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Exploration of the Binding Mode of Indanesulfonamides as Selective Inhibitors of Human Carbonic Anhydrase Type VII by Targeting Lys 91

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Convulsions are common neurological disorders in clinical medicine and are triggered by several mechanisms. The enhancement of neuronal excitability can be related, among other factors, to GABAergic depolarization. Carbonic anhydrase (CA) VII contributes to this electrophysiological behavior by providing bicarbonate anion, which can mediate current through channels coupled to $GABA_A$ receptors. Among the cytosolic CAs, the mechanism of action and inhibition of CA VII is less understood. We present herein the pharmacological evaluation of both enantiomers of an indanesulfonamide compound substituted by a pentafluorophenyl moiety against CA VII and five other human CA isoforms to evaluate their selectivity. The investigated compounds are powerful inhibitors of hCA VII, with K_i values in the range of 1.7–3.3 nM, but their selectivity needs to be improved. A molecular modeling study was conducted to rationalize the structure–activity relationships and provide useful insight into the future design of selective hCA VII inhibitors.

Introduction

Carbonic anhydrases (CAs) are widespread zinc-dependent enzymes present in eukaryotes and prokaryotes.[1] At this point, 16 mammalian isozymes have been discovered and are characterized by their cellular localization, tissue distribution, and catalytic activity. The 13 active isozymes catalyze the reversible hydration of carbon dioxide to bicarbonate ($CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$). They play crucial physiological roles in such processes as acid–base homeostasis, electrolyte secretion, ion transport, and biosynthetic reactions. Herein we focused on the human cytosolic isoform VII, which plays a role in neuronal excitability and therefore has a direct implication in seizures and epilepsy.[2] CA VII is localized in the cytosol and has high catalytic activity. Like CA II, it is inhibited by sulfonamides[3] and metal-complexing anions.[4] Among the cytosolic CAs, the mechanism of action and inhibition of CA VII is less understood. Recently, data reported by Ruusuvuori et al. implied the involvement of intracellular CA VII in neuronal excitation.[5] In fact, CA VII provides bicarbonate anion, which is able to mediate a current through channels coupled to $GABA_A$ receptors. The GABAergic transmission is therefore not always inhibitory, but can be excitatory in certain circumstances, such as synchronous firing during neuronal plasticity or epileptic activity.[5,6] $GABA_A$-mediated responses become depolarizing during periods of intense neuronal firing and are associated with bicarbonate efflux by way of $GABA_A$ receptors. The depolarizing GABAergic potentials may contribute highly to the enhancement of excitability owing to the increase in extracellular potassium that occurs directly after GABAergic depolarization (excitation).[7,8] Moreover, the study by Perez Velazquez supports the role of CA VII in maintaining the bicarbonate gradient, resulting in an efflux of HCO_3^- ions through $GABA_A$ receptors, possibly upon the breakdown of the chloride gradient.[6] This bicarbonate flux is cancelled by membrane-permeating drugs that suppress the activity of cytosolic carbonic anhydrase, supporting the implication of CA VII in neuronal excitation.[6]

Notably, however, seizures cannot be attributed to only one CA isozyme in particular. Indeed, Halmi et al. showed that CA II and XII are strongly induced in rat brain after status epilepticus induced by kainic acid.[9] Moreover, variation in pH has fundamental importance in regulating neuronal excitability.[7] Small changes in intra- and extracellular pH have been shown to alter the function of ligand-gated and voltage-gated channels. Studies with CA XIV knockout mice have revealed that this isozyme can regulate activity-dependent pH shifts, particularly alkaline transients.[10]

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We have been engaged in the synthesis of racemic indanesulfonamides, which were previously tested against the physiologically relevant CA I and II and against the cancer-related CA IX.[11,12] In a continuation of our research in the field of CA and to extend our knowledge of hCA VII in particular, the inhibitory potency of both *R* and *S* enantiomers of compound **1** against hCA VII was evaluated (Figure 1). To our knowledge, the impact of optical isomerism has yet to be described for hCA VII inhibition, and stereoselectivity in biological molecular recognition processes is extremely important for rational drug design. To assess the selectivity of both enantiomers, inhibitory activities were also determined against five other hCA isoforms (hCA I, II, IX, XII, and XIV). hCA I and II are considered to have physiological relevance, whereas the others have been attributed to pathologies such as epilepsy. In addition to in vitro enzymatic evaluation, a maximal electroshock seizure test was also performed in mice to evaluate the anticonvulsant properties.

In an attempt to rationalize the structure–activity relationships (SARs) obtained, we carried out docking studies of (*R*)-**1** and (*S*)-**1** with CA VII and five other isozymes. For this purpose, homology modeling was first performed to predict the 3D structure of the hCA VII protein, for which crystallographic data are not yet available. Subsequently, this allowed us to compare the active site of the six isoforms to highlight specific residues of CA VII and then to focus on the design of selective CA VII inhibitors. Such compounds will be used as pharmacological tools to better understand the functional relevance of hCA VII to seizures and excitatory activity.

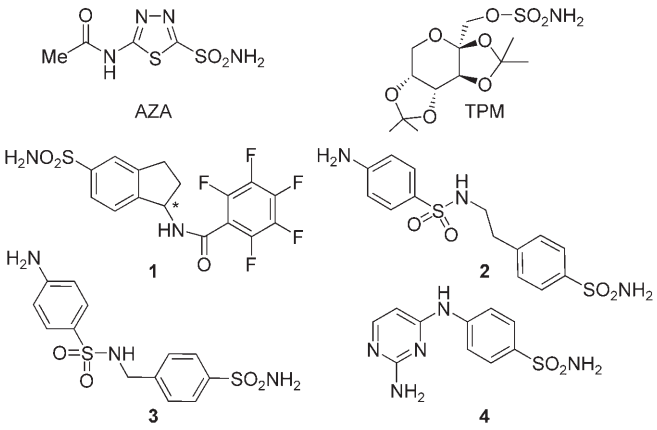

Figure 1. Structures of carbonic anhydrase inhibitors.

Table 1. Inhibition data for **1**–**4** and anticonvulsant sulfonamides in clinical use.

	K_i [nM][a]					
Compd	hCA I	hCA II	hCA VII	hCA IX	hCA XII	hCA XIV
AZA	250[13,14]	12.3[15]	2.5[13,14]	25[16]	5.7[16]	41[16]
TPM	250[13,14]	10.3[15]	0.87[13,14]	1590[16]	3800[16]	1460[16]
1	770[14]	490[14]	1.9	3.5[14]	5.5	7.3
(*R*)-**1**	150	60	3.3	4.0	1.0	106
(*S*)-**1**	1299	3459	1.7	14	6.0	38
2	185[3]	50[3]	6.5[3]	NT[b]	NT[b]	NT[b]
3	164[3]	46[3]	5.6[3]	NT[b]	NT[b]	NT[b]
4	109[3]	33[3]	6.8[3]	NT[b]	NT[b]	NT[b]

[a] Errors are in the range of 3–5% of the reported values. [b] NT = not tested.

Results and Discussion

CA inhibition and anticonvulsant studies

The inhibitory potency of (*R*)-**1** and (*S*)-**1** was first evaluated against hCA VII and then against five isoforms of hCA, namely hCA I, II, IX, XII and XIV, to assess their selectivity. These were compared with the inhibitory potency of three sulfonamide compounds (**2**–**4**, Figure 1) described recently as hCA VII inhibitors[3] and of standard CA inhibitors used in the treatment of epilepsy: acetazolamide (AZA) and topiramate (TPM). For TPM, inhibition of CA does not always constitute its primary mechanism of action.[2] Indeed, it blocks the voltage-gated Na^+ channel, potentiates GABAergic transmission, and blocks the kainate/AMPA receptor. The data are listed in Table 1.

The racemic compound **1** is characterized by powerful inhibitory potency against hCA VII, similar to the potencies of the reference inhibitors. However, **1** is less selective than TPM, as **1** also inhibits CA IX, XII, and XIV. Selectivity is observed only for the physiologically relevant CA I and II, as weak inhibition is observed for these isoforms. The relative potency of drug stereoisomers can have many permutations: there may be equipotency or differing potency with additive agonistic, partial, or antagonistic effects. The separation of the racemic mixture of **1** was therefore necessary to study 1) the impact of optical isomerism on hCA VII inhibition and 2) the stereoselectivity towards other CA isoforms. Both (*S*)-**1** and (*R*)-**1** share the same inhibition profile against hCA VII, though (*S*)-**1** is shown to be more selective for CA VII than (*R*)-**1**. However, (*S*)-**1** is still quite active against CA IX, XII, and XIV.

Regarding the activity of each separate enantiomer and of the racemic mixture against hCA I and hCA II, we observe that the very weak inhibition of (*S*)-**1** coupled with the better inhibition of (*R*)-**1** leads to an intermediate K_i value. Surprisingly, the racemate improves the K_i value against hCA XIV relative to the K_i values of each separate enantiomer. A synergistic effect by both enantiomers in the racemic mixture is therefore expected. The inhibitory potency of (*R*)-**1** towards hCA IX is greater than its corresponding enantiomer (*S*)-**1**, confirming our previous hypothesis of the binding mode predicted by docking studies.[11]

Because compound **1** showed good inhibitory potency against several hCA isoforms implicated in excitatory electrophysiological current, particularly against hCA VII, we decided to evaluate its anticonvulsant properties through the maximal electroshock seizures test. Our model was first validated with two reference anticonvulsant drugs: AZA and TPM. AZA acts only through the inhibition of CA, whereas several mechanisms of action are attributed to TPM. At a dose of 50 mg kg^{-1} body weight for a period of 2 h, TPM protected 68.7% of the mice and AZA protected only 12.5%. Our compounds **1** (racemate), (*R*)-**1**, and (*S*)-**1** tested at the same dose led to the same extent

of protection by AZA (12.5%). At a dose of 150 mg kg^{-1}, TPM and AZA protected 100% of the mice, whereas **1**, (*R*)-**1**, and (*S*)-**1** still exhibited weak anticonvulsant effects (12.5% of protected mice). However, these indanesulfonamide compounds display a Clog *P* value of 1.54, which suggests they are sufficiently lipophilic to cross the blood brain barrier. It is too early to conclude that CAs may not be involved at all in the etiology of convulsions. Indeed, data published by Masereel et al. showed that parameters other than CA inhibition and lipophilicity, such as protein binding and pK_a, may also strongly influence in vivo anticonvulsant properties.[17] Several other sulfonamide compounds that specifically target each CA isoform implicated in convulsions should be designed and synthesized to highlight the exact role(s) of CA in epileptic disorders.

Molecular modeling

Docking studies were conducted to rationalize the observed SARs of both enantiomers of **1** against hCA VII and five other isoforms. Crystal structures of human carbonic anhydrase I (PDB code: 1HCB),[18] II (1A42),[19] and XII (1JD0)[20] were used, whereas hCA IX, XIV, and VII were first modeled, because crystallographic information is not yet available from the PDB[21] for these three isoenzymes. In a second step, and to highlight specific residues of hCA VII, sequence alignment and active site superposition were performed. Finally, from the simulated binding modes of (*R*)-**1**, (*S*)-**1**, and reference hCA VII inhibitors (Figure 1),[3] structural modifications were proposed to enhance the selectivity of **1**.

Homology modeling of hCA XIV and VII

A model of human CA isoform IX was built from the available X-ray crystal structure of murine CA XIV as previously described.[11] The human CA isoform XIV was modeled from the murine homologue (PDB code: 1RJ5; 2.8 Å).[22] They share 82.5% sequence identity, and the model obtained is therefore reliable, as shown by the Ramachandran plot (see Supporting Information); 92.2% of the residues are situated in the allowed regions, and the remaining 7.8% are in disallowed space.

Human CA II (PDB code 1A42; 2.25 Å)[19] provides the highest sequence identity (56%) with the human VII isoform and was used to build the hCA VII model; 94.5% of the residues are in the allowed region, and 5% in the disallowed one. These results show that the different models are sufficiently reliable for further docking studies.

Sequence alignment and superposition of the six isoforms

To identify the conserved and specific residues of each isoform, and particularly those of CA VII, sequence alignments (Figure 2) and 3D structure superimpositions were performed (Figure 3). In general, the overall fold of the six isozymes (Figure 3a) is quite similar, as observed by the root mean square deviation (RMSD) ranging from 0.43 to 1.31 Å (Table 2), and the residues of the active site being relatively conserved (Figures 2–3b). The active site of each isoform is cone-like, characterized by two sides: polar and hydrophobic. The active site of CA I differs most from the others and is smaller and narrower than the other enzymes.[1] Several changed residues can be highlighted and are suspected to be important for the binding of inhibitors to hCA VII in comparison with the other isoforms (Table 3). From this analysis, it is notable that Phe 131 and Gly 132 are present only in hCA II and hCA VII, whereas Lys 91 and Ser 204 constitute specific residues of the hCA VII active site. All these amino acids are located in the hydrophobic portion of the active site, except Lys 91, which is at the interface between the hydrophilic and hydrophobic pockets. These data are relevant for the design of future selective hCA VII inhibitors.

Docking

To examine the reliability of our approach, we performed docking validation studies on two carbonic anhydrase–ligand complexes selected from the PDB (1JD0 and 1A42). Both ligands (acetazolamide (AZA) in CA XII and brinzolamide in CA II) of known crystallographic structures were successfully placed (RMSD < 2 Å) by the GOLD docking protocol (data not shown).

(*R*)-**1** and (*S*)-**1** were docked in the active site of the six isoforms using the program GOLD.[23] The resulting complexes were then minimized with Discover3.[24] As the sulfonamide inhibitors are known to bind the zinc ion of CA, the nitrogen atom of the sulfonamide moiety of (*R*)-**1** and (*S*)-**1** was complexed to the zinc in a tetrahedral coordination geometry. In each complex, hydrogen bond interactions are observed between the sulfonamide group and Thr 199, as observed in most of the crystal structures.

Globally, both enantiomers lie in an extended conformation along the hydrophilic pocket of the active site towards Trp 5 in the six isoforms. In isoform VII, the indane ring of (*S*)-**1** is surrounded by His 94, Gln 92, Gln 67, Thr 200, and Ser 65 (Figure 4a, b). A shared H bond between the carbonyl group, Gln 67, and Asn 62 is observed. The pentafluorophenyl moiety lies close to Pro 202, Pro 201, Trp 5, His 64, and Ser 65, making hydrophobic contact with Trp 5. Even if the indane group of (*R*)-**1** has a different position (Figure 4a, b), the same interactions are observed for both enantiomers. The calculated interaction energies are -36.8 and -36.2 kcal mol^{-1} for (*S*)-**1** and (*R*)-**1**, respectively. This observation is in accordance with the inhibition data (Table 1).

In contrast, (*S*)-**1** and (*R*)-**1** do not share the same interactions in binding the hydrophilic pockets of hCA I and II. For example, in both hCA I and hCA II, the pentafluorophenyl group of (*R*)-**1** is in contact with Trp 5, which is not the case for (*S*)-**1**. In both isoforms, the calculated interaction energy is better for (*R*)-**1** (-47.0 and -37.1 kcal mol^{-1} in hCA I and II, respectively) than it is for (*S*)-**1** (-43.8 and -34.4 kcal mol^{-1} in hCA I and II, respectively), explaining the greater inhibitory potency of (*R*)-**1**.

The differences between the enantiomers in isoform IX were described previously.[11] In hCA XII, there is a slight difference in positioning between the two enantiomers, but their interaction energy is quite similar (-39.3 and -40.4 kcal mol^{-1} for (*S*)-**1** and (*R*)-**1**, respectively). A shared H bond is observed be-

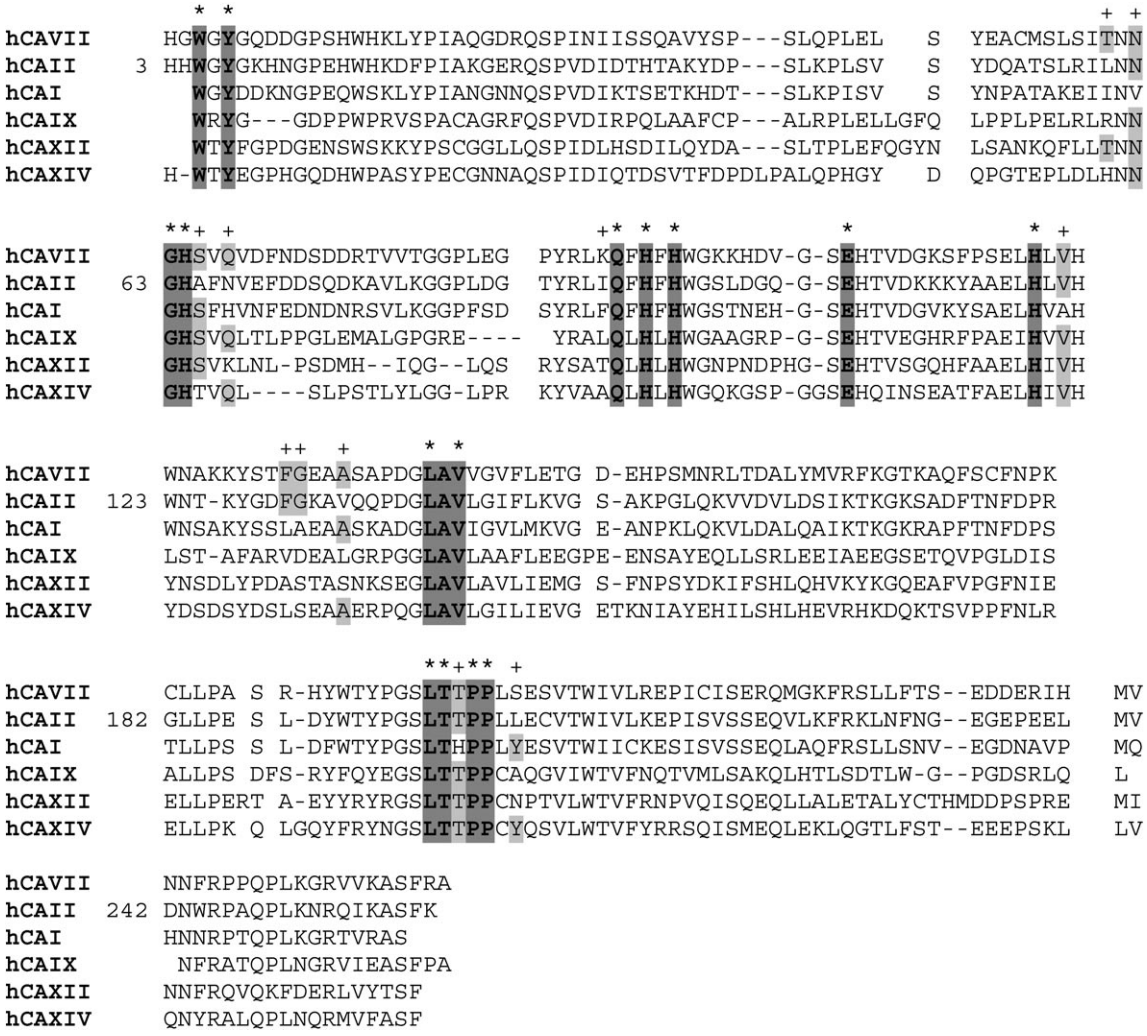

Figure 2. Sequence alignment of human CA isoforms I, II, VII, IX, XII, and XIV. The conserved active site residues are marked with * and colored in dark gray. Non-conserved active site residues are marked with + and colored in light gray.

Table 2. RMSD of the trace of the six isoforms.

	RMSD [Å]				
	hCA I	hCA II	hCA VII	hCA IX	hCA XII
hCA I	–	–	–	–	–
hCA II	0.99	–	–	–	–
hCA VII	1.01	0.43	–	–	–
hCA IX	1.2	1.01	0.93	–	–
hCA XII	1.31	1.10	1.11	0.87	–
hCA XIV	1.14	0.97	0.93	0.54	0.96

tween the carbonyl moiety of (*R*)-**1** and Asn62 and Gln92, whereas (*S*)-**1** is hydrogen bonded to Thr200.

The differences in binding mode of a specific enantiomer between the various CA isoforms are quite subtle. For example, in hCA I, some interactions such as the H bond with Gln67 are lost; this is expected because the active site is quite different from the other isoforms (Q67H, T200H, N62V). Owing to the Q67N variation of hCA II, this H bond is also lost.

These analyses show that residues such as Asn62, Gln67, Gln92, and Trp5, involved in specific interactions, seem important in the binding of indanesulfonamide compounds. Unfortunately, their binding mode does not involve specific residues of hCA VII, such as Lys91 or Ser204. To design more selective compounds, it would be interesting to add groups that orient the inhibitor towards these residues of the hydrophobic pocket.

Structural modifications

Three potent CA VII inhibitors (**2**–**4**, Figure 1), recently described,[3] were also docked in the active site of hCA VII to compare them with the indanesulfonamide family and to provide useful insight for structural modifications to enhance activity and selectivity towards hCA VII.

Compounds **2** and **3** bind similarly in the hydrophilic pocket (data not shown), and the sulfone of the *N*-alkyl sulfonamide group is hydrogen bonded with Asn62 and Gln67. Moreover, another H bond is observed between Tyr7 and the NH_2

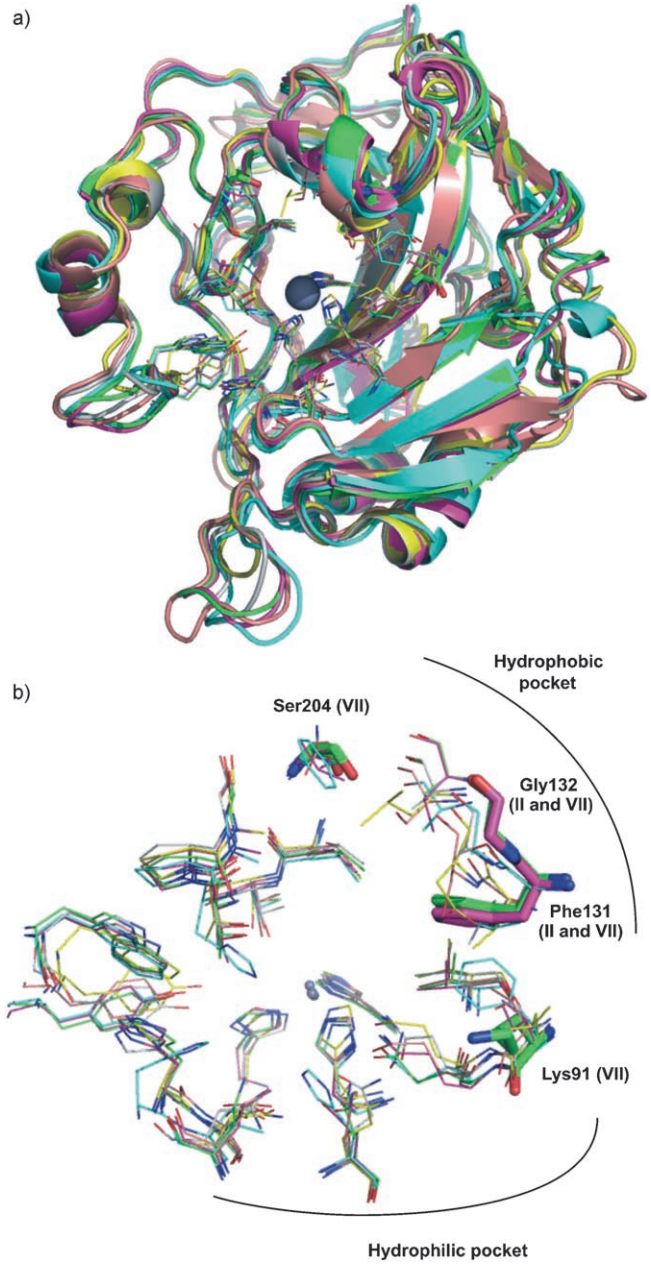

Figure 3. a) Superimposition of the six CA isoforms, with VII (green), I (cyan), II (magenta), IX (yellow), XII (salmon), and XIV (gray); b) active site of CA VII, I, II, IX, XII, and XIV.

moiety. In contrast with the extended conformation of the studied compound, **2** and **3** are bent with the terminal NH_2 group pointing inside the active site towards Tyr 7, His 64, Ser 65, and His 96. These inhibitors do not seem to target specific residues of hCA VII.

The aminopyrimidine group of compound **4** stacks with Phe 131, orienting the inhibitor towards the hydrophobic part (Figure 4c). This specific interaction was already observed for other molecules in complex with the CA II isozyme.[25,26] In addition, one of the nitrogen atoms of the pyrimidine is hydrogen bonded to Lys 91, which is a specific residue of isoform VII.

Table 3. Residue variation of isoforms I, II, IX, XII, and XIV with respect to VII.

Isoform	Variation from Consensus[a]
I	V62N, H67Q, ***F91K***, A121V, L131F, A132G, H200T, ***Y204S***
II	A65S, N67Q, ***I91K***, V135A, ***L204S***
IX	***L91K***, V131F, D132G, L135A, ***A204S***
XII	K67Q, ***T91K***, A131F, S132G, S135A, ***N204S***
XIV	H60T, T65S, ***A91K***, L131F, S132G, ***Y204S***

[a] Residue numbers are those of isoform I. Variations in bold italics are specific to isoform VII.

To enhance the selectivity of (*S*)-**1** towards hCA VII, and based on the binding mode of **4**, we suggest substitution of the indane ring of (*S*)-**1** with an aminopyridine group (Figure 4d). This additional moiety would establish interactions with the hydrophobic pocket. The proposed molecule was docked in hCA VII and shows a better interaction energy than (*S*)-**1** ($-41.9\ kcal\ mol^{-1}$). The pyridine group stacks with Phe 131, and the nitrogen atom of the new pyridine ring is hydrogen bonded to Lys 91, enhancing the selectivity for hCA VII (Figure 4e).

Conclusions

Convulsions are among the most common neurological disorders in clinical medicine and are triggered by several mechanisms. The enhancement of neuronal excitability can be related, among others, to GABAergic depolarization (excitation). In this context, carbonic anhydrase (CA) VII has been highlighted for its contribution to this electrophysiological behavior. The design of selective hCA VII inhibitors would help our understanding of the particular role of CA VII in epilepsy.

In this context, the in vitro inhibitory potency of both enantiomers of indanesulfonamide **1** towards hCA VII was evaluated and compared with that toward five other isoforms, namely hCA I, II, IX, XII, and XIV. This analysis determined (*S*)-**1** to be more selective than (*R*)-**1**, and therefore the best candidate to be modified to enhance CA VII selectivity.

A model of human CA isoform VII was built from the available X-ray crystal structure of hCA II. This allowed a comparison of the active sites of various isoforms to highlight specific residues of CA VII. Docking studies of (*R*)-**1**, (*S*)-**1**, and reference inhibitors in the hCA VII model provided ideas for targeting the CA VII-specific residue, Lys 91. We therefore propose, as an example, to substitute the indanesulfonamide by an aminopyridine group, which is able to target this particular residue.

Experimental Section

Synthesis: The synthesis of compound **1** has already been described.[11] The synthesis scheme of (*R*)- and (*S*)-indanesulfonamides is similar to those described previously.[11] The (*R*)- and (*S*)-1-aminoindanes were purchased from Fluka and used in the preliminary step of the synthesis. All optical rotations were determined at the sodium D line using a PerkinElmer polarimeter 343 (1 dm cell).

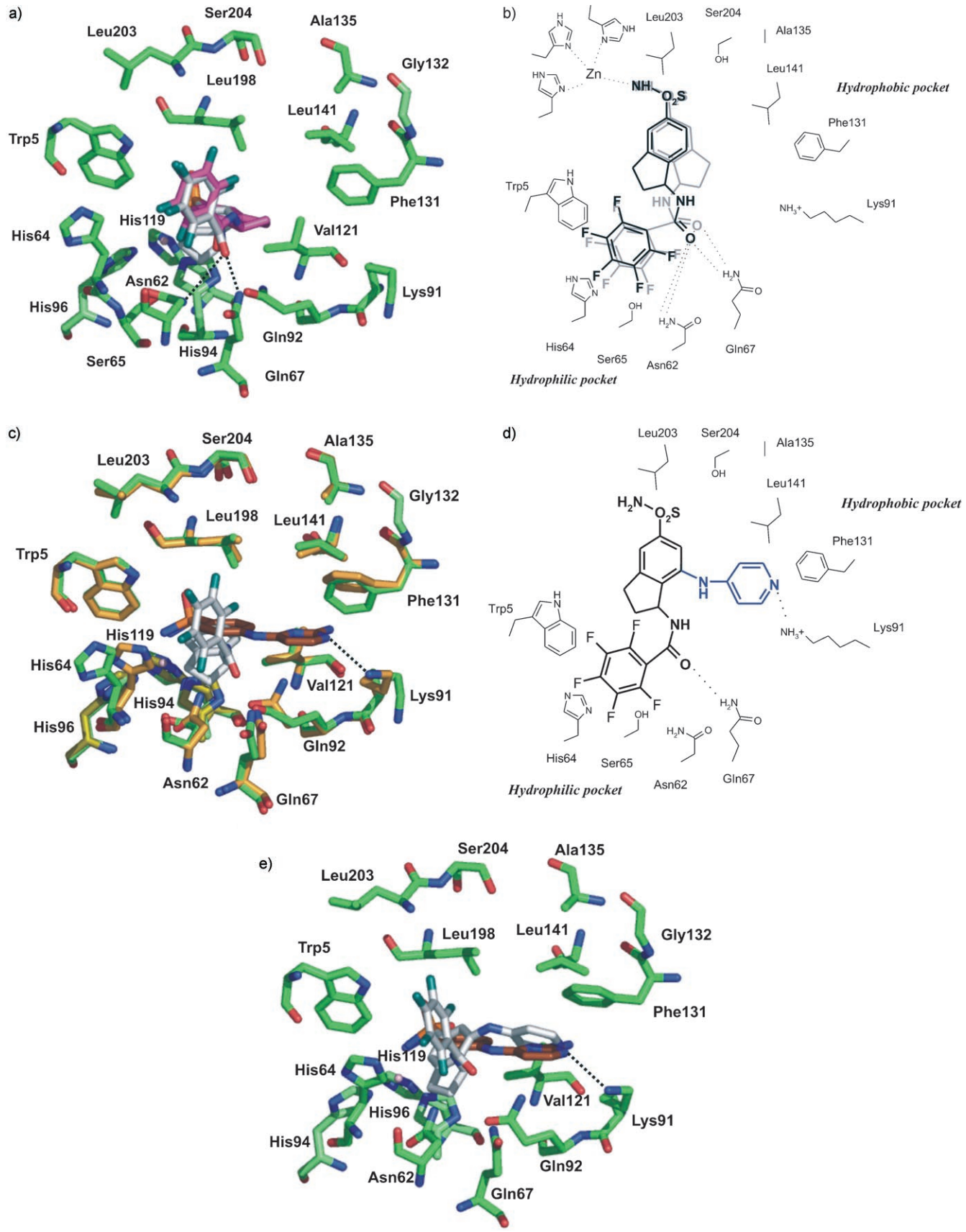

Figure 4. a) View of the active site of hCA VII (green) with (*S*)-**1** (light gray) and (*R*)-**1** (magenta). The zinc ion and catalytic histidine residues are colored in light pink and light green, respectively; b) schematic representation of (*S*)-**1** (dark) and (*R*)-**1** (gray) inside the hCA VII active site; c) binding mode of **4** (dark orange) in the active site of hCA VII in comparison with that of (*S*)-**1** (light gray); d) schematic representation of a proposed new selective compound inside the hCA VII active site; e) binding mode of the proposed new compound (light gray) in the active site of hCA VII compared with that of **4** (dark orange).

(*R*)-1-pentafluorophenylamido-5-sulfonamide ((*R*)-1): The title compound was formed according to the general procedure.[11] Yield: 70.7%; $[\alpha]_D^{20} = -0.147$ ($c = 10$ mg mL^{-1} in EtOH); mp: 233.6–235.2 °C; ^{1}H NMR (400 MHz; [D$_6$]DMSO): $\delta_H = 9.45$–9.40 (1H, br, N*H*), 7.71–7.68 (1H, m, Ar-*H*), 7.44–7.41 (2H, br, SO_2NH_2), 7.37 (1H, s, Ar-*H*), 7.32 (1H, s, Ar-*H*), 5.51–5.47 (1H, m, C*H*), 2.98–2.88 (2H, m, CH_2), 2.56 −2.53 (1H, m, CH_2), 1.92–1.86 ppm (1H, m, CH_2); anal. calcd for $C_{16}H_{11}F_5N_2O_3S$: C 47.29, H 2.73, N 6.89 S 7.89; found: C 47.95, H 2.73, N 6.97, S 7.97.

(*S*)-1-pentafluorophenylamido-5-sulfonamide ((*S*)-1): The title compound formed according to the general procedure.[11] Yield: 57.5%; $[\alpha]_D^{20} = +0.150$ ($c = 10$ mg mL^{-1} in EtOH); mp: 233.8–235.7 °C; ^{1}H NMR (400 MHz; [D$_6$]DMSO): $\delta_H = 9.48$–9.43 (1H, br, N*H*), 7.71 (1H, m, Ar-*H*), 7.47–7.45 (2H, br, SO_2NH_2), 7.40 (1H, s, Ar-*H*), 7.35 (1H, s, Ar-*H*), 5.55–5.52 (1H, m, C*H*), 3.1–2.90 (2H, m, CH_2), 2.56 −2.54 (1H, m, CH_2), 1.93–1.92 ppm (1H, m, CH_2); anal. calcd for $C_{16}H_{11}F_5N_2O_3S$: C 47.29, H 2.73, N 6.89, S 7.89; found: C 47.59, H 3.09, N 6.75, S 7.93.

Pharmacological evaluation: *Carbonic anhydrase inhibition*: CA I and II were supplied by Sigma–Aldrich. The recombinant CA VII, IX, XII, and XIV enzymes were obtained as reported previously.[3,4,13,27,28] A stopped-flow instrument (SX.18MV-R, Applied Photophysics) was used for the carbonic anhydrase CO_2 hydration activity assays. This method was described previously by our research group.[8]

Maximal electroshock seizure test: Compounds were tested for their anticonvulsant activity against the maximal electroshock seizure (MES) test. The experiments were carried out on male OF1 mice (28–40 g, Charles River Laboratories) after acclimatization for at least one week. All experimental procedures applied in this study were conducted at the University of Namur (Belgium) and were approved by the Ethics Committee of the University of Namur. The animals were housed under standard laboratory conditions (ambient temperature of 20 °C, natural light–dark cycles). Tap water and pellets were freely available before the experiment. Each experimental group consisted of eight animals. The synthesized compounds and the reference antiepileptic drugs (topiramate) were suspended in an aqueous solution of 1% Tween 80 (Acros Organics) administered intraperitoneally (ip) 2 h before stimulation in a standard volume of 3 mL kg^{-1} at doses of 50 mg kg^{-1} or 150 mg kg^{-1} body weight for the other compounds. Control animals received appropriate volumes of carrier alone. Topiramate and acetazolamide were purchased from Sigma.

The electroconvulsions were produced by a Hugo Sachs generator (15 mA, 50 Hz, 500 V, 200 ms, Rodent Shocker Type 221, Freiburg, Germany) and delivered via saline-moistened eye electrodes. A drop of Unicaïne (oxybuprocaïne·HCl 4 mg mL^{-1}, Théa Pharma, Belgium) was instilled in the eye prior to application of the electrodes in order to induce local anesthesia and to ensure good conductivity of the electroshock current. Abolition of the hind-leg tonic extension component of the seizure is defined as protection.

Computational methods: All computational experiments were conducted on a Silicon Graphics Octane2 workstation, running under the IRIX 6.5 operating system.

*Clog*P *calculations*: The lipophilicity of the target compounds used for the in vivo measurements reported was calculated with the program ChemDraw Ultra 6.0.1.

Homology modeling: The human carbonic anhydrase (CA) VII and XIV sequences were obtained from the Swiss-Prot database. Sequence analysis was performed using BLAST (BLOSUM62 matrix).[29] Human CA II (PDB code: 1A42) and murine CA XIV (PDB code: 1RJ5) were selected as the most appropriate templates. The Esypred3D program (available at http://www.fundp.ac.be/urbm/bioinfo/esypred/) was used.[30] This automated homology modeling tool compares results from various multiple alignment algorithms to derive a "consensus" alignment between the target sequence and the template. Then, a 3D model (built with Modeler) is provided. The resulting model was energy minimized using the ESFF force field (Discover3[24]/InsightII[31]) after the addition of H atoms fixed at physiological pH 7.4 and the coordination of the zinc ion in a tetrahedral geometry. Quality verification of the model was performed with Procheck 3.0 with a pseudo-resolution of 2.0 Å.[32]

Docking studies: Docking of the inhibitors into the six isoforms was performed using the GOLD program.[23] GOLD is based on a genetic algorithm, performing docking of flexible ligands into proteins with partial flexibility in the neighborhood of the active site (Ser, Thr, Tyr, and Lys). Default settings were used for the genetic algorithm parameters. A tetrahedral geometry was imposed on the zinc binding site; 20 solutions were generated and ranked by GOLD score. To take protein flexibility into account, the complexes were then refined with Discover3 (ESFF force field). The energy-minimization process is performed in two steps: the steepest descent algorithm, reaching a convergence of 10.0 kcal mol^{-1} Å^{-1}, followed by the conjugate gradient to reach a final convergence of 0.01 kcal mol^{-1} Å^{-1}.

Pictures: The figures were produced using PyMOL.[33]

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[1] a) C. T. Supuran in *Carbonic Anhydrase. Its Inhibitors and Activators*, CRC, London, **2004**, pp. 1–23; b) C. T. Supuran, A. Scozzafava, A. Casini, *Med. Res. Rev.* **2003**, *23*, 146–189.
[2] A. Thiry, J.-M. Dogné, C. T. Supuran, B. Masereel, *Curr. Top. Med. Chem.* **2007**, *7*, 855–864.
[3] D. Vullo, J. Voipio, A. Innocenti, C. Rivera, H. Ranki, A. Scozzafava, K. Kaila, C. T. Supuran, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 971–976.
[4] D. Vullo, E. Ruusuvuori, K. Kaila, A. Scozzafava, C. T. Supuran, *Bioorg. Med. Chem. Lett.*. **2006**, *16*, 3139–3143.
[5] E. Ruusuvuori, H. Li, K. Huttu, J. M. Palva, S. Smirnov, C. Rivera, K. Kaila, J. Voipio, *J. Neurosci.* **2004**, *24*, 2699–2707.
[6] J. L. Perez Velazquez, *Eur. J. Neurosci.* **2003**, *18*, 1337–1342.
[7] A. M. Aribi, J. L. Stringer, *Epilepsy Res.* **2002**, *49*, 143–151.
[8] J. Voipio, K. Kaila, *Prog. Brain Res.* **2000**, *125*, 329–338.
[9] P. Halmi, S. Parkkila, J. Honkaniemi, *Neurochem. Int.* **2006**, *48*, 24–30.
[10] G. N. Shah, B. Ulmasov, A. Waheed, T. Becker, S. Makani, N. Svichar, M. Chesler, W. S. Sly, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 16771–16776.
[11] A. Thiry, M. Ledecq, A. Cecchi, J.-M. Dogné, J. Wouters, C. T. Supuran, B. Masereel, *J. Med. Chem.* **2006**, *49*, 2743–2749.
[12] C. Chazalette, B. Masereel, S. Rolin, A. Thiry, A. Scozzafava, A. Innocenti, C. T. Supuran, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5781–5786.
[13] D. Vullo, A. Innocenti, I. Nishimori, J. Pastorek, A. Scozzafava, S. Pastorekova, C. T. Supuran, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 963–969.
[14] I. Nishimori, D. Vullo, T. Minakuchi, K. Morimoto, S. Onishi, A. Scozzafava, C. T. Supuran, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2182–2188.

[15] G. De Simone, A. Di Fiore, V. Menchise, C. Pedone, J. Antel, A. Casini, A. Scozzafava, M. Wurl, C. T. Supuran, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2315 – 2320.
[16] I. Nishimori, D. Vullo, A. Innocenti, A. Scozzafava, A. Mastrolorenzo, C. T. Supuran, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3828 – 3833.
[17] B. Masereel, S. Rolin, F. Abbate, A. Scozzafava, C. T. Supuran, *J. Med. Chem.* **2002**, *45*, 312 – 320.
[18] V. Kumar, K. K. Kannan, *J. Mol. Biol.* **1994**, *241*, 226 – 232.
[19] T. Stams, Y. Chen, P. A. Boriack-Sjodin, J. D. Hurt, J. Liao, J. A. May, T. Dean, P. Laipis, D. N. Silverman, D. W. Christianson, *Protein Sci.* **1998**, *7*, 556 – 563.
[20] D. A. Whittington, A. Waheed, B. Ulmasov, G. N. Shah, J. H. Grubb, W. S. Sly, D. W. Christianson, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 9545 – 9550.
[21] H. M. Berman, T. Battistuz, T. N. Bhat, W. F. Bluhm, P. E. Bourne, K. Burkhardt, Z. Feng, G. L. Gilliland, L. Iype, S. Jain, P. Fagan, J. Marvin, D. Padilla, V. Ravichandran, B. Schneider, N. Thanki, H. Weissig, J. D. Westbrook, C. Zardecki, *Acta Crystallogr. Sect. D* **2002**, *58*, 899 – 907.
[22] D. A. Whittington, J. H. Grubb, A. Waheed, G. N. Shah, W. S. Sly, D. W. Christianson, *J. Biol. Chem.* **2004**, *279*, 7223 – 7228.
[23] G. Jones, P. Willett, R. C. Glen, A. R. Leach, R. Taylor, *J. Mol. Biol.* **1997**, *267*, 727 – 748.
[24] *Discover3*, version 2.98, Accelrys Inc., San Diego, CA (USA).
[25] a) V. Menchise, G. De Simone, V. Alterio, A. Di Fiore, C. Pedone, A. Scozzafava, C. T. Supuran, *J. Med. Chem.* **2005**, *48*, 5721 – 5727; b) A. Thiry, J.-M. Dogné, B. Masereel, C. T. Supuran, *Trends Pharmacol. Sci.* **2006**, *27*, 566 – 573.
[26] A. Di Fiore, C. Pedone, K. D'Ambrosio, A. Scozzafava, G. De Simone, C. T. Supuran, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 437 – 442.
[27] O. Ozensoy, I. Nishimori, D. Vullo, L. Pucetti, A. Scozzafava, C. T. Supuran, *Bioorg. Med. Chem.* **2005**, *13*, 6089 – 6093.
[28] D. Vullo, M. Franchi, E. Gallori, J. Antel, A. Scozzafava, C. T. Supuran, *J. Med. Chem.* **2004**, *47*, 1272 – 1279.
[29] S. F. Altschul, T. L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller, D. J. Lipman, *Nucleic Acids Res.* **1997**, *25*, 3389 – 3402.
[30] C. Lambert, N. Leonard, X. De Bolle, E. Depiereux, *Bioinformatics* **2002**, *18*, 1250 – 1256.
[31] *InsightII*, version 2005, Accelrys Inc., San Diego, CA (USA).
[32] R. A. Laskowski, *J. Appl. Crystallogr.* **1993**, *26*, 283 – 291.
[33] W. L. DeLano, *The PyMOL Molecular Graphics System*, version 0.99, DeLano Scientific, San Carlos, CA (USA), **2002**.

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