ChemMedChem

Chemistry Europe
European Chemical Societies Publishing

Accepted Article

Title: Benzylaminoethylureido-tailed benzenesulfonamides show potent inhibitory action against bacterial carbonic anhydrases

Authors: Majid Ali, Murat Bozdag, andrea angeli, Fabrizio Carta, Clemente Capasso, Umar Farooq, and Claudiu T. Supuran

To be cited as: *ChemMedChem* 10.1002/cmdc.202000680

Link to VoR: https://doi.org/10.1002/cmdc.202000680

WILEY-VCH

WILEY-VCH

FULL PAPER

Benzylaminoethylureido-tailed benzenesulfonamides show potent inhibitory action against bacterial carbonic anhydrases

Majid Ali,[a,b] Andrea Angeli,*[a,c] Murat Bozdag, [a] Fabrizio Carta, [a] Clemente Capasso,* [d] Umar Farooq,[b] and Claudiu T. Supuran[a]

[a] M. Ali, A. Angeli, M. Bozdag, F. Carta, C.T. Supuran
University of Florence
NEUROFARBA Dept., Sezione di Scienze Farmaceutiche,
Via Ugo Schiff 6, 50019 Sesto Fiorentino (Florence), Italy.
[b] M. Ali, U. Farooq
Department of Chemistry, COMSATS University Islamabad, Abbottabad Campus, 22060, KPK, Pakistan
[c] A. Angeli
Centre of Advanced Research in Bionanoconjugates and Biopolymers Department, "Petru Poni"
Institute of Macromolecular Chemistry,
700487 Iasi, Romania.
E-mail: andrea.angeli@unifi.it
[d] C. Capasso
Institute of Biosciences and Bioresources, CNR,
Via Pietro Castellino 111, 80131 Napoli, Italy
E-mail: clemente.capasso@ibbr.cnr.it

Abstract: A series of benzylaminoethylureido-tailed benzenesulfonamides was analyzed for their inhibition potential against bacterial carbonic anhydrases (CAs) such as VhCA α, β, and γ from *Vibrio cholerae*, and BpsCA β and γ-CAs from *Burkholderia pseudomallei*. Growing drug-resistance against antibiotics demands alternative targets and mechanisms of action. As CA is essential for the survival of bacteria, such enzymes have the potential for developing new antibiotics. Most of the compounds presented excellent inhibition potential against VhCA γ as compared to α and β, with K_is in the range of 82.5 – 191.4 nM. Several sulfonamides presented excellent inhibition against BpsCA β with K_is in the range of 394 – 742.8 nM. Recently it has been demonstrated that sufonamides CA inhibitors are effective against vancomycin-resistant enterococcus. Such data proved that CA inhibition of pathogenic bacteria may lead to a new class of antibiotics.

Introduction

Bacterial resistance is defined by the WHO as critical issue for the public health at global scale, and it is mainly due to pathogenic strains no longer responding to classical antibiotic treatments [1-3]. As response, the scientific community has engaged a deep search of new druggable targets which are definite, essential for the virulence and/or for the life cycle of pathogens considered [3-5]. In this context the metalloenzymes Carbonic Anhydrases (CAs, EC 4.2.1.1) are particularly interesting [2, 6-8]. Bacteria encode four different CA genetic classes namely the *α*-, *β*-, *γ*- and *ι*-CAs with variegate distribution among the various strains [2, 7b]. Besides the apoenzymes structural differences, the *α*- and *β*-CAs contain a zinc (II) ion within the active site whereas the *γ*-CAs possess an iron (II) ion or cobalt (II) instead. As for the *α*- and *γ*-CAs the metal ion is coordinated by three His residues, whilst one His and two Cys residue constitute the *β*-CA coordination sphere [6]. Although the prosthetic ion for the *ι*-CAs is not reported yet, Mn (II) and/or Zn (II) are the suited candidates [7b]. In the last few years, numerous reports on pathogenic bacterial CAs *in vitro* inhibition profiles were produced [2, 9-14]. Among the strains explored *Helicobacter pylori*, *Mycobacterium tuberculosis*, *Vibrio cholerae*, *Streptococcus mutans*, *Burkholderia pseudomallei*, *Legionella pneumophila*, *Clostridium perfringens*, and *Porphyromonas gingivalis* were the most important [2, 9-12]. All the investigations produced were aimed at identifying new CA inhibitors (CAIs) endowed with potential antibacterial activity [9-14]. Although this study has the same scientific objectives, we propose a series of compounds obtained by means of a modular design strategy though to grant: *i*) easy access to variegate chemical diversities potentially useful for the identification of valuable CAIs as well as for structure-activity relationship (SAR) refinements; ii) low cost and high efficient scale-up syntheses of selected products. All compounds obtained were investigated *in vitro* for the possible inhibition properties against the *V. cholerae* (i.e. *α*-CA and *β*-CA) and *B. pseudomallei* (*β*-CA and *γ*-CA) expressed CAs with the aim to identify the chemical determinants useful to inhibit efficiently such bacterial strains expressing CAs.

Results and Discussion

Compounds **1-20** were synthesized according to our previous reported procedures [15]. Briefly, the commercially available sulfanilamide (**SA**) and metanilamide (**MA**) were reacted with phenylchloroformate under mild conditions and the isolated phenylcarbamates (**A**,**B**) were subjected to addition with mono-*N*-Boc-ethylene diamine to afford the Boc-aminoethylureidobenzene sulfonamide **C** and **D.** Removal of the *N*-Boc protection (**E, F**), exposed the terminal primary amine which were reacted according to a standard reductive amination protocol with a series of commercially available benzaldehydes to afford the final compounds **1-20** as outlined in **Figure 1** [15].

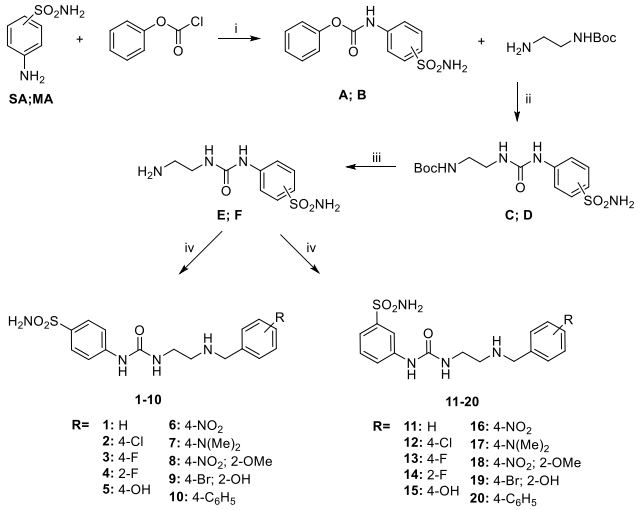


Figure 1. Synthesis of sulfonamides **1-20.** i) THF, DIPEA; ii) Acetonitrile reflux; iii) DCM, TFA; iv) MeOH, benzaldehyde, Et3N 0°C 3h followed by addition of $NaBH_4$ [15].

The potential inhibitory activity of the compounds **1-20** against the *α*-, *β*-, *γ*-CA from *V. cholerae*, and *β*-, *γ*-CA from *B. pseudomallei* was explored by means of the stopped flow CO_2 hydrase assay [16]. The inhibition values (K_i) obtained were referred to the standard sulfonamide drug Acetazolamide (**AAZ**) and are all reported in **Table 1**.

Table 1. Inhibition data of bacterial CAs: *V. cholerae* (*α*-CA, *β*-CA, and *γ*-CA) and *B. pseudomallei* (*β*-CA and *γ*-CA), with compounds **1-20** and **AAZ** by the stopped flow CO_2 hydrase assay [16].

		K_I (nM)[a]				
Cmp	**R**	**VhCAα**	**VhCAβ**	**VhCAγ**	**BpsCAβ**	**BpsCAγ**
1	H	695.0	535.1	3448	394.0	2023
2	4-Cl	514.2	7307	191.4	730.0	372.4
3	4-F	871.8	>10000	2714	501.7	2567
4	2-F	477.7	6858	97.4	2573	432.2
5	4-OH	769.3	>10000	5213	741.2	2593
6	4-NO_2	>10000	>10000	5563	895.8	3275
7	4-MeN_2	>10000	>10000	7245	>10000	340.4
8	4-NO_2, 2-OMe	>10000	>10000	5590	2918	422.0
9	4-Br, 2-OH	395.5	>10000	92.8	5500	3213
10	4-C_6H_5	>10000	5235	82.5	3785	1531
11	H	>10000	2868	3821	2605	360.7
12	4-Cl	391.0	3005	93.3	5787	411.7
13	4-F	631.2	4476	89.1	648.8	2428
14	2-F	705.0	2526	392.5	471.4	357.4
15	4-OH	254.5	4735	89.3	6931	3621
16	4-NO_2	234.6	>10000	242.7	3753	2235
17	4-Me_2N	423.0	3627	95.0	742.8	2366
18	4-NO_2, 2-OMe	419.3	3455	915.8	3273	324.6
19	4-Br, 2-OH	40.7	4966	95.2	7261	350.0
20	4-C_6H_5	>10000	3518	535.0	4000	351.7
AAZ	-	6.8	451.0	473.0.	745.0	149.0

[a] Mean from 3 different assays, by the stopped flow technique (errors were in the range of ± 5-10 % of the reported values).

The following SARs can be draw from data in **Table 1**.

i) Overall, compounds **1-20** showed excellent inhibition values against the VhCA γ when compared to the *α* and *β* isoforms expressed from the same strain. In particular **4, 9, 10, 12, 13, 15, 17** and **19** showed K_I values for the VhCA *γ* below 100 nM and considerably lower than the reference drug **AAZ** (K_I of 473.0 nM). The most VhCA *γ* effective inhibitor among the series was compound **10**, with K_i value of 82.5 nM. Its **MA** analogue bearing the same 4-C_6H_5 substitution resulted 6.5-fold less active. It is worth noting that the substitution at the phenyl ring among the SA (i.e. **1-10**) and MA (i.e. **11-20**) series seriously affected the VhCA *γ* kinetic profile regardless the electronic and/or bulky features of the groups introduced. Compound **13** bearing at position 4 a fluorine, **15** an -OH, and compound **12** a chloro atom reported K_i values of 89.1, 89.3, and 93.3 nM respectively, whereas their SA analogues (i.e. **3**, **5** and **2**) resulted far less effective (i.e. K_is of 2714, 5213, 191.4 nM respectively). Compound **9** of the SA series with a 4-Br, 2-OH substitution and

compound **19** which belongs to the MA series had almost similar inhibition potencies (i.e. K_i values of 92.8 nM and 95.2 nM). Compound **19** was the only effective inhibitor of the VhCA *α* (K_i of 40.7 nM) whereas compound **1** showed the best inhibition value against the VhCA *β* isoform (K_i of 535.1 nM).
ii) Compounds **1-20** showed rather complex inhibition profiles against *B. pseudomallei* CAs (i.e. BpsCA *β* and BpsCA γ). Compounds **1**, **3, 13** and **14,** resulted the best performing CAIs against BpsCA *β* with K_is of 394.0, 501.7, 648.8 and 471.4 nM, respectively, whereas the 4-OH derivative **5** and the 4-*N,N*-dimethyl amino substituted **17** resulted almost equal in inhibiting the such an isoenzyme when compared to the reference **AAZ** (K_i values of 741.2, 742.8 and 745.0 nM respectively). Again, the introduction of various moieties at the phenyl ring tail resulted critical for the kinetic profile. For instance, the MA analogue of **1** (i.e. compound **11**) showed its K_i reduced by 6.6-fold (K_is of 394.0 and 2605 nM respectively). Among the most effective BpsCA *β* inhibitors are the fluorine containing **13** and **14.** It is worth noting that the regioisomer **14** was 1.4-fold more potent than **13**, whereas opposite kinetic trend, with comparable intensity, was observed for the same regioisomers belonging to the SA series (i.e. compounds **3** and **4**). As for the BpsCA *γ* the CAI reference **AAZ** was the most effective (K_I of 149.0 nM), whereas **18** resulted the most potent among the compounds synthesized (K_i value of 324.6 nM). A clear SAR for the BpsCA *γ* by means of the data in table 1 resulted quite difficult although the MA series still confirmed to be the most efficient in inhibiting such an isoform. Overall, most of the compounds from the **1-20** series were found selective and effective CAIs against the VhCA *γ* and BpsCA *β* over their bacterial CAs expressed strains.

Conclusions

This study makes use of an *ad hoc* modular design strategy to obtain a set of compounds useful to investigate for the first time their ability to inhibit CAs expressed from *V. Cholerae* (i.e. α-CA and β-CA) and *B. Pseudomallei* (β-CA and γ-CA). Most of the compounds presented excellent and selective inhibition potencies against VhCA *γ* and BpsCA *β* with K_is in the range of 82.5 – 191.4 nM and 394 – 742.8 nM, respectively. Although an exhaustive SAR is not feasible at this stage, for the scope of this study is acceptable to define as general trend that compounds **11-20**, which belong to the MA series, are far more efficient in inhibiting the VhCA *γ* and BpsCA *β* when compared to their SA counterpart (i.e. compounds **1-10**). More importantly the data obtained in this study represent a solid starting point for the design of potential new therapeutics useful reduce bacterial resistance to conventional and clinical used antibiotics.

Experimental Section

General

All compounds were obtained according to previous reported procedures [15].

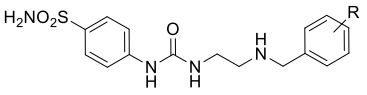
Carbonic anhydrase inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalysed CO_2 hydration activity [16] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng–Prusoff equation, as reported earlier [17-19], and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier. [17-19]

Keywords: bacterial carbonic anhydrases, benzene-sulfonamides, *V. cholerae*, *B. pseudomallei,* inhibition studies

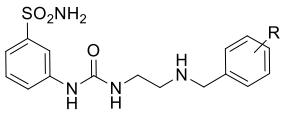
[1] https://www.who.int/news-room/fact-sheets/detail/antibiotic resistance#:~:text=Antibiotic%20resistance%20occurs%20when%20bacteria,caused%20by%20non%2Dresistant%20bacteria. (last access 28/08/2020)
[2] C.T. Supuran, C. Capasso Metabolites 2017, 7, 56.
[3] G.H. Furtado, D.P. Nicolau, Expert Opin. Ther. Pat. 2010, 20, 1273-1276.
[4] C.T. Supuran, C. Capasso Expert Opin Ther Pat. 2020; doi: 10.1080/13543776.2020.1811853
[5] J. Kaur, X. Cao, N.S. Abutaleb, A. Elkashif, A.L. Graboski, A.D. Krabill, A.H. AbdelKhalek, W. An, A. Bhardwaj, M.N. Seleem, D.P. Flaherty. J Med Chem. 2020; doi: 10.1021/acs.jmedchem.0c00734.
[6] M. Ferraroni, S. Del Prete, D. Vullo, C. Capasso, C.T. Supuran Acta Crystallogr. D. Biol. Crystallogr. 2015, 71, 2449-2456
[7] a) G. De Simone, S.M. Monti, V. Alterio, M. Buonanno, V. De Luca, M. Rossi, V. Carginale, C.T. Supuran, C. Capasso, A. Di Fiore *Bioorg. Med. Chem. Lett.* **2015**, 25, 2002-2006; b) S. Del Prete, A. Nocentini, C.T. Supuran, C. Capasso *J Enzyme Inhib Med Chem.* **2020**;35:1060-1068.
[8] I. Nishimori, T. Minakuchi, A. Maresca, F. Carta, A. Scozzafava, C.T. Supuran, *Curr. Pharm. Des.* **2010**, 16, 3300-3309.
[9] D. Vullo, S. Del Prete, P. Di Fonzo, V. Carginale, W. Donald, C.T. Supuran, C. Capasso *Molecules* **2017**, 22, 421.
[10] J.K. Modak, Y.C. Liu, C.T. Supuran, A. Roujeinikova, *J. Med. Chem.* **2016**, 59, 11098-11109.
[11] N. Dedeoglu, V. DeLuca, S. Isik, H. Yildirim, F. Kockar, C. Capasso, C.T. Supuran, *Bioorg. Med. Chem. Lett.* **2015**, 25, 2291-2297.
[12] I. Nishimori, S. Onishi, H. Takeuchi, C.T. Supuran, *Curr. Pharm. Des.* **2008**, 14, 622-630.
[13] M.V. Buchieri, L.E. Riafrecha, O.M. Rodriguez, D. Vullo, H.R. Morbidoni, C.T. Supuran, P.A. Colinas. *Bioorg. Med. Chem. Lett.* **2013**, 23, 740-743.
[14] C.T. Supuran, C. Capasso, *Pathogens* **2017**, 6, 30.
[15] M. Ali, M. Bozdag, U. Farooq, A. Angeli, F. Carta, P. Berto, G. Zanotti, C.T. Supuran, *Inter. J. Mol. Sci.* **2020**, 21, 2560.
[16] R.G. Khalifah J. Biol. Chem. 1971, 246, 2561.
[17] M. Abdoli, A. Angeli, M. Bozdag, F. Carta, A. Kakanejadifard, H. Saeidian, C.T. Supuran, *J. Enzyme. Inhib. Med. Chem.* **2017**, 32, 1071–1078.
[18] D. Tanini, A. Capperucci, M. Scopelliti, A. Milaneschi, A. Angeli, C.T. Supuran, *Bioorg Chem.* **2019**, 89, 102984.
[19] D. De Vita, A. Angeli, F. Pandolfi, M. Bortolami, R. Costi, R. Di Santo, E. Suffredini, M. Ceruso, S. Del Prete, C. Capasso, L. Scipione, C.T. Supuran, *J Enzyme Inhib Med Chem.* **2017**, 32, 798-804.

Entry for the Table of Contents



1-10

R= **1:** H; **2:** 4-Cl; **3:** 4-F; **4:** 2-F; **5:** 4-OH; **6:** 4-NO_2; **7:** 4-$N(Me)_2$; **8:** 4-NO_2; 2-OMe; **9:** 4-Br; 2-OH; **10:** 4-C_6H_5



11-20

R= **11:** H; **12:** 4-Cl; **13:** 4-F; **14:** 2-F; **15:** 4-OH; **16:** 4-NO_2; **17:** 4-$N(Me)_2$; **18:** 4-NO_2; 2-OMe; **19:** 4-Br; 2-OH; **20:** 4-C_6H_5

Potent inhibition against VhCAγ and BpsCAβ