Carbonic Anhydrase Inhibitors. A General Approach for the Preparation of Water-Soluble Sulfonamides Incorporating Polyamino−Polycarboxylate Tails and of Their Metal Complexes Possessing Long-Lasting, Topical Intraocular Pressure-Lowering Properties

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Reaction of polyamino-polycarboxylic acids or their dianhydrides with aromatic/heterocyclic sulfonamides possessing a free amino/imino/hydrazino/hydroxy group afforded mono- and bis-sulfonamides containing polyamino-polycarboxylic acid moieties in their molecule. The acids/anhydrides used in synthesis included IDA, NTA, EDDA, EDTA and EDTA dianhydride, DTPA and DTPA dianhydride, EGTA and EGTA dianhydride, and EDDHA, among others. All the newly prepared derivatives showed strong affinity toward isozymes I, II, and IV of carbonic anhydrase (CA). Metal complexes of the new compounds have also been prepared. Metal ions used in such preparations included di- and trivalent main-group and transition cations, such as Zn(II), Cu(II), Al(III), etc. Some of the new sulfonamides/disulfonamides obtained in this way, as well as their metal complexes, behaved as nanomolar CA inhibitors against isozymes II and IV, being slightly less effective in inhibiting isozyme I. Some of these sulfonamides as well as their metal complexes strongly lowered intraocular pressure (IOP) when applied topically, directly into the normotensive/glaucomatous rabbit eye, as 1−2% water solutions/suspensions. The good water solubility of these sulfonamide CA inhibitors, correlated with the neutral pH of their water solutions used in the ophthalmologic applications and the long duration of action of the IOP-lowering effect, makes them interesting candidates for developing novel types of antiglaucoma drugs devoid of serious topical side effects.

Introduction

Sulfonamides possessing carbonic anhydrase (CA, EC 4.2.1.1) inhibitory properties such as acetazolamide (AAZ), methazolamide (MZA), ethoxzolamide (EZA), and dichlorophenamide (DCP) (Chart 1) have been used for more than 40 years as systemic eye-pressure-lowering drugs in the treatment of open-angle glaucoma as well as other diseases associated with acid/base secretory disequilibria.[1−3] Their ocular effects are due to inhibition of at least two CA isozymes present within ciliary processes of the eye, i.e., CA II and CA IV, followed by a diminished secretion of bicarbonate and a 25−30% reduction of aqueous humor secretion.[1−3] The main drawback of such agents is constituted by side effects such as augmented diuresis, fatigue, paresthesias, anorexia, etc. due to CA inhibition in other tissues/organs than the target one (CA, in the form of 14 isozymes, is ubiquitous in vertebrates).[4]

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,[5] was shortly followed by the development of the first agents of this type, dorzolamide DZA (clinically launched in 1995),[6] followed soon thereafter (in 1999) by the structurally related brinzolamide BRZ[7] (Chart 1). These topically acting antiglaucoma sulfonamides incorporate secondary amine moieties because the required water solubility needed for effective topical action is achieved by using their hydrochloride salts.[6,7] Still, this represents an undesired problem because the pH of such solutions is rather acidic (pH 5.5) and consequently produces eye irritation after the topical administration of the drug, as already reported for many patients treated with dorzolamide (the use of brinzolamide is rather recent, and few studies of the side effects of this drug are available for the moment).[1,6,7] In fact, the most common adverse effects after topical dorzolamide treatment are local burning and stinging of the eyes, reddening of the eyes, and blurred vision and bitter taste,[1,6,7] but more serious side effects, such as contact allergy, nephrolithiasis, anorexia, depression, and dementia, as well as irreversible corneal decompensation in patients already presenting corneal problems, were also reported with dorzolamide.[8−11]

The clinical importance of the two existing topical antiglaucoma sulfonamides, and also their imperfections due to many undesired topical side effects, fostered

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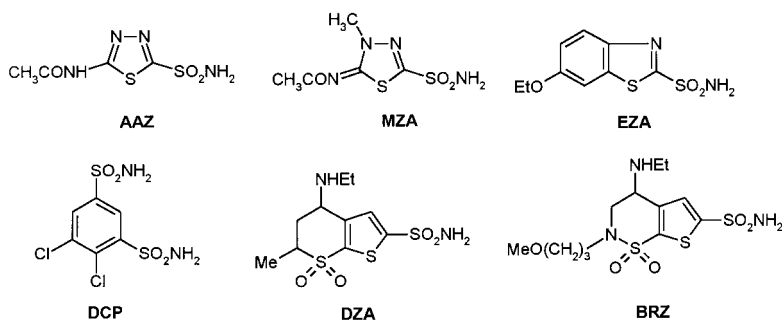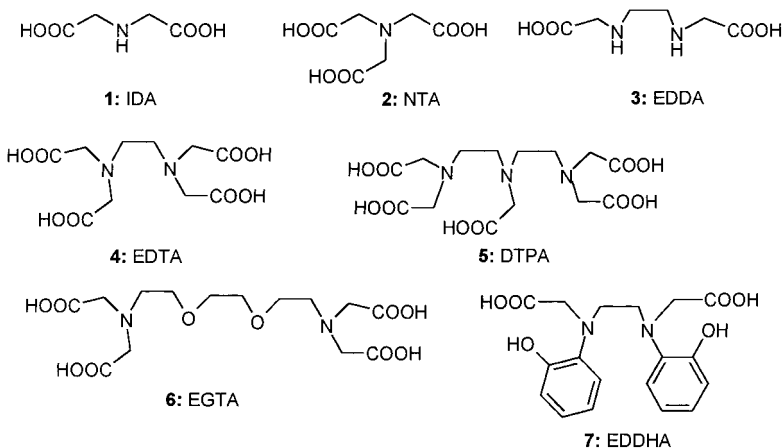ll CSGI.

Chart 1

Chart 2

much research in the synthesis and evaluation of novel generation of such derivatives.[12–18] The major side effects of dorzolamide mentioned above might be avoided for compounds that should not be administered as hydrochloride salts, but this generally leads to a drastical diminution of water solubility of such a sulfonamide.[1,2,12] Here we propose a general approach for obtaining water-soluble, high-affinity sulfonamide CA inhibitors, which do not owe their water solubility to formation of hydrochloride salts. Thus, in a preliminary communication[19] we recently showed that by attaching diethylenetriaminopentaacetic acid moieties to the molecules of aromatic/heterocyclic sulfonamides, strong, water-soluble (at pH 7–7.5) CA inhibitors may be obtained, which showed promising properties as topically acting antiglaucoma agents in normotensive and glaucomatous rabbits. Here, we extend the above-mentioned approach, showing that by attachment of different polyamino-polycarboxylic acid moieties to the amino/imino/hydrazino/hydroxy moieties of aromatic/ heterocyclic sulfonamides (by their reaction with acids/ anhydrides such as IDA (iminodiacetic acid), NTA (nitrilotriacetic acid), EDDA (ethylenediaminodiacetic acid), EDTA (ethylenediaminotetraacetic acid) and EDTA dianhydride, DTPA (diethylenetriaminopentaacetic acid) and DTPA dianhydride, EGTA (ethylenebis(oxyethyl-enenitrilo)tetraacetic acid) and EGTA dianhydride, and EDDHA (ethylenediaminodihydroxyphenyldiacetic acid)), both mono- as well as bis-sulfonamides with excellent water solubility (at neutral pH values) may easily be prepared. Furthermore, metal complexes of such derivatives were also obtained because it was recently shown that some Zn(II) or Cu(II) complexes of sulfonamides devoid of topical activity as intraocular pressure-lower-

ing (IOP) drugs show themselves to be very strong in such properties in experimental animals.[20]

Results

Chemistry. The chemical structures of the carboxylic acids **1**–**7** as well as those of sulfonamides **A**–**T** used in the synthesis of the new compounds reported here are shown in Charts 2 and 3, respectively. Because a large number of such derivatives (monoamides/esters, as well as diamides/esters) have been prepared, in the following they will be abbreviated by using both a figure designating the carboxylic acid from which they were derived and a letter designating the sulfonamides at which the polyamino-polycarboxylate moiety has been attached. For example, **1A** is the monoamide of imino-diacetic acid **1** with orthanilamide **A**; **6M** is the monoa-mide of EGTA **6** with 5-amino-1,3,4-thiadiazole-2-sulfonamide **M**; **6M$_2$** is the bisamide of EGTA with the sulfonamide **M**; **4S** is the monoester of EDTA **4** with 4-(2-hydroxymethyl)benzenesulfonamide **S**; **4S$_2$** is the diester of EDTA **4** with 4-(2-hydroxymethyl)benzene-sulfonamide **S** (see these in Chart 4). The whole series of the monosulfonamides containing the seven carboxy-lic acids and the 20 sulfonamides have been prepared (140 derivatives). Bis-amides/esters derived from the above-mentioned 20 sulfonamides and the following acids EDTA, DTPA, and EGTA (60 compounds) have also been obtained.

Two synthetic approaches have been used for the preparation of the new sulfonamides reported here: (i) reaction of polyamino-polycarboxylic acids **1**–**7** with sulfonamides **A**–**T** in the presence of carbodiimides, as reported previously by Whitesides[17] or by this group;[15,18] (ii) reaction of dianhydrides (such as EDTA dianhydride,

Chart 3
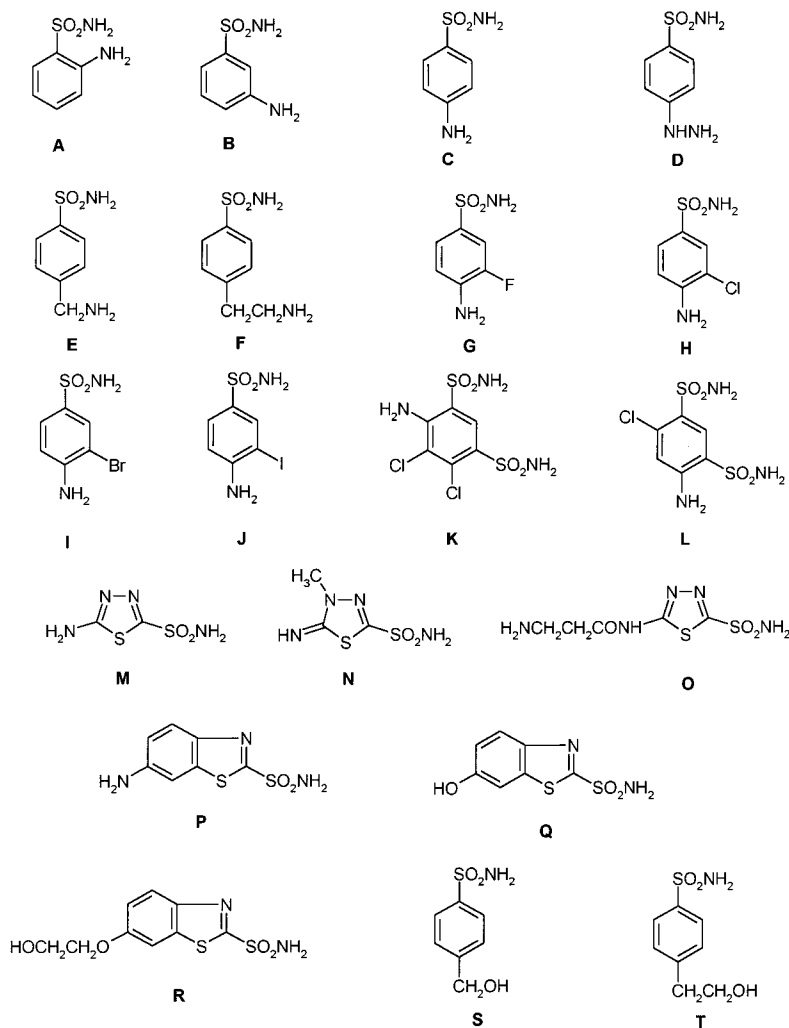
DTPA dianhydride, and EGTA dianhydride) with sulfonamides **A–T**, in molar ratios of 1:2, when bis-sulfonamides were obtained.[19] The metal complexes were then prepared by using sodium salts of the above-mentioned sulfonamides as ligands and transition metal salts (such as zinc(II) chloride, copper(II) sulfate, aluminum(III) sulfate, etc.), as reported previously.[20–22]

Carbonic Anhydrase 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 aqueous 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).[1]

Transcorneal Penetration of Drugs. Some physicochemical properties of several of the new sulfonamides reported here that are relevant for their pharmacological activity, such as buffer solubility and chloroform–buffer partition coefficient, are shown in Table 2. The in vitro transcorneal accession rates (k_{in}) of classical sulfonamides and topically acting derivatives, such as dorzolamide 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
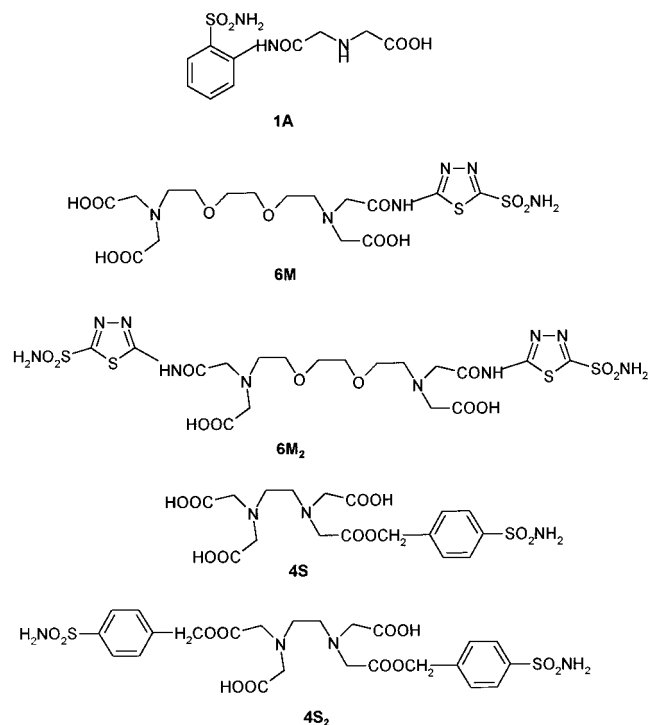
Chart 4

Table 1. Inhibition Data for Some Derivatives Reported in the Present Paper (Data in Parentheses Represent Inhibition by the Parent Sulfonamide **A**−**T**)

inhibitor	K_I (nm)			inhibitor	K_I (nm)		
	hCA I[a]	hCA II[a]	bCA IV[b]		hCA I[a]	hCA II[a]	bCA IV[b]
acetazolamide	900	12	220	**4C**	6300 (28000)	124 (300)	330 (3000)
methazolamide	780	14	240	**4D**	16000 (78500)	310 (320)	750 (3200)
ethoxzolamide	25	8	13	**4E**	900 (25000)	52 (170)	83 (2800)
dichlorophenamide	1200	38	380	**4F**	650 (21000)	47 (160)	75 (2500)
dorzolamide	>50000	9	43	**4G**	360 (8300)	31 (60)	40 (180)
brinzolamide		3	45	**4H**	700 (9800)	52 (110)	69 (320)
1A	27000 (45400)	280 (295)	1240 (1310)	**4I**	130 (6500)	25 (40)	50 (66)
1B	23000 (25000)	233 (240)	1500 (2200)	**4J**	123 (6000)	36 (70)	78 (125)
1C	21300 (28000)	214 (300)	450 (3000)	**4K**	130 (6100)	19 (28)	96 (175)
1D	32700 (78500)	313 (320)	980 (3200)	**4L**	210 (8400)	73 (75)	105 (160)
1E	3600 (25000)	125 (170)	279 (2800)	**4M**	225 (8600)	12 (60)	37 (540)
1F	3450 (21000)	90 (160)	257 (2500)	**4N**	215 (9300)	9 (19)	26 (355)
1G	1300 (8300)	54 (60)	145 (180)	**4O**	200 (455)	2 (3)	23 (125)
1H	975 (9800)	80 (110)	165 (320)	**4P**	48 (70)	3 (9)	11 (19)
1I	610 (6500)	38 (40)	57 (66)	**4Q**	43 (55)	2 (8)	10 (17)
1J	515 (6000)	55 (70)	114 (125)	**4R**	39 (50)	1.5 (7)	8 (15)
1K	405 (6100)	24 (28)	107 (175)	**4S**	850 (24000)	104 (125)	335 (560)
1L	400 (8400)	69 (75)	145 (160)	**4T**	640 (18000)	89 (110)	310 (450)
1M	415 (8600)	50 (60)	265 (540)	**4A₂**	8100 (45400)	230 (295)	650 (1310)
1N	710 (9300)	13 (19)	120 (355)	**4B₂**	7650 (25000)	190 (240)	620 (2200)
1O	280 (455)	1.5 (3)	48 (125)	**4C₂**	5000 (28000)	87 (300)	230 (3000)
1P	65 (70)	6 (9)	14 (19)	**4D₂**	12500 (78500)	300 (320)	510 (3200)
1Q	50 (55)	5 (8)	14 (17)	**4E₂**	510 (25000)	36 (170)	62 (2800)
1R	46 (50)	6 (7)	12 (15)	**4F₂**	400 (21000)	33 (160)	54 (2500)
1S	1260 (24000)	110 (125)	350 (560)	**4G₂**	240 (8300)	20 (60)	35 (180)
1T	1200 (18000)	97 (110)	340 (450)	**4H₂**	225 (9800)	31 (110)	50 (320)
2A	25800 (45400)	280 (295)	1200 (1310)	**4I₂**	105 (6500)	15 (40)	34 (66)
2B	23000 (25000)	234 (240)	1520 (2200)	**4J₂**	89 (6000)	23 (70)	45 (125)
2C	20600 (28000)	210 (300)	450 (3000)	**4K₂**	65 (6100)	13 (28)	51 (175)
2D	30500 (78500)	318 (320)	930 (3200)	**4L₂**	72 (8400)	24 (75)	69 (160)
2E	3500 (25000)	101 (170)	256 (2800)	**4M₂**	80 (8600)	6 (60)	12 (540)
2F	3300 (21000)	88 (160)	240 (2500)	**4N₂**	105 (9300)	7 (19)	17 (355)
2G	1000 (8300)	53 (60)	136 (180)	**4O₂**	125 (455)	1 (3)	10 (125)
2H	960 (9800)	82 (110)	150 (320)	**4P₂**	33 (70)	2 (9)	9 (19)
2I	620 (6500)	36 (40)	53 (66)	**4Q₂**	37 (55)	1.5 (8)	8 (17)
2J	510 (6000)	57 (70)	110 (125)	**4R₂**	25 (50)	1 (7)	6 (15)
2K	345 (6100)	26 (28)	100 (175)	**4S₂**	460 (24000)	78 (125)	200 (560)
2L	300 (8400)	66 (75)	148 (160)	**4T₂**	450 (18000)	71 (110)	165 (450)
2M	410 (8600)	52 (60)	240 (540)	Zn-**4C₂**	450	20	210
2N	520 (9300)	14 (19)	106 (355)	Zn-**4E₂**	45	6	10
2O	268 (455)	2 (3)	45 (125)	Zn-**4F₂**	39	8	13
2P	62 (70)	7 (9)	13 (19)	Zn-**4M₂**	40	0.8	5
2Q	48 (55)	6 (8)	13 (17)	Zn-**4N₂**	40	0.7	8
2R	44 (50)	5 (7)	10 (15)	Cu-**4M₂**	39	0.6	6
2S	1200 (24000)	103 (125)	350 (560)	Al-**4M₂**	51	0.9	8
2T	1100 (18000)	94 (110)	320 (450)	**5A**	8600 (45400)	210 (295)	550 (1310)
3A	20500 (45400)	286 (295)	1020 (1310)	**5B**	7200 (25000)	180 (240)	500 (2200)
3B	13000 (25000)	235 (240)	1005 (2200)	**5C**	4100 (28000)	75 (300)	180 (3000)
3C	12000 (28000)	200 (300)	365 (3000)	**5D**	12000 (78500)	250 (320)	630 (3200)
3D	21800 (78500)	315 (320)	950 (3200)	**5E**	550 (25000)	23 (170)	40 (2800)
3E	1760 (25000)	81 (170)	245 (2800)	**5F**	290 (21000)	15 (160)	36 (2500)
3F	1540 (21000)	80 (160)	250 (2500)	**5G**	180 (8300)	13 (60)	29 (180)
3G	780 (8300)	49 (60)	103 (180)	**5H**	200 (9800)	20 (110)	32 (320)
3H	900 (9800)	78 (110)	155 (320)	**5I**	96 (6500)	12 (40)	27 (66)
3I	380 (6500)	35 (40)	54 (66)	**5J**	79 (6000)	16 (70)	29 (125)
3J	430 (6000)	50 (70)	115 (125)	**5K**	58 (6100)	10 (28)	36 (175)
3K	290 (6100)	24 (28)	110 (175)	**5L**	87 (8400)	12 (75)	52 (160)
3L	250 (8400)	68 (75)	135 (160)	**5M**	55 (8600)	0.9 (60)	28 (540)
3M	250 (8600)	53 (60)	130 (540)	**5N**	62 (9300)	2 (19)	19 (355)
3N	275 (9300)	12 (19)	76 (355)	**5O**	110 (455)	1 (3)	16 (125)
3O	240 (455)	2 (3)	39 (125)	**5P**	36 (70)	0.8 (9)	9 (19)
3P	56 (70)	7 (9)	12 (19)	**5Q**	32 (55)	0.8 (8)	6 (17)
3Q	45 (55)	5 (8)	11 (17)	**5R**	30 (50)	0.6 (7)	5 (15)
3R	43 (50)	4 (7)	9 (15)	**5S**	510 (24000)	80 (125)	265 (560)
3S	1020 (24000)	106 (125)	350 (560)	**5T**	360 (18000)	61 (110)	190 (450)
3T	1000 (18000)	95 (110)	330 (450)	**5A₂**	5400 (45400)	175 (295)	330 (1310)
4A	9200 (45400)	280 (295)	800 (1310)	**5B₂**	6400 (25000)	155 (240)	425 (2200)
4B	10000 (25000)	230 (240)	750 (2200)	**5C₂**	3750 (28000)	64 (300)	156 (3000)

Table 1. (Continued)

inhibitor	K_I (nm) hCA I[a]	hCA II[a]	bCA IV[b]	inhibitor	K_I (nm) hCA I[a]	hCA II[a]	bCA IV[b]
$5D_2$	9800 (78500)	215 (320)	430 (3200)	$6C_2$	3800 (28000)	60 (300)	150 (3000)
$5E_2$	380 (25000)	19 (170)	37 (2800)	$6D_2$	9500 (78500)	210 (320)	400 (3200)
$5F_2$	170 (21000)	8 (160)	25 (2500)	$6E_2$	350 (25000)	15 (170)	39 (2800)
$5G_2$	135 (8300)	7 (160)	18 (180)	$6F_2$	160 (21000)	7 (160)	20 (2500)
$5H_2$	155 (9800)	15 (110)	27 (320)	$6G_2$	155 (8300)	7 (60)	16 (180)
$5I_2$	79 (6500)	10 (40)	21 (66)	$6H_2$	150 (9800)	9 (110)	22 (320)
$5J_2$	70 (6000)	9 (70)	24 (125)	$6I_2$	85 (6500)	12 (40)	25 (66)
$5K_2$	43 (6100)	9 (28)	25 (175)	$6J_2$	76 (6000)	8 (70)	20 (125)
$5L_2$	63 (8400)	11 (75)	41 (160)	$6K_2$	49 (6100)	10 (28)	21 (175)
$5M_2$	50 (8600)	1 (60)	7 (540)	$6L_2$	50 (8400)	13 (75)	36 (160)
$5N_2$	54 (9300)	1.5 (19)	9 (355)	$6M_2$	55 (8600)	2 (60)	4 (540)
$5O_2$	102 (455)	0.6 (3)	8 (125)	$6N_2$	36 (9300)	1.1 (19)	5 (355)
$5P_2$	25 (70)	0.5 (9)	6 (19)	$6O_2$	76 (455)	0.5 (3)	6 (125)
$5Q_2$	21 (55)	0.6 (8)	5 (17)	$6P_2$	23 (70)	0.5 (9)	4 (19)
$5R_2$	16 (50)	0.5 (7)	4 (15)	$6Q_2$	21 (55)	0.6 (8)	5 (17)
$5S_2$	325 (24000)	43 (125)	170 (560)	$6R_2$	15 (50)	0.6 (7)	5 (15)
$5T_2$	290 (18000)	34 (110)	115 (450)	$6S_2$	335 (24000)	38 (125)	150 (560)
Zn-$5C_2$	350	16	100	$6T_2$	300 (18000)	33 (110)	96 (450)
Zn-$5E_2$	39	9	21	Zn-$6C_2$	300	11	76
Zn-$5F_2$	36	7	16	Zn-$6E_2$	35	9	16
Zn-$5M_2$	40	0.5	4	Zn-$6F_2$	30	6	12
Zn-$5N_2$	43	0.4	5	Zn-$6M_2$	27	0.7	8
Cu-$5M_2$	31	0.3	3	Zn-$6N_2$	32	0.8	7
Al-$5M_2$	44	0.8	6	Cu-$6M_2$	21	0.2	2
6A	8900 (45400)	200 (295)	545 (1310)	Al-$6M_2$	19	0.7	3
6B	7450 (25000)	195 (240)	500 (2200)	7A	12500 (45400)	280 (295)	800 (1310)
6C	4000 (28000)	79 (300)	170 (3000)	7B	10000 (25000)	235 (240)	860 (2200)
6D	12500 (78500)	265 (320)	620 (3200)	7C	11500 (28000)	175 (300)	320 (3000)
6E	610 (25000)	30 (170)	48 (2800)	7D	21000 (78500)	310 (320)	840 (3200)
6F	290 (21000)	18 (160)	32 (2500)	7E	1200 (25000)	69 (170)	135 (2800)
6G	210 (8300)	17 (60)	31 (180)	7F	1050 (21000)	61 (160)	215 (2500)
6H	230 (9800)	16 (110)	30 (320)	7G	450 (8300)	43 (60)	79 (180)
6I	78 (6500)	10 (40)	32 (66)	7H	870 (9800)	67 (110)	108 (320)
6J	76 (6000)	13 (70)	24 (125)	7I	175 (6500)	34 (40)	56 (66)
6K	50 (6100)	12 (28)	35 (175)	7J	220 (6000)	47 (70)	90 (125)
6L	93 (8400)	16 (75)	65 (160)	7K	250 (6100)	23 (28)	105 (175)
6M	54 (8600)	2 (60)	21 (540)	7L	240 (8400)	65 (75)	123 (160)
6N	66 (9300)	2 (19)	16 (355)	7M	245 (8600)	54 (60)	105 (540)
6O	120 (455)	1.5 (3)	13 (125)	7N	250 (9300)	13 (19)	57 (355)
6P	39 (70)	1 (9)	7 (19)	7O	210 (455)	3 (3)	35 (125)
6Q	33 (55)	0.9 (8)	5 (17)	7P	52 (70)	6 (9)	13 (19)
6R	30 (50)	0.8 (7)	6 (15)	7Q	47 (55)	4 (8)	12 (17)
6S	500 (24000)	72 (125)	250 (560)	7R	41 (50)	3 (7)	7 (15)
6T	430 (18000)	56 (110)	155 (450)	7S	945 (24000)	110 (125)	320 (560)
$6A_2$	5600 (45400)	160 (295)	320 (1310)	7T	760 (18000)	94 (110)	300 (450)
$6B_2$	6150 (25000)	145 (240)	400 (2200)				

[a] Human (cloned) isozymes. [b] From bovine lung microsomes, by the esterase method.[4,5]

administration of the drug, are shown in Tables 3 and 4, respectively.

The full time dependence of the IOP after dorzolamide 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 compound in ocular tissues and fluids after the topical administration in normotensive rabbits are shown in Table 5.

Discussion

Chemistry. The preparation of sulfonamide CA inhibitors incorporating amino acid moieties has thoroughly been studied by this,[1,15] Whitesides,[17] and Blackburn's groups.[12] Still, compounds incorporating polyamino-polycarboxylic acid moieties in their molecule (and more specifically DTPA moieties) have only recently been reported in our preliminary communication.[19] Here we extend that work, including a large series of such derivatives in our study (of types **1**–**7**),

to better understand the structure–activity relationship in this class of CA inhibitors. Thus, several well-known polyamino-polycarboxylic acids possessing metal complexing moieties were chosen to be incorporated in the molecules of the new sulfonamides reported here for at least three reasons. (i) It was previously observed in this laboratory[23] that CA activators possessing "sticky" tails such as EDTA and DTPA attached to the aliphatic amino moiety of histamine (a much investigated CA activator)[24,25] possessed a very high affinity for isozymes CA I, II, and IV. This has been explained as due to the fact that the many heteroatoms present in these moieties confer to the obtained compounds "sticky" properties; i.e., they are able to participate in a host of interactions with amino acid residues from the rim of the active site, ensuring thus the formation of very stable E–A (enzyme–activator) adducts. Thus, it appeared of interest to apply the same approach to the design of CA inhibitors, and the strategy proved to be of interest because the compounds incorporating DTPA moieties reported in our preliminary communication[19]

Table 2. Solubility, Chloroform−Buffer Partition Coefficients, and in Vitro Corneal Permeability of Some Sulfonamide CA Inhibitors Reported in the Paper and Dorzolamide as Standard

| | | | | $k_{in} \times 10^3$ (h^{-1})[c] | |
| | | solubility,[a] | | cornea | no |
compound	pH	mM	log P[b]	intact	epithelium
dorzolamide **DZA**	5.5	60[d]	2.0[e]	3.0	5.2
1O	7.4	62[e]	1.230	2.5	4.6
2R	7.4	37[e]	1.925	5.1	10.3
3Q	7.4	44[e]	1.166	4.7	8.4
4N	7.4	65[e]	1.750	4.8	9.5
4N₂	7.4	41[e]	1.333	3.1	5.5
4K₂	7.4	32[e]	1.764	3.6	7.0
4O₂	7.4	46[e]	1.150	3.0	5.9
5C	7.4	48[e]	0.983	3.8	6.9
5C₂	7.4	35[e]	1.456	3.6	6.5
5M	7.4	59[e]	1.375	4.3	8.1
5M₂	7.4	54[e]	1.961	3.9	6.2
6I	7.4	50[e]	1.747	3.3	6.5
6M	7.4	52[e]	1.446	4.6	8.4
6G₂	7.4	47[e]	1.534	4.5	8.3
7O	7.4	44[e]	2.354	5.1	10.7

[a] Solubility in pH 7.40 buffer, at 25 °C. [b] Chloroform−buffer partition coefficient.[13] [c] Determined as described in ref 13. [d] As hydrochloride, at pH 5.8, from ref 6. [e] As sodium salts.

Table 3. IOP Lowering in Normotensive Rabbits (23.0 ± 1.5 mmHg) after Treatment with 1 Drop (50 μL) of 2% Solution/Suspension of CA Inhibitor (pH of the Solution Shown), at 30, 60, and 90 min after Administration Directly into the Eye

| | | ΔIOP[a] (mmHg) | | | |
inhibitor	pH	$t = 0$	$t = 30$ min	$t = 60$ min	$t = 90$ min
dorzolamide	5.5	0	1.9 ± 0.2	4.0 ± 0.3	2.1 ± 0.2
brinzolamide[b]	5.5	0	2.9 ± 0.1	3.2 ± 0.3	6.3 ± 0.4
1O	7.0	0	2.2 ± 0.2	4.5 ± 0.3	5.7 ± 0.3
2R[c]	7.0	0	2.6 ± 0.2	4.9 ± 0.3	6.2 ± 0.3
4K	7.0	0	1.8 ± 0.2	2.5 ± 0.3	3.4 ± 0.3
4N	7.4	0	6.0 ± 0.1	9.3 ± 0.2	12.2 ± 0.4
Zn-**4N**[b]	6.5	0	4.9 ± 0.1	9.5 ± 0.2	13.1 ± 0.5
5C	7.0	0	3.1 ± 0.2	4.5 ± 0.2	7.2 ± 0.3
5C₂	7.0	0	4.4 ± 0.2	7.5 ± 0.2	9.5 ± 0.3
5M	7.4	0	3.1 ± 0.5	6.8 ± 0.2	4.5 ± 0.3
5M₂	7.5	0	6.5 ± 0.1	13.0 ± 0.2	8.1 ± 0.6
Zn-**5M₂**[b]	7.0	0	7.4 ± 0.2	14.5 ± 0.2	9.5 ± 0.3
5N	7.5	0	6.7 ± 0.1	9.2 ± 0.3	13.0 ± 0.4
6I	7.0	0	1.2 ± 0.1	3.9 ± 0.15	7.2 ± 0.2
Zn-**6I**	6.2	0	2.9 ± 0.1	5.6 ± 0.1	8.6 ± 0.2
6M	7.0	0	4.6 ± 0.2	8.9 ± 0.4	12.1 ± 0.4
Zn-**6M**[b]	6.0	0	5.2 ± 0.1	10.3 ± 0.3	13.6 ± 0.5
Cu-**6M**[b]	6.0	0	4.7 ± 0.2	9.6 ± 0.2	13.5 ± 0.3
7O	7.0	0	2.5 ± 0.3	4.9 ± 0.3	8.0 ± 0.4

[a] ΔIOP = IOP$_{control\ eye}$ − IOP$_{treated\ eye}$; mean ± SEM ($n = 3$). [b] Suspension. [c] Eye reddening.

showed indeed very good CA inhibitory properties. (ii) Sulfonamides incorporating polyamino-polycarboxylic acid moieties would possess good water solubility in the neutral pH range because of the buffering properties of such moieties and the easy formation of water-soluble sodium salts. This hypothesis was in fact verified during the course of the preparation of the DTPA derivatives mentioned above,[19] which showed indeed excellent water solubility at pH 7. (iii) The presence of polyamino-polycarboxylic acid moieties in the molecules of such sulfonamides leads to good metal-complexing properties of these derivatives, and this may be important for the design of coordination compounds as CA inhibitors.[20,21] Indeed, it was recently reported that some Zn(II) and Cu(II) complexes of heterocyclic sulfonamides possess excellent IOP-lowering properties via the topical route, although the parent sulfonamides from which the

Table 4: Fall of IOP of Glaucomatous Rabbits (34.5 ± 2.0 mmHg) after Treatment with 1 Drop (50 μL) of Solution/Suspension of 2% of CA Inhibitor (as Hydrochloride or Sodium Salt, with the pH Value Shown below) Directly into the Eye at 30, 60, and 90 min after Administration

| | | ΔIOP[a] (mmHg) | | | |
inhibitor	pH	$t = 0$ min	$t = 30$ min	$t = 60$ min	$t = 90$ min
dorzolamide	5.5	0	3.6 ± 0.2	6.7 ± 0.3	4.2 ± 0.2
4N	7.4	0	7.3 ± 0.1	11.3 ± 0.4	15.2 ± 0.4
5C₂	7.0	0	6.0 ± 0.4	12.5 ± 0.2	11.0 ± 0.2
5M₂	7.5	0	7.4 ± 0.3	13.0 ± 0.5	14.9 ± 0.5
5N	7.5	0	8.8 ± 0.6	16.0 ± 0.4	17.5 ± 0.3
Zn-**5M₂**[b]	7.0	0	19.8 ± 0.7	19.2 ± 0.8	18.9 ± 0.5
6M	7.0	0	7.6 ± 0.4	12.9 ± 0.5	15.1 ± 0.5

[a] ΔIOP = IOP$_{control\ eye}$ − IOP$_{treated\ eye}$; mean ± SEM ($n = 3$). [b] Suspension.
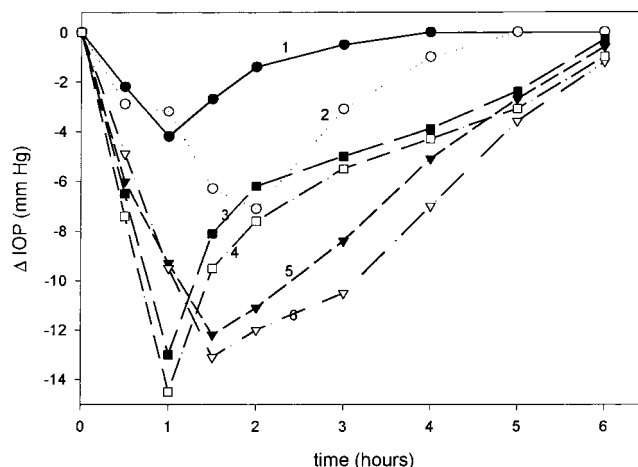
Figure 1. Effect of topically administered sulfonamide inhibitors (2% water solutions/suspensions) on the IOP of normotensive albino rabbits: (1) dorzolamide (hydrochloride salt, pH 5.5); (2) brinzolamide (suspension, hydrochloride salt, pH 5.5); (3) compound **5M₂** (trisodium salt, pH 7.0); (4) compound Zn-**5M₂** (suspension, pH 7.0); (5) compound **4N** (trisodium salt, pH 7.0); (6) compound Zn-**4N** (suspension, pH 7.0).

Table 5. Ocular Tissue Concentrations (μM) after 1 and 2 h, Following Corneal Application of 1 Drop (50 μL) of 2% Solution of Compounds **5M₂** and **6M** in Normotensive Albino Rabbits

| | | drug concentration[a] (μM) | | |
compound	time (h)	cornea	aqueous humor	ciliary process
5M₂	1	150 ± 10	241 ± 13	50 ± 3
	2	45 ± 5	39 ± 3	19 ± 1
6M	1	173 ± 12	268 ± 16	59 ± 4
	2	66 ± 5	53 ± 3	37 ± 3

[a] Mean ± SEM ($n = 3$).

complexes have been prepared are devoid of such properties.[20,21] It must be mentioned that metal complexes of sulfonamides are generally 10−100 times stronger CA inhibitors than the sulfonamides from which they are prepared, a fact explained mechanistically at the molecular level by this group.[1,2]

Two series of derivatives incorporating polyamino-polycarboxylic acid moieties are reported here: bis- and monosulfonamides (Scheme 1). The following strategy has been employed for preparing these compounds. It was originally investigated whether the reaction of anhydrides **8** (such as EDTA dianhydride; EGTA dianhydride and DTPA dianhydride) with aromatic/heterocyclic sulfonamides **A**−**T** in molar ratios of 1:1 leads to monosulfonamides. When these reactions were per-

Scheme 1
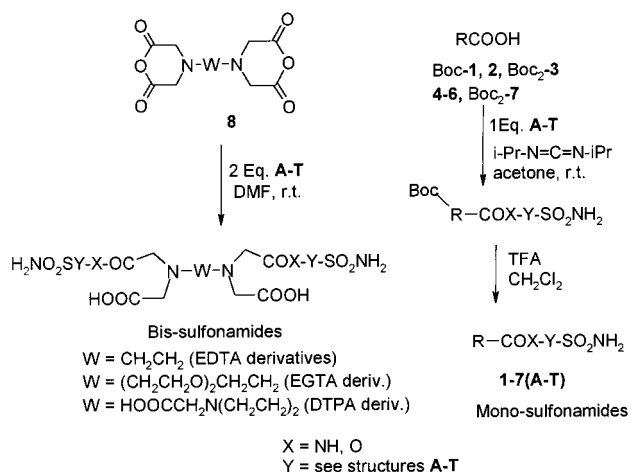
formed in a variety of conditions, it was observed that only bis-sulfonamides (and unreacted anhydride/acid) could be isolated. Thus, this strategy has been employed only for the preparation of bis-sulfonamides, working at molar ratios of anhydride **8** to sulfonamide **A–T** of 1:2 in DMF at room temperature (Scheme 1). To obtain the monosulfonamides, a different strategy has been employed. The conjugation of the sulfonamides **A–T** with the polyamino-polycarboxylic acid **1–7** has been performed in the presence of diisopropylcarbodiimide, as previously reported for the synthesis of CA inhibitors incorporating amino acyl or oligopeptidyl moieties.[15] This reaction led to the preparation of monosulfonamides of types **1–7 (A–T)** in high yields, without other undesired side reactions (Scheme 1). The only complication consisted of the need to protect the secondary amino moieties present in IDA **1** and EDDA **3**, as well as the phenolic OH moieties of EDDHA **7**. This has been done by using the *tert*-butoxycarbonyl (Boc) moiety as the protecting group (the Boc derivatives were prepared from Boc-On and compounds **1**, **3**, and **7**),[26] whereas the deprotection (after the condensation step) has been effected in the usual way, with trifluoroacetic acid (TFA) in methylene chloride at room temperature.[15] All 140 derivatives incorporating the seven distinct polyamino-polycarboxylic acids **1–7** and the 20 sulfonamides **A–T** were prepared according to this strategy (Table 1).

Metal complexes of some of the new sulfonamides reported here and Zn(II), Cu(II), and Al(III) ions have also been obtained. The synthesis has been done by using the di- or trisodium carboxylate salts of the sulfonamides and Zn(II), Cu(II), or Al(III) inorganic salts (chlorides or sulfates) in molar ratios of sulfonamide ligand to metal ion of 1:1, 1:2, or 1:3. The metal ions included in these studies included Zn(II) and Cu(II) because it was originally shown that complexes containing such cations act as efficient IOP-lowering agents.[20] Al(III) complexes have also been prepared because we recently showed that some Al(III) derivatives of heterocyclic sulfonamides exhibit interesting pharmacological properties as antacids in gastric acid disequilibria in experimental animals.[21] In all cases investigated here, only complexes containing one metal ion per sulfonamide ligand moiety have been obtained, as determined by elemental analysis and physicochemical investigations with these new complexes (see Experimental Protocols for details). Presumably, only the carboxylate

moieties of the ligand are involved in the interaction with the metal ion in such compounds, although it is well established that the sulfonamide moiety, as well as heteroatoms present in the heterocyclic moieties of some of these derivatives, may indeed participate in coordinating metal ions.[20–22,27]

Carbonic Anhydrase Inhibitory Activity. The data of Table 1 show that the new inhibitors prepared by attaching polyamino-polycarboxylic acid moieties to aromatic orheterocyclic sulfonamides **A–T** are more effective compared to the parent sulfonamides from which they were prepared toward the three investigated isozymes hCA I, hCA II, and bCA IV. The enhanced inhibitory power of these compounds is presumably due to the interaction of the (long) polyamino-polycarboxylic acid moiety incorporated in their molecules with hydrophilic patches at the entrance of the enzyme active site as observed for inhibitors previously reported by Whitesides'[17] and our groups,[1,2,13,15] and explained by detailed QSAR models.[28]

The nature of the sulfonamide attached to the polyamino-polycarboxylic acid moiety in the new derivatives reported here greatly influenced the CA inhibitory power of these compounds. Among the synthesized compounds, the heterocyclic sulfonamide derivatives were the most active, followed by the aromatic sulfonamide derivatives. The efficiency of the obtained inhibitor generally varied in the following way based on the parent sulfonamide from which it was prepared: the derivatives of *p*-hydrazino-benzenesulfonamide < the orthanilamides < the metanilamides < the sulfanilamides < the homosulfanilamides < the *p*-aminoethyl-benzenesulfonamides ≃ the halogeno-substituted sulfanilamides ≃ the 1,3-benzene-disulfonamides < the 1,3,4-thiadiazole-2-sulfonamides ≃ 4-methyl-δ^2-1,3,4-thiadiazoline-2-sulfonamide ≃ the benzothiazole-2-sulfonamides. The monoamides or esters were generally less active than the corresponding bis-amides or esters. The metal complexes of some bis-sulfonamides that were also prepared were more active than the corresponding ligands, as already reported in the literature.[20,27] The Cu(II) complexes were more effective than the Zn(II) derivatives, which in turn were more inhibitory than the corresponding Al(III) complexes. The nature of the acylating moiety also influenced the inhibitory properties of these new sulfonamides, with the following range of inhibitory effects for the corresponding sulfonamides incorporating polyamino-polycarboxylic acid moieties: IDA derivatives < NTA derivatives < EDDA derivatives < EDDHA derivatives < EDTA derivatives < EGTA derivatives ≃ DTPA derivatives. All three CA isozymes investigated here were susceptible to inhibition with this type of sulfonamide, with hCA II and bCA IV the most sensitive, whereas hCA I was generally less susceptible to inhibition compared to the first two isozymes. Mention should be made that the two susceptible isozymes (CA II and CA IV) are just those involved in aqueous humor secretion,[1] whereas CA I inhibition may explain the presence or the lack of side effects due to inhibitor washed out from the eye.[19] Thus, our compounds, in contrast to dorzolamide, also inhibit the slow isozyme CA I, which may be a positive property because once in the systemic circulation (after being washed out from the eye after a

prolonged use of eye drops containing such CA inhibitors), they should bind to the predominant isozyme present in blood, i.e., hCA I, and will thus inhibit to a slighter degree the rapid isozyme hCA II, which is involved in bicarbonate transport, electrolyte secretion, and other vital physiological processes.[1,2] Indeed, the blood contains up to 150 μM of hCA I, whereas the hCA II concentration is much lower, of about 20 μM.[1,4] The fact that dorzolamide shows so many systemic side effects[8–11] after topical administration may be due in part to its low affinity for just hCA I.

Transcorneal Penetration of Drugs. The data of Table 2 show that many of the new derivatives reported here possess excellent water solubility in the neutral pH range (pH around 7.4) as mono-, di-, tri-, or tetrasodium salts. This is correlated with a balanced hydrophilicity and lipophilicity and, as a consequence, optimal accession rates across the cornea, which is typical for the effective topically acting sulfonamides.[1,3,5] The prepared metal complexes of such derivatives were, on the other hand, less water-soluble (data not shown), but because brinzolamide BRZ is administered as a suspension (because of its poor water solubility), we also used water suspensions of some of these compounds for in vivo experiments.

IOP Measurements. In vivo, in normotensive rabbits, some of the new mono- and bis-sulfonamides reported here, such as **4N** and its Zn(II) complex, **5C**, **5C₂**, **5M**, **5M₂** and its Zn(II) complex, **5N**, **6M** and its Zn(II) and Cu(II) complexes, etc., showed very effective IOP lowering after topical administration, with pressure reductions of 3.1–7.4 mmHg at half an hour (compared to 1.9 mmHg for dorzolamide and 2.9 mmHg for brinzolamide), 4.5–14.5 mmHg at 1 h (4.0 for the first standard drug and 3.2 mmHg for the second one), and 7.2–13.5 mmHg at 90 min after administration (compared to 2.1 for dorzolamide and 6.3 for brinzolamide) (Table 3 and Figure 1). Other derivatives, such as **1O**, **2R**, **4K**, **6I**, and **7O** among others, showed more modest IOP lowering, which was comparable to or better with those produced by dorzolamide or brinzolamide. An important feature of the new class of CA inhibitors reported here is that IOP remained at much lower values for longer periods (3–6 h) after their topical administration, as compared to the standard drugs (Figure 1). This was particularly true for some Zn(II) complexes of these new sulfonamide CA inhibitors. IOP generally returned to the baseline values after 5–6 h after administration of these new topically acting sulfonamides/metal complexes reported here. The above findings also apply to the glaucomatous rabbits experiments (Table 4), in which the IOP reductions were much more important compared to those seen in normotensive rabbits. Thus, in glaucomatous animals, IOP reductions of 6.0–19.8 mmHg were generally observed after 30 min postadministration, whereas at 1 h, these amounted to 11.3–19.2 mmHg, and in the case of the Zn(II) complex Zn-**5M₂**, they remained at these low values for rather prolonged periods (Table 4). Thus, all these derivatives are longer lasting and much more effective IOP-lowering agents compared to the clinically available drugs dorzolamide and brinzolamide.

Distribution of Drugs in Ocular Fluids and Tissues. Table 5 shows ex vivo data obtained in normotensive rabbits after the topical administration of two of the most potent topical inhibitors in the prepared series. It can be observed that at 1 and 2 h after topical administration of drug high levels of inhibitors were found in the cornea, aqueous humor, and ciliary processes. On the basis of the inhibition constant of these compounds, the fractional inhibition estimated in these tissues or fluids is 99.5–99.9%, indicating the fact that the powerful IOP decrease observed is due to CA inhibition.

Conclusions

We report here a novel class of water-soluble, topically acting antiglaucoma sulfonamides obtained by the "tail" approach,[1] which incorporate polyamino-polycarboxylic moieties in their molecules, such as those of iminodiacetic acid, NTA, EDDA, e EDTA, DTPA, EGTA, and EDDHA. Metal complexes (containing Zn(II), Cu(II), and Al(III) ions) of such sulfonamides have also been synthesized. Many of the newly reported derivatives showed very good water solubility at nearly neutral pH values, whereas their lipid solubility, hydrophobicity (log *P*), and accession rates across the cornea were those appropriate for efficient topical IOP-lowering agents. Some of these new CA inhibitors, as well as their metal complexes, possessed affinities in the low nanomolar range for isozymes hCA II and bCA IV, acting as effective enzyme inhibitors in vitro. Several of the most active inhibitors strongly lowered IOP pressure in normotensive and glaucomatous rabbits, showing a prolonged duration of action compared to dorzolamide or brinzolamide. The new compounds reported here might lead to the development of more efficient antiglaucoma drugs from the class of the sulfonamide CA inhibitors, with fewer side effects compared to the presently available drugs.

Experimental Protocols

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 or TFA as solvents, with a Bruker CPX200 or Varian 300 instrument. Chemical shifts are reported as δ values relative to Me₄Si as an internal standard. Elemental analyses were done by combustion for C, H, and N with an automated Carlo Erba analyzer, and gravimetrically for the metal ions, and were ±0.4% of the theoretical values. High-resolution mass spectroscopy (HRMS) was recorded with an AEI-MS 902 spectrometer, using fast-atom bombardment (FAB) or electrospray techniques. 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 C₁₈ Bondapack column, with a Beckman EM-1760 instrument. Sulfonamides **A–T** used in the syntheses were either commercially available compounds (from Sigma-Aldrich, Milan, Italy, or Acros, Milan, Italy) or were prepared as described previously.[13,15,16] Dorzolamide was from Merck & Co. (Trusopt eye drops), whereas brinzolamide was from Alcon Laboratories (Azopt eye drops). Polyamino-polycarboxylic acids and anhydrides, carbodiimides, metal salts, and other inorganic reagents were from Sigma-Aldrich, Fluka, or E. Merck and were used without additional purification. Acetone, acetonitrile, DMF, and other solvents (E. Merck) used in the synthesis/chromatography were doubly distilled and kept on molecular sieves in order to maintain them under anhydrous conditions.

General Procedure for the Preparation of Bis-sulfonamides. An amount of 15 mmol of EDTA, EGTA, or DTPA

dianhydride was added to a solution of 30 mmol of sulfonamide **A–T** dissolved in 100 mL of anhydrous DMF. The mixture was magnetically stirred at room temperature for 4 h, then the reaction mixture was poured in 300 mL of methylene chloride and the obtained solid was filtered and thoroughly washed with methylene chloride and then with acetone. HPLC purification was necessary in some cases and was done by elution with potassium phosphate buffer–MeCN 2:1, v/v (10 mL/min), on a reversed-phase C_{18} Bondapack column.

General Procedure for the Preparation of Compounds 1–7 (A–T). An amount of 1 mM sulfonamide **A–T** was dissolved/suspended in 25 mL of anhydrous acetone and then treated with the stoichiometric amount (1 mM) of (Boc-protected) polyamino-polycarboxylic acid **1–7**. An amount of 190 mg (1 mM) of EDCI·HCl (or the equivalent amount of diisopropylcarbodiimide) was then added, and the reaction mixture was magnetically stirred at room temperature for 15 min. Then a total of 30 μL (2 mM) of triethylamine was added, and stirring was continued for 8–16 h at 4 °C (TLC control). The solvent was evaporated in vacuo, and the residue was taken up in ethyl acetate (5 mL), poured into a 5% solution of sodium bicarbonate (5 mL), brought to pH 7 with 1 N HCl, and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate and filtered, and the solvent was removed in vacuo. The obtained oils were directly used in the deprotection step or the intermediates were purified by preparative HPLC as described below. The removal of the protecting groups has been performed as described in the literature.[15–17] The crude Boc-protected intermediate was dissolved in 20 mL of CH_2Cl_2, treated with 4 mL of trifluoroacetic acid (TFA), and stirred for 10 min at 0 °C. The solvent was removed in vacuo, and the residue was concentrated from water twice to remove excess TFA, giving thus the unprotected derivative as a colorless syrup. The pure compounds **1–7 (A–T)** were obtained in pure form by means of preparative HPLC (C_{18} reversed-phase μ-Bondapack or Dynamax-60A (25 mm × 250 mm) columns, 90% acetonitrile/8% methanol/2% phosphate buffer, pH 7.4, 10 mL/min).

General Procedure for the Preparation of the Coordination Compounds. The metal complexes of some of the above-mentioned sulfonamides were prepared as exemplified below for **5F₂** as ligand. A suspension of 14-[4-(aminosulfonyl)-phenyl]-3-[2-[[2-[4-(aminosulfonyl)phenyl]ethyl]amino]-2-oxo-ethyl]-6,9-bis(carboxymethyl)-11-oxo-3,6,9,12-tetraazatetrade-canoic acid (**5F₂**, 1.51 g, 2 mmol) in 50 mL of water was treated with a stoichiometric amount of 1 N NaOH solution in order to obtain the disodium carboxylate salt. The obtained solution was treated with a solution of $ZnCl_2$ (1 mmol) in 5 mL of water, maintaining the pH at 6.5. The reaction was monitored by HPLC on a stationary phase of Lichrospher 100 RP-18.5 μm, with a 250 mm × 4 mm column packed by E. Merck, at 40 °C. Isocratic elution with a premixed mobile phase (1 g of octylamine was added to 100 mL of acetonitrile mixed with 900 mL of water) has been performed. The eluent was buffered with phosphoric acid, maintaining the pH at 6, and the flow rate was 1.5 mL/min. After 4 h the solution was loaded onto an Amberlite XAD 1600 resin column (250 mL) and eluted with MeCN/water (1:10, v/v). The fractions containing the complex were evaporated to give a white solid of the complex with an overall yield of 95%. Similarly prepared were the Cu(II) and Al(III) complexes of this and other sulfonamides, by using Cu-(II) sulfate or Al(III) sulfate instead of Zn(II) chloride and working at different ratios of metal salt to sulfonamide ligand (between 1:1 and 1:3). In all cases only complexes with the stoichiometry metal/sulfonamide of 1:1 were obtained.

1N. Mp 184–5 °C (dec). IR (KBr), cm^{-1}: 1175 (SO_2sym), 1381 (SO_2as), 1560 (amide II), 1603 (amide I), 1760 (COOH), 3334 (NH, NH_2). ^{1}H NMR (TFA), 300 MHz, δ, ppm: 3.30 (2H, s, CH_2COOH); 3.42 (2H, s, CH_2CONH); 3.51 (s, 3H, N-Me); 7.25 (br s, 3H, CONH + SO_2NH_2); the NH and COOH protons are not seen in this solvent, being in fast exchange. ^{13}C NMR (TFA), δ, ppm: 26.8 (Me); 53.24 (*C*H₂CH₂); 53.70 (CH₂*C*H₂); 60.18 (*C*H₂CONH); 61.34 (*C*H₂COOH); 158.7 (C-2 of thiadia-

zole); 170.9 (C-5 of thiadiazole); 176.41 (CONH); 180.82 (COOH). Anal. ($C_7H_{11}N_5O_5S_2$) C, H, N.

4M. Mp 155–6 °C (dec). IR (KBr), cm^{-1}: 1164 (SO_2sym), 1360 (SO_2as), 1561 (amide II), 1600 (amide I), 1765 (COOH), 3330 (NH, NH_2). ^{1}H NMR (D_2O–KOD), 300 MHz, δ, ppm: 3.35 (6H, s, 3CH_2COOH); 3.54 (4H, t, CH₂CH₂); 3.56 (2H, s, CH_2-CONH); the SO_2NH_2, CONH, and COOH protons are not seen in this solvent mixture, being in fast exchange. ^{13}C NMR (D_2O–KOD), δ, ppm: 53.24 (*C*H₂CH₂); 53.70 (CH₂*C*H₂); 60.18 (*C*H₂CONH); 61.34 (*C*H₂COOH); 159.5 (C-2 of thiadiazole); 170.3 (C-5 of thiadiazole); 176.41 (CONH); 180.82 (COOH). Anal. ($C_{12}H_{18}N_6O_9S_2$) C, H, N.

4M₂. Mp 176–8 °C (dec). IR (KBr), cm^{-1}: 1163 (SO_2sym), 1356 (SO_2as), 1563 (amide II), 1600 (amide I), 1764 (COOH), 3330 (NH, NH_2). ^{1}H NMR (D_2O–KOD), 300 MHz, δ, ppm: 3.33 (4H, s, 2CH_2COOH); 3.54 (4H, t, CH₂CH₂); 3.60 (4H, s, 2CH_2-CONH); the SO_2NH_2, CONH and COOH protons are not seen in this solvent mixture, being in fast exchange. ^{13}C NMR (D_2O–KOD), δ, ppm: 53.19 (*C*H₂CH₂); 53.76 (CH₂*C*H₂); 60.23 (*C*H₂CONH); 61.13 (*C*H₂COOH); 159.3 (C-2 of thiadiazole); 170.5 (C-5 of thiadiazole); 176.28 (CONH); 180.70 (COOH). Anal. ($C_{14}H_{20}N_{10}O_{10}S_4$) C, H, N.

5F₂. Mp 121–2 °C (dec). IR (KBr), cm^{-1}: 1173 (SO_2sym), 1340 (SO_2as), 1560 (amide II), 1600 (amide I), 1760 (COOH), 3335 (NH, NH_2). ^{1}H NMR (D_2O–KOD), 300 MHz, δ, ppm: 2.90 (t, 2H, CH_2 of aminoethyl-benzenesulfonamide, 7.2), 3.47 (q, 2H, CH_2 of aminoethylbenzenesulfonamide 6.5), 3.20 (4H, t, ethylenic CH_2 near lateral nitrogens); 3.35 (4H, s, CH_2 of the lateral acetates); 3.48 (4H, t, ethylenic CH_2 near central nitrogen); 3.56 (4H, s, CH_2 of the acetamido groups); 3.91 (2H, s, CH_2 of the central acetate); 7.42 (d, 2H, AA′BB′, 8.2); 7.75 (d, 2H, AA′BB′, 8.2). ^{13}C NMR (D_2O–KOD), δ, ppm: 36.15 (C-10); 42.07 (C-9); 53.12 (C-4); 53.68 (C-3); 60.03 (C-2); 60.15 (C-7); 61.05 (C-5); 128.12 (C-13); 132.47 (C-12); 146.79 (C-14); 147.20 (C-11); 177.04 (C-8); 180.53 (C-1); 181.20 (C-6). Anal. ($C_{30}H_{43}N_7O_{12}S_2$) C, H, N.

The Zn-5F₂ Complex. Mp >300 °C. IR(KBr), cm^{-1}: 1170 (SO_2sym), 1336 (SO_2as), 1560 (amide II), 1610 (amide I), 1745 (COO^-), 3335 (NH_2). Anal. ($C_{30}H_{41}N_7O_{12}S_2Zn$) Zn, C, H, N.

Enzyme Preparations. 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 et al.[29] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by this group,[30] and enzymes were purified by affinity chromatography according to the method of Khalifah et al.[31] 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, respectively, based on M_r = 28.85 kDa for CA I and 29.30 kDa for CA II, respectively.[32,33] CA IV was isolated from bovine lung microsomes as described by Maren et al., and its concentration has been determined by titration with ethoxzolamide.[34]

Initial rates of 4-nitrophenyl acetate hydrolysis catalyzed by different CA isozymes were monitored spectrophotometrically, at 400 nm, with a Cary 3 instrument interfaced with an IBM-compatible PC.[35] 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, under the conditions of the experiments (pH 7.40), as reported in the literature.[35] 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.[35] Enzyme concentrations were

3.5 nM for hCA II, 12 nM for hCA I, and 36 nM for bCA IV (this isozyme has a decreased esterase activity,[36] and higher concentrations had to be used for the measurements).

Measurement of Tonometric IOP. 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 conform 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 or suspensions of inhibitors (2% by weight as hydrochlorides or sodium carboxylates) were obtained in distilled−deionized water. The pH values of these solutions were in the range 5.5−7.4.

IOP was measured using a Digilab 30R pneumatonometer (BioRad, Cambridge, MA) as described by Maren's group.[37,38] 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 were 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 the 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.[37,38] All data are expressed as the 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 Sugrue et al.[39] The IOP of operated animals was checked after approximately 4 weeks, and animals with an elevated pressure of 30−36 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 has been followed.[37,38] The animals were killed with an intracardiac air injection. Aqueous humor (both posterior and anterior chamber fluids) were 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 has been 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 the drug from the E−I complex), diluted, and then incubated with a known amount of enzyme. The activity of the free enzyme and in the presence of the inhibitor was determined as described above. A calibration curve has been used in order to determine the fractional inhibition in the different tissues, as described in refs 37 and 38.

Determination of Water (Buffer) Solubility. A standard solution was prepared by dissolving a precisely weighed amount (generally 1 mg) of inhibitor in 10 mL of methanol. The UV absorption maximum of each compound has been 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 was scanned by UV at the wavelength of the absorption maximum previ-

ously determined. Total solubility was determined by the relationship $C' = A'C/A$, where C is the concentration of standard solution (mg/mL), A is the absorbance of standard solution, A' is the absorbance of the saturated solution, and C' is the concentration of the saturated solution (mg/mL).[40]

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.[15,16]

Transcorneal Penetration of Drugs. The method of Maren et al.[5] with the modifications of Pierce's group[41,42] (for the HPLC assay of sulfonamides) has been used. Excised rabbit corneas with either intact or denuded epithelium were used in these experiments. The pH was 7.4, and the exposed area was 1.2 cm². Drugs of concentrations 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.[41,42] or enzymatically.[5] The results of the drug analyses were used to calculate the rate constant of transfer across the cornea (k_{in}). As described by Pierce,[41,42] this value was determined by using the formula

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

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

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