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SHORT COMMUNICATION

## N-glycosyl-N-hydroxysulfamides as potent inhibitors of *Brucella suis* carbonic anhydrases

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### Abstract

We investigated a series of N-hydroxysulfamides obtained by Ferrier sulfamidoglycosylation for the inhibition of two bacterial carbonic anhydrases (CAs, EC 4.2.1.1) present in the pathogen *Brucella suis*. bsCA I was moderately inhibited by these compounds with inhibition constants ranging between 522 and 958 nM and no notable differences of activity between the acetylated or the corresponding deacetylated derivatives. The compounds incorporating two *trans*-acetates and the corresponding deprotected ones were the most effective inhibitors in the series. bsCA II was better inhibited, with inhibition constants ranging between 59.8 and 799 nM. The acetylated derivatives were generally better bsCA II inhibitors compared to the corresponding deacetylated compounds. Although these compounds were not highly isoform-selective CA inhibitors (CAIs) for the bacterial over the human CA isoforms, some of them possess inhibition profiles that make them interesting leads for obtaining better and more isoform-selective CAIs targeting bacterial enzymes.

### Keywords

*Brucella suis*, carbonic anhydrase, glycoinhibitor, glycosyl derivative, N-hydroxysulfamide

### History

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### Introduction

Antibiotic resistance is currently a major public health problem. The discovery of new antibiotics having novel mechanisms of action for fighting pathogenic bacteria efficiently is therefore a priority. So the current challenge is to identify and validate new pharmaceutical targets in bacteria, which would serve as a basis for discovering new classes of antibacterial agents. With the huge progress in bacterial genomics and genome sequencing, many potential new drug targets have been identified, among which the zinc metalloenzyme carbonic anhydrase (CA; EC 4.2.1.1), present with at least three genetic families in bacteria, the  $\alpha$ -,  $\beta$ - and  $\gamma$ -CAs<sup>1–3</sup>.

In the past few years, bacterial CAs started to be investigated in detail and several CAs belonging to classes mentioned above have been cloned and characterized from pathogenic bacteria such as *Vibrio cholerae*, *Escherichia coli*, *Helicobacter pylori*, *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, *Salmonella enterica*, *Haemophilus influenzae*, *Legionella pneumophila*, *Porphyromonas gingivalis* and *Brucella suis*<sup>1–3</sup>.

Considering the important role of these proteins in the survival, invasion and pathogenicity as well as their role as

virulence factors of some of these microorganisms, a campaign of *in vitro* and *in vivo* screening with various classes of CA inhibitors (CAIs) such as anions, sulfonamides and sulfamates has been initiated in the past years<sup>1–3</sup>.

Our research groups participated with its work to this challenging task, focusing on the bacterial pathogen *B. suis*<sup>4–9</sup>. *Brucella* spp. are facultative intracellular coccobacilli responsible of brucellosis, the major bacterial zoonosis worldwide, in a variety of mammals including ruminants and humans<sup>4–9</sup>. The genome of the human pathogen *B. suis* contains two CAs belonging to the  $\beta$ -class: bsCA I and bsCA II, which have been discovered and characterized by our groups<sup>6,9</sup>. These two CAs were shown to be catalytically efficient, with activity for the CO<sub>2</sub> hydration reaction similar to that of the human (h) isoform hCA II, and were inhibited by many sulfonamides/sulfamates<sup>4–9</sup>. Recently, our group has shown that a glycosyl sulfanilamide derivative also inhibited the *B. suis* growth in cell cultures<sup>8</sup>, demonstrating thus that CA glycoinhibitors may lead to new and potent anti-infectives with a mechanism of action that could bypasses the drug resistance problems of clinically used antibiotics<sup>8</sup>.

The use of glycomimetics was proven to be a successful approach in the design of CAIs<sup>10,11</sup>, constituting now one of the most attractive ways to develop new generations of effective and selective such agents. In this article, we report the activity of carbohydrate-based CAs inhibitors of types **1a–5b**, recently reported by our group, against bsCA I and bsCA II<sup>12</sup> (Figure 1).

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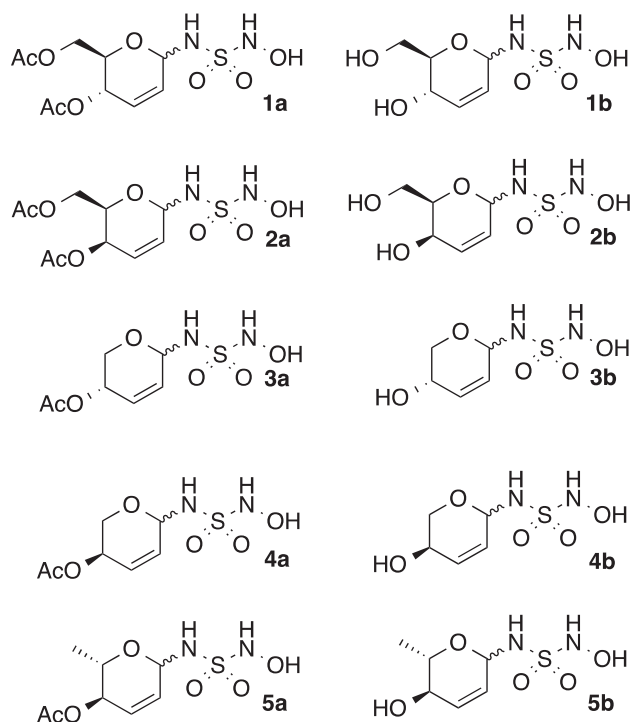


Figure 1. Structure of glyco-inhibitors belonging to the hydroxysulfamide series<sup>12</sup> (mixture of anomers,  $\alpha/\beta$  of 77/23) investigated as bsCA I and II inhibitors.

## Materials and methods

### Chemistry

Compounds **1a–5b** were reported earlier<sup>12</sup>, whereas acetazolamide (AAZ) used in this study was commercially available from Sigma-Aldrich (Milan, Italy).

### CA inhibition

A stopped-flow instrument (SX.18MV-R Applied Photophysics model, Surrey, UK) was used for assaying the CA-catalyzed CO<sub>2</sub> hydration activity<sup>13</sup>. Inhibitor and enzyme were preincubated for 15 min for allowing the complete formation of the enzyme-inhibitor adduct<sup>14–16</sup>. IC<sub>50</sub> values were obtained from dose-response curves working at seven different concentrations of test compound (from 0.1 nM to 50  $\mu$ M), by fitting the curves using PRISM (www.graphpad.com) (La Jolla, CA) and non-linear least squares methods, the obtained values representing the mean of at least three different determinations<sup>17–19</sup>. The inhibition constants (K<sub>i</sub>) were derived from the IC<sub>50</sub> values by using the Cheng–Prusoff equation as follows:  $K_i = IC_{50}/(1 + [S]/K_m)$ , where [S] represents the CO<sub>2</sub> concentration at which the measurement was carried out and K<sub>m</sub> the concentration of substrate at which the enzyme activity is at half maximal<sup>20–23</sup>. All enzymes used were recombinant, produced in *E. coli* as reported earlier<sup>6,9</sup>. The concentrations of enzymes used in the assay were in the range of 10.5 nM for bsCA I and of 12.1 nM for bsCA II.

## Results and discussion

A large number of CAs isolated from bacterial<sup>24–26</sup> or fungal pathogens<sup>27</sup> started to become investigated recently in detail as drug targets for developing anti-infectives with a new mechanism of action<sup>28–30</sup>. As mentioned above, the two *Brucella*  $\beta$ -CAs bsCA I and bsCA II are among those enzymes of great interest, and their inhibition has been investigated with inorganic anions, and primary aromatic, heterocyclic or glycosidic sulfonamides<sup>4–9</sup>.

Table 1. Inhibitory activity of compounds **1a–5a** and **1b–5b** against the human CA isoform hCA II and the *Brucella suis* CA: bsCA I and bsCA II, determined by a stopped-flow, CO<sub>2</sub> hydration assay method at pH 8.4<sup>13</sup>.

Inhibitors	K <sub>i</sub> (nM)*		
	hCA II†	bsCA I	bsCA II
AAZ	12	96.5	67.2
<b>1a</b>	94	565	59.8
<b>1b</b>	91	522	241
<b>2a</b>	86	732	76.2
<b>2b</b>	>10 000	845	472
<b>3a</b>	>10 000	>10 000	92.1
<b>3b</b>	>10 000	>10 000	635
<b>4a</b>	>10 000	958	755
<b>4b</b>	85	811	89.5
<b>5a</b>	94	883	338
<b>5b</b>	>10 000	656	799

\*Mean from three different assays, errors in the range of  $\pm 10\%$  of the reported values (data not shown).

†See reference 12.

However, these CAIs strongly inhibit the human, offtarget isoforms hCA I and II; and this is the reason why we investigate in this study the inhibition profile of the two bacterial enzymes with a series of newly reported N-hydroxysulfamides<sup>12</sup>, of types **1a–5b**, some of which do not significantly inhibit the human enzymes (Table 1).

The N-hydroxysulfamides **1a–5b** and AAZ (5-acetamido-1,3,4-thiadiazole-2-sulfonamide) as standard were assayed for the inhibition of bsCA I and II (hCA II inhibitory data were reported in the earlier work<sup>12</sup>). The following structure–activity relationship (SAR) can be drawn from data of Table 1:

(i) bsCA I was weakly inhibited by most N-hydroxysulfamides investigated in this study, with inhibition constants ranging between 522 and 958 nM (except **3a** and **3b**, which were in fact not inhibitory up to concentrations of 10  $\mu$ M in the assay system). There were no notable differences of activity between the acetylated or the corresponding deacetylated derivatives, but the compounds incorporating two *trans*-acetate (or the corresponding ones with two *trans*-OH) moieties, **1a** and **1b**, were the most effective inhibitors in the series.

(ii) bsCA II was better inhibited by the N-hydroxysulfamides investigated in this study, compared to the isoform bsCA I, with inhibition constants ranging between 59.8 and 799 nM (Table 1). SAR was in this case more interesting, with the acetylated derivatives being better bsCA II inhibitors compared with the corresponding deacetylated derivatives (except for the pair **4a/4b**, case in which the deprotected alcohol was a better inhibitor compared to the acetylated derivative). There were no important differences of activity between the *cis* and *trans* isomers **1a** and **2a**, whereas for the corresponding deprotected derivatives, **1b** and **2b**, the differences in the inhibition constants were relevant, with the *trans* derivative **1b** being roughly two times a better bsCA II inhibitor compared with the corresponding *cis*-isomer **2b** (Table 1). These data clearly show that small structural differences in the scaffold of the N-hydroxysulfamides investigated in this study lead to important differences of affinity for the cognate enzyme.

(iii) No interesting selectivity profiles for the inhibition of the bacterial over the human CA isoforms were observed for the investigated N-hydroxysulfamides, most of which were better hCA II than bsCA I/II inhibitors. However, **2b** did not inhibit hCA II significantly, but was a medium potency inhibitor of the bacterial enzymes bsCA I and II, whereas **3a** was not inhibitory against hCA II and bsCA I, being on the other hand a <100 nM inhibitor of bsCA II. This is a bsCA II-specific inhibitor, and both



these derivatives may be considered interesting leads for obtaining better and more isoform-selective CAIs targeting bacterial enzymes.

In conclusion, we investigated a series of N-hydroxysulfamides obtained by Ferrier sulfamidoglycosylation for the inhibition of two bacterial CAs involved in the pathogenesis of brucellosis. bsCA I was moderately inhibited by these compounds, with inhibition constants ranging between 522 and 958 nM, and no notable differences of activity between the acetylated or the corresponding deacetylated derivatives. The compounds incorporating two *trans*-acetate and the corresponding ones with two *trans*-OH moieties were the most effective inhibitors in the series. bsCA II was better inhibited, compared to isoform I, with inhibition constants ranging between 59.8 and 799 nM. The acetylated derivatives were generally better bsCA II inhibitors compared with the corresponding deacetylated compounds. For some of the de-acetylated derivatives, the differences in the inhibition constants were relevant between the *trans* and *cis* derivatives, with the *trans* one being two times a better bsCA II inhibitor compared to the corresponding *cis*-isomer. Although these compounds were not highly isoform-selective CAIs for the bacterial over the human CA isoforms, some of them possess inhibition profiles, which make them interesting leads for obtaining better and more isoform-selective CAIs targeting bacterial enzymes.

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### Declaration of interest

The authors report no conflict of interest.

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