Supporting Information

Benzoxaboroles as efficient inhibitors of the β-carbonic anhydrases from pathogenic fungi: activity and modelling study.

Alessio Nocentini, Roberta Cadoni, Sonia del Prete, Clemente Capasso, Pascal Dumy, Paola Gratteri, Claudiu T. Supuran, and Jean-Yves Winum

Table of contents

CA inhibition assay

An applied photophysics stopped-flow instrument has been used for assaying the CA catalysed CO_2 hydration activity.[1] Bromothymol blue (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 10–20 mM TRIS (pH 8.3) as buffer, and 20 mM $NaBF_4$ for maintaining constant the ionic strength, following the initial rates of the CA-catalysed 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 uncatalysed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (10 mM) were prepared in distilled-deionized water and dilutions up to 0.01 μM 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 the Cheng–Prusoff equation whereas the kinetic parameters for the uninhibited enzymes from Lineweaver–Burk plots, as reported earlier,[2,3] and represent the mean from at least three different determinations. All CAs were recombinant proteins obtained as reported earlier by these groups. [4, 5,6, 8]

Molecular modelling

The dimeric form of the homology built model of MgCA[7] and CAN2 (PDB 2W3N)[8] crystal structures were prepared for docking using the Schrodinger preparation wizard protocol. It consists of preliminary pre-treatment by adjusting the bond orders, metal ions and cofactors, evaluating the ionization states, adding hydrogen atoms, refining loop region and energy minimization.[9a-d]
3D ligand structures were prepared by Maestro MM[9a] and their atomic electrostatic charges were computed with Jaguar MM[9e] fitting them to an electrostatic potential calculated at the B3LYP/6-31G*+ level of theory. ESP atomic charges were used in docking simulations.
Grids for docking analysis were centered in the centroid of the catalytic cavity residues. Docking studies were carried out with the program Glide.[9f] The standard precision (SP) mode of the GlideScore function was applied to evaluate the predicted binding poses.
The pictures were generated with Maestro.

References

(1) Khalifah, R.G. 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.
(2) (a) Sahin, A.; Isık, S.; Arslan, O.; et al. A new affinity gel for the purification of α-carbonic anhdrases. *J. Enzyme Inhib. Med. Chem.* **2015**, *30*, 224–8; (b) De Luca, V.; Del Prete, S.; Supuran, CT.; et al. Protonography, a new technique for the analysis of carbonic anhydrase activity. *J. Enzyme Inhib. Med. Chem.* **2015**, *30*, 277–82; (c) Del Prete, S.; Vullo, D.; De Luca, V.; et al. Biochemical characterization of recombinant β-carbonic anhydrase (PgiCAb) identified in the genome of the oral pathogenic bacterium *Porphyromonas gingivalis*. *J Enzyme Inhib Med Chem* **2015**, *30*, 366–70, (d) Eldehna, W.M.; Al-Ansary, G.H.; Bua, S.; et al. Novel indolin-2-one-based sulfonamides as carbonic anhydrase inhibitors: synthesis, in vitro biological evaluation against carbonic anhydrases isoforms I, II, IV and VII and molecular docking studies. *Eur J. Med. Chem.* **2017**, *127*, 521–30.
(3) (a) Migliardini, F. ; De Luca, V. ; Carginale, V. ; et al. Biomimetic CO_2 capture using a highly thermostable bacterial α-carbonic anhydrase immobilized on a polyurethane foam. *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 146–50; (b) Nocentini, A.; Ferraroni, M.; Carta, F.; et al. Benzenesulfonamides incorporating flexible triazole moieties are highly effective carbonic anhydrase inhibitors: synthesis and kinetic, crystallographic, computational, and intraocular pressure lowering investigations. *J. Med. Chem.* **2016**, *59*, 10692–704, (c) Abdel-Aziz, A.A.; El-Azab, A.S.; Ekinci, D.; et al. Investigation of arenesulfonyl-2-imidazolidinones as potent carbonic anhydrase inhibitors. *J. Enzyme Inhib. Med. Chem.* **2015**, *30*, 81–4; (d) Akdemir, A.; De Monte, C.; Carradori, S.; Supuran, C.T. Computational investigation of the selectivity of salen and tetrahydrosalen compounds towards the tumor-associated hCA XII isozyme. *J. Enzyme Inhib. Med. Chem.* **2015**, *30*, 114–18.
(4) Del Prete, S.; De Luca, V.; Vullo, D.; et al. A new procedure for the cloning, expression and purification of the β-carbonic anhydrase from the pathogenic yeast *Malassezia globosa*, an anti-dandruff drug target. *J. Enzyme Inhib. Med. Chem.* **2016**, *31*, 1156–61.
(5) Supuran, C. T.; Mincione, F.; Scozzafava, A.; Briganti, F.; Mincione, G.; Ilies, M. A. Carbonic anhydrase inhibitors. Part 52. Metal complexes of heterocyclic sulfonamides: a new class of strong topical intraocular pressure-lowering agents in rabbits. *Eur. J. Med. Chem.* **1998**, *33*, 247-254.

(6) Vullo, D.; Leewattanapasuk, W.; Mühlschlegel, F.A.; Mastrolorenzo, A.; Capasso, C.; Supuran, C.T. Carbonic anhydrase inhibitors: inhibition of the β-class enzyme from the pathogenic yeast *Candida glabrata* with sulfonamides, sulfamates and sulfamides. Bioorg. Med. Chem. Lett. **2013**, *23*, 2647-52.
(7) Vullo, D.; Del Prete, S.; Nocentini, A.; Osman, S.; AlOthman, Z.; Capasso, C.; Bozdag, M.; Carta, F.; Gratteri, P.; Supuran, C. T. Dithiocarbamates effectively inhibit the β-carbonic anhydrase from the dandruff-producing fungus *Malassezia globosa*. *Bioorg. Med. Chem.* **2017**, *25*, 1260–1265.
(8) Schlicker, C.; Hall, R. A.; Vullo, D.; Middelhaufe, S.; Gertz, M.; Supuran, C. T.; Muehlschlegel, F. A.; Steegborn, C. Structure and Inhibition of the CO_2-Sensing Carbonic Anhydrase Can2 from the Pathogenic Fungus *Cryptococcus neoformans. J. Mol. Biol.* **2009**, *385*, 1207-1220.
(9) Schrödinger Suite Release 2016-1, Schrödinger, LLC, New York, NY, 2016: (a) Maestro v.10.5; (b) Epik, v.3.5; (c) Impact, v.7.0; (d) Prime, v.4.3; (e) Jaguar, v.9.1; (f) Glide, v.7.0.