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CARBONIC ANHYDRASE INHIBITORS. METAL COMPLEXES OF 5-(2-CHLOROPHENYL)-1,3,4-THIADIAZOLE-2-SULFONAMIDE WITH TOPICAL INTRAOCULAR PRESSURE LOWERING PROPERTIES: THE INFLUENCE OF METAL IONS UPON THE PHARMACOLOGICAL ACTIVITY*

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Metal complexes of a sulfonamide possessing strong carbonic anhydrase (CA) inhibitory properties, 5-(2-chlorophenyl)-1,3,4-thiadiazole-2-sulfonamide (chlorazolamide) have been obtained from the sodium salt of the sulfonamide and the following metal ions: Mg(II), Zn(II), Mn(II), Cu(II), Co(II), Ni(II), Be(II), Cd(II), Pb(II), Al(III), Fe(III) and La(III). The original sulfonamide and its complexes were assayed for the *in vitro* inhibition of three CA isozymes, CA I, II, and IV, some of which play a critical role in ocular fluid secretion. All these compounds (the sulfonamide and its metal complexes) behaved as powerful inhibitors against the three investigated isozymes. The parent sulfonamide possessed an extremely weak topical pressure lowering effect when administered as a 1–2% suspension into the rabbit eye, but some of its metal complexes, such as the Mg(II), Zn(II), Mn(II) and Cu(II) derivatives, lower intraocular pressure (IOP) in experimental animals very well. *Ex vivo* data showed a 99.5–99.9% CA II inhibition in

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ocular fluids and tissues of rabbits treated with these agents, proving that the observed IOP lowering is due to CA inhibition. The influence of the different metal ions upon the efficiency of the obtained complexes as pressure lowering drugs are discussed, leading to the possibility of designing more selective/potent pharmacological agents from this class.

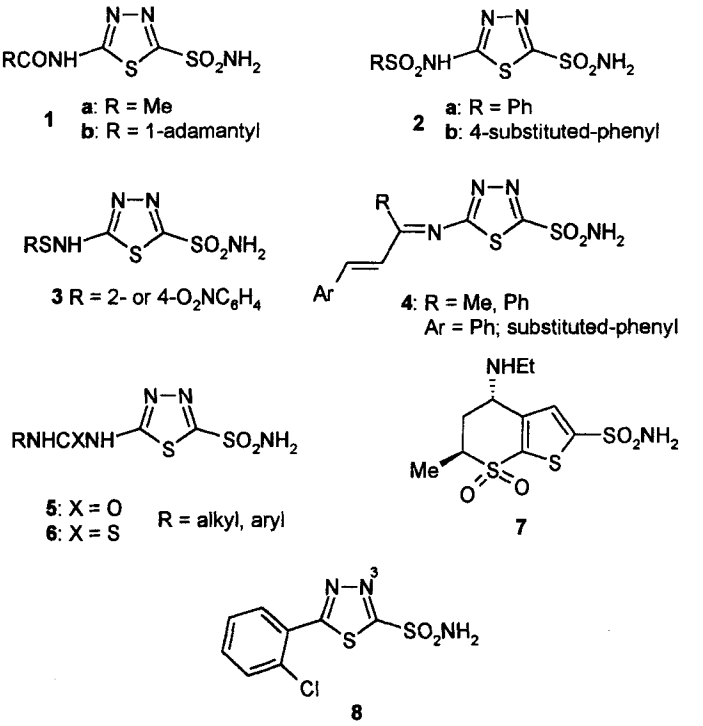
Keywords: Topical sulfonamide; Chlorazolamide; Carbonic anhydrase; Metal complex; Intraocular pressure lowering; Antiglaucoma drug

INTRODUCTION

1,3,4-Thiadiazole-2-sulfonamide derivatives[2–7] played a critical role in the development of several important classes of pharmacological agents, such as the diuretics with saluretic action,[8,9] the benzothiadiazine[10] and high-ceiling diuretics,[11] as well as the antiglaucoma drugs with carbonic anhydrase (CA) inhibitory action amongst others.[12,13] The prototype for all these drugs was acetazolamide **1a**, the first non-mercurial diuretic,[2,8] used for more than 45 years in clinical medicine as diuretic,[8] antiglaucoma,[8,12,13] antiepileptic[14] and antiulcer compound.[15] It is still used nowadays, mainly as a diagnostic tool in NMR imaging,[16,17] and in many physiological studies.[18–20] The major biological action of acetazolamide and related heterocyclic/aromatic sulfonamides is connected with the powerful inhibition of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1), of which at least fourteen isozymes are presently known in higher vertebrates.[6–8,12]

Many structural variants were derived using acetazolamide **1a** as lead molecule, such as 5-aryl/alkyl-sulfonylamido-1,3,4-thiadiazole-2-sulfonamides **2**[21,22] of which benzolamide **2a** is the most important representative,[23] whereas other derivatives of this type, such as **2b**, were recently reported for the development of diagnostic tools in positron emission tomography (PET),[22] sulfenamido-sulfonamides of type **3**,[24] some of which could display omeprazole-like activation in acidic media *in vivo*,[24] Schiff bases of type **4** which showed increased affinity for the membrane-associated isozyme CA IV,[25,26] as well as ureido/thioureido derivatives of type **5** and **6**, which were recently shown to possess very strong affinities for the physiologically relevant isozymes CA I, II and IV.[27,28]

Metal complexes of sulfonamides of type **1** and **2**, containing a large number of main group or transition metal ions, were shown to possess very strong CA inhibitory properties,[29–35] which were explained mechanistically as being due to a dual process: binding of the undissociated complex to the histidine cluster of isozyme II[36] as well as inhibition due to the dissociation of the complex in dilute solution in the assay system.[37] Such dissociation processes lead to the formation of sulfonamide anions which subsequently bind to the Zn(II) ion within the CA active site, and metal ions which bind



STRUCTURES **1–8**

to critical histidine residues or the histidine cluster itself in isozymes in which this is present (CA II and CA III).[36,37] The result of these interactions is that, generally, metal complexes of heterocyclic sulfonamides are 10–100 times more active as CA inhibitors than the sulfonamides from which they were obtained, with affinities for the receptor in the 10^{10}–10^{12} range.[29–35,37] Recently we have reported[38] the unexpected finding that metal complexes of a sulfonamide structurally related to acetazolamide, **1b**, which does not itself possess intraocular pressure (IOP) lowering effects when applied directly into the rabbit eye, were extremely potent topical IOP lowering agents. In the above mentioned study,[38] only the Zn(II) and Cu(II) complexes of 5-adamantylcarboxamido-1,3,4-thiadiazole-2-sulfonamide **1b** were tested for their antiglaucoma action. They showed much better properties than dorzolamide **7**, the recently introduced clinic topical sulfonamide with antiglaucoma action,[11–13] which anyhow shows many undesired side-effects in many treated patients (such as irreversible corneal decompensation).[13c] It appeared thus of great interest to prepare metal complexes of other sulfonamides and determine whether the entire class of such derivatives possesses

these valuable biological/pharmacological properties, in order to evaluate the possibility of obtaining less toxic and more efficient antiglaucoma drugs of this type. The influence of different metal ions on the putative IOP lowering properties of such derivatives has not been investigated in detail previously, although we hypothesized that this factor was extremely important for this type of biological action.[38] Continuing our previous research on metal complexes with antiglaucoma action, in this paper we report the synthesis of coordination compounds of a sulfonamide derived from the classical ring system leading to strong CA inhibitors, i.e., 5-(2-chlorophenyl)-1,3,4-thiadiazole-2-sulfonamide **8**. A large number of di- and trivalent metal ions, such as: Mg(II), Zn(II), Mn(II), Cu(II), Co(II), Ni(II), Be(II), Cd(II), Pb(II), Al(III), Fe(III) and La(III) were included in the present study. The obtained new complexes were characterized by standard procedures in order to assign their structures, and were assayed as CA inhibitors against three isozymes, CA I, II, and IV. *In vivo* studies in rabbits allowed us to determine the influence of diverse metal ions upon the IOP lowering properties of the new complexes, so as to detect the best candidates for the development of novel types of antiglaucoma drugs.

MATERIALS AND METHODS

IR spectra were recorded in KBr pellets with a Perkin Elmer 1012 FTIR instrument. Magnetic susceptibility measurements were carried out at room temperature with a fully automated AZTEC DSM8 pendulum-type susceptometer. Mercury(II) tetrakis-(thiocyanato)cobaltate(II) was used as susceptibility standard. Corrections for the diamagnetism were estimated from Pascal's constants.[39] Conductimetric measurements were done at room temperature (1 mM concentration of complex) in DMSO solution with a Fisher conductimeter. Elemental analyses were done by combustion for C, H, N with an automated Carlo Erba analyzer, and gravimetrically or volumetrically for the metal ions, and were $\pm 0.4\%$ of the theoretical values. Thermogravimetric measurements were done in air, at a heating rate of 10°C/min, with a Perkin Elmer 3600 thermobalance.

Sulfonamides used as standards in the enzymatic assay (except for **7** and **8**), acetazolamide, solvents as well as inorganic reagents were from Sigma, E. Merck and Carlo Erba. 5-(2-chlorophenyl)-1,3,4-thiadiazole-2-sulfonamide **8** was from Lederle Laboratories. Dorzolamide hydrochloride **7** was from Merck, Sharp and Dohme. Metal salts used in the syntheses as well as solvents were analytical grade (from E. Merck).

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 Forsman *et al.*[40] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by Lindskog's group,[41] and enzymes were purified by affinity chromatography according to the method of Khalifah *et al.*[42] Enzyme concentrations were determined spectrophotometrically at 280 nm, utilizing a molar absorptivity of $49\,mM^{-1}\cdot cm^{-1}$ for hCA I and $54\,mM^{-1}\cdot cm^{-1}$ for hCA II, respectively, based on $M_r = 28.85$ kDa for hCA I, and 29.3 kDa for hCA II, respectively.[43,44] bCA IV was isolated from bovine lung microsomes as described by Maren *et al.*, and its concentration was determined by titration with ethoxzolamide.[45]

General Procedure for the Preparation of Compounds 10–21

An amount of 6 mmol of the sodium salt of **8** was prepared *in situ* by reacting the sulfonamide with the required amount of an alcoholic 1 N NaOH solution, in ethanol as solvent. To this solution was added the metal salt (Be(II), Mg(II), Zn(II), Pb(II), Mn(II), Cu(II), Co(II), Ni(II) and Cd(II) as chlorides, Fe(III) as perchlorate and La(III) and Al(III) as nitrates) in aqueous solution, working in molar ratios $RSO_2NH^-:M^{n+}$ of 2:1 for the divalent cations and 3:1 for the trivalent cations. The aqueous-alcoholic reaction mixture was heated on a steam bath for 1 h and after being cooled at 0°C the precipitated complexes were filtered and thoroughly washed with alcohol–water 1:1 (v/v) and air dried. Yields were in the range of 85–90%. The powders of the complexes obtained melt with decomposition at temperatures higher than 300°C, and are poorly soluble in water and alcohol, but have good solubilities in DMSO, DMF as well as mixtures of DMSO–water, DMF–water.

Carbonic Anhydrase Inhibition

Initial rates of 4-nitrophenyl-acetate hydrolysis catalysed by different CA isozymes were monitored spectrophotometrically, at 400 nm, with a Cary 3 instrument interfaced with an IBM compatible PC.[46] Solutions of substrate were prepared in anhydrous acetonitrile; the substrate concentrations varying between $2\cdot10^{-2}$ and $1\cdot10^{-6}$ M, working at 25°C. A molar absorption coefficient ε of $18{,}400\,M^{-1}\cdot cm^{-1}$ was used for the 4-nitrophenolate formed by hydrolysis, under the conditions of the experiments (pH 7.40), as

reported in the literature.[46] Non-enzymatic 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, in order to allow for the formation of the E–I complex. The inhibition constant K_I was determined as described by Pocker and Stone.[46] Enzyme concentrations were 3.6 nM for hCA II, 11 nM for hCA I and 26 nM for bCA IV (this isozyme has a decreased esterase activity and higher concentrations had to be used for the measurements).[47]

Measurement of Tonometric IOP

Adult male New Zealand albino rabbits weighing 2–3 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 : 12 h light/dark cycle in a temperature controlled room, at 22–26°C. Solutions of inhibitors (2%, by weight) were obtained in DMSO–water (1 : 4, v/v) due to the low water solubility of some of these derivatives. Control experiments with DMSO (at the same concentration as that used for obtaining the inhibitors solutions showed that it does not possess IOP lowering or increasing effects.

IOP was measured using a Digilab 30R pneumatonometer (BioRad, Cambridge, MA, USA) as described by Maren's group.[48–50] The pressure readings were matched with two-point standard pressure measurements at least twice each day using a Digilab Calibration verifier. The same investigator did all IOP measurements 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 several hours. 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.[48–50] All data are expressed as mean ± SE, using a one-tailed *t*-test.

Drug Distribution in Ocular Fluids and Tissues

The general procedure of Maren's group was followed.[48–50] The animals were sacrificed with an intracardiac air injection. Aqueous humour (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 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 denaturate 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 in the presence of the inhibitor were determined as described above. A calibration curve was used in order to determine the fractional inhibition in the different tissues, as described by Maren's group.[48–50]

RESULTS AND DISCUSSION

Metal complexes of chlorazolamide **8** were prepared from its sodium salt **9** (obtained *in situ* from **8** and sodium ethoxide) and salts of di- and trivalent transition and main group metal ions (reactions (1) and (2)):

$$R\text{–}SO_2\text{–}NH_2 + EtONa \longrightarrow R\text{–}SO_2\text{–}NH^- + Na^+ + EtOH \quad (1)$$

$$nR\text{–}SO_2\text{–}NH^- + M^{n+} + xH_2O \longrightarrow [M(R\text{–}SO_2\text{–}NH)_n(OH_2)_x] \quad (2)$$

The prepared new complexes of type **10–21** as well as their elemental analysis data are shown in Table I. Mention should be made that metal ions which were previously shown[29–35] to lead to powerful complex CA inhibitors were included in the study, such as Zn(II), Cu(II), Co(II), Ni(II), Fe(III) and Al(III).

TABLE I Metal complexes **10–21** containing the conjugate base of 5-(2-chlorophenyl)-1,3,4-thiadiazole-2-sulfonamide **8** (LH) as ligand and their elemental analysis data

No.	*Complex*	*Analysis (calcd./found)*			
		%*M*[a]	%*C*[b]	%*H*[b]	%*N*[b]
10	$[BeL_2]$	1.61/1.39	34.41/34.75	1.80/2.03	15.05/14.76
11	$[MgL_2] \cdot 3H_2O$	3.87/4.10	30.61/30.75	2.57/2.49	13.39/13.12
12	$[ZnL_2]$	10.63/10.95	31.26/31.12	1.64/1.55	10.63/10.47
13	$[CdL_2(OH_2)_2]$	16.11/15.90	27.54/27.61	2.02/1.87	12.04/11.89
14	$[MnL_2(OH_2)_2]$	8.58/8.76	30.01/30.24	2.20/2.25	13.12/12.93
15	$[CoL_2(OH_2)_2]$	9.15/9.34	29.82/30.14	2.19/2.40	13.04/12.87
16	$[NiL_2(OH_2)_2]$	9.11/8.98	29.83/29.97	2.19/2.05	13.05/12.90
17	$[CuL_2(OH_2)_2]$	9.79/10.03	29.61/29.54	2.17/2.40	12.95/12.69
18	$[PbL_2(OH_2)_2]$	26.14/25.91	24.24/24.36	1.78/1.95	10.60/10.43
19	$[FeL_3]$	6.335/6.51	32.76/32.62	1.72/1.90	14.32/14.09
20	$[AlL_3]$	3.17/3.32	33.87/34.08	1.78/1.95	14.81/14.56
21	$[LaL_2(OH_2)_5] \cdot 3H_2O$	16.68/16.54	23.06/22.94	2.40/2.51	10.09/9.71

[a]By gravimetric or volumetric analysis. [b]By combustion.

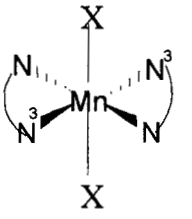
TABLE II IR, solution electronic spectral, and thermogravimetric (TG) analysis data for sulfonamide **8**, its sodium salt **9** and its metal complexes **10–21**

Comp.	*IR spectra*[a] (cm^{-1})			*UV spectra*[b] λ_{max}, nm (lg ϵ)	*TG analysis*[c] (*calcd./found*)
	$\nu(SO_2^s)$	$\nu(SO_2^{as})$	ν(C=N)		
8	1180	1365	1650	275 (3.90); 321 (4.17)	[d]
9	1175	1360	1650	265 (4.07); 305 (3.48)	[d]
10	1155	1340	1640	265 (4.32); 305 (3.64)	[d]
11	1165	1340	1645	265 (4.35); 305 (3.59)	8.60/8.33[e]
12	1150	1315	1650	265 (4.24); 305 (3.55)	[d]
13	1150	1320	1630	265 (4.26); 305 (3.60)	5.15/5.09[f]
14	1160	1320	1635	265 (4.16); 305 (3.54)	5.62/5.34[f]
15	1140	1330	1630	265 (4.24); 305 (3.59)	5.58/5.69[f]
16	1160	1330	1640	265 (4.33); 305 (3.57)	5.58/5.42[f]
17	1160	1330	1640	265 (4.29); 305 (3.67)	5.54/5.51[f]
18	1165	1340	1635	265 (4.25); 305 (3.68)	4.54/4.61[f]
19	1165	1330	1650	265 (4.29); 305 (3.76)	[d]
20	1140	1330	1640	265 (4.24); 305 (3.70)	[d]
21	1145	1330	1640	265 (4.26); 305 (3.74)	6.48/6.31[e]; 10.81/10.59[g]

[a]In KBr pellets. [b]In DMSO. [c]% weight loss in the temperature range of 100–200°C. [d]No weight loss under 280–300°C. [e]Corresponding to three uncoordinated water molecules, lost between 100–110°C. [f]Corresponding to two coordinated water molecules, lost between 150–180°C. [g]Corresponding to five coordinated water molecules, lost in one step, between 170–190°C.

The new compounds **10–21** were characterized by elemental analysis and physico-chemical methods (UV, IR spectroscopy, magnetic, thermogravimetric (TG) and conductimetric data) which allowed us to propose their formulas (Tables II–V).

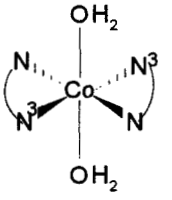
TABLE III Electronic spectroscopic data for the Mn(II) complex **14** (X = H_2O) by the diffuse reflectance technique in MgO as standard and its proposed structure



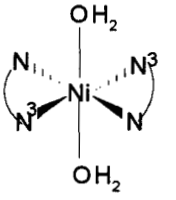
14

Complex (colour)	*Wavenumber ν* (cm^{-1})	*Assignments*
14 (white)	25,550	$^6A_{1g} \rightarrow {}^4T_{2g}(D)$
	24,700	$^6A_{1g} \rightarrow {}^4A_{1g}, E_g(G)$
	19,650	$^6A_{1g} \rightarrow {}^4T_{2g}$
	16,200	$^6A_{1g} \rightarrow {}^4T_{1g}$

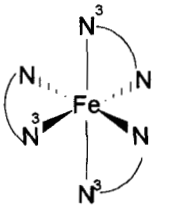
TABLE IV Electronic spectroscopic data for complexes **15**, **16** and **19** by the diffuse reflectance technique in MgO as standard and the proposed structures



15



16

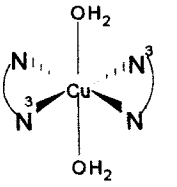


19

Complex (colour)	*Wavenumber ν* (cm^{-1})	*Assignments*
15 (pink)	26,300	$(\pi \rightarrow \pi^*)$
	19,540	$^4T_{1g} \rightarrow {}^4T_{1g}(P)$ (Oh)
	17,300	$^4T_{1g} \rightarrow {}^4T_{1g}(P)$ (Oh)
	9450	$^4T_{1g} \rightarrow {}^4T_{2g}$ (Oh)
16 (blue)	26,300	$(\pi \rightarrow \pi^*) + (^3A_{2g} \rightarrow {}^3T_{1g}(P))$
	24,000	$(\pi \rightarrow \pi^*)$
	17,500	$^3A_{2g} \rightarrow {}^3T_{1g}$
	11,600	$^3A_{2g} \rightarrow {}^1E_g$
	9050	$^3A_{2g} \rightarrow {}^3T_{2g}$
19 (yellow)	26,350	$\pi \rightarrow \pi^* + CT$ [a]
	21,700	$^6A_{1g} \rightarrow {}^4T_{2g}$ (Oh high spin)
	18,000	$^6A_{1g} \rightarrow {}^4T_{1g}$ (Oh high spin)

[a]CT = charge transfer band.

TABLE V Room temperature magnetic moment and electronic spectroscopic data of the Cu(II) complex **17** by the diffuse reflectance technique in MgO as standard and the proposed structures



17

Complex (colour)	*Magnetic moment BM*	*Wavenumber* ν (cm^{-1})	*Assignments*
17 (gray-blue)	0.756	23,590	$\pi \rightarrow \pi^*$
		16,300	$d_{yz}, d_{xz} \rightarrow d_{x-y}$
		13,550	$CT^a + d_{yz}, d_{xz} \rightarrow d_z$
		10,600	$d_{yz}, d_{xz} \rightarrow d_{xy}$

[a]CT = charge transfer band.

Elemental analysis data (obtained by combustion for C, H, N, and gravimetrically or volumetrically for the metal ions) were within ±0.4% of the theoretical data, calculated for the proposed formulae (Table I).

IR and solution electronic spectroscopic data afforded the determination of the coordination mode of the sulfonamidate anion of **8** in the new complexes **10–21** reported here. Thus, similarly to other 1,3,4-thiadiazole-2-sulfonamide derivatives (such as **1a**, **b**[31–35,38] or **2a**[51]) for which metal complexes have been prepared and characterized by X-ray crystallography and spectroscopic methods, the donor system of sulfonamide **8** is constituted by the sulfonamidic (ionized) nitrogen and the endocyclic N-3 atom of the 1,3,4-thiadiazole ring (shown schematically as "N" and "N^3", respectively, in the structures proposed for the new complexes). The conjugate base of **8** acts in this way as a bidentate ligand, similarly to acetazolamide, benzolamide or other such derivatives previously investigated.[30–38,51] Several changes in the spectra of complexes **10–21** as compared to the corresponding spectrum of **8** (or its sodium salt **9**) confirm the above assumptions. Thus, in these IR spectra the following features were noted: (i) the intense sulfonamide vibrations, at 1180 and 1365 cm^{-1} in the spectrum of **8** were shifted to 1140–1165 cm^{-1} and 1315–1340 cm^{-1}, respectively, in the IR spectra of complexes **10–21**, due to the involvement of this moiety in the interaction with the metal ions[29–38,51] (Table II), (ii) the thiadiazole C=N stretching vibration from 1650 cm^{-1} in sulfonamide **8** underwent shifts in the spectra of complexes **10–21**, where generally it appeared at lower

wavenumbers (1630–1640 cm^{-1}) for reasons identical to those mentioned at (i) (Table II), (iii) vibrations in the region 320–480 cm^{-1} were identified in the spectra of the metal complexes, which were not present in the spectrum of the ligand, and were assigned as being due to Metal–N (or Metal–O) vibrations[29–38] (data not shown).

Complexes **10–21** possessed UV spectra highly similar to those of the sodium salt of the ligand **8** (Table II), proving the presence of sulfonamide anionic moieties in their molecule. Thus, the ligand **8** has two strong absorption maxima in the UV spectrum, one at 275 nm and the other at 321 nm, similarly to other 1,3,4-thiadiazole-2-sulfonamide derivatives previously investigated,[9,24,25,29] whereas the sodium salt and its metal complexes show a hypsochromic shift of these bands, which generally appear at 265 and 305 nm, respectively (Table II).

TG analysis showed the presence of lattice water molecules in compounds **11** and **21**, as well as coordinated water molecules in many of the prepared complexes (**13–18**, **21**). In the first case, the lattice water was lost in one step between 100–110°C, whereas the coordinated water molecules were lost also in one step, but at higher temperatures (160–200°C) (Table I).

Diffuse reflectance electronic spectroscopic and magnetic data (for paramagnetic transition metal ions) helped us to establish the geometry of the cations in the prepared complexes (Tables III–V).[52–55] Thus, octahedral structures were proposed for the majority of the prepared complexes (such as the Cd(II), Mn(II), Ni(II), Co(II), Cu(II), Pb(II), Fe(III) and Al(III) derivatives, whereas tetrahedral structures were detected for the Be(II), Mg(II) and Zn(II) complexes. The La(III) derivative **21** is probably in its preferred nine-coordinated geometry.[56] For all these derivatives the expected transitions were evidenced in the electronic spectra, which are assigned in detail in Tables III–V, and which are in agreement with literature data for similar system.[29–34] Conductimetric data have shown all complexes to be non-electrolytes, with molar conductibilities in the range of 0.5–3.6 $\Omega^{-1} \cdot cm^2 \cdot mol^{-1}$ (data not shown).

The new compounds **10–21** and standard CA inhibitors were assayed for CA inhibition against three isozymes, hCA I, hCA II and bCA IV (Table VI). As seen from the data in Table VI, chlorazolamide **8** and its metal complexes **10–21** behave as very strong inhibitors against all the three investigated isozymes, hCA I, hCA II and bCA IV. The original sulfonamide **8** is initially more inhibitory than acetazolamide, methazolamide and benzolamide, having a potency similar to that of dorzolamide or ethoxzolamide against the red cell isozyme hCA II and the membrane-bound isozyme CA IV. A remarkable finding (which was previously reported by us[29] for the

TABLE VI CA inhibition data with standard inhibitors **1–7**, chlorazolamide **8** and its metal complexes **10–21**

Inhibitor		K_I (nM)		
		hCA I[a]	*hCA II*[a]	*bCA IV*[b]
1a	Acetazolamide	900	12	220
	Methazolamide	780	14	240
	Ethoxzolamide	25	8	13
2a	Benzolamide	15	9	12
7	Dorzolamide	50,000	9	43
8	Chlorazolamide	18	3	15
10		15	2.0	10.5
11		12	2.4	10.1
12		5.0	1.4	6.9
13		4.2	0.9	6.1
14		5.5	1.5	6.7
15		5.4	1.5	5.9
16		6.5	1.7	6.5
17		4.0	0.4	4.6
18		2.5	0.5	4.2
19		3.2	0.5	3.0
20		3.9	0.9	4.6
21		3.2	0.5	3.6

[a]Human (cloned) isozymes. [b]From bovine lung microsomes.

class of ureas/thioureas of type **5** and which also applies to chlorazolamide **8**) was that these compounds possess a high affinity for the slow red cell isozyme, hCA I, which is generally less susceptible to inhibition by sulfonamides.[6–8] The metal complexes **10–21**, being even more inhibitory than **8**, and than all other simple sulfonamides assayed, also present this feature mentioned above, inhibiting to a high degree isozyme hCA I. They also show the same behaviour as the metal complexes of acetazolamide, methazolamide or dorzolamide previously reported by this group, which were all more inhibitory than the parent sulfonamide from which they were obtained.[29–35,37,38,51] Particularly strong inhibition was observed again for the Cu(II), Zn(II), Co(II), Mn(II) and La(III) derivatives of sulfonamide **8**.

IOP pressure measurements after the topical administration of one drop (50 μL) of 2% solutions/suspensions (in DMSO–water 1 : 4 (v/v)) of inhibitors of type **8**, **10–21** or a 2% solution of the clinical drug dorzolamide **7** into the eyes of normotensive albino rabbits are shown in Table VII.

Chlorazolamide **8** possesses a reduced water solubility, and it was applied as a 1% or 2% suspension in DMSO–water 1 : 4 (v/v). The other compounds (**7** or the metal complexes of type **10–21**) were applied as 2% solutions in the same solvent system mentioned above. Mention should be made that a new drug from this class recently introduced in clinical medicine in

TABLE VII Fall of IOP (21.4 ± 1.5 mmHg) following topical application of CA inhibitors, at 0.5, 1 and 1.5 h after instillation into the normotensive rabbit eye of a drop (50 μL) of 2% solution of inhibitor **10–21**, 2% solution of the standard inhibitor dorzolamide **7**, or 2% suspension of sulfonamide **8**. The pH of the solutions/suspensions (obtained in 1 : 4 (v/v) DMSO–water) is also shown

Inhibitor	*pH*	$\Delta IOP \pm SEM$[a] (mmHg)		
		0.5 h	1 h	1.5 h
7	5.5	2.2 ± 0.10	4.1 ± 0.15	3.6 ± 0.10
8	7.5	0	0.5 ± 0.25	0.3 ± 0.10
11	7.5	3.6 ± 0.45	6.2 ± 0.25	5.4 ± 0.20
12	7.5	5.4 ± 0.30	9.5 ± 0.20	9.0 ± 0.40
14	7.5	3.1 ± 0.20	6.8 ± 0.30	6.1 ± 0.35
15	7.5	3.0 ± 0.25	6.1 ± 0.40	5.5 ± 0.30
16	7.5	2.5 ± 0.15	5.5 ± 0.30	4.0 ± 0.35
17	7.0	3.4 ± 0.20	7.0 ± 0.35	5.5 ± 0.20
19	7.0	1.4 ± 0.20	3.5 ± 0.20	2.4 ± 0.10
20	7.0	1.5 ± 0.15	3.2 ± 0.15	2.7 ± 0.15

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

USA, brinzolamide, is also available as a 1% suspension (not as a solution) due to its reduced water solubility.[57]

As seen from data of Table VII, chlorazolamide **8** is ineffective as a topical IOP lowering agent, whereas important reductions of IOP have been observed with many of its metal complexes. Compounds such as **16** (containing Ni(II)), **19** (containing Fe(III)) or **20** (containing Al(III)) were slightly less or equally active to dorzolamide **7** in lowering IOP in this animal model of glaucoma. The Mg(II), Mn(II), Co(II) and Cu(II) derivatives on the other hand (compounds **11**, **14**, **15** and **17**, respectively) were all more active than dorzolamide **7**, with IOP lowering of 3.0–3.6 mmHg at 30 min after administration (versus 2.2 mmHg after dorzolamide), which increased to 6.1–7.0 mmHg after 1 h (when the maximal effect of dorzolamide was 4.1 mmHg). A prolonged IOP lowering was observed with all these derivatives at longer periods after administration (90 min–3 h), whereas the effect of dorzolamide was much less pronounced (see also Figure 1). The most effective agent in the prepared series was the Zn(II) complex **12**, which produced an IOP lowering of 5.4 mmHg at 30 min after administration, of 9.5 mmHg after 1 h, and this important lowering was maintained for a prolonged period of 4–5 h, in contrast to dorzolamide whose effects completely vanished after 3 h (Figure 1).

The amount of CA inhibitor present in ocular fluids and tissues after the topical administration of the Zn(II) complex of sulfonamide **8** (compound **12**) is shown in Table VIII. It is seen from these data that 1 and 2 h after

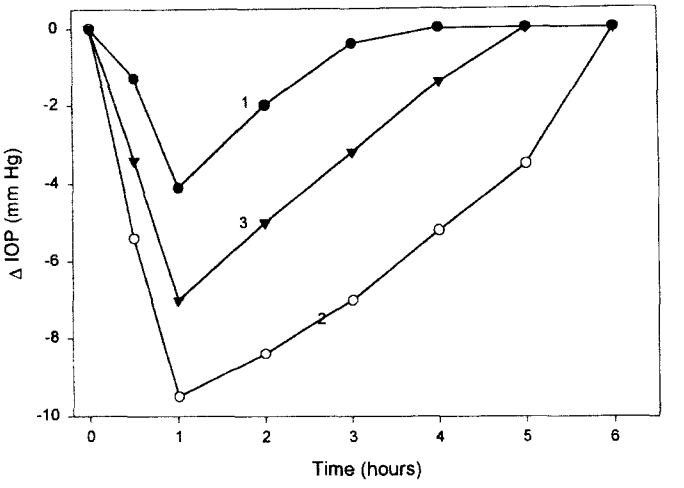


FIGURE 1 Effect of topically administered sulfonamide inhibitors (2% DMSO–water (1:4; v/v) solutions) on the IOP of normotensive albino rabbits. Curve 1, dorzolamide **1** (hydrochloride salt, pH 5.5); curve 2, the Zn(II) complex **12** (pH 7.5); curve 3, the Cu(II) complex **17** (pH 7.0).

TABLE VIII Ocular tissue concentrations (μM) after 1 and 2 h, following corneal application of one drop (50 μL) of 2% solution of the Zn(II) complex **12** and the standard sulfonamide **7**, in albino rabbits

Time (h)	*Inhibitor*	*Cornea*	*Drug concentration* (μM)*	
			Aqueous humour	*Ciliary process*
1	**7**	106 ± 5	32 ± 3	15 ± 3
2	**7**	39 ± 5	21 ± 3	6 ± 1
1	**12**	150 ± 4	262 ± 21	46 ± 5
2	**12**	54 ± 5	59 ± 5	12 ± 0.9

*Mean ± standard deviation ($n = 3$).

topical administration of the drug, high levels of **12** were found in the cornea, aqueous humour and ciliary processes. Based on the inhibition constant of this compound (1.4 nM for CA II, and 6.9 nM for CA IV), the fractional inhibition estimated in these tissues/fluids is 99.5–99.9%,[48,49] proving that the IOP decrease is indeed due to CA inhibition.

We stress again that the parent ligand of the prepared metal complexes, chlorazolamide **8**, is a very weak IOP lowering agent, whereas many of its metal complexes act as efficient IOP lowering agents. Many of them act even better than the clinical drug dorzolamide. Thus, the presence of some metal ions (such as Zn(II), Cu(II), Mg(II) or Mn(II) amongst others) seem to induce strong IOP lowering properties due to important changes in the

physico-chemical and pharmacological properties of the compounds connected with the presence of the metal ions.

In conclusion we report here a study relating the influence of metal ions upon the IOP lowering properties of the metal complexes containing as ligand a sulfonamide with strong CA inhibitory properties which is ineffective as a topical antiglaucoma drug. The best metal ion for the biological activity of such compounds seem to be Zn(II) and correlated with the general lack of toxicity of Zn(II) compounds, this suggests that some of the complexes reported here might be good candidates for developing novel types of antiglaucoma drugs.

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