is particularly crucial for VPA as on November 21st, 2014, the EMA's Coordination Group for Mutual Recognition and Decentralized Procedures (CMDh) decided to strengthen the warning on the use of VPA in women and girls because of the risk of malformation and developmental problems in children exposed to VPA in the womb that might be associated with autistic spectrum disorder and childhood autism [29]. In contrast to VPA, SID and TED did not cause a statistically significant increase of NTDs at doses of 141 and 283 mg/kg (Table 5). These doses are 4–7 times higher than their anticonvulsant ED_{50} values. Hereon, we demonstrate that SID and TED failed to induce NTDs in SWV mice at the dose of 283 mg/kg (1.8 mmol/kg). Thus, SID and TED are superior to VPA not only by exhibiting a more potent anticonvulsant activity but also by their reproductive safety.

Preclinical strategies in new drug (AEDs) discovery used to identify potential drug candidates include target-based screening, phenotypic screening utilizing animal models, and modifications of existing drugs or natural substances [30,31]. Screening in rodent models has been the engine in AED discovery since phenytoin (1938). Anticonvulsant animal models are effective in identifying new AEDs, are nonselective with respect to the mechanism of action, and provide insight into AEDs' PK–PD relations including the ability to penetrate the brain and exert a CNS effect [6,32]. Animal models with a similarly high predictive value do not exist in other nonepileptic CNS disorders. In light of the highly heterogeneous nature of seizure disorders in humans and the complexity of seizure phenotypes, it is unlikely that any single animal model will predict the full therapeutic potential of an investigational AED [33]. Consequently, promising investigational new AEDs have to demonstrate activity in a battery of anticonvulsant models that may indicate a wide antiepileptic spectrum in humans. Bialer et al. demonstrated a correlation between AEDs' ED_{50} values in the mouse and rat MES models and AEDs' therapeutic dose and steady-state plasma concentrations in patients with epilepsy [34]. This analysis showed VPA to be the least potent AED in anticonvulsant rodent models. The constitutional isomers of SPD or VCD designed and evaluated in the current study by utilizing the phenotypic approach preserve VPA's wide anticonvulsant spectrum but are significantly more potent than VPA and, thus, may have a potential to be efficacious in patients who have seizures resistant to VPA.

5. Conclusions

In rats, SID, TID (ip), and TED exhibited a broad spectrum of anticonvulsant activity at nonteratogenic doses. However, combined anticonvulsant analysis in mice and rats shows SID as the most potent compound with similar activity to that of SPD, demonstrating that substitution of SPD's isobutyl moiety by *tert*-butyl as well as a propyl-to-isopropyl replacement in the SPD or VCD molecule did not majorly affect the anticonvulsant activity. The lack of significant difference in the anticonvulsant activity of VCD and SPD and their constitutional isomers may indicate that the anticonvulsant activity of these chemically, closely related compounds is due to multiple mechanisms of action. The choice between SID and SPD or TED and VCD could be done after a toxicological analysis coupled with additional pharmacological testing.

Abbreviations

CNS	central nervous system
AED	antiepileptic drug
MES	maximal electroshock seizure
scMet	subcutaneous metrazol
SE	status epilepticus
PI	protective index
VPA	valproic acid

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Disclosure/conflict of interest

Dr. Meir Bialer has received in the last three years speakers' or consultancy fees from Bial, CTS Chemicals, Desitin, Janssen-Cilag, Johnson & Johnson, Medgenics, Rekah, Sepracor, Teva, UCB Pharma, and Upsher-Smith. Dr. Bialer has been involved in the design and development of new antiepileptic and CNS drugs as well as new formulations of existing drugs.

None of the other authors has any conflict of interest to disclose.

We, the authors, confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

References

[1] Barel S, Yagen B, Schurig V, Soback S, Pisani F, Perucca E, et al. Stereoselective pharmacokinetic analysis of valnoctamide in healthy subjects and in patients with epilepsy. Clin Pharmacol Ther 1997;61:442–9.
[2] Bialer M, Yagen B. Valproic acid — second generation. Neurotherapeutics 2007;4:130–7.
[3] Bialer M. Chemical properties of antiepileptic drugs (AEDs). Adv Drug Deliv Rev 2012;64:887–95.
[4] Bialer M, Haj-Yehia A, Barzaghi N, Pisani F, Perucca E. Pharmacokinetics of a valpromide isomer, valnoctamide, in healthy subjects. Eur J Clin Pharmacol 1990;38:289–91.
[5] Bialer M. Clinical pharmacology of valpromide. Clin Pharmacokinet 1991;20:114–22.
[6] Bialer M, White HS. Key factors in the discovery and development of new antiepileptic drugs (AEDs). Nat Rev Drug Discov 2010;9:68–83.
[7] White HS, Alex AB, Pollock A, Hen N, Shekh-Ahmad T, Wilcox KS, et al. A new derivative of valproic acid amide possesses a broad-spectrum antiseizure profile and unique activity against status epilepticus and organophosphate neuronal damage. Epilepsia 2012;53:134–46.
[8] Pouliot W, Bialer M, Hen N, Kaufmann D, Yagen B, Ricks K, et al. Electrographic analysis of the effect of *sec*-butyl-propylacetamide on pharmacoresistant status epilepticus. Neuroscience 2013;231:145–56.
[9] Hen N, Shekh-Ahmad T, Yagen B, McDonough JH, Finnell RH, Wlodarczyk B, et al. Stereoselective pharmacodynamic and pharmacokinetic analysis of *sec*-propyl-butylacetamide (SPD), a new CNS-active derivative of valproic acid with unique activity against status epilepticus. J Med Chem 2013;56:6467–77.
[10] Bersudsky Y, Applebaum J, Gaiduk Y, Sharony L, Mishory A, Podberezsky A, et al. Valnoctamide as valproate substitute with low teratogenic potential in mania: double blind controlled clinical trial. Bipolar Disord 2010(12):376–82.
[11] Bialer M, Johannessen SI, Levy RH, Perucca E, Tomson T, White HS. Progress report on new antiepileptic drugs: a summary of the eleventh Eilat Conference (EILAT XI). Epilepsy Res 2013(103):2–30.

[12] Bialer M, Johannessen SI, Levy RH, Perucca E, Tomson T, White HS. Progress report on new antiepileptic drugs: a summary of the twelfth Eilat Conference (EILAT XII). Epilepsy Res 2015(111):85–141.
[13] Shekh-Ahmad T, Hen N, McDonough JH, Yagen B, Bialer M. Valnoctamide and sec-propyl-butylacetamide (SPD) for acute seizures and status epilepticus. Epilepsia 2013;54:98–101.
[14] Shekh-Ahmad T, Hen N, Yagen B, McDonough JH, Finnell RH, Wlodarczyk BJ, et al. Stereoselective anticonvulsant and pharmacokinetic analysis of valnoctamide, a new CNS-active derivative of valproic acid with low teratogenic potential. Epilepsia 2014;55:353–61.
[15] Kaufmann D, Yagen B, Minert A, Wlodarczyk B, Finnell RH, Schurig V, 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.
[16] Finnell RH, Bielec B, Nau H. Anticonvulsant drugs: mechanisms and pathogenesis of teratogenicity. Handbook of experimental pharmacology 124 (drug toxicity in embryonic development II). Berlin: Springer-Verlag; 1997. p. 121–59.
[17] 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.
[18] Kaufmann D, Bialer M, Shimshoni JA, Devor M, Yagen B. Synthesis and evaluation of the antiallodynic of novel amide and urea derivatives of valproic acid analogues. J Med Chem 2009;52:7236–48.
[19] Finney DJ. Statistical logic in the monitoring of reactions to therapeutic drugs. Methods Inf Med 1971;10:237–45.
[20] Blotnik S, Bergman F, Bialer M. The disposition of valpromide, valproic acid and valnoctamide in brain, liver, plasma and urine of rats. Drug Metab Dispos 1996; 24:560–4.
[21] Sobol E, Yagen Y, White HS, Wilcox KS, Lamb JG, Pappo O, et al. Preclinical evaluation of 2,2,3,3-tetramethylcyclopropanecarbonylurea, a novel second generation to valproic acid, antiepileptic drug. Neuropharmacology 2006;51:933–46.
[22] Pessah N, Bialer M, Wlodarczyk B, Finnell RH, Yagen B. α-Fluoro-2,2,3,3-teteramethylcylcopropanecarboaxamide a novel potent anticonvulsant derivative of a cyclic analogue of valproic acid. J Med Chem 2009(52):2233–42.
[23] Spampanato J, Dudek EF. Valnoctamide enhances phasic inhibition: a potential target mechanism for the treatment of benzodiazepine-refectory status epilepticus. Epilepsia 2014;55:e94–8.
[24] Haj-Yehia A, Bialer M. Structure–pharmacokinetic relationship in a series of valpromide isomers with antiepileptic activity. Pharm Res 1989;6:683–9.
[25] Haj-Yehia A, Bialer M. Structure pharmacokinetic relation in a series of short fatty acid amides that posses anticonvulsant activity. J Pharm Sci 1990;79:719–24.
[26] Spiegelman O, Yagen B, Levy RH, Finnell RH, Bennett GD, Roeder M, et al. Strereoselective pharmacokinetics and pharmacodynamics of propylisopropyl acetamide — a CNS-active chiral amide derivative of valproic acid. Pharm Res 1999;16:1582–8.
[27] Meador KJ, Baker GA, Browning N, Clayton-Smith J, Combs-Cantrell DT, Cohen M, et al. Cognitive function at 3 years of age after fetal exposure to antiepileptic drugs. N Engl J Med 2009;360:1597–605.
[28] Tomson T, Battino D, Bonizzoni E, Craig J, Lindhout D, Sabers A, et al. Dose-dependent risk of malformations with antiepileptic drugs: an analysis of data from the EURAP epilepsy and pregnancy registry. Lancet Neurol 2011(10): 609–6017.
[29] European Medicines Agency (EMA). website: http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2014/10/news_detail_002186.jsp&mid=WC0b01ac058004d5c1.
[30] Enna SJ, Willaims M. Challenged in the search for drugs to treat central nerve system disorders. J Pharmacol Exp Ther 2009;329:404–11.
[31] Swinney DC, Anthony J. How were new medicines discovered? Nat Rev Drug Discov 2011;10:507–19.
[32] Rogawski MA. Diverse mechanisms of antiepileptic drugs in development. Epilepsy Res 2006;69:272–84.
[33] Smith M, Wilcox KS, White HS. Discovery of antiepileptic drugs. Neurotherapeutics 2007;4:12–7.
[34] Bialer M, Twyman RE, White HS. Correlation analysis between anticonvulsant ED_{50} values of antiepileptic drugs in mice and rats and their therapeutic doses and plasma levels. Epilepsy Behav 2004;5:866–72.