

# Carbonic anhydrase inhibitors. Synthesis and inhibition of cytosolic/tumor-associated carbonic anhydrase isozymes I, II, and IX with boron-containing sulfonamides, sulfamides, and sulfamates: Toward agents for boron neutron capture therapy of hypoxic tumors

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Received 7 March 2005; revised 31 March 2005; accepted 12 April 2005

Available online 23 May 2005

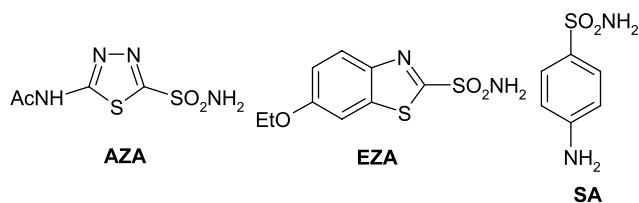
**Abstract**—A library of boron-containing carbonic anhydrase (CA, EC 4.2.1.1) inhibitors, including sulfonamides, sulfamides, and sulfamates is reported. The new compounds have been synthesized by derivatization reactions of 4-carboxy-/amino-/hydroxy-phenylboronic acid pinacol esters with amino/isothiocyanato-substituted aromatic/heteroaromatic sulfonamides or by sulfamoylation reactions with sulfamoyl chloride. The new derivatives have been assayed for the inhibition of three physiologically relevant CA isozymes, the cytosolic CA I and II, and the transmembrane, tumor-associated isozyme CA IX. Effective inhibitors were detected both among sulfonamides, sulfamates, and sulfamides. Against the human isozyme hCA I the new compounds showed inhibition constants in the range of 34–94 nM, against hCA II in the range of 3.1–48 nM, and against hCA IX in the range of 7.3–89 nM, respectively. As hypoxic tumors highly overexpress CA IX, the design of boron-containing inhibitors with high affinity for the tumor-associated CA isozymes may lead to important advances in boron neutron capture therapy (BNCT) applications targeting such tumors, which are non-responsive to both classical chemo- and radiotherapy.

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## 1. Introduction

The carbonic anhydrases (CAs, EC 4.2.1.1) are widespread metalloenzymes, present in mammals in a multitude (at least 15) of isoforms, and catalyze the interconversion between carbon dioxide and bicarbonate at the physiological pH (a proton is also formed in this reaction).<sup>1,2</sup> Some of these isozymes are cytosolic (CA I, CA II, CA III, CA VII, and CA XIII), others are membrane-bound (CA IV, CA IX, CA XII, and CA XIV), CA VA, and CA VB are present in mitochondria

throughout the body, whereas CA VI is secreted in the saliva and milk.<sup>1–3</sup> Three cytosolic acatalytic forms are also known (CARP VIII, CARP X, and CARP XI).<sup>1</sup> The catalytically active isoforms, which play important physiological and patho-physiological functions in all tissues/organs in which the three chemical species mentioned above are present (i.e., CO<sub>2</sub>, bicarbonate, and



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H<sup>+</sup> ions), are also strongly inhibited by aromatic and heterocyclic sulfonamides, sulfamates, sulfamides, and some of their derivatives.<sup>3–7</sup> The catalytic and inhibition mechanisms of these enzymes are understood in great detail, and this was helpful for the design of potent inhibitors, some of which possess clinical applications for the treatment or prevention of a multitude of diseases.<sup>1–3</sup>

Recently, the involvement of some CAs and their sulfonamide inhibitors in cancer has been investigated: many potent CA inhibitors (CAIs) derived from acetazolamide **AZA**, ethoxzolamide **EZA**, and sulfanilamide (**SA**) as lead molecules among others, were shown to inhibit the growth of several tumor cell lines in vitro and in vivo, constituting thus interesting candidates for developing novel antitumor therapies.<sup>8–14</sup> Indeed, Svastova et al.<sup>15</sup> showed that the acidic extracellular pH, which is a typical attribute of the tumor microenvironment, is generated by the activity of one of the tumor-associated CA isozymes, that is, CA IX, and that this acidification can be perturbed by deletion of the enzyme active site and inhibited by CA IX-selective inhibitors of the sulfonamide type (some thioureido- or pyridinium derivatives of **SA** among others have been employed in such experiments),<sup>7,16</sup> which bind only to hypoxic cells containing CA IX. The involvement of the other tumor-associated isozyme, that is, CA XII, in such processes has not been investigated in detail for the moment. Thus, it appears of critical importance to continue the development of CAIs targeting the tumor-associated CA isozymes CA IX and XII, eventually belonging to novel classes of compounds, less investigated up to now.<sup>7,8</sup> This may lead to novel possibilities for the management (imaging and treatment) of hypoxic tumors in which these enzymes are highly overexpressed.<sup>17–19</sup>

Boron neutron capture therapy (BNCT) is based on the preferential targeting of tumor cells with <sup>10</sup>B-containing compounds and subsequent activation with thermal neutrons to produce a highly localized radiation.<sup>20,21</sup> This therapy relies on a binary process in which the capture of a slow neutron by the <sup>10</sup>B nucleus leads to an energetic nuclear fission reaction, with the formation of one <sup>7</sup>Li nucleus and one <sup>4</sup>He<sup>2+</sup> nucleus, accompanied by about 2.4 MeV of energy. The fleeting of these two nuclei travel a distance of only about the diameter of one cell, being deadly to any cell in which they have been produced.<sup>22–24</sup> Thus, provided that a boron-containing drug is being delivered preferentially to the tumor cells, there is a real possibility for the design of highly innovative techniques for the management of tumors. Hypoxic tumors highly overexpress CA IX<sup>19,25</sup> (and to some extent also CA XII),<sup>14,15</sup> isozymes showing high affinity for sulfonamide/sulfamate CA inhibitors.<sup>1–8</sup> As a consequence, the design of boron-containing CAIs with high affinity for the tumor-associated isozymes IX and XII, and eventually a lower affinity for other CA isozymes (such as the ubiquitous CA I and II) may lead to important advances in BNCT targeting hypoxic tumors, which are non-responsive to both classical chemo- and radiotherapy.<sup>15,18</sup>

## 2. Chemistry

Since it has previously been shown, mainly by this group that potent hCA I, II, and IX inhibitors can be designed possessing various zinc-binding groups, such as the sulfonamide, sulfamide, or sulfamate one,<sup>1–4,6–8</sup> we here report all these three types of derivatives incorporating boron-containing moieties, which, as stressed above, may be useful for BNCT applications of these compounds. The chemistry used for the design of these inhibitors is illustrated in Scheme 1.

A first group of derivatives were obtained from the pinacol ester of 4-carboxy-phenylboronic acid, which were treated with aromatic or heteroaromatic amino-sulfonamides in the presence of BOP, leading to carboxamides of type **1a–c** and **2**. It has been previously shown that acylated such sulfanilamide, homosulfanilamide, 4-aminoethylbenzenesulfonamide, or 5-amino-1,3,4-thiadiazole-2-sulfonamide derivatives show enhanced hCA I, II, IV, and V inhibitory properties over the free amino sulfonamides from which they were obtained,<sup>26,27</sup> and this constitutes one of the rationales of performing this type of coupling reaction.

Sulfamoylation of the pinacol ester of 4-amino- or 4-hydroxy-phenylboronic acid with sulfamoyl chloride afforded the sulfamide derivative **3** and the corresponding sulfamate **4**, respectively, by the procedure already applied for the preparation of both aliphatic/aromatic sulfamide/sulfamate CAIs (Scheme 1).<sup>5,12,13</sup>

Finally, a third type of chemistry used for the preparation of some boron-containing CAIs, takes advantage of the coupling reactions between isothiocyanato-substituted sulfonamides and the pinacol ester of 4-amino-phenylboronic acid (Scheme 1). We have previously shown that this type of thioureido-containing sulfonamides show excellent CA I, II, IV, and IX inhibitory properties, both in the aromatic and heteroaromatic sulfonamides series.<sup>16,28,29</sup> In such work, we have investigated the reactions of isothiocyanato sulfonamides with diverse amino acids, some of their derivatives as well as simple amines, mainly in the search of water-soluble sulfonamides with applications as antiglaucoma drugs.<sup>28,29</sup> However, this type of chemistry is quite versatile and may be applied also for the design of inhibitors with applications in BNCT. Thus, reaction of the pinacol ester of 4-amino-phenylboronic acid with 4-isothiocyanato-sulfanilamide, its 3-halogenated derivatives, the isomeric metanilamide isothiocyanate as well as the sulfanilyl-sulfanilamide isothiocyanate or aminobenzolamide isothiocyanate, afforded the small library of thiureas **5a–h** (Scheme 1).<sup>30</sup>

## 3. CA inhibition data

Inhibition data against isozymes hCA I, II, and IX with compounds **1–5** as well as standard CA inhibitors are presented in Table 1.<sup>31</sup>

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