



An anion and small molecule inhibition study of the β -carbonic anhydrase from *Staphylococcus aureus*

Linda J. Urbanski, Daniela Vullo, Seppo Parkkila & Claudiu T. Supuran

To cite this article: Linda J. Urbanski, Daniela Vullo, Seppo Parkkila & Claudiu T. Supuran (2021) An anion and small molecule inhibition study of the β -carbonic anhydrase from *Staphylococcus aureus*, Journal of Enzyme Inhibition and Medicinal Chemistry, 36:1, 1088-1092, DOI: [10.1080/14756366.2021.1931863](https://doi.org/10.1080/14756366.2021.1931863)

To link to this article: <https://doi.org/10.1080/14756366.2021.1931863>



© 2021 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.



Published online: 31 May 2021.



Submit your article to this journal [↗](#)



Article views: 1274



View related articles [↗](#)





View Crossmark data [↗](#)



Citing articles: 10 View citing articles [↗](#)

An anion and small molecule inhibition study of the β -carbonic anhydrase from *Staphylococcus aureus*

Linda J. Urbanski^a, Daniela Vullo^b, Seppo Parkkila^{a,c}  and Claudiu T. Supuran^b 

^aFaculty of Medicine and Health Technology, Tampere University, Tampere, Finland; ^bNeurofarba Department, Sezione di Chimica Farmaceutica e Nutraceutica, Università degli Studi di Firenze, Firenze, Italy; ^cFimlab Ltd, Tampere University Hospital, Tampere, Finland

ABSTRACT

Pathogenic bacteria resistant to most antibiotics, including the methicillin-resistant *Staphylococcus aureus* (MRSA) represent a serious medical problem. The search for new anti-infectives, possessing a diverse mechanism of action compared to the clinically used antibiotics, has become an attractive research field. *S. aureus* DNA encodes a β -class carbonic anhydrase, SauBCA. It is a druggable target that can be inhibited by certain aromatic and heterocyclic sulphonamides. Here we investigated inorganic anions and some other small molecules for their inhibition of SauBCA. The halides, nitrite, nitrate, bicarbonate, carbonate, bisulphite, sulphate, stannate, and *N,N*-diethyldithiocarbamate were submillimolar SauBCA inhibitors with K_i s in the range of 0.26–0.91 mM. The most effective inhibitors were sulfamide, sulfamate, phenylboronic acid, and phenylarsonic acid with K_i s of 7–43 μ M. Several interesting inhibitors detected here may be considered lead compounds for the development of even more effective derivatives, which should be investigated for their bacteriostatic effects.

ARTICLE HISTORY

Received 22 April 2021
Revised 11 May 2021
Accepted 14 May 2021

KEYWORDS

β -carbonic anhydrase;
Staphylococcus aureus;
kinetics; inhibition; anions





1. Introduction

Staphylococcus aureus is a Gram-positive bacterium that infects nearly all host tissues in many mammalian species, including humans and livestock, causing severe morbidity and mortality¹. It belongs to the sadly famous ESKAPE group of bacterial pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp.) that are resistant to many clinically used antibiotics including methicillin and vancomycin². As a consequence, the treatment of such infection remains particularly challenging if not impossible in severe cases^{1,2}. Thus, there is an urgent need for new classes of antibiotics which can either inhibit the growth of these pathogens and subsequently kill them, or of compounds which can restore the sensitivity of resistant bacteria to the various classes of clinically used agents^{3–5}. The inhibitors of the widespread metalloenzyme, carbonic anhydrase (CA, EC 4.2.1.1), were recently shown to be effective in inhibiting the growth (possessing a significant bactericidal activity) of some drug-resistant pathogens, such as vancomycin-resistant *Enterococci*⁵ and *Neisseria gonorrhoeae*⁶.

In fact, CAs are present in most microorganisms including bacteria and are encoded by at least four genetic families (although new ones may still exist to be reported), which are the α -, β -, γ -, and ι -CAs^{4,7,8}. In some bacteria, such as *Escherichia coli*, the CAs are essential for the survival of the organism⁸. For others, such as *Helicobacter pylori*⁴, the CAs assure the acclimation of the bacterium in the specific niches (gastric and duodenal mucosa) in which it thrives, whereas for others, such as *Vibrio cholerae*, these enzymes participate in the secretion of bicarbonate which is a

virulence factor of this pathogen⁷. In the last decade, many representatives of these enzymes, belonging to all four classes present in bacteria, were cloned and characterised both biochemically and structurally in the search for inhibitors. This can eventually lead to the development of new antibacterial agents. Among the various species which have been characterised in this way are *E. coli*, *H. pylori*, *Mycobacterium tuberculosis*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Porphyromonas gingivalis*, *Streptococcus* spp., *Staphylococcus aureus*, etc.^{4,7–15}. Although the scientific community was rather sceptical for a long time that bacterial CA inhibition may lead to significant growth inhibition of pathogenic bacteria, Flaherty's group recently published the long-awaited^{5,6} proof-of-concept that inhibition of bacterial CAs may lead to antibiotics with novel mechanisms of action. They showed that the sulphonamide CA inhibitor (CAI) acetazolamide and some of its derivatives, as well as dorzolamide, outperformed the current drug of choice, linezolid, both *in vitro* and *in vivo*, for inhibiting the growth of vancomycin-resistant enterococci (VRE)⁵ and *N. gonorrhoeae*⁶. Furthermore, other groups have demonstrated that CAIs may exhibit reduced potential for the development of drug resistance, as in the case of *H. pylori* and ethoxzolamide as CAI. Mutations were observed in several bacterial genes, including the bacterial α -CA gene, but the pathogen remained susceptible to the drug at clinically relevant concentrations⁹.

Recently, we cloned and characterised a β -CA of *S. aureus* (SauBCA), an enzyme that possesses a high catalytic activity for the physiologic CO₂ hydration reaction to bicarbonate and protons, with the following kinetic parameters: k_{cat} of $1.46 \times 10^5 \text{ s}^{-1}$ and a k_{cat}/K_M of $2.56 \times 10^7 \text{ s}^{-1} \text{ M}^{-1}$. This enzymatic function was

CONTACT Seppo Parkkila  seppo.parkkila@tuni.fi  Faculty of Medicine and Health Technology, Tampere University, Arvo Ylpön katu 34, FI-33520, Tampere, Finland; Claudiu T. Supuran  claudiu.supuran@unifi.it  Neurofarba Department, Sezione di Chimica Farmaceutica e Nutraceutica, Università degli Studi di Firenze, Via U. Schiff 6, Firenze, Sesto Fiorentino, I-50019, Italy

© 2021 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

inhibited by various sulphonamide derivatives, which represent one of the main classes of inhibitors of these enzymes¹⁶. In this study, we continue the exploration of the inhibitors of SauBCA, reporting its inhibition profile with anions and other small molecules known to inhibit CAs.

2. Materials and methods

2.1. Chemistry

Anions and small molecules were commercially available reagents of the highest available purity from Sigma-Aldrich (Milan, Italy). Purity of tested compounds was higher than 99%.

2.2. Enzymology

SauBCA was a recombinant enzyme obtained in-house as described earlier¹⁵.

2.3. CA activity and inhibition measurements

An Applied Photophysics stopped-flow instrument was used for assaying the CA catalysed CO₂ hydration activity¹⁷. Phenol red at a concentration of 0.2 mM was used as a pH indicator (working at the absorbance maximum of 557 nm) with 10 mM Hepes (pH 7.4) as a buffer, and in the presence of 10 mM NaClO₄ for maintaining constant ionic strength. The initial rates of the CA-catalysed CO₂ hydration reaction were followed for a period of 10–100 s. The CO₂ 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 were used for determining the initial velocity. The uncatalysed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitors (10–20 mM) were prepared in distilled-deionized water and dilutions up to 0.01 μM 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 enzyme-inhibitor complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng–Prusoff equation, whereas the kinetic parameters for the uninhibited enzymes were obtained from Lineweaver–Burk plots, as reported earlier^{18–20}. The results represent the mean from at least three different determinations (data not shown). The SauBCA concentration in the assay system was 9.7 nM.

3. Results and discussion

Inorganic anions represent a well-characterised class of CAIs²¹. Our study included anions known to have a high affinity in solution for complexing metals, such as halides and especially pseudo-halides (cyanide, cyanate, thiocyanate, azide, etc.), as well as those which do not easily form complexes with transition metal ions (e.g. sulphate, selenate, tellurate, tetraborate, etc.). Both groups of anions have been shown to possess inhibitory action against all classes of CAs investigated so far, from prokaryotes to eukaryotes^{21–23}. Furthermore, small molecules such as sulfamide, sulphamic acid, phenylboronic, and phenylarsonic acid also possess such properties²⁴. In this study, we investigated a panel of such anions and small molecules for the inhibition of SauBCA (Table 1). The inhibition data of the abundant human (h) isoforms hCA I and II as well as those of another bacterial CA, NgCA from *N. gonorrhoeae*²⁵ are also shown in Table 1 for comparison.

Table 1. Inhibition constants (K_i s) of anion inhibitors against hCA I, II and the bacterial enzymes NgCA and SauBCA, measured by a stopped-flow CO₂ hydration assay¹⁷.

Anion ^b	K_i (mM) ^a			
	hCA I	hCA II	NgCA	SauBCA
F ⁻	>300	>300	8.3	0.48
Cl ⁻	6	200	4.8	0.69
Br ⁻	4	63	4.0	0.26
I ⁻	0.3	26	9.6	0.72
CNO ⁻	0.0007	0.03	0.43	3.7
SCN ⁻	0.2	1.6	0.92	28.6
CN ⁻	0.0005	0.02	1.0	4.1
N ₃ ⁻	0.0012	1.51	2.1	7.4
NO ₂ ⁻	8.4	63	0.59	0.56
NO ₃ ⁻	7	35	0.85	0.41
HCO ₃ ⁻	12	85	1.3	0.42
CO ₃ ²⁻	15	73	2.9	0.76
HSO ₃ ⁻	18	89	0.66	0.90
SO ₄ ²⁻	63	>200	0.83	0.91
HS ⁻	0.0006	0.04	0.55	19.3
NH ₂ SO ₂ NH ₂	0.31	1.13	0.058	0.009
NH ₂ SO ₃ H	0.021	0.39	0.024	0.043
PhAsO ₃ H ₂	31.7	49	0.74	0.007
PhB(OH) ₂	58.6	23	0.15	0.008
ClO ₄ ⁻	>200	>200	>100	>100
SnO ₃ ²⁻	0.57	0.83	1.7	0.32
SeO ₄ ²⁻	118	112	0.87	4.8
TeO ₄ ²⁻	0.66	0.92	0.76	42.0
OsO ₅ ²⁻	0.92	0.95	2.3	6.0
P ₂ O ₇ ²⁻	25.8	48	4.9	>100
V ₂ O ₇ ²⁻	0.54	0.57	2.8	>100
B ₄ O ₇ ²⁻	0.64	0.95	0.65	8.7
ReO ₄ ⁻	0.11	0.75	0.96	>100
RuO ₄ ⁻	0.101	0.69	1.9	>100
S ₂ O ₈ ²⁻	0.107	0.084	0.79	>100
SeCN ⁻	0.085	0.086	0.66	5.5
NH(SO ₃) ₂ ²⁻	0.31	0.76	0.25	>100
FSO ₃ ⁻	0.79	0.46	0.61	8.9
CS ₃ ²⁻	0.0087	0.0088	0.088	11.4
EtNCS ₂ ⁻	0.00079	0.0031	0.0051	0.64
PF ₆ ⁻	>100	>100	>100	>100
CF ₃ SO ₃ ⁻	>100	>100	5.7	>100

^aMean from three different assays, measured by a stopped-flow technique (errors were in the range of ± 5–10% of the reported values); ^bAs sodium salts, except sulphamide and phenylboronic acid.

The following observations can be delineated from the data presented in Table 1 regarding the inhibition of SauBCA with anions and small molecules:

- anions with a rather low propensity for complexing metal ions, such as perchlorate and hexafluorophosphate, and triflate, did not inhibit SauBCA significantly with concentrations up to 100 mM in the assay system. This is also the case for their interaction with hCA I and II, as well as many other CAs belonging to all known classes. For this reason, we used perchlorate at 10 mM concentration for maintaining constant ionic strength in the stopped-flow assays, as mentioned in Materials and methods. Other anions, such as pyrodiphosphate, divanadate, perruthenate, perrhenate, peroxydisulfate and iminidissulfonate, were also in this category of non-inhibiting anions. It should be noted, however, that some of them act as rather efficient anion inhibitors of other enzymes than SauBCA, as shown in Table 1.
- The following anions showed weak inhibitory action against SauBCA: thiocyanate, hydrogensulfide, tellurate, and trithiocarbonate, with inhibition constants in the range of 11.4–42 mM (Table 1). Except for tellurate, which is not a high-affinity ligand for metal ions, the other three anions mentioned here are either very good coordinating agents for

transition metal ions (thiocyanate, hydrogensulfide, and trithiocarbonate) or quite effective CAs (see the trithiocarbonate data for hCA I, II and NgCA in Table 1). Additionally, in some cases, the X-ray crystal structure of their complexes with hCA II is also available^{26,27}. Thus, these low inhibition constants against SauBCA deserve a better investigation in order to understand the structural features of this enzyme active site, which for the moment has not been crystallised.

- iii. Effective, millimolar inhibition was observed for the following anions: cyanate, cyanide, azide, selenate, perosmate, tetraborate, selenocyanate, and fluorosulfonate, with K_i s in the range of 3.7–8.9 mM. It should be noted that some of these anions (e.g. cyanide, cyanate) are extremely potent, micromolar hCA I inhibitors, whereas their activity against hCA II and NgCA are usually in the millimolar or submillimolar range.
- iv. The halides, nitrite, nitrate, bicarbonate, carbonate, bisulphite, sulphate, stannate, and *N,N*-diethyldithiocarbamate were even more effective as SauBCA inhibitors with K_i s in the range of 0.26–0.91 mM (Table 1). Among the halides, bromide was the most effective inhibitor, whereas the isosteric/ isoelectronic nitrate and bicarbonate had very similar inhibitory behaviour. Sulphate, which is an extremely weak hCA I and II inhibitor, is on the other hand much more effective as an inhibitor of bacterial CAs. In fact, many such bacterial enzymes have been purified in the presence of extremely high concentrations of sulphate and showed no catalytic activity due to inhibition by the anion present in the buffer or the assay system¹⁴.
- v. The most effective inhibitors detected in the current study were sulfamide, sulfamate, phenylboronic acid, and phenylarsonic acid, which showed K_i s in the range of 7–43 μ M. In fact, these compounds are known to inhibit many CAs of different classes, and X-ray crystal structures have even been reported for some of the enzyme-inhibitor complexes^{12,28}.

4. Conclusions

SauBCA is a high activity β -CA present in the genome of the bacterial pathogen *S. aureus*, known for its extensive drug resistance to classical antibiotics. We investigated here its inhibition with a series of inorganic and organic anions. Perchlorate, hexafluorophosphate, triflate, pyrodiphosphate, divanadate, perruthenate, perrhenate, peroxydisulfate, and iminidissulfonate did not show any significant inhibitory action against this enzyme with concentrations up to 100 mM in the assay system. Thiocyanate, hydrogensulfide, tellurate, and trithiocarbonate were weak inhibitors with K_i s in the range of 11.4–42 mM, whereas cyanate, cyanide, azide, selenate, perosmate, tetraborate, selenocyanate, and fluorosulfonate showed K_i s in the range of 3.7–8.9 mM. The halides, nitrite, nitrate, bicarbonate, carbonate, bisulphite, sulphate, stannate, and *N,N*-diethyldithiocarbamate were more effective as SauBCA inhibitors with K_i s in the range of 0.26–0.91 mM, but the most effective inhibitors were sulfamide, sulfamate, phenylboronic acid, and phenylarsonic acid, which showed K_i s in the range of 7–43 μ M. Several inhibitors detected here may be considered as lead compounds for the development of even more effective derivatives, which should thereafter be investigated for their bacteriostatic effects.

Acknowledgements

CTS thanks the Italian Ministry for University and Research (MIUR), project FIS2019_04819 BacCAD. SP is thankful for the funding from the Academy of Finland and Jane & Aatos Erkkö Foundation.

Disclosure statement

The authors have no relevant affiliations or financial involvement with any organisation or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

Funding

The research program was also funded by the Academy of Finland, and Jane & Aatos Erkkö Foundation and Italian Ministry for University and Research (MIUR) [FIS2019_04819 BacCAD].

ORCID

Seppo Parkkila  <http://orcid.org/0000-0001-7323-8536>

Claudio T. Supuran  <http://orcid.org/0000-0003-4262-0323>

References

1. (a) Ford CA, Hurford IM, Cassat JE. Antivirulence strategies for the treatment of *Staphylococcus aureus* infections: a mini review. *Front Microbiol* 2020;11:632706. (b) Cheung GYC, Bae JS, Liu R, et al. Bacterial virulence plays a crucial role in MRSA sepsis. *PLoS Pathog* 2021;17:e1009369.
2. (a) Mahros MA, Abd-Elghany SM, Sallam KI. Multidrug-, methicillin-, and vancomycin-resistant *Staphylococcus aureus* isolated from ready-to-eat meat sandwiches: an ongoing food and public health concern. *Int J Food Microbiol* 2021; 346:109165. (b) Côrtes MF, Botelho AMN, Bandeira PT, et al. Reductive evolution of virulence repertoire to drive the divergence between community- and hospital-associated methicillin-resistant *Staphylococcus aureus* of the ST1 lineage. *Virulence* 2021;12:951–67.
3. (a) Chojnacki M, Cao X, Flaherty DP, Dunman PM. Optimization of 2-acylaminocycloalkylthiophene derivatives for activity against *Staphylococcus aureus* RnpA. *Antibiotics* 2021;10:369. (b) Piras C, Di Ciccio PA, Soggiu A, et al. *S. aureus* biofilm protein expression linked to antimicrobial resistance: a proteomic study. *Animals* 2021;11:966. (c) Princiotta S, Mazzini S, Musso L, et al. New antimicrobials based on the adarotene scaffold with activity against multi-drug resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*. *Antibiotics* 2021;10:126.
4. (a) Supuran CT, Capasso C. Antibacterial carbonic anhydrase inhibitors: an update on the recent literature. *Expert Opin Ther Pat* 2020;30:963–82. (b) Supuran CT. Bacterial carbonic anhydrases as drug targets: toward novel antibiotics? *Front Pharmacol* 2011;2:34. (c) Campestre C, De Luca V, Carradori S, et al. Carbonic anhydrases: new perspectives on protein functional role and inhibition in *Helicobacter pylori*. *Front Microbiol* 2021;12:629163.
5. (a) Kaur J, Cao X, Abutaleb NS, et al. Optimization of acetazolamide-based scaffold as potent inhibitors of vancomycin-resistant *Enterococcus*. *J Med Chem* 2020;63:9540–62. (b)

- Abutaleb NS, Elkashif A, Flaherty DP, Seleem MN. In vivo antibacterial activity of acetazolamide. *Antimicrob Agents Chemother* 2021;65:e01715–20. (c) Abutaleb NS, Elhassanny AEM, Flaherty DP, Seleem MN. In vitro and in vivo activities of the carbonic anhydrase inhibitor, dorzolamide, against vancomycin-resistant enterococci. *PeerJ* 2021;9:e11059.
6. Hewitt CS, Abutaleb NS, Elhassanny AEM, et al. Structure-activity relationship studies of acetazolamide-based carbonic anhydrase inhibitors with activity against *Neisseria gonorrhoeae*. *ACS Infect Dis* 2021;(in press).
 7. (a) Smith KS, Jakubzick C, Whittam TS, Ferry JG. Carbonic anhydrase is an ancient enzyme widespread in prokaryotes. *Proc Natl Acad Sci USA* 1999;96:15184–9. (b) 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. (c) Abuaita BH, Withey JH. Bicarbonate induces *Vibrio cholerae* virulence gene expression by enhancing ToxT activity. *Infect Immun* 2009;77:4111–20.
 8. (a) Merlin C, Masters M, McAteer S, Coulson A. Why is carbonic anhydrase essential to *Escherichia coli*? *J Bacteriol* 2003;185:6415–24. (b) Vullo D, Kumar RSS, Scozzafava A, et al. Sulphonamide inhibition studies of the β -carbonic anhydrase from the bacterial pathogen *Clostridium perfringens*. *J Enzyme Inhib Med Chem* 2018;33:31–6. (c) Supuran CT, Capasso C. Biomedical applications of prokaryotic carbonic anhydrases. *Expert Opin Ther Pat* 2018;28:745–54. (d) Del Prete S, Nocentini A, Supuran CT, Capasso C. Bacterial γ -carbonic anhydrase: a new active class of carbonic anhydrase identified in the genome of the Gram-negative bacterium *Burkholderia territorii*. *J Enzyme Inhib Med Chem* 2020;35:1060–8.
 9. (a) Rahman MM, Tikhomirova A, Modak JK, et al. Antibacterial activity of ethoxzolamide against *Helicobacter pylori* strains SS1 and 26695. *Gut Pathog* 2020;12:20. (b) Modak JK, Tikhomirova A, Gorrell RJ, et al. Anti-*Helicobacter pylori* activity of ethoxzolamide. *J Enzyme Inhib Med Chem* 2019;34:1660–7. (c) Modak JK, Liu YC, Supuran CT, Roujeinikova A. Structure-activity relationship for sulfonamide inhibition of *Helicobacter pylori* α -carbonic anhydrase. *J Med Chem* 2016;59:11098–109.
 10. (a) Aspatwar A, Kairys V, Rala S, et al. *Mycobacterium tuberculosis* β -carbonic anhydrases: novel targets for developing antituberculosis drugs. *Int J Mol Sci* 2019;20:5153. (b) Wani TV, Bua S, Khude PS, et al. Evaluation of sulphonamide derivatives acting as inhibitors of human carbonic anhydrase isoforms I, II and *Mycobacterium tuberculosis* β -class enzyme Rv3273. *J Enzyme Inhib Med Chem* 2018;33:962–71. (c) Aspatwar A, Hammarén M, Koskinen S, et al. β -CA-specific inhibitor dithiocarbamate Fc14-584B: a novel antimycobacterial agent with potential to treat drug-resistant tuberculosis. *J Enzyme Inhib Med Chem* 2017;32:832–40. (d) Carta F, Maresca A, Covarrubias AS, et al. Carbonic anhydrase inhibitors. Characterization and inhibition studies of the most active beta-carbonic anhydrase from *Mycobacterium tuberculosis*, Rv3588c. *Bioorg Med Chem Lett* 2009;19:6649–54. (e) Nishimori I, Minakuchi T, Vullo D, et al. Carbonic anhydrase inhibitors. Cloning, characterization, and inhibition studies of a new beta-carbonic anhydrase from *Mycobacterium tuberculosis*. *J Med Chem* 2009;52:3116–20.
 11. (a) Del Prete S, Bua S, Supuran CT, Capasso C. *Escherichia coli* γ -carbonic anhydrase: characterisation and effects of simple aromatic/heterocyclic sulphonamide inhibitors. *J Enzyme Inhib Med Chem* 2020;35:1545–54. (b) Nocentini A, Del Prete S, Mastrolorenzo MD, et al. Activation studies of the β -carbonic anhydrases from *Escherichia coli* with amino acids and amines. *J Enzyme Inhib Med Chem* 2020;35:1379–86. (c) Del Prete S, De Luca V, Bua S, et al. The effect of substituted benzene-sulfonamides and clinically licensed drugs on the catalytic activity of CynT2, a carbonic anhydrase crucial for *Escherichia coli* life cycle. *Int J Mol Sci* 2020;21:4175.
 12. (a) Lotlikar SR, Kayastha BB, Vullo D, et al. *Pseudomonas aeruginosa* β -carbonic anhydrase, psCA1, is required for calcium deposition and contributes to virulence. *Cell Calcium* 2019;84:102080. (b) Murray AB, Aggarwal M, Pinard M, et al. Structural mapping of anion inhibitors to β -carbonic anhydrase psCA3 from *Pseudomonas aeruginosa*. *ChemMedChem* 2018;13:2024–9. (c) Aggarwal M, Chua TK, Pinard MA, et al. Carbon dioxide “trapped” in a β -carbonic anhydrase. *Biochemistry* 2015;54:6631–8. (d) Pinard MA, Lotlikar SR, Boone CD, et al. Structure and inhibition studies of a type II beta-carbonic anhydrase psCA3 from *Pseudomonas aeruginosa*. *Bioorg Med Chem* 2015;23:4831–8.
 13. (a) Bonardi A, Nocentini A, Osman SM, et al. Inhibition of α -, β - and γ -carbonic anhydrases from the pathogenic bacterium *Vibrio cholerae* with aromatic sulphonamides and clinically licensed drugs – a joint docking/molecular dynamics study. *J Enzyme Inhib Med Chem* 2021;36:469–79. (b) Gitto R, De Luca L, Mancuso F, et al. Seeking new approach for therapeutic treatment of cholera disease via inhibition of bacterial carbonic anhydrases: experimental and theoretical studies for sixteen benzenesulfonamide derivatives. *J Enzyme Inhib Med Chem* 2019;34:1186–92. (c) Bua S, Berrino E, Del Prete S, et al. Synthesis of novel benzenesulfamide derivatives with inhibitory activity against human cytosolic carbonic anhydrase I and II and *Vibrio cholerae* α - and β -class enzymes. *J Enzyme Inhib Med Chem* 2018;33:1125–36. (d) Ferraroni M, Del Prete S, Vullo D, et al. Crystal structure and kinetic studies of a tetrameric type II β -carbonic anhydrase from the pathogenic bacterium *Vibrio cholerae*. *Acta Crystallogr D Biol Crystallogr* 2015;71:2449–56. (e) Del Prete S, Isik S, Vullo D, et al. DNA cloning, characterization, and inhibition studies of an α -carbonic anhydrase from the pathogenic bacterium *Vibrio cholerae*. *J Med Chem* 2012;55:10742–8.
 14. (a) Matsumoto Y, Miyake K, Ozawa K, et al. Bicarbonate and unsaturated fatty acids enhance capsular polysaccharide synthesis gene expression in oral streptococci, *Streptococcus anginosus*. *J Biosci Bioeng* 2019;128:511–7. (b) Capasso C, Supuran CT. An overview of the carbonic anhydrases from two pathogens of the oral cavity: *Streptococcus mutans* and *Porphyromonas gingivalis*. *Curr Top Med Chem* 2016;16:2359–68. (c) Dedeoglu N, De Luca V, Isik S, et al. Cloning, characterization and anion inhibition study of a β -class carbonic anhydrase from the caries producing pathogen *Streptococcus mutans*. *Bioorg Med Chem* 2015;23:2995–3001. (d) Burghout P, Vullo D, Scozzafava A, et al. Inhibition of the β -carbonic anhydrase from *Streptococcus pneumoniae* by inorganic anions and small molecules: toward innovative drug design of anti-infectives? *Bioorg Med Chem* 2011;19:243–8.
 15. Urbanski LJ, Bua S, Angeli A, et al. Sulphonamide inhibition profile of *Staphylococcus aureus* β -carbonic anhydrase. *J Enzyme Inhib Med Chem* 2020;35:1834–9.

16. (a) Supuran CT. Exploring the multiple binding modes of inhibitors to carbonic anhydrases for novel drug discovery. *Expert Opin Drug Discov* 2020;15:671–86. (b) Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. *Nat Rev Drug Discov* 2008;7:168–81. (c) Mishra CB, Tiwari M, Supuran CT. Progress in the development of human carbonic anhydrase inhibitors and their pharmacological applications: where are we today? *Med Res Rev* 2020;40:2485–565.
17. 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.
18. (a) Angeli A, Pinteala M, Maier SS, et al. Inhibition of α -, β -, γ -, δ -, ζ - and η -class carbonic anhydrases from bacteria, fungi, algae, diatoms and protozoans with famotidine. *J Enzyme Inhib Med Chem* 2019;34:644–50. (b) Urbański LJ, Di Fiore A, Azizi L, et al. S. Biochemical and structural characterisation of a protozoan beta-carbonic anhydrase from *Trichomonas vaginalis*. *J Enzyme Inhib Med Chem* 2020;35:1292–9.
19. (a) Petreni A, De Luca V, Scaloni A, et al. Anion inhibition studies of the Zn(II)-bound ι -carbonic anhydrase from the Gram-negative bacterium *Burkholderia territorii*. *J Enzyme Inhib Med Chem* 2021;36:372–6. (b) De Luca V, Petreni A, Nocentini A, et al. Effect of sulfonamides and their structurally related derivatives on the activity of ι -carbonic anhydrase from *Burkholderia territorii*. *Int J Mol Sci* 2021;22:571.
20. (a) Bonardi A, Nocentini A, Bua S, et al. Sulfonamide inhibitors of human carbonic anhydrases designed through a three-tails approach: improving ligand/isoform matching and selectivity of action. *J Med Chem* 2020;63:7422–44. (b) Bouzina A, Berredjem M, Nocentini A, et al. Ninhydrins inhibit carbonic anhydrases directly binding to the metal ion. *Eur J Med Chem* 2021;209:112875.
21. (a) De Simone G, Supuran CT. (In)organic anions as carbonic anhydrase inhibitors. *J Inorg Biochem* 2012;111:117–29. (b) Ozensoy Guler O, Capasso C, Supuran CT. A magnificent enzyme superfamily: carbonic anhydrases, their purification and characterization. *J Enz Inhib Med Chem* 2016;31:689–94. (c) Nocentini A, Angeli A, Carta F, et al. Reconsidering anion inhibitors in the general context of drug design studies of modulators of activity of the classical enzyme carbonic anhydrase. *J Enzyme Inhib Med Chem* 2021;36:561–80. (d) Supuran CT. Structure-based drug discovery of carbonic anhydrase inhibitors. *J Enzyme Inhib Med Chem* 2012;27:759–72. (e) Supuran CT. How many carbonic anhydrase inhibition mechanisms exist? *J Enzyme Inhib Med Chem* 2016;31:345–60.
22. (a) Urbański LJ, Angeli A, Hytönen VP, et al. Inhibition of the newly discovered β -carbonic anhydrase from the protozoan pathogen *Trichomonas vaginalis* with inorganic anions and small molecules. *J Inorg Biochem* 2020;213:111274. (b) Haapanen S, Bua S, Kuuslahti M, et al. Cloning, characterization and anion inhibition studies of a β -carbonic anhydrase from the pathogenic protozoan *Entamoeba histolytica*. *Molecules* 2018;23:3112. (c) Vullo D, Lehneck R, Donald WA, et al. Anion inhibition studies of the β -class carbonic anhydrase CAS3 from the filamentous ascomycete *Sordaria macrospora*. *Metabolites* 2020;10:93.
23. (a) Vullo D, Syrjänen L, Kuuslahti M, et al. Anion inhibition studies of a beta carbonic anhydrase from the malaria mosquito *Anopheles gambiae*. *J Enzyme Inhib Med Chem* 2018;33:359–63. (b) Perfetto R, Del Prete S, Vullo D, et al. Sequence analysis, kinetic constants, and anion inhibition profile of the nacrein-like protein (CgiNAP2X1) from the pacific oyster *Magallana gigas* (ex-*Crassostrea gigas*). *Mar Drugs* 2017;15:270. (c) Del Prete S, Vullo D, Di Fonzo P, et al. Anion inhibition profiles of the γ -carbonic anhydrase from the pathogenic bacterium *Burkholderia pseudomallei* responsible of melioidosis and highly drug resistant to common antibiotics. *Bioorg Med Chem* 2017;25:575–80.
24. (a) Supuran CT. Bortezomib inhibits bacterial and fungal β -carbonic anhydrases. *Bioorg Med Chem* 2016;24:4406–9. (b) Supuran CT. Bortezomib inhibits mammalian carbonic anhydrases. *Bioorg Med Chem* 2017;25:5064–7.
25. Nocentini A, Hewitt CS, Mastrolorenzo MD, et al. Anion inhibition studies of the α -carbonic anhydrases from *Neisseria gonorrhoeae*. *J Enzyme Inhib Med Chem* submitted.
26. (a) 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. (b) Innocenti A, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors. Inhibition of transmembrane isoforms IX, XII, and XIV with less investigated anions including trithiocarbonate and dithiocarbamate. *Bioorg Med Chem Lett* 2010;20:1548–50.
27. Huang S, Xue Y, Sauer-Eriksson E, et al. Crystal structure of carbonic anhydrase from *Neisseria gonorrhoeae* and its complex with the inhibitor acetazolamide. *J Mol Biol* 1998;283:301–10.
28. Abbate F, Supuran CT, Scozzafava A, et al. Nonaromatic sulfonamide group as an ideal anchor for potent human carbonic anhydrase inhibitors: role of hydrogen-bonding networks in ligand binding and drug design. *J Med Chem* 2002;45:3583–7.