Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 673–676

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Carbonic Anhydrase Inhibitors: Topical Sulfonamide Antiglaucoma Agents Incorporating Secondary Amine Moieties

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Received 10 September 1999; accepted 3 February 2000

Abstract—Reaction of aromatic/heterocyclic sulfonamides possessing free amino, imino or hydrazino moieties with 7-chloro-4-chloromethylcoumarin afforded a series of *N*-[(7-chloro-4-coumarinyl)-methyl]- derivatives which showed effective inhibition of three carbonic anhydrase (CA) isozymes. Topical application within the rabbit eye of some of these compounds led to effective intraocular pressure lowering due to CA inhibition within the ocular tissues, and reduced aqueous humor production.

Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1),[1–5] have been the target for drug design since the early 1950s, with the discovery of the first sulfonamide diuretics.[6] Sulfonamides of the type RSO_2NH_2, acting as powerful CA inhibitors are now widely used drugs for the treatment or prevention of a variety of diseases,[4–8] among which glaucoma,[4,5,7] is one of the most important. CA inhibition in ocular tissues (mainly the ciliary processes) with systemically or topically administered sulfonamide CA inhibitors, is followed by an effective reduction of intraocular pressure (IOP) due to the reduced rate of bicarbonate secretion within the aqueous humor.[9–11] Since the systemic inhibitors generally produce undesired side-effects, recently, many efforts have been made for the development of water-soluble sulfonamide CA inhibitors that might be administered via the topical route.[12–16]

In previous works[12–16] we showed that by attaching water-solubilizing tails (such as pyridinecarboxamido; quinolinesulfonylamido, etc.) to the molecules of well-known sulfonamide inhibitors of the type **1**–**20**, it is possible to obtain compounds (as salts of strong acids/bases) with long lasting topical antiglaucoma activity in an animal model of this disease. Here we extend our previous study, proving that secondary amines derived from the mentioned sulfonamides **1**–**20** are useful for obtaining compounds with good antiglaucoma properties.

Reaction of 7-chloro-4-chloromethylcoumarin with sulfonamides **1**–**20** in the presence of triethylamine afforded the secondary amines **A1**–**A19**, as well as the tertiary amine **A20**, possessing *N*-[(7-chloro-4-coumarinyl)]-methyl moieties in their molecule. Mention should be made that sulfonamides **20** (dorzolamide) and **21** (brinzolamide) are the only clinically used topical antiglaucoma agents at the moment.[12] The substituted coumarinyl moiety has been chosen to be introduced in the molecules of these CA inhibitors due to the presence of both hydrophilic as well as hydrophobic structural elements in it, which should presumably be reflected also in the new compounds. It is in fact well established that the best topically acting antiglaucoma sulfonamides possess a high water solubility (due to their hydrophilic character) balanced by a moderate but not insignificant liposolubility (that assures good penetrability within the ocular tissues).[7,12–16] Salts of the new derivatives (useful in order to study the IOP lowering effects of these compounds) were then prepared by reacting the secondary/tertiary amines **A1**–**A20** with a methanolic HCl solution. Similarly were obtained the triflate salts, by reaction of the previously mentioned free bases with triflic acid in water as solvent.[12,13]

Physico-chemical properties for several of these new derivatives as well as for the previously reported,[13] structurally related 2,3-pyridinecarboximido compound **B13** are shown in Table 1, and these data prove that the

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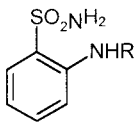
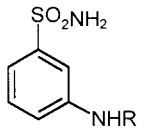


1: R = H
A1: R = 7-Ccm



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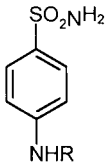
A2: R = 7-Ccm



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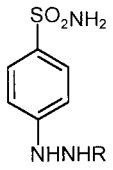
A3: R = 7-Ccm



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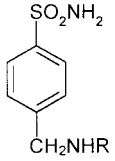
A4: R = 7-Ccm



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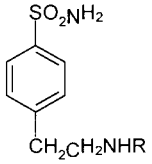
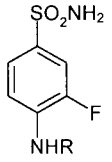
A5: R = 7-Ccm



6: R = H
A6: R = 7-Ccm



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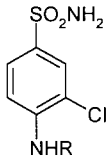
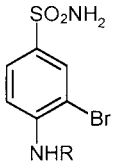
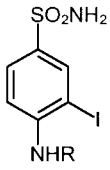
A7: R = 7-Ccm



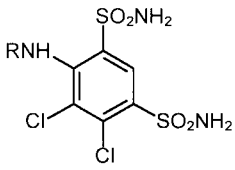
8: R = H
A8: 7 = β-Ccm



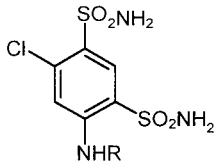
9: R = H
A9: R = 7-Ccm



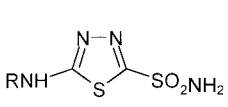
10: R = H
A10: R = 7-Ccm



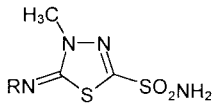
11: R = H
A11: R = 7-Ccm



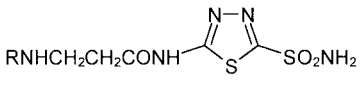
12: R = H
A12: R = 7-Ccm



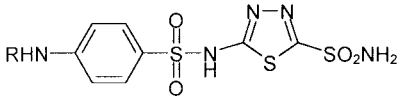
13: R = H
A13: R = 7-Ccm; **B13**: R =2,3-PC



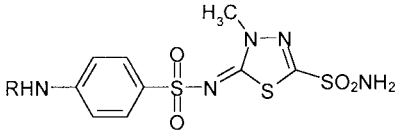
14: R = H
A14: R = 7-Ccm



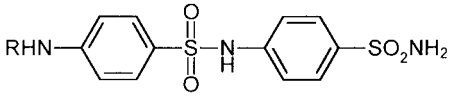
15: R = H
A15: R = 7-Ccm



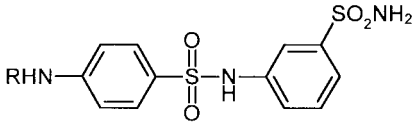
16: R = H
A16: R = 7-Ccm



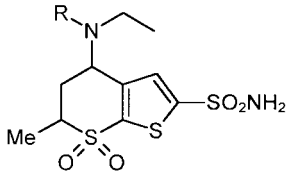
17: R = H
A17: R = 7-Ccm



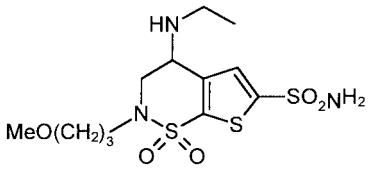
18: R = H
A18: R = 7-Ccm



19: R = H
A19: R = 7-Ccm



20: R = H
A20: R = 7-Ccm



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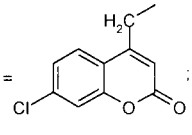
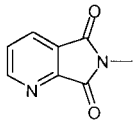
In the above formulas 7Ccm =



; 2,3-PC =



right balance between lipo- and hydrosolubility mentioned above has indeed been achieved.

Inhibition data against three CA isozymes, hCA I, hCA II and bCA IV (h = human; b = bovine isozyme) with the new derivatives **A1**–**A20** (Table 2) proved that the *N*-[(7-chloro-4-coumarinyl)-methyl]- derivatives reported here behave as strong inhibitors, with increased affinities as compared to the parent compounds from which they were prepared (the sulfonamides **1**–**20**,[13] data not shown). The affinities of the obtained inhibitor generally varied as for the previously reported pyridinecarboxamido derivatives,[13] with the heterocyclic compounds more active than the aromatic ones. The coumarinyl derivatives reported here were slightly more active than the pyridine-carboximido CA inhibitors reported previously[13] (compare for example data for **A13** and **B13** in Table 2). All three CA isozymes investigated here were susceptible to inhibition with this type of sulfonamides, with hCA II and bCA IV the most sensitive, whereas hCA I was generally less susceptible to inhibition as compared to the first two isozymes (Table 2).

The following facts should be noted regarding the in vivo data presented in Table 3. Some of the new compounds investigated here, such as **A13**, **A14** and **A16**, showed IOP lowering effects slightly better than those of dorzolamide **20**. Thus, after half an hour or 1 h after the administration, these were of around 2.9–3.3 mm Hg, and 5.5–6.0 mm Hg, respectively. An important difference between the two groups of drugs appears at longer periods after the administration, since unlike dorzolamide, which drastically diminishes its power of action to an IOP lowering of 2.7 mm Hg after 90 min, the new compounds mentioned above maintained a much more effective IOP lowering, in the range of 4.0–4.9 mm Hg, comparable to that observed at one hour after their administration. Another inhibitor in the investigated series, such as **A15**, showed much more effective IOP lowering effects as compared to dorzolamide **20**, both after 30 min from the administration of the inhibitor within the rabbit eye, as well as at longer times (1, 1.5 and 2–6 h, respectively). After 30 min, the IOP lowering was in the range of 5.1 mm Hg with the new compound; at one hour after the administration the new compound fared doubly as well as the clinically used drug **20** (8.3 mm Hg for the new derivative, versus 4.1 mm Hg for dorzolamide) and this strong effect was maintained after another half an hour (whereas it is halved in the case of **20**, where the pressure decrease amounts to 2.7 mm Hg after 90 min). But one of the most interesting findings of this report is that IOP remains low for longer periods (5-6 hours; data not shown) after the topical administration of the new type of compounds reported in this paper, as compared to the standard drug dorzolamide (see data at 4 h in Table 3).

Table 1. Solubility, chloroform-buffer partition coefficients and in vitro transcorneal accession rates (k_{in}) of some sulfonamides CA inhibitors. Data for dorzolamide **20** were also included for comparison

Inhibitor	Solubility[a] (mM)	Log P[b]	$k_{in}\times10^3$ (h^{-1})[c]	
			Intact cornea	No epithelium
20	60[d,e]	2.0[e]	3.0	5.2
B13	72[d,e]	0.315	2.5	7.6
A13	54[e]	0.558	2.8	6.5
A15	62[e]	0.683	3.3	8.4
A20	41[e]	1.762	2.1	5.9

[a]Solubility in pH 7.40 buffer, at 25 °C.
[b]Chloroform-buffer partition coefficient.
[c]Determined as described in ref 13.
[d]Data from ref 13.
[e]As hydrochloride salts.

Table 2. CA inhibition data with the standard inhibitor **20**, and the new derivatives **A1**–**A20** reported in the present study, against isozymes I, II and IV[17,a,b]

Inhibitor	K_I (nM)		
	hCA I[a,c]	hCA II[c]	bCA IV[d]
Dorzolamide **20**	50,000	9	45
A1	21,500	270	300
A2	20,000	250	280
A3	15,000	125	170
A4	21,800	275	320
A5	1060	145	240
A6	950	120	225
A7	540	30	110
A8	520	45	96
A9	600	33	78
A10	610	36	70
A11	500	18	49
A12	400	12	50
A13	36	7	15
B13[d]	39	8	18
A14	29	6	16
A15	12	2	16
A16	5	2	4
A17	1	0.5	0.6
A18	33	3	14
A19	36	6	30
A20	18,000	8	25

[a]Mean from at least three determinations by the esterase method.[17]
[b]Standard error was in the range of 5–10%. Human cloned isozyme.
[c]Purified from bovine lung microsomes.[18]
[d]From ref 13.

In order to test the hypothesis that the water solubility (of the hydrochlorides or triflates of these secondary amines) is a critical parameter for their topical IOP lowering properties (obviously together with other features, such as a high affinity for hCA II), we also prepared two compounds which cannot form this type of salts with strong acids, but which also contain the substituted coumarinyl moiety of interest. Compounds **C13** and **C14** were obtained by routine procedures involving the reaction of 7-chloro-4-chloromethyl-coumarine with sodium sulfite in methanol, followed by conversion of the obtained sodium sulfonate to the corresponding sulfonyl chloride (with $POCl_3$ in chloroform). The synthesized sulfonyl chloride was then reacted with amines **13** and **14**, affording thus the two derivatives **C13** and **C14**.[13–15]

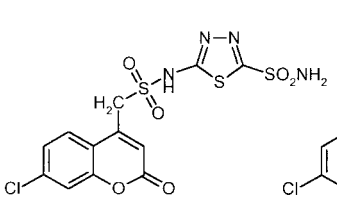
Although the two compounds mentioned above behave as very strong CA inhibitors (affinities in the nanomolar range for hCA II and bCA IV, data not shown), they

Table 3. Reduction of IOP in normotensive rabbits (20.4 ± 2.5 mm Hg), after treatment with one drop (50 μL) solution 2% of CA inhibitors (as hydrochloride or triflate salt, with the pH value shown below) directly into the eye, after 30, 60 and 240 min from administration.[10] The data of dorzolamide **20** are also shown for comparison[a]

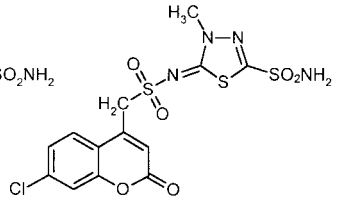
Inhibitor	pH	ΔIOP (mm Hg)[b]				
		$t=0$	$t=30$ min	$t=60$ min	$t=90$ min	$t=240$ min
20[c]	5.5	0	2.2 ± 0.20	4.1 ± 0.30	2.7 ± 0.25	0
A13[c]	5.5	0	3.9 ± 0.25	5.8 ± 0.45	4.9 ± 0.30	1.5 ± 0.30
A14[c]	6.5	0	3.3 ± 0.30	6.0 ± 0.15	5.5 ± 0.30	1.9 ± 0.35
A15[d]	5.5	0	5.1 ± 0.20	8.3 ± 0.40	7.2 ± 0.35	3.4 ± 0.25
A16[d]	6.0	0	2.9 ± 0.25	5.5 ± 0.35	4.0 ± 0.25	2.1 ± 0.20
A20[c]	6.0	0	1.9 ± 0.30	3.7 ± 0.30	1.6 ± 0.30	0

[a]Mean ± average spread ($n=3$).
[b]$IOP = IOP_{control\ eye} - IOP_{treated\ eye}$.
[c]As HCl salt.
[d]As triflate salt.

were devoid of topical IOP lowering effects in normotensive rabbits, probably due to their reduced water solubility (data not shown) as compared to the structurally related secondary amines **A13** and **A14**.



C13



C14

In conclusion, we report here a novel approach for the design of high affinity, water soluble sulfonamide CA inhibitors. Reaction of amino-, imino- or hydrazino-containing aromatic/heterocyclic sulfonamides with 7-chloro-4-chloromethyl-coumarine afforded a series of water soluble compounds (as salts of strong acids, such as hydrochloric or trifluoromethanesulfonic) which showed stronger and more prolonged IOP lowering in normotensive rabbits as compared to the clinically used compound dorzolamide.

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