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Carbonic Anhydrase Inhibitors: Synthesis of Sulfonamides Incorporating dtpa Tails and of their Zinc Complexes with Powerful Topical Antiglaucoma Properties

Andrea Scozzafava,[a] Luca Menabuoni,[b] Francesco Mincione,[c] Giovanna Mincione[a] and Claudiu T. Supuran[a,*]

[a]*Università degli Studi, Laboratorio di Chimica Inorganica e Bioinorganica, Via Gino Capponi 7, I-50121, Firenze, Italy*
[b]*Ospedale San Giovanni di Dio, S. O. Oculistica, Via Torregalli 3, I-50123, Florence, Italy*
[c]*Università degli Studi, Institute of Ophthalmology, Viale Morgagni 85, I-50134, Firenze, Italy*

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Abstract—Reaction of diethylenetriamino pentaacetic acid (dtpa) dianhydride with aromatic/heterocyclic sulfonamides possessing a free amino/imino/hydrazino/hydroxy group afforded bis-sulfonamides containing metal-complexing, polyamino-polycarboxylic acid moieties in their molecule. The corresponding mono-sulfonamide derivatives of dtpa were also obtained by an alternative method, from the free acid. Zn(II) complexes of these new sulfonamides were then prepared. Many of these derivatives showed nanomolar affinity towards isozymes I, II and IV of carbonic anhydrase (CA). Some of the best inhibitors were applied as 2% water solutions/suspensions into the eye of normotensive or glaucomatous albino rabbits, when strong and long-lasting intraocular pressure (IOP) lowering was observed.

Introduction

Inhibitors of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1) are clinically used agents in the treatment of diverse diseases such as glaucoma; gastroduodenal ulcers; acid–base disequilibria and diverse neurological/neuromuscular disorders.[1]

Since the discovery that sulfanilamide **1** and related aromatic/heterocyclic sulfonamides of types **2**–**5** act as specific CA inhibitors,[2] several such compounds were utilized clinically for more than 45 years in the treatment and prevention of the above-mentioned diseases, or as physiological tools.[1,2] Although systemically administered sulfonamide inhibitors of types **4** and **5** are highly effective antiglaucoma drugs, their main drawback is constituted by severe side-effects due to CA inhibition in tissues other than the eye.[1,2] Only recently some water soluble, topically effective sulfonamide CA inhibitors, such as dorzolamide **6** and brinzolamide **7** have been developed and successfully introduced in clinical medicine.[3] They represent a radically new approach in treating glaucoma with CA inhibitors, as the undesired side effects observed with the systemically administered compounds are less pronounced.[1–3]

The clinical success of dorzolamide and brinzolamide fostered much research in the design and evaluation of novel agents from this class of enzyme inhibitors.[4,5] The two topically acting antiglaucoma drugs mentioned above, **6** and **7**, are both secondary amines, and the required water solubility needed for their topical action is achieved by using their hydrochloride salts. But in some cases this represents an undesired problem, since the pH of such solutions becomes rather acidic, and consequently produces eye irritation after the topical administration, as already reported for many patients treated with dorzolamide.[6] The most common adverse effects after topical treatment with dorzolamide consist in local burning or stinging of the eyes, pruritus and bitter taste, but some more serious problems, such as nephrolithiasis, anorexia, depression and irreversible corneal decompensation were also reported.[6] Such side-effects might be avoided for compounds that should not be administered as hydrochloride salts, but this generally leads to a drastic diminution of water solubility of

*Corresponding author. Tel.: +39-55-275-7551; fax: +39-055-275-7555; e-mail: cts@bio.chim.unifi.it

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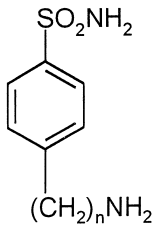

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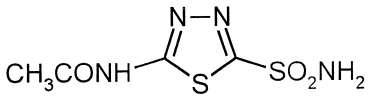

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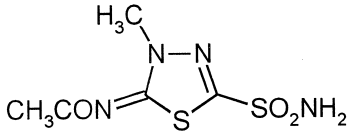

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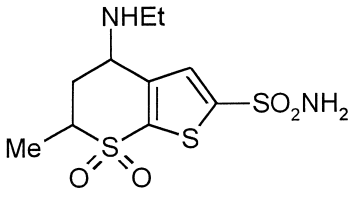

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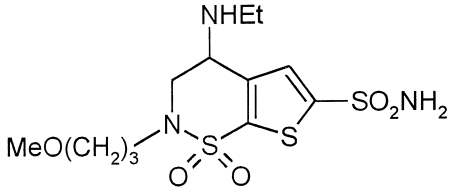

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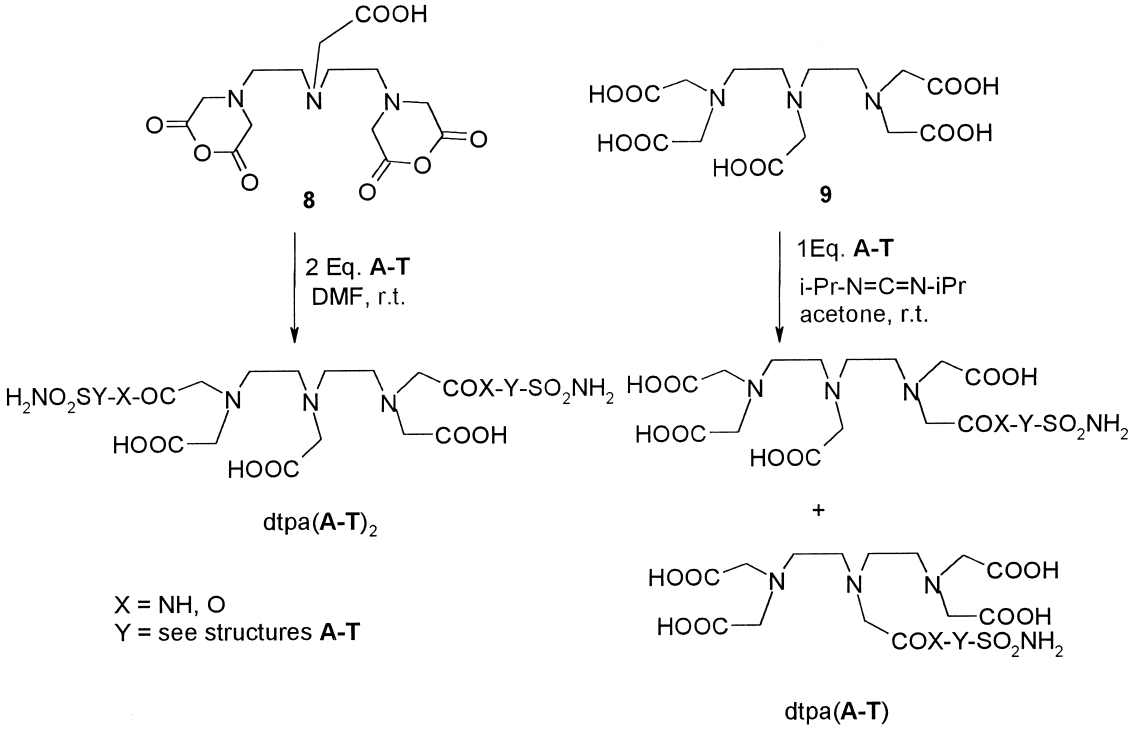
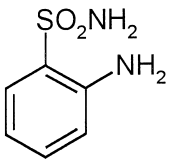
a sulfonamide drug. Thus, it is clear that novel generation, more effective antiglaucoma sulfonamides acting topically are needed for an efficient treatment of glaucoma.

In recent contributions from this laboratory[4,5] it was shown that by attaching water-solubilizing tails (such as 8-quinoline-sulfonyl-; nicotinoyl-; isonicotinoyl-; 6-carboxy-pyridine-2-carboxamido-; amino acyl-, etc.) to the molecules of aromatic/heterocyclic sulfonamides of types **A**–**T**, efficient CA inhibitors were obtained, some of which also showed excellent water solubility in the neutral pH range, and promising anti-glaucoma activity via the topical route in experimental animals. Here we extend that approach for obtaining water-soluble, high affinity sulfonamide CA inhibitors, which do not owe their water solubility to formation of hydrochloride salts. We report here a series of compounds obtained by reaction of diethylenetriamino pentaacetic acid (dtpa) dianhydride with aromatic/heterocyclic sulfonamides of types **A**–**T**, possessing free amino/imino/hydrazino or hydroxy groups, leading to bis-sulfonamides



Scheme 1.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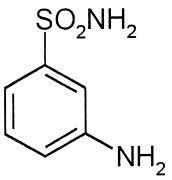
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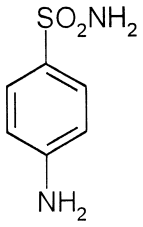
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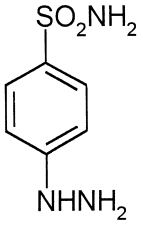
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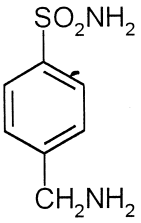
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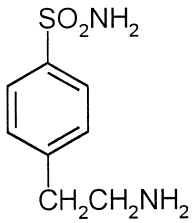
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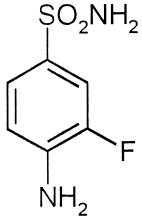
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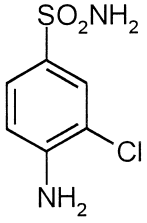
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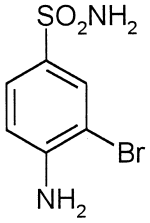
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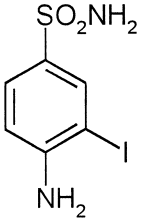
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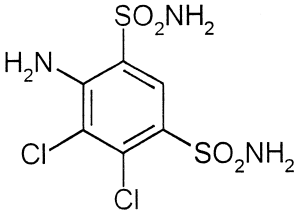
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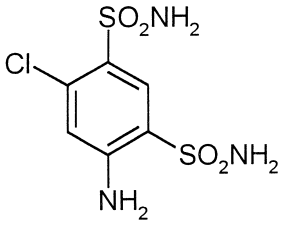
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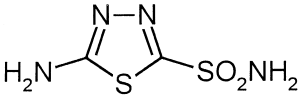
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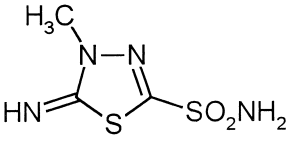
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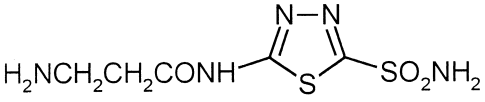
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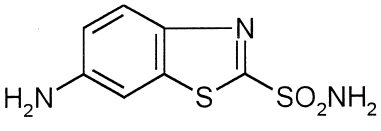
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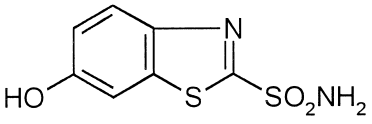
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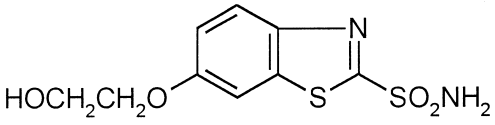
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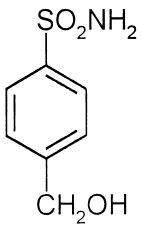
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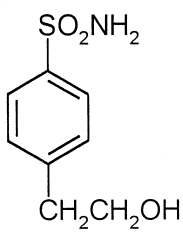
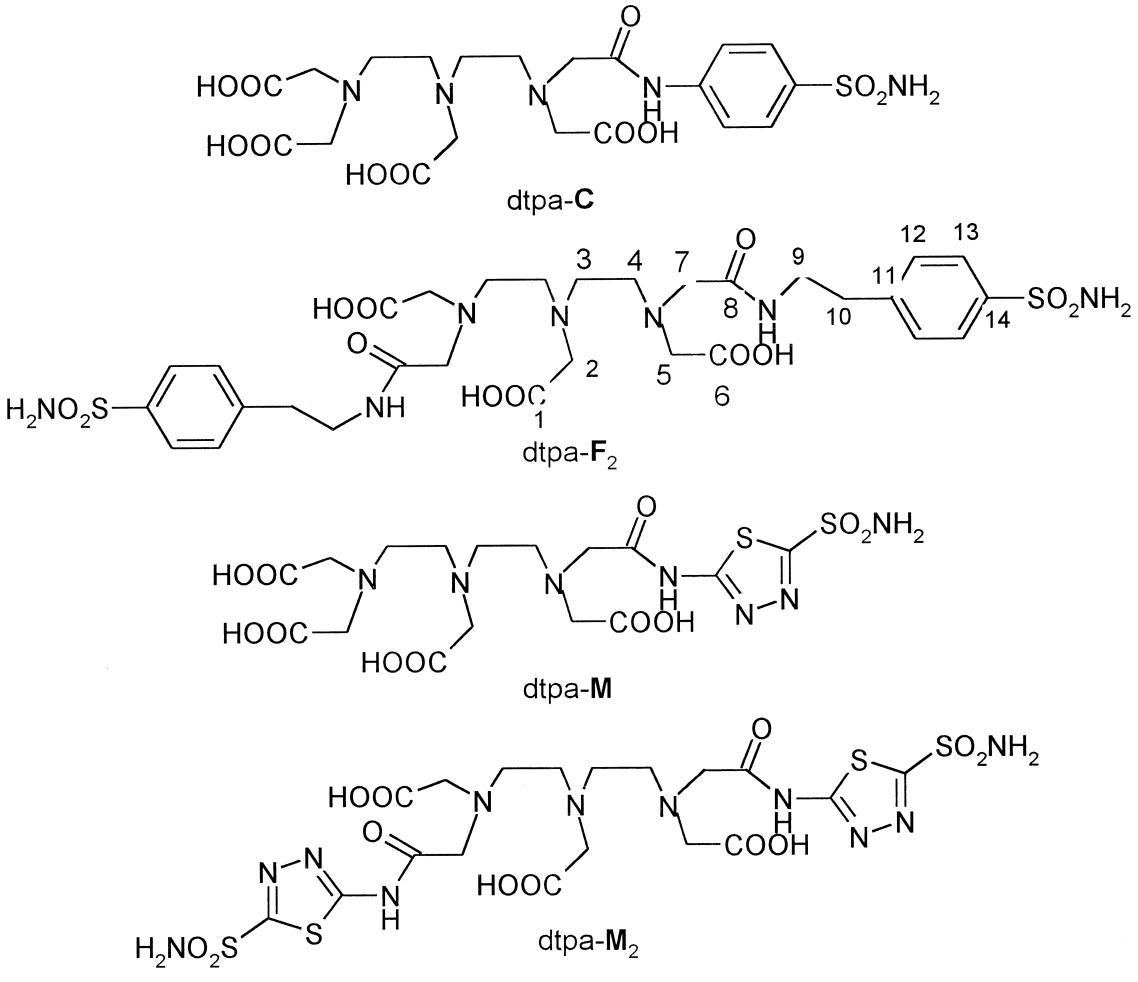

T

incorporating polyamino-polycarboxylic acid moieties in their molecule. Another series of compounds, more precisely the corresponding mono-sulfonamide dtpa derivatives, has been prepared by condensing the free acid (dtpa) with the sulfonamides **A**–**T** in the presence of carbodiimides. Some Zn(II) complexes of some of these ligands have also been obtained.

Reaction of dtpa dianhydride **8** or of the free acid (dtpa) **9** with aromatic/heterocyclic sulfonamides **A**–**T** possessing a free amino/imino/hydrazino/hydroxy group in molar ratios of 1:2, and 1:1, respectively, afforded the bis-sulfonamides of type dtpa(**A**–**T**)$_2$ and the mono-sulfonamides dtpa(**A**–**T**) (Scheme 1). The procedure for the synthesis of bis-amides of this type has been reported previously by Aime's group for aniline derivatives.[7] When dianhydride **8** was reacted with the aromatic/heterocyclic sulfonamides **A**–**T** in molar ratios of 1:1, in the same conditions as above, derivatives of type dtpa(**A**–**T**) could not be isolated from the reaction mixture (a mixture of bis-derivatized compounds dtpa(**A**–**T**)$_2$ and unreacted dtpa were the reaction products in such cases), and this prompted us to synthesize the mono-derivatives by an alternative procedure, as shown in Scheme 1. Since dtpa contains four equivalent carboxylic acid moieties, and the central one, which is non-equivalent with the other four, the above mentioned reaction afforded a mixture of two isomeric sulfonamides (in a molar ratio of about 4:1) which could not be purified by HPLC due to their very similar retention times (data not shown), and such a mixture has been used in the enzyme assay as well as for some in vivo experiments described shortly. The new compounds reported here were characterized by standard procedures (elemental analysis, spectroscopic data) which confirmed the proposed structures.[8] Due to the metal coordinating moieties incorporated in these new compounds, and since metal complexes of sulfonamides were recently shown to possess interesting topical anti-glaucoma properties,[9] some Zn(II) complexes of several new bis-sulfonamides of type dtpa(**A**–**T**)$_2$ were also prepared, characterized and investigated for their CA inhibition properties in vitro and IOP lowering in experimental animals (see Tables 1–5).

Since a relatively large number of derivatives are reported here, each compound will be designated by a letter identifying the sulfonamide from which it was obtained (**A**–**T**) with its stoichiometry (1 or 2, corresponding to one or two moieties of sulfonamide incorporated), and 'dtpa' preceding it, corresponding to the poly-aminopolycarboxylic acid moiety contained in its molecule. For instance, dtpa-**C** is the sulfanilamide derivative incorporating the dtpa tail, whereas dtpa-**C**$_2$ corresponds to the bisamide, containing the dtpa moiety bound to two sulfanilamide groups. Correspondingly, the 'acetazolamide-like' compounds dtpa-**M** (mono-amide) and dtpa-**M**$_2$ are also shown below.



The data of Table 1 show that the new inhibitors prepared by attaching dtpa moieties to aromatic/heterocyclic sulfonamides **A**–**T**, are more effective as compared to the parent sulfonamides from which they were prepared, towards 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 dtpa moiety incorporated in their molecules with hydrophilic patches at the entrance of the enzyme active site as observed for inhibitors previously reported by Whitesides'[10] and our groups,[4,5,11] and explained by detailed QSAR models.[12]

The nature of the sulfonamide attached to the dtpa moiety in the new derivatives reported here greatly influenced the CA inhibitory power of these sulfonamides. Among the synthesized derivatives, 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-

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)		
	hCA I[a]	hCA II[a]	bCA IV[b]
Acetazolamide **4**	900	12	220
Methazolamide **5**	780	14	240
Dorzolamide **6**	>50,000	9	43
dtpa-**A**	8600 (45,400)	210 (295)	550 (1310)
dtpa-**B**	7200 (25,000)	180 (240)	500 (2200)
dtpa-**C**	4100 (28,000)	75 (300)	180 (3000)
dtpa-**D**	12,000 (78,500	250 (320)	630 (3200)
dtpa-**E**	550 (25,000)	23 (170)	40 (2800)
dtpa-**F**	290 (21,000)	15 (160)	36 (2500)
dtpa-**G**	180 (8300)	13 (60)	29 (180)
dtpa-**H**	200 (9800)	20 (110)	32 (320)
dtpa-**I**	96 (6500)	12 (40)	27 (66)
dtpa-**J**	79 (6000)	16 (70)	29 (125)
dtpa-**K**	58 (6100)	10 (28)	36 (175)
dtpa-**L**	87 (8400)	12 (75)	52 (160)
dtpa-**M**	55 (8600)	0.9 (60)	28 (540)
dtpa-**N**	62 (9300)	2 (19)	19 (355)
dtpa-**O**	110 (455)	1 (3)	16 (125)
dtpa-**P**	36 (70)	0.8 (9)	9 (19)
dtpa-**Q**	32 (55)	0.8 (8)	6 (17)
dtpa-**R**	30 (50)	0.6 (7)	5 (15)
dtpa-**S**	510 (24,000)	80 (125)	265 (560)
dtpA–T	360 (18,000)	61 (110)	190 (450)
dtpa-$\mathbf{A_2}$	5400 (45,400)	175 (295)	330 (1310)
dtpa-$\mathbf{B_2}$	6400 (25,000)	155 (240)	425 (2200)
dtpa-$\mathbf{C_2}$	3750 (28,000)	64 (300)	156 (3000)
dtpa-$\mathbf{D_2}$	9800 (78,500)	215 (320)	430 (3200)
dtpa-$\mathbf{E_2}$	380 (25,000)	19 (170)	37 (2800)
dtpa-$\mathbf{F_2}$	170 (21,000)	8 (160)	25 (2500)
dtpa-$\mathbf{G_2}$	135 (8300)	7 (60)	18 (180)
dtpa-$\mathbf{H_2}$	155 (9800)	15 (110)	27 (320)
dtpa-$\mathbf{I_2}$	79 (6500)	10 (40)	21 (66)
dtpa-$\mathbf{J_2}$	70 (6000)	9 (70)	24 (125)
dtpa-$\mathbf{K_2}$	43 (6100)	9 (28)	25 (175)
dtpa-$\mathbf{L_2}$	63 (8400)	11 (75)	41 (160)
dtpa-$\mathbf{M_2}$	50 (8600)	1 (60)	7 (540)
dtpa-$\mathbf{N_2}$	54 (9300)	1.5 (19)	9 (355)
dtpa-$\mathbf{O_2}$	102 (455)	0.6 (3)	8 (125)
dtpa-$\mathbf{P_2}$	25 (70)	0.5 (9)	6 (19)
dtpa-$\mathbf{Q_2}$	21 (55)	0.6 (8)	5 (17)
dtpa-$\mathbf{R_2}$	16 (50)	0.5 (7)	4 (15)
dtpa-$\mathbf{S_2}$	325 (24,000)	43 (125)	170 (560)
dtpA-$\mathbf{T_2}$	290 (18,000)	34 (110)	115 (450)
Zn-dtpa-$\mathbf{C_2}$	350	16	100
Zn-dtpa-$\mathbf{E_2}$	39	9	21
Zn-dtpa-$\mathbf{F_2}$	36	7	16
Zn-dtpa-$\mathbf{M_2}$	40	0.5	4
Zn- dtpa-$\mathbf{N_2}$	43	0.4	5

[a]Human (cloned) isozymes.

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

Compound	Solubility[a] (mM)	Log P[b]	$k_{in}\times10^3$ (h^{-1})[c]	
			Cornea intact	No epithelium
Dorzolamide **6**	60[d]	2.0[e]	3.0	5.2
dtpa-C	48[e]	0.983	3.8	6.9
dtpa-C_2	35[e]	1.456	3.6	6.5
dtpa-M	59[e]	1.375	4.3	8.1
dtpa-M_2	54[e]	1.961	3.9	6.2

[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 4.
[e]As sodium salts.

Table 3. IOP lowering in normotensive rabbits (23.5 ± 2.6 mmHg) after treatment with one drop (50 μL) 2% solution/suspension of CA inhibitor (the pH of the solution shown), at 30, 60 and 90 min after administration directly into the eye

Inhibitor	ΔIOP (mmHg)[a]				
	pH	$t=0$	$t=30$ min	$t=60$ min	$t=90$ min
Dorzolamide **6**	5.5	0	1.9 ± 0.2	4.0 ± 0.3	2.1 ± 0.2
dtpa-C	7.0	0	3.1 ± 0.2	4.5 ± 0.2	7.2 ± 0.3
dtpa-C_2	7.0	0	4.4 ± 0.2	7.5 ± 0.2	9.5 ± 0.3
dtpa-M	7.4	0	3.1 ± 0.5	6.8 ± 0.2	4.5 ± 0.3
dtpa-M_2	7.5	0	6.5 ± 0.1	13.0 ± 0.2	8.1 ± 0.6
dtpa-N	7.5	0	6.7 ± 0.1	9.2 ± 0.3	13.0 ± 0.4
Zn- dtpa-M_2[b]	7.0	0	7.4 ± 0.2	14.5 ± 0.2	9.5 ± 0.3

[a]$\Delta IOP = IOP_{control\ eye} - IOP_{treated\ eye}$; Mean ± SEM ($n=3$).
[b]Suspension.

Table 4. Fall of IOP of glaucomatous rabbits (33.5 ± 3.0 mmHg), after treatment with one drop (50 μL) 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

Inhibitor	pH	ΔIOP (mmHg)[a]			
		$t=0$	$t=30$ min	$t=60$ min	$t=90$ min
Dorzolamide	5.5	0	3.6 ± 0.2	6.7 ± 0.3	4.2 ± 0.15
dtpa-C_2	7.0	0	6.0 ± 0.4	12.5 ± 0.15	11.0 ± 0.2
dtpa-M_2	7.5	0	7.4 ± 0.3	13.0 ± 0.5	14.9 ± 0.5
dtpa-N	7.5	0	8.8 ± 0.6	16.0 ± 0.4	17.5 ± 0.3
Zn-dtpa-M_2[b]	7.0	0	19.8 ± 0.7	19.2 ± 0.8	18.9 ± 0.5

[a]$\Delta IOP = IOP_{control\ eye} - IOP_{treated\ eye}$; Mean SEM ($n=3$).
[b]Suspension.

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/esters were generally less active than the corresponding bis-amides/esters. The Zn(II) complexes of some bis-sulfonamides that were also prepared, were much more active than the corresponding ligands, as already reported in the literature.[9] 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 as 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.[14] Thus, our compounds, in contrast to dorzolamide also inhibit the slow isozyme, CA I, which may be a positive property, since 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.[1,14] Indeed, the blood contains up to 150 μM of hCA I, whereas the hCA II concentration is much lower, of about 20 μM.[15] The fact that dorzolamide shows so many systemic side effects[6] after topical administration may be due in part to its low affinity just for hCA I.

Table 5. Ocular tissue concentrations (μM) after 1 and 2 h, following corneal application of one drop (50 μL) of 2% solution of compounds dtpa-M_2 and dtpa-*N* in normotensive albino rabbits

Compound	Time (h)	Drug concentration (μM)[a]		
	Cornea	Aqueous humor		Ciliary process
dtpa-M_2	1 h	150 ± 10	241 ± 13	50 ± 3
	2 h	45 ± 5	39 ± 3	19 ± 1
dtpa-N	1 h	164 ± 9	250 ± 10	46 ± 5
	2 h	56 ± 5	49 ± 4	23 ± 2

[a]Mean ± SEM ($n = 3$).

Some physico-chemical properties of several strong in vitro inhibitors were investigated in detail (Table 2), showing that many of these new derivatives possess excellent water solubility, at pH values in the range of 7.0–7.5 pH units. This is correlated with a balanced hydro- and lipophilicity, and in consequence optimal accession rates across the cornea, which is typical for the effective topically acting sulfonamides.[1–5] The prepared Zn(II) complexes of such derivatives were on the other hand less water soluble (data not shown), but brinzolamide **6** is administered as a suspension (due to its poor water solubility), and thus we also used water suspensions of these compounds for the in vivo experiments.

In vivo, in normotensive rabbits, some of the new mono- and bis-sulfonamides reported here showed very effective IOP lowering after topical administration, with pressure reductions of 3.1–6.7 mmHg at half an hour

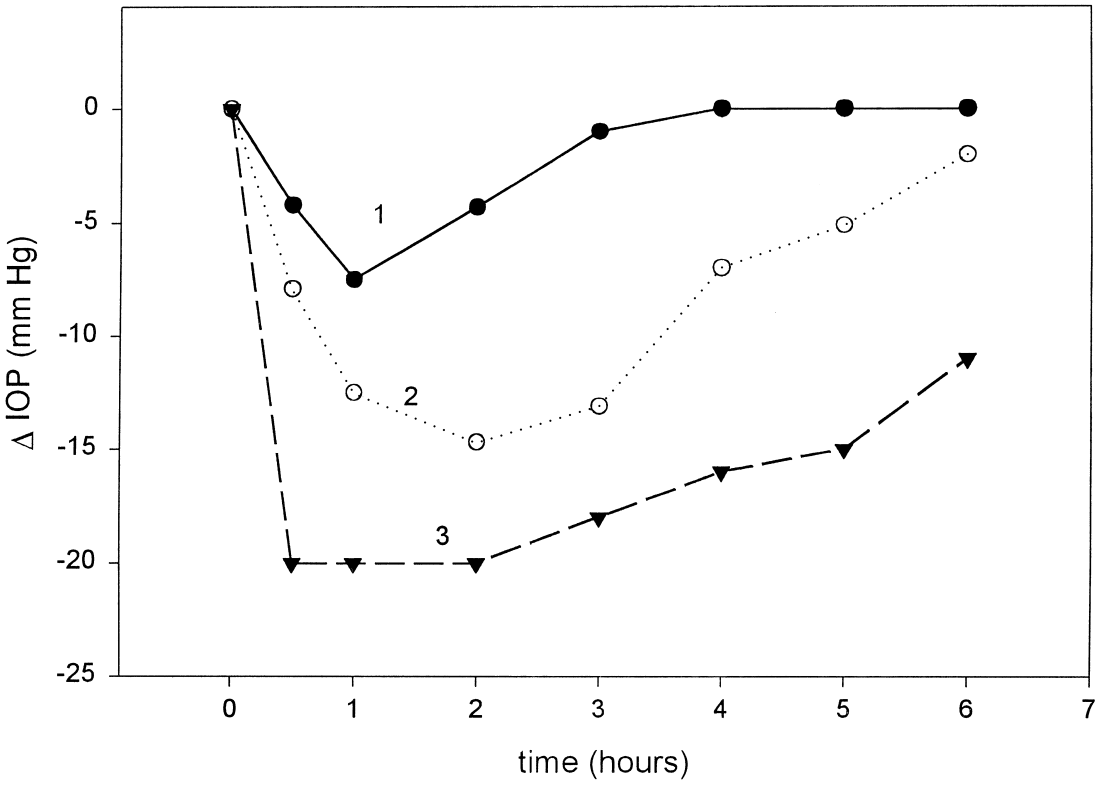


Figure 1. Effect of topically administered IOP lowering drugs (50 μL solutions/suspensions) on the IOP of glaucomatous albino rabbits. Curve 1: Dorzolamide hydrochloride solution 2% (standard); Curve 2: dtpa-M_2 2% solution (pH 7.0) Curve 3: Zn-dtpa-M_2 suspension 2% (pH 7.0).

(compared to 1.9 mmHg for dorzolamide), 4.5–13.0 mmHg at 1 h (4.0 for the standard drug), and 4.5–13 mmHg at 90 min after administration (compared to 2.1 for dorzolamide) (Table 3). An important feature of the new class of CA inhibitors reported here is that IOP remained low for longer periods (3–6 h) after their topical administration, as compared to the standard drug (data not shown). IOP generally returned at the baseline values after 5–6 h after administration of the drug. The above findings also apply for the Zn(II) complexes as well as to the glaucomatous rabbit experiments (Table 4 and Fig. 1) but the IOP reductions were much more important as compared to those seen in normotensive rabbits. Thus, IOP reductions of 6.0–19.8 mmHg were generally observed after 30 min, whereas at 1 h, these amounted to 12.0–19.2 mmHg, and in the case of the Zn(II) complex Zn-dtpa-$\mathbf{M_2}$ remained at these low values for prolonged periods (Fig. 1). Thus, all these derivatives are longer lasting and much more effective IOP lowering agents as compared to the clinically available drug dorzolamide.

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. Based on the inhibition constant of these compounds, the fractional inhibition estimated in these tissues/fluids is of 99.5–99.9%, indicating the fact that the powerful IOP decrease observed is indeed due to CA inhibition.

In conclusion, we report here a novel class of very powerful, water soluble, topically acting sulfonamide CA inhibitors, incorporating metal-complexing dtpa moieties in their molecules, as well as some of their zinc(II) complexes. Some of these inhibitors were very efficient IOP lowering agents in normotensive and glaucomatous rabbits after topical administration as water solutions/suspensions.

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8. For example: 14-[4-(Aminosulfonyl)phenyl]-3-[2-[2-[4-(aminosulfonyl)phenyl]ethyl]amino]-2-oxoethyl]-6,9-bis(carboxymethyl)-11-oxo-3,6,9,12-tetraazatetradecanoic acid, dtpa-F_2: An amount of 5.35 g (15 mmol) dtpa dianhydride (*N,N*-bis-[2-(2,6-dioxo-4-morpholinyl)ethyl]glycine was added to a solution of 6.0 g (30 mmol) of 4-(2-aminoethyl)-benzenesulfonamide F 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 by elution with potassium phosphate/MeCN 2:1, v/v (1 mL/min); mp 121–122 °C (dec.); IR (KBr), cm^{-1}: 1173 (SO_2^{sym}), 1340 (SO_2^{as}), 1560 (amide II), 1600 (amide I), 1760 (COOH), 3335 (NH, NH_2); ^1H NMR (300 MHz, D_2O–KOD), δ, ppm: 2.90 (t, 2H, CH_2 of aminoethylbenzenesulfonamide, 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: (see structural formula dtpa-F_2 for the numbering of the carbon atoms) 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); Elemental analysis, found: C, 47.13; H, 5.89; N, 12.61; S, 8.30%; $C_{30}H_{43}N_7O_{12}S_2$ requires: C 47.55; H, 5.72; N, 12.94; S, 8.46%. The Zn(II) complex of the above ligand was prepared as follows: A suspension of 14-[4-(aminosulfonyl)phenyl]-3-[2-[2-[4-(aminosulfonyl)phenyl]ethyl]amino]-2-oxoethyl]-6,9-bis(carboxymethyl)-11-oxo-3,6,9,12-tetra-azatetradecanoic acid (1.51 g, 2 mmol) in 50 mL water was treated with the stoichiometric amount of 1 N NaOH solution in order to obtain the disodium 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 250×4 mm column packed by E. Merck, at 40 °C. Isocratic elution with premixed mobile phase (1 g 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, 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%; mp >300 °C; IR (KBr), cm^{-1}: 1170 (SO_2^{sym}); 1336 (SO_2^{as}); 1560 (amide II); 1610 (amide I); 1745 (COO^-); 3335 (NH_2); Elemental analysis, found: Zn, 8.14; C, 43.84; H, 4.75; N, 11.67; S, 7.54%; $C_{30}H_{41}N_7O_{12}S_2Zn$ requires: Zn, 7.94; C, 43.73; H, 4.98; N, 11.90; S, 7.77%.

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