



HAL
open science

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

Joanna Ombouma, Daniela Vullo, Stephan Köhler, Pascal Dumy, Claudiu T.
Supuran, Jean-Yves Winum

► **To cite this version:**

Joanna Ombouma, Daniela Vullo, Stephan Köhler, Pascal Dumy, Claudiu T. Supuran, et al.. N-glycosyl-N-hydroxysulfamides as potent inhibitors of Brucella suis carbonic anhydrases. Journal of Enzyme Inhibition and Medicinal Chemistry, 2014, 30 (6), pp.1010-1012. 10.3109/14756366.2014.986119 . hal-02425102

HAL Id: hal-02425102

<https://hal.science/hal-02425102>

Submitted on 8 Jan 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



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

Joanna Ombouma, Daniela Vullo, Stephan Köhler, Pascal Dumy, Claudiu T. Supuran & Jean-Yves Winum

To cite this article: Joanna Ombouma, Daniela Vullo, Stephan Köhler, Pascal Dumy, Claudiu T. Supuran & Jean-Yves Winum (2015) N-glycosyl-N-hydroxysulfamides as potent inhibitors of *Brucella suis* carbonic anhydrases, *Journal of Enzyme Inhibition and Medicinal Chemistry*, 30:6, 1010-1012, DOI: [10.3109/14756366.2014.986119](https://doi.org/10.3109/14756366.2014.986119)

To link to this article: <http://dx.doi.org/10.3109/14756366.2014.986119>



Published online: 20 Mar 2015.



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



Article views: 50



View related articles [↗](#)



View Crossmark data [↗](#)

SHORT COMMUNICATION

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

Joanna Ombouma¹, Daniela Vullo², Stephan Köhler³, Pascal Dumy¹, Claudiu T. Supuran⁴, and Jean-Yves Winum¹

¹Institut des Biomolécules Max Mousseron (IBMM) UMR 5247 CNRS-ENSCM-UM1-UM2, Bâtiment de Recherche Max Mousseron, Ecole Nationale Supérieure de Chimie de Montpellier, Montpellier Cedex, France, ²Laboratorio di Chimica Bioinorganica, Polo Scientifico, Università degli Studi di Firenze, Sesto Fiorentino, Florence, Italy, ³Centre d'Études d'Agents Pathogènes et Biotechnologies pour la Santé (CPBS), UMR5236, CNRS-Université Montpellier I-Université Montpellier II, Montpellier Cedex, France, and ⁴Neurofarba Department, Section of Pharmaceutical and Nutriceutical Sciences, Università degli Studi di Firenze, Via Ugo Schiff, Florence, Italy

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

Received 24 October 2014
Revised 4 November 2014
Accepted 6 November 2014
Published online 20 March 2015

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 α -, β - and γ -CAs^{1–3}.

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*^{1–3}.

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^{1–3}.

Our research groups participated with its work to this challenging task, focusing on the bacterial pathogen *B. suis*^{4–9}. *Brucella* spp. are facultative intracellular coccobacilli responsible of brucellosis, the major bacterial zoonosis worldwide, in a variety of mammals including ruminants and humans^{4–9}. The genome of the human pathogen *B. suis* contains two CAs belonging to the β -class: bsCA I and bsCA II, which have been discovered and characterized by our groups^{6,9}. These two CAs were shown to be catalytically efficient, with activity for the CO₂ hydration reaction similar to that of the human (h) isoform hCA II, and were inhibited by many sulfonamides/sulfamates^{4–9}. Recently, our group has shown that a glycosyl sulfanilamide derivative also inhibited the *B. suis* growth in cell cultures⁸, 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 antibiotics⁸.

The use of glycomimetics was proven to be a successful approach in the design of CAIs^{10,11}, 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 II¹² (Figure 1).

Address for correspondence: Jean-Yves Winum, Institut des Biomolécules Max Mousseron (IBMM) UMR 5247 CNRS-ENSCM-UM1-UM2, Bâtiment de Recherche Max Mousseron, Ecole Nationale Supérieure de Chimie de Montpellier, 8 rue de l'École Normale, 34296 Montpellier Cedex, France. E-mail: jean-yves.winum@univ-montp2.fr

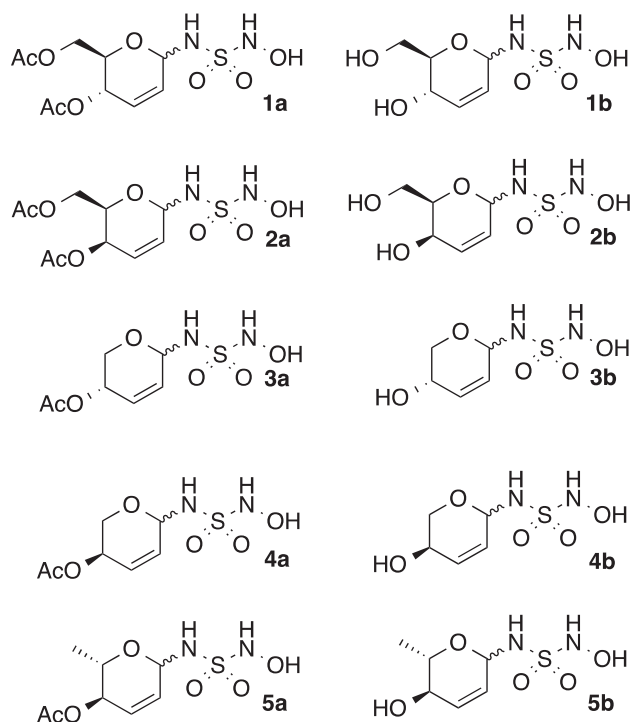


Figure 1. Structure of glycoinhibitors belonging to the hydroxysulfamide series¹² (mixture of anomers, α/β of 77/23) investigated as bsCA I and II inhibitors.

Materials and methods

Chemistry

Compounds **1a–5b** were reported earlier¹², 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 CO₂ hydration activity¹³. Inhibitor and enzyme were preincubated for 15 min for allowing the complete formation of the enzyme-inhibitor adduct^{14–16}. IC₅₀ values were obtained from dose-response curves working at seven different concentrations of test compound (from 0.1 nM to 50 μ 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^{17–19}. The inhibition constants (K_i) were derived from the IC₅₀ values by using the Cheng–Prusoff equation as follows: $K_i = IC_{50}/(1 + [S]/K_m)$, where [S] represents the CO₂ concentration at which the measurement was carried out and K_m the concentration of substrate at which the enzyme activity is at half maximal^{20–23}. All enzymes used were recombinant, produced in *E. coli* as reported earlier^{6,9}. 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^{24–26} or fungal pathogens²⁷ started to become investigated recently in detail as drug targets for developing anti-infectives with a new mechanism of action^{28–30}. As mentioned above, the two *Brucella* β -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^{4–9}.

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, CO₂ hydration assay method at pH 8.4¹³.

| Inhibitors | K _i (nM)* | | |
|------------|----------------------|---------|---------|
| | hCA II† | bsCA I | bsCA II |
| AAZ | 12 | 96.5 | 67.2 |
| 1a | 94 | 565 | 59.8 |
| 1b | 91 | 522 | 241 |
| 2a | 86 | 732 | 76.2 |
| 2b | >10 000 | 845 | 472 |
| 3a | >10 000 | >10 000 | 92.1 |
| 3b | >10 000 | >10 000 | 635 |
| 4a | >10 000 | 958 | 755 |
| 4b | 85 | 811 | 89.5 |
| 5a | 94 | 883 | 338 |
| 5b | >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-hydroxysulfamides¹², 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 work¹²). 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 μ 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.

Acknowledgements

The authors would like to thank the Gabonese Ministry of Research for PhD fellowship (to J. O.) and the CNRS/CNR (CoopIntEER program, grant no. 131999, to J.-Y. W.) for financial support.

Declaration of interest

The authors report no conflict of interest.

References

- Supuran CT. Bacterial carbonic anhydrases as drug targets: toward novel antibiotics? *Front Pharmacol* 2011;2:1–6.
- Supuran CT. Structure-based drug discovery of carbonic anhydrase inhibitors. *J Enzyme Inhib Med Chem* 2012;27:759–72.
- 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* 2014. [Epub ahead of print]. doi:10.3109/14756366.2014.910202.
- Maresca A, Scozzafava A, Köhler S, et al. Inhibition of beta-carbonic anhydrases from the bacterial pathogen *Brucella suis* with inorganic anions. *J Inorg Biochem* 2012;110:36–9.
- Lopez M, Köhler S, Winum JY. Zinc metalloenzymes as new targets against the bacterial pathogen *Brucella*. *J Inorg Biochem* 2012;111:138–45.
- Joseph P, Ouahrani-Bettache S, Montero JL, et al. A new β -carbonic anhydrase from *Brucella suis*, its cloning, characterization, and inhibition with sulfonamides and sulfamates, leading to impaired pathogen growth. *Bioorg Med Chem* 2011;19:1172–8.
- Winum JY, Köhler S, Supuran CT. *Brucella* carbonic anhydrases: new targets for designing anti-infective agents. *Curr Pharm Des* 2010;16:3310–16.
- Vullo D, Nishimori I, Scozzafava A, et al. Inhibition studies of a beta-carbonic anhydrase from *Brucella suis* with a series of water soluble glycosyl sulfanilamides. *Bioorg Med Chem Lett* 2010;20:2178–82.
- Joseph P, Turtaut F, Ouahrani-Bettache S, et al. Cloning, characterization, and inhibition studies of a beta-carbonic anhydrase from *Brucella suis*. *J Med Chem* 2010;53:2277–85.
- Winum JY, Colinas PA, Supuran CT. Glycosidic carbonic anhydrase IX inhibitors: a sweet approach against cancer. *Bioorg Med Chem* 2013;21:1419–26.
- Winum JY, Poulsen SA, Supuran CT. Therapeutic applications of glycosidic carbonic anhydrase inhibitors. *Med Res Rev* 2009;29:419–35.
- Ombouma J, Vullo D, Supuran CT, Winum JY. Ferrier sulfamidoglycosylation of glycals catalyzed by nitrosonium tetrafluoroborate: towards new carbonic anhydrase glycoinhibitors. *Bioorg Med Chem* 2014;22:6353–9.
- 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.
- Bonneau A, Maresca A, Winum JY, Supuran CT. Metronidazole-coumarin conjugates and 3-cyano-7-hydroxy-coumarin act as isoform-selective carbonic anhydrase inhibitors. *J Enzyme Inhib Med Chem* 2013;28:397–401.
- Sharma A, Tiwari M, Supuran CT. Novel coumarins and benzocoumarins acting as isoform-selective inhibitors against the tumor-associated carbonic anhydrase IX. *J Enzyme Inhib Med Chem* 2014;29:292–6.
- Alp C, Özsoy S, Alp NA, et al. Sulfapyridine-like benzenesulfonamide derivatives as inhibitors of carbonic anhydrase isoenzymes I, II and VI. *J Enzyme Inhib Med Chem* 2012;27:818–24.
- Koz O, Ekinçi D, Perrone A, et al. Analysis of saponins and phenolic compounds as inhibitors of α -carbonic anhydrase isoenzymes. *J Enzyme Inhib Med Chem* 2013;28:412–17.
- Avram S, Milac AL, Carta F, Supuran CT. More effective dithiocarbamate derivatives inhibiting carbonic anhydrases, generated by QSAR and computational design. *J Enzyme Inhib Med Chem* 2013;28:350–9.
- Singh S, Supuran CT. 3D-QSAR CoMFA studies on sulfonamide inhibitors of the Rv3588c β -carbonic anhydrase from *Mycobacterium tuberculosis* and design of not yet synthesized new molecules. *J Enzyme Inhib Med Chem* 2014;29:449–55.
- Maresca A, Vullo D, Scozzafava A, Supuran CT. Inhibition of the alpha- and beta- carbonic anhydrases from the gastric pathogen *Helicobacter pylori* with anions. *J Enzyme Inhib Med Chem* 2013;28:388–91.
- Maresca A, Scozzafava A, Vullo D, Supuran CT. Dihalogenated sulfanilamides and benzolamides are effective inhibitors of the three β -class carbonic anhydrases from *Mycobacterium tuberculosis*. *J Enzyme Inhib Med Chem* 2013;28:384–7.
- Maresca A, Carta F, Vullo D, Supuran CT. Dithiocarbamates strongly inhibit the beta-class carbonic anhydrases from *Mycobacterium tuberculosis*. *J Enzyme Inhib Med Chem* 2013;28:407–11.
- Allouche F, Chabchoub F, Carta F, Supuran CT. Synthesis of aminocyanopyrazoles via a multi-component reaction and anti-carbonic anhydrase inhibitory activity of their sulfamide derivatives against cytosolic and transmembrane isoforms. *J Enzyme Inhib Med Chem* 2013;28:343–9.
- Capasso C, Supuran CT. Antiinfective carbonic anhydrase inhibitors: a patent and literature review. *Expert Opin Ther Pat* 2013;23:693–704.
- Del Prete S, De Luca V, Scozzafava A, et al. Biochemical properties of a new α -carbonic anhydrase from the human pathogenic bacterium *Vibrio cholerae*. *J Enzyme Inhib Med Chem* 2014;29:23–7.
- Supuran CT. Carbonic anhydrase inhibitors: an editorial. *Expert Opin Ther Pat* 2013;23:677–9.
- Bilginer S, Unluer E, Gul HI, et al. Carbonic anhydrase inhibitors. Phenols incorporating 2- or 3-pyridyl- ethenylcarbonyl and tertiary amine moieties strongly inhibit *Saccharomyces cerevisiae* β -carbonic anhydrase. *J Enzyme Inhib Med Chem* 2014;29:495–9.
- Capasso C, Supuran CT. Sulfa and trimethoprim-like drugs-antimetabolites acting as carbonic anhydrase, dihydropteroate synthase and dihydrofolate reductase inhibitors. *J Enzyme Inhib Med Chem* 2014;29:379–87.
- Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. *Nat Rev Drug Disc* 2008;7:168–81.
- Supuran CT. Carbonic anhydrases: from biomedical applications of the inhibitors and activators to biotechnologic use for CO₂ capture. *J Enzyme Inhib Med Chem* 2013;28:229–30.