Carbonic Anhydrase Inhibitors: Anticonvulsant Sulfonamides Incorporating Valproyl and Other Lipophilic Moieties

Bernard Masereel,[†] Stéphanie Rolin,[†] Francesco Abbate,[‡] Andrea Scozzafava,[‡] and Claudiu T. Supuran*[,‡,§]

Department of Pharmacy, University of Namur, FUNDP, 61 rue de Bruxelles, B-5000 Namur, Belgium, Laboratorio di Chimica Inorganica e Bioinorganica, Università degli Studi di Firenze, Via Gino Capponi 7, I-50121, Florence, Italy, and CSGI, c/o Università degli Studi di Firenze, Via Gino Capponi 7, I-50121 Florence, Italy

Received May 7, 2001

A series of aromatic/heterocyclic sulfonamides incorporating valproyl moieties were prepared to design antiepileptic compounds possessing in their structure two moieties known to induce such a pharmacological activity: valproic acid, one of the most widely used antiepileptic drugs, and the sulfonamide residue included in acetazolamide and topiramate, two carbonic anhydrase inhibitors with antiepileptic properties. Some of these derivatives showed very high inhibitory potency against three carbonic anhydrase (CA) isozymes, such as CA I, CA II, and CA IV, involved in important physiological processes. Topiramate, a recently developed antiepileptic drug possessing a sulfamate moiety, also shares this property, although earlier literature data reported this compound to be a weak−moderate CA I, II, and IV inhibitor. The valproyl derivative of acetazolamide (5-valproylamido-1,3,4-thiadiazole-2-sulfonamide, **6M**) was one of the best hCA I and hCA II inhibitor in the series and exhibited very strong anticonvulsant properties in an MES test in mice. In consequence, other 1,3,4-thiadiazolesulfonamide derivatives possessing potent CA inhibitory properties and substituted with different alkyl/arylcarboxamido/sulfonamido/ureido moieties in the 5 position have been investigated for their anticonvulsant effects in the same animal model. It was observed that some lipophilic derivatives, such as 5-benzoylamido-, 5-toluenesulfonylamido-, 5-adamantylcarboxamido-, and 5-pivaloylamido-1,3,4-thiadiazole-2-sulfonamide, show promising in vivo anticonvulsant properties and that these compounds may be considered as interesting leads for developing anticonvulsant or selective cerebrovasodilator drugs.

Introduction

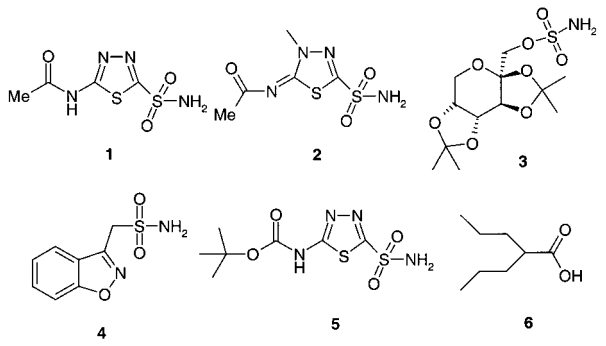
Carbonic anhydrase (CA, EC 4.2.1.1), a widely occurring enzyme in higher vertebrates, is also quite abundant in the brain, being present in the glia but not neurons, mainly as the cytosolic isozymes CA II, CA VII, and the mebrane-bound isoform CA XIV.[1−3] The function of this enzyme in the brain is not well defined, but since the choroid plexus of all vertebrates has a 10 times higher concentration of CA than the eye (a tissue very rich in this enzyme) and since the cerebrospinal fluid (CSF) contains a high amount of bicarbonate, it is obvious that CAs (catalyzing with high efficiency the reversible interconversion between carbon dioxide and bicarbonate) are involved in the secretion of this fluid (as they are analogously involved in the secretion of the ocular fluid).[1−3] It has been proved that inhibition of the brain CAs causes a selective increase of the cerebral blood flow, with the concomitant raising of the carbon dioxide partial pressure.[1−4] As a consequence, CA inhibitors are useful in the treatment of conditions associated with increased intracranial pressure,[1−5] as well as different neurological/neuromuscular pathologies such as epilepsy,[1,4,6] genetic hemiplegic migraine and ataxia,[7] tardive dyskinesia,[8] hypokalemic periodic paralysis,[9,10] essential tremor and Parkinson's disease,[11] and mountain sickness,[12,13] among others.

Several sulfonamide CA inhibitors such as acetazolamide **1**,[1,4] methazolamide **2**,[1,4] topiramate **3**,[14] and zonisamide **4**[15] were and are still used as antiepileptic drugs. The anticonvulsant effects of these or related sulfonamides are probably due to CO_2 retention secondary to inhibition of the red cell and brain enzymes,[1,4] but other mechanisms of action, such as blockade of sodium channels and kainate/AMPA receptors, as well as enhancement of GABA-ergic transmission, were also hypothesized/proved for some of these drugs.[14] Lipophilic derivatives, such as methazolamide **2**[1,4] or the *tert*-butoxycarbonyl derivative of acetazolamide **5**[16] are more effective anticonvulsants than acetazolamide itself, proving in this way that the penetrability of the drug to brain is an important factor influencing biological activity. Acetazolamide and methazolamide are still clinically used nowadays in some forms of epilepsy, but they are considered to belong to a minor class of antiepileptic agents.[16] The recently developed drug, topiramate **3**, is a very effective antiepileptic,[14] and it also acts as a strong CA inhibitor with a potency similar to that of acetazolamide against the physiologically important

* To whom correspondence should be addressed. Phone: +39-055-2757551. Fax: +39-055-2757555. E-mail: claudiu.supuran@unifi.it.
[†] University of Namur.
[‡] Università degli Studi di Firenze.
[§] CSGI.

10.1021/jm0109199 CCC: $22.00
Published on Web 12/19/2001

isozyme CA II (unpublished results from this laboratory; see later in the text).



A widely used antiepileptic drug, on the other hand, is valproic acid (or its sodium salt), VPA **6**.[17] A large variety of VPA derivatives or related compounds with potent antiepileptic properties have been reported,[18−21] among which are many VPA amides, esters, and phosphonate analogues, but no sulfonamides incorporating this moiety have been prepared and assayed as anticonvulsants up to now.

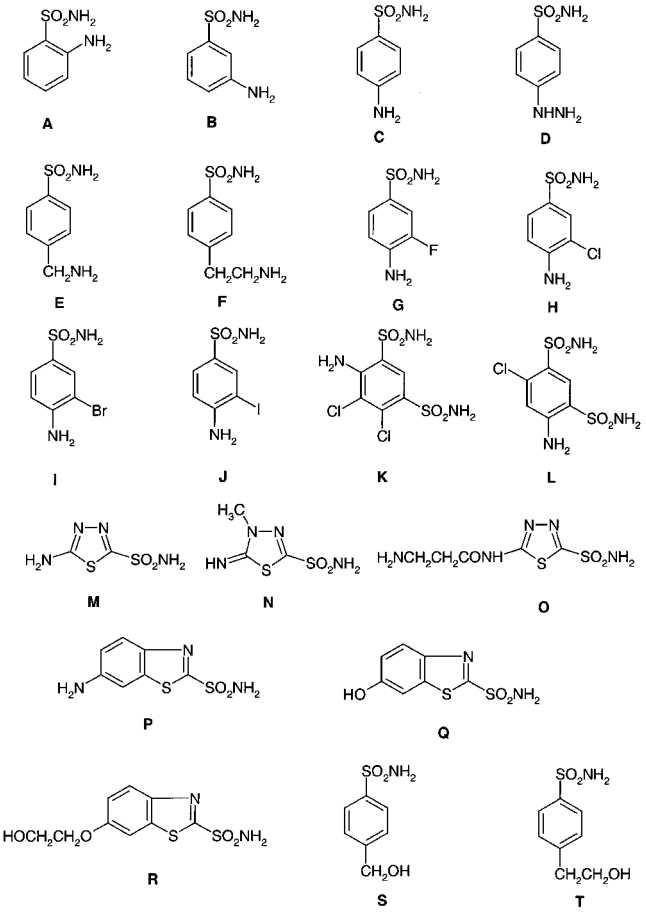
Considering our interest in developing sulfonamide CA inhibitors with different biological activity,[22−26] we investigated the possibility of designing anticonvulsant sulfonamides by using acetazolamide **1**, topiramate **3**, and valproic acid **6** as lead molecules. Thus, aromatic/heterocyclic sulfonamides incorporating VPA moieties in their molecule are reported here, together with their inhibitory properties against several physiologically relevant CA isozymes. Some of the best in vitro CA inhibitors were then tested in vivo for their anticonvulsant activity by a maximal electroshock seizure (MES) test in rats. Structure−activity relationship data were then used for the evaluation of other anticonvulsant sulfonamides incorporating lipophilic moieties other than the valproyl one. Among the new derivatives investigated here, some compounds showed a more potent anticonvulsant activity compared to the clinically used drug topiramate.

Results

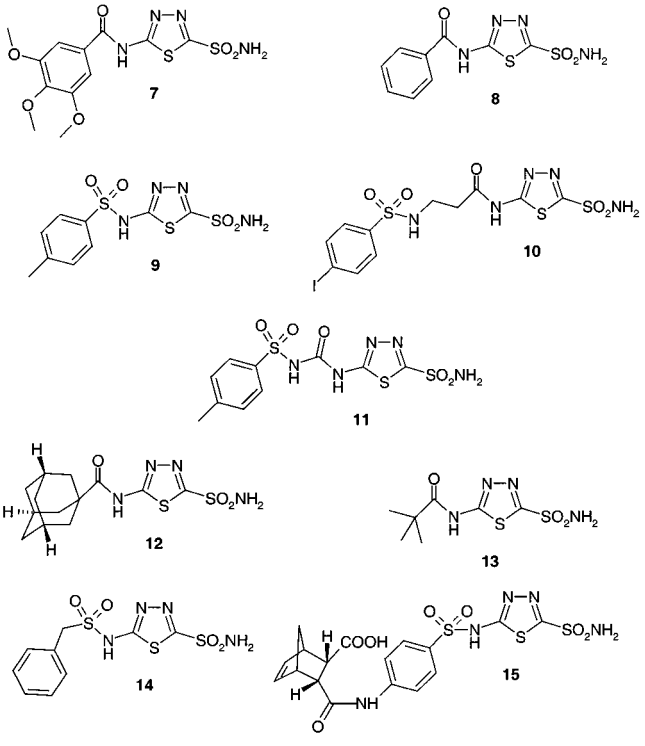
Chemistry. The chemical structures of sulfonamides **A**−**T** on which the valproyl moiety has been attached are shown. The sulfonamides obtained by attaching the valproyl moiety of **6** to the amino/hydroxy group of sulfonamides **A**−**T** will be designated as **6A**−**6T**.

Two synthetic approaches have been used for the preparation of the new sulfonamides reported here: (i) reaction of valproic acid **6** with sulfonamides **A**−**T** in the presence of carbodiimides, as reported previously by this group,[23] leading to amides (**6A**−**6P**) or esters (**6Q**−**6T**) (Woltersdorf et al.[28b] showed that in the conditions used here, only the *O*-acylation reaction occurs, without the concomitant acylation of the sulfonamide moiety) and (ii) reaction of valproyl chloride with sulfonamides **A**−**T** in the presence of base such as tertiary amines, sodium bicarbonate, etc.[25−28] By similar procedures, some other sulfonamides of types **7**−**15** investigated here for their anticonvulsant properties were also obtained.

Carbonic Anhydrase Inhibitory Activity. The new sulfonamides reported here were assayed for



inhibition of three CA isozymes (CA I, II, and IV)[29−34] known to play a critical role in electrolyte secretion/CO_2 transport processes in a variety of tissues,[1] using the



esterase activity of these isozymes with 4-nitrophenylacetate as substrate, and the inhibition data are shown in Table 1.[35,36]

Table 1. Inhibition Data of Carbonic Anhydrase for Derivatives Reported in the Present Paper[a]

no.	inhibitor	K_I (nM) hCA I[b]	hCA II[b]	bCA IV[c]	mp (°C)
1	acetazolamide	900	12	220	
2	methazolamide	780	14	240	
3	topiramate	250	5	54	
4	zonisamide	nt[d]	4300[d]	nt[d]	
	5	460	9	130	
	6A	23000 (45400)	255 (295)	950 (1310)	204−6
	6B	19000 (25000)	221 (240)	740 (2200)	233−5
	6C	22500 (28000)	240 (300)	630 (3000)	278−9
	6D	35600 (78500)	300 (320)	950 (3200)	288−9
	6E	3700 (25000)	96 (170)	210 (2800)	255−6
	6F	3500 (21000)	84 (160)	200 (2500)	234−6
	6G	1000 (8300)	45 (60)	110 (180)	240−1
	6H	950 (9800)	50 (110)	125 (320)	246−7
	6I	600 (6500)	36 (40)	49 (66)	244−5
	6J	550 (6000)	43 (70)	85 (125)	252−4
	6K	970 (6100)	26 (28)	155 (175)	298−9
	6L	800 (8400)	70 (75)	133 (160)	281−3
	6M	50 (8600)	6 (60)	25 (540)	274−6
	6N	70 (9300)	10 (19)	42 (355)	218−20
	6O	360 (455)	9 (3)	76 (125)	>300
	6P	62 (70)	7 (9)	16 (19)	189−91
	6Q	50 (55)	7 (8)	15 (17)	176−8
	6R	50 (50)	7 (7)	13 (15)	164−5
	6S	1500 (24000)	115 (125)	333 (560)	213−4
	6T	1300 (18000)	844 (110)	313 (450)	201−3
	7[28]	900	10	51	
	8[41]	38	2	21	
	9[26a]	5	4	3	
	10[42]	260	1.2	120	
	11[43]	3	4	12	
	12[25b]	850	10	65	
	13[44]	110	9	46	
	14[26a]	7	5	6	
	15[45]	5.5	1.5	4	

[a] The data in parentheses represent inhibition by the parent sulfonamide **A**−**T**). [b] Human (cloned) isozymes. [c] From bovine lung microsomes, by the esterase method. [d] nt = not tested. Data from ref 37.

Maximal Electroshock Seizures Test. The anticonvulsant activity of the most active CA inhibitors was examined at 10 and 30 mg/kg (ip) in mice by using the MES test, which detects drugs that prevent spread of tonic-clonic seizures.[37] At the desired time following ip administration (0.5 or 3 h), the electrical stimulus was delivered and the extent of protected mice was noted (Table 2). The time course activity of the most active compound (**6M**) was then evaluated up to 6 h after injection (Table 3).

ClogP Calculations. The lipophilicity of some of the target compounds used for the in vivo measurements reported above has been calculated (Table 4) with the program ChemDraw Ultra 6.0.1.

Discussion

Chemistry. Few sulfonamides with anticonvulsant properties have been reported. Thus, Ganz et al.[27] showed that some substituted benzenesulfonamides such as 3-chloro-4-phenacetamidobenzenesulfonamide exhibited good anticonvulsant activity in experimental animals, activity that was decreased by substituting the SO_2NH_2 moiety and increased by introducing lipophilic substituents to the aromatic ring. More recently, Chufan et al.[16] demonstrated that the more liphophilic acetazolamide analogue **5** shows better anticonvulsant properties than the lead molecule **1** itself. All these data prompted us to prepare the even more lipophilic valproic acid derivatives of the aromatic/heterocyclic sulfonamides **A**−**T**, previously used to design CA inhibitors with topical antiglaucoma activity.[22−26]

The valproyl moiety was introduced into the molecules of these new derivatives either by reacting the free acid **6** with amines (**A**−**P**) or alcohols (**Q**−**T**) in the presence of carbodiimides[23,28] or by reacting valproyl chloride (obtained from **6** and $SOCl_2$)[28] with amines/alcohols **A**−**T** in the presence of bases (triethylamine, pyridine, or sodium bicarbonate).[23,28] The amides/esters **6A**−**6T** were obtained in good yields by both these very simple methods. It has been demonstrated by Woltersdorf et al.[28b] that under such conditions only the O-acylation reaction takes place without the concomitant N-acylation of the sulfonamide moiety.

Carbonic Anhydrase Inhibitory Activity. The data of Table 1 show that the new inhibitors prepared by attaching valproyl moieties to aromatic/heterocyclic sulfonamides **A**−**T** are generally more effective, compared to their parent sulfonamide from which they were prepared, toward the three investigated isozymes hCA I, hCA II, and bCA IV (h = human; b = bovine isozyme). The enhanced inhibitory power of these compounds is

Table 2. Extent of Protected Mice (in %) against Convulsions in the MES Test at 0.5 and 3 h following Intraperitoneal Injection of 30 or 10 mg/kg of CA Inhibitor[a]

compound	30 mg/kg 0.5 h	30 mg/kg 3 h	10 mg/kg 0.5 h	10 mg/kg 3 h
methazolamide **2**	83 (12)	100 (12)	75 (8)	88 (8)
topiramate **3**	100 (6)	83 (6)	62 (8)	50 (8)
5[b]	75 (4)	60 (5)		
6M	96 (14)	93 (14)	25 (8)	100 (14)
7	25 (6)	67 (6)		
8	83 (6)	25 (6)		
9	92 (6)	75 (6)	12 (8)	62 (8)
10	33 (6)	25 (6)		
11	8 (6)	33 (6)		
12	83 (6)	96 (12)	25 (8)	93 (8)
13	75 (6)	100 (12)	44 (8)	87 (8)
14	67 (6)	38 (12)		
15	25 (6)	33 (6)		

[a] Rate of mice (n = 6−14 for each group) protected against seizures induced by an electroshock (50 mA; 0.2 s). [b] Data from ref 16 (obtained at a dose of 315 μmol/kg of compound **5**).

Table 3. Time-Course Activity of **6M** (30 mg/kg ip) in the ME Test with Six to Eight OF1 Male Mice for Each Time

time (h)	MES test	% of protected mice
0.5	7.5/8 + 6/6	96
1	6.5/8	81
2	8/8	100
3	7/8 + 6/6	93
4	4/8	50
6	5/8	63

Table 4. Lipophilicity (CLogP) Data for Compounds **1**−**15** Investigated in This Work

compound	ClogP[a]	compound	ClogP[a]
1	−1.1308	**9**	0.4788
2	0.0880	**10**	1.3691
3	0.0415	**11**	0.9413
4	−0.3630	**12**	1.5281
5	0.3178	**13**	0.1061
6M	1.8231	**14**	0.0388
7	−0.3342	**15**	−0.2233
8	0.3581		

[a] Calculated with the program ChemDraw Ultra 6.0.1.

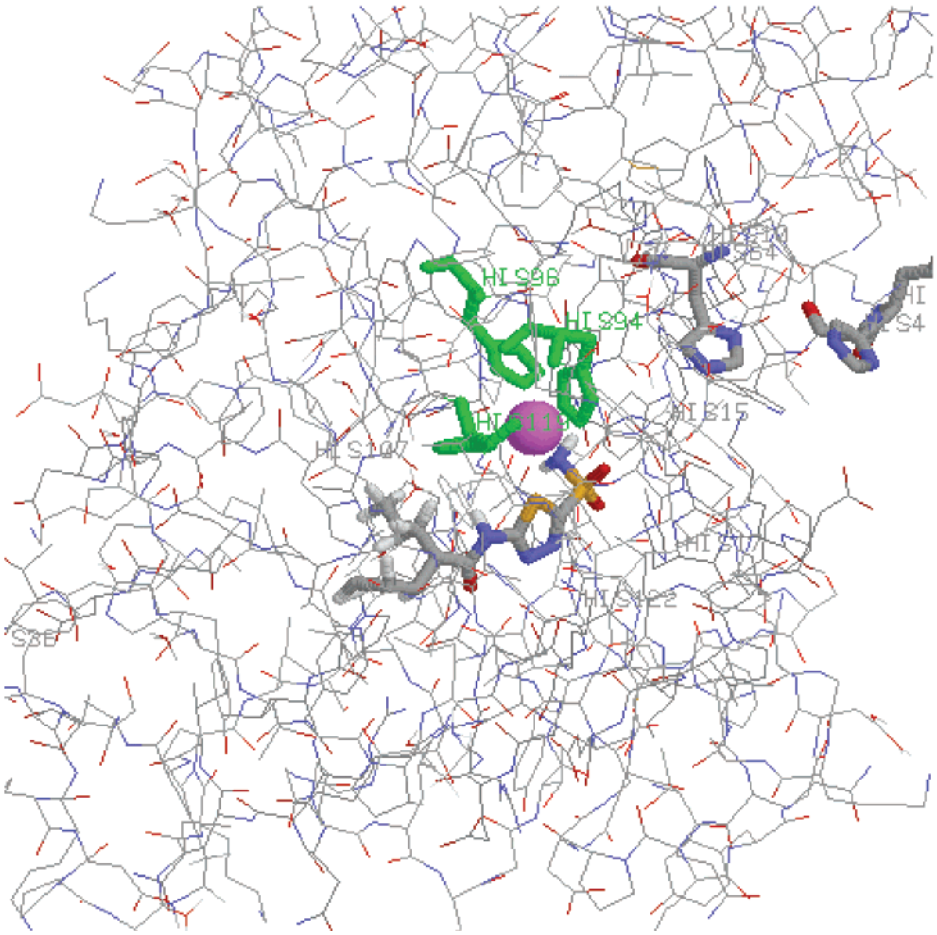


Figure 1. hCA II/**6M** adduct. The inhibitor has been docked within the enzyme active site by using the acetazolamide/hCA II adduct (PDB file 1AZM).[38a] The Zn(II) ion (central violet sphere), its three histidine ligands (in green, His 94, His 96, and His 119), and the proton shuttle residues His 64 and His 4 (situated at the entrance of the active site) are evidenced. The valproyl moiety of the inhibitor points toward the hydrophobic half of the active site.

presumably due to the interaction of the relatively long valproyl moiety incorporated in their molecules with hydrophobic patches within the enzyme active site, as observed for inhibitors previously reported by this group,[1,2,22−25] a fact explained thereafter by detailed QSAR models.[26] This is particularly well exemplified for the inhibitor **6M**, one of the most potent in the new series reported here, which has been docked within the hCA II active site, using the X-ray coordinates of the acetazolamide/hCA II adduct (Figure 1).[38a] It may be seen that the deprotonated sulfonamide moiety of the inhibitor is coordinated to the Zn(II) ion of the enzyme and its NH moiety donates a hydrogen bond to the Oγ of Thr 199, which in turn donates a hydrogen bond to the carboxylate group of Glu 106, as for other hCA II/sulfonamide adducts previously reported.[1,2,38] One of the oxygen atoms of the SO_2NH moiety also participates in a hydrogen bond with the backbone NH moiety of Thr 199.[1,2,38] This binding is in fact expected because the new inhibitor **6M** has a carboxamidothiadiazole sulfonamide moiety similar to acetazolamide (**1**) and its derivative (**5**). In contrast to acetazolamide, whose acetamido moiety does not participate in any particular interaction with active site residues,[38a] the long hydrophobic valproyl moiety of the inhibitor **6M** is oriented toward the hydrophobic half of the active site, being in van der Waals contact with the following amino acid residues: Val 121, Phe 130, Val 135, Leu 141, Val 143, Val 207, and Pro 201, all of which line the hydrophobic half of the CA active cavity.[38,39a] The two hydrophilic, catalytically relevant residues His 64 and His 4 (the CA II proton shuttle residues)[39b] are also shown in Figure 1, since they are two of the most important residues situated on the hydrophilic half of the active site. Thus, it is obvious that the extensive hydrophobic interactions between the valproyl moiety of **6M** and amino acid residues situated in the hydrophobic half of the active site cavity have as an effect that **6M** ($K_I = 6$ nM) is twice more potent a hCA II inhibitor compared to acetazolamide **1** ($K_I = 12$ nM) (see Table 1).

The nature of the sulfonamide attached to the valproyl moiety in the new derivatives **6A**−**6T** reported here greatly influenced the CA inhibitory power of these compounds. Among the synthesized derivatives, the heterocyclic sulfonamides (**6M**−**6R**) were more active than the aromatic ones (**6A**−**6L**, **6S**, **6T**). The efficiency of the obtained inhibitor generally varied in the following way based on the parent sulfonamide from which it was prepared: the derivatives of *p*-hydrazinobenzenesulfonamide < the orthanilamides < the metanilamides < the sulfanilamides < the homosulfanilamides < the *p*-aminoethylbenzenesulfonamides ≅ the halogenosubstituted sulfanilamides ≅ the 1,3-benzene-disulfonamides < 4-methyl-δ^2-1,3,4-thiadiazoline-2-sulfonamide < the benzothiazole-2-sulfonamides ≅ the 1,3,4-thiadiazole-2-sulfonamides. All three CA isozymes investi-

gated here were susceptible to inhibition with this type of sulfonamide. hCA II and bCA IV were the most sensitive, whereas hCA I was generally less susceptible to inhibition compared to the first two isozymes.

It must also be stressed that topiramate **3**, a lipophilic sugar derivative possessing a sulfamate moiety, tested under the same conditions as the other sulfonamides investigated here, behaved as a very strong CA inhibitor, being together with the valproyl derivative **6M** one of the most effective inhibitor in the investigated series (Table 1). Thus, both **3** and **6M** are more effective inhibitors than acetazolamide **1** or methazolamide **2** against all three CA isozymes investigated in the present study. Against isozyme II the two compounds practically possess the same efficacy (K_I values of 5 nM for **3** and 6 nM for **6M**), whereas the latter compound is a much more potent CA I and CA IV inhibitor compared to topiramate (Table 1). Our results are thus in contradiction with the early report of Shank's group that topiramate is a weak CA inhibitor but consistent with the presence of CA II in the brain.[14a] It is also true that the same group recently reported different results, showing that **3** is a much stronger CA inhibitor, but with an efficacy 10 times lower than that of acetazolamide, against a large number of CA isozymes.[40] Obviously, the assay methods of this and the above-mentioned study[40] are different, as different as the source of enzymes. Our data clearly show that topiramate is a potent CA II inhibitor, and this statement is also very much supported by the side effects seen in many patients treated with this antiepileptic drug,[14d] which are typical for the strong sulfonamide CA inhibitors used as systemic antiglaucoma agents (such as acetazolamide, methazolamide) and include paresthesias, nephrolithiasis, and weight loss, among others.[1] We must also mention that zonisamide **4** has also been reported to act as a weak CA inhibitor,[37] but since this compound is not available yet in Europe, we did not test its effect on the three CA isozymes of our interest and the inhibition data of Table 1 are taken from the literature.[37] Being an aliphatic sulfonamide derivative, **4** is expected to act as a weaker CA inhibitor compared to the aromatic/heterocyclic sulfonamides or the sulfamate investigated here.[1b]

Since the most effective CA inhibitors **3** and **6M** showed very good anticonvulsant properties in the MES test (see later in the text and Table 2), several other thiadiazolesulfonamides (**7**−**15**) structurally related to **6M** were also included in the study. The CA inhibitory properties of these derivatives (Table 1) show all of them to be low nanomolar inhibitors of hCA II (K_I values ranging from 1.2 to 10 nM), similar to derivative **5** reported by Chufan et al.,[16] and to appreciably inhibit the other two isozymes hCA I and bCA IV. These compounds (**7**−**15**), reported previously by our group,[25b,26a,28,39,41−45] were generally designed in order to obtain novel antiglaucoma sulfonamides, and they possess different alkyl/arylcarboxamido, arylsulfonamido, or arylsulfonylureido moieties in the 5 position of the thiadiazole ring. These moieties were chosen in such a way as to ensure a wide range of lipophilicities for these thiadiazolesulfonamides (ClogP data in the range 1.8 to −0.36), since this property seems to be crucial[1] for the biological activity/pharmacology of this class of derivatives and thus to better understand SAR for obtaining anticonvulsant sulfonamides/sulfamates (Table 4).

Maximal Electroshock Seizures Test. The MES test has been performed with compounds **6M** and **7**−**15** as well as methazolamide **2** and topiramate **3** as standards. Thus, compounds with powerful CA inhibitory properties (K_I values against hCA II ranging from 1 to 14 nM) and characterized by a wide range of physicochemical properties (ClogP in the range 1.8 to −0.36) were included in these experiments (Tables 2 and 3). Each CA inhibitor (30 or 10 mg/kg) was injected intraperitoneally to mice at a dose volume of 3 mL/kg. At the desired time following injection (0.5 or 3 h), an electrical stimulus was delivered and the protection extent against convulsions was registered.

The data of Table 2 show that several of the investigated compounds, such as **6M**, **8**, **9**, **12**, and **13**, similar to methazolamide **2** and topiramate **3**, efficiently protect mice against seizures induced by electroshock at a dosage of 30 mg/kg. The protection rate was of 75−100% at 0.5 h and 25−100% at 3 h after drug administration. All these compounds were quite lipophilic, possessing ClogP in the range 0.35−1.82. Other investigated sulfonamides, such as **7**, **10**, **14**, and **15**, showed less effective protection against electroshock-induced seizures, with a protection rate ranging from 25% to 67% at 0.5 h and from 25% to 67% at 3 h drug injection (Table 2). These derivatives were generally less lipophilic than the first subseries mentioned above (CLogP of −0.33 to 0.038) except for **10**, which has a lipophilicity comparable to that of the compounds discussed earlier (CLogP of 1.36). The least effective sulfonamide was **11**, which produced a protection of 8% at 0.5 h and 33% at 3 h following administration, although this is a rather lipophilic derivative, possessing a ClogP of 0.94. The compounds of the first group (**2**, **3**, **6M**, **9**, **12**, **13**) were also investigated at a decreased dosage of 10 mg/kg (Table 2). In these experiments, methazolamide produced a protection of 75% at 0.5 h and 88% at 3 h postadministration, being slightly more effective than topiramate (protection of 62% at 0.5 h and 50% at 3 h postadministration). The most effective compounds in the investigated series were **6M**, **12**, and **13**, which showed a rate of protection in the range 25−44% at 0.5 h and 87−100% at 3 h after administration. Less effective was **9**, showing a protection of 12% after 0.5 h and 62% after 3h. The correlation of the lipophilicity (ClogP), in vitro CA inhibition, and in vivo anticonvulsant activity of these compounds is thus not really straightforward. It seems that a strong CA inhibitor (with an inhibition constant against hCA II in the 1−15 nM range) also possessing a good lipophilicity should intrinsically lead to powerful anticonvulsant activities (such as in the case of **6M**, the best anticonvulsant in this series, possessing a ClogP of 1.82, or **12**, with a ClogP of 1.52). On the other hand, our data also show that parameters other than CA inhibition and lipophilicity may strongly influence the in vivo anticonvulsant properties, since some less lipophilic compounds (such as **13**, ClogP of 0.10) showed in vivo activity similar to that of **6M** and **12**, whereas many quite lipophilic compounds (for example, **10**, ClogP of 1.36) possessed a rather diminished anticonvulsant activity. Plasma pro-

tein binding of these sulfonamides is probably another important parameter influencing their bioavailability, since it is known, for example, that acetazolamide or methazolamide indeed binds to plasma proteins to different degrees.[4] It can be envisaged, on the other hand, that all these compounds possess pK_a values in the range 7.4−7.6 (for the deprotonation of the SO_2NH_2 moiety),[2,4] and thus, we think that this parameter may not vary considerably in the series of derivatives investigated in vivo.

The excellent anticonvulsant activity of the derivative **6M**, which incorporates in its structure moieties present in two antiepileptic drugs (acetazolamide and valproic acid), prompted us to investigate the time course activity up to 6 h after injection in mice (Table 3). The obtained data clearly show this sulfonamide to be highly effective in protecting mice from electroshock-induced seizures over a long period of time, with a protection rate of 93−100% in the first 3 h and 50−63% in the next 3 h.

Although other mechanisms of action should be not excluded, we suggest that the interesting anticonvulsant properties of some of these sulfonamides investigated here are due to inhibition of brain CAs, particulary CA II. In this regard, it is also important to observe that some of the most lipophilic inhibitors, such as **6M**, **8**, **9**, **12**, and **13**, showed the best anticonvulsant properties, which constitutes clear-cut proof that penetration through the blood−brain barrier is an important factor influencing bioavailability of the drug, although other factors (such as drug binding to plasma proteins) may also considerably influence activity in vivo.

At this point, we also want to stress that inhibition of brain CAs causes a selective increase of cerebral blood flow, with a concomitant increase of the carbon dioxide partial pressure.[46] For instance, administration of 12 mg/kg of acetazolamide to rabbits induced an 80% increase in the brain blood flow.[47] In consequence, these vasodilatory properties of acetazolamide are increasingly being used as a diagnostic tool for testing the cerebrovascular reserve capacity in patients with chronic cerebrovascular disease by using both magnetic resonance imaging (MRI) and positron emission tomographic (PET) methods, devised in order to develop diagnostic tools that exploit these valuable properties of the sulfonamide CA inhibitors.[47−51] The use of more potent CA inhibitors and more lipophilic derivatives than acetazolamide may be of interest for developing novel diagnostic tools for monitoring cerebrovascular diseases too.

Conclusions

We prepared a series of aromatic/heterocyclic sulfonamides incorporating valproyl moieties to design anticonvulsant compounds possessing in their structure moieties known to induce such a biological action (it is well-known that valproic acid is one of the most widely used antiepileptic drug, whereas some sulfonamides, such as acetazolamide and topiramate, also possess this pharmacological action). Some of these derivatives showed very good inhibitory properties against three CA isozymes involved in important physiological processes, such as CA I, CA II, and CA IV. A particularly interesting discovery was that topiramate also shares this property, although earlier literature data reported this compound to be a weak or moderate CA inhibitor. The valproyl derivative of acetazolamide (5-valproylamido-1,3,4-thiadiazole-2-sulfonamide, **6M**) was the best inhibitor in the series and exhibited very strong anticonvulsant properties in the MES test in mice. In consequence, several other 1,3,4-thiadiazole-sulfonamide derivatives possessing potent CA inhibitory properties and substituted with different alkyl/arylcarboxamido/sulfonamido/ureido moieties in the 5 position have been investigated for their anticonvulsant effects in the same animal model. It was observed that some derivatives, such as 5-benzoylamido-, 5-toluenesulfonylamido-, 5-adamantylcarboxamido-, and 5-pivaloylamido-1,3,4-thiadiazole-2-sulfonamide, show promising in vivo anticonvulsant properties and that these compounds may be considered as interesting leads for developing anticonvulsant or selective cerebrovasodilator drugs. A straightforward correlation among anticonvulsant activity, CA inhibition, and lipophilicity could not be rationalized for this series of compounds, proving thus that many of the enigmas surrounding sulfonamide antiepileptic drugs still remained unresolved for the moment.

Experimental Protocols

Chemistry. Melting points were recorded with a heating plate microscope and are not corrected. IR spectra were recorded in KBr pellets with a Carl Zeiss IR-80 instrument. ^{1}H NMR spectra were recorded in DMSO-d_6 or TFA as solvents, with a Bruker CPX200 or Varian 300 instrument. Chemical shifts are reported as δ values relative to Me_4Si as internal standard. Elemental analyses were done by combustion for C, H, N with an automated Carlo Erba analyzer and were ±0.4% of the theoretical values. All reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm precoated silica gel plates (E. Merck). Sulfonamides **A**−**T** used in the syntheses were either commercially available compounds (from Sigma-Aldrich, Milan, Italy, or Acros, Milan, Italy) or were prepared as described previously.[22−26] Topiramate was from Janssen Pharmaceutica (Topamax), whereas acetazolamide, methazolamide, and valproic acid were from Sigma-Aldrich (Milan, Italy). Other organic/inorganic reagents were from Sigma-Aldrich, Fluka, or E. Merck and were used without additional purification. Acetone, acetonitrile, DMF, and other solvents (E. Merck) used in the synthesis/chromatography were doubly distilled and kept on molecular sieves in order to maintain them under anhydrous conditions.

General Procedure for the Preparation of Compounds 6A−6T. Method A. An amount of 2 mmol of sulfonamide **A**−**T** was dissolved/suspended in 50 mL of anhydrous acetonitrile and then treated with a stoichiometric amount (290 mg = 322 μL, 2 mmol) of valproic acid **6.** An amount of 380 mg (2 mmol) of EDCI·HCl (or the equivalent amount of diisopropylcarbodiimide) was then added, and the reaction mixture was magnetically stirred at room temperature for 15 min. Then a total of 30 μL (2 mmol) of triethylamine was added and stirring was continued for 8−16 h at 4 °C (TLC control). The solvent was evaporated in vacuo and the residue taken up in 50 mL of water when the lipophilic valproic acid derivatives precipitated and were filtered. The obtained crude sulfonamides were crystallized from ethanol or mixtures of EtOH/water. Yields were in the range 85−95%.

Method B. An amount of 2 mmol of sulfonamide **A**−**T** was dissolved/suspended in 50 mL of anhydrous acetonitrile and then treated with a stoichiometric amount (360 mg, 2 mmol) of valproyl chloride[28] dissolved in a small volume of the same solvent. A stoichiometric amount of base (Et_3N, pyridine, or sodium bicarbonate) was then added, and the reaction mixture was stirred at room temperature for 3−6 h (TLC control). The solvent was then evaporated in vacuo, the residue was taken up in 50−100 mL of cold water, and the crude precipitate

obtained was filtered and crystallized from the same solvents mentioned above. Yields were in the range 90−95%.

4-(Valproylamido)benzenesulfonamide, 6C (Method A). White crystals, mp 278−9 °C (EtOH). IR (KBr), cm^{-1}: 1160 (SO_2sym), 1335 (SO_2as), 1585 (amide II), 1640 (amide I). ^1H NMR (DMSO-d_6), δ, ppm; J, Hz: 0.89 (t, 6H, 2Me), 1.10−1.70 (m, 8H, 2 CH_2CH_2), 2.55 (m, 1H, CH), 7.23 (s, 2H, SO_2NH_2), 7.78 (d, 2H, AA′BB′, 8.9), 7.91 (d, 2H, AA′BB′, 8.9), 10.03 (s, 1H, CONH). ^{13}C NMR (DMSO-d_6), δ, ppm: 6.35 (CH_3), 20.36 (CH_2), 20.59 (CH_2), 30.66 (CH), 119.20 (C2/C3 −Ph), 126.5 (C3/C2 −Ph), 137.92 (C1/C4 −Ph), 142.39 (C4/C1 −Ph), 159.27 (CONH). Anal. ($C_{14}H_{22}N_2O_3S$) C, H, N.

4-(Valproylamidomethyl)benzenesulfonamide, 6E (Method B). White crystals, mp 255−6 °C (EtOH). IR (KBr), cm^{-1}: 1160 (SO_2sym), 1330 (SO_2as), 1560 (amide II), 1630 (amide I). ^1H NMR (DMSO-d_6), δ, ppm; J, Hz: 0.89 (t, 6H, 2Me), 1.10−1.70 (m, 8H, 2 CH_2CH_2), 2.55 (m, 1H, CH), 4.49 (d, 2H, 6.0, CH_2−C_6H_4), 7.31 (s, 2H, SO_2NH_2), 7.46 (d, 2H, AA′BB′, 8.3), 7.78 (d, 2H, AA′BB′, 8.3), 8.62 (t, 1H, NHCO, 6.1). ^{13}C NMR (DMSO-d_6), δ, ppm: 6.38 (CH_3), 20.33 (CH_2), 20.65 (CH_2), 30.62 (CH), 41.58 (CH_2), 125.68 (C2/C3 −Ph), 121.56 (C5-pyrrole), 127.41 (C3/C2 −Ph), 142.53(C1/C4 −Ph), 144.19 (C4/C1 −Ph), 160.75 (CONH). Anal. ($C_{15}H_{24}N_2O_3S$) C, H, N.

4-(Valproylamidoethyl)benzenesulfonamide, 6F (Method A). White crystals, mp 234−6 °C (EtOH/water 1:1). IR (KBr), cm^{-1}: 1160 (SO_2sym), 1330 (SO_2as), 1560 (amide II), 1610 (amide I). ^1H NMR (DMSO-d_6), δ, ppm; J, Hz: 0.89 (t, 6H, 2Me), 1.10−1.70 (m, 8H, 2 CH_2CH_2 of valproic acid), 2.55 (m, 1H, CH), 2.90 (t, 2H, 7.2), 3.47 (q, 2H, 6.5), 7.29 (s, 2H, SO_2NH_2), 7.42 (d, 2H, AA′BB′, 8.2), 7.75 (d, 2H, AA′BB′, 8.2), 8.08 (t, 1H, NHCO, 5.7). ^{13}C NMR (DMSO-d_6), δ, ppm: 6.30 (CH_3), 20.35 (CH_2), 20.58 (CH_2), 30.72 (CH), 35.14 (CH_2−Ph), 39.70, (N−CH_2), 125.68 (C2/C3 −Ph), 129.14 (C3/C2 −Ph), 141.99 (C1/C4 −Ph), 143.89 (C4/C1 −Ph), 160.66 (CONH). Anal. ($C_{16}H_{26}N_2O_3S$) C, H, N.

4-(Valproylamido)-3-fluorobenzenesulfonamide, 6G (Method B). White crystals, mp 240−1 °C (EtOH/water, 1:2). IR (KBr), cm^{-1}: 1160 (SO_2sym), 1320 (SO_2as), 1600 (amide II), 1650 (amide I). ^1H NMR (DMSO-d_6), δ, ppm; J, Hz: 0.89 (t, 6H, 2Me), 1.10−1.70 (m, 8H, 2 CH_2CH_2), 2.55 (m, 1H, CH), 7.46 (s, 2H, SO_2NH_2), 7.65 (ddd, 1H, H2−Ph, 6.1, 2.0), 7.68 (dtr, 1H, H6−Ph, 8.4, 2.0), 7.92 (ddtr, 1H, H5−Ph, 8.3, 4.1, 1.6), 9.84 (s, 1H, NHCO). ^{13}C NMR (DMSO-d_6), δ, ppm; $J_{C,F}$, Hz: 6.30 (CH_3), 20.28 (CH_2), 20.55 (CH_2), 30.68 (CH), 113.40 (d, C2−Ph, 22.9), 121.13 (C5−Ph), 123.28 (C6−Ph), 129.39 (d, C4−Ph, 11.8), 140.81 (d, C1−Ph, 6.2), 153.89 (d, C3−Ph, 249.9), 159.01 (CONH). Anal. ($C_{14}H_{21}FN_2O_3S$) C, H, N.

4-(Valproylamido)-3-chlorobenzenesulfonamide, 6H (Method A). White crystals, mp 246−7 °C (EtOH). IR (KBr), cm^{-1}: 1160 (SO_2sym), 1320 (SO_2as), 1580 (amide II), 1660 (amide I). ^1H NMR (DMSO-d_6), δ, ppm; J, Hz: 0.89 (t, 6H, 2Me), 1.10−1.70 (m, 8H, 2 CH_2CH_2), 2.55 (m, 1H, CH), 7.49 (s, 2H, SO_2NH_2), 7.78 (dd, 1H, H6−Ph, 8.4, 2.1), 7.89 (d, 1H, H5−Ph, 8.5), 7.92 (d, 1H, H2−Ph, 2.1), 9.64 (s, 1H, NHCO). ^{13}C NMR (DMSO-d_6), δ, ppm: 6.37 (CH_3), 20.29 (CH_2), 20.61 (CH_2), 30.75 (CH), 123.33 (C6−Ph), 125.08 (C4−Ph), 126.82 (C5−Ph), 127.09 (C2−Ph), 138.14 (C1−Ph), 141.39 (C3−Ph), 158.95 (CONH). Anal. ($C_{14}H_{21}ClN_2O_3S$) C, H, N.

5-Valproylamido-1,3,4-thiadiazole-2-sulfonamide, 6M (Method B). White crystals, mp 274−6 °C (EtOH). IR (KBr), cm^{-1}: 1170 (SO_2sym), 1350 (SO_2as), 1580 (amide II), 1630 (amide I). ^1H NMR (DMSO-d_6), δ, ppm; J, Hz: 0.89 (t, 6H, 2Me), 1.10−1.70 (m, 8H, 2 CH_2CH_2), 2.55 (m, 1H, CH), 8.31 (s, 2H, SO_2NH_2), 13.07 (s, 1H, CONH). ^{13}C NMR (DMSO-d_6), δ, ppm: 6.35 (CH_3), 20.36 (CH_2), 20.59 (CH_2), 30.66 (CH), 158.07 (CONH), 161.52 (C2 thiadiazole), 163.38 (C5 thiadiazole). Anal. ($C_{10}H_{18}N_4O_3S_2$) C, H, N.

2-Sufamoyl-6-benzothiazolyl Valproate 6Q (Method B). White crystals, mp 176−8 °C (EtOH). IR (KBr), cm^{-1}: 1180 (SO_2sym), 1373 (SO_2as), 1748 (COO). ^1H NMR (DMSO-d_6), δ, ppm: 0.88 (t, 6H, 2Me), 1.10−1.73 (m, 8H, 2 CH_2CH_2), 2.54 (m, 1H, CH), 6.69 (m, 2H, H5, and H6 of benzothiazole), 7.24 (s, 1H, H-7 of benzothiazole), 8.15 (s, 2H, SO_2NH_2). Anal. ($C_{15}H_{20}N_2O_4S_2$) C, H, N.

Enzyme Preparations. Human CA I and CA II cDNAs were expressed in *Escherichia coli* strain BL21 (DE3) from the plasmids pACA/hCA I and pACA/hCA II described by Lindskog et al.[29] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by this group,[30] and enzymes were purified by affinity chromatography according to the method of Khalifah et al.[31] Enzyme concentrations were determined spectrophotometrically at 280 nm, utilizing a molar absorptivity of 49 mM^{-1} cm^{-1} for CA I and 54 mM^{-1} cm^{-1} for CA II on the basis of M_r = 28.85 kDa for CA I and M_r = 29.30 kDa for CA II.[32,33] CA IV was isolated from bovine lung microsomes as described by Maren et al., and its concentration has been determined by titration with ethoxzolamide.[34]

Initial rates of 4-nitrophenyl acetate hydrolysis catalyzed by different CA isozymes were monitored spectrophotometrically at 400 nm with a Cary 3 instrument interfaced with an IBM compatible PC.[35] Solutions of substrate were prepared in anhydrous acetonitrile; the substrate concentrations varied between 2×10^{-2} and 1×10^{-6} M, working at 25 °C. A molar absorption coefficient ϵ of 18 400 M^{-1} cm^{-1} was used for the 4-nitrophenolate formed by hydrolysis under the conditions of the experiments (pH 7.40) as reported in the literature.[35] Nonenzymatic hydrolysis rates were always subtracted from the observed rates. Duplicate experiments were done for each inhibitor concentration, and the values reported throughout the paper are the mean of such results. 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 0.01 nM were done thereafter with distilled−deionized water. Inhibitor and enzyme solutions were preincubated together for 10 min at room temperature prior to assay to allow for the formation of the E/I complex. The inhibition constant K_I was determined as described by Pocker and Stone.[35] Enzyme concentrations were 3.5 nM for hCA II, 12 nM for hCA I, and 36 nM for bCA IV (this isozyme has a decreased esterase activity,[36] and higher concentrations had to be used for the measurements).

Maximal Electroshock Seizures Test. Each compound was intraperitoneally injected to OF1 male mice (25−28 g; Iffa-Credo, Les Oncins, France) at a dose volume of 3 mL/kg. At the desired time, an electrical stimulus (50 mA; 60 Hz) was delivered for 0.2 s through corneal electrodes. Protection against seizures was defined as the abolition of the hind limb tonic extension. The preliminary screening was conducted with groups of 6−14 mice at an intraperitoneal dose of 30 mg/kg. For the most active compounds, the groups contained eight mice each and the dosage was 10 mg/kg. The electroshock was applied at 0.5 or 3 h after injection. Then the time-course activity of **6M** was examined up to 6 h after injection of 30 mg/kg.[52−54]

Calculations and Docking. All the molecular mechanics and dynamics calculations were done with the program MOE (Molecular Operating Environment).[55] The starting point for modeling the binding of inhibitor **6M** within the CA active site was constituted by the X-ray structure of the adduct of the structurally related acetazolamide **1** with hCAII reported by Liljas's group.[38] A total of 30 minimization cycles have been performed with a tether constraint on the heavy atoms gradually lowered till complete relaxation.[39a] ClogP qjdata were calculated by using the program ChemDraw Ultra 6.0.1.[56]

Acknowledgment. This research was financed by a grant from the Italian CNR−Target Project Biotechnology and by CSGI (Florence, Italy). Thanks are addressed to Drs. M.A. Ilies, A. Casini, and M. Barboiu for expert technical assistance.

References

(1) (a) Supuran, C. T.; Scozzafava, A. Carbonic anhydrase inhibitors and their therapeutic potential. *Expert Opin. Ther. Pat.* **2000**, *10*, 575−600. (b) Supuran, C. T.; Scozzafava, A. Carbonic anhydrase inhibitors. *Curr. Med. Chem.: Immunol., Endoc., Metab. Agents* **2001**, *1*, 61−97.

(2) Supuran, C. T. Carbonic anhydrase inhibitors. In *Carbonic Anhydrase and Modulation of Physiologic and Pathologic Processes in the Organism*; Puscas, I., Ed.; Helicon: Timisoara, Roumania, 1994; pp 29−111.

(3) Chegwidden, W. R., Edwards, Y., Carter, N., Eds. *The Carbonic Anhydrases—New Horizons*; Birkhäuser Verlag: Basel, Switzerland, 2000 (and references therein).

(4) Maren, T. H. Carbonic anhydrase: chemistry, physiology and inhibition. *Physiol. Rev.* **1967**, *47*, 595−781.

(5) Scarrow, A. M.; Segal, R.; Medsger, T. A.; Wasko, M. C. Communicating hydrocephalus secondary to diffuse meningeal spread of Wegener's granulomatosis: case report and literature review. *Neurosurgery* **1998**, *43*, 1470−1473.

(6) Reiss, W. G.; Oles, K. S. Acetazolamide in the treatment of seizures. *Ann. Pharmacother.* **1996**, *30*, 514−519.

(7) Battistini, S.; Stenirri, S.; Piatti, M.; Gelfi, C.; Righetti, P. G.; Rocchi, R. A new CACNA1A gene mutation in acetazolamide-responsive familial hemiplegic migraine and ataxia. *Neurology* **1999**, *13*, 38−43.

(8) Cowen, M. A.; Green, M.; Bertollo, D. N.; Abbott, K. A treatment for tardive dyskinesia and some other extrapyramidal symptoms. *J. Clin. Psychopharmacol.* **1997**, *17*, 190−193.

(9) Griggs, R. C.; Moxley, R. T.; Riggs, J. E.; Engel, W. K. Effects of acetazolamide on myotonia. *Ann. Neurol.* **1978**, *3*, 531−537.

(10) Links, T. P.; Smit, A. J.; Molenaar, W. M.; Zwarts, M. J.; Oosterhuis, H. J. Familial hypokalemic periodic paralysis. Clinical, diagnostic and therapeutic aspects**.** *J. Neurol. Sci.* **1994**, *122*, 33−43.

(11) Uitti, R. J. Medical treatment of essential tremor and Parkinson's disease. *Geriatrics* **1998**, *53*, 46−48.

(12) Bernhard, W. N.; Schalik, L. M.; Delaney, P. A.; Bernhard, T. M.; Barnas, G. M. Acetazolamide plus low-dose dexamethasone is better than acetazolamide alone to ameliorate symptoms of acute mountain sickness. *Aviat., Space Environ. Med.* **1998**, *69*, 883−886.

(13) Bradwell, A. R.; Wright, A. D.; Winterborn, M.; Imray, C. Acetazolamide and high altitude disease. *Int. J. Sports Med.* **1992**, *13* (Suppl. 1), 63−64.

(14) (a) Shank, R. P.; Gardocki, J. F.; Vaught, J. L.; Davis, C. B.; Schupsky, J. J.; Raffa, R. B.; Dodgson, S. J.; Nortey, S. O.; Marianoff, B. E. Topiramate: preclinical evaluation of structurally novel anticonvulsant. *Epilepsia* **1994**, *35*, 450−460. (b) Edmonds, H. L.; Jiang, Y. D.; Zhang, P. Y., Shank, R. P. Anticonvulsant activity of topiramate and phenytoin in a rat model of ischemia-induced epilepsy. *Life Sci.* **1996**, *59*, 127−131. (c) Stringer, J. L. A comparison of topiramate and acetazolamide on seizure duration and paired-pulse inhibition in the dentate gyrus of the rat. *Epilepsy Res.* **2000**, *40*, 147−153. (d) Sabers, A.; Gram, L. Newer anticonvulsants: Comparative review of drug interactions and adverse effects. *Drugs* **2000**, *60*, 23−33. (e) Bourgeois, B. F. D. Pharmacokinetics and pharmacodynamics of topiramate. *J. Child. Neurol.* **2000**, *15*, S27−S30.

(15) Bialer, M.; Johannessen, S. I.; Kupferberg, H. J.; Levy, R. H.; Loiseau, P.; Perucca, E. Progress report on new antiepileptic drugs: summary of the Fifth Eilat Conference. *Epilepsy Res.* **2001**, *43*, 11−18.

(16) Chufan, E. E.; Pedregosa, J. C.; Baldini, O. N.; Bruno-Blanch, L. Anticonvulsant activity of analogues of acetazolamide. *Farmaco* **1999**, *54*, 838−841.

(17) (a) Johannessen, C. U. Mechanism of action of valproate: a commentary. *Neurochem. Int.* **2000**, *37*, 103−110. (b) Wheless, J. W.; Venkataraman, V. New formulations of drugs in epilepsy. *Expert Opin. Pharmacother.* **1999**, *1*, 49−60.

(18) (a) Redecker, C.; Altrup, U.; Hoppe, D.; Dusing, R.; Speckmann, E. J. Effects of valproate derivatives. I. Antiepileptic efficacy of amides, structural analogs and esters. *Neuropharmacology* **2000**, *39*, 254−266. (b) Redecker, C.; Altrup, U.; Hoppe, D.; Hense, T.; Kreier, A.; Rabe, A.; Dusing, R.; Speckmann, E. J. Effects of valproate derivatives. II. Antiepileptic efficacy in relation to chemical structures of valproate sugar esters. *Neuropharmacology* **2000**, *39*, 267−281.

(19) Kehler, J.; Hansen, H. I.; Sanchez, C. Novel phosphonic and phosphonic acid analogues of the anticonvulsant valproic acid. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2547−2548.

(20) (a) Krogsgaard-Larsen, P.; Frolund, B.; Frydenvang, K. GABA uptake inhibitors. Design, molecular pharmacology and therapeutic aspects. *Curr. Pharm. Des.* **2000**, *6*, 1193−1209. (b) Krogsgaard-Larsen, P.; Falch, E.; Larsson, O. M.; Schousboe, A. GABA uptake inhibitors: relevance to antiepileptic drug research. *Epilepsy Res.* **1987**, *1*, 77−93. (c) Krogsgaard-Larsen, P.; Froelund, B.; Joergensen, F. S.; Schousboe, A. GABAA receptor agonists, partial agonists, and antagonists. Design and therapeutic prospects. *J. Med. Chem.* **1994**, *37*, 2489−2505.

(21) (a) Kehler, J.; Stensboel, T. B.; Krogsgaard-Larsen, P. Piperidinyl-3-phosphinic acids as novel uptake inhibitors of the neurotransmitter gamma-aminobutyric acid (GABA). *Bioorg. Med. Chem. Lett.* **1999**, *9*, 811−814. (b) Peyron, R.; Le Bars, D.; Cinotti, L.; Garcia-Larrea, L.; Galy, G.; Landais, P.; Millet, P.; Lavenne, F.; Froment, J. C.; Krogsgaard-Larsen P. Effects of GABAA receptors activation on brain glucose metabolism in normal subjects and temporal lobe epilepsy (TLE) patients. A positron emission tomography (PET) study. Part I: Brain glucose metabolism is increased after GABAA receptors activation. *Epilepsy Res.* **1994**, *19*, 45−54. (c) Gonsalves, S. F.; Twitchell, B.; Harbaugh, R. E.; Krogsgaard-Larsen, P.; Schousboe, A. Anticonvulsant activity of the glial GABA uptake inhibitor, THAO, in chemical seizures. *Eur. J. Pharmacol.* **1989**, *168*, 265−268.

(22) (a) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C. T. Carbonic anhydrase inhibitors. Perfluoroalkyl/aryl-substituted derivatives of aromatic/heterocyclic sulfonamides as topical intraocular pressure lowering agents with prolonged duration of action. *J. Med. Chem.* **2000**, *43*, 4542−4551. (b) Casini, A.; Scozzafava, A.; Mincione, F.; Menabuoni, L.; Ilies, M. A.; Supuran, C. T. Carbonic anhydrase inhibitors. Water soluble 4-sulfamoylphenyl-thioureas as topical intraocular pressure lowering agents with long lasting effects. *J. Med. Chem.* **2000**, *43*, 4884−4892.

(23) (a) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C. T. Carbonic anhydrase inhibitors: Synthesis of water-soluble, topically effective intraocular pressure lowering aromatic/heterocyclic sulfonamides containing cationic or anionic moieties: is the tail more important than the ring? *J. Med. Chem.* **1999**, *42*, 2641−2650. (b) Scozzafava, A.; Briganti, F.; Mincione, G.; Menabuoni, L.; Mincione, F.; Supuran, C. T. Carbonic anhydrase inhibitors: Synthesis of water-soluble, amino acyl/dipeptidyl sulfonamides possessing long-lasting intraocular pressure-lowering properties via the topical route. *J. Med. Chem.* **1999**, *42*, 3690−3700.

(24) (a) Borras, J.; Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C. T. Carbonic anhydrase inhibitors: Synthesis of water-soluble, topically effective intraocular pressure lowering aromatic/heterocyclic sulfonamides containing 8-quinolinesulfonyl moieties: is the tail more important than the ring? *Bioorg. Med. Chem.* **1999**, *7*, 2397−2406. (b) Renzi, G.; Scozzafava, A.; Supuran, C. T. Carbonic anhydrase inhibitors: Topical sulfonamide antiglaucoma agents incorporating secondary amine moieties. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 673−676. (c) Supuran, C. T.; Briganti, F.; Tilli, S.; Chegwidden, W. R.; Scozzafava, A. Carbonic anhydrase inhibitors: Sulfonamides as anticancer agents? *Bioorg. Med. Chem.* **2001**, *9*, 703−714.

(25) (a) Ilies, M.; Supuran, C. T.; Scozzafava, A.; Casini, A.; Mincione, F.; Menabuoni, L.; Caproiu, M. T.; Maganu, M.; Banciu, M. D. Carbonic anhydrase inhibitors. Sulfonamides incorporating furan-, thiophene- and pyrrole-carboxamido groups possess strong topical intraocular pressure lowering properties as aqueous suspensions. *Bioorg. Med. Chem.* **2000**, *8*, 2145−2155. (b) Supuran, C. T.; Mincione, F.; Scozzafava, A.; Briganti, F.; Mincione, G.; Ilies, M. A. Carbonic anhydrase inhibitors. Part 52. Metal complexes of heterocyclic sulfonamides: A new class of strong topical intraocular pressure-lowering agents with potential use as antiglaucoma drugs. *Eur. J. Med. Chem.* **1998**, *33*, 247−254.

(26) (a) Supuran, C. T.; Clare, B. W. Carbonic anhydrase inhibitors. Part 57. Quantum chemical QSAR of a group of 1,3,4-thiadiazole and 1,3,4-thiadiazoline disulfonamides with carbonic anhydrase inhibitory properties. *Eur. J. Med. Chem.* **1999**, *34*, 41−50. (b) Clare, B. W.; Supuran, C. T. Carbonic anhydrase inhibitors. Part 61. Quantum Chemical QSAR of a group of benzenedisulfonamides. *Eur. J. Med. Chem.* **1999**, *34*, 463−474.

(27) Ganz A. J.; Waser, P. G.; Pfirrmann, R. W. Die Entwicklung neuer Antiepileptika. V. Mitteilung: Pharmakologische Wirkungen einiger Sulfanilamidderivate. *Arzneimittelforschung* **1978**, *28*, 1331−1334.

(28) (a) Supuran, C. T.; Popescu, A.; Banciu, M. D. Carbonic anhydrase inhibitors. Part 10. New derivatives of 1,3,4-thiadiazole-2-sulfonamide and 4-methyl-2-sulfonamido-δ^2-1,3,4-thiadiazoline. *Rev. Roum. Chim.* **1992**, *37*, 289−297. (b) Woltersdorf, W.; Schwam, H.; Bicking, J. B.; Brown, S. L.; deSolms, S. J.; Fishman, D. R.; Graham, S. L.; Gautheron, P. D.; Hoffman, J. M.; Larson, R. D.; Lee, W. S.; Michelson, S. R.; Robb, C. M.; Share, C. N.; Shepard, K. L.; Smith, A. M.; Smith, R. L.; Sondey, J. M.; Strohmeyer, K. M.; Sugrue, M. F.; Viader, M. P. Topically active carbonic anhydrase inhibitors. 1. *O*-Acyl derivatives of hydroxybenzothiazole-2-sulfonamide. *J. Med. Chem.* **1989**, *32*, 2486−2492.

(29) Lindskog, S.; Behravan, G.; Engstrand, C.; Forsman, C.; Jonsson, B. H.; Liang, Z.; Ren, X.; Xue, Y.. Structure−function relations in human carbonic anhydrase II as studied by site-directed mutagenesis. In *Carbonic anhydrase—from biochemistry and genetics to physiology and clinical medicine*; Botrè, F., Gros, G., Storey, B. T., Eds.; VCH: Weinheim, Germany, 1991; pp 1−13.
(30) Behravan, G.; Jonsson, B. H.; Lindskog, S. Fine tuning of the catalytic properties of carbonic anhydrase. Studies of a Thr200-His variant of human isoenzyme II. *Eur. J. Biochem*. **1990**, *190*, 351−357.
(31) Khalifah, R. G.; Strader, D. J.; Bryant, S. H.; Gibson, S. M. Carbon-13 nuclear magnetic resonance probe of active site ionization of human carbonic anhydrase B. *Biochemistry* **1977**, *16*, 2241−2247.
(32) Lindskog, S.; Coleman, J. E. The catalytic mechanism of carbonic anhydrase. *Proc. Natl. Acad Sci. U.S.A*. **1964**, *70*, 2505−2508.
(33) Steiner, H.; Jonsson, B. H.; Lindskog, S. The catalytic mechanism of carbonic anhydrase. Hydrogen-isotope effects on the kinetic parameters of the human C isoenzyme. *Eur. J. Biochem*. **1975**, *59*, 253−259.
(34) Maren, T. H.; Wynns, G. C.; Wistrand, P. J. Chemical properties of carbonic anhydrase IV, the membrane-bound enzyme. *Mol. Pharmacol*. **1993**, *44*, 901−906.
(35) Pocker, Y.; Stone, J. T. The catalytic versatility of erythrocyte carbonic anhydrase. III. Kinetic studies of the enzyme-catalyzed hydrolysis of *p*-nitrophenyl acetate. *Biochemistry* **1967**, *6*, 668−678.
(36) Baird, T. T.; Waheed, A.; Okuyama, T.; Sly, W. S.; Fierke, C. A. Catalysis and inhibition of human carbonic anhydrase IV. *Biochemistry* **1997**, *36*, 2669−2678.
(37) Masuda, Y.; Karasawa, T. Inhibitory effect of zonisamide on human carbonic anhydrase in vitro. *Arzneimittelforschung* **1993**, *43*, 416−418.
(38) (a) Vidgren, J.; Liljas, A.; Walker, N. P. Refined structure of the acetazolamide complex of human carbonic anhydrase II at 1.9 Å. *Int. J. Biol. Macromol*. **1990**, *12*, 342−344. (b) Stams, T.; Christianson, D. W. X-ray crystallographic studies of the carbonic anhydrase isozymes. In *The Carbonic Anhydrases—New Horizons*; Chegwidden W. R., Edwards, Y., Carter, N., Eds.; Birkhäuser Verlag: Basel, Switzerland, 2000; pp 159−174.
(39) (a) Chazalette, C.; Rivière-Baudet, M.; Scozzafava, A.; Abbate, F.; Maarouf, Z. B.; Supuran, C. T. Carbonic anhydrase inhibitors. Interaction of boron derivatives with isozymes I and II: a new binding site for hydrophobic inhibitors at the entrance of the active site evidenced by docking studies. *J. Enzyme Inhib*. **2001**, *16*, 125−133. (b) Briganti, F.; Mangani, S.; Orioli, P.; Scozzafava, A.; Vernaglione, G.; Supuran, C. T. Carbonic anhydrase activators: X-ray crystallographic and spectroscopic investigations for the interaction of isozymes I and II with histamine. *Biochemistry* **1997**, *36*, 10384−10392.
(40) Dodgson, S. J.; Shank, R. P.; Maryanoff, B. E. Topiramate as an inhibitor of carbonic anhydrase isoenzymes. *Epilepsia* **2000**, *41* (Suppl. 1), S35−S39.
(41) Jitianu, A.; Ilies, M. A.; Scozzafava, A.; Supuran, C. T. Complexes with biologically active ligands. Part 8. Synthesis and carbonic anhydrase inhibitory activity of 5-benzoylamido- and 5-(3-nitrobenzoylamido)-1,3,4-thiadiazole-2-sulfonamide and their metal complexes. *Main Group Met. Chem*. **1997**, *20*, 151−156.
(42) Barboiu, M.; Supuran, C. T.; Menabuoni, L.; Scozzafava, A.; Mincione, F.; Briganti, F.; Mincione, G. Carbonic anhydrase inhibitors. Synthesis of topically effective intraocular pressure lowering agents derived from 5-(ω-aminoalkylcarboxamido)-1,3,4-thiadiazole-2-sulfonamide. *J. Enzyme Inhib*. **1999**, *15*, 23−46.
(43) Scozzafava, A.; Supuran, C. T. Carbonic anhydrase inhibitors. Arylsulfonylureido and arylureido-substituted aromatic and heterocyclic sulfonamides: towards selective inhibitors of carbonic anhydrase isozyme I. *J. Enzyme Inhib*. **1999**, *14*, 343−363.
(44) Supuran, C. T.; Almajan, G. L. Carbonic anhydrase inhibitors. Part 34. Synthesis and biological activity of coordination compounds of 5-pivaloylamido-1,3,4-thiadiazole-2-sulfonamide with divalent metal ions. *Main Group Met. Chem*. **1995**, *18*, 347−351.
(45) Casini, A.; Mincione, F.; Ilies, M. A.; Menabuoni, L.; Scozzafava, A.; Supuran, C. T. Carbonic anhydrase inhibitors: Synthesis and inhibition against isozymes I, II and IV of topically acting antiglaucoma sulfonamides incorporating *cis*-5-norbornene-*endo*-3-carboxy-2-carboxamido moieties. *J. Enzyme Inhib*. **2001**, *16*, 113−123.
(46) Lassen, N. A.; Friberg, L.; Kastrup, J.; Rizzi, D.; Jensen, J. J. Effects of acetazolamide on cerebral blood flow and brain tissue oxygenation. *Postgrad. Med. J*. **1987**, *63*, 185−187
(47) Taki, K.; Hirahara, K.; Tomita, S.; Totoki, T. Acetazolamide-induced increase in blood flow to rabbit organs is confirmed using colored microspheres. *Heart Vessels* **1998**, *13*, 63−67.
(48) Grossmann, W. M.; Koeberle, B. The dose−response relationship of acetazolamide on the cerebral blood flow in normal subjects. *Cerebrovasc. Dis*. **2000**, *10*, 65−69.
(49) Detre, J. A.; Samuels, O. B.; Alsop, D. C.; Gonzalez, J. B.; Kasner, S. E.; Raps, E. C. Noninvasive magnetic resonance imaging evaluation of cerebral blood flow with acetazolamide challenge in patients with cerebrovascular stenosis. *Magn. Reson. Imaging* **1999**, *10*, 870−875.
(50) Hirano, T.; Minematsu, K.; Hasegawa, Y.; Tanaka, Y.; Hayashida, K.; Yamaguchi, T. Acetazolamide reactivity on ^{123}I−IMP single photon emission computed tomography in patients with major cerebral artery occlusive disease: correlation with positron emission tomography parameters. *J. Cereb. Blood Flow Metab*. **1994**, *14*, 763−770.
(51) Takeuchi, R.; Matsuda, H.; Yonekura, Y.; Sakahara, H.; Konishi, J. Noninvasive quantitative measurements of cerebral blood flow using technetium-99m-L,L-ECD SPECT activated with acetazolamide: quantification analysis by equal-volume-split ^{99m}Tc-ECD consecutive SPECT method. *J. Cereb. Blood Flow Metab*. **1997**, *17*, 1020−1032.
(52) Porter, R. J.; Cerghino, J. J.; Gladding, G. D.; Hessie, B. J.; Kupferberg, H. J.; Scoville, B.; White, B. G. Antiepileptic drug development program. *Cleveland Clin. Q*. **1984**, *51*, 293−305.
(53) Masereel, B.; Lambert, D. M.; Dogné, J. M.; Poupaert, J. H.; Delarge, J. Anticonvulsant activity of pyrid-3-yl sulfonyl urea and thiourea. *Epilepsia* **1997**, *38*, 334−337.
(54) Masereel, B. Design, synthesis, and anticonvulsant activity of 1-(pyrid-3-ylsulfonamido)-2-nitroethylenes. *J. Med. Chem*. **1998**, *41*, 3239−3244.
(55) MOE, available from Chemical Computing Groups Inc., 1255 University Street, Suite 1600, Montreal, Quebec H3B 3X3, Canada.
(56) ChemDraw Ultra 6.0.1, available from CambridgeSoft Com, Cambridge, MA.

JM0109199