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Carbonic Anhydrase Inhibitors: 4-Sulfamoyl-benzenecarboxamides and 4-Chloro-3-sulfamoyl-benzenecarboxamides with Strong Topical Antiglaucoma Properties

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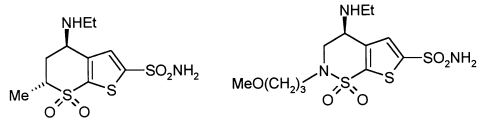
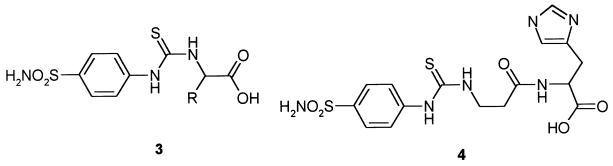
Abstract—Reaction of 4-carboxy-benzenesulfonamide or 4-chloro-3-sulfamoyl benzoic acid with carboxy-protected amino acids/dipeptides, or aromatic/heterocyclic sulfonamides/mercaptans afforded the corresponding benzene-carboxamide derivatives. These were tested as inhibitors of three carbonic anhydrase (CA) isozymes, CA I, II and IV. Some of the new derivatives showed affinity in the low nanomolar range for isozymes CA II and IV, involved in aqueous humor secretion within the eye, and were tested as topically acting anti-glaucoma agents, in normotensive and glaucomatoous rabbits. Good in vivo activity and prolonged duration of action has been observed for some of these derivatives, as compared to the clinically used drugs dorzolamide and brinzolamide. Some of the 4-chloro-3-sulfamoyl benzenecarboxamides reported here showed higher affinity for CA I than for the sulfonamide avid isozyme CA II.

Introduction

Inhibition of carbonic anhydrase (CA, EC 4.2.1.1) with sulfonamides constitutes one of the most physiological approaches for the treatment of glaucoma, a condition associated with elevated intraocular pressure (IOP) due to excessive bicarbonate secretion within the aqueous humor.[1–3] This secretion is mediated by isozymes CA II and CA IV present within ciliary processes of the eye, and its inhibition by topically acting sulfonamides, such as dorzolamide **1** or brinzolamide **2** (which are low nanomolar CA II inhibitors) are widely used clinically, alone or in combination with other agents, such as β-blockers, prostaglandin derivatives or α-adrenergic agonists among others.[1–3]

The presently available topically acting sulfonamides **1** and **2** are imperfect due to many topical side effects observed after their administration. These are mainly due either to the acidic pH of their solutions (in the case of **1**) or to their low solubility and need to be administered as suspensions (in the case of **2**).[1–3] Thus, much research has been done in the last years for developing alternative agents, devoid of the major draw-backs of the dorzolamide-type agents.[4–8]





A successful approach recently designed by us[4a] in order to avoid many of the side effects of dorzolamide due to the high acidity of its ophthalmologic solutions,

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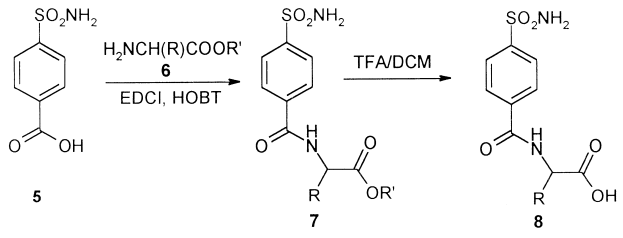
consisted of preparing water soluble sulfonamides incorporating a 4-thioureido-benzenesulfonamide scaffold. Some of these derivatives, such as **3** and **4**, showed high affinity for the enzyme, very good water solubility (as sodium carboxylate salts) at pH values of 7.0–7.5, and excellent IOP lowering properties in animal models of glaucoma.[4a] Using derivatives **3** and **4** as lead molecules, we report here a new approach for obtaining nanomolar CA II and CA IV inhibitors with good water solubility in the neutral pH range, also characterized by good and long lasting in vivo properties as IOP lowering agents in glaucoma animal models.

Reaction of 4-carboxybenzenesulfonamide **5** with carboxy-protected (as *tert*-butyl esters) amino acids/dipeptides **6** in the presence of carbodiimides (EDCI) and hydroxybenzotriazole, afforded the corresponding carboxamides **7**, which were deprotected with TFA following the standard procedure,[6,7] leading to the derivatives **8** (Scheme 1 and Table 1). Several structurally related derivatives were obtained by using aromatic/heterocyclic sulfonamides/mercaptans possessing free amino groups instead of amino acids in the coupling reactions mentioned above (Table 1). Finally, by using 4-chloro-3-sulfamoyl benzoic acid instead of **5**, a related series of amides **9** has been prepared (Table 1).[10] It must be mentioned that structurally related derivatives to **8** and **9** have been previously reported by Whitesides'[7] and Baldwin's group,[9] but as far as we know, such derivatives were not investigated for their IOP lowering properties (although some of them showed excellent in vitro CA II inhibitory properties).[7,9]

The following may be commented regarding the inhibition data with the new sulfonamides **8** and **9** reported here: (i) the dipeptide derivatives of 4-carboxybenzenesulfonamide **8x**–**8z**, **8aa** and **8ab**, as well as the sulfonamide/mercapto derivatives in both series (**8ac**–**8ag** and **9af**, **9ag**) behave as very potent CA II and CA IV inhibitors, with affinities in the low nanomolar range for these isozymes (3–16 nM for hCA II, and 13–25 nM for bCA IV, respectively) whereas generally they are slightly less effective CA I inhibitors (this isozyme is known to possess a lower affinity for sulfonamides);[1–3,11] (ii) less effective inhibitors are the 4-sulfamoylbenzenecarboxamides derived from GABA, Ala, Val, Leu, Ile, α-phenyl-glycine, Met and dicarboxylic amino acids as well as aromatic/heterocyclic amino acids (compounds **8r**–**8u**), together with several derivatives belonging to the second series, such as **9x**–**9z**, **9aa**–**9ae**. All these inhibitors possessed affinities of 26–79 nM against hCA II and 50–160 nM against bCA IV, whereas their hCA I affinity was much lower, of around 270–560 nM [for compounds of the first series; see discussion for the second series of derivatives (of type **9**) against hCA I later in the text]; (iii) several derivatives of the first series, such as the Gly, β-Ala, Ser, Thr and Cys derivatives, as well as the largest majority of the carboxamides of the second series (such as **9a**–**9i** and **9k**–**9v**) were only moderate hCA II/bCA IV inhibitors, with affinities in the 85–735 nM against the first isozyme and 170–600 nM against the second one. The diminished affinity of derivatives of type **9** as compared to the corresponding inhibitors **8**, towards isozymes II and IV, may be due to the chlorine atom *ortho* to the sulfamoyl moiety present in the first compounds, which is known to lead to a steric hindrance when binding to the Zn(II) within the enzyme active site,[12,13] but it seems that this phenomenon is beneficial for the selectivity of such inhibitors towards the isozyme I; (iv) a fascinating discovery regards the activity of the second series of sulfonamides—some compounds of type **9**—(as an entire class of CA inhibitors) against isozyme I: these derivatives are among the very few reported CA inhibitors[1,2] that possess a higher affinity for isozyme CA I as compared to isozyme CA II. Indeed, as seen from the data of Table 1, many of these compounds possess inhibition constants of 21–305 nM against hCA I, and of 26–490 nM towards hCA II, respectively. Sometimes, a quite spectacular 'selectivity' is achieved, with the His, Tyr and DOPA derivatives possessing an hCA I inhibition constant around 100 nM, whereas the same parameter against hCA II is of around 250 nM. We consider these data highly significant, especially considering the fact that hCA I is a very abundant blood protein (85% of the blood CA is constituted by this isozyme) whose physiological role is largely unknown for the moment.[1,2,11]

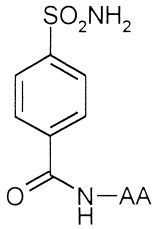


Scheme 1.

Some physicochemical properties of several strong in vitro inhibitors were investigated in detail (Table 2), showing that many of these new derivatives possess excellent water solubility, at pH values in the range of 7.0–7.5 pH units. This is correlated with a balanced hydro- and lipophilicity, and in consequence optimal accession rates across the cornea, which is typical for the effective topically acting sulfonamides.[1–3] Two of the investigated compounds (**8ae** and **9af**) showed a very low water solubility, but all the other physicochemical properties were appropriate for acting as topical IOP lowering agents. These compounds have been applied in suspensions (similarly to brinzolamide **1**) and indeed they showed very good antiglaucoma activity in an animal model of the disease (Tables 3 and 4).

In vivo, in normotensive rabbits, some of the new sulfonamides reported here showed very effective IOP lowering after topical administration, with pressure reductions of 2.5–7.0 mmHg at 30 min (compared to 1.9 mmHg for dorzolamide and 2.9 mmHg for brinzolamide), 5.0–7.1 mmHg at 60 min (4.0 for **1**, and 3.2 for **2**, respectively), 3.1–9.1 mmHg at 90 min, and 2.1–9.5 mmHg at 120 min after administration (compared to the much lower values for the standard drugs **1** and **2**) (Table 3). An important feature of the new class of CA

Table 1. Inhibition data for some derivatives **8** and **9** reported in the paper, and standard inhibitors



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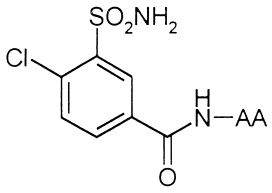

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No.	Inhibitor AA	K_I (nM)		
		hCA I[a]	hCA II[a]	bCA IV[b]
1	Dorzolamide	50,000	9	43
2	Brinzolamide	—	3	45
8a	Gly	500	85	200
8b	β-Ala	435	85	190
8c	GABA	415	73	120
8d	Ala	425	79	75
8e	Val	420	74	150
8f	Leu	405	53	90
8g	Ile	350	48	86
8h	α-Ph-Gly	250	36	75
8i	Ser	440	120	325
8j	β-Ph-Ser	54	21	62
8k	Thr	475	115	350
8l	Cys	450	110	230
8m	Met	400	66	150
8n	Asp	425	73	150
8o	Asn	410	72	155
8p	Glu	430	74	160
8q	Gln	425	75	160
8r	His	270	40	125
8s	Phe	560	52	130
8t	Tyr	535	45	120
8u	DOPA	520	43	105
8v	Gly-Gly	350	42	50
8x	β-AlaHis	270	16	21
8y	HisGly	380	10	19
8z	HisPhe	240	9	23
8aa	AlaPhe	320	12	24
8ab	LeuGly	400	9	16
8ac	$-C_6H_4-SO_2NH_2$ (*p*)	38	10	25
8ad	$CH_2-C_6H_4-SO_2NH_2$ (*p*)	40	7	16
8ae	$(CH_2)_2C_6H_4-SO_2NH_2$ (*p*)	40	5	13
8af	-1,3,4-thiadiazole–SO_2NH_2	35	3	15
8ag	-1,3,4-thiadiazole–SH	38	5	14
9a	Gly	210	450	600
9b	β-Ala	205	450	610
9c	GABA	200	400	540
9d	Ala	175	420	475
9e	Val	130	330	360
9f	Leu	125	305	330
9g	Ile	150	280	360
9h	α-Ph-Gly	105	160	240
9i	Ser	170	290	395
9j	β-Ph-Ser	37	62	98
9k	Thr	320	520	735
9l	Cys	300	490	720
9m	Met	240	410	650
9n	Asp	285	300	355
9o	Asn	240	275	330
9p	Glu	305	340	400
9q	Gln	250	305	410
9r	His	103	210	350
9s	Phe	135	250	340
9t	Tyr	115	250	325
9u	DOPA	100	240	330
9v	Gly-Gly	69	85	170
9x	β-AlaHis	30	36	50

(*Continued on next page*)

Table 1 (*continued*)

No.	Inhibitor AA	K_I (nM)		
		hCA I[a]	hCA II[a]	bCA IV[b]
9y	HisGly	41	50	73
9z	HisPhe	27	38	45
9aa	AlaPhe	36	44	72
9ab	LeuGly	39	51	80
9ac	$-C_6H_4-SO_2NH_2$ (*p*)	24	30	66
9ad	$CH_2-C_6H_4-SO_2NH_2$ (*p*)	24	28	61
9ae	$(CH_2)_2C_6H_4-SO_2NH_2$ (*p*)	21	26	63
9af	-1,3,4-thiadiazole-SO_2NH_2	13	10	22
9ag	-1,3,4-thiadiazole–SH	15	11	21

[a]Human (cloned) isozymes.
[b]From bovine lung microsomes, by the esterase method.[14]

Table 2. Solubility, chloroform–buffer partition coefficients and in vitro corneal permeability of some sulfonamide CA inhibitors reported in the paper and dorzolamide **1** as standard

Compound	Solubility[a] (nM)	Log P[b]	$k_{in}\times10^3$ (h^{-1})[c]	
			Cornea intact	No epithelium
Dorzolamide **1**	60[d]	2.0	3.0	5.2
8z	37[e]	1.735	2.9	5.8
8ab	34[e]	1.821	3.3	6.4
8ae	<0.1	1.433	2.5	6.3
9af	<0.1	1.893	3.1	7.0

[a]Solubility in pH 7.40 buffer, at 25 °C, determined as described in ref 13.
[b]Chloroform–buffer partition coefficient, determined as described in ref 13.
[c]Determined as described in ref 4.
[d]As hydrochloride, at pH 5.8, from ref 3.
[e]As sodium salts.

inhibitors reported here is that IOP remained low for longer periods (3–5 h) after their topical administration, as compared to the standard drug (data not shown). The above findings also apply to the glaucomatous rabbits experiments (Table 4) but the IOP reductions were much more important as compared to those seen in normotensive rabbits. Thus, IOP reductions of 6.4–9.5 mmHg were observed after 30 min, whereas, at 60 min these amounted to 12.0–13.0 mmHg, at 90 min of 11.0–14.2 mmHg, and at 120 min of 10.0–13.3 mmHg, respectively. Thus, these derivatives are longer lasting and more effective IOP lowering agents as compared to the clinically available drugs dorzolamide and brinzolamide.

In conclusion, we report here a novel class of powerful, topically acting sulfonamide CA inhibitors, incorporating

Table 3. IOP lowering in normotensive rabbits (21.8±2.1 mmHg) after treatment with one drop (50 μL) 2% solution/suspension of CA inhibitor (the pH of the solution also shown), at 30, 60, 90 and 120 min after administration directly into the eye[14]

Inhibitor	pH	ΔIOP (mmHg)[a]				
		$t=0$	$t=30$ min	$t=60$ min	$t=90$ min	$t=120$ min
Dorzolamide **1**	5.5	0	1.9±0.2	4.0±0.3	2.1±0.2	1.3±0.3
Brinzolamide **2**[b]	5.5	0	2.9±0.1	3.2±0.3	6.3±0.4	7.0±0.2
8z	7.0	0	4.2±0.2	6.5±0.2	9.1±0.3	9.5±0.4
8ab	7.0	0	3.3±0.3	5.2±0.4	7.8±0.3	8.7±0.2
8ae[b]	7.0	0	3.1±0.1	5.3±0.2	6.7±0.3	5.3±0.2
8ag	7.0	0	7.0±0.1	5.0±0.2	3.1±0.2	2.1±0.1
9af[b]	7.5	0	2.5±0.3	7.1±0.3	8.3±0.4	7.5±0.3

[a]$\Delta IOP = IOP_{control\ eye} - IOP_{treated\ eye}$; mean±SEM ($n=3$ rabbits for each compound). Drug was administered in the left eye, whereas the contralateral eye was used as control.
[b]Suspension.

Table 4. Fall of IOP of glaucomatous rabbits (30.5±3.0 mmHg), after treatment with one drop (50 μL) solution/suspension of 2% of CA inhibitor (the pH value shown below) directly into the eye, at 30, 60, 90 and 120 min after administration

Inhibitor	PH	ΔIOP (mmHg)[a]				
		$t=0$	$t=30$ min	$t=60$ min	$t=90$ min	$t=120$ min
Dorzolamide	5.5	0	3.6±0.2	6.7±0.3	4.2±0.2	3.6±0.3
8z	7.0	0	7.1±0.3	12.0±0.3	11.0±0.2	10.0±0.3
8ae[b]	7.0	0	9.5±0.3	12.3±0.5	11.9±0.5	10.5±0.2
9af[b]	7.5	0	6.4±0.2	13.0±0.5	14.2±0.3	13.3±0.4

[a]$\Delta IOP = IOP_{control\ eye} - IOP_{treated\ eye}$; mean±SEM ($n=3$ rabbits for each compound). Drug was administered in the left eye, whereas the contralateral eye was used as control.
[b]Suspension.

sulfamoyl-benzenecarboxamide moieties in their molecules. Some of these inhibitors were very efficient IOP lowering agents in normotensive and glaucomatous rabbits after topical administration as water solutions/suspensions. Furthermore, an entire class of sulfonamides possessing higher affinity for the slow isozyme CA I as compared to the sulfonamide 'avid' one CA II is reported.

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10. For example: 258 mg (1 mmol) Phe *t*-butyl ester hydrochloride, 200 mg (1 mmol) of 4-carboxybenzenesulfonamide, 140 mg HOBT (1.1 mM) and 200 mg EDCI·HCl were suspended in 50 mL of acetonitrile, and the required amount of triethylamine added to the reaction mixture, which was stirred at room temperature for 16 h (TLC control). The solvent was evaporated in vacuo and the residue taken up in ethyl acetate (5 mL), poured into a 5% solution of sodium bicarbonate (5 mL) and extracted with ethyl acetate. The oils obtained after evaporation of the solvent were directly used in the deprotection step. The removal of the *t*-Bu groups has been performed by treating the crude intermediate dissolved in 20 mL of CH_2Cl_2, with 4 mL of trifluoroacetic acid (TFA) and stirring for 60 min at 0 °C. The solvent was removed in vacuo and the residue concentrated from water twice to remove excess TFA, giving thus the carboxamide as a colorless syrup. The pure compound was obtained by means of preparative HPLC (C_{18} reversed-phase μ-Bondapack or Dynamax-60A (25×250 mm) columns; 90% acetonitrile/8% methanol/2% water, 30 mL/min). Mp 229–231 °C (ethanol–water 1:1, v/v). IR (KBr), cm^{-1}: 1148 (SO_2^{sym}), 1256 (amide III), 1370 (SO_2^{as}), 1550 (amide II), 1695 (amide I), 1770 (COOH), 3065 (NH); ^1H NMR (DMSO-d_6), δ, ppm: 3.10–3.52 (m, 2H, CH_2CH of Phe), 4.11 (dd, $^3J_{HH}=5.0$ Hz, $^3J_{HH}=7.8$ Hz, 1H, CH_2CH of Phe), 7.29–7.51 (m, 5H, H_{arom} of Phe), 7.50 (s, 2H, SO_2NH_2), 7.61 (d, $^3J_{HH}=8.1$, 2H, H_{ortho} of $H_2NO_2SC_6H_4$), 7.94 (d, $^3J_{HH}=8.1$ Hz, 2H, H_{meta} of $H_2NO_2SC_6H_4$), 8.12 (br s, 1H, CONH), 10.71 (br s, 1H, COOH); ^{13}C NMR (DMSO-d_6), δ, ppm: 41.3 (s, CH_2CH of Phe), 59.8 (s, CH_2CH of Phe), 131.5 (s, C_{meta} of $H_2NO_2SC_6H_4$), 133.9 (s, C_{meta} of Phe), 134.6 (s, C_{ortho} of Phe), 135.2 (s, C_{ortho} of $H_2NO_2SC_6H_4$), 141.7 (s, C_{ipso} of Phe), 144.2 (s, C_{ipso} of $H_2NO_2SC_6H_4$), 145.6 (s, C_{para} of Phe), 147.3 (s, C_{para} of $H_2NO_2SC_6H_4$), 158.9 (s, CONH), 179.3 (COOH). Anal. found: C, 55.33; H, 4.60; N, 9.07%; $C_{16}H_{16}N_2O_5S$ requires C, 55.16; H, 4.63; N, 9.20%.
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