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Carbonic anhydrase inhibitors: Inhibition of the human isozymes I, II, VA, and IX with a library of substituted difluoromethanesulfonamides

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Abstract—An inhibition study of the human cytosolic isozymes I, and II, the mitochondrial isoform VA, and the tumor-associated, transmembrane isozyme IX of carbonic anhydrase (CA, EC 4.2.1.1) with a library of aromatic/heteroaromatic/polycyclic difluoromethanesulfonamides is reported. Most of the inhibitors were derivatives of benzenedifluoromethanesulfonamide incorporating substituted-phenyl moieties, or were methylsulfonamide and difluoromethyl-sulfonamide derivatives of the sulfamates COUMATE and EMATE, respectively. Except for the methylsulfonamide-COUMATE derivative which behaved as a potent CA II inhibitor (K_I of 32 nM), these sulfonamides were moderate inhibitors of all isozymes, with inhibition constants in the range of 96–5200 nM against hCA I, of 80–670 nM against hCA II, and of 195–9280 nM against hCA IX, respectively. Remarkably, some derivatives, such as 3-bromophenyl-difluoromethanesulfonamide, showed a trend to selectively inhibit the mitochondrial isoform CA VA, showing selectivity ratios for inhibiting CA VA over CA II of 3.53; over CA I of 6.84 and over CA IX of 9.34, respectively, although it is a moderate inhibitor (K_I of 160 nM). Some of these derivatives may be considered as leads for the design of isozyme selective CA inhibitors targeting the mitochondrial isozyme CA VA, with potential use as anti-obesity agents.

In contrast to the aromatic or heteroaromatic sulfonamides which generally act as very potent inhibitors of the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1),[1–3] the aliphatic derivatives have been considered for a long time to be ineffective CA inhibitors (CAIs).[4] Maren and Conroy[5] then showed that perfluoro-/perchloro-alkylsulfonamides of the general formula $C_nX_{2n+1}SO_2NH_2$, (X = F, Cl; n = 1, 2, 4) act as potent CAIs against the physiologically most relevant CA isozyme, the cytosolic CA II, but the best inhibitor detected in their study, trifluoromethanesulfonamide, was observed to be chemically unstable, being easily hydrolyzed to triflic acid and ammonia. Perfluoroalkylsulfonamido-/carboxamido-tails were also attached to the molecules or aromatic/heterocyclic sulfonamides, leading to highly potent CAIs targeting isoforms I, II and IV,[6] which showed excellent water- and liposolubility, and good activity as antiglaucoma agents in an animal model of the disease.[6,7] Furthermore, very recently we have also shown that aliphatic sulfamates of the type C_nH_{2n+1}-OSO_2NH_2 (n = 2–18)[8] and aliphatic *N*-hydroxysulfamides of the type C_nH_{2n+1}-$NHSO_2NHOH$ (n = 8–12),[9] also act as very potent inhibitors of several physiologically important isozymes, such as the cytosolic CA I and CA II or the tumor-associated, transmembrane isoforms CA IX and CA XII. Thus, it is now possible to design potent CAIs belonging to a variety of classes of aliphatic sulfonamides, sulfamates or sulfamides among others, with many such compounds useful for the design of antiglaucoma, antitumor or antiobesity pharmacological agents among others.[1–3,10,11]

Keywords: Carbonic anhydrase; Isozymes I, II, VA, IX; Sulfonamides; Difluoromethanesulfonamide; Sulfamate; Antiobesity agent; Enzyme inhibitors.
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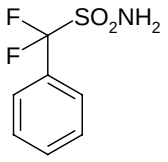
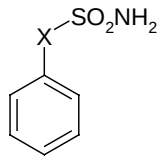


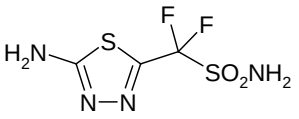
1

(58 nM, bCA II)



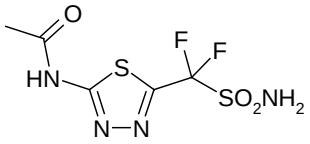
1a: X = CH_2

1b: X = O



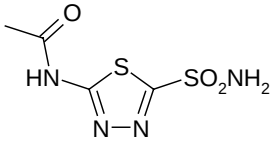
2

(15 nM, hCA II)



3

(208 nM, hCA II)

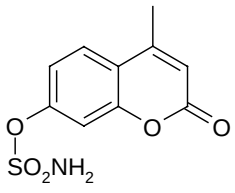


4

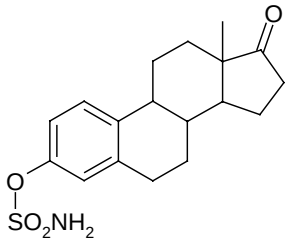
(10 nM, hCA II)

Recently, Taylor's[12] and Blackburn's[13,14] groups reported the synthesis of difluoromethane-sulfonamides possessing the general formula RCF_2-SO_2NH_2 (R = aromatic or heteroaromatic moiety). Blackburn's group demonstrated that a small number of such derivatives, such as **1–3**, were moderate to good inhibitors of the bovine or human isozyme CA II exhibiting inhibition constants in the range of 15–208 nM, though, in general, being somewhat less efficient CAIs as compared to acetazolamide **4**, the inhibitor *par excellence* of this class of enzymes (K_I of 10 nM).[13,14] Considering the interesting biological activity of derivatives **1–3**, we decided to investigate in more detail this new type of CAIs. Here, we report an inhibition study of four physiologically relevant CA isoforms, i.e., the cytosolic human isozymes hCA I and hCA II, the mitochondrial isozyme hCA VA,[15] and the transmembrane, tumor-associated isozyme hCA IX,[16–19] with a library of substituted difluoromethanesulfonamides incorporated into phenyl, coumarin or steroidal platforms.

Acetazolamide **4** (**AZA**) was from Sigma–Aldrich (Milan, Italy), whereas EMATE **15** was prepared as previously reported.[20] Compounds **1**, **5–13** were prepared as previously reported.[12] Most of them (**5–9**) are derivatives of the benzenedifluoromethanesulfonamide **1** reported by Blackburn's group to act as a moderate bCA II inhibitor,[13] in which the phenyl moiety was substituted in the 2-, 3- or 4-position with diverse groups such as methyl-, nitro- or bromo. Derivatives **10** and **11** are analogous to COUMATE **14**, a group of sulfamates developed by Potter's and Reed's groups[21] as inhibitors of steroid sulfatase,[22] but which also showed good CA inhibitory properties.[21] In the new coumarin derivatives investigated here, the sulfamate moiety of COUMATE has been replaced by the methylsulfonamide and difluoromethylsulfonamide moieties. Similarly, the new steroidal derivatives investigated here, compounds **12** and **13**, may be considered as being derived from EMATE **15**, another steroid sulfatase inhibitor[21,22] that was shown to act as a very potent CAI against isozymes I, II, and IX,[22] and for which the X-ray crystal structure in the adduct with hCA II has recently been reported by our group.[23] Again, the sulfamate moiety of EMATE has been replaced by the isosteric moieties methylsulfonamide and difluoromethylsulfonamide in derivatives **12** and **13**.[24]



14: COUMATE

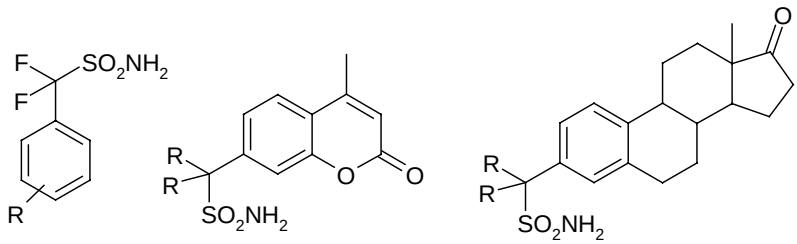


15: EMATE

Inhibition data of four physiologically relevant CA isozymes, i.e., hCA I, II, VA, and IX with sulfonamides/sulfamates **1**, **4–13**, and **15** and, the structurally related to **1** phenylmethanesulfonamide ($C_6H_5CH_2SO_2NH_2$) **1a** and phenylsulfamate ($C_6H_5OSO_2NH_2$) **1b**, and standard, clinically used inhibitors are shown in Table 1.[25]

The following should be noted regarding CA inhibition data with this series of compounds: (i) the slow red cell cytosolic isoform hCA I was inhibited by derivatives **1**, and **5–13**, with inhibition constants in the range of 96–5200 nM. The polycyclic methanesulfonamide **10** and difluoromethanesulfonamide **13** were the strongest inhibitors (K_I-s of 96–98 nM), being more efficient than acetazolamide (K_I of 250 nM) but approximately one-third as effective as the sulfamate inhibitor EMATE, a strong CA I inhibitor (K_I of 37 nM). The structurally related (to **10** and **13**) compounds, **11** and **12**, as well as the 4-bromophenyl-difluoromethanesulfonamide **9** and the lead molecule **1**, were moderate inhibitors (K_I-s in the range of 116–415 nM). Other substitution patterns of the phenyl moiety in **1** (derivatives **5–8**) lead to a drastic decrease of the inhibitory potency (K_I-s in the range of 952–5200 nM). It is interesting to note that for compounds **1**, **1a**, and **1b** possessing the isosteric replacements CF_2, CH_2 and O, the most potent CA I inhibitor was the sulfamate **1b** (one of the most effective

Table 1. hCA I, II, VA and IX inhibition data with sulfonamides/sulfamates **1**, **4**–**15**



5-9 **10, 11** **12, 13**

Compound	R	K_I (nM) [a]			
		hCA I[b]	hCA II[b]	hCA VA[b]	hCA IX[c]
4 (AZA)	—	250	12	60	25
1	H	357	156	785	8500
1a	—	1250	645	1580	9300
1b	—	2.1	1.3	nt	63
5	4-Me	952	348	128	250
6	4-O_2N	1130	113	153	2420
7	2-Br	5200	112	714	9280
8	3-Br	1095	565	160	1495
9	4-Br	273	98	982	7730
10	H	98	32	8600	240
11	F	415	104	1516	195
12	H	116	80	1464	208
13	F	96	670	129	1725
14 (COUMATE)	—	nt	25[d]	nt	nt
15 (EMATE)	—	37	10	nt	30

[a] Mean from three assays (errors in the range of 5–10% of the reported value); nt = not tested.
[b] Human, full-length recombinant enzymes.
[c] Catalytic domain of the human cloned isozyme.
[d] From Ref. 21.

CA I inhibitors ever reported),[20] whereas the phenyldifluoromethanesulfonamide **1** and phenylmethanesulfonamide **1a** were much less effective inhibitors; (ii) against the cytosolic rapid isoform hCA II (one of the physiologically most important CA isozymes),[1–4] derivatives **1**, **5**–**13**, showed inhibition constants in the range of 32–670 nM. Only the methanesulfonamide analogue of COUMATE, compound **10**, showed properties of potent CAI against this isozyme, but its inhibition constant is 2.6–3.2 times higher than that of acetazolamide or EMATE, two very potent CA II inhibitors (Table 1). It is also important to note that COUMATE itself had a slightly better potency (K_I of 25 nM)[21] as compared to the sulfamoylmethyl derivative **10**. It is surprising that replacing the OSO_2NH_2 functionality of EMATE/COUMATE with the isosteric $CH_2SO_2NH_2$ moiety (inhibitors **10** and **12**) leads to a loss of activity (eight-fold in the case of the EMATE derivative, and only 1.3 times in the case of the COUMATE derivative), whereas replacement with the $CF_2SO_2NH_2$ moiety (inhibitors **11** and **13**) leads to an even greater (67-fold for EMATE and approximately 4-fold for COUMATE) loss of activity. Also, for the COUMATE type of compounds **10** and **11**, the difluoromethanesulfonamide **11** was 3.25 times less effective as a CA II inhibitor as compared to the corresponding dihydro derivative **10**. Our data also show phenyldifluoromethanesulfonamide **1** to be a modest hCA II inhibitor, with an inhibition constant around three times higher than that reported earlier with the bovine isozyme by Blackburn's group[13,14] (Table 1). This may be due to the different source of enzymes used (human in our study, bovine in the previous study)[13] or to the different assay methods employed in the two studies. We have recently documented that bovine and human CAs sometimes show quite diverse inhibition profiles by sulfonamides.[26] It is interesting to note that as for hCA I, for compounds **1**, **1a**, and **1b**, the best inhibitor is the sulfamate **1b** (K_I of 1.3 nM), followed by the difluoro derivative **1**, which in turn was much more active as compared to the dihydro derivative **1a**. Also, the substituted-phenyl congeners of **1**, compounds **5–9**, did not show very good CA II inhibitory properties, the best inhibitors being the 4-bromo-, 2-bromo-, and 4-nitrophenyl-derivatives (K_I-s in the range of 98–113 nM), which were approximately 10 times less effective than acetazolamide **4**. The 3-bromo- and 4-methylphenyl-derivatives **5** and **8** were the least effective hCA II inhibitors in this subset of compounds (K_I-s in the range of 348–565 nM); (iii) The mitochondrial isoform hCA VA was moderately inhibited by the compounds investigated here with K_I-s in the range of 128–8600 nM. The best inhibitors were the 4-methyl-, 4-nitro-, and 3-bromophenyl-difluoromethanesulfonamides **5**, **6**, and **8**, as well as the difluoromethyl analogue of EMATE **13**, which showed K_I-s in the range of 128–160 nM, being 2–3 times less effective as acetazolamide (K_I of 60 nM).

However, an important observation was made regarding the inhibition profiles of **5** and **8**, which showed the highest selectivity for the mitochondrial isoform hCA VA over the cytosolic or transmembrane isozymes hCA I, II, and IX. In fact, these are the first CAIs ever reported to show this type of selectivity trend for the inhibition of the mitochondrial isozyme, which may be the target of the anti-obesity agents of the topiramate/zonisamide type.[27,28] Indeed, for **8**, the selectivity ratios for inhibiting CA VA over CA II, CAI, and CAIX were 3.53, 6.84, and 9.34, respectively. Usually, CA II has a much higher affinity for sulfonamide/sulfamate inhibitors as compared to the mitochondrial isozyme (for example, the selectivity ratio of acetazolamide for inhibiting CA VA over CA II is 0.2). As isozyme-selective or isozyme-specific CAIs have rarely been reported,[1–3] this type of difluoromethanesulfonamide derivatives may be extremely useful for the possible design of CA VA-selective inhibitors, even if the potency of the compounds investigated up to now is moderate. Much more modest CA VA inhibitory properties were exhibited by compounds **1**, **7**, and **9** (K_I-s in the range of 714–982 nM), whereas derivatives **10**–**12** behaved as quite weak inhibitors (in the micromolar range, see Table 1); (iv) the transmembrane, tumor-associated isozyme hCA IX was also modestly inhibited by the new sulfonamides investigated here. The best inhibitors were **5**, and **10**–**12**, showing K_I-s in the range of 195–250 nM, typically 7.8–10 times higher than those of acetazolamide, which, similarly to EMATE, behave as potent CA IX inhibitors (K_I-s in the range of 25–30 nM). The other investigated derivatives inhibited CA IX in the high micromolar range (K_I-s in the range of 1495–9280 nM). It is again important to note that for the structurally related derivatives **1**, **1a**, and **1b**, the best hCA IX inhibitor was the sulfamate **1b** (K_I of 63 nM),[20] whereas phenylmethanesulfonamide and phenyldifluoromethanesulfonamide were quite ineffective inhibitors (135–147 times less potent as compared to **1b**) (Table 1).

In conclusion, an inhibition study of the human cytosolic isozymes CA I, and II, the mitochondrial isoform CA VA, and the tumor-associated, transmembrane isozyme CA IX with a library of aromatic/heteroaromatic/polycyclic difluoromethanesulfonamides is reported. Most of the inhibitors were derivatives of benzenedifluoromethanesulfonamide incorporating substituted-phenyl moieties, or were methylsulfonamide and difluoromethyl-sulfonamide derivatives of the sulfamates COUMATE and EMATE, respectively. Except for the methylsulfonamide-COUMATE derivative which behaved as a potent CA II inhibitor (K_I of 32 nM), these sulfonamides were moderate inhibitors against all investigated CA isozymes, with inhibition constants in the range of 96–5200 nM for hCA I, of 80–670 nM for hCA II, and of 195–9280 nM for hCA IX, respectively. Remarkably, some derivatives, such as 3-bromophenyldifluoromethanesulfonamide, showed a trend to selectively inhibit the mitochondrial isoform CA VA, showing selectivity ratios for inhibiting CA VA over CA II of 3.53; over CA I of 6.84 and over CA IX of 9.34, respectively, although the compound is a moderate CA VA inhibitor (K_I of 160 nM). Some of these derivatives may thus be considered as leads for the design of isozyme selective CA inhibitors targeting the mitochondrial isozyme CA VA, with potential use as anti-obesity agents.

Acknowledgments

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