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Lead Development of Thiazolylsulfonamides with Carbonic Anhydrase Inhibitory Action

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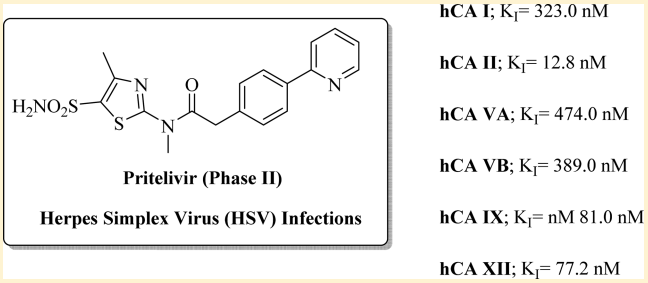
Supporting Information

ABSTRACT: A series of congeners structurally related to pritelivir, *N*-[5-(aminosulfonyl)-4-methyl-1,3-thiazol-2-yl]-*N*-methyl-2-[4-(2-pyridinyl)phenyl]acetamide, a helicase-primase inhibitor for the treatment of herpes simplex virus infections, was prepared. The synthesized primary and secondary sulfonamides were investigated as inhibitors of six physiologically and pharmacologically relevant human (h) carbonic anhydrase (hCA, EC 4.2.1.1) isoforms, the cytosolic enzymes hCA I and II, the mitochondrial ones hCA VA and VB, and the transmembrane, tumor associated hCA IX and XII. Low nanomolar inhibition K_I values were detected for all of them, with a very interesting and well-defined structure−activity relationship. As many CAs are involved in serious pathologies, among which are cancer, obesity, epilepsy, glaucoma, etc., sulfonamide inhibitors as those reported here may be of interest as drug candidates. Furthermore, pritelivir itself is an effective inhibitor of some CAs, also inhibiting whole blood enzymes from several mammalian species, which may be a favorable pharmacokinetic feature of the drug which can be transported throughout the body bound to blood CA I and II.



INTRODUCTION

Pritelivir, (*N*-[5-(aminosulfonyl)-4-methyl-1,3-thiazol-2-yl]-*N*-methyl-2-[4-(2-pyridinyl)phenyl]acetamide), previously known as BAY 57-1293 and AIC316 (Figure 1),[1] is an antiviral agent in phase II clinical development, useful for the treatment of herpes simplex virus (HSV) infections.[1−3] Its mechanism of action is totally different from that of other antiherpetic agents such as acyclovir, penciclovir, and other nucleoside analogs (which inhibit the herpesviral DNA polymerase after becoming activated by the viral thymidine kinase), as pritelivir acts as a helicase-primase inhibitor that does not need to become activated.[1−3] The phase I and II clinical trials were promising, with the compound being well tolerated and effective showing superiority over placebo and the nucleoside analog valacyclovir.[2,3]

One of the interesting features of pritelivir is that the compound incorporates a primary sulfonamide moiety not present in any other antiviral agent. However, this functionality is well-known for its affinity for the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1),[4−10] with many aromatic, heterocylic, aliphatic, and sugar derivatives incorporating this moiety, acting as highly effective CA inhibitors (CAIs).[11−15] In line with this, initial activity on carbonic anhydrase in the micromolar range has been reported previously for pritelivir by means of a carbonic anhydrase catalyzed CO_2 hydration assay.[1]

There are six genetic families encoding CAs in virtually all organisms known to date, the α-, β-, γ-, δ-, ζ-, and η-CAs.[6−9] All CAs known so far are metal-ion-dependent enzymes, with a metal hydroxide species within the enzyme cavity acting as a nucleophile in the catalytic cycle and a second step (usually rate-determining) involving a proton transfer reaction from a water molecule coordinated to the active site metal ion to the environment, for regenerating the nucleophile.[4,7−10] Metal ions employed at the active site of the different CAs include Zn(II) (in all classes), Cd(II) (in ζ-CAs), Co(II) (in the δ class), or Fe(II) (for γ-CAs, in anaerobic conditions).[4] This ping-pong mechanism makes some of the members of the CA superfamily among the most effective enzymes known in nature, with k_{cat}/K_M values close to the limit of the diffusion-controlled processes.[10,11]

The CAs possess crucial physiologic functions, as the reaction products obtained from the hydration of CO_2 are involved in pH regulation (bicarbonate and protons) but also in many biosynthetic processes (lipogenesis, ureagenesis, gluconeogenesis) or in other important phenomena such as chemosensing,[4] sexual development (in pathogenic fungi),[8] pH and CO_2-sensing, pathogenicity, and survival in ambient air of many bacteria, fungi, and/or protozoa.[7−10]

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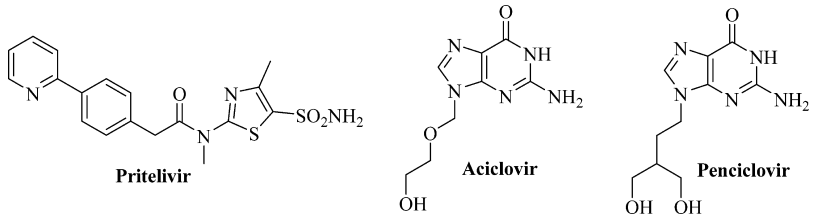

Figure 1. Chemical structures of the anti-HSV helicase-primase inhibitor pritelivir and herpesviral DNA polymerase inhibitors acyclovir and penciclovir.

In humans, 15 α-CAs isoforms are known to date, CA I to CA VA, CA VB, CA VI to CA XIV, with 12 of them being catalytically active and three (CA VIII, X and XI) devoid of activity but still playing significant functions in tumorigenesis and other physiologic as well as pathologic processes.[4]

As CO_2, bicarbonate, and protons are simple molecules/ions involved in a host of physiologic processes, some of which are briefly mentioned above, their up- or down-regulation is associated with a range of diseases.[12−15] Indeed, the CAIs of the primary sulfonamide type (but many other chemotypes were reported, such as the coumarins,[16] sulfocoumarins,[17] mono- and dithiocarbamates,[18] etc.) are clinically used for decades as diuretics,[4,11] antiepileptics,[12] antiobesity agents,[13] antiglaucoma drugs,[4,15] or more recently antitumor agents, with one such compound in clinical development for the treatment of hypoxic, metastatic tumors.[4,19] Although many new chemotypes with CA inhibitory properties and with various mechanisms of actions were reported in the past 10 years,[16−18] the sulfonamides remain the most investigated class of such compounds with many interesting representatives being reported constantly.[19,20]

One of the main hurdles connected with the use of CAIs in the treatment of diverse conditions as those mentioned above is related to the off-target inhibition of isoforms other than the desired one.[4] In fact the various pharmacological applications of the CAIs are due to the high number of isoforms and their involvement in different pathologies.[11−15]

RESULTS AND DISCUSSION

Compound Design and Synthesis. Many sulfonamide CAIs incorporate an elongated scaffold which contains a five-membered heterocyclic ring on which the SO_2NH_2 zinc binding group moiety and a tail are attached on the two sides of the cycle in such a way that the tail extends as much as possible within the enzyme active site and makes interactions with amino acid residues at the entrance of the cavity, which are the least conserved residues among the many mammalian isoforms known.[4−11,21] Most of the time the five-membered heterocyclic ring was an 1,3,4-thiadiazole,[21a,b] a thiophene,[21c] and rarely a thiazole nucleus.[21d] This type of scaffold leads to a multitude of contacts between the inhibitor and the enzyme, as shown schematically in Figure 2 for 5-[1-(naphthalen-1-yl)-1*H*-1,2,3-triazol-4-yl]thiophene-2-sulfonamide bound to human (h) isoform hCA II, as determined by X-ray crystallography.[21c] The sulfamoyl zinc binding group (ZBG) is observed bound to the catalytically crucial Zn(II) ion, whereas the organic scaffold of the inhibitor is in contact with many amino acid residues involved in inhibitor binding, such as Phe131, Val135, Leu204, Pro202, extending throughout the cavity.[21c]

Such a binding for sulfonamide CAIs with elongated scaffolds affords the possibility of obtaining compounds with very high affinities for the enzyme (usually low nanomolar or subnanomolar), and also many cases of isoform-selective inhibitors were detected in this way.[19−21] Considering that pritelivir (Figure 1) also has this type of scaffold, with the thiazole ring substituted with the sulfonamide ZBG and the (2-pyridinyl)phenyl]acetamide fragment at positions 5 and 2, respectively, we decided to first confirm the CA inhibitory properties of this primary sulfonamide in our laboratory and thereafter to use the molecule as a lead for obtaining new sulfonamide CAIs.

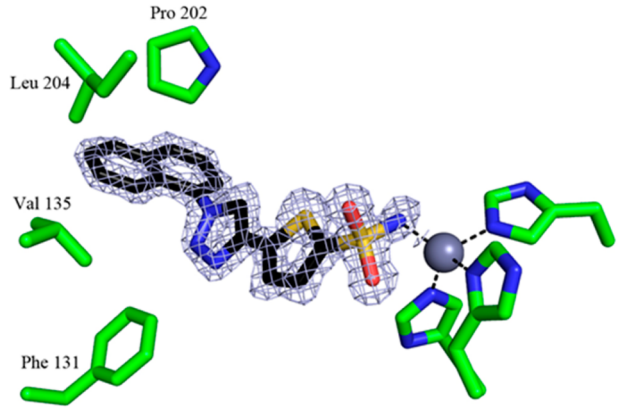

Figure 2. Binding of a thiophene-2-sulfonamide derivative to hCA II as determined by X-ray crystallography (PDB file 4BF6).[21c]

In Schemes 1 and 2 the synthetic strategy to obtain a series of thiazolyl-5-sulfonamides (including pritelivir) is shown.

2-Chlorothiazoles **1a**,**b** were transformed to the corresponding sulfonyl chlorides **2a**,**b**. Reaction of **2a**,**b** with ammonia or primary amines led to sulfonamides **3a**−**e**, which were thereafter reacted again with amines in order to obtain the 2-aminothiazole-5-sulfonamide derivatives **4a**−**h**, incorporating a series of different substituents at the 2-amino-, thiazole-4-position and *N*-sulfonamide fragments of the molecule, in order to generate chemical diversity (Scheme 1). Derivative **4a**, incorporating a 4-methyl moiety at the thiazole ring, was converted into the corresponding 4-hydroxymethylene derivative **6** via monobromination with *N*-bromosuccinimide, followed by displacement of the bromide **5** with water, thus leading to the desired 2-aminomethyl-5-sulfonamidethiazole **6** incorporating the hydroxymethylene group at position **4** (Scheme 1).

In order to obtain the pritelivir-like compounds, the intermediates **4a**−**h** obtained as shown in Scheme 1 were coupled with 4-substituted phenylacetic acids **7a**−**i** in the presence of carbodiimides leading to sulfonamides **8a**−**t** (derivative **8b** is pritelivir). The chemical diversity was achieved by varying the nature of the aromatic/heterocyclic moieties which substitute the phenylacetic ring **7** at position 4, with phenyl, 2-pyridyl, pyridinium, pyrazyl, pyrazoyl, and sub-

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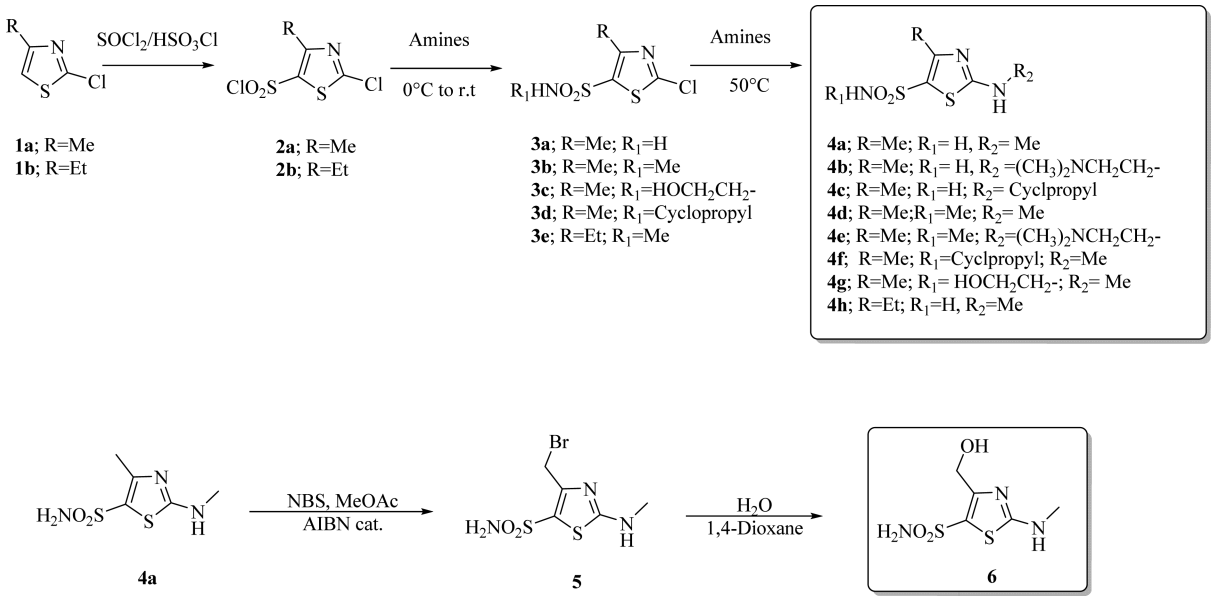
Scheme 1. Synthesis of Sulfonamides 4a−h and 6



stituted-phenyl groups included in the study (Scheme 2).[1b,23,24] The 4-ethyl (**8s**) and 4-hydroxymethyl (**8t**) analogs of pritelivir (which has a 4-methyl for such moiety) were also prepared as depicted in Scheme 2.

All compounds were properly characterized by spectroscopic methods which confirmed their structure (^{1}H NMR, ^{13}C NMR, MS, and IR). Purity was controlled by HPLC. All compounds reported here were >98% pure.

Carbonic Anhydrase Inhibition. Sulfonamide reported here were tested in vitro for their inhibition profiles against six physiologically relevant hCA enzymes,[25] the cytosolic isoforms I and II, the mitochondrial ones hCA VA and VB, and the transmembrane, tumor associated hCA IX and XII (Table 1).

The following structure−activity relationship (SAR) can be drawn by considering data of Table 1. It should be stressed from the beginning that most of these derivatives are primary sulfonamides (**8a−l**, **8s**, and **8t**), four derivatives incorporate the SO_2NHMe moiety (**8m−p**), whereas **8q** and **8r** possess bulkier substituents at the sulfamoyl nitrogen (see Scheme 2).

(I) The slow cytosolic isoform hCA I, widely present in the blood, gastrointestinal tract, and many other tissues in humans,[4−11] was effectively inhibited by sulfonamides **8** investigated here, with K_I values ranging between 26.6 and 543 nM (Table 1). Most of these sulfonamides were thus more effective hCA I inhibitors compared to acetazolamide (AAZ, 5-acetamido-1,3,4-thiadiazolesulfonamide), a clinically used drug (K_I of 250 nM).[4] The nature of the Het moiety present in the final part of the tail seems to be the most important factor influencing activity. For example, the phenyl-substituted compound **8a** is a 12 times more effective hCA I inhibitor compared to pritelivir **8b** which has a 2-pyridyl moiety instead of the phenyl one. Thus, the replacement of one CH group by an N atom leads to important changes in the inhibitory activity, a phenomenon already documented by us for many classes of CAIs by means of kinetic and X-ray crystallographic studies.[15−21] Compact R2 moieties (Me and cyclopropyl) led to better hCA I inhibition in compounds incorporating them (e.g., **8h−l**) compared to the derivative incorporating a bulkier such group (**8g**). There are no major differences of activity between primary and secondary sulfonamides, and also the nature of the group in position 4 of the thiazole (methyl, ethyl, or hydroxyethyl) does not influence much the inhibitory power of these sulfonamides (Table 1).

(II) The physiologically dominant isoform hCA II, widely spread all over the body and a drug target for diuretic and antiglaucoma agents,[4,11,15] was also potently inhibited by most sulfonamides **8** investigated here, with K_I values ranging between 0.9 and 341 nM (Table 1). Several compounds were much more effective than AAZ as hCA II inhibitors (e.g., **8a**, **8i**, **8s**, K_I values ranging between 0.9 and 4.9 nM versus 12.1 nM for AAZ), whereas pritelivir **8b** and many of its congeners (**8c**, **8d**, **8l**, **8t**) showed comparable (K_I values of 10.0−32.9 nM) or slightly weaker (**8g**, **8h**, **8m**, **8n**, **8p**, **8r**) activity compared to the standard drug (Table 1). SAR is rather similar to what was stressed above for hCA I inhibition, with the nature of the Het fragment of the molecule being the most important structural feature influencing activity. Again phenyl and substituted phenyl (**8a** and **8i**) seem to be more effective than the heterocyclic such moieties, except for **8s**, the case in which the 4-Et instead of 4-Me leads to 12.8 times better activity of **8s** compared to pritelivir **8b** (the two compounds differ only by one CH_2 group).

(III) The mitochondrial isoform hCA VA was also effectively inhibited by most sulfonamides reported here, which showed K_I values ranging between 29.0 and 816 nM (Table 1). It should be mentioned that hCA VA (and probably also the second mitochondrial isoform hCA VB) are drug targets for antiobesity agents.[13] The only compound in clinical use for treating obesity based on CAIs is topiramate, an antiepileptic for which this second use was approved recently.[13] Several sulfonamides reported here, such as **8a**, **8e**, **8f**, **8g**, **8j**, **8l**, **8n**, **8o**, **8s**, and **8t** showed better or comparable hCA VA inhibitory activity compared to AAZ, with K_I values ranging between 29.0 and 64.8 nM, being thus of considerable interest as antiobesity drug candidates. Pritelivir and some of its congeners (**8b−d**, **8k**) showed weaker hCA VA inhibitory activity, with K_I values ranging between 354 and 816 nM (Table 1). SAR is more complicated for the inhibition of this isoform, and it is interesting to note that the most effective inhibitor was **8g** which incorporates a bulky R2 moiety, which was detrimental

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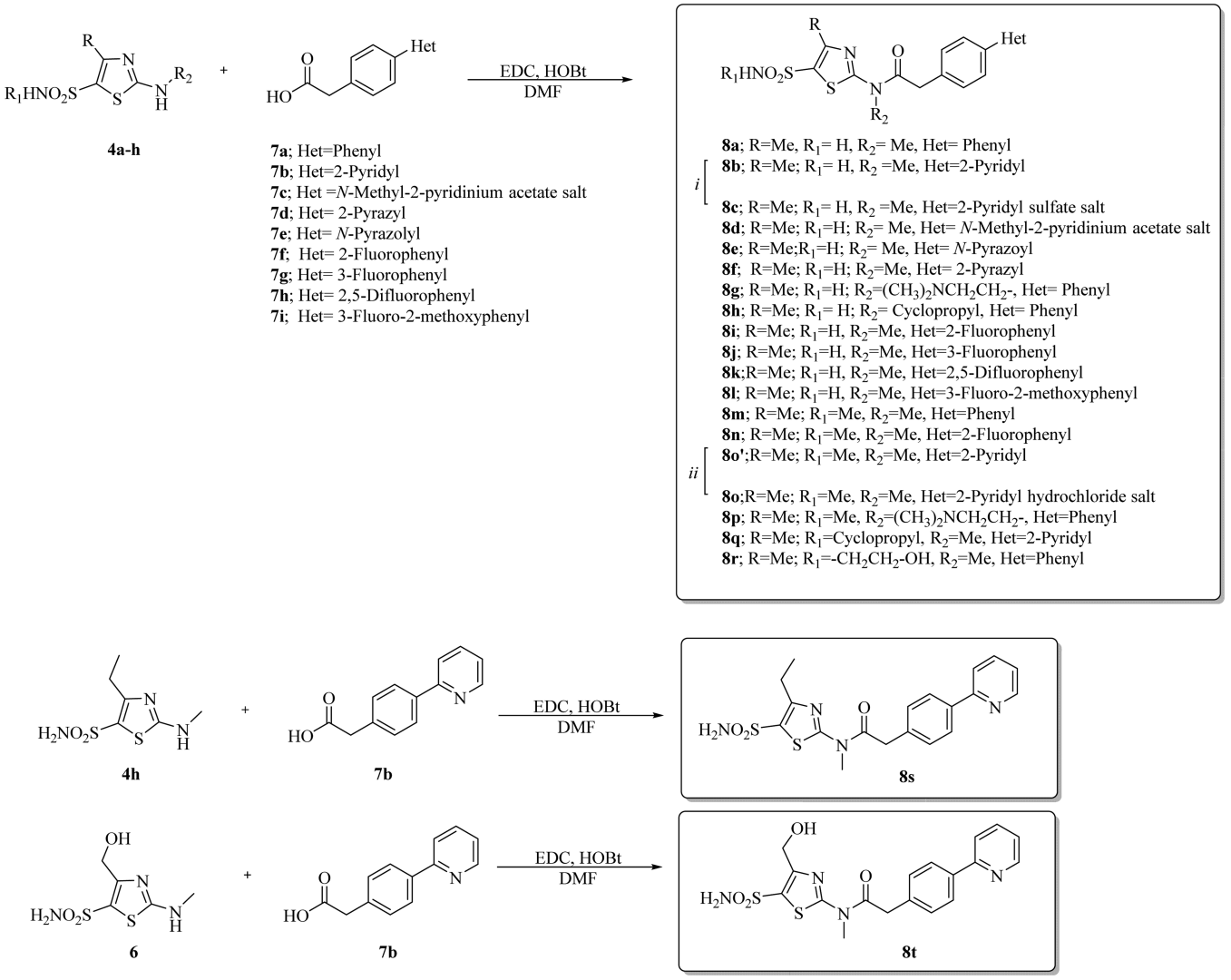
Scheme 2. Synthesis of Sulfonamides 8a–t[a]



[a]Reagents: (i) H_2SO_4 2% aq; (ii) 1.25 M HCl/MeOH.

to inhibition of isoforms hCA I and II. Other structural aspects which lead to effective inhibition (e.g., the nature of the Het and R1 moietiess from inhibitors **8**) are similar to what discussed above for their inhibition of hCA I and II.

(IV) The second mitochondrial isoform, hCA VB, was more sensitive to inhibition with sulfonamides **8** compared to hCA VA, showing K_I values in the range of 23.8–441 nM. Many of these sulfonamides were better or comparable hCA VB inhibitors compared to AAZ, among which are **8a**, **8e–g**, **8j**, **8l**, **8o–s** (K_I values ranging between 23.8 and 58.2 nM). Pritelivir **8b** and its congeners **8c** and **8k** were the least effective hCA VB inhibitors with inhibition constants of 251–441 nM (Table 1).

(V) The tumor associated isoform hCA IX, a validated antitumor drug target[4,14] was effectively inhibited by sulfonamides **8**, with K_I values ranging between 0.9 and 464 nM (Table 1). Only two compounds (**8c** and **8d**) showed K_I > 100 nM, whereas the remaining ones were highly effective hCA IX inhibitors (pritelivir is one of the least effective in the group of good inhibitors, with a K_I of 81.0 nM; see Table 1). Again the most important structural feature influencing activity is the nature of the Het moiety at the terminal part of the tail, with pyridyl (as sulfate, thus probably pyridinium) and *N*-methylpyridinium (in **8c** and **8d**) leading to the least effective inhibitors. The best substitution patterns in this part of the molecule include the Ph (**8a**, **8h**) and 3-fluorophenyl (**8j**) moieties. Again primary and secondary sulfonamides showed similar inhibitory properties, and the nature of the group in position 4 of the thiazole ring was not very influential for the inhibitory activity (Table 1).

(VI) Powerful inhibitory activity was registered also against hCA XII, a second transmembrane isoform investigated here (target for antiglaucoma and anticancer agents)[4,14,15] with sulfonamides **8** showing K_I values ranging between 3.1 and 77.2 nM. Pritelivir **8b** was the least effective hCA XII inhibitor in the series, but several of its congeners (**8a**, **8h**, **8j**, **8l**) have a K_I of 3.1–5.5 nM, being highly effective inhibitors of this isoform. SAR is similar to what was discussed above for hCA IX inhibition, but it is interesting to note that the most effective hCA XII inhibitor possesses the cyclopropyl moiety as R_2 group (Table 1 and Scheme 2).

Since mammalian blood is very rich in CAs (mainly isoforms I and II),[4b] we also investigated the inhibition of whole blood

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Table 1. CA Inhibition Data against Isoforms Human Carbonic Anhydrases (hCA) I, II, VA, VB, IX, and XII with Compounds 8a–t and Acetazolamide (AAZ) as Standard, by a Stopped-Flow CO_2 Hydrase Assay[25]

	K_I (nM)[a]					
compd	hCA I	hCA II	hCA VA	hCA VB	hCA IX	hCA XII
8a	26.9	0.9	67.6	55.1	0.9	4.9
8b	323.0	12.8	474.0	389.0	81.0	77.2
8c	262.0	14.0	816.0	251.0	464.0	61.3
8d	436.0	15.6	354.0	78.0	261.0	36.7
8e	378.0	248.0	32.0	58.0	35.0	77.0
8f	29.3	203	61.7	45.6	69.1	61.5
8g	264.0	79.0	29.0	47.0	22.0	59.0
8h	55.4	74.8	61.2	67.8	1.0	3.1
8i	56.1	4.9	53.5	62.0	10.1	46.5
8j	37.0	104	58.9	49.1	0.9	5.5
8k	58.5	180	560	441	51.4	75.1
8l	47.8	32.9	37.4	56.7	21.8	4.2
8m	47.4	81.5	75.4	92.3	40.8	57.3
8n	54.1	74.3	32.8	79.4	25.6	52.8
8o	44.2	341	64.8	37.8	24.7	67.0
8p	543.0	82.0	73.0	33.0	14.0	60.0
8q	73.0	122	88.1	34.9	41.0	31.6
8r	26.6	94.5	98.1	58.2	44.5	67.6
8s	48.4	1.0	60.9	23.8	28.1	48.3
8t	64.7	10.0	51.1	66.0	42.9	43.1
AAZ	250.0	12.1	63.1	54.2	25.4	5.6

[a]Mean from three different assays, and errors were within ±10% of the reported values, by a stopped-flow, CO_2 hydrase assay.[25]

CAs from three species, mouse, rat, and human with the antiviral drug pritelivir (Table 2).

Table 2. IC_{50} for the Inhibition of Mouse, Rat and Human Whole Blood with Pritelivir 8b

species	IC_{50} (nM)[a]
mouse	134
rat	115
human	65.3

[a]Mean from three different assays, errors within ±10% of the reported values, stopped-flow, CO_2 hydrase assay.[25]

From data of Table 2 it can be seen that there are no substantial differences of inhibitory power of pritelivir against the blood CA of the different mammals included in the investigation (all in the same order of magnitude), with the human enzymes being the most inhibited ones (IC_{50} of 65.3 nM), followed by the rodent blood enzymes (see Table 2).

CONCLUSIONS

A series of thiazole-5-sulfonamide derivatives was prepared by an original procedure. These compounds are congeners of the helicase-primase inhibitor pritelivir, *N*-[5-(aminosulfonyl)-4-methyl-1,3-thiazol-2-yl]-*N*-methyl-2-[4-(2-pyridinyl)phenyl]-acetamide, currently in phase II clinical development. The synthesized primary and secondary sulfonamides were investigated as inhibitors of six physiologically and pharmacologically relevant hCA isoforms, the cytosolic isoforms I and II, the mitochondrial ones hCA VA and VB, and the transmembrane, tumor associated hCA IX and XII. Low nanomolar inhibitors were detected for all of them, with a very interesting and well-defined structure–activity relationship, typical for all these different isoforms. As many CAs are involved in serious pathologies, among which are cancer, obesity, epilepsy, glaucoma, etc., sulfonamide inhibitors such as those reported here may be of interest as drug candidates for all these pathologies. Furthermore, we could confirm that pritelivir itself is an effective inhibitor of some CA isoforms in vitro, whereas our IC_{50} values were reproducibly lower than those previously reported (Kleymann 2002). However, there are considerable differences between the test system that was used by Kleymann et al. and our assay leading to this discrepancy, first of all by monitoring the dehydratase but not the hydratase enzymatic reaction and second because of our automated (stopped-flow) versus manual measurements.

Since pritelivir is in phase II clinical development, a variety of in vivo data and data from treatment in humans are available. In animal studies only the development of urinary bladder hyperplasia in rats has been attributed to the CA inhibitory effect of pritelivir so far.[2] This proliferation of the transitional cells is a transient, rodent-specific effect of CA inhibition which is well-known and generally considered as not relevant for humans. Neither adverse findings or beneficial pharmacological effects that could be related to CA inhibition in humans have been reported for healthy subjects or patients treated with pritelivir as of today. However, these studies were not designed to show effects on diseases or conditions targeted by CA inhibitors such as cancer or obesity.

Finally, we could show that pritelivir interacts with whole blood enzymes from several mammalian species, which may be a favorable pharmacokinetic feature of a drug which can be transported throughout the body bound to blood enzymes such as CA I and II. In fact, pritelivir has a long half-life in the body of up to 80 h allowing even once weekly dosing for suppression of genital herpes.[3]

In summary, by investigating a series of primary and secondary sulfonamides, we could identify several compounds with one-digit nanomolar or even subnanomolar activity on certain CA isoenzymes. A potential use of these potent inhibitors for CA associated conditions and diseases remains to be investigated.

EXPERIMENTAL PROTOCOLS

Chemistry. General. Anhydrous solvents and all reagents were purchased from Sigma-Aldrich, Alfa Aesar, and TCI. All reactions involving air- or moisture-sensitive compounds were performed under a nitrogen atmosphere using dried glassware and syringes techniques to transfer solutions. Nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR, ^{19}F NMR) spectra were recorded using a Bruker Advance III 400 MHz spectrometer in DMSO-d_6. Chemical shifts are reported in parts per million (ppm), and the coupling constants (J) are expressed in hertz (Hz). Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; brs, broad singlet; dd, doublet of doublets. The assignment of exchangeable protons (O*H* and N*H*) was confirmed by the addition of D_2O. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel F-254 plates. Flash chromatography purifications were performed on Merck silica gel 60 (230–400 mesh ASTM) as the stationary phase, and ethyl acetate and *n*-hexane were used as eluents. Melting points (mp) were measured in open capillary tubes with a Gallenkamp MPD350.BM3.5 apparatus and are uncorrected. HPLC was performed by using a Waters 2690 separation module coupled with a photodiode array detector (PDA Waters 996) and, as column, a Nova-Pak C18 4 μm, 3.9 mm × 150 mm (Waters), silica-based reverse phase column. Sample was dissolved in acetonitrile 10%, and an injection volume of 45 μL was used. The mobile phase, at a flow rate of 1 mL/min, was a

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gradient of water + trifluoroacetic acid (TFA) 0.1% (A) and acetonitrile + TFA 0.1% (B), with steps as follows: (A%:B%), 0−10 min 90:10, 10−25 min gradient to 60:40, 26:28 min isocratic 20:80, 29−35 min isocratic 90:10. TFA 0.1% in water as well in acetonitrile was used as counterion. All compounds reported here showed more than 96% HPLC purity. The solvents used in MS measures were acetone, acetonitrile (Chromasolv grade), purchased from Sigma-Aldrich (Milan, Italy), and mQ water 18 MΩ, obtained from Millipore's Simplicity system (Milan, Italy). The mass spectra were obtained using a Varian 1200L triple quadrupole system (Palo Alto, CA, USA) equipped with electrospray source (ESI) operating in both positive and negative ions. Stock solutions of analytes were prepared in acetone at 1.0 mg mL^{-1} and stored at 4 °C. Working solutions of each analyte were freshly prepared by diluting stock solutions in a mixture of mQ H_2O/ACN 1/1 (v/v) up to a concentration of 1.0 μg mL^{-1} The mass spectra of each analyte were acquired by introducing, via syringe pump at 10/L min^{-1}, the working solution. Raw data were collected and processed by Varian Workstation version 6.8 software.

Synthesis of 2-Chloro-4-methylthiazole-5-sulfonyl Chloride (2a) and 2-Chloro-4-ethylthiazole-5-sulfonyl Chloride (2b).[1b,22] 2-Chloro-4-methylthiazole (**1a**) or 2-chloro-4-ethylthiazole (**1b**) (1.0 equiv) was added dropwise to a solution of thionyl chloride (2.5 equiv) and chlorosulfonic acid (5.0 equiv). The reaction mixture was stirred at 120 °C for 48 h, cooled down, quenched with slush, and extracted with DCM (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered-off, and concentrated under vacuo to give a residue that was purified by fractional distillation (**2a**, 17 mbar, 85−95 °C; **2b**, 0.9 mbar, 59−64 °C).

2-Chloro-4-methylthiazole-5-sulfonyl chloride (**2a**): *m*/*z* (ESI positive) 231.90 $[M + H]^+$.

2-Chloro-4-ethylthiazole-5-sulfonyl chloride (**2b**): *m*/*z* (ESI positive) 247.12 $[M + H]^+$.

Experimental data are in agreement with reported data.[1b,22]

Synthesis of 2-Chloro-4-alkylthiazole-5-sulfonylamides 3a−e.[22] 2-Chloro-4-methylthiazole-5-sulfonyl chloride (**2a**) or 2-chloro-4-ethylthiazole-5-sulfonyl chloride (**2b**) (1.0 equiv) was treated with the proper amine (1.0 equiv) in THF and stirred until consumption of the starting material occurred (TLC monitoring). Then the solvents were removed under vacuo and the obtained residue was immediately used for the next step without further purification.

2-Chloro-4-methylthiazole-5-sulfonamide **3a**: *m*/*z* (ESI positive) 212.95 $[M + H]^+$.[22]

2-Chloro-*N*,4-dimethylthiazole-5-sulfonamide **3b**: *m*/*z* (ESI positive) 226.96 $[M + H]^+$.

2-Chloro-*N*-(2-hydroxyethyl)-4-methylthiazole-5-sulfonamide **3c**: *m*/*z* (ESI positive) 256.97 $[M + H]^+$.

2-Chloro-*N*-cyclopropyl-4-methylthiazole-5-sulfonamide **3d**: *m*/*z* (ESI positive) 252.98 $[M + H]^+$.

2-Chloro-4-ethyl-*N*-methylthiazole-5-sulfonamide **3e**: *m*/*z* (ESI positive) 240.98 $[M + H]^+$.

Synthesis of 4-Alkyl-2-(alkylamino)thiazole-5-sulfonamido Derivatives 4a−h.[22] 2-Chloro-4-alkylthiazole-5-sulfonylamides **3a−e** (1.0 equiv) were dissolved in acetonitrile and treated with the appropriate amine (3.3 equiv) at 50 °C until consumption of the starting material (TLC monitoring). The reaction solution was cooled down to rt, and the solvent was removed under vacuo to give a residue that was treated with H_2O. The solid formed was collected by filtration and dried under vacuo to afford the title compounds which did not require further purification.

4-Methyl-2-(methylamino)thiazole-5-sulfonamide (**4a**): mp 192 °C;[22] *m*/*z* (ESI positive) 208.01 $[M + H]^+$.

2-((2-(Dimethylamino)ethyl)amino)-4-methylthiazole-5-sulfonamide (**4b**): *m*/*z* (ESI positive) 265.07 $[M + H]^+$.

2-(Cyclopropylamino)-4-methylthiazole-5-sulfonamide (**4c**): *m*/*z* (ESI positive) 234.03 $[M + H]^+$.

N,4-Dimethyl-2-(methylamino)thiazole-5-sulfonamide (**4d**): *m*/*z* (ESI positive) 222.03 $[M + H]^+$.

2-((2-(Dimethylamino)ethyl)amino)-*N*,4-dimethylthiazole-5-sulfonamide (**4e**): *m*/*z* (ESI positive) 279.09 $[M + H]^+$.

N-Cyclopropyl-4-methyl-2-(methylamino)thiazole-5-sulfonamide (**4f**): *m*/*z* (ESI positive) 248.04 $[M + H]^+$.

N-(2-Hydroxyethyl)-4-methyl-2-(methylamino)thiazole-5-sulfonamide (**4g**): *m*/*z* (ESI positive) 252.04 $[M + H]^+$.

4-Ethyl-2-(methylamino)thiazole-5-sulfonamide (**4h**): *m*/*z* (ESI positive) 222.03 $[M + H]^+$.

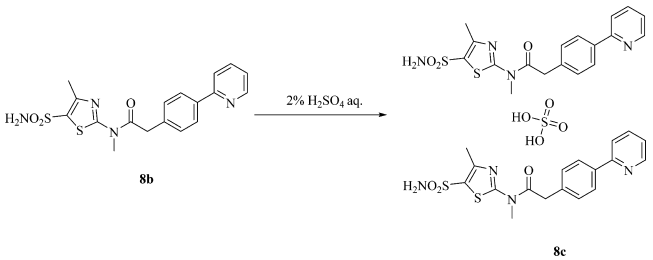
Synthesis of 4-(Hydroxymethyl)-2-(methylamino)thiazole-5-sulfonamide (6). 4-Methyl-2-(methylamino)thiazole-5-sulfonamide (**4a**) (1.0 equiv) was dissolved in MeOAc and treated with NBS (1.0 equiv) and AIBN cat. at rt for 3 h. Then the solvents were removed under vacuo and the residue was purified by silica gel column chromatography, eluting with 30% ethyl acetate in *n*-hexane followed by trituration in DCM to afford 4-(bromomethyl)-2-(methylamino)-thiazole-5-sulfonamide (**5**) which was treated with a 1/1 solution of H_2O/1,4-dioxane at 100 °C. The solvents were removed in vacuo and the obtained residue was triturated from DCM to afford the title compound (**6**) in 98% yield: δ_H (400 MHz, DMSO-d_6) 3.10 (3H, d, *J* 6.2, *N*-CH_3), 4.72 (2H, d, *J* 6.4, CH_2), 4.80 (2H, t, *J* 6.4, exchange with D_2O, O*H*), 7.20 (1H, brs, exchange with D_2O, N*H*), 7.56 (2H, s, exchange with D_2O, SO_2NH_2); δ_C (100 MHz, DMSO-d_6) 29.8, 58.6, 110.1, 148.2, 165.0; *m*/*z* (ESI positive) 224.01 $[M + H]^+$.

Synthesis of *N*-Alkyl-*N*-(4-alkyl-5-sulfamoylthiazol-2-yl)-2-(4-aryl-2-ylphenyl)acetamides 8a−t.[1b] The proper acid **7a−i** (1.0 equiv) was dissolved in dry DMF an treated with 1-hydroxy-1*H*-benzotriazole (HOBT; 1.0 equiv) for 10 min at rt, followed by addition of the corresponding 4-alkyl-2-(alkylamino)thiazole-5-sulfonamido derivatives **4a−h**, **6** (1.1 equiv), and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI·HCl; 1.1 equiv). The reaction mixture was stirred at rt under a nitrogen atmosphere until consumption of the starting material. Then the solvent was removed under vacuo and the obtained residue was triturated from DCM or H_2O to afford the titled compounds in the pure form.

2-([1,1′-Biphenyl]-4-yl)-*N*-methyl-*N*-(4-methyl-5-sulfamoylthiazol-2-yl)acetamide (**8a**) was obtained according to the previously reported general procedure, by using **4a** and **7a**, in 83% yield: mp 193 °C; δ_H (400 MHz, DMSO-d_6) 2.48 (3H, s, CH_3), 3.71 (3H, s, N-CH_3), 4.23 (2H, s, CH_2), 7.38 (1H, m, Ar-H), 7.48 (2H, d, *J* 8.4, Ar-*H*), 7.50 (2H, d, *J* 8.4, Ar-*H*), 7.65 (2H, s, exchange with D_2O, SO_2NH_2), 7.80 (2H, d, *J* 8.4, Ar-*H*); δ_C (100 MHz, DMSO-d_6) 16.5, 35.0, 42.3, 127.5, 128.0, 128.5, 129.0, 130.6, 134.2, 139.8, 140.1, 148.0, 148.1, 160.2, 171.0; *m*/*z* (ESI positive) 402.09 $[M + H]^+$.

N-Methyl-*N*-(4-methyl-5-sulfamoylthiazol-2-yl)-2-(4-pyridin-2-ylphenyl)acetamide (**8b**) was obtained according to the previously reported general procedure, by using **4a** and **7b**, in 74% yield: mp 190 °C; δ_H (400 MHz, DMSO-d_6) 2.48 (3H, s, CH_3), 3.71 (3H, s, N-CH_3), 4.23 (2H, s, CH_2), 7.32 (1H, m, Ar-H), 7.63 (2H, d, *J* 8.4, Ar-*H*), 7.67 (2H, s, exchange with D_2O, SO_2NH_2), 7.85 (2H, appt, *J* 8.8, Ar-*H*), 8.00 (1H, d, *J* 8.8, Ar-*H*), 8.10 (2H, d, *J* 8.4, Ar-*H*), 8.66 (1H, d, *J* 8.8, Ar-*H*); δ_C (100 MHz, DMSO-d_6) 16.6, 34.7, 42.0, 120.6, 123.0, 127.0, 128.7, 130.6, 135.4, 137.7, 137.8, 148.6, 150.0, 156.2, 158.9, 172.3; *m*/*z* (ESI positive) 403.08 $[M + H]^+$.

Bis [*N*-Methyl-*N*-(4-methyl-5-sulfamoyl-thiazol-2-yl)-2-(4-pyridin-2-yl-phenyl)-acetamide] sulfate salt (**8c**) was obtained by the following method.



N-Methyl-*N*-(4-methyl-5-sulfamoylthiazol-2-yl)-2-(4-pyridin-2-ylphenyl)acetamide (**8b**) (1.0 equiv) was treated at 0 °C with a 2% w/w aqueous solution of H_2SO_4 (0.6 equiv). The precipitate formed was collected by filtration, washed with H_2O, and dried under vacuo to afford **8c** in 90% yield. δ_H (400

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MHz, DMSO-d_6) 2.48 (3H, s, CH_3), 3.71 (3H, s, N-CH_3), 4.23 (2H, s, CH_2), 7.32 (1H, m, Ar-H), 7.63 (2H, d, J 8.4, Ar-H), 7.67 (2H, s, exchange with D_2O, SO_2NH_2), 7.85 (2H, appt, J 8.8, Ar-H), 7.99 (1H, d, J 8.8, Ar-H), 8.14 (2H, d, J 8.4, Ar-H), 8.68 (1H, d, J 8.8, Ar-H); δ_C (100 MHz, DMSO-d_6) 16.6, 34.7, 42.0, 120.6, 123.0, 127.0, 128.7, 130.6, 135.4, 137.7, 137.8, 148.6, 150.0, 156.2, 158.9, 172.3; m/z (ESI positive) 403.08 [M − HSO_4^-]$^+$.

1-Methyl-2-(4-(2-(methyl(4-methyl-5-sulfamoylthiazol-2-yl)-amino)-2-oxoethyl)phenyl)pyridin-1-ium acetate salt (**8d**) was obtained according to the previously reported general procedure, by using **4a** and **7c**, in 89% yield: δ_H (400 MHz, DMSO-d_6) 2.15 (3H, s, CH_3-CO_2), 2.25 (3H, s, CH_3), 3.52 (3H, s, N-CH_3), 4.14 (3H, s, N^+-CH_3), 4.26 (2H, s, CH_2), 7.63 (2H, d, J 8.4, Ar-H), 7.59 (2H, s, exchange with D_2O, SO_2NH_2), 7.80 (2H, d, J 8.4, Ar-H), 7.82 (1H, appt, J 8.8, Ar-H), 8.38 (1H, appt, J 8.8, Ar-H), 8.52 (1H, d, J 8.8, Ar-H), 9.30 (1H, d, J 8.8, Ar-H); δ_C (100 MHz, DMSO-d_6) 17.0, 22.0, 23.1, 33.9, 39.8, 42.0, 121.0, 123.0, 125.1, 128.7, 130.0, 132.1, 136.5, 143.2, 146.0, 148.8, 150.4, 159.0, 171.9, 172.3, 175.0; m/z (ESI positive) 417.10 [M − $CH_3CO_2^-$]$^+$.

N-Methyl-*N*-(4-methyl-5-sulfamoylthiazol-2-yl)-2-(4-pyrazol-1-ylphenyl)acetamide (**8e**) was obtained according to the previously reported general procedure, by using **4a** and **7e**, in 60% yield: δ_H (400 MHz, DMSO-d_6) 2.46 (3H, s, CH_3), 3.69 (3H, s, N-CH_3), 4.20 (2H, s, CH_2), 6.42 (1H, s, Ar-H), 7.42 (2H, d, J 8.4, Ar-H), 7.48 (1H, s, Ar-H), 7.58 (2H, d, J 8.4, Ar-H), 7.63 (2H, s, exchange with D_2O, SO_2NH_2), 8.00 (1H, s; Ar-H); δ_C (100 MHz, DMSO-d_6) 15.8, 32.0, 42.0, 110.1, 112.4, 125.7, 126.8, 130.2, 131.1, 138.6, 141.0, 147.9, 159.0, 170.1; m/z (ESI positive) 391.08 [M + H]$^+$.

N-Methyl-*N*-(4-methyl-5-sulfamoylthiazol-2-yl)-2-(4-(pyrazin-2-yl)-phenyl)acetamide (**8f**) was obtained according to the previously reported general procedure, by using **4a** and **7d**, in 63% yield: mp 220 °C; δ_H (400 MHz, DMSO-d_6) 2.30 (3H, s, CH_3), 3.7 (3H, s, N-CH_3), 4.08 (2H, s, CH_2), 7.53 (2H, s, exchange with D_2O, SO_2NH_2), 7.65 (2H, d, J 8.4, Ar-H), 8.10 (2H, d, J 8.4, Ar-H), 8.76 (1H, s, Ar-H), 8.80 (1H, d, J 8.8, Ar-H), 8.82 (1H, d, J 8.8, Ar-H); δ_C (100 MHz, DMSO-d_6) 15.4, 32.0, 41.9, 111.0, 126.5, 130.3, 135.8, 142.4, 143.4, 144.8, 148.0, 153.2, 160.4, 172.3; m/z (ESI positive) 404.08 [M + H]$^+$.

2-([1,1′-Biphenyl]-4-yl)-*N*-(2-(dimethylamino)ethyl)-*N*-(4-methyl-5-sulfamoylthiazol-2-yl)acetamide (**8g**) was obtained according to the previously reported general procedure, by using **4b** and **7a**, in 72% yield: δ_H (400 MHz, DMSO-d_6) 2.30 (3H, s, CH_3), 2.35 (6H, s, 2 × N-CH_3), 2.71 (2H, t, J 6.4 CH_2-CH_2-$N(CH_3)_2$), 3.64 (2H, t, J 6.4 CH_2-CH_2-$N(CH_3)_2$), 3.92 (2H, s, CH_2), 7.40 (1H, m, Ar-H), 7.50 (2H, d, J 8.4, Ar-H), 7.54 (2H, d, J 8.4, Ar-H), 7.68 (2H, s, exchange with D_2O, SO_2NH_2), 7.79 (2H, d, J 8.4, Ar-H); δ_C (100 MHz, DMSO-d_6) 16.0, 42.4, 48.0, 48.1, 60.2, 110.4, 128.0, 128.5, 129.0, 130.6, 134.2, 139.8, 140.1, 148.0, 148.1, 160.2, 172.0; m/z (ESI positive) 459.14 [M + H]$^+$.

2-([1,1′-Biphenyl]-4-yl)-*N*-cyclopropyl-*N*-(4-methyl-5-sulfamoylthiazol-2-yl)acetamide (**8h**) was obtained according to the previously reported general procedure, by using **4c** and **7a**, in 82% yield: mp 164 °C; δ_H (400 MHz, DMSO-d_6) 1.06 (2H, m), 1.32 (2H, m), 2.16 (3H, s, CH_3) 3.98 (2H, s, CH_2), 4.18 (1H, m), 7.36 (2H, d, J 8.4; Ar-H), 7.39 (1H, appt, J 8.4, Ar-H), 7.49 (2H, d, J 8.4, Ar-H), 7.53 (2H, s, exchange with D_2O, SO_2NH_2), 7.70 (2H, d, J 8.4, Ar-H), 7.78 (2H, d, J 8.4; Ar-H); δ_C (100 MHz, DMSO-d_6) 10.4, 15.9, 35.0, 38.4, 110.1, 127.8, 128.0, 128.3, 129.1, 130.4, 135.0, 139.9, 141.1, 148.0, 162.3, 169.4; m/z (ESI positive) 428.10 [M + H]$^+$.

2-(2′-Fluoro-[1,1′-biphenyl]-4-yl)-*N*-methyl-*N*-(4-methyl-5-sulfamoylthiazol-2-yl)acetamide (**8i**) was obtained according to the previously reported general procedure, by using **4a** and **7f**, in 94% yield: mp 209 °C; δ_H (400 MHz, DMSO-d_6) 2.20 (3H, s, CH_3) 3.78 (s, N-CH_3), 4.12 (2H, s, CH_2), 7.27 (1H, appt, J 8.7, Ar-H), 7.38 (2H, d, J 8.4; Ar-H), 7.49 (1H, m, Ar-H), 7.53 (2H, s, exchange with D_2O, SO_2NH_2), 7.63 (2H, d, J 8.4, Ar-H), 7.69 (1H, appt, J 8.6, Ar-H), 7.75 (2H, d, J 8.6; Ar-H); δ_C (100 MHz, DMSO-d_6) 16.2, 32.0, 39.2, 110.4, 115.8 (d, J_{C-F} 23.5), 125.0, 128.2, 129.1, 129.3 (d, J_{C-F} 23.5), 130.2, 130.9, 134.6, 135.0, 148.0, 158.9, (d, J_{C-F} 247) 159.1, 171.3; m/z (ESI positive) 420.08 [M + H]$^+$.

2-(3′-Fluoro-[1,1′-biphenyl]-4-yl)-*N*-methyl-*N*-(4-methyl-5-sulfamoylthiazol-2-yl)acetamide (**8j**) was obtained according to the previously reported general procedure, by using **4a** and **7g**, in 84% yield; mp 148 °C; δ_H (400 MHz, DMSO-d_6) 2.20 (3H, s, CH_3) 3.78 (s, N-CH_3), 4.12 (2H, s, CH_2), 7.21 (1H, m, Ar-H), 7.28 (1H, d, J 8.4; Ar-H), 7.38 (1H, d, J 8.4; Ar-H), 7.53 (2H, s, exchange with D_2O, SO_2NH_2), 7.54 (2H, m, Ar-H), 7.65 (2H, d, J 8.4, Ar-H); δ_C (100 MHz, DMSO-d_6) 16.0, 32.4, 40.1, 114.1 (d, J_{C-F} 24.0), 116.1 (d, J_{C-F} 24.0), 123.6, 127.8, 128.1, 130.2, 130.9, 134.5, 139.9, 141.2, 148.0, 159.1, 162.1 (d, J_{C-F} 247), 171.0; m/z (ESI positive) 420.08 [M + H]$^+$.

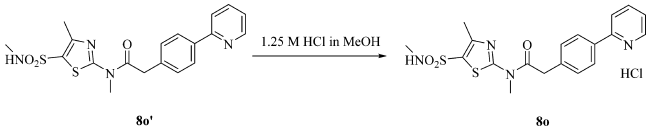
2-(2′,5′-Difluoro-[1,1′-biphenyl]-4-yl)-*N*-methyl-*N*-(4-methyl-5-sulfamoylthiazol-2-yl)acetamide (**8k**) was obtained according to the previously reported general procedure, by using **4a** and **7h**, in 80% yield: mp 188 °C; δ_H (400 MHz, DMSO-d_6) 2.19 (3H, s, CH_3) 3.80 (s, N-CH_3), 4.12 (2H, s, CH_2), 7.23 (1H, m, Ar-H), 7.36 (1H, m; Ar-H), 7.37 (2H, d, J 8.4; Ar-H), 7.50 (1H, m; Ar-H), 7.52 (2H, s, exchange with D_2O, SO_2NH_2), 7.64 (2H, d, J 8.4, Ar-H); δ_C (100 MHz, DMSO-d_6) 16.0, 32.0, 40.3, 110.1, 116.0, 116.1 (d, J_{C-F} 24.0), 118.0 (d, J_{C-F} 24.0), 128.2, 130.0, 132.9, 134.4, 135.4, 148.0, 154.5, 157.4 (d, J_{C-F} 247), 158.0 (d, J_{C-F} 247), 159.1, 171.3; m/z (ESI positive) 438.07 [M + H]$^+$.

2-(3′-Fluoro-2′-methoxy-[1,1′-biphenyl]-4-yl)-*N*-methyl-*N*-(4-methyl-5-sulfamoylthiazol-2-yl)acetamide (**8l**) was obtained according to the previously reported general procedure, by using **4a** and **7i**, in 65% yield; δ_H (400 MHz, DMSO-d_6) 2.20 (3H, s, CH_3), 3.78 (s, N-CH_3), 3.80 (3H, s, O-CH_3), 4.11 (2H, s, CH_2), 7.21 (1H, m, Ar-H), 7.30 (1H, m; Ar-H), 7.38 (2H, d, J 8.4; Ar-H), 7.52 (2H, s, exchange with D_2O, SO_2NH_2), 7.63 (2H, d, J 8.4, Ar-H), 7.74 (1H, m, Ar-H); δ_C (100 MHz, DMSO-d_6) 16.0, 32.1, 40.2, 58.1, 110.1, 115.4 (d, J_{C-F} 24.0), 119.3, 126.2, 127.3, 128.2, 130.1, 134.6, 136.4, 148.0 (d, J_{C-F} 24), 148.1, 152.4 (d, J_{C-F} 247), 156.0, 171.4; m/z (ESI positive) 450.09 [M + H]$^+$.

2-([1,1′-Biphenyl]-4-yl)-*N*-methyl-*N*-(4-methyl-5-(*N*-methylsulfamoyl)thiazol-2-yl)acetamide (**8m**) was obtained according to the previously reported general procedure, by using **4d** and **7a**, in 93% yield: mp 177 °C; δ_H (400 MHz, DMSO-d_6) 2.21 (3H, s, CH_3), 2.50 (3H, s, SO_2NH-CH_3), 3.80 (s, N-CH_3), 4.12 (2H, s, CH_2), 7.41 (1H, appt, J 6.84, Ar-H), 7.38 (2H, d, J 8.4; Ar-H), 7.49 (2H, d, J 8.4; Ar-H), 7.63 (2H, d, J 8.4, Ar-H), 7.72 (2H, d, J 8.4, Ar-H), 7.75 (1H, s, exchange with D_2O, SO_2NH-); δ_C (100 MHz, DMSO-d_6) 16.4, 30.1, 32.0, 40.2, 110.4, 127.4, 127.6, 128.2, 129.0, 129.8, 134.5, 139.6, 140.1, 148.0, 159.1, 172.3; m/z (ESI positive) 416.10 [M + H]$^+$.

2-(2′-Fluoro-[1,1′-biphenyl]-4-yl)-*N*-methyl-*N*-(4-methyl-5-(*N*-methylsulfamoyl)thiazol-2-yl)acetamide (**8n**) was obtained according to the previously reported general procedure, by using **4d** and **7f**, in 87% yield: mp 182 °C; δ_H (400 MHz, DMSO-d_6) 2.20 (3H, s, CH_3), 2.50 (3H, s, SO_2NH-CH_3), 3.78 (s, N-CH_3), 4.12 (2H, s, CH_2), 7.27 (1H, appt, J 8.7, Ar-H), 7.38 (2H, d, J 8.4; Ar-H), 7.49 (1H, m, Ar-H), 7.63 (2H, d, J 8.4, Ar-H), 7.69 (1H, appt, J 8.6, Ar-H), 7.75 (2H, d, J 8.6; Ar-H), 7.76 (1H, s, exchange with D_2O, SO_2NH-); δ_C (100 MHz, DMSO-d_6) 16.2, 30.4, 32.0, 39.2, 110.4, 115.8 (d, J_{C-F} 23.5), 125.0, 128.2, 129.1, 129.3 (d, J_{C-F} 23.5), 130.2, 130.9, 134.6, 135.0, 148.0, 158.9, (d, J_{C-F} 247) 159.1, 171.3; m/z (ESI positive) 434.09 [M + H]$^+$.

N-Methyl-*N*-(4-methyl-5-(*N*-methylsulfamoyl)thiazol-2-yl)-2-(4-(pyridin-2-yl)phenyl)acetamide hydrochloride salt (**8o**) was obtained according to the following procedure.



8o′ **8o**

N-Methyl-*N*-(4-methyl-5-(*N*-methylsulfamoyl)thiazol-2-yl)-2-(4-(pyridin-2-yl)phenyl)acetamide (**8o′**) was treated with a commercially available 1.25 M hydrochloric acid solution in methanol. The precipitate formed was collected by filtration

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and dried under vacuo to afford the compound **8o** in 98% yield: mp 240 °C; δ_H (400 MHz, DMSO-d_6) 2.20 (3H, s, CH_3), 2.50 (3H, s, SO_2NH-CH_3), 3.78 (s, N-CH_3), 4.15 (2H, s, CH_2), 7.32 (1H, m, Ar-H), 7.63 (2H, d, *J* 8.4, Ar-*H*), 7.74 (1H, s, exchange with D_2O, SO_2NH-), 7.85 (2H, appt, *J* 8.8, Ar-*H*), 7.99 (1H, d, *J* 8.8, Ar-*H*), 8.14 (2H, d, *J* 8.4, Ar-*H*), 8.68 (1H, d, *J* 8.8, Ar-*H*); δ_C (100 MHz, DMSO-d_6) 16.6, 30.2, 34.7, 42.0, 120.6, 123.0, 127.0, 128.7, 130.6, 135.4, 137.7, 137.8, 148.6, 150.0, 156.2, 158.9, 172.2; *m*/*z* (ESI positive) 432.12 $[MH - Cl^-]^+$.

2-([1,1′-Biphenyl]-4-yl)-*N*-(2-(dimethylamino)ethyl)-*N*-(4-methyl-5-(*N*-methylsulfamoyl)thiazol-2-yl)acetamide (**8p**) was obtained according to the previously reported general procedure, by using **4e** and **7a**, in 81% yield: δ_H (400 MHz, DMSO-d_6) 2.21 (3H, s, CH_3), 2.35 (6H, s, 2 × N-CH_3), 2.50 (3H, s, SO_2NH-CH_3), 2.71 (2H, t, *J* 6.4 CH_2-CH_2-N(CH_3)$_2$), 3.64 (2H, t, *J* 6.4 CH_2-CH_2-N(CH_3)$_2$), 3.80 (s, N-CH_3), 4.13 (2H, s, CH_2), 7.41 (1H, appt, *J* 6.84, Ar-*H*), 7.38 (2H, d, *J* 8.4; Ar-*H*), 7.49 (2H, d, *J* 8.4; Ar-*H*), 7.63 (2H, d, *J* 8.4, Ar-*H*), 7.72 (2H, d, *J* 8.4, Ar-*H*), 7.74 (1H, s, exchange with D_2O, SO_2NH-); δ_C (100 MHz, DMSO-d_6) 16.4, 32.1, 42.4, 48.0, 48.1, 110.3, 127.6, 127.8, 128.3, 129.0, 130.0, 134.5, 139.8, 141.0, 148.0, 159.0, 172.2; *m*/*z* (ESI positive) 473.16 $[M + H]^+$.

N-(5-(*N*-Cyclopropylsulfamoyl)-4-methylthiazol-2-yl)-*N*-methyl-2-(4-(pyridin-2-yl)phenyl)acetamide (**8q**) was obtained according to the previously reported general procedure, by using **4f** and **7b**, in 77% yield: δ_H (400 MHz, DMSO-d_6) 1.06 (2H, m), 1.32 (2H, m), 2.48 (3H, s, CH_3), 3.71 (3H, s, N-CH_3), 4.20 (2H, s, CH_2), 4.22 (1H, m), 7.32 (1H, m, Ar-H), 7.63 (2H, d, *J* 8.4, Ar-*H*), 7.74 (1H, s, exchange with D_2O, SO_2NH-), 7.85 (2H, appt, *J* 8.8, Ar-*H*), 8.00 (1H, d, *J* 8.8, Ar-*H*), 8.10 (2H, d, *J* 8.4, Ar-*H*), 8.66 (1H, d, *J* 8.8, Ar-*H*); δ_C (100 MHz, DMSO-d_6) 14.2, 16.7, 20.0, 31.2, 38.0, 110.2, 121.0, 123.2, 126.4, 130.1, 134.2, 137.2, 138.0, 148.4, 150.0, 155.7, 159.0, 172.0; *m*/*z* (ESI positive) 443.11 $[M + H]^+$.

2-([1,1′-Biphenyl]-4-yl)-*N*-(5-(*N*-(2-hydroxyethyl)sulfamoyl)-4-methylthiazol-2-yl)-*N*-methylacetamide (**8r**) was obtained according to the previously reported general procedure, by using **4g** and **7a**, in 88% yield: mp 170 °C; δ_H (400 MHz, DMSO-d_6) 2.21 (3H, s, CH_3), 3.00 (2H, dd, *J* 6.4, 6.6, SO_2NH-CH_2-), 3.43 (2H, m, -CH_2-OH), 3.82 (s, N-CH_3), 4.10 (2H, s, CH_2), 4.45 (1H, t, *J* 6.4, exchange with D_2O, O*H*), 7.38 (2H, d, *J* 8.4; Ar-*H*), 7.41 (1H, appt, *J* 6.84, Ar-*H*), 7.49 (2H, d, *J* 8.4; Ar-*H*), 7.63 (2H, d, *J* 8.4, Ar-*H*), 7.72 (2H, d, *J* 8.4, Ar-*H*), 7.73 (1H, s, exchange with D_2O, SO_2NH-), 7.75 (2H, d, *J* 8.4, Ar-*H*); δ_C (100 MHz, DMSO-d_6) 16.2, 30.1, 38.4, 45.6, 59.9, 110.2, 127.6, 128.0, 128.2, 129.0, 130.0, 134.5, 140.0, 140.9, 148.1, 159.8, 172.2; *m*/*z* (ESI positive) 446.11 $[M + H]^+$.

N-(4-Ethyl-5-sulfamoylthiazol-2-yl)-*N*-methyl-2-(4-(pyridin-2-yl)-phenyl)acetamide (**8s**) was obtained according to the previously reported general procedure, by using **4h** and **7b**, in 82% yield: δ_H (400 MHz, DMSO-d_6) 1.35 (3H, d, *J* 6.4, CH_2-CH_3), 3.00 (2H, q, *J* 6.4, CH_2-CH_3), 3.71 (3H, s, N-CH_3), 4.22 (2H, s, CH_2), 7.32 (1H, m, Ar-H), 7.63 (2H, d, *J* 8.4, Ar-*H*), 7.66 (2H, s, exchange with D_2O, SO_2NH_2), 7.85 (2H, appt, *J* 8.8, Ar-*H*), 8.00 (1H, d, *J* 8.8, Ar-*H*), 8.10 (2H, d, *J* 8.4, Ar-*H*), 8.66 (1H, d, *J* 8.8, Ar-*H*); δ_C (100 MHz, DMSO-d_6) 14.2, 19.9, 34.7, 42.0, 120.6, 123.0, 127.0, 128.7, 130.6, 135.4, 137.7, 137.8, 148.6, 150.0, 156.2, 159.1, 172.0; *m*/*z* (ESI positive) 417.10 $[M + H]^+$.

N-(4-(Hydroxymethyl)-5-sulfamoylthiazol-2-yl)-*N*-methyl-2-(4-(pyridin-2-yl)phenyl)acetamide (**8t**) was obtained according to the previously reported general procedure, by using **6** and **7b**, in 64% yield: δ_H (400 MHz, DMSO-d_6) 3.72 (3H, s, N-CH_3), 4.20 (2H, s, CH_2), 4.60 (2H, d, *J* 6.2, CH_2OH), 4.78 (1H, t, *J* 6.2, O*H*), 7.32 (1H, m, Ar-H), 7.63 (2H, d, *J* 8.4, Ar-*H*), 7.66 (2H, s, exchange with D_2O, SO_2NH_2), 7.85 (2H, appt, *J* 8.8, Ar-*H*), 8.00 (1H, d, *J* 8.8, Ar-*H*), 8.10 (2H, d, *J* 8.4, Ar-*H*), 8.66 (1H, d, *J* 8.8, Ar-*H*); δ_C (100 MHz, DMSO-d_6) 14.2, 19.9, 34.7, 42.0, 120.6, 123.0, 127.0, 128.7, 130.6, 135.4, 137.7, 137.8, 148.6, 150.0, 156.2, 159.1, 172.0; *m*/*z* (ESI positive) 417.10 $[M + H]^+$.

CA Inhibition. An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity.[25] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10−100 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, and dilutions up to 0.01 nM were done thereafter with the assay buffer. 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 nonlinear least-squares methods using PRISM 3 and the Cheng−Prusoff equation, as reported earlier,[26−29] and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier,[26−29] and their concentrations in the assay system were in the range of 7.1−12.3 nM.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.7b00183.

SMILES representation for compounds (CSV)

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Notes

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■ ABBREVIATIONS USED

CA, carbonic anhydrase; CAI, carbonic anhydrase inhibitor; K_I, inhibition constant

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