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# Sulfonamides incorporating boroxazolidone moieties are potent inhibitors of the transmembrane, tumor-associated carbonic anhydrase isoforms IX and XII

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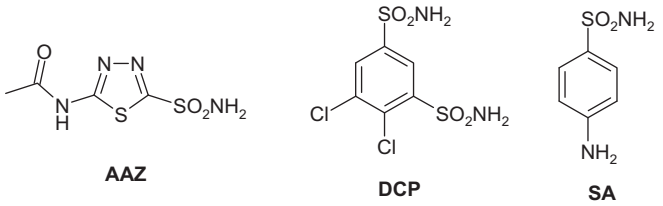
ABSTRACT

A new series of sulfonamides was synthesized by the reaction of the boroxazolidone complex of L-lysine with isothiocyanates incorporating sulfamoyl moieties and diverse organic scaffolds. The obtained thioureas have been investigated as inhibitors of four physiologically relevant human carbonic anhydrase (hCA, EC 4.2.1.1) isoforms, hCA I, II, IX and XII. Inhibition between the low nanomolar to the micromolar range has been observed against them, with several low nanomolar and tumor-CA selective inhibitors detected. These boron-containing compounds might be useful for the management of hypoxic tumors overexpressing hCA IX/XII by means of boron neutron capture therapy, a technique not investigated so far with inhibitors of this enzyme.



Carbonic anhydrase (CA, EC 4.2.1.1) isoforms IX (CA IX) and XII (CA XII) have recently been shown to be druggable targets for imaging and treatment of hypoxic tumors.[1–7] CA IX is one of the most strongly overexpressed genes in response to hypoxia in a high number of human cancer cells.[2–4] This enzyme is a multidomain protein[1,6] with the CA subdomain situated outside the cell and possessing a very high $CO_2$ hydrase catalytic activity.[1,6,7] This makes CA IX a key player in the regulation of the tumor pH.[1–7] CA IX expression is strongly increased in many types of solid tumors, such as gliomas/ependymomas, mesotheliomas, papillary/follicular carcinomas, as well as carcinomas of the bladder, uterine cervix, kidneys, esophagus, lungs, head and neck, breast, brain, vulva, and squamous/basal cell carcinomas, among others.[8,9] Furthermore, such hypoxic tumors do not generally respond to the classic chemo- and radiotherapy, and the strong acidification produced by CA IX overexpression also triggers the development of metastases.[8,9] Another isoform which is sometimes associated with cancers is CA XII, which similar to CA IX is a transmembrane enzyme with an extracellular active site and good activity for the hydration of $CO_2$ to bicarbonate and protons.[10] Recently, it has been shown by several groups[1–3] that the genetic silencing[2b] or pharmacologic inhibition[1,2a,c,d,11] of CA IX and XII have a strong anticancer effect, with growth delay of both the primary tumor and the metastases.[11]




Sulfonamides represent one of the classical chemotypes associated with potent CA inhibition.[1,12–15] They bind in deprotonated form to the Zn(II) ion from the enzyme active site, and also make a wide range of polar, hydrophobic and/or stacking interactions with amino acid residues from the enzyme cavity, leading to highly stable enzyme-inhibitor adducts.[1,12–15] Indeed, many aromatic, heterocyclic, aliphatic and sugar sulfonamides show activities in the range of the low micromolar to the low nanomolar for

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inhibiting various CA isoforms, among which also CA IX and XII.[1,12–15] However, the mammalian CA family comprises 16 isoforms, 13 of which are catalytically active and most of which are indiscriminately inhibited by classical sulfonamide CA inhibitors (CAIs) such as acetazolamide **AAZ**, dichlorophenamide **DCP** or sulfanilamide **SA**.[1,12–15] These and other sulfonamides are in clinical use as diuretics, antiglaucoma, anticonvulsant or antiinfective drugs for more than 50 years.[1] The strong correlations between CA IX/XII and tumors, and the possibility to inhibit these enzymes by sulfonamides and other CAIs, envisages the use of specific CA IX/XII inhibitors as antitumor drugs and diagnostic agents for hypoxic tumors, a rather new and unexpected application for CAIs.[1–4] Thus, there is a stringent need to design specific CAIs for the tumor-associated isoforms CA IX and XII, which should possess decreased affinity for the main offtarget CA isoforms (CA I and II, which are rather abundant in many tissues and participate in important physiological processes).[1,12–15]

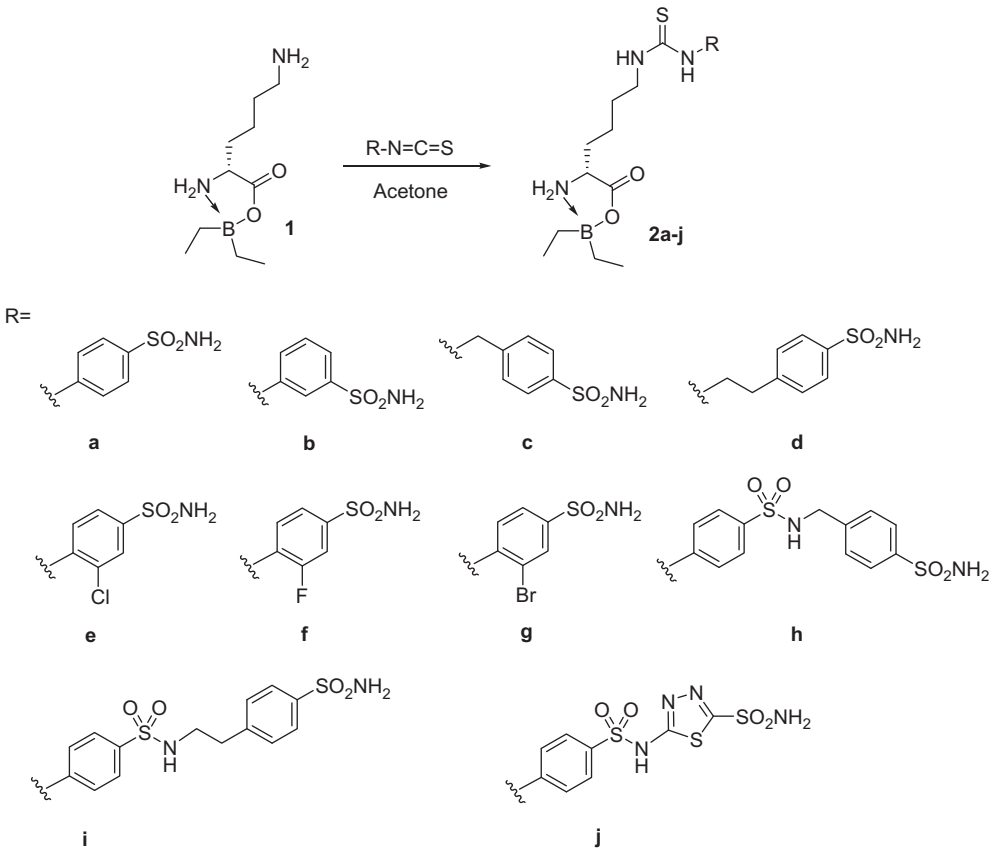
Continuing our interest in the design of various classes of CAIs, we report here the synthesis and evaluation as enzyme inhibitors of a new class of boron-containing compounds,[16] obtained starting from the boroxazolidone complex of L-lysine **1** (Scheme 1).[17]

The key intermediate **1** has only the ε-amino group enough reactive to be derivatized with isothiocyanates incorporating sulfamoyl moieties of the type RNCS, leading to thioureas of types **2a–2j** which have been investigated earlier as CAIs. Indeed, many compounds incorporating a thiourea linker between the aromatic benzenesulfonamide moiety and the tail have been reported by our groups to possess potent CA inhibitory effects against several physiologically relevant isoforms, such as CA II, IV, IX and XII among others.[18] Furthermore, the X-ray crystal structure of one such thioureido sulfonamide in complex with CA II has been reported,[19] giving interesting hints for the design of CAIs incorporating this functionality. We were interested to design CAIs incorporating boron-containing moieties, such as derivatives **2a–2j** reported here, due to their potential use as agents for boron neutron capture therapy of hypoxic tumors, a field not explored up until now in detail.[20] The new derivatives incorporate various aromatic (3-amino- and 4-aminobenzenesulfonamide derivatives) and heterocyclic (1,3,4-thiadizole-2-sulfonamide) sulfonamide heads and linkers of diverse nature/length, in order to generate chemical diversity and obtain a complete structure–activity relationship (SAR) for the inhibition of CAs. Compounds **2a–2j** have been characterized in detail by physico-chemical methods which confirmed their structures.[16]

Compounds **2a–2j** reported here and standard CAIs of the sulfonamide type were screened for the inhibition of the tumor-associated isoforms hCA IX and XII (h = human isoform) as well as the offtarget, cytosolic hCA I and II (Table 1).[21]

The following SAR has been observed for the inhibition of these four CA isozymes with sulfonamides **2a–2j**:

(i) Activities from the low nanomolar to the micromolar have been observed for the inhibition of the slow cytosolic isoform hCA I with derivatives **2a–2j**. The inhibition constants of these compounds were in the range of 3.2–6390 nM (Table 1). Derivatives **2h–2**i were very potent, low nanomolar hCA I inhibitors ($K_I$s of 3.2–6.8 nM). They incorporate a longer scaffold compared to other sulfonamides investigated here, which was shown[18] to be associated with very potent CA inhibitory activity against many isoforms, this fact being confirmed also for the boroxazolidone-containing derivatives investigated in the present paper. The sulfanilamide,




**Scheme 1.** Synthesis of the sulfonamides incorporating boroxazolidone moieties of type **2a–2j**.