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A thiabendazole sulfonamide shows potent inhibitory activity against mammalian and nematode α-carbonic anhydrases ☆

Letizia Crocetti [a], Alfonso Maresca [a], Claudia Temperini [a], Rebecca A. Hall [b], Andrea Scozzafava [a], Fritz A. Mühlschlegel [b], Claudiu T. Supuran [a,*]

[a] Università degli Studi di Firenze, Laboratorio di Chimica Bioinorganica, Rm. 188, Via della Lastruccia 3, I-50019 Sesto Fiorentino (Firenze), Italy
[b] Department of Biosciences, University of Kent, Canterbury CT2 7NJ, UK

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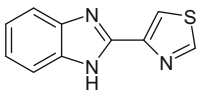
ABSTRACT

A sulfonamide derivative of the antihelmintic drug thiabendazole was prepared and investigated for inhibition of the zinc enzyme carbonic anhydrase CA (EC 4.2.1.1). Mammalian isoforms CA I–XIV and the nematode enzyme of *Caenorhabditis elegans* CAH-4b were included in this study. Thiabendazole-5-sulfonamide was a very effective inhibitor of CAH-4b and CA IX (K_Is of 6.4–9.5 nm) and also inhibited effectively isozymes CA I, II, IV–VII, and XII, with K_Is in the range of 17.8–73.2 nM. The high resolution X-ray crystal structure of its adduct with isozyme II evidenced the structural elements responsible for this potent inhibitory activity.

Thiabendazole **1** is a broad-spectrum antihelmintic used for the treatment of parasitic infections in animals and humans and as an agricultural fungicide for postharvest treatment of fruits and vegetables, as it inhibits the growth of many pathogenic and saprobic fungi, such as among others *Aspergillus* spp., *Cladosporium cladosporioides, Cunninghamella echinulata, Fusarium roseum, Gliocladium* sp., *Mortierella isabellina, Mucor plumbeus, Rhizopus arrhizus*, and *Trichothecium roseum*.[1,2] The mechanism of action of this drug is poorly understood but at least its antihelmintic actions seems to be due to its selective binding to the parasite β-tubulin with subsequent prevention of microtubule formation.[3]

Helminths and other nematodes infect 25% of the world's population.[4] In addition to the widespread parasitic nematode species *Onchocerca volvulus*, *Wuchereria bancrofti*, and *Brugia malayi*, for which few effective therapies are presently known,[5] *Mansonella perstans* filariasis is widely present in Africa and equatorial America,[5a] *Strongyloides stercoralis*, an intestinal nematode acquired in the tropics or subtropics, is emerging as a widespread infection in the Western countries.[5b] Although there are effective drugs for some of these infections (thiabendazole is effective against larva migrans, mebendazole for ascariasis, trichiuriasis and hookworms, albendazole for inoperable cases of cystic hydatid disease, diethylcarbamazine for *Toxocara* induced visceral larva migrans and loiasis, ivermectin for onchocerciasis, praziquantel for schistosomiasis and niridazole for *Dracunculus medinensis*)[4,5] the widespread resistance to many of them,[6] both in humans and animals, may lead to



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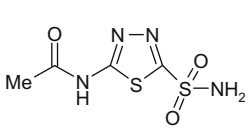
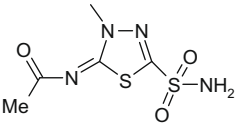
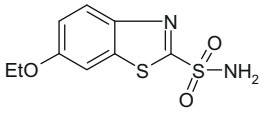


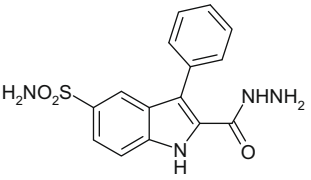
AZA



MZA



EZA



GUZ

☆ The X-ray coordinates of the hCA II–thiabendazole-5-sulfonamide adduct are available in PDB with the ID 3FFP.

* Corresponding author. Tel.: +39 055 457 3005; fax: +39 055 4573385.
E-mail address: claudiu.supuran@unifi.it (C.T. Supuran).

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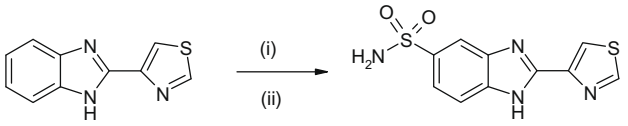
serious medical problems. It is thus critically important to design agents targeting other metabolic pathways in these organisms, which may circumvent the resistance/toxicity problems of the currently used antihelmintics. One of the enzymes present in many worm species is the carbonic anhydrase, CA (EC 4.2.1.1).[5c] Indeed, CAs are widespread all over the phylogenetic tree, with several different evolutionarily unrelated gene families encoding them.[7–10] CAs are catalysts for the interconversion of carbon dioxide and bicarbonate and are involved in pH regulation and function in several metabolic pathways.[7–10] In mammals there are 16 CAs known to date. These include several cytosolic isoforms (CA I–III, CA VII, and CA XIII), five membrane-bound isozymes (CA IV, CA IX, CA XII, CA XIV, and CA XV), two mitochondrial forms (CA VA and VB), as well as one secreted CA isozyme, CA VI. Three acatalytic isozymes are also known, that is, CA VIII, CA X, and CA XI.[7–10] In mammals, these enzymes are involved in crucial physiological processes connected with respiration and transport of CO_2/bicarbonate between metabolizing tissues and lungs, pH and CO_2 homeostasis, electrolyte secretion in a variety of tissues/organs, biosynthetic reactions (such as gluconeogenesis, lipogenesis and ureagenesis), bone resorption, calcification, tumorigenicity, and several other physiologic/pathologic processes.[7–13]

Sulfonamide CA inhibitors (CAIs) such as acetazolamide **AZA**, methazolamide **MZA** or ethoxzolamide **EZA** among others, are clinically used agents for the management of a variety of disorders connected to CA disbalances, such as glaucoma[7,8]; in the treatment of edema due to congestive heart failure,[11] or for drug-induced edema; as mountain sickness drugs,[11] whereas other agents of this pharmacological class show applications as anticonvulsants,[12] antiobesity,[7] or antitumor drugs/tumor diagnostic agents.[7,10] As there are few isoforms-selective inhibitors to date, many new sulfonamides (such as **GUZ** exemplified here)[14] are constantly being reported in the search of derivatives with better inhibition profiles as compared to the promiscuous, first generation inhibitors such as **AZA, MZA**, or **EZA**.[7]

There are only a few reports regarding the CAs present in nematodes.[5c] An α-CA, subject to environmental pH regulation denominated CAH-4b (or ceCA) was recently cloned and characterized in the model organism *Caenorhabditis elegans* (which contains at least 6 CA isoforms) by Hall et al.[13] In addition, DeRosa et al. investigated another α-CA isoform homologous to *C. elegans* CAH-6 (78% homology) and hCA III (55% homology) for its involvement in exsheathment of *Ostertagia ostertagi* nematodes, that infect intestines of cattle.[15] Although DeRosa et al. did not directly confirm that this CA isoform was involved in the process of exsheathment, the transcriptional regulation of the enzyme suggests that it may function in developmental processes related to this.[15] Furthermore, the strong α-CA inhibitor ethoxzolamide (**EZA**) is useful as an anti-infective in cattle infected with *Ostertagia ostertagi*.[15] CAH-4b was shown by our group to be susceptible to inhibition by sulfonamides such as **AZA, MZA**, and **EZA**.[13] Thus, considering the fact that nematodes contain CAs in their genome (although scantly investigated to date) and the fact that these enzymes seem to be susceptible to inhibition by sulfonamides, we decided to incorporate the sulfamoyl moiety responsible for the binding to the zinc ion from the CA active site, in the molecule of the antihelmintic compound **1**. The rationale for this drug design study is the following one: (i) we hypothesize that the thiabendazole scaffold (responsible for the antihelmintic biological activity of **1**) of the sulfonamide derivative may bind to β-tubulin similarly with the parent derivative **1**; (ii) the additional sulfonamide group should lead to interactions with CAs present in the parasite. Obviously, we are also interested in this new sulfonamide scaffold for its interaction with mammalian CA isoforms, which as mentioned above, constitute drug targets for many types of applications.



1 2

Scheme 1. Preparation of sulfonamide **2** from thiabendazole **1**. Reagents and conditions: (i) $ClSO_3H$ at 0 °C; (ii) aqueous NH_3, room temperature.

2-(4′-Thiazolyl)-1*H*-benzimidazole (thiabendazole) **1** was treated with chlorosulfonic acid and the obtained sulfonyl chloride with ammonia, leading to the 5-sulfamoyl derivative **2** (Scheme 1).[16] Only one sulfonamide isomer (**2**) has been obtained in good yield, by the sulfamoylation illustrated in Scheme 1, without the need to protect/deprotect the endocyclic NH from the benzimidazole ring. Sulfonamide **2** has been investigated for the inhibition of mammalian CA isozymes hCA I–XIV as well as the nematode CA from *C. elegans*, ceCA (Table 1).[17] Inhibition data of the standard compounds A**ZA, MZA**, and **EZA**,[7] as well as that of the bicyclic sulfonamide **GUZ**[14] are also provided in Table 1, for comparison reasons.[18–22]

The following should be noted regarding the inhibition data of Table 1:

(i) Thiabendazole-5-sulfonamide **2** behaved as a strong inhibitor of isoforms hCA I, IV, VB, VI, IX, XII, and ceCA, with inhibition constants in the range of 6.4–50.9 nM. Especially potent inhibition (K_Is < 10 nM) was observed for hCA IX and the nematode enzyme ceCA. Indeed, **2** is the most potent nematode CA inhibitor evidenced so far,[13] being 3.7-fold more efficient than **AZA**, the compound showing the second most potent inhibition against ceCA, discovered in our first study on this enzyme.[13]

(ii) Isoforms hCA II, hCA VA, and hCA VII were also efficiently inhibited by **2**, with K_I values in the range of 72.5–73.2 nM. These data are indeed very interesting, since usually CA II and VII are the isoforms with the highest affinity for sulfonamide inhibitors such as **AZA, MZA**, and **EZA** (Table 1). In the case of **2**, other isoforms than hCA II and VII are much better inhibited, as shown above.

Table 1
Inhibition data with some of the clinically used sulfonamides **AZA–GUZ** and **2** against mammalian isozymes I–XIV (the isoforms CA VIII, X, and XI are devoid of catalytic activity and probably do not bind sulfonamides as they do not contain Zn(II) ions) and nematode enzyme ceCA[7]

Isozyme*	K_I** (nM)				
	AZA	**MZA**	**EZA**	**GUZ**	**2**
hCA I[a]	250	50	25	7.5	50.9
hCA II[a]	12	14	8	7.2	72.5
hCA III[a]	2.0×10^5	7.0×10^5	1.1×10^6	1.4×10^6	34,500
hCA IV[a]	74	6200	93	9000	48.9
hCA VA[a]	63	65	25	1100	72.7
hCA VB[a]	54	62	19	1100	40.9
hCA VI[a]	11	10	43	2650	25.0
hCA VII[a]	2.5	2.1	0.8	89	73.2
hCA IX[b]	25	27	34	102	6.4
hCA XII[b]	5.7	3.4	22	110	17.8
mCA XIII[a]	17	19	50	2633	4850
hCA XIV[a]	41	43	25	48	424
ceCA	35	42	40	339	9.5

[a] Full length enzyme.
[b] Catalytic domain.
* h, human; m, murine isozyme.
** Mean value from at least three different measurements.[14] Errors were in the range of ±5% of the obtained value (data not shown).