Carbonic Anhydrase Inhibitors: Perfluoroalkyl/Aryl-Substituted Derivatives of Aromatic/Heterocyclic Sulfonamides as Topical Intraocular Pressure-Lowering Agents with Prolonged Duration of Action

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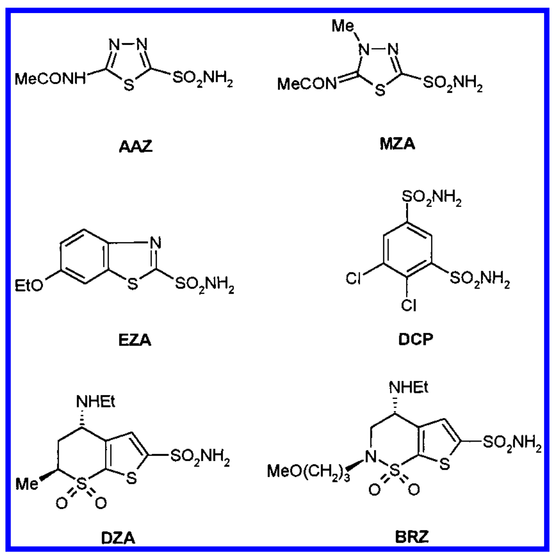
Reaction of perfluoroalkyl/arylsulfonyl chlorides or perfluoroalkyl/arylcarbonyl chlorides with aromatic/heterocyclic sulfonamides possessing a free amino/imino/hydrazino/hydroxy group afforded compounds with the general formula C_xF_yZ-A-SO_2NH_2, where Z = SO_2NH, SO_3, CONH, or CO_2 and A = aromatic/heterocyclic moiety. The sulfonyl chlorides used in synthesis included: CF_3SO_2Cl, *n*-$C_4F_9SO_2Cl$, *n*-$C_8F_{17}SO_2Cl$, and $C_6F_5SO_2Cl$, whereas the acyl chlorides were $C_8F_{17}COCl$ and C_6F_5COCl. A total of 25 different sulfonamides have been derivatized by means of the above-mentioned perfluorosulfonyl/acyl halides. These new series of sulfonamides showed strong affinities toward isozymes I, II, and IV of carbonic anhydrase (CA). For a given sulfonamide derivatized by the above procedures, inhibitory power was greater for the alkyl/arylsulfonylated compounds, as compared to the corresponding perfluoroalkyl/arylcarbonylated ones. In vitro inhibitory activity generally increased with the number of carbon atoms in the molecule of the acylating/sulfonylating agent, with a maximum for the perfluorophenylsulfonylated and perfluorobenzoylated derivatives. Some of the prepared CA inhibitors displayed very good water solubility (in the range of 2%) and strongly lowered intraocular pressure (IOP) when applied topically, directly into the normotensive/glaucomatous rabbit eye, as 2% water solutions. The good water solubility of these new classes of CA inhibitors, correlated with the neutral pH of their solutions used in the ophthalmologic applications, makes them attractive candidates for developing novel types of antiglaucoma drugs devoid of unpleasant ocular side effects.

Introduction

Sulfonamides possessing carbonic anhydrase (CA; EC 4.2.1.1) inhibitory properties[1,2] such as acetazolamide (AAZ), methazolamide (MZA), ethoxzolamide (EZA), and dichlorophenamide (DCP) have been used for more than 45 years as pressure-lowering systemic drugs in the treatment of open-angle glaucoma as well as other diseases associated with acid base/secretory disequilibria.[3,4] Recently, they started to show increasing interest as potential agents for the treatment of macular edema, a condition for which no effective therapy was known up to now.[1] The ocular effects of such sulfonamides are due to inhibition of at least two CA isozymes present within cilliary processes of the eye, i.e., CA II and CA IV, which is followed by a diminished secretion of bicarbonate and reduction of aqueous humor secretion.[5−7] Their main drawback is constituted by systemic side effects such as fatigue, augmented diuresis, or paresthesias, due to CA inhibition in other tissues/organs than the target one, i.e., the eye.[8]

The above-mentioned side effects are absent in the case in which the inhibitor has topical activity and is applied directly into the eye. This route, discovered in 1983 by Maren's group,[9] was shortly followed by the development of the first clinical agents of this type, dorzolamide (DZA)[10] as well as the structurally related brinzolamide (BRZ), several years later.[11] The clinical success of topical antiglaucoma CA inhibitors fostered much research in the synthesis and evaluation of other types of such compounds.[12−16] These two drugs mentioned above are effective in reducing intraocular pres-



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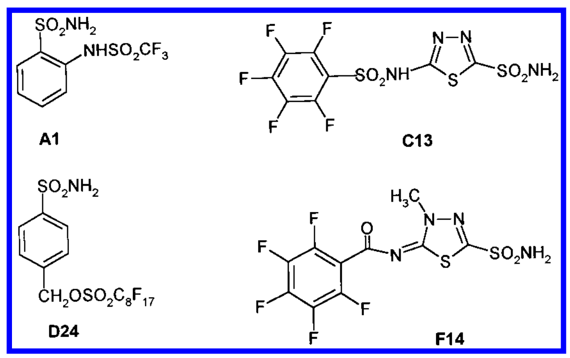
sure (IOP) and show fewer side effects as compared to the systemically applied sulfonamides. The observed side effects after topical sulfonamides include stinging, burning or reddening of the eye, blurred vision, pruritus, and bitter taste.[6,7] All but the last are probably due to the fact that DZA (the best studied topical CA inhibitor) is the salt of a weak base with a very strong acid, so that the pH of the drug solution is rather acidic (generally around 5.5). The last side effect mentioned above is probably due to drug-laden lachrymal fluid draining into the oropharynx and inhibition of CA present in the saliva (CA VI) and the taste buds (CA II and CA VI), with the consequent accumulation of bicarbonate, and was seen with both systemic as well as topical CA inhibitors.[6,7] Less is known for the moment regarding the side effects of BRZ, but it seems that this drug produces less stinging (being administered at pH 7.5) but more blurred vision as compared to DZA.[7] Unfortunately, DZA also showed some more serious side effects, such as contact allergy, nephrolithiasis, anorexia, depression, and dementia as well as irreversible corneal decompensation in patients who already had corneal problems.[17] Thus, new types of topically effective CA inhibitors as possible antiglaucoma agents are being investigated,[12−16] and an alternative approach (to the thienothiopyran sulfonamides and their analogues) was recently reported, consisted in attaching water-solubilizing moieties to the molecules of aromatic/heterocyclic sulfonamides.[12,13] Such moieties included pyridinecarboximido, carboxypyridinecarboxamido, quinolinesulfonamido, picolinoyl, and isonicotinoyl, as well as amino acyl groups among others, whereas ring systems which have been derivatized by using the above-mentioned moieties included: 2-, 3-, or 4-aminobenzenesulfonamides, 4-(ω-aminoalkyl)benzenesulfonamides, 3-halogeno-substituted-sulfanilamides, 1,3-benzenedisulfonamides, 1,3,4-thiadiazole-2-sulfonamides, benzothiazole-2-sulfonamides, and thienothiopyran-2-sulfonamides among others, and were chosen in such a way as to demonstrate that the proposed "tail" approach is a general one.[12,13]

As mentioned above, the two topically active antiglaucoma drugs used clinically, DZA and BRZ, are both secondary amines, and the required water solubility needed for effective topical action is achieved by using their hydrochloride salts. Still, in some cases this represents an undesired problem, since the pH of such solutions is acidic enough and produces eye irritation after topical administration, which is in fact the side effect most frequently reported after the use of these drugs. Such side effects might be avoided for compounds that do not need to be administered as hydrochloride salts, but this generally leads to a drastical diminution of water solubility of such sulfonamides. Here we propose a novel approach for obtaining water-soluble, high-affinity sulfonamide CA inhibitors, which do not owe their water solubility to formation of hydrochloride salts. Thus, during our work[18] for the synthesis of benzolamide-like sulfonamide CA inhibitors with applications in positron emission tomography (PET), it was observed that perfluorobenzolamide possesses an unexpectedly high water solubility. Starting from this compound, a large series of derivatives were obtained by reaction of perfluoroalkyl/arylsulfonyl halides or perfluoroalkyl/arylcarbonyl chlorides (of types **A**−**F**) with aromatic/heterocyclic sulfonamides containing a free amino/imino/hydrazino/hydroxy group in their molecule of type **1**−**25** (the synthesis of perfluorobenzolamide **C13** has not been reported previously in the mentioned work) (Chart 1).[18]

Results

Synthesis. As a large number of derivatives (150 compounds) have been prepared, in the following, they will be abbreviated by using both a letter, designating the perfluorosulfonic/carboxylic acid moiety, as well as a number, designating the sulfonamide from which the new compounds were derived. For instance: **A1** is the trifluoromethylsulfonyl derivative of orthanilamide **1**; **C13** is the perfluorophenylsulfonylamido derivative of 5-amino-1,3,4-thiadiazole-2-sulfonamide **13**, (perfluorobenzolamide); **D24** is the perfluorooctylsulfonylated derivative of 4-(2-hydroxymethyl)benzenesulfonamide (**24**); **F14** is the perfluorobenzoyl derivative of 5-imino-4-methyl-2-sulfonamido-δ^2-1,3,4-thiadiazoline (see the structures of these derivatives below). The whole series of compounds from 6 acylating/sulfonylating agents and the 25 initial sulfonamides **1**−**25** has been prepared by reported procedures from Whitesides' laboratory[14,15] or from our laboratory,[12,13] in order to select valuable inhibitors for the in vivo studies (Table 1).



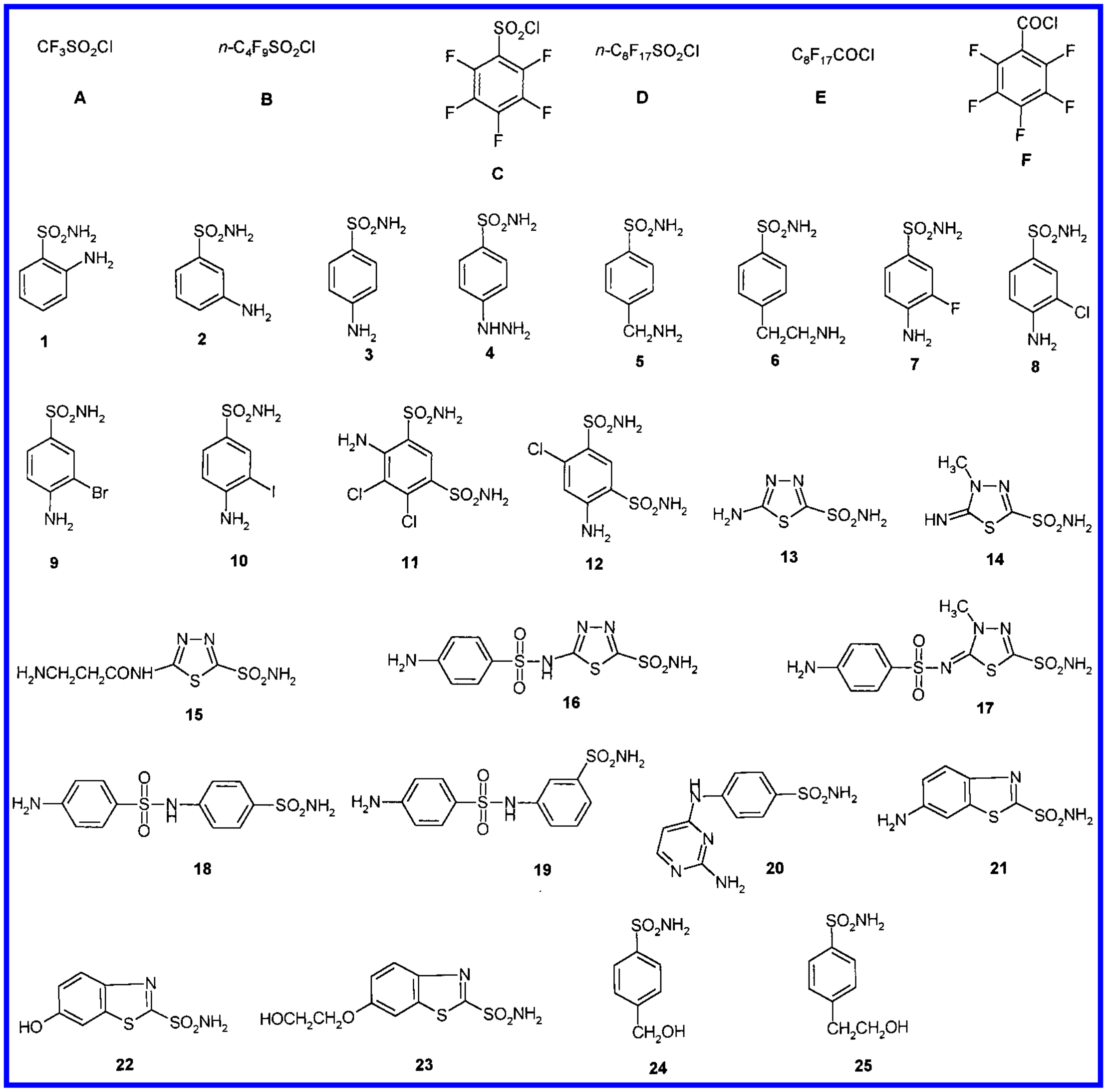
CA Inhibitory Activity. The new sulfonamides reported here were assayed for inhibition of three CA isozymes, two of them known to play a critical role in acqueous humor formation (CA II and CA IV), whereas the other one, CA I, is known to be important for the possible systemic side effects of such drugs (Table 1).

Transcorneal Penetration of Drugs. Some physicochemical properties of several of the new compounds reported here, relevant for their pharmacological activity, such as buffer solubility or chloroform−buffer partition coefficient, are shown in Table 2. The in vitro transcorneal accession rates (k_{in}) of classical sulfonamides and topically active derivatives, such as DZA and some of the new compounds reported in the present study, are also shown in Table 2.

IOP Measurements. In vivo IOP-lowering data with some of the most active CA inhibitors reported here, in normotensive and glaucomatous rabbits, after topical administration of the drug, are shown in Tables 3 and 4, respectively. The full time dependence of the IOP after DZA and some of the new compounds reported here in normotensive albino rabbits is shown in Figure 1.

Distribution of Drugs in Ocular Fluids and Tissues. Ex vivo distribution data of some active com-

Chart 1



pounds in ocular tissues and fluids after topical administration in normotensive rabbits are shown in Table 5.

Discussion

Chemistry. Although the lead molecule for obtaining this series of CA inhibitors was perfluorobenzolamide **C13** (initially prepared in a study[18] in which sulfonamides for applications in PET studies were being designed), historically, the topical route for administration of sulfonamides has been discovered by Maren's group working with trifluoromethazolamide **26**.[9] Still, trifluoromethazolamide, as well as the structurally related trifluoroacetazolamide **27**, is unstable, undergoing spontaneous hydrolysis with a half-life of 45 min at neutral pH and could not be developed for clinical use.[9] Also having a similar fate is the other fluoro-containing sulfonamide investigated up to now, trifluoromethanesulfonamide ($CF_3SO_2NH_2$, **28**) which has been shown by Maren's group[19] and by Kvam[20] to act as an effective IOP-lowering agent but which also proved to be chemically unstable, being hydrolyzed to triflic acid (CF_3SO_3H) which is a very strong acid.[19] In the same study, Maren and Conroy[19] showed that other perfluoroalkylsulfonamides as well as perchloroalkylsulfonamides of the general formula $C_nX_{2n+1}SO_2NH_2$ (X = F, Cl; n = 1, 2, 4) act as potent CA inhibitors, but only the trifluoromethanesulfonamide mentioned previously has been investigated for its IOP-lowering effects in normotensive rabbits. One must also mention that CA inhibitors

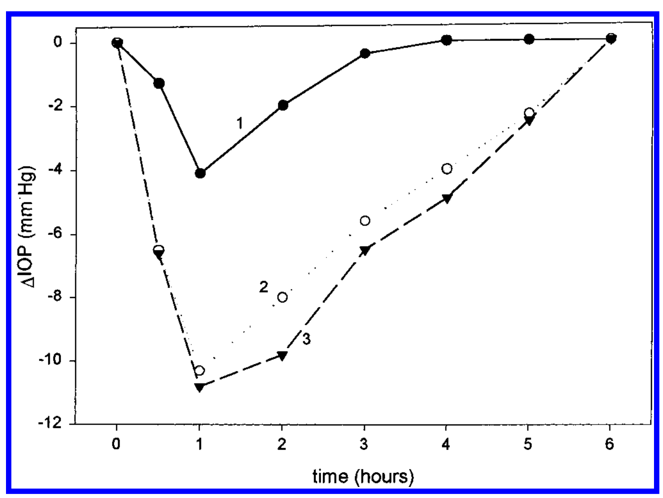


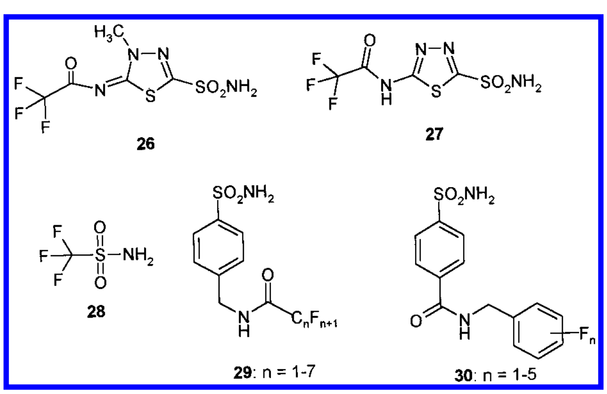
Figure 1. Effect of topically administered sulfonamide inhibitors (2% water solutions) on the IOP of normotensive albino rabbits: curve 1, DZA **1** (hydrochloride salt, pH 5.5); curve 2, compound **C14** (pH 6.50); curve 3, compound **C13** (pH 7.5).

Table 1. CA Inhibition Data with Standard Inhibitors and the New Sulfonamides Reported in the Present Study

	K_i (nM)				K_i (nM)				K_i (nM)		
inhibitor	hCA I[a]	hCA II[a]	bCA IV[b]	inhibitor	hCA I[a]	hCA II[a]	bCA IV[b]	inhibitor	hCA I[a]	hCA II[a]	bCA IV[b]
AZA	900	12	220	**B23**	19	0.7	6	**D24**	15000	540	10900
MZA	780	14	240	**B24**	24500	460	10550	**D25**	22000	440	12300
EZA	25	8	13	**B25**	20900	385	9200	**E1**	1800	103	1500
DCP	1200	38	380	**C1**	1390	24	680	**E2**	1600	69	935
DZA	50000	9	43	**C2**	1100	10	500	**E3**	1400	66	850
A1	175000	20450	97000	**C3**	890	10	410	**E4**	1850	107	840
A2	188400	18700	95300	**C4**	1000	16	500	**E5**	1000	62	750
A3	165500	10900	64600	**C5**	790	15	240	**E6**	950	55	520
A4	155500	14800	60780	**C6**	760	15	230	**E7**	1400	51	550
A5	137800	5000	24500	**C7**	710	9	140	**E8**	2300	55	600
A6	81300	600	50000	**C8**	1450	110	540	**E9**	2500	69	620
A7	100000	500	29000	**C9**	1500	125	550	**E10**	3000	70	680
A8	180000	730	42000	**C10**	1755	150	675	**E11**	7000	72	830
A9	200000	1040	47000	**C11**	1500	15	142	**E12**	810	35	130
A10	210000	1300	49000	**C12**	300	5	26	**E13**	300	5	13
A11	5500	430	725	**C13**	210	0.3	6	**E14**	330	6	18
A12	550	90	150	**C14**	250	0.3	9	**E15**	300	2	8
A13	59800	100	600	**C15**	200	0.4	9	**E16**	600	1.5	13
A14	2000	24	32	**C16**	24	1.0	8	**E17**	900	2	12
A15	3000	13	40	**C17**	21	1.5	9	**E18**	2400	11	25
A16	1000	3	25	**C18**	125	8	15	**E19**	3000	16	30
A17	1000	5	33	**C19**	150	8	16	**E20**	4400	22	36
A18	4000	21	40	**C20**	325	11	24	**E21**	45	1	9
A19	5000	23	45	**C21**	41	0.2	5	**E22**	37	3	14
A20	8000	25	39	**C22**	25	0.3	6	**E23**	24	2	7
A21	70	0.9	8	**C23**	18	0.5	7	**E24**	15000	550	11000
A22	55	0.9	10	**C24**	290	40	100	**E25**	20000	410	12000
A23	50	1	7	**C25**	300	35	103	**F1**	1560	35	810
A24	135000	5100	24100	**D1**	1500	96	1200	**F2**	1400	19	650
A25	80000	550	45000	**D2**	1400	67	870	**F3**	950	17	600
B1	22000	250	900	**D3**	1140	62	760	**F4**	1500	23	980
B2	18400	170	590	**D4**	1680	110	860	**F5**	980	20	350
B3	15200	160	480	**D5**	1000	60	705	**F6**	900	17	310
B4	15000	180	600	**D6**	965	54	470	**F7**	760	15	185
B5	13000	150	255	**D7**	1450	125	500	**F8**	1080	125	590
B6	12500	150	240	**D8**	2480	550	10300	**F9**	1870	156	865
B7	1070	98	200	**D9**	2550	680	12500	**F10**	2560	230	1040
B8	10200	425	880	**D10**	3400	755	15000	**F11**	1900	38	420
B9	20300	485	900	**D11**	8000	98	765	**F12**	750	12	54
B10	23400	650	1040	**D12**	890	32	155	**F13**	250	2	10
B11	5100	51	160	**D13**	400	3	12	**F14**	270	1.5	8
B12	400	8	43	**D14**	380	3	14	**F15**	300	2	10
B13	360	2	9	**D15**	300	2	9	**F16**	1500	8	32
B14	350	2	10	**D16**	800	2	21	**F17**	1600	7	29
B15	300	3	11	**D17**	1100	3	25	**F18**	6600	18	39
B16	900	2	17	**D18**	3000	12	29	**F19**	8000	36	55
B17	1000	4	24	**D19**	3600	21	33	**F20**	7700	27	62
B18	3600	15	31	**D20**	4500	20	34	**F21**	120	0.5	8
B19	4000	20	36	**D21**	49	0.6	7	**F22**	105	0.6	9
B20	6000	20	30	**D22**	28	0.5	8	**F23**	65	0.7	11
B21	54	0.5	6	**D23**	17	0.6	5	**F24**	1200	54	410
B22	29	0.5	7					**F25**	1250	50	400

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

containing fluorine atoms were recently investigated by Whitesides' [15b] and Jain's[21] groups, who reported derivatives of types **29** and **30**, respectively, showing good hCA II inhibitory properties, but which were not tested for their efects on the IOP.



Considering these data, it appeared of interest to design inhibitors that would contain perfluoroalkyl/arylsulfonylamido and perfluoroacyl moieties attached to an aromatic/heterocyclic ring also bearing an unsubstituted SO_2NH_2 group. Mention should be made that based on empirical considerations, one should expect increased hydrolytic stability for the $C_nF_{2n+1}SO_2NH$ moiety when attached to an aromatic/heterocyclic ring, as compared to a CF_3CONH one (which precluded with the clinical development of derivatives **26** and **27**). Fortunately these initial hypotheses proved to be correct (see later in the text).

Reactions of sulfonyl chlorides/sulfonic acid anhydrides or acyl chlorides with amino-containing sulfonamides were previously reported, both in the heterocyclic series (such as 5-amino-1,3,4-thiadiazole-2-sulfonamide **13** or 5-imino-4-methyl-2-sulfonamido-δ^2-1,3,4-thiadiazoline **14**) by Vaughan et al.[22] as well as in the aromatic series by

Table 2. Solubility, Chloroform–Buffer Partition Coefficients, and in Vitro Corneal Permeability of Some Sulfonamide CA Inhibitors

compd	solubility[a] (mM)	log P^b	$k_{in} \times 10^3$ (h^{-1})[c] cornea intact	no epithelium
AAZ[d]	3.2	0.001	0.37	7.0
MZA[d]	12	0.06	1.90	13
DZA	60[e]	2.0[e]	3.0	5.2
A14	69	1.012	3.1	4.8
B13	50	1.525	4.3	7.0
C13	62	2.113	4.5	8.7
C14	66	2.347	4.9	8.8
D16	47	1.689	4.1	7.5
E16	54	1.436	3.2	8.0
F13	43	2.245	4.8	10.1

[a] Solubility in pH 7.40 buffer, at 25 °C. [b] Chloroform–buffer partition coefficient. [c] Determined as described in refs 12, 44, 45. [d] Data from ref 9. [e] As hydrochloride, at pH 5.8, from ref 12.

Table 3. Fall of IOP of Normotensive Rabbits (21.5 ± 2.0 mmHg) after Treatment with 1 drop (50 μL) of a 2% Solution of CA Inhibitor Directly into the Eye[a]

inhibitor	pH	ΔIOP ± SE[b] (mmHg) 30 min	60 min	90 min
DZA	5.5	2.2 ± 0.15	4.1 ± 0.15	2.7 ± 0.10
A14	6.5	3.2 ± 0.15	7.3 ± 0.25	4.9 ± 0.25
B13	7.0	2.8 ± 0.20	8.5 ± 0.25	5.6 ± 0.30
B14	7.0	3.2 ± 0.20	8.9 ± 0.30	7.1 ± 0.30
C13	7.5	4.9 ± 0.30	12.5 ± 0.40	10.2 ± 0.40
C14	6.5	4.7 ± 0.25	11.3 ± 0.35	9.1 ± 0.50
C15	7.0	4.0 ± 0.15	7.5 ± 0.20	6.1 ± 0.35
C21[c]	7.0	3.0 ± 0.20	8.0 ± 0.30	6.6 ± 0.25
D13	7.0	4.6 ± 0.30	8.9 ± 0.35	7.2 ± 0.40
D14	7.0	5.2 ± 0.30	10.3 ± 0.10	8.1 ± 0.35
F13	7.0	2.2 ± 0.25	7.3 ± 0.35	6.5 ± 0.20
F14	7.0	3.2 ± 0.15	8.6 ± 0.25	7.7 ± 0.40

[a] Solution was the hydrochloride salt in the case of DZA and neutral compounds for the other derivatives, with the pH value shown. Decrease in IOP measured 30, 60, and 90 min after administration. [b] $\Delta IOP = IOP_{control\ eye} - IOP_{treated\ eye}$ ($n = 3$). [c] Eye reddening was observed.

Table 4. Fall of IOP of Glaucomatous Rabbits (31.9 ± 3.0 mmHg) after Treatment with 1 drop (50 μL) of a 2% Solution of CA Inhibitor Directly into the Eye[a]

inhibitor	pH	ΔIOP ± SE[b] (mmHg) 30 min	60 min	90 min
DZA[c]	5.5	3.6 ± 0.20	6.7 ± 0.30	4.2 ± 0.15
C13	7.5	8.3 ± 0.30	15.7 ± 0.25	17.4 ± 0.30
C14	6.5	7.1 ± 0.25	14.2 ± 0.30	16.9 ± 0.40
D14	7.0	6.8 ± 0.30	13.0 ± 0.45	15.5 ± 0.30

[a] Solution had the pH value shown. Decrease in IOP measured 30, 60, and 90 min after administration. [b] $\Delta IOP = IOP_{control\ eye} - IOP_{treated\ eye}$ ($n = 3$). [c] As hydrochloride salt.

Table 5. Ocular Tissue Concentrations after 1 and 2 h following Corneal Application of 1 drop (50 μL) of a 2% Solution of Inhibitors **C13**,**14** in Albino Rabbits

time (h)	drug concentration (μM)[a] cornea	aqueous humor	ciliary process
		DZA·HCl	
1	105 ± 5	32 ± 3	15 ± 3
2	39 ± 4	21 ± 2	6 ± 1
		C13	
1	176 ± 15	324 ± 18	45 ± 6
2	69 ± 5	42 ± 3	12.5 ± 1
		C14	
1	150 ± 11	296 ± 10	38 ± 3
2	36 ± 5	39 ± 3	7.1 ± 0.8

[a] Mean ± standard deviation ($n = 3$). With DZA·HCl as standard.

this group,[12,13,18,23] and were applied for the synthesis of derivatives (**A–F**)**1–25** described in the present paper. Generally, in the aromatic series, best results were obtained working in anhydrous pyridine as solvent, for both acylation and alkyl/arylsulfonylation reactions, whereas in the heterocyclic sulfonamides series things were more complicated. Thus, Schotten–Baumann conditions were efficient only when working with aromatic sulfonyl/acyl halides (case in which the reactions performed in pyridine led to formation of tar and small yields in the desired acylated/arylsulfonylated compounds). Conversely, aliphatic sulfonyl/acyl halides reacted better in anhydrous pyridine than in Schotten–Baumann conditions. In the present study both these methods have been used, together with a new procedure developed by us, consisting of a combination of the two methods described above. Thus, we observed that a large number of amino/imino/hydrazino/hydroxy-containing aromatic/heterocyclic sulfonamides are easily derivatized by sulfonyl/acyl halides in acqueous acetone, working at temperatures between 4 and 25 °C. This strategy has been perticularly useful for obtaining the fluoro-containing sulfonamides reported here (see Experimental Section for details). Derivatization of amino, alcoholic, phenolic, or imino/hydrazino moieties present in the raw material sulfonamides **1–25** has been achieved the above-mentioned procedures too, but generally, the reactions of amines could be controlled better than those of the corresponding phenols/alcohols, leading to fewer side products. The new compounds reported here were characterized by standard chemical (elemental analysis) and spectroscopic (IR, ^{1}H NMR, ^{13}C NMR, ^{19}F NMR) procedures that confirmed their structure (see Experimental Section for details).

In Vitro CA Inhibition. CA inhibition data against three isozymes (hCA I, hCA II, and bCA IV) for the prepared compounds and standard inhibitors are shown in Table 1. All these compounds act as better CA inhibitors than the parent sulfonamides from which they were obtained (data not shown), the most efficient ones including the heterocyclic sulfonamide derivatives, such as the 1,3,4-thiadiazole-2-sulfonamides **13** and **15** and the corresponding thiadiazolines **14**, as well as the benzothiazole-2-sulfonamide derivatives **21–23**. In the aromatic sulfonamide series, compounds with different types of activity were obtained, from moderately active (derivatives of orthanilamide **1**, metanilamide **2**, sulfanilamide **3**, etc.) to active (derivatives of halogenosulfanilamides **7–10** or the benzene-1,3-disulfonamides **12** and **13**). The same pattern of inhibition has in fact been observed previously, with other sulfonamides designed by the above-mentioned tail strategy, and this fact has been discussed previously in greater detail.[12,13] The most important factor influencing the biological activity of these compounds was the nature of the acylating/sulfonylating moiety containing the perfluoroalkyl/aryl groups. Thus, for a given sulfonamide, inhibitory activity generally varied in the following manner: $C_6F_5SO_2 > C_4F_9SO_2 \cong C_6F_5CO > C_8F_{17}SO_2 \cong C_8F_{17}CO > CF_3SO_2$.

Among the three CA isozymes investigated here, the most susceptible to inhibition was hCA II, followed by bCA IV and then hCA I. This is of great importance, since the sensitive isozymes are just those involved in aqueous humor secretion within the eye, i.e., CA II and CA IV.

Quantitative detailed measurements of water solubility, log *P*, and corneal accession rates were performed for several compounds reported here: **A14**, **B13**, **C13**,**14**, **D16**, **E16**, **F13**,**14**. An important factor influencing the pharmacological properties of a topical CA inhibitor is represented by its water solubility. The presence of perfluoroalkyl/arylsulfonyl moieties in the compounds reported here led to highly increased water solubility as compared to that of the starting sulfonamide (data not shown) from which it was prepared. Inhibitors containing the perfluoroalkyl/arylcarbonyl moiety were slightly less hydrosoluble than the corresponding sulfonylated ones, but they possessed good enough solubility for their ocular topical application. As seen from the data of Table 2, the newly obtained compounds also possess moderate lipid solubility, similar to or slightly better than that of DRZ, and this is also of great importance for the topical antiglaucoma effect. In fact, Maren[4] noted in his classical review that one of the conditions needed for a sulfonamide to act as an effective IOP-lowering agent is to possess (modest) lipid solubility (attributable to its un-ionized form), accompanied by good water solubility (eventually conferred by the presence of ionizable groups of appropriate pK_a). As seen from data of Table 2, the compounds reported here possessed just this type of balanced physicochemical properties. The very good hydrosolubility of these perfluoro derivatives is rather surprising (considering their liposolubility), but a similar case was also encountered by Maren's group for analogues of MZA possessing longer aliphatic chains instead of the acetyl moiety, with the propionyl derivative 3 times more soluble than the acetyl one. The accession rates across the cornea of the new compounds investigated here were of the same order of magnitude (or slightly better) as those of DZA, as a consequence of their favorable physicochemical properties mentioned above. Thus, 2% water solutions of some of these new inhibitors could be obtained without any problems, and these solutions were stable for months at room temperature, with no hydrolysis of the sulfonylated compound detected by means of TLC or HPLC (data not shown). Thus, in contrast to trifluoromethazolamide **26**, the compounds reported here are chemically stable in water solutions.

IOP Lowering in Normotensive and Glaucomatous Rabbits. In vivo IOP-lowering experiments were done in normotensive and glaucomatous rabbits, with several of the new compounds (**A14**, **B13**,**14**, **C13**–**15**,**21**, **D13**,**14**, **F13**,**14**, etc.) which were among the strongest hCA II and bCA IV inhibitors in the obtained series. From data of Table 3 it is seen that similarly to DZA, the new perfluorosulfonamides possess strong topical activity in lowering IOP in normotensive rabbits. IOP lowering observed with some of the most active CA inhibitors reported here was much more accentuated than that with the standard, clinically used inhibitor DZA. Thus, DZA reaches a maximal IOP lowering at 60 min postadministration, which is of 4.1 mmHg, but after 90 min, this effect is further reduced to an IOP lowering of only 2.7 mmHg. The compounds reported here also showed their maximal IOP lowering after 60 min, which was in the range of 7.3–12.5 mmHg. At later times after the topical administration their effect was much more pronounced than that of the clinically used drug (for instance at 90 min the IOP lowering was in the range of 4.9–10.2 mmHg with derivatives such as **A14**, **B13**,**14**, **C13**–**15**, **D13**,**14**, etc.; see Table 3) and in several cases the return at the basal IOP occurred after 5–6 h post-administration, in contrast to DZA for which the return occurred after 3 h (Figure 1). This fact might be extremely important for the pharmacology of a putative drug of the class described here, as it would imply administration of the eye drops only two times daily, in contrast to DZA which was initially administered four times a day and then three times daily (which remains the mostly used administration schedule presently).[7]

The same powerful IOP lowering after administration of perfluorosulfonamide derivatives has been observed in glaucomatous rabbits (Table 4). In this case, at 30 min after topical administration IOP was lowered with 6.8–8.3 mmHg by the new sulfonamides (with DZA the IOP lowering was of 3.6 mmHg); at 60 min the IOP lowering with the first derivatives mentioned above was in the range of 13.0–15.7 mmHg (6.7 with DZA), whereas at 90 min it was 15.5–17.4 mmHg (versus 4.2 mmHg with DZA). We stress again that the pH of all solutions of the new inhibitors described here, used in the in vivo experiments, were in the range of 6.5–7.5 pH units and that no eye irritation has been observed (except for a benzothiazole derivative, **C21**, in which probably an allergic reaction occurred, as reported for similar benzothiazole- or benzothiophenesulfonamides by the Merck group).[25] In fact it has been established that such electrophilic sulfonamides undergo a nucleophilic substitution reaction of the sulfonamido group by reduced glutathione in vivo, which leads to the abovementioned allergy, which in our case has been observed only with the benzothiazole derivative **C21** but not with other perfluorosulfonamides. Thus, the prerequisites that solutions of such perfluorosulfonamides will provoke less (or no) eye irritation as compared to the very acidic DZA solutions are met, as one may expect from these in vivo data in experimental animals.

Drug Distribution in Ocular Fluids and Tissues. In Table 5 the drug distribution in ocular fluids and tissues is shown, after the topical administration of compounds **C13**,**14**. It is seen that 1 and 2 h after topical administration of the drug, high levels of inhibitors were found in the cornea, aqueous humor, and ciliary processes. Based on the inhibition constant of these compounds, the fractional inhibition estimated in these tissues/fluids is of 99.5–99.9%,[4] proving the fact that the IOP decrease is indeed due to CA inhibition. Furthermore, as seen from the data of Table 5, the new compounds reported here tend to concentrate in the aqueous humor (concentrations of around 295–325 μM were detected 1 h after administration), whereas DZA reaches much lower concentrations (32 μM after 1 h). High concentrations of the inhibitor were maintained 2 h after administration too. Concentrations of the new compounds **C13**,**14** in the cornea and ciliary processes are also enhanced as compared to those of DZA, but the differences are not so dramatic as those from the aqueous humor. Thus, the strong and long-lasting IOP-lowering properties of the new compounds reported here are probably due to this concentrating effect reached mainly in the aqueous humor, but which is also present in the cornea and ciliary processes. The mechanism by which such high concentrations of active compounds reach these tissues is unknown at the moment.

Conclusions

We report here a novel class of water-soluble, topically very effective antiglaucoma sulfonamides, obtained by attaching perfluoroalkyl/arylsulfonyl or perfluoroalkyl/arylcarbonyl moieties to well-known aromatic/heterocyclic sulfonamides incorporating free amino, imino, hydrazino, or hydroxy groups. Ring systems which have been derivatized by the above-mentioned procedures included: 2-, 3-, or 4-aminobenzenesulfonamides, 4-(ω-aminoalkyl)benzenesulfonamides, 3-halogeno-substituted-sulfanilamides, 1,3-benzenedisulfonamides, 1,3,4-thiadiazole-2-sulfonamides, benzothiazole-2-sulfonamides, and thienothiopyran-2-sulfonamides, among others. Many of the newly reported derivatives showed very good water solubility, in the range of 2−5% at neutral pH values, whereas their lipid solubility, hydrophobicity (log *P*), and accession rates across the cornea were those appropriate for acting as efficient topical IOP-lowering agents. Many such inhibitors possessed affinities in the nanomolar range for isozymes hCA II and bCA IV, acting as effective enzyme inhibitors in vitro. Some of the most active inhibitors strongly lowered IOP in normotensive and glaucomatous rabbits, showing a prolonged duration of action as compared to DZA. The new compounds reported here might lead to the development of more efficient antiglaucoma drugs from the class of sulfonamide CA inhibitors.

Experimental Section

Chemistry. Melting points were recorded with a heating plate microscope and are not corrected. IR spectra were recorded in KBr pellets with a Carl Zeiss IR-80 instrument. ^{1}H NMR spectra were recorded in DMSO-d_6 as solvent, with a Bruker CPX200 or Varian 300 instrument. Chemical shifts are reported as δ values, relative to Me_4Si as internal standard. Elemental analyses were done by combustion for C, H, N with an automated Carlo Erba analyzer and were ±0.4% of the theoretical values. All reactions were monitored by thin-layer chromatography (TLC) using 0.25-mm precoated silica gel plates (E. Merck). Analytical and preparative HPLC was performed on a reversed-phase C_{18} Bondapack column, with a Beckman EM-1760 instrument. Sulfonamides **1**−**25** used in synthesis were either commercially available compounds (from Sigma-Aldrich or Acros) or were prepared as described previously: 4-hydrazinobenzenesulfonamide **4** by diazotization of sulfanilamide followed by reduction of the diazonium salt with tin(II) chloride;[26] halogenosulfanilamides **9**−**12** by halogenation of sulfanilamide as reported in the literature;[27] compound **15** from 5-amino-1,3,4-thiadiazole-2-sulfonamide (obtained from AAZ)[28] by acylation with the phthalimido derivative of β-alanine, followed by hydrazinolysis;[29] whereas imine **14** by deprotection of MZA with concentrated hydrochloric acid.[30] The benzothiazole-2-sulfonamide derivatives **18**−**20** were prepared as described in ref 31, whereas the alcohols **21** and **22** from the corresponding amines by diazotization followed by hydrolysis of the diazonium salts. DZA was prepared as described in the literature.[32] Perfluorosulfonyl halides and perfluoroacyl halides were from Sigma-Aldrich. Acetonitrile (E. Merck) or other solvents used in the synthesis were doubly distilled and kept on molecular sieves in order to maintain them in anhydrous conditions.

General Procedure for Preparation of Perfluorinated Compounds. Method A (Schotten−Baumann synthesis): 5 mmol of sulfonamide **1**−**25** (such as 5-amino-1,3,4-thiadiazole-2-sulfonamide **13** or 5-imino-4-methyl-2-sulfonamido-δ^2-1,3,4-thiadiazoline **14**) was dissolved in a 15-mL solution of 2.5 M NaOH and cooled to 2−5 °C in a salt−ice bath. 5 mmol of sulfonyl/acyl chloride **A**−**F** 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), 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, the reaction mixture was poured in 50 mL of ice + water, and the precipitated sulfonamides were recrystallized as described above or purified by preparative HPLC.

Method C: The sulfonamide to be derivatized was suspended/dissolved in a 1:1 mixture of acetone−water and the stoichiometric amounts of sulfonyl chloride and base were added concomitantly. The used base was 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, then the solvent evaporated and the reaction products recrystallized as described above.

4-Trifluoromethanesulfonamidobenzenesulfonamide, A3: white crystals, mp 290−1 °C dec; IR (KBr) cm^{-1} 1152 and 1176 (SO_2sym), 1345 and 1382 (SO_2as), 3360 (NH, NH_2); ^{1}H NMR (DMSO-d_6) δ 7.33 (s, 2H, SO_2NH_2), 7.80 (d, 2H, AA′BB′, 8.9), 7.95 (d, 2H, AA′BB′, 8.9), 8.15 (br s, SO_2NH); ^{13}C NMR (DMSO-d_6) δ 45.8 (CF_3), 126.67 (C2/C3−Ph), 128.26 (C3/C2−Ph), 139.49 (C1/C4−Ph), 141.80 (C4/C1−Ph). Anal. ($C_7H_7F_3N_3O_4S_2$) C, H, N.

4-(Perfluorobutylsulfonylamidoethyl)benzenesulfonamide, B6: white crystals, mp 234−6 °C; IR (KBr) cm^{-1} 1158 and 1169 (SO_2sym), 1355 and 1374 (SO_2as), 3365 (NH, NH_2); ^{1}H NMR (DMSO-d_6) δ 2.91 (t, 2H, 7.2, αCH_2), 3.49 (q, 2H, 6.4, βCH_2), 7.30 (s, 2H, SO_2NH_2), 7.44 (d, 2H, AA′BB′, 8.3), 7.78 (d, 2H, AA′BB′, 8.1), 8.19 (br s, SO_2NH); ^{13}C NMR (DMSO-d_6) δ 34.80 (CH_2−Ph), 40.28 (N−CH_2), 127.99 (C2/C3−Ph), 129.13 (C3/C2−Ph), 139.90 (C1/C4−Ph), 143.73 (C4/C1−Ph). Anal. ($C_{12}H_{11}F_9N_2O_4S_2$) C, H, N.

5-Perfluorophenylsulfonylamido-1,3,4-thiadiazole-2-sulfonamide, C13: white crystals, mp 260−2 °C; IR (KBr) cm^{-1} 1151 and 1180 (SO_2sym), 1363 and 1376 (SO_2as), 3060 (NH), 3365 (NH_2); ^{1}H NMR (DMSO-d_6) δ 7.25 (br s, 2H, SO_2NH_2), 9.15 (br s, 1H, SO_2NH); ^{13}C NMR (DMSO-d_6) δ 124.8, 127.9, 138.6, 159.5 (C-2 of thiadiazole), 170.0 (C-5 of thiadiazole); ^{19}F NMR (DMSO-d_6) δ −134.0 (2,6-F_2), −143.7 (4-F), −158.9 (3,5-F_2). Anal. ($C_8H_3F_5N_4O_4S_3$) C, H, N.

4-Methyl-5-perfluorophenylcarboximido-δ^2-1,3,4-thiadiazoline-2-sulfonamide, F14: white crystals, mp 243−4 °C; IR (KBr) cm^{-1} 1146 (SO_2sym), 1375 (SO_2as), 1550 (amide II), 1610 (amide I); 3060 (NH), 3365 (NH_2); ^{1}H NMR (DMSO-d_6) δ 3.54 (s, 3H, Me), 7.13 (br s, 2H, SO_2NH_2), 9.15 (br s, 1H, SO_2NH); ^{13}C NMR (DMSO-d_6) δ 31.5 (Me), 120.7, 124.3, 135.9, 164.91 (C-thiadiazoline), 164.94 (C-thiadiazoline); ^{19}F NMR (DMSO-d_6) δ −137.8 (2,6-F_2), −149.1 (4-F), −158.2 (3,5-F_2). Anal. ($C_{10}H_5F_5N_4O_3S_2$) 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.[33] Cell growth conditions were those described in ref 34 and enzymes were purified by affinity chromatography according to the method of Khalifah et al.[35] Enzyme concentrations were determined spectrophotometrically at 280 nm, utilizing a molar absorptivity of 49 mM^{-1} cm^{-1} for CA I and 54 mM^{-1} cm^{-1} for CA II, based on M_r = 28.85 kDa for CA I and 29.3 kDa for CA II.[36,37] bCA IV was isolated from bovine lung microsomes as described by Maren et al., and its concentration has been determined by titration with EZA.[38]

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.[39] 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.[39] Nonenzymatic hydrolysis rates were always subtracted from the observed rates. Duplicate 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.[39] Enzyme concentrations were 3.3 nM for CA II, 10 nM for CA I, and 34 nM for CA IV (this isozyme has a decreased esterase activity[40] and higher concentrations had to be used for the measurements). Adult male New Zealand albino rabbits weighing 3−3.5 kg were used in the experiments (three animals were used for each inhibitor studied). The experimental procedures conformed to the Association for Research in Vision and Ophthalmology Resolution on the use of animals. The rabbits were kept in individual cages with food and water provided ad libitum. The animals were maintained on a 12 h:12 h light/dark cycle in a temperature-controlled room, at 22−26 °C. Solutions of inhibitors (2%, by weight, as hydrochlorides, triflates, or sodium carboxylates) were obtained in distilled−deionized water. The pH of these solutions was in the range of 5.5−8.4.

Measurement of Tonometric IOP. IOP was measured using a Digilab 30R pneumatonometer (BioRad, Cambridge, MA) as described by Maren's group.[41,42] The pressure readings were matched with two-point standard pressure measurements at least twice each day using a Digilab Calibration verifier. All IOP measurements were done by the same investigator with the same tonometer. One drop of 0.2% oxybuprocaine hydrochloride (novesine; Sandoz) diluted 1:1 with saline was instilled in each eye immediately before each set of pressure measurements. IOP was measured three times at each time interval and the means reported. IOP was measured first immediately before drug administration, then at 30 min after the instillation of the pharmacological agent, and then each 30 min for a period of 4−6 h. For all IOP experiments drug was administered to only one eye, leaving the contralateral eye as an untreated control. The ocular hypotensive activity is expressed as the average difference in IOP between the treated and control eye, in this way minimizing the diurnal, seasonal, and interindividual variations commonly observed in the rabbit.[41,42] All data are expressed as mean ± SE, using a one-tailed *t*-test.

Ocular hypertension was elicited in the right eye of albino rabbits by the injection of α-chymotrypsin (from Sigma) as described by Melena et al.[43] The IOP of operated animals was checked after approximately 4 weeks, and animals with an elevated pressure of 30−35 mmHg were used at least 1 month after the injection of α-chymotrypsin.

Drug Distribution in Ocular Fluids and Tissues. The general procedure of Maren's group was followed.[41,42] The animals were killed with an intracardiac injection. Aqueous humor (both posterior and anterior chamber fluids) was withdrawn. Then, the cornea and anterior uvea (iris plus attached ciliary body) were dissected, rinsed well with water, blotted, weighed and put into 1−2 mL of water. For isolation of the ciliary processes, intact anterior uvea rings were placed on a Parafilm-covered piece of polystyrene foam in a Petri dish. The tissue was wetted with normal saline and dissected under a microscope, when ciliary processes were liberated from their attachment to the iris, cut, weighed and put in 0.5 mL of distilled water. The tissue from four eyes (average weight of 8 mg/eye) was pooled for drug analysis. Samples were boiled for 5 min (in order to denature CA and free drug from the E·I complex), diluted and then incubated with a known amount of enzyme. The activity of the free enzyme and the activity in the presence of inhibitor were determined as described above. A calibration curve was used in order to determine the fractional inhibition in the different tissues, as described in refs 41, 42.

Determination of Water (Buffer) Solubility. A standard solution was prepared by dissolving a precisely weighted amount (generally 1 mg) of inhibitor in 10 mL of methanol. The UV absorption maximum of each compound was determined (with a Cary 3 spectrophotometer) eventually diluting the solution (with MeOH) as necessary. A saturated solution of each compound was then prepared by stirring magnetically a small volume of 0.039 M phosphate buffer (pH 7.4) in the presence of excess inhibitor for 3 h. The obtained saturated solution was filtered in order to remove solid compound through a Millipore 0.45-μm filter and scanned by UV at the wavelength of the absorption maximum previously determined. Total solubility was determined by the relationship:

$$C' = A'C/A$$

where C = concentration of standard solution (mg/mL), A = absorbance of standard solution, A' = absorbance of the saturated solution, C' = concentration of the saturated solution (mg/mL).[18]

Partition Coefficient Determinations. Chloroform−buffer partition coefficients were obtained by equilibrating the test compound between chloroform and 0.1 ionic strength pH 7.4 phosphate buffer. The concentration in each phase was determined by UV spectrophotometry or HPLC.[18]

Transcorneal Penetration of Drugs. The method of Maren et al.[9] with the modifications of Pierce's group[44,45] (for the HPLC assay of sulfonamides) was used. Excised rabbit corneas with either intact or denuded epithelium were used in these experiments. The pH was 7.4 and exposed area was 1.2 cm^2. Concentrations of drug of 40−2000 μM were placed in the epithelial chamber and samples of fluid were collected from the endothelial chamber at different intervals, up to 4 h. Both chambers contained 6 mL. Drugs present in these fluids were assayed by the HPLC method of Pierce et al.[44,45] or enzymatically.[9] The results of the drug analyses were used to calculate the rate constant of transfer across the cornea (k_{in}). As described by Pierce,[44,45] this value was determined by using the formula:

$$k_{in}\ (\times 10^3\ h^{-1}) = [drug]_{endo}/[drug]_{epi} \times 60/t \times 1000$$

where $[drug]_{endo}$ = concentration of drug on endothelial side, $[drug]_{epi}$ = concentration of drug on epithelial side, t = time (in min).

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