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Bortezomib inhibits bacterial and fungal β-carbonic anhydrases

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ABSTRACT

Inhibition of the β-carbonic anhydrases (CAs, EC 4.2.1.1) from pathogenic fungi (*Cryptococcus neoformans*, *Candida albicans*, *Candida glabrata*, *Malassezia globosa*) and bacteria (three isoforms from *Mycobacterium tuberculosis*, Rv3273, Rv1284 and Rv3588), as well from the insect *Drosophila melanogaster* (DmeCA) and the plant *Flaveria bidentis* (FbiCA1) with the boronic acid peptidomimetic proteosome inhibitor bortezomib was investigated. Bortezomib was a micromolar inhibitor of all these enzymes, with K_Is ranging between 1.12 and 11.30 μM. Based on recent crystallographic data it is hypothesized that the $B(OH)_2$ moiety of the inhibitor is directly coordinated to the zinc ion from the enzyme active site. The class of boronic acids, an under-investigated type of CA inhibitors, may lead to the development of anti-infectives with a novel mechanism of action, based on the pathogenic organisms CA inhibition.

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1. Introduction

The β-carbonic anhydrase (CAs, EC 4.2.1.1) family is widespread in bacteria, archaea, fungi, protozoa, plants and in the mitochondria of arthropods such as the insect *Drosophila melanogaster*.[1–5] In these and other organisms these enzymes are crucial for a large number of physiologic processes such as pH regulation, provision of bicarbonate/CO_2 for carboxylating reactions, several biosynthetic pathways, and photosynthesis among others.[1–5] Recently, the inhibition of β-CAs from pathogenic organisms was proposed as a new strategy to design anti-infectives devoid of the resistance problems of all currently used antibiotics, antifungals and antiprotozoan agents.[6–10]

Indeed, many fungal pathogens encode for such enzymes which were recently investigated for their druggability and in some cases, whether their inhibition interferes with the life cycle of the pathogen.[1–5] The enzymes from *Cryptococcus neoformans* (Can2), *Candida albicans* (CalCA), *Candida glabrata* (CglCA) and *Malassezia globosa* (MgCA) were investigated for such purposes, being shown that their inhibition with sulfonamides,[3,4] aromatic/aliphatic carboxylates[4b,e] or inorganic anions[4c] has antifungal effects.[6–10] As the β-CA genetic family is not present in mammals (which encode for α-CAs),[1a] its inhibition may provide an innovative way of designing antifungals with less toxicity and a novel mechanism of action.[4,5]

The β-CA class is also widespread in bacteria,[6a] with many new enzymes cloned, purified and characterized in pathogenic species such as *Helicobacter pylori*, *Escherichia coli*, *Mycobacterium tuberculosis*, *Brucella* spp., *Streptococcus pneumoniae*, *Salmonella enterica*, *Haemophilus influenzae*, *Legionella pneumophila*, *Vibrio cholerae*, *Porphyromonas gingivalis*, *Streptococcus mutans*, *Clostridium perfringens*, *Pseudomonas aeruginosa*, etc.[11–20] Many of these enzymes are highly inhibited by several classes of CA inhibitors (CAIs) such as the sulfonamides and their isosteres,[11–20] the dithio- and monothiocarbamates[21,22] and the inorganic anions.[23,24] For *H. pylori*, *Brucella suis* and *S. pneumoniae* enzymes it has been possible to evidence inhibition of bacterial growth in vivo when some of these inhibitors were present in the culture medium.[9] Considering such preliminary but encouraging results, bacterial CAs represent promising targets for obtaining anti-bacterials devoid of the resistance problems to the clinically used antibiotics, but few studies are presently available in this field. However one of the main issues regards the fact that the potent such inhibitors detected so far, which are constituted mainly by the primary sulfonamides and their isosteres, also potently inhibit human (h) CAs (hCAs), with the potential to lead to severe side effects. Thus, the exploration of different CA inhibitory chemotypes[25] is warranted in order to identify possible inhibitors with good activity and selectivity for the inhibition of β- over α-CAs. The boronic acids (and their derivatives) may represent such novel chemotypes, which were for the moment rarely considered for their interaction with these enzymes, apart some studies reported by our group on aromatic boronic acids of the type $ArB(OH)_2$.[26]

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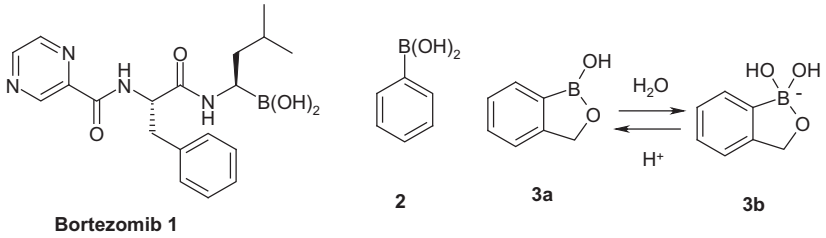

The peptidomimetic boronic acid bortezomib **1** is a clinically used 26S proteosome inhibitor for the treatment of haematological malignancies, such as multiple myeloma and mantle cell lymphoma, also being in clinical trials alone or in combination therapy for the management of other tumors.[27–29] Its mechanism of action involves binding to the proteosome, which leads to interferences with cellular homeostasis mechanisms by blocking regulatory proteins degradation, many of which are involved in tumor growth and progression (e.g., cyclins, cyclin-dependent kinase inhibitors, tumor suppressors, and NF-κB inhibitors among others).[27,28] Bortezomib reacts with an OH moiety of Thr or Ser residues from various subunits of the proteosome, inactivating it.[29] On the other hand, as mentioned above, we have investigated the interaction of boronic acids (such as the aryl derivative $PhB(OH)_2$ **2** and some of its congeners),[26] with CAs belonging to the α- and β-CA classes, proposing as inhibition mechanism the hypothetic binding showed in Figure 1, which involves coordination of the boronic acid moiety to the zinc ion.

This hypothesis was criticized by some colleagues, but recently we were able to prove its correctedness by the report of the X-ray crystal structure of the adduct of benzoxaborole **3a** bound to human isoform hCA II, in which the boron was found indeed in a tetrahedral geometry (compound **3b** was generated, probably through the nucleophilic attack of the CA active site zinc hydroxide to **3a**), with one of the oxygen atoms directly coordinated to the zinc ion from the CA active site, as proposed in Figure 1A.[30]

These encouraging results prompted us to investigate whether the clinically used, aliphatic, peptidomimetic boronic acid **1**, may also act as an inhibitor of β-CAs. Here we report a study of β-CA inhibition with bortezomib, including in it several pathogenic fungal and bacterial enzymes, as well as one insect and one plant such enzymes.

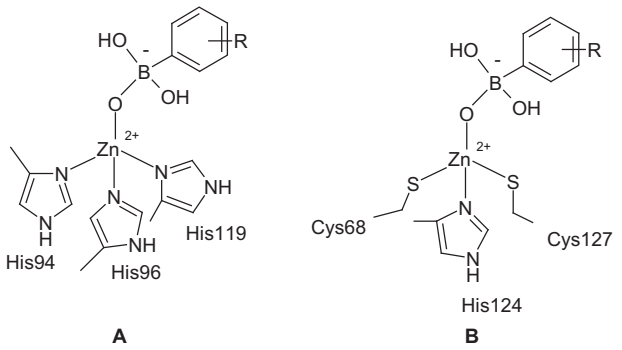

Figure 1. Proposed binding of aromatic boronic acids to α-(A) and β-CAs (B). The α-class enzymes have the Zn(II) ion coordinated by three His residues (hCA I numbering system),[1a] the fourth ligand being a water molecule/hydroxide ion, which being a strong nucleophile may react with the electrophilic boronic acid leading to the adduct A. In β-CAs, the Zn(II) is coordinated by one His and two Cys residues (Can2 numbering system used in B),[4b] and the fourth ligand is similar to the α-CAs one, leading thus to a similar tetrahedral inhibited species of the enzyme, depicted schematically in B.

2. Results and discussion

2.1. Chemistry

Apart boronic acid **2**, few of its congeners substituted in the *para* position with alkyl, methoxy, halogens and aryl moieties were investigated as β-CA inhibitors against the enzymes from *Cryptococcus neoformans* (Can2) and *Candida albicans*, (CalCA) in our previous wor.[26b] We reported that although **2** itself was a quite weak inhibitor against both enzymes (K_Is >100 μM), several of the *para*-substituted derivatives acted as low micromolar inhibitors against the two enzymes. However, as stressed above, no aliphatic boronic acids were investigated so far for the inhibitory effects of β-CAs. Bortezomib **1** is a clinically used, commercially available compound that was thus included in this study.

2.2. CA inhibition

The following enzymes were included in this study: the human isoforms hCA I and hCA II (belonging to the α-class, which are the main off-targets when considering CAIs as anti-infective agents,[5–10] since they are the physiologically dominant, widespread host enzymes), as well as a collection of β-CAs cloned and investigated earlier in our and other laboratories, among which: CalCA (from *C. albicans*);[2a,2b] CglCA (from *C. glabrata*);[2d] Can2 (from *C. neoformans*);[4b] MgCA (from *M. globosa*);[3e] Rv3273, Rv1284 and Rv3588 (from *M. tuberculosis*);[13,20] DmeCA (from the insect *Drosophila melanogaster*),[5e] and FbiCA1 (from the C4 plant *Flaveria bidentis*).[5f] We included in our study enzymes from well-known pathogenic fungi and bacteria, but also the two insect and plant β-CAs in order to understand whether there may be differences in the behavior of the inhibitors against enzymes originating from so different branches of the phylogenetic tree (all the β-CAs investigated here have a similar Zn(II) coordination, with two Cys, one His and a water molecule bound to the catalytically crucial metal ion).[5]

Table 1
CA inhibition data against human isoforms hCA I and II, as well as β-CAs from fungi, bacteria, insects and plants with boronic acids **1**, **2**, and acetazolamide **AAZ** as standard inhibitor, by a CO_2 hydrase stopped-flow assay[31]

Isoform	K_I (μM) *		
	1	**2**	**AAZ**
hCA I	1.29	>100	0.25
hCA II	1.16	>100	0.012
CalCA	1.12	>100	0.132
CglCA	5.73	100	0.011
Can2	4.70	>100	0.010
MgCA	3.24	89	74
Rv3273	6.50	67	0.104
Rv1284	7.30	58	0.480
Rv3588	4.20	48	0.010
DmeCA	11.30	>100	0.049
FbiCA1	8.45	8.00	0.027

* Mean from three different determinations. Errors were in the range of ±10% of the reported data.

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