Carbonic Anhydrase Inhibitors. Inhibition of Mitochondrial Isozyme V with Aromatic and Heterocyclic Sulfonamides

Daniela Vullo,[†] Marco Franchi,[‡] Enzo Gallori,[‡] Jochen Antel,[§] Andrea Scozzafava,[†] and Claudiu T. Supuran*[,†]

Laboratorio di Chimica Bioinorganica, Università degli Studi di Firenze, Rm. 188, via della Lastruccia 3, I-50019 Sesto Fiorentino (Firenze), Italy, Dipartimento di Biologia Animale e Genetica, Università degli Studi di Firenze, via Romana 17-19, 50122 Firenze, Italy, and Solvay Pharmaceuticals GmbH, Hans Böckler-Allee 20, D-30173 Hannover, Germany

Received October 6, 2003

The first inhibition study of the mitochondrial isozyme carbonic anhydrase (CA) V (of murine origin) with a series of aromatic and heterocyclic sulfonamides is reported. Inhibition data of the cytosolic isozymes CA I and CA II and the membrane-bound isozyme CA IV with these inhibitors are also provided for comparison. Several low nanomolar CA V inhibitors were detected (K_I values in the range of 4−15 nM), most of them belonging to the acylated sulfanilamide, ureido-benzenesulfonamide, 1,3,4-thiadiazole-2-sulfonamide, and aminobenzolamide type of compounds. The clinically used inhibitors acetazolamide, methazolamide, ethoxzolamide, dorzolamide, brinzolamide, and topiramate on the other hand were less effective CA V inhibitors, showing inhibition constants in the range of 47−63 nM. Some of the investigated sulfonamides, such as the ureido-benzenesulfonamides and the acylated sulfanilamides showed higher affinity for CA V than for the other isozymes, CA II included, which is a remarkable result, since most compounds investigated up to now inhibited the cytosolic isozyme CA II better. These results prompt us to hypothesize that the selective inhibition of CA V, or the dual inhibition of CA II and CA V, may lead to the development of novel pharmacological applications for such sulfonamides, for example in the treatment or prevention of obesity, by inhibiting CA-mediated lipogenetic processes.

Introduction

At least 14 different α-carbonic anhydrase (CA, EC 4.2.1.1) isoforms were isolated in higher vertebrates, where these zinc enzymes play crucial physiological roles.[1−3] Some of these isozymes are cytosolic (CA I, CA II, CA III, CA VII), others are membrane-associated (CA IV, CA IX, CA XII and CA XIV), CA V is mitochondrial, and CA VI is secreted in saliva.[1−3] Three acatalytic forms are also known, which are denominated CA-related proteins (CARP), CARP VIII, CARP X, and CARP XI.[1−3] Representatives of the β- and γ-CA family are highly abundant in plants, bacteria, and archaea.[4] These enzymes are very efficient catalysts for the reversible hydration of carbon dioxide to bicarbonate, but at least the α-CAs possess a high versatility, being able to catalyze different hydrolytic processes, such as the hydration of cyanate to carbamic acid, or of cyanamide to urea, the aldehyde hydration to *gem*-diols, and the hydrolysis of carboxylic or sulfonic acids esters, as well as other less investigated hydrolytic processes, such as hydrolysis of halogeno derivatives, arylsulfonyl halides, etc.[1,2] It is not known whether reactions catalyzed by CAs other than the hydration of CO_2/dehydration of HCO_3^- may have physiological relevance in systems where these enzymes are present.[1] The catalytic mechanism of the α-CAs is understood in great detail: the active site consists of a Zn(II) ion coordinated by three histidine residues and a water molecule/hydroxide ion. The latter is the active species, acting as a potent nucleophile.[1,2] For β- and γ-CAs, the zinc hydroxide mechanism is valid too, although at least some β-class enzymes do not have water directly coordinated to the metal ion.[4] CAs are inhibited primarily by two main classes of inhibitors: the inorganic anions (such as cyanide, cyanate, thiocyanate, azide, hydrogen sulfide, etc.) and the unsubstituted sulfonamides possessing the general formula RSO_2NH_2 (R = aryl, hetaryl, perhaloalkyl).[1,2] Several important physiological and physiopathological functions are played by the CA isozymes present in organisms all over the phylogenetic tree, related to respiration and transport of CO_2/bicarbonate between metabolizing tissues and the lungs, pH and CO_2 homeostasis, electrolyte secretion in a variety of tissues/organs, biosynthetic reactions, such as the lipogenesis, gluconeogenesis, and ureagenesis among others (in animals), CO_2 fixation (in plants and algae), etc.[1,2,4] The presence of these ubiquitous enzymes in so many tissues and in so many different isoforms represents an attractive goal for the design of inhibitors or activators with biomedical applications.[1,2]

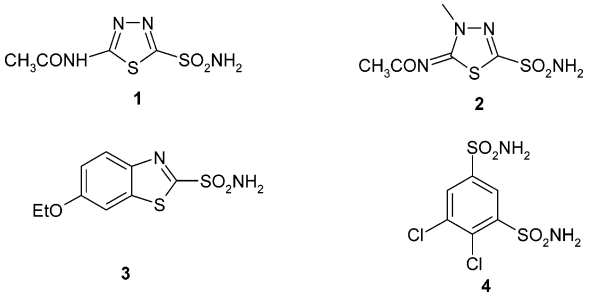
Only CA V is present in mitochondria, among the many α-CA isoforms found in animals. This isozyme was shown to be involved in several biosynthetic processes, such as ureagenesis,[5] gluconeogenesis,[6] and lipogenesis, both in vertebrates (rodents) and in invertebrates (locust).[7−10] Indeed, in several important biosynthetic processes involving pyruvate carboxylase, acetyl CoA carboxylase, and carbamoyl phosphate synthetases I and II, bicarbonate, not carbon dioxide, is the real substrate of these carboxylating enzymes, and the

* Correspondence author. Tel. +39−055−4573005: Fax: +39−055−4573385; E-mail: claudiu.supuran@unifi.it.
[†] Laboratorio di Chimica Bioinorganica, Università degli Studi di Firenze.
[‡] Dipartimento di Biologia Animale e Genetica, Università degli Studi di Firenze.
[§] Solvay Pharmaceuticals Gmb.

10.1021/jm031057+ CCC: $27.50
Published on Web 01/29/2004

provision of enough bicarbonate is assured mainly by the catalysis involving the mitochondrial isozyme CA V (probably assisted by the high activity cytosolic isozyme CA II).[8,11−14]

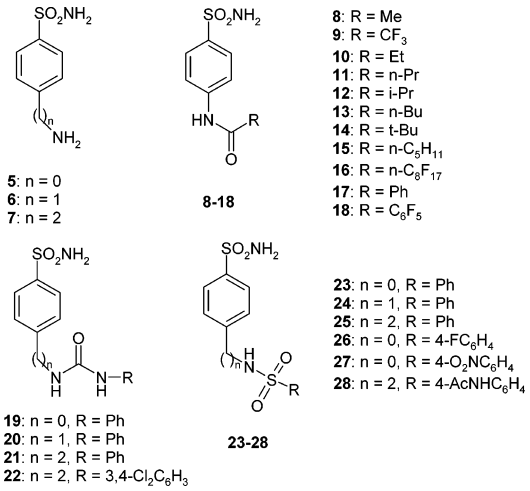
Considering that inhibition of the mitochondrial enzyme with sulfonamides was not investigated up to now, and that this information may be important for obtaining inhibitors with new pharmacological applications,[15] we conducted a detailed such study, using the murine full length isozymes mCA V. Inhibition data for clasical sulfonamide CA inhibitors (CAIs) used clinically, such as acetazolamide **1**, methazolamide **2**, ethoxzolamide **3**, and dichlorophenamide **4**, were obtained, together with



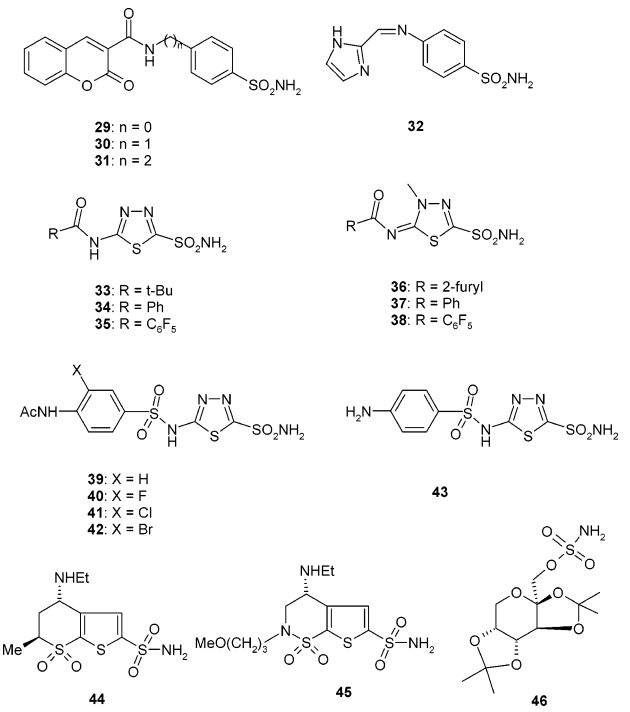
inhibition data for aromatic/heterocyclic sulfonamides possessing varied structures, designed in order to investigate the best inhibition/selectivity profiles against the mitochondrial isoform with respect to the cytosolic isozymes CA I and II and the membrane-associated isozyme CA IV, considered up to now to be responsible for most of the physiological functions of CAs in higher vertebrates.[1−3,14] Such compounds may be useful for the development of novel antiobesity therapies.[15]

Results

Chemistry. The structure of sulfonamides **1**−**45** and sulfamate **46** investigated for their interaction with mCA V (as well as with the cytosolic, physiologically relevant isozymes hCA I and II, and the membrane-bound isozyme bCA IV) is shown below.



Most of these sulfonamides were previously reported by our group,[16−24] except for **8**−**15**, **17**, **29**−**31**, and **37**, which are new and were prepared by acylation of sulfanilamide **5**, its homologues **6** and **7**, or 5-imino-4-methyl-δ^2-1,3,4-thiadiazoline-2-sulfonamide[25] with acyl chlorides in the presence of bases, as reported earlier for structurally related compounds.[16−25]



CA Inhibition. Inhibition data of four CA isozymes, i.e., the cytosolic hCA I and II, the membrane-bound bCA IV, and the mitochondrial mCA V with compounds **1**−**46** is shown in Table 1 (h denotes enzyme of human, b of bovine, and m of murine origin).

Discussion

Chemistry. Sulfonamides[1,2] and sulfamates[26,27] are the most potent CAIs reported up to now against the physiologically relevant isozymes CA I, II, and IV or the tumor-associated isozyme CA IX. Thus, we decided to explore a large series of sulfonamides of types **1**−**45** as well as the most potent sulfamate CA II inhibitor detected up to now, the clinically used antiepileptic drug topiramate **46**,[26] against the murine, full length mitochondrial isozyme, mCA V. Both aromatic and heterocyclic sulfonamides were included in this study, since the inhibition profile against the other investigated isozymes (i.e., CA I, II, IV, and IX) of these compounds is very different.[1,2] A major concern when designing CAIs is constituted by the general lack of selectivity of such compounds for the target isozyme, and as a consequence, the probable side effects of drugs based on them.[1,2] With very few exceptions (reviewed in ref 1) most sulfonamides undiscriminately inhibit all CA isozymes, although the affinity of particular isoforms for these inhibitors vary in a well defined manner, with CA II showing the highest affinity, generally followed by CA IX and IV,[1,2,26,27] whereas CA I is usually less inhibited by sulfonamides but has a higher affinity for metal-complexing anions.[28] Up to now, only the mCA V inhibition data of one sulfonamide inhibitor, acetazolamide **1** has been published,[29] and thus it is necessary to explore as many as possible aromatic/heterocyclic sulfonamides in order to detect both potent inhibitors, as well as leads that may show a favorable selectivity profile toward the mitochondrial isozyme with respect

Table 1. Inhibition Data of Compounds **1**−**46** against Isozymes CA I, II, IV, and V, at 20 °C

inhibitor	K_I[a] [nM] hCA I[b]	hCA II[b]	bCA IV[c]	mCA V[d]	selectivity ratio K_I (hCA II)/ K_I (mCA V)	inhibitor	K_I[a] [nM] hCA I[b]	hCA II[b]	bCA IV[c]	mCA V[d]	selectivity ratio K_I (hCA II)/ K_I (mCA V)
1	900	12	220	58	0.206	**24**	81	40	64	24	1.666
2	780	14	240	63	0.222	**25**	69	28	39	26	1.076
3	25	8	13	47	0.170	**26**	40	9	15	20	0.450
4	1200	38	380	640	0.059	**27**	31	10	22	18	0.555
5	28000	300	3000	1360	0.220	**28**	187	75	119	103	0.728
6	25000	170	2800	920	0.184	**29**	74	13	nt[e]	20	0.650
7	21000	160	2500	913	0.175	**30**	69	12	nt	20	0.600
8	21400	246	2850	212	1.160	**31**	66	10	nt	18	0.555
9	14600	133	2100	74	1.797	**32**	1000	120	130	245	0.489
10	19700	232	2630	210	1.104	**33**	645	10	185	54	0.185
11	19300	227	2540	206	1.101	**34**	900	15	163	76	0.197
12	23500	258	2840	233	1.107	**35**	250	2	10	13	0.153
13	17650	214	2370	85	2.517	**36**	290	6	30	87	0.068
14	20600	230	2550	96	2.395	**37**	850	17	174	83	0.204
15	14200	63	980	18	3.500	**38**	270	1.5	8	14	0.107
16	1400	66	850	13	5.076	**39**	10	3	8	9	0.333
17	3300	37	176	17	2.176	**40**	6	1	4	5	0.200
18	950	17	600	10	1.700	**41**	5	1	4	5	0.200
19	1200	240	460	21	11.428	**42**	5	2	4	4	0.500
20	800	105	140	20	5.250	**43**	6	2	5	6	0.333
21	430	75	150	15	5.000	**44**	50000	9	43	48	0.187
22	12	13	20	36	0.361	**45**	nt	3	45	51	0.058
23	103	49	85	23	2.130	**46**	250	5	54	63	0.079

[a] Errors in the range of 5−10% of the reported value (from three different assays). [b] Human (cloned) isozymes, by the 4-nitrophenyl acetate esterase method. [c] Bovine isozyme, isolated from lung microsomes, by the esterase method. [d] Full length murine, cloned isozyme, by the CO_2 hydration method. [e] nt = not tested.

to the cytosolic or membrane-bound isoforms mentioned above. The series of compounds included in our study comprises sulfonamides **1**−**45** and sulfamate **46**. Most of these sulfonamides were previously reported by this group, mainly in the search of topically acting antiglaucoma drugs based on CAIs,[16−24] but 13 compounds, such as **8**−**15**, **17**, **29**−**31**, and **37**, are new and were obtained from sulfanilamide **5**, its close analogues **6** and **7**, or 5-imino-4-methyl-δ^2-1,3,4-thiadiazoline-2-sulfonamide[25] by acylation reactions with aliphatic or aromatic acyl chlorides in the presence of bases (such as triethylamine or pyridine), as previously reported by this group for structurally related CAIs.[16−25] The new compounds reported here were characterized by standard physicochemical procedures that confirmed their structures (see Experimental Section for details).

CA Inhibition. Inhibition data of the four CA isozymes of interest here, i.e., CA I, II, IV, and V with compounds **1**−**46** are shown in Table 1. Since most CA I, II, and IV inhibition data were previously reported, only the CA V data will be discussed in detail, stressing on the other hand the selectivity issue of these sulfonamides with regards to the mitochondrial isozyme versus the cytosolic/membrane-associated ones. The following observations can be made: (i) several highly potent CA V inhibitors have been detected among the investigated sulfonamides, such as **15**−**27**, **29**−**31**, **35**, and **38**−**43**, these compounds showing inhibition constants in the range of 6−36 nM. These inhibitors both belong to the aromatic sulfonamide and heterocyclic compounds subseries. Thus, the acylated sulfanilamides **15**−**18**, the ureas derived from sulfanilamide, homosulfanilamide and 4-aminoethylbenzenesulfonamide **19**−**22**, the disulfonamides **23**−**27**, and the coumarinyl-substituted benzenesulfonamides **29**−**31** belong to the first subseries of potent, low nanomolar CA V inhibitors. It is obvious from the data of Table 1 that the best CA V inhibitory activity for the 4-acylated-sulfanilamide derivatives investigated here is associated with long aliphatic acyl (**15**), long perfluoroacyl (**16**), or aromatic acyl (**17**, **18**, **29**) moieties, whereas shorter aliphatic acyls lead to less effective CA V inhibitors (compounds **8**−**14**). For ureas **19**−**22**, CA V inhibitory activity increased from the sulfanilamide derivative **19** to the longer chain analogue **21**, with the phenyl moiety of the second urea nitrogen atom (for example in **21**) being more effective than the corresponding 3,4-dichlorophenyl-substituted analogue **22**. For the disulfonamides **23**−**27**, good CA V inhibitory activity was correlated with the presence of 4-phenylsufonylamido- or 4-substituted-phenylsulfonylamido moieties both for sulfanilamide (**23**, **26**, and **27**) and its homologues (**24** and **25**). Less effective was only the 4-acetylamidobenzenesulfonylamido-substituted compound **28**. Thus, a first structure−activity relationship (SAR) conclusion is that sulfanilamides, homosulfanilamides, or 4-aminoethylbenzenesulfonamides incorporating long acyl/perfluoroacyl, aromatic acyl, aryl ureas, or arylsulfonamido moieties substituting the free amino moiety of the leads **5**−**7** show low nanomolar affinity for mCA V. For the second subseries, of heterocyclic sulfonamides showing low nanomolar affinity for mCA V, i.e., compounds **35** and **38**−**43**, one may observe that the presence of perfluorobenzoyl moieties (in **35** and **38**) is beneficial for inducing strong inhibitory properties, whereas the corresponding benzoyl derivatives **34** and **37**, or the two compounds incorporating other acyls (**33** and **36**), show decreased CA V inhibitory properties. The best CA V inhibitors were, on the other hand, the aminobenzolamide derivatives **39**−**43**, which showed K_I values in the range of 4−9 nM. The lead itself, aminobenzolamide **43**, is already a very potent CA V inhibitor (K_I of 6 nM), whereas its acetylated derivative **39** is slightly less effective (K_I of 9 nM). The halogenated acetylated aminobenzolamides **40**−**42** are, on the other hand, more effective CA V inhibitors as compared to the parent,

unsubstituted compound **39**. One should stress that all inhibitors discussed at this paragraph show much stronger CA V inhibitory properties as compared to the clinically used CAIs, compounds **1**−**4** (see later in the text); (ii) a second group of compounds, such as **1**−**3**, **9**, **13**, **14**, **33**, **34**, **36**, **37**, and **44**−**46**, are effective mCA V inhibitors, with K_I values in the range of 47−96 nM, but they are less potent inhibitors as compared to the derivatives discussed above. In this group we find the clinically used CAIs acetazolamide **1**, methazolamide **2**, ethoxzolamide **3**, dorzolamide **44**, brinzolamide **45**, and topiramate **46**, as well as several close analogues of acetazolamide and methazolamide (**33**, **34**, **36**, and **37**). The remaining compounds are acylated sulfanilamides incorporating trifluoromethyl (**9**) *n*-butyl and *tert*-butyl moieties (**13** and **14**). The main SAR conclusion here is that surprisingly, it seems that fused bicyclic sulfonamides (such as **3**, **44** and **45**) or sugar-sulfamates (although only one such compound has been tested, and maybe the conclusion for this type of derivatives should be taken with caution) do not lead to the most effective CA V inhibitors, although for CA II, usually this type of inhibitors was the most effective (ethoxzolamide, dorzolamide, brinzolamide, and topiramate are among the most effective CA II inhibitors detected). Another conclusion is that the acylating moieties in position 5 for 1,3,4-thiadiazole-2-sulfonamide and the corresponding thiadiazoline-2-sulfonamide derivatives is of critical importance for the CA V inhibitory properties. These data show that both acylamido (such as in **35** and **38**) and especially arylsulfonamido (in **39**−**43**) moieties are highly beneficial for the CA V inhibitory properties, with aromatic moieties being preferred over the aliphatic ones; (iii) a third group of compounds, including **8**, **10**−**12**, **28**, and **32**, showed moderate CA V inhibitory properties, with K_I values in the range of 100−245 nM. These compounds include the acylated sulfanilamides (derivatives **8**, **10**−**12**) incorporating short acyl moieties (C_1−C_3), one of the disulfonamides (compound **28**) and the only Schiff's base investigated here, and another sulfanilamide derivative, compound **32**; (iv) the last group of sulfonamides (compounds **4**−**7**) include the weak CA V inhibitors demonstrated here, which showed K_I values in the range of 640−1360 nM. All these compounds are simple aromatic sulfonamides or bis-sulfonamides, such as dichlorophenamide **4**, sulfanilamide **5**, and its two close homologues **6** and **7**. The most ineffective CA V inhibitor in the entire series of 46 derivatives investigated here was sulfanilamide **5**, whereas the elongation of the molecule in **6** and **7** led to an enhanced inhibitory activity. The presence of two sulfonamide moieties, such as in dichlorophenamide **4**, further increased the CA V inhibitory properties. It should also be noted that derivatization of the simple aminosulfonamides **5**−**7** by a variety of acyl, arylsulfonyl, or ureido moieties, led to highly potent CA V inhibitors as compared to the parent sulfonamides, as illustrated well for compounds **8**−**32** discussed earlier.

A last important aspect regards the inhibition profiles of the different CA isozymes investigated here with these derivatives and the CA V selectivity issues related to it. Thus, at a first glance, the general trend observed is that hCA II has the highest affinity for these sulfonamide CAIs, followed by mCA V and bCA IV, whereas isozyme hCA I has the lowest affinity. Still, many exceptions from this general behavior are observed (see discussion later in the text). First of all, by defining a selectivity ratio between isozymes II and V as the ratio of the corresponding inhibition constants, one may see (Table 1) that most compounds have this ratio less than 1, obviously meaning that they better inhibit the cytosolic isozyme II and not the mitochondrial one, CA V. But 17 compounds in the series investigated here, i.e., **8**−**21** and **23**−**25**, show a selectivity ratio in the range of 1.1−11.4, possessing thus a higher affinity for the mitochondrial CA V, over the rapid cytosolic isozyme CA II. The most CA V selective inhibitor was urea **19**, followed by its close congeners **20** and **21** and the acylated sulfanilamides **15** and **16**. Since some of these compounds are also relatively efficient CA V inhibitors, with inhibition constants in the range of 13−20 nM, one may affirm that the acylated sulfanilamides and the ureido-benzenesulfonamides tend to be more CA V selective than the other derivatives investigated here. The most efficient CA V inhibitors detected on the other hand, such as **39**−**43**, all possess selectivity ratios in the range of 0.2−0.5, being much more efficient CA II inhibitors than CA V inhibitors, similarly with the clinically used derivatives **1**−**3** and **44**. The most CA II selective inhibitors (over CA V) were dichlorophenamide **4**, the furyl analogue of methazolamide **36**, brinzolamide **45**, and topiramate **46**, all of them showing selectivity ratios of 0.058−0.079. One of the investigated compounds, **22**, showed very efficient CA I inhibitory properties, possessing K_I values of the same order of magnitude for both cytosolic isozymes CA I and II, a behavior previously observed for several other ureido-sulfonamide derivatives.[1,17] Finally, most of these sulfonamides showed higher affinity for CA V over CA IV (such as **1**−**21**, **23**−**28**, **33**, **34**, and **37**), but some derivatives, such as **22**, **32**, **35**, **36**, and **39**−**46**, were more effective CA IV inhibitors and not CA V inhibitors (Table 1).

The compounds investigated here which possess potent inhibitory activity against CA II and CA V, may be useful for the prevention and treatment of obesity.[15] Indeed, in a recent patent it has been shown that topiramate, a potent CA II inhibitor and also an efficient CA V inhibitor, may be useful for this purpose.[15] It was in fact noted that many obese epilepsy patients treated with this antiepileptic drug lost 10−15% of their body weight,[39] and the same effect has also been demonstrated in an animal model of obesity,[40] but this "side effect" of the drug was never explained before the above-mentioned patent was published.[15,41] Antel et al.[15] demonstrated that the antiobesity activity of topiramate may be due among others to the CA II/CA V inhibition which has as a consequence a diminished de novo lipogenesis in adipocytes. Since many of the compounds investigated here show more potent CA II/V inhibitory properties in vitro, we expect that they may show better antiobesity activity, and thus a potential for clinical development.[41]

The supposed mechanism by which CA V inhibitors may reduce fat biosynthesis and thus be useful as antiobesity agents is schematically shown in Figure 1. Thus, in several important biosynthetic processes in-

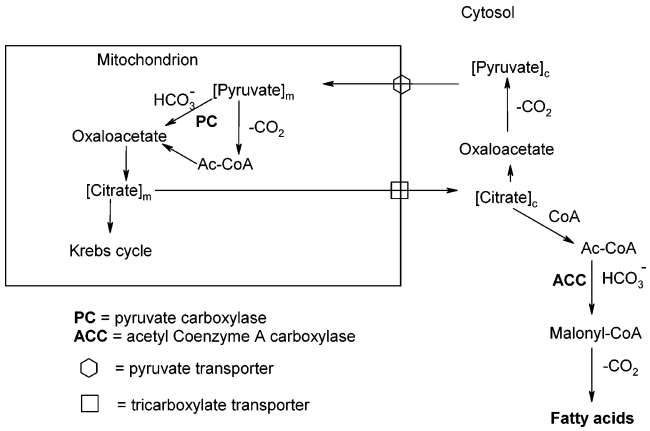


Figure 1. The transfer of acetyl groups from the mitochondrion to the cytosol (as citrate) for the provision of substrate for de novo lipogenesis.[41] All steps involving bicarbonate also need the presence of at least two CA isozymes: CA V in the mitochondrion and CA II in the cytosol (see discussion in the text).

volving pyruvate carboxylase (PC), acetyl CoA carboxylase (ACC), and carbamoyl phosphate synthetases I and II, bicarbonate, not carbon dioxide, is the real substrate of these carboxylating enzymes, and the provision of enough bicarbonate is assured mainly by the catalysis involving the mitochondrial isozyme CA V (probably assisted by the high activity cytosolic isozyme CA II). Mitochondrial PC is needed for the efflux of acetyl groups from the mitochondria to the cytosol where the fatty acid biosynthesis takes place.[8,41] Practically, pyruvate is carboxylated to oxaloacetate in the presence of bicarbonate and PC. The bicarbonate needed for this process is generated under the catalytic influence of the mitochondrial CA V. The mitochondrial membrane is impermeant to acetyl-CoA which condenses with oxaloacetate to form citrate, which is thereafter translocated to the cytoplasm by means of the tricarboxylic acid transporter. In the cytosol, the citrate is cleaved and regenerates acetyl-CoA and oxaloacetate. As oxaloacetate also cannot cross the mitochondrial membrane, its decarboxylation regenerates pyruvate which can be then transported into the mitochondria by means of the pyruvate transporter (Figure 1).[8,41] The acetyl-Co A thus generated in the cytosol is in fact used for the de novo lipogenesis, by carboxylation in the presence of ACC and bicarbonate, with formation of malonyl-Co A. The bicarbonate needed in this process is furnished by the CA II catalyzed conversion of CO_2 to bicarbonate. Subsequent steps involving the sequential transfer of acetyl groups lead to longer chain fatty acids.[8,41] As a whole, two CA isozymes are critical to the entire process of fatty acid biosynthesis: CA V within the mitochondria (to provide enough substrate to PC), and CA II within the cytosol (for providing sufficient substrate to ACC). It was in fact demonstrated that inhibition of CAs by sulfonamides (such as for example trifluoromethanesulfonamide (TFM), a very potent but unstable CA inhibitor[1]) can decrease lipogenesis in adipocytes in cell culture.[8−10] In such experiments, an undiscriminate inhibition of all CA isozymes present in these tissues is achieved by the used inhibitor, such as for example TFM or acetazolamide.[8−10] With more specific CA V inhibitors, as those described here, the process of fatty acid biosynthesis inhibition may be enhanced even more, and may thus explain the rationale of using CAIs in the treatment and prophylaxis of obesity.[15,41]

Conclusions

We investigated a large series of aromatic/heterocyclic sulfonamide and a sulfamate for their inhibitory properties against the mitochondrial isozyme CA V, comparing these inhibition data with those for the cytosolic isozymes CA I and II and the membrane-associated isoform CA IV. Several low nanomolar CA V inhibitors were detected (K_I values in the range of 4−15 nM), most of them belonging to the acylated sulfanilamide, ureido-benzenesulfonamide, 1,3,4-thiadiazole-2-sulfonamide, and aminobenzolamide type of compounds. The clinically used CAIs acetazolamide, methazolamide, ethoxzolamide, dorzolamide, brinzolamide, and topiramate on the other hand were less effective CA V inhibitors, showing inhibition constants in the range of 47−63 nM. Some of the investigated sulfonamides, such as the ureido-benzenesulfonamides and the acylated sulfanilamides showed higher affinity for CA V than for the other isozymes, CA II included, which is a remarkable result, since most investigated up to now CAIs inhibited better the cytosolic isozyme CA II. These results prompt us to hope that the selective inhibition of CA V, or the dual inhibition of CA II and CA V, may lead to the development of novel pharmacological applications of CAIs, for example for the treatment or prevention of obesity, by inhibiting CA-mediated lipogenetic processes.

Experimental Section

General. Melting points: heating plate microscope (not corrected); IR spectra: KBr pellets, 400−4000 cm^{-1} Karl Zeiss Jena UR-20 spectrometer; NMR spectra: Varian Gemini 300BB apparatus, operating at 300 MHz for ^1H NMR and at 75 MHz for ^{13}C NMR (chemical shifts are expressed as δ values relative to Me_4Si as internal standard for proton spectra and to the solvent resonance for carbon spectra). Attributions are given based on APT, COSY ($^1H-^1H$), and HETCOR ($^1H-^{13}C$) experiments. Elemental analysis (±0.4% of the theoretical values, calculated for the proposed structures): Carlo Erba Instruments CHNS Elemental Analyzer, Model 1106. All reactions were monitored by thin-layer chromatography (TLC), using 0.25 mm-thick precoated silica gel plates (E. Merck) eluted with MeOH:$CHCl_3$ 1:4 v/v unless specified otherwise. Sulfonamides/sulfamate **1**−**7** and **44**−**46** are commercially available and were from Sigma-Aldrich, Merck, Alcon, and Johnson & Johnson. Acyl halides used in the syntheses of compounds **8**−**15**, **17**, and **37** were commercially available (from Sigma-Aldrich or Fluka) and were used without additional purification. Coumarin-3-carboxylic acid was a gift from prof. G. Renzi (University of Florence). Acetonitrile, triethylamine, pyridine, methanol, ethanol, chloroform, ethyl acetate (E. Merck, Darmstadt, Germany), or other solvents/reagents used in the synthesis were double distilled and kept on molecular sieves in order to maintain them in anhydrous conditions. Sulfonamides **16**, **18**, **19**−**28**, **32**−**36**, and **38**−**43** were previously reported by this group.[16−24]

Chemistry. General Procedure for the Synthesis of Sulfonamides 8−15, 17, 29−31, and 37. Method A (Schotten−Baumann synthesis): Amino/imino-sulfonamide (5 mmol) (such as 5-imino-4-methyl-2-sulfonamido-δ^2-1,3,4-thiadiazoline,[25] sulfanilamide **5**, homo-sulfanilamide **6**, or 4-aminoethylbenzenesulfonamide **7**) was dissolved in 15 mL of 2.5 M NaOH and cooled to 2−5 °C in a salt-ice bath. The corresponding acyl chloride (5 mmol) was added in small portions, concomitantly with 10 mL of a 2 M NaOH solution, maintaining the temperature under 10 °C. The reaction mixture was then

stirred at room temperature for 5−10 h (TLC control) and then the pH was adjusted to 2 with 5 N HCl, and the precipitated sulfonamides were filtered and recrystallized from aqueous ethanol.

Method B: As above, but pyridine was used as solvent and no other base was necessary. After the reaction was performed, the excess pyridine was evaporated in vacuo and the reaction mixture poured in 50 mL of ice−water, and the precipitated sulfonamides were recrystallized as described above.

Method C: The sulfonamide to be derivatized was suspended/dissolved in a 1:1 mixture of acetone−water, and the stoichiometric amount of acyl chloride and base were added concomitantly. The base used may be NaOH, $NaHCO_3$, Et_3N, pyridine, etc. Good results were generally obtained when working with sodium bicarbonate as base. The reaction mixture was magnetically stirred at room temperature for several hours, the solvent was evaporated, and the reaction products were recrystallized as described above.

Coumarin-3-carboxylic acid was converted to the corresponding acyl chloride by reaction with thionyl chloride in benzene and was used for the preparation of derivatives **29**−**31** as described above (best yields were obtained by applying Method C).

4-Acetamido-benzenesulfonamide **8**, white crystals, mp 203−4 °C; IR (KBr), cm^{-1}: 1163 (SO_2^{sym}), 1375 (SO_2^{as}), 1672 (CONH); 3360 (NH, NH_2); ^1H NMR (DMSO-d_6), δ, ppm: 2.54 (s, 3H, Me); 7.09 (s, 2H, SO_2NH_2), 7.81 (d, 2H, AA′BB′, 8.9 Hz), 7.94 (d, 2H, AA′BB′, 8.9 Hz), 8.07 (br s, CONH); ^{13}C NMR (DMSO-d_6), δ, ppm: 21.4 (Me); 126.56 (C2/C3-Ph), 128.45 (C3/C2-Ph), 139.60 (C1/C4-Ph), 141.37 (C4/C1-Ph); 162.39 (CO); Anal. ($C_8H_{10}N_2O_3S$) C, H, N.

4-Trifluoroacetamido-benzenesulfonamide **9**, white crystals, mp 185−6 °C; IR (KBr), cm^{-1}: 1159 (SO_2^{sym}), 1374 (SO_2^{as}), 1673 (CONH); 3360 (NH, NH_2); ^1H NMR (DMSO-d_6), δ, ppm: 7.33 (s, 2H, SO_2NH_2), 7.80 (d, 2H, AA′BB′, 8.9 Hz), 7.95 (d, 2H, AA′BB′, 8.9 Hz), 8.04 (br s, CONH); ^{13}C NMR (DMSO-d_6), δ, ppm: 44.2 (CF_3); 126.67 (C2/C3-Ph), 128.26 (C3/C2-Ph), 139.49 (C1/C4-Ph), 141.80 (C4/C1-Ph); 163.62 (CO); Anal. ($C_8H_7F_3N_2O_3S$) C, H, N.

4-Propionamido-benzenesulfonamide **10**, white crystals, mp 190−1 °C; IR (KBr), cm^{-1}: 1162 (SO_2^{sym}), 1375 (SO_2^{as}), 1675 (CONH); 3360 (NH, NH_2); ^1H NMR (DMSO-d_6), δ, ppm: 1.33 (t, 3H, Me); 3.27 (q, 2H, CH_2); 7.09 (s, 2H, SO_2NH_2), 7.82 (d, 2H, AA′BB′, 8.9 Hz), 7.94 (d, 2H, AA′BB′, 8.9 Hz), 8.05 (br s, CONH); ^{13}C NMR (DMSO-d_6), δ, ppm: 16.1 (Me); 42.5 (CH_2); 126.60 (C2/C3-Ph), 128.13 (C3/C2-Ph), 139.07 (C1/C4-Ph), 141.45 (C4/C1-Ph); 162.13 (CO); Anal. ($C_9H_{12}N_2O_3S$) C, H, N.

4-*n*-Butanamido-benzenesulfonamide **11**, white crystals, mp 187−8 °C; IR (KBr), cm^{-1}: 1162 (SO_2^{sym}), 1376 (SO_2^{as}), 1675 (CONH); 3360 (NH, NH_2); ^1H NMR (DMSO-d_6), δ, ppm: 1.26 (t, 3H, J = 7.3 Hz); 1.62 (m, 2H), 3.43 (t, 2H, J = 6.2 Hz), 7.11 (s, 2H, SO_2NH_2), 7.80 (d, 2H, AA′BB′, 8.9 Hz), 7.92 (d, 2H, AA′BB′, 8.9 Hz), 8.05 (br s, CONH); ^{13}C NMR (DMSO-d_6), δ, ppm: 12.1 (Me); 22.7 (CH_2); 42.5 (CH_2); 126.19 (C2/C3-Ph), 128.17 (C3/C2-Ph), 139.34 (C1/C4-Ph), 141.59 (C4/C1-Ph); 162.76 (CO); Anal. ($C_{10}H_{14}N_2O_3S$) C, H, N.

4-Isobutanamido-benzenesulfonamide **12**, white crystals, mp 164−6 °C; IR (KBr), cm^{-1}: 1158 (SO_2^{sym}), 1374 (SO_2^{as}), 1673 (CONH); 3360 (NH, NH_2); ^1H NMR (DMSO-d_6), δ, ppm: 1.28 (d, 6H, J = 6.2 Hz); 3.87 (m, 1H), 7.13 (s, 2H, SO_2NH_2), 7.76 (d, 2H, AA′BB′, 8.9 Hz), 7.95 (d, 2H, AA′BB′, 8.9 Hz), 8.02 (br s, CONH); ^{13}C NMR (DMSO-d_6), δ, ppm: 22.4 (Me); 67.8 (CH); 126.24 (C2/C3-Ph), 128.36 (C3/C2-Ph), 139.19 (C1/C4-Ph), 141.46 (C4/C1-Ph); 162.50 (CO); Anal. ($C_{10}H_{14}N_2O_3S$) C, H, N.

4-*n*-Pentanamido-benzenesulfonamide **13**, white crystals, mp 186−7 °C; IR (KBr), cm^{-1}: 1163 (SO_2^{sym}), 1370 (SO_2^{as}), 1675 (CONH); 3360 (NH, NH_2); ^1H NMR (DMSO-d_6), δ, ppm: 0.92 (t, 3H, J = 7.1 Hz); 1.35 (m, 2H), 1.58 (m, 2H), 3.87 (t, 2H, J = 6.4 Hz), 7.08 (s, 2H, SO_2NH_2), 7.80 (d, 2H, AA′BB′, 8.9 Hz), 7.94 (d, 2H, AA′BB′, 8.9 Hz), 8.05 (br s, CONH); ^{13}C NMR (DMSO-d_6), δ, ppm: 14.0 (Me); 19.1 (CH_2); 31.1, (CH_2); 42.3 (CH_2); 126.18 (C2/C3-Ph), 128.21 (C3/C2-Ph), 139.30 (C1/C4-Ph), 141.64 (C4/C1-Ph); 162.60 (CO); Anal. ($C_{11}H_{16}N_2O_3S$) C, H, N.

4-Pivaloylamido-benzenesulfonamide **14**, white crystals, mp 162−4 °C; IR (KBr), cm^{-1}: 1158 (SO_2^{sym}), 1374 (SO_2^{as}), 1672 (CONH); 3360 (NH, NH_2); ^1H NMR (DMSO-d_6), δ, ppm: 0.95 (s, 9H, t-Bu); 7.02 (s, 2H, SO_2NH_2), 7.80 (d, 2H, AA′BB′, 8.9 Hz), 7.91 (d, 2H, AA′BB′, 8.9 Hz), 8.01 (br s, CONH); ^{13}C NMR (DMSO-d_6), δ, ppm: 9.4 (Me); 40.3 (C tertiary); 126.24 (C2/C3-Ph), 128.33 (C3/C2-Ph), 139.32 (C1/C4-Ph), 141.51 (C4/C1-Ph); 162.39 (CO); Anal. ($C_{11}H_{16}N_2O_3S$) C, H, N.

4-*n*-Hexanamido-benzenesulfonamide **15**, white crystals, mp 132−5 °C; IR (KBr), cm^{-1}: 1159 (SO_2^{sym}), 1368 (SO_2^{as}), 1675 (CONH); 3360 (NH, NH_2); ^1H NMR (DMSO-d_6), δ, ppm: 0.89 (t, 3H, J = 6.8 Hz); 1.30 (m, 4H), 1.62 (m, 2H), 3.94 (t, 2H, J = 6.4 Hz), 7.07 (s, 2H, SO_2NH_2), 7.79 (d, 2H, AA′BB′, 8.9 Hz), 7.90 (d, 2H, AA′BB′, 8.9 Hz), 8.04 (br s, CONH); ^{13}C NMR (DMSO-d_6), δ, ppm: 14.2 (Me); 19.9 (CH_2); 28.7 (CH_2); 28.9 (CH_2); 42.1 (CH_2); 126.24 (C2/C3-Ph), 128.35 (C3/C2-Ph), 139.33 (C1/C4-Ph), 141.70 (C4/C1-Ph); 162.54 (CO); Anal. ($C_{12}H_{18}N_2O_3S$) C, H, N.

4-Benzoylamino-benzenesulfonamide **17**, white crystals, mp 209−10 °C; IR (KBr), cm^{-1}: 1154 (SO_2^{sym}), 1373 (SO_2^{as}), 1673 (CONH); 3360 (NH, NH_2); ^1H NMR (DMSO-d_6), δ, ppm: 7.12 (s, 2H, SO_2NH_2), 7.20−7.55 (m, 5H, Ph); 7.78 (d, 2H, AA′BB′, 8.9 Hz), 7.92 (d, 2H, AA′BB′, 8.9 Hz), 8.06 (br s, CONH); ^{13}C NMR (DMSO-d_6), δ, ppm: 123.05 (Ph); 126.24 (C2/C3-Ph), 127.41(Ph); 128.35 (C3/C2-Ph), 130.62 (Ph); 139.33 (C1/C4-Ph), 141.70 (C4/C1-Ph); 151.13 (Ph); 166.59 (CO); Anal. ($C_{13}H_{12}N_2O_3S$) C, H, N.

4-(Coumarin-3-yl-carboxamido)benzenesulfonamide **29**, white crystals, 166−8 °C; IR (KBr), cm^{-1}: 1159 (SO_2^{sym}), 1362 (SO_2^{as}), 1676 (CONH); 3360 (NH, NH_2); ^1H NMR (DMSO-d_6), δ, ppm: 6.84 (s, 1H, 4H of coumarin); 7.12 (s, 2H, SO_2NH_2), 7.16−7.58 (m, 4H, coumarin); 7.79 (d, 2H, AA′BB′, 8.9 Hz), 7.90 (d, 2H, AA′BB′, 8.9 Hz), 8.04 (br s, CONH); ^{13}C NMR (DMSO-d_6), δ, ppm: 123.45; 126.24 (C2/C3-Ph), 127.40; 128.35 (C3/C2-Ph), 130.13; 139.33 (C1/C4-Ph), 141.70 (C4/C1-Ph); 151.13; 153.34; 158.50, 166.59 (CONH); 176.33 (CO−O); Anal. ($C_{16}H_{12}N_2O_5S$) C, H, N.

4-(Coumarin-3-yl-carboxamidomethyl)benzenesulfonamide **30**, white crystals, 169−70 °C; IR (KBr), cm^{-1}: 1159 (SO_2^{sym}), 1364 (SO_2^{as}), 1675 (CONH); 3360 (NH, NH_2); ^1H NMR (DMSO-d_6), δ, ppm: 4.49 (d, 2H, 6.0 Hz), 6.84 (s, 1H, 4H of coumarin); 7.10 (s, 2H, SO_2NH_2), 7.16−7.57 (m, 4H, coumarin); 7.79 (d, 2H, AA′BB′, 8.9 Hz), 7.92 (d, 2H, AA′BB′, 8.9 Hz), 8.04 (br s, CONH); ^{13}C NMR (DMSO-d_6), δ, ppm: 41.54 (CH_2); 123.40; 126.24 (C2/C3-Ph), 127.46; 128.43 (C3/C2-Ph), 130.25; 139.14 (C1/C4-Ph), 141.62 (C4/C1-Ph); 151.76; 153.67; 158.48, 166.52 (CONH); 176.40 (CO−O); Anal. ($C_{17}H_{14}N_2O_5S$) C, H, N.

4-(Coumarin-3-yl-carboxamidoethyl)benzenesulfonamide **31**, white crystals, 160−1 °C; IR (KBr), cm^{-1}: 1158 (SO_2^{sym}), 1363 (SO_2^{as}), 1675 (CONH); 3360 (NH, NH_2); ^1H NMR (DMSO-d_6), δ, ppm: 2.91 (t, 2H, 7.2 Hz), 3.45 (q, 2H, 6.5 Hz), 6.83 (s, 1H, 4H of coumarin); 7.11 (s, 2H, SO_2NH_2), 7.18−7.58 (m, 4H, coumarin); 7.79 (d, 2H, AA′BB′, 8.9 Hz), 7.92 (d, 2H, AA′BB′, 8.9 Hz), 8.05 (br s, CONH); ^{13}C NMR (DMSO-d_6), δ, ppm: 35.14 (CH_2), 39.70, (N−CH_2), 123.40; 126.21 (C2/C3-Ph), 127.45; 128.58 (C3/C2-Ph), 130.36; 139.09 (C1/C4-Ph), 141.55 (C4/C1-Ph); 151.41; 153.38; 158.44, 166.19 (CONH); 176.32 (CO−O); Anal. ($C_{18}H_{16}N_2O_5S$) C, H, N.

5-Benzoylimino-4-methyl-Δ^2-1,3,4-thiadiazoline-2-sulfonamide, **37**, white crystals, mp 280−1 °C (MeOH). IR (KBr), cm^{-1}: 1175 (SO_2^{sym}), 1325 (SO_2^{as}), 1600 (CONH); ^1H NMR (d_6-DMSO), δ, ppm; J, Hz: 4.90 (s, 3H, Me); 7.20−7.55 (m, 5H, Ph); 8.51 (s, 2H, SO_2NH_2); ^{13}C NMR (DMSO-d_6), δ, ppm: 42.13 (Me); 123.13 (Ph); 127.30 (Ph); 130.48 (Ph); 151.04 (Ph); 157.91 (CONH), 164.91 (C-thiadiazole), 164.94 (C-thiadiazole); Anal. ($C_{10}H_{10}N_4O_3S_2$) C, H, N.

CA Inhibition. Human CA I and CA II cDNAs were expressed in *Escherichia coli* strain BL21 (DE3) from the plasmids pACA/hCA I and pACA/hCA II described by Lindskog's group.[30] Cell growth conditions were those described in the literature,[31] and enzymes were purified by affinity chromatography according to the method of Khalifah et al.[32] Enzyme concentrations were determined spectrophotometri-

cally at 280 nm, utilizing a molar absorptivity of 49 mM^{-1} cm^{-1} for CA I and 54 $mmol^{-1}$ cm^{-1} for CA II, respectively, based on M_r = 28.85 kDa for CA I, and 29.3 kDa for CA II, respectively.[33,34] bCA IV was isolated from bovine lung microsomes as described by Maren et al., and its concentration has been determined by titration with ethoxzolamide.[35]

The mCAV cDNAs (a gift of Prof. P. Laipis, University of Florida) was amplified by using PCR and specific primers for the vector pCAL-n-FLAG (from Stratagene, Milan, Italy). The obtained construct was inserted in the pCAL-n-FLAG vector and then cloned and expressed in *E. coli* strain BL21-GOLD-(DE3) (from Stratagene) as described by Heck et al.[29] In contrast to the report of Heck et al.,[29] no CA V activity was found in the cell lysate, but all enzyme was present in inclusion bodies from which it was purified as described below. The bacterial cells were lysed by sonification and homogenated in a buffered solution (pH 8) of 4 M urea and 2% Triton X-100. The homogenate thus obtained was extensively centrifuged (11 000*g*) in order to remove soluble and membrane-associated proteins as well as other cellular debris. The resulting pellet was washed by repeated homogenation and centrifugation in water, to remove the remaining urea and Triton X-100. Purified CA V inclusion bodies were denaturated in 6 M guanidine hydrochloride and refolded into the active form by snap dilution into a solution of 100 mM MES (pH 6), 500 mM L-arginine, 2 mM $ZnCl_2$, 2 mM EDTA, 2 mM reduced glutathione, and 1 mM oxidized glutathione. Active mCA V were extensively dialyzed into a solution of 10 mM Hepes (pH 7.5), 10 mM Tris HCl, 100 mM Na_2SO_4, and 1 mM $ZnCl_2$ and further purified by sulfonamide affinity chromatography.[32] The amount of protein was determined by spectrophometric measurements and its activity by stopped-flow enzymatic assays, with CO_2 as substrate.

Initial rates of 4-nitrophenylacetate hydrolysis catalyzed by different CA isozymes were monitored spectrophotometrically, at 400 nm, with a Cary 3 instrument interfaced with an IBM-compatible PC.[36] Solutions of substrate were prepared in anhydrous acetonitrile; the substrate concentrations varied between 2×10^{-2} and 1×10^{-6} M, working at 25 °C. A molar absorption coefficient ϵ of 18 400 M^{-1} cm^{-1} was used for the 4-nitrophenolate formed by hydrolysis, in the conditions of the experiments (pH 7.40), as reported in the literature.[36] Non-enzymatic hydrolysis rates (which were around 0.5−0.9% of the catalytic rates) were always subtracted from the observed rates. Triplicate experiments were done for each inhibitor concentration, and the values reported throughout the paper are the mean of such results. Stock solutions of inhibitor (1 mM) were prepared in distilled−deionized water with 10−20% (v/v) DMSO (which is not inhibitory at these concentrations) and dilutions up to 0.01 nM were done thereafter with distilled−deionized water. Inhibitor and enzyme solutions were preincubated together for 10 min at room temperature prior to assay, to allow for the formation of the E−I complex. The inhibition constant K_I was determined as described by Pocker and Stone, for isozymes I, II, and IV.[36] Enzyme concentrations were 3.0 μM for CA II, 10.5 μM for CA I and 25 μM for CA IV (this isozyme has a decreased esterase activity[37] and higher concentrations had to be used for the measurements).

An SX.18MV-R Applied Photophysics stopped-flow instrument has been used for assaying the CA IX CO_2 hydration activity.[38] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 10 mM Hepes (pH 7.5) as buffer, 0.1 M Na_2SO_4 (for maintaining constant the ionic strength), following the CA-catalyzed CO_2 hydration reaction for a period of 10−100 s. Saturated CO_2 solutions in water at 20 °C were used as substrate. Stock solutions of inhibitors were prepared at a concentration of 1−3 mM (in DMSO−water 1:1, v/v) and dilutions up to 0.1 nM done with the assay buffer mentioned above. Enzyme concentration was 0.1 μM, and inhibition constants were calculated as described in the literature.[38]

Acknowledgment. Thanks are addressed to Dr. P. Laipis (University of Florida, Gainesville) for the gift of the mCA V cDNA and to Prof. G. Renzi (University of Florence) for the gift of coumarin-3-carboxylic acid.

References

(1) (a) Supuran, C. T.; Scozzafava, A.; Conway, J. (Eds.) *Carbonic Anhydrase − Its Inhibitors and Activators*; CRC Press: Boca Raton, FL, 2004; pp 1−369. (b) Supuran, C. T.; Scozzafava, A.; Casini, A. Carbonic anhydrase inhibitors. *Med. Res. Rev.* **2003**, *23*, 146−189.

(2) (a) Supuran, C. T.; Scozzafava, A. Carbonic anhydrase inhibitors and their therapeutic potential. *Exp. Opin. Ther. Pat.* **2000**, *10*, 575−600. (b) Supuran, C. T.; Scozzafava, A. Applications of carbonic anhydrase inhibitors and activators in therapy. *Exp. Opin. Ther. Pat.* **2002**, *12*, 217−242.

(3) Hewett-Emmett, D. Evolution and distribution of the carbonic anhydrase gene families. In *The Carbonic Anhydrases − New Horizons*, Chegwidden, W. R.; Carter, N.; Edwards, Y., Eds.; Birkhauser Verlag: Basel, Switzerland, 2000; pp 29−78.

(4) Smith, K. S.; Ferry, J. G. Prokaryotic carbonic anhydrases. *FEMS Microbiol. Rev.* **2000**, *24*, 335−366.

(5) Dodgson, S. J. Inhibition of mitochondrial carbonic anhydrase: a discrepancy examined. *J. Appl. Physiol.* **1987**, *63*, 2134−2141.

(6) Dodgson, S. J.; Cherian, K. Mitochondrial carbonic anhydrase is involved in rat renal glucose synthesis. *Am. J. Physiol.* **1989**, *257*, E791−E796.

(7) (a) Spencer, I. M.; Hargreaves, I.; Chegwidden, W. R. Effect of the carbonic anhydrase inhibitor acetazolamide on lipid synthesis in the locust. *Biochem. Soc. Trans.* **1988**, *16*, 973−974. (b) Chegwidden, W. R.; Spencer, I. M. Carbonic anhydrase provides bicarbonate for de novo lipogenesis in the locus. *Compd. Biochem. Physiol.* **1996**, *115B*, 247−254.

(8) Chegwidden, W. R.; Dodgson, S. J.; Spencer, I. M. The roles of carbonic anhydrase in metabolism, cell growth and cancer in animals. In *The Carbonic Anhydrases − New Horizons*; Chegwidden, W. R., Edwards, Y., Carter, N., Eds.; Birkhäuser Verlag: Basel, 2000; pp 343−363.

(9) Lynch, C. J.; Fox, H.; Hazen, S. A.; Stanley, B. A.; Dodgson, S. J.; Lanoue, K. F. Role of hepatic carbonic anhydrase in de novo lipogenesis. *Biochem. J.* **1995**, *310*, 197−202.

(10) Hazen, S. A.; Waheed, A.; Sly, W. S.; Lanoue, K. F.; Lynch, C. J. Differentiation-dependent expression of CA V and the role of carbonic anhydrase isozymes in pyruvate carboxylation in adipocytes. *FASEB J.* **1996**, *10*, 481−490.

(11) Atwood, P. V. The structure and mechanism of action of pyruvate carboxylase. *Int. J. Biochem. Cell Biol.* **1995**, *27*, 231−249.

(12) Alldred, J. B.; Reilly, K. E. Short-term regulation of acetyl CoA carboxylase in tissues of higher animals. *Prog. Lipid Res.* **1997**, *35*, 371−385.

(13) Forster, R. E.; Dodgson, S. J. Membrane transport and provision of substrates for carbonic anhydrase in vertebrates. In *The Carbonic Anhydrases − New Horizons*; Chegwidden, W. R., Edwards, Y., Carter, N., Eds.; Birkhäuser Verlag: Basel, 2000; pp 263−280.

(14) (a) Parkkila, S. An overview of the distribution and function of carbonic anhydrase isozymes in mammals. In *The Carbonic Anhydrases − New Horizons*; Chegwidden, W. R., Edwards, Y., Carter, N., Eds.; Birkhäuser Verlag: Basel, 2000; pp 79−94. (b) Parkkila, S.; Parkkila, A. K.; Kivelä, J. Role of carbonic anhydrase and its inhibitors in biological science related to gastroenterology, neurology and nephrology. In *Carbonic anhydrase − Its inhibitors and activators*; Supuran, C. T.; Scozzafava, A.; Conway, J., Eds., CRC Press: Boca Raton, FL, 2004; in press.

(15) Hebebrand, J.; Antel, J.; Preuschoff, U.; David, S.; Sann, H.; Weske, M. Method for locating compounds which are suitable for the treatment and/or prophylaxis of obesity. WO Patent 07821, 2002.

(16) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C. T. Carbonic anhydrase inhibitors: Perfluoroalkyl/aryl-substituted derivatives of aromatic/heterocyclic sulfonamides as topical intraocular pressure lowering agents with prolonged duration of action. *J. Med. Chem.* **2000**, *43*, 4542−4551.

(17) Supuran, C. T.; Scozzafava, A.; Jurca, B. C.; Ilies, M. A. Carbonic anhydrase inhibitors. Part 49. Synthesis of substituted ureido- and thioureido derivatives of aromatic/heterocyclic sulfonamides with increased affinities for isozyme I. *Eur. J. Med. Chem.* **1998**, *33*, 83−93.

(18) Clare, B. W.; Supuran, C. T. Carbonic anhydrase inhibitors. Part 61. Quantum chemical QSAR of a group of benzenedisulfonamides. *Eur. J. Med. Chem.* **1999**, *34*, 463−474.

(19) Supuran, C. T.; Briganti, F.; Scozzafava, A. Sulfenamido-sulfonamides as inhibitors of carbonic anhydrase isozymes I, II and IV. *J. Enzyme Inhib.* **1997**, *12*, 175−190.

(20) Supuran, C. T.; Nicolae, A.; Popescu, A. Carbonic anhydrase inhibitors. Part 35. Synthesis of Schiff bases derived from sulfanilamide and aromatic aldehydes: the first inhibitors with equally high affinity towards cytosolic and membrane-bound isozymes. *Eur. J. Med. Chem.* **1996**, *31*, 431−438.
(21) Almajan, L. G.; Supuran, C. T. Carbonic anhydrase inhibitors. Part 30. Complexes of 5-pivaloylamido-1,3,4-thiadiazole-2-sulfonamide with trivalent metal ions. *Rev. Roumaine Chim.* **1997**, *42*, 593−597.
(22) Jitianu, A.; Ilies, M. A.; Briganti, F.; Scozzafava, A.; Supuran, C. T. Complexes with biologically active ligands. Part 9. Metal complexes containing of 5-benzoylamino- and 5-(3-nitrobenzoylamino)-1,3,4-thiadiazole-2-sulfonamide as carbonic anhydrase inhibitors. *Metal Based Drugs* **1997**, *4*, 1−8.
(23) Ilies, M.; Supuran, C. T.; Scozzafava, A.; Casini, A.; Mincione, F.; Menabuoni, L.; Caproiu, M. T.; Maganu, M.; Banciu, M. D. Carbonic anhydrase inhibitors. Sulfonamides incorporating furan-, thiophene- and pyrrole-carboxamido groups possess strong topical intraocular pressure lowering properties as aqueous suspensions. *Bioorg. Med. Chem.* **2000**, *8*, 2145−2155.
(24) Ilies, M. A.; Vullo, D.; Pastorek, J.; Scozzafava, A.; Ilies, M.; Caproiu, M. T.; Pastorekova, S.; Supuran, C. T. Carbonic anhydrase inhibitors. Inhibition of tumor-associated isozyme IX with halogenosulfanilamide and halogeno-aminophenylbenzolamide derivatives. *J. Med. Chem.* **2003**, *46*, 2187−2196.
(25) (a) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C. T. Carbonic anhydrase inhibitors: Synthesis of water-soluble, topically effective intraocular pressure lowering aromatic/heterocyclic sulfonamides containing cationic or anionic moieties: is the tail more important than the ring? *J. Med. Chem.* **1999**, *42*, 2641−2650. (b) Scozzafava, A.; Briganti, F.; Mincione, G.; Menabuoni, L.; Mincione, F.; Supuran, C. T. Carbonic anhydrase inhibitors: Synthesis of water-soluble, amino acyl/dipeptidyl sulfonamides possessing long-lasting intraocular pressure-lowering properties via the topical route. *J. Med. Chem.* **1999**, *42*, 3690−3700.
(26) Casini, A.; Antel, J.; Abbate, F.; Scozzafava, A.; David, S.; Waldeck, H.; Schäfer, S.; Supuran, C. T. Carbonic anhydrase inhibitors: SAR and X-ray crystallographic study for the interaction of sugar sulfamates/sulfamides with isozymes I, II and IV. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 841−845.
(27) Winum, J.-Y.; Vullo, D.; Casini, A.; Montero, J.-L.; Scozzafava, A.; Supuran, C. T. Carbonic anhydrase inhibitors: Inhibition of cytosolic isozymes I and II and the transmembrane, tumor-associated isozyme IX with sulfamates including EMATE also acting as steroid sulfatase inhibitors. *J. Med. Chem.* **2003**, *46*, 2197−2204.
(28) Franchi, M.; Vullo, D.; Gallori, E.; Antel, J.; Wurl, M.; Scozzafava, A.; Supuran, C. T. Carbonic anhydrase inhibitors. Inhibition of human and murine mitochondrial isozymes V with anions. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2857−2861.
(29) Heck, R. W.; Tanhauser, S. M.; Manda, R.; Tu, C.; Laipis, P. J.; Silverman, D. N. Catalytic properties of mouse carbonic anhydrase V. *J. Biol. Chem.* **1994**, *269*, 24742−24746.
(30) Lindskog, S.; Behravan, G.; Engstrand, C.; Forsman, C.; Jonsson, B. H.; Liang, Z.; Ren, X.; Xue, Y. Structure−function relations in human carbonic anhydrase II as studied by site-directed mutagenesis. In *Carbonic anhydrase − From biochemistry and genetics to physiology and clinical medicine*; Botrè, F., Gros, G., Storey, B. T., Eds., VCH: Weinheim, 1991; pp 1−13.
(31) Behravan, G.; Jonsson, B. H.; Lindskog, S. Fine-tuning of the catalytic properties of carbonic anhydrase. Studies of a Thr200-His variant of human isoenzyme II. *Eur. J. Biochem.* **1990**, *190*, 351−357.
(32) Khalifah, R. G.; Strader, D. J.; Bryant, S. H.; Gibson, S. M. Carbon-13 nuclear magnetic resonance probe of active site ionization of human carbonic anhydrase B. *Biochemistry*, **1977**, *16*, 2241−2247.
(33) Lindskog, S.; Coleman, J. E. The catalytic mechanism of carbonic anhydrase. *Proc. Natl. Acad Sci. U.S.A.* **1964**, *70*, 2505−2508.
(34) Steiner, H.; Jonsson, B. H.; Lindskog, S. The catalytic mechanism of carbonic anhydrase. Hydrogen-isotope effects on the kinetic parameters of the human C isoenzyme. *Eur. J. Biochem.* **1975**, *59*, 253−259.
(35) Maren, T. H.; Wynns, G. C.; Wistrand, P. J. Chemical properties of carbonic anhydrase IV, the membrane-bound enzyme. *Mol. Pharmacol.* **1993**, *44*, 901−906.
(36) Pocker, Y.; Stone, J. T. The catalytic versatility of erythrocyte carbonic anhydrase. III. Kinetic studies of the enzyme-catalyzed hydrolysis of *p*-nitrophenyl acetate. *Biochemistry* **1967**, *6*, 668−678.
(37) Baird, T. T.; Waheed, A.; Okuyama, T.; Sly, W. S.; Fierke, C. A. Catalysis and inhibition of human carbonic anhydrase IV. *Biochemistry* **1997**, *36*, 2669−2678.
(38) Khalifah, R. G. The carbon dioxide hydration activity of carbonic anhydrase. *J. Biol. Chem.* **1971**, *246*, 2561−2573.
(39) Gordon, A.; Price, L. H. Mood stabilization and weight loss with topiramate. *Am. J. Psychiatry* **1999**, *156*, 968−969.
(40) Picard, F.; Deshaies, Y.; Lalonde, J.; Samson, P.; Richard, D. Topiramate reduces energy and fat gains in lean (*Fa*/?) and obese (*fa*/*fa*) Zucker rats. *Obesity Res.* **2000**, *8*, 656−663.
(41) Supuran, C. T. Carbonic anhydrase inhibitors in the treatment and prophylaxis of obesity. *Exp. Opin. Ther. Pat.* **2003**, *13*, 1545−1550.

JM031057+