ACS Medicinal Chemistry Letters

pubs.acs.org/acsmedchemlett Letter

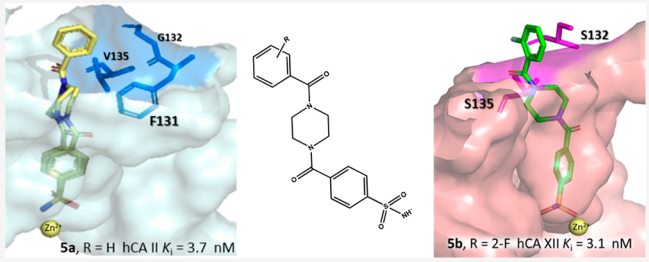
Looking toward the Rim of the Active Site Cavity of Druggable Human Carbonic Anhydrase Isoforms

Francesca Mancuso, Anna Di Fiore, Laura De Luca, Andrea Angeli, Simona M. Monti, Giuseppina De Simone, Claudiu T. Supuran, and Rosaria Gitto*

Cite This: ACS Med. Chem. Lett. 2020, 11, 1000−1005 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: We report the synthesis and biochemical evaluation of a series of substituted 4-(4-aroylpiperazine-1-carbonyl)benzenesulfonamides (**5a−s**) developed as inhibitors of druggable carbonic anhydrase (CA) isoforms, as tools for the identification of new therapeutics. X-ray crystallography confirmed that this class of benzenesulfonamides binds CAs through the canonical anchoring of the benzenesulfonamide moiety to the metal ion and a *tail-mediated* recognition of the middle/top area of the active site cavity. Compound **5e** (R = 2-Cl) demonstrated relevant selectivity toward brain-expressed hCA VII. The best balancing in binding affinity and selectivity toward tumor-expressed hCA IX/hCA XII over ubiquitous hCA I/hCA II was found for inhibitor **5o** (R = 3-NO_2). Notably **5b** (R = 2-F) proved to be the most efficacious inhibitor of hCA XII for which computational studies elucidated the CA recognition process.



KEYWORDS: *Carbonic anhydrases, CA inhibitors, benzenesulfonamides, tumor-associated CA isoforms, docking studies, X-ray crystallography*

Carbonic anhydrases (CAs, EC 4.2.1.1) catalyze the reversible hydration of carbon dioxide in bicarbonate and proton.[1] The 12 catalytically active human (h) α-CAs are zinc-containing isoforms differing in catalytic properties, oligomeric structure, tissue and cellular distribution.[2] Some of them are known to play a relevant role in different pathological processes related to cancer, epilepsy, obesity, glaucoma, etc.[3] Therefore, several hCAs have become well-established targets for designing hCA inhibitors (hCAIs) endowed with biomedical applications (see Figure 1).[1,4−11]

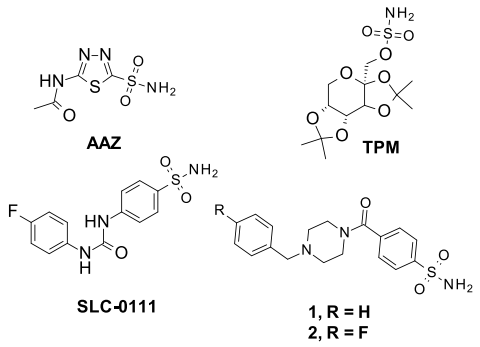


Figure 1. Chemical structures of well-known hCA inhibitors: AAZ, TPM, SLC-0111, and 4-(4-benzylpiperazine-1-carbonyl)benzenesulfonamides (**1** and **2**).

Particularly, acetazolamide (AAZ) and topiramate (TPM) are well-known CAIs targeting hCA VII isoform so that they are considered useful for the treatment of epilepsy and others neurological disorders,[12−14] whereas the 4-ureidobenzenesulfonamide derivative SLC-0111 (WBI-5111) entered clinical trials for the treatment of hypoxic tumors in the metastatic pancreatic ductal cancer.[9,15−22] Indeed, SLC-0111 is a selective inhibitor of hCA IX and hCA XII, which are crucial in controlling tumor growth, invasiveness, proliferation, metastasis, and resistance to radio- and chemotherapy.[18,23]

These CAIs (Figure 1) are capable of binding the catalytic metal ion through a zinc binder group (ZBG), namely, the sulfonamide/sulfamate moiety in the deprotonated form. In this context, the benzenesulfonamide moiety has been extensively explored as a crucial binding motif for the CA active site cavity close to the zinc ion.[23] To improve the isoform selectivity of CAIs, efforts have been generally addressed to introduce an additional fragment linked to benzenesulfonamide as a "tail" for enhancing the interaction

Special Issue: In Memory of Maurizio Botta: His Vision of Medicinal Chemistry

Received: February 4, 2020
Accepted: March 4, 2020
Published: March 4, 2020

with hydrophobic/hydrophilic residues paving the middle and/or top region of the active site cavity.[2,12,13,24−35]

On the basis of this approach, we have previously developed a small library of 4-(4-benzylpiperazine-1-carbonyl)benzenesulfonamides (e.g., **1** and **2**, Figure 1) as a class of potent inhibitors of several druggable CA isoforms (e.g., hCA VII, hCA IX, hCA XII, and hCA XIV).

This series of compounds was studied in depth by means of X-ray crystallography highlighting the network of relevant interactions within the hCA active site.[25] In particular, the high-resolution crystal structure of compound **2** in complex with hCA II showed that the inhibitor was bound to the enzyme establishing the canonical interactions of the benzenesulfonamide moiety with the CA active pocket. Moreover, polar and hydrophobic interactions between the carbonyl-piperazine moiety and residues delimiting the middle region of the cavity were detectable. Finally, the 4-fluorobenzyl substituent was oriented toward a rather unexplored region of the active site located at the border of its hydrophobic region. Due to this unusual feature, in this paper we decided to further investigate these piperazine derivatives, substituting the *N*-benzyl moiety with the *N*-benzoyl one. Our idea was to explore the effect of converting the amine moiety in an amide one which, differing in terms of geometrical and chemical properties, could affect the interaction with the residues located on the rim of the active site cavity characterized by the highest diversity.[36] For instance, hCA IX and hCA XII isoforms bear in this region smaller residues (V131 and A131 in hCA IX and XII, respectively) when compared to hCA II (F131), thus forming a more accessible active site. Searching for isoform selectivity over hCA II, we further decorated the benzoyl *tail* by incorporating hydrophobic/hydrophilic substituents in order to find additional interactions with the top area of catalytic site.[36] The inhibitory activity/selectivity of all obtained benzenesulfonamide derivatives was assayed against selected isoforms (hCA VII, hCA IX, hCA XII, and hCA XIV) over the ubiquitous hCA I and hCA II. Furthermore, crystallographic data and docking experiments helped us to analyze in-depth the main interactions of these new compounds with the CA catalytic site.

The synthesis of designed 4-(4-aroylpiperazine-1-carbonyl)-benzenesulfonamide derivatives was carried out through the procedure described in Scheme 1.

Initially, a *one-pot* procedure involved the coupling of suitable benzoyl chlorides **3a−p** with 1-Boc-piperazine followed by removal of protecting group without purification of intermediates, thus giving amides **4a−p** in good yields. In turn, the intermediates **4a−p** reacted with the 4-sulfamoylbenzoic acid to give the desired 4-(4-aroylpiperazine-1-carbonyl)benzenesulfonamides **5a−p**. Finally, the nitroreduction of compounds **5n−p** gave the corresponding amine derivatives **5q−s**. The chemical characterization of the synthesized compounds was supported by spectroscopic measurements (see Supporting Information).

The CA inhibitory effects of synthesized 4-(4-aroylpiperazine-1-carbonyl)benzenesulfonamides (**5a−s**) were then measured toward selected CA isoforms by means of a stopped-flow carbon dioxide hydration assay. The obtained results are summarized in Table 1 and compared with K_i values

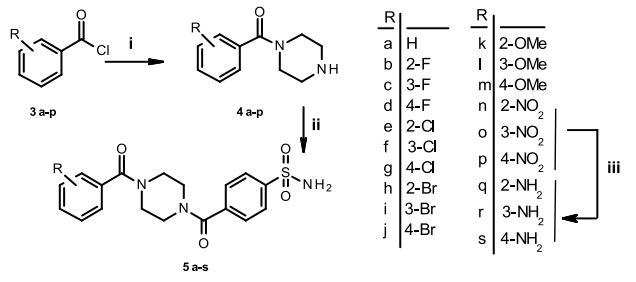
Table 1. K_i Values (nM) against hCA I, hCA II, hCA VII, hCA IX, hCA XII, and hCA XIV Isoforms Shown by New Benzenesulfonamide Derivatives 5a−s and Reference Compounds 1 and 2, AAZ, and SLC-0111

	K_i (nM)[a]					
	hCA I	hCA II	hCA VII	hCA IX	hCA XII	hCA XIV
1	6.8	3.0	10.4	33.1	3.8	34.6
2	0.69	0.5	7.8	45.1	5.6	15.2
5a	69.1	3.7	70.7	37.1	8.5	85.5
5b	22.4	28.4	6.8	68.7	3.1	6.9
5c	0.84	0.41	66.1	3.8	29.1	35.7
5d	7.7	32.4	39.5	332.4	26.4	69.6
5e	94.4	5.6	2.9	62.2	63.1	86.1
5f	72.5	22.5	7.5	54.4	66.9	58.3
5g	87.5	7.2	61.7	29.2	6.3	63.4
5h	58.0	3.0	45.3	60.4	48.7	73.9
5i	460	0.6	3.5	16.2	8.5	85.3
5j	85.1	4.4	9.1	8.0	37.4	86.0
5k	81.9	19.8	27.7	250.0	39.6	78.1
5l	87.6	36.3	46.7	360.3	26.5	48.0
5m	81.9	5.5	9.3	223.0	86.3	9.7
5n	81.2	42.4	39.2	9.8	6.9	61.6
5o	93.8	28.6	41.7	2.2	8.2	66.5
5p	60.8	23.9	64.2	11.2	6.2	46.1
5q	9.4	4.1	9.0	19.1	27.4	86.4
5r	404.9	51.1	65.0	15.2	7.7	91.7
5s	173.5	78.8	69.3	14.3	8.2	66.5
AAZ	250	12.5	2.5	25.8	5.7	41.0
SLC-0111	5080	960.0	8550	45.0	4.5	ND

[a]Errors are in the range of ±10% of the reported value, from three different assays. ND: not determined.

of the structurally related compounds **1** and **2**, AAZ, and SLC-0111. By analysis of Table 1, some structure−activity relationships (SARs) can be drawn for this new class of CAIs. Most of the synthesized 4-(4-aroylpiperazine-1-carbonyl)benzenesulfonamides displayed inhibitory effects at low nanomolar concentration against druggable CA isoforms; however, no clear correlation was found between K_i inhibition values and the nature/position of hydrophilic/hydrophobic substituents on the phenyl ring. In more detail, this new series of compounds generally exhibited medium inhibitory effects toward physiologically dominant hCA I isoform except for compounds **5c**, **5d**, and **5q** for which the K_i values fell in the low nanomolar range. Moreover, tested compounds inhibited hCA II with K_i values ranging from 0.41 to 78.8 nM; the best inhibitors were compounds **5c** (K_i = 0.41 nM) and **5i** (K_i = 0.6

Scheme 1[a]



[a]Reagents and conditions: (i) (a) *N*-Boc-piperazine, DCM, EDIPA, rt, 3 h; (i) (b) TFA, 0 °C to rt, 4 h; (ii) DMF, HBTU, 4-sulfamoylbenzoic acid, DIPEA, rt, overnight; (iii) EtOH, $NH_2-NH_2\cdot H_2O$, Pd/C, rt to reflux, 1 h.

https://dx.doi.org/10.1021/acsmedchemlett.0c00062
ACS Med. Chem. Lett. 2020, 11, 1000−1005

nM) bearing fluorine or bromine substituent at the meta-position of the benzoyl moiety.

Concerning the brain-expressed hCA VII isoform, it can be observed that all compounds displayed significant inhibitory activity with K_i values ranging from 6.8 to 70.7 nM. Interestingly, the enzymatic activities of two tumor-associated isoforms, hCA IX and hCA XII, were affected by all tested compounds with K_i values spanning from the low-nanomolar to the medium-nanomolar range (3.1−360 nM). The methoxy-substituted compounds **5k**, **5l**, and **5m** were found to be less active hCA IX inhibitors when compared to unsubstituted compound **5a**; a significant reduction of hCA IX affinity resulted also for the 4-fluorine substituted compound **5d**. Conversely, no relevant impact on hCA XIV binding affinity was generally observed for the different pattern of aromatic substituents of tested compounds **5a−s**. The main features of the binding of this new series of inhibitors to the CA active site were elucidated by means of a detailed crystallographic study. In particular, the crystal structure of hCA II in complex with unsubstituted compound **5a** was determined at 1.0 Å (Table S1 in Supporting Information). hCA II was chosen as a model isoform for crystallization, since it readily forms crystals and many studies have been reported on its adducts with different classes of inhibitors.[37] From the first steps of the crystallographic refinement, inspection of (F_o − F_c) and ($2F_o$ − F_c) electron density maps revealed the presence of inhibitor **5a** within the hCA II active site. Two different conformations of the inhibitor were modeled differing only for the orientation of the carbonyl group between benzenesulfonamide and the piperazine ring (Figure 2A).

Electron density maps were well-defined for the entire inhibitor molecule with the exception of phenyl tail that showed a greater conformational variability. Inhibitor binding did not alter hCA II three-dimensional structure. Indeed, the rmsd values calculated by superposition of all the Cα atoms of the hCA II/**5a** complex with those of the native protein[38] were very low (rmsd value of 0.3 Å). Similar to other benzenesulfonamide-based hCAIs,[37] compound **5a** was anchored to the active site by means of the sulfonamide moiety, which coordinated the catalytic zinc ion and formed two hydrogen bonds with residue Thr199, and of the inner phenyl ring which established strong hydrophobic interactions (distance of <4.0 Å) with residues Q92, V121, L198, and T199. The carbonyl group of one conformer formed a weak hydrogen bond with a Q92NE2 atom, whereas in the case of the other conformer the oxygen atom was involved in a polar interaction with a glycerol molecule. Moreover, in both conformers the 4-carbonylpiperazine moiety was stabilized by van der Waals interactions with F131, V135, P202, and L204 residues. Finally, the phenyl tail was located as expected on the border of the active site cavity establishing only few and weak hydrophobic interactions (distance of <4.2 Å) with F131 and G132 (Figure 2A and Figure 2B). These structural findings were in good agreement with previous achievements for an analogue bearing the 2-benzylpiperazine core.[39]

Apart from clarifying the inhibitory mechanism of this class of substituted 4-(4-aroylpiperazine-1-carbonyl)benzenesulfonamides (**5a−s**), our interest was focused on their selectivity over ubiquitous hCA I/hCA II isoforms. Interestingly, a careful analysis of the data collected in Table 1 reveals that some compounds show a certain selectivity. As an example, compound **5f** (R = 3-Cl) prefers the brain-expressed hCA VII isoform and compounds **5n** (R = 2-NO_2), **5o** (R = 3-NO_2), **5p** (R = 4-NO_2), **5r** (R = 3-NH_2), and **5s** (R = 4-NH_2) display the best selectivity toward the tumor-expressed hCA IX/hCA XII. By comparing the K_i values measured for amides **5a** and **5d** with corresponding previously synthesized amine-derivatives **1** and **2**, it is also evident that the introduced structural modification significantly affects the affinity for selected druggable hCAs. Specifically, the binding affinities of compound **5d** (R = 4-F) toward hCA VII and hCA IX were about 7-fold lower than those of compound **2**. Furthermore, the introduction of a 3-nitro substituent on the phenyl ring significantly improved hCA IX affinity so that the inhibitor **5o** (K_i = 2.2 nM) was more active than SLC-0111 (K_i = 45.0 nM). Interestingly, the 2-fluorosubstituted derivative **5b** was very active inhibitor of hCA XII isoform (K_i = 3.1 nM) displaying an inhibitory activity similar to that of the promising antitumor

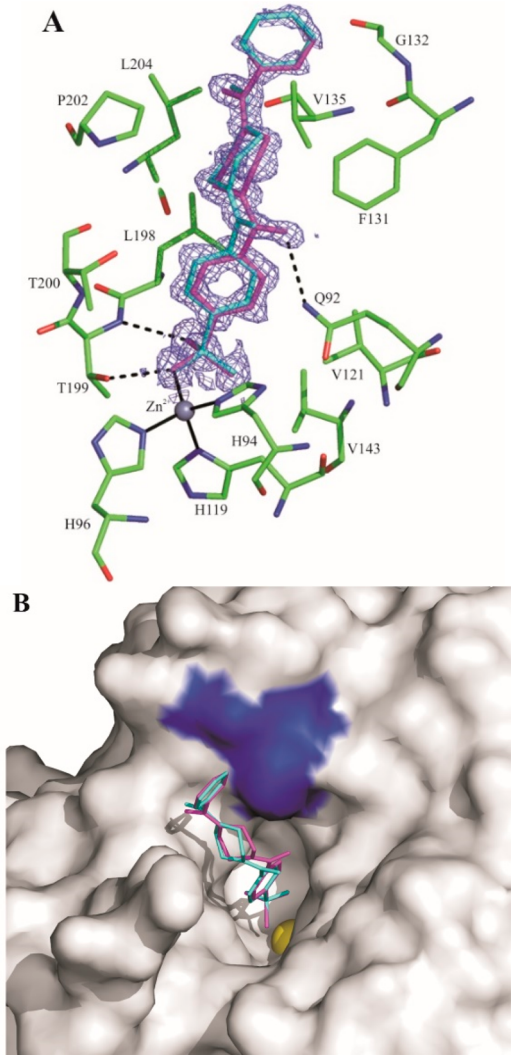


Figure 2. (A) hCA II/5a (PDB code 6XXT) active site showing the σ_A-weighted $|2F_o - F_c|$ electron density map (contoured at 1.0σ) relative to the inhibitor with the conformer A colored in cyan and the conformer B in magenta. The zinc ion coordination and residues involved in polar and hydrophobic interactions with the **5a** molecule are also depicted. (B) Representation of hCA II surface showing the inhibitor **5a** located in the enzyme active site. The Zn^{2+} ion within the catalytic cavity is represented as a yellow sphere. The phenyl tail of the inhibitor is positioned on the rim of the cavity forming weak hydrophobic interactions with F131 and G132 (colored in blue). The figure was made using PyMol.

https://dx.doi.org/10.1021/acsmedchemlett.0c00062
ACS Med. Chem. Lett. 2020, 11, 1000−1005

agent SLC-0111 (K_i = 4.5 nM). Compound **5b** demonstrated also an improved selectivity over off-target isoforms hCA I/hCA II with respect to amine parent compounds **1** and **2**. Therefore, to decipher the structural requirements for optimized hCA XII affinity, we carried out docking calculations between hCA XII and compounds **5b** and SLC-0111. In detail, the crystallographic structure of hCA XII in complex with the inhibitor AAZ (PDB code 1JD0) was used for docking simulation by Gold Suite 5.7.1, and the results are displayed in Figure 3.

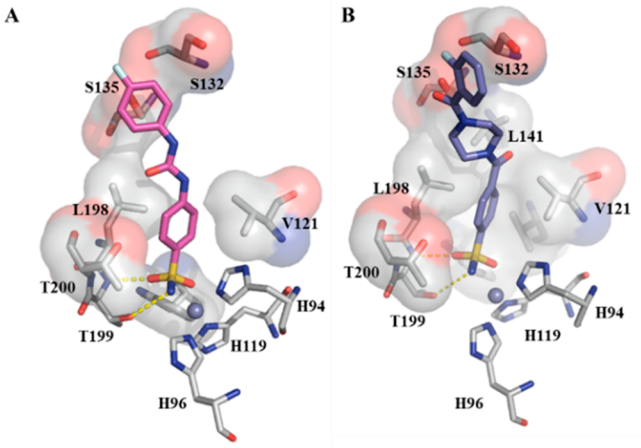


Figure 3. Suggested binding modes of compounds SLC-0111 (magenta, panel A) and **5b** (purple, panel B) docked into the hCA XII structure (PDB code 1JD0). The key residues of the pocket are presented, and the hydrogen-bond interactions are shown by dotted lines. The interactions between hCA XII and inhibitors SLC-0111 and **5b** were examined using PyMOL (https://pymol.org) and LIGPLUS.[41]

Docking studies suggested that some similarities are present between the binding mode of SLC-0111 (colored in magenta) and compound **5b** (colored in purple) to the CA XII active site. Indeed, in both cases, the benzenesulfonamide moiety engages canonical H-bond contacts with T199 and T200 residues and interacts with V121, L141, and L198 residues of the active site, whereas the fluoro-substituted tail is oriented toward two specific residues S132 and S135 at the entrance of catalytic cavity, confirming the important role of this region in the design of selective CAIs.[37,40]

In conclusion, we designed a series of benzenesulfonamides as CAIs. Crystallography and docking studies revealed that the aromatic tail is generally directed toward the rim and makes few key contacts with the enzyme. Compound **5o** represents a potent inhibitor that combines excellent hCA IX/hCA XII inhibitory effects and isoform selectivity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.0c00062.

Synthetic procedures for preparation of compounds and analytical data. Conditions for CA inhibition assay; crystallographic data collection and refinement statistics for hCA II/**5a** crystal structure; docking study methods; selected representative ^{1}H and ^{13}C NMR spectra (PDF)

Accession Codes

The final model of hCA II bound to inhibitor **5a** is deposited in the Protein Data Bank (6XXT). Authors will release the atomic coordinates and experimental data upon article publication.

■ AUTHOR INFORMATION

Corresponding Author

Rosaria Gitto − *Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali (CHIBIOFARAM), Università degli Studi di Messina, 98168 Messina, Italy;* orcid.org/0000-0003-0002-2253; Email: rosaria.gitto@unime.it

Authors

Francesca Mancuso − *Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali (CHIBIOFARAM), Università degli Studi di Messina, 98168 Messina, Italy;* orcid.org/0000-0002-2209-957X

Anna Di Fiore − *Istituto di Biostrutture e Bioimmagini—CNR, 80134 Napoli, Italy;* orcid.org/0000-0003-2924-5194

Laura De Luca − *Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali (CHIBIOFARAM), Università degli Studi di Messina, 98168 Messina, Italy;* orcid.org/0000-0003-0614-5713

Andrea Angeli − *Dipartimento NEUROFARBA, Università di Firenze, 50019 Sesto Fiorentino, Italy;* orcid.org/0000-0002-1470-7192

Simona M. Monti − *Istituto di Biostrutture e Bioimmagini—CNR, 80134 Napoli, Italy*

Giuseppina De Simone − *Istituto di Biostrutture e Bioimmagini—CNR, 80134 Napoli, Italy;* orcid.org/0000-0001-9783-5431

Claudiu T. Supuran − *Dipartimento NEUROFARBA, Università di Firenze, 50019 Sesto Fiorentino, Italy;* orcid.org/0000-0003-4262-0323

Complete contact information is available at: https://pubs.acs.org/10.1021/acsmedchemlett.0c00062

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

MIUR: PRIN2017 (Grant 201744BN5T). MIUR-PON: "Ricerca e Innovazione" 2014−2020 (Grant MOLIM ONCO-BRAIN LAB). Regione Campania: PO FESR 2014−2020 (Grant eMORFORAD).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge the financial support for this research by MIUR and Regione Campania.

■ ABBREVIATIONS

AAZ, acetazolamide; CA, carbonic anhydrase; CAI, carbonic anhydrase inhibitor; TPM, topiramate; ZBG, zinc binder group

■ REFERENCES

(1) Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Supuran, C. T.; De Simone, G. Multiple binding modes of inhibitors to carbonic

anhydrases: how to design specific drugs targeting 15 different isoforms? *Chem. Rev.* **2012**, *112* (8), 4421−4468.
(2) Supuran, C. T. Advances in structure-based drug discovery of carbonic anhydrase inhibitors. *Expert Opin. Drug Discovery* **2017**, *12* (1), 61−88.
(3) Supuran, C. T. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. *Nat. Rev. Drug Discovery* **2008**, *7* (2), 168−181.
(4) Monti, S. M.; Supuran, C. T.; De Simone, G. Anticancer carbonic anhydrase inhibitors: a patent review (2008 - 2013). *Expert Opin. Ther. Pat.* **2013**, *23* (6), 737−749.
(5) Nishimori, I.; Vullo, D.; Innocenti, A.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, C. T. Carbonic anhydrase inhibitors: inhibition of the transmembrane isozyme XIV with sulfonamides. *Bioorg. Med. Chem. Lett.* **2005**, *15* (17), 3828−3833.
(6) Nishimori, I.; Vullo, D.; Innocenti, A.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, C. T. Carbonic anhydrase inhibitors. The mitochondrial isozyme VB as a new target for sulfonamide and sulfamate inhibitors. *J. Med. Chem.* **2005**, *48* (24), 7860−7866.
(7) Ruusuvuori, E.; Kaila, K. Carbonic anhydrases and brain pH in the control of neuronal excitability. *Subcell. Biochem.* **2014**, *75*, 271−290.
(8) Supuran, C. T. Carbonic anhydrase inhibitors as emerging drugs for the treatment of obesity. *Expert Opin. Emerging Drugs* **2012**, *17* (1), 11−15.
(9) Bonardi, A.; Falsini, M.; Catarzi, D.; Varano, F.; Di Cesare Mannelli, L.; Tenci, B.; Ghelardini, C.; Angeli, A.; Supuran, C. T.; Colotta, V. Structural investigations on coumarins leading to chromeno[4,3-c]pyrazol-4-ones and pyrano[4,3-c]pyrazol-4-ones: New scaffolds for the design of the tumor-associated carbonic anhydrase isoforms IX and XII. *Eur. J. Med. Chem.* **2018**, *146*, 47−59.
(10) Supuran, C. T.; Di Fiore, A.; De Simone, G. Carbonic anhydrase inhibitors as emerging drugs for the treatment of obesity. *Expert Opin. Emerging Drugs* **2008**, *13* (2), 383−392.
(11) Waheed, A.; Sly, W. S. Carbonic anhydrase XII functions in health and disease. *Gene* **2017**, *623*, 33−40.
(12) Bruno, E.; Buemi, M. R.; De Luca, L.; Ferro, S.; Monforte, A. M.; Supuran, C. T.; Vullo, D.; De Sarro, G.; Russo, E.; Gitto, R. In Vivo Evaluation of Selective Carbonic Anhydrase Inhibitors as Potential Anticonvulsant Agents. *ChemMedChem* **2016**, *11* (16), 1812−1818.
(13) De Luca, L.; Ferro, S.; Damiano, F. M.; Supuran, C. T.; Vullo, D.; Chimirri, A.; Gitto, R. Structure-based screening for the discovery of new carbonic anhydrase VII inhibitors. *Eur. J. Med. Chem.* **2014**, *71*, 105−111.
(14) Gitto, R.; Agnello, S.; Ferro, S.; Vullo, D.; Supuran, C. T.; Chimirri, A. Identification of potent and selective human carbonic anhydrase VII (hCA VII) inhibitors. *ChemMedChem* **2010**, *5* (6), 823−826.
(15) Abo-Ashour, M. F.; Eldehna, W. M.; Nocentini, A.; Ibrahim, H. S.; Bua, S.; Abdel-Aziz, H. A.; Abou-Seri, S. M.; Supuran, C. T. Novel synthesized SLC-0111 thiazole and thiadiazole analogues: Determination of their carbonic anhydrase inhibitory activity and molecular modeling studies. *Bioorg. Chem.* **2019**, *87*, 794−802.
(16) Andreucci, E.; Ruzzolini, J.; Peppicelli, S.; Bianchini, F.; Laurenzana, A.; Carta, F.; Supuran, C. T.; Calorini, L. The carbonic anhydrase IX inhibitor SLC-0111 sensitises cancer cells to conventional chemotherapy. *J. Enzyme Inhib. Med. Chem.* **2019**, *34* (1), 117−123.
(17) Boyd, N. H.; Walker, K.; Fried, J.; Hackney, J. R.; McDonald, P. C.; Benavides, G. A.; Spina, R.; Audia, A.; Scott, S. E.; Libby, C. J.; Tran, A. N.; Bevensee, M. O.; Griguer, C.; Nozell, S.; Gillespie, G. Y.; Nabors, B.; Bhat, K. P.; Bar, E. E.; Darley-Usmar, V.; Xu, B.; Gordon, E.; Cooper, S. J.; Dedhar, S.; Hjelmeland, A. B. Addition of carbonic anhydrase 9 inhibitor SLC-0111 to Temozolomide treatment delays glioblastoma growth in vivo. *JCI Insight* **2017**, *2* (24), e92928.
(18) Bozdag, M.; Carta, F.; Ceruso, M.; Ferraroni, M.; McDonald, P. C.; Dedhar, S.; Supuran, C. T. Discovery of 4-Hydroxy-3-(3-(phenylureido)benzenesulfonamides as SLC-0111 Analogues for the Treatment of Hypoxic Tumors Overexpressing Carbonic Anhydrase IX. *J. Med. Chem.* **2018**, *61* (14), 6328−6338.
(19) Carta, F.; Vullo, D.; Osman, S. M.; AlOthman, Z.; Supuran, C. T. Synthesis and carbonic anhydrase inhibition of a series of SLC-0111 analogs. *Bioorg. Med. Chem.* **2017**, *25* (9), 2569−2576.
(20) Eldehna, W. M.; Abo-Ashour, M. F.; Berrino, E.; Vullo, D.; Ghabbour, H. A.; Al-Rashood, S. T.; Hassan, G. S.; Alkahtani, H. M.; Almehizia, A. A.; Alharbi, A.; Abdel-Aziz, H. A.; Supuran, C. T. SLC-0111 enaminone analogs, 3/4-(3-aryl-3-oxopropenyl) aminobenzenesulfonamides, as novel selective subnanomolar inhibitors of the tumor-associated carbonic anhydrase isoform IX. *Bioorg. Chem.* **2019**, *83*, 549−558.
(21) Eldehna, W. M.; Abo-Ashour, M. F.; Nocentini, A.; El-Haggar, R. S.; Bua, S.; Bonardi, A.; Al-Rashood, S. T.; Hassan, G. S.; Gratteri, P.; Abdel-Aziz, H. A.; Supuran, C. T. Enhancement of the tail hydrophobic interactions within the carbonic anhydrase IX active site via structural extension: Design and synthesis of novel N-substituted isatins-SLC-0111 hybrids as carbonic anhydrase inhibitors and antitumor agents. *Eur. J. Med. Chem.* **2019**, *162*, 147−160.
(22) Lomelino, C. L.; Mahon, B. P.; McKenna, R.; Carta, F.; Supuran, C. T. Kinetic and X-ray crystallographic investigations on carbonic anhydrase isoforms I, II, IX and XII of a thioureido analog of SLC-0111. *Bioorg. Med. Chem.* **2016**, *24* (5), 976−981.
(23) Supuran, C. T.; Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Carta, F.; Monti, S. M.; De Simone, G. Inhibition of carbonic anhydrase IX targets primary tumors, metastases, and cancer stem cells: Three for the price of one. *Med. Res. Rev.* **2018**, *38* (6), 1799−1836.
(24) Bruno, E.; Buemi, M. R.; Di Fiore, A.; De Luca, L.; Ferro, S.; Angeli, A.; Cirilli, R.; Sadutto, D.; Alterio, V.; Monti, S. M.; Supuran, C. T.; De Simone, G.; Gitto, R. Probing Molecular Interactions between Human Carbonic Anhydrases (hCAs) and a Novel Class of Benzenesulfonamides. *J. Med. Chem.* **2017**, *60* (10), 4316−4326.
(25) Buemi, M. R.; Di Fiore, A.; De Luca, L.; Angeli, A.; Mancuso, F.; Ferro, S.; Monti, S. M.; Buonanno, M.; Russo, E.; De Sarro, G.; De Simone, G.; Supuran, C. T.; Gitto, R. Exploring structural properties of potent human carbonic anhydrase inhibitors bearing a 4-(cycloalkylamino-1-carbonyl)benzenesulfonamide moiety. *Eur. J. Med. Chem.* **2019**, *163*, 443−452.
(26) Gitto, R.; Ferro, S.; Agnello, S.; De Luca, L.; De Sarro, G.; Russo, E.; Vullo, D.; Supuran, C. T.; Chimirri, A. Synthesis and evaluation of pharmacological profile of 1-aryl-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1H)-sulfonamides. *Bioorg. Med. Chem.* **2009**, *17* (10), 3659−3664.
(27) Gitto, R.; Agnello, S.; Ferro, S.; De Luca, L.; Vullo, D.; Brynda, J.; Mader, P.; Supuran, C. T.; Chimirri, A. Identification of 3,4-Dihydroisoquinoline-2(1H)-sulfonamides as potent carbonic anhydrase inhibitors: synthesis, biological evaluation, and enzyme−ligand X-ray studies. *J. Med. Chem.* **2010**, *53* (6), 2401−2408.
(28) Gitto, R.; De Luca, L.; Ferro, S.; Agnello, S.; Russo, E.; De Sarro, G.; Chimirri, A. Synthesis and structure-active relationship of 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline anticonvulsants. *Chem. Pharm. Bull.* **2010**, *58* (12), 1602−1605.
(29) Gitto, R.; Damiano, F. M.; De Luca, L.; Ferro, S.; Vullo, D.; Supuran, C. T.; Chimirri, A. Synthesis and biological profile of new 1,2,3,4-tetrahydroisoquinolines as selective carbonic anhydrase inhibitors. *Bioorg. Med. Chem.* **2011**, *19* (23), 7003−7007.
(30) Mader, P.; Brynda, J.; Gitto, R.; Agnello, S.; Pachl, P.; Supuran, C. T.; Chimirri, A.; Rezacova, P. Structural basis for the interaction between carbonic anhydrase and 1,2,3,4-tetrahydroisoquinolin-2-ylsulfonamides. *J. Med. Chem.* **2011**, *54* (7), 2522−2526.
(31) Gitto, R.; Damiano, F. M.; Mader, P.; De Luca, L.; Ferro, S.; Supuran, C. T.; Vullo, D.; Brynda, J.; Rezacova, P.; Chimirri, A. Synthesis, structure-activity relationship studies, and X-ray crystallographic analysis of arylsulfonamides as potent carbonic anhydrase inhibitors. *J. Med. Chem.* **2012**, *55* (8), 3891−3899.
(32) De Simone, G.; Alterio, V.; Supuran, C. T. Exploiting the hydrophobic and hydrophilic binding sites for designing carbonic anhydrase inhibitors. *Expert Opin. Drug Discovery* **2013**, *8* (7), 793−810.

https://dx.doi.org/10.1021/acsmedchemlett.0c00062
ACS Med. Chem. Lett. 2020, 11, 1000−1005

(33) Buemi, M. R.; De Luca, L.; Ferro, S.; Bruno, E.; Ceruso, M.; Supuran, C. T.; Pospisilova, K.; Brynda, J.; Rezacova, P.; Gitto, R. Carbonic anhydrase inhibitors: Design, synthesis and structural characterization of new heteroaryl-N-carbonylbenzenesulfonamides targeting druggable human carbonic anhydrase isoforms. *Eur. J. Med. Chem.* **2015**, *102*, 223−232.
(34) De Luca, L.; Mancuso, F.; Ferro, S.; Buemi, M. R.; Angeli, A.; Del Prete, S.; Capasso, C.; Supuran, C. T.; Gitto, R. Inhibitory effects and structural insights for a novel series of coumarin-based compounds that selectively target human CA IX and CA XII carbonic anhydrases. *Eur. J. Med. Chem.* **2018**, *143*, 276−282.
(35) Gitto, R.; De Luca, L.; Mancuso, F.; Del Prete, S.; Vullo, D.; Supuran, C. T.; Capasso, C. 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* (1), 1186−1192.
(36) Lomelino, C. L.; Andring, J. T.; McKenna, R. Crystallography and Its Impact on Carbonic Anhydrase Research. *Int. J. Med. Chem.* **2018**, *2018*, 9419521.
(37) Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Supuran, C. T.; De Simone, G. Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms? *Chem. Rev.* **2012**, *112* (8), 4421−4468.
(38) Hakansson, K.; Carlsson, M.; Svensson, L. A.; Liljas, A. Structure of native and apo carbonic anhydrase II and structure of some of its anion-ligand complexes. *J. Mol. Biol.* **1992**, *227* (4), 1192−1204.
(39) Chiaramonte, N.; Bua, S.; Ferraroni, M.; Nocentini, A.; Bonardi, A.; Bartolucci, G.; Durante, M.; Lucarini, L.; Chiapponi, D.; Dei, S.; Manetti, D.; Teodori, E.; Gratteri, P.; Masini, E.; Supuran, C. T.; Romanelli, M. N. 2-Benzylpiperazine: A new scaffold for potent human carbonic anhydrase inhibitors. Synthesis, enzyme inhibition, enantioselectivity, computational and crystallographic studies and in vivo activity for a new class of intraocular pressure lowering agents. *Eur. J. Med. Chem.* **2018**, *151*, 363−375.
(40) Alterio, V.; Hilvo, M.; Di Fiore, A.; Supuran, C. T.; Pan, P.; Parkkila, S.; Scaloni, A.; Pastorek, J.; Pastorekova, S.; Pedone, C.; Scozzafava, A.; Monti, S. M.; De Simone, G. Crystal structure of the catalytic domain of the tumor-associated human carbonic anhydrase IX. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106* (38), 16233−16238.
(41) Laskowski, R. A.; Swindells, M. B. LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. *J. Chem. Inf. Model.* **2011**, *51* (10), 2778−2786.

https://dx.doi.org/10.1021/acsmedchemlett.0c00062
ACS Med. Chem. Lett. 2020, 11, 1000−1005