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Pharmacophore model for antiepileptic drugs acting on sodium channels

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Abstract Fifteen antiepileptic drugs (AED), active against the maximal electroshock seizure test and able to block the neuronal voltage-dependent sodium channel, have been studied by means of a similarity analysis. Structural and electronic, quantum chemically derived characteristics are compared. Rigid analogs are included, because of the flexibility of some structures, in order to discern the conformational requirements associated with these ligands in the moment of the interaction. An inactive compound (ethosuximide) helps in the definition of the structural factors that are important for the activity. We propose a pharmacophore model that, giving an interpretation of the biological activity, allows the design of new AED with a well-defined mechanism of interaction.

Keywords Pharmacophore · Sodium channel · Antiepileptic · Rational drug design · Quantum chemical molecular design

Introduction

In the field of rational drug design, there are two different approaches that allow searching for new structures with improved biological activity, targeting not only an increase in the potency, but also the simultaneous decrease of adverse side effects. [1] New structures can be modeled on the basis of knowledge of the characteristics of the receptor site, or by means of comparison of different ligands, selected from those that interact with the receptor.

For the applicability of the latter approach, all the molecules should be assumed to bind to the same domain of a given type of receptor protein. Within this framework, the complexity of the study is partially determined by the flexibility of the molecules, which defines many energetically accessible conformations that can coexist in equilibrium. When designing from the ligand, the goal is to identify the geometric and electronic features that, being shared by all the molecules, are most likely involved in receptor recognition and activation. Under these conditions, a comparative analysis, which should include active and non-active compounds, leads to the identification of the minimal requirements associated with the pharmacophoric pattern for a manifested activity. [2]

For the majority of antiepileptic drugs (AED) that are presently in clinical use, the mechanism of action responsible of the whole biological response remains unknown, mainly due to the fact that one receptor site cannot be uniquely associated with a given action. [3]

Voltage-dependent blockade of sodium channels is a mechanism held in common by several structurally diverse compounds. [4] A large number of ligands known to act as sodium-channel blockers are currently marketed as AED, such as phenytoin, [1, 3, 5, 6, 7, 8, 9, 10] carbamazepine, [1, 3, 5, 6, 9, 10, 11] topiramate, [1, 3, 6, 9, 12, 13, 14] and lamotrigine, [1, 3, 5, 6, 9, 14, 15] although for topiramate, as for many others, several mechanisms, such as those involving modulation of $GABA_A$ receptor or inhibition of carbonic anhydrase, have been also found. [16] These ligands are active in several animal models of epilepsy. The maximal electroshock seizure (MES) test is that most frequently used to identify antiepileptic activity and constitutes a procedure useful for the discovery of drugs acting through an Na^+ channel-related mechanism. [4] The MES test is valuable for anticonvulsant preclinical evaluation, due to its high predictability of clinical efficacy. It is included in the initial phases of the Anticonvulsant Screening Project of the

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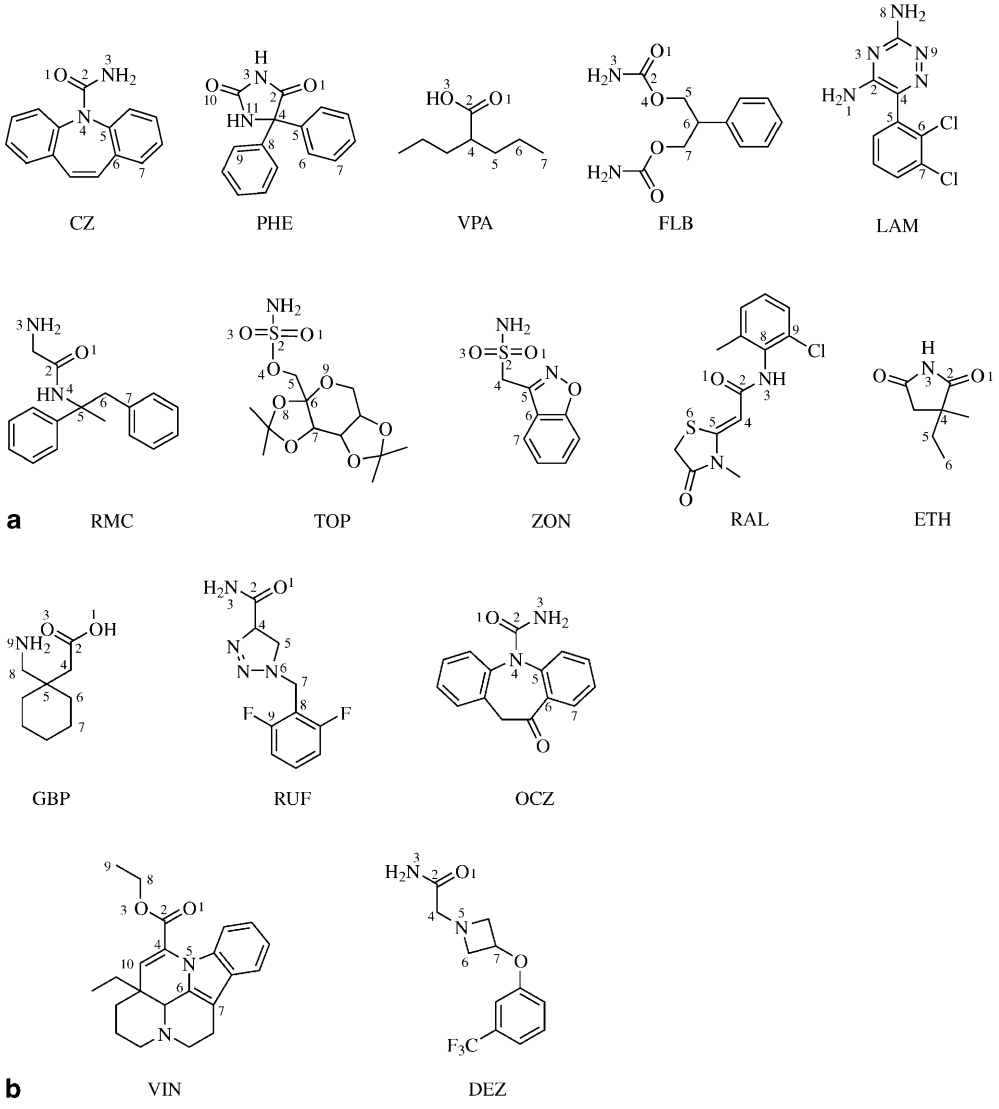

Fig. 1 **a** Structures of the compounds that define the training set: carbamazepine (CZ), phenytoin (PHE), valproic acid (VPA), felbamate (FLB), lamotrigine (LAM), remacemide(RMC), topiramate (TOP), zonisamide (ZON), ralitoline (RAL), ethosuximide (ETT). The representative portion used for HF-MP2 calculations is in *boldface*. Definition of dihedral angles: τ_1=1–2–4–5, τ_2=2–4–5–6, τ_3=4–5–6–7, τ_4=2–4–8–9 for PHE, τ_4=1–2–3–8 and τ_5=2–3–8–9 for RAL. **b** Structures of the compounds that define the validation set: gabapentin (GBP), rufinamide (RUF), oxcarbazepine (OCZ), vinpocetine (VIN), dezinamide (DEZ). The representative portion used for HF-MP2 calculations is in bold face. Definition of dihedral angles: τ_1=1–2–4–5, τ_2=2–4–5–6, τ_3=4–5–6–7, τ_4=4–5–8–9 for GBP, τ_4=5–6–7–8 and τ_5=6–7–8–9 for RUF, τ_4=1–2–3–8 and τ_5=2–3–8–9 for VIN

Antiepileptic Drug Development program of the National Institute of Neurological and Communicative Disorders and Stroke. [17, 18]

On the basis of our previous experience related to MES-active AED, [19, 20] we seek to find a pharmacophoric pattern that, capable of giving an interpretation for the biological activity, allows the design of new AED that interact with neuronal voltage-dependent Na^+ channels. Previous attempts have been recently reported. Unverferth et al. [5] compared nine structures associated with those effects, and proposed a pharmacophore that shows some similarities to but also differences from ours. A COMFA-derived model, [20] restricted to 5-phenylhydantoins, has been demonstrated to be predictive within that set.

We report, in this article, a comparative analysis of several ligands, characterized by an Na^+ channel-blocking effect, that show activity against the MES test. The strategy is based on the determination of similarities among the molecules under study considering, to this end, quantum chemically derived descriptors. We propose a pharmacophore associated with the activity in the Na^+ channels, which complies, at the same time, with the requirements previously found when a different set was analyzed in relation to the anti-MES potency. [21] Structures for which the Na^+ channel effect has not been determined were also included in that set.

Considering the previously reported model in a comparative fashion, we stress the relevance of an accurate definition of a pharmacophore that allows the design of new anti-MES drugs, that, in addition to other mechanisms that might be involved, are capable of blocking the neuronal voltage-dependent Na^+ channel. The development of new agents acting like phenytoin, the prototypic antiepileptic Na^+ channel blocker, leads to the specific control of epileptic episodes related to partial and generalized tonic-clonic seizures in humans. [6, 9]

Experimental

Fifteen different structures were chosen for the present research. Whereas most of them are currently marketed

as AED [4] (carbamazepine – CZ, phenytoin – PHE, valproic acid – VPA, felbamate – FLB, lamotrigine – LAM, topiramate – TOP, zonisamide – ZON, ethosuximide – ETH, gabapentin – GBP, oxcarbazepine – OCZ), others (remacemide [4] – RMC, rufinamide [4] – RUF, ralitoline [14] – RAL, dezinamide [14] – DEZ) are under clinical study. For all of them, as well as for vinpocetine [22] (VIN), their capability of blocking Na^+ channels is well documented.

CZ, PHE, VPA, FLB, LAM, RMC, TOP, ZON, RAL and ETH (chart in Fig. 1a) constitute the training set and have been used for the initial comparison, oriented to identify the pharmacophore for the Na^+ channel-blocking activity. Additional structures (GBP, RUF, OCZ, VIN and DEZ, chart in Fig. 1b) that show similar pharmacological characteristics have been used to support the definition of the pharmacophore, and constitute the validation set.

All the antiepileptic active analogs that define the training and validation sets, except ETH, share a common mechanism of action at a molecular level: they can act by blockade of the neuronal Na^+ channel. [1, 3, 5, 6, 8, 9, 10, 14, 23, 24, 25] They also show a common pharmacological profile: they are active against the MES test. [1, 9, 10, 14, 26] ETH (chart in Fig. 1a) does not show activity in the sodium channels [27, 28] and also has a different pharmacological behavior, being inactive against MES. [29] On the consideration that it is one of the major antiepileptics used in absence seizures, [30] it has been selected as an inactive analog, helping in the definition of the minimal requirements associated with the activity.

The molecules have been compared in their structural and electronic characteristics for their active conformation, which is defined as the conformation capable of interacting efficiently with the receptor. As a consequence of the high degree of flexibility present in the majority of the structures, a rigid analog is used for the definition of conformational requirements.

Similarity analysis

The similarity analysis was based on graphical superposition techniques and focuses on the identification of portions that are shared by all the active members of the set. When flexible structures are included, not only the lowest energy conformation of each compound, but many others very close in energy to them are very likely to accommodate to the requirements imposed by the interaction with the receptor site. The rigid and highly active analogs (PHE and CZ, Fig. 2) define the active conformation, and can be used as templates.

In order to discern the feasibility of the different compounds to become active, the energy that is necessary to evolve from their most stable conformation to the active one, defined by the structural characteristics of the rigid analog, was evaluated by means of semiempirical AM1 calculations. [31] These energy requirements are mainly associated with modifications of τ_1, τ_2 and τ_3 (charts in Fig. 1a and b). For their evaluation, each of these angles was modified in 4–10° steps until the values of the rigid analog were reached, keeping its value frozen at each step of the walk, while the other degrees of freedom were fully optimized. These active conformations have been compared, when they are energetically accessible, in the superposition analysis.

Fig. 2 Superposition of the rigid analogs (CZ in *yellow* and PHE in *violet*)

The pharmacophoric portion is defined by the atoms of the different structures that overlap within a distance no larger than 0.7 Å. This pharmacophoric group has been further analyzed in its electronic description by means of the comparison of the local charges on the atomic centers, derived from calculated electrostatic potentials. The results derived from AM1 calculations (Spartan [32]) were compared with those from density functional theory (DFT) B3LYP (Gaussian 98, [33] 6-31+G(d,p)). For LAM and TOP, ab initio HF-MP2 calculations (Gaussian 98, [33] 6-31+G(d,p)) have been performed in order to check the reliability of the DFT-derived electronic description. The local density charges on the polar moiety of the pharmacophore (see below) has been also evaluated for each structure at a correlated HF-MP2 level, considering a smaller portion of the molecule which is, however, capable of giving a good representation of this moiety (8–11 atoms, shown in boldface in the charts in Fig. 1a and b).

Conformational analysis

The definition of the pharmacophore is based on the comparison of the active conformations of the molecules of the training set. However, the most stable conformations have to be known for the different ligands, in order to calculate the feasibility to evolve from it to the active one. A conformational analysis has been performed, thence, using an AM1 Hamiltonian for the geometry optimization procedure. Because we are dealing with both rigid and flexible structures, systematic and stochastic procedures have been used, respectively, to generate the starting geometries.

In the systematic procedure the initial conformations were generated by means of modifications of the dihedral angles. The angles and their modifications were chosen, for each structure, as the minimum number and step values compatible with a complete search of the conformational space. Whereas τ_1 was varied for CZ, VPA, OCZ and LAM, and τ_2 for ETH, they were simul-

taneously modified for ZON (charts in Fig. 1a and b). These two angles were varied, together with τ_3, for TOP, and together with τ_4 and τ_5 for RAL (chart in Fig. 1a). τ_2 and τ_4 were modified for PHE and GBP (charts in Fig. 1a and b), and τ_1, τ_4 and τ_5 were varied for RUF and VIN (chart in Fig. 1b). Step values varied from 30° to 90° depending on the flexibility.

A stochastic procedure was applied to generate the initial geometries of RMC, DEZ and FLB. Molecular dynamics calculations (T=300 K in the case of FLB and T=600 K in the case of RMC and DEZ, heat time=0.1 ps, step size=0.0005 ps, run times=0.5, 1, 2, 3, 5, 7, 9, 10, 13, 15, 16, 20 ps, cooling times=0.1, 0.5, 1, 1.5, 2, 2.5, 3 ps) rendered 7 differentiable stable structures in the case of FLB, 4 in the case of RMC and 11 in the case of DEZ. From each of the conformations generated in this way, AM1 full geometry optimizations have been performed in order to identify the global minimum.

In the case of TOP, the conformational analysis showed stable conformations when hydrogen bonds were established between either O_8 or O_9 and N (chart in Fig. 1a), the first conformation being 0.3 kcal mol^{-1} more stable than the second one. The reliability of these bonds was further studied by means of geometry optimizations at an ab initio SCF 3-21G level (Gaussian 98 [33]) for a representative portion that keeps the local characteristics of its environment. The influence of the solvent (physiological media) was modeled as water within an Onsager approach.

Results and discussion

Structural and conformational requirements of the pharmacophore

Two different portions can be identified in the structures, on the basis of their different hydrophobicity: a polar end and a hydrophobic portion.

The polar end is defined by atoms 1, 2 and 3 in the structures shown in the chart in Fig. 1a. Because PHE is the less flexible molecule in this moiety, according to the conformational analysis, we consider it in order to define the rigid analog that imposes the characteristics of the active conformation in this portion. The rigid carboxamide portion in PHE imposes a narrow set of accessible values to τ_1 after optimization of the geometry. Values between –57° and –69° characterize the more stable conformations, and are within a 2 kcal mol^{-1} range from the minima, associated with a τ_1 value of –64°. These conformations differ in the orientation of the phenylic substituents in position 4. The non-rigid structures (CZ, VPA, FLB, ZON, TOP, RMC, LAM) have rotational freedom around the 4–2 bond in the polar end. They can easily accommodate to the conformation defined by PHE (ΔE_1, Table 1).

The hydrophobic moiety is associated with either aliphatic or aromatic groups. FLB helps in defining the characteristics of the pharmacophore in this portion, as it demonstrates that only one hydrocarbon chain bonded to position 4 is necessary to actively interact with the receptor. Notice that in some cases, where position 4 corresponds to either an N or a C atom, two hydrophobic portions are bonded to it. ETH, the inactive analog, shows that this hydrophobic group should contain at least three atoms, as no difference in the polar end of this molecule is found relative to the others that can help to justify its lack of activity. The geometrical characteristics of the hydrophobic portion are defined by CZ, in whose structure both phenylic groups are bonded together and prevent rotation around τ_2 and τ_3, offering a rigid definition of this moiety. The global minimum is associated with a τ_2 value of –152° and τ_3 value of 178°. CZ has been chosen, thence, as a rigid analog for the hydrophobic moiety.

The active conformation is defined by the superposition of both rigid analogs, PHE and CZ (Fig. 2). It is associated with a characteristic spatial arrangement of both the polar and the hydrophobic groups, mainly described

Table 1 Most relevant conformational characteristics of the ligands of the training set defined by the torsion angles τ_1, τ_2, τ_3 and the distance between atoms 1 and 7 (d_{1-7}). The flexibility of the polar and hydrophobic ends is expressed by ΔE_1, ΔE_2 and ΔE_3

	τ_1		τ_2		τ_3		ΔE[a]	d_{1-7} (Å)
	Most stable	ΔE_1[a]	Most stable	ΔE_2[a]	Most stable	ΔE_3[a]		
CZ	–70	2.68	*–152*	–	*178*	–	2.68	5.47
PHE	*–64*	–	–1	0.43	*179*	–	0.43	5.52
VPA	–64	0.00	–72	0.99	175	–	0.99	5.51
FLB	9	1.85	171	2.15	–80	0.87	4.87	5.31
LAM	–3	3.29	–115	1.87	180	–	5.16	5.27
RMC	5	1.47	173	1.13	–56	4.47	7.07	5.51
TOP	–23	0.03	–87	6.56	–171	0.43	7.02	5.57
ZON	–144	0.24	–107	1.31	–179	–	1.55	5.22
RAL	61	1.69	–178	3.44	–178	–	5.13	5.60
ETH	–59	0.04	178	1.74	–	–	1.78	4.55[b]

[a] ΔE_i : energy (kcal mol^{-1}) involved in the evolution from the most stable conformation of each structure to the one defined by the rigid analog (Fig. 1). τ1, τ2 and τ3 have been analyzed separately. The simultaneous influence of the three angles defines the total energy cost (ΔE). Italics indicate those dihedral angles that are constrained in the rigid analogs

[b] Distance between O_1 and the last hydrophobic atom (C_6)

by the τ_1, τ_2 and τ_3 dihedral angle values. This also establishes a distance of 5.5 Å (d_{1-7}, Table 1) between the polar group (specifically atom 1, chart in Fig. 1a) and the last atom of the hydrophobic moiety (atom 7, chart in Fig. 1a).

In order to learn how feasible it is for the other compounds of the set to achieve the active conformation, the energies involved in the torsional movements around 4–2, 5–4 and 5–6 bonds have been evaluated, until reaching the τ_1, τ_2 and τ_3 values associated with it, as described in the experimental section. Taking into account that both the ligand and the receptor site should accommodate to each other for a better docking, and that we are only evaluating the flexibility of the ligand side when considering the coincidence among dihedrals angles, a range of 10° is considered to give a good description of the dynamics of the interaction process. Superpositions are considered, thence, acceptable when the values of the dihedral angles of the pharmacophoric portion of the different compounds are within this range of the one defined by the rigid structures.

Our calculated data for the energy involved in the accommodation of each structure to the active conformation, calculated according to the previous description (ΔE, Table 1), are low enough for all the structures under consideration. They can fit to this superposition scheme and accommodate, then, to the requirements of the receptor, becoming capable of triggering the biological response. According to these data, total energy values larger than 7 kcal mol^{-1} for the conformational change are considered not compatible with the activity.

In the case of TOP, the energy involved in the rotation around τ_2 (approximately 6 kcal mol^{-1}, Table 1) is made up of two contributions. It accounts for intramolecular repulsive interactions in the rotational process. It has also a contribution of the energy involved in the process of breaking the hydrogen bond, established between N and O_8 (chart in Fig. 1a) in the lowest energy conformation, that is not kept in the active one.

The active conformations of all the compounds have been superimposed as shown in Fig. 3. The structural portions that are shared by all the active compounds of the training set define the structural requirements associated with the pharmacophoric pattern, which is shown in Fig. 4. The conformational requirements, associated with τ_1 (1–2–4–5), τ_2 (2–4–5–6) and τ_3 (4–5–6–7) values close to –64°, –152° and 178° respectively, are compatible with a distance no longer than 0.7 Å between overlapping centers.

Local density charges

Table 2 shows the agreement between DFT and HF-MP2 calculated charges, derived from electrostatic potentials, when the complete molecule is considered, taking TOP and LAM as representative elements of the set. This supports the electronic comparison used for the other molecules, which is based, for the complete structure, on DFT

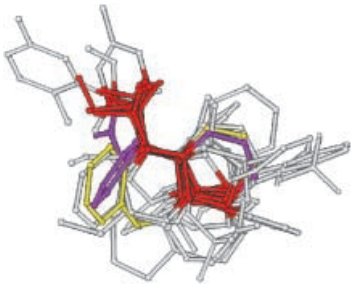

Fig. 3 Superposition of the compounds included in both training and validation sets. The pharmacophoric group, shown in *red*, has been built on the consideration of the overlapping portion of the structures of the training set. The rigid analogs are shown in *yellow* (CZ) and *violet* (PHE)

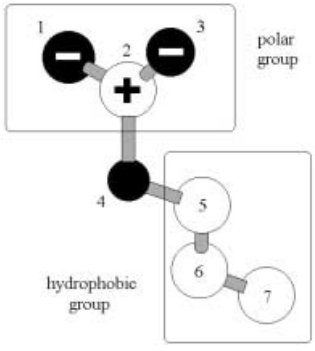

Fig. 4 The definition of the pharmacophoric group includes a polar group comprising two negative atoms and one positive atom, and a hydrophobic group that should be no smaller than three atoms

Table 2 Comparison between DFT and MP2 levels of calculation for the local charges (q) on two compounds of the training set, LAM and TOP

LAM	q (MP2)	q (DFT)	TOP	q (MP2)	q (DFT)
N_1	–0.827	–0.734	O_1	–0.548	–0.551
C_2	0.580	0.557	S_2	1.439	1.433
N_3	–0.738	–0.768	O_3	–0.517	–0.535
C_4	0.180	0.120	O_4	–0.415	–0.423
C_5	–0.082	–0.069	C_5	–0.037	–0.041
C_6	0.068	0.023	C_6	0.471	0.474
C_7	0.094	0.161	C_7	0.351	0.396
N	–0.227	–0.123	N	–1.080	–1.044
N	–0.468	–0.517	C	0.319	0.294
C	1.067	1.052	C	0.299	0.257
N	–1.104	–0.963	C	0.089	–0.061
C	–0.086	–0.121	O	–0.422	–0.339
C	–0.117	–0.137	O	–0.498	–0.483
C	–0.003	–0.034	C	0.495	0.476
Cl	–0.068	–0.051	O	–0.552	–0.539
Cl	–0.111	–0.134	C	–0.306	–0.280
			C	–0.291	–0.260
			O	–0.479	–0.498
			C	0.499	0.515
			O	–0.594	–0.609
			C	–0.250	–0.262
			C	–0.255	–0.272

Table 3 Local charges (q) on the atomic centers of the polar end involved in the definition of the pharmacophore, for the training set

	AM1			6-31+G(d,p) B3LYP			6-31+G(d,p) MP2[a]		
	q_1	q_2	q_3	q_1	q_2	q_3	q_1	q_2	q_3
CZ	–0.57	+0.87	–0.67	–0.64	+1.07	–1.05	–0.69	+1.13	–1.16
PHE	–0.50	+0.71	–0.67	–0.52	+0.65	–0.67	–0.63	+0.91	–1.04
VPA	–0.59	+0.91	–0.62	–0.63	+0.87	–0.71	–0.61	+0.87	–0.70
FLB	–0.60	+0.99	–0.89	–0.65	+1.03	–0.97	–0.63	+1.07	–1.16
LAM	–0.90	+0.62	–0.79	–0.73	+0.56	–0.77	–0.83	+0.58	–0.74
RMC	–0.52	+0.81	–0.82	–0.57	+0.52	–1.01	–0.61	+0.74	–1.06
TOP	–1.07	+3.18	–1.25	–0.54	+1.43	–0.55	–0.52	+1.44	–0.55
ZON	–1.03	+3.04	–1.20	–0.59	+1.42	–0.61	–0.61	+1.46	–0.61
RAL	–0.52	+0.88	–0.76	–0.59	+0.84	–0.69	–0.61	+0.89	–1.09
ETH	–0.49	+0.65	–0.60	–0.56	+0.60	–0.62	–0.63	+0.91	–1.04

[a] MP2 calculations were performed for a representative structural portion of each of the compounds (chart in Fig. 1a) in the case of PHE, CZ, FLB, VPA, RAL, ZON, RMC and ETH

calculations. DFT calculations, which are not orbital based, use iteratively converged functionals to model the electronic density. As they include electronic correlations, they are compared with correlated (MP2) Hartree–Fock calculations.

Table 3 shows the local density charges restricted to the atoms that define the polar end of the pharmacophore most strongly related to electronic requirements. For both the AM1 and ab initio (B3LYP, HF-MP2) levels of calculation, they derive from a fitting to calculated electrostatic potentials. This electronic description corresponds to the active conformation of the derivatives. The pattern of separation in positive and negative ends of the polar moiety is retained along the series at semiempirical (AM1) and correlated HF-MP2 and DFT levels. There is a remarkable agreement in the description of a charge separation in this end, which is not dependent on the calculation level and even holds for the semiempirical case. Quantitative agreement is only found when ab initio (DFT, HF-MP2) results are compared (Table 3). This is a consequence of the dependence of the calculated charges on the basis set. Only for the ab initio case can the same basis be used. Whereas for the DFT case the complete structure has been considered for each compound of the training set, a representative structural portion has been modeled for the HF-MP2 calculations (portions of 8–11 atoms, charts in Fig. 1a and b). An apparent disagreement in the description of q_2 and q_3 for molecules like RAL, PHE and ETH when DFT and HF-MP2 levels are compared, has to be evaluated taking into account the difference between both models.

The charge separation on the atoms of the polar end is compatible with the characteristics of this moiety. In all the cases the positive center is placed over a carbon or a sulfur atom, and the negative centers over nitrogen or oxygen ones. In the case of RMC, the position of one of the negative charges of the polar end (N_3) implies two instead of one interatomic bonds counted from the positive pole (chart in Fig. 1a). Even the consideration of RMC renders a good superposition of the centers bearing the charges (Fig. 3).

The similarity of the results derived from different calculation levels supports the accuracy of the electronic description. Moreover, the importance of finding electronic requirements is related to the fact that, in the event of the interaction, the receptor site perceives electronic distributions approaching it. [2] On the basis of this consideration, the repeated distribution of the charge on the atomic centers of the polar group points to it as the relevant portion for an effective ligand–receptor attraction. This will result in an efficient docking provided that the other structural and conformational requirements are also met. This is in agreement with previous results derived from a QSAR analysis that points to the carbonylic group of the polar end as the main group responsible for the anti-MES activity of a series of N-valpromide derivatives and Na^+ channel-blocking drugs. [22]

Definition of the pharmacophore

The structural and electronic requirements imposed by the pharmacophore involve (Fig. 4):

- A 3-atom portion characterized by a large polarization of the interatomic bonds (negative charge on atoms 1 and 3, positive charge on atom 2), bonded to an sp^3 hybridized atom, that can be nitrogen (CZ, RMC), carbon (VPA, ZON, RAL, LAM, PHE), or oxygen (TOP, FLB)
- A hydrophobic portion coordinated through atom 4 to the positive end of the polar group, which comprises at least three atoms, which can belong to aromatic (PHE, CZ, RAL, ZON, LAM) or aliphatic moieties (VPA, FLB, TOP, RMC)

The conformational requirements are associated with the spatial orientation of the hydrophobic moiety relative to the polar group (Table 1), that can be defined by values of the dihedral angles τ_1, τ_2 and τ_3 close to –64°, –152° and 178° respectively. Both the negative center 1 and the hydrophobic moiety are oriented in the same di-

Table 4 Most relevant conformational characteristics of the ligands of the validation set defined by the torsion angles τ1, τ2 and τ3 and the distance between atoms 1 and 7 (d_{1-7}). The flexibility of the polar and hydrophobic ends is expressed by ΔE_1, ΔE_2 and ΔE_3

	τ_1		τ_2		τ_3		ΔE[a]	d_{1-7} (Å)
	Most stable	ΔE_1[a]	Most stable	ΔE_2[a]	Most stable	ΔE_3[a]		
GBP	98	1.27	–64	3.85	–170	–	5.12	5.74
RUF	1	2.67	179	2.10	174	–	4.77	5.73
OCZ	6	2.63	–138	0.39	180	–	3.02	5.42
VIN	–54	–	–172	1.11	153	4.35	5.46	5.40
DEZ	134	4.88	178	–	122	0.28	5.16	5.12

[a] ΔE_i: energy (kcal mol^{-1}) involved in the evolution from the most stable conformation of each structure to the one defined by the rigid analog (Fig. 1). τ1, τ2 and τ3 have been analyzed separately. The simultaneous influence of the three angles defines the total energy cost (ΔE)

Table 5 Local charges (q) on the atomic centers of the polar end involved in the definition of the pharmacophore, for the validation set

[a] MP2 calculations were performed for a representative structural portion of each of the compounds (chart in Fig. 1b)

	AM1			6-31+G(d,p) B3LYP			6-31+G(d,p) MP2[a]		
	q_1	q_2	q_3	q_1	q_2	q_3	q_1	q_2	q_3
GBP	–0.61	+0.89	–0.59	–0.65	+0.89	–0.66	–0.61	+0.87	–0.70
RUF	–0.52	+0.54	–0.59	–0.62	+0.75	–0.93	–0.61	+0.74	–1.06
OCZ	–0.58	+0.84	–0.66	–0.61	+1.03	–1.14	–0.69	+1.17	–1.16
VIN	–0.58	+0.89	–0.46	–0.55	+0.80	–0.47	–0.57	+0.82	–0.42
DEZ	–0.23	+0.31	–1.17	–0.58	+0.75	–0.91	–0.61	+0.74	–1.06

rection, being capable of interacting simultaneously with an electron acceptor group and a hydrophobic pocket of the receptor face. When all the structures are considered, the distance between the polar and the hydrophobic groups (measured from atom 1 to atom 7), previously mentioned for the rigid analog, lies within 5.1–5.7 Å. The other negative end of the polar group which points in opposite direction, is not involved in the interaction, but plays a role in the stabilization of the charge separation in the polar end. It is included then, in the definition of the pharmacophore, although in some cases is structurally hindered, and its possible interaction precluded.

Validation of the pharmacophore. Validation set

The consideration of the structures (GBP, RUF, OCZ, VIN, DEZ) that were kept for further validation of the proposed pharmacophore successfully confirms both the structural and electronic requirements.

Table 4 shows the geometric characteristics of the most stable (lowest energy) conformer of each member of the validation set. As has previously been discussed, the superposition analysis is based on the comparison of the active conformations: those complying as closely as possible with the requirements imposed by the pharmacophore. Table 4 also reports the energy values involved in the evolution from the most stable to the active conformer for each member of the set (ΔE_i).

For RUF and VIN, the active conformations (close to 5 kcal mol^{-1} from the most stable one, ΔE) define τ values that are within 10° of those previously given in the definition of the rigid analog (Table 1, italic). For DEZ, the active conformation corresponds to one that overlaps the rigid analog in the pharmacophoric portion within distances shorter than 0.7 Å between corresponding atoms from different molecules. For GBP and OCZ, perfect overlapping with the rigid analog is achieved.

The electronic analysis shows the same charge distribution previously described when the training set was considered (Table 5). According to it, polar and hydrophobic moieties can be identified. The considerations previously discussed when comparing the results presented in Table 3 have to be taken into account.

Antiepileptic activity – sodium channel blockade

The pharmacophoric pattern defines the requirements for the Na^+ channel-blocking activity shared by the active structures of both the training and the validation sets.

Our model can be compared with pharmacophoric patterns previously proposed by others to fit both anti-MES and Na^+ channel-blocking activities. We center on the comparison in the model proposed by Unverferth et al. [5], as being the most closely related to ours. These authors have compared the essential structural characteristics of CZ, PHE, LAM, ZON, RMC, RUF, VIN, DEZ and a representative 3-aminopirrol, and suggested a model pharmacophore (Fig. 5) that comprises an electron donor D group in distance ranges of 3.2–5.1 Å to an aryl ring or other hydrophobic unit R, and of 3.9–5.5 Å to a hydrogen bond acceptor–donor unit HAD. The distance between R and HAD spans a wider range of 4.2–8.5 Å. The hydrogen bond donor–acceptor group can be almost correlated with the polar end of our model, although we define our polar end as mainly H acceptor. This mismatching is due to the fact that, in some cases, as in the case of LAM, PHE and VIN, this

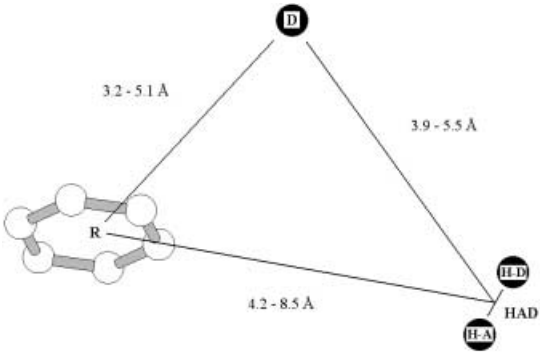

Fig. 5 Pharmacophore model proposed by Unverferth et al. [5]

portion of the model implies a different set of atoms[1], although in close proximity, when defined by us or by Unverferth et al. [5] We can accept, however, that H donor capabilities are always associated with atom 3 (charts in Fig. 1a and b) within our scheme, if we relate it to N instead of O in TOP and ZON. Although we are not losing too much in accuracy with this consideration, recent studies on anticonvulsant sugar sulfamates related to topiramate [16] have shown that the activity is kept after disubstitution of both N-bonded sulfonamide hydrogen atoms. This substitution, that would prevent interaction of N with the receptor, gives further support to our previous assignment of the polar moiety of TOP and ZON. We agree with Unverferth et al. [5] on the presence of hydrophobic moiety. No other portion that can be interpreted as a donor group derives from our superposition analysis. We extended the comparison a little further, including the consideration of the calculated distances between the representative groups. We calculate, for CZ, a distance of 4.45 Å from R (defined as the center of the aryl ring) to O_1, and of 5.22 Å from R to N_3. These distances, which should be compared with the 4.2–8.5 Å values reported by Unverferth et al., [5] do not belong to the most stable but to the active conformation, and represent more accurately the structural requirements in the event of binding to the receptor site. On the other hand, the hydrophobic portion that we define is not restricted to an aryl moiety (DEZ, Fig. 3), and the comparison between the distance from HAD to this portion is not straightforward. We define the condition of hydrophobicity as associated with the presence of a hydrocarbon portion that, counted from atom 4, should be larger than two bonds (on the basis of the lack of activity of ETH). This implies a distance of 5.5 Å measured from the negative end corresponding to the atom 1 of the polar group to the last atom of the hydrophobic moiety of the pharmacophore.

In spite of the similarities, the two models do not agree fully. In some cases, such as CZ, they differ only in the presence of the donor group, which does not become evident when a larger set of more dissimilar compounds is considered, as in the case of the present research. For our model, based on a thorough structural and electronic analysis, we have to mention that the conformational requirements that it suggests have been already tested, and further confirmed in their AE predictability, through the synthesis of more potent valpromide derivatives that comply with it (Moon SCh, Bruno-Blanch L, personal communication, 2000). QSAR studies based on a training set that include compounds synthesized and tested in our laboratory, [21] have pointed to the electron donor moiety of the polar end (atom 1 in charts of Fig. 1) as determinant for the AE activity.

[1] In the case of LAM, N_1 and N_3 in our pharmacophore versus N_8 and N_9 in [5], for PHE, O_1 and N_3 instead of O_{10} and N_{11}, and in the case of VIN the O_3 is replaced by a C_{10} in [5] (charts in Fig. 1a, b).

Conclusions

A comparative analysis performed for a series of structurally diverse antiepileptic structures allowed us to define a pharmacophoric model involving structural, conformational and electronic characteristics. Two main different portions can be identified in the structures: a polar end and a hydrophobic portion, defining a spatial arrangement where both groups are able to interact simultaneously with receptor complementary points.

The active conformation, defined by the rigid analogs, can be achieved by all the compounds of the training set, allowing the definition of the pharmacophore. The pharmacophore postulated is validated when a new set of compounds is considered.

The pharmacophore model discussed in this article is aimed to help in the design of new AED with a mechanism of action related to an interaction with voltage-dependent sodium channels, leading to the specific control of epileptic episodes related to partial and generalized tonic-clonic seizures in humans.

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