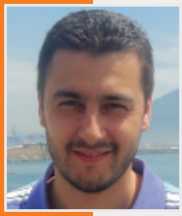
About the Authors



Fabrizio Carta

Fabrizio Carta received his degree in 2002 in pharmaceutical chemistry and technology from the University of Sassari (Italy). In 2008, he received his PhD in chemistry at the School of Chemistry of the Bristol University (UK), was appointed as postdoctoral fellow at MIT (USA), and finally joined Andrea Scozzafava's and Claudiu T Supuran's groups at the University of Florence (Italy). His research interests are on the synthesis of modulators the carbonic anhydrases and other zinc metalloenzymes.

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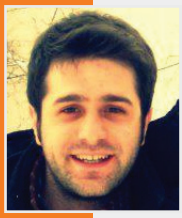
Murat Bozdag received his BSc in 2007 in chemistry from the University of Balikesir (Turkey). In 2010, he received his MSc in chemistry at the Institute of Science of the Balikesir University. In 2012, he started his PhD in Scozzafava's and Supuran's groups at the University of Florence. His research interests are on the synthesis of modulators of the carbonic anhydrases and protein arginine deiminases.



Muhammet Tanc

In 2012, Muhammet Tanc graduated from the Department of Chemistry Education MSc and BSc integrated program from Middle East Technical University in Ankara (Turkey). In 2012, he started his PhD under Claudiu T Supuran's supervision at the University of Florence. His research intrests are on synthesis of new classes of carbonic anhydrases inhibitors and activators.

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Claudiu T Supuran received his BSc in chemistry from the Polytechnic University of Bucharest (Romania) in 1987, and PhD in chemistry from the same university in 1991. In 1990, he became Assistant and then Associate Professor of Chemistry at the University of Bucharest. In 1995, he moved to the University of Florence, where he is currently a Professor. He has published two books and more than 820 papers in these fields. He is the Editor-in-Chief of *Journal of Enzyme Inhibition and Medicinal Chemistry*, and of *Expert Opinion on Therapeutic Patents*.

Chapter 8

Next-generation dithiocarbamate carbonic anhydrase inhibitors

Fabrizio Carta, Murat Bozdag, Muhammet Tanc & Claudiu T Supuran

Dithiocarbamates (DTCs) are chemically referred to as functional groups analogs to the carbamates, containing sulfur instead of oxygen atoms. Herein we report the kinetic results of a series of DTC-containing compounds tested on α and β-carbonic anhydrases (EC 4.2.1.1). The DTCs binding mode was also investigated by means of crystallographic experiments of the selected DTC **10**, **11** and **14** in adduct with human carbonic anhydrase II.

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Dithiocarbamates (DTCs) are chemically referred to as functional group analogs to the carbamates, containing two sulfur atoms instead of oxygen atoms. DTCs are stable in the form of both inorganic and organic salts or esters, and are easily obtained by coupling reactions of primary/secondary amines (aliphatic or aromatic) with carbon disulfide in the presence of an appropriate base. The introduction of an alkyl halide in the reaction medium affords the corresponding esters [1]. The use of DTC-bearing compounds in large amounts began in the early 20th century, with their industrial application as accelerators in the rubber vulcanization process [1]. This method proved to be much more efficient than the primitive, sulfur-based procedures introduced by Goodyear and Hancock approximately 60 years earlier [1]. Actually many DTCs are widely used in different fields such as in the analytical, macromolecular and organic chemistry, medicine and biology [1]. Undoubtedly the plant disease management is the area in which DTCs are predominantly employed [1]. The role DTCs play nowadays is heading for strengthening in the near future, due to the global growth population estimations (~9 billion in 2050) and the necessity to control plant diseases by means of chemical agents [1,2].

DTCs & the carbonic anhydrases

The main feature of DTCs is the ability to form stable complexes with metal ions, and plenty of reports accounting for the characterization of such derivatives are available in the literature [1]. However, the first study dealing with DTCs and metalloenzymes only appeared in 1983 with the investigation of the interaction between *N*,*N*-diethyldithiocarbamate **1** and various metal-containing bovine carbonic anhydrases (bCAs) [3]. In particular, the study reported the enzymatic inhibition of **1** with the native zinc-containing bCA, the ability of **1** to extrude the catalytic metal ion from the enzymatic site, and the authors proposed a trigonal bipyramidal geometry for the **1**-Co(II)-bCA adduct [3]. However the scientific community ignored the topic until the kinetic profiles of a series of unexplored inorganic anions on the human (h) and murine (m) CAs (EC 4.2.1.1) were recently reported by our group [4,5]. Such results demonstrated that most common inorganic anions explored [6], possess inhibition constants (K_is) between the millimolar to the sub-millimolar range. It is worth mentioning that the cyanide, azide and hydrogen sulfide were the only known exceptions [6]. Among the newly investigated inorganic species, trithiocarbonate **2** showed the highest affinities (Kis 8.7, 9.9,

Aa Dithiocarbamates (DTCs): chemically referred as functional groups analogs to the carbamates, containing two sulfur instead of oxygen atoms.

Carbonic anhydrase inhibitors (CAIs): in general referred as chemical species able to inhibit the activity of the carbonic anhydrase enzymes. Classical CAIs include the sulfonamides and their bioisosters the sulfamates and the sulfamides.

9.7 and 120 μM) for various CA isoforms, such as hCA I, II, IX and XII, respectively **(Table 8.1)** [4,5]. These studies validated trithiocarbonate **2** as a new inorganic CA inhibitor (CAI) and its binding mode was then explored by means of x-ray crystallography on the **2**-hCA II adduct [7]. Along with trithiocarbonate **2** the kinetic studies were also extended to the organic, structurally related compound *N,N*-diethyldithiocarbamate **1**. The rationale of this investigation was to explore whether organic compounds incorporating the trithiocarbonate moiety merged into organic scaffolds, such as the DTCs, might act as CAIs. The known metal chelating properties of the DTCs and the preliminary investigations by Morpurgo *et al.* previously discussed, indeed represented an interesting starting point to validate such a hypothesis. Indeed, we definitely proved that *N,N*-diethyldithiocarbamate **1** not only efficiently inhibits hCAs but it is even a better inhibitor when compared with its inorganic lead trithiocarbonate **2**, having K_i values as low as 24.6-times those for the hCA VII [4,5]. Afterwards, we reported a series of new DTC derivatives **3–28**, which have been prepared and screened for their inhibition activities on several α- and β-CAs. The biological implications as well as structure–activity relationship (SAR) investigations will be developed later in this chapter, now it is important to focus on the DTCs binding mode, which was explored by means of x-ray crystallography on adducts of several such DTCs, that is, **10**, **11** and **14,** bound to hCA II **(Figure 8.1)** [8,9].

The DTCs were found deeply buried into the enzyme active site. The CS_2^- groups upon displacement of the zinc-bound water/hydroxide, directly interacted through a sulfur atom with the catalytic zinc ion at a distance Zn-S of 2.3 Å. The angles defined by the zinc-coordinating histidines (His94, His96 and His119), the metal ion and the DTCs sulfur atom closely associated, account for a distorted tetrahedral geometry, which resembles the binding mode of the classical CAIs, such as the sulfonamides and their bioisosters sulfamates and sulfamides **(Figure 8.1A–8.1C)** [8,9]. The major differences in the interaction modes of **10**, **11** and **14** with the hCA occur at the organic scaffold level. The benzyl ring of compound **14** engages a T-shaped π-staking interaction with the imidazole ring of the His64, which, as a result, was frozen in the 'in' conformation. Furthermore, **14** is stabilized by the weaker van der Waals interactions occurring with the side chains of Asn62, Glu92, His94, Val121, Phe131, Leu198, Thr200 and Pro202 **(Figure 8.1C)** [8,9]. On the other hand, compound **10** positioned its phenyl ring onto a hydrophobic pocket located at the opposite site of the enzymatic tunnel bearing the His64 residue **(Figure 8.1A)** [8,9]. The pendant His64 imidazole ring was at a distance of 5 Å from **10**, being thus not involved in any valuable interaction with the inhibitor and is allowed to flip

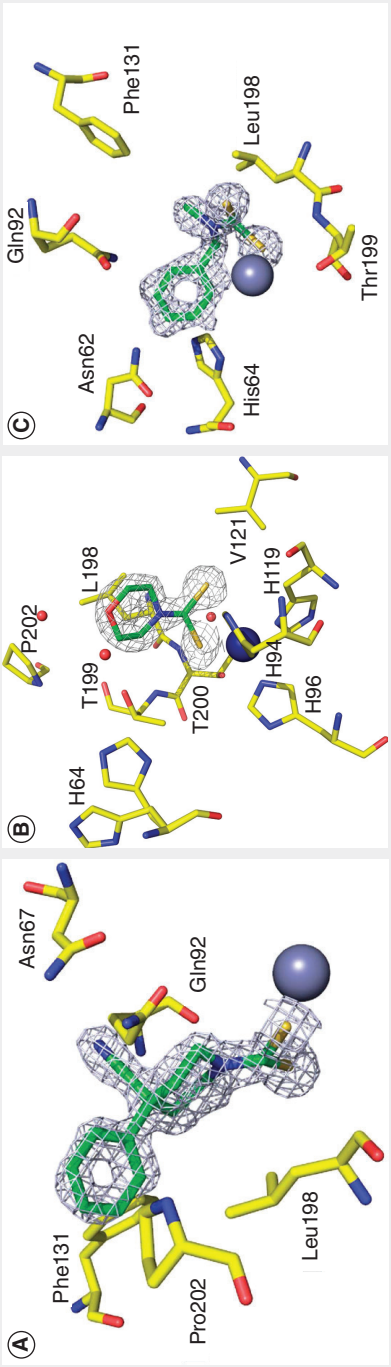

Figure 8.1. Electron densities for dithiocarbamates (A) 10, (B) 11 and (C) 14 in adduct with human carbonic anhydrase II.

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adopting both the 'in' and 'out' conformations. The phenyl ring of **10** headed towards the rim of the catalytic cleft, interacting with Phe131 and Pro202, whereas the cyano group hydrogen bonds with Asn67 and Gln 92 and a water molecule locate nearby **(Figure 8.1A)** [8,9]. Compound **11** was the deepest buried among all, and, as for compound **10**, no interactions with the His64 side chain occurred. The only contacts detected for the compound **11** scaffold in adduct with hCA II were with two water molecules, Wat393 and 540 **(Figure 8.1B)** [8,9].

The inhibition profiles of the DTCs **1** and **3–28** on hCA I, II, IX and XII are reported in **Table 8.1** and compared with the acetazolamide (**AAZ**) as CAI reference compound [8].

Compounds **1** and **3** are less effective as CAIs against all the screened isoforms. The elongation of both the aliphatic chains, as for **4–6**, ensures a progressive amelioration in the affinities toward all hCA isozymes tested, with K_is in the range of 5.8–1838 nM. A slight selectivity of **4** and **5** for the tumor-associated isoform hCA XII is observed (Kis 7.0 and 5.8 nM for **4** and **5,** respectively). The introduction of terminal hydroxide groups, as in **7**, results into an overall improvement of the CA inhibitions (Ki 4.0–9.2 nM) and further enhancements are gained when the linear side chains are substituted with branched analogs as in **8**. The K_i values for the *i*-Bu **8** were lower of one or two order of magnitude compared with the parent linear compound **5** (Kis 0.97, 0.95, 4.5 and 0.99 nM for **8** and K_is 43.1, 50.9, 50.3 and 5.8 nM for **5** on hCA I, II, IX and XII, respectively). The idea to join the linear alkyl

Table 8.1. Human carbonic anhydrase I, II, IX and XII inhibition data with dithiocarbamates 1, 3–28 and trithiocarbonate 2.

Compound	R^1	R^2	K_I (nM)			
			hCA I	*hCA II*	*hCA IX*	*hCA XII*
1	Et	Et	790	3100	1413	11
2†	CS_3^-		8.7×10^3	9.9×10^3	9.7×10^3	120×10^3
3	Me	Me	699	6910	714	798
4	*n*-Pr	*n*-Pr	1838	55.5	53.8	7.0
5	*n*-Bu	*n*-Bu	43.1	50.9	50.3	5.8
6	*n*-Hex	*n*-Hex	48.0	51.3	27.4	16.1
7	$HOCH_2CH_2$	$HOCH_2CH_2$	9.2	4.0	4.3	4.2
8	*i*-Bu	*i*-Bu	0.97	0.95	4.5	0.99
9	$(CH_2)_5$		0.96	27.5	70.4	46.1
10	$(NC)(Ph)C(CH_2CH_2)_2$		48.4	40.8	757	169
11	$O[(CH_2CH_2)]_2$		0.88	0.95	6.2	3.4
12	Et	*n*-Bu	157	27.8	25.9	7.5
13	Me	Ph	39.6	21.5	28.2	7.7
14	Me	$PhCH_2$	69.9	25.4	53.0	3.0
15	H	*sec*-butyl	21.1	29.4	4.6	31.7
16	H	Ph	4.8	4.5	4.2	4.3
17	H	2-thiazolyl	3.9	4.6	12.6	22.0
18	H	$PhCH_2$	4.1	0.7	19.2	11.5
19	H	4-PyridylCH_2	3.5	16.6	26.0	24.1
20	H	Imidazol-1-yl-$(CH_2)_3$	8.6	24.7	4.3	6.5
21	H	$O[(CH_2CH_2)]_2N$	4.8	3.6	29.1	9.2
22	H	$O[(CH_2CH_2)]_2N(CH_2)_2$	31.8	36.3	4.5	4.2
23	H	$[(CH_2)_5N]CH_2CH_2$	4.5	20.3	3.6	20.5
24	H	$MeN[(CH_2CH_2)]_2N$	33.5	33.0	22.1	17.5
25	$NaS(S=C)N[(CH_2CH_2)]_2$		12.6	0.92	37.5	0.78
26	H	$KOOCCH_2$	13.1	325	57.1	6.7
27	(*S*)-$[CH_2CH_2CH_2CH(COONa)]$		2.5	17.3	4.1	4.0
28	H	$N[(CH_2CH_2)N]_3$	31.9	13.5	27.4	9.3
AAZ			250	12	25	5.7

†hCA III, VII and XIII not shown.
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chains by means of a -CH_2- (compound **9**), ends in affinity values similar to the best alkyl derivative **8** only for the cytosolic hCA I, whereas the other isoforms keep their K_i closer to the linear DTCs **4–6** (Kis 0.96, 27.5, 70.4 and 46.1 nM for **9** on hCA I, II, IX and XII, respectively) **(Table 8.1)**. The substitution of the joint -CH_2-protons with bulkier groups (compound **10**) causes a sensible drop in the inhibitory activities (Kis 48.4, 40.8, 757 and 169 nM for **10** on hCA I, II, IX and XII, respectively). On the other end, replacement of the -CH_2- with a sp^3-hybridized oxygen, as in **11**, greatly enhances the enzymatic affinities (Kis 0.88, 0.95, 6.2 and 3.4 nM on hCA I, II, IX and XII, respectively). The asymmetric *N,N*-disubstituted DTCs **12**, **13** and **14** preferentially inhibit the tumor-associated hCA XII isoform (Kis 7.5, 7.7 and 3.0 nM for **12**, **13** and **14,** respectively, on hCA XII). The monosubstituted *sec*-butyl derivative **15** has inhibition values comprised between the parent linear *N,N*-dialkylated **5** and the branched **8** (Kis 21.1, 29.4, 4.6 and 31.7 nM for **15** on hCA I, II, IX and XII, respectively). When an aromatic moiety is introduced, such as the phenyl ring in **16** or the heterocyclic thiazolyl group in **17,** the K_i values get flattened to the low nanomolar levels, and only a minor selectivity for the hCA I and hCA II is observed for **17** **(Table 8.1)**. The insertion of a spacer between the aromatic/heteroaromatic tail and the DTC nitrogen (compounds **18**, **19** and **20**) did not improve the inhibition profiles **(Table 8.1)**. Interestingly, the morpholine compound **21** showed preferential inhibition for the cytosolic hCA I and II, whereas the introduction of an ethylenic spacer (compound **22**) shifted the selectivity toward the tumor-associated isoforms hCA IX and XII. The replacement of the morpholinic oxygen in **22** with a -CH_2- , to give **23**, lowers the hCA I and IX K_i values of 4.5- and 5.7-fold compared with the corresponding cytosolic hCA II and tumor-associated hCA XII isoforms. The bis DTC **25** had a complementary kinetic profile to **23**, as shows stronger affinity for the hCA II and XII over the corresponding hCA I and IX **(Table 8.1)**. The aminoacidic derivatives glycine **26** and *S*-proline **27** were screened for their inhibitory activities. In particular **26** badly interacts with the hCA II (Ki of 325 nM). Despite the *S*-proline **27** reported K_i values at the low nanomolar level for all the tested hCAs, in analogy to **26**, keeps the inhibition for hCA II weaker **(Table 8.1)**. In conclusion the SAR of compounds **1** and **3–28** validated the DTCs as new class of CAIs, having inhibition constants spanning from the micro- to the nano-molar range.

Aa Glaucoma: consists of a group of progressive optic neuropathies characterized by a slow and progressive degeneration of retinal ganglion cells and their axons. It shows a broad spectrum of clinical presentation and etiologies, which lead to a permanent loss of visual function due to the damage of the optical nerve. Most types of glaucoma are characterized by an elevated intraocular pressure.

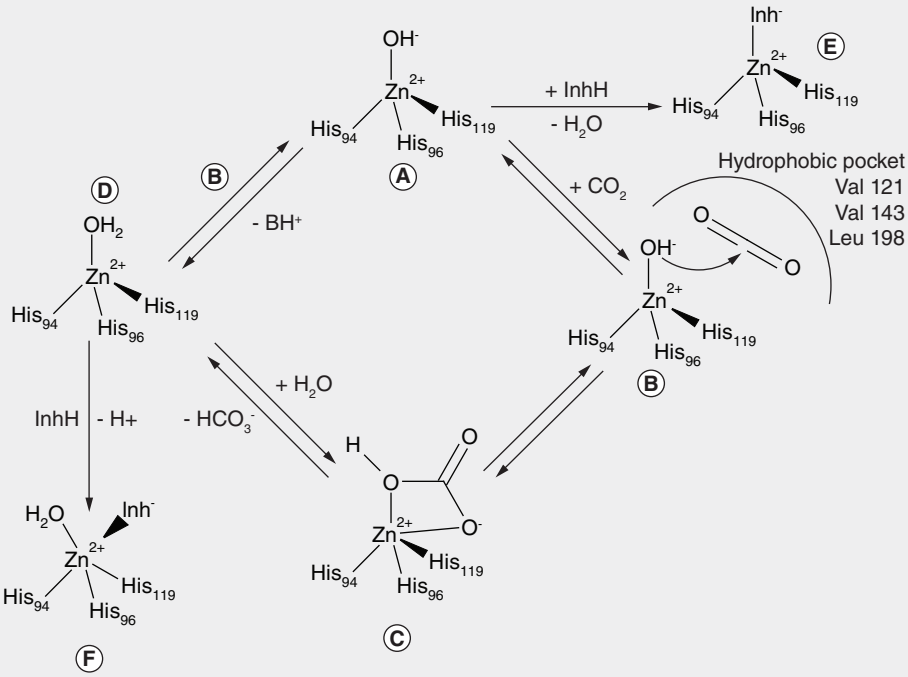
In light of such considerations DTCs **11** and **25** were investigated for their intraocular pressure (IOP) inhibition properties on the carbomer-induced glaucoma model on

rabbits, and compared with the standard clinically used dorzolamide (**DRZ**) [8]. Glaucoma is estimated to affect more than 70 million people worldwide and is clinically characterized by a series of neuropathic diseases, which progressively affect the retinal systems toward irreversible visual impairment and blindness [6,10,11]. The majority of glaucomas are characterized by elevated IOPs as result of unbalance between flow-out and production of the aqueous humor [6,10,11]. The metalloenzyme CAs plays a crucial role in the production of the aqueous humor, which is particularly rich in the bicarbonate ions [6,10], and the pioneering report of Becker in 1955 created the base for the use of CAIs as antiglaucoma agents [12].

As previously discussed DTCs take part in the CA catalytic cycle in the same manner as the classical CAIs such as sulfonamides and their bioisosters, to afford the intermediate **E** in **Figure 8.2** [13]

The result is the interruption of the CAs catalytic cycle with a diminished production of bicarbonate and protons as in agreement with $CO_2 + H_2O \leftrightharpoons HCO_3^- + H^+$. The biological consequences are a lower production of the

Figure 8.2. Catalytic cycle of human carbonic anhydrases.



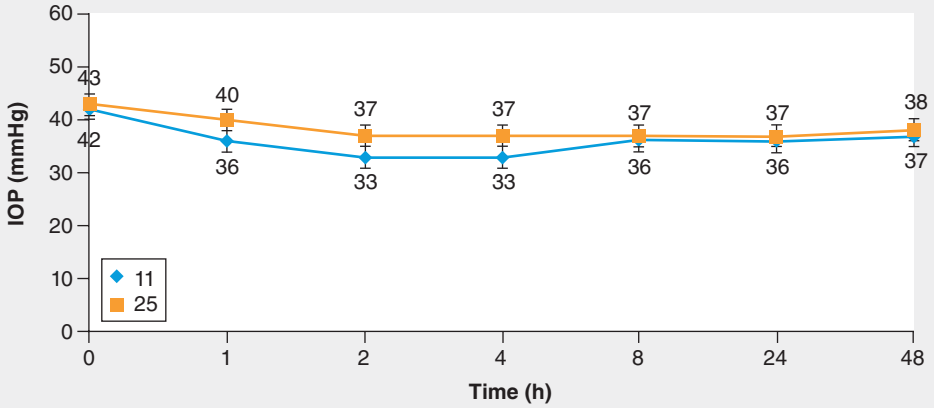
Data taken from [13].

humor liquid and IOP lowering as demonstrated by the **Figure 8.3** referred to **11** and **25** used at 2% concentration [8]. Both compounds show a persistent IOP lowering effect up to 8 h after local administration with **11** been slightly the more potent. In any case both DTCs **11** and **25** proved to be superior in terms of potency and lasting to the clinically used **DRZ** at the same concentration [8].

DTCs & α-CAs from *Drosphila melanogaster*

The arthropod *Drosophila melanogaster*, also known as fruit fly, became of particular attention as it represented the first animal, along with the nematode *Caenorhabditis elegans*, in which the presence of a β-class CA (DmBCA) was reported [14,15]. CA enzymes are classified into five unrelated gene families (α-, β-, γ-, δ- and ζ) and usually it is assumed that each CA-class is expressed only within specific species [6]. Despite the new scientific findings, little is known about the properties of the α-CAs abundantly expressed in *D. melanogaster.* A study of this kind recently appeared [16], and it reported the expression, purification and characterization of the *D. melanogaster* CAH1 and CAH2 along with their tissue distributions. Both enzymes were also considered for their carbon dioxide hydration reaction activities by means of stopped-flow measurements. Interestingly CAH1 and CAH2 revealed high catalytic turnovers with values similar to the highly active hCA II, but slightly lower when compared with the DmBCA (6.4×10^6 for CAH1,

Figure 8.3. 48 h intraocular pressure monitoring of compounds 11 and 25 at 2% concentration in aqueous solution.



Vehicle treatment not shown.
IOP: Intraocular pressure.
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6.0×10^6 for CAH1, 9.5×10^5 for DmBCA and 1.4×10^6 for hCA II) [14,16]. CAH1 and CAH2 are efficiently inhibited by the CAI acetazolamide (**AAZ**), even if with K_is higher than the hCA II and DmBCA values (Kis 106, 78, 49 and 12 nM for **AAZ** on CAH1, CAH1, DmBCA and hCA II, respectively) [14,16].

The DTCs **1** and **3–28** previous reported were also screened for their inhibition profiles on both CAH1 and CAH2, and their data were compared with the hCA II K_i values obtained in the same conditions **(Table 8.2)** [16]. A general overview reveals that all DTCs tested have good inhibition properties, with the only exceptions of **1** and **3** been the less active in the series **(Table 8.2)**.

Elongation of the aliphatic chains as in **4–6** drives the selectivity toward CAH2 over CAH1 and hCA II **(Table 8.2)**. Similarly **10**, the *N*-monosubstituted DTC **15**, and the tris-DTC **28** show analogous inhibition profiles. On the other hand the heterocyclic thiazolyl derivative **17**, the methyl piperazine **24** and *S*-proline **27** have preferential inhibition for the CAH1 over the other tested isozymes. The kinetic investigation reported is of particular importance as subcellular localization predictions account for the cytoplasmic and extracellular membrane-bounded expression of CAH1 and CAH2, respectively [16]. Moreover, in support of the localization predictions, the phylogenetic tree reveals CAH1 sharing a common ancestor with the cytosolic hCA I and hCA II, whereas CAH2 been related to the extracellular human isoforms [16]. Thus compounds, as the DTCs, which show selectivity for specific arthropod CAs, are of particular relevance for the management of parasites or organisms acting as vectors for potential diseases.

DTCs as new β-CAIs

The β-CAs, in agreement with their primordial origins, are largely encoded in archaea, bacteria, algae and fungi. In this contest is important to consider the problems associated to the management of diseases directly provoked of transmitted by such organisms [6,17]. Moreover the appearance of drug-resistant (DR) and multiple drug-resistant (MDR) bacteria are the principal causes of failure in the treatment of infections. Nevertheless, the US FDA registered a drop in the approval of new drugs as antibacterials up to 56% in the last 20 years [17]. Therefore, the selective targeting of the β-CAs represents a very promising approach for the development of new anti-infectives, and the validation of the DTCs as new CAIs indeed might provide such opportunity.

The *Mycobacterium tuberculosis* genome encodes for three β-CAs (mtCA1, mtCA2 and mtCA3) [18,19]. The bacterial enzymes have been cloned, characterized and screened for their kinetic profiles with

Aa Anti-infectives: molecules of natural or synthetic origin able to suppress microorganisms dangerous for human health.

Table 8.2. hCA II, CAH1 and CAH2 inhibition data with dithiocarbamates 1 and 3–28.

Compound	R^1	R^2	K_i (nM)		
			hCA II	*CAH1*	*CAH2*
1	Et	Et	3100	2355	654
3	Me	Me	6910	3461	769
4	*n*-Pr	*n*-Pr	55.5	23.4	3.9
5	*n*-Bu	*n*-Bu	50.9	17.1	0.5
6	*n*-Hex	*n*-Hex	51.3	1.4	3.8
7	$HOCH_2CH_2$	$HOCH_2CH_2$	4.0	3.2	134
8	*i*-Bu	*i*-Bu	0.95	20.2	0.9
9	$(CH_2)_5$		27.5	135	4.7
10	$(NC)(Ph)C(CH_2CH_2)_2$		40.8	12.8	3.7
11	$O[(CH_2CH_2)]_2$		0.95	13.8	0.8
12	Et	*n*-Bu	27.8	10.5	21.9
13	Me	Ph	21.5	67.1	5.9
14	Me	$PhCH_2$	25.4	50.2	12.4
15	H	*sec*-butyl	29.4	325	0.9
16	H	Ph	4.5	21.4	33.9
17	H	2-thiazolyl	4.6	0.5	3.9
18	H	$PhCH_2$	0.7	0.8	213
19	H	4-PyridylCH_2	16.6	35.1	58.8
20	H	Imidazol-1-yl-$(CH_2)_3$	24.7	0.9	24.1
21	H	$O[(CH_2CH_2)]_2N$	3.6	13.1	20.7
22	H	$O[(CH_2CH_2)]_2N(CH_2)_2$	36.3	12.1	10.4
23	H	$[(CH_2)_5N]CH_2CH_2$	20.3	57.1	26.7
24	H	$MeN[(CH_2CH_2)]_2N$	33.0	0.9	13.8
25	$NaS(S=C)N[(CH_2CH_2)]_2$		0.92	0.9	21.6
26	H	$KOOCCH_2$	325	12.3	76.5
27	(*S*)-$[CH_2CH_2CH_2CH(COONa)]$		17.3	1.4	13.6
28	H	$N[(CH_2CH_2)N]_3$	13.5	134	1.5
AAZ			12	106	78

classical CAIs as the sulfonamides [19–21]. DTCs **1** and **3–27** were investigated for their ability to inhibit mtCA1 and mtCA3 and the results are reported below in comparison with the cytosolic human hCA I and hCA II **(Table 8.3)** [22].

Table 8.3 shows that almost all tested DTCs have good affinities for both the mtCA1 and mtCA3, with K_is spanning from 0.9 to 893 nM. Similarly to the human cytosolic isozymes hCAI and hCA II, compounds **1** and **3** were the less active (Kis 431–893 nM). Chain elongation, as for **4–6**, accounts for a slightly preferential inhibition of the mtCA3 isoform over the mtCA1 **(Table 8.3)**. Such effect becomes more evident when the branched *i*-Bu substituents are present (Kis 86.2, 43.0 nM for **8** on mtCA1 and mtCA3, respectively). The introduction of the hydroxyl functionalities, as for compound **7**, results in an overall enhancement of the mtCAs inhibitory properties (Kis 7.5, 6.0 nM for **7** on mtCA1 and mtCA3, respectively). Inclusion of the DTC nitrogen into a cyclohexane ring, as in **9**, shifts the inhibition toward the mtCA3 isozyme over the mtCA1 (Kis 90.5, 4.1 nM for **9** on mtCA1 and mtCA3, respectively). Interestingly the substitution in **9** of the distal -CH_2- with an oxygen (compound **11**) lowers both the mtCAs K_i values, and it completely abolishes any preferential inhibition. Also the asymmetric *N,N*-disubstituted DTCs **12–14** show good inhibition with K_is comprised between 25.2 and 91.6 nM. The *N*-monosubstituted DTCs **15**, **16**, **19**, **20–24** reveal better profiles among all the series having K_is in the low nanomolar range and comprised between 2.4 and 9.1 nM. Interestingly, **17** and **18** are 9.4- and 12.3-fold more selective toward the mtCA3 and mtCA1, respectively **(Table 8.3)**. The bis-DTC **25** offers better selectivity when compared with **17**, as possesses a K_i for the mtCA3 48-times lower to the corresponding for mtCA1. Finally the amino acidic derivatives **26**, **27** and the tris-DTC **28** are good mtCAs inhibitors with K_is between 4.0 and 8.0 nM

As for the *M. tuberculosis*, the β-CAs from the pathogenic fungi *Cryptococcus neoformans*, *Candida albicans* and *Candida glabrata* (Can2, CaNce103 and CgNce103) were investigated for their inhibition profile with DTCs **1**, **3–28** **(Table 8.4)** [23].

In general, all tested compounds show good inhibition profiles towards all three fungal CAs, with the smaller derivatives **1** and **3** being the less active in the series (K_is 962–802 nM). Again the chain elongation, as for compounds **4–6**, or the introduction of the branched *i*-Bu substituents (as for **8**) lowered the enzymatic K_is up to low nanomolar values **(Table 8.4)**. In particular the *n*-hexyl-disubstituted DTC derivative **6** had the lowest K_i ever reported for Can2 (K_i 0.75 nM). Among the remaining *N,N*-disubstituted derivatives **7** and **9–14**, compounds **10** and **14** have the better affinities (K_is

Table 8.3. hCAI, II, mtCA 1 and mtCA 3 inhibition data with dithiocarbamates 1, 3–28.

Compound	R^1	R^2	K_i (nM)			
			hCA I	*hCA II*	*mtCA 1*	*mtCA 3*
1	Et	Et	790	3100	615	431
3	Me	Me	699	6910	893	659
4	*n*-Pr	*n*-Pr	1838	55.5	74.8	80
5	*n*-Bu	*n*-Bu	43.1	50.9	81.7	72.8
6	*n*-Hex	*n*-Hex	48.0	51.3	95.4	51.7
7	$HOCH_2CH_2$	$HOCH_2CH_2$	9.2	4.0	7.5	6.0
8	*i*-Bu	*i*-Bu	0.97	0.95	86.2	43.0
9	$(CH_2)_5$		0.96	27.5	90.5	4.1
10	$(NC)(Ph)C(CH_2CH_2)_2$		48.4	40.8	93	61.2
11	$O[(CH_2CH_2)]_2$		0.88	0.95	0.94	0.91
12	Et	*n*-Bu	157	27.8	91.6	63.5
13	Me	Ph	39.6	21.5	25.2	46.8
14	Me	$PhCH_2$	69.9	25.4	72	62.5
15	H	*sec*-butyl	21.1	29.4	6.0	3.6
16	H	Ph	4.8	4.5	5.6	2.5
17	H	2-thiazolyl	3.9	4.6	89.4	9.5
18	H	$PhCH_2$	4.1	0.7	7.1	87.3
19	H	4-PyridylCH_2	3.5	16.6	5.4	5.7
20	H	Imidazol-1-yl-$(CH_2)_3$	8.6	24.7	5.3	8.7
21	H	$O[(CH_2CH_2)]_2N$	4.8	3.6	6.1	2.4
22	H	$O[(CH_2CH_2)]_2N(CH_2)_2$	31.8	36.3	7.1	2.8
23	H	$[(CH_2)_5N]CH_2CH_2$	4.5	20.3	9.1	8.8
24	H	$MeN[(CH_2CH_2)]_2N$	33.5	33.0	4.7	2.6
25	$NaS(S=C)N[(CH_2CH_2)]_2$		12.6	0.92	37.5	0.78
26	H	$KOOCCH_2$	13.1	325	7.7	8.0
27	(*S*)-$[CH_2CH_2CH_2CH(COONa)]$		2.5	17.3	7.1	6.4
28	H	$N[(CH_2CH_2)N]_3$	31.9	13.5	4.2	4.0
AAZ			250	12	481	104

Table 8.4. hCA II, Can2, CaNce103 and CgNce103 inhibition data with dithiocarbamates 1, 3–28.

Compound	R^1	R^2	K_i (nM)			
			hCA II	*Can2*	*CaNce103*	*CgNce103*
1	Et	Et	3100	802	950	874
3	Me	Me	6910	876	962	913
4	*n*-Pr	*n*-Pr	55.5	88.1	69.4	76.2
5	*n*-Bu	*n*-Bu	50.9	75.8	67.0	73.2
6	*n*-Hex	*n*-Hex	51.3	0.75	6.3	3.9
7	$HOCH_2CH_2$	$HOCH_2CH_2$	4.0	65.5	69.0	70.3
8	*i*-Bu	*i*-Bu	0.95	6.2	6.0	5.7
9	$(CH_2)_5$		0.96	64.5	62.1	72.9
10	$(NC)(Ph)C(CH_2CH_2)_2$		48.4	7.3	7.2	7.6
11	$O[(CH_2CH_2)]_2$		0.88	5.4	4.2	7.8
12	Et	*n*-Bu	27.8	7.6	5.7	7.1
13	Me	Ph	21.5	15.2	16.8	14.9
14	Me	$PhCH_2$	25.4	8.3	6.5	7.4
15	H	*sec*-butyl	29.4	71.3	50.8	88.2
16	H	Ph	4.5	4.8	6.4	7.8
17	H	2-thiazolyl	4.6	64.4	58.5	72.3
18	H	$PhCH_2$	0.7	21.7	37.3	34.5
19	H	4-PyridylCH_2	16.6	5.7	5.3	8.7
20	H	Imidazol-1-yl-$(CH_2)_3$	24.7	6.7	5.3	7.2
21	H	$O[(CH_2CH_2)]_2N$	3.6	5.6	6.6	9.2
22	H	$O[(CH_2CH_2)]_2N(CH_2)_2$	36.3	6.6	6.0	8.4
23	H	$[(CH_2)_5N]CH_2CH_2$	20.3	5.1	7.5	6.0
24	H	$MeN[(CH_2CH_2)]_2N$	33.0	7.1	7.5	8.4
25	$NaS(S=C)N[(CH_2CH_2)]_2$		12.6	6.3	7.4	7.7
26	H	$KOOCCH_2$	325	50.8	61.3	47.0
27	(*S*)-$[CH_2CH_2CH_2CH(COONa)]$		2.5	6.4	6.2	7.2
28	H	$N[(CH_2CH_2)N]_3$	13.5	60.5	466	85.9
AAZ			12	10.5	132	11

4.2–8.3 nM). Also the monosubstituted DTCs **16** and **19–24** possess very good inhibitory properties for all the fungal CAs (K_is 4.8–9.2 nM) **(Table 8.4)**. The only exceptions are derivatives **15**, **17** and **18** (K_is 21.7–88.2 nM). The bis derivative **25** reports good inhibition profile too (K_i 6.3–7.7 nM). Among the amino acid derivatives **26** and **27** it is interestingly to note they differ for an order of magnitude in inhibiting all the fungal CAs, with the latter been the more active **(Table 8.4)**. Finally the tris DTC **28** showed a slightly preference for the inhibition of the Can2 and CgNce103 isozymes over the CaNce103 with a selectivity of 7.7 and 5.4, respectively.

Conclusion

The DTCs are indeed a new potent class of CAIs. Their salt character provides them good solubility in aqueous media, thus allowing for a vast array of biomedical applications such as anti-glaucoma and anti-infectives (antibacterial, antifungal agents) or in agriculture.

The crystallographic experiments of several DTCs in adduct with hCA II revealed a new binding mode of these compounds to the enzyme, which although similar to that of the classical CAIs, the sulfonamides, is quite distinct. The DTCs are monodentate ligands of the Zn(II) ion from the enzyme active site, binding to it by means of a sulfur atom at a distance Zn-S of 2.3 Å.

In the series of approximately 30 DTCs investigated so far as CAIs, it has been observed that small structural modifications in the inhibitor scaffold lead to different location of the molecules within the enzyme active site, leading also to quite variable SAR for the various isoforms or enzyme classes (α- and β-CAs of mammalian, insect, fungal or bacterial origin). Such observations are in agreement with the reported kinetic profiles as small modifications in the DTC scaffolds account for sensible changes in the inhibition potency and selectivity over the CA isozymes tested so far. We estimate that in the future more classes and variants of DTCs will be investigated as CAIs and that all enzyme classes (apart from the α- and β-ones) will be included in such studies.

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Summary.

- Dithiocarbamates (DTCs) are chemically referred as functional groups analogs to the carbamates, containing sulfur instead of oxygen atoms.
- The main feature of DTCs is the ability to form stable complexes with metal and alkali-metal ions.
- The rationale of this investigation was to explore whether organic compounds incorporating the trithiocarbonate moiety merged into organic scaffolds, such as the DTCs, might act as carbonic anhydrase inhibitors (CAIs).
- In any case both DTCs tested proved to be superior in terms of potency and lasting to the clinically used DRZ at the same concentration.
- The selective targeting of the β-CAs represents a very promising approach for the development of new anti-infectives and the validation of the DTCs as new CAIs indeed might provide such opportunity.
- Their salt character provides them good solubility in aqueous media, thus allowing for a vast array of biomedical applications such as glaucoma and anti-infectives or agriculture.
- The crystallographic experiments of DTC **10**, **11** and **14** in adduct with hCA II reveal a binding mode similar to the classical CAIs, as the sulfonamides, and also show that small structural modifications in the inhibitor determine dissimilar allocation of the molecule portions within the enzymatic cleft.
- Such observations are in agreement with the reported kinetic profiles as small modifications in the DTC organic scaffolds account for sensible changes in the inhibition potency and selectivity over the CA isozymes tested.

References

1 Gullino ML, Tinivella F, Garibaldi A *et al*. Mancozeb: past, present, and future. *Plant Disease* 94, 1076–1087 (2010).

2 Southgate D. Population growth, increases in agricultural production and trends in food prices. *Electronic J. Sustain. Devel.* 1, 31–35 (2009).

3 Morpurgo L, Desideri A, Rigo A *et al*. Reaction of N,N-diethyldithiocarbamate and other bidentate ligands with Zn, Co and Cu bovine carbonic anhydrases. Inhibition of the enzyme activity and evidence for stable ternary enzyme–metal–ligand complexes. *Biochim. Biophys. Acta*. 746, 168–175 (1983).

4 Innocenti A, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors. Inhibition of cytosolic isoforms I, II, III, VII and XIII with less investigated inorganic anions. *Bioorg. Med. Chem. Lett*. 19, 1855–1857 (2009).

5 Innocenti A, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors. Inhibition of transmembrane isoforms IX, XII and XIV with less investigated inorganic anions including thritiocarbonate and dithiocarbamate. *Bioorg. Med. Chem. Lett.* 20, 1548–1550 (2010).

6 Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. *Nat. Rev. Drug Discov.* 7 168–181 (2008).

7 Temperini C, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors. X-ray crystal studies of the carbonic anhydrase II-thritiocarbonate adduct-An inhibitio mimicking the sulfoanmide and urea binding mode to the enzyme. *Bioorg. Med. Chem. Lett*. 20, 474–478 (2010).

8 Carta F, Aggarwal M, Maresca A *et al*. Dithiocarbamates strongly inhibit carbonic anhydrases and show antiglaucoma action *in vivo*. *J. Med. Chem*. 55, 1721–1730 (2012).

9 Carta, F, Aggarwal M, Maresca A *et al*.

Dithiocarbamates: a new class of carbonic anhydrase inhibitors. Crystallographic and kinetic investigations. *Chem. Commun. (Cambridge)* 48, 1868–1870 (2012).

10 Masini E, Carta F, Scozzafava A *et al*. Antiglaucoma carbonic anhydrase inhibitors: a patent review. *Expert Opin. Ther. Patents*. 23, 705–716 (2013)

11 Zhang K, Zhang L, Weinreb RN. Ophthalmic drug discovery: novel targets and mechanisms for retinal disease and glaucoma. *Nat. Rev. Drug Discov.* 11, 541–559 (2012).

12 Becker B. The mechanism of the fall in intraocular pressure by the carbonic anhydrase inhibitor Diamox. *Am. J. Ophthalmol*. 39, 177–183 (1955).

13 Supuran CT. Structure-based drug discovery of carbonic anhydrase inhibitors. *J. Enzyme Inhib. Med. Chem.* 27, 759–772 (2012).

14 Syrjänen L, Tolvanen MEE, Hilvo M *et al*. Characterization of the first beta-class carbonic anhydrase from an arthropod (*Drosophila melanogaster*) and phylogenetic analysis of beta-class carbonic anhydrases in invertebrates. *BMC Biochem*. 11, 1–13 (2010).

15 Fasseas MK, Tsikou D, Flemetakis E *et al*. Molecular and biochemical analysis of the b class carbonic anhydrases in *Caenorhabditis elegans*. *Mol. Biol. Rep*. 37, 2941–2950 (2010).

16 Syrjänen L, Tolvanen MEE, Hilvo M *et al*. Characterization, bioinformatic analysis and dithiocarbamate inhibition studies of two new α-carbonic anhydrase, CAH1 and CAH2 from the fruit fly *Drosophila melanogaster*. *Bioorg. Med. Chem.* 21, 1516–1521 (2013).

17 Supuran CT, Carta F, Scozzafava A. Metalloenzyme inhibitors for the treatment of Gram-negative bacterial infections: a patent review (2009–2012). *Expert Opin. Ther. Patents* 23, 777–788 (2013).

18 Covarrubias AS, Larsson AM, Högbom M *et al*. Structure and function of carbonic anhydrases from *Mycobacterium tuberculosis*. *J. Biol. Chem*. 280, 18782–18789 (2005).

19 Nishimori I, Minakuchi T, Vullo D *et al*. Carbonic anhydrase inhibitors. Cloning, characterization, and inhibition studies of a new beta-carbonic anhydrase from *Mycobacterium tuberculosis*. *J. Med. Chem*. 52, 3116–3120 (2009).

20 Minakuchi T, Nishimori I, Vullo D *et al*. Molecular cloning, characterization, and inhibition studies of the Rv1284 beta-carbonic anhydrase from *Mycobacterium tuberculosis* with sulfonamides and a sulfamate. *J. Med. Chem*. 52, 2226–2232 (2009).

21 Carta F, Maresca A, Covarrubias AS *et al*. Carbonic anhydrase inhibitors. Characterization and inhibition studies of the most active beta-carbonic anhydrase from *Mycobacterium tuberculosis*, Rv3588c. *Bioorg. Med. Chem. Lett*. 19, 6649–6654 (2009).

22 Maresca A, Carta F, Vullo D *et al*. Dithiocarbamates strongly inhibit the β-class carbonic anhydrases from *Mycobacterium tuberculosis*. *J. Enzyme Inhib. Med. Chem.* 28, 407–411 (2013).

23 Monti SM, Maresca A, Viparelli F *et al*. Dithiocarbamates are strong inhibitors of the beta-class fungal carbonic anhydrases from *Cryptococcus neoformans*, *Candida albicans* and *Candida glabrata*. *Bioorg. Med. Chem. Lett*. 22, 859–862 (2009).