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Carbonic anhydrase inhibitors. Inhibition of isoforms I, II, IV, VA, VII, IX, and XIV with sulfonamides incorporating fructopyranose–thioureido tails

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Abstract—A series of aromatic/heterocyclic sulfonamides incorporating 2,3:4,5-bis-*O*-(isopropylidene)-β-D-fructopyranosyl-thioureido moieties has been synthesized and assayed for the inhibition of seven human isoforms of the zinc enzyme carbonic anhydrase (hCA, EC 4.2.1.1). The new derivatives behaved as weak hCA I inhibitors (K_Is of 9.4 −13.3 μM), were efficient hCA II inhibitors (K_Is of 6–750 nM), and slightly inhibited isoforms hCA IV and hCA VA. Only the sulfanilamide derivative showed efficient and selective inhibition of hCA IV (K_I of 10 nM). These derivatives also showed excellent hCA VII inhibitory activity (K_Is of 10–79 nM), being less efficient as inhibitors of the transmembrane isoforms hCA IX (K_Is of 10–4500 nM) and hCA XIV (K_Is of 21–3500 nM). Two of the new compounds showed anticonvulsant action in a maximal electroshock seizure test in mice, with the fluorosulfanilamide derivative being a more efficient anticonvulsant than the antiepileptic drug topiramate.

Several carbonic anhydrase (CA, EC 4.2.1.1) isoforms are known to be present in the vertebrate central nervous system (CNS), being located in various cell populations, where they play a variety of functions.[1,2] For a long time, the ubiquitous, catalytically superefficient isoform CA II was reported to be the only isozyme within this organ.[1] Recently, many other such isoforms of the 16 actually known in vertebrates[3–5] have been demonstrated to be present in the brain, being located in precise regions of this organ, and playing critical physiological functions, some of which started to be understood only in the last period (Fig. 1).[6–11] Thus, epithelial cells of the choroid plexus possess CA II, III, XII, and the acatalytic CA related protein (CARP) VIII and CARP XI. CA IV is located in endothelial cells of blood vessels. Astrocytes express both CA V and CARP XI, whereas CARP X is present in the myelin sheath. CA II is also expressed in oligodendrocytes, whereas neurons contain CA II, CA V, CA VII, XIV, and CARP XI. The presence of CA III and CA XII was demonstrated in the rat, whereas CARP X, XI, and CA XIV were observed in the mouse.[6–11] In situ hybridization in rat hippocampal pyramidal has demonstrated a steep increase in intrapyramidal CA VII expression at around postnatal day 12. This developmental expression promotes excitatory responses evoked by intense GABAergic activity.[11] It should be mentioned that CA VII is the unique isoform present only in the CNS.[11] Various physiological roles are attributed to mammalian CAs in the brain such as fluid and ion compartmentation,[12] formation of cerebrospinal fluid,[1] regulation of its pH and ionic constituents,[1,11] epileptogenesis,[1,13] regulation of GABAergic signaling,[11] respiratory response to carbon dioxide,[14] generation of bicarbonate for biosynthetic reactions,[15] proliferation, and differentiation.[10]

Keywords: Carbonic anhydrase; Sulfonamide; Sulfamate; Topiramate; Anticonvulsant; MES test.
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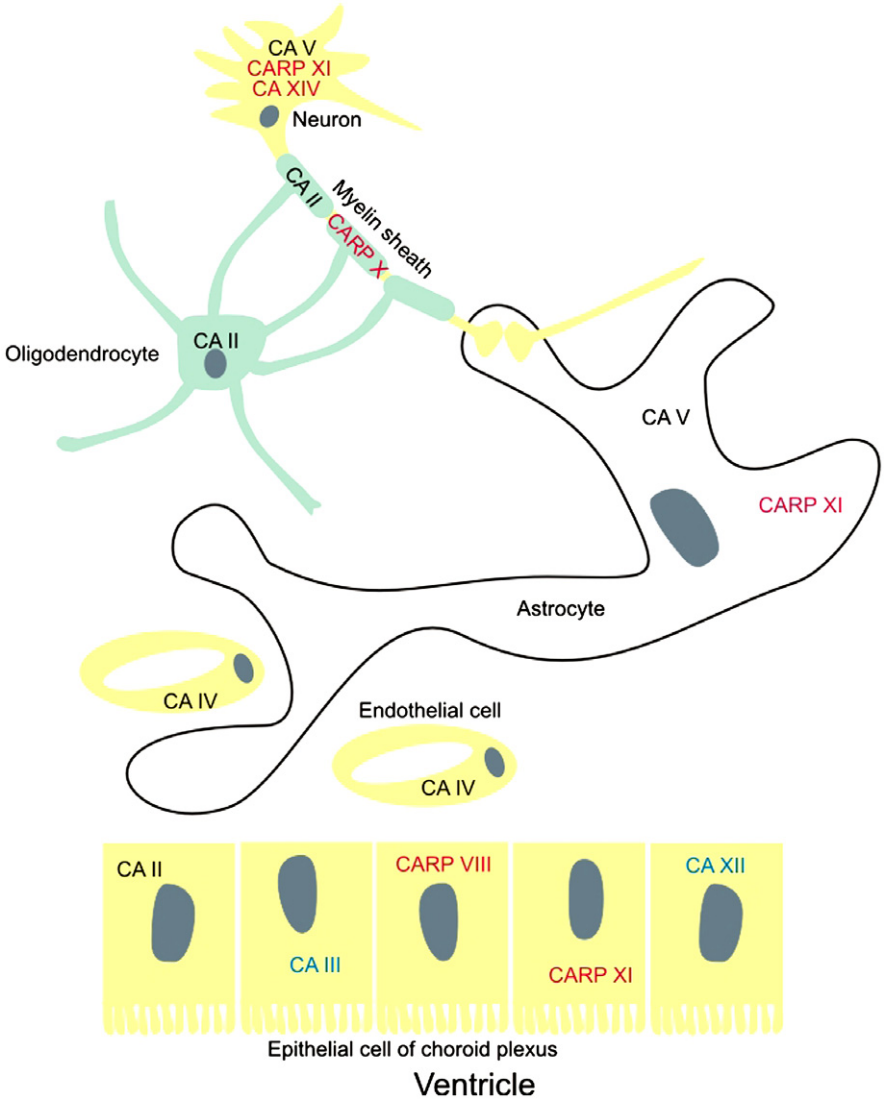
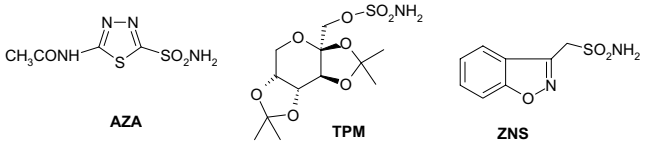


Figure 1. Schematic localization of CA isozymes in the choroid plexus within CNS. Epithelial cells of the choroid plexus possess CA II, III, XII, and CARP VIII and XI. CA IV is located in endothelial cells of blood vessels. Astrocytes express both CA V and CARP XI; CARP X is present in the myelin sheath. CA II is also expressed in oligodendrocytes. Neurons contain CA V, XIV, and CARP XI. The presence of CA III and CA XII was demonstrated in the rat, whereas CARP X, XI, and CA XIV were observed in the mouse. For all the other isozymes, experiments were done both in the rat and the mouse.[6–11] CA VII is only found in intrapyramidal neurons which have no projections in the choroid plexus.[11]

Acetazolamide AZA and structurally related sulfonamides known as potent CA inhibitors (CAIs) do show anticonvulsant activity in humans.[16,17] Consequently, acetazolamide was approved in 1953 for the treatment of epilepsy.[17] This drug is primarily used in combination therapy with other antiepileptic medications, being also employed in the management of partial, myoclonic, absence and primary generalized tonico–clonic seizures in refractory epilepsy.[1,17] However, CAI sulfonamide derivatives do not generally provide effective, long-term benefits to epileptic patients, leading to the development of tolerance and subsequent lack of clinical efficacy.[17] The recent discovery that some widely used, newer antiepileptic drugs such as topiramate TPM[18] and zonisamide ZNS[19] also act as effective inhibitors of several CA isozymes specifically expressed in the brain stimulated further research for the development of CAIs as novel anticonvulsants (possibly acting on refractory epilepsy), and characterized by fewer side-effects as compared to the conventional antiepileptic drugs.[20–22]



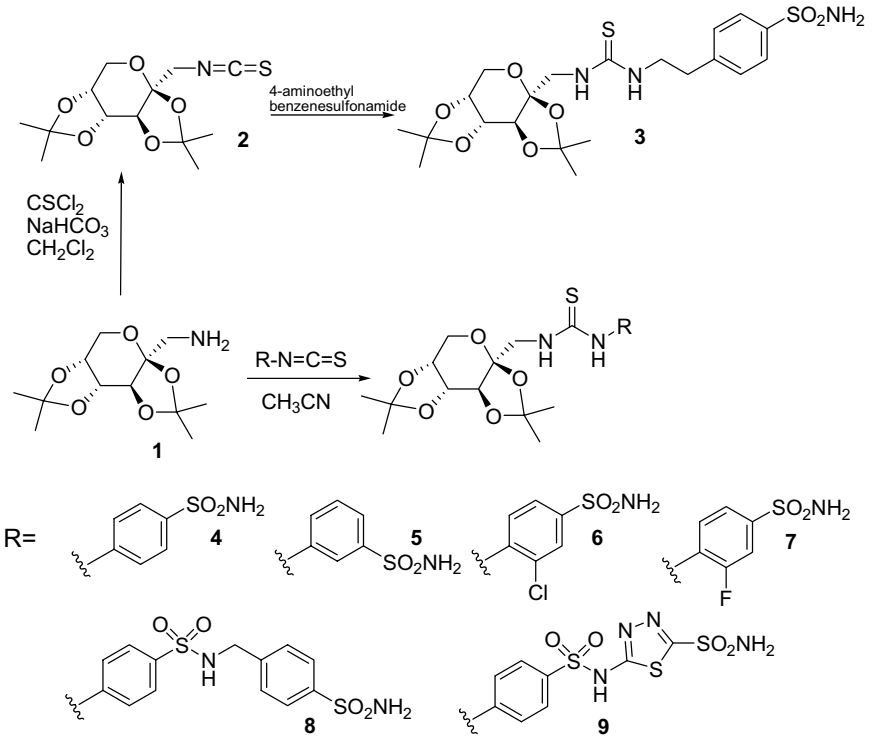
Considering the interesting CA inhibitory properties of topiramate[18] and its sulfamide analogue recently investigated by this group,[22] which is the first derivative showing decreased inhibition of the ubiquitous isozyme CA II (due to a clash between a methyl group of the inhibitor and the methyl of Ala65 from the CA II active site),[22] we report in this paper the synthesis of a series of derivatives incorporating the protected fructopyranose moiety present in topiramate. This tail has been attached to the

scaffold of aromatic/heterocyclic sulfonamides[23] by means of the thioureido functionality, shown earlier to lead to potent CAIs belonging to several classes.[24] The new derivatives reported here were thus designed for two purposes: (i) to evidence possible compounds with selectivity toward some isozymes with medicinal chemistry applications (such as among others CA II—the target of anticonvulsant and antiglaucoma drugs,[23] CA VA—the target of antiobesity CAIs,[15] CA VII—a possible target of anticonvulsants;[21a] CA IX—the validated target for the design of antitumor sulfonamide inhibitors,[25] or CA XIV,[26] an isoform whose physiologic functions are less understood, but which is abundant in the brain[1,2]); and (ii) to verify whether the presence of the derivatized fructopyranose tail (of TPM) present in these new CAIs may lead to possible anticonvulsant activity. In fact the mechanism of anticonvulsant action of topiramate is not well understood at this moment.[27] It is assumed that the drug shows a multifactorial mechanism, involving among others: blockade of Na^+ channels and kainate/AMPA receptors,[1] enhancement of GABAergic transmission,[27] and CA inhibition.[18,22] In fact topiramate (in micromolar concentrations) shows a positive modulatory effect on some types of GABA-A receptors, antagonizes kainate/AMPA receptors, and inhibits the generation of action potentials in neurons via antagonizing the activation of Na^+ channels.[27]

The thiourea derivatives reported here were prepared starting from the amine **1** obtained as previously described.[22] The amine **1** was either transformed to the corresponding isothiocyanate **2** (by reaction with thiophosgene)[28] and then converted to thiourea **3** by reaction with 4-aminoethylbenzenesulfonamide[28] (Scheme 1), or was reacted with a series of aromatic/heterocyclic sulfonamide isothiocyanates,[28] leading thus to thioureas **4–9** in good yields (Scheme 1).[29] All these derivatives were extensively characterized by NMR spectroscopy and MS, their structures being thus confirmed.[29]

Sulfonamides **3–9** reported in the present paper were assayed for the in vitro inhibition of seven physiologically relevant human CA (hCA) isoforms, that is, the cytosolic hCA I, II, and VII; the membrane-associated isozyme hCA IV, the mitochondrial one hCA VA, as well as the transmembrane isoforms hCA IX and XIV (Table 1). Inhibition data of the standard sulfonamide/sulfamate CA inhibitors acetazolamide and topiramate are also provided for comparison in Table 1.[30]

The following should be noted regarding the inhibition data of Table 1: (i) against the kinetically slow cytosolic isoform hCA I, all the sulfonamides **3–9** reported here showed a modest inhibitory activity, with K_Is in the range of 9.4–13.3 μM, being thus weaker inhibitors than AZA and TPM (K_Is of 250 nM). This behavior is typical for many sulfonamides possessing a rather bulky scaffold,[24b] and is due to the fact that the active site cavity of hCA I is smaller as compared to that of isozymes hCA II, VII, IX, and XII among others, because of the presence of two extra His residues (i.e., His200 and His67), characteristic only to this isozyme;[31] (ii) the physiologically ubiquitous and highly relevant isoform hCA II was on the other hand well inhibited by most of these compounds. Indeed, derivatives **3** and **6–9** were quite effective inhibitors, with K_Is in the range of 6–11 nM, similarly with the clinically used drug AZA



Scheme 1. Preparation of derivatives **2–9** described in the paper.

Table 1. Inhibition of cytosolic isozymes hCA I, II, and VII, membrane-bound isoform hCA IV, mitochondrial isoform hCA VA, and transmembrane isozymes hCA IX and hCA XIV with acetazolamide AZA, topiramate TPM, and the new sulfonamides **3–9**

Isozymes	K_I^* (nM)								
	AZA	TPM	**3**	**4**	**5**	**6**	**7**	**8**	**9**
hCA I[a]	250	250	10700	11000	10200	12300	11000	13300	9400
hCA II[a]	12	10	10	62	750	11	6	8	9
hCA IV[b]	74	4900	850	10	30700	1040	43700	670	89300
hCA VA[c]	63	63	39800	43000	122	940	710	1040	850
hCA VII[a]	2.5	0.9	24	41	79	18	10	23	25
hCA IX[d]	25	1590	10	82	4500	134	110	11	76
hCA XIV[c]	41	1460	2350	1320	3500	630	540	800	21

[a] Human, recombinant isozymes.
[b] Human truncated (−20 aminoterminal residues) recombinant isozyme.
[c] Full length human recombinant isozyme.
[d] Catalytic domain of human, cloned isoform.[20–26]
[*] Errors in the range of 5–10% of the shown data, from three different assays, by a CO_2 hydration stopped-flow assay.[30]

and TPM (K_Is of 10–12 nM), one compound (**4**) was a moderate inhibitor (K_I of 62 nM), whereas derivative **5** was a weak inhibitor (K_I of 750 nM). SAR is thus rather clear: the sulfanilamide (in **4**) and metanilamide (in **5**) heads to which the topiramate–thioureido tail has been attached lead to moderate-weak inhibitors, whereas all other derivatives incorporating halogenosulfanilamide (**6** and **7**), 4-aminoethylbenzenesulfonamide (**3**) or sulfanylated–sulfonamide (in **8** and **9**) heads led to a rather similar behavior, of very potent inhibitors. Topiramate is known to bind in a compact manner to the hCA II active site,[18] coordinating to the Zn(II) ion by means of the deprotonated sulfamate nitrogen, and making an extended network of seven hydrogen bonds with amino acid residues Asn62, Gln92, Thr199 and Thr200, and several water molecules (as well as many favorable van der Waals contacts with various amino acid residues from the active site). In this way, the entire bottom of the active site cavity of hCA II is filled by the inhibitor molecule.[18] Derivatives **3–9** reported here possess the derivatized fructopyranose tail of topiramate connected to the sulfonamide scaffold in such a way that the interactions mentioned above cannot occur. However, probably other favorable binding modes can be assured between the inhibitor scaffold and the enzyme active site,[22] explaining thus the very good inhibitory activity manifested by most of these compounds (i.e., derivatives **3** and **6–9**). On the other hand, the metanilamide head is known to generally lead to weaker hCA II inhibitors by the tail approach derivatization,[23] as compared to other *para*-substituted-benzenesulfonamide derivatives, probably due to steric constraints related to the *meta* position between the sulfonamide moiety and the remaining part of the organic scaffold; (iii) against the membrane-associated isoform hCA IV, only one compound, the sulfanilamide derivative **4**, showed very effective inhibition, with a K_I of 10 nM, whereas all other compounds investigated here were ineffective inhibitors, with K_Is in the range of 670 nM–43.7 μM. It has been in fact shown by us earlier that hCA IV is less inhibited by many clinically used sulfonamides/sulfamates as compared to the corresponding bovine isozyme, bCA IV.[32] However, we must note that compound **4** is the first derivative ever reported—as far as we know—showing selectivity for inhibiting hCA IV over the sulfonamide-avid isozyme hCA II. Indeed, the selectivity ratio (i.e., the ratio of the corresponding inhibition constants) of this compound for the inhibition of the membrane-bound isoform IV over the cytosolic isozyme II is of 6.2 (that of acetazolamide is of 0.16 and of topiramate of 0.002)—Table 1. This compound (see discussion later in the text) is a moderate inhibitor of hCA VII (and II), it inhibits rather well CA IX, being a weak or very weak inhibitor of isozymes I, VA, and XIV; (iv) the mitochondrial isozyme hCA VA was also not well inhibited by the new derivatives reported here (K_Is in the range of 122 nM–43 μM) as compared to acetazolamide and topiramate which acted as good inhibitors (K_I of 63 nM). Surprisingly, the best hCA VA inhibitor was just the metanilamide derivative **5** (K_I of 122 nM) which behaved as a weak inhibitor of all other isoforms (except hCA VII, see later in the text); (v) the brain-specific cytosolic isozyme hCA VII was shown previously by us to be highly susceptible to inhibition by aromatic/heterocyclic sulfonamides and by the sulfamate topiramate.[21a] The sulfonamides **3–9** reported here are no exception to this rule, showing inhibition constants in the range of 10–79 nM, being thus slightly less effective than acetazolamide and especially topiramate (a subnanomolar hCA VII inhibitor).[21a] It must be observed that similarly to hCA II, this isoform is also well inhibited by most of these new derivatives, the best inhibitor being the halogenosulfanilamide derivatives **6** and **7**, whereas the weakest one being the metanilamide derivative **5**. However, the differences of inhibitory capacity between these sulfonamides are much less accentuated as compared to the case of hCA II, since the selectivity ratio between the worst and the best inhibitor was of 7.9 for hCA VII, and of 125 for hCA II (Table 1); (vi) for the tumor-associated transmembrane isozyme hCA IX, a more diverse behavior of these new sulfonamides has been observed. Thus, compounds **3** and **8** acted as very efficient inhibitors (K_Is of 10–11 nM), being thus much more effective than the clinically used drugs AZA and TPM. Derivatives **4** and **9** showed also effective inhibition (K_Is of 76–82 nM), **6** was a moderate inhibitor (K_I of 134 nM), whereas **5**, similarly to topiramate, a quite weak hCA IX inhibitor (K_Is of 1590–4500 nM); (vii) against the transmembrane isoform hCA XIV again only one compound (**9**) showed effective inhibitory

power (K_I of 21 nM, stronger than acetazolamide), whereas all the new derivatives **3–8**, similarly to topiramate, behaved as weak inhibitors (K_Is in the range of 540–2350 nM) (Table 1). It may be thus stated that the first goal of our research, that is, detection of compounds with interesting activity and possibly selectivity for some CA isozymes, has been fulfilled, since some of these sulfonamides possess indeed a unique inhibition profile against the seven physiologically relevant investigated CA isozymes.

As topiramate was discovered by means of a maximal electroshock seizure (MES) test,[27a] we decided to investigate compounds **3–9** reported here for their possible anticonvulsant effects in mice (Table 2).[33] The standard drug topiramate TPM as well as two other widely used antiepileptics possessing different mechanisms of action as compared to topiramate, that is, phenytoine and carbamazepine, were also included in these tests for comparison.

Data of Table 2 show that only one compound of the small library of derivatives reported here possesses significant anticonvulsant effects in mice. Indeed, the fluorosulfanilamide derivative **7** was more effective in providing protection in the MES test than topiramate (87.5% for **7**, vs 68.75% for TPM) at a dose of 50 mg/kg, the only other derivative with some activity being **8** (20% protection). All other new compounds reported in the paper were devoid of anticonvulsant activity, whereas the clinically used drugs phenytoine and carbamazepine provided a 100% protection from electroshock at a dose of 30 mg/kg. It may be observed that **7** is the best CA VII inhibitor (K_I of 10 nM) after TPM (and acetazolamide), being also an excellent CA II inhibitor (K_I of 6 nM).

In conclusion, a series of aromatic/heterocyclic sulfonamides incorporating 1-thioureido-2,3:4,5-bis-*O*-(isopropylidene)-β-D-fructopyranosyl moieties has been synthesized by reacting sulfonamide isothiocyanates with the amino precursor of topiramate. These derivatives were assayed for the inhibition of seven human isoforms of the zinc enzyme CA. The new derivatives behaved as weak hCA I inhibitors (K_Is of 9.4–13.3 μM), were efficient CA II inhibitors (K_Is of 6–750 nM), and slightly inhibited isoforms hCA IV and hCA VA. Only the sulfanilamide derivative showed efficient and selective inhibition of CA IV (K_I of 10 nM). These derivatives also showed excellent hCA VII inhibitory activity (K_Is of 10–79 nM), being less efficient as inhibitors of the transmembrane isoforms hCA IX (K_Is of 10–4500 nM) and hCA XIV (K_Is of 21–3500 nM). Two of the new compounds showed anticonvulsant action in a maximal electroshock seizure test in mice, with the fluorosulfanilamide derivative being a more efficient anticonvulsant than the antiepileptic drug topiramate.

Table 2. Anticonvulsant results of several antiepileptic drugs (phenytoine, carbamazepine, and topiramate) and compounds **3–9** reported in the paper, in mice, evaluated by the maximal electroshock seizures (MES) test[33]

Compound[a,b]	% of protected mice (n = 8)	
Vehicle	0	(0/8)
Phenytoine[c]	100	(8/8)
Carbamazepine[c]	100	(8/8)
TPM[d]	68.75	(6/8)
3[d]	0	(0/8)
4[d]	0	(0/8)
5[d]	0	(0/8)
6[d]	0	(0/8)
7[d]	87.5	(7/8)
8[d]	20	(1/5)
9[d]	0	(0/8)

[a] All test compounds were suspended in an aqueous solution of 1% Tween 80.
[b] The maximal electroshock test was carried out 2 h after ip administration of the test compound.
[c] At 30 mg/kg.
[d] At 50 mg/kg.

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29. 1-[(4-Sulfamoylphenylethyl)-thioureido]-2,3:4,5-bis-O-(isopropylidene)-*β*-D-fructo pyranose **(3)**: ^{1}H NMR (400 MHz, DMSO d^6)δ 1.27/1.30/1.38/1.44 (4s, 12H), 2.87 (s, 2H), 3.38 (t, 2H), 3.76 (m, 4H), 4.26 (dd, 2H), 4.54 (d, 1H), 7.30 (s, 2H), 7.48 (d, 4H), 7.75 (d, 2H). MS, ESI^+ *m/z* 502 $(M+H)^+$; 524 $(M+Na)^+$; 540 $(M+K)^+$.
1-[(4-Sulfamoylphenyl)-thioureido]-2,3:4,5-bis-*O*-(isopropylidene)-β-D-fructopyranose **(4)**: ^{1}H NMR (400 MHz, DMSO d^6) δ 1.28/1.36/1.47 (3s, 12H), 3.7 (dd, 2H), 3.9 (m, 2H), 4.25 (d, 1H), 4.40 (s, 1H), 4.56 (d, 1H), 7.27 (s, 2H), 7.74 (m, 4H), 7.94 (s, 1H), 9.97 (s, 1H). MS, ESI^+ *m/z* 474 $(M+H)^+$, 496 $(M+Na)^+$; ESI^- *m/z* 472 $(M-H)^-$.
1-[(3-Sulfamoylphenyl)-thioureido]-2,3:4,5-bis-*O*-(isopropylidene)-β-D-fructopyranose **(5)**: ^{1}H NMR (400 MHz, DMSO d^6) 1.28/1.37/1.47 (3 s, 12H), 3.41 (dd, 2H), 3.9 (m, 2H), 4.24 (d, 1H), 4.40 (s, 1H), 4.56 (d, 1H), 7.38 (s, 2H), 7.5 (m, 2H), 7.81 (m, 2H), 8.05 (s, 1H), 9.88 (s, 1H). MS ESI^+ *m/z* 474 $(M+H)^+$, 496 $(M+Na)^+$, 512 $(M+K)^+$.
1-[(2-Chloro-4-sulfamoylphenyl)-thioureido]-2,3:4,5-bis-*O*-(isopropylidene)-β-D-fructopyranose **(6)**: ^{1}H NMR (400 MHz, DMSO d^6) δ 1.28/1.38/1.39/1.48 (4s, 12H), 3.7 (dd, 2H), 3.9 (dd, 2H), 4.25 (d, 1H), 4.4 (d, 1H), 4.5 (d, 1H), 7.6 (m, 1H), 7.7 (dd, 1H), 7.8 (m, 1H), 8.23 (m, 1H), 8.6 (m, 1H), 9.64 (s, 1H). MS, ESI^+ *m/z* 508 $(M+H)^+$, 530 $(M+Na)^+$; ESI^- *m/z* 506 $(M-H)^-$.
1-[(2-Fluoro-4-sulfamoylphenyl)-thioureido]-2,3:4,5-bis-*O*-(isopropylidene)-β-D-fructopyranose **(7)**: ^{1}H NMR (400 MHz, DMSO d^6) δ 1.28/1.37/1.38/1.48 (4s, 12H), 3.7 (dd, 1H), 3.9 (dd, 1H), 4.25 (d, 1H), 4.40 (s, 1H), 4.57 (d, 1H), 7.43 (s, 2H), 7.60 (t, 2H), 8.38 (m, 2H), 9.64 (s, 1H). MS, ESI^+ *m/z* 492 $(M+H)^+$, 514 $(M+Na)^+$; ESI^- *m/z* 490 $(M-H)^-$.
1-[(4-(4-Sulfamoyl-benzylsulfamoyl)-phenyl)-thioureido]-2,3:4,5-bis-*O*-(isopropylidene)-β-D-fructopyranose **(8)**: ^{1}H NMR (400 MHz, DMSO d^6) δ 1.28/1.37/1.48 (3s, 12H), 3.7 (dd, 2H), 3.9 (dd, 2H), 4 (s, s, 2H), 4.26 (d, 1H), 4.40 (d, 1H), 4.5 (d, 1H), 7.3 (s, 2H), 7.7 (m, 4H), 7.8 (m, 4H), 8 (m, 1H), 8.1 (t, 1H), 10 (s, 1H). MS, ESI^+ *m/z* 643 $(M+H)^+$; 665 $(M+Na)^+$, ESI^- *m/z* 641 $(M-H)^-$.
1-[(4-(5-Sulfamoyl-[1,3,4]thiadiazol-2-ylsulfamoyl)-phenyl)-thioureido-2, 3:4,5-bis-*O*-(isopropylidene)-β-D-fructopyranose **(9)**: ^{1}H NMR (400 MHz, DMSOd^6) δ 1.26/1.35/1.46 (3 s, 12H), 3.66 (dd, 2H), 3.9 (m, 2H), 4.24 (d, 1H), 4.4 (d, 1H), 4.55 (d, 1H), 7.40 (d, 2H), 7.6 (m, 2H), 7.7 (d, 2H), 7.8 (m, 2H), 9.8 (m, 1H). MS, ESI^+ *m/z* 637 $(M+H)^+$; 659 $(M+Na)^+$; 675 $(M+K)^+$.
30. Khalifah, R. G. *J. Biol. Chem.* **1971**, *246*, 2561, An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity. Phenol red (at a concentration of 0.2 mM) has been 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 constant the ionic strength), following the CA-catalyzed CO_2 hydration reaction for a period of 5–30 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water/DMSO (3:1, v/v) and dilutions up to 0.1 nM were done thereafter with distilled-deionized water. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E–I complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3, from Lineweaver–Burk plots, as reported earlier,[22] and represent the mean from at least three different determinations. All recombinant enzymes were obtained as described earlier.[20–26,31,32]
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32. Innocenti, A.; Firnges, M. A.; Antel, J.; Wurl, M.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1149.
33. The compounds were tested for their anticonvulsant activity by the maximal electroshock seizure (MES) test. The experiments were carried out on male OF1 mice (weighing 28–40 g, from Charles River Laboratories, Belgium) after at least one-week acclimatization. All experimental procedures applied in this study were conducted at the University of Liège (Belgium) and were approved by the Ethics Committee of the University of Liège. The animals were housed under standard laboratory conditions (ambient temperature of 20 °C, natural light–dark cycle). 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 (carbamazepine, phenytoine, and topiramate) were suspended in an aqueous solution of 1% Tween 80 (Acros Organics) administered intraperitoneally (ip) 2 h before the stimulation in a standard volume of 3mL/kg at 30 mg/kg body weight dose for phenytoine and carbamazepine and at 50 mg/kg body weight dose for the other compounds. Control animals received appropriated volumes of the solvent. Carbamazepine and phenytoine were purchased from Acros Organics. Topiramate was purchased from Sigma. The electroconvulsions were produced by a Hugo Sachs generator (15 mA, 50 Hz, 500 V, 200 ms, Rodent Shocker Type-221, Feiburg, Germany) and delivered via saline moistened eye electrodes. A drop of Unicaine (oxybuprocaine HCl 4 mg/mL, Théa Pharma, Belgium) is instilled in the eye prior to application of the electrodes in order to induce local anesthesia and ensure a good conductivity of the electroshock current. Abolition of the hind-leg tonic extension component of the seizure is defined as protection.