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Bioorganic & Medicinal Chemistry 12 (2004) 3857–3869
Bioorganic & Medicinal Chemistry

Characterization of the anticonvulsant profile of valpromide derivatives

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Received 23 December 2003; revised 2 May 2004; accepted 4 May 2004
Available online 7 June 2004

Abstract—The antiepileptic activity of nine derivatives of valpromide is discussed. They comply with a pharmacophore model that establishes the essential structural and electronic features responsible for the protection against the MES test. The model results from the comparison of 17 structures, using density functional methodologies combined with an active analog approach. The derivatives of valpromide have been tested for anticonvulsant activity in mice. These compounds displayed a phenytoin-like profile, being active in the MES test and inactive in the PTZ test. 4-(Valproylamido)benzenesulfonamide is the most active compound, with an ED_{50} of 53 μmol/kg and no neurotoxicity at doses up to 1000 μmol/kg. The pharmacological behavior of the drugs points to a sodium channel blocking effect as one of the associated mechanisms. This mechanism was tested positive for N-ethylvalpromide through its competition with the binding of [^{3}H]batrachotoxin-A-20α-benzoate to the voltage-dependent sodium channels from rat brain synaptosomes.

1. Introduction

Among the different neurological disorders that affect human condition, epilepsy has been largely studied during the last century,[1–4] becoming a dynamic research field in recent years.[4–13] It describes disorders characterized by recurrent seizure attacks due to synchronous neuronal firing. The main drawback related to its therapy is associated with the fact that antiepileptic drugs (AEDs) fail to control seizures in 20–25% of patients.[9–11,14,15] Moreover, even the new generation of AEDs causes sizeable side effects, which include ataxia, diplopia, mental dulling, rash, blood dyscrasias, and hepatotoxicity.[7] Thus, new AEDs with better safety, less toxicity, and higher efficacy in difficult-to-control patients are urgently needed.[8]

The broad spectra of mechanisms, which may occur during drug action in the epileptic patient, include, among others, voltage-dependent blockade of Na^+ channels, modulation of GABA synthesis, or degradation, inhibition of cellular GABA uptake, modulation of $GABA_A$ receptors, modulation of various excitatory amino acid receptors, and modulation of adenosine metabolism.[3,4,9] For this reason, several seizure models have been developed to evaluate the anticonvulsant (AC) activity during the research process. Among them, the maximal electroshock (MES) and the subcutaneous pentylenetetrazol (PTZ) tests are the most widely used.[2] These models respond well to neuronal voltage-gated sodium channel (VGSC) inhibitors and gabaergic compounds, respectively.[5,7]

A large number of ligands that are presently marketed (or in the process of being marketed) as AEDs exhibit a similar pharmacological profile, being quite potent in the MES test and inactive in the PTZ test. This is the case of the widely used phenytoin (PHE), as well as carbamazepine (CZ), topiramate (TOP), lamotrigine (LAM), zonisamide (ZON), ralitoline (RAL), and oxcarbazepine (OCZ).[4,5,9,15–17] The drugs sharing the PHE-like profile of activity are accepted today to prevent seizures because they block Na^+ channels in the brain.[7,15] Na^+ channel blockers are front-runners in the treatment of epilepsy. This mechanism is not, however, unique for the above mentioned drugs. As an example,

Keywords: Anticonvulsant activity; Pharmacophore; Valpromide derivatives; Antiepileptic drugs.
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doi:10.1016/j.bmc.2004.05.003

the variety of molecular actions displayed by valproic acid (VPA) includes Na^+ channel blockade, increase of GABA levels in the brain by glutamate decarboxylase activation and GABA transaminase inhibition, increase of postsynaptic GABA responses, direct membrane effects on the neurons, and blockade of Ca^{++} channels.[9,15,16,18]

In spite of the large amount of attempts of postulating a general pharmacophore for the antiepileptic activity, only one has been reported, to our knowledge, for the Na^+ channel blocking activity of dissimilar antiepileptic compounds.[19] The model reported is based on the structural comparison of CZ, PHE, LAM, ZON, and rufinamide (RUF), and identifies three groups of different characteristics that should be present, at well-defined distances, in order for the structures to be active. These different groups involve an aryl ring, an electron donor atom and a second donor atom which, close to an NH group, defines a hydrogen bond acceptor/donor unit. On the other hand, several model pharmacophores have been developed from the comparison of complexes that belong to a given family. Some of them bear some similarities in relation to the position of H-bond donor/acceptor and lipophilic groups. In this way, from the comparative analysis of compounds structurally related to N-benzylamides of γ-hydroxybutyric acid (GMB),[20] the requirements for activity have been associated with the presence of an N-benzylamide fragment, an aryl ring, and a H-bond donor group located between them (see Fig. 1).

For the case of ureylenes and semicarbazones, a model pharmacophore has been proposed, which points to the existence of an H-bonding site located between two hydrophobic areas (Fig. 2). The H-bonding site has been further disclosed in H-bonding and electron donor groups.[21]

Computational analysis has also contributed in other cases to design novel antiepileptic ligands, but the studies were limited to compounds of a given family.[22] We have recently analyzed several AEDs, which are recognized to bind to Na^+ channels.[23] On the basis of this analysis, we have proposed a pharmacophore that, showing some similarities with the one previously described,[19] mainly differs in the necessity of an electron donor group. According to our analysis, based on a larger set of structures, the presence of an acceptor/donor unit comprising two closely located H-donor–acceptor groups, together with a lipophilic portion, defines enough conditions to attain activity.

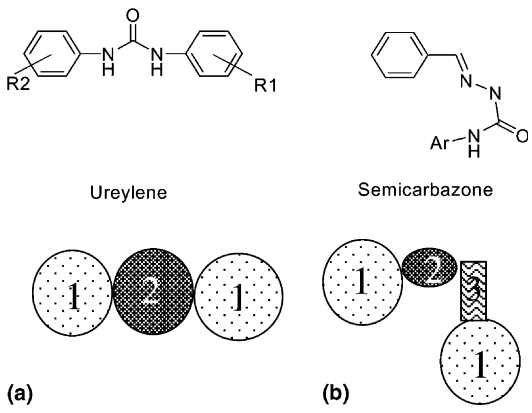

Figure 2. (a) Schematic representation of the ureylenes and suggested pharmacophore models for the anticonvulsant activity.[21] (b) Schematic representation and proposed pharmacophore model for the anticonvulsant activity of the semicarbazones.[21] (1) Hydrophobic binding areas, (2) hydrogen bonding site, (3) electron donor group.

On the basis of this pattern, we have considered a set of AEDs, structurally related to VPA. They bear a high degree of flexibility and we speculate this might help to accommodate the receptor pocket more easily. Showing a PHE-like profile, they manifest higher anti-MES potency than the parent compound. As a further test of the relation between the PHE-like activity and the Na^+ channel blocking capability, in vitro tests have been performed for a representative member of the newly designed set, which meets the requirements of potency and solubility.

We present, in the first part of this article, a validation of the previous pharmacophore, using a training set that extends the previous one, including AEDs developed in previous years. Some of them derive from the already marketed PHE and LAM,[22,24,25] but also structures as diverse as γ-butyrolactams, semicarbazides, and 3-aminopyrrole are included.[19,26–30] The second part of the article deals with the synthesis and biological evaluation of the VPD-related AEDs, thoroughly discussing their pharmacological profile.

2. Results and discussion

2.1. Pharmacophore identification

The activity data for the compounds that define the training set (Fig. 3, Table 1) quantify their response to

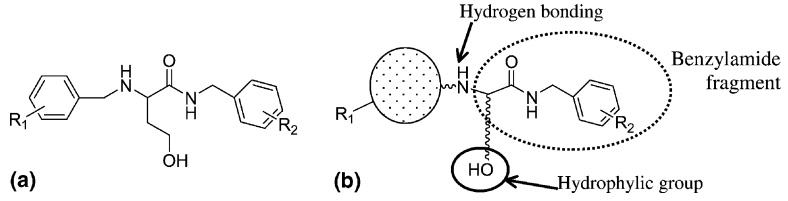

Figure 1. (a) Schematic representation of the compounds related to N-benzylamides of γ-hydroxybutyric acid derivatives (GMB) considered in Ref. 20. (b) Pharmacophore model proposed for the anticonvulsant activity of substituted GMB.

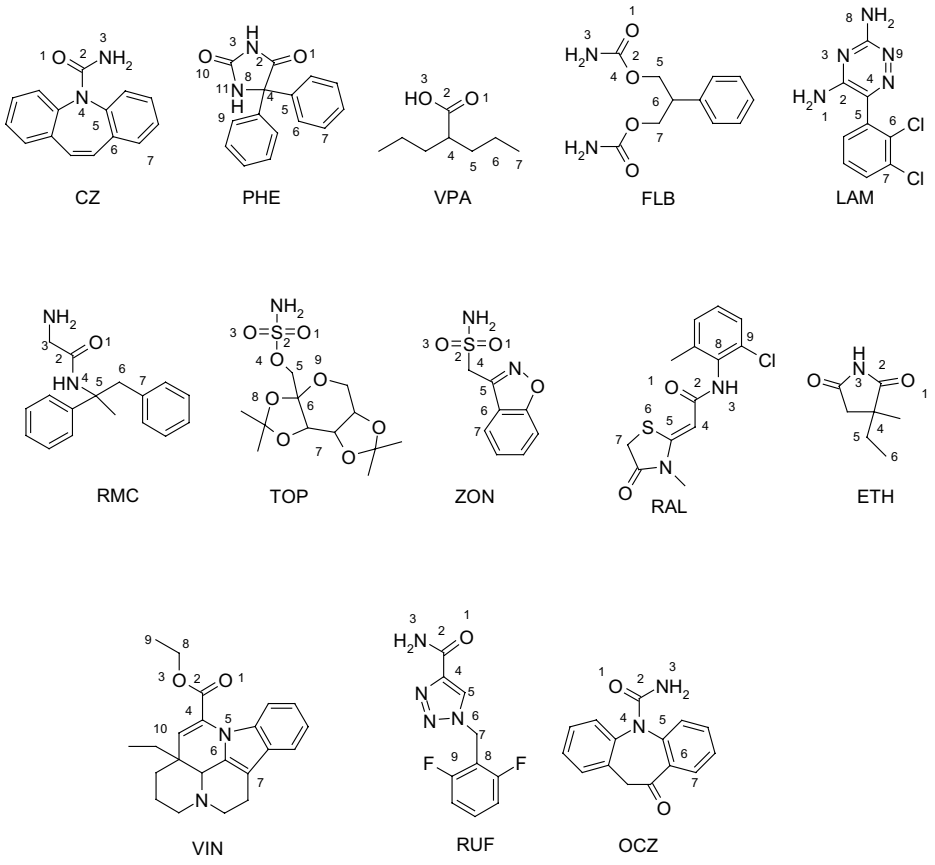

Figure 3. Schematic representation of the compounds that define the training set.

Table 1. Biological data of the compounds that constitute the training set and those new ones selected for their Na^+ channel activity

Compound	Log *P*	ED_{50} (μmol/kg) MES test (mice)	Ref.	ED_{50} (μmol/kg) PTZ test (mice)	Ref.	Na^+ channel binding	Ref.
CZ	2.72	37	32	NE	1,32	Yes	5,26,31,33,34
PHE	2.26	38	32	NE	32	Yes	5,7,27,31,34,35
VPA	2.6	1886	32	1033	32	Yes	27,31
FLB	0.78	210	1	621	1	Yes	3,9
LAM	2.66	7	1,5	NE	1	Yes	5,10,26,34
RMC	2.66	292	1,4	NE	1	Yes	4
TOP	5.16	112	1,5	NE	1	Yes	5
ZON	0.64	92	1	NE		Yes	5
RAL	1.85	9	1,31,33,34,37	NE	1	Yes	5
ETH	0.55	>7000	32	921	32	NA	31
VIN	2.42	77	38			Yes	5
RUF	0.65	21–71	3,9			Yes	5
OCZ	1.66	79	1	NE	1	Yes	33
SSDG	6.86					Yes	36
3BEP	2.99	341	28,29	193	27	Yes	29
AWD	1.03	7	30			Yes	30
PNU-151774E	2.45	26	10,27	89	27	Yes	10,27

The main references that report their activities are given. The partition coefficients (log *P*) of the compounds were calculated using the program HyperChem QSAR Properties, version 7.01 Hypercube, Inc.[39]

the MES and PTZ tests. The majority of them show a PHE-like profile, being active against the MES test and inactive against the PTZ test. Some of them (felbamate—FLB, remacemide—RMC, ethosuximide—ETH, vinpocetina—VIN) are also active in the PTZ test. They have been selected on the basis of their common capability of blocking the VGSC, as either established or newer drugs that have overcome the development stage.

The majority of the active structures bear the carboxylic acid or amide (sulfonamide) function that may be either substituted or not. ETH has been included as an inactive analog, because it is one of the major antiepileptics used

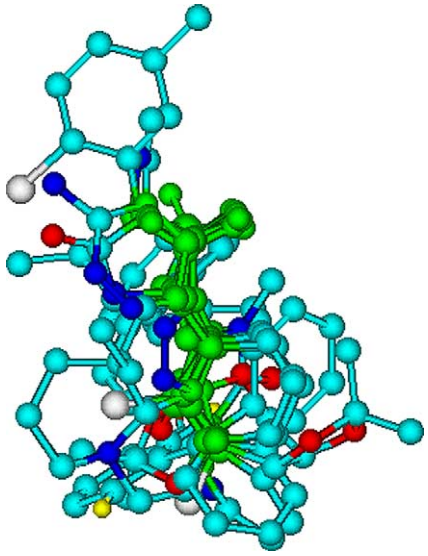

Figure 4. Superposition of the molecules that define the training set. Color pattern as follows: nitrogen atoms blue, oxygen atoms red, sulfur atoms yellow, carbon atoms light blue. H atoms are not shown for clarity. The dissimilarity of the structures becomes evident. Pharmacophoric points (green atoms), defined as those that are common to all the overlapping structures.

in absence seizures.[31] It does not show activity in the sodium channels[40,41] and also has a different pharmacological behavior, being inactive against the MES test.[42]

The structures of the training set have been aligned (Fig. 4) after a search of the conformational space through torsional modifications of the geometries initially optimized at a density functional (DFT) level. The alignment procedure followed an active analog approach (SYBYL)[43] where the torsion angles were varied in 5° steps.

CZ and PHE were used as starting structures. Because of their rigidity and activity, the consideration of their overlapping portions (Fig. 5) becomes enough to define the active conformation (rigid analog). Whereas PHE determines the orientation of the polar moiety, that is present in all the structures, CZ does the same for the lipophilic portion. They comply, on the other hand, with the requirements of high antiepileptic potency and Na^+ channel binding efficiency.

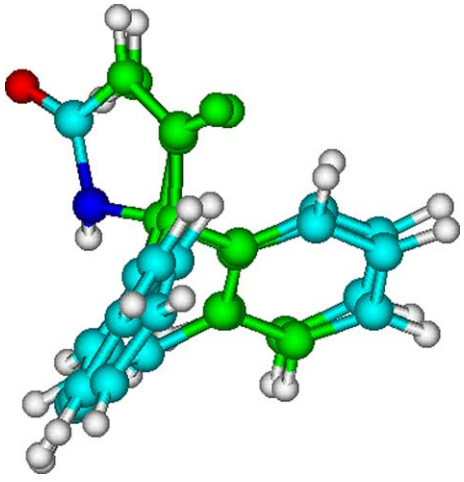

Figure 5. Superposition of CZ and PHE. The overlapping atoms are highlighted in green and define the rigid analog. Atom colors as in Figure 2.

From the structure shown in Figure 5, the constrains have been defined as the nonbonded distances in the PHE–CZ overlapping structure, considering only three consecutive atoms of the aryl ring on the basis of the activity and the chain length of VPA.

Three main steps have been followed in the computations:

(i) The initial optimization of each structure by means of DFT calculations. It allows the identification of the most stable conformer, associated with the absolute minima in the potential energy hypersurface. The optimized conformers represent the most probable structures when far enough from the receptor. It is assumed that the conformational change in the event of the interaction only modifies the torsion angles, keeping the bond lengths and planar angles equal to the DFT optimized values.
(ii) The alignment of the resultant structures with the template, defined by CZ and PHE. This allows one to identify the conformation capable of interacting with the active site (bioactive conformation). The aligned structures are shown in Figure 4.
(iii) The refinement of the geometry of the bioactive conformers by means of constrained optimizations, where the dihedral angles defined by overlapping atoms were kept frozen to the pharmacophore values. The resulting conformations, associated with relative minima in the potential energy hypersurface, have been used to evaluate the electronic characteristics of the ligands.

Density functional calculations have been used for the first and third steps, maintaining the same level of theory. The last step is oriented to find a stable structure, close to the active one, optimized at a level of theory that allows the evaluation of the electronic properties. In the active analog approach, used for the second step, root mean square (rms) values were lower than 0.5 but for remacemide (RMC). For the latter, the superposition of the polar moiety involves an additional atom linking the positive and negative ends (Fig. 3), rising the rms to 0.7. The conformational flexibility of some ligands, as VPA and FLB, increases the likelihood of finding numerous conformations that match the distance requirements. To reduce the size of the conformational space to be explored, rotable bonds have been only defined for the portion that should adapt to the pharmacophore requirements, allowing the rest of the molecule to maintain the DFT optimized geometry.

In the superimposed structures, the overlapping bonds are highlighted in green (Fig. 4). This common pattern allows us to propose certain requirements for the structures to manifest AC activity. The requirements

Table 2. Local density charges (Q_i) on the atomic centers of the polar moiety derived from a fitting to calculated electrostatic potentials within B3LYP/6-31+G** calculations

	Q_1	Q_2	Q_3
CZ	−0.634	1.065	−1.048
PHE	−0.524	0.650	−0.669
VPA	−0.627	0.868	−0.712
FLB	−0.646	1.032	−0.971
LAM	−0.734	0.557	−0.768
RMC	−0.568	0.521	−1.011
TOP	−0.535	1.433	−0.551
ZON	−0.589	1.419	−0.608
RAL	−0.586	0.838	−0.690
ETH	−0.558	0.595	−0.615
VIN	−0.551	0.797	−0.473
RUF	−0.620	0.746	−0.926
OCZ	−0.606	1.028	−1.136

The index i denotes the atom number in Figure 3.

can be summarized in: (1) a hydrophobic chain (atoms 5–7, Fig. 3), (2) a polar moiety (atoms 1–3, Fig. 3). The polar nature of the second portion is shown by the local density charges depicted in Table 2, which have been derived from a fitting to calculated electrostatic potentials at the DFT level. The electronic description corresponds to the active conformation of the derivatives.

In all the cases included in the present study, the negative end is defined by N or O atoms, whereas C or S atoms are comprised in the positive one (only in the case of RMC the negative center in position 3 is placed over a carbon atom). The electronic distribution is always compatible with the polar characteristics of this moiety. The charges on atoms 5, 6, and 7, on the other hand, remain close to zero (data not shown), defining the hydrophobic region. For this region, a minimum lipophilicity seems to be required, as it is demonstrated by ETH which, bearing the polar portion is not active. Although the lipophilic moiety is aromatic in a large number of compounds of the training set, this condition does not define a requirement for the activity, as it is mainly demonstrated by VPA. The hydrophobic requirements cannot be easily quantified as the electronic ones. Whereas some compounds show two hydrophilic regions, like RMC, PHE, and even VPA, only one hydrophobic tail is present in ligands as FLB, RAL, ZON. The calculated log P values (see Table 1) seem to indicate that, whenever a minimum lipophilicity is attained, the other requirements dictate the AE potency. Nevertheless, this minimum value cannot be easily quantified. ETH can be considered to somehow define the limiting region. The AC activity of VPA and related acids has been largely studied.[44,45] Whereas no correlation with calculated log P values has been found when different hydrocarbon chains were considered, a requirement for activity has been associated with the existence of ramifications.[45] Nevertheless, the activity of carboxylic acids related to VPA is originated in several different mechanisms,[46] and no simple interpretation of the different anticonvulsant potencies has been yet provided.

The overlapping region comprises strictly the green portion (Fig. 4) when all the molecules of the training set are considered. However, Figure 4 shows a lipophilic portion larger than this, which extends a region in the space pointing opposite to the polar moiety. Although the atoms of different ligands do not comply with a regular pattern in this region, it renders information on the possible size of the lipophilic moiety. One of the negative ends of the polar end, free from steric hindrance, is more easily accessible than the other for receptor interaction.

The previous discussion allows one to postulate the position of complementary sites in the receptor, associated with H-donor–acceptor groups and, at least, one lipophilic pocket, at well defined relative orientations. This orientation is imposed by the bioactive conformation of the ligands, shown in Figure 5. Pair wise distances between the overlapping atoms (see Fig. 6) helps in the definition of the shape of the proposed pharmacophore.

The lipophilic pocket should be wide enough to allow the interaction of ligands as vinpocetine (VIN).

Neuronal VGSCs have emerged as useful targets for anticonvulsant therapy in the last decade, and a large number of families of promising ligands have been designed, which are still under development. It is interesting to analyze how these newly designed ligands fit the requirements of the proposed pharmacophore. For this purpose, we have chosen four ligands from different families, which are shown in Figure 7.

3BEP[27] belongs to a series of 2-piperidinones, synthesized as noncompetitive $GABA_A$ antagonists, and was recently found to modulate sodium channels in a use-dependent manner.[28] PNU-151774E is a propanamide derivative developed from milacemide,[26,27] whereas AWD-140-190 is a 3-aminopyrrole derivative.[29] SSDG is the more active member of a family of conformationally constrained analogs of *N*,*N*-diaryl-guanidines, locked into the SS^+ conformation.[43] All of them share the Na^+ channel blocking activity as one of their related mechanisms of action. The superposition of the active conformation of each of these structures with the CZ–PHE template is shown in Figure 8. The local density

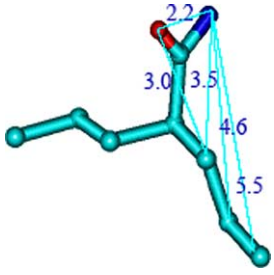

Figure 6. Distance map for the pharmacophore proposed for the MES antiepileptic activity. Hydrogen atoms not shown. Color pattern as follows: nitrogen atoms blue, oxygen atoms red, sulfur atoms yellow, carbon atoms light blue.

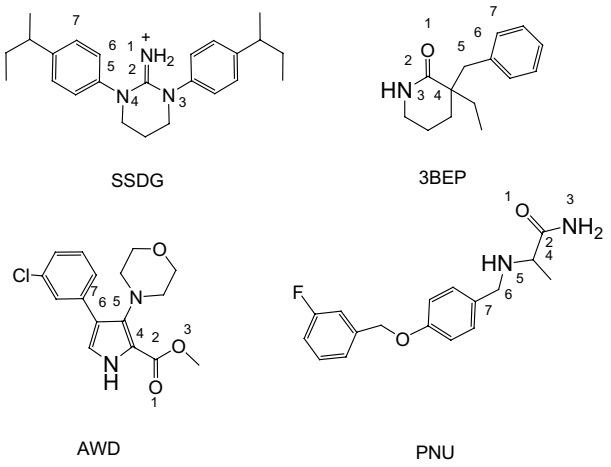

Figure 7. Schematic representation of the structure of some newly designed anticonvulsant compounds associated with a Na^+ channel blocking mechanism of action.

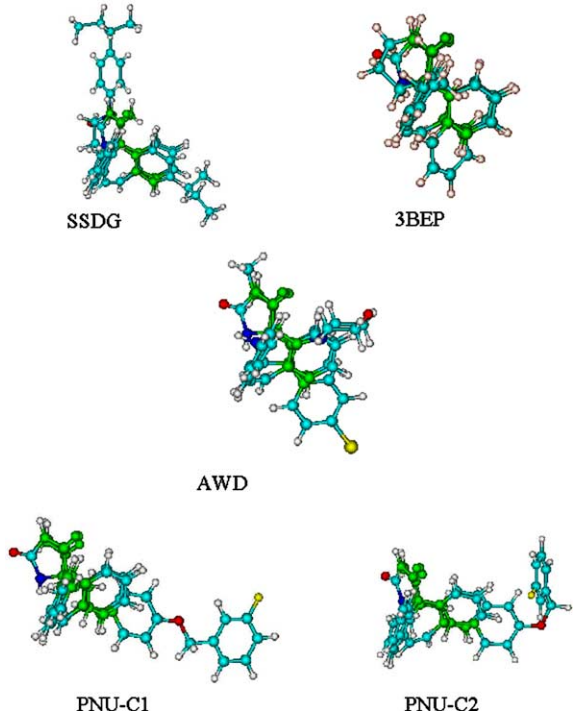

Figure 8. Superposition of SSDG, 3BEP, AWD, and PNU with the pharmacophore template (in green), defined by CZ and PHE. For the case of PNU, two different conformations (C1, C2) are considered.

charges on the polar moieties are shown in Table 2 for 3-BEP. For the case of PNU-151774E, its interaction with the lipophilic pocket may imply a conformational change (Fig. 8).

2.2. Design and synthesis of valpromide derivatives

The step that follows the identification of a pharmacophore is its further validation by means of the consideration of other ligands, which comply with its requirements, usually obtained from modifications of a member of the training set. VPA is an ideal test compound for this objective, as a large number of modifications can be designed, which can lead to either structural or functional derivatives.[18,48–50] We have simultaneously considered the AE activity and the side effects related to VPA therapy: sedation, hepatotoxicity, teratogenicity, and microsomal epoxide hydrolase (mEH) inhibition.[47] The propyl chains have been retained on the consideration that VPA possess the optimal chemical structure with regard to the margin between its anticonvulsant activity and its sedative and hypnotic side effects.[51–53] Functionalization to the amide, valpromide (VPD), appears as promising, as a higher anticonvulsant potency and lower teratogenicity are attained in mice after this transformation.[54–56] However, VPD is actually a prodrug of VPA when administered in human.[56] This condition is not necessarily retained after structural modifications on the amide moiety, that will certainly modify the kinetics of biotransformation to VPA. This hypothesis is sustained by the results of pharmacokinetic studies of various N-mono and N,N-disubstituted VPD derivatives,[50,57,58] which has demonstrated that they act as intact entities, following a very low hydrolysis to VPA. For Valrocemide (N-valproylglycinamide-TV1901), a N-substituted VPD that has reached phase IIb of clinical trails,[4] it is well-known that only a 4% fraction of the drug is metabolized to valproic acid in humans.[4] Another advantage of N-substituted derivatives of VPD, over VPA and VPD, is that they probably do not inhibit the human mEH, an enzyme responsible for detoxifying reactive epoxide intermediates formed by oxidative metabolism of xenobiotics.[59]

The advantages associated with N-substitution of valpromide can be considered together with the high antiepileptic potency of RAL and LAM, which are N-substituted in the polar end. To this end we have analyzed nine N-substituted VPD derivatives in order to validate the proposed pharmacophore and to evaluate the importance of a lipophilic substituent in the polar moiety. In this way, we are simultaneously evaluating the agreement with the pharmacophore previously proposed by Stables and co-workers[20] (Fig. 1), which locates the H-bond donor/acceptor group between two lipophilic regions. The structures (Fig. 9) comply with the pharmacophore previously identified.

The superposition of the valpramides with the pharmacophore template is shown in Figure 10. The capability of interaction with the Na^+ channels has been tested for ETVPD, which, being more potent than VPD, has the same pharmacological profile, and matches the solubility requirements for the in vitro assay.

2.2.1. Chemistry. The general procedure implies the reaction of the amine with valproyl chloride in anhydrous conditions. Particular details for each reaction are given in Section 4.

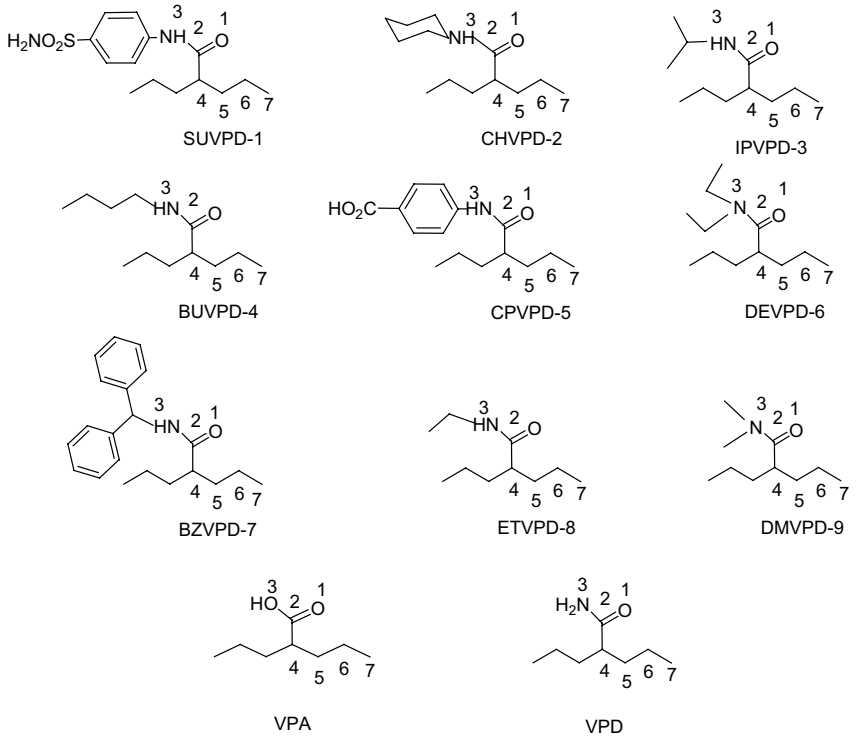

Figure 9. Schematic representation of the structures of the valpromide derivatives.

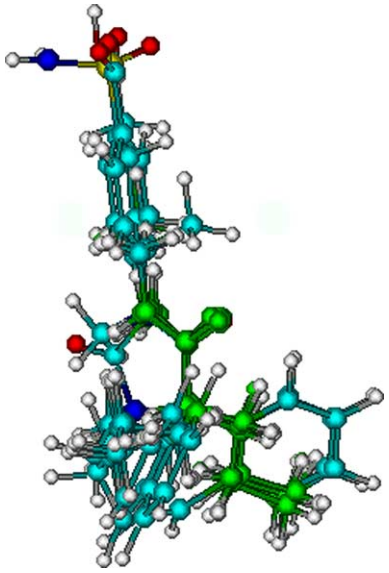

Figure 10. Superposition of the valpromide derivatives with the pharmacophore template (in green), defined by CZ and PHE.

2.2.2. Pharmacology. The N-substituted valpromides were tested for anticonvulsant activity by their ability to suppress experimentally induced convulsions in laboratory animals. The tests used were the Maximal ElectroShock Seizure test (MES test), related to electrical induction, and the pentylenetetrazol test (PTZ test), based on chemical induction of the seizure episode. The RotoRod test was used to determine the possible neurotoxic effects. The results obtained are listed in Table 3.

With exception of CPVPD-5, the VPD's derivatives show protection against the MES test, but not against the PTZ test (Table 3). The MES test is a valuable tool to identify drugs that are active against generalized tonic clonic and focal seizures.[2,67] The PHE-like profile, characteristic of the majority of the VPD's, indicates an effectiveness against generalized seizures and is considered as indicative of a Na^+ channel blocking activity.

In some cases (SUVPD-1, CHVPD-2, ETVPD-8, DMVPD-9), there is an important increase in the AE potency, and in the protective index (PI), relative to VPA (Table 3). SUVPD-1 is the most promising derivative, being 19 times more potent than VPA and almost seven times more than VPD, when considering the anti-MES activity. It is also remarkably safer (more than 11 times) than the parent drugs VPA and VPD.

Intraperitoneal testing becomes difficult in several cases, due to the low solubility of the drugs. This is the case of DEVPD-6 and BZVPD-7, for which protection percentage is reported.

SUVPD-1 opens a promising family of substituted valpromides, showing an ED_{50} value close to that of PHE or CZ. We have previously analyzed the AC of sulfonamides, including acetazolamide (**1**) and two analogs (compounds **2** and **3**, Fig. 11), testing also their ability to inhibit carbonic anhydrase (CA).[68] The compounds exerted a PHE-like profile, being active in the MES test and inactive against PTZ-induced convulsions. At that time, it was noted that bulky substituents in position 5 (Fig. 11) increases the anti-MES potency.

Table 3. Biological data determined (mice) for the valpromide derivatives designed in this research

Compound	TPE (h)	ED_{50} (μmol/kg) MES test	ED_{50} (μmol/kg) PTZ test	TPE_N (h)	TD_{50} (μmol/kg) RotoRod test	PI[a]	Pot.[b]	Cat.[c]
SUVPD-1	4	53 (33–87)	0% at 1000	—	0% at 1000	>19	19	1
CHVPD-2	2	61 (44–85)	0% at 65	—	0% at 65	>1.1	17	1
IPVPD-3	1	384 (300–492)	0% at 250	—	0% at 450	>1.2	2.6	1
BUVPD-4	1	487 (412–576)	0% at 600	0.50	40% at 600	>1.2	2.1	1
CPVPD-5	2	1225 (1107–1355)	1817 (1669–1978)	—	0% at 1500	>1.2	0.8	2
DEVPD-6	1	50% at 300[e]	0% at 375[e]	—	0% at 300[e]	>1.0	3.4	—
BZVPD-7	2	0% at 200[e]	0% at 200[e]	—	0% at 200[e]	—	—	—
ETVPD-8	0.5	200 (148–272)	0% at 800	0.5	632 (550–727)	3.2	5.0	1
DMVPD-9	0.5	347 (266–452)	0% at 1000	—	0% at 700	>2.0	2.9	1
VPA	0.25	1008 (792–1282)	1261 (1155–1377)	0.25	17% at 1700	1.1–1.7[d]	1	2
VPD	1	353 (267–466)	490 (447–537)	2	25% at 600	1.3–1.4[d]	2.9	1

TPE: time of peak effect, TPE_N: Neurotoxic time of peak effect.
[a] PI (protective index) = TD_{50}/ED_{50}, calculated for the MES test.
[b] Pot. (Relative Potency) = ED_{50VPA}/ED_{50drug}, calculated for the MES test.
[c] Category according to the classification of Phase 1 of the Anticonvulsant Screening Project of the ADD program.
[d] According to bibliography.[52,60–66]
[e] Not soluble at higher concentration.

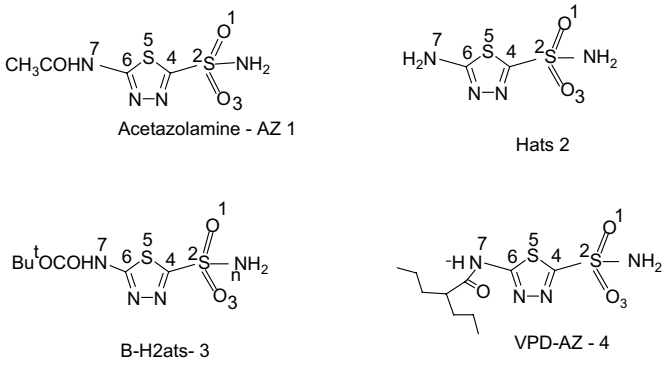

Figure 11. Acetazolamide (**1**) and derivatives previously analyzed (**2**, **3**, Ref. 68); (**4**), Ref. 67.

That research led to the design of SUVPD-1, whose anticonvulsant activity has already been discussed by us.[69] The same drug has been considered two years later by Masereel et al.[67] These authors have combined valproic acid derivatives and sulfonamide functionalities, thoroughly analyzing the resulting compounds in their inhibitory activity of CA in the brain, and further relating it to the AE activity.[68] For the drugs showing a higher performance in the in vitro CA inhibition, among which SUVPD-1 was not included, the in vivo anti-MES AE activity has been determined. In this way, they have succeeded in synthesizing other sulfonamide-related AEDs, which showed protection comparable to that of TOP[67] and SUVPD-1[69] (Table 3). Among them, 5-valproylamido-1,3,4-thiadiazole-2-sulfonamide (VPD-AZ 4, Fig. 11) demonstrated to be highly effective in protecting mice from MES over a long period of time (3 h) We have also found protection over a long period of time for SUVPD-1. It is a remarkable that this drug develops its maximum activity 4 h after its administration, in contrast to VPD, whose activity is higher after an hour. At no time was protection against PTZ noted. This fact cancels out the possible hydrolysis of SUVPD-1 to either VPD or valproic acid, as both of them shows activity in both animal models. The outstanding protection of SUVPD-1 against MES convulsions may be not only restricted to a Na^+ channel blocking mechanism. Its functionality resembles TOP, whose multiple mechanisms of action include the inhibition of the generation of action potentials in neurons antagonizing the activation of Na^+ channels, the positive modulation of some types of $GABA_A$ receptors, and the antagonism of kainate/AMPA receptors.[9,16,70] The capability to interact with CA[67] is not enough ($K_i = 22,500$ nM vs 250 nM for TOP), to justify the remarkable activity of SUVPD-1. Thus, in addition to the testing of CA inhibition, it would be certainly interesting to monitor other possible mechanisms in the development of this class of compounds over the next few years.

The pharmacological profile of ETVPD-3 is also attractive, as it almost duplicates the protection of VPD. In order to further test the capability of the PHE-like drugs to block the Na^+ channels, ETVPD-3 has been tested in its competition with the binding of [^{3}H]batrachotoxinin A-20α-benzoate to the VGSCs from rat brain synaptosomes. It was found to block the Na^+ channels in a dose dependent manner. Due to its limited solubility, an IC_{50} could not be calculated. It would be interesting to do the same test for SUVPD-1, as it is the

most active drug of the set. However, it does not meet the solubility requirements that are necessary to perform the binding assay.

2.2.3. Structure–activity relationships. The valpromide derivatives are shown in Figure 10, aligned with the CZ–PHE template. The modifications of the parent VPD structure do not influence the interaction with the lipophilic pocket, as they involve substitution in the N-atom of the polar moiety.

The protection against MES increases from VPD to ETVPD-8. Two carbon atoms in the N-alkyl chain seems to define the optimum size for this kind of substituents, as its increase to three or four centers (IPVPD-3, BUVPD-4) shows a negative effect. An inverse correlation with the length of the alkyl chain seems to hold for N-substituted VPD's when no additional function is present in the substituent (VPD, ETVPD-8, IPVPD-3, BUVPD-4). However, an alkyl bulky substituent (CHVPD-2) surprisingly increases the antiepileptic activity. It may be inferred that a lipophilic effect helps in the delivery of the drug, whereas a linear increase of the chain counterbalance this effect due to steric repulsions. We have synthesized N-aryl substituted VPD derivatives in order to analyze the effect of this substituent on the activity, but no definite conclusions have been extracted. A sulfonamidophenyl substituent, bearing a $–SO_2NH_2$ function, increases the MES protection, approaching the ED_{50} value of PHE (Tables 1 and 3).

Surprisingly, substitution of the $–SO_2NH_2$ group by –COOH (CPVPD-5) markedly reduces the activity, changing, at the same time, the pharmacological profile of the drug, which turns out to show protection against both MES and PTZ tests. The pharmacological response of CPVPD-5 closely resembles that of VPA, being 40% more active against MES than against PTZ test (Table 3). This fact leads to consider the possible hydrolysis of **5** to VPA. In order to confirm this inference, the hydrolysis has been analyzed in vitro, following the procedure described in Ref. 78. VPA and *p*-aminobenzoic acid were obtained from the reaction. On the other hand, diaryl substitution leads to a derivative (BZVPD-11) not soluble enough to allow the evaluation of its response in the animal models.

The consideration of N-valproylglycinamide (TV1901),[3] opens an optimistic insight on the set of compounds presented in this paper. TV1901 is less active than VPD, but more than VPA, when administered to rodents. However, its resistance to hydrolysis makes this drug more effective than VPD in humans, as the latter biotransforms to VPA. N-Substituted VPD's, with improved performance in animal models become, then, highly promising for the development of new drugs for antiepileptic therapy, based on the grounds of the higher resistance to hydrolysis induced by N-substitution.

The lipophilic substitution of the polar moiety, which is present in RAL and LAM, as well as in the substituted valpromides, resembles the second lipophilic moiety that has been proposed within the pharmacophore previously proposed for semicarbazides and urelylene anticonvulsants.[21] According to our analysis, based on a broader set of dissimilar structures, the lipophilic portion (Fig. 2) does not define a requirement, but certainly helps in improving the activity.

3. Conclusions

The pharmacological profile of a set of valpromide derivatives has been extensively analyzed. The pharmacological behavior resembles PHE being active against MES and inactive against PTZ. The derivatives comply with the requirements imposed by a pharmacophore proposed for the anti-MES activity. The pharmacophoric pattern, on the other hand, has been shown to represent the structural and electronic features compatible with a Na^+ channel blocking mechanism.

SUVPD-1 appears as the most potent anti-MES drug of the set. The efficacy of SUVPD-1 to prevent electrically induced convulsions, and the demonstrated resistance of N-substituted valpromides to biotransformation, point to this set of structures as promising candidates for the development of new drugs efficient for antiepileptic therapy.

On the basis of the largely accepted assumption that the drugs with a PHE-like profile block the VGSC as one of their related mechanisms, we have tested this behavior by means of in vitro tests. This mechanism has been tested positive for ETVPD-8, which is the most potent derivative, among those that meet the solubility requirements for the binding assay.

4. Experimental

4.1. Chemistry

^1H and ^{13}C NMR spectra were recorded on a Bruker AC-200 spectrometer with tetramethylsilane as an internal standard. Melting points were determined on an Electrothermal IA6304 apparatus and are uncorrected. Elemental analyses were measured on a Carlo Erba EA 1108. Solvents were purified and dried by standard procedures. The evolution of the reaction was monitored by TLC (thin layer chromatography). All TLC data were determined using aluminum sheets with silica gel 60 F_{254} (Merck-Darmstadt, Germany). Cyclohexylamine, benzhydryl amine, ethyl amine, and valproic acid were from Aldrich (Milwaukee, USA). N-butyl amine, isopropyl amine, and diethyl amine were from Eastman Kodak. 4-aminobenzoic acid and sulfanilamide were from Merck, whereas dimethylamine was from Carlo Erba. The amines were distilled previous use. Valpromide has been prepared from valproic acid by standard procedures.

4.2. General procedure for the preparation of compounds

4.2.1. Compounds 1–7. Method A. A solution of valproyl chloride (33 mmol) in anhydrous acetone (10 mL, compounds **1** and **5**) or anhydrous benzene (10 mL, compounds **2**–**4**, **6**, and **7**) was added dropwise to a solution of 30 mmol of the reactant in anhydrous acetone (or benzene), containing anhydrous pyridine (70.0 mmol). (Reactant: 4- sulfanilamide: compound **1**; cyclohexylamine: compound **2**; isopropyl amine: compound **3**; *n*-butyl amine: compound **4**; 4-aminobenzoic acid: compound **5**; diethyl amine: compound **6**; benzhydryl amine: compound **7**). The reaction mixture was stirred for a period of 24–48 h at room temperature and concentrated in vacuo. The residue was purified by recrystallization (**1**–**5** and **7**) or distillation under reduced pressure (**6**).

4.2.2. Compounds 8 and 9. Method B. The amine was bubbled into a solution of valproyl chloride (3 mL, 18.2 mmol) in anhydrous benzene (10 mL). The mixture was stirred at 0–4 °C for 1 h and at room temperature for 48 h. The solid was filtered and washed twice with 3 mL of benzene. The filtrate was evaporated under reduced pressure to give a white solid and the residue was purified by recrystallization (**8**) or distillation under reduced pressure (**9**).

4.2.3. 4-(Valproylamido)benzenesulfonamide (1). Yield 65% (acetone), mp: 278–279 °C.[59] TLC R_f 0.5 (dichloromethane/acetone, 5:1). ^{1}H NMR (acetone-d_6) δ 0.89–0.93 (t, 6H, 2Me), 1.33–1.70 (m, 8H, $2CH_2CH_2$), 2,45–2.49 (m, 1H, CH), 6.44 (s, 2H, SO_2NH_2), 7.82–7.87 (m, 4H, C_6H_4), 9.43 (s, 1H, CONH); ^{13}C NMR (acetone-d_6) δ 13.9 (CH_3), 20.9 (CH_2), 35.5 (CH_2), 47.8 (CH), 119.2, 127.4, 138.9, and 142.9 (C_6H_4), 175.3 (CONH). Anal. Calcd for $C_{14}H_{22}N_2O_3S$: C, 56.38%; H, 7.38%; N, 9.40%; S, 10.74%. Found: C, 56.56%; H, 7.32%; N, 9.40%; S, 10.79%.

4.2.4. N-Cyclohexylvalpromide (2). Yield 72% (MeOH), mp: 172.0–172.5 °C. ^{1}H NMR (chloroform-d_3) δ 0.85–0.96 (m, 6H, 2Me), 1.03–1.19 (m, 18H, $9CH_2$), 1.97–2.03 (m, 1H, CH), 3.71–3.89 (m, 1H, cyclohexyl CH), 5.41 (br, 1H, NH); ^{13}C NMR (chloroform-d_3) δ 14.1 ($2CH_3$), 20.7 (CH_2), 24.8, 25.5, 33.3 (cyclohexyl) 35.3 (CH_2), 47.8 (CH), 174.8 (COO). Anal. Calcd for $C_{14}H_{27}NOC$, 74.66%; H, 12.00%; N, 6.22%. Found: C, 74.82%; H, 12.02%; N, 6.29%.

4.2.5. N-Isopropylvalpromide (3). Yield 88% (acetone), mp 131–132 °C. TLC R_f 0.69 (dichloromethane/methanol, 15:1). ^{1}H NMR (chloroform-d_3) δ 0.86–0.93 (t, 6H, $J = 7.0$, 2Me), 1.13 and 1.16 (2s, 6H, isopropyl $2CH_3$), 1.21–1.65 (m, 8H, $4CH_2$), 1.90–1.96 (m, 1H, CH), 4.06–4.17 (m, 1H, isopropyl CH), 5.22 (br, 1H, NH); ^{13}C NMR (chloroform-d_3) δ 14.0 (CH_3), 20.7 (CH_2), 22.8 (isopropyl CH_3), 35.3 (CH_2), 40.9 (CHNH), 47.6 (CH), 174.9 (COO). Anal. Calcd for $C_{11}H_{23}NO$: C, 71.35%; H, 11.35%; N, 7.57%. Found: C, 71.63%; H, 11.40%; N, 7.61%.

4.2.6. N-Butylvalpromide (4). Yield 58% (acetone), mp: 70.0–71.0 °C. ^{1}H NMR (chloroform-d_3) δ 0.82–0.93 (m, 9H, 3Me), 1.15–1.69 (m, 12H, $6CH_2$), 1.94–2.05 (m, 1H, CH), 3.17–3.27 (m, 2H, CH_2N), 5.72 (br, 1H, NH); ^{13}C NMR (chloroform-d_3) δ 13.6 (butyl CH_3), 14.0 (CH_3), 20.0 (butyl CH_2), 20.7 (CH_2), 31.8 (butyl CH_2), 35.3 (CH_2), 38.9 (CNH), 47.6 (CH), 175.8 (COO). Anal. Calcd for $C_{12}H_{25}NO$: C, 72.28%; H, 12.66%; N, 7.02%. Found: C, 72.53%; H, 12.55%; N, 6.99%.

4.2.7. 4-(Valproylamido)benzoic acid (5). Yield 70% (acetone), mp: 233.0–235.0 °C. TLC R_f 0.59 (chloroform/ethanol/water/ammonia, 18:20:2:1). IR (KBr): 3301 cm^{-1} (NH), 1688 cm^{-1} (COO). ^{1}H NMR (chloroform-d_3) δ 0.89–0.96 (m, 6H, 2Me), 1.25–1.75 (m, 8H, $4CH_2$), 2.38–2.51 (m, 1H, CH), 4.88 (br, 1H, NH), 7.67–7.73 (m, 2H, ar), 7.94–8.01 (m, 2H, ar); ^{13}C NMR (chloroform-d_3) δ 16.7 (CH_3), 24.1 (CH_2), 38.8 (CH_2), 51.1 (CH), 122.7, 129.3, 134.1, 146.5 (C_6H_4), 171.8 (CONH), 180.4 (COOH). Anal. Calcd for $C_{15}H_{21}NO_3$: C, 68.38%; H, 7.98%; N, 5.32%. Found: C, 68.22%; H, 7.94%; N, 5.36%.

4.2.8. N,N-Diethylvalpromide (6). Yield (54%), bp 92 °C 1.8 mmHg. TLC R_f 0.73 (dichloromethane/methanol, 20:1). ^{1}H NMR (chloroform-d_3) δ 0.84–0.93 (m, 6H, 2Me), 1.06–1.70 (m, 14H, $4CH_2$ and ethyl $2CH_3$), 2,50–2.61 (m, 1H, CH), 3.28–3.43 (m, 4H, CH_2 NCH_2); ^{13}C NMR (chloroform-d_3) δ 13.0 (ethyl CH_3), 14.8 (ethyl CH_3), 13.9 (CH_3), 14.2 (CH_3), 20.5 (CH_2), 20.8 (CH_2), 34.4 (CH_2), 35.4 (CH_2), 40.4 (NCH_2), 45.0 (NCH_2), 41.8 (CH), 175.6 (CONH). Anal. Calcd for $C_{12}H_{25}NO$: C, 72.36%; H, 12.56%; N, 7.04%. Found: C, 72.60%; H, 12.55%; N, 7.07%.

4.2.9. N-Benzhydrylvalpromide (7). Yield 30% (MeOH), mp: 174–176 °C. TLC R_f 0.77 (dichloromethane). ^{1}H NMR (chloroform-d_3) δ 0.85–0.92 (t, 6H, $J = 7.0$, 2Me), 1.20–1.70 (m, 8H, $4CH_2$), 2.09–2.16 (m, 1H, CH), 5.99–6.04 (d, 1H, $J = 7.8$, NH), 6.29–6.33 (d, 1H, $J = 8.0$, benzhydryl CH), 7.21–7.39 (m, 10H, benzhydryl $5CH_2$); ^{13}C NMR (chloroform-d_3) δ 14.0 (CH_3), 20.7 (CH_2), 35.3 (CH_2), 47.6 (CH), 56.6 (CHNH), 127.3, 127.4, 128.5, 141.7 (C_6H_5), 175.0 (COO). Anal. Calcd for C_{21} H_{27} NO: C, 81.55%; H, 8.74%; N, 4.53%. Found: C, 81.57%; H, 8.77%; N, 4.53%.

4.2.10. N-Ethylvalpromide (8). Yield 52% (MeOH), mp 93–95 °C. TLC R_f 0.65 (dichloromethane/methanol, 20:1). ^{1}H NMR (chloroform-d_3) δ 0.86–0.97 (m, 6H, 2Me), 1.10–1.17 (t, 3H, $J = 7.26$ ethyl CH_3), 1.21–1.71 (m, 8H, $4CH_2$), 1.92–2.04 (m, 1H, CH), 3.24–3.37 (m, 2H, CH_2N), 5.42 (br, 1H, NH); ^{13}C NMR (chloroform-d_3) δ 14.0 (CH_3), 14.9 (ethyl CH_3), 20.7 (CH_2), 34.0 (ethyl CH_2), 35.2 (CH_2), 47.5 (CH),175.8 (CO). Anal.

Calcd for $C_{10}H_{21}NO$: C, 70.17%; H, 12.28%; N, 8.19%. Found: C, 70.37%; H, 12.26%, N, 8.16%.

4.2.11. N,N-Dimethylvalpromide (9). Yield (30%), bp 75–6 °C 1.2 mmHg. TLC R_f 0.67 (dichloromethane/methanol, 20:1). ^{1}H NMR (chloroform-d_3) δ 0.85–0.92 (t, 6H, $J = 7.0$, 2Me), 1.21–1.72 (m, 8H, $4CH_2$), 2.62–2.75 (m, 1H, CH), 2.96 and 3.06 (2s, 6H, CH_3NCH_3); ^{13}C NMR (chloroform-d_3) δ 14.1 (CH_3), 20.8 (CH_2), 31.1 (NCH_3), 35.2 (NCH_3), 35.5 (CH_2), 47.7 (CH), 174.3 (CONH). Anal. Calcd for $C_{10}H_{21}NO$: C, 70.17%; H, 12.28%; N, 8.19%. Found: C, 70.06%; H, 12.31%; N, 8.16%.

4.2.12. Log *P* calculations. The log *P* calculated values were estimated using the HyperChem QSAR Properties, version 7.01 Hypercube, Inc.[39]

4.3. Pharmacological methods

4.3.1. In vivo experiments. The pharmacological tests were performed according to standard procedures provided by the Antiepileptic Drug Development (ADD) Program of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS).[60]

Albino mice BALB/cN weighing between 25 and 30 g at time of testing, were used as experimental animals. Mice are housed in colony cages on a 12 h light/dark cycle, and allowed free access to both commercial rodent chow and water, except when they are removed from their cages and placed on individual ones for the experimental testing.

4.3.2. Preparation and injection of the testing compounds. The compounds were dissolved in 30% PEG 400.[51] A volume of 10 mL/kg of the freshly made preparations was injected intraperitoneally (ip). When higher doses of the compounds were tested, compromising the solubility, the proportion of PEG 400 was increased, maintaining the same maximum volume of 3 mL/kg of PEG 400 and reducing the total volume to less than 10 mL/kg.

4.3.3. Anticonvulsant tests. Maximal electroshock seizures were elicited in mice by delivering a 60 Hz/50 mA electrical stimulus for 0.2 s via ear clip electrodes. A drop of saline solution applied on each ear before placing the electrodes ensures adequate electrical contact. In these conditions, maximal seizures are produced in virtually all normal mice. The maximal seizure typically consists of a short period of tonic flexion followed by a longer period of tonic extension of the hind limbs and a final clonic episode. Blockade of the hind limbs tonic extensor component due to the drug treatment is taken as the end point. The tonic component is considered abolished if the hindleg tonic extension does not exceed a 90° angle with the trunk.[42]

The PTZ tests identify substances that raise the seizure threshold. The freshly made solution of PTZ (1.7% in 0.9% saline solution) is administered subcutaneously (sc) into a loose fold of skin in the midline of the neck in a volume of 5 mL/kg body weight. This amount of PTZ induces convulsions in more than 97% of mice. Animals are observed for at least 30 min after sc injection of PTZ for the presence or absence of a convulsive episode persisting for at least 5 s. Absence of a clonic seizure indicates protection.[42]

4.3.4. Determination of the time of peak effect (TPE). The test compounds were administered to groups of three–five animals each that were tested at different times (usually 30 min, 1, 2, and 4 h, or when the maximum effect has been passed). The percentages of protection or neurotoxicity are recorded and plotted against time and the TPEs are determined by visual inspection of the graphs.

4.3.5. Estimation of the ED_{50}s. Groups of eight animals per dose are injected via ip at least four doses, between those that induce no protection (0% of the animals) and total protection (100% of the animals), were assayed at the TPE determined as previously described. The percentages of protection at each dose (converted to probit) were plotted against log-doses. These data were then subjected to statistical analysis and the ED50s with the 95% confidence intervals, slopes of the regression lines, and standard errors of the slopes were estimated by the method of Litchfield and Wilcoxon.[71]

4.3.6. Neurotoxicity tests. The RotoRod test is used exclusively in mice to assess minimal neurotoxicity. A normal mouse can maintain its equilibrium on a rotating rod (6 rpm) for long periods of time. Neurological deficit is indicated by failure to maintain balance on a rotating rod in each of three trials of 1 min each.

4.3.7. Estimation of the neurotoxic effects. Groups of eight animals per dose were injected and tested with the RotoRod test. The percentages of animals showing minimal neurotoxicity were recorded and the higher dose producing neurotoxic effects is reported. TD50 estimations could not be completed due to solubility problems with high doses.

4.4. In vitro experiments

4.4.1. Sodium channel binding assay. The assay was performed in Novascreen Biosciences Co. (7170 Standard Drive, Hanover, Maryland 21076-1334) according to reported methods[72,73] with modifications. [^{3}H]Batrachotoxin ($K_D = 32$ nM) with a specific activity of 30–60 Ci/mmol was used in a final concentration of 2.0 nM. Rat forebrain membranes were the source of receptors ($B_{max} = 52$ fmol/mg tissue as wet weight). The experiments were performed in triplicate. The

nonspecific determinant was aconitine in a concentration of 1.0 mM. Reactions were carried out in 50 nM HEPES (pH 7.4) containing 130 mM choline chloride at 37 °C for 60 min. The reaction was terminated by rapid vacuum filtration of the reaction contents onto glass fiber filters. Radioactivity trapped onto the filters was determined and compared to control values in order to ascertain any interactions of test compound with the sodium channel, site 2 binding site.

4.4.2. Computational procedure. The structures of all the ligands have been geometry optimized with no constrains at the same level of theory. Density functional calculations have been performed using GAUSSIAN 98 (G98)[74] and the Becke's three-parameter hybrid functional[75] with LYP correlation functional (B3LYP).[76] Basis were of double zeta split valence plus polarization quality (6-31G**) The electronic characteristics have been derived from a fitting to applied electrostatic potentials (CHELPG),[77] using the same level of theory and the same program package. The superposition analysis has been performed using the SYBYL[43] software version 6.6 on a Silicon Graphics Iris Octane MIPS R12000 computer.

Acknowledgements

G.L.E. has performed this research as a member of the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina (CONICET), L.E.B.-B. and S.Ch.M. are researchers of the Facultad de Ciencias Exactas, Universidad Nacional de La Plata. S.M.T. is a fellowship holder of CONICET. This work was supported in part through grants from Agencia de Promoción Científica y Tecnológica (PICT 98-06-03237) CONICET (PIP No 02207 Res 1478) and Universidad Nacional de La Plata, Argentina.

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