



# Click-tailed benzenesulfonamides as potent bacterial carbonic anhydrase inhibitors for targeting *Mycobacterium tuberculosis* and *Vibrio cholerae*

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## ABSTRACT

A series of 1,2,3-triazole-bearing benzenesulfonamides was assessed for the inhibition of carbonic anhydrases (CA, EC 4.2.1.1) from bacteria *Vibrio cholerae* (VchCA $\alpha$  and VchCA $\beta$ ) and *Mycobacterium tuberculosis* ( $\beta$ -mtCA3). Growing resistance phenomena against existing antimicrobial drugs are globally spreading and highlight a urgent need of agents endowed with alternative mechanisms of action. Two global WHO strategies aim to reduce cholera deaths by 90% and eradicate the tuberculosis epidemic by 2030. The derivatives here reported represent interesting leads towards the optimization of new antibiotic agents showing excellent inhibitory efficiency and selectivity for the target CAs over the human (h) off-target isoform hCA I. In detail, the first subset of derivatives potently inhibits VchCA $\alpha$  in a low nanomolar range ( $K_i$ s between 0.72 and 22.6 nM). Compounds of a second subset, differing from the first one for the position of the spacer between benzenesulfonamide and triazole, preferentially inhibit VchCA $\beta$  ( $K_i$ s in the range 54.8–102.4 nM) and  $\beta$ -mtCA3 ( $K_i$ s in the range 28.2–192.5 nM) even more than the clinically used AAZ, used as the standard.

## 1. Introduction

To date, many sulfonamide compounds are marketed for the treatment of different diseases [1–3]. The main prerogative of this category of derivatives is a remarkable inhibitory activity against the zinc enzymes carbonic anhydrases (CA, EC 4.2.1.1) [3–5]. Distinct, evolutionarily non-related gene families of CAs are present in organisms throughout all the tree of life and encode for  $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\delta$ -,  $\zeta$ -,  $\eta$ -, and  $\theta$ -CAs [6–8], of which the  $\alpha$ -class is solely present in humans (h) in the form of 15 different isozymes (hCA I–XIV) [4]. hCAs possess various roles in physiological events such as carbon dioxide and bicarbonate transport processes, respiration, pH balancing, CO<sub>2</sub> homeostasis, electrolyte secretion, biosynthetic reactions [4]. Sulfonamide derivatives such as acetazolamide, methazolamide, ethoxzolamide, dichlorphenamide, dorzolamide and brinzolamide have been clinically used for decades as CA inhibitors for the treatment of different human diseases, e.g., glaucoma, cancer, epilepsy and diabetes [6]. In recent years the druggability of CAs from pathogens as anti-microbial targets have emerged for designing anti-infective drugs with a novel

mechanism of action [4]. These enzymes are indeed essential in the life cycle (pH homeostasis and biosynthetic reactions) as well as in the virulence of many bacterial, fungal and protozoan pathogens [4]. As described in the literature, many data show that interference with CA activity in various parasites leads to an impairment of parasite growth and virulence, with sulfonamides being again the most studied and effective among the screened inhibitors to yield the anti-infective action [4,9,10].

Tuberculosis (TB) is a highly contagious infection induced by *Mycobacterium tuberculosis* that quickly spreads through airborne droplets. The latest estimates show that 2 billion people worldwide are currently infected with the latent form of TB [11]. The anti-tuberculosis drugs entered the market 40 years ago have become less effective due to the development of drug resistance [11].

Cholera is another human infectious with a very high incidence, especially in developing countries [12]. It targets the small intestine and is caused by the gram-negative bacterium *Vibrio cholerae*. In 2016, WHO estimated worldwide 132121 cholera cases with 2420 deaths [12]. Thus, the treatment of these two infective diseases needs of safe

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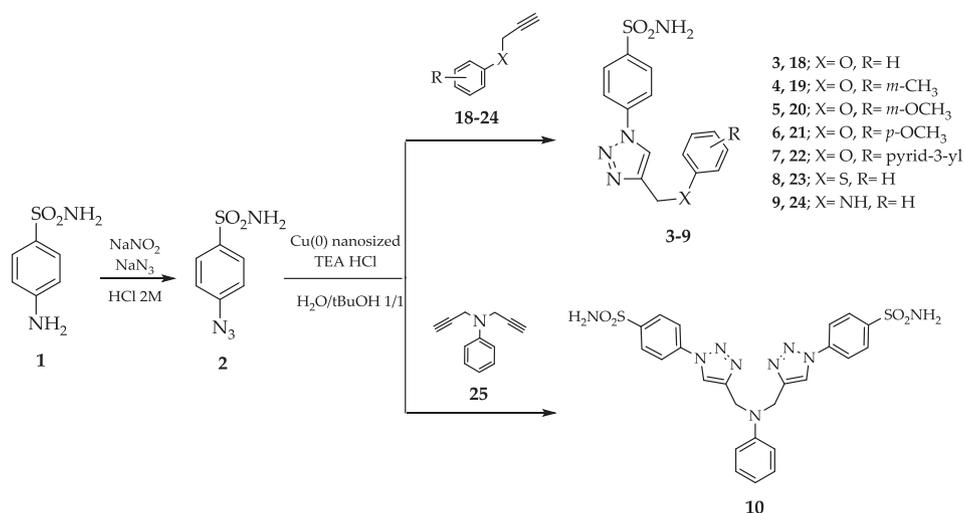
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**Scheme 1.** General synthetic procedure for compounds 3–10.

and potent new drugs to overcome, especially, the worldwide growing resistance phenomena [11–13]. The discovery of new potential antimicrobial targets and drugs is in line with two global strategies: the first, launched in 2017 by Global Task Force on Cholera Control aims to reduce cholera deaths by 90% [14] and the second one schedules the eradication of the TB epidemic by 2030 (Sustainable Development Goals) [15].

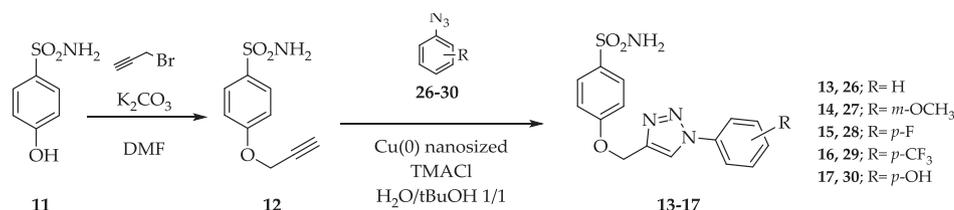
In this article, we report the evaluation of a series of sulfonamide compounds (Schemes 1 and 2) as inhibitors of some of the bacterial isoforms of CA that have been identified in *Mycobacterium tuberculosis* ( $\beta$ -mtCA3, encoded by the gene Rv32738) [16,17] and *Vibrio cholera* (VchCA $\alpha$  and  $\beta$ ) [18–20] looking for new agents suitable for the treatment of the related infections.

The choice to incorporate a triazole moiety in the compound structure relies on the intrinsic activity of the scaffold to perform a wide range of effects, such as anti-microbial [21,22], anti-inflammatory [23], analgesic [24], antitumor [25], and anticonvulsive [26]. Of note, Thirumal Yempala et al. reported a series of dibenzo[*b,d*]furan-1,2,3-triazoles as agents against *M. tuberculosis* with cytotoxicity studies revealing that some such derivatives possess good antitubercular action [27].

## 2. Results and discussion

### 2.1. Chemistry

Two different subsets of compounds were designed to vary the spacer connecting the ligand portions and were prepared by copper-catalyzed azide-alkyne cycloadditions (CuAAC), using the azides **2**, **26–30** and the alkynes **12**, **18–25** (Schemes 1 and 2) [28,29]. Copper (0) nanosized was used as a source of Cu (I), the necessary catalyst for the progress of the reaction, whose production *in situ* was aided by the presence of TEA hydrochloride or TMACl. The mixture H<sub>2</sub>O/*t*BuOH was used as solvent. A unique ditriazole derivative was yielded by reaction of 4-azidobenzenesulfonamide **2** with *N,N*-dipropargylaniline **25** [28].



**Scheme 2.** General synthetic procedure for compounds 13–17.

### 3. Carbonic anhydrase inhibition

Inhibition studies of VchCA $\alpha$ , VchCA $\beta$  and mtCA3 with sulfonamides **3–10**, **13–17** were performed to detect possible candidates as anti-infective drugs and carried out using a stopped-flow carbon dioxide hydration assay [30] in addition to the standard, clinically used CAI acetazolamide (AAZ) (Table 1). The displayed inhibitory activities were compared to those against the off-target widely distributed hCA I and hCA II.

The following structure-activity relationship (SAR) can be drawn from the data of this table:

- The first set of sulfonamides **3–10** reported potent inhibitory activity against VchCA $\alpha$  with inhibition constants in the low nanomolar range (the  $K_i$ s between 0.72 and 22.6 nM), except for compound **10**, which was inactive. It is interesting to note that compound **9**, with a  $K_i$  of 0.72 nM, is the most potent inhibitor among those tested whereas its triazole benzenesulfonamide double analogue **10** showed no efficacy below 10  $\mu$ M against this isoform and hCA I and II. Small structural differences in the linker moiety, such as the substitution of an oxygen atom (**3**,  $K_i$  = 6.0 nM) with different heteroatoms, in particular, nitrogen (**9**,  $K_i$  = 0.72 nM) or sulfur (**8**,  $K_i$  = 8.0 nM) did not lead to significant effects on the biological activity. Small substitutions on the outer phenyl ring are well tolerated not eliciting substantial variations in the compounds inhibitory profiles. The sulfonamides belonging to the second set showed diminished inhibitory potency against VchCA $\alpha$  ( $K_i$ s ranged between 50.3 and 121.7 nM) with respect to the first subset. Among the substitutions on the aromatic ring, solely the introduction of a methoxy group in a *para* position showed a negative effect on the inhibitory properties of compound **14** ( $K_i$  = 121.7 nM), which turned out to be the worst inhibitor of this set.
- Unexpectedly, an opposite situation was found in the case of VchCA $\beta$ : compounds of the second set ( $K_i$ s ranging between 54.8 and 102.4 nM) showed better inhibition constants compared with

**Table 1**

Inhibition data of  $\alpha$ -CAs VchCA $\alpha$ , hCA I, hCA II and  $\beta$ -CAs VchCA $\beta$  and mtCA3, with sulfonamides **3–10**, **13–17** reported here and the standard inhibitor acetazolamide (AAZ) by a Stopped Flow CO<sub>2</sub> hydrase assay [30].

| Cmpd      | X  | R                          | K <sub>i</sub> (nM) |               |        |          |          |
|-----------|----|----------------------------|---------------------|---------------|--------|----------|----------|
|           |    |                            | VchCA $\alpha$      | VchCA $\beta$ | mtCA3  | hCAI     | hCAII    |
| <b>3</b>  | O  | H                          | 6.0                 | 393.4         | 278.2  | 349.9    | 1.0      |
| <b>4</b>  | O  | <i>m</i> -CH <sub>3</sub>  | 18.5                | 472.7         | 648.2  | 406.8    | 1.5      |
| <b>5</b>  | O  | <i>m</i> -OCH <sub>3</sub> | 22.6                | 531.0         | 691.1  | 351.1    | 1.5      |
| <b>6</b>  | O  | <i>p</i> -OCH <sub>3</sub> | 13.7                | 489.7         | 260.2  | 512.6    | 4.3      |
| <b>7</b>  | O  | pyridyl-3-yl               | 4.8                 | 367.6         | 228.5  | 123.0    | 1.4      |
| <b>8</b>  | S  | H                          | 8.0                 | 409.3         | 344.9  | 195.7    | 1.5      |
| <b>9</b>  | NH | H                          | 0.72                | 283.6         | 340.0  | 7.9      | 0.83     |
| <b>10</b> | –  | –                          | > 10,000            | 4916.1        | 9479.5 | > 10,000 | > 10,000 |
| <b>13</b> | –  | H                          | 63.9                | 54.8          | 71.7   | 565.6    | 1.2      |
| <b>14</b> | –  | <i>m</i> -OCH <sub>3</sub> | 121.7               | 90.9          | 192.5  | 278.1    | 12.4     |
| <b>15</b> | –  | <i>p</i> -F                | 50.3                | 85.0          | 34.1   | 949.8    | 2.6      |
| <b>16</b> | –  | <i>p</i> -CF <sub>3</sub>  | 57.2                | 102.4         | 28.2   | > 10000  | 15.7     |
| <b>17</b> | –  | <i>p</i> -OH               | 77.8                | 60.9          | 81.1   | 92.8     | 1.0      |
| AAZ       | –  | –                          | 6.8                 | 451           | 104    | 250      | 12       |

\* Mean from 3 different assays, by a stopped flow technique (errors were in the range of  $\pm$  5–10% of the reported values).

those of the first set (K<sub>i</sub>s ranging between 283.6 and 4916.1 nM). The active site of  $\beta$ -CAs is known to be narrower than that of  $\alpha$ -CAs, resulting in better efficacy of derivatives endowed with greater flexibility after the portion that binds the zinc [4]. Also against this isoform, the introduction of the methoxy group in *meta* or *para* position to the outer phenyl ring slightly reduced the inhibitory potency of compounds **5**, **6** and **14** (K<sub>i</sub> = 531.0, 489.7 and 90.9 nM, respectively) compared to the unsubstituted analogs. Within the second series of derivatives, the incorporation of a fluorine atom or trifluoromethyl group in *para* position to the aromatic ring led to a decrease of the inhibitory activity of compounds **15** (K<sub>i</sub> = 85.0 nM) and **16** (K<sub>i</sub> = 102.4 nM), respectively, with respect to the best compound of second set, the unsubstituted **13** that showed a K<sub>i</sub> of 54.8 nM. The structural variations on the linker or substitutions on the aromatic ring did not lead to significant improvements in the inhibitory trend of the first series derivatives with respect to the lead **3** (K<sub>i</sub> = 393.4 nM).

(iii) The general tendencies described above are also applicable for isoform mtCA3,  $\beta$ -CA from *M. tuberculosis*. In fact, compounds of the second set (**13–17**) reported better inhibitory activity than compounds of the first set (**3–9**). For instance, a significant inhibition difference can be noted, regardless of the nature of the XCH<sub>2</sub> linker, within two couples of derivatives: **3** and **13** (K<sub>i</sub>s = 278.2 and 71.7 nM, respectively) and **5** and **14** (K<sub>i</sub>s = 691.1 and 192.5 nM, respectively), bearing an unsubstituted and *m*-CH<sub>3</sub> substituted phenyl ring, respectively. An interesting inhibition profile was observed for compounds **15** and **16**. The combination of a more flexible linker and a *para*-substitution with a fluorine atom or trifluoromethyl group had a positive effect on the derivatives inhibitory activity, such to make compounds **15** and **16** the best inhibitors among those studied with K<sub>i</sub>s of 34.1 and 28.2 nM, respectively. Whereas the substitution of the outer phenyl ring with a pyridyl one (**7**) seems to be effective in inducing strong  $\alpha$ -CA inhibitory effects, in contrast, it reduced the activity against  $\beta$ -CAs with K<sub>i</sub> in high nanomolar range (K<sub>i</sub> = 367.6 for VchCA $\beta$  and K<sub>i</sub> = 228.5 for mtCA3). Compound **10** reported a weak inhibitory activity against VchCA $\beta$  and mtCA3 with K<sub>i</sub>s ranging between 4.9 and 9.5  $\mu$ M.

(iv) All compounds (except **10**) showed an improved efficacy in inhibiting VchCA $\alpha$  in comparison to hCA I, whereas only compounds of the second set inhibited VchCA $\beta$  and mtCA3 significantly more efficiently than the same ubiquitous isoform that is responsible for most side effects related to the use of non-selective hCAIs. Isoform hCA II is conversely potentially inhibited by the reported triazole

sulfonamides with K<sub>i</sub>s significantly lower than those observed against the pathogens CAs. Of note, the most active inhibitor against the target CAs, that is **9** against VchCA $\alpha$ , solely showed a competitive K<sub>i</sub> (0.72 nM) with that measured against hCA II (K<sub>i</sub> of 0.83 nM).

#### 4. Conclusions

A series of 1,2,3-triazole-bearing benzenesulfonamides was assessed for the inhibition of CAs from bacteria *Vibrio cholerae* (VchCA $\alpha$  and VchCA $\beta$ ) and *Mycobacterium tuberculosis* ( $\beta$ -mtCA3). There is an urgent need of new antimicrobial agents exploiting alternative mechanisms of action because of the globally spreading resistance phenomena against existing antimicrobial drugs. The discovery of new potential anti-microbial targets and drugs is in line with two global strategies: the first, launched in 2017 by Global Task Force on Cholera Control aims to reduce cholera deaths by 90% [14] and the second one schedules the eradication of the TB epidemic by 2030 (Sustainable Development Goals) [15]. The first subset of derivatives potently inhibits VchCA $\alpha$  in a low nanomolar range (K<sub>i</sub>s between 0.72 and 22.6 nM). The compounds of a second subset that possess a different kind of connection between the molecular portions, preferentially inhibit VchCA $\beta$  (K<sub>i</sub>s in the range 54.8–102.4 nM) and  $\beta$ -mtCA3 (K<sub>i</sub>s in the range 28.2–192.5 nM) even more than the clinically used AAZ, used as the standard. All these derivatives represent interesting leads towards the optimization of new antibiotic agents showing excellent inhibitory efficiency and selectivity for the target CAs over the human (h) off-target isoform hCA I.

#### 5. Experimental sections

##### 5.1. Chemistry

The synthesis and characterization of sulfonamides **3–10**, **13–17** was reported earlier by our group [28].

#### 6. Carbonic anhydrase inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA-catalysed CO<sub>2</sub> hydration activity [30]. 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<sub>2</sub>SO<sub>4</sub> (for maintaining constant the ionic strength), following the initial rates of the CA-catalysed CO<sub>2</sub> hydration reaction for a period of 10–100 s. The CO<sub>2</sub> 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-deionised water and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E–I complex. The inhibition constants were obtained by nonlinear least-squares methods using PRISM 3 and the Cheng-Prusoff equation, as reported earlier, and represent the mean from at least three different determinations [4,31]. All CA isoforms were recombinant ones obtained in-house as reported earlier [32,33].

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bioorg.2019.01.065>.

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