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Synthesis of a new series of dithiocarbamates with effective human carbonic anhydrase inhibitory activity and antiglaucoma action

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ABSTRACT

A new series of dithiocarbamates (DTCs) was prepared from primary/secondary amines incorporating amino/hydroxyl-alkyl, mono- and bicyclic aliphatic ring systems based on the quinuclidine, piperidine, hydroxy-/carboxy-/amino-substituted piperidine, morpholine and piperazine scaffolds, and carbon disulfide. The compounds were investigated for the inhibition of four mammalian α-carbonic anhydrases (CAs, EC 4.2.1.1) of pharmacologic relevance, that is, the human (h) hCA I, II, IX and XII, drug targets for antiglaucoma (hCA II and XII) or antitumor (hCA IX/XII) agents. The compounds were moderate or inefficient hCA I inhibitors (off-target isoform for both applications), efficiently inhibited hCA II, whereas some of them were low nanomolar/subnanomolar hCA IX/XII inhibitors. One DTC showed excellent intraocular pressure (IOP) lowering properties in an animal model of glaucoma, with a two times better efficiency compared to the clinically used sulfonamide dorzolamide.

1. Introduction

There are several classes of inhibitors of the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1), among which: (i) metal ion binders (inorganic anions,[1,2] sulfonamides, sulfamates and sulfamides;[1–6] dithiocarbamates and xanthates;[7,8] aromatic/heterocyclic thiols[9] and hydroxamates[10]); (ii) compounds anchoring to the zinc-coordinated water molecule/hydroxide ion from the enzyme active site (e.g., phenols,[11] carboxylates,[12] polyamines,[13] esters[14] and sulfocoumarins[15]); (iii) coumarins/lactones and related compounds (thiocoumarins, dithiocoumarins, etc.), which bind even further away from the metal ion, towards the exit of the active site cavity, in hydrolyzed form as hydroxycinnamic acid derivatives.[16,17] These inhibitors are classified according to their distance and/or direct interaction with the metal ion from the enzyme active site cavity. The zinc binders were the first CA inhibitors (CAIs) to be investigated in detail, with some of these compounds (sulfonamides and sulfamates) clinically used as antiglaucoma agents, diuretics, antiepileptics, antiobesity drugs and more recently, as theragnostics for hypoxic tumors.[1–6,18,19]

The dithiocarbamates were recently discovered to act as efficient CAIs of α- and β-class CAs from various organisms.[7,8] In fact there are six genetic families (α-, β-, γ-, δ-, ζ- and η-CAs)[20] encoding such enzymes in organisms widely distributed over the phylogenetic tree. This is due to the fact that CAs catalyze (usually very efficiently)[21] the interconversion between CO_2 and bicarbonate, which otherwise is a slow process at physiologic pH values. This reaction also leads to the formation of species involved in acid-base equilibria and buffering (e.g., bicarbonate and H^+ ions), which further pleas for a crucial role played by CAs in a host of physiologic and pathologic states, in very diverse organisms.[1–6,18–20] It has been demonstrated for a long time that CA inhibition elicits pharmacologic effects which was exploited by using sulfonamide or sulfamate CAIs for the treatment and prevention of diseases as those mentioned above.[1–6,18,19]

The dithiocarbamates (DTCs) have been rationally discovered as CAIs after our report of the inorganic ion trithiocarbonate (CS_3^{2-}) as an interesting but inefficient (milli-micromolar) CAI.[22] In the X-ray crystal structure of this anion bound to the widespread and physiologically dominant cytosolic human (h) isoform CA II, hCA II, it has been observed a monodentate coordination of the inhibitor via one sulfur atom to the zinc ion from the enzyme active site. Another hydrogen bond between another sulfur atom of trithiocarbonate and the OH of Thr199 was also evidenced, which further stabilized

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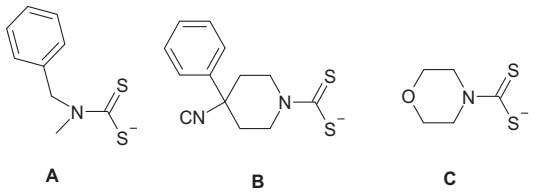
the enzyme-inhibitor adduct.[22] Thr199 is an amino acid residue crucial for the catalytic cycle of α-CAs but also for the binding of inhibitors, being conserved in all α-CAs.[1–3] Thus, with the help of this inorganic anion, the CS_2^- moiety was detected as a new zinc binding group (ZBG) in the design of CAIs. As DTCs incorporate this new ZBG, a first series of such compounds was shortly thereafter prepared and evaluated for their inhibitory activity against several mammalian, fungal and bacterial CAs.[7,8] Several low nanomolar/subnanomolar CAIs were thus detected against all these isoforms.[7,8]



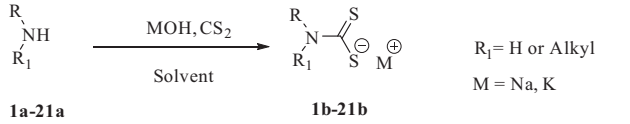
X-ray crystal structures were also reported for several of these DTCs complexed to hCA II, such as for example compounds **A**–**C**. These DTCs (**A**–**C**) effectively inhibited various human isoforms, such as hCA II and hCA IX, with inhibition constants in the nanomolar range.[7a] Their binding mode was rather identical to that of trithiocarbonate, regarding the ZBG, with one sulfur coordinated to the metal ion and the second one interacting with Thr199, but the organic scaffold present in the DTC was observed to make extensive contacts with amino acid residues/water molecules from the active site, which explained the wide range of inhibitory power of these derivatives (from the subnanomolar to the micromolar, for the entire series of around 30 DTCs reported earlier).[7,8] Interestingly, the highly water soluble compound **C** was also effective in vivo as an antiglaucoma agent when administered topically, directly into the eye of hypertensive rabbits, a widely used animal model of glaucoma.[7b]

However only one series of such CAIs of the DTC type was reported so far, by our group.[7,8] Here we extend the earlier investigations and report new DTCs, possessing diverse scaffolds compared to the first series of such compounds, investigating their inhibitory action against CA isoforms involved in severe pathologies, such as glaucoma (CA II and XII) and cancer (CA IX and XII). One of the new compounds also showed effective antiglaucoma activity in an animal model of the disease.

2. Results and discussion

2.1. Chemistry

In the previous work[7,8] on DTCs as CAIs, we investigated both primary and secondary derivatives which incorporated simple alkyl, aralkyl, aryl and hetaryl moieties, as well as two amino acid scaffolds (the Gly and Pro DTC derivatives). In the present paper we extended the series of DTCs, including again both primary as well as secondary derivatives, which were obtained in such a way as to explore novel chemical space. Indeed, the starting amines (**1a**–**21a**) used to synthesize DTCs **1b**–**21b** reported here (Scheme 1) included *N,N*-dimethylaminoethylenediamine **1a**, aminoalcohols with 3-5 carbon atoms in their molecule **2a**–**4a**, the bicyclic quinuclidine-3-amine (both the racemate as well as the *R*- and *S*-enantiomeric DTCs **5b**–**7b** incorporating this scaffold were obtained), piperidine **8a** and several of its derivatives with hydroxyl-, carboxy-, acetamido- and boc-amido functionalities in various positions of the heterocyclic ring, of types **9a**–**16a**; morpholine and peiparazine derivatives **17a**–**19a**, as well as phenethylamine **20a** and its sulfamoylated derivative, 4-aminoethylbenzenesulfonamide **21a**, a well know CAI which binds to the enzyme through the sulfamoyl moiety[1] (Scheme 1). The choice of these scaffolds was motivated by the fact that the structure–activity relationship (SAR) for the inhibition of the various CA isoforms with the DTCs reported earlier[7,8] was primarily influenced by the organic scaffold present in the inhibitor molecule. Furthermore, important differences of activity were observed between the primary and secondary DTCs (i.e., the compounds prepared from primary or secondary amines, respectively) and, between the aliphatic derivatives and the compounds incorporating aromatic/heterocyclic groups in their molecules.[7,8] Thus, we report here compounds belonging to the two DTCs type, with a variety of substitution patterns, based on several general scaffolds, among which: (i) primary, aliphatic aminoalkyl- or hydroxyalkyl derivatives (**1b**–**4b**); (ii) primary, bicyclic, bulky DTCs (**5b**–**7b**), with various stereochemistries at the C-3 of the quinuclidine ring; (iii) secondary, piperidine, morpholine and piperazine-based DTCs, of types **8b**–**19b**, for which the lead compound was the morpholine-DTC (compound **C**) reported earlier,[7] which showed excellent in vitro CA inhibitory properties and also antiglaucoma activity in an animal model of this disease. Indeed, the largest number of the new DTCs investigated here belongs to this subgroup. We also explored whether the presence and position of various functionalities on the six-membered heterocyclic amine scaffold (such as OH, COOH, AcNH, BocNH, etc.) is beneficial or not for the inhibitory properties of the new DTCs. Thus the DTC derivatives of 3-hydroxy-piperidine **9b**, pipecolic acid **10b**, nipecotic acid **11b** as well as isonipecotic acid **14b** (together with other structurally related such compounds) were synthesized (Scheme 1, Table 1). For the nipecotic acid **11b**, both the racemic as well as the two stereoisomeric DTCs **12b** and **13b** were prepared in order to investigate possible sterochemical requirements for effective CA inhibition; and (iv) primary, phenethylamine-based DTCs, **20b** and **21b**. In the earlier communications[7] we observed that the benzylamine-DTC, $PhCH_2NHCS_2Na$ (and the corresponding xanthate, $PhCH_2OCS_2Na$)[8d] were highly effective CAIs. As the homolog with one more carbon atoms was not reported in the first DTC series, we prepared here this compound, **20b**.[23] Unfortunately, due to lack of reactivity of the aniline, the phenyl-substituted compound $PhNHCS_2Na$ could not be obtained. As the sulfamoylated derivative of phenethylamine, **21a**, is a widely used CAI,[1] we also performed the reaction of its amino moiety with CS_2 and report the corresponding DTC, compound **21b**. The interest for this compound possessing two different ZBGs, the sulfonamide and the dithiocarbamate ones, was just the possible competition between them for binding to the metal ion within the CA active site, and whether such effects may lead to more effective CAIs.



Scheme 1. Synthesis of DTCs **1b**–**21b** from amines **1a**–**21a** and carbon disulfide.

2.2. CA inhibition

We investigated the CA inhibitory properties[24] of compounds **1b**–**21b** reported here, as well as the sulfonamide CAI acetazolamide (**AAZ**) as standard inhibitor, against four physiologically relevant human (h) CA isoforms, the cytosolic, hCA I and II, as well as the transmembrane, tumor-associated hCA IX and XII (the last isoform is however also present in normal tissues, being up-regulated in the eyes of glaucoma patients).[25] The reasons why we investigated these isoforms are the following: hCA II and XII are targets