COMPLEXES WITH BIOLOGICALLY ACTIVE LIGANDS. Part 5 [1] Zn(II) AND Cd(II) COORDINATION COMPOUNDS OF HYDRAZINE AND HETEROCYCLIC SULFONAMIDES AS INHIBITORS OF THE ZINC ENZYME CARBONIC ANHYDRASE[#]

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Abstract: Zn(II) and Cd(II) complexes with hydrazine and six heterocyclic sulfonamide drugs possessing carbonic anhydrase (CA) inhibitory properties, were prepared and characterized by elemental analysis, spectroscopic (IR, electronic and ^{1}H-NMR), thermogravimetric, and conductimetric measurements. The complexes behave as strong inhibitors for two isozymes (I and II) of carbonic anhydrase.

Introduction

Metal complexes of heterocyclic sulfonamides constitute a novel class of inhibitors of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1). [2,3] Their potencies towards inhibiting different CA isozymes is very high, a fact that has been rationalized as being due to a dual mechanism of action, by means of sulfonamido anions, and metal ions formed by dissociation of the complexes in dilute solutions (equation *1*) in assay systems.[2-5]

$$[M^{n+}(RSO_2NH^-)_n] \rightleftharpoons M^{n+} + n\,RSO_2NH^- \qquad (1)$$

This fact has been confirmed by stability constant determinations for such complexes,[6,7] which proved the relatively low stability in aqueous solution of the complexes (values for this parameter were in the range 10^2-10^{10} for the Cu(II); Ni(II); Zn(II) or Pd(II) complexes of some sulfonamides discussed bellow). The sulfonamido anions formed in this way bind thereafter at the Zn(II) ion from the enzyme active site,[7,8] which is critical for catalysis,[9] whereas metal ions probably bind in the neighborhood of the histidine cluster at the entrance of the active site (consisting of residues His-3, His-4, His-10, His-15, His-17 and His-64[10] for isozyme CA II, or to residues His-64 and His-200 for CA I). It should be noted that these histidines were shown to possess an important role in catalysis, acting as proton shuttle residues, which assure transfer of protons out of the active site. This proton transfer from the metal-bound water molecule to the environment is the rate-determining step in all catalytic processes involving CAs.[9,10]

Coordination compounds containing di-, tri-, tetra- and hexavalent metal ions were reported up to now (the M^{n+} in equation *1*), with a large range of heterocyclic sulfonamides (possessing well-established CA inhibitory properties),[2,3,11] such as acetazolamide **1** (H_2aaz); methazolamide **2** (Hmza); ethoxzolamide **3** (Heza), sezolamide **4** (Hsza).[12] The last one is member of a new class of clinical agents used for the topical treatment of glaucoma, recently developed by Merck[13] and introduced in clinical medicine in USA. Some derivatives of type **4** were recently used by us for the preparation of metal complexes possessing very strong CA inhibitory properties[14] In addition to **1-4**, the other two sulfonamides included in the present study as ligands, **5** and **6**, have never been used previously for the preparation of complexes, although they possess

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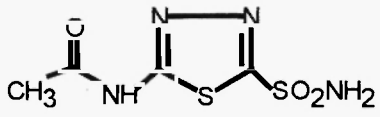
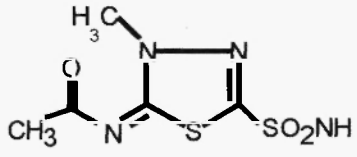
Thus, benzolamide **5** (H_2bza) has the status of orphan drug[15] being an extremely potent CA inhibitor, and also having a very acidic proton (pK_a of the SO_2NH moiety is 3.8)[11], which confers to this drug interesting pharmacological properties, such as impaired penetration through biological membranes (when ionized, as anion, at certain pH values). In fact, benzolamide is one of the few systemic CA inhibitors which in certain concentrations is able to selectively inhibit only some CA isozymes,[16] acting thus as isozyme-specific or organ-selective inhibitor.[2,16] The other very strong inhibitor included in our study, chlorzolamide, **6** (Hcza) is chemically similar to the previously mentioned derivatives **1,2,5**. This also leads to very different biological properties, such as increased liposolubility, and good penetrability through membranes.[11] It is thus quite clear how relatively small chemical modifications in these 1,3,4-thiadiazole-2-sulfonamide derivatives (of the types **1,2,5,6**) lead to drastical modifications of pharmacological properties of the drugs.



1: H_2aaz



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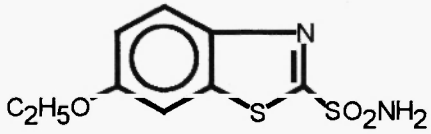

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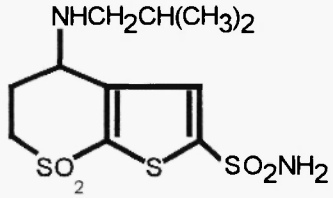
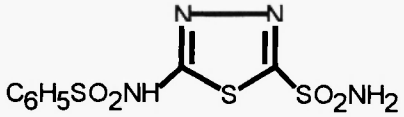
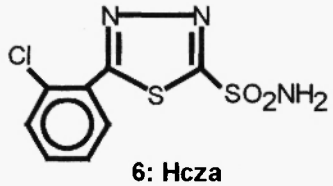


4: Hsza



5: H_2bza



6: Hcza

In addition to complexes containing only heterocyclic sulfonamides as ligands,[2,3] Borras' group reported the preparation and characterization, by means of X-ray crystallography and spectroscopic methods, of metal complexes of sulfonamides of type **1** and **2**, also containing ammonia, pyridine, and diamines (ethylenediamine and 1,3-propylenediamine) as ligands.[17,18] Some of these derivatives mimic quite well the inhibited CA active site, when complexed with the sulfonamide type inhibitors and might bring novel insights regarding the mechanism of inhibition of these enzymes.[18]

Although different amines were included in these studies,[17,18] except for ammonia, simple such derivatives were not used for the preparation of ternary metal complexes (of sulfonamides, and the amine-type ligand). Thus, here we report the Zn(II) and Cd(II) complexes of heterocyclic sulfonamides **1-6** and hydrazine as ligands, which were characterized by standard procedures and assayed as inhibitors of red cell isozymes CA I and CA II.

Materials and Methods

IR spectra were obtained in KBr pellets with a Perkin Elmer 1600 spectrometer, in the range 200 - 4000 cm^{-1}. Electronic spectra were obtained in acetonitrile solutions with a Cary 3 instrument. Thermogravimetric measurements were done in air, at a heating rate of 10°C/min., with a Perkin Elmer 3600 thermobalance. Conductimetric measurements were done in DMF solutions, at 25°C (solution 0.1 mM of complex) with a Fisher conductimeter. ^{1}H-NMR spectra were obtained in DMSO-d_6 as solvent, with a Varian Gemini 300 spectrometer. Chemical shifts are expressed as δ values relative to tetramethylsilane as

internal standard. Elemental analyses were done by combustion for C,H,N with an automated Carlo Erba analyzer, and gravimetrically for the metal ions, and were ± 0.4% of the theoretical values.

Sulfonamides **1** and **2**, metal salts (zinc and cadmium nitrates) and solvents were from Aldrich and were used without further purification. Hydrazine hydrate (80% aqueous solution) was from Merck. Derivatives **3,4** and **6** were synthesized by literature procedures,[12-19] whereas benzolamide **5** was a gift from Dr. T.H.Maren (University of Florida, Gainesville). Human isozymes CA I and II and buffers were from Sigma. Inhibitors were assayed by Maren's micromethod[20] for inhibition of CO_2 hydration reaction catalyzed by the two isozymes, at 0°C in barbital buffer.

Synthesis of coordination compounds 7-18

10 mMoles of sulfonamide **1-6** were dissolved in the minimum amount of ethanol (generally 50-200 mL). The obtained solution was mixed with 10 mL of aqueous solution containing 5 mMoles of Zn(II) or Cd(II) nitrate, and the obtained solution was treated with an excess (3 mL) of hydrazine hydrate 80%. The mixture was stirred magnetically at room temperature for 2 hours, the obtained white precipitates were filtered and air-dried. Yields were in the range of 56-95%.

Results and Discussion

The prepared Zn(II) and Cd(II) complexes containing sulfonamides **1-6** and hydrazine as ligands, are shown in Table I, together with their elemental analysis data (± 0.4% of the theoretical values calculated for the proposed formulas).

Table 1: Prepared Zn(II) and Cd(II) coordination compounds containing sulfonamides **1-6** (in monodeprotonated form) and hydrazine as ligands, and their elemental analysis data.

No.	Compound	Yield (%)	Analysis, (calculated/found)			
			%M[a]	%C[b]	%H[b]	%N[b]
7	$[Zn(Haaz)_2(N_2H_4)_2]$	76	11.4/11.5	16.8/16.5	3.1/3.1	29.4/29.3
8	$[Zn(mza)_2(N_2H_4)_2]$	79	11.1/11.2	20.5/20.5	3.7/3.3	28.7/28.5
9	$[Zn(eza)_2(N_2H_4)_2]$	86	10.1/10.0	33.5/33.3	4.0/3.7	17.4/17.3
10	$[Zn(sza)_2(N_2H_4)_2]$	56	8.4/8.4	30.8/30.7	5.3/5.5	14.3/14.3
11	$[Zn(Hbza)_2(N_2H_4)_2]$	95	8.5/8.3	25.0/25.2	2.8/2.7	21.9/22.0
12	$[Zn(cza)_2(N_2H_4)_2]$	89	9.6/9.5	28.3/28.5	2.6/2.8	20.6/20.5
13	$[Cd(Haaz)_2(N_2H_4)_2]$	68	18.1/18.0	15.5/15.1	2.9/3.1	27.1/26.8
14	$[Cd(mza)_2(N_2H_4)_2]$	77	17.7/17.5	18.9/18.5	3.4/3.3	26.5/26.6
15	$[Cd(eza)_2(N_2H_4)_2]$	91	16.2716.4	31.2/31.1	3.7/3.5	16.2/16.0
16	$[Cd(sza)_2(N_2H_4)_2]$	58	13.6/13.5	29.0/28.9	5.0/5.1	13.5/13.1
17	$[Cd(Hbza)_2(N_2H_4)_2]$	90	13.8/13.5	23.5/23.3	2.7/2.5	20.6/20.5
18	$[Cd(cza)_2(N_2H_4)_2]$	84	15.5/15.4	26.4/26.1	2.4/2.5	19.3/19.2

[a] By gravimetry; [b] By combustion.

The new derivatives were also characterized by means of spectroscopic (IR, electronic and ^{1}H-NMR spectra), thermogravimetric and conductimetric measurements (Table II).

In the IR spectra of complexes **7-18**, the main difference as compared to the corresponding spectrum of the ligand involves the two sulfonamido vibrations, in the region 1100-1400 cm^{-1}. Thus, for both Zn(II) as well as Cd(II) complexes, a shift towards lower wavenumbers with 12-40 cm^{-1} was detected for these two bands, which proves the direct interaction between the sulfonamido moiety of the ligand and the metal ions. This behavior is well documented for complexes of sulfonamides **1-4** with a large range of

metal ions, previously investigated by our groups.[2-7,17,18] Moreover, amide bands (around 1650 cm^{-1}) of ligands possessing this moiety (such as **1** and **2**) appear at the same frequency in the complexes and the ligands, proving that they are not involved in complexation.

Table II: Spectroscopic, thermogravimetric and conductimetric data for compounds **1-18**.

Comp.	IR Spectra[a] ,cm^{-1}		Electronic Spectra[b]	TG analysis[c]	Conductimetry[d]
	$\nu(SO_2)^S$	$\nu(SO_2)^{as}$	λ_{max}, nm (Log ε)	calc./found	Λ_M (Ω^{-1} x cm^2 x mol^{-1})
	1170	1318	265 (3.92)	-	13
7	1138	1296	302 (3.98)	11.2/10.9	18
13	1135	1300	302 (4.03)	10.3/10.4	15
2	1153	1366	270 (3.65)	-	17
8	1126	1332	294 (3.84)	10.9/10.5	17
14	1124	1334	295 (3.79)	10.1/9.8	20
3	1180	1372	272 (3.87)	-	15
9	1140	1360	300 (3.95)	9.9/9.6	21
15	1137	1360	301 (3.95)	9.2/8.8	22
4	1162	1368	282 (3.41)	-	114[e]
10	1140	1347	297 (3.76)	8.2/8.3	24
16	1139	1348	296 (3.79)	7.7/7.4	28
5	1170	1340	270 (3.90)	-	23
11	1147	1326	310 (4.11)	8.3/8.0	19
17	1145	1329	310 (4.09)	7.8/7.7	24
6	1172	1369	268 (3.74)	-	10
12	1154	1348	297 (3.85)	9.4/9.2	15
18	1152	1349	295 (3.82)	8.8/8.5	14

[a] In KBr; [b]In MeCN; [c]Weight loss between 130-175 °C, corresponding to two coordinated hydrazine molecules; [d] 10^{-3} M solution, in DMF, at 25°C, [e] As hydrochloride, being a 1:1 electrolyte.

The hydrazine vibration, around 970 cm^{-1}, were also detected in the IR spectra of complexes **7-18**, but not in those of the original ligands (data not shown).

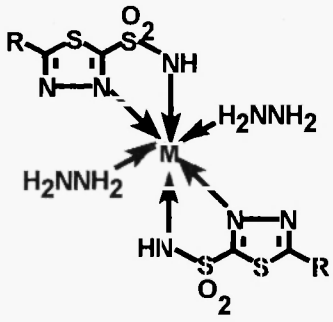
In the solution electronic spectra of the prepared complexes, the ligand absorption maxims (characteristic for the corresponding heterocyclic ring conjugated with the sulfonamido moiety) undergo a bathochromic shift accompanied by a hyperchromic effect (Table II), as in the spectra of the sodium salts of such ligands[21] (data not shown). This fact proves that in the presence of excess hydrazine, deprotonation of the sulfonamido groups of ligands **1-6** occurred, and the resulting SO_2NH^- moiety interacted thereafter with the metal ions, leading to complexes **7-18**. It should be mentioned that in many cases complexes of heterocyclic sulfonamides were prepared directly from the sodium salts of the ligands,[3-5] whereas when ternary complexes were intended to be obtained, an excess of amine-type ligand was used, which in addition to participation to complexation, also assured deprotonation of the sulfonamido ligand.[17,18]

^{1}H-NMR spectra of complexes **7-18** (recorded in DMSO-d_6) were quite similar to the spectra of the corresponding ligand, except for the absence of the signal of SO_2NH_2 protons, which in the ligands appeared in the region 6.4-7.7 ppm (data not shown). This is another proof that the deprotonated sulfonamido moiety is directly interacting with the metal ions.

Conductimetric data (Table II) proved the original ligands as well as their Zn(II) and Cd(II) complexes to be non-electrolytes, excepting for sezolamide **4** which was hydrochloride salt, and behaved as 1:1 electrolyte (but its complexes were non-electrolytes too).

TG data showed that no decomposition of the ligands **1-6** occurred in the temperature range 130-200 °C, whereas all complexes **7-18** showed a weight loss corresponding to two hydrazine molecules in the range 130-175 °C (Table II). This is a direct evidence that in addition to the two sulfonamidate ligands, each complex contains two coordinated hydrazine molecules.

As shown in earlier work, sulfonamides of this type possess a complicated coordination chemistry[2,3] but generally in mononuclear complexes they act either as bidentate ligands (interacting with the metal ions by means of the sulfonamido nitrogen and an endocyclic atom, usually the N-3 of the 1,3,4-thiadiazole or benzothiazole rings, or S-1 in the thienothiopyran ring[2-7 17 18]) or as monodentate ligands, interacting with the metal ions by means of the deprotonated sulfonamido moiety. Taking into account these data and considering hydrazine a monodentate ligand, complexes **7-18** reported here may possess either an octahedral structure of type **19** (with a ligand of the 1,3,4-thiadiazole type shown in a simplified manner bellow, but the same type of structure apply for the benzothiazole derivative **3**) or the tetrahedral geometry **20** (with the organic ligand in deprotonated state shown schematically as $ArSO_2NH^-$).



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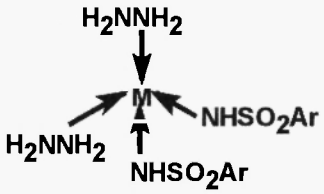

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Although appropriate crystals for X-ray diffraction experiments could not be obtained in order to assign the precise structure to the synthesized complexes, by comparing their spectroscopic properties with those of compounds structurally characterized by the above mentioned technique,[17,18] the geometry of type

Table III : CA I and II inhibition data, for CO_2 hydration, with compounds **1-18**, determined by Maren's method. [20]

Compound	IC_{50} (nM)[a]	
	CA I	CA II
1	200	7
2	10	9
3	1	0.6
4	$>5x10^6$	2
5	2	1
6	1	1
7	110	3
8	7	4
9	0.8	0.2
10	5,800	0.5
11	1	0.4
12	0.7	0.2
13	60	2
14	4	3
15	0.1	0.1
16	5,300	0.4
17	0.8	0.1
18	0.4	0.1

[a]Molarity of inhibitor producing a 50% decrease of enzyme specific activity for CO_2 hydration at 0°C.

20 appear most probable for all the Zn(II) complexes prepared in the present study, whereas for the Cd(II) derivatives both octahedral (**19**) as well as tetrahedral (**20**) geometries are possible. Mention should be made that derivatives of type **20** mimic the metal center in inhibited CAs, as previously reported by Vahrenkamp's[18a] and Borras' [18b-d] X-ray crystallographic determinations of Zn(II) derivatives containing ammonia, acetazolamide or methazolamide as ligands. What we also want to stress is the fact that benzolamide **5** and chlorzolamide **6** seem to have a similar coordination chemistry in their zinc and cadmium complexes with acetazolamide and methazolamide, to which they are structurally related.

CA inhibition data with the prepared complexes and the original ligands, towards human red cell isozymes CA I and CA II, are shown in Table III.

As seen from the above data, all complexes **7-18** behave as extremely effective inhibitors for both investigated isozymes. Although the original ligands **1-6** act themselves as strong inhibitors, complexes are more effective due to their dual mechanism of action mentioned above (see Introduction). Generally the Cd(II) derivatives were more effective than the corresponding Zn(II) ones, but differences are not very significant, and depended more upon the efficiency of the original ligand contained in the complex.

Although clinical applications were not envisaged yet for this type of CA inhibitors, it should be mentioned that their strong inhibitory properties as well as the presence of metal ions, might be exploited in states in which this phenomenon is desirable, such as inhibition of gastric acid secretion. It was shown that high doses of acetazolamide constitute a good treatment of gastric ulcer, due to CA inhibition in gastric mucosa.[22] Complexes as these described here (the Zn(II) derivatives presumably having a low toxicity) or Al(III) derivatives reported previously[23] might constitute a good approach in treating ulcer, since in addition to powerful CA inhibition, such compounds would also possess antacidic properties. Work is in progress in these laboratories for the pharmacological evaluation of some of these complexes as possible antiulcer drugs.

References

1. Part 4 of this series: C.T.Supuran, *Main Group Metal Chem., submitted.*
2. C.T.Supuran, "Carbonic anhydrase inhibitors", in "Carbonic Anhydrase and Modulation of Physiologic and Pathologic Processes in the Organism", I.Puscas Ed., Helicon, Timisoara, **1994**, pp. 29-113.
3. G.Alzuet, S.Ferrer, J.Borràs and C.T.Supuran, *Roum.Chem.Quart.Rev.*, **1994**, *2*, 283-300.
4. C.T.Supuran, *Rev.Roum.Chim.*, **1992**, *37*, 849-855.
5. C.T.Supuran, G.Manole and M.Andruh, *J.Inorg.Biochem.*, **1993**, *49*, 97-103.
6. S.Ferrer, G.Alzuet and J. Borràs, *J.Inorg.Biochem.*, **1989**, *37*, 163-174.
7. C.Luca, M. Bârboiu and C.T.Supuran, *Rev.Roum.Chim.*, **1991**, *36*, 1169-1173.
8. T.H.Maren, B.W.Clare and C.T.Supuran, *Roum. Chem. Quart. Rev.*, **1994**, *2*, 259-282.
9. D.N.Silverman and S.Lindskog, *Acc.Chem.Res.*, **1988**, *21*, 30-36.
10. C.T.Supuran, *Metal Based Drugs, submitted.*
11. a)T.H.Maren, *Physiol.Rev.*, **1967**, *47*, 595-782; b) T.H.Maren, *J.Glaucoma*, **1995**, *4*, 49-62.
12. J.J.Baldwin, G.S.Ponticello, P.S.Anderson, M.E.Christy, M.A.Murcko, W.C.Randall, H.Schwamm, M.F. Sugrue,J.P. Springer, P. Gautheron, J. Grove, P. Mallorga, M.P.Viader, B.M.McKeever, and M.A. Navia, *J.Med.Chem.*, **1989**, *32*, 2510-2513.
13. W.F.Brechue, *Roum.Chem.Quart.Rev.*, **1994**, *2*, 301-311.
14. C.T.Supuran *Metal-Based Drugs*, **1995**, *2* (in press).
15. T.H.Maren, in "Orphan Drugs", F.E..Karch Ed., M.Dekker, New York, 1982, pp. 89-115.
16. S.Lindskog and P.J.Wistrand, in "Design of Enzyme Inhibitors as Drugs", M.Sandler and H.J.Smith Eds., Oxford Univ.Press, Oxford, 1989, pp. 698-723.
17. a) S.Ferrer, J.G.Haasnoot, R.A.G. de Graaff, J.Reedijk and J.Borràs, *Inorg.Chim.Acta*, **1992**, *192*, 129-138; b) G.Alzuet, S.Ferrer, J.Borràs, A.Castineiras, X.Solans and M.Font-Bardia, *Polyhedron*, **1992**, *11*, 2849-2856.
18.a) U.Hartmann and H.Vahrenkamp, *Inorg.Chem.*, **1991**, *30*, 4676; b) U.Hartmann and H.Vahrenkamp, *Chem.Ber.*, **1994**, *127*, 2381-2385; c) U.Hartmann and H.Vahrenkamp, *Z.Naturforsch.*, **1994**, *49b*, 1725-1729; d) G.Alzuet, L.Casella, A. Perotti and J.Borràs, *J.Chem.Soc.Dalton Trans.*, **1994**, 2347-2351. e)

G.Alzuet, J.Casanova, J.A.Ramirez, J.Borràs and O.Carugo, *J.Inorg.Biochem.*, **1995**, *57*, 219-234; f) L. Sumalan, J.Casanova, G.Alzuet, J.Borràs, A.Castineiras and C.T.Supuran, *J.Inorg.Biochem.*, *in press*
19. a) J.Korman, *J.Org.Chem.*, **1958**, *23*, 1768-1772; b) R.O.Roblin, and J.W.Clapp, *J.Am.Chem.Soc.*, **1950**, *72*, 4890-4892.
20. T.H.Maren, *J.Pharmacol.Exp.Ther.*, **1960**, *130*, 26 -30.
21. H.Abdine, M.A.Elsayed and Y.M.Elsayed, *J.Assoc.Off.Anal.Chem.*, **1978**, *61*, 695-701.
22. I.Puscas, in "Carbonic Anhydrase and Modulation of Physiologic and Pathologic Processes in the Organism", I.Puscas Ed., Helicon, Timisoara, **1994**, pp. 438-450.
23. C.T.Supuran and M.Andruh, *Rev.Roum.Chim.*, **1994**, *39*, 1229-1234.

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