A class of carbonic anhydrase I – selective activators
Erol Licsandru, Muhammet Tanc, Istvan Kocsis, Mihail Barboiu, Claudiu T. Supuran

To cite this version:
Erol Licsandru, Muhammet Tanc, Istvan Kocsis, Mihail Barboiu, Claudiu T. Supuran. A class of carbonic anhydrase I – selective activators. Journal of Enzyme Inhibition and Medicinal Chemistry, Informa Healthcare, 2017, 32 (1), pp.37 - 46. 10.1080/14756366.2016.1232254. hal-01671828

HAL Id: hal-01671828
https://hal.umontpellier.fr/hal-01671828
Submitted on 1 Jun 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License
A class of carbonic anhydrase I – selective activators

Erol Licsandru, Muhammet Tanc, Istvan Kocsis, Mihail Barboiu & Claudiu T. Supuran

To cite this article: Erol Licsandru, Muhammet Tanc, Istvan Kocsis, Mihail Barboiu & Claudiu T. Supuran (2017) A class of carbonic anhydrase I – selective activators, Journal of Enzyme Inhibition and Medicinal Chemistry, 32:1, 37-46, DOI: 10.1080/14756366.2016.1232254

To link to this article: https://doi.org/10.1080/14756366.2016.1232254

© 2016 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group

Published online: 01 Nov 2016.
A class of carbonic anhydrase I – selective activators

Erol Licsandru\textsuperscript{a}, Muhammet Tanc\textsuperscript{b}, Istvan Kocsis\textsuperscript{a}, Mihail Barboiu\textsuperscript{a} and Claudiu T. Supuran\textsuperscript{b}

\textsuperscript{a}Adaptive Supramolecular Nanosystems Group, Institut Europeen des Membranes, University of Montpellier ENSCM-UMR CNRS 5635, Montpellier, France; \textsuperscript{b}Department of Neurofarba and Laboratorio di Chimica Bioinorganica, Sezione di Chimica Farmaceutica e Nutraceutica, Università degli Studi di Firenze, Sesto Fiorentino (Florence), Italy

ABSTRACT

A series of ureido and bis-ureido derivatives were prepared by reacting histamine with alkyl/aryl-isocyanates or di-isocyanates. The obtained derivatives were assayed as activators of the enzyme carbonic anhydrase (CA, EC 4.2.1.1), due to the fact that histamine itself has this biological activity. Although inhibition of CAs has pharmacological applications in the field of antiglaucoma, anticonvulsant, anticancer, and anti-infective agents, activation of these enzymes is not yet properly exploited pharmacologically for cognitive enhancement or Alzheimer’s disease treatment, conditions in which a diminished CA activity was reported. The ureido/bis-ureido histamine derivatives investigated here showed activating effects only against the cytosolic human (h) isoform hCA I, having no effect on the widespread, physiologically dominant isofrom hCA II. This is the first report in which CA I-selective activators were identified. Such compounds may constitute interesting tools for better understanding the physiological/pharmacological effects connected to activation of this widespread CA isoform, whose physiological function is not fully understood.

Introduction

The carbonic anhydrases (CAs, EC 4.2.1.1) represent a superfamilly of metalloenzymes, with six distinct genetic families known to date, the $\alpha$, $\beta$, $\gamma$, $\delta$, $\zeta$, and $\eta$-CAs, all of which efficiently catalyze the reaction between CO\textsubscript{2} and water, with the formation of bicarbonate and protons\textsuperscript{1–11}. The inhibition and the activation of CAs are well-understood processes: most types of classical inhibitors bind to the metal center within the enzyme active site\textsuperscript{12–21}, whereas the activators bind at the entrance of the active site cavity and participate in proton shuttling processes after the metal ion-bound water molecule and the environment\textsuperscript{22,24}. This leads to enhanced formation of the metal hydroxide, catalytically active species of the enzyme\textsuperscript{24}.

The substrates/reaction products involved in the CA catalyzed reaction, i.e. CO\textsubscript{2}, bicarbonate and protons, are essential molecules/ions in many important physiologic processes in all life kingdoms (Bacteria, Archaea, and Eukarya), throughout the tree of life, and for this reason, relatively high amounts of these enzymes are present in different tissues/cell compartments of most investigated organisms\textsuperscript{1–77}.

Sulfonamides are the most important class of CA inhibitors (CAs)\textsuperscript{75–81}, with at least 20 such compounds in clinical use for decades, or in clinical development\textsuperscript{84–79}. Sulfonamide/sulfamate CAs are used as diuretics, antiglaucoma, anticonvulsant, and anti-obesity agents\textsuperscript{80–87}, whereas the anticancer and anti-infective use of such derivatives started to be investigated only recently\textsuperscript{88,89}. Furthermore, in the last period, the use of CAs for the management of neuropathic pain\textsuperscript{88}, organ preservation without ischemia reperfusion injury\textsuperscript{89}, and the management of cerebral ischemia\textsuperscript{90} were also reported, extending thus the applications of these pharmacological agents. However, the activators of CAs (CAAs), although known for decades\textsuperscript{23,24}, do not have at this moment pharmacological applications. This is due to several reasons, the first of which has to do with the catalyzed reaction (Scheme 1).

As mentioned above, the catalytically effective species of all CAs has a metal hydroxide species within the active site, which for the $\alpha$-CAs is a zinc hydroxide species generated from a water molecule bound to the Zn\textsuperscript{2+} ion (Equation (1) in Scheme 1). This is also the rate-determining step for the catalytic cycle of many CAs and it is assisted by buffers present in the medium as well as by an amino acid residue from the middle of the active site cavity, His64, which has a $pK_a$ of about 7 and may shuttle protons between the active site and the environment\textsuperscript{23,24}.

The second step (Equation (2) in Scheme 1) involves the nucleophilic attack of the zinc hydroxide to the CO\textsubscript{2} molecule bound in a hydrophobic pocket, with formation of bicarbonate coordinated to zinc, which is thereafter replaced by an incoming water molecule, with formation of the acidic species of the enzyme, with water as the fourth zinc ligand\textsuperscript{1–3,23,24}. Many CAs are highly effective catalysts, with turnover numbers of $>10^8$ s\textsuperscript{-1}, close to the limit of diffusion-controlled processes\textsuperscript{1–3}. Thus, many researchers in the period starting with 50s until the 90s were reluctant to admit that CAs may have activators. Only in 1997, we reported the first X-ray crystal structure of an activator bound to the human (h) CA isoform hCA II. This activator was histamine\textsuperscript{89}. The activator was found bound at the entrance of the CA active site cavity, with the imidazole moiety participating in shuttling protons between the active site and
the bulk solvent, thus acting as a second proton shuttle of the enzyme in addition to His64, whereas the amino group from the aminomethyl moiety of histamine did not participate in any interaction with the enzyme active site.

The second reason why CAAs do not have for the moment pharmacological applications is due to their difficult pharmacology. Indeed, it has been reported that some CAAs (such as phenylalanine or imidazole) administered to experimental animals may produce an important pharmacological enhancement of synaptic efficacy, spatial learning, and memory, proving that this class of relatively unexplored enzyme modulators may have important applications in conditions in which learning and memory are impaired, such as for example aging or Alzheimer’s disease.

One must also mention that it was reported that the levels of CA are significantly diminished in the brain of patients affected by Alzheimer’s disease and these facts strongly support the involvement of different brain CA isozymes in cognitive functions. However, no clinical trials for the use of CAAs for the management of these conditions were done at this moment. One should mention that the chemistry and biochemistry of this class of derivatives was thoroughly investigated, with a large number of activator adducts reported and several CA – selective CAAs, based on the histamine scaffold, which has been derivatized by using cyanate/dicyanate chemistry.

**Materials and methods**

**Chemistry**

All the compounds were synthesized following the general procedure described below. The amine (30 mmol) was mixed with the corresponding amount of isocyanate, under sonication (1 eq.: 1eq. for the monoure a compounds and 2 eq. for the diurea compounds). The mixture was solubilized in 10 ml of tetrahydrofuran (THF), 5 ml of ethylacetate, and 10 ml of dimethylacetamide. The reaction mixture was heated to 120°C for 15 min. When the precipitation began, 5 ml of acetonitrile were added and the heating was maintained for another hour. The resulting product (a white powder) was filtered and washed with methanol. The exceptions of the protocol were compounds 1, 6, 10, and 11 for which the reaction temperature was 60°C and compounds 2, 7, 12, and 13 for which the reaction temperature was 80°C. Compound 1 is soluble in the reaction mixture and, therefore, the purification procedure consisted in evaporation of the solvent under vacuum in a round bottomed flask and recrystallization from CHCl3. Alternatively a microwave reactor has been used. The procedure was the following: the isocyanate was dissolved in 5 ml of acetonitrile and added over the amine in the microwave reactor. The reaction was performed at 140°C under energetic stirring, for 15 min. The product was filtered and washed with methanol. In the case of compounds 1, 6, 10, and 11, the temperature was 50°C and for compounds 2, 7, 12, and 13 was 90°C. For compound 1, the purification method was the same. Ureas 1–22 were characterized using 1H NMR methods and mass spectrometry.

1-(2-(1H-imidazol-4-yl)ethyl)-3-butylurea 1: (mass spectrometry, ES M++ = 211.1)

1H-NMR (DMSO-d6, 300 MHz) δ (ppm) = 0.86 (t, 3H, CH3CH2); 1.30 (m, 4H, CH3CH2CH2CH2); 2.58 (t, 2H, NHCH2CH2); 2.96 (g, 2H, CH2CH2NH); 3.21 (q, 2H, CH2CH2NH), 5.75 (s mod, 1H, NHCH2); 5.83 (s mod, 1H, NHCH2); 6.78 (s, 1H, CHN CHN imidazole); 7.55 (s, 1H, N CHN imidazole).

1-(2-(1H-imidazol-4-yl)ethyl)-3-hexylurea 2: (mass spectrometry, ES M++ = 239.1)

1H-NMR (DMSO-d6, 300 MHz) δ (ppm) = 0.86 (t, 3H, CH3CH2); 1.24–1.34 (m, 8H, CH3(CH2)4CH2); 2.57 (t, 2H, NHCH2CH2); 2.96 (q, 2H, CH2CH2NH); 3.21 (q, 2H, CH2CH2NH), 5.75 (s mod, 1H, NHCH2); 5.84 (s mod, 1H, NHCH2); 6.75 (s, 1H, C CHN imidazole); 7.54 (s, 1H, N CHN imidazole).

1-(2-(1H-imidazol-4-yl)ethyl)-3-octylurea 3: (mass spectrometry, ES M++ = 267.1)

1H-NMR (DMSO-d6, 300 MHz) δ (ppm) = 0.86 (t, 3H, CH3CH2); 1.25–1.34 (m, 12H, CH3(CH2)6CH2); 2.58 (t, 2H, NHCH2CH2); 2.95 (q, 2H, CH2CH2NH); 3.21 (q, 2H, CH2CH2NH), 5.74 (s mod, 1H, NHCH2); 5.82 (s mod, 1H, NHCH2); 6.76 (s, 1H, C CHN imidazole); 7.52 (s, 1H, N CHN imidazole).

1-(2-(1H-imidazol-4-yl)ethyl)-3-dodecylurea 4: (mass spectrometry, ES M++ = 323.2)

1H-NMR (DMSO-d6, 300 MHz) δ (ppm) = 0.85 (t, 3H, CH3CH2); 1.25–1.33 (m, 20H, CH3(CH2)10CH2); 2.57 (t, 2H, NHCH2CH2); 2.93 (q, 2H, CH2CH2NH); 3.21 (q, 2H, CH2CH2NH), 5.75 (s mod, 1H, NHCH2); 5.83 (s mod, 1H, NHCH2); 6.75 (s, 1H, C CHN imidazole); 7.50 (s, 1H, N CHN imidazole).

1-(2-(1H-imidazol-4-yl)ethyl)-3-octadecylurea 5: (mass spectrometry, ES M++ = 407.3)

1H-NMR (DMSO-d6, 300 MHz) δ (ppm) = 0.86 (t, 3H, CH3CH2); 1.24–1.33 (m, 32H, CH3(CH2)16CH2); 2.58 (t, 2H, NHCH2CH2); 2.95 (q, 2H, CH2CH2NH); 3.21 (q, 2H, CH2CH2NH), 5.75 (s mod, 1H, NHCH2); 5.82 (s mod, 1H, NHCH2); 6.78 (s, 1H, C CHN imidazole); 7.56 (s, 1H, N CHN imidazole).

1-(Butane-1,4-diyl)bis(3-(2-(1H-imidazol-4-yl)ethyl)urea) 6: (mass spectrometry, ES M++ = 363.2)

1H-NMR (DMSO-d6, 300 MHz) δ (ppm) = 1.32 (m, 4H, CH3(CH2)2CH2); 2.58 (t, 4H, NHCH2CH2); 2.95 (q, 4H, CH2CH2NH);
The provided text is related to NMR and mass spectrometry studies on various compounds. Here is a summary of the key findings:

1-1-(Hexane-1,6-diyl)bis(3-(2-(1H-imidazol-4-yl)ethyl)urea) (mass spectrometry, ES $M^+ = 479.3$)

- 1H-NMR (DMSO-d$_6$, 300 MHz) δ (ppm) = 1.24–1.33 (m, 16H, CH$_3$(CH2)8CH2); 2.59 (t, 4H, NHCH2CH2); 2.95 (q, 4H, CH2CH2NH); 3.22 (q, 4H, CH2CH2NH); 5.77 (s mod, 2H, NHCH2); 5.83 (s mod, 2H, NHCH2); 6.82 (s, 2H, $N$ CHNH imidazole).

1-1-(Octane-1,8-diyl)bis(3–(2-(1H-imidazol-4-yl)ethyl)urea) (mass spectrometry, ES $M^+ = 419.3$)

- 1H-NMR (DMSO-d$_6$, 300 MHz) δ (ppm) = 1.24–1.36 (m, 12H, CH$_3$(CH2)6CH2); 2.58 (t, 4H, NHCH2CH2); 2.95 (q, 4H, CH2CH2NH); 3.21 (q, 4H, CH2CH2NH); 5.76 (s mod, 2H, NHCH2); 5.84 (s mod, 2H, NHCH2); 6.77 (s, 2H, $N$ CHNH imidazole).

1-1-(Dodecane-1,12-diyl)bis(3-(2-(1H-imidazol-4-yl)ethyl)urea) (mass spectrometry, ES $M^+ = 475.2$)

- 1H-NMR (DMSO-d$_6$, 300 MHz) δ (ppm) = 0.81 (q, 6H, CH$_3$CH$_3$CH$_3$); 0.92 (d, 3H, CH$_3$CH$_2$CH$_3$); 1.57 (h, 1H, CH$_3$CHCH$_3$); 2.60 (t, 2H, NHCH$_2$CH$_2$); 3.21 (q, 2H, CH$_2$CH$_2$NH); 3.45 (m, 1H, CH$_2$CH$_2$CH$_2$NH); 5.68 (s mod, 1H, NHCH$_2$); 5.71 (s mod, 1H, NHCH$_2$); 6.82 (s, 1H, $N$ CHNH imidazole).

(R)-1-1-(2-(1H-imidazol-4-yl)ethyl)-3-(3-methylbutan-2-yl)urea (mass spectrometry, ES $M^+ = 225.2$)

- 1H-NMR (DMSO-d$_6$, 300 MHz) δ (ppm) = 0.81 (q, 6H, CH$_3$CH$_3$CH$_3$); 0.92 (d, 3H, CH$_3$CH$_2$CH$_3$); 1.56 (h, 1H, CH$_3$CH$_2$CH$_3$); 2.57 (t, 2H, NHCH$_2$CH$_2$); 3.20 (q, 2H, CH$_2$CH$_2$NH); 3.45 (m, 1H, CH$_2$CH$_2$CH$_2$NH); 5.68 (s mod, 1H, NHCH$_2$); 5.70 (s mod, 1H, NHCH$_2$); 6.76 (s, 1H, $N$ CHNH imidazole).

(S)-1-1-(2-(1H-imidazol-4-yl)ethyl)-3-(3-methylbutan-2-yl)urea (mass spectrometry, ES $M^+ = 225.2$)

- 1H-NMR (DMSO-d$_6$, 300 MHz) δ (ppm) = 0.81 (q, 6H, CH$_3$CH$_3$CH$_3$); 0.92 (d, 3H, CH$_3$CH$_2$CH$_3$); 1.56 (h, 1H, CH$_3$CH$_2$CH$_3$); 2.57 (t, 2H, NHCH$_2$CH$_2$); 3.20 (q, 2H, CH$_2$CH$_2$NH); 3.45 (m, 1H, CH$_2$CH$_2$CH$_2$NH); 5.68 (s mod, 1H, NHCH$_2$); 5.70 (s mod, 1H, NHCH$_2$); 6.76 (s, 1H, $N$ CHNH imidazole).

(R)-1-1-(2-(1H-imidazol-4-yl)ethyl)-3-(hexan-2-yl)urea (mass spectrometry, ES $M^+ = 239.3$)

- 1H-NMR (DMSO-d$_6$, 300 MHz) δ (ppm) = 0.86 (t, 3H, CH$_3$CH$_2$); 0.97 (d, 3H, CH$_3$CH$_2$CH$_3$); 1.24 (m, 6H, CH$_3$(CH2)3CH2); 2.57 (t, 2H, NHCH$_2$CH$_2$); 2.91 (q, 2H, CH$_2$CH$_2$NH); 3.54 (m, 1H, CH$_3$CH($N$H)$N$ imidazole); 7.52 (s, 1H, $N$ CHNH imidazole).

(S)-1-1-(2-(1H-imidazol-4-yl)ethyl)-3-(hexan-2-yl)urea (mass spectrometry, ES $M^+ = 239.2$)

- 1H-NMR (DMSO-d$_6$, 300 MHz) δ (ppm) = 0.84 (t, 3H, CH$_3$CH$_2$); 0.97 (d, 3H, CH$_3$CH$_2$CH$_3$); 1.23 (m, 6H, CH$_3$(CH2)3CH2); 2.56 (t, 2H, NHCH$_2$CH$_2$); 2.91 (q, 2H, CH$_2$CH$_2$NH); 3.54 (m, 1H, CH$_3$CH($N$H)$N$ imidazole); 7.52 (s, 1H, $N$ CHNH imidazole).

(R)-1-1-(2-(1H-imidazol-4-yl)ethyl)-3-(octan-2-yl)urea (mass spectrometry, ES $M^+ = 267.2$)

- 1H-NMR (DMSO-d$_6$, 300 MHz) δ (ppm) = 0.85 (t, 3H, CH$_3$CH$_2$); 0.97 (d, 3H, CH$_3$CH$_2$CH$_3$); 1.23 (m, 10H, CH$_3$(CH2)5CH2); 2.57 (t, 2H, NHCH$_2$CH$_2$); 3.20 (q, 2H, CH$_2$CH$_2$NH); 3.53 (m, 1H, CH$_2$CH$_2$CH$_2$NH); 5.67 (s mod, 1H, NHCH$_2$); 5.69 (s mod, 1H, NHCH$_2$); 6.82 (s, 1H, $N$ CHNH imidazole).
where \( [A]_f \) is the free concentration of activator.

\[ v = \frac{v_{\text{max}}}{1 + K_A/[S](1 + [A]_t/K_A)} \]  

(4)

where \([A]_t\) is the free concentration of activator.

Working at substrate concentrations considerably lower than \( K_m([S] \ll K_m) \), and considering that \([A]_t\) can be represented in the form of the total concentration of the enzyme \([E]_t\) and activator \([A]_t\), the obtained competitive steady-state equation for determining the activation constant is given by the following equation \(^{23,24,95-104}\):

\[ v = \frac{v_0 K_A}{[A]_t + ([A]_t + [E]_t + K_A) - ([A]_t + [E]_t + K_A)^2 - 4 [A]_t [E]_t^{1/2}} \]  

(5)

where \( v_0 \) represents the initial velocity of the enzyme-catalyzed reaction in the absence of activator. All CA isozymes used in the experiments were purified recombinant proteins obtained as reported earlier by our group \(^{23,24}\).

Results and discussion

Chemistry

The rationale for designing new CAAs reported in this paper is based on the reported X-ray crystal structure for the hCA II – histamine adduct \(^{29}\). As mentioned above, the aminoethyl moiety of the activator does not make relevant contacts with the enzyme and is free to be derivatized as it points out towards the exit of the active site. In this way, the imidazole moiety of the activator can participate to the proton shuttling processes crucial for enhancing the catalytic efficiency of the enzyme, whereas the derivatized amino group may lead to a further stabilization of the enzyme-activator adduct. In an earlier work \(^{106}\), we showed that sulfonamido, carbamamido, and ureido/thiourea derivatives of histamine (at the aliphatic portion of the molecule) act as efficient activators of several CA isoforms, such as hCA I, hCA II, and bCA IV (b = bovine isofrom). As only a few (more exactly 5) ureido derivatives of histamine were reported, all of them incorporating aromatic R moieties, here we decided to investigate a larger such series of ureas and diureas, obtained by reacting histamine with alkyl/aryl isocyanates and di-isocyanates, as described in Scheme 2.

A rather large number of such derivatives were obtained (Table 1), which incorporate various alkyl moieties of variable length, cycloalkyl, and aryl moieties. All compounds were thoroughly characterized by physico-chemical procedures which conformed their structure (see Materials and methods for details).

CA activation

Ureas 1–22 and histamine were assayed for the activation of the physiologically most important cytosolic isoforms hCA I and II (Table 1). It should be mentioned that these are widespread isoforms in many tissues (e.g. red blood cells contain approximately 150 \( \mu \)M of hCA I and 20 \( \mu \)M of hCA II) \(^{2}\), including not only the blood but also the gastro-intestinal tract, kidneys, lungs, and the brain \(^{5,7}\).

Data of Table 1 show some very interesting structure-activity relationship (SAR) data for the activation of these two isoforms with histamine and its ureido/bisureido derivatives 1–22. The most salient feature is that unlike histamine, which is a poor hCA II activator \((K_A < 125 \mu M)\) but a rather efficient hCA I activator \((K_A \approx 2.0 \mu M)\), the ureas 1–22 do not activate at all hCA II, but are all of them effective hCA I activators, with \( K_A \)S in the range of 0.73–3.4 \( \mu M \) (Table 1). The second rather interesting feature of this class of CAAs is the fact that the activation constants against hCA I show a rather modest range, with a minimal variation of potency, irrespective of the rather diverse substitution pattern at the ureido moiety, or whether they are mono- or bis-urea derivatives (and as a consequence they contain one or two imidazole moieties able to participate in proton shuttling processes). Thus, the most

---

**Scheme 2.** Synthesis of ureas 1–22 from histamine and isocyanates/diisocyanates.
Table 1. CA activation against isoforms hCA I and II with ureas 1–22 and histamine as standard, by a stopped-flow CO₂ hydrase assay. $K_a$ = activation constant.

| No. | Structure | $K_a$ hCA I ($\mu$M)$^a$ | $K_a$ hCA II ($\mu$M)$^a$ |
|-----|-----------|--------------------------|---------------------------|
| 1   |           | 3.1                      | >200                      |
| 2   |           | 2.7                      | >200                      |
| 3   |           | 3.0                      | >200                      |
| 4   |           | 3.4                      | >200                      |
| 5   |           | 2.8                      | >200                      |
| 6   |           | 2.9                      | >200                      |
| 7   |           | 3.1                      | >200                      |
| 8   |           | 2.2                      | >200                      |
| 9   |           | 1.7                      | >200                      |
| 10  |           | 1.6                      | >200                      |
| 11  |           | 1.5                      | >200                      |
| 12  |           | 3.0                      | >200                      |
| 13  |           | 2.2                      | >200                      |
| 14  |           | 3.1                      | >200                      |
| 15  |           | 3.0                      | >200                      |
| 16  |           | 1.6                      | >200                      |
| 17  |           | 1.1                      | >200                      |
| 18  |           | 0.73                     | >200                      |

(continued)
effective activator is indeed a bis-urea (compound 18), which contains two imidazoles in its molecule and showed a $K_A$ of 0.73 $\mu$M, being thus 2.74 times a more effective hCA I activator compared to histamine. However, the other submicromolar CAAs detected here, compounds 21 and 22 ($K_A$ of 0.97–0.98 $\mu$M) were monoureas, containing only one imidazole moiety. Several of the ureas investigated here (e.g. 1–8 and 12–15) were slightly less effective hCA I activators compared with histamine, with $K_A$s in the range of 2.2–3.4 $\mu$M. It is difficult to explain this loss of activity, also considering the fact that some aryl-ureido histamines reported earlier106, possessing a very diverse substitution pattern compared with these compounds, possessed a much more efficient activating profile against hCA I (and also activated hCA II and bCA IV). Probably the rather long aliphatic moieties present in the ureas investigated here were detrimental for the binding of the activator at the entrance of the active site cavity, a region of the enzyme rich in hydrophilic amino acid residues1,18. However, although slightly less effective than histamine, these compounds did show activating properties against this isoform, but not at all against hCA II, which is probably even more difficult to explain. However, as explained earlier by us23, the entrance of the active site cavity of the two isoforms are very diverse, with hCA II possessing a cluster of at least 6 histidine residues (His3, 4, 10, 15, 17, and 64) which is absent in hCA I. The much more hydrophilic environment at the entrance of hCA II active site probably explains why the hydrophobic ureas reported here 1–22 do not efficiently bind to this enzyme, and do not show any CA activating effect. This is, as far as we know, the only example of isoform-selective CAA, and may be of relevance for better understanding the physiology/pharmacology of hCA I.

Conclusions

By catalyzing the simple but highly important hydration of carbon dioxide to bicarbonate and protons, CAs are involved in critical steps of the life cycle of many organisms, including eukaryotes, Bacteria and Archaea. A large number of CA inhibitors have pharmacological applications in the field of antiglaucoma, anticonvulsant, anticancer, and anti-infective agents, whereas activation of these enzymes is not yet properly exploited pharmacologically for cognitive enhancement or Alzheimer’s disease, conditions in which a diminished CA activity was reported. We report here a series of ureido/bis-ureido histamine derivatives which were investigated for their activating effects against the cytosolic human (h) isoform hCA I and II. We observed that all these compounds, unlike histamine or other activator classes, show no activating effects on the widespread, physiologically dominant isoform hCA II and II. We observed that all these compounds, unlike histamine or other activator classes, show no activating effects on the widespread, physiologically dominant isoform hCA II, but were rather effective hCA I activators. This is the first report in which CA I-selective activators were identified. Such compounds may constitute interesting tools for better understanding the physiological/pharmacological effects connected to activation of this widespread CA isoform.

Disclosure statement

One author (C. T. S.) declares conflict of interest, being author of several patents in the field of CA inhibitors/activators. This research was financed by several EU projects (Euroxy, Metoxia, DeZnIt, and Dynano). The other authors do not declare conflict of interest.

References

1. Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nat Rev Drug Discov 2008;7:168–81.
2. Neri D, Supuran CT. Interfering with pH regulation in tumours as a therapeutic strategy. Nat Rev Drug Discov 2011;10:767–77.
3. Capasso C, Supuran CT. An overview of the alpha-, beta- and gamma-carbonic anhydrases from bacteria: can bacterial carbonic anhydrases shed new light on evolution of bacteria? J Enzyme Inhib Med Chem 2015;30:325–32.

4. Supuran CT, Capasso C. The η-class carbonic anhydrases as drug targets for antimalarial agents. Expert Opin Ther Targets 2015;19:551–63.

5. Supuran CT. Structure-based drug discovery of carbonic anhydrase inhibitors. J Enzyme Inhib Med Chem 2012;27:759–72.

6. Supuran CT. Carbonic anhydrase inhibitors. Bioorg Med Chem Lett 2010;20:3467–74.

7. Pastorekova S, Parkkila S, Pastorek J, Supuran CT. Carbonic anhydrases: current state of the art, therapeutic applications and future prospects. J Enzyme Inhib Med Chem 2004;19:199–229.

8. Supuran CT. Carbonic anhydrase inhibitors and activators for novel therapeutic applications. Future Med Chem 2011;3:1165–80.

9. Supuran CT. Bacterial carbonic anhydrases as drug targets: toward novel antibiotics? Front Pharmacol 2011;2:34.

10. Supuran CT, Scozzafava A, Casini A. Carbonic anhydrase inhibitors. Med Res Rev 2003;23:146–89.

11. Luca C, Barboiu M, Supuran CT. Stability constant of complex inhibitors and their mechanism of action. Rev Roum Chim 1991;36:1169–73.

12. De Simone G, Supuran CT. (In)organic anions as carbonic anhydrase inhibitors. J Inorg Biochem 2012;111:177–29.

13. Del Prete S, Vullo D, De Luca V, et al. Biochemical characterization of recombinant beta-carbonic anhydrase (PgICab) identified in the genome of the oral pathogenic bacterium Porphyromonas gingivalis. J Enzyme Inhib Med Chem 2015;30:366–70.

14. Supuran CT. Carbonic anhydrase inhibitors: an editorial. Expert Opin Ther Pat 2013;23:677–9.

15. Supuran CT. Carbonic anhydrases: from biomedical applications of the inhibitors and activators to biotechnological use for CO(2) capture. J Enzyme Inhib Med Chem 2013;28:229–30.

16. Capasso C, Supuran CT. Anti-infective carbonic anhydrase inhibitors: a patent and literature review. Expert Opin Ther Pat 2013;23:693–704.

17. Capasso C, Supuran CT. Sulfur and threomethoprim-like drugs – antimetabolites acting as carbonic anhydrase, dihydropteroylate synthase and dihydrofolate reductase inhibitors. J Enzyme Inhib Med Chem 2014;29:379–87.

18. Supuran CT. Structure and function of carbonic anhydrases. Biochem J 2016;473:2023–32.

19. Barboiu M, Supuran CT, Menabuoni L, et al. Carbonic anhydrase inhibitors, synthesis of topically effective intraocular pressure lowering agents derived from 5-(aminoalkylcarboxamido)-1,3,4-thiadiazole-2-sulfonamide. J Enzyme Inhib Med Chem 1999;15:23–46.

20. Zimmerman S, Innocenti A, Casini A, et al. Carbonic anhydrase inhibitors. Inhibition of the prokaryotic beta and gamma-class enzymes from Archea with sulfonamides. Bioorg Med Chem Lett 2004;14:6001–6.

21. Alterio V, Di Fiore A, D’Ambrosio K, et al. Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms? Chem Rev 2012;112:4241–68.

22. Temperini C, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors. X-ray crystal studies of the carbonic anhydrase II – trithiocarbonate adduct – an inhibitor mimicking the sulfonamide and urea binding to the enzyme. Bioorg Med Chem Lett 2010;20:474–8.

23. Briganti F, Mangani S, Orioli P, et al. Carbonic anhydrase activators: X-ray crystallographic and spectroscopic investigations for the interaction of isozymes I and II with histamine. Biochemistry 1997;36:10384–92.

24. Temperini C, Scozzafava A, Vullo D, Supuran CT. Carbonic anhydrase inhibitors. Activation of isozymes I, II, IV, VA, VII and XIV with l- and d-histidine and crystallographic analysis of their adducts with isofem II: engineering proton transfer processes within the active site of an enzyme. Chemistry 2006;12:7057–66.

25. Vullo D, De Luca V, Del Prete S, et al. Sulfinamide inhibition studies of the γ-carbonic anhydrase from the Antarctic cyanobacterium Nostoc commune. Bioorg Med Chem 2015;23:1728–34.

26. Schlicker C, Hall RA, Vullo D, et al. Structure and inhibition of the CO2-sensing carbonic anhydrase Can2 from the pathogenic fungus Cryptococcus neoformans. J Mol Biol 2009;385:1207–20.

27. Pacchiano F, Carta F, Vullo D, et al. Inhibition of β-carbonic anhydrases with ureido-substituted benzenesulfonamides. Bioorg Med Chem Lett 2010;20:102–5.

28. Carta F, Aggarwal M, Maresca A, et al. Dithiocarbamates strongly inhibit carbonic anhydrases and show antiglaucoma action in vivo. J Med Chem 2012;55:1721–30.

29. Monti SM, Maresca A, Viparelli F, et al. Dithiocarbamates strongly inhibit the beta-class fungal carbonic anhydrases from Cryptococcus neoformans, Candida albicans and Candida glabrata. Bioorg Med Chem Lett 2012;22:859–62.

30. Maresca A, Carta F, Vullo D, Supuran CT. Dithiocarbamates strongly inhibit the beta-class carbonic anhydrases from Mycobacterium tuberculosis. J Enzyme Inhib Med Chem 2013;28:407–11.

31. Carta F, Innocenti A, Hall RA, et al. Carbonic anhydrase inhibitors. Inhibition of the β-class enzymes from the fungal pathogens Candida albicans and Cryptococcus neoformans with branched aliphatic/aromatic carboxylates and their derivatives. Bioorg Med Chem Lett 2011;21:2521–6.

32. Nishimori I, Onishi S, Takeuchi H, Supuran CT. The alpha and beta classes carbonic anhydrases from Helicobacter pylori as novel drug targets. Curr Pharm Des 2008;14:622–30.

33. Minakuchi T, Nishimori I, Vullo D, et al. Molecular cloning, characterization and inhibition studies of the Rv1284 β-carbonic anhydrase from Mycobacterium tuberculosis with sulfonamides and a sulfamate. J Med Chem 2009;52:2226–32.

34. Nishimori I, Minakuchi T, Vullo D, et al. Carbonic anhydrase inhibitors: cloning, characterization, and inhibition studies of a new β-carbonic anhydrase from Mycobacterium tuberculosis. J Med Chem 2009;52:3116–20.

35. Güzel O, Maresca A, Scozzafava A, et al. Discovery of low nanomolar and subnanomolar inhibitors of the mycobacterial beta-carbonic anhydrases Rv1284 and Rv3273. J Med Chem 2009;52:4063–7.

36. Carta F, Maresca A, Suarez Covarrubias A, et al. Carbonic anhydrase inhibitors. Characterization and inhibition studies of the most active β-carbonic anhydrase from Mycobacterium tuberculosis, Rv3588c. Bioorg Med Chem Lett 2009;19:6649–54.

37. Nishimori I, Minakuchi T, Maresca A, et al. The β-carbonic anhydrases from Mycobacterium tuberculosis as drug targets. Curr Pharm Des 2010;16:3300–9.
Winum JY, Kohler S, Supuran CT. Brucella carbonic anhydrases: new targets for designing anti-infective agents. Curr Pharm Des 2010;16:3310–16.

Vullo D, Nishimori I, Minakuchi T, et al. Inhibition studies with anions and small molecules of two novel β-carbonic anhydrases from the bacterial pathogen Salmonella enterica serovar Typhimurium. Bioorg Med Chem Lett 2011;21:3591–5.

Maresca A, Vullo D, Scozzafava A, Supuran CT. Inhibition of the alpha- and beta-carbonic anhydrases from the gastric pathogen Helicobacter pylori with anions. J Enzyme Inhib Med Chem 2013;28:388–91.

Maresca A, Vullo D, Scozzafava A, et al. Inhibition of the beta-class carbonic anhydrases from Mycobacterium tuberculosis with carboxylic acids. J Enzyme Inhib Med Chem 2013;28:392–6.

Vullo D, Nishimori I, Scozzafava A, et al. Inhibition studies of a β-carbonic anhydrase from Brucella suis with a series of water soluble glycosylsulfanilamides. Bioorg Med Chem Lett 2010;20:2178–82.

Nishimori I, Minakuchi T, Kohsaki T, et al. Carbonic anhydrase inhibitors. The β-carbonic anhydrase from Helicobacter pylori is a new target for sulfonamide and sulfamate inhibitors. Bioorg Med Chem Lett 2007;17:3585–94.

Weber A, Casini A, Heine A, et al. Unexpected nanomolar inhibition of carbonic anhydride by COX-2 selective Celecoxib: new pharmacological opportunities due to related binding site recognition. J Med Chem 2004;47:550–7.

Di Fiore A, Pedone C, D’Ambrosio K, et al. Carbonic anhydrase inhibitors: valdecoxib binds to a different active site region of the human isozyme II as compared to the structurally related, cyclooxygenase II “selective” inhibitor celecoxib. Bioorg Med Chem Lett 2006;16:437–42.

Köhler K, Hillebrecht A, Schulze Wischeler J, et al. Saccharin inhibits carbonic anhydrases: possible explanation for its unpleasant metallic aftertaste. Angew Chem Int Ed Engl 2007;46:7697–9.

Casini A, Scozzafava A, Mincione F, et al. Carbonic anhydrase inhibitors: water-soluble 4-sulamoylphenylthioureas as topical intraocular pressure-lowering agents with long-lasting effects. J Med Chem 2000;43:4884–92.

Scozzafava A, Menabuoni L, Mincione F, Supuran CT. Carbonic anhydrase inhibitors. A general approach for the preparation of water soluble sulfonamides incorporating polyamino-polycarboxylate tails and of their metal complexes possessing long lasting, topical intraocular pressure lowering properties. J Med Chem 2002;45:1466–76.

Fabrizi F, Mincione F, Somma T, et al. A new approach to antiglaucoma drugs: carbonic anhydrase inhibitors with or without NO donating moieties. Mechanism of action and preliminary pharmacology. J Enzyme Inhib Med Chem 2012;27:138–47.

Ebbesen P, Pettersen EO, Gorr TA, et al. Taking advantage of tumor cell adaptations to hypoxia for developing new tumor markers and treatment strategies. J Enzyme Inhib Med Chem 2009;24:1–39.

Svávestová E, Hulíková A, Rafajová M, et al. Hypoxia activates the capacity of tumor-associated carbonic anhydrase IX to acidify extracellular pH. FEBS Lett 2004;577:439–45.

Dubois L, Liewes NG, Maresca A, et al. Imaging of CA IX with fluorescent labelled sulfonamides distinguishes hypoxic and (re)-oxygenated cells in a xenograft tumour model. Radiother Oncol 2009;92:423–8.

Ahlskog JKJ, Dumelin CE, Trüssel S, et al. In vivo targeting of tumor-associated carbonic anhydrases using acetazolamide derivatives. Bioorg Med Chem Lett 2009;19:4851–6.

Pacchiano F, Carta F, McDonald PC, et al. Ureido-substituted benzensulfonamides potently inhibit carbonic anhydrase IX and show antimitastatic activity in a model of breast cancer metastasis. J Med Chem 2011;54:1896–902.

Mincione F, Scozzafava A, Supuran CT. The development of topically acting carbonic anhydrase inhibitors as anti-glaucoma agents. Curr Top Med Chem 2007;7:849–54.

Carta F, Supuran CT, Scozzafava A. Novel therapies for glaucoma: a patent review 2007–2011. Expert Opin Ther Pat 2012;22:79–88.

Steele RM, Batugo MR, Benedini F, et al. Nitric oxide-donating carbonic anhydrase inhibitors for the treatment of open-angle glaucoma. Bioorg Med Chem Lett 2009;19:6556–70.

Mincione F, Benedini F, Biondi S, et al. Synthesis and crystallographic analysis of new sulfonamides incorporating NO-donating moieties with potent antiglaucoma action. Bioorg Med Chem Lett 2011;21:3216–21.

Alterio V, Vitale RM, Monti SM, et al. Carbonic anhydrase inhibitors: X-ray and molecular modeling study for the interaction of a fluorescent antitumor sulfonamide with isozyme II and IX. J Am Chem Soc 2006;128:8329–35.

Alterio V, De Simone G, Monti SM, et al. Carbonic anhydrase inhibitors: inhibition of human, bacterial, and archaean isozymes with benzene-1,3-disulfonamides – solution and crystallographic studies. Bioorg Med Chem Lett 2007;17:4201–7.

Wagner J, Avvaru BS, Robbins AH, et al. Coumarinyl-substituted sulfonamides strongly inhibit several human carbonic anhydrase isoforms: solution and crystallographic investigations. Bioorg Med Chem 2010;18:4873–8.

Biswas S, Aggarwal M, Guzel O, et al. Conformational variability of different sulfonamide inhibitors with thienyl-acetamido moieties attributes to differential binding in the active site of cytosolic human carbonic anhydrase isoforms. Bioorg Med Chem 2011;19:3732–8.

Pacchiano F, Aggarwal M, Avvaru BS, et al. Selective hydrophobic pocket binding observed within the carbonic anhydrase II active site accommodate different 4-substituted-ureido-benzenesulfonamides and correlate to inhibitor potency. Chem Commun (Camb.) 2010;46:8371–3.

Carta F, Garaj V, Maresca A, et al. Sulfonamides incorporating 1,3,5-triazine moieties selectively and potently inhibit carbonic anhydrase transmembrane isoforms IX, XII and XIV over cytosolic isoforms I and II: solution and X-ray crystallographic studies. Bioorg Med Chem 2011;19:3105–19.

Hen N, Blaler M, Yagen B, et al. Anticonvulsant 4-amino-benzenesulfonamide derivatives with branched-alkylamide moieties: X-ray crystallography and inhibition studies of human carbonic anhydrase isoforms I, II, VII and XIV. J Med Chem 2011;54:3977–81.

Kolayli S, Karahalil F, Sahin H, et al. Characterization and inhibition studies of an α-carbonic anhydrase from the endangered sturgeon species Acipenser gueldenstaedti. J Enzyme Inhib Med Chem 2011;26:895–900.

Guzel Ö, Innocenti A, Scozzafava A, et al. Carbonic anhydrase inhibitors. Phenacyetyl-, pyridylacetyl- and thienylacetyl-substituted aromatic sulfonamides act as potent and
selective isoform VII inhibitors. Bioorg Med Chem Lett 2009;19:3170–3.
68. Guzel Ö, Innocenti A, Scozzafava A, et al. Carbonic anhydrase inhibitors. Aromatic/heterocyclic sulfonamides incorporating phenacetyl-, pyridylacetyl- and thienylacetyl-tails act as potent inhibitors of human mitochondrial isoforms VA and VB. Bioorg Med Chem 2009;17:4894–9.
69. Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors. Arylsulfonylureido and arylureido-substituted aromatic and heterocyclic sulfonamides: towards selective inhibitors of carbonic anhydrase isozyme I. J Enzyme Inhib 1999;14:343–63.
70. Garaj V, Puccetti L, Fasolis G, et al. Carbonic anhydrase inhibitors: synthesis and inhibition of cytosolic/tumor-associated carbonic anhydrase isozymes I, II and IX with sulfonamides incorporating 1,2,4-triazine moieties. Bioorg Med Chem Lett 2004;14:5427–33.
71. Garaj V, Puccetti L, Fasolis G, et al. Carbonic anhydrase inhibitors. Novel sulfonamides incorporating 1,3,5-triazine moieties as inhibitors of the cytosolic and tumor-associated carbonic anhydrase isozymes I, II and IX. Bioorg Med Chem Lett 2005;15:3102–8.
72. McDonald PC, Winum JY, Supuran CT, Dedhar S. Recent developments in targeting carbonic anhydrase IX for cancer therapeutics. Oncotarget 2012;3:84–97.
73. Carta F, Scozzafava A, Supuran CT. Sulfonamides (RSO2NH2): a patent review 2008–2012. Expert Opin Ther Pat 2012;22:747–58.
74. Masini E, Carta F, Scozzafava A, Supuran CT. Antiglaucoma carbonic anhydrase inhibitors: a patent review. Expert Opin Ther Pat 2013;23:705–16.
75. Monti SM, Supuran CT, De Simone G. Anticancer carbonic anhydrase inhibitors: a patent review (2008–2013). Expert Opin Ther Pat 2013;23:737–49.
76. Carta F, Supuran CT. Diuretics with carbonic anhydrase inhibitory action: a patent and literature review (2005–2013). Expert Opin Ther Pat 2013;23:681–91.
77. Supuran CT. Carbonic anhydrase inhibitors as emerging drugs for the treatment of obesity. Expert Opin Emerg Drugs 2012;17:11–15.
78. Scozzafava A, Supuran CT, Carta F. Antiobesity carbonic anhydrase inhibitors: a literature and patent review. Expert Opin Ther Pat 2013;23:725–35.
79. Supuran CT. The safety and clinical efficacy of acetazolamide for the treatment of idiopathic intracranial hypertension. Expert Rev Neurother 2015;15:851–6.
80. De Simone G, Alterio V, Supuran CT. Exploiting the hydrophobic and hydrophilic binding sites for designing carbonic anhydrase inhibitors. Expert Opin Drug Discov 2013;8:793–810.
81. Scozzafava A, Menabuoni L, Mincione F, et al. 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;42:2641–50.
82. Borras J, Scozzafava A, Menabuoni L, et al. Carbonic anhydrase inhibitors. Part 73. Synthesis of water-soluble, topically effective intraocular pressure lowering aromatic/heterocyclic sulfonamides containing 8-quinoline-sulfonil moieties: is the tail more important than the ring? Bioorg Med Chem 1999;7:2397–406.
83. Supuran CT. Carbonic anhydrase inhibition with natural products: novel chemotypes and inhibition mechanisms. Mol Divers 2011;15:305–16.
84. Karioti A, Ceruso M, Carta F, et al. New natural product carbonic anhydrase inhibitors incorporating phenol moieties. Bioorg Med Chem 2015;23:7219–25.
85. Winum JY, Supuran CT. Recent advances in the discovery of zinc-binding motifs for the development of carbonic anhydrase inhibitors. J Enzyme Inhib Med Chem 2015;30:321–4.
86. Briganti F, Pierattelli R, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors. Part 37. Novel classes of carbonic anhydrase inhibitors and their interaction with the native and cobalt-substituted enzyme: kinetic and spectroscopic investigations. Eur J Med Chem 1996;31:1001–10.
87. Casini A, Antel J, Abbate F, et al. Carbonic anhydrase inhibitors: SAR and X-ray crystallographic study for the interaction of sugar sulfonamides/sulfamides with isozymes I, II and IV. Bioorg Med Chem Lett 2003;13:841.
88. Carta F, Di Cesare Mannelli L, Pinard M, et al. A class of sulfonamide carbonic anhydrase inhibitors with neuropathic pain modulating effects. Bioorg Med Chem 2015;23:1828–40.
89. Bejaoui M, Panatzi E, De Luca V, et al. Acetazolamide protects steatotic liver grafts against cold ischemia reperfusion injury. J Pharmacol Exp Ther 2015;355:191–8.
90. Di Cesare Mannelli L, Micheli L, Carta F, et al. Carbonic anhydrase inhibition for the management of cerebral ischamia: in vivo evaluation of sulfonamide and coumarin inhibitors. J Enzyme Inhib Med Chem 2016. [Upb ahead of print]. doi: 10.3109/14756366.2015.1113407.
91. Sun MK, Alkon DL. Carbonic anhydrase gating of attention: memory therapy and enhancement. Trends Pharmacol Sci 2002;23:83–92.
92. Illes M, Scozzafava A, Supuran CT, Carbonic anhydrase activators. In: Supuran CT, Scozzafava A, Conway J, eds. Carbonic anhydrase – its inhibitors and activators. Boca Raton, FL: CRC Press; 2004:317–52.
93. Meier-Ruge W, Iwangoff P, Reichlmeyer K. Neurochemical enzyme changes in Alzheimer’s and Pick’s disease. Arch Gerontol Geriatr 1984;3:161–5.
94. Temperini C, Innocenti A, Scozzafava A, Supuran CT. Carbonic anhydrase activators: kinetic and X-ray crystallographic study for the interaction of d- and l-tryptophan with the mammalian isoforms I–XIV. Bioorg Med Chem 2008;16:8373–8.
95. Temperini C, Scozzafava A, Puccetti L, Supuran CT. Carbonic anhydrase activators: X-ray crystal structure of the adduct of human isozyme II with l-histidine as a platform for the design of stronger activators. Biorg Med Chem Lett 2005;15:5136–41.
96. Temperini C, Innocenti A, Scozzafava A, et al. Carbonic anhydrase activators: l-adrenaline plugs the active site entrance of isozyme II, activating better isoforms I, IV, VA, VII and XIV. Bioorg Med Chem Lett 2007;17:628–35.
97. Temperini C, Vullo D, Scozzafava A, Supuran CT. Carbonic anhydrase activators. Activation of isoforms I, II, IV, VA and XIV with l- and d-phenylalanine and crystallographic analysis of their adducts with isozyme II: sterospecific recognition within the active site of an enzyme and its consequences for the drug design. J Med Chem 2006;49:3019–27.
98. Nishimori I, Onishi S, Vullo D, et al. Carbonic anhydrase activators. The first activation study of the human secretory isoform VI. Bioorg Med Chem 2007;15:5351–7.
99. Abdo MR, Vullo D, Saada MC, et al. Carbonic anhydrase activators: activation of human isozymes I, II and IX with phenylsulfonylhydrazido l-histidine derivatives. Bioorg Med Chem Lett 2009;19:2440–3.

100. Scozzafava A, Supuran CT. Carbonic anhydrase activators: high affinity isozymes I, II, and IV activators, incorporating a beta-alanyl-histidine scaffold. J Med Chem 2002;45:284–91.

101. Scozzafava A, Supuran CT. Carbonic anhydrase activators: human isozyme II is strongly activated by oligopeptides incorporating the carboxyterminal sequence of the bicarbonate anion exchanger AE1. Bioorg Med Chem Lett 2002;12:1177–80.

102. Illies M, Banciu MD, Illies MA, et al. Carbonic anhydrase activators: design of high affinity isozymes I, II and IV activators, incorporating tri-/tetrasubstituted-pyridinium-azole moieties. J Med Chem 2002;45:504–10.

103. Supuran CT, Scozzafava A, Carbonic anhydrase activators as potential anti-Alzheimer’s disease agents. In: Smith HJ, Simons C, Sewell RDE, eds. Protein misfolding in neurodegenerative diseases: mechanisms and therapeutic strategies. Boca Raton, Florida: CRC Press; 2007:265–88.

104. Clare BW, Supuran CT. Carbonic anhydrase activators. 3: structure-activity correlations for a series of isozyme II activators. J Pharm Sci 1994;83:768–73.

105. Khalifah RG. The carbon dioxide hydration activity of carbonic anhydrase. I. Stop-flow kinetic studies on the native human isoenzymes B and C. J Biol Chem 1971;246:2561–73.

106. Scozzafava A, Supuran CT. Carbonic anhydrase activators – Part 21. Novel activators of isozymes I, II and IV incorporating carboxamido and ureido histamine moieties. Eur J Med Chem 2000;35:31–9.