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Carbonic anhydrase inhibitors: inhibition of human cytosolic isozyme II and mitochondrial isozyme V with a series of benzene sulfonamide derivatives

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Abstract—Among the 14 human isozymes of carbonic anhydrase (CA, EC 4.2.1.1) presently known, the cytosolic hCA II is the most active and plays a host of physiological functions, whereas the mitochondrial hCA V is unique due to its role in several biosynthetic reactions. An inhibition study of these isozymes with a series of sulfonamides is reported here, with the scope to detect lead molecules for the design of isozyme-specific CA inhibitors (CAIs) targeting the mitochondrial isoform. Indeed, recently it has been shown that CA V is a novel target for the drug design of anti-obesity agents among others. Compounds included in this study were mainly *ortho*-, *meta*-, and *para*-substituted-benzenesulfonamides, together with several halogeno-substituted sulfanilamides and disubstituted-benzene-1,3-disulfonamide derivatives. Isozyme V showed an inhibition profile with these sulfonamides different of that of hCA II. Thus, IC_{50} values in the range of 80 nM to 74 μM against hCA II, and 0.78–63.7 μM against hCA V with these derivatives have been obtained. Only one compound, 2-carboxymethyl-benzenesulfonamide, was more active against hCA V over hCA II (selectivity ratio of 1.39), whereas all other derivatives investigated here were much better hCA II inhibitors (selectivity ratios CA II/CA V in the range of 0.0008–0.73) than hCA V inhibitors.

1. Introduction

By catalyzing a very simple physiological reaction, CO_2 hydration to bicarbonate and H^+ ions, carbonic anhydrases (CAs, EC 4.2.1.1), of which five genetically distinct families (α–ε) are presently known, are fundamental enzymes in all organisms over the phylogenetic tree.[1–5] In vertebrates, including humans, the 14 different α-CA isozymes characterized so far are involved in a host of physiological and pathological processes, and modulation of their activity by means of specific inhibitors or activators leads to important pharmacological responses.[1–5] CA inhibitors (CAIs)—systemic or topically acting—are clinically used ophthalmologic drugs for the management of glaucoma, cystoid macular edema, and retinopathies of diverse nature, being used alone or in combination therapies with other agents.[1–5] In the last period, a large number of sulfonamides/sulfamates targeting the tumor-associated CA isozymes (CA IX and XII) have also been reported,[6–10] with one such derivative, indisulam (E-7070) being in Phase II clinical trials for the treatment of solid tumors.[11,12]

Only one isozyme, CA V, is present in mitochondria, among the many α-CA isoforms isolated in animals (humans included). This isozyme was shown to be involved in several biosynthetic processes, such as ureagenesis,[13] gluconeogenesis,[14] and lipogenesis, both in vertebrates (rodents) as well as invertebrates (locust).[15–19] Indeed, in several crucial biosynthetic processes involving pyruvate carboxylase, acetyl CoA carboxylase, and carbamoyl phosphate synthetases I and II, bicarbonate not CO_2, is the real substrate of these carboxylating enzymes, and the provision of enough bicarbonate is assured by the mitochondrial isozyme CA V.[18–22]

In a recent study,[23] our groups showed that this isozyme (CA V) is a novel target for the drug design of agents

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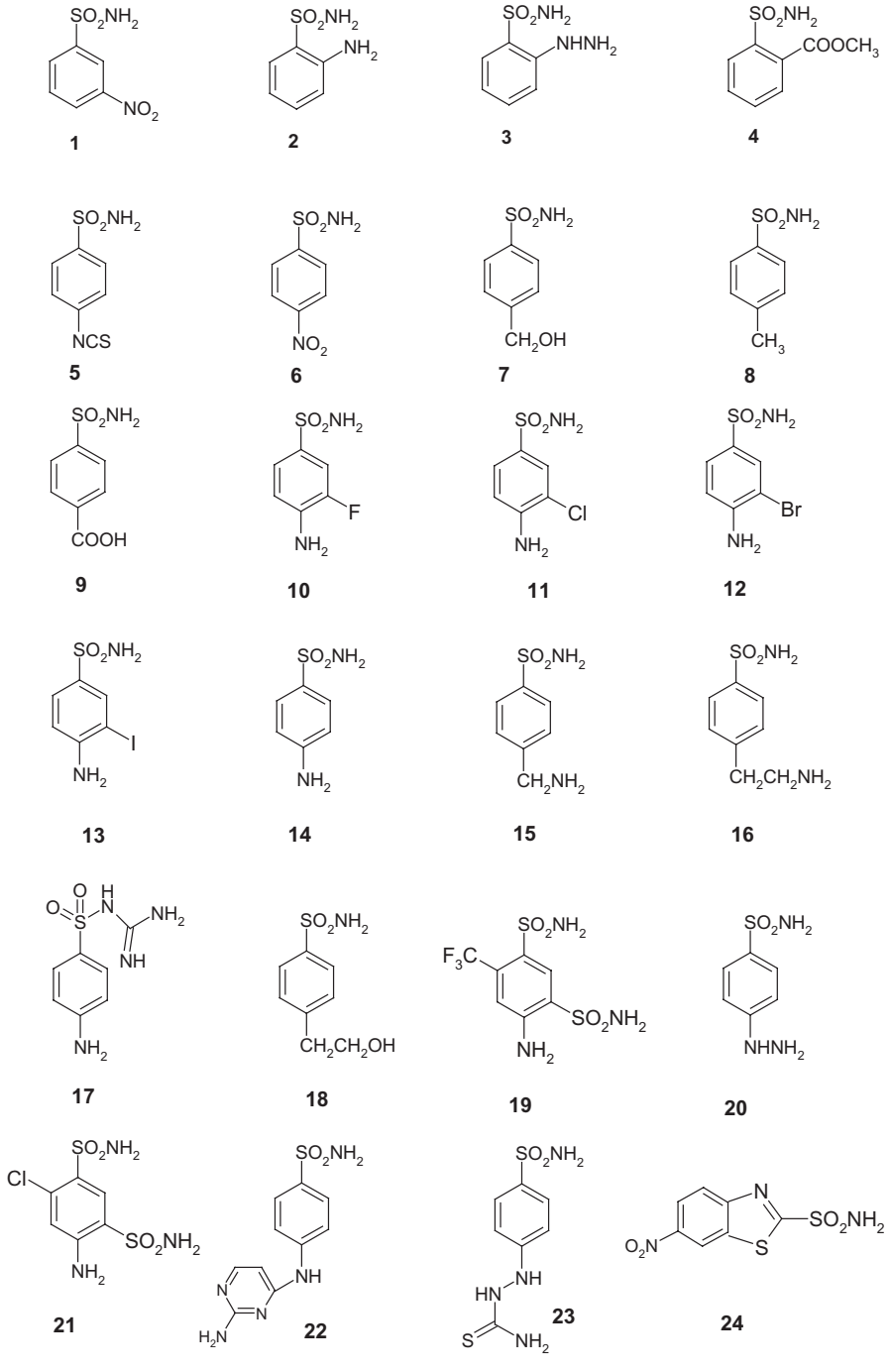
with possible use in the treatment and prophylaxis of obesity,[22–24] due to their effects in the inhibition of lipogenesis mentioned above. In the previous study,[23] a number of ureido-, carboxamido-, and sulfonylated-aromatic/heterocyclic sulfonamides reported by this group were investigated for their interaction with the murine isozyme mCA V, and some potent inhibitors have been detected. But a major problem in the design of such CAIs regards the selectivity toward the target isozyme. Both in our study of the anti-obesity sulfonamides,[22–24] as well as for the design of novel anti-tumor agents targeting isozymes CA IX or CA XII (predominantly found in tumor cells),[6–10] it was rather difficult to identify inhibitors with selectivity for CA V (or CA IX/XII) over CA II. Indeed, isozyme CA II, which is an abundant, widespread, and physiologically relevant cytosolic isozyme, shows a very high affinity for these classes of inhibitors,[1–5] and most low nanomolar CA V (or CA IX) inhibitors detected up to now, also consistently inhibited CA II. Thus, the main challenge in the drug design of such agents is represented by the need to identify lead molecules from which such isozyme-specific CAIs can then be obtained, targeting other isozymes than the ubiquitous CA II. Since inhibition of the human isozyme hCA V has never been investigated with sulfonamides (in fact only one CA V inhibition study with such compounds has been reported, in which the murine isoform has been employed),[23] we decided to investigate a



series of benzenesulfonamide derivatives, possibly substituted in different positions with simple groups, such as amino, hydroxy, nitro, halogeno, carboxy, etc., for their interaction with hCA V. Our main goal was not to detect low nanomolar CA V inhibitors (since the derivatives included in the study are too simple from a chemical point of view in order to show this potent activity), but compounds that may show selectivity toward CA V over CA II, amenable to chemical derivatization, from which to obtain in a second moment such potent, and possibly isozyme-specific inhibitors.

2. Chemistry

A series of sulfonamides, of type **1**–**24**, have been included in our study. Some of them were commercially available (compounds **1**, **2**, **4**, **6**, **8**, **9**, **14**–**17**, **19**, **21**, and **22**) whereas derivatives **3**,[25] **5**,[26] **7**,[27] **10**–**13**,[7] **18**,[27] **20**,[25] **23**,[28] and **24**[27] were prepared as reported previously by our group.

Except **17** (which was included just for this feature) all other derivatives investigated here possess unsubstituted SO_2NH_2 moieties, as this is one of the best zinc-binding functions present in CAIs.[1–5] Sulfaguanidine **17** possesses on the other hand a substituted sulfonamide group, which generally is unable to bind to the Zn(II) ion within the CA active site.[1–5] In consequence, substituted sulfonamides are generally much weaker CAIs as compared to compounds possessing SO_2NH_2 moieties.[1–5] Since substituted sulfonamides have never been tested for their interaction with CA V, we included **17** in our study in order to check that this situation is also valid for the mitochondrial isozyme. Other sulfonamides investigated here were generally *ortho*-, *meta*-, or *para*-substituted benzenesulfonamide derivatives incorporating diverse moieties such as amino, nitro, isothiocyanato, hydroxy, methyl, hydrazino, carboxy, etc. Trisubstituted derivatives were also considered, such as, for example, the halogeno-sulfanilamides **10**–**13**, together with tetrasubstituted derivatives, such as the 1,3-benzenedisulfonamide derivatives **19** and **21**. Finally, a benzothiazole-2-sulfonamide derivative, **24**, was also included in our study, being the only compound with a heterocyclic scaffold.

3. CA inhibition

Inhibition data of the two isozymes of interest, the cytosolic hCA II, and the mitochondrial hCA V, with sulfonamides **1**–**24** are shown in Table 1.

As seen from data of Table 1, except for sulfaguanidine **17**, possessing a substituted sulfonamide group, which cannot bind to the Zn(II) ion within the CA active site, all other compounds investigated here, possessing primary SO_2NH_2 moieties, showed inhibitory properties against the two investigated isozymes. IC_{50} values were in the range of 80 nM to 73.9 μM against hCA II, and of 0.78–63.7 μM against hCA V. The following structure–activity relationship can be deduced from these data. For isozyme hCA II: (i) the *ortho*-substituted derivatives (except for **3**) were the most inefficient inhibitors (IC_{50} values in the range of 7.1–73.9 μM), with the ester **4** being a 925 times less effective CA II inhibitor as compared to the hydrazine **3**. Thus, bulky *ortho*-substitution of benzenesulfonamides is clearly detrimental to the CA inhibitory properties, presumably due to steric hindrance and unfavored coordination to the zinc ion within the enzyme active site (ii) another group of sulfonamides, such as **2**, **5**, **10**, **13**–**16**, and **18** were more effective inhibitors, with IC_{50} values in the range of 1.73–4.0 μM. These compounds possessed a rather variable substitution pattern at the benzene ring, with both *ortho*-, *para*-, or trisubstituted compounds; (iii) another group of derivatives, including **1**, **6**, **8**, **9**, **11**, **12**, **19**, **23**, and **24**, showed IC_{50} values in the range of 0.25–0.88 μM. Again, the substitution pattern and the nature of moieties substituting the benzene ring were rather variable. On one hand, electron withdrawing groups such as nitro, carboxy, and sulfamoyl, are known to favor CA inhibitory properties due to their acidifying effect on the (first) sulfamoyl group.[1–5] Thus, such an effect is clearly seen in the case of compounds incorporating such groups (e.g., **1**, **6**, **9**, **19**, **24**). Still, the acidifying effect is not the only factor responsible for the binding to the CA active site, since by comparing compounds with substitution patterns that lead to opposing effects on acidification of the sulfamoyl group (for example, compare **8** and **9** or **9** and **11**/**12**) a very similar IC_{50}

Table 1. IC_{50} values of sulfonamide inhibitors against isozymes hCA II and hCA V, for the CO_2 hydration reaction, at 20 °C[29]

Inhibitor	IC_{50} (μM)		Selectivity ratio (CA II/CA V)
	hCA II[a]	hCA V[b]	
1	0.41	0.78	0.52
2	2.64	15.3	0.17
3	0.080	9.6	0.083
4	73.9	53.2	1.39
5	3.60	8.60	0.41
6	0.40	4.20	0.09
7	5.7	9.3	0.61
8	0.58	4.7	0.12
9	0.88	2.65	0.33
10	3.0	9.3	0.32
11	0.56	7.4	0.07
12	0.62	7.6	0.08
13	1.73	9.4	0.18
14	2.4	32.0	0.075
15	4.0	27.6	0.14
16	3.1	42.4	0.073
17	>100	>100	—
18	3.0	17.0	0.17
19	0.34	9.4	0.036
20	0.081	7.7	0.010
21	0.096	4.17	0.023
22	0.089	29.9	0.002
23	0.29	63.7	0.004
24	0.25	44.9	0.005

Enzyme concentrations were [hCA II] = 1 μM (the enzyme from Sigma–Aldrich had a rather low catalytic activity); [hCA V] = 1 μM (CA V is a much less active enzyme as compared to CA II).[23]

[a] Human purified red cell enzyme from Sigma–Aldrich.

[b] Recombinant, truncated form of hCA V lacking the first 51 amino-terminal residues,[31] obtained as described in Ref. 23.

value was measured; (iv) a last group of four compounds, **3** and **20**–**22**, showed very efficient CA II inhibitory activity, with IC_{50} values in the range of 80–96 nM. Again the substitution pattern is the most important factor influencing biological activity. The best inhibitors were the rather simple 2- and 4-hydrazino-benzenesulfonamides **3** and **20**, whereas the bicyclic derivative **22** was slightly less effective. It is interesting to note that the benzene-1,3-disulfonamide derivative **21** is closely related to **19** (which was less effective). Thus, the chlorine in *ortho* leads to more potent CA II inhibitors as compared to the trifluoromethyl group, in this small subseries of disulfonamides investigated here.

For the hCA V inhibition, the following observations can be made: (i) a rather large number of the compounds investigated, such as **2**–**5**, **7**, **10**–**16**, **18**–**20**, and **22**–**24**, showed a moderate inhibitory activity toward hCA V, with IC_{50} values in the range of 7.4–63.7 μM. It is thus clear at a first look that this isozyme is generally less prone to be inhibited by sulfonamides as compared to the cytosolic isoform discussed above. Only one compound, the ester **4** showed a better activity toward hCA V as compared to hCA II, but unfortunately this is a rather weak inhibitor. Still, this is an important result, because it proves that the vicinity of Zn(II) in the active site of hCA V may accommodate some bulkier groups as compared to the same environment in hCA II, and leaves space for improvements in this type of inhibitors with enhanced affinity for isozyme V over isozyme II. It may be seen that the substitution pattern of these derivatives is not the main factor influencing activity, since *ortho*-, *meta*-, *para*-, or polysubstituted derivatives showed rather similar activity. On the other hand, the nature of the substituents present on the benzene nucleus strongly influenced activity. In fact, in this subseries of sulfonamides the most active were the halogenosulfanilamides **10**–**13**, the disulfonamide **19**, and the hydrazine derivative **20**, together with the isothiocyanate **5**. The least active derivatives were the isothiohydrazido derivative **23** and the ester **4**. It is noteworthy the very high difference of activity between the hydrazine **20** and its thioureido derivative **23**, with the first compound being 8.3 times more effective a CA V inhibitor as compared to the latter one. It is difficult to explain these data presently, since the X-ray crystal structure of hCA V is unknown (work is in progress in this laboratory for the crystallization of this enzyme, alone and in complex with inhibitors); (ii) a small number of derivatives, for example, **1**, **6**, **8**, **9**, and **21** behaved as more effective hCA V inhibitors, with IC_{50} values in the range of 0.78–4.7 μM. The best inhibitor was the *m*-nitrobenzenesulfonamide **1**, followed by the benzoic acid derivative **9**. It should be also mentioned that the chloro-substituted benzene-1,3-disulfonamide derivative **21** was about two times more effective a CA V inhibitor as compared to the closely structurally related trifluoromethyl derivative **19**.

The selectivity of the tested CAIs against these two isozymes is the last important issue to be dealt with. As may be seen from the data of Table 1, only one compound, the ester **4**, behaved as a better CA V inhibitor than CA II inhibitor, showing a rather modest selectivity ratio of 1.4. On the other hand, quite a lot of very selective CA II versus CA V inhibitors were detected, such as **22**, which is 500 times more potent as a CA II inhibitor than a CA V inhibitor, **23** (250 times) or **24** (200 times difference of activity between the two isozymes). Certainly, our intention was to detect CA V specific not CA II specific inhibitors, but work is in progress in our laboratory to crystallize the two isozymes with some of these inhibitors in order to understand at molecular level the interactions contributing to this activity. This may indeed be very helpful for the drug design of isozyme-more-specific CAIs.

In conclusion, in a series of simple, variably substituted benzenesulfonamide derivatives, tested for the inhibition of the cytosolic isozyme hCA II and the mitochondrial isozyme hCA V (for the first time investigated for its interaction with sulfonamide inhibitors), interesting inhibitory activities have been detected. Isozyme V showed an inhibition profile with these sulfonamides different of that of hCA II. IC_{50} values in the range of 81 nM to 74 μM against hCA II, and 0.78–63.7 μM against hCA V with these derivatives have been obtained. Only one compound, 2-carboxymethyl-benzenesulfonamide, was more active against hCA V over hCA II (selectivity ratio of 1.39), whereas all other derivatives investigated here were much better hCA II inhibitors (selectivity ratios CA II/CA V in the range of 0.002–0.73) than hCA V inhibitors. These data may be helpful for the future drug design of sulfonamide CAIs with selectivity for a target isozyme, such as, for example, the mitochondrial or the tumor-associated ones (CA V and CA IX/XII, respectively).

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