COMPLEXES WITH BIOLOGICALLY ACTIVE LIGANDS. Part 3 [1] SYNTHESIS OF COORDINATION COMPOUNDS OF CHLOROTHIAZIDE WITH DIVALENT METAL IONS

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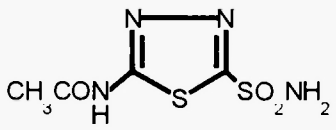
Abstract: Complexes of chlorothiazide (6-chloro-7-sulfamoyl-1,2,4-benzothiadiazine-1,1-dioxide) - a widely used diuretic and antihypertensive agent - with divalent metal ions (Be(II); Mg(II); Zn(II); Cd(II); Hg(II) and Pb(II)) were prepared and characterized by elemental analysis, spectroscopic, thermogravimetric, and conductimetric measurements. The complexes were tested as inhibitors of the enzyme carbonic anhydrase (CA).

Introduction

A large number of coordination compounds of heterocyclic and aromatic sulfonamides[2,3] containing transition and main-group metal ions were recently reported, in connection with our interest to develop novel types of inhibitors of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1).[4]

Derivatives used in such studies included clinically employed CA inhibitors, of the heterocyclic sulfonamide type, such as acetazolamide **1**;[5] methazolamide **2**;[6] ethoxzolamide **3**;[7] or the thienothiopyran sulfonamide **4**[8] among others. Recently we also reported[9] the complexes of diazoxide **5** (HDZO), a benzothiadiazine possessing interesting pharmacological activity and used clinically as hyperglycemic drug.[10]



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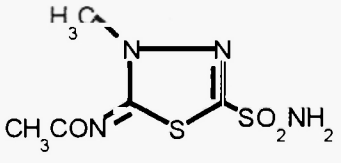

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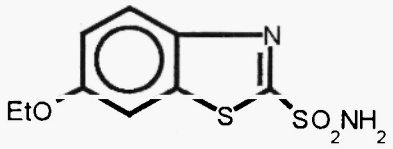

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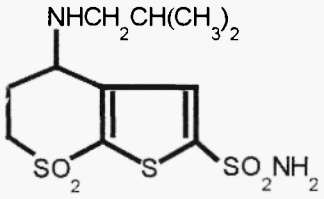

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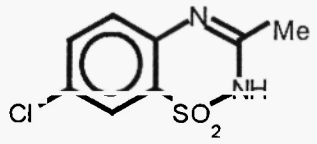
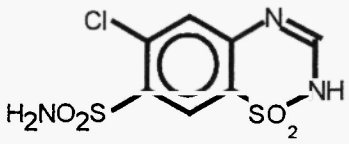


5: HDZO



6: HCTZ

As 1,2,4-benzothiadiazine-1,1-dioxide derivatives possess prominent biological activity and many clinical applications,[10,11] this paper is an extension of the chemistry of this class of derivatives and reports the preparation and characterization of divalent metal complexes of chlorothiazide 6, HCTZ, a

compound related to **5**, which is an important diuretic drug, widely employed clinically as antihypertensive agent.[11]

Coordination compounds of chlorothiazide with divalent metal ions such as Be(II); Mg(II); Zn(II); Cd(II); Hg(II) and Pb(II) were prepared and characterized by elemental analysis, IR-, electronic and ^{1}H-NMR spectroscopy, as well as thermogravimetric and conductimetric measurements. The new compounds were tested for inhibition against two CA isozymes (human I and II red cell CAs), and they behave as stronger inhibitors as compared to the parent ligand.

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.

Chlorothiazide, metal salts and solvents were from Aldrich and were used without further purification. Human isozymes CA I and II, buffers and 4-nitrophenyl acetate were from Sigma. Inhibitors were assayed spectrophotometrically at 400 nm, by the method of Pocker and Stone[12] for the inhibition of 4-nitrophenyl acetate hydrolysis catalyzed by the two CA isozymes. The buffer used in the enzymatic assay (Tris-H_2SO_4 10 mM) was brought to an ionic strength μ = 0.1, by addition of K_2SO_4. Enzyme concentrations used in the experiments were 10 μM for CA I and 1.4 μM for CA II. The total acetonitrile concentration in the assay cell was 10% (v/v) both in the absence and in the presence of complex inhibitors, and this should not lead to errors due to CA inhibition by MeCN.

Initial rates of 4-nitrophenyl acetate hydrolysis were monitored spectrophotometrically, at 400 nm and 25°C, with a Cary 3 apparatus interfaced with an IBM compatible PC. Solutions of substrate were prepared in anhydrous acetonitrile; the substrate concentrations varied between 10^{-2} and 10^{-4} M. A molar absorption coefficient ε = 18400 $M^{-1}.cm^{-1}$ was used for the 4-nitrophenolate formed by hydrolysis, in the conditions of the experiments (pH 7.80), as reported by Pocker and Stone.[12] Non-enzymatic hydrolysis rates were always subtracted from the observed rates. Duplicate experiments were done for each inhibitor, and the values reported throughout the paper are the averages of such results.

Synthesis of coordination compounds 8-12

A *cold* solution of chlorothiazide sodium salt (NaCTZ, 7) was prepared by suspending HCTZ in the stoichiometric amount of 2N NaOH solution at 0-5°C. Mention should be made that the benzothiadiazine ring is not very stable in the presence of bases, being cleaved to orthanilamide derivatives.[11a,13] Still, at room temperature and in 2N NaOH, this ring is cleaved in about 150 hours,[13] so that, presumably, no decomposition occurred during the experiments reported here, in which complexes were prepared in about 0.5 - 1 hours. The cold solution obtained above, was mixed with a methanolic-aqueous solution of metal salts MCl_2 in molar ratios of 2:1. The obtained reaction mixture was stirred magnetically at room temperature for 0.5 - 1 hours. The obtained complexes were collected by filtration and air-dried. Mention should be made that preparation of the monosodium salt of chlorothiazide **7** is essential for obtaining the type of complexes described here. Chlorothiazide, possessing two sulfonamido moieties (the endocyclic SO_2NH and the 7-substituent, a SO_2NH_2 group), is a diprotic acid, with pK_{a1} of 8.4-8.5, and pK_{a2} of 10.3-10.4. [14] We assign the most acidic pK_a to the SO_2NH moiety (as in the related diazoxide **5**[9]), in contrast to earlier reports from Malmstrom's group[15] that considered the primary sulfonamido moiety as the stronger acid. Obviously, with two equivalents of NaOH, a disodium salt of chlorothiazide can be obtained, which may participate in formation of complexes different of those reported here.

Results and Discussion

Starting from the monosodium salt of chlorothiazide, NaCTZ **7** , prepared *in situ* from **6** and the stoichiometric amount of NaOH (the pK_a of the SO_2NH moiety of diazoxide is around 8.5)[14] both transition- as well as main-group metal complexes were obtained. Cations which led to strong CA inhibitors in complexes with sulfonamides **1-5**[1,2,3-9] were included in the present study, such as Be(II); Mg(II); Zn(II); Cd(II); Hg(II); Pb(II). The synthesized compounds **8-13** and their elemental analysis data (± 0.4% of the theoretical values, for C,H,N, by combustion, and for M by gravimetry) are shown in Table I.

Table 1: The prepared chlorothiazide complexes **8-13**, and their elemental analysis and TG data (CTZ stands for the endocyclic SO_2NH deprotonated species of **6**).

No.	Compound	Analysis (calc./found)				
		%M[a]	%C[b]	%H[b]	%N[b]	$\%H_2O$[c]
8	$[Be(CTZ)_2(OH_2)_2]$	1.3/1.5	29.0/28.8	3.0/3.1	12.6/12.5	5.4/5.2[d]
9	$[Mg(CTZ)_2(OH_2)_4]$	3.4/3.5	26.9/26.7	3.3/3.3	11.7/11.5	10.1/10.0[e]
10	$[Zn(CTZ)_2(OH_2)_2]$	9.1/9.2	26.7/26.6	2.7/2.6	11.7/11.7	5.0/5.1[d]
11	$[Cd(CTZ)_2(OH_2)_4]]$	14.0/14.0	23.9/23.7	3.0/3.0	10.4/10.5	8.9/8.7[e]
12	$[Hg(CTZ)_2(OH_2)_4]$	22.5/22.3	21.5/21.2	2.7/2.5	9.4/9.1	8.1/8.0[e]
13	$[Pb(CTZ)_2(OH_2)_4]$	23.1/23.0	21.4/21.2	2.6/2.5	9.3/9.1	8.0/7.8[e]

[a]By gravimetry; [b]By combustion; [c]By TG analysis; [d] Corresponding to 2 H_2O, lost between 150-175°C; [e] Corresponding to 4 H_2O, lost between 150-180°C.

The new complexes **8-13** were also characterized by IR-, electronic and ^{1}H-NMR spectroscopy as well as by thermogravimetric and conductimetric measurements (Table II).

In the IR spectra of the new complexes, the following differences were evidenced as compared to the spectra of the original sulfonamide **6** or its sodium salt **7**: (i) important changes in the region 1100-1400 cm^{-1}, where the sulfonamido vibrations are located. Thus, in the spectrum of chlorothiazide four such vibrations appear, two symmetrical at 1117 and 1180 cm^{-1},and two antisymmetrical, at 1325 and 1377 cm^{-1} respectively. In the spectrum of the sodium salt **7**, these vibrations undergo little changes, consisting in small shifts (3-5 cm^{-1}) towards higher wavenumbers. In complexes **8-13** (see Table II) one of these bands (more precisely the first symmetrical and the first antisymmetrical ones) appear shifted with 20-32 cm^{-1} towards lower wavenumbers, proving that the corresponding sulfonamido moiety interacts with the metal ions. This behavior was previously documented for the metal complexes of sulfonamides **1-5** described by us and Borras' group.[1-3,5-9] But in contrast to derivatives of type **1-5**, chlorothiazide contains two sulfonamido groups, and probably this is the reason why two bands were detected in the IR spectra both for symmetrical as well as antisymmetrical vibrations. As seen from data of Table II, only one of these bands was shifted towards lower wavenumbers in the IR spectra of the prepared complexes. We propose that the first of these bands (the ones undergoing the shift) is due to the SO_2NH moiety, which when deprotonated interacts with the metal ions in complexes **8-13**, whereas the bands at 1180 and 1377 cm^{-1}, respectively are due to the primary sulfonamido moiety, which does not participate to coordination; (ii) the NH vibrations appearing at 3080 cm^{-1} in **6** undergo minor changes in complexes **8-13** (as the band now seen is broader and not so well resolved in the spectra of the coordination compounds), whereas for the monosodium salt this vibration appears at 3065 cm^{-1}; (iii) the appearance of bands in the region 350-380 cm^{-1}, tentatively assigned as due to M-N and/or M-O vibrations.

Table II: Spectroscopic and conductimetric data of compounds **6-12**.

No.	IR Spectra [a]			UV Spectra [b]	Conductimetry [c]
	$\nu_{(M-N)}$	$\nu_{(SO_2)}^{s}$	$\nu_{(SO_2)}^{as}$	$\nu_{(NH)}$	λ_{max} (log ε)	Λ_M
	[cm^{-1}]			[nm] ([L x mol^{-1} x cm^{-1}])	[$\Omega^{-1}xcm^2xmol^{-1}$]
6	-	1117, 1180	1325, 1377	3080	278 (3.82); 328 (2.13)	12
7	-	1121, 1185	1328, 1382	3065	323 (4.18)	110
8	370	1085, 1180	1305, 1376	3080, br	323 (4.70)	11
9	380	1091, 1180	1302, 1377	3080, br	323 (4.65)	17
10	380	1092, 1180	1300, 1376	3080, br	323 (4.69)	21
11	365	1095, 1178	1304, 1375	3080, br	323 (4.57)	19
12	358	1093, 1179	1298, 1377	3080, br	323 (4.72)	15
13	350	1094, 1180	1295, 1377	3080, br	323 (4.71)	17

[a] In KBr pellets; [b] In MeCN solution; [c] Solution 10^{-4} M, in DMF, at 25°C

In the solution electronic spectrum of chlorothiazide (in acetonitrile) two absorption maxima were detected, at 278 and 328 nm, respectively, which in the case of the monosodium salt led to only one large band at 323 nm, as documented for related systems.[16] The same broad band was detected in complexes **8-13** too, proving that **6** interacts in the monodeprotonated state with the metal ions. From conductimetric data (Table II) it can be seen that the ligand and the complexes are non-electrolytes, whereas the monosodium salt is a 1:1 electrolyte. Another proof that only the SO_2NH moiety interacts with the metal ions in the prepared complexes, is constituted by the fact that in the ^{1}H-NMR spectra of compounds **6-13**, the broad signal of the SO_2NH_2 protons, at 7.64 ppm, remains unchanged, whereas the signal of the SO_2NH moiety, which in the spectrum of **6** appeared at 7.88 ppm is absent in the spectra of compounds **7-13** (the two mentioned signals disappear when D_2O is added into the NMR tube). The signals of the aromatic protons of **6** (consisting of three singlets, at 7.14, 7.32 and 7.51 ppm, respectively) appeared at the same chemical shifts (± 0.02 ppm) in the ^{1}H-NMR spectra of complexes **8-13**.

All these data, as well as the TG measurements reported in Table I, prompted us to propose the chlorothiazidate anion as a monodentate ligand in complexes **8-13**, interacting with metal ions by means of the N-2 nitrogen, similarly to diazoxide **5**, previously investigated by us.[9] The presence of coordinated water molecules in complexes **8-13** leads to the conclusion that Be(II) in **8** as well as Zn(II) in **10** are in tetrahedral geometries with two chlorothiazidate and two water molecules as ligands, whereas the other complexes contain octahedral M(II) ions, probably with the two organic ligands coordinated in *trans* to each other and the four water molecules occupying the equatorial positions. In these complexes the primary sulfonamido group does *not* participate to coordination of the metal ions, although working in slightly different conditions (i.e., with the disodium salt of **6**), presumably complexes in which this moiety participates to coordination might be obtained. This is also obvious from enzyme inhibition data presented bellow.

Inhibition of the two CA isozymes with compounds **6-13** as well as standard CA inhibitors of the type **1** and **5** (for comparison) are shown in Table III.

From these data it can be seen that the benzothiadiazine derivatives **5** (diazoxide) and **6** (chlorothiazide) are weaker inhibitors as compared to acetazolamide **1** (the standard and one of the most powerful CA inhibitors) towards both investigated isozymes, with CA I less susceptible to inhibition with these sulfonamides, as compared to the fast isozyme, CA II.[2,4] On the other hand it is also obvious that **6** is much more effective as CA inhibitor as compared to **5**, due to the fact that the latter compound does not possess a free SO_2NH_2 moiety.[2,4] The coordination compounds of **6** are all much more powerful inhibitors against both isozymes, with the Hg(II) derivatives reaching a 10-fold improvement of inhibition. This is obviously due to the dual mechanism of action of these inhibitors[2,3] and their interference with both steps of the catalytic mechanism. Lately, another putative mechanism to explain this powerful inhibition with coordination compounds emerged, as we evidenced[17] in the rapid isozyme, CA II (the most susceptible to

Table III : CA I and II inhibition data for 4-nitrophenyl acetate hydrolysis reaction,[12] in the presence of compounds **1, 5-13.**

Inhibitor	IC_{50} (μM)[a]	
	CA I	CA II
1	11	0.4
5	115	58
6	104	20
8	74	17
9	93	18
10	54	10
11	19	8.1
12	8	1.9
13	12	3.7

[a] Molarity of inhibitor producing a 50% decrease of enzyme specific activity for the esterasic activity of these enzymes.

inhibition with this class of inhibitors[3]) a cluster of histidine residues at the entrance of the active site. This cluster contains the following residues: His 3, His 4, His 10, His 15 and His 17, which in addition to His 64, probably constitutes the shuttle which allows protons to be transferred from the active site to the reaction medium (the proton transfer is the rate-limiting step in CA catalysis[18]). All these residues are exposed to the solvent (Fig. 1) and is probable that they can easily interact too with metal-containing derivatives such as the coordination compounds of heterocyclic/aromatic sulfonamides.

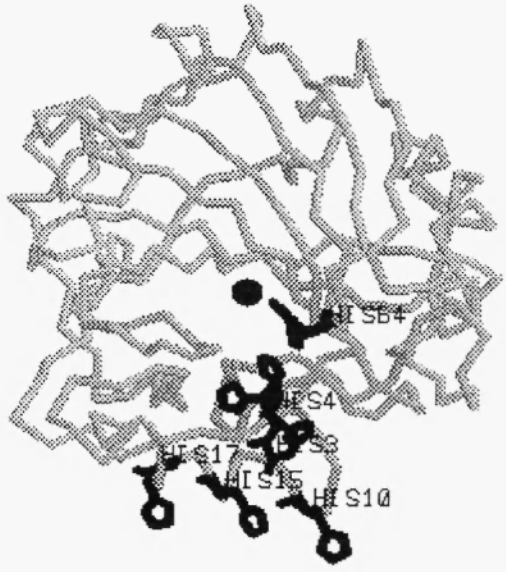


Fig. 1. The histidine cluster at the entrance of CA II active site, consisting of residues 3, 4, 10, 15, 17 and 64 (in black). The catalytically vital Zn(II) ion is shown at the center of the molecule. The crystallographic coordinates of CA II are those of Hakansson et al.[19] (at 1.54 Å resolution) and were taken from Brookhaven Protein Data Base via Internet. The programme RasMol (version 2.6) was used for simulations and generation of the above figure.

This would also explain why CA I (not possessing such a cluster) is less susceptible to inhibition with metal complexes and is also a much slower enzyme.[2,18] It is obvious thus that in order to design strong dual inhibitors both the sulfonamide ligand as well as the metal ion play an important role. Choosing metal ions having a strong affinity for imidazole-type ligands would lead to more potent inhibitors, and this is well illustrated for the Hg(II) derivatives reported here or the Cu(II) complexes of chlorothiazide reported in a separate paper.[20]

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