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Original article

Indanesulfonamides as carbonic anhydrase inhibitors and anticonvulsant agents: Structure–activity relationship and pharmacological evaluation

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Abstract

A small library of indanesulfonamides was screened for the inhibition of the human carbonic anhydrase (CA, EC 4.2.1.1) isoforms involved in neuronal excitation, that is, isoforms VII, XII and XIV. These CA isoforms are becoming interesting target for the design of agents useful for the treatment of epilepsy. The inhibition pattern of these indanesulfonamide compounds towards these three isoforms was excellent, with many nanomolar inhibitors detected (K_Is in the range of 0.78–10 nM against hCA VII; 0.32–56 nM against hCA XII, and 0.47–1030 nM against hCA XIV, respectively). The maximal electroshock seizure (MES) test performed on mice showed a good anticonvulsant activity for some compounds which protected the mice against convulsions in the 50–62.5% range at a dose of 50 mg/kg. In parallel, the blood–brain barrier passive permeation of these sulfonamides was also estimated by using a computational approach.

Keywords: Carbonic anhydrase; Epilepsy; Maximal electroshock seizure; Blood–brain barrier permeation

1. Introduction

Epilepsy is one of the most common serious neurological disorders characterized by recurrent seizures. It results from a temporary electrical disturbance of the brain due to an imbalance between excitatory and inhibitory neurotransmitters. The mechanisms of action of the antiepileptic drugs (AEDs) consist in the blockade of voltage-dependent Na^+ channels or T-type Ca^{++} channels, inhibition of glutamatergic transmission and facilitation of gamma aminobutyric acid (GABA) inhibitory neurotransmission [1]. However, 30% of epileptic patients continue to have seizures despite optimized treatment with classical AEDs [2]. Moreover, many serious side effects are reported in many patients treated with presently available AEDs [1]. Therefore, there is a growing interest for new AEDs acting on novel therapeutic targets with a pharmacological profile characterized by enhanced efficacy and minimal side effects. This needs to be coupled with a better understanding of generation and propagation of seizures [2].

Since several decades, the carbonic anhydrase (CA, EC 4.2.1.1) inhibitor acetazolamide (**AZA**) is used as an anticonvulsant agent in the treatment of epilepsy [3,4]. Despite the development of a rapid tolerance consisting in diminished therapeutic efficacy after the initial response of the patients, **AZA** is still used in combination therapy with other AEDs or in refractory epilepsies [3]. Zonisamide (**ZNS**) another sulfonamide with CA inhibitory properties is also used as adjunctive therapy for refractory partial seizures in adult [5,6]. Its sulfamoyl group was expected to suppress seizures in a similar way to **AZA** through the inhibition of carbonic anhydrase [7,8]. However, this does not appear to be its only primary mechanism of action. The sulfamate topiramate (**TPM**) is a recent antiepileptic drug which has been shown to be clinically effective against different types of seizures [8]. Several mechanisms of action are attributed to **TPM** including its ability to

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inhibit CA (Table 1) [9]. CAs are metalloenzymes which catalyse the hydration reaction of carbon dioxide into bicarbonate ($H_2O + CO_2 \leftrightarrow HCO_3^- + H^+$) and are therefore involved in various physiological functions such as pH regulation, respiration, bone resorption, etc [10–12]. Among the 16 CA isozymes presently known in mammals, the expression of some of them has been recently reported in the brain, being demonstrated their involvement in the physiology/physiopathology of central nervous system, including epileptogenesis [13]. Different physiological roles are attributed to CAs in the brain such as fluid and ion compartmentation, formation of cerebrospinal fluid, regulation of pH, proliferation, differentiation and seizure activity. The cytosolic isoform CA VII and the transmembrane isoforms CA XII and CA XIV have been pointed out for their contribution to generating neuronal excitation [8]. In fact, CA VII can establish a GABAergic transmission functionally excitatory by providing bicarbonate [8,14,15]. CA XIV can regulate activity-dependent pH shifts which affect neuronal excitability [8,16]. CA XII is overexpressed during status epilepticus produced by kainic acid and could also be implicated in epilepsy [8,13]. Isoform-selective carbonic anhydrase inhibitors (CAi) are of interest in order to better understand the physiopathology of seizures and the functional relevance of CAs to seizures [8].

In the present work, we investigated the inhibition of a series of racemic indanesulfonamides previously described against three CA isoforms possibly involved in epilepsy [17,18]. Structure–activity relationships will provide information for the design of more selective inhibitors. The anticonvulsant activities of these derivatives were tested through an in vivo rodent model of convulsions (the maximal electroshock, MES test). In order to predict the blood–brain barrier (BBB) permeation which is an important factor in central nervous system (CNS) drug design, we used the VolSurf method to project our indanesulfonamides in the chemical space represented by the quantitative model for BBB permeation.

2. Results and discussion

2.1. CA inhibition

The structures of the racemic synthezised indanesulfonamides are displayed in Fig. 1. The inhibitory activity of indanesulfonamides was evaluated against the hCA VII, XII and XIV and is reported in Table 1. **AZA** and **TPM** were used as standard inhibitors for comparison.

The indanesulfonamide scaffold (**1**) exhibits powerful inhibitory properties against the three investigated isoforms, similarly to those found earlier against CA I, II and IX [18]. The same range of K_I is observed for hCA XIV and VII but the inhibition is better against hCA XII. The following structure–activity relationships should be noted: (i) against hCA XII, all the derivatives showed a good inhibitory potency ($K_I = 0.32$–52 nM). The sulfonamide **2** provides a better inhibition than the corresponding sulfonic acid **3**. Nevertheless, the sulfonamide **4** and the sulfonic acid **5** exhibit a similar inhibitory potency. A methyl, a 4-heptyl and a cyclohexyl moiety lead to a better inhibition when it is substituted in position 1 of the indane (**6** vs **4**, **8** vs **7** and **10** vs **9**). The same range of inhibitory potency is observed with a pentafluorophenyl (**11**, **12**) or a tetrafluorophenyl (**13**) moiety. The elongation of the side chain improves the K_I from 9.0 to 0.69 nM (**14**, **15** and **17**). Surprisingly a *n*-butyl moiety (**16**) has a higher K_I value (52 nM). The *n*-nonyl side chain increases the inhibitory potency when located in position 2 of indane (**17** vs **18**). The inhibition pattern of compound **19** and **20** is similar. (ii) The K_I against hCA XIV is ranging from 0.47 to 1030 nM. As observed for hCA XII, it should be noted that the sulfonamide **2** exhibits a stronger inhibition than its corresponding sulfonic acid **3**. An opposite behaviour is observed with the sulfonamide **4** and the sulfonic acid **5**. The methyl (**4**, **6**) or the 4-heptyl moiety (**7**, **8**) reveals a weak inhibition. Differences can be observed between the two investigated positions of the substituent. Indeed, cyclohexyl side chain improves the activity when it is bound in position 2 (**9** vs **10**). A pentafluorophenyl group exhibits a good

Table 1
CA inhibition data of indanesulfonamides and several inhibitors (AZA and TPM); mice anticonvulsant activity and calculated lipophilicity

Compound	K_I (nM)[a]			MES test Protected mice (%) [b]	*C* log *P*
	hCA XII	hCA XIV	hCA VII		
Vehicle injected	–	–	–	0	
PHEN	–	–	–	100	2.08
CARB	–	–	–	100	1.98
TPM	3800[c]	1460[c]	0.87[d]	68.75	−2.03
AZA	5.7	41	2.5	12.5	−1.13
1	0.39	6.5	5.1	0	1.32
2	1.4	5.8	1.6	NT	−0.52
3	4.8	82	8.0	NT	−3.74
4	5.4	675	10	50	−0.93
5	4.7	68	7.1	37.5	−1.88
6	0.32	842	1.7	25	−0.47
7	28	658	9.3	62.5	2.02
8	6.2	251	1.7	12.5	2.48
9	9.1	48	0.78	12.5	1.10
10	2.8	341	8.2	0	1.56
11	2.3	24	6.8	0	1.14
12	5.5	7.3	1.9	12.5	1.54
13	3.1	0.47	3.7	NT	0.99
14	9.0	61	3.9	12.5	−0.40
15	5.9	5.0	4.5	37.5	0.12
16	52	40	6.5	25	0.65
17	0.69	2.2	1.9	0	3.30
18	5.7	1030	1.5	0	3.76
19	0.36	5.6	2.7	62.5	1.99
20	0.44	2.7	1.5	0	1.92

NT, not tested; **PHEN**, phenytoine; **CARB**, carbamazepine; **TPM**, topiramate; **AZA**, acetazolamide.

[a] Errors were in the range of 3–5% of the reported values, from three different assays.

[b] All of tested compounds were suspended in an aqueous solution of Tween 80 (1% v/v); the maximal electroshock test was carried out 2 h after administration of the tested compounds ip 50 mg/kg; $n = 8$ mice except for compounds **1** and **17** where $n = 7$.

[c] From Nishimori et al. [26].

[d] From Vullo et al. [28].

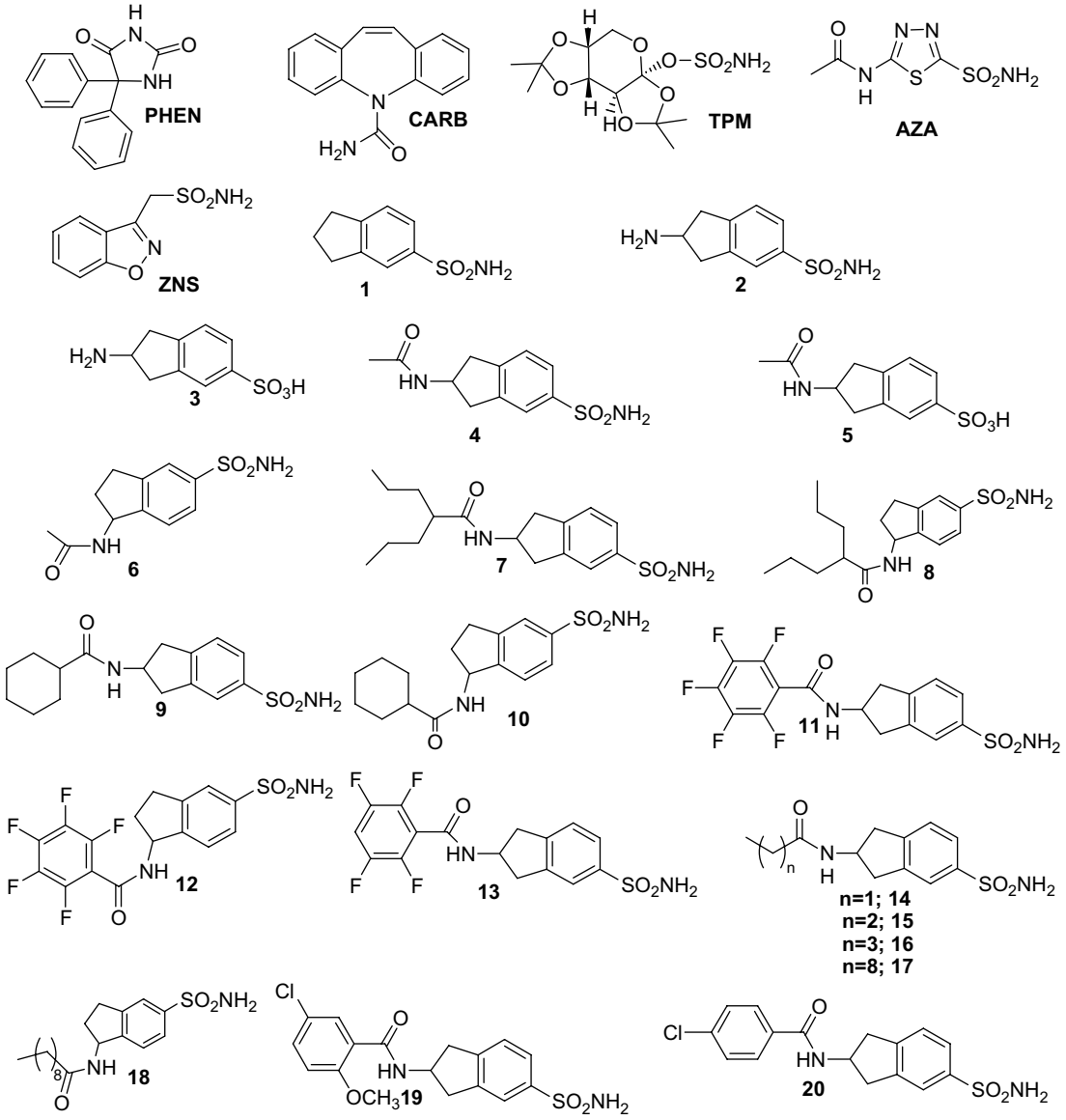

Fig. 1. Structure of anticonvulsant drugs (**PHEN**: phenytoine, **CARB**: carbamazepine, **TPM**: topiramate, **AZA**: acetazolamide) and indanesulfonamide derivatives (**1**–**20**).

inhibition (**11**, **12**); nevertheless, the position 1 seems to enhance the inhibitory potency to a K_I value of 7.3 nM. Regarding compounds **11** and **13**, tetrafluorophenyl offers a better inhibition than pentafluorophenyl. The elongation of the side chain gives a similar trend than that observed for hCA XII. Indeed, the *n*-ethyl chain (**14**) shows a K_I value of 61 nM, the *n*-propyl (**15**) improves the inhibition, but the *n*-butyl, (**16**) decreases the inhibitory potency. The *n*-nonyl derivative (**17**) inhibits strongly hCA XIV. If the *n*-nonyl is substituted in position 1 of the indane scaffold (**18**), the inhibitory potency is strongly decreased by comparison with the corresponding regioisomer **17**. Finally, a parachlorophenyl (**20**) and a 5-chloro-2-methoxyphenyl moiety (**19**) exhibit a good K_I (2.7 and 5.6 nM, respectively). (iii) In general all the compounds show a high inhibitory potency against hCA VII ($K_I = 0.78$–10 nM). As previously described, the sulfonamide function (**2**) exhibits a better inhibition to compare with the corresponding sulfonic acid function (**3**). It is the opposite for compounds **4** and **5**, where a sulfonic acid function (**5**) increases the inhibition. The influence of the substitution pattern of indanesulfonamide scaffold reveals that a methyl, a 4-heptyl and a pentafluorophenyl moiety provide a strong inhibition when they are introduced in position 1 (**6** vs **4**, **8** vs **7**, **12** vs **11**). Position 2 seems to be favorable for a cyclohexyl group (**9** vs **10**). Elongation of the side chain preserves the inhibitory potency (**14**–**18**). A *n*-nonyl side chain gives the same K_I whatever the substituted position (**17** vs **18**). The tetrafluorophenyl **13** slightly increases the K_I in comparison with the corresponding pentafluorophenyl **11**. Compounds **19** and **20** inhibit hCA VII with the same range of K_I.

2.2. Anticonvulsant activity and brain permeability

Since a large number of indanesulfonamides share a good inhibition profile against CA isoforms involved in epileptogenesis, we examined their anticonvulsant activity in the maximal

electroshock seizure (MES) test. The standard drug topiramate **TPM**, and two other widely used AEDs possessing different mechanisms of action, phenytoine (**PHEN**) and carbamazepine (**CARB**), were also included in this test for comparison. **TPM** has several mechanisms of action including: inhibition of CA, inhibition of the voltage Na^+-channel, antagonism of the kainate/AMPA receptor and improving the GABAergic transmission [8,19]. The anticonvulsive activity of **PHEN** and **CARB** is mainly due to the inhibition of voltage Na^+-channel. Injected at 30 mg/kg (data not shown) and 50 mg/kg (Table 1), both compounds showed a complete protection in the MES test. **AZA** was also evaluated through the MES test since it suppresses seizures through inhibition of CA and it is used to treat refractory epilepsy. At body weight dose of 50 mg/kg, **AZA** protected only 12.5% of the mice in the MES test model. A dose–response was conducted with **AZA** and led to determine the dose protecting 50% of the mice against MES (ED_{50}). **AZA** demonstrated an anticonvulsive property with an ED_{50} value corresponding to 96 mg/kg. The data of Table 1 show that, at 50 mg/kg, three indanesulfonamide derivatives (**4**, **7** and **19**) protected at least 50% of the mice against convulsions. Compounds **7** and **19** were characterized by a similar protection to **TPM** at an intraperitoneal dose of 50 mg/kg (62.5% for **7** and **19** vs 68.75% for **TPM**). Two molecules (**5** and **15**) showed a moderate anticonvulsant activity (protection of 37.5%). Other derivatives were poor anticonvulsants but might be compared to **AZA** which was not able at a body weight dose of 50 mg/kg to protect mice against convulsions. From these experiments, we can assert that compounds which are powerful CA inhibitors do not constantly allow protection against convulsions through the MES test. The exact mechanisms that trigger seizures are not completely elucidated [8]. Complex phenomena involving pH shifts, increase in extracellular potassium concentration, modification of the extracellular ionic environment and imbalance between excitatory and inhibitory neurotransmitter are also involved in the etiology of convulsive activity [8]. Targeting only carbonic anhydrase will probably not completely resolve seizures due to the implication of complex and multifactorial molecular factors.

To be effective as antiepileptic agents, AEDs have to cross the BBB [20]. Entry into the brain is therefore a crucial step in developing effective drug therapies for cerebral disorders. In vitro determination of brain–blood partitioning is difficult, time-consuming, expensive and not suitable to screen a large collection of chemicals. Therefore, we used alternative method based on computerized models. Lipophilic substances are able to permeate into the brain interstitium in a relatively easy way [21–23]. First, we calculated the log *P* value (*C* log *P*) for each compound in order to reflect the overall lipophilicity of the indanesulfonamides (Table 1). It is postulated that a log *P* value of at least 2.0 is required to cross the BBB [19,24]. **PHEN** and **CARB** have an optimal *C* log *P* but it is not the case for **TPM** and **AZA**. However, **TPM** and **AZA** appear to cross the BBB readily [19,25]. Since the brain also needs water-soluble nutrients which can not diffuse through the lipid layer, several transporters are expressed along the BBB [21–23]. Those transporters can also carry out drugs. Table 1 shows that compounds with a weak *C* log *P* (i.e.: compounds **4** and **5**) are able to protect the mice against convulsions. Compounds **7** and **19** share an optimal *C* log *P* value and exhibit also a good anticonvulsant effect. In the opposite, other compounds with an optimal *C* log *P* were not able to protect the mice (i.e.: compounds **1**, **8**, **10**, **12** and **20**). Therefore, the correlation between the *C* log *P* and the in vivo anticonvulsant activity is not really straightforward.

Secondly, in addition to the *C* log *P* parameter, we used the VolSurf software to predict the passive blood–brain permeation of the indanesulfonamides [20]. The basic concept of VolSurf is to transform the information present in 3D molecular field maps into a limited number of numerical descriptors which are easy to understand and to interpret. Ninety-four molecular descriptors are available and some of them refer to molecular size and shape, to size and shape of hydrophilic and lipophilic regions and to the balance between them [20]. The other principal descriptors are (i) hydrogen-bonding, (ii) amphiphilic moment which is defined as a vector pointing from the center of the hydrophobic domain to the center of the hydrophilic domain around a molecule, (iii) integy moment characterized as a vector pointed from the center of mass to the center of hydrophilic/hydrophobic regions, (iv) capacity factor which represents the ratio between the hydrophilic regions and the molecular surface and (v) critical packing parameters defined as a ratio between the hydrophilic and the lipophilic part of a molecule in contrast to the hydrophilic–lipophilic balance where this ratio refers only to molecular shape [20]. The VolSurf methodology has been used by Crivori et al. to perform a quantitative model for BBB permeation [20]. This model has been set up from a relation between the 3D structure and the BBB permeation of 313 compounds which penetrate strongly, moderately or weakly into the brain. Chemometric tools such as PLS (Partial Least Squares) were used to correlate the data and build the BBB permeation model. The 2D-PLS score model offers a good discrimination between the BB+ (brain-penetrating) and BB− (non-penetrating) compounds since it assigned a correct BBB profile to more than 90% of the compounds [20]. The model has been used to project indanesulfonamide compounds (Fig. 2) and to project the reference antiepileptic drugs (Fig. 5) in order to predict their BBB permeation. Fig. 2 illustrates the projection of the simple indanesulfonamide scaffold (**1**) and each *R* enantiomer of indanesulfonamides. We observed that compound **1**, the simple indanesulfonamide scaffold, is able to cross the BBB (BB+) while it is not the case for all the *R*-enantiomers except for **10** which is close to the limit of BB+ and BB−.

Fig. 3 reports the contribution of each VolSurf descriptor for compound **1**, where we have indicated only the principal descriptors which are implied in the BBB permeation. The vertical bars represent the contribution of each single descriptor, with a short bar (positive or negative value) being an unimportant descriptor and a long bar an important descriptor. The size and shape descriptor have no marked impact on BBB permeation. Descriptors of polarity such as hydrophilic

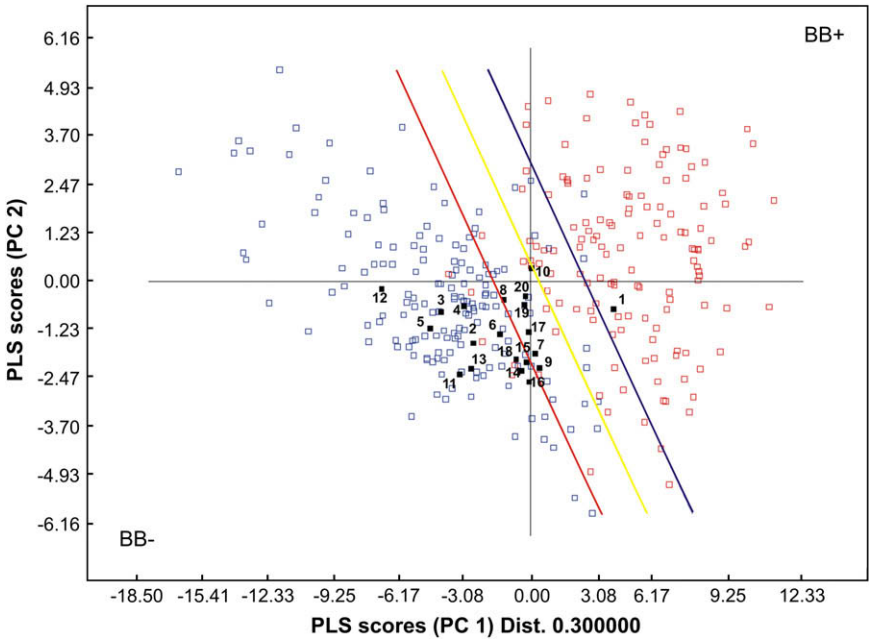

Fig. 2. Blood–brain barrier permeation of compound **1** and *R*-indanesulfonamide derivatives. Red points refer to brain-penetrating compounds (BB+) and blue points are non-penetrating compounds (BB−). The confidence level has been set to 0.3.

regions, capacity factors and H-bonding are inversely correlated with BBB permeability [20]. Hydrophilic regions' descriptors refer to polar water-accessible surface areas, indicating that BBB permeation decreases when polar surface increases [20]. The descriptors of hydrophobic interactions are directly correlated with BBB permeation. Positive values of integy moments and critical packing are favourable to a BBB permeation while amphiphilic moments and hydrophilic–lipophilic balance should exhibit negative values. Globally, it is the balance of all descriptors which are seen to control BBB permeation [20]. In the case of compound **1**, hydrophilic regions, capacity factors and H-bonds exhibit negative values and are consequently favourable to BBB permeation. However, negative values are observed for the hydrophobic regions and may influence negatively the BBB permeation. Their role appears smaller than that of the polar permeation [20]. In comparison to compound **1** which crosses the BBB in the model, compound **12**-*R* does not cross the BBB passively (Fig. 2). The corresponding contribution of each VolSurf descriptor of **12**-*R* is represented in Fig. 4 and can easily

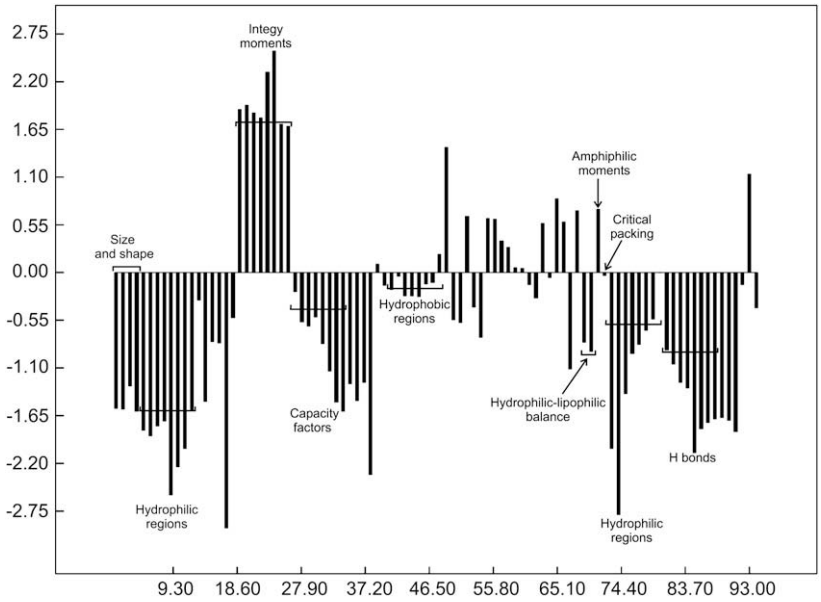

Fig. 3. Variable plot showing the original VolSurf descriptors for compound **1**.

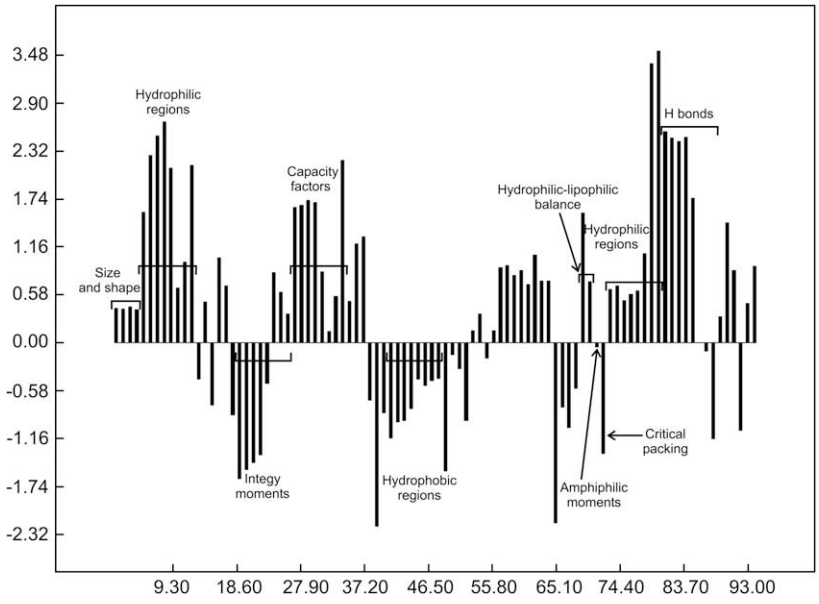

Fig. 4. Variable plot for compound **12**-*R* representing the original VolSurf descriptors.

explain the BB− feature by positive values obtained for H-bond, capacity factor, hydrophilic−lipophilic balance and hydrophilic regions descriptors (Fig. 4).

As expected, the BBB permeation of *S*-enantiomers is similar to that of *R* enantiomers, assuming negligible stereoselectivity in permeation, a reasonable assumption for passive permeation. Most of *S*-indanesulfonamides show a weak BBB permeability except the *S* compounds **9**, **15**−**17** which are found close to the limit BB+ and BB−.

Unfortunately, we observed through this study that the active anticonvulsant compounds (**4**, **7** and **19**) detected by the MES test do not seem to penetrate the BBB passively. However, mechanisms by which molecules cross the BBB include free diffusion (passive BBB permeation) and also several active transports [21,23]. All the active transports are not completely known and own specific interaction between therapeutic and certain BBB transport system. We can hypothesize that the active compounds are carried out by active transport which can explain their anticonvulsant effect.

Fig. 5 shows that the AEDs **CARB** and **PHEN** penetrate the BBB while **AZA** and **TPM** are non-penetrating. **TPM** is completely out of the graphic and it can be explained by the

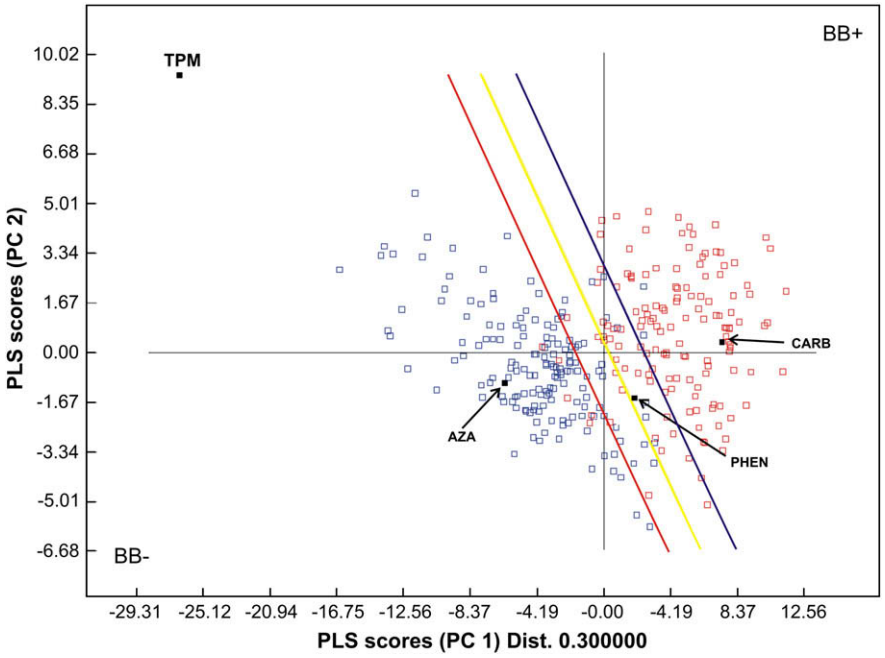

Fig. 5. Blood−brain barrier permeation of reference antiepileptic drugs.

fact that its structure is highly unusual [19]. Oxygen atoms account for nearly 40% of the total mass. Many of these oxygen atoms are available to serve as proton acceptors for hydrogen-bond formation, but the amide group in expected to be important as a donor for hydrogen-bond formation. However, **TPM** and **AZA** are effective anticonvulsants and cross surely the BBB by active transport [19,24,25]. The VolSurf theoretical model of BBB penetration fails if specific active transport mechanisms are involved in permeation.

3. Conclusions

In this work, a series of indanesulfonamides was evaluated against three CA isoforms possibly implicated in epileptogenesis. All the compounds showed strong inhibition against the three isoforms, hCA VII, hCA XII and hCA XIV, namely. Several structure–activity relationships were determined according both to the position of the substituent on the indanesulfonamide scaffold and to the length of the side chain.

The compounds were also evaluated in the maximal electroshock seizure test in order to determine their anticonvulsant activity. At a single body weight dose of 50 mg/kg, three compounds (**4**, **7** and **19**) protected at least 50% of the mice against convulsions.

Finally, we have estimated the passive blood–brain barrier permeation through a computational method. Our compounds do not cross passively the except the unsubstituted indanesulfonamide (**1**). As this theoretical method does not take into account existing active transport along the BBB, an in vitro BBB model should be used to assess if active transport are involved in the BBB permeation of our compounds.

4. Experimental section

4.1. Synthesis

Almost all the indanesulfonamides derivatives were previously described [17,18] except for compound **18**. It has been synthesized following the same procedure as before [18].

4.1.1. 1-Nonylamido-5-sulfonamidoindane (*18*)

The title compound reacted according to the general procedure described earlier by our group [18]. Yield: 55.4%; Mp: 167.9–169.6 °C; RMN ^{1}H (400 MHz; DMSO-d_6) 8.26–8.24 (1H, br, N*H*), 7.67 (1H, d, Ar-*H*, $J = 7.6$), 7.60 (1H, s, Ar-*H*), 7.4 (1H, d, Ar-*H*, $J = 8.4$), 7.35 (2H, br, SO_2NH_2), 5.34 (1H, mult, C*H*), 3.0–2.8 (2H, mult, CH_2), 2.44–2.36 (1H, mult, CH_2), 2.15–2.09 (2H, t, CH_2–C=O), 1.87–1.77 (1H, mult, CH_2), 1.54–1.52 (2H, CH_2–CH_2–C=O), 1.25 (12H, m, CH_3–$(CH_2)_6$–CH_2–CH_2–C=O), 0.87–0.84 (3H, t, CH_3, $J = 6.6$); MS (electrospray): $MH^+ = 367$, $MNa^+ = 389.2$, $MMH^+ = 733$, $MMNa^+ = 755.3$; Anal. ($C_{19}H_{30}N_2O_3S$) C: calcd, 62.26; found, 61.26; H: calcd, 8.25; found, 8.46; S: calcd, 8.75; found, 8.16; N: calcd, 7.64; found, 7.89.

4.2. Pharmacological evaluation

4.2.1. Expression and purification of the recombinant hCA VII, XII and XIV

The three recombinant CA isozymes were produced in *Escherichia coli* as previously published [26–28] and purified accordingly by sulphonamide affinity chromatography.

4.2.2. CA inhibition

An SX.18MV-R Applied Photophysics stopped-flow instrument has been used for assaying the CA-catalysed CO_2 hydration activity [29]. Phenol red (at a concentration of 0.2 mM) is used as indicator, working at the absorbance maximum of 557 nm, with 10 mM HEPES (pH 7.5) as buffer, 0.1 M Na_2SO_4 (for maintaining a constant ionic strength), following the CA-catalysed CO_2 hydration reaction for a period of 10–100 s. Satured CO_2 solutions in water at 20 °C were used as substrate. Stock solutions of inhibitor (1 mM) were prepared in distilled–deionized water with 10–20% (v/v) DMSO (which is not inhibitory at these concentrations), and dilutions up to 0.1 nM were done thereafter with distilled–deionized water. Inhibitor and enzyme solutions were preincubated during 15 min at room temperature prior to assay, to allow for the formation of the *E–I* complex. Each experiment was performed in triplicate. The values reported throughout the paper are calculated as described in the literature [29].

4.2.3. Animals

Experiments were performed on male OF1 mice (28–35 g, purchased from Charles River Laboratories). The animals were kept on a 12/12 h light/dark cycle and allowed free access to both standard pellet food and water. Animals were housed in a temperature-controlled room. Each experimental group consisted of 8 animals (excepted for compounds **1** and **17** where the group consisted of 7 animals). All the experimental procedures were approved by the Ethics Committee of the FUNDP, University of Namur (Belgium).

4.2.4. Maximal electroshock seizures in mice

The compounds were tested for their anticonvulsant activity against the maximal electroshock seizure (MES) test. The synthesized compounds and the reference AEDs (carbamazepine, phenytoine, acetazolamide and topiramate) were suspended in an aqueous solution of 1% (v/v) Tween 80 (Acros Organics) and administered intraperitoneally 2 h before the stimulation in a standard volume of 3 mL/kg at 50 mg/kg body weight dose for phenytoine, carbamazepine and the indanesulfonamides. The body weight dose of 30 mg/kg was also used for **PHEN** and **CARB**. Control animals received appropriated volumes of the solvent. Carbamazepine and phenytoine were purchased from Acros Organics. Acetazolamide and topiramate were purchased from Sigma.

The electroconvulsions were induced by a Hugo Sachs generator (15 mA, 50 Hz, 500 V, 200 ms, Rodent Shocker Type-221) which delivered direct current via saline moistened eye electrodes. A drop of Unicaïne (oxybuprocaïne HCl 4 mg/mL, Théa Pharma, Belgium) is instilled in the eyes prior to

the application of the electrodes in order to induce local anaesthesia and to ensure a good conductivity of the electroshock current. Abolition of the hind-leg tonic extension component of the seizure is defined as protection [30].

The ED_{50} value corresponds to the dose protecting 50% of mice against MES induced convulsions and was determined from the effect of 5 doses of **AZA** by a non-linear regression analysis (GraphPad Prism Software 3.0).

4.3. Computational method

4.3.1. C log P calculations

The lipophilicity of the target compounds used for the in vivo measurements reported has been calculated with the program ChemDraw Ultra 6.0.1.

4.3.2. VolSurf

This computational procedure explores the physicochemical property of a molecule starting from 3D maps of interaction energies between the molecule and chemical probes [20]. The basic concept of VolSurf is to compress the information present in 3D maps into few numerical descriptors without any superposition of molecules. VolSurf descriptors are specifically designed for the optimisation of *in silico* pharmacokinetics properties (ADME). The interaction of molecules with biological membranes is mediated by surface properties such as shape, electrostatic, hydrogen-bonding and hydrophobicity. Therefore, the GRID force-field [31,32] was chosen to characterize potential polar and hydrophobic interaction sites by the OH2, DRY and carbonyl O probe, respectively, and to transform this information into a quantitative scale by calculating the volume or the surface of the interaction contours. The BBB model is a qualitative model containing 313 related, but chemically diverse compounds extracted from the literature and in house data from Crivori and colleagues which are either brain-penetrating (BB+ score +1), have a moderate permeation (BB score 0) or have a little if any ability to cross the blood–brain barrier (BB− score −1) [20]. The overall procedure contained the following major steps: (1) The 3D structure of the compounds was built with BUILDER implemented in INSIGHT II. (2) The compounds were submitted to multivariate characterization based on their interaction energy with chemical probes (hydrophobic (DRY), water (OH2) and carbonyl oxygen atom (O)). We used GRID program (grid spacing of 0.5 Å) to calculate the 3D molecular interaction fields. (3) Molecular descriptors were calculated using the VolSurf program. (4) Chemometric tools (PCA, Principal Components Analysis; PLS, Partial Least Squares) were used to extract and rationalize the information from any multivariate description of a biological system. PLS condenses the overall information into score plot. PCA and PLS discriminant analysis were used to build the statistical model and two significant latent variables (PC, principal component) emerged with cross validation. Steps 2–4 were performed automatically by the VolSurf program [20]. The model has been used to project external compounds in the chemical space represented by the model in order to rank the BB behaviour of external compounds.

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